Keynote Address

Smokeless tobacco use is increasing throughout the United States, especially among the most vulnerable of our citizens—the children.

With more than 30,000 new cases of oral cancer reported in 1991 for the United States alone, it is time that use of smokeless tobacco take its rightful place next to cigarette smoking, in National and world consciousness, as a serious health risk that must be stopped.

As many of you recall, smokeless tobacco use as a health issue first became a subject of focus in the United States about 6 years ago. The media reported extensively at that time on the tragic consequences of smokeless tobacco use—particularly on cases of oral cancer among young men.

In 1986, the Surgeon General released a report, *The Health Consequences of Using Smokeless Tobacco*, which concluded that oral use of smokeless tobacco represents a significant health risk, is not a safe substitute for cigarette smoking, can cause cancer and a number of noncancerous oral conditions, and can lead to nicotine addiction and dependence. Also, Congress passed the Comprehensive Smokeless Tobacco Act of 1986, which banned smokeless tobacco advertising on television and radio and required that three health warnings be rotated on smokeless tobacco packages and in advertisements.

Regrettably, the subsequent downturn in sales of smokeless tobacco products was not sustained. Statistics from the 1991 Federal Trade Commission Report to Congress on smokeless tobacco sales and advertising expenditures paint a grim picture: The weight of smokeless tobacco products sold overall rose by more than 2 million pounds in 1989, to 117.5 million pounds, following a 3-year decline. In 1990, sales increased by 5 million pounds, to 122.5, and in 1991, sales again rose by almost 3 million pounds, to 125.1 million. Three companies—U.S. Tobacco, Conwood, and Pinkerton—together control over 80 percent of the smokeless tobacco market share in the United States.

The most disturbing trend is in sales of moist snuff, now the most popular—and the most dangerous—form of smokeless tobacco. While oral cancer has been shown to occur several times more frequently among snuff users than among non-users, for some cancer sites in the mouth, the disease may occur as much as 50 times more frequently among long-term snuff users.

Sales of moist snuff have risen steadily in recent years, while sales of other forms of smokeless tobacco have fluctuated or have declined. Sales of moist snuff climbed to 45.0 million pounds in 1989, a 25 percent increase

---

Based on HHS Secretary Sullivan's keynote address to the First International Conference on Smokeless Tobacco, Columbus, Ohio, April 10-13, 1991.

over the 35.9 million pounds sold in 1986. In 1990 and 1991, sales reached 52.9 million pounds, a 47 percent increase over 1986 (Figure 1). The *U.S. Distribution Journal* reported that the volume of moist snuff sales has risen 70 percent in the last 10 years.

Our most recent surveys of smokeless tobacco use nationwide revealed that in 1988, 25.8 percent of males between the ages of 12 and 17 had tried some form of smokeless tobacco, and 6.6 percent had used smokeless tobacco in the preceding month.

Newer evidence suggests the problem is becoming much more serious. For example, according to a 1991 survey by the Illinois Department of Public Health, nearly half of all high school-aged males across the state have tried smokeless tobacco in some form. About 16 percent of Illinois high school juniors are regular users, and in rural communities, the figure rises to 28 percent. Some children begin ST use as early as the second, third, or fourth grade!

The culture of smokeless tobacco use in the United States has to a large extent centered on sports, particularly baseball. When impressionable youngsters see their heroes openly using smokeless tobacco on the playing field, there is a powerful incentive to try it. These young people also begin to correlate smokeless tobacco use with athletic excellence.

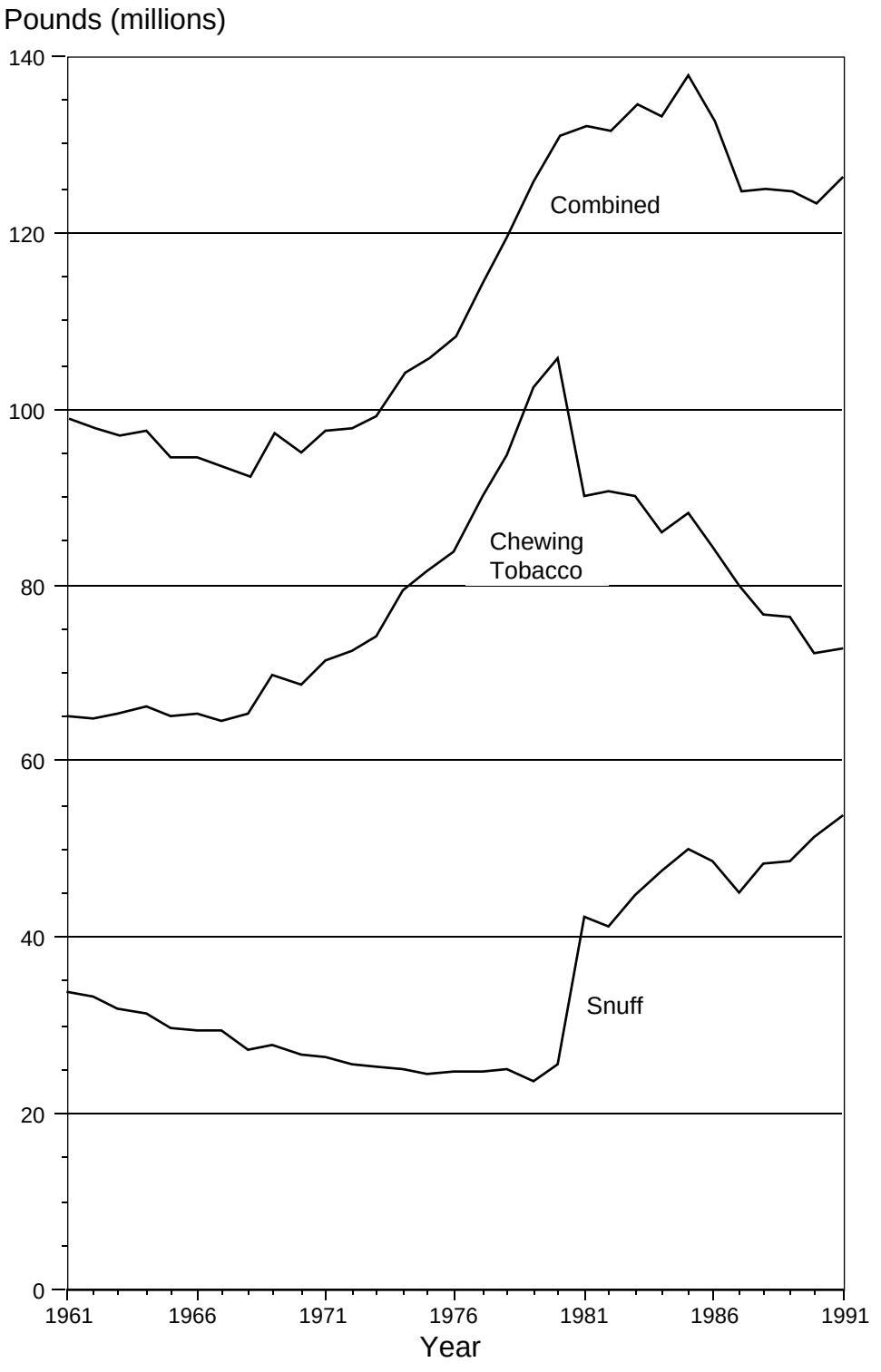
When I addressed the Major League Baseball team physicians and trainers in December of 1990, I suggested that no effort aimed at ending the use of smokeless tobacco in America would succeed without the support of the Major League Baseball community. I called upon baseball officials to develop a program with the goal of eventually eliminating smokeless tobacco use in professional baseball and in America as a whole.

I am pleased to report that the baseball community has responded positively and boldly. On March 7, 1991, as a first step in the effort to disassociate professional baseball from smokeless tobacco use, Baseball Commissioner Fay Vincent announced that the use of smokeless tobacco will be forbidden in four rookie and class A minor leagues—the levels at which most young men enter professional baseball.

The Commissioner also announced stepped-up efforts to educate players about the health risks of smokeless tobacco use and to help users quit. Major League Baseball and the National Cancer Institute have teamed up to produce "Beat the Smokeless Habit," a guide for baseball players of all ages to learn about the dangers of smokeless tobacco use and how to quit. Copies of the guide have been distributed to every major and minor league player as well as to 40,000 college baseball players. I commend Commissioner Vincent for his actions and will be following the progress of his efforts with great interest.

Now we must urge officials and coaches at the college, high school, and youth league levels to ban the use of smokeless tobacco in baseball and in other sports activities under their jurisdictions.

Figure 1
Annual consumption of spitting tobacco, United States, 1961-1991



This brings us to another significant element that serves to encourage young people to begin using smokeless tobacco—promotion and advertising by the tobacco companies.

I would like to note that expenditures by the tobacco companies to promote and advertise smokeless tobacco use rose sharply—by more than 19 percent—in 1989, according to the Federal Trade Commission. The largest single category of expenditure, nearly a quarter of total promotion and advertising expenditures for smokeless tobacco, was devoted to sports and sporting events. Another 18.5 percent was devoted to expenses involved in the insidious practice of distributing free samples.

The tobacco companies indeed play a major role in the promotion of sporting events. Philip Morris, known for its Virginia Slims Tennis Tournaments, is now the number one event sponsor in sports, according to *The Sporting News*, spending about $100 million annually. RJR Nabisco spends about $40 million. The tobacco product these two companies promote, of course, is cigarettes.

It is not surprising, therefore, that when *The Sporting News* last year compiled a list of who they believe to be the 100 most powerful people in sports in the United States, the marketing vice presidents of Philip Morris and RJR Nabisco were ranked 29th and 49th, respectively.

This represents a sad commentary on the state of sports in America. Without question, by sponsoring sporting events, the tobacco companies are trading on the prestige and image of the athletes to barter their deadly products. They are using the vigor and energy of these athletes as a subtle—but incorrect and dishonest—message that tobacco use is compatible with good health. And, all too often, that message is aimed at young people, women, minorities, and blue-collar workers.

We must question seriously the values that allow activities ostensibly representing the essence of fun, fitness, and health to be exploited to such a large degree by the merchants of suffering, disease, and death.

The time has come to end the association of tobacco and sports in this country and around the world. The time has come for promoters of and participants in sporting events to be held accountable for the fact that when they accept money from the tobacco companies, they are promoting not only fun and games—they are promoting disease and death.

It is immoral for civilized societies to condone the promotion and advertising of products that, when used as intended, cause disability and death.

I am all too aware that such activities are allowed under current law in the United States and elsewhere. And I am, quite frankly, disgusted that this remains the case. We react with horror and outrage when we hear of suffering and death overseas. Yet, at the same time, we not only allow our citizens to suffer and die from the poisonous chemicals contained in tobacco, we condone expenditures of vast amounts of money by the tobacco companies to encourage it!

In 1990, I urged the tobacco industry to voluntarily withdraw from direct sponsorship of sporting events. Obviously, my plea fell on indifferent ears.

If the tobacco companies will not adhere to this country's strong philosophy of voluntary corporate responsibility, then it is up to our citizens to provide the incentive. As individuals, Americans can send a message to the tobacco companies in the only language they appear to understand—the language of money. The message is that we will no longer financially support promoters of sporting events and others who would encourage our children to use addictive substances that will ruin their health and send them to an early grave. And I urge public and private institutions to refrain from allowing their facilities to be used for tobacco company-sponsored sporting events.

The disgraceful tradeoff in America between profits and good health must stop! But it will stop only when our citizens rise up and say, "Enough—no more!"

I urge individuals and organizations throughout the Nation and the world to join me in the expression of anger and resolve. Let this be the beginning of an all-out effort.

Make no mistake: the continuing battle against tobacco use will be long and difficult. But it is a battle that can be won, must be won, and will be won. Together, we will win it.

Louis W. Sullivan, M.D.
Secretary
Department of Health
and Human Services

Foreword

Tobacco use in the United States has gone through many stages. Initially tobacco was used by Native Americans in religious ceremonies, with apparently little or no routine usage. The colonists, however, adopted tobacco for secular uses and quickly established it as a trading commodity. Toward the end of the 19th century, smoking tobacco was found primarily in cigars, pipes, or roll-your-own cigarettes; less than 3 percent of all tobacco consumed was in the form of manufactured cigarettes. By contrast, more than 50 percent was consumed as chewing tobacco.

The changing social acceptance of the manufactured cigarette, together with the use of milder, blended tobaccos, beginning around 1913, allowed tobacco users to absorb nicotine more quickly and efficiently through inhalation than via absorption through the oral mucosa. Thus, by the end of World War I, cigarette smoking had become not only socially acceptable but even fashionable—at least for men. By the early 1920's, tobacco consumed in the form of cigarettes had increased considerably, accounting for fully 25 percent of all tobacco being used in the United States.

The 1920's and 1930's saw a major increase in the number of male smokers, with the 1940's and 1950's characterized as the beginning of this country's lung cancer epidemic. These time frames illustrate the 20- to 30-year lag time between the initiation of regular smoking and a clinical diagnosis of lung cancer. This lag time was observed again with the increased use of cigarettes by women in the 1940's and 1950's and the subsequent increase in lung cancer among women in the 1970's.

Social norms have played a major role in the history of tobacco use. In the early part of this century, many antitobacco laws were based on morals and religion.[1] During the latter half of the 20th century, however, medical science has provided the foundation for a rational public health policy on tobacco use. When the landmark 1964 Surgeon General's Report identified smoking as the major cause of lung cancer, smoking rates dropped. Subsequent reports from the Surgeon General have reinforced the public's consciousness of the multitude of hazards in exposure to tobacco smoke—for both the smoker and the nonsmoker.

Paradoxically, it was the heightened awareness of smoking hazards in the 1970's and early 1980's that prompted some people, looking for a safe alternative to cigarettes, to begin using a product that the industry labeled "smokeless" tobacco. Early advertising campaigns pitched these products as a safe alternative because they did not contain the major health-threatening

[1] The use of spitting tobacco fell into social disfavor immediately after the turn of the century because of concern that exposure to saliva increased the spread of tuberculosis, a major uncontrolled cause of death at that time. Many towns and cities enacted local ordinances against spitting in public.

ingredient of cigarettes—*smoke*. Millions of consumers succumbed to this faulty logic, and the use of spitting tobacco (chewing tobacco and snuff, or ST) spread rapidly, particularly among young adult and adolescent males, as Figure 1 illustrates.

Unfortunately, the tobacco industry's ability to increase demand through new and innovative marketing strategies has always outpaced the ability of the scientific community to document the adverse health effects resulting from product use. The ST industry began to aggressively advertise and promote new product lines, often using well-known figures in sports and entertainment to pitch these products on television during prime time. The use of TV and radio for cigarette advertising had been banned by the Congress since 1971, but the legislation did not address other tobacco products.

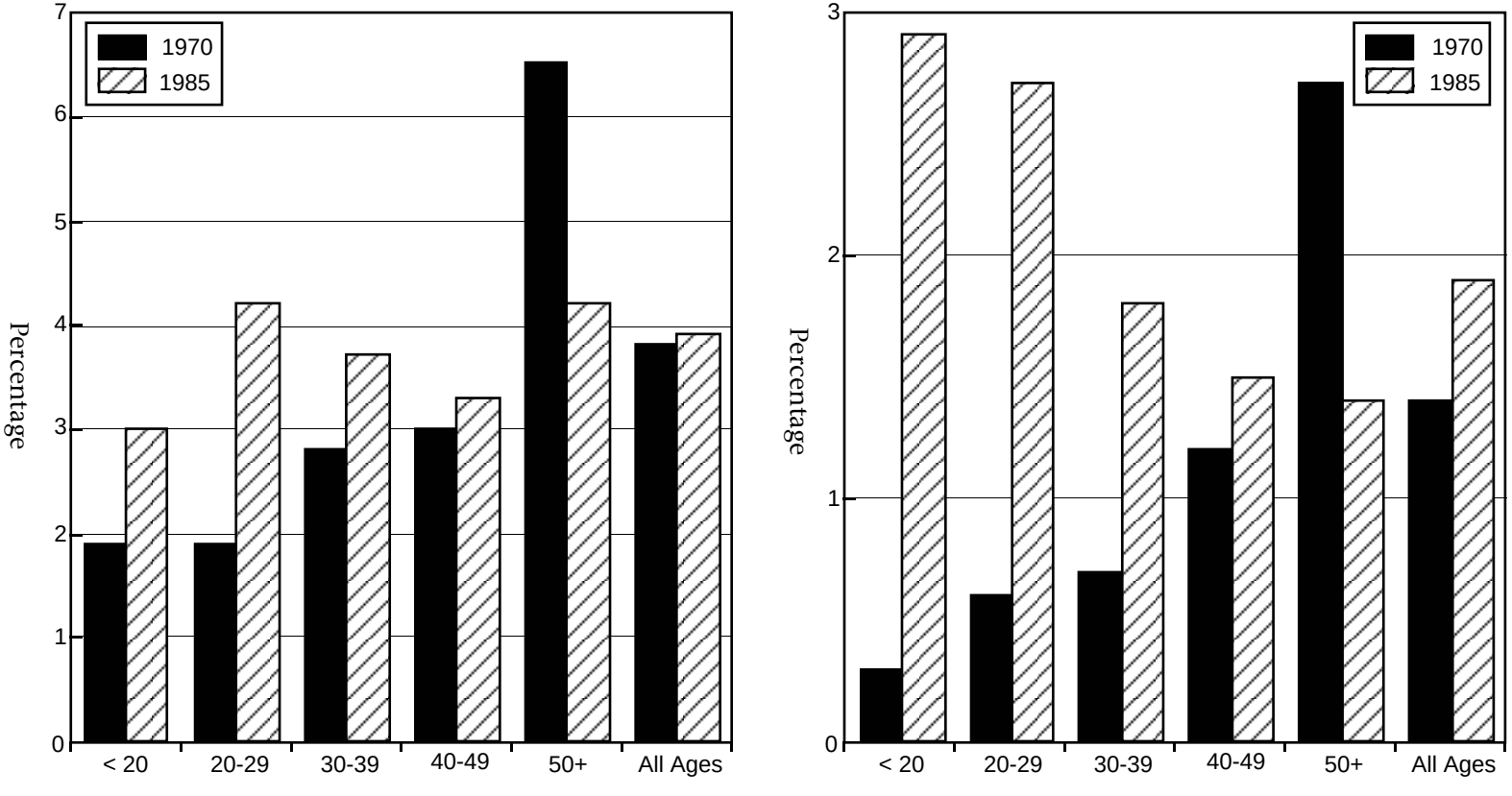
Particularly alarming is that ST advertising has been most effective with boys and young men. Although the marketing of ST products is legal, current marketing strategies are persuading many individuals to become regular users before reaching 18, the legal age to buy the products in most states. One advertising campaign (see Figure 2) used National Football League stars to teach "beginners" how to work up to stronger brands of spitting tobacco. In the wake of these promotions, the consumption of spitting tobacco, especially moist snuff, has continued to rise. From 1972 to 1991, total U.S. consumption of spitting tobacco has risen from 99 million pounds per year to 125 million pounds. There has been an alarming 40 percent increase in consumption of moist snuff, the most dangerous form of spitting tobacco.

The increase in ST consumption stands in sharp contrast to the significant decline in consumption observed in all other tobacco categories. Over the past decade alone, total cigarette sales have decreased by more than 130 billion cigarettes, and our national per capita consumption is at its lowest level since the early 1940's (Figure 3). Because of the 20- to 30-year lag time between large-scale exposure to a carcinogenic agent and subsequent increases in cancer mortality, the trends in ST consumption predict a public health problem in the making, unless significant behavior changes can be achieved soon.

In 1981 National Cancer Institute (NCI) investigator Deborah Winn and colleagues published a seminal study that pointed to a link between oral snuff dipping and oral cancer. Later that year, after reviewing the evidence, the Institute announced that it believed that snuff use was a cause of oral cancer. In 1984 the Smoking and Tobacco Control Program of NCI issued a Request for Applications, soliciting research on the use of spitting tobacco, and the following year NCI funded the first clinical trials aimed at intervening in ST use.

The year 1985 saw a flurry of activity related to spitting tobacco. In February, the National Cancer Advisory Board issued a resolution on smokeless tobacco, in which it stated that the NCAB "considers the use of smokeless tobacco to pose a serious and increasing health risk." The board also

Figure 1
Prevalence of the use of chewing tobacco (left) and snuff (right) among males, 1970 and 1985



Source: 1970 National Health Interview Survey and 1985 Current Population Survey

Figure 2
ST ad aimed at "beginners"

Walt Garrison answers your questions about smokeless tobacco.

Q: *Walt, just what is Moist Smokeless Tobacco?*
A: It's just what it says: Tobacco you enjoy without lighting up.

Q: *How do you use it?*
A: First, you don't chew it. Just take a small pinch between your thumb and forefinger, put it between your cheek and gum, and leave it there. The tobacco will slowly release its great flavor to give you real tobacco satisfaction.

Q: *Is it hard to use?*
A: Not really. When you first try it, the tobacco may move around in your mouth more than it should, and your mouth may water a bit more than you're used to, but getting the hang of "going smokeless" is all part of the fun. In a couple of weeks you'll be a "pro."

Q: *Is there a difference between the three most popular brands?*
A: Sure...**HAPPY DAYS** is a mild, satisfying blend of mint-flavored tobacco, while **SKOAL** is full-bodied with the added good taste of wintergreen. **COPENHAGEN** is a stronger, natural blend of choice tobaccos.

All three are packed in convenient cans and each is dated for freshness.

Q: *Is the date on the can the expiration date?*
A: No, it's the date of manufacture. It's our way of letting you know how fresh and moist our tobacco is.

Q: *How much does "Going Smokeless" cost?*
A: An average user "dips" about 1½ cans per week, and that's about a dollar's worth. Not bad, when you think how much everything else costs these days.

Q: *Do a lot of people use smokeless tobacco?*
A: A lot more than you think. Last year we sold over 325 million cans. And more and more people from every part of the country are "going smokeless" all the time. (Even loose-leaf chewers are mixing it in with their brands for extra flavor.)

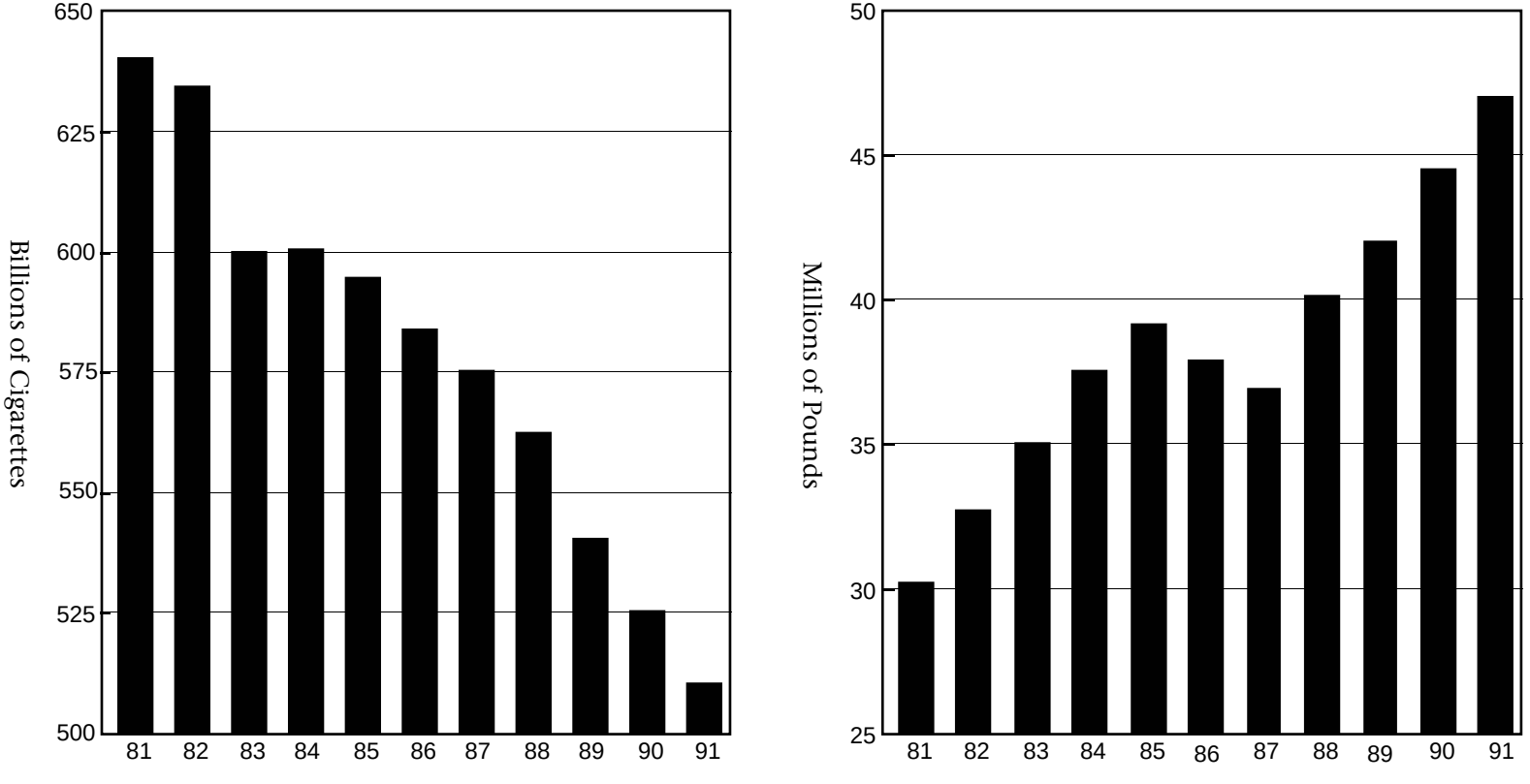
Q: *Where can I buy it?*
A: Ask for it at your favorite tobacco counter; or mail the coupon below and you'll get a free can of **HAPPY DAYS** to try.
Thanks a lot, Walt....

A pinch is all it takes!™

recommended that the Surgeon General undertake a complete review of the scientific evidence on ST's health effects, and that the Office on Smoking and Health include ST use as part of its ongoing surveillance activities. Subsequently, the Office on Smoking and Health sponsored a supplement to the Current Population Survey to collect data on current usage of smokeless tobacco. The Surgeon General at that time, Dr. C. Everett Koop, appointed a PHS Advisory Committee to report on ST's health consequences.

In September 1985, the International Agency for Research on Cancer (IARC) in Lyon, France, issued a report that concluded, "In aggregate, there is sufficient evidence that oral use of smokeless tobacco is carcinogenic to humans." Then, in January 1986, an NIH-NCI Consensus Development Conference on Smokeless Tobacco was held; the participants concluded that there was strong evidence from human studies to link snuff use with cancer.

Figure 3
U.S. tobacco consumption of cigarettes (left) and moist snuff (right), 1981 to 1991



Source: U.S. Department of Agriculture

The Surgeon General's Advisory Committee Report, compiled and published under NCI auspices, was issued in April and concluded that

> *"oral use of smokeless tobacco represents a significant health risk. It is not a safe substitute for smoking cigarettes. It can cause cancer and a number of noncancerous oral conditions and can lead to nicotine addiction and dependence."*

The committee also concluded that evidence related to the carcinogenic potential of chewing tobacco was limited, but the evidence on oral use of snuff was more than sufficient to conclude that it is carcinogenic to humans.

The PHS Advisory Committee also noted that snuff contained *N'*-nitrosamines at levels 100 times higher than the levels permitted under Federal regulations for all other ingested consumer products in the United States. The levels of nicotine absorbed during ST use were found to be high and to remain elevated for most of the day. Later in the same year, Congress passed the Comprehensive Smokeless Tobacco Health Education Act of 1986, which banned advertising on broadcast media and required rotating warning labels on ST products and in print advertising.

In 1989, NCI issued its first monograph on this subject, *Smokeless Tobacco Use in the United States*, which reinforced the overall conclusion of the 1986 Report of the Surgeon General, that "there is no safe form of tobacco use." The monograph also contained results from the Current Population Survey sponsored by the Office on Smoking and Health, which found that ST use varied considerably by region of the country, with rates among adult males in many southern states exceeding 12 percent, reaching 23 percent in West Virginia.

In April 1991, NCI cosponsored the First International Conference on Smokeless Tobacco. From this conference, the world learned that ST use is a problem in many countries, and the types of ST used are diverse, but the potential for addiction and oral cancer and other untoward health effects are omnipresent. This monograph presents a selection of peer-reviewed papers from that valuable conference.

We have made significant advances in our understanding of the health consequences associated with ST use, but much more remains to be done. NCI is committed to the continued support of research on spitting tobacco, with the eventual goal of making the use of this deadly and addictive product an extinct behavior. Research alone, however, will not solve this growing health threat. Thus, the reduction of ST use is a high priority within ASSIST—the American Stop Smoking Intervention Study for Cancer Prevention, a 17-state, NCI-funded, demonstration project that began last October. Over the next 7 years, ASSIST is expected to reach more than 90 million individuals, including 20 million tobacco users. While ASSIST is aimed primarily at cigarette smokers, an estimated 1 million or more regular ST users will be targeted for the project interventions.

We have made great progress over the past 40 years in making the United States a smoke-free society. Our task will not be complete however, until we make the nation free of tobacco use in *all form*s. To accomplish this will require the full cooperation of public and private agencies, research and service organizations, professionals and laypeople, working together toward this common goal. To do otherwise would invite unnecessary suffering, disease, and death.

Antonia C. Novello, M.D., M.P.H.
Surgeon General

Contents

Chapter 2 (continued)

Chapter 3 (continued)

Chapter 3 (continued)

Acknowledgments

Smokeless Tobacco or Health: An International Perspective was developed under the general editorship of the Smoking and Tobacco Control Program (STCP), **Donald R. Shopland**, Coordinator, National Cancer Institute, Bethesda, Maryland. The senior scientific editor was **R. Craig Stotts**, Dr.P.H., R.N., Research Fellow, NCI Division of Cancer Prevention and Control. The consulting scientific editors were **Kathleen L. Schroeder**, D.D.S., M.Sc., West Virginia University Schools of Medicine and Dentistry, and **David M. Burns**, M.D., Professor of Medicine, University of California, San Diego.

Special recognition is due Dr. Schroeder for her role in planning and organizing the First International Conference on Smokeless Tobacco, from which these monograph papers were developed. Members of the committee that assisted Dr. Schroeder in planning the conference are listed near the end of this section.

The staff of the NCI Smoking and Tobacco Control Program gratefully acknowledges the authors who made this monograph possible. Attributions for each chapter, in alphabetical order, are as follows:

Chapter 1 Epidemiology

Virginia L. Ernster, Ph.D.
Department of Epidemiology and Biostatistics
School of Medicine
Department of Dental Public Health and Hygiene
School of Dentistry
University of California, San Francisco
San Francisco, California

Elbert D. Glover, Ph.D.
Tobacco Research Center
Mary Babb Randolph Cancer Center
West Virginia University Health Sciences Center
Morgantown, West Virginia

Penny N. Glover, M.Ed.
Tobacco Research Center
Mary Babb Randolph Cancer Center
West Virginia University Health Sciences Center
Morgantown, West Virginia

Chapter 1 (continued)

Deborah G. Grady, M.D.
Department of Epidemiology and Biostatistics
Department of Medicine, School of Medicine
Department of Dental Public Health and Hygiene
School of Dentistry
University of California, San Francisco
San Francisco, California

John C. Greene, D.M.D., M.P.H., I.A.C.D.
Department of Dental Public Health and Hygiene
Dean, School of Dentistry
University of California, San Francisco
San Francisco, California

Prakesh C. Gupta, D.Sc., F.A.C.E.
Basic Dental Research Unit and
WHO Collaborating Centre for
Oral Cancer Prevention
Tata Institute of Fundamental Research
Colaba, Bombay, India

Linda A. Stillman, M.P.H.
Department of Dental Public Health and Hygiene
University of California, San Francisco
San Francisco, California

Deborah M. Winn, Ph.D.
National Center for Health Statistics
Centers for Disease Control
Hyattsville, Maryland

Chapter 2 Clinical and Pathological Effects

Jan M. Bergström, Odont. Dr.
Department of Periodontology
Karolinska Institutet
School of Dentistry
Huddinge, Sweden

R.B. Bhonsle, B.D.S.
Basic Dental Research Unit and
WHO Collaborating Centre for
Oral Cancer Prevention
Tata Institute of Fundamental Research
Colaba, Bombay, India

Laisheng Chou
Division of OIral Pathology
Department of Stomatology
School of Dentistry
University of California, San Francisco
San Francisco, California

Chapter 2 (continued)

D.K. Daftary, M.D.S.
Basic Dental Research Unit and
WHO Collaborating Centre for
Oral Cancer Prevention
Tata Institute of Fundamental Research
Colaba, Bombay, India

Troy E. Daniels, D.D.S., M.S.
Division of Oral Pathology
Department of Stomatology
School of Dentistry
University of California, San Francisco
San Francisco, California

Virginia L. Ernster, Ph.D.
Department of Epidemiology and Biostatistics
School of Medicine
Department of Dental Public Health and Hygiene
School of Dentistry
University of California, San Francisco
San Francisco, California

Deborah G. Grady, M.D.
Department of Epidemiology and Biostatistics
Department of Medicine, School of Medicine
Department of Dental Public Health and Hygiene
School of Dentistry
University of California, San Francisco
San Francisco, California

John C. Greene, D.M.D., M.P.H., I.A.C.D.
Department of Dental Public Health and Hygiene
Dean, School of Dentistry
University of California, San Francisco
San Francisco, California

John S. Greenspan, B.D.S., Ph.D.
Division of Oral Pathology
Department of Stomatology
School of Dentistry
University of California, San Francisco
San Francisco, California

Prakesh C. Gupta, D.Sc., F.A.C.E.
Basic Dental Research Unit and
WHO Collaborating Centre for
Oral Cancer Prevention
Tata Institute of Fundamental Research
Colaba, Bombay, India

Chapter 2 (continued)

Louis S. Hansen, D.D.S.
Division of Oral Pathology
Department of Stomatology
School of Dentistry
University of California, San Francisco
San Francisco, California

Walter W. Hauck, Ph.D.
Department of Epidemiology and Biostatistics
School of Medicine
University of California, San Francisco
San Francisco, California

F.S. Mehta, D.M.D.
Basic Dental Research Unit and
WHO Collaborating Centre for
Oral Cancer Prevention
Tata Institute of Fundamental Research
Colaba, Bombay, India

P.R. Murti, M.D.S.
Basic Dental Research Unit and
WHO Collaborating Centre for
Oral Cancer Prevention
Tata Institute of Fundamental Research
Colaba, Bombay, India

J.J. Pindborg, D.D.S., Dr. Odont.
Department of Oral Pathology
Royal Dental College
Rigshospitalet
Copenhagen, Denmark

Paul B. Robertson, D.D.S.
Dean, Faculty of Dentistry
University of British Columbia
Vancouver, Canada

Linda A. Stillman, M.P.H.
Department of Dental Public Health and Hygiene
School of Denistry
University of California, San Francisco
San Francisco, California

Margaret M. Walsh, Ed.D.
Department of Dental Public Health and Hygiene
School of Denistry
University of California, San Francisco
San Francisco, California

Chapter 3 Carcinogenesis

Lucy M. Anderson, Ph.D.
Division of Cancer Etiology
National Cancer Institute
Frederick, Maryland

Jennifer Berline
Division of Oral Pathology,
Department of Stomatology
School of Dentistry
University of California, San Francisco
San Francisco, California

Klaus D. Brunnemann, Ph.D.
Naylor Dana Institute for Disease Prevention
American Health Foundation
Valhalla, New York

Steven G. Carmella
Naylor Dana Institute for Disease Prevention
American Health Foundation
Valhalla, New York

Suzanne L. Cartwright
Head and Neck Tumor Biology Program
Departments of Microbiology/Immunology
and Otalaryngology-Head and Neck Surgery
Norfolk, Virginia

Henry M. Cherrick, D.D.S., M.S.D.
School of Dentistry
University of California, Los Angeles
Los Angeles, California

Louise K. Crosby, M.A.
Division of Oral Pathology and Oncology
University of Colorado
Denver, Colorado

Troy E. Daniels, D.D.S., M.S.
Division of Oral Pathology
Department of Stomatology
School of Dentistry
University of California, San Francisco
San Francisco, California

Madhev G. Deo, M.D., Ph.D., F.A.M.S., F.N.A.
Cancer Research Institute
Parel, Bombay, India

Thomas R. Dirksen, D.D.S., M.S., Ph.D.
Department of Oral Biology
School of Dentistry
Medical College of Georgia
Augusta, Georgia

Chapter 3 (continued)

Jay Doniger, Ph.D.
Georgetown University School of Medicine
Washington, D.C.

John F. Erbland, Ph.D., D.M.D.
Department of Oral Biology
Medical College of Georgia
School of Dentistry
Augusta, Georgia

Virginia L. Ernster, Ph.D.
Department of Epidemiology and Biostatistics
School of Medicine
Department of Dental Public Health and Hygiene
School of Dentistry
University of California, San Francisco
San Francisco, California

Suzanne E. Glickman
Head and Neck Tumor Biology Program
Departments of Microbiology/Immunology
and Otolaryngology-Head and Neck Surgery
Eastern Virginia Medical School
Norfolk, Virginia

Deborah G. Grady, M.D.
Department of Epidemiology and Biostatistics
Department of Medicine, School of Medicine
Department of Dental Public Health and Hygiene
School of Dentistry
University of California, San Francisco
San Francisco, California

John S. Greenspan, B.D.S., Ph.D.
Division of Oral Pathology
Department of Stomatology
School of Dentistry
University of California, San Francisco
San Francisco, California

Robert O. Greer, Jr., D.D.S., Sc.D.
Department of Pathology, School of Medicine
Division of Oral Pathology and Oncology
School of Dentistry
University of Colorado
Denver, Colorado

Stephen S. Hecht, Ph.D.
Naylor Dana Institute for Disease Prevention
American Health Foundation
Valhalla, New York

Chapter 3 (continued)

Dietrich K. Hoffmann, Ph.D.
Naylor Dana Institute for Disease Prevention
American Health Foundation
Valhalla, New York

Georgia K. Johnson
Dows Institute for Dental Research
College of Dentistry
University of Iowa
Iowa City, Iowa

Sheng-Lin Li, D.D.S.
School of Dentistry
University of California, Los Angeles
Los Angeles, California

Scott Lubas
Department of Oral Biology
Medical College of Georgia
School of Dentistry
Augusta, Georgia

Stella L. Maurizio
Head and Neck Tumor Biology Program
Departments of Microbiology/Immunology
and Otolaryngology-Head and Neck Surgery
Eastern Virginia Medical School
Norfolk, Virginia

W. Frederick McGuirt, M.D.
Department of Otolaryngology
Bowman Gray School of Medicine
Wake Forest University
Winston-Salem, North Carolina

Byung-Moo Min, D.D.S., Ph.D.
School of Dentistry
University of California, Los Angeles
Los Angeles, California

Sharon E. Murphy, Ph.D.
Naylor Dana Institute for Disease Prevention
American Health Foundation
Valhalla, New York

Joel M. Palefsky, M.D.
Division of Oral Pathology,
Department of Stomatology
School of Dentistry
Department of Laboratory Medicine
School of Medicine
University of California, San Francisco
San Francisco, California

Chapter 3 (continued)

No-Hee Park, D.M.D., Ph.D.
School of Dentistry and
Jonsson Comprehensive Cancer Center
University of California, Los Angeles
Los Angeles, California

Jerry M. Rice, Ph.D.
Division of Cancer Etiology
National Cancer Institute
Frederick, Maryland

Abraham Rivenson, M.D.
Naylor Dana Institute for Disease Prevention
American Health Foundation
Valhalla, New York

Dhananjaya Saranath, Ph.D.
Cancer Research Institute
Parel, Bombay, India

Gary L. Schechter, M.D.
Head and Neck Tumor Biology Program
Departments of Microbiology/Immunology
and Otolaryngology-Head and Neck Surgery
Eastern Virginia Medical School
Norfolk, Virginia

George S. Schuster, D.D.S., M.S., Ph.D.
Department of Oral Biology
Medical College of Georgia
School of Dentistry
Augusta, Georgia

Kenneth R. Shroyer, M.D., Ph.D.
Department of Pathology
University of Colorado School of Medicine
Denver, Colorado

Kenneth D. Somers, Ph.D.
Head and Neck Tumor Biology Program
Departments of Microbiology/Immunology
and Otolaryngology-Head and Neck Surgery
Eastern Virginia Medical School

Christopher A. Squier, Ph.D.
Dows Institute for Dental Research
College of Dentistry
University of Iowa
Iowa City, Iowa

Chapter 3 (continued)

Bryan G. Stoll
Head and Neck Tumor Biology Program
Departments of Microbiology/Immunology
and Otolaryngology-Head and Neck Surgery
Eastern Virginia Medical School
Norfolk, Virginia

Anna M. Wray, M.D.
Department of Otolaryngology
Bowman Gray School of Medicine
Wake Forest University
Winston-Salem, North Carolina

Chapter 4 Nicotine Effects and Addiction

Neal L. Benowitz, M.D.
University of California, San Francisco
San Francisco, California

Paul B.S. Clarke, M.A., Ph.D.
Department of Pharmacology and Therapeutics
McGill University
Montreal, Canada

Martin J. Jarvis, M.Phil.
Imperial Cancer Research Fund
Health Behaviour Unit
Institute of Psychiatry
London, U.K.

Chapter 5 Prevention

Carol N. D'Onofrio, Dr.P.H.
School of Public Health
University of California, Berkeley
Berkeley, California

Richard I. Evans, Ph.D.
Department of Psychology
University of Houston
Houston, Texas

J. Greg Getz, Ph.D.
Department of Psychology
University of Houston
Houston, Texas

Bettye E. Raines
Department of Psychology
University of Houston
Houston, Texas

Chapter 6 Cessation

Mira B. Aghi, Ph.D.
Basic Dental Research Unit
Tata Institute of Fundamental Research
Bombay, India

Chapter 6 (continued)

Elbert D. Glover, Ph.D.
Tobacco Research Center
Mary Babb Randolph Cancer Center
West Virginia University Health Sciences Center
Morgantown, West Virginia

Penny N. Glover, M.Ed.
Tobacco Research Center
Mary Babb Randolph Cancer Center
West Virginia University Health Sciences Center
Morgantown, West Virginia

Prakesh C. Gupta, D.Sc., F.A.C.E.
Basic Dental Research Unit and
WHO Collaborating Centre for
Oral Cancer Prevention
Tata Institute of Fundamental Research
Colaba, Bombay, India

David E. Heisel, D.D.S., M.B.A.
Columbus Health Department
Community Dental Programs
Columbus, Ohio

F.S. Mehta, D.M.D.
Basic Dental Research Unit and
WHO Collaborating Centre for
Oral Cancer Prevention
Tata Institute of Fundamental Research
Colaba, Bombay, India

J.J. Pindborg, D.D.S., Dr. Odont.
Department of Oral Pathology
Royal Dental College
Rigshospitalet, Copenhagen, Denmark

Kathleen L. Schroeder, D.D.S., M.Sc.
Tobacco Research Center
Mary Babb Randolph Cancer Center
Department of Oral Pathology
West Virginia University Schools of
Medicine and Dentistry
Morgantown, West Virginia

Herbert H. Severson, Ph.D.
Oregon Research Institute
Eugene, Oregon

Chapter 7 Policy

Roberto Masironi, Ph.D.
Tobacco or Health Programme
World Health Organization
Geneva, Switzerland

Chapter 7 (continued)

Robert E. Mecklenburg, D.D.S., M.P.H.
Consultant, National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Seamus O'Hickey, M.Sc., B.D.S., F.I.C.D., D.P.A.
Department of Health
Dublin, Ireland

Chapter 8 Recommendations

Robert E. Mecklenburg, D.D.S., M.P.H.
Consultant, National Cancer Institute
National Institutes of Health
Bethesda, Maryland

R. Craig Stotts, Dr.P.H., R.N.
Division of Cancer Prevention and Control
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

The editors gratefully acknowledge also the following distinguished scientists, researchers, and others, both in and outside government, who contributed critical reviews:

Jerry E. Bouquot, D.D.S., M.S.D.
Department of Oral Pathology
Head and Neck Diagnosis of West Virginia
West Virginia University Schools of Medicine and Dentistry
Morgantown, West Virginia

Moon S. Chen, Jr., Ph.D., M.P.H.
Department of Preventive Medicine
Ohio State University
Columbus, Ohio

Arden G. Christen, D.D.S., M.S.D, M.A., F.A.C.D.
Preventive/Community Dentistry
Indiana University School of Dentistry
Indianapolis, Indiana

Richard I. Evans, Ph.D.
Department of Psychology
University of Houston
Houston, Texas

Jacquelyn L. Fried, R.D.H., M.S.
Department of Dental Hygiene
College of Dental Surgery
University of Maryland at Baltimore, Dental School
Baltimore, Maryland

Elbert D. Glover, Ph.D.
Tobacco Research Center
Mary Babb Randolph Cancer Center
West Virginia University Health Sciences Center
Morgantown, West Virginia

John C. Greene, D.M.D., M.P.H., F.A.C.D.
Department of Dental Public Health and Hygiene
Dean, School of Dentistry
University of California, San Francisco
San Francisco, California

Robert O. Greer, Jr., D.D.S., Sc.D.
Division of Oral Pathology and Oncology
University of Colorado Health Sciences Center
Denver, Colorado

Stephen S. Hecht, Ph.D.
Naylor Dana Institute for Disease Prevention
American Health Foundation
Valhalla, New York

David E. Heisel, D.D.S., M.B.A.
Community Dental Programs
Columbus Health Department
Columbus, Ohio

Jack E. Henningfield, Ph.D.
Clinical Pharmacology Branch
National Institute on Drug Abuse
Addiction Research Center
Baltimore, Maryland

Dietrich K. Hoffmann, Ph.D.
Naylor Dana Institute for Disease Prevention
American Health Foundation
Valhalla, New York

Elizabeth C. Lenz, D.D.S.
Department of Oral Pathology
West Virginia University School of Dentistry
Morgantown, West Virginia

James L. McDonald, Jr., Ph.D.
Preventive/Community Dentistry
Indiana University School of Dentistry
Indianapolis, Indiana

Robert E. Mecklenburg, D.D.S., M.P.H.
Consultant, National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Rawhi Omar, M.D., Ph.D.
Department of Pathology
West Virginia Universoty School of Medicine
Morgantown, West Virginia

No-Hee Park, D.M.D., Ph.D.
School of Dentistry and
Jonsson Comprehensive Cancer Center
University of California, Los Angeles
Los Angeles, California

Kathleen L. Schroeder, D.D.S., M.Sc.
Tobacco Research Center
Mary Babb Randolph Cancer Center
Department of Oral Pathology
West Virginia University Schools of
Medicine and Dentistry
Morgantown, West Virginia

George S. Schuster, D.D.S., Ph.D., M.S.
Department of Oral Biology/Microbiology
School of Dentistry, Medical College of Georgia
Augusta, Georgia

Herbert H. Severson, Ph.D.
Oregon Research Institute
Eugene, Oregon

Christopher A. Squier, Ph.D.
Dows Institute for Dental Research
College of Dentistry
University of Iowa
Iowa City, Iowa

R. Craig Stotts, Dr.P.H., R.N.
Division of Cancer Prevention and Control
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Deborah M. Winn, Ph.D.
National Center for Health Statistics
Centers for Disease Control
Hyattsville, Maryland

Following are members of the Conference Planning Committee, who participated in organizing the First International Conference on Smokeless Tobacco:

Donald F. Bowers, D.D.S., M.S.D., F.A.C.D.
Ohio State University
College of Dentistry
Columbus, Ohio

Moon S. Chen, Jr., Ph.D., M.P.H.
Department of Preventive Medicine
Ohio State University
Columbus, Ohio

Arden G. Christen, D.D.S., M.S.D., M.A., F.A.C.D.
Preventive/Community Dentistry
Indiana University
School of Dentistry
Indianapolis, Indiana

Michael W. Easley, D.D.S., M.P.H., F.A.C.D.
Health Care Division—Oral Products
Clinical Operations and Biometrics Section
Procter & Gamble Company
Sharon Woods Technical Center
Cincinnati, Ohio

Richard I. Evans, Ph.D.
Department of Psychology
University of Houston
Houston, Texas

Elbert D. Glover, Ph.D.
Tobacco Research Center
Mary Babb Randolph Cancer Center
West Virginia University Health Sciences Center
Morgantown, West Virginia

John C. Greene, D.M.D., M.P.H., F.A.C.D.
University of California
School of Dentistry
San Francisco, California

Prakash C. Gupta, D.Sc., F.A.C.E.
Basic Dental Research Unit and
WHO Collaborating Centre for
Oral Cancer Prevention
Tata Institute of Fundamental Research
Bombay, India

Dietrich K. Hoffmann, Ph.D.
Naylor Dana Institute for Disease Prevention
American Health Foundation
Valhalla, New York

James L. McDonald, Ph.D.
Preventive/Community Dentistry
Indiana University
School of Dentistry
Indianapolis, Indiana

Robert E. Mecklenburg, D.D.S., M.P.H.
Consultant, National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Herbert H. Severson, Ph.D.
Oregon Research Institute
Eugene, Oregon

Margaret M. Titterington, M.S.
Public Education Department
American Cancer Society
Ohio Division, Inc.
Dublin, Ohio

David S. Yohn, Ph.D., M.P.H.
Ohio State University
Comprehensive Cancer Center
Columbus, Ohio

Sponsors of the smokeless tobacco conference were the following:

American Fund for Dental Health

Marion Merrell Dow, Inc.

National Institute of Dental Research (grant no. R13 DE/CA-09884-01)

National Cancer Institute (grant no. R13 DE/CA-09884-01)

U.S. Public Health Service, Office of International Health

Ohio State University Comprehensive Cancer Center

American Cancer Society, Ohio Division

West Virginia University, Mary Babb Randolph Cancer Center

Nationwide Insurance

Ohio Dental Association

The editor acknowledges the contributions of the following staff members of R.O.W. Sciences, Inc., Rockville, Maryland, who provided technical and editorial assistance in the preparation of this monograph:

Richard H. Amacher, Project Manager

Jill Aughenbaugh, Indexer

Douglas Bishop, Art Director

Margaret Bowman, Copy Editor

Jean Brady, Research Assistant

Karen Bray, Technical Assistant

Caroline Caldwell, Graphics Specialist

Rebecca Charton, Librarian

Traci Cherrier, Research Assistant

Catherine Godfrey, Word Processing Specialist

Faye Grant, Administrative Secretary

Chris Louthan, Research Assistant

Steve Luckabaugh, Word Processing Specialist

Kay Marshall, Copy Editor

Chérie Melat, Graphics Specialist

Frances Nebesky, Copy Editor

Adrienne Plater, Word Processing Specialist

Theodora Radcliffe, Copy Editor, Senior Indexer

Barbara Shine, Managing Editor

Neil Swan, Science Writer

Donna Tharpe, Copy Editor

Sonia Van Putten, Word Processing Specialist

Debra Waugh, Copy Editor

Larry Wentzel, Graphics Specialist

Ronald W. Wolf, Coordinator, Senior Copy Editor

Overview and Summary

BRIEF HISTORICAL PERSPECTIVE

Large-scale consumption of tobacco has been a significant lifestyle factor in America for centuries (Robert, 1967; US DHHS, 1992). Prior to the beginning of the 20th century, tobacco was consumed in the form of spitting tobacco (chewing tobacco and snuff), smoked as cigars, or loose tobacco smoked in pipes or in hand-rolled cigarettes (Shopland and Haenlein, in press; US DHHS, 1990). Consumption of machine-made cigarettes was almost nonexistent, and spitting tobacco was the dominate form of use, accounting for nearly 60 percent of all tobacco consumed on a per capita basis.

Figure 1 depicts tobacco consumption in the United States, by major product category, for the past 100 years (Milmore and Conover, 1956; US Department of Agriculture). This data is in pounds of tobacco consumed per adult rather than in individual units such as cigars or cigarettes, to allow direct comparisons between categories. The data are also presented in a cumulative fashion to show better the change in individual product use over time and in relation to other categories.

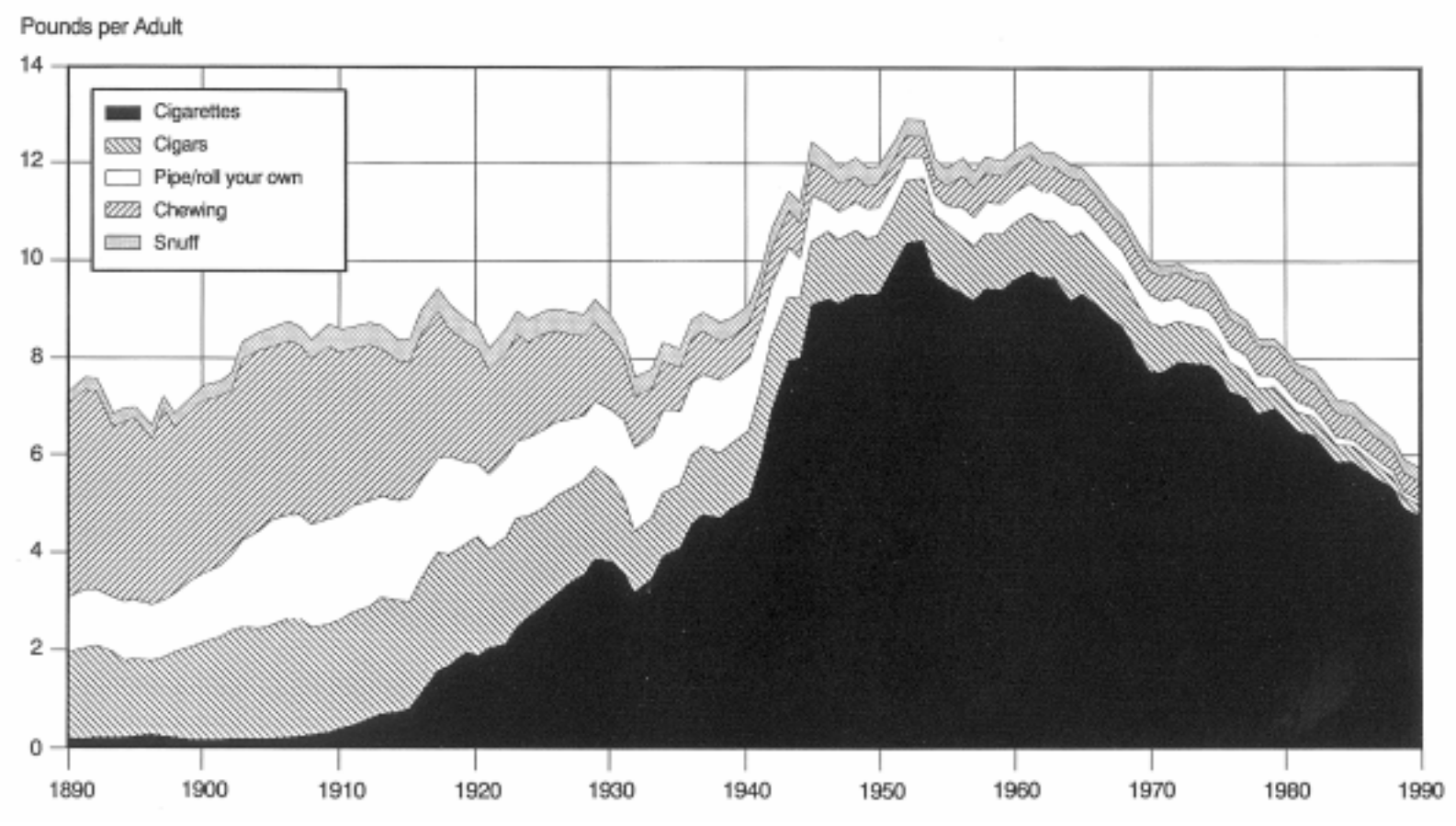
What is clearly evident is that, for much of the latter part of the 19th century and the early decades of the 20th, chewing tobacco was the single predominate product (Milmore and Conover, 1956). When one recognizes that, during the early part of this century, tobacco use was primarily a male phenomena, if these data were expressed in terms of male consumption only, total pounds per person would be nearly double. In 1890, on a per adult basis, chewing tobacco accounted for 4 lbs of the 7.3 lbs of total tobacco used annually. What is also evident is that cigarette smoking did not begin to penetrate significantly into American society until after 1910, when R.J. Reynolds introduced the first blended cigarette—Camel. Camel cigarettes, first marketed in 1913, differed substantially from other brands at that time in that they contained different types and blends of tobacco and had a pH that prevented absorption of nicotine through the oral mucosa (National Cancer Institute, 1991). In contrast to smokeless tobacco users, smokers of these new cigarettes had to inhale the smoke to achieve sufficient quantities of nicotine in the blood stream.

Camel was also the first nonregional cigarette, and Camel's introduction was accompanied by a then massive $1.5 million national advertising and marketing campaign. Consumption of Camel cigarettes immediately increased, providing one of the first documented cases to demonstrate that advertising could create demand for a product where no previous demand existed. Other manufacturers followed by introducing such brands as Lucky Strike and Chesterfield; for decades these brands dominated the domestic U.S. cigarette market.

Consumption of machine-manufactured cigarettes increased dramatically following World War I; by 1935, more tobacco was being consumed in the form of cigarettes than all other products combined (US DHHS, 1990).

Figure 1

Trends in per capita tobacco consumption by major product category, United States, 1890 to 1990



As the popularity of cigarettes continued to increase, consumption of noncigarette tobacco products, especially smokeless tobacco, declined.

Per capita cigarette tobacco consumption peaked in the early 1950's (as did total per capita tobacco consumption), probably in response both to the first scientific studies linking cigarette smoking to lung cancer and to the introduction and aggressive promotion of filter cigarettes by the cigarette industry in response to these early scientific findings (US DHHS, 1990). Filter cigarettes, which had previously accounted for less than 1 percent of all cigarettes consumed in the United States in 1950, had captured 50 percent of the market by the end of the decade, and today account for over 90 percent of all cigarette sales (US DHHS, 1989a). In comparison to their nonfilter counterparts, filter cigarettes generally contain less tobacco.

For decades following the introduction of the mass-produced and mass-marketed cigarettes, consumption and prevalence of smokeless tobacco had been on the decline (Milmore and Conover, 1956). However, the latter part of the 1970's and the early part of the 1980's, saw major increases in ST use (Connolly, 1986; US DHHS, 1986). This increase was the result of renewed and more aggressive advertising by the ST industry that accompanied the introduction of innovative products such as Skoal Bandits—and the use of well-known sport and entertainment personalities in ST promotions (Ernster, 1989). Personalities such as football stars Walt Garrison and Terry Bradshaw; baseball greats George Brett, Sparky Lyle, Carlton Fisk, and Bobby Murcer; and country-and-western singer Charlie Daniels, obviously appealed to a very young and impressionable audience (Ernster, 1989). Furthermore, use of broadcast media to promote ST was not prohibited under the existing Congressional legislation that had governed cigarettes since 1971; thus, the ST industry was free to use television to recruit a large and relatively untapped market of new users. Because the themes and images used appealed primarily to children and adolescents, increases in ST consumption that occurred during the 1980's was primarily confined to these age groups (Marcus et al., 1989; Rouse, 1989). Where previously little or no use of ST was seen among adolescents, prevalence of ST use among older teens increased between 250 and 300 percent between 1970 and 1985 (Marcus et al., 1989) (See Figures 2 and 3 in the Foreword).

Compared with cigarettes, we know much less about the factors influencing ST use. Only in the past few years have the research results elucidated the many facets of ST use and means to intervene in this process (US DHHS, 1986, 1989a, and 1990).

This monograph represents the most recent major attempt to bring together the important research findings of the last few years. Previous compilations of ST research have been the U.S. Surgeon General's Report in 1986 (US DHHS, 1986) and a monograph published by the National Cancer Institute in 1989, titled *Smokeless Tobacco Use in the United States* (US DHHS, 1989b). The present monograph will use the model established by the two previous publications in presenting as broad a picture of the ST problem as possible. Sections in this monograph describe the epidemiology, clinical and pathological effects, carcinogenesis, nicotine effects and addiction,

prevention, cessation, and policy research findings in the area of ST use. Finally, recommendations based on research and compiled by experts in the field is presented.

EPIDEMIOLOGY From an international perspective, the use of ST is not as prevalent as cigarette smoking, although some countries experience relatively high ST use rates. Although ST use in Europe is practically nonexistent, Sweden has one of the highest rates of use in the world. Prevalence is also high in India, the United States, and Canada. Most countries, however, unless they have banned ST, are experiencing growth in ST consumption.

ST use is typically a male habit, but is prevalent in women in certain geographic areas as well as within some cultures and populations. For example, some Native American tribes have 45 percent ST prevalence rates among adolescent females (Schinke et al., 1989; US DHHS, 1986). Among some elderly women in rural southeastern United States, high prevalence rates of dry snuff contributed to a 50-fold increase in risk for oral cancer among long-term users, according to one study (Winn et al., 1981). Nationally, use among women in the United States is less than 0.5 percent, whereas among adult males regular use exceeds 6 percent. Among younger white males, 30 to 33 percent are current users in many regions of the country (Rouse, 1989).

Baseball players in the United States are often ST users. Ernster and colleagues (chapter 1) found that 37 percent had used ST in the past month. This high-profile group of ST users is highly influential, because they act as role models for youth.

Historically, the highest rates of use in the United States have been among residents in rural areas, but recent studies have documented an increasing prevalence rate among urban and suburban residents. White males are the most likely group to use ST, whereas Hispanics and Asians traditionally have had very low rates of use. However, given ST's recent reach into urban areas and into all education and income strata, this problem is becoming more difficult to relegate to only a few segments of society. ST use is rapidly becoming a significant public health problem not only for the United States but also for other countries of the world.

CLINICAL AND PATHOLOGICAL EFFECTS The presence of oral lesions is common place among many ST users. Among snuff users, oral lesions have been found in 67 percent of baseball players who were year-round users and in 32 percent of baseball players who used snuff only during the baseball season (Greene et al., chapter 2). Other common findings among ST users are gingival recession, loss of tooth structure, and leukoplakia. In Swedish data on snuff use, Bergström (chapter 2) found five- to sixfold increases in risk of developing cancer at the site of snuff placement and a frequent occurrence of irreversible gingival recession.

CARCINOGENESIS Earlier studies have conclusively established the use of ST as a cause of oral cancer. The studies in this monograph on carcinogenesis build earlier studies and expand our knowledge of possible mechanisms of tumor development.

Carcinogenesis is a complex, multistage process, and data are just now beginning to emerge that permit a better understanding of this complex process for ST use. This monograph describes the effects of tobacco-specific *N*-nitrosamines on live tissue, specifically the development of tumors. ST contains much higher levels of *N*-nitrosamines than are permitted in any other consumer product (US DHHS, 1986) and are believed, from a number of studies, to play a major role in ST carcinogenesis.

Anderson and Rice (chapter 3) have reported results from their study suggesting that 4-(methylnitrosamino)-1-(3-pyridyl)-1-bitanone in ST may be a potential source of cancer in the children of ST users. Their findings clearly warrant additional investigation including longitudinal epidemiological studies to determine whether the father's use of ST carries a risk of cancer for descendants.

Nicotine's main activity in the system of a smoker or ST user has been thought to be in its addictive properties. However, Hoffmann and colleagues and Squier (chapter 3) found nicotine to also have a role as a potentiator in the carcinogenic process in ST users.

Other studies in this monograph have found oral cancers in ST users among whom some had human papillomavirus, some had herpes simplex virus, and some had neither virus present. These results may indicate that, in some individuals, the viruses may be co-factors in carcinogenicity, but for others the viruses are not necessary for tumors to develop. The role of two other ST constituents, *N*-nitrosonornicotine , a tobacco-associated nitrosamine, and 12-*O*-tetradecanoylphorbol-13-acetate , a tumor promoter, also have been found to affect cell growth and behavior.

NICOTINE EFFECTS AND ADDICTION The multiple adverse health effects of smoking, including nicotine addiction (US DHHS, 1988), have been documented in a series of authoritative reports by the U.S. Surgeon General and others. This monograph presents new research results on nicotine consumption in ST users. Although many studies have found cigarettes and ST to be similarly addictive, some differences remain in these two major forms of nicotine administration.

Among ST users, the blood level of nicotine is very steady following initial administration and remains quite constant during the day—probably the result of the prolonged contact of tobacco with the oral cavity. For this reason, the transdermal nicotine patch may be an improved cessation method for ST users compared with nicotine gum, as the patch more closely mimics the stable nicotine levels commonly seen in ST users.

PREVENTION Typical health education methods may not work when applied to ST prevention. D'Onofrio (chapter 5) describes the success of marketing by the ST industry and recommends that ST prevention efforts incorporate some of these strategies, including the narrow targeting of messages for high-risk groups, attractive packaging of educational programs, product diversification (focusing on "micro-markets"), using educators who are culturally acceptable to the target group, and providing education programs that react

quickly to changes in promotions and marketing strategies by the tobacco industry.

CESSATION One example of the strategies that D'Onofrio recommends is found in the work of Evans and colleagues who used the Little League as a channel for delivering messages about the dangers of ST use (chapter 5). Based on the social inoculation theory originally used by these investigators in their work on smoking, the current study makes extensive use of formative evaluations to guide midcourse corrections in materials, educational strategies, and methods for maintaining a high level of participation in the study.

Cessation interventions for ST use have demonstrated mixed results. Although the withdrawal symptoms appear to be less severe for ST users than for smokers, other factors hamper higher success rates. One factor is the practice of combining ST use with smoking; when one form of tobacco use is prohibited or discouraged, the user often temporarily switches to the other. Thus, ST cessation programs must also include smoking as a targeted behavior.

One advantage that ST cessation efforts have over smoking cessation is the presence of an easily observable clinical marker. A lesion in the mouth where the ST users places tobacco is very common; showing the user this lesion serves as a strong impetus to quit and remain abstinent.

Pharmacological adjuncts hold some promise for improving cessation rates. Although nicotine gum has provided some degree of success, especially when used as part of a comprehensive effort, one problem with gum appears to be using it correctly. The transdermal nicotine patch has several advantages over the gum, including its ease of use and its ability to maintain a steady level of nicotine with only minimal effort by the user. Like nicotine gum, however, the patch's potential as a cessation aid is greatest only when used as part of a complete program that addresses behavioral and physiological aspects of ST addiction.

The role of health care professionals in ST cessation interventions deserves much more attention than it has previously received. Dentists and oral hygienists see many people who are at high risk for becoming ST users or who are already users. Yet, very few dentists have someone in their office teach prevention of ST use, refer patients to an outside cessation program, or prescribe nicotine replacement therapy.

In a survey by Schroeder and Heisel (chapter 6), dentists reported a willingness to provide pamphlets to their patients using ST, they also reported three major barriers to ST cessation counseling: (1) lack of training in counseling, (2) lack of insurance coverage for this activity, and (3) frustration from attempting to counsel about ST cessation.

Some researchers have found that "low-intensity" counseling by health care professionals is very effective and efficient (US DHHS, 1990). The low-intensity approach requires asking whether the patient uses tobacco and, if so, recommending that the patient quit, giving reasons related to that specific patient's health, suggesting the patient set a quit date, providing

self-help materials, and scheduling a follow-up visit or phone call. In about 3 minutes, this intervention can provide a cue to action, strategies for quitting, and plans for follow-up—all considered essential elements of any full-fledged cessation program.

POLICY

As mentioned previously, ST use is relatively common in some countries but in many, its use is unknown. The European Community is considering a total ban on ST products, which may be feasible since the industry has not been able to develop a strong consumer base. The World Health Organization (WHO) recommends that ST products be banned in countries where ST use is nonexistent and that, in all countries, a social climate unfavorable to ST use should be established and maintained. Other strategies recommended by WHO include (1) the use of taxation as a means of reducing ST accessibility to young people and providing a financial base for health education programs, (2) restrictions on use in public places and worksites, and (3) prominent health warnings on packages.

In January 1991, NCI convened an expert panel of ST researchers, health care, and public health professionals. This panel developed a set of recommendations that was later approved by the First International Conference on Smokeless Tobacco and that presents the most important actions that are needed to prevent and control the use of ST. This document is presented in this monograph and is offered as a resource for persons who want to become involved in this very important area of tobacco control (chapter 8).

COMPREHENSIVE STRATEGIES TO CONTROL ST USE

In the United States, the National Cancer Institute has led the federal government's efforts in preventing ST use among youth and in helping adult users to quit (US DHHS, 1990). NCI has funded eight major research studies, six on prevention and two on cessation, that have resulted in improvements in our knowledge and understanding of ST problem in general, as well as the efficacy of specific intervention approaches.

As the scientific understanding of ST use has grown, one fact has become increasingly clear: Those factors that encourage, establish and maintain cigarette smoking are essentially identical for ST. Thus, the same strategies now being recommended to control cigarette smoking should also be applied to reduce ST use. These strategies employ a dual approach that targets both the user and the social environment. This approach was detailed in the National Cancer Institutes first Smoking and Tobacco Control Monograph titled *Strategies to Control Tobacco Use in the United States: A Blueprint for Public Health Action in the 1990's*.

The state of the art in controlling tobacco use combines multiple environmental changes with multiple programs directed to individuals in different stages of the initiation and cessation process. Just as is the case in cigarette smoking, this approach recognizes that no single approach is best for all ST users and that different users are most attracted to and most affected by different programs. Perhaps most important, it recognizes that no single intervention channel can reach all users and that no single time is

best for individuals to make an attempt to quit. Comprehensive strategies are characterized by the delivery of persistent and inescapable messages to quit, or not start, coupled with continuously available support for individual cessation efforts provided through multiple channels, and reinforced by environmental incentives to not use ST.

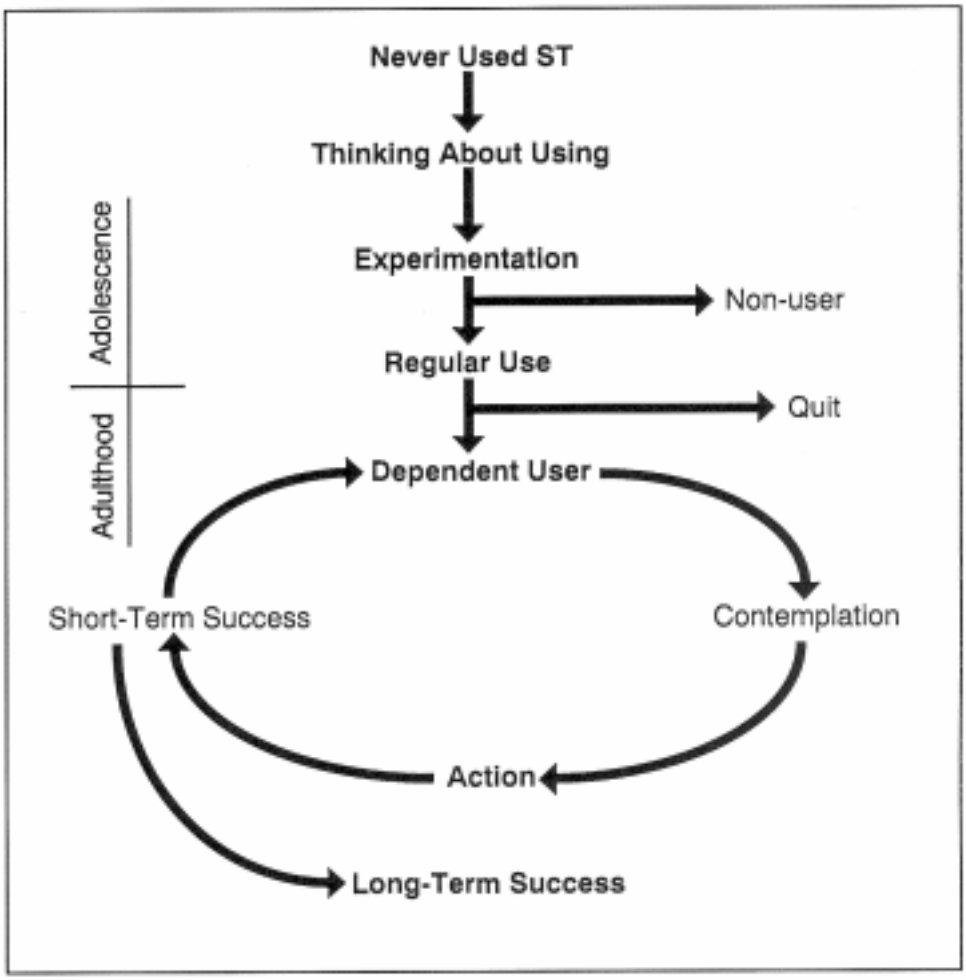
As with cigarette smoking, ST use primarily starts during adolescence. Unlike cigarette smoking, however, ST use is almost exclusively a male phenomena. The processes involved in ST initiation and cessation can be viewed as a cycle (Figure 2) with transition from regular use to dependence occurring during late adolescence and early adulthood. Experimentation and initial use are heavily influenced by issues, such as peer pressure and role modeling, during adolescent development. Dependent use develops when the individual incorporates the personal psychological and sociological utility of ST use into methods by which they function in and cope with the adult world. Although a significant number of adolescents may experiment with ST—including a certain fraction of adolescent females—most never become regular ST users and some who do adopt regular use quit before they become dependent.

Just as the process of quitting smoking is cyclical with smokers making many attempts to stop before finally succeeding, users of ST follow a similar cycle with many attempts to quit. Among cigarette smokers in the United States, about one-third attempt to quit each year but only about 10 percent of these succeed (Pierce and Hatziandreu, 1989). Although comparable national data on ST behavior is unavailable, data from NCI's eight intervention studies on ST use indicate ST users report experiencing the same withdrawal symptoms and problems of relapse as those seen in cigarette smokers (National Cancer Institute, 1990). Clearly, then, ST users who have unsuccessfully tried to quit need to be motivated to try again. A useful conceptualization of the cessation process is one whereby users cycle through various stages of cessation, and with each attempt, gain additional experience that can ultimately contribute to long-term success.

One goal in the overall strategy to control ST use, therefore, is to move users from one stage of the cycle to another, rather than using long-term cessation as the only goal and outcome measure. ST dependency is not a sudden process but one that begins gradually with experimentation starting in early adolescence or even preadolescence. Although the process is nearly identical to that seen in cigarette smoking, a number of regional studies have reported first use of ST occurring much earlier, especially among certain populations of Native Americans where even pre-school-aged children use ST (Schinke et al., 1989)

As shown in Figure 3, the first step in the process is thinking about using ST, and as individuals move through their teenage years, a certain fraction change from believing they will never use ST to contemplation of use. The images presented by advertising as well as the examples of older siblings and adults—especially adult role models such as athletes and well-known entertainers—are powerful inducements for children to perceive ST

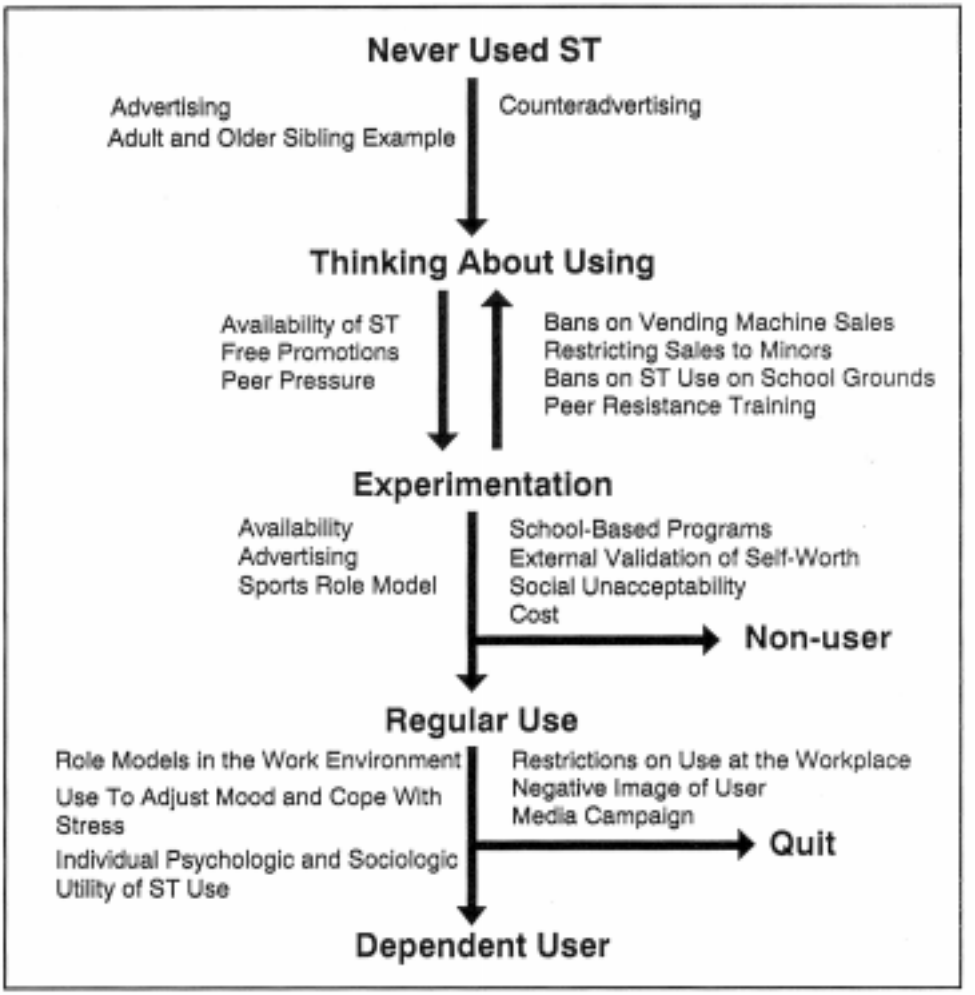
Figure 2
Processes of ST initiation and cessation



use as a means of entry into the adult world. Counter-advertising that creates a negative image of the user can often help offset these influences.

The change from thinking about using ST to experimentation does not necessarily lead to regular use, but as the individual gains experience with ST, this transition clearly becomes more likely. Factors that help move the individual from thinking to experimenting include the wide-spread availability of ST products, the ease with which children can purchase ST, promotional distribution of free samples—many of which are given to teens despite local laws, and advertising. The change from occasional experimentation to regular use is critical, because with regular use the adolescent develops experience that imparts psychological and sociological utility to the user. The ability to purchase ST products easily, the personal and social rewards, and peer acceptance, are all critical to establishing regular use.

Figure 3
Factors that influence adolescent progression into adult ST use



Progressing from regular to dependent use requires that the utility of ST to persist after the pervasive anxieties of adolescents dissipate. For utility to continue, however, ST use has to be allowed in those situations when the user wants to use the product, such as during work as a means to relieve stress. If ST use is not permitted in the workplace or the user is in a white-collar occupation, the behavior may be frowned on by coworkers. In these instances the user must develop alternative methods for handling stress.

For the confirmed smoker, the cyclical pattern of not thinking about quitting (precontemplation), thinking about quitting (contemplation), and attempting to quit, generates a new set of nonsmokers each time they pass through the cycle (Prochaska and DiClemente, 1986). The same process is

Figure 4
ST cessation process

true for ST users. This process is illustrated in Figure 4 and identifies specific intervention processes that can influence these stages. Interventions are provided as examples to show possible interactions between intervention approaches and not meant to be all inclusive.

Many environmental influences and programs for controlling tobacco use (both cigarettes and ST) are intended to influence the user at different stages in this cycle. Public information campaigns that present the risks associated with ST use move individuals from the precontemplation to contemplation, as is personalizing the risks through physicians' or dentists' warnings. Other reasons important to the user include concern about addiction and setting a good example to others, especially children if the user is a parent.

The move from thinking about quitting to making a serious attempt is often triggered by a variety of environmental stimuli. Two factors that contributed to the decline in ST use in the mid-1980's, even though this decline was temporary, was concern about the health risks and Congressionally mandated warning labels on ST products and in advertising. As with cigarettes, cost is an important consideration that contributes to cessation attempts. Another powerful inducement to quit is a dentist's warning to a patient when the dentist finds leukoplakia at the site of ST placement.

Social factors may also play different roles in motivating quitting behavior among ST users compared with cigarette smokers. Increasingly, smokers are feeling more socially ostracized as fewer public settings permit smoking indoors (Pertschuk and Shopland, 1989; US DHHS, 1991). ST users can be surreptitious users, thereby avoiding social sanctions, resulting in less motivation to quit.

The major barriers to long-term cessation remain difficult to alter and, with the exception of addiction, are part of the user's social and economic environment. The barriers include social norms and workplace rules that encourage ST use; the positive behavior of peers, family members, and friends toward ST use; and periods of stress that facilitate relapse.

R. Craig Stotts, Dr. P.H., R.N.
Donald R. Shopland
Division of Cancer Prevention and Control
National Cancer Institute

REFERENCES

Connolly, G.N., Winn, D.M., Hecht, S.S., Henningfield, J.E., Walker, B., Hoffmann, D. The reemergence of smokeless tobacco. *New England Journal of Medicine* 314: 1020-1027, 1986.

Ernster, V.L. Advertising and promotion of smokeless tobacco products. *National Cancer Institute Monographs* No. 8: 87-94, 1989.

Marcus, A.C., Crane, L.A., Shopland, D.R., Lynn, W.R. Use of smokeless tobacco in the United States: Recent estimates from the Current Population Survey. *National Cancer Institute Monographs* 8: 17-23, 1989.

Milmore, B.K., Conover, A.G. Tobacco consumption in the United States, 1880-1955. *Tobacco Smoking patterns in the United States*, W. Haenszel, M.B. Shimkin, and H.P. Miller (editors). Washington, DC: U.S. Government Printing Office, 1956. (Public Health Monograph No. 45).

Novotny, T.E., Pierce, J.P., Fiore, M.C., Davis, R.M. Smokeless tobacco use in the United States: The Adult Use of Tobacco Surveys. *National Cancer Institute Monographs* 8: 25-28, 1989.

Pertschuk, M., Shopland, D.R. (editors). *Major Local Smoking Ordinances in the United States. A Detailed Matrix of the Provisions of Workplace, Restaurant, and Public Places Smoking Ordinances*. U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute. NIH Publication No. 90-479, 1989.

Pierce, J., Hatziandreu, E. *Report of the 1986 Adult Use of Tobacco Survey*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control. Publication No. 10491, 1989.

Prochaska, J.O., DiClemente, C.C. Towards a comprehensive model of change. In: *Treating Behaviors*, W.R. Miller and H. Heather (editors). New York: Plenum Press, 1986, pp. 3-27.

Robert, J.C. The Story of Tobacco in America. Chapel Hill, NC: The University of North Carolina Press, 1967.

Rouse, B.A. Epidemiology of smokeless tobacco use: A national study. *National Cancer Institute Monographs* 8: 29-33; 1989.

Schinke, S.P., Schilling, R.F., Gilchrist, L.D., Ashby, M.R., Kitajima, E. Native youth and smokeless tobacco: Prevalence rates, gender differences, and descriptive characteristics. *National Cancer Institute Monographs* 8: 39-42, 1989.

Shopland, D.R., Haenlein, M. Use of epidemiology in lung cancer control. *Prevention and Treatment of Lung Cancer*. In press.

U.S. Department of Agriculture. *Tobacco Situation and Outlook Report*. U.S. Department of Agriculture, Economic Research Service, Series TS 218. Various years, 1955 to 1992.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

U.S. Department of Health and Human Services. *The Health Consequences of Smoking: Nicotine Addiction. A Report of the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Health Promotion and Education, Office on Smoking and Health. DHHS Publication No. (CDC) 88-846, 1988.

U.S. Department of Health and Human Services. *Reducing the Health Consequences of Smoking: 25 Years of Progress. A Report of the Surgeon General, 1989*. U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. DHHS Publication No. (CDC) 89-8411, 1989a.

U.S. Department of Health and Human Services. *Smokeless Tobacco Use in the United States*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. NIH Publication No. 89-3055, 1989b.

U.S. Department of Health and Human Services. *Smoking, Tobacco and Cancer Program Status Report, 1985-1989*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. NIH Publication No. 90-3107, 1990.

U.S. Department of Health and Human Services. *Strategies to Control Tobacco Use in the United States: A Blueprint for Public Health Action in the 1990's*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. NIH Publication No. 92-3316, 1991.

U.S. Department of Health and Human Services. *Smoking and Health in the Americas. A 1992 Report of the Surgeon General in Collaboration with the Pan American Health Organization*. U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, DHHS Publication No. (CDC) 92-8419, 1992.

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Fraumeni, J.F., Jr. Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

Chapter 1

Epidemiology

CONTENTS

The Smokeless Tobacco Problem: Risk Groups in North America

Elbert D. Glover and Penny N. Glover

ABSTRACT Tobacco consumption—most notably smokeless tobacco use—has become one of the fastest growing detrimental health habits in North America. Adolescents still perceive ST as a safe alternative to cigarettes. This paper provides state-specific information about the prevalence of ST use in the United States. A review of the literature reveals that the groups at highest risk are white youth and young adults, aged 10 to 30, with the most vulnerable being those living in the southern United States. It is suggested that further research in several areas is needed for the effectiveness of smokeless tobacco control to be enhanced.

INTRODUCTION Perhaps one of the fastest growing detrimental health habits in North America over the past few years has been the use of smokeless tobacco. There has been an upswing in the popularity of ST among young adults and children. As a result, the topic has captured the attention of the North American press and public as health professionals and legislators seek to alert the populace about health problems associated with the use of smokeless tobacco (Glover et al., 1988).

There are two types of ST—snuff and chewing tobacco. Snuff is a finely ground tobacco of which the user places a pinch (called a dip or rub) in the gingival groove. Snuff can be dry, moist, or in sachets (tea bag-like pouches). The most common position to place snuff is in the mandibular labial mucosa (cuspid to cuspid); however, this is more common in many European countries, especially Sweden. In European countries, sniffing (inhaling) dry snuff through the nostrils is more common than in North America. Chewing tobacco comes in the form of loose leaf, plug, or twist, and the user places a bolus of tobacco (a golf ball-sized piece) inside the cheek. Typically, whenever a user chews tobacco, one will see an extended cheek. This manuscript provides state-specific information regarding the prevalence of ST and reviews the groups at risk for ST use (Christen and Glover, 1987; Christen et al., 1982; Penn, 1902; Smokeless Tobacco Research Council, 1984; USDA, 1969; Vogues, 1984).

HISTORICAL PERSPECTIVE The use of ST in North America appears to have originated with Native Americans. On his trip to the New World, Christopher Columbus discovered Native Americans using tobacco in various forms (e.g., dipping, chewing, rubbing, smoking), and on his return, he introduced tobacco to the Old World. Actually many of his crew were viewed as being possessed by the devil when they expelled smoke from their nostrils; consequently, they were imprisoned (Christen and Glover, 1987; Christen et al., 1982; Penn, 1902). Once Britain established its colonies in North America, the use of ST became deeply entrenched. Before 1900, the dominant form of tobacco used in North America was smokeless tobacco. Three events occurred that began to move North American tobacco users from smokeless tobacco to cigarette smoking: (1) the invention of the cigarette rolling machine, which allowed for mass production of cigarettes; (2) the

postulation of the germ theory, which at the time created a fear that tuberculosis could be spread by spitting tobacco juices; and (3) World War I. Cigarettes were offered and given freely to American combat soldiers, allowing a nation to become hooked on tobacco. Since the turn of the century, smokeless tobacco use declined until the 1970's when, through clever advertising by the tobacco industry, it began to experience a resurgence (Glover et al., 1984; Harper, 1980; Smight, 1981). The annual increase of 10 to 11 percent continued until 1986, when two significant events occurred: (1) a consensus development conference on smokeless tobacco was held in Bethesda, Maryland (Consensus Conference, 1986); and (2) the Surgeon General's first report on smokeless tobacco was published (US DHHS, 1986). These reports helped create a national awareness of ST's effect on health for the first time, along with the much publicized case of Sean Marsee, product labeling, and an advertisement ban. As a result, tobacco sales declined over the next 18 mo (1986 to 1987). Sales and usage bounced back by 1988.

Today, it appears that tobacco use continues to increase, especially among young people. Specifically, moist snuff (e.g., Copenhagen, Skoal), sachets (e.g., Skoal Bandits, Renegades), and loose leaf tobacco (e.g., Red Man, Chattanooga Chew, Levi Garrett) are the smokeless tobacco products that young adults and youth are using (Glover et al., 1988). On the other hand, dry snuff (e.g., Bruton, Dental Snuff), plug (e.g., Bull of the Woods, Red Man Plug), and twist (e.g., Mammoth Cave, Samson) are declining in use. These last products are used primarily by older adults (Glover et al., 1988).

There are an estimated 10 to 12 million ST users in the United States alone (Consensus Conference, 1986; US DHHS, 1986). Youth are using smokeless tobacco products at alarming rates. This resurgence of popularity is attributed to innovative advertising campaigns by tobacco companies. Sports figures promote the product in an attempt to erase the old, unsanitary image of the habit and replace it with a "macho" image (Christen and Glover, 1981; Glover et al., 1981, 1982, and 1988). Because of public pressure, the tobacco companies stopped using current and former sports personalities to promote their products in 1985 (Consensus Conference, 1986; US DHHS, 1986). Today, the tobacco companies focus their advertisements on young white males, using masculine role models in activities such as fishing, hunting, rock climbing, and white-water rafting (Glover et. al., 1988). Children tend to model the behavior and accept the values of significant others (e.g., parents, teachers, coaches); consequently, these sport figures are contemporary role models (Glover, 1978).

PREVALENCE The average consumer of ST is in the 18- to 30-yr age bracket, with substantial emphasis by advertisers on the 18- to 20-yr-old group (Maxwell, 1980). However, younger people (as young as 10 to 12) also appear to be influenced by the trend of ST use (Christen, 1980; Hunter et al., 1986; Marty et al., 1986; Schroeder et al., 1987). Some reports indicate that smokeless tobacco is sometimes consumed by individuals younger than age 10 (US DHHS, 1986). A statewide study of 5,392 Texas children in grades 7 through 12 reported that approximately 9 percent were regular ST users (Schaefer et al., 1985). In an Oklahoma statewide study, approximately 13 percent of

third grade males and 22 percent of fifth grade males reported regular use of smokeless tobacco (Glover, 1990). These percentages increased to approximately 22, 33, and 39 percent among the 7th, 9th, and 11th grade males, respectively. In two studies in Oregon, 9 percent of 7th grade males, 19 percent of 9th grade males, and 23 percent of 11th grade males reported using smokeless tobacco daily (Lichtenstein et al., 1985; Severson et al., 1985).

In all the aforementioned studies, females reported a low level of ST use— approximately 1 to 3 percent—which is typical of female use in prevalence studies. However, North American Indians report a much higher use among both males and females (Schinke et al., 1989). Actually, gender is not a predictor of smokeless tobacco use among Native Americans. African Americans and Mexican Americans also report a low rate of smokeless tobacco use. The typical user can be described as a white, rural or suburban, young adult male (Hunter et al., 1980; Marty et al., 1986). These data are not limited to the South and West; northern and eastern states on occasion have reported similar rates of use. However, the use of smokeless tobacco tends to be regional in that some areas report higher rates of use. In Ohio, for example, a survey of adults indicated that 10 percent of the males regularly use some form of snuff or chewing tobacco, with another 5 percent being previous smokeless tobacco users (Schroeder and Chen, 1985). In a Massachusetts study of 5,078 students, 16 percent of the males and 2 percent of the females reported using smokeless tobacco "once or twice" (McCarty and Krakow, 1985). In the only national survey among college students, it was reported that 22 percent of collegiate males are users of smokeless tobacco, whereas 2 percent of females reported using smokeless tobacco (Glover et al., 1986). Although there are no national youth surveys on ST use, a summary of self-reported data on 43,000 students in grades 4 through 11 in 16 locations in the United States and 1 in Canada reported that 40 to 60 percent of males had tried smokeless tobacco and 10 to 20 percent of older males reported recent use (Boyd et al., 1987).

Eight surveys conducted in Canada, Colorado, Georgia, Nebraska, and Oregon indicated that about 8 to 10 percent of the young males (aged 5 to 19) were regular users of smokeless tobacco (Glover et al., 1984). A survey in Pitt County, North Carolina (the county that produces more flue-cured tobacco than any other county in the United States), noted a prevalence of 15 percent (Glover et al., 1987).

As shown in Table 1, recent population surveys estimate the rate of smokeless tobacco use for U.S. males age 16 and older (1985 data) at 5.5 percent and for U.S. males age 18 and older (1987 data) at 6.2 percent (Marcus et al., 1989).

The lowest reported use of smokeless tobacco in the United States was in the Northeast (2.3 percent), and the highest reported use was in the South (8.3 percent) (Marcus et al., 1989). As shown in Tables 2 and 3, West Virginia had the highest prevalence (23.1 percent), and Washington, D.C., showed the lowest (Marcus et al., 1989).

Table 1
Prevalence rates from population surveys

	1985	1987
Snuff	1.9%	3.2%
ChewingTobacco	3.9	4.1
All ST	5.5	6.2

Source: Marcus et al., 1989.

Table 2
States with the highest prevalence of ST use

State	Prevalence
West Virginia	23.1%
Mississippi	16.5
Wyoming	15.7
Arkansas	14.7
Montana	13.7
Kentucky	13.6
Oklahoma	11.0
Tennessee	10.3
New Mexico	10.2

Table 3
States with the lowest prevalence of ST use

State	Prevalence
Washington, D.C.	0.0%
New Jersey	0.1
Hawaii	0.2
Massachusetts	0.2
Connecticut	0.3
Maryland	0.4
Rhode Island	0.5
New York	0.5
Delaware	0.6

The previous data by Marcus, although reported in an NCI monograph in 1989, were collected in 1985. More recently, patterns of tobacco use were surveyed by the Youth Risk Behavior Surveillance System (Morbidity and Mortality Weekly Report, 1991). This survey used a three-stage sample design to obtain a probability sample of 11,631 students in grades 9 through 12 in 50 states, the District of Columbia, Puerto Rico, and the Virgin Islands. Twenty-two states are noted and reported current use (30 d preceding the survey) at 10 percent. Tables 4 and 5 illustrate the highest and the lowest ST use among states.

GROUPS AT RISK From gleaning the literature, it becomes obvious that the groups at highest risk are white youth and young adults, aged 10 to 30. The tobacco companies aim their advertisements and marketing at these groups (Maxwell, 1980). Moreover, the profile indicates that the southern region is the most vulnerable (Marcus et al., 1989). In addition, smokeless tobacco

Table 4
Highest reported ST use among states in Youth Risk Behavior Surveillance System (1990)

State	Prevalence
West Virginia	20%
South Dakota	19
Oklahoma	16
Kentucky	15
Alabama	14
Nebraska	14
Colorado	13
New Mexico	13
Pennsylvania	13

Table 5
Lowest reported ST use among states in Youth Risk Behavior Surveillance System (1990)

State	Prevalence
Washington, D.C.	1%
Massachusetts	7
New York	7
New Hampshire	8
North Carolina	8
Utah	8
South Carolina	9
Wisconsin	10

use is two to three times more prevalent among blue-collar workers than among white-collar workers.

In our concern for young, white, blue-collar males, it is important that we not ignore females. Virtually all the studies reported ST use by females with only one line and moved on to males' use. At one time we had few female smokers because smoking was neither fashionable nor acceptable. Today, women are smoking at the same rates as men, and it is estimated that there will be more female smokers than male by the year 1996. Today, certain brands of cigarettes are marketed specifically to women; in the future, women may have their own snuff and chewing brands.

Finally, Native Americans (in Canada and the United States, especially Alaska) report disturbing rates of smokeless tobacco use without regard to gender. It appears that Native Americans are in need of education and funds to combat this major health problem.

FURTHER RESEARCH

Several areas must be addressed if we are to understand better the health menace of smokeless tobacco.

- There is a need for well-designed studies of the prevalence of ST use based on continuing national and international probability samples. This would allow us to monitor trends.
- These studies should include the following: (1) Identifying the type of tobacco used as moist, dry, or sachet snuff tobacco or loose leaf, plug, or twist chewing tobacco allows us to determine where the problem exists. Because of a lack of knowledge of ST, many researchers lump all products under one category. Yet, in research on smoking tobacco, we know exactly where we stand relative to cigarettes, pipes, and cigars. If data are collected for ST generically, we do not know where the problem exists relative to the type. (2) Age of users. (3) Gender of users. It is critical to recognize women as potential users and collect data on women as well as men. Currently, women do not use ST products in significant numbers. However, if we delay until we have a problem, we then become crisis-oriented. As we collect data, it is critical to collect data on female usage to monitor trends. (4) Region of users. (5) Concomitant cigarette smoking.
- Longitudinal studies are important.
- Operational definitions of users, regular users, heavy users, light users, and ex-users should be set to permit useful comparisons. Only one study has attempted to quantify these terms on the basis of nicotine consumption (Marcus et al., 1989; Schroeder et al., 1988).
- Studies should validate self-reports.
- A national survey for persons aged 12 and under would be beneficial; however, this type of national study is complicated and filled with compliance problems. Moreover, it would be a monumental task to convince an agency to fund such a study.

REFERENCES

Boyd, G., Ary, D.V., Wirt, R., et al. Use of smokeless tobacco among children and adolescents in the United States. *Preventive Medicine* 16: 402-421, 1987.

Christen, A.G. The case against smokeless tobacco: Five facts for the health professional to consider. *Journal of the American Dental Association* 101: 464-469, 1980.

Christen, A.G., Glover, E.D. Smokeless tobacco: Seduction of youth. *World Smoking and Health* 6: 20-24, 1981.

Christen, A.G., Glover, E.D. History of smokeless tobacco use in the United States. *Health Education* 18(3): 6-11, 13, 1987.

Christen, A.G., Swanson, B.Z., Glover, E.D., Henderson, A.H. Smokeless tobacco: The folklore and social history of snuffing, sneezing, dipping, and chewing. *Journal of the American Dental Association* 105: 821-829, 1982.

Consensus Conference. Health implications of smokeless tobacco use. *Journal of the American Medical Association* 255(8): 1045-1048, 1986.

Glover, E.D. Modeling: A powerful change agent. *Journal of School Health* 48: 175-176, 1978.

Glover, E.D. Current research in smokeless tobacco. Presented at the 14th Commonwealth and International Conference on Physical Education, Sport, Health, and Dance, Auckland, New Zealand, 1990.

Glover, E.D., Christen, A.G., Henderson, A.H. Just a pinch between the cheek and gum. *Journal of School Health* 51: 415-418, 1981.

Glover, E.D., Christen, A.G., Henderson, A.H. Smokeless tobacco and the adolescent male. *Journal of Early Adolescence* 2: 1-13, 1982.

Glover, E.D., Edwards, S.W., Christen, A.G., Finnicum, P. Smokeless tobacco research: An interdisciplinary approach. *Health Values* 8: 21-25, 1984.

Glover, E.D., Johnson, R., Laflin, M., Edwards, S.W., Christen, A.G. Smokeless tobacco use trends among college students in the United States. *World Smoking and Health* 11(1): 4-9, 1986.

Glover, E.D., O'Brien, K., Holbert, D. Prevalence of smokeless tobacco use in Pitt County, North Carolina. *International Journal of Addictions* 22(6): 557-565, 1987.

Glover, E.D., Schroeder, K.L., Henningfield, J.E., Severson, H.H., Christen, A.G. An interpretative review of smokeless tobacco research in the United States: Part I. *Journal of Drug Education* 18(4): 285-310, 1988.

Harper, S. In tobacco, where there's smokeless fire. *Advertising Age* 85, 1980.

Hunter, S.M., Croft, J.B., Burke, G.L., Parker, F.C., Webber, L.S., Berenson, G.S. Longitudinal patterns of cigarette smoking and smokeless tobacco use in adolescents: The Bogalusa Heart Study. *American Journal of Public Health* 76: 193-195, 1986.

Hunter, S.M., Webber, L.S., Berenson, G.S. Cigarette smoking: Bogalusa Heart Study. *Preventive Medicine* 9: 710-712, 1980.

Lichtenstein, E., Severson, H.H., Friedman, L.S., Ary, D.V. Chewing tobacco use by adolescents: Prevalence and relation to cigarette smoking. *Addictive Behaviors* 9: 351-355, 1985.

Marcus, A.C., Crane, L.A., Shopland, D.R., Lynn, W.R. Use of smokeless tobacco in the United States: Recent estimates from the Current Population Survey. *National Cancer Institute Monographs* 8: 17-24, 1989.

Marty, P.J., McDermott, R.J., Williams, T. Patterns of smokeless tobacco use in a population of high school students. *American Journal of Public Health* 76(17): 190-192, 1986.

Maxwell, J.C. Maxwell Manufactured Products: Chewing snuff is growth segment. *Tobacco Reporter* 107: 32-33, 1980.

McCarty, D., Krakow, M. More than "just a pinch": The use of smokeless tobacco among Massachusetts students. Report by the Massachusetts Department of Public Health, Division of Drug Rehabilitation. January 28, 1985.

Morbidity and Mortality Weekly Report. Current tobacco, alcohol, marijuana, and cocaine use among high school students: United States. *Morbidity and Mortality Weekly Report* 40(38): 659-663, 1991.

Penn, W.A. *The Soverane Herbe: A History of Tobacco*. New York: Grant Richards, 1902, pp. 1-326.

Schaefer, S.P., Henderson, A.H., Glover, E.D., Christen, A.G. Patterns of use and incidence of smokeless tobacco consumption in school-aged children. *Archives of Otolaryngology* 111: 639-642, 1985.

Schinke, S.P., Schilling, R.F., II, Gilchrist, L.D., Ash, M.R., Kitajima, E. Native youth and smokeless tobacco: Prevalence rates, gender differences, and descriptive characteristics. *National Cancer Institute Monographs* 8: 39-42, 1989.

Schroeder, K.L., Chen, M.S. Smokeless tobacco and blood pressure. *New England Journal of Medicine* 312: 919, 1985.

Schroeder, K.L., Chen, M.S., Iaderosa, G.R., Glover, E.D., Edmundson, E.W. Proposed definition of a smokeless tobacco user based on potential nicotine consumption. *Addictive Behaviors* 13: 395-400, 1988.

Schroeder, K.L., Iaderosa, G.B., Chen, M.S., Glover, E.D., Edmundson, E.W. Bimodal initiation of smokeless tobacco usage: Implications for cancer education. *Journal of Cancer Education* 2(1): 1-7, 1987.

Severson, H.H., Lichtenstein, E., Gallison, C. A pinch instead of a puff: Implications of chewing tobacco for addictive processes. *Bulletin of Social Psychology and Addictive Behaviors* 4: 85-92, 1985.

Smight, T.A. A man's chew. *Nutshell* 43, 1981.

Smokeless Tobacco Research Council. *Smokeless Tobacco*. Peekskill, NY: Smokeless Tobacco Research Council, 1984.

U.S. Department of Agriculture. *Tobacco in the United States*. Misc. Publ. 867. Washington, DC: U.S. Department of Agriculture, 1969.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

Vogues, E. *Tobacco Encyclopedia*. Mainz: Germany Tobacco International, 1984, p. 293.

Surveillance of and Knowledge About Cancer Associated With Smokeless Tobacco Use

Deborah M. Winn

ABSTRACT Epidemiological studies of smokeless tobacco and cancer continue to show that smokeless tobacco increases oral cancer risk and possibly the risk of other head and neck cancers, suggesting a continuing need to monitor oral cancer trends and to communicate health risks to the public. Cancers of the gum and buccal mucosa, the sites most strongly associated with smokeless tobacco, show no change in incidence since ST use increased in the 1970's; however, it may be premature to expect a rise in oral cancer as a result of smokeless tobacco use. Soft tissue lesions, including oral precancers, are common in ST users. The majority of adult U.S. male users and non-users of smokeless tobacco know that smokeless tobacco use increases the risk of mouth and throat cancer, although older men (age 65 and older) are less informed. A few studies suggest that many adolescents are aware of cancer risks from ST. However, smokeless tobacco use remains high among youth and young men despite their knowledge of health consequences.

INTRODUCTION Epidemiological evidence, as well as studies of carcinogenesis involving the tobacco-specific nitrosamines contained in smokeless tobacco (Hecht and Hoffmann, 1988), have implicated ST as a cause of cancers of the oral cavity and pharynx. The epidemiological evidence is well documented in reviews and evaluations, including the report of an advisory committee to the U.S. Surgeon General (US DHHS, 1986a), the International Agency for Research on Cancer (IARC, 1985), and other documents written up to about 1986 (US DHHS, 1986b), when Federal legislation was passed requiring warning labels on smokeless tobacco products and banning advertising on radio and TV. Since the mid-1980's, work on carcinogenesis has continued, including the development of potential biological markers of exposure to smokeless tobacco (Carmella, 1990).

In addition to the case control and other studies used as the basis for conclusions in the mid-1980's about the carcinogenicity of ST products, several recent epidemiological case control studies of oral cancer (Blot et al., 1988; Spitz et al., 1988; Stockwell and Lyman, 1986), summarized elsewhere (Winn, in press), have examined cancer risks from smokeless tobacco use. All three studies showed that oral cancer risks were elevated among users of smokeless tobacco, with relative risks, the estimate of the ratio of oral and pharyngeal cancer risks in smokeless tobacco users vs. non-users, ranging from 2.3 to 11.2, suggesting moderate to strong associations between ST use and oral cancer sites. However, in one study (Spitz et al., 1988) use of smoking tobacco could not be ruled out as responsible for the association, and the findings had only marginal statistical significance. The other two, both using population-based cancer registries for case ascertainment (ensuring complete enumeration of cases in specific geographic areas), could rule out cigarette smoking and chance as explanations for the findings. In the study that examined numerous head and neck anatomic sites (Stockwell and

Lyman, 1986), ST use was linked to elevated risks in all cancer sites studied, controlling for smoking and other factors; statistically significant excesses were observed for the gum and buccal mucosa, salivary glands, and larynx. In that report, tobacco use histories were obtained through medical record review. Most patients had tobacco use recorded. However, no information was given to determine whether tobacco use data were obtained with equal accuracy in the control group, consisting of patients with other cancers, as for the cases with a head and neck cancer. The third report used interview data for information on tobacco use.

Concern over health effects from smokeless tobacco use has prompted interest in whether oral cancer is becoming epidemic in areas where ST use is common. An additional area of importance to the public health community is the prevention of new smokeless tobacco use and cessation by current users. One aspect of the effort is public education, and public education relies on information about gaps in public knowledge. Both of these issues are examined in greater depth in this report.

TRENDS IN ORAL CANCER INCIDENCE AND MORTALITY

Examination of geographic and time trends in cancer incidence and mortality has been useful in identifying or highlighting causes of cancer. For example, the higher mortality rates for malignant melanoma and other skin cancers as latitude decreases (and sun exposure increases) are clearly evident from U.S. maps of cancer mortality based on deaths from 1950 to 1980 (Pickle et al., 1987).

Maps also were important in identifying an epidemic area of oral cancer mortality among women in the southeastern United States (Mason et al., 1975). Interest in this epidemic of high oral cancer mortality among women led to an ecological study (Blot and Fraumeni, 1977) correlating oral cancer mortality with industrial and demographic characteristics and subsequently to a case control study. In that case control study (Winn et al., 1981), it was found that ST use was associated with a fourfold increased risk of oral and pharyngeal cancer. Risks for the gum and buccal mucosa were especially elevated and estimated to increase the risk by close to fiftyfold. Smokeless tobacco was thought to account for 87 percent of the gum and buccal mucosal cancer among women in the epidemic area.

This epidemic is on the downswing as shown by maps of cancer mortality that cover a longer period, for example, from 1950 to 1980, and highlight trends across decades (Mason et al., 1975). These maps show that oral cancer death among women is becoming less common in the southeastern United States and more common in some urban areas elsewhere in the United States. One may infer that the use of snuff, the probable cause of the epidemic in the southeastern United States, is no longer common among women there. U.S. production figures show a decline in demand for dry snuff (US DHHS, 1986a), the type of smokeless tobacco most commonly used by the women in the study.

Incidence as well as mortality data are available for examination of time trends. According to data from the Surveillance, Epidemiology, and End Results (SEER) program from 1947 to 1984 (Devesa et al., 1987), the incidence of oral cancer among women increased by more than 50 percent

during the four decades to 5.3/100,000 women. Although the incidence rate during this interval remained fairly constant for men across all ages, at about 12.3 to 13.5/100,000 men, this stability obscures an increase in incidence among younger cohorts and a decrease among older cohorts. Devesa and coworkers (1987) suggest that adoption of cigarette smoking among women might account for the rise in incidence of oral cancer. They also suggest that the decrease in older men was consistent with gradual declines in ST use and pipe smoking during the century, and that cigarettes and, possibly, alcohol might account for increases among the younger age groups.

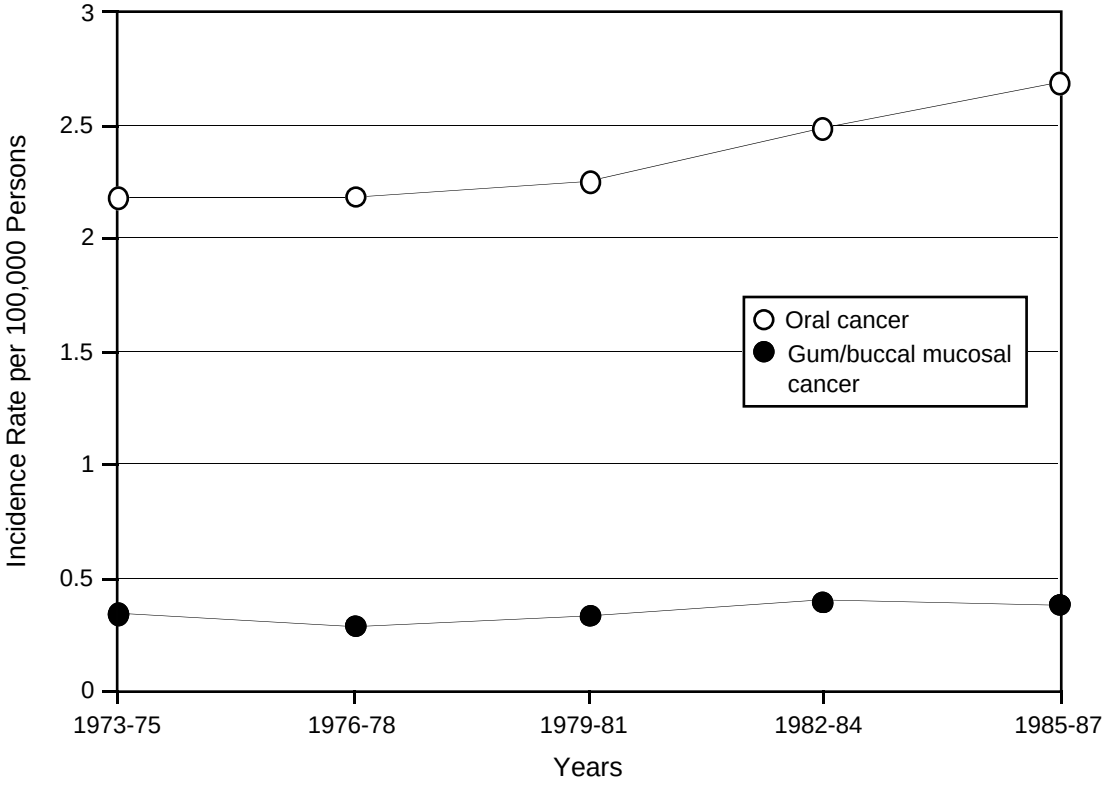
It is difficult to correlate tobacco use patterns with oral cancer incidence and mortality because of the relatively sparse data on tobacco use patterns from small geographic units such as counties. Also, the long latency for cancers makes it difficult to know when to expect a change in incidence after a population changes its risk-factor habits. Nevertheless, available cancer surveillance data have been examined for changes in cancer occurrence.

Partly inspired by concern over potential rises in oral cancer associated with ST use by youth, several letters to the editors of medical journals were published presenting data on tongue cancer from cancer hospital records and from U.S. incidence and mortality data bases (Davis and Severson, 1987; Depue, 1986; Schantz et al., 1984; Shemen, 1984). In all of these reports, tongue cancer rose when measured by increases in incidence or mortality or as a proportion of cancers seen at cancer centers. However, it has not been possible to elucidate the causes of this increase.

If smokeless tobacco use among young men is leading to an epidemic of oral cancer, then it might be expected that a rise in incidence might be detected or be clearly evident for cancers of the gum and buccal mucosa because of the strong relative risks associated with ST and this cancer site. Figure 1 shows the incidence of oral cancer and the incidence of gum and buccal mucosal cancer (a subset of all oral cancers) from 1973 to 1987 among men under age 50 from the SEER program, which includes data from population-based cancer registries covering about 10 percent of the U.S. population. Gum and buccal mucosal cancer incidence essentially was unchanged during this 15-yr period, in spite of a corresponding rise in ST use, although oral cancer in general has been increasing among younger men. Although there was no increase in the incidence of gum and buccal mucosal cancer during this period, it should be noted that baseline rates of these cancers are very low in younger adults (< 0.5/100,000 new cancers per year in men under age 50); oral cancer typically occurs at older ages (62.4 is the median age of diagnosis [Young et al., 1981]); and case control studies suggest that most individuals with oral cancer associated with ST use have used the products for a long time. This would suggest that it may be too early to detect any cancer consequences of increased ST use.

Oral mucosal lesions are common in ST users. In surveys of adolescents, lesions ranging from small local mucosal changes involving slight color and texture modifications to more significant color changes and deep

Figure 1
Incidence of oral cancer: males under age 50, 1973 to 1987



Source: SEER Program, 1973 to 1987.

furrowing have been observed in between 23 and 63 percent of smokeless tobacco users (Centers for Disease Control, 1988; Greer and Poulson, 1983; Offenbacher and Weathers, 1985; Poulson et al., 1984), far exceeding those among non-users (Offenbacher and Weathers, 1985). The more serious lesion, leukoplakia, has been noted in small proportions of young users. In one study, leukoplakia was observed in 5.0 percent of high school football players in Alabama, in contrast to only 0.1 percent among non-users (Creath et al., 1988). Leukoplakia had a prevalence of 46.0 percent among ST-using professional baseball players, whereas only 1.4 percent of non-users had leukoplakia (Ernster et al., 1990).

The transformation of leukoplakia to frank carcinoma is a concern. In one study in India (Gupta et al., 1980), malignant transformation of leukoplakia occurred at a rate of 0.9/1,000 among persons ages 35 to 54 and 10.2/1,000/yr among older persons. The authors summarized the literature on transformation rates, which suggests that between 0.13 and 10.0 percent of leukoplakias will transform, but the studies involved differing followup periods and lesion definitions.

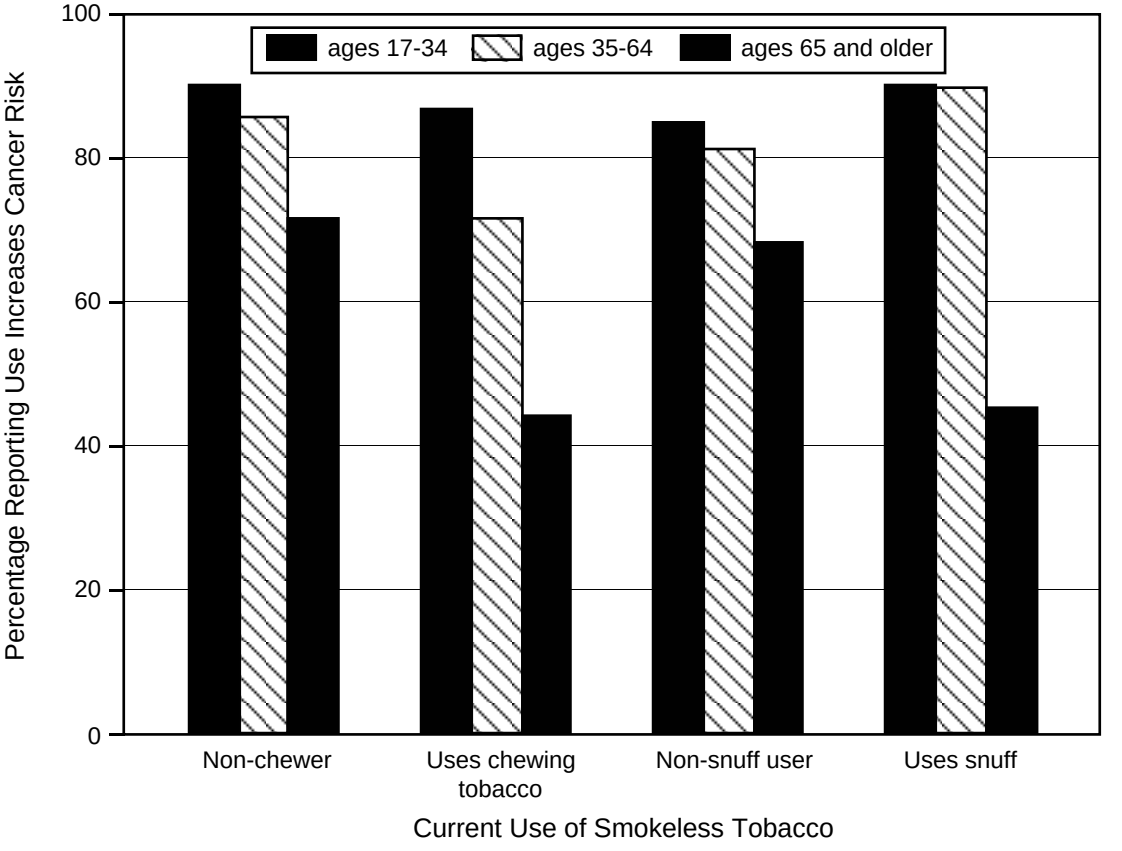
Few other recent studies on this subject have been conducted. There are variations in the risk of transformation to cancer by type of leukoplakia (Gupta et al., 1989), and precise estimates of transformation rates for non-Asian populations are lacking. Some information on the prevalence of oral soft tissue lesions from 1957 to 1973 is available, based on data from large oral cancer screening clinics, some of which covered more than half of the target communities (Bouquot and Gorlin, 1986). However, currently in the United States, leukoplakia (or other precancerous lesions) is not reportable to population-based cancer or other registries, so statistical data on prevalence, incidence, and trends over time for leukoplakia are not obtainable. National prevalence estimates for leukoplakia and ST-associated lesions will be available on the completion of the Third National Health and Nutrition Examination Survey conducted by the Centers for Disease Control's National Center for Health Statistics.

PUBLIC KNOWLEDGE OF ST AND CANCER LINKS

About 6 percent of U.S. adult men use smokeless tobacco, according to data from the National Health Interview Survey (NHIS) of Cancer Epidemiology and Control conducted in 1987 by the National Center for Health Statistics; the survey indicated that smokeless tobacco use by women overall was negligible (0.5 percent) (Schoenborn and Boyd, 1989). The NHIS is a continuous, multipurpose, cross-sectional survey used to obtain national estimates of health characteristics through household interviews. The survey has a multistage, probability, cluster sample design. In the 1987 NHIS, members of the U.S. public were asked questions about whether they thought that snuff and chewing tobacco increase the risk of mouth and throat cancer. The percentages of U.S. men reporting that these products increase cancer risks were similar for snuff (79.9 percent) and for chewing tobacco (83.8 percent). Among tobacco chewers, 71.5 percent thought that their habit increases risk, compared to 85.1 percent among non-users of chewing tobacco. The corresponding figures concerning risks due to snuff use were 85.1 percent and 80.6 percent for snuff users and non-users, respectively. Figure 2 shows that knowledge was inversely related to age, with more younger men being informed; 80 percent or more of those under age 65 knew of mouth and throat cancer risks, compared to about 70 percent of older men.

The NHIS survey covered adults only, but several regional studies, generally conducted in areas where ST use among youth is common, suggest that two-thirds or more of adolescents understand that ST use can cause cancer. Missouri users in grades 5, 8, and 12 were similar to non-users in knowing that smokeless tobacco causes mouth cancer (75 and 80 percent, respectively) (Brownson et al., 1990); furthermore, knowledge of health risks improved with age. A Texas survey (Schaefer et al., 1985) showed that 67 percent of high school students surveyed thought that snuff and chewing tobacco cause cancer. In another study, in Alabama (Creath et al., 1988), 93.7 percent of male high school football players and 92.5 percent of those who used ST were aware that using smokeless tobacco could be harmful to health; "cancer" was the most common response to a question about how health was affected by these products. These data suggest a fairly high level

Figure 2
Males who report that smokeless tobacco increases cancer risk, United States, 1987



Source: NCHS, NHIS, 1987.

of knowledge among all ages that smokeless tobacco causes cancer. However, the relation of health knowledge to behavior is uncertain; it seems clear that knowledge alone is insufficient to reduce significant ST use in these populations.

RESEARCH NEEDS This report suggests some important research needs:

- Longitudinal studies of persons with ST-associated oral lesions are needed to examine the natural history of these lesions, including their continuance, regression, and progression. Factors influencing the natural history of the lesions must be determined.
- Definitions and classifications of oral lesions vary in studies involving mucosal examinations and should be standardized, possibly through an international consensus conference. The same definitions should be used by all investigators to allow comparison of results across studies.

- Cancer incidence and mortality surveillance systems should continue to monitor oral cancer trends in view of the increasing numbers of smokeless tobacco users and the potential for rises in oral cancer incidence rates.
- Better data are needed on the transformation rates for leukoplakia in U.S. populations.
- Finally, more data are needed on how knowledge of health risks and health education can be used to prevent ST use and encourage effective cessation efforts.

REFERENCES

Blot, W.J., Fraumeni, J.F., Jr. Geographic patterns of oral cancer in the United States: Etiologic implications. *Journal of Chronic Disease* 30: 745-757, 1977.

Blot, W.J., McLaughlin, J.K., Winn, D.M., et al. Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Research* 48: 3282-3287, 1988.

Bouquot, J.E., Gorlin, R.J. Leukoplakia, lichen planus, and other oral keratoses in 23,616 white Americans over the age of 35 years. *Oral Surgery, Oral Medicine, Oral Pathology* 61: 373-381, 1986.

Brownson, R.C., Dilorenzo, T.M., Tuinen, M.V., Finder, W.W. Patterns of cigarette and smokeless tobacco use among children and adolescents. *Preventive Medicine* 19: 170-180, 1990.

Carmella, S.G., Kagan, S.S., Kagan, M., et al. Mass spectrometric analysis of tobacco-specific nitrosamine hemoglobin adducts in snuff dippers, smokers and non-smokers. *Cancer Research* 50: 5438-5445, 1990.

Centers for Disease Control. Prevalence of oral lesions and smokeless tobacco use in Northern Plains Indians. *Morbidity and Mortality Weekly Report* 37: 608-611, 1988.

Creath, C.J., Shelton, W.O., Wright, J.T., Bradley, D.H., Feinstein, R.A., Wisniewski, J.F. The prevalence of smokeless tobacco use among adolescent male athletes. *Journal of the American Dental Association* 116: 43-48, 1988.

Davis, S., Severson, R.K. Increasing incidence of cancer of the tongue in the United States among young adults. *Lancet* 2: 910-911, 1987.

Depue, R.H. Rising mortality from cancer of the tongue in young white males. *New England Journal of Medicine* 315: 647, 1986.

Devesa, S.S., Silverman, D.T., Young, J.L., Pollack, E.D., Brown, C.C., Horm, J.W., Percy, C.L., Myers, M.H., McKay, F.W., Fraumeni, J.F., Jr. Cancer incidence and mortality trends among whites in the United States, 1947-1984. *Journal of the National Cancer Institute* 79: 701-770, 1987.

Ernster, V.L., Grady, D.G., Greene, J.C., Walsh, M., Robertson, P.B., Daniels, T., Benowitz, N., Seigel, D., Gerbert, B., Hauck, W. Smokeless tobacco use and health effects among baseball players. *Journal of the American Medical Association* 264: 218-224, 1990.

Greer, R.O., Poulson, T.C. Oral tissue alterations associated with the use of smokeless tobacco by teen-agers: Part 1. Clinical findings. *Oral Surgery* 56: 275-284, 1983.

Gupta, P.C., Bhonsle, R.B., Murti, P.R., et al. An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. *Cancer* 63: 2247-2252, 1989.

Gupta, P.C., Mehta, F.S., Daftary, D.K., et al. Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. *Community Dental and Oral Epidemiology* 8: 287-333, 1980.

Hecht, S.S., Hoffmann, D. Tobacco-specific nitrosamines: An important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis* 9: 875-884, 1988.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985.

Mason, T.J., McKay, F.W., Hoover, R., et al. *Atlas of Cancer Mortality for U.S. Counties: 1950-1969*. U.S. Department of Health, Education, and Welfare; Public Health Service; National Institutes of Health. NIH Publication No. 75-780, 1975.

Offenbacher, S., Weathers, D.R. Effects of smokeless tobacco on the periodontal, mucosal, and caries status of adolescent males. *Journal of Oral Pathology* 14: 169-181, 1985.

Pickle, L.W., Mason, T.J., Howard, N., et al. *Atlas of U.S. Cancer Mortality Among Whites: 1950-1980*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH Publication No. 87-2900, 1987.

Poulson, T.C., Lindenmuth, J.E., Greer, R.O., Jr. A comparison of the use of smokeless tobacco in rural and urban teenagers. *CA—A Cancer Journal for Clinicians* 34: 248-261, 1984.

Schaefer, S.D., Henderson, A.H., Glover, E.D., Christen, A.G. Patterns of use and incidence of smokeless tobacco consumption in school-age children. *Archives of Otolaryngology* 111: 639-642, 1985.

Schantz, S.P., Byers, R.M., Goepfert, J. Tobacco and cancer of the tongue in young adults. *Journal of the American Medical Association* 259: 1943-1944, 1988.

Schoenborn, C.A., Boyd, G. Smoking and other tobacco use: United States, 1987. National Center for Health Statistics. *Vital Health Statistics* 10(169): 9, 1989.

Shemen, L.J., Klotz, J., Schottenfeld, D., Strong, E.W. Increase of tongue cancer in young men. *Journal of the American Medical Association* 252: 1857, 1984.

Spitz, M.R., Fueger, J.J., Goepfert, J., et al. Squamous cell carcinoma of the upper aerodigestive tract: A case comparison analysis. *Cancer* 61: 203-208, 1988.

Stockwell, H.G., Lyman, G.H. Impact of smoking and smokeless tobacco on the risk of cancer of the head and neck. *Head and Neck Surgery* 9: 104-110, 1986.

U.S. Department of Health and Human Services. *Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH Publication No. 86-2874, 1986a.

U.S. Department of Health and Human Services. *Health Implications of Smokeless Tobacco Use: National Institutes of Health Consensus Development Conference* (volume 6, no. 1). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Office of Medical Applications of Research, 1986b.

Winn, D.M. Recent research directions: Smokeless tobacco and aerodigestive cancers. In: *The Biology and Prevention of Aerodigestive Tract Cancer*, G. Newell and W.K. Hong (Editors). New York: Plenum Publishing, 1992 (in press).

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Fraumeni, J.F., Jr. Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

Young, J.L., Jr., Percy, C.L., Asire, A.J. (Editors). Surveillance, Epidemiology, and End Results: Incidence and Mortality Data, 1973-1977. *National Cancer Institute Monographs* 57: 66, 1981. DHHS Publication No. (NIH) 81-2330.

Smokeless Tobacco Use in India[1]

P.C. Gupta

ABSTRACT Smokeless tobacco use is very common in India and neighboring countries. The betel quid chewing habit and its variants predominate, but several other forms of ST also are popular. The major use of ST is in the form of custom-made preparations from individual ingredients for immediate use. In recent years several commercial ST products have been marketed, with backing by intense advertising and promotion campaigns. It has been possible to educate rural Indian populations and thereby persuade them to reduce their tobacco use; such education has significantly decreased the risk of oral cancer.

INTRODUCTION Smokeless tobacco use may be of two kinds: oral use and nasal use. In India and Southeast Asia, nasal use is uncommon; therefore, only oral use is described in this paper.

In India, the neighboring countries, and some other countries of the southeast region, smokeless tobacco use is very common, although it has declined over time. Trend data on the prevalence of ST use are not available, but inferences may be drawn from the data on total tobacco consumption. Analysis of the per capita amount of tobacco consumed in both smoked and smokeless tobacco forms in India over the past 40 years has shown that smoking has increased considerably, but smokeless tobacco has declined from its position as the dominant form (Sanghvi, 1989).

Reliable prevalence data from some selected parts of India became available during the late 1960's and early 1970's, when large cross-sectional, house-to-house surveys of tobacco habits were conducted in rural populations (Mehta et al., 1969 and 1972). Table 1 shows the sample size and the prevalence of overall tobacco use (smoking and chewing), by sex, among individuals aged 15 and over. It is clear that tobacco use is very common in India. Among men it is uniformly high, but among women there is great variability. The areas surveyed were selected with specific objectives and thus may not represent the whole of India. They are, however, widely dispersed areas and do represent a large part of the country.

Table 2 shows the prevalence of smokeless tobacco use among nonsmokers and smokers, and the overall prevalence of use. The prevalence of ST use varied markedly in different regions, although in general it was comparable between smokers and nonsmokers. In most places, ST use was found to be more common among women.

A high prevalence of smokeless tobacco use in India may be somewhat surprising because tobacco was introduced in India, like everywhere else, by the Europeans about 400 years ago, primarily as a substance for smoking. To learn the reasons for the popularity of ST use, one has to look at some ancient cultural practices of this region.

[1] Supported solely by the National Institutes of Health, under Indo-U.S. Fund Research Agreement no. 01-022-N.

Table 1
Prevalence of tobacco use (smoking and smokeless) in India, from population-based, house-to-house studies

Area	Sample Size	Tobacco Users, Percentage		
		Men	Women	All Over Age 15
Bhavnagar	10,071	71%	15%	44%
Ernakulam	10,287	81	39	59
Strikakulam	10,169	81	33	56
Singhbhum	10,048	81	33	56
Darabhanga	10,340	78	51	64
Pune	101,761	62	49	64

TYPES OF ST USED

The most common methods of ST use in India are betel quid chewing and its variants. Betel quid chewing is an ancient practice—several millennia old. Betel quid is mentioned in ancient religious texts, medicinal treatises, literary works, and old stone inscriptions. It has always been a part of religious, social, and cultural rituals, and the practice enjoys complete social acceptance, even today.

Traditionally betel quid consisted of betel leaf, pieces of areca nut, a few drops of lime (calcium hydroxide), several condiments, sweetening, and flavoring agents, depending on regional practices and individual preferences. After tobacco was introduced in India in the 17th century, it became an ingredient of the betel quid. Through its association with a socially accepted practice, ST use became widespread. Currently almost all habitual users of betel quid use it with tobacco. The habit is widespread in all parts of India and is practiced by both men and women.

Until recently, the habit of betel quid chewing was not recognized as an ST habit. In the literature, betel quid chewing was generally referred to as "betel nut" chewing. This terminology was probably responsible for the erroneous impression that the nut used in the betel quid was the main active substance for carcinogenesis. The term "betel nut" is a misnomer, because the nut used in the betel quid is from the palm *Areca catachu* and therefore should be called "areca nut" (Burton-Bradly, 1979).

The betel quid chewing habit evolved into several variants such as chewing of *mawa*, *khaini*, *mainpuri* tobacco, and, more recently, various brands of commercially manufactured and marketed *pan masala*. Probably a major reason for the popularity of these variants is the perishability of the betel leaf, a fresh green leaf from the betel vine, in which various ingredients of the quid are smeared and wrapped. The tenderness and freshness of the leaf are highly prized, and therefore betel leaf does not last for more than a few days. Betel vine is said to be a delicate plant, requiring much care and attention, and cannot be grown everywhere. The leaf is thus difficult to obtain in places distant from betel-growing areas.

Table 2
ST use among men and women, smokers and nonsmokers

Area	Men, Percentage			Women, Percentage		
	Smokers	Nonsmokers	Overall	Smokers	Nonsmokers	Overall
Gujurat	10%	24%	15%	0%	15%	15%
Kerala	33	42	36	43	38	38
Srikakulam	16	16	16	4	8	6
Darabhanga	52	56	54	8	12	11
Singhbhum	22	47	31	25	28	27
Maharashtra	30	58	56	0	49	49

Although the betel quid itself, as well as various combinations of its ingredients, can be chewed with or without the inclusion of tobacco, most habitual chewers include tobacco in their quid. This is understandable because tobacco is the only addictive substance among the betel quid ingredients. This point was not recognized, however, until evidence emerged from house-to-house, cross-sectional studies of large samples of rural populations (Mehta et al., 1969 and 1972).

Table 3 shows the prevalence of chewing habits in some areas where chewing was reported to be popular. The table shows the prevalence of chewing among nonsmokers, the proportion of chewers who exclude tobacco from their quid, and those who include tobacco. Smokers are excluded from the table. The data readily confirm that, although chewing habits were popular, the proportion of those who excluded tobacco from their quid was minuscule. In different areas, different kinds of chewing habits prevailed; for example, the predominant chewing substance in Ernakulam district was betel quid, whereas in Pune district it was tobacco and lime.

The tobacco-plus-lime mixture is probably the most common variant of the betel quid. The mixture is known as *khaini* in the northern part of India, and it is popular in other parts as well. Tobacco-plus-lime is the most common form of tobacco used in Pune district.

A user typically carries a double-mouth box, the larger part containing tobacco flakes and the smaller one lime (calcium hydroxide) paste. To prepare the quid, the user places a small amount of tobacco in the palm; a dash of lime is flicked by a thumb or forefinger, and it is mixed and rubbed vigorously with the tobacco in the hand. The mixture is then ready for use and is placed in the mouth. Some individuals may add pieces of areca nut as well.

The exact placement of the tobacco-plus-lime mixture in the mouth varies by geographic area. In Pune district, the mixture is often placed in the canine region; in Darabhanga, in the labial groove; and in Singhbhum district, many users prefer to keep it on the tongue. The most common sites of oral cancers and precancers also vary correspondingly in those regions.

Table 3
Comparison of individuals who use tobacco in their chewing quid with those who do not

	In Ernakulam (n=10,287)	In Singhbhum (n=10,048)	In Maharashtra (n=101,761)
Number of Chewers[a]	2,699	1,334	51,835
With tobacco	99%	97%	99%
Without tobacco	1%	3%	1%

[a] *Without any smoking habit.*

Mawa is another variant of betel quid that contains areca nut, tobacco, and lime. *Mawa* is popular in Bhavnagar district and nearby areas, but in other areas it may be known by different names. By weight, more than 90 percent of *mawa* is areca nut. It is prepared immediately prior to use and is generally purchased from kiosks that sell betel quid and other tobacco products. The vendor places small pieces of sun-cured areca nut (5.5 g) on a piece of cellophane (10 to 13 cm), adds tobacco flakes (0.4 g), and sprinkles a few drops of a solution of calcium hydroxide. The mixture is then tied as a round ball in the cellophane wrapper and given to the customer. A user typically rubs the cellophane ball vigorously on the palm for a couple of minutes, ostensibly to homogenize the *mawa* mixture. (More likely, this action produces greater availability of free nicotine through the action of calcium hydroxide on tobacco.) The user then opens the cellophane, removes any veins of tobacco leaf, and puts the mixture into the mouth. One quid may be chewed for 10 to 20 min. Some users may chew only half of the quid at one time (Sinor et al., 1990).

The most recent variant on betel quid is *pan masala*, a manufactured item containing areca nut and other ingredients common in betel quid; some brands contain dehydrated and powdered betel leaves. *Pan masala* is generally available in two types—with tobacco and without tobacco—sold under the same brand name. The single name for both choices affords a significant marketing advantage to the manufacturer. Since there are no restrictions on advertising a consumer product that contains no tobacco, *pan masala* without tobacco is vigorously advertised and promoted, without restriction, even on the government-controlled electronic media. *Pan masala* with tobacco, however, carries the same brand name and therefore gets considerable benefit from the unrestricted advertisement and promotion of the nontobacco counterpart.

The vigorous, high-profile advertising of *pan masala* has prompted manufacturers who rarely advertised their chewing products before to start advertising heavily as well. As a result, advertisements of commercially manufactured and marketed ST products are common on the roads, in magazines, and in videotapes. It is easy to discern the target group: urban individuals with education, traditional values, and disposable income. Recently, working women and middle-class housewives also seem to have become a specific target of such advertisements.

New and more effective ways of advertising and promoting are constantly explored and employed. For example, in the videocassette versions of Indian films, animation is used to superimpose dancing tobacco products on the movie so that viewers cannot avoid the commercial. The March 1991 issue of several magazines contained not only an advertisement of a particular brand of chewing tobacco, but also, for the first time, a free product sample pasted on the advertisement.

As a result of such high-profile advertising, ST use is increasing rapidly in the stratum of society from which it had almost disappeared—among individuals with college education who are in business and in middle and high-level management positions. No hard data are yet available, but cans and sachets of smokeless tobacco are becoming more common in public places where well-educated people are seen, for example, in airport lounges. Medical practitioners are reporting a rapid increase in the incidence of oral submucous fibrosis, a chronic debilitating disease with no known cure, believed to be caused by areca-nut chewing (Bhonsle et al., 1987; Sinor et al., 1990).

There are also several methods of ST use that cannot be termed variants of betel quid chewing. One of them is use of manufactured snuff, which is common in the Western Region. Dry snuff is meant for nasal use, but oral use is more common in India. There is a difference in variety as well; finer snuff is for nasal use and coarser snuff for oral use. Manufacturers market snuff as *tapkeer*, but local names may differ. One method of using dry snuff is oral application with a dry twig, identical to the snuff dipping described among women in North Carolina (Winn et al., 1981).

Mishri is a powdered form of roasted tobacco. It is common in Maharashtra and central regions of India, especially among women. People begin using *mishri* as a dentifrice, but it soon turns into an addiction. A typical user applies *mishri* to the teeth and gums several times a day.

Tobacco is also used in the form of *gudakhu*, a paste made of tobacco and molasses. This is common in the eastern region. Creamy snuff, common in Goa, is a manufactured item marketed in toothpaste-like tubes. Its marketing technique exploits the prevailing misconception that tobacco is good for the teeth and gums. There are several herbal and medicinal tooth powders that contain tobacco.

EFFECTS OF ST USE

The most extensively studied and best documented health consequence of ST use in India is oral cancer. Numerous case-control and some cohort studies have clearly demonstrated the causal role of smokeless tobacco in oral cancer. This subject has already been reviewed and evaluated in depth (IARC, 1984). Interestingly, the experimental as well as epidemiological evidence with respect to areca nut chewing has been assessed as inadequate to demonstrate carcinogenicity (Gupta et al., 1982; IARC, 1984).

A less studied health consequence is the effect of smokeless tobacco use on reproduction. Two studies have indicated that ST use during pregnancy leads to significantly lower birth weight, higher placenta weight, and higher infant mortality (Krishnamurty, 1989).

Overall health consequences of smokeless tobacco use in India have been assessed through examination of the relative risk of all-cause mortality among tobacco users. This was done through cohort studies in two areas. In one area where *bidi* smoking and betel quid chewing were prevalent, the age-adjusted excess risk among smokers was 40 percent ($p < 0.05$). Surprisingly, even among chewers who did not smoke, the excess risk was 30 percent ($p < 0.05$). There was no clue, however, as to specific causes of this excess mortality among chewers (except oral cancer) because of a lack of information on the causes of death (Gupta et al., 1984a).

In another area, where reverse smoking was practiced, the age-adjusted excess mortality was close to 100 percent (Gupta et al., 1984b). An attempt has been made to estimate excess mortality attributable to tobacco use. The estimate is that every year, 630,000 adult deaths occur prematurely because of tobacco use in India (Gupta, 1988). Because of insufficient information, it is possible to categorize only 56 percent of these excess deaths according to cause (Notani et al., 1989).

EFFECTS OF EDUCATION

Can the tobacco habits in rural populations be changed through educational efforts, and would such efforts result in any health benefit? An answer to this question has been provided by an intervention study among rural Indian populations in three areas of India. More than 36,000 tobacco users were interviewed about tobacco use and examined for the presence of oral cancer and precancerous lesions. All these individuals were educated about the health hazards of tobacco use. Personal communication was provided by the examining dentist and a social scientist. Subjects also were educated through the use of documentary films, posters, newspaper articles, radio messages, and folk-art theater.

Considerable social science research was carried out to assess why people begin using tobacco, why they continue using it, what they perceive as the health effects of using tobacco, and what influences could help them discontinue its use. This research was continuous, and feedback was incorporated into the education program. The educational campaign was also continuous; interviews and examinations were carried out yearly. An assessment after 5 yr of followup showed that a significantly higher percentage of people in the intervention cohort stopped or reduced their tobacco use (smoking as well as ST use) than in the control cohort. Consequently, the incidence of precancerous lesions decreased substantially in the intervention cohort compared to the control cohort. Higher quit rates were achieved among smokeless tobacco users and thus the reduction in risk was also greater (Gupta et al., 1986a and 1986b). Similar results were reported after 8 yr of followup (Gupta et al., 1989).

CONCLUSION

On the whole, this discussion shows that the problem of smokeless tobacco use in India is quite different from that in industrialized countries. The health consequences of ST use are very serious but, except for oral cancer, are not as well understood as the consequences of cigarette smoking. It is possible to educate the population about the health risks of smokeless tobacco. Such an effort would result in a significant health benefit to the population.

REFERENCES

Bhonsle, R.B., Murti, P.R., Gupta, P.C., Mehta, F.S., Sinor, P.N., Irani, R.R., Pindborg, J.J. Regional variations in oral submucous fibrosis in India. *Community Dentistry and Oral Epidemiology* 15: 225-229, 1987.

Burton-Bradly, B.G. Is betel chewing carcinogenic? *Lancet* 2: 903, 1979.

Gupta, P.C. Health consequences of tobacco use in India. *World Smoking and Health* 13: 5-10, 1988.

Gupta, P.C., Aghi, M.B., Bhonsle, R.B., et al. Intervention study of chewing and smoking habits for primary prevention of oral cancer among 12,212 Indian villagers. In: *Tobacco: A Major International Health Hazard*, D.G. Zaridze and R. Peto (Editors). IARC Scientific Publications, No. 74. Lyon: International Agency for Research on Cancer, 1986b.

Gupta, P.C., Bhonsle, R.B., Mehta, F.S., Pindborg, J.J. Mortality experience in relation to tobacco chewing and smoking habits from a 10-year follow-up study in Ernakulam district, Kerala. *International Journal of Epidemiology* 13: 184-187, 1984a.

Gupta, P.C., Mehta, F.S., Pindborg, J.J. Mortality among reverse chutta smokers in South India. *British Medical Journal* 289: 865-866, 1984b.

Gupta, P.C., Mehta, F.S., Pindborg, J.J., et al. Intervention study for primary prevention of oral cancer among 36,000 Indian tobacco users. *Lancet* 1: 1235-1238, 1986a.

Gupta, P.C., Mehta, F.S., Pindborg, J.J., et al. A primary prevention study of oral cancer among Indian villagers: Eight-year follow-up results. In: *Evaluating Effectiveness of Primary Prevention of Cancer*, M. Hakama, V. Beral, J.W. Cullen, and D.M. Parkin (Editors). IARC Scientific Publications, No. 103. Lyon: International Agency for Research on Cancer, 1989, pp. 149-156.

Gupta, P.C., Pindborg, J.J., Mehta, F.S. Comparison of carcinogenicity of betel quid with and without tobacco: An epidemiological review. *Ecology of Disease* 1: 213-219, 1982.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1984.

Krishnamurty, S. Tobacco use in pregnancy and reproductive outcome. In: *Tobacco and Health: The Indian Scene*, L.D. Sanghvi and P.N. Notani (Editors). Bombay: UICC-Tata Memorial Centre, 1989.

Mehta, F.S., Gupta, P.C., Daftary, D.K., Pindborg, J.J., Choksi, S.K. An epidemiologic study of oral cancer and precancerous conditions among 101,761 villagers in Maharashtra, India. *International Journal of Cancer* 10: 134-141, 1972.

Mehta, F.S., Pindborg, J.J., Gupta, P.C., Daftary, D.K. Epidemiologic and histologic study of oral cancer and leukoplakia among 50,915 villagers in India. *Cancer* 24: 832-849, 1969.

Notani, P.N., Jayant K., Sanghvi, L.D. Assessment of mortality and morbidity due to tobacco use in India. In: *Tobacco and Health: The Indian Scene*, L.D. Sanghvi and P.N. Notani (Editors). Bombay: UICC-Tata Memorial Centre, 1989.

Sanghvi, L.D. Tobacco related cancer. In: *Tobacco and Health: The Indian Scene*, L.D. Sanghvi and P.N. Notani (Editors). Bombay: UICC-Tata Memorial Centre, 1989.

Sinor, P.N., Gupta, P.C., Bhonsle, R.B., Murti, P.R., Daftary, D.K., Mehta, F.S., Pindborg, J.J. A case-control study of oral submucous fibrosis with special reference to the etiologic role of areca nut. *Journal of Oral Pathology and Medicine* 19: 94-98, 1990.

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Fraumeni, J.F. Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

Smokeless Tobacco in Professional Baseball: Patterns of Players' Use[1]

V.L. Ernster, D.G. Grady, L. Stillman, M. Walsh, and J.C. Greene

ABSTRACT Smokeless tobacco use was examined in 2,009 professional baseball players as part of a study of oral and other health effects of ST use conducted at spring training in 1988, 1989, and 1990. According to questionnaire data, 37.5 percent of participants had used ST within the previous week. Among current-week users, the median age at first ST use was 17 yr, median duration of use was 5 yr, median amount of use per day was 1 h, and median cotinine level was 92.8 ng/mL. The reported ST product usually used was more often a snuff than a chewing tobacco brand (77.1 vs. 19.7 percent, respectively). Of current-week users, 49 percent were year-round users. The latter used ST more hours per day, had used ST more recently, were more likely to use snuff, and had higher serum cotinine levels than did seasonal users. Similar differences were seen in comparisons of snuff to chewing tobacco users. Most ST users considered ST harmful to health; however, snuff users were more likely to report ST as "very" harmful, and year-round snuff users most likely to have noticed oral effects of use. Snuff users appeared more motivated to quit than did chewing tobacco users.

INTRODUCTION Over the 3-yr period from 1988 through 1990, we collected data on smokeless tobacco use among professional baseball players as part of a study of oral and other health effects of ST use. A description of the overall study design and highlights of the findings from the first year of the study have been published elsewhere (Ernster et al., 1990). Here we provide a detailed description of ST use patterns in professional baseball players, based on the combined data from all 3 yr of the study.

METHODS During spring training of 1988, 1989, and 1990, all players and coaching staff of the seven Major League Baseball teams and their associated minor league teams that conduct spring training in Phoenix and Tucson, Arizona, were eligible to participate in the study. Participants completed a questionnaire that provided detailed information about their patterns of ST use, as well as demographic and other data. In all 3 yr of the study, information was collected on age at initiation of ST use, duration of use, amount used, and type and brand of ST used most often. Analyses of type (snuff or chewing tobacco), brand, and amount (cans of snuff or pouches of chewing tobacco used per week) were based on the reported type usually used. Because some participants who usually used one type of ST (snuff or chew) also used the other type, we also calculated the number of hours of ST use per day as a combined measure of amount of use. Information about differences in ST use during the baseball season compared with the off-season were collected only in years 2 and 3 of the study, and data on perceptions of health effects and attempts to quit using ST were collected only in year 2. A question about perceived readiness to quit using ST was added in year 3.

[1] Supported by National Institute of Dental Research grant no. DE-08547-02.

On the basis of their self-reported ST use, we classified participants as non-users (those who had never used ST or who had never used it more than once a month in the past), former users (those who had used ST more than once a month in the past but not within the previous month), and current users (those who had used ST more than once per month and had used it within the previous month). Current users were further divided into current-month users (those who had used ST within the past month but not within the past week) and current-week users (those who had used ST within the past week). Unless otherwise indicated, the results reported here for current ST users pertain only to current-week users.

During the first 2 yr of the study, blood was drawn for biochemical validation of self-reported ST use. Low serum cotinine levels (< 12 ng/mL) together with normal serum thiocyanate levels (< 85 mmol/L) were considered biochemical evidence of no use of tobacco. According to this standard, 95 percent of participants in the first year of the study from whom blood samples were available and who reported that they neither used ST nor smoked cigarettes (357 of 376) were classified biochemically as non-users. Given the accuracy of self-reported use status and because blood was collected from only about 80 percent of players in the first year of the study, 50 percent of players in the second year, and none of the players in the third year, ST use status in this paper is based on self-report. Data on cotinine levels are for those players from whom blood was obtained in the first 2 yr of the study. Finally, information on most recent ST use was collected only in the first 2 yr of the study.

For univariate statistical analyses, standard t tests and χ^2 tests were performed, as appropriate. We have based the p values for the multivariate analyses on the standard errors of log odds ratios, adjusted for covariates by means of multiple logistic analysis (Hosner and Lemeshow, 1989).

RESULTS The distribution of the participants by age, race, education, cigarette smoking, and alcohol use at the time of entry into the study is shown in Table 1. These are the combined baseline data from players who were new to the study in each of the 3 yr, a total of 2,039 players (1,109 first seen in 1988, 532 first seen in 1989, and 398 first seen in 1990). A majority of the players were aged 20 to 29 yr (76.3 percent), were white (68.1 percent), and had had at least some college education (75.2 percent). Current cigarette smoking was rare (3.1 percent), and alcohol consumption was generally moderate (21.3 percent were nondrinkers and 72.5 percent consumed fewer than 14 drinks per week).

At baseline, 37.5 percent of participants reported having used ST within the past week and 3.4 percent within the past month but not the past week; 12.6 percent reported themselves to be former ST users and 46.5 percent non-users (Figure 1). We deleted from the analysis 30 players for whom ST use status was not determined. Characteristics of ST use among current-week users, again based on the combined baseline data, are shown in Table 2. The median age at first use of ST was 17 yr, median duration of use was 5 yr, and median time ST was used per day was 1 h. With respect to brand usually used, 77.1 percent of players named a snuff brand and only

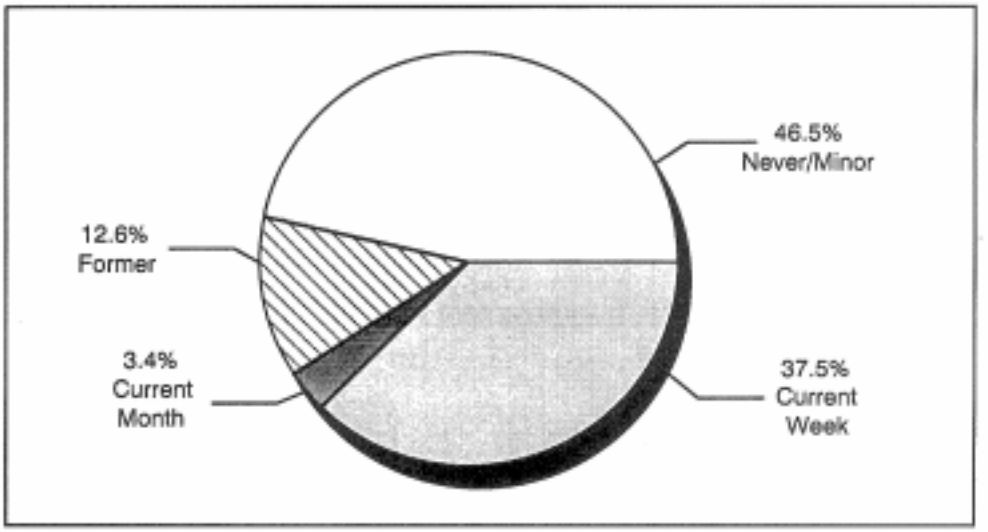
Table 1
Baseline demographic and other characteristics from all 3 yr combined

	Number[a]	Percentage[b]
Age		
< 20 yr	241	11.9%
20 to 24	1,125	55.6
25 to 29	419	20.7
30 to 34	129	6.4
35 to 39	48	2.4
≥ 40	63	3.1
Total	2,025	100.1
Race		
White	1,387	68.1
Hispanic	350	17.2
Black	269	13.2
Asian	25	1.2
Other	5	0.2
Total	2,036	99.9
Education		
Elementary	43	2.2
Some high school	76	3.8
High school graduate	375	18.8
Some college	1,041	52.3
College graduate	455	22.9
Total	1,990	100.0
Cigarette Smoking		
Never-smoker	1,779	88.1
Former smoker	177	8.8
Current smoker	63	3.1
Total	2,019	100.0
Alcohol Consumption		
Nondrinker	429	21.3
< 14 drinks per week	1,459	72.5
14 to 20 drinks per week	85	4.2
> 20 drinks per week	40	2.0
Total	2,013	100.0

[a] *Totals vary because of missing data.*
[b] *Totals vary from 100 percent because of rounding.*

19.7 percent a brand of chewing tobacco; 3.1 percent listed both. Among those who usually used snuff, Copenhagen and Skoal (69.5 percent and 20.8 percent, respectively) were the most popular brands named, and among those who usually used chew, Levi Garrett and Red Man (46.9 percent and 40.7 percent, respectively) were the most popular. Among snuff users, 39.7 percent used less than one can per week, 35.8 percent used one to three

Figure 1
Distribution of subjects (n=2,009) at baseline examination, by ST use



cans per week, and 24.4 percent used more than three cans per week (median = two cans per week). Among chewing tobacco users 48.9 percent used less than one pouch per week, 31.1 percent used one to three pouches per week, and 20.0 percent used more than three pouches per week (median = 1.5 pouches per week). Data on most recent ST use and cotinine levels were available for participants who entered the study in 1988 or 1989. Among current-week users, nearly one-third (30.7 percent) reported using ST within the preceding hour (median = 3.8 h since last use), and 76.1 percent had serum cotinine levels 25 ng/mL (median = 92.8 ng/mL).

Because data on seasonal use were collected only in years 2 and 3 of the study, analyses of characteristics associated with seasonal vs. year-round use are confined to all current ST users seen in year 2 of the study, whether or not they had been in the study in year 1 (n=397), plus all current users new to the study in year 3 (n=160). These data showed that about one-half of current-week users (51 percent) used ST almost exclusively during the baseball season ("I rarely use it during off-season").

Given the accepted addictiveness of tobacco use, it was curious to us that fully half of the current-week users were able to refrain from ST during the off-season. We therefore decided to compare characteristics of seasonal and year-round ST users in our study (Table 3). There were no significant differences in age or race, but year-round users were significantly more likely to have started using ST at an early age (mean age at initiation of use was 16.2 vs. 17.4 yr for year-round and seasonal users, respectively), to have used ST for a longer period of time (7.0 vs. 5.1 yr), to currently use ST more hours per day (2.1 vs. 0.9 h), to have used ST more recently (10.5 vs. 25.8 h since last use), to use snuff (90.0 percent vs. 66.4 percent), and to be white (87.4 vs. 77.0 percent).

Table 2
ST use characteristics in current-week users[a]

	Number[b]	Percentage[c]
Age at First Use		
< 10 yr	18	2.4%
10 to 14	141	18.7
15 to 19	444	59.0
≥ 20	150	19.9
Total	753	100.0
Duration of Use		
≤ 3 yr	206	27.9
4 to 6	275	37.2
7 to 9	119	16.1
≥ 10	139	18.8
Total	739	100.0
Hours in Mouth		
0.0 to 0.5 h/d	186	27.2
> 0.5 to 1.0	157	23.0
> 1.0 to 1.5	93	13.6
> 1.5 to 2.0	67	9.8
> 2.0 to 4.0	123	18.0
≥ 4.0	57	8.3
Total	683	99.9
Type of ST Usually Used		
Snuff	567	77.1
Chew	145	19.7
Both equally	23	3.1
Total	735	99.9
Brand of Snuff Usually Used		
Copenhagen	394	69.5
Skoal	118	20.8
Hawken	37	6.5
Other	18	3.2
Total	567	100.0
Brand of Chew Usually Used		
Levi Garrett	68	46.9
Red Man	59	40.7
Other	18	12.4
Total	145	100.0
Amount of Use		
Snuff (cans/wk)[d]		
≤ 1	216	39.7
> 1 to 3	195	35.8
> 3	133	24.4
Total	544	99.9

Footnotes at end of table.

Table 2 (continued)

	Number[b]	Percentage[c]
Amount of Use (continued)		
Chew (pouches/wk)[e]		
≤ 1	66	48.9%
> 1 to 3	42	31.1
> 3	27	20.0
Total	135	100.0
Time Since Last Use[f]		
> 24 h	86	15.9
> 12 to 24	134	24.8
> 1 to 12	155	28.6
≤ 1	166	30.7
Total	541	100.0
Cotinine (ng/mL)[f]		
0 to 25	99	23.9
> 25 to 75	87	21.0
> 75 to 200	133	32.0
> 200	96	23.1
Total	415	100.0

[a] *Includes baseline data for current-week users from all 3 yr combined for all variables except cotinine, most recent use, and seasonal use.*
[b] *Totals vary because of missing data.*
[c] *Totals vary from 100 percent because of rounding.*
[d] *Includes only subjects who usually use snuff.*
[e] *Includes only subjects who usually use chew.*
[f] *Includes all current-week users seen in year 1 plus all new current-week players from year 2.*

We then attempted to determine which of these ST use variables were independently associated with seasonal use by constructing a multiple logistic regression model with seasonal use as the dependent variable and age, race, age at first use, amount, type, duration, and most recent use as the predictor variables. This analysis was confined to year 2 users, the only group with data on both seasonal use and most recent use. Only hours of use per day ($p < 0.0001$) and type of ST usually used ($p=0.0008$) were independently associated with seasonal use in the multivariate model. Duration of use may also be independently associated with seasonal use ($p=0.066$). Analyses of brand and amount used in relation to seasonality of use were performed separately for snuff and for chewing tobacco users (Table 4). Among year-round snuff users, Copenhagen was more commonly used and Hawken less commonly used than among seasonal snuff users; and year-round users were much more likely than seasonal users to report use of more than three cans per week. There were no significant brand differences between year-round and seasonal chewing tobacco users, but the former were much more likely to report use of more than three pouches per week.

Table 3
Characteristics of seasonal and year-round ST users seen in years 2 and 3 and association of each characteristic with year-round use

	Year-Round		Seasonal		Univariate Analysis[a]	Multivariate Analysis[b]
	(n)	Mean	(n)	Mean	p	
Age, yr	(270)	24.1	(282)	24.1	(0.92)	(0.13)
Age at First Use, yr	(271)	16.2	(281)	17.4	(0.0001)	(0.29)
Duration of Use, yr	(266)	7.0	(275)	5.1	(< 0.0001)	(0.066)
Hours of Use/Day	(256)	2.1	(251)	0.9	(< 0.0001)	(0.0001)
Time Since Last Use, h[c]	(162)	10.5	(178)	25.8	(0.0001)	(0.096)
	(n)	Percentage	(n)	Percentage		
Race[d]					(0.009)	(0.785)
White	(235)	87.4%	(213)	77.0%		
Black	(19)	7.0	(27)	9.9		
Latino	(14)	5.2	(32)	11.4		
Other	(1)	0.4	(5)	1.8		
Type of ST Usually Used[e]					(< 0.0001)	(0.0008)
Snuff	(238)	90.0	(180)	66.4		
Chew	(22)	8.2	(82)	30.4		
Both	(5)	1.8	(9)	3.2		

[a] *Univariate p values are from two-tailed t tests for continuous variables (age, age at first use, duration of use, hours of use per day, and time since last use) and from χ^2 tests for categorical variables (race and type usually used).*

[b] *Multivariate p values are from the multivariate logistic regression model with seasonal use as the dependent variable and all variables in the table as predictors. The multivariate analysis was confined to current-week ST users seen in year 2 (n=298), because information on recency of use was not available for players seen in year 3.*

[c] *Includes only current-week ST users seen in year 2.*

[d] *Players in the "other" category were excluded from both univariate and multivariate analyses.*

[e] *Players who reported using both snuff and chewing tobacco were excluded from both univariate and multivariate analyses.*

Finally, serum cotinine levels were significantly higher in year-round users than in seasonal users (199.2 vs. 71.4 ng/mL, respectively).

The fact that year-round users were more likely than seasonal users to use snuff suggested that it might be a more addictive product than chewing tobacco. We therefore compared snuff and chew users in terms of several surrogate measures of addiction, using the combined baseline data from all 3 yr of the study. Compared with chewing tobacco users, snuff users used ST more hours per day, had used ST more recently, had higher serum cotinine levels, and were more likely to be year-round users (Table 5).

Table 4
Additional characteristics of seasonal and year-round ST users

	Seasonal	Year-Round	p[a]
Brand ST Usually Used			
Snuff	(n=186)	(n=243)	0.0001
Copenhagen	62.9%	76.5%	
Skoal	22.0	18.9	
Hawken	12.4	2.0	
Other	2.7	2.5	
Chew	(n=85)	(n=22)	0.68
Levi Garrett	51.8	45.4	
Red Man	29.4	27.3	
Other	18.8	27.3	
Amount Used			
Snuff (cans/wk)	(n=182)	(n=241)	< 0.0001
≤ 1	63.2	24.1	
1 to 3	29.7	39.0	
> 3	7.1	36.9	
Chew (pouches/wk)	(n=84)	(n=22)	0.023
≤ 1	56.0	36.4	
1 to 3	32.1	27.3	
> 3	11.9	36.4	
Serum Cotinine (ng/mL)	(n=121) mean=71.4	(n=95) mean=199.2	< 0.0001

[a] *Based on χ^2 test.*

We found no significant differences in age, race, age at initiation, or duration of ST use between snuff and chewing tobacco users among these professional baseball players.

Finally, we examined perceptions of health effects of ST and attitudes toward quitting among current-week users, distributed by seasonality of use and type of ST used (Table 6). Year-round snuff users were more likely to report having noticed sores, white patches, or gum problems where ST is placed in the mouth (39.5 percent) than were chew users or seasonal snuff users. When asked how harmful to their health they thought ST use to be, only a small proportion indicated that they didn't know or that it was "not at all" harmful. However, both year-round and seasonal snuff users were more likely than chewing tobacco users to think that ST is "very harmful" and nearly twice as likely to indicate that they were thinking about quitting ST use in the next 12 mo. Snuff users reported more quit attempts to date than chewing tobacco users and scored somewhat higher on a quit ladder, a scale of 1 to 10 that measures readiness to quit.

Table 5
Characteristics of current snuff and chewing tobacco users

	Type of ST Usually Used		
	Snuff	Chewing Tobacco	
	Mean		p[a]
Use/Day, h	1.9	1.1	0.0004
Hours Since Last Use	14.1	29.3	< 0.0001
Serum Cotinine, ng/mL[b]	149.6	46.7	< 0.0001
Age, yr	24.1	24.6	0.29
Age at Initiation	17.1	17.0	0.80
Duration of Use, yr	6.1	6.1	0.93
	Percentage		
Year-Round Users[c]	56.6%	20.6%	
Race			
White	83.2	80.6	0.87
Black	8.3	9.0	
Latino	7.8	9.7	
Other	0.7	0.7	

[a] *Based on t test for continuous variables; χ^2 test for race.*
[b] *Serum cotinine analysis is based on all current-week ST users seen in year 1 plus all current-week users seen for the first time in year 2.*
[c] *Seasonal use analysis is based on all current-week ST users seen in year 2 plus all current-week users seen for the first time in year 3.*

DISCUSSION This report, based on combined baseline data collected from 2,009 Major League and minor league baseball players from 1988 through 1990, confirms our earlier findings, based on the 1988 data alone (Ernster et al., 1990), of a high rate of ST use in this population (37.5 percent). Two other studies of Major League Baseball players surveyed in 1987 reported comparable results: Connolly and coworkers (1988) found that 34 percent of 265 players who completed questionnaires at spring training in Florida were self-reported current ST users, and Wisniewski and Bartolucci (1989) found that 45.6 percent of the 528 players on 25 teams who responded to a mail survey were current users. In all three studies, snuff was found to be the preferred form of ST; a snuff brand was reported as the product usually used by 77 percent of ST users in our study and 71 percent of users surveyed by Connolly and coworkers.

The prevalence of ST use found among professional baseball players is much higher than that reported for young men in the general population (5.9 percent among men aged 20 to 29 in 1986, with much regional variation) (Bauman et al., 1989; Marcus et al., 1989; Novotny et al., 1989; Rouse, 1989), and generally much higher than reported in school-based studies of adolescent males (Ary, 1989; Ary et al., 1987; Boyd et al., 1987; Brownson

Table 6
Perceptions of health effects and attitudes toward quitting among current ST users, by seasonality of use and type of ST used[a]

	Seasonal Use		Year-Round Use	
	Chew	Snuff	Chew	Snuff
"How harmful do you think that chewing/dipping tobacco is for your health?"	(n=65)	(n=131)	(n=18)	(n=172)
Not at all harmful/don't know	4.6%	12.2%	5.6%	5.8%
Slightly/somewhat harmful	72.3	56.5	72.2	61.1
Very harmful	23.1	31.3	22.2	33.1
"Have you ever noticed sores, white patches, or gum problems where you hold the tobacco in your mouth?"	(n=65)	(n=131)	(n=18)	(n=172)
Affirmative responses	16.9	22.1	22.2	39.5
"Are you seriously thinking about quitting chewing/ dipping tobacco in the next 12 months?"	(n=64)	(n=129)	(n=18)	(n=172)
Affirmative responses	34.4	58.1	33.3	60.2
"How many times have you seriously tried to quit?"	(n=60)	(n=111)	(n=18)	(n=157)
	0.4	1.7	1.1	1.7
Score on quitting ladder[b]	(n=47)	(n=93)	(n=10)	(n=142)
	4.7	5.8	4.0	5.7

[a] *Results are based on all current-week users seen in year 2, with the exception of "score on quitting ladder," which is based on all current-week smokeless tobacco users seen in year 3.*

[b] *Measures readiness to quit using smokeless tobacco; values range from 0 ("no thought of quitting") to 10 ("taking action to quit").*

et al., 1990; Colburn et al., 1989; Creath et al., 1988; Glover et al., 1986; Jones and Moberg, 1988; Kegeles et al., 1989; Leopardi et al., 1989; Murray et al., 1988). Only Native Americans have comparably high rates in the United States (Bruerd, 1990; Hall and Dexter, 1988; Schinke et al., 1989). However, it appears that about one-half of users in our study who reported use within the past week were seasonal users, which means that the prevalence of year-round use may be closer to 18 or 19 percent in this group. On the one hand, the ability to "take or leave" ST during the off-season might be seen as an indication that many individuals can use ST without becoming addicted. On the other hand, year-round users differ from seasonal users in ways that suggest ST is an addictive product for many individuals. Year-round users use ST more hours per day, use more cans of snuff or pouches of chew, have used ST more recently, and have higher serum cotinine levels than seasonal users.

Year-round users in our study were also more likely to usually use snuff than were seasonal users, which suggests that snuff may be more addictive than chewing tobacco. When we compared current ST users who usually

used snuff to those who usually used chewing tobacco, our findings were similar to those reported in the comparison of year-round to seasonal users. Snuff users used ST more hours per day than chewing tobacco users, had used ST more recently, and had higher serum cotinine levels. Not surprisingly, they were also more likely to be year-round users. Among snuff users, those who used ST year-round were more likely to use the Copenhagen brand and less likely to use the Hawken brand than seasonal users. Thus, snuff users, particularly users of the most popular brands, seem much less able to restrict their ST use to the baseball season than chewing tobacco users. These findings are interesting in light of our earlier report of a much higher risk of leukoplakia in snuff users than in chewing tobacco users, and the lower risk of leukoplakia in users of Hawken than in users of other snuff brands (Grady et al., 1990).

Finally, snuff users seem to have a greater awareness of the health hazards of ST use and to be more motivated to quit than chewing tobacco users. Snuff users were more likely to think their ST use might be "very" harmful to their health, were much more likely to indicate that they were seriously thinking about quitting in the next 12 mo, and had higher scores on readiness to quit. Compared with chewing tobacco users and seasonal snuff users, year-round snuff users were also more likely to report having noticed sores, white patches, or gum problems where they held tobacco in the mouth. Given their own subjective experience, and our earlier findings of a much greater risk of oral lesions for snuff users, the perception of greater adverse health risks on the part of snuff users than of chewing tobacco users may be well founded.

Our findings underscore the importance, for future studies, of distinguishing between snuff and chewing tobacco users and, where applicable, between year-round and seasonal ST users. If only because intensity of use appears to differ markedly by ST type and seasonal use, separate analyses should be performed to distinguish health risks associated with these different types of use.

ACKNOWLEDGMENTS We gratefully acknowledge the support of the management, trainers, players, coaches, and medical staff of the following professional baseball teams: California Angels, Chicago Cubs, Cleveland Indians, Milwaukee Brewers, Oakland Athletics, San Francisco Giants, and Seattle Mariners. We also thank Jana Murray, Patricia Lee, and Maureen Morris for their invaluable efforts.

REFERENCES

Ary, D.V. Use of smokeless tobacco among male adolescents: Concurrent and prospective relationships. *National Cancer Institute Monographs* 8: 49-55, 1989.

Ary, D.V., Lichtenstein, E., Severson, H.H. Smokeless tobacco use among male adolescents: Patterns, correlates, predictors, and the use of other drugs. *Preventive Medicine* 16: 385-401, 1987.

Bauman, K.E., Koch, G.G., Fisher, L.A., Bryan, E.S. Use of smokeless tobacco by age, race, and gender in ten standard metropolitan statistical areas of the southeast United States. *National Cancer Institute Monographs* 8: 35-37, 1989.

Boyd, G.D., et al. Use of smokeless tobacco among children and adolescents in the United States. *Preventive Medicine* 16: 402-421, 1987.

Brownson, R.C., Dilorenzo, T.M., Van Tuinen, M., et al. Patterns of cigarette and smokeless tobacco use among children and adolescents. *Preventive Medicine* 19: 170-180, 1990.

Bruerd, B. Smokeless tobacco use among Native American school children. *Public Health Reports* 105: 196-201, 1990.

Colborn, J.W., Cummings, K.M., Michalek, A.M. Correlates of adolescents' use of smokeless tobacco. *Health Education Quarterly* 16: 91-100, 1989.

Connolly, G.N., Orleans, C.T., Kogan, M. Use of smokeless tobacco in major-league baseball. *New England Journal of Medicine* 318: 1281-1284, 1988.

Creath, C.J., Bradley, D.H., Shelton, W.O., et al. The prevalence of smokeless tobacco use among adolescent male athletes. *Journal of the American Dental Association* 116: 43-48, 1988.

Ernster, V.L., Grady, D.G., Greene, J.C., Walsh, M., Robertson, P.B., Daniels, T., Benowitz, N., Siegel, D., Gerbert, B., Hauck, W. Smokeless tobacco use and health effects among baseball players. *Journal of the American Medical Association* 264: 218-224, 1990.

Glover, E.D., Johnson, R., Laflin, M., et al. Smokeless tobacco use trends among college students in the United States. *World Smoking and Health* 11: 4-9, 1986.

Grady, D., Greene, J., Daniels, T.E., Ernster, V.L., Robertson, P.B., Hande, W., Greenspan, D., Greenspan, J.S., Silverman, S. Oral mucosal lesions found in smokeless tobacco users. *Journal of the American Dental Association* 121: 117-123, 1990.

Hall, R.L., Dexter, D. Smokeless tobacco use and attitudes toward smokeless tobacco among Native Americans and other adolescents in the northwest. *American Journal of Public Health* 78: 1586-1588, 1988.

Hosner, D.W., Lemeshow, S. *Applied Logistic Regression*. New York: John Wiley and Sons, 1989.

Jones, R.B., Moberg, D.P. Correlates of smokeless tobacco use in a male adolescent population. *American Journal of Public Health* 78: 61-63, 1988.

Kegeles, S.S., Burleson, J.A., Miozza, J. Cigarette and smokeless tobacco use among Connecticut adolescents. *American Journal of Public Health* 79: 1413-1414, 1989.

Leopardi, E.A., Poulson, T.C., Neiger, B.L., et al. A report of two smokeless tobacco surveys and associated intervention strategies among Utah adolescents. *Journal of Cancer Education* 4: 125-134, 1989.

Marcus, A.C., Crane, L.A., Shopland, D.R., Lynn, W.R. Use of smokeless tobacco in the United States: Recent estimates from the Current Population Survey. *National Cancer Institute Monographs* 8: 17-23, 1989.

Murray, D.M., Roche, L.M., Goldman, A.I., Whitbeck, J. Smokeless tobacco use among ninth graders in a north-central metropolitan population: Cross-sectional and prospective associations with age, gender, race, family structure, and other drug use. *Preventive Medicine* 17: 449-460, 1988.

Novotny, T.E., Pierce, J.P., Fiore, M.C., Davis, R.M. Smokeless tobacco use in the United States: The Adult Use of Tobacco Surveys. *National Cancer Institute Monographs* 8: 25-28, 1989.

Rouse, B.A. Epidemiology of smokeless tobacco use: A national study. *National Cancer Institute Monographs* 8: 29-33, 1989.

Schinke, S.P., Schilling, R.F. II, Gilchrist, L.D., Ashby, M.R., Kitajima, E. Native youth and ST: Prevalence rates, gender differences, and descriptive characteristics. *National Cancer Institute Monographs* 8: 39-42, 1989.

Wisniewski, J.F., Bartolucci, A.A. Comparative patterns of smokeless tobacco usage among major league baseball personnel. *Journal of Oral Pathology and Medicine* 18: 322-326, 1989.

Chapter 2

Clinical and Pathological Effects

CONTENTS

Oral Mucosal Lesions: Clinical Findings In Relation to Smokeless Tobacco Use Among U.S. Baseball Players[1]

John C. Greene, Virginia L. Ernster, Deborah G. Grady, Paul B. Robertson, Margaret M. Walsh, and Linda A. Stillman

ABSTRACT We have been conducting a 3-yr study of the health effects of smokeless tobacco use by members of Major League and minor league baseball teams. Findings from the first year (1988), involving 1,109 players, were previously reported. This report presents data about ST usage and associated oral mucosal lesions among 894 additional players who first entered the study in 1989 (n=523) and 1990 (n=371). The 1989 and 1990 data were obtained by the same study team, using the same study methods, as in 1988. Findings in the two sets of data are highly consistent. There was a significant association between the prevalence of oral mucosal lesions and the frequency, amount, and recency of ST use and the type and brand used. Players who used ST year round had a much higher prevalence of lesions (67 percent) than those who used it primarily during the baseball season (32 percent). Among those who used snuff year round, 72 percent had lesions, compared to 44 percent of seasonal users. Year-round snuff users who used four or more cans per week had a lesion prevalence of 88 percent.

INTRODUCTION We previously reported findings from the first year (1988) of a 3-yr study of the health effects of smokeless tobacco use among members of Major League and minor league baseball teams (Ernster et al., 1990; Grady et al., 1990 and 1991). Our primary purpose has been to study the association of ST use with various oral health and other health measures: oral mucosal lesions; gingival inflammation and recession; dental caries, erosion, attrition, and staining; pulse and blood pressure; and total and high-density lipoproteins. The study was designed to detect the effects of the type and brand of ST used and the amounts and length of use, controlling for potentially confounding variables such as age, alcohol use, cigarette smoking, and oral hygiene practices. For efficiency, we chose an adult population known to have a high prevalence of ST use—professional baseball players.

Ernster and coworkers (1990) described the overall study design and provided an overview of findings involving 1,109 players examined during the first year. Grady and colleagues (1990) presented a more extensive analysis of the mucosal lesion findings from the same study as they relate to a number of variables. Because the long-term use of ST has been strongly associated with oral cancer in previous studies (IARC, 1985; US DHHS, 1986; Winn et al., 1981), the oral mucosal lesions that are associated with ST use are of special interest and concern.

In this paper we present new data on oral mucosal lesions from 894 players examined for the first time in the second and third years of the study. We also examine the effects of seasonality of ST use on the prevalence and severity of oral mucosal lesions.

[1] Supported by National Institute of Dental Research grant no. DE-08547-02.

METHODS A detailed description of the study methods is presented by Ernster and coworkers elsewhere in this volume. The study population consisted of professional baseball players and other male team personnel from seven Major League teams and their associated minor league teams who participated in spring training in and around Phoenix, Arizona. People who agreed to take part in the study were asked to complete a questionnaire; a study staff member was present to assist in filling out the questionnaire, if needed. Players then were asked to rinse their mouths with water to remove any traces of tobacco, and to undergo an oral examination. The participants were instructed not to inform the examiners of their ST use status. The examination consisted of an inspection of the oral mucosa and a standardized clinical examination of the periodontium and the dentition by trained examiners calibrated for diagnostic uniformity. Unless otherwise indicated, the present analysis is confined to players who first entered the study in 1989 (n=523) and 1990 (n=371).

Because self-reports of ST use were more than 90 percent accurate, according to serum cotinine and thiocyanate verification in year 1, the smokeless tobacco use status reported here is derived from questionnaire data. On the basis of self-reports, the players were classified as never or minor users, former users, current-month users, or current-week users. Abnormalities of the oral mucosa were categorized and recorded as (1) leukoplakia/erythroplakia—any white, opaque, or leathery-appearing plaque not clinically characteristic of another type of white lesion, or any red patch not clinically identifiable as another type of red lesion, or (2) other mucosal abnormalities—i.e., interdental hyperkeratosis, retromolar hyperkeratosis, other white changes, aphthous ulcer, other mucosal ulcers, pigmented lesions, fibroma, or tongue changes. The leukoplakia/erythroplakia lesions were also classified into four degrees of severity: (1) no or slight color change, with a change in texture; (2) color and texture change, but no thickening; (3) color and texture change, with mild to moderate thickening; (4) no normal color, severe texture change, and heavy thickening. These categories are similar to the ones developed by Greer and Poulson (1983) from a system published by Axéll and coworkers (1976). Because erythroplakia was not noted in the current study group, we refer to the oral mucosal lesions either as leukoplakia or, simply, as lesions.

Study participants were asked to indicate the type and brand of ST they usually used. They were also asked when they first started using ST and how long they had been using it. Participants' exposure to ST was recorded in terms of cans per week for snuff, and pouches per week for chew. When a player reported using both snuff and chew, but used one type usually and the other only occasionally, the exposure was counted as the dominant type for purposes of determining dose-response. We calculated hours per day that either snuff or chew was kept in the mouth as a combined measure of ST use. Recency of use was reported in hours since ST was last used. Because recency of use was not recorded in the third year of the study, multivariate logistic regression analysis of recency data was performed on second-year data only.

During the first year of the study, it became obvious that some players used ST only during the regular baseball season, whereas others used it year round. Therefore, seasonality of use was included in the questionnaire for the second and third years, and the effects of this variable are reported here. Seasonality analyses include all current-week ST users examined in year 2 (n=389), some of whom had been examined for the other variables in year 1 and are not otherwise included in this report, and all current-week users examined in year 3 but not in year 2 (n=156).

RESULTS Baseline demographic and other characteristics (age, race, educational level, cigarette smoking, alcohol consumption) of the 894 study participants were very similar in percentages to those of the entire study population from all 3 yr combined (see Ernster et al., this volume). Most of the study subjects (76 percent) were between the ages of 20 and 29, nearly 70 percent were white, and 77 percent had some college education. Only 2.0 percent of subjects reported that they were current smokers, and only 4.7 percent reported heavy alcohol consumption. Approximately two-thirds indicated that they visited their dentist to have their teeth cleaned at least once every 12 mo.

As we reported earlier (Ernster et al., 1990; Grady et al., 1990), the prevalence of ST use among professional baseball players is very high. Data on ST use status were available for 879 players in this study. Nearly 37 percent of these players (323) reported using ST within the 7 d prior to the examination and thus were classified as current-week users. Most of those had used ST in the previous 24 h. Oral mucosal lesions were found in 51.7 percent of the current-week users, 3.5 percent of former users, and 2.9 percent of non-users (odds ratio = 36.0 for current-week users vs. non-users). The association between severity of the most severe lesion and ST use status is shown in Table 1. Most of the lesions (76.7 percent) were degree 1 or 2 in severity. All of the degree 3 and degree 4 lesions were found in current-week users.

The prevalence of lesions according to characteristics of ST use in current-week users is shown in Table 2. Prevalence of lesions increased with earlier first use, with longer duration of use, and with more recent use. Lesion prevalence increased as the exposure to ST increased, whether measured by the amount used per week or by hours ST was held in the mouth each day. Among snuff users, 40 percent of those who used one can or less than two cans per week had lesions, whereas 84.7 percent of those who used more than three cans per week had lesions. A similar trend was seen among tobacco chewers: 8 percent of those who used up to one pouch per week had lesions, while 27 percent of those who used more than three pouches per week had lesions. When prevalence of lesions was analyzed according to the number of hours of ST use per day, 30 percent of those using ST for 0.5 h/d or less had lesions, whereas 85 percent of those using ST for more than 4.0 h/d had lesions.

The prevalence of lesions in current-week users, according to the type and brand of ST used, is shown in Table 3. Most of the ST users usually used snuff, and the snuff users had a much higher risk of developing oral lesions

Table 1
Severity of most severe lesion, by ST use status

	Degree of Severity of Lesions[a]								
	1		2		3		4		
	n	(%)	n	(%)	n	(%)	n	(%)	Total
Use Status									
Never/minor	10	(83.3)	2	(16.7)	0	(0.0)	0	(0.0)	12
Former	3	(75.0)	1	(25.0)	0	(0.0)	0	(0.0)	4
Current month	2	(100.0)	0	(0.0)	0	(0.0)	0	(0.0)	2
Current week	63	(37.7)	61	(36.5)	40	(24.0)	3	(1.8)	167
Total	78		64		40		3		185

[a] *Lesions were graded from 1 to 4 by increasing degree of severity.*

than chew users (61.2 percent vs. 14.8 percent). Of the players who used Copenhagen brand snuff, 72 percent had a lesion at the time of the examination, compared with only 11 percent of the Hawken brand users.

Multivariate logistic regression analyses were based on year 2 data only, because recency of use was not recorded in year 3. Variables included in the analysis were duration, recency, and amount of use; age when ST use began; type and brand of ST; and seasonality of use. Amount of use (hours per day), recency of use, and use of Copenhagen and Skoal brands were independently and significantly associated with the presence of oral lesions (Table 4). Lesion prevalence increased with increasing amounts of use (odds ratio [OR]=1.46 per hour of use per day; p=0.009). Lesion prevalence decreased with time since last use of ST (OR=0.96 for each additional hour since last use; p=0.0012). The odds of having a lesion were eightfold higher for users of Copenhagen snuff than for users of chew (OR=8.05; $p < 0.0001$), and more than sixfold higher for users of Skoal than for users of chew (OR=6.78; p=0.0003). Duration of use and age when ST use was begun showed no independent effect on lesion prevalence. The odds ratio for lesion prevalence among year-round users compared to seasonal users was elevated but was not statistically significant (OR=1.46; p=0.23).

Of the current-week users, 277 (51.2 percent) used ST only during the baseball season, and 264 (48.8 percent) used it year round. Of the seasonal users, about one-third (32.1 percent) had lesions, in contrast to two-thirds (66.7 percent) of the year-round users. The distribution of seasonal and year-round ST users with lesions according to various ST use characteristics is shown in Table 5. The percentage of participants with lesions was greater for year-round users than for seasonal users at virtually all levels of each use characteristic.

Table 6 shows the prevalence of lesions in seasonal and year-round current-week users, according to type and brand of ST usually used. Except for Hawken brand snuff, for which there were only 5 year-round users,

Table 2
Prevalence of leukoplakia, by ST use characteristics in current-week users (n=323)

	n[a]	Percentage With Leukoplakia
Age at First Use		
< 10 yr	13	69.2%
10-14	75	57.3
15-19	185	51.9
≥ 20	49	36.7
Duration of Use		
≤ 3 yr	98	37.8
4-6	122	52.5
7-9	45	68.9
≥ 10	52	59.6
Time Since Last Used[b]		
> 24 h	26	11.5
> 12-24	36	22.2
> 1-12	37	51.4
≤ 1	57	79.0
Amount of Use		
Snuff[c] (cans/wk)		
≤ 1	95	40.0
> 1-3	89	65.2
> 3	72	84.7
Chew[d] (pouches/wk)		
≤ 1	26	7.7
> 1-3	17	17.6
> 3	11	27.3
Hours ST in Mouth/Day		
0.0-0.5	76	30.3
> 0.5-1.0	65	36.9
> 1.0-1.5	37	54.0
> 1.5-2.0	26	61.5
> 2.0-4.0	66	74.2
> 4.0	26	84.6

[a] *Category totals differ because of missing values.*
[b] *Information not collected in year 3, so these values are for year 2 subjects only.*
[c] *Includes only subjects who usually used snuff.*
[d] *Includes only subjects who usually used chew.*

prevalence was greater in the year-round user group for each type and brand usually used. Nearly three-fourths (72 percent) of those who used snuff year round had lesions, compared to 44 percent of seasonal snuff users. Players who used chew were more likely to be seasonal users, while those who used snuff were more likely to be year-round users.

Table 3
Prevalence of leukoplakia, by type and brand of ST used, current-week users (n=323)

	n[a]	Percentage With Leukoplakia
Type of ST Usually Used		
Snuff	258	61.2%
Chew	54	14.8
Brand of ST Usually Used		
Snuff		
Copenhagen	177	72.3
Skoal	54	42.6
Hawken	18	11.1
Chew		
Red Man	21	14.3
Levi Garrett	24	16.7

[a] *Category totals differ because of missing values.*

Table 4
Association of various risk factors with the presence of oral lesions in current ST users[a]

	Risk per:	Odds Ratio	p
Duration	Year of ST use	1.00	0.91
Amount	Hours/day of ST use	1.46	0.0093
Initiation	Year of age ST use begun	1.03	0.49
Recency	Hours since ST last used	0.96	0.0012
Type/Brand			
Chew	(referent)	1.00	—
Copenhagen		8.05	< 0.0001
Skoal		6.78	0.0003
Hawken		3.24	0.16
Seasonality			
Seasonal	(referent)	1.00	—
Year round		1.46	0.23

[a] *This multivariate regression analysis includes all current-week users seen in year 2 for whom data on all variables were available; n=290.*

Table 5
Prevalence of leukoplakia in current-week ST users (n=545),[a] by seasonality of use and use characteristics

	Seasonal Current-Week ST Users		Year-Round Current-Week ST Users	
	n[b]	Percentage With Leukoplakia	n[b]	Percentage With Leukoplakia
All Current-Week ST Users[c]	277	32.1%	264	66.7%
Age at First Use				
< 10 yr	3	66.7	12	75.0
10-14	34	20.6	72	65.3
15-19	174	32.8	137	67.2
≥ 20	65	33.8	43	65.1
Duration of Use				
≤ 3 yr	104	36.5	46	54.4
4-6	105	31.4	102	64.7
7-9	28	28.6	47	76.6
≥ 10	34	26.5	64	70.3
Time Since Last Used[d]				
> 24 h	46	10.9	14	21.4
> 12-24	47	27.8	33	30.3
> 1-12	36	34.0	46	71.7
≤ 1	47	51.1	64	87.5
Hours ST in Mouth/Day				
0.0-0.5	111	26.1	42	40.5
> 0.5-1.0	70	35.7	48	56.2
> 1.0-1.5	25	40.0	41	65.8
> 1.5-2.0	15	33.3	26	76.9
> 2.0-4.0	21	52.4	64	79.7
> 4.0	5	20.0	28	82.1
Amount of Use				
Snuff[e] (cans/wk)				
≤ 1	112	33.9	55	52.7
> 1-3	55	61.8	91	70.3
> 3	13	61.5	88	87.5
Chew[f] (pouches/wk)				
≤ 1	47	4.3	8	0.0
> 1-3	26	11.5	6	50.0
> 3	9	22.2	8	25.0

[a] Includes some players examined in year 1 who are not otherwise included in this report.
[b] Category totals differ because of missing values.
[c] Seasonality data were not available for four current-week users.
[d] Information not collected in year 3, so these values are for year 2 subjects only.
[e] Includes only persons who usually used snuff.
[f] Includes only persons who usually used chew.

Table 6
Prevalence of leukoplakia in current-week users (n=545), by seasonality of use and by type and brand of ST used

	Seasonal Current-Week ST Users		Year-Round Current-Week ST Users	
	n[a]	Percentage With Leukoplakia	n[a]	Percentage With Leukoplakia
Type of ST Usually Used				
Snuff	184	44.0%	236	72.0%
Chew	82	8.5	22	22.7
Brand of ST Usually Used				
Snuff				
Copenhagen	117	52.1	179	76.5
Skoal	39	33.3	46	63.0
Hawken	23	17.4	5	0.0
Chew				
Red Man	24	12.5	6	16.7
Levi Garrett	42	4.8	10	30.0
Other	16	12.5	6	16.7

[a] *Category totals differ because of missing values.*

Univariate analysis showed that lesion prevalence was significantly increased among year-round ST users (OR=4.17; $p < 0.0001$), compared to seasonal users. However, as shown in Table 4, seasonality showed no statistically significant independent effect on lesion prevalence.

Finally, lesions found in year-round ST users were more likely to be severe (29.6 percent were degree 3 or 4 leukoplakia) than those in seasonal users (6.7 percent degree 3 or 4 leukoplakia).

DISCUSSION Questionnaire and clinical examination data from 894 new participants in the second and third years of this 3-yr study of professional baseball players yielded findings that were highly consistent with the previously published results from the first year (Ernster et al., 1990; Grady et al., 1990). The prevalence of current-week ST use in this new group of players was 36.7 percent, and oral lesions were found in 51.7 percent of current-week users, yielding an odds ratio of 36.0 for users compared with non-users. The prevalence of oral lesions increased with the amount used (whether expressed in hours per day or in cans or pouches per week), with recency of last use, with use of snuff instead of chew, and with use of certain brands of snuff. All of these trends are very consistent with and confirmatory of our earlier reports on first-year findings from our study of 1,109 different players.

That ST users are at increased risk of oral lesions has been shown by other investigators (US DHHS, 1986). However, no other group has had as large a sample of adults or examined the effects of so many variables related to patterns of use.

Lesion prevalence associated with the use of Copenhagen and Skoal brands of snuff continued to be several times greater than prevalence with the use of chew, as we have noted previously. Our previously published first-year results were the first to show an increased risk of oral lesions associated with recency of use and with type and brand used. Other investigators have previously reported an increased risk of oral lesions with increasing amounts of ST used (Greer and Poulson, 1983; Hirsch et al., 1982; Poulson et al., 1984; Wolfe and Carlos, 1987).

Information on the prevalence and severity of oral mucosal lesions related to seasonality of ST use has not previously been reported. The data gathered on seasonality of use in years 2 and 3 of this study show that about half of the current ST users in this population are year-round users, while the other half use ST only during the baseball season. Year-round users were much more likely to have lesions (66.7 percent) than were seasonal users (32.1 percent). It might be expected that, because the oral exams were made at the beginning of the season, seasonal users would naturally be less likely to have developed a white keratotic mucosal response. However, when the effects of other variables are taken into account, seasonality was not a statistically significant predictor of lesion prevalence in the multivariate model shown in Table 4; in that model the ST use variables of amount, recency, and type of ST were shown to have statistically significant independent effects. These findings are not unexpected, because there is no real reason to believe that seasonality should have an effect on lesion prevalence apart from the amount and type of ST used. It is interesting to note that of those who reported using ST year round, 91.5 percent used snuff, while of those who used ST only during the baseball season, 69.2 percent used snuff. Furthermore, of the year-round snuff users, 77.9 percent used Copenhagen, whereas 65.4 percent of the seasonal snuff users did so.

Thus, the greater prevalence of lesions among year-round users compared to seasonal users appears to result largely from increased and recent exposure to certain types and brands of ST. In keeping with this, the lesions found in year-round users tended to be more severe. From other evidence gathered in this study (Ernster et al., this volume), it appears that year-round users are more likely to have difficulty quitting and to be addicted. For these reasons, year-round ST users should be preferentially targeted for documenting long-term health effects of such use and for cessation intervention.

REFERENCES

Axéll, T., Mörnstad, H., Sundström, B. The relation of the clinical picture to the histopathology of snuff dipper's lesions in a Swedish population. *Journal of Oral Pathology* 5: 229-236, 1976.

Ernster, V.L., Grady, D.G., Greene, J.C., Walsh, M., Robertson, P.B., Daniels, T., Benowitz, N., Siegel, D., Gerbert, B., Hauck, W. Smokeless tobacco use and health effects among baseball players. *Journal of the American Medical Association* 264: 218-224, 1990.

Grady, D.G., Ernster, V.L., Stillman, L., Greene, J.C., Daniels, T.E., Silverman, S. A surprise with smokeless tobacco oral lesions. *Journal of the American Dental Association* 122: 62-64, 1991.

Grady, D.G., Greene, J.C., Daniels, T.E., Ernster, V.L., Robertson, P.B., Hauck, W., Greenspan, D., Greenspan, J.S., Silverman, S. Oral mucosal lesions found in smokeless tobacco users. *Journal of the American Dental Association* 121: 117-123, 1990.

Greer, R.O., Jr., Poulson, T.C. Oral tissue alterations associated with the use of smokeless tobacco by teenagers. *Oral Surgery, Oral Medicine, Oral Pathology* 56: 275-284, 1983.

Hirsch, J.M., Neyden, G., Thilander, H. A clinical, histomorphological and histochemical study on snuff-induced lesions of varying severity. *Journal of Oral Pathology* 11: 387-398, 1982.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985, pp. 37-135.

Poulson, T.C., Lindenmuth, J.E., Greer, R.O., Jr. A comparison of the use of smokeless tobacco in rural and urban teenagers. *CA—A Cancer Journal for Clinicians* 34: 248-261, 1984.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication (NIH) No. 86-2874, 1986.

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Fraumeni, J.F., Jr. Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

Wolfe, M.D., Carlos, J.P. Oral health effects of smokeless tobacco use in Navajo Indian adolescents. *Community Dentistry and Oral Epidemiology* 15: 230-235, 1987.

Smokeless Tobacco Use in India: Effects on Oral Mucosa[1]

P.R. Murti, P.C. Gupta, R.B. Bhonsle, D.K. Daftary,
F.S. Mehta, and J.J. Pindborg

ABSTRACT Smokeless tobacco is used in diverse forms in India for chewing, sucking in the mouth until it becomes bland, or applying over the teeth and gums. Tobacco is chewed most commonly in betel quid. ST use results in oral cancer, precancer, and other oral lesions. Tobacco smoking and other factors such as nutrition and viruses like HPV and HSV may modify the effect of ST use. There is a need to study the exact role of other factors in the causation of oral cancer. Long-term studies have demonstrated that in most instances oral cancer arises from precancerous lesions or conditions. The methods currently available to identify cancer potential in precancer, however, have limited usefulness. Therefore, research should be extended in that direction.

INTRODUCTION Tobacco was introduced to India by Portuguese traders about 1600. Although tobacco was initially smoked, it later was used in smokeless form as well. Of the 400 million individuals aged 15 and over in India, 47 percent use tobacco and 16 percent use tobacco in smokeless forms; of the 250 million kg of tobacco consumed each year, 86 percent is used for smoking, 13 percent in smokeless forms other than snuff, and 1 percent as snuff (Sanghvi, 1989).

Smokeless tobacco is used in diverse forms in different regions of India for chewing, holding in the mouth, or applying over teeth and gums. ST is chewed, more often in betel quid (*pan*), consisting of betel leaves (*Piper betle*), areca nut (*Areca catechu*), slaked lime, and catechu (*Acacia catechu*); with areca nut and slaked lime (e.g., *mainpuri* tobacco, *mawa*, *pan masala*); and less commonly by itself. A mixture of tobacco and slaked lime (*khaini*) is kept in the mouth and sucked. Other products like roasted and powdered tobacco (*mishri*), dry snuff (*bajjar*), or tobacco paste with molasses (*gudhaku*) are applied over teeth and gums. Creamy snuff is used initially as a dentifrice but soon turns into an addiction.

Betel quid chewing is widespread all over India, whereas most other uses are popular in specific geographic regions. A link between betel quid chewing and oral cancer was suspected as early as 1908 (Bentall, 1908; Fells, 1908), and by the late 1960's, several studies had demonstrated the association between betel quid chewing and other forms of tobacco use with oral cancer in India (Orr, 1933; Sanghvi et al., 1955; Shanta and Krishnamurthy, 1959; Wahi, 1968).

Several oral lesions are associated with ST use, of which oral cancer and precancer are the most serious. Since 1966 we have conducted several cross-sectional and prospective epidemiological studies on oral cancer and

[1] Supported solely by funds from the National Institutes of Health, through Indo-U.S. Fund Research Agreement no. 01-022-N.

precancer (Bhonsle et al., 1976; Daftary et al., 1980; Gupta et al., 1980; Mehta et al., 1971 and 1972) in different geographic areas chosen for specific tobacco habits. In this paper, we review oral mucosal lesions associated with some common forms of ST use in India.

This paper discusses oral cancer and precancerous lesions and conditions associated with chewing betel quid with tobacco in Ernakulam district in Kerala State. The effects of chewing *mainpuri* tobacco, *mawa*, the use of tobacco-plus-lime mixture, and other habits practiced elsewhere in India are also described briefly.

PREVALENCE OF ST USE

There are no nationwide surveys on the prevalence of tobacco use in India. Table 1 gives the prevalence of ST use reported among 255,194 individuals in 9 areas in some major studies: The reported prevalence ranges from 11 to 49 percent. Except in Singhbhum, Pune, and Mainpuri districts, chewing betel quid with tobacco was widespread. Chewing tobacco without any other ingredient was practiced in two areas by 3 and 7 percent of the individuals. However, there are differences in the selection of samples, methodology, and the age groups of the individuals in these studies.

EFFECTS OF BETEL QUID CHEWING

Chewing of betel quid (betel leaf, areca nut, slaked lime, sweeteners, and flavoring) is an Indian habit dating back more than 2,000 years. Sometime after its introduction, tobacco became an important constituent of betel quid, and currently most habitual users chew betel quid with tobacco (Figure 1). Various tobacco preparations (e.g., *zarda* or *kiwam*) that can be used in the betel quid are commercially available. Regional differences in the choice of material and the way in which betel quid is chewed abound.

Betel quid chewing inevitably stains the mucosa bright red as a result of the formation of *o*-quinone from water-soluble polyphenols, notably leucocynidins, at an alkaline pH of 8 to 9 via secondary reactions (Jayalakshmi and Mathew, 1980). These stains can be washed clean or will disappear if the habit is discontinued. It is not uncommon, however, to find habitual chewers with perpetually stained mucosa.

***Pan* Chewer's Lesion**

Among heavily addicted betel quid chewers, a thick, brownish-black encrusted lesion was observed on the buccal mucosa and the mandibular groove corresponding to the site of the quid placement. This could be scraped off with a piece of gauze. The annual age-adjusted incidence rate of this lesion was 28.0 per 1,000 male betel quid chewers and 17.4 per 1,000 among females (Gupta et al., 1980).

These lesions show a pale-staining, parakeratin-like surface layer of epithelium containing round nuclear remnants, ballooning vacuolated cells, and consistent epithelial hyperplasia. *Pan* chewer's lesion is a thick encrustation, but it disappears when the habit is discontinued. The lesion does not seem to exhibit any cancer potential. Of the 532 lesions observed over a 3-yr period, 26 percent remained persistent, 45 percent regressed spontaneously, and 29 percent recurred (Gupta et al., 1980).

Table 1
Prevalence of some ST habits in India, by area

	n	Prevalence	Predominant Habit[a]
Ernakulam	10,287	37%	Betel quid
Srikakulam	10,169	11	Betel quid
Singhbhum	10,048	28	Tobacco-plus-lime
Darbhanga	10,340	16	Betel quid
Bhavnagar	10,071	13	Betel quid, dry snuff
Pune	101,761	49	Tobacco-plus-lime, *mishri*
Mainpuri	35,000	30[b]	*Mainpuri* tobacco, *pattiwala*
Lucknow	10,000	13[c]	Betel quid
Ahmedabad	57,718	47[d]	Betel quid

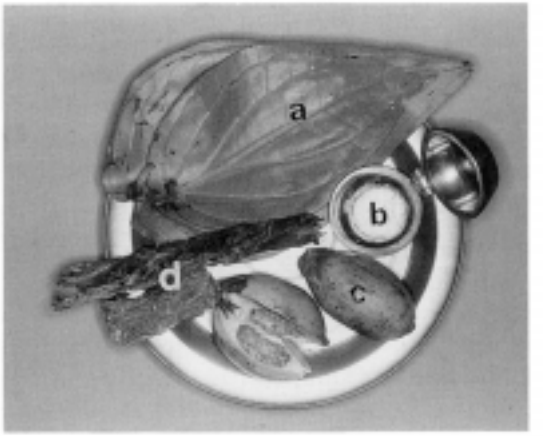
[a] *Many smoked as well.*
[b] *7 percent* mainpuri *tobacco.*
[c] *3 percent tobacco alone.*
[d] *7 percent tobacco alone.*
Source: Mehta et al., 1971 and 1972; Wahi, 1968; Pindborg et al., 1972; Smith et al., 1975.

Epithelial Dysplasia Epithelial dysplasia is generally assumed to indicate the malignant potential of a lesion. Dysplastic lesions exhibited malignant transformation rates that were 15 times higher than nondysplastic lesions (Gupta et al., 1980). In a 10-yr followup study of 16 dysplastic lesions in betel quid chewers, 8 dysplastic lesions in those who chewed and smoked, and 18 dysplastic lesions in smokers only, 19 percent, 25 percent, and 6 percent, respectively, progressed to cancer, and 25 percent, 50 percent, and 22 percent, respectively, regressed. The remaining dysplastic lesions either remained stationary or changed their clinical characteristics.

Leukoplakia Leukoplakia is the most common precancerous lesion, initially hypothesized as precancerous mainly because of its coexistence with cancer. In a study of 650 oral cancers in India, 32 percent had coexistent leukoplakia (Paymaster, 1956). Furthermore, leukoplakias and oral cancers share the same etiologic agent, tobacco, and they occur at strikingly similar locations. For example, oral cancer and leukoplakias among betel quid chewers generally occur on the buccal mucosa. The most important evidence of leukoplakia's precancerous nature, however, is its excess risk for cancer as demonstrated in prospective studies (see below).

Epidemiology The prevalence of leukoplakia in Ernakulam district was 17 per 1,000. Prevalence was 18 per 1,000 among betel quid chewers and 61 per 1,000 among those who chewed and smoked (Mehta et al., 1969). The annual age-adjusted incidence rate was 2.1 per 1,000 person-years among men and 1.3 per 1,000 among women; among betel quid chewers, the incidence was 2.5 per 1,000 among men and 3.0 per 1,000 among women. The incidence rate was highest (6.0) in those who smoked and chewed (Gupta et al., 1980).

Figure 1
Constituents of betel quid in Kerala: (a) betel leaf; (b) shell lime; (c) raw areca nut; (d) tobacco. Note that catechu is not used in Kerala.

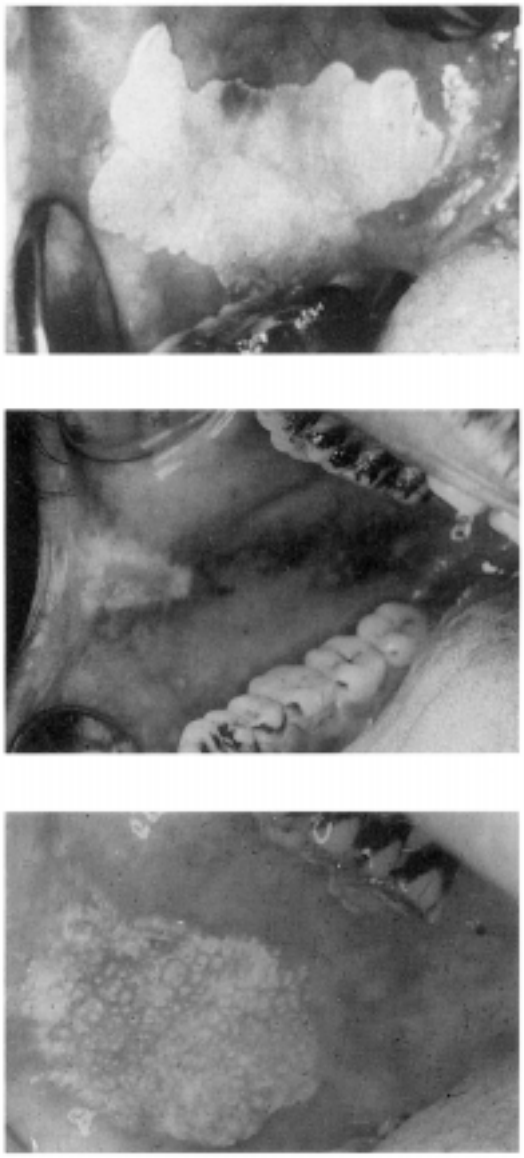


Clinical Aspects Leukoplakia is a raised white patch on the oral mucosa that measures 5 mm or more and that cannot be scraped off and cannot be attributed to any other diagnosable disease (Mehta et al., 1969). Leukoplakias are classified as homogeneous, ulcerated, or nodular types. Homogeneous leukoplakia is characterized by raised plaque formation consisting of plaque (Figure 2, top) or groups of plaques varying in size and with irregular edges. Ulcerated leukoplakia consists of an area of ulceration that is sometimes surrounded by keratinized areas, pigmentation, or both (Figure 2, middle) and generally occurs among smokers of *bidi* (a kind of cheap smoking stick). Nodular leukoplakia consists of many small white nodules on an erythematous base (Figure 2, bottom). About 94 percent of the leukoplakias among betel quid chewers are the homogeneous type. The risk for cancer varies with different clinical types of leukoplakia (see below).

In Ernakulam district, leukoplakias occurred more often among men, with the male-to-female ratio being 4:1 (Mehta et al., 1971). The average age of individuals with this lesion was 47.8 yr. Generally the location of leukoplakias varies according to where tobacco is held in the mouth. For example, those associated with betel quid chewing occurred more often on the posterior part of the buccal mucosa and the mandibular groove, whereas leukoplakias associated with tobacco-plus-lime and *mishri* occur in the premolar region of the buccal mucosa and the labial mucosa.

Histology Leukoplakias associated with betel quid chewing showed hyperorthokeratosis in 82 percent and hyperparakeratosis in 12 percent. Hyperparakeratosis was seen somewhat more frequently in those who chewed betel quid and smoked (23 percent) or who smoked only (24 percent) (Mehta et al., 1969). Epithelial dysplasia was observed in 15 percent of the betel quid-associated leukoplakias compared with 5 and 8 percent in those associated with the combined habit of chewing and smoking or smoking only. In regard to epithelial thickness, atrophy was observed in 75 percent of the

Figure 2
Homogeneous leukoplakia in a betel quid chewer (top); ulcerated leukoplakia in a *bidi* smoker (middle); nodular leukoplakia in a betel quid chewer (bottom).



betel quid-associated leukoplakias compared with 40 and 31 percent, respectively, in those associated with the combined habit or with smoking only. Orthokeratinization occurred generally in combination with epithelial atrophy, whereas parakeratinization was more commonly associated with hyperplastic epithelium.

Natural History In a 10-yr followup study of 94 betel quid-associated leukoplakias, 7 percent progressed to oral cancer, in contrast to only 4 percent of the 85 leukoplakias associated with combined betel quid chewing and smoking and none of the 45 leukoplakias associated with smoking only (Gupta et al., 1980). The higher rate of malignant transformation in betel quid-associated leukoplakias may be caused by epithelial atrophy. The malignant transformation rate was highest in nodular leukoplakias (21.0 percent) and in homogeneous leukoplakias (1.7 percent) (Gupta et al., 1980). Leukoplakias associated with betel quid chewing were less persistent (25 percent), regressed more often (57 percent), and showed higher recurrence rates than did smoking-associated leukoplakias. In another 8-yr followup study, the relative risk (RR) for the development of oral cancer among all oral precancerous lesions and conditions was highest (RR=3,243.2) for nodular leukoplakia (Gupta et al., 1989); the RR for homogeneous (RR=25.6) and ulcerated (RR=43.6) leukoplakia were also high and significant.

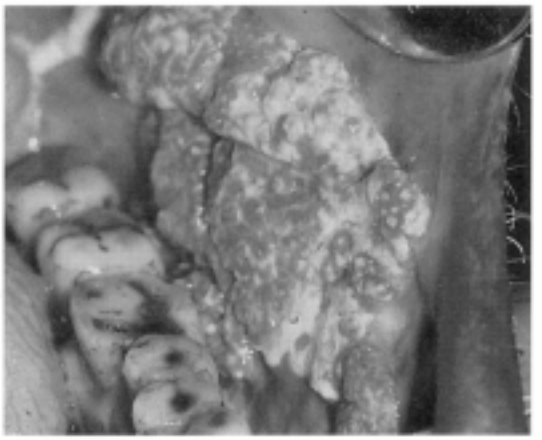
Oral Cancer Oral cancer is common in India. According to weighted averages from six cancer registries in India, oral cancer is the second and third most common cancer among men and women, respectively (Indian Council of Medical Research, 1989). Contributing to the problem of high morbidity, patients with this disease often seek medical attention in a late clinical stage, consequently having a poor prognosis.

Epidemiology In a cross-sectional study among 10,287 villagers in Ernakulam district, all 12 oral cancers occurred among those who chewed betel quid; 6 of these patients smoked as well (Mehta et al., 1969). In a 10-yr followup study of the above sample, the overall age-adjusted incidence was 16 per 100,000 person-years, 23 per 100,000 among betel quid chewers, and 32 per 100,000 in those who chewed and smoked (Gupta et al., 1980). Several extensive independent assessments have confirmed the causal role of betel quid chewing and oral cancer (IARC, 1985; National Institutes of Health, 1986; US DHHS, 1986; WHO, 1984). About 30 percent of the oral cancers in this part of the world are attributable to betel quid chewing and an additional 50 percent to the combined habit of chewing and smoking (WHO, 1984).

In India, most of the habitual betel quid chewers include tobacco. Betel quid, however, may be chewed without tobacco. As several investigators in the past were not explicit about this, there was some confusion about the carcinogenicity of betel quid with and without tobacco. The question has been extensively reviewed, and the conclusions are that the relative risk for oral cancer in those who chewed betel quid without tobacco was insignificant or significantly lower than for those who chewed betel quid with tobacco (Gupta et al., 1982). Overall, there is inadequate evidence that the habit of chewing betel quid without tobacco is carcinogenic for humans (IARC, 1985).

Clinical Aspects Oral cancer occurs more frequently among men than among women. In a sample of 2,007 oral cancers seen at the main cancer treatment center in Kerala, the male-to-female ratio was 1.8:1 (Nair et al., 1988); the mean age for men was 57.1 yr and for women, 58.6. The bucccal mucosa is the most commonly involved location (Figure 3), and in a sample of 2,007 oral

Figure 3
Buccal carcinoma in a betel quid chewer



cancers, 50 percent were located in the buccal mucosa and 24 percent on the tongue. The frequency of buccal mucosal involvement among men was slightly higher (53 percent) than that among women (45 percent). This may be because men smoke and chew, whereas women generally chew betel quid only. The mean ages of patients with cancer of the buccal mucosa and the tongue were quite similar, 56.9 and 55.8 yr, respectively, among men, and 58.5 and 56.5 yr among women.

Squamous cell carcinoma is the most common cancer, accounting for 95 to 98 percent of all oral malignancies in India. In Kerala, 7 percent of the 2,007 oral cancers reported were verrucous carcinomas (Nair et al., 1988). This is a variant of squamous cell carcinoma that is broad based and locally invasive; generally it does not metastasize.

Natural History In most instances oral cancer arises from precancerous lesions or conditions. In a 10-yr followup study in Ernakulam district, all 12 oral cancers arose from a precancerous condition (Gupta et al., 1980), whereas in another 8-yr followup study, 15 of the 19 oral cancers developed from precancer (RR=69.2) (Gupta et al., 1989).

Unfortunately, patients in India seek medical attention in the later stages of disease. Of the 2,007 oral cancers described above, only 12 percent were localized lesions and most of the remaining ones were extensive (Nair et al., 1988).

Intervention Oral cancer is amenable to primary and secondary prevention. In Ernakulam district, after 5 yr of educational intervention among 12,000 tobacco users, 10 percent of the men and 15 percent of the women discontinued their betel quid chewing, and 26 percent of men and 31 percent of women reduced betel quid use (Gupta et al., 1986). This resulted in a significant drop in the incidence of leukoplakia among betel quid chewers. The rate ratios that indicate the protective effect of educational intervention were 0.51 for men and 0.19 for women. After 8 yr the cessation rate

increased to 13 percent among men and 18 percent among women, and the rate of reduction increased to 35 percent among men and 39 percent among women (Gupta et al., 1990). Correspondingly, the number of observed leukoplakias among betel quid chewers was 27.0 among men vs. 79.7 predicted, and 49.0 among women vs. 163.9 predicted. A further increase in the rates of cessation and reduction in tobacco use and a decrease in the incidence of leukoplakia also were observed after 10 yr of intervention.

Submucous Fibrosis

Submucous fibrosis is a chronic condition marked by mucosal rigidity of various intensity caused by a fibroelastic transformation of the juxtaepithelial layer that results in progressive inability to open the mouth (Figure 4). When the tongue is involved, it is shrunken, and its mobility may be restricted. Occasional pharyngeal and esophageal involvement also has been observed. Diagnosis of the condition is based on the presence of palpable fibrous bands. Submucous fibrosis occurs predominantly among Indians at home and abroad and, to a lesser extent, other Asiatics. Areca nut chewing in any form is currently believed to be involved in the pathogenesis of submucous fibrosis (Bhonsle et al., 1987; Mehta et al., 1972; Sinor et al., 1990). Because areca nut, like tobacco, is an ingredient in betel quid, and tobacco is known to contain carcinogens, this condition is discussed here. Furthermore, submucous fibrosis is a high-risk precancerous condition (Gupta et al., 1989) in which the malignant potential is the result of epithelial atrophy and the action of carcinogens (Pindborg et al., 1984).

Epidemiology

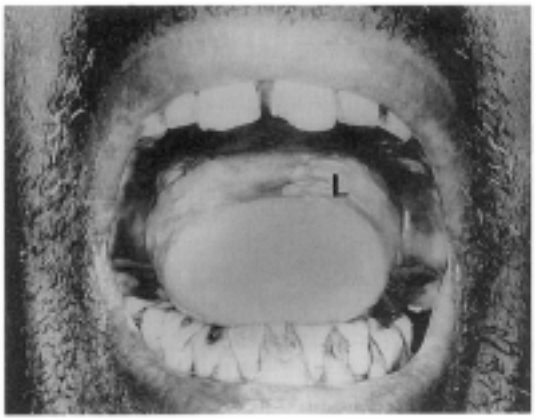
The prevalence and the incidence rates of submucous fibrosis are high in Ernakulam district relative to rates in other areas of India. The overall prevalence was 351 per 100,000, and prevalence was highest among betel quid chewers (1,090 per 100,000) (Pindborg et al., 1968). This condition was not seen among people who do not chew betel quid. The overall age-adjusted incidence rate was 7 per 100,000 person-years among men and 17 per 100,000 among women; all new cases of submucous fibrosis developed among betel quid chewers (Gupta et al., 1980). In comparison to the incidence rates, the prevalence rates seem too large (prevalence = incidence x duration).

Submucous fibrosis affects both sexes, but a definite female predominance was observed in Ernakulam district (Gupta et al., 1980; Pindborg et al., 1968). Such predominance, however, seemed to depend on the type and extent of areca nut chewing habits among men and women in different areas (Bhonsle et al., 1987). Although there is some regional variation, this condition generally occurs between the ages of 20 and 40 yr. The mean age of patients in Ernakulam district (51 yr) was higher than the mean age of patients in Pune district (37 yr) (Bhonsle et al., 1987).

Clinical Aspects

Submucous fibrosis commonly affects the buccal mucosa, retromolar areas, and the soft palate. The frequency of their involvement, however, varies from one geographic area to another, probably depending on the variations in the method of areca nut chewing (Bhonsle et al., 1987). The earliest and most common sign of this condition is blanching of the oral mucosa that imparts a marblelike appearance (Pindborg et al., 1980). When the disease is fully developed, palpable fibrous bands develop in the buccal

Figure 4
Limited oral opening in a patient with submucous fibrosis. Note the shrunken appearance of the tongue, the absence of lingual papillae, and associated leukoplakia (L).



mucosa, soft palate, and the rima oris. The bands run vertically in the buccal mucosa and are circular around the rima oris. As the disease progresses, the mucosa becomes stiff and the oral opening may be restricted. Petechial spots resulting from the breakdown of connective tissue support to the vasculature were observed in 11 percent of patients, according to Bhonsle and coworkers (1981). Furthermore, submucous fibrosis is often associated with leukoplakia (Figure 4), oral cancer, and pigmentation changes (Pindborg et al., 1968 and 1984). Most of the patients complained of a burning sensation, often aggravated by spicy food; another common complaint is excessive or decreased salivation.

Histology Epithelial atrophy, juxtaepithelial hyalinization, and varying density of collagen are the common features in submucous fibrosis. A notable feature is the presence of epithelial dysplasia in 26 percent of the cases (Pindborg et al., 1984).

Natural History Unlike other precancerous lesions, submucous fibrosis does not regress, either spontaneously or with cessation of betel quid chewing. The most serious aspect of this condition is its malignant potential. In a 17-yr followup study of 66 patients with submucous fibrosis, malignant transformation was observed in 0.4 percent at the end of 10 yr (Gupta et al., 1980), which increased to 4.5 percent at the end of 15 yr (Pindborg et al., 1984) and to 7.6 percent at the end of 17 yr (Murti et al., 1985). All five individuals in whom cancer developed were women who chewed betel quid with tobacco. In another 8-yr followup study of 12,000 tobacco users, the RR of malignant transformation for submucous fibrosis, compared with that for tobacco users without any oral mucosal lesion or condition, was 397.3 (Gupta et al., 1989). There is no effective cure for submucous fibrosis; however, the condition appears to be amenable to primary prevention (Murti et al., 1990).

Oral Lichen Planus Lichen planus is primarily a dermatologic disorder. Mucosal surfaces may be affected along with cutaneous lesions; the mucosal lesions may occur alone; or one may precede the other. The oral mucosa is more commonly affected, and oral lesions are suspected of having some cancer potential. In Ernakulam district, oral lichen planus was found to be strongly associated with betel quid chewing (Bhonsle et al., 1979; Gupta et al., 1980; Pindborg et al., 1972). This paper describes oral lichen planus as a result of betel quid use and its malignant potential.

Epidemiology Oral lichen planus was diagnosed on the basis of presence of Wickham's striae. The overall prevalence of oral lichen planus was 1.5 percent; it was highest (3.2 percent) among betel quid chewers, and lowest (0.3 percent) in non-users of tobacco (Pindborg et al., 1972). The annual age-adjusted incidence rate per 1,000 person-years was 2.1 among men and 2.5 in women who chewed betel quid vs. 0.6 among men and 0.9 in women who were not users of tobacco (Gupta et al., 1980). The RR for oral lichen planus among betel quid chewers was 6.2 for men and 4.9 for women (Bhonsle et al., 1979). Oral lesions thus showed a strong association with betel quid chewing, although tobacco use is not regarded as an etiologic factor for oral lichen planus.

Clinical Aspects Oral lichen planus occurred predominantly among women. The buccal mucosa was the most favored location; the lesions occurred in diverse morphological forms such as reticular, annular, linear, erosive or ulcerated, and pigmented forms. Of these, 20 percent were erosive or ulcerated lesions (Pindborg et al., 1972).

Natural History The malignant potential of oral lichen planus over a 10-yr period (mean, 5.1 yr) was assessed in 722 individuals with the condition. Oral cancer developed in three patients (0.4 percent) who had erosive (atrophic) lesions; two of them were betel quid chewers and the third was a smoker (Murti et al., 1986). In another 8-yr followup study of 344 individuals with oral lichen planus, the RR for malignant transformation was 15.8, which was not significant ($p > 0.05$) (Gupta et al., 1989). Oral lichen planus often regressed and sometimes recurred. The regression rates were highest in non-users of tobacco and lowest in those with the combined habits of chewing and smoking (Gupta et al., 1980). These observations support the hypothesis that tobacco plays some role in this disease.

OTHER ST USE AND EFFECTS

Chewing tobacco without any other ingredient does not seem to be common in India. In two cross-sectional studies, the prevalence of leukoplakia among tobacco chewers was 7.3 percent in Lucknow (Pindborg et al., 1972) and 12.7 percent in Ahmedabad (Smith et al., 1975).

Tobacco Chewing

Mainpuri Tobacco *Mainpuri* tobacco contains tobacco, slaked lime, finely cut areca nut, cloves, camphor, and other flavoring agents. This product is widely used in Mainpuri district in Uttar Pradesh and nearby areas, where it is known by different names, and is strongly associated with leukoplakia and oral cancer.

Leukoplakia The overall prevalence of leukoplakia in Mainpuri district was 5,160 per 100,000; it was 26,740 per 100,000 in *mainpuri* tobacco users and 15,160 among those who used *pattiwalla* tobacco (sun-cured tobacco chewed with or without slaked lime and without areca nut), which is another popular brand of ST used in that region. Among those who used both types of tobacco, the prevalence was 80,000 per 100,000 (Wahi et al., 1970).

Oral Cancer The prevalence of oral cancer in Mainpuri district was 99 per 100,000. Prevalence was 781 per 100,000 among *mainpuri* tobacco users, 117 per 100,000 in *pattiwalla* tobacco users, and 413 per 100,000 in those who used both forms. Prevalence was 36 per 100,000 in non-users of tobacco (Wahi, 1968).

***Mawa* Chewing** *Mawa* consists of areca nut shavings sprinkled with watery slaked lime. A little sun-cured tobacco is added, and the product is packed in a cellophane paper and sold. In recent years *mawa* chewing has gained considerable popularity in Bhavnagar district and adjoining areas in Gujarat.

Mawa chewing is strongly associated with oral submucous fibrosis (Sinor et al., 1990). For example, the RR for submucous fibrosis for all forms of areca nut chewing was 109.6, 106.4 for *mawa* chewing, and 780.0 for chewing *mawa* and betel quid. Clinically, submucous fibrosis in *mawa* chewers differs in regard to age, sex, and location from those observed among betel quid chewers.

Tobacco-Plus-Lime Sun-cured tobacco and slaked lime is used in Maharashtra and other states in northern and eastern India where it is known as *khaini*. A small quantity of tobacco and slaked lime is held in the palm and rolled with the thumb. In Maharashtra, the mixture is placed in the premolar region of the mandibular groove (Bhonsle et al., 1979); in Bihar and Uttar Pradesh, the mixture is held in the lower labial groove. It is sucked from time to time until it becomes bland.

Tobacco-Plus-Lime User's Lesion A thick, yellowish-white lesion, occasionally with loose tags of tissue, occurs where tobacco-plus-lime is held in the mouth by patients in Maharashtra. Prevalence among 101,761 villagers in Maharashtra was 2.9 percent (Bhonsle et al., 1979), and use occurred more often among men. As this lesion is similar in appearance to leukoplakia, it may be misdiagnosed. However, unlike leukoplakia this lesion can be scraped off; it will disappear if tobacco-plus-lime use is discontinued.

The histological characteristics include pale, parakeratin-like surface layers of epithelium containing round nuclear remnants, ballooning vacuolated cells, and epithelial hyperplasia (Bhonsle et al., 1979).

Tobacco-plus-lime user's lesion as a specific entity appears to be a counterpart of the *pan* chewer's lesion (described above). This lesion does not show any malignant potential.

Leukoplakia Tobacco-plus-lime use is associated with both leukoplakia and oral cancer among patients in Maharashtra (Mehta et al., 1972). The prevalence of leukoplakia was 1,442 per 100,000 among tobacco-plus-lime users and

6,337 per 100,000 among those who also smoked. Generally, leukoplakias associated with this habit occurred in the premolar region of the mandibular groove where the tobacco-plus-lime mixture is held.

Oral Cancer Oral cancer was also observed among tobacco-plus-lime users. Prevalence was 28 per 100,000 among tobacco-plus-lime users and 186 per 100,000 in those who also smoked. As with leukoplakia, cancer generally occurred at the site of placement of the tobacco-plus-lime, and sometimes leukoplakia coexisted with it.

Mishri Compared with use of betel quid or tobacco-plus-lime, *mishri* use produced fewer leukoplakias and no oral cancers. The prevalence of leukoplakia among *mishri* users in Maharashtra was 190 per 100,000. Although no oral cancers were detected among *mishri* users, short-term experimental tests have indicated mutagenic potential of *mishri* (Kulkarni et al., 1987).

Bajjar In Gujarat, dry snuff known as *bajjar* (described above as *mishri*) is applied over teeth and gums by 8 percent of 10,071 individuals (Mehta et al., 1971). Very few leukoplakias were observed among *bajjar* users; the prevalence was 280 per 100,000, and these leukoplakias did not show any cancer potential.

Gudhaku *Gudhaku* is a tobacco paste with molasses. Initially, it is used to clean teeth, but the product becomes addictive. *Gudhaku* was used by 8.3 percent of the 10,048 individuals in Singhbhum district in Bihar, but no oral lesions were observed among them.

Miscellaneous Products Among other ST products, *pan masala* and creamy snuff are increasingly becoming popular all over the country. *Pan masala* contains pieces of areca nut, slaked lime, catechu, and powdered tobacco. *Pan masala* comes in attractive tins and foil packs, making it appealing and convenient to use. Undoubtedly the aggressive advertising in the print and the electronic media contribute to its popularity. Animal experimental studies indicated the genotoxic nature of *pan masala*. Creamy snuff is initially used as a dentifrice but becomes an addictive substance. There are, however, no studies to date on the effects of these products on human oral mucosa.

DISCUSSION Betel quid chewing is the most common form of ST use in India. Extensive investigations in several cross-sectional and prospective epidemiological studies showed a strong association between many forms of ST use, oral cancer, precancer, and other oral mucosal lesions. Of the several ST forms, *mawa* and *pan masala* are of relatively recent origin, but there is an upswing in their use. *Mawa* chewing was demonstrated to cause oral submucous fibrosis (Sinor et al., 1990). There is practically no information, however, on the effects of *pan masala* on human oral mucosa.

Smokeless tobacco is an independent risk for oral cancer and precancer. There is also an additive effect of using more than one type of ST product and a synergistic effect with smoking (Gupta et al., 1980; Mehta et al., 1969; Pindborg et al., 1984; Sinor et al., 1990; Wahi et al., 1968 and 1970; WHO, 1984).

Although the pathogenic effects of ST in oral cancer and precancerous lesions are clear, the role of other factors that may modify the effect of ST use in India is not fully understood. Such factors may include nutrition, viruses, especially HPV and HSV, and the role of oncogenes. Furthermore, several animal studies and epidemiological studies have shown that vitamins A, C, and E, retinoic acid, carotenoids, riboflavin, selenium, and zinc may influence the risk of oral cancer independently or by modifying the effect of tobacco. There are several hospital-based studies with short-term followup on chemoprevention with vitamin A and beta-carotene. Population-based studies with long-term followup, however, are highly desirable and will be helpful in the development of practical chemopreventive measures.

The finding that oral cancer is generally preceded by precancer facilitates the early detection of cancer. Although nodular leukoplakia and submucous fibrosis are demonstrated to be a high-risk lesion and condition, respectively, the difficulty lies in identifying which particular lesion would progress to cancer. The conventional approach of using epithelial dysplasia as a marker has been very helpful. There is, however, a need to develop other markers based on advanced methodologies. In recent times flowcytometric methods have been tried and reported to be promising, but additional long-term studies are essential to demonstrate the potential of such methods.

REFERENCES

Bentall, W.C. Cancer in Travancore, South India. A summary of 1,700 cases. *British Journal of Medicine* 2: 1428-1431, 1908.

Bhonsle, R.B., Murti, P.R., Daftary, D.K., Mehta, F.S. An oral lesion in tobacco-lime users in Maharashtra, India. *Journal of Oral Pathology* 8: 47-52, 1979.

Bhonsle, R.B., Murti, P.R., Gupta, P.C., Mehta, F.S. Reverse *dhumti* smoking in Goa: An epidemiologic study of 5,449 villagers for oral precancerous lesions. *Indian Journal of Cancer* 13: 301-305, 1976.

Bhonsle, R.B., Murti, P.R., Gupta, P.C., Mehta, F.S., Sinor, P.N., Irani, R.R., Pindborg, J.J. Regional variations in oral submucous fibrosis in India. *Community Dentistry and Oral Epidemiology* 15: 225-229, 1987.

Bhonsle, R.B., Murti, P.R., Pindborg, J.J., Daftary, D.K., Mehta, F.S. Focal vascular dilatation and petechiae in oral submucous fibrosis. *Scandinavian Journal of Dental Research* 89: 270-274, 1981.

Bhonsle, R.B., Pindborg, J.J., Gupta, P.C., Murti, P.R., Mehta, F.S. Incidence rate of oral lichen planus among Indian villagers. *Acta Dermato-Venereologica* (Stockholm) 59: 255-257, 1979.

Daftary, D.K., Bhonsle, R.B., Murti, P.R., Pindborg, J.J., Mehta, F.S. An oral lichen planus-like lesion in Indian betel-tobacco chewers. *Scandinavian Journal of Dental Research* 88: 244-249, 1980.

Fells, A. Cancer of the mouth in southern India, with an analysis of 209 operations. *British Journal of Medicine* 2: 1357-1358, 1908.

Gupta, P.C., Bhonsle, R.B., Murti, P.R., Daftary, D.K., Mehta, F.S., Pindborg, J.J. An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. *Cancer* 63: 2247-2252, 1989.

Gupta, P.C., Mehta, F.S., Daftary, D.K., Pindborg, J.J., Bhonsle, R.B., Jalnawalla, P.N., Sinor, P.N., Pitkar, V.K., Murti, P.R., Irani, R.R., Shah, H.T., Kadam, P.M., Iyer, K.S.S., Iyer, H.M., Hegde, A.K., Chandrashekar, V.K., Shroff, B.C., Sahiar, B.E., Mehta, M.N. Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. *Community Dentistry and Oral Epidemiology* 8: 287-333, 1980.

Gupta, P.C., Mehta, F.S., Pindborg, J.J., Aghi, M.B., Bhonsle, R.B., Daftary, D.K., Murti, P.R., Shah, H.T., Sinor, P.N. Intervention study for primary prevention of oral cancer among 36,000 Indian tobacco users. *Lancet* 1(8492): 1235-1239, 1986.

Gupta, P.C., Mehta, F.S., Pindborg, J.J., Aghi, M.B., Bhonsle, R.B., Murti, P.R., Daftary, D.K. A primary prevention study of oral cancer among Indian villagers: 8-year follow-up results. In: *Evaluating Effectiveness of Primary Prevention of Cancer*, M. Hakama, V. Beral, J.W. Cullen, and D.M. Parkin (Editors). Lyon: IARC, 1990, pp. 149-156.

Gupta, P.C., Pindborg, J.J., Mehta, F.S. Comparison of carcinogenicity of betel quid with and without tobacco: An epidemiological review. *Ecology of Disease* 1: 213-219, 1982.

Indian Council of Medical Research. *National Cancer Registry*: *Annual Report*. New Delhi: Indian Council of Medical Research, 1989.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985.

Jayalakshmi, A., Mathew, A.G. Chemical composition and processing. In: *The Areca Nut Palm*, K.V.A. Bavappa, M.K. Nair, and T. Premkumar (Editors). Kasargod, India: Central Plantation Crops Research Institute, 1980, p. 241.

Kulkarni, J.R., Sarkar, S., Bhide, S.V. Mutagenecity of abstracts of brown and black mishri, pyrolysed products of tobacco using short-term tests. *Mutagenesis* 2: 263-266, 1987.

Mehta, F.S., Gupta, P.C., Daftary, D.K., Pindborg, J.J. Choksi, S.K. An epidemiologic study of oral cancer and precancerous conditions among 101,761 villagers in Maharashtra, India. *International Journal of Cancer* 10: 134-141, 1972.

Mehta, F.S., Pindborg, J.J., Gupta, P.C., Daftary, D.K. Epidemiologic and histologic study of oral cancer and leukoplakia among 50,915 villagers in India. *Cancer* 24: 832-849, 1969.

Mehta, F.S., Pindborg, J.J., Hamner, J.E. III., et al. *Report on Investigations of Oral Cancer and Precancerous Conditions in Indian Rural Populations, 1966-69*. Copenhagen: Munksgaard, 1971.

Murti, P.R., Bhonsle, R.B., Pindborg, J.J., Daftary, D.K., Gupta, P.C., Mehta, F.S. Malignant transformation rate in oral submucous fibrosis over a 17-yr period. *Community Dentistry and Oral Epidemiology* 13: 340-341, 1985.

Murti, P.R., Daftary, D.K., Bhonsle, R.B., Gupta, P.C., Mehta, F.S., Pindborg, J.J. Malignant potential of oral lichen planus: Observations in 722 patients from India. *Journal of Oral Pathology* 15: 71-77, 1986.

Murti, P.R., Gupta, P.C., Bhonsle, R.B., Daftary, D.K., Mehta, F.S., Pindborg, J.J. Effect on the incidence of oral submucous fibrosis of intervention in the areca nut chewing habit. *Journal of Oral Pathology and Medicine* 19: 99-100, 1990.

Nair, M.K., Sankaranarayanan, R., Padmanabhan, T.K., Padmakumari, G. Clinical profile of 2,007 oral cancers in Kerala, India. *Annals of Dentistry* 47: 23-26, 1988.

National Institutes of Health. *Health Implications of Smokeless Tobacco Use*. National Institutes of Health Consensus Development Conference Statement. Vol. 6. No. 1., 1986.

Orr, I.M. Oral cancer in betel nut-chewers in Travancore: Its aetiology, pathology and treatment. *Lancet* 2: 575-580, 1933.

Paymaster, J.C. Cancer of the buccal mucosa: I. Clinical study of 650 cases in Indian patients. *Cancer* 9: 431-435, 1956.

Pindborg, J.J., Bhonsle, R.B., Murti, P.R., Gupta, P.C., Daftary, D.K., Mehta, F.S. Incidence rate and early forms of oral submucous fibrosis. *Oral Surgery, Oral Medicine, Oral Pathology* 50: 40-44, 1980.

Pindborg, J.J., Kiaer, J., Gupta, P.C., Chawla, T.N. Studies in oral leukoplakia among 10,000 persons in Lucknow, India, with special reference to tobacco and betel nut. *Bulletin of the World Health Organization* 47: 13-19,1972.

Pindborg, J.J., Mehta, F.S., Daftary, D.K., Gupta, P.C., Bhonsle, R.B. Prevalence of oral lichen planus among 7,639 Indian villagers in Kerala, South India. *Acta Dermato-Venereologica* (Stockholm) 52: 216-220, 1972.

Pindborg, J.J., Mehta, F.S., Gupta, P.C., Daftary, D.K. Prevalence of oral submucous fibrosis among 50,915 Indian villagers. *British Journal of Cancer* 22: 646-654, 1968.

Pindborg, J.J., Murti, P.R., Bhonsle, R.B., Gupta, P.C., Daftary, D.K., Mehta, F.S. Oral submucous fibrosis as a precancerous condition. *Scandinavian Journal of Dental Research* 92: 224-229, 1984.

Sanghvi, L.D. Tobacco-related cancers. In: *Tobacco and Health: The Indian Scene*, L.D. Sanghvi and P. Notani (Editors). Bombay: Tata Memorial Center, 1989, pp. 9-15.

Sanghvi, L.D., Rao, K.C.M., Khanolkar, V.R. Smoking and chewing of tobacco in relation to the cancer of the upper alimentary tract. *British Journal of Cancer* 1: 111-114, 1955.

Shanta, V., Krishnamurthy, S.A. A study of aetiological factors in oral squamous cell carcinoma. *British Journal of Cancer* 13: 382-388, 1959.

Sinor, P.N., Gupta, P.C., Murti, P.R., Bhonsle, R.B., Daftary, D.K., Mehta, F.S., Pindborg, J.J. A case-control study of oral submucous fibrosis with special reference to the etiologic role of areca nut. *Journal of Oral Pathology and Medicine* 19: 94-98, 1990.

Smith, L.W., Bhargava, K., Mani, N.J., Malaowalla, A.M., Silverman, S., Jr. Oral cancer and precancerous lesions in 57,518 industrial workers of Gujarat, India. *Indian Journal of Cancer* 12: 118-123, 1975.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

Wahi, P.N. The epidemiology of oral and oropharyngeal cancer: A report of the study in Mainpuri district, Uttar Pradesh, India. *Bulletin of the World Health Organization* 38: 495-521, 1968.

Wahi, P.N., Mittal, V.P., Lahiri, B., Luthra, U.K., Seth, R.K., Arora, G.D. Epidemiological study of precancerous lesions of the oral cavity: A preliminary report. *Indian Journal of Medical Research* 58: 1361-1391, 1970.

World Health Organization. Control of oral cancer in developing countries: Report of WHO meeting. *Bulletin of the World Health Organization* 62: 817-830, 1984.

Smokeless Tobacco-Associated Epithelial And Langerhans Cell Changes[1]

Troy E. Daniels, Louis S. Hansen, John S. Greenspan, Laisheng Chou, Deborah G. Grady, Walter W. Hauck, John C. Greene, and Virginia L. Ernster

ABSTRACT Smokeless tobacco-associated oral mucosal lesions from professional baseball players were studied for histopathologic changes and changes in Langerhans cell (LC) density and antigen expression. Four types of epithelial change were found: hyperparakeratosis, hyperorthokeratosis, pale surface staining, and basal cell hyperplasia. These changes were associated with the type of ST used (snuff or chewing tobacco) but not with the duration (years) or amount (hours per day) of ST use. The thickness of the hyperkeratotic layer in a specimen correlated directly with the amount of ST use. Immunohistochemical analysis with four monoclonal antibodies detected fewer LC in lesion specimens than in autologous control specimens. The average reduction in LC was 58 percent (range, 3 to 95 percent). There were no significant differences in the LC reduction identified by the four different marker antigens. Specimens with all types of epithelial change showed similar reductions in LC. Our data indicate that snuff causes a greater variety and severity of epithelial change than does chewing tobacco, and that smokeless tobacco reduces the number of LC at its site of contact with the oral mucosa.

INTRODUCTION Smokeless tobacco contains carcinogens (Hoffmann et al., 1987), and an association between snuff use and oral carcinoma has been observed (Peacock et al., 1960; Sundström et al., 1982; US DHHS, 1986; Winn et al., 1981). ST use results in oral mucosal lesions, usually described as leukoplakia (Grady et al., 1990; Pindborg and Renstrup, 1963), that can become dysplastic (Andersson et al., 1989; Greer et al., 1986; Hirsh et al., 1982; Jungell and Malmström, 1985; Kaugars et al., 1989; Roed-Petersen and Pindborg, 1973; Smith et al., 1970) and can undergo malignant transformation (Roed-Petersen and Pindborg, 1973; Sundström et al., 1982). Histopathologic studies of ST-associated oral mucosal lesions have found hyperkeratosis in most specimens and various frequencies of dysplasia and carcinoma (Andersson et al., 1989; Axéll et al., 1976; Greer et al., 1986; Hirsh et al., 1982; Jungell and Malmström, 1985; Kaugars et al., 1989; Pindborg and Renstrup, 1963; Pindborg et al., 1980; Roed-Petersen and Pindborg, 1973; Smith et al., 1970).

Langerhans cells are dendritic cells that migrate from the bone marrow to reside in epithelium throughout the body. They perform important immunologic functions by participating in cutaneous and mucosal immune reactions that can have both local and systemic effects. Changes in the number of LC can affect systemic immune responses (Toews et al., 1980), and the localized absence of LC in the mucosa may be associated with colonization by viruses and fungi (Daniels et al., 1987). Localized reductions in the number of LC might impair skin or mucosal immunologic

[1] This study was supported by National Institute of Dental Research grant no. DE-08547.

protection against mutagens or opportunistic viruses and have a role in epithelial dysplasia and carcinoma (Viac et al., 1990).

A 3-yr study of the oral and systemic effects of ST use on professional baseball players provided us an opportunity to examine patterns of histopathologic change, and possible effects on LC numbers and antigen expression, in oral epithelial lesions associated with the use of ST (Ernster et al., 1990). This paper summarizes the results of studies presented in detail elsewhere (Daniels et al., 1992 and in press).

SUBJECTS AND METHODS

We conducted oral examinations among 1,632 members of seven Major League and minor league professional baseball teams during spring training in 1988 (Ernster et al., 1990) and 1989. Participants were asked to identify all ST products they used and to indicate the types and brands they used most often. Of the players examined, 40 percent had used ST within the past week and were classified as current ST users. Previous detailed analysis of the 1988 data showed that 75 percent of current users reported a brand of snuff as their usual ST product, 21 percent reported chewing tobacco as their usual product, and the remainder did not indicate a preference (Ernster et al., 1990).

At oral examination, 350 of the players (21 percent) had mucosal lesions, usually consisting of an ill-defined white area on the upper or lower labial, alveolar, or buccal mucosa; 94 percent of the lesions were in mucosal sites adjacent to the lower jaw. There were 138 biopsy specimens from 129 current ST users that were satisfactory for morphological evaluation.

The average age of the players who had biopsies was 25 yr. By self-report, 126 of the players who had biopsies stated their usual brand of ST as follows: 6 (5 percent) used chewing tobacco; 90 (71 percent) used Copenhagen brand snuff; 27 (21 percent) used other brands of snuff; and 3 (2 percent) used a combination of ST products. The average duration of ST use by the players whose lesions were biopsied was 7.3 yr. Other details about these players and methods of histopathologic examination and grading are described elsewhere (Daniels et al., in press).

We selected 17 pairs of lesion and autologous control specimens for LC studies. LC were identified by the use of monoclonal antibodies against the T6 antigen, usually considered to be intrinsically expressed by LC, and against three class II *HLA-D* gene products: *HLA-DR*, *HLA-DP*, and *HLA-DQ*. We expressed mean LC counts as number of LC per millimeter of epithelial surface (LC/mm) and as number of LC per square millimeter of epithelial cross-section (LC/mm^2). The calculation in terms of sectional area of epithelium compensates for the difference in thickness between some of the lesion specimens and their autologous controls. We expressed the numbers of LC in the paired lesion and control specimens from each individual as a percentage reduction of LC in the lesion specimen compared with its control. The selection of cases and immunohistochemical methods are described elsewhere (Daniels et al., 1989 and 1992).

EPITHELIAL CHANGES Each specimen had a predominant pattern of epithelial change:

- Hyperparakeratosis (HPK), characterized by increased epithelial thickness and keratinization, with nuclei remaining in most of the surface epithelial cells;
- Hyperorthokeratosis (HOK), characterized by increased keratinization, the presence of a granular cell layer, and absence of nuclei in the surface epithelial cells;
- Pale surface staining (PSS) of the epithelium, characterized by a pale staining zone of hyperkeratosis having ill-defined epithelial cell boundaries with few nuclei, no granular cell layer, and frequent vacuoles; or
- Basal cell hyperplasia (BCH), characterized by cells with a basilar pattern extending through a larger-than-usual portion of the epithelium, widened rete processes, thinning of the epithelium over the connective tissue papillae, and little or no hyperkeratosis.

Of the 132 specimens from snuff users, 56 percent had HPK, 35 percent had PSS, 5 percent had HOK, and 4 percent had BCH. The thickness of hyperkeratosis was grade 1 (slight) in 32 percent of the lesions, grade 2 (moderate) in 50 percent, and grade 3 (severe) in 18 percent. Of the six specimens from chewing tobacco users, all had HPK and the hyperkeratosis was grade 1 in five and grade 2 in the sixth. The grade of hyperkeratosis and the amount of ST use, in estimated hours per day, were highly correlated ($p=0.005$, one-way analysis of variance). No significant correlations were observed between the four patterns of epithelial change in the specimens and the age of the players, the duration of ST use, or the number of hours of ST use per day.

None of the HPK, PSS, or HOK specimens showed any signs of dysplasia. Among the five BCH specimens, we observed slight epithelial dysplasia (BCH and cellular atypia) in only one, taken from a subject who had used snuff for 3 yr. Of the remaining BCH specimens, two were from players who reported using snuff for 3 yr or less, and two were from players who used snuff for 5 yr. All five used Copenhagen brand snuff.

In addition to the changes in epithelial maturation, we observed koilocytic cells (Koss and Durfee, 1956) in 12 percent of the specimens, which resemble those seen in papillomavirus-infected epithelium of the uterine cervix (zur Hausen and Schneider, 1987). We also observed a chevron pattern of keratinization (Pindborg et al., 1980) in 6 percent of the specimens, all of the PSS type.

LC CHANGES Langerhans cells were readily identified within the epithelium by their intense staining, dendrites, and generally suprabasal location. Those identified by their *HLA-DP* and *-DQ* antigens tended to have shorter, blunter dendritic processes than those identified by their T6 and *HLA-DR* antigens. Epithelial cell expression of *HLA-DR* antigen was not observed in either lesion or control specimens.

The number of LC expressing each of the four antigens was fairly uniform in each of the four types of pathological lesions. Comparison of the mean number of LC in all types of lesions identified by all four antigens showed that there were fewer LC in the lesion specimens (means of 6 LC/mm and 10 LC/mm^2) than in the control specimens (means of 14 LC/mm and 30 LC/mm^2).

The 17 pairs of lesion and autologous control specimens showed an average reduction in LC per millimeter of 58 percent (range, 3 to 95 percent). That each pair showed a difference in the same direction is highly significant ($p < 0.0001$ by the sign test) (Conover, 1980). In all but one pair of specimens, there were fewer LC per square millimeter in each lesion specimen than in its corresponding control, by an average of 60 percent. Control specimens averaged 0.4 mm in thickness. The lesion specimens with PSS were the thickest at 0.7 mm, and those with HPK were the next thickest at 0.6 mm. Both of those types showed slightly greater reductions in LC per square millimeter than in LC per millimeter, as a result of differences in epithelial thickness between lesion and control specimens. However, similar reductions in LC were seen in HOK specimens, which were not thicker than their autologous controls, and in BCH specimens, which do not have hyperkeratosis.

DISCUSSION

Epithelial Changes

Our findings suggest that the epithelial response in snuff users is different from that in chewing tobacco users. Snuff was associated with a greater variety and severity of epithelial change than chewing tobacco. Snuff has been reported to be more likely than chewing tobacco to cause oral lesions and to cause more severe lesions (Grady et al., 1990), possibly because of differences in pH, chemical composition, or moisture content (Hoffmann et al., 1977). However, caution should be used in interpreting our findings about possible differences between snuff and chewing tobacco, because there were only six chewing tobacco users among the subjects who had biopsies.

Chewing tobacco users have a lower rate of mucosal lesions than snuff users, and a smaller percentage of players use chewing tobacco than use snuff (Ernster et al., 1990). Previous histopathologic studies of ST lesions in Europe and North America have mainly examined snuff-associated lesions. Two studies (Greer et al., 1986; Kaugars et al., 1989) included snuff-associated and chewing tobacco-associated lesions but did not analyze the findings separately (Table 1).

Some of these previous histopathologic studies showed the prevalence of epithelial dysplasia to range from 2 to 18 percent (Andersson et al., 1989; Greer et al., 1986; Hirsch et al., 1982; Jungell and Malmström, 1985; Roed-Petersen and Pindborg, 1973; Smith et al., 1970), whereas other studies did not note dysplasia (Axéll et al., 1976; Pindborg et al., 1980; Pindborg and Renstrup, 1963), and one included only specimens having dysplasia (Kaugars et al., 1989). Several authors have described ST-associated oral carcinomas (Peacock et al., 1960; Roed-Petersen and Pindborg, 1973; Smith et al., 1970; Sundström et al., 1982; Winn et al., 1981) (Table 1). The prevalence of dysplasia in our study was < 1 percent. It is not known,

Table 1
Data summary: histopathologic studies of snuff-associated oral lesions

Authors	Year	Country	n	Mean Age	Epithelial Chevrons	Epithelial Dysplasia	Carcinoma
Pindborg and Renstrup	1963	Denmark	12	60		0	0
Smith et al.	1970	United States	657	62		12 (2%)	2 (< 1%)
Roed-Petersen and Pindborg	1973	Denmark	31	58	yes	1 (3%)	1 (3%)
Axéll et al.	1976	Sweden	114	50	yes	0	0
Pindborg et al.	1980	Denmark	12	59	12 (100%)	0	0
Sundström et al.	1982	Sweden	23	76		0	23 (100%)
Hirsch et al.	1982	Sweden	50	41		9 (18%)	0
Jungell and Malmström	1985	Finland	21	19	1 (5%)	1 (5%)	0
Greer et al.[a]	1986	United States	45	41	42 (93%)	1 (2%)	0
Kaugars et al.[a]	1989	United States	108	56		108 (100%)	0
Andersson et al.	1989	Sweden	252	36	yes	"few"	0
Daniels et al.[a]	1991	United States	129	25	8 (6%)	1 (< 1%)	0

[a] *Includes chewing tobacco use.*

however, whether similar criteria were used for the diagnosis of dysplasia in the other studies. Differences in rates of dysplasia might also be attributable to differences in the populations studied, types of ST used, duration and amount of ST use, placement site of ST, and variations in concurrent smoking and alcohol use.

Although the establishment of consistent and reproducible morphological criteria for epithelial dysplasia is not yet possible, evidence of disturbed epithelial maturation and the presence of atypical cells are considered risks for malignant transformation (WHO Collaborating Centre for Oral Precancerous Lesions, 1978). BCH, as observed alone in four of the five BCH specimens in this study, does not by itself define dysplasia but is a commonly observed feature in dysplastic epithelium. All of the BCH specimens in this study were associated with a single brand of snuff, but the significance of this observation is not yet established.

We have demonstrated a variety of morphological changes in the epithelium that contacts ST. Although the lesions from this group of generally young men were all benign and only one exhibited signs of dysplasia, that specimen was from a player who had been using snuff for only 3 yr. Our data indicate that of the various forms of ST, snuff is more frequently associated with development of oral mucosal lesions and with a greater variety and severity of epithelial changes than chewing tobacco.

Langerhans Cells Our present data are consistent with previous observations that mucosal or cutaneous lesions that are or may develop into carcinoma are associated with a significant reduction in T6+ (CD1+) Langerhans cells (Barton et al., 1989; Meissner et al., 1986; Pitigala-Arachchi et al., 1989; Smolle et al., 1986; Viac et al., 1990). In vivo chemical carcinogenesis experiments have suggested that loss of LC during tumor promotion may impair immunologic protection against skin tumors (Halliday et al., 1988).

We have shown that the number of LC per millimeter in ST-associated oral lesions is on average 58 percent less than the number in autologous control tissue. This reduction in the number of LC in lesional tissue is not accounted for by differences in epithelial thickness, because the number of LC is similarly reduced when measured per square millimeter. Nonkeratinized areas of the oral mucosa generally have more LC than keratinized areas have (Cruchley et al., 1989; Daniels, 1984), but no study has investigated changes in the number of LC at sites undergoing increasing keratinization.

Epidemiological studies have shown that cigarette smoking is associated with an increased risk for cancer of the uterine cervix (Winkelstein, 1990). A recent study (Barton et al., 1988) observed a dose-response relationship between the number of cigarettes smoked daily and reduction in LC counts in the cervical epithelium. The counts were reduced in both clinically normal and lesional epithelium in the cervix. The amounts of reduction noted in the above studies were similar to those noted in our study.

The pathogenesis of oral mucosal carcinoma is unknown, but viruses and immunologic changes may be cofactors. There is clear evidence of participation by a few types of human papillomavirus (HPV) in the

development of most cervical carcinomas (zur Hausen and Schneider, 1987), but a consistent relationship has not been established for oral carcinoma (Scully, 1988). Koilocytic epithelial cells (Koss and Durfee, 1956) are associated with HPV infection (zur Hausen and Schneider, 1987), and those cells have been observed in some of the specimens from previous studies of ST-associated lesions (Andersson et al., 1989; Daniels et al., in press; Greer et al., 1986; Hirsch et al., 1982; Pindborg and Renstrup, 1963) but without examination for HPV. Viral DNA from diverse types of HPV has been identified by in situ hybridization methods in only small proportions of ST lesions, oral mucosal dysplasias and carcinomas (Greer et al., 1990), and by polymerase chain reaction methods in less than half of oral carcinomas (Palefsky et al., 1991). LC are intraepithelial antigen-processing cells that are crucial in epithelial immunity, and their number is greatly reduced at the site of contact with ST (Daniels et al., 1989 and 1992). This localized reduction or loss of immunologic function may contribute to colonization of the mucosal sites by opportunistic viruses as noted in oral hairy leukoplakia (Daniels et al., 1987).

ST reduces the number of LC at the site of its contact with the mucosa. This illustrates one form of host response to the constituents of that complex material. More knowledge is needed about the constituents of various types of ST that are responsible for morphological epithelial changes, the immunologic sequelae of ST-induced LC reduction, and the presence of cofactors that may lead to the development of malignant neoplasia.

REFERENCES

Andersson, G., Axéll, T., Larsson, Å. Histologic changes associated with the use of loose and portion-bag packed Swedish moist snuff: A comparative study. *Journal of Oral Pathology and Medicine* 18: 491-497, 1989.

Axéll, T., Mörnstad, H., Sundström, B. The relation of the clinical picture to the histopathology of snuff dipper's lesions in a Swedish population. *Journal of Oral Pathology* 5: 229-236, 1976.

Barton, S.E., Hollingworth, A., Maddox, P.H., et al. Possible cofactors in the etiology of cervical intraepithelial neoplasia: An immunopathologic study. *Journal of Reproductive Medicine* 34: 613-616, 1989.

Barton, S.E., Maddon, P.H., Jenkins, D., et al. Effect of cigarette smoking on cervical epithelial immunity: A mechanism for neoplastic change? *Lancet* 2: 652-654, 1988.

Conover, W.J. *Practical Nonparametric Statistics* (2nd edition). New York: John Wiley and Sons, 1980, pp. 122-128.

Cruchley, A.T., Williams, D.M., Farthing, P.M., et al. Regional variation in Langerhans cell distribution and density in normal human oral mucosa determined using monoclonal antibodies against CD1, HLADR, HLADQ and HLADP. *Journal of Oral Pathology and Medicine* 18: 510-516, 1989.

Daniels, T.E. Human mucosal Langerhans cells: Postmortem identification of regional variations in oral mucosa. *Journal of Investigative Dermatology* 82: 21-24, 1984.

Daniels, T., Chou, L., Greenspan, J., et al. Langerhans cells in smokeless tobacco-associated oral mucosal lesions (abstract). *Journal of Dental Research* 68: 947, 1989.

Daniels, T.E., Chou, L.S., Greenspan, J.S., et al. Reduction of Langerhans cells in smokeless tobacco-associated oral mucosal lesions. *Journal of Oral Pathology and Medicine* 21: 100, 1992.

Daniels, T.E., Greenspan, D., Greenspan, J.S., et al. Absence of Langerhans cells in oral hairy leukoplakia, an AIDS-associated lesion. *Journal of Investigative Dermatology* 89: 178-182, 1987.

Daniels, T.E., Hansen, L.S., Greenspan, J.S., et al. Histopathology of smokeless tobacco lesions in professional baseball players: Associations with different types of tobacco. *Oral Surgery, Oral Medicine, Oral Pathology*, in press.

Ernster, V.L., Grady, D., Greene, J.C., Walsh, M., Robertson, P.B., Daniels, T., Benowitz, N., Siegel, D., Gerbert, B., Hanck, W. Smokeless tobacco use and health effects among professional baseball players. *Journal of the American Medical Association* 264: 218-224, 1990.

Grady, D., Greene, J.C., Daniels, T.E., Ernster, V.L., Robertson, P.B., Hauck, W., Greenspan, D., Greenspan, J.S., Silverman, S. Oral mucosal lesions found in smokeless tobacco users. *Journal of the American Dental Association* 121: 117-123, 1990.

Greer, R.O., Eversole, L.R., Crosby, L.K. Detection of human papillomavirus-genomic DNA in oral epithelial dysplasias, oral smokeless tobacco-associated leukoplakias, and epithelial malignancies. *Journal of Oral and Maxillofacial Surgery* 48: 1201-1205, 1990.

Greer, R.O., Poulson, T.C., Boone, M.E., et al. Smokeless tobacco-associated oral changes in juvenile, adult and geriatric patients: Clinical and histomorphologic features. *Gerodontics* 2: 87-98, 1986.

Halliday, G.M., Odling, K.A., Ruby, J.C., et al. Suppressor cell activation and enhanced skin allograft survival after tumor promoter but not initiator induced depletion of cutaneous Langerhans cells. *Journal of Investigative Dermatology* 90: 293-297, 1988.

Hirsch, J.M., Heyden, G., Thilander, H. A clinical, histomorphological and histochemical study on snuff-induced lesions of varying severity. *Journal of Oral Pathology* 11: 387-398, 1982.

Hoffmann, D., Adams, J.D., Lisk, D., et al. Toxic and carcinogenic agents in dry and moist snuff. *Journal of the National Cancer Institute* 79: 1281-1286, 1987.

Jungell, P., Malmström, M. Snuff-induced lesions in Finnish recruits. *Scandinavian Journal of Dental Research* 93: 442-447, 1985.

Kaugars, G.E., Mehailescu, W.L., Gunsolley, J.C. Smokeless tobacco use and oral epithelial dysplasia. *Cancer* 64: 1527-1530, 1989.

Koss, L.G., Durfee, G.R. Unusual patterns of squamous epithelium of the uterine cervix: Cytologic and pathologic study of koilocytic atypia. *Annals of the New York Academy of Science* 63: 1245-1261, 1956.

Meissner, K., Haftek, M., Arlot, M., et al. Quantitative analysis of T6-positive Langerhans cells in human skin cancers. *Virchows Archives A [Pathology and Anatomy]* 410: 57-63, 1986.

Palefsky, J., Berline, J., Seid, K., et al. Heterogeneity of HPV types detected in invasive oral cancer (abstract). *Journal of Dental Research* 70: 271, 1991.

Peacock, E.E., Greenberg, B.G., Brawley, B.W. The effect of snuff and tobacco on the production of oral carcinoma: An experimental and epidemiological study. *Annals of Surgery* 151: 542-550, 1960.

Pindborg, J.J., Renstrup, G. Studies in oral leukoplakias: II. Effect of snuff on oral epithelium. *Acta Dermato-Venereologica* 43: 271-276, 1963.

Pindborg, J.J., Reibel, J., Roed-Petersen, B., et al. Tobacco-induced changes in oral leukoplakic epithelium. *Cancer* 45: 2330-2336, 1980.

Pitigala-Arachchi, A., Crane, I.J., Scully, C., et al. Epithelial dendritic cells in pathological human oral tissues. *Journal of Oral Pathology and Medicine* 18: 11-16, 1989.

Roed-Petersen, B., Pindborg, J.J. A study of Danish snuff-induced oral leukoplakias. *Journal of Oral Pathology* 2: 301-313, 1973.

Scully, C., Cox, M.F., Prime, S.S., et al. Papillomaviruses: The current status in relation to oral disease. *Oral Surgery* 65: 526-532, 1988.

Smith, J.F., Mincer, H.A., Hopkins, K.P., et al. Snuff-dipper's lesion, a cytological and pathological study in a large population. *Archives of Otolaryngology* 92: 450-456, 1970.

Smolle, J., Soyer, H.P., Ehall, R., et al. Langerhans cell density in epithelial skin tumors correlates with epithelial differentiation but not with the peritumoral infiltrate. *Journal of Investigative Dermatology 87*: 477-479, 1986.

Sundström, B., Mörnstad, H., Axéll, T. Oral carcinomas associated with snuff dipping. *Journal of Oral Pathology* 11: 245-251, 1982.

Toews, G.B., Bergstresser, P.R., Streilein, J.W., et al. Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *Journal of Immunology* 124: 445-453, 1980.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

Viac, J., Guérin-Reverchon, I., Chardonnet, Y., et al. Langerhans cells and epithelial cell modifications in cervical intraepithelial neoplasia: Correlation with human papillomavirus infection. *Immunobiology* 180: 328-338, 1990.

WHO Collaborating Centre for Oral Precancerous Lesions. Definition of leukoplakia and related lesions: An aid to studies on oral precancer. *Oral Surgery* 46: 518-539, 1978.

Winkelstein, W. Smoking and cervical cancer—current status: A review. *American Journal of Epidemiology* 131: 945-957, 1990.

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Fraumeni, J.F., Jr. Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

zur Hausen, H., Schneider, A. The role of papillomaviruses in human anogenital cancer. In: *Papovaviridae: The Papillomaviruses* (volume 2), N.P. Salzman and P.M. Howley (Editors). New York: Plenum Press, 1987, pp. 245-263.

The Use of Smokeless Tobacco In Sweden

Jan Bergström

ABSTRACT Twenty-five years of observation of increasing consumption of moist snuff in Sweden have undoubtedly offered some experience. Use of moist snuff is inevitably associated with a more or less severe mucosal lesion, with a typically wrinkled appearance at the site of placement of the tobacco quid. The rate of aggravation and the risk for malignant change are conclusions not readily deduced from the information presently available. For a better understanding of such complex problems, prospective studies are warranted.

INTRODUCTION Smokeless tobacco in the form of moist snuff seems to be a variety of tobacco that by tradition is related to Sweden. From a historical point of view, the use of smokeless tobacco or snuff has been known in Sweden since the early 1600's. Snuff seems to have been appreciated since, in a report dated 1638, it is stated that "there is nobody neither man nor woman who does not snuff or drink tobacco" (P. Brake, report to Axel Oxenstierna, Royal Library, Stockholm). At that time, however, snuffing tobacco probably meant to inhale it into one's nose, as was the custom elsewhere in Europe. Later on, from the early 1800's and onward, moist snuff for intraoral use became a specifically Swedish fashion.

At first, the use of moist oral snuff had social implications: whereas nasal snuff was used among the upper class and the bourgeoisie, oral moist snuff was the habit of the simple people. Later, however, use of the former variety declined, while the latter increased and became a widespread habit, remaining so into the 1920's when it was gradually replaced by cigarette smoking. In some parts of the country, snuff dipping remained a frequent habit. In the mid-1960's there was a revival of the habit, starting among young people, especially high school students and team athletes. It is not well known why this old habit reemerged, but it may be speculated that it replaced cigarette smoking among those who were either theoretically aware of the risks of smoking (students) or practically aware that smoking actually interfered with their physical performance (sportsmen).

EPIDEMIOLOGICAL ASPECTS Since 1965 there has been a steady increase in the use of moist snuff in Sweden. It is further evident that the increase has been concentrated among young men. According to a study among Stockholm schoolchildren in 1970, 4 percent of 12- and 13-yr-olds, 10 percent of 15- and 16-yr-olds, and 35 percent of students aged 16 to 18 dipped snuff (Bergström et al., 1975); all of the snuff dippers were male. Furthermore, 80 percent of the students exhibited typical lesions of the mucosa—sometimes including gingival recession at the site of placement.

According to the Swedish National Smoking and Health Association, 20 to 25 percent of Swedish men used snuff daily or occasionally during the period 1985 to 1988 (Ramström, 1985; Ramström and Tibblin, 1986, 1987, and 1988). The most recent report, based on 1988 data, shows that 35 percent of males in the 16 to 24 age group are regular or occasional users

(Table 1). The frequency of snuff use decreases with increasing age. Still, the habit is nearly totally limited to males, with a predominance among the lower educational stratum of the population (Table 2). As observed by Nordgren and Ramström (1990), the trend in Sweden over the past two-and-a-half decades clearly indicates an emerging pattern of oral snuff use among young Swedish males.

According to reports over the past 5 yr, snuff dipping among young men is usually not associated with regular smoking. Only 13 percent are regular consumers of both smoked and smokeless tobacco. Interestingly, it appears that snuff dipping has, to some extent, replaced smoking: 37 percent of the 18- to 34-yr-old and 61 percent of the 35- to 70-yr-old dippers claim to be former smokers. Ramström (1990) found that, among smokers who turn to snuff dipping, nearly twice as many quit smoking as compared to "pure smokers." Thus there seems to be some evidence that snuff dipping in Sweden may be a result of shifting tobacco habits, serving as a substitute for smoking and thus as a means of smoking cessation. The number of young dippers who later turn to smoking seems to be relatively small. Some 15 percent of current smokers report that they started their tobacco use as snuff dippers. Among older smokers, the percentage of those starting as snuff dippers is even smaller (4 percent).

EFFECTS ON ORAL HEALTH

The most common adverse oral effect associated with the use of moist snuff is a lesion at the site of the quid placement, which has been described by several investigators (Andersson and Axéll, 1989; Axéll et al., 1976; Frithiof et al., 1983; Mörnstad and Axéll, 1989). The clinical severity of the lesion is usually scored on a four-point scale based mainly on the wrinkling pattern of the mucosa. There is some evidence that the severity of lesions associated with moist snuff increases with age, with duration of use, with exposure, and to some extent with brand (some brands seem to be more hazardous than others) (Axéll et al., 1976; Hirsch et al., 1982). A variety of snuff available on the Swedish market is the portion-packed snuff in paper bags. The bag allows diffusion of the active components, without being dissolved itself. Although presently only 10 percent of dippers, at most, regularly use portion-packed snuff, and they may not be fully comparable to dippers who use ordinary loose snuff, it appears that use of the portion packs may be less harmful (Andersson and Axéll, 1989; Andersson et al., 1989).

Another clinical feature associated with Swedish use of oral snuff is localized gingival recession at the buccal aspect of teeth adjacent to the place of the quid—usually the lateral incisor. Although repeatedly observed, gingival recession has been the object of little study. This condition should be further studied because it is an irreversible sign and probably reflects the accumulated impact on the mucosa. According to Andersson and Axéll (1989) there seems to be a positive correlation between the severity of the lesion and the magnitude of the gingival recession. It might be speculated that gingival recession is an indirect manifestation secondary to contractile actions within the mucosal lesion, rather than a direct reaction of the marginal gingiva. This needs to be substantiated, however.

Table 1
Snuff habits among Swedish men (1988), by age group

	Percentage at Each Level of Use			
	Age 16 to 24 (n=213)	Age 25 to 34 (n=196)	Age 35 to 54 (n=433)	Age 55 to 74 (n=312)
Daily User	29%	20%	13%	14%
Occasional User	6	8	5	1
Non-User	65	72	82	83

Source: Ramström and Tibblin, 1988.

Table 2
Snuff habits among Swedish men (1988), by age and education

	Percentage at Each Level of Use					
	Age 18 to 29		Age 30 to 49		Age 50 to 70	
	Low Education[a] (n=69)	High Education[b] (n=101)	Low Education[a] (n=133)	High Education[b] (n=151)	Low Education[a] (n=260)	High Education[b] (n=63)
Daily User	37%	18%	18%	10%	21%	0%
Occasional User	10	1	6	6	2	0
Non-User	54	81	77	84	77	100

[a] *Low = 9 yr schooling.*
[b] *High = ≥12 yr schooling.*
Source: Ramström and Tibblin, 1988.

The histopathological pattern associated with snuff-induced lesions has been investigated by Axéll et al. (1976) and Hirsch et al. (1982). They found a high incidence of slight dysplasia, but the histomorphologic pattern was not predictable from the clinical state. The occurrence of oral cancer associated with snuff dipping in Swedish populations has been addressed by Axéll et al. (1978) and Sundström et al. (1982), who report a five- or sixfold increase in the risk of developing cancer at the site of snuff placement. Prospective, systematic studies are needed to yield more information about the development and aggravation of snuff-induced lesions and their potential transformation into dysplastic precancerous or cancerous manifestations.

REFERENCES

Andersson, G., Axéll, T. Clinical appearance of lesions associated with the use of loose and portion-bag packed Swedish moist snuff: A comparative study. *Journal of Oral Pathology and Medicine* 18: 2-7, 1989.

Andersson, G., Axéll, T., Larsson, Å. Histologic changes associated with the use of loose and portion-bag packed Swedish moist snuff: A comparative study. *Journal of Oral Pathology and Medicine* 18: 491-497, 1989.

Axéll, T., Mörnstad, H., Sundström, B. The relation of the clinical picture to the histopathology of snuff dipper's lesions in a Swedish population. *Journal of Oral Pathology* 5: 229-236, 1976.

Axéll, T., Mörnstad, H., Sundström, B. Snuff and cancer of the oral cavity. *L Kartidningen* 75: 2224-2226, 1978.

Bergström, J., Larsson, K., Pschorn, E., Randahl, P., Öman, P. *Occurrence of Snuff Dipping Among Children in Stockholm and Its Periodontal Effects* (Swedish). Stockholm: School of Dentistry, Karolinska Institutet, 1975.

Frithiof, L., Anneroth, G., Larson, U., Sederholm, C. The snuff-induced lesion: A clinical and morphological study of Swedish material. *Acta Odontologica Scandinavica* 41: 53-64, 1983.

Hirsch, I.M., Heyden, G., Thilander, H. A clinical histomorphological and histochemical study on snuff-induced lesions of varying severity. *Journal of Oral Pathology* 11: 387-398, 1982.

Mörnstad, H., Axéll, T. Clinical picture of snuff dipper's lesion in Swedes. *Community Dentistry and Oral Epidemiology* 17: 97-101, 1989.

Nordgren, P., Ramström, L. Moist snuff in Sweden: Tradition and evolution. *British Journal of Addiction* 85: 1107-1112, 1990.

Ramström, L. *Tobacco Habits in Sweden 1985* (Swedish). Stockholm: Swedish National Smoking and Health Association, 1985.

Ramström, L. Smokeless tobacco: A potential gateway to smoking? In: *Tobacco and Health 1990: The Global War. Proceedings of the 7th World Conference on Tobacco and Health*, B. Durston and K. Jamrozik (Editors). Perth: Health Department of Western Australia, 1990.

Ramström, L., Tibblin, H. *Tobacco Habits in Sweden 1986* (Swedish). Stockholm: Swedish National Smoking and Health Association, 1986.

Ramström, L., Tibblin, H. *Tobacco Habits in Sweden 1987* (Swedish). Stockholm: Swedish National Smoking and Health Association, 1987.

Ramström, L., Tibblin, H. *Tobacco Habits in Sweden 1988* (Swedish). Stockholm: Swedish National Smoking and Health Association, 1988.

Sundström, B., Mörnstad, H., Axéll, T. Oral carcinomas associated with snuff dipping. *Journal of Oral Pathology* 11: 245-251, 1982.

Periodontal Effects Associated With The Use of Smokeless Tobacco: Results After 1 Year[1]

P.B. Robertson, V. Ernster, M. Walsh, J. Greene,
D. Grady, and W. Hauck

ABSTRACT This report of the oral consequences of smokeless tobacco use among 280 professional baseball players examined in both 1988 and 1989 compares findings in 133 non-users with no mucosal changes, 63 users without ST-induced mucosal lesions, and 84 users with mucosal lesions. Subjects completed questionnaires on smokeless tobacco use, rinsed their mouths under supervision, and were cautioned not to discuss their use of tobacco with the dental examiners. They then received an oral examination that included recording of all mucosal abnormalities, missing teeth, decayed and filled surfaces, extrinsic stain, plaque index, gingival index, pocket depth, attachment loss, and gingival recession. In addition, loss of cervical tooth structure was assessed at the 1989 examinations. In both years, about 92 percent of mucosal lesions in users affected the mandibular teeth at sites where the ST quid was placed. These sites exhibited a significantly greater frequency of recession than did the sites not adjacent to lesions in users or similar sites in non-users. By 1989, 36 percent of sites adjacent to lesions in users showed at least 1 mm of recession and 25 percent showed at least 2 mm of recession, compared with 12 and 3 percent, respectively, in non-users. Moreover, an apical shift in the position of the gingival margin of users in sites adjacent to lesions averaged -0.36 mm during the 1-yr period, whereas sites of non-users and users without lesions were unchanged. Loss of tooth structure in areas of recession appeared to result from mechanical abrasion rather than chemical erosion from products of smokeless tobacco, because exposed root surfaces of non-users were affected by cervical depressions as often as sites adjacent to lesions. Compared with sites of non-users, extrinsic stain was more frequent in sites adjacent to lesions in users, but more than 80 percent of these sites did not show stain. Missing teeth, previous caries experience, levels of plaque and gingivitis, pocket depths, and occurrence of severe forms of periodontitis were not related to ST use.

INTRODUCTION We previously described the oral consequences of smokeless tobacco use among baseball players examined during the 1988 spring training season in Arizona (Ernster et al., 1990; Grady et al., 1990; Robertson et al., 1990). More than 50 percent of these team members reported using ST, and 39 percent were using ST during the week prior to the examinations. Among these current-week users, 46 percent had oral mucosal lesions, primarily in mandibular sites where the smokeless tobacco quid was placed. Poor oral hygiene and gingivitis were not related to the development of oral lesions. All periodontal measurements on mesial surfaces were similar, and severe forms of periodontal disease were equally rare in non-users, users without lesions, and users with lesions. However, buccal sites adjacent to mucosal lesions in ST users showed significantly greater recession and related attachment loss than in sites not adjacent to lesions in users or comparable sites in non-users.

[1] Supported by National Institute of Dental Research grant no. DE-08547.

The study reported here compared changes in oral and periodontal measurements among smokeless tobacco non-users, ST users without mucosal lesions, and ST users with mucosal lesions, who were examined in both 1988 and 1989.

MATERIALS AND METHODS

The subjects were 280 Major League and minor league baseball team members who were examined during both the 1988 and 1989 spring training seasons in Arizona. The study protocol has been described in detail (Ernster et al., 1990; Robertson et al., 1990). Briefly, each of the subjects completed a comprehensive questionnaire that included a basic demographic profile and dental history and elicited information on the use of smokeless tobacco or other tobacco products. The 1988 examinations included cotinine and thiocyanate determinations to confirm self-reported tobacco use and showed that the self-report was highly accurate in defining use status. Before entering the oral examination area, the subjects were asked not to discuss their use of tobacco with the oral examiners, and all subjects rinsed their mouths repeatedly with water to ensure that no traces of ST remained in the mouth. Mucosal and gingival pathology were recorded, including a complete description of oral lesions. This analysis considers measurements obtained on the buccal surfaces of a subset of 12 teeth: maxillary and mandibular first molars (tooth numbers 3, 14, 19, 30), maxillary and mandibular first premolars (tooth numbers 5, 12, 21, 28), and maxillary and mandibular central incisors (tooth numbers 8, 9, 24, 25). Measurements in both years included the presence or absence of caries, restorations, and extrinsic stain. The plaque index (PI) (Silness and Löe, 1964), gingival index (GI) (Löe and Silness, 1963), pocket depth measured from the gingival margin, and position of the gingival margin measured from the cementoenamel junction were recorded. All probing measurements were recorded to the nearest millimeter. Attachment levels were derived from the pocket depth and gingival margin measurements. Recession was defined as displacement of the gingival margin at least 1 mm and at least 2 mm apical to the cementoenamel junction. In addition, the presence of cervical erosion or abrasion was recorded in 1989 because loss of tooth structure was frequently observed in association with areas of recession during the 1988 examinations. We recorded all buccal sites that showed a loss of tooth structure resulting in smooth, hard depressions adjacent to the cementoenamel junction. No distinction was made between loss of tooth substance by a chemical process (erosion) or by excessive mechanical wear (abrasion).

Prior to both spring seasons, the examiners participated in an extensive training and calibration exercise that included patients with stain, cervical caries, and moderate adult periodontitis with some areas of recession. Differences between examiners were discussed and resolved and the examination process was repeated until at least 90 percent agreement between any two examiners was achieved for all measurements. A final calibration session was conducted with 12 patients in 1988 and 10 patients in 1989. Average percentage of agreement between all examiners for buccal surfaces was essentially equal in both years and ranged from 98 percent (cervical caries) to 93 percent (PI). Average kappa statistics (Fleiss and Chilton, 1983) for any measurement were never lower than 0.78.

Measurements were expressed as the percentage of total sites that showed caries, stain, cervical depressions, visible plaque (PI > 1), and gingival bleeding (GI > 1), and the mean and standard deviation for age, missing teeth, percentage of decayed and filled teeth, pocket depth, attachment loss, and position of the gingival margin. Where players were used as the unit of analysis (non-users, users without lesions, and users with lesions), differences between categories were evaluated by χ^2, and differences between mean scores were evaluated by Wilcoxon tests.

To assess the effects of ST on the tooth and adjacent tissue where the tobacco quid was held, the data were also analyzed by tooth site in four categories: sites in the mouths of subjects who were not using ST and did not have any oral mucosal lesions in either 1988 or 1989 (non-user, no lesion); sites in the mouths of subjects who used ST in 1988 and 1989 but did not have any oral mucosal lesions in 1989 (user, no lesion); sites in the mouths of subjects who used ST in 1988 and 1989 and had a mucosal lesion in 1989 not adjacent to the site (user, nonadjacent lesion); and sites in the mouths of subjects who used ST in 1988 and 1989 and had a lesion on the mucosa in 1989 that was immediately adjacent to the site (user, adjacent lesion). The analyses of differences between sites were evaluated by χ^2, which was corrected for the interclass correlation that resulted from use of multiple tooth sites in the same player (Donner and Donald, 1988).

RESULTS Of 280 team members who were seen in 1988 and 1989, 133 subjects were non-users of ST and showed no mucosal lesions, 63 subjects were users but did not show a mucosal lesion in 1989, and 84 subjects were users and did show a mucosal lesion in 1989. Of the 63 users without lesions in 1989, 46 had also been without a lesion in 1988, and 54 of the 84 users with lesions in 1989 also had shown lesions in 1988. For 1989, the age, race, average number of missing teeth, and percentage of all buccal surfaces that were decayed or filled are shown in Table 1. There were no differences in age among the three subject groups. A higher proportion of users than non-users—with and without lesions—were white. Values for missing teeth and previous caries experience were very low and reflected the excellent general dental health and regular dental care exhibited by these team members. About 65 percent of all subjects showed no missing teeth, and most of the tooth loss in remaining subjects was associated with premolar extraction prior to orthodontic therapy.

A total of 104 oral lesions were identified in the 84 ST users with lesions in 1989, 67 of whom had 1 lesion, and 17 of whom had 2 or more separate lesions. These lesions were characterized by white, slightly raised, and irregularly corrugated changes in the mucobuccal fold, typical of ST use (Grady et al., 1990; Greer and Poulson, 1983; Greer et al., 1986; Pindborg and Renstrup, 1963). Although most lesions terminated coronally at the mucogingival junction, the color of the adjacent gingiva was usually more pale than surrounding areas. On average, each lesion involved 4.6 ±1.9 teeth. Lesion patterns among teeth of ST users with lesions in 1988 and 1989 are shown in Table 2. The majority of lesions in both years showed a bilateral pattern involving the mandibular central incisors. The second most common site for lesions was in the mandibular left quadrant, followed

Table 1
Age, race, missing teeth, and decayed and filled teeth in 1989 among team members examined in 1988 and 1989

	n	Age[a]	Race			Missing Teeth[a]	Decayed and Filled Teeth[a]
			White	Black	Latino		
Non-Users	133	25.7 ± 5.8	57%	26%	16%	3.0 ± 3.5	2.4 ± 3.8%
Users Without Lesions	63	25.5 ± 3.9	83	8	10	2.8 ± 3.6	2.9 ± 3.9
Users With Lesions	84	25.3 ± 5.5	82	14	4	3.0 ± 3.8	3.6 ± 4.6

[a] *Mean ± SD.*

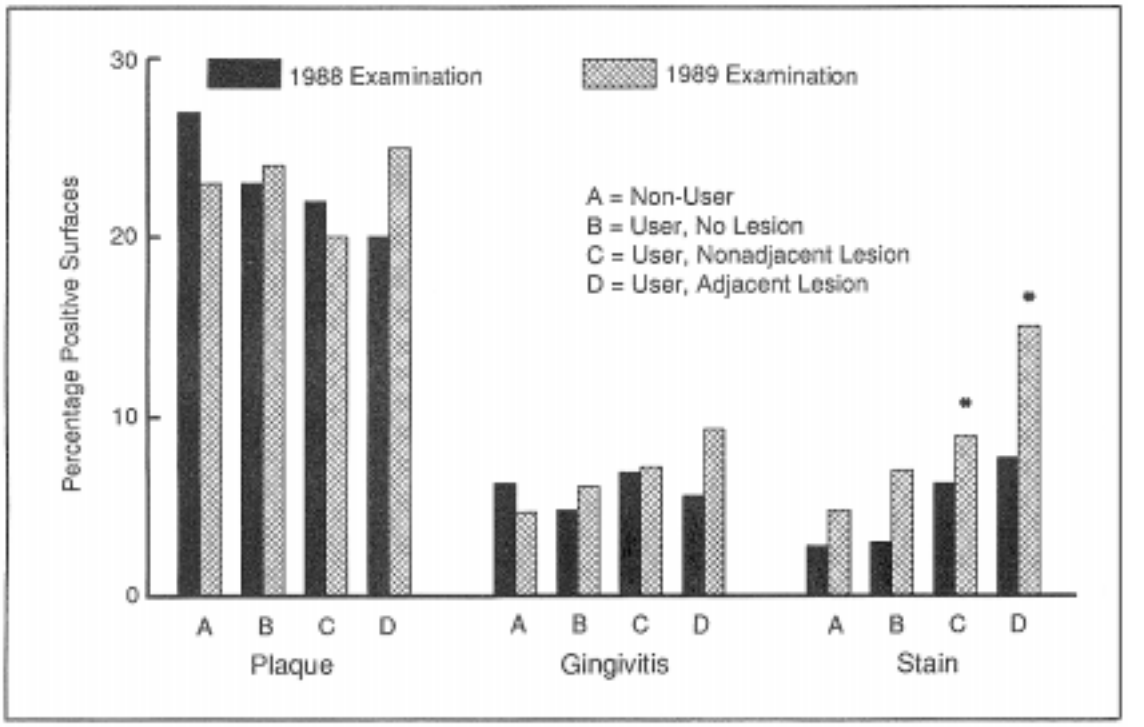
Table 2
Lesion patterns among teeth of smokeless tobacco users with lesions in 1988 and 1989

	1988	1989
Bilateral Mandibular Anterior	46%	45%
Bilateral Mandibular Posterior	2	9
Unilateral Mandibular Right	16	18
Unilateral Mandibular Left	32	20
Maxillary	4	8

by the mandibular right quadrant. The maxilla was not a preferred site to hold the tobacco quid, and maxillary lesions were infrequent. Despite comments from users that they tried to move the quid around the mouth in anticipation of the 1989 examinations, the general patterns of lesions in both years remained fairly constant, although in 1989 there was a slight shift of lesions to the maxilla.

The remaining dental and periodontal results are given only for the mandibular teeth where more than 90 percent of mucosal lesions were located. The percentage of mandibular surfaces that showed visible plaque, gingival bleeding, and extrinsic stain for each site group in 1988 and 1989 is shown in Figure 1. About 20 to 28 percent of all surfaces showed visible plaque, and there were no large or statistically significant differences between the groups in either year, nor were there significant changes from 1988 to 1989 in any group. A similar lack of significant differences was observed in gingival bleeding, which occurred in < 10 percent of

Figure 1
The percentage of mandibular buccal surfaces in 1988 and 1989 that showed visible plaque (PI > 1), gingival bleeding (GI > 1), and extrinsic stain for sites in non-users with no ST-induced mucosal lesions in 1989 (A), sites in users with no lesion (B), sites in users with a lesion elsewhere but not adjacent to the site (C), and sites in users with a lesion immediately adjacent to the site (D).



** Significant difference from non-user with no lesion sites.*

surfaces in all groups. Severe forms of gingivitis were observed infrequently and were equally distributed among subject groups. However, sites in users with lesions had a significantly greater frequency of extrinsic stain than sites in non-users at both the 1988 (p=0.04) and 1989 (p=0.03) examinations. Extrinsic stain was seen most frequently in sites with an adjacent lesion, and the number of these sites that exhibited stain increased significantly (p=0.01) from the 1988 to 1989 examination.

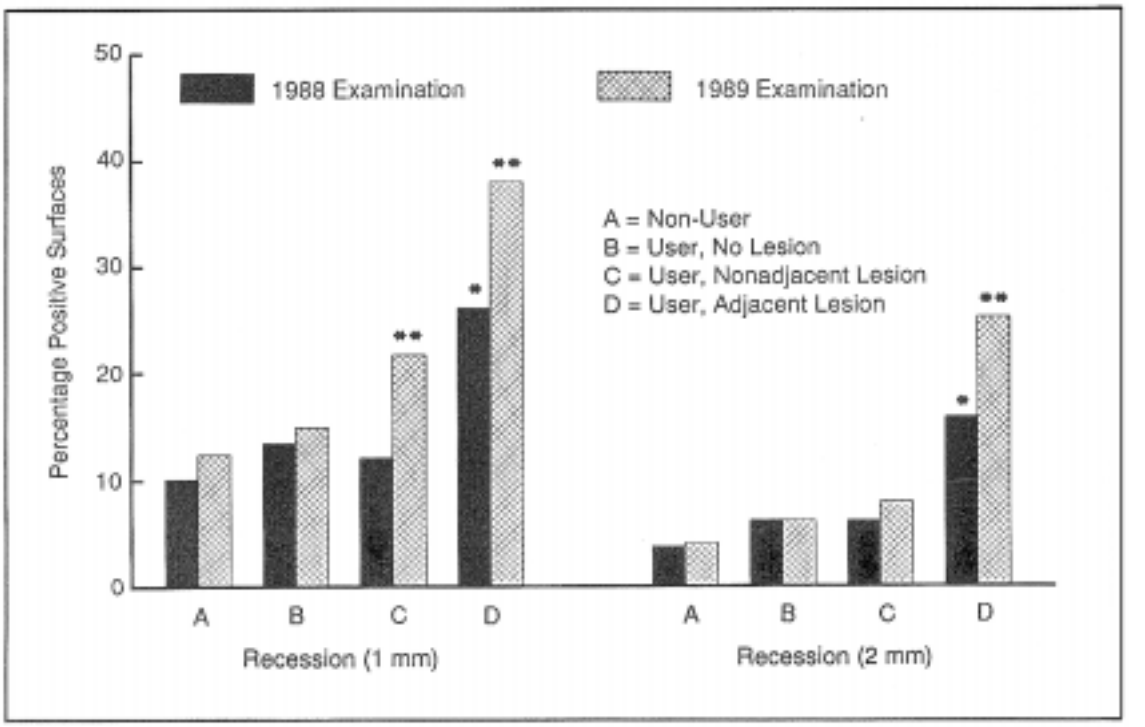
Because probing measurements were made to the nearest millimeter, we were concerned that, in cases where the gingival margin was positioned near the cementoenamel junction, very slight apical or coronal movement might distort the change in number of sites defined as showing recession. Thus, in Figure 2 we have expressed recession as the percentage of all mandibular surfaces with at least 1 mm, and at least 2 mm of displacement of the gingival margin apical to the cementoenamel junction for each site group at both examinations. Using the 1-mm threshold, we observed a significant increase (p=0.04) relative to non-users in the percentage of sites with recession in sites of users with nonadjacent lesions in 1989 and sites of users with

an adjacent lesion in 1988 and 1989 (p=0.02). In addition, nonadjacent and adjacent sites showed significant increases (p=0.01) in the frequency of recession from 1988 to 1989. Using the 2-mm threshold, we found sites adjacent to lesions at both examinations showed a significant increase (p=0.03) in the frequency of recession compared with non-users. The frequency of recession in these sites also increased significantly (p=0.01) from 1988 to 1989. In general, incisor and premolar teeth showed more recession than molar teeth in all site categories. However, patterns of recession for incisor, premolar, and molar teeth were similar to those shown in Figure 2, at both the 1-mm and 2-mm thresholds. All tooth types adjacent to ST-induced lesions showed a significantly greater frequency of recession than did the same tooth types in non-users, users without lesions, and users with a lesion not adjacent to the tooth.

The average position of the gingival margin relative to the cemento-enamel junction in 1988 was 0.5±0.8 mm for sites of non-users, 0.4±0.8 mm for sites of users without lesions, 0.4±0.9 mm for sites not adjacent to lesions in users, and 0.0±0.8 mm for sites adjacent to lesions in users. In 1989, no changes were observed in the average position of the gingival margin in the same sites of non-users or users without lesions. Sites in users with lesions that were not adjacent to the lesion showed a slight apical displacement (-0.11±0.5 mm) during the 1-yr period. However, a significant (p=0.02) apical shift in the gingival margin, which averaged -0.36±0.6 mm during the 1-yr period, was observed in sites adjacent to lesions in users. By 1989, 36 percent of sites adjacent to lesions in users showed at least 1 mm of recession and 25 percent showed 2 mm of recession compared with 12 and 3 percent, respectively, of sites with recession in non-users. About 98 percent of sites in non-users and all sites in users with 2 mm of recession in 1988 also showed recession in 1989.

Loss of cervical tooth structure recorded at the 1989 examination occurred primarily on the root surface in areas of recession. The majority showed hard, smooth, and often highly polished depressions in the root surface that were clinically consistent with mechanical abrasion. The percentage of all mandibular buccal surfaces with loss of cervical tooth substance and the percentage of mandibular buccal surfaces with recession that also showed loss of tooth substance is given in Figure 3. Cervical depressions were more frequent in sites of users with an adjacent lesion than all other sites, although differences were not statistically significant. The finding was consistent with the higher frequency of recession in sites adjacent to lesions. However, sites with recession that also showed loss of cervical tooth substance occurred at least as often in non-users as in users with and without lesions, and there appeared to be no relationship between ST use and cervical depressions in sites with existing recession. Because these cervical depressions were common on exposed root surfaces of all subjects and were seen as often in sites that were not adjacent to ST-induced mucosal lesions as in lesion-adjacent sites, we have used the term *abrasion* in Figure 3 to suggest that this loss of tooth substance is not related to chemical decalcification from products of smokeless tobacco.

Figure 2
The percentage of mandibular buccal surfaces in 1988 and 1989 that showed recession of 1 mm or greater and recession of 2 mm or greater for the categories of tooth sites described in Figure 1.

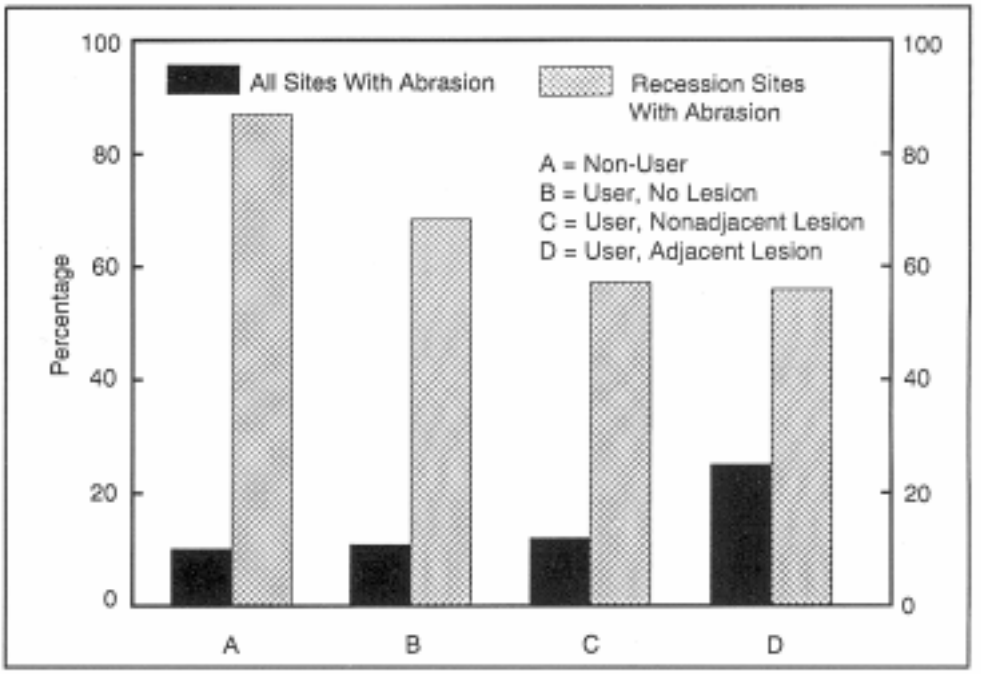


** Significant difference from non-user with no lesion sites.*
*** Significant difference from non-user with no lesion sites as well as from 1988 to 1989.*

No significant differences in pocket depth were found among the four groups of sites, and the average pocket depth for all subjects was 1.7±0.6 mm. Fewer than 1 percent of all buccal sites showed pocket depths > 6 mm, which were distributed proportionally among sites in non-users, and in users with and without lesions. About 95 percent of attachment loss observed in the subjects was a function of recession, and no severe forms of early onset or rapidly progressive periodontal disease were observed in any of the team members.

DISCUSSION The primary oral consequences of using ST for at least 1 yr are increased risk of mucosal lesions among mandibular teeth where the quid is most often placed and increased frequency of gingival recession affecting buccal tooth surfaces adjacent to ST-induced lesions. Compared with sites of non-users, extrinsic stain was also more frequent in ST users, adjacent to lesions, but more than 80 percent of these sites did not show stain. Missing teeth, previous caries experience, levels of plaque and gingivitis, pocket depths, and occurrence of severe forms of periodontitis were not related to the use of smokeless tobacco.

Figure 3
Percentage of mandibular buccal sites that showed abrasion in 1989 and the percentage of mandibular buccal sites with recession ≥ 1 mm that also showed abrasion in 1989 for the tooth sites in Figure 1.



Recession associated with ST use is permanent and progressive, and it affects about one-quarter to one-third of sites with adjacent mucosal lesions. In the 1-yr period, few new areas of recession were observed in non-users, whereas about 20 percent of sites adjacent to lesions in ST users showed recession in 1989 but not 1988. The recession presumably results from localized damage to gingival tissue by products of the ST quid, particularly in areas with a thin or absent alveolar housing (Löst, 1984; O'Leary et al., 1971; Robertson et al., 1990). In addition to esthetic problems, these areas of recession may develop thermal sensitivity and root caries (Gorman, 1967).

Exposed root surfaces are also at risk for abrasion or erosion. Abrasion is the loss of tooth substance by abnormal mechanical wear, primarily as a result of toothbrushing or use of highly abrasive dentifrices. Erosion is the loss of tooth substance by chemical processes that do not involve bacterial action. We had hypothesized that products of the ST quid, held against the surfaces of the teeth for considerable periods of time, might result in loss of some tooth structure by erosion. This seems not to be the case, however, as loss of enamel was rare in all groups, and exposed root surfaces of non-users and users without lesions were affected by cervical depressions at least as often as sites adjacent to lesions. Thus, this loss of root structure appears to be caused by abrasion.

We conclude that, although ST use in young subjects with good oral hygiene and regular professional care does not result in severe forms of periodontal disease, such use is associated with a significantly increased risk for permanent and progressive recession in areas adjacent to ST-induced mucosal lesions.

ACKNOWLEDGMENTS We gratefully acknowledge the support of the Office of the Commissioner of Baseball and the management, trainers, coaches, and players of the California Angels, Chicago Cubs, Cleveland Indians, Milwaukee Brewers, Oakland Athletics, San Francisco Giants, and Seattle Mariners. We also acknowledge the invaluable efforts of Jana Murray for coordinating the study, Joan Drues for data entry and management, and students in the periodontology graduate and dental hygiene programs at UCSF who played a major role in all phases of the studies in Arizona.

REFERENCES

Donner, A., Donald, A. The statistical analysis of multiple binary measurements. *Journal of Clinical Epidemiology* 9: 899-904, 1988.

Ernster, V.L., Grady, D., Greene, J.C., Walsh, M., Robertson, P.B., Daniels, T., Benowitz, N., Siegel, D., Gerbut, B., Hauck, W. Smokeless tobacco: Prevalence of use and health effects among professional baseball players. *Journal of the American Medical Association* 264: 218-224, 1990.

Fleiss, J.L., Chilton, N.W. The measurement of interexaminer agreement on periodontal disease. *Journal of Periodontal Research* 18: 601-608, 1983.

Gorman, W.J. Prevalence and etiology of gingival recession. *Journal of Periodontology* 38: 316-340, 1967.

Grady, D., Greene, J., Daniels, T.E., Ernster, V.L., Robertson, P.B., Hauck, W., Greenspan, D., Greenspan, J.S., Silverman, S. Oral mucosal lesions in smokeless tobacco users. *Journal of the American Dental Association* 121: 117-123, 1990.

Greer, R.O., Poulson, T.C. Oral tissue alterations associated with the use of smokeless tobacco by teenagers. *Oral Surgery, Oral Medicine, Oral Pathology* 56: 275-284, 1983.

Greer, R.O., Poulson, T.C., Boone, M.E., et al. Smokeless tobacco-associated oral changes in juvenile adult and geriatric patients: Clinical and histomorphic features. *Gerodontics* 2: 87-98, 1986.

Löe, H., Silness, J. Periodontal disease in pregnancy: I. Prevalence and severity. *Acta Odontologica Scandinavica* 21: 533-551, 1963.

Löst, C. Depth of alveolar bone dehiscences in relation to gingival recessions. *Journal of Clinical Periodontology (Copenhagen)* 11: 583-589, 1984.

O'Leary, T.J., Drake, R.B., Crump, P.P., et al. The incidence of recession in young males: A further study. *Journal of Periodontology* 42: 264-275, 1971.

Pindborg, J.J., Renstrup, G. Studies in oral leukoplakias: II. Effect of snuff on oral epithelium. *Acta Dermato-Venereologica (Stockholm)* 43: 271-276, 1963.

Robertson, P.B., Walsh, M., Greene, J., et al. Periodontal effects associated with the use of smokeless tobacco. *Journal of Periodontology* 61: 438-443, 1990.

Silness, J., Löe, H. Periodontal disease in pregnancy: II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* 22: 121-135, 1964.

Chapter 3

Carcinogenesis

CONTENTS

Oral Carcinoma and Smokeless Tobacco Use: A Clinical Profile

W. Frederick McGuirt and Anna Wray

ABSTRACT The clinical profile of 116 patients with oral cavity cancer who used smokeless tobacco exclusive of other known carcinogens, such as smoking tobacco and alcohol, is discussed. The patients, whose average age was 78.4 yr and average use 55.5 yr, had a 1:23 male-to-female ratio and a 14.5:1 white-to-black ratio, both different from national rates for patients with oral cavity cancers. The 116 patients' course illustrates the field cancerization concept: In addition to the referral lesion, 55 percent (64/116) had leukoplakia, erythroplasia, dysplasia, or carcinoma in situ previously, at the time of presentation, or after initial therapy. A second malignant oral lesion developed in 18 percent (21/116); 35 percent (41/116) had local recurrence despite predominantly clear margins earlier. Of the 91 patients with documented followup, 45 died of or with cancer. These distressing figures may, unfortunately, be duplicated in another 20 to 40 years in a different population group—current young male smokeless tobacco users.

INTRODUCTION Not only is tobacco an integral part of the local economy in North Carolina, but also its use in all forms is pervasive in the population served by the Wake Forest University Medical Center. Smokeless tobacco use, especially, is endemic to the three or four generations living in our geographic region. Review of our tumor registry files yielded 150 cases of oral cavity cancer in smokeless tobacco users—4.9 percent of our total head and neck cancer caseload. Smokeless tobacco was used exclusive of other known oral carcinogens, such as smoking tobacco and alcohol, by 116 of the 150 patients. These 116 cases form the basis of this report, in which we profile the patients, the nature of the disease process, its treatment, and outcome.

PATIENTS The patient population reported here differed in several ways from the national profile of oral cancer patients. Oral cavity carcinoma in the United States has a 2:1 male-to-female ratio (Boring et al., 1991). This ratio is consistent with our new head and neck cancer patient population (1,393/699) but higher than our oral cancer male-to-female ratio of 483/361 (1.3:1). This oral cavity cancer ratio was strongly influenced (that is, reduced) by our patients who used smokeless tobacco only. This group of patients had a 1:23 male-to-female ratio, representing disease occurrence almost exclusively in females.

A second epidemiological aberration seen in the pool of smokeless tobacco-associated oral cancers relates to the most common tumor sites. The mobile tongue and floor of the mouth are the most common sites of oral cavity carcinoma. In our series of smokeless-tobacco-only users, the cancers occurred most frequently in the buccal and gingival areas, the regions where the quid was held. Ninety-four percent of patients (109/116) had their cancers at these sites.

The average age of these patients was 78.4 yr, with 83 percent (96/116) older than 65 yr and 27 percent (31/116) older than 80 yr. These patients were older than the overall head and neck cancer patients seen in our institution, who averaged 63 yr during the same study period.

The duration of smokeless tobacco use before presentation for treatment averaged 55.5 yr, with 81 percent of patients (86/106) having a history of use > 40 yr.

A further epidemiological aberration occurred in the racial distribution of patients. A 14.5:1 (116:8) ratio of white to black was seen, compared with a 7.5:1 (1,845:247) ratio of all head and neck cancer cases and a population distribution frequency of 3.5:1 in the geographic region served (U.S. Census figures, 1990 to 1991). These figures support the epidemiological study of Winn et al. (1981) from the same geographic region but a different referral base. The use of snuff—and cancer cases attributed to it—was reported by Winn and coworkers to be a phenomenon of white women.

These factors of sex, race, and age are results of sociological trends well recognized and reported previously. Snuff use in the region until the mid-1970's was predominantly a practice of white females, who began to use snuff at an early age in the 1930's and 1940's. This was a period of declining smokeless tobacco use and increased smoking. The social attitudes of that time discouraged women's smoking, but in the rural South, women continued privately to use smokeless tobacco. Dry, powdered snuff (i.e., Scotch snuff) has been the predominant form used by these women, in contrast to the flavored moist strips of tobacco preferred by today's young male users.

Whereas the now elderly women with long-term smokeless tobacco use form the patient population reported here, the obvious concern is that the young males currently using smokeless tobacco may well be the future patients who will be profiled if they continue their chronic use. The addictive qualities of the product would imply this to be the expected outcome.

Oral carcinoma in a high school athlete from Oklahoma who was a regular user of smokeless tobacco has become the focal point for an educational effort spearheaded by the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS). Oral carcinoma in the male population under age 30 has not yet been seen by personal experience or become a national problem, according to a questionnaire mailed to members of the American Society of Head and Neck Surgery and selected AAO-HNS members, who are the physicians most likely to be treating such patients. The youngest patient to date in our experience was aged 40; the next youngest, aged 43.

FIELD CANCERIZATION

Patients with cancers attributed to smokeless tobacco illustrate the field cancerization concept. In addition to the referral lesion, adjacent and distant mucosal lesions exist synchronously or develop metachronously. In the field cancerization model, a diffuse surface area of mucous membrane is bathed by the carcinogens and placed at risk. Logically, the highest risk area for smokeless tobacco use is where the quid is held, but adjacent areas and distal sites are continuously bathed by saliva containing the carcinogenic agents that leach from the quid. Our patients with smokeless tobacco-related oral cancers often exhibited adjacent and distant mucous membrane changes. Varying degrees of abnormality, compatible with the evolutionary transformation to malignancy, were seen.

These abnormal changes included clinical leukoplakia and erythroplasia as well as histologically varying degrees of dysplasia and carcinoma in situ. Fifty-five percent of patients in our series (64/116) had had these types of lesions excised previously, had them at the time of their presentation, or developed them subsequent to therapy. Further evidence for an evolutionary transformation of a condemned mucosa was the high incidence (18 percent) of second or metachronous malignant oral lesions that developed in these patients. A third indicator of this evolutionary transformation was the 35 percent incidence (41/116) of local cancer recurrence, despite predominantly clear margins on histological examination (28 of 33 surgical specimens, 85 percent). These lesions were most likely not recurrences of the original cancer but, rather, malignant transformations of previously exposed mucous membranes at risk for new cancers in the same region.

DISCUSSION The disease course is most often silently and indolently progressive. Patients may note a change or abnormality within the mouth, but the symptoms are usually minimal and a delay in seeking evaluation occurs. Forty-three percent of patients (48/111) had symptoms for 3 mo or longer before presentation. The silent but progressive nature of the disease is further illustrated by the 53 percent (62/116) incidence of advanced stage III and stage IV lesions at the time of diagnosis, of which one-third (34 percent, 21/62) had been symptomatic for less than 2 mo.

Although the premalignant changes of leukoplakia and erythroplasia have a slow transformation and progression, with only minimal symptoms, once the smokeless tobacco-related cancers are established, they act in aggressive fashion, similar to other oral cavity cancers. This is confirmed by the high incidence of stage III and stage IV disease. The advanced stages were attributable to regional metastases in 27 percent of the cases, to locally advanced disease (T3-T4) in 43 percent of the cases, and in many cases to both characteristics.

Although many of these smokeless tobacco-related tumors have a verruciform appearance, they should never be considered, on clinical grounds alone, to be the less aggressive verrucous carcinoma described histologically by Ackerman (1948). Although 24 percent (28/116) of our patients had verruciform lesions, less than one-fifth of those, or 4.3 percent overall (5/116), had verrucous carcinomas by the histological criteria of Ackerman.

Localized stage I and stage II carcinomas may be treated equally well with either surgery or irradiation. Therapy of advanced stage III and stage IV cases usually required both surgery and irradiation. The surgical therapy in advanced stage lesions may result in a significant alteration in form and function of the oral cavity. The major problems of postsurgical resection relate to the loss of the mandibular-dental function of mastication and the oral-buccal-lingual function of deglutition. The addition of irradiation to surgery in these advanced cases or as the primary modality of therapy for early lesions causes morbidity related to the resulting xerostomia and the lifelong need for frequent dental care and rehabilitation. The use of irradiation and the advanced age of the patients reduce the success rate of mandibular reconstruction.

Mandibular resection results in cosmetic disfigurement, the degree usually being directly proportional to the amount of bone resected and how far anteriorly the resection proceeds. In general, lateral mandibular defects are usually left unreconstructed. Anterior defects, though, require reconstruction, not only to correct severe cosmetic problems, but also to correct basic functional problems. Various reconstructive methods and synthetic replacement prostheses have been used. Our current preference is to use microvascular anastomotic techniques for restoration by a composite free flap. This provides soft-tissue support and bone with its own direct blood supply.

There is a general lack of success in fitting dentures that allow adequate mastication for solid food, even when the mandibular defect is restored by bone grafting or a metal bar. The future use of implanted prosthetic devices holds promise for nonirradiated cases, but such devices are still contraindicated in irradiated bone.

Intraoral soft tissue loss has been handled in various ways. In the majority of our cases, primary closure of the defect is performed, because patients have better function if sensate, normally lubricated, moist membranes are present. Tethering of the tongue is to be avoided if possible. The tongue dysfunction is more often seen secondary to reconstruction of the floor of the mouth. Lateral tongue flaps and skin grafts used to close the anterior and lateral floor-of-mouth defects frequently contract and form scar tissue that restricts movement of the oral structures. When oral tissue must be replaced, use of the platysma myocutaneous flap is our most effective method because of the pliability and thinness of the flap tissue. Unless the resection has been quite extensive, other myocutaneous flaps often result in an adynamic and obstructive tissue mass in the oral cavity.

Long-term followup was available for 91 patients; 49.5 percent of these (45/91) have died of or with cancer. There was a linear survival rate correlating to the stage of lesion, and survival was better for patients with buccal lesions than for those with alveolar lesions.

In summary, oral cavity carcinoma associated with smokeless tobacco use has been a disease of elderly, white women with histories of long-term (> 40 yr) snuff use. The disease is indolent and progressive, and it manifests a high rate of associated leukoplakia, metachronous second primary cancers of the oral cavity, and a high local recurrence rate, all manifestations of the field cancerization phenomenon. The tumor is aggressive, with a high incidence of advanced local disease, bony mandibular involvement, and regional metastases. A midrange (43 percent) 3-yr cure rate results. Therapy should be aggressive, but it is associated with significant morbidity related to cosmetic effects, mastication, deglutition, xerostomia, and dental complications. Prevention through education of young potential users of smokeless tobacco promises to be the most effective therapeutic measure.

REFERENCES

Ackerman, L.V. Verrucous carcinoma of the oral cavity. *Surgery* 23: 670-678, 1948.

Boring C.C., Squires T.S., Tung T. Cancer statistics, 1991. *CA—A Journal for Clinicians* 41: 19-36, 1991.

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Fraumani, J.F., Jr. Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

Chemical Composition of Smokeless Tobacco Products[1]

Klaus D. Brunnemann and Dietrich Hoffmann

ABSTRACT To date, 28 carcinogens have been identified in smokeless tobacco. In addition to certain volatile aldehydes, traces of benzo[*a*]pyrene, certain lactones, urethan, hydrazine, metals, polonium-210, and uranium-235 and -238 can be found in ST. However, the major contributors to the carcinogenicity of chewing tobacco and of snuff are the *N*-nitrosamines, especially the tobacco-specific *N*-nitrosamines. The latter are formed from the *Nicotiana* alkaloids during tobacco processing. In the United States, daily exposure to carcinogenic nitrosamines for snuff users is at least 250 times higher than for those who do not use tobacco. Although there has been a decline in the concentrations of nitrosamines in U.S. and Swedish ST products during the past decade, this trend is not evident for all snuff brands. One new snuff brand contains extremely high concentrations of carcinogenic nitrosamines. This observation adds to the urgency of the recommendation of the World Health Organization to regulate harmful substances in chewing tobacco and snuff. Similarly, flavorants and additives to tobacco should be controlled.

INTRODUCTION In the United States, we differentiate between four primary types of smokeless tobacco: Three are chewing tobaccos, namely loose leaf (scrap leaf), plug, and twist or roll; the fourth is oral snuff. Loose leaf chewing tobacco accounted for 52.7 percent of the U.S. output of total ST products in 1988 (124.5 million lb) (USDA, 1990). Loose leaf chewing tobacco consists primarily of air-cured tobacco and, in most cases, is heavily treated with licorice and sugars. Plug tobacco (7.2 percent of 1988 ST production) is the oldest form of chewing tobacco. Plug tobacco is produced from the heavier grades of leaves harvested from the top of the plant, freed from stems, immersed in a mixture of licorice and sugar, pressed into a plug, covered by a wrapper leaf, and reshaped. Plug tobacco is kept between cheek and gum and is chewed in bites. Twist or roll tobacco is less important (1.1 percent of 1988 U.S. production). Twist tobacco is made from cured burley, and air- and fire-cured leaves, which are flavored and twisted to resemble a decorative rope or pigtail.

The only U.S. tobacco product with increasing consumption is oral snuff (39.0 percent of the U.S. smokeless tobacco production in 1988). Dry snuff is made primarily from Kentucky and Tennessee fire-cured tobaccos. The initial curing requires several weeks and goes through multiple phases. In contrast to most other tobacco products, snuff undergoes an additional fermentation process. Dry snuff is processed into a powdered substance that may contain flavor and aroma additives, including spices. U.S. dry snuff, which is taken orally, is similar to European nasal snuff.

Moist snuff consists primarily of air- and fire-cured tobaccos and contains tobacco stems as well as leaves that are powdered into fine particles (containing between 20 and 55 percent moisture). Many brands of moist

[1] Supported by National Cancer Institute grant no. CA-29580.

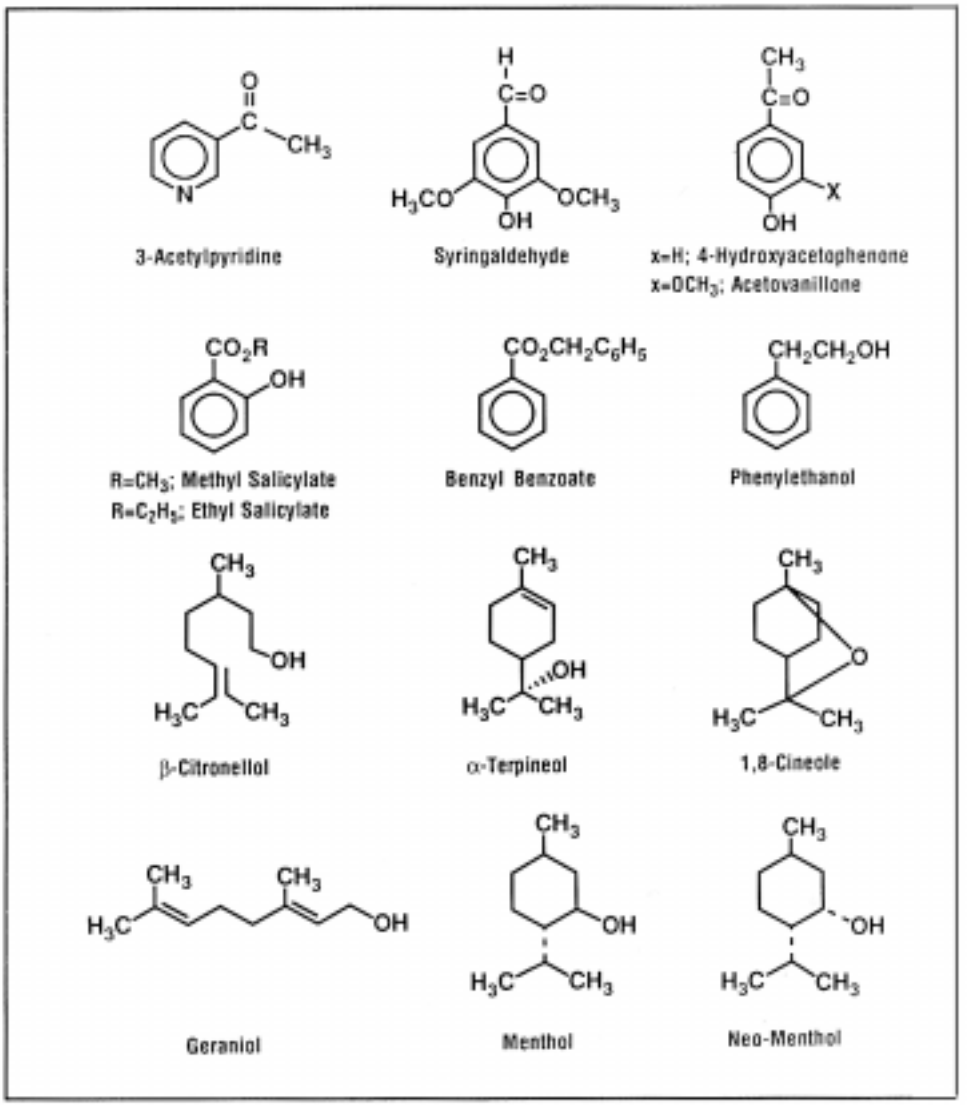
snuff are flavored with wintergreen, but mint and raspberry also are popular. Since about 1975, the consumption of moist snuff has been steadily growing in all parts of the United States, except for a temporary decline immediately after the Surgeon General's report on smokeless tobacco in 1986 (US DHHS, 1986). During the past 3 yr, the manufacture of moist snuff has again steadily risen by more than 13 percent (Smyth, 1989; USDA, 1990). Oral use of snuff, also termed "snuff dipping," means placing a pinch of the tobacco between the cheek or lip and the gums or beneath the tongue.

CHEMICAL COMPOSITION Extensive literature on the chemistry of tobacco, beginning with Brückner (1936), has led to our current knowledge that natural tobacco contains at least 3,050 different components (Robert, 1988). The quantitative composition of tobacco undergoes substantial changes during processing for smokeless tobaccos. In curing, the starch content of the leaves declines drastically, and the reducing sugars increase by 100 percent. Protein and nicotine decrease slightly. Fermentation of cured tobacco causes the contents of carbohydrates and polyphenols in the leaves to diminish. The bulk of the processed tobacco leaf before fermentation consists of carbohydrates (about 50 percent) and proteins. Other major components are alkaloids (0.5 to 5.0 percent) with nicotine as the predominant compound (85 to 95 percent of total alkaloids), terpenes (0.1 to 3.0 percent), polyphenols (0.5 to 4.5 percent), phytosterols (0.1 to 2.5 percent), carboxylic acids (0.1 to 0.7 percent), alkanes (0.1 to 0.4 percent), aromatic hydrocarbons, aldehydes, ketones, amines, nitriles, *N*- and *O*-heterocyclic hydrocarbons, pesticides, alkali nitrates (0.01 to 5.00 percent), and at least 30 metallic compounds (International Agency for Research on Cancer, 1985; Wynder and Hoffman, 1967). The given percentages apply to the *Nicotiana tabacum* species, which is grown in North America and throughout the world, but not to *N. rustica*, which is cultivated in parts of Eastern Europe and Asia Minor. The leaves of *N. rustica* may contain up to 12 percent nicotine (McMurtrey et al., 1942). Many ST formulations use plant extracts or chemicals as flavoring agents (LaVoie et al., 1989; Mookherjee, 1988; Robert, 1988; Sharma et al., 1991). Such additives may include methyl or ethyl salicylate, β-citronellol, 1,8-cineole, menthol, benzyl benzoate, and possibly coumarin (Figure 1) (LaVoie et al., 1989; Sharma et al., 1991). However, most of the flavor additives are present in only small amounts; their formulations remain trade secrets.

CARCINOGENIC AGENTS IN ST Until now, 28 tumorigenic agents have been isolated and identified in smokeless tobacco products (Table 1). These include some carcinogenic polynuclear aromatic hydrocarbons (PAH), especially benzo[*a*]pyrene (B[*a*]P). PAH originate primarily from polluted air (Campbell and Lindsay, 1956 and 1957; Wynder and Hoffmann, 1967) and, in the case of plug tobacco and snuff, probably also from fire-curing. In fact, the highest reported values for B[*a*]P were found in snuff at levels of up to 90 ppb (Ough, 1976).

The α- and β-angelica lactones have been reported in natural tobacco (Robert, 1988). These tumorigenic agents may also be added to ST as part of the flavoring mixtures made from plant extracts. A minor group of polyphenols in tobacco are the coumarins, of which scopoletin is the major

Figure 1
Flavor compounds identified in snuff tobacco



representative (Figure 2) (Wynder and Hoffmann, 1967). Thus, it is not surprising that tobacco also contains the parent compound, coumarin (≤ 600 ppm). It is known that the fermentation of food and beverages leads to the formation of urethan (Ough, 1976). Therefore, it is not unexpected that burley tobacco, which is fermented, contains up to 400 ppm of urethan (Schmoltz et al., 1978).

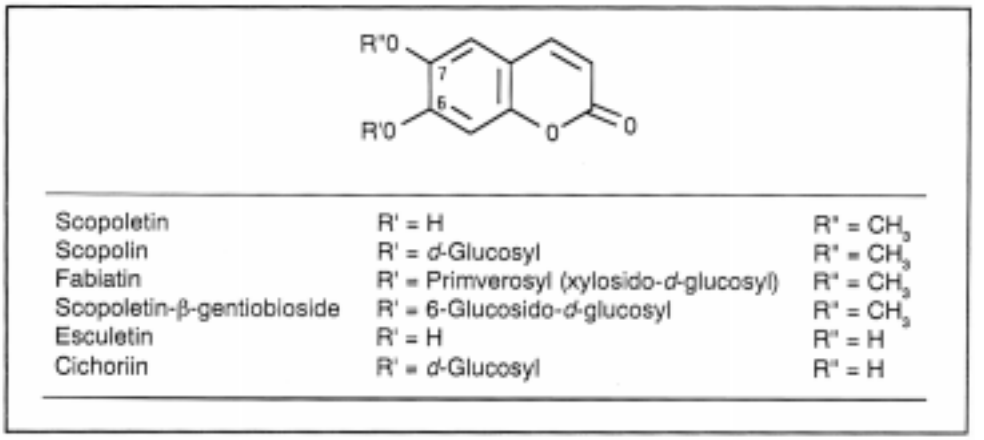
The most abundant carcinogens in smokeless tobacco are some volatile aldehydes (Table 1). Although formaldehyde, acetaldehyde, and croton aldehyde are weakly carcinogenic, they contribute most likely to the carcinogenic potential of smokeless tobacco (Weybrew and Stephens, 1962). It is

Table 1
Carcinogenic agents in tobacco

	Type of Tobacco[a]	Concentration[b] (ng/g)
Benzo[*a*]pyrene	NT, S	> 0.1 - 90.0
α-Angelica Lactone	NT	present
β-Angelica Lactone	NT	present
Coumarin	NT	600
Ethylcarbamate	CT	310 - 375
Volatile Aldehydes		
Formaldehyde	NT, S	1,600 - 7,400
Acetaldehyde	NT, S	1,400 - 27,400
Croton aldehyde	S	200 - 2,400
Nitrosamines		
Nitrosodimethylamine	CT, S	ND - 270
Nitrosopyrrolidine	CT, S	ND - 760
Nitrosopiperidine	CT, S	ND - 110
Nitrosomorpholine	CT, S	ND - 690
Nitrosodiethanolamine	CT, S	40 - 6,800
Nitrosamino Acids		
Nitrososarcosine	S	ND - 2,500
3-(Methylnitrosamino)-propionic acid	CT, S	200 - 65,700
4-(Methylnitrosamino)-butyric acid	CT, S	ND - 9,100
Nitrosoazetadine-2-carboxylic acid	CT	4 - 140
Tobacco-Specific Nitrosamines		
N'-Nitrosonornicotine	CT, S	400 - 147,000
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	CT, S	ND - 18,000
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol	S	present
N'-Nitrosoanabasine	SM, S	present - 560
Inorganic Compounds		
Hydrazine	SM	14 - 51
Arsenic	NT	500 - 900
Nickel	SM, S	180 - 2,700
Cadmium	SM	700 - 790
		(pCi/g)
Polonium-210	NT, S	0.16 - 1.22
Uranium-235	S	2.4
Uranium-238	S	1.91

[a] *NT, natural tobacco; SM, smoking tobacco; S, snuff; CT, chewing tobacco.*
[b] *ND, not detected.*

Figure 2
Coumarins in tobacco



known that tobacco contains a sizeable spectrum of alkyl aldehydes, which contribute to its scent. In commercial U.S. snuff brands, formaldehyde and acetaldehyde were each found up to 7,400 ppb, and croton aldehyde up to 2,400 ppb (Sharma et al., 1991).

Both air- and fire-cured tobaccos contain hydrazine. In burley leaves treated with the sucker growth inhibitor maleic hydrazide, the hydrazine content was significantly higher (Liu et al., 1974). Like other plant products, tobacco contains trace amounts of nickel, cadmium, and arsenic. These animal carcinogens were found in concentrations up to 2,700 ppb. Uranium-235 and -238 were reported only in Indian snuff, each at about 2 pCi/g tobacco (Sharma et al., 1985). The radioactive polonium-210, which decays to yield the human carcinogen radon, originates in U.S. tobacco from soil that is fertilized with phosphates rich in radium-226 (Tso et al., 1986), or from airborne particles that were taken up by the glandular hair (trichomes) of the tobacco leaf (Martell, 1974). In U.S. commercial snuff, we found between 0.16 and 1.22 pCi/g of polonium-210 (Hoffmann et al., 1987).

CARCINOGENIC *N*-NITROSAMINES The most detailed studies on carcinogens in smokeless tobacco have been reported for *N*-nitrosamines. These agents are present in fresh green leaf in only minute amounts and are primarily formed during curing, fermentation, and aging from secondary or tertiary amines and nitrite or nitrogen oxides. Basically, in smokeless tobacco there are three types of nitroso compounds: volatile nitrosamines, nitrosamino acids, and tobacco-specific *N*-nitrosamines (TSNA). In addition, smokeless tobacco contains *N*-nitrosodiethanolamine (NDELA), which is formed from diethanolamine, a contamination product in tobacco. Table 2 presents data on carcinogenic volatile *N*-nitrosamines (VNA) in various smokeless tobacco types from the United States, Sweden, and other European countries (Andersen et al., 1989; Brunnemann et al., 1985; Chamberlain et al., 1988; Hoffman et al., 1987; International Agency for Research on Cancer, 1985;

Table 2
Major volatile *N*-nitrosamines in smokeless tobacco, 1981 to 1990

Country	Tobacco Type	Samples (n)	NDMA[a] (µg/kg)	NPYR[a] (µg/kg)	NMOR[a] (µg/kg)
United States	Moist snuff	32	3.8 -215.0	7.4 - 360.0	ND - 690.0
	Dry snuff	3	ND - 19.0	72.0 - 148.0	ND - 39.0
	Chewing tobacco	6	64.0	0.8	0.6
Sweden	Moist snuff	98	0.1 - 50.0	ND - 95.0	ND - 44.0
	Chewing tobacco	4	0.2	0.8	0.4
Norway	Moist snuff	2	130.0	8.9	32.0
Denmark	Chewing tobacco	8	5.5	16.0	ND
United Kingdom	Nasal snuff	5	4.5 - 82.0	1.5 - 130.0	ND
	Moist snuff	7	6.0 - 82.0	64.0 - 860.0	ND - 1.5
Germany	Nasal snuff	7	2.0 - 42.0	5.0 - 75.0	ND

[a] *NDMA, nitrosodimethylamine; NPYR, nitrosopyrrolidine; NMOR, nitrosomorpholine; ND, not detected. Single numbers represent mean of all samples.*

Tricker and Preussmann, 1989). In general, the highest amounts of VNA are found in moist and dry snuff, *N*-nitrosodimethylamine up to 265 ppb and *N*-nitrosopyrrolidine up to 760 ppb. *N*-Nitrosomorpholine (NMOR), a strong animal carcinogen, has been detected only in those U.S. snuff brands that were packed in containers lined with a morpholine-containing wax coating (Brunnemann et al., 1982).

Like volatile amines, the amino acids in tobacco, and probably also the proteins with secondary amino groups, are amenable to *N*-nitrosation. Since 1983, numerous studies have reported the presence of nitrosamino acids in smokeless tobacco (Brunnemann et al., 1983; Djordjevic, 1989; Ohshima, 1985; Tricker and Preussmann, 1989 and 1990). Until now, 10 nitrosamino acids have been identified in smokeless tobacco. Of these, nitrosoproline, nitrosothioproline, and iso-NNAC are not carcinogenic; nitrososarcosine, 3-(methylnitrosamino)propionic acid, 4-(methylnitrosamino)butyric acid, and *N*-nitrosoazetadine-2-carboxylic acid are known carcinogens; and the remainder of the identified nitrosamino acids have so far not been bioassayed. (See Table 3.) The concentration of the nitrosamino acids depends on the nitrate or nitrite content of the tobacco as well as on the processing and aging of the tobacco.

TSNA

The most powerful carcinogens in smokeless tobacco derive from the *N*-nitrosation of the *Nicotiana* alkaloids, especially from nicotine and nornico-tine. They are formed during the curing, fermentation, and aging of tobacco. These carcinogens are present in tobacco, tobacco smoke, and

Table 3
Major *N*-nitrosamino acids in smokeless tobacco, 1989 to 1991[a]

Country and Tobacco Type	Samples (n)	NSAR[b] (μg/g)	MNPA[b] (μg/g)	MNBA[b] (μg/g)	NPRO[b] (μg/g)	iso-NNAC[b] (μg/g)
United States						
Moist snuff	10	ND - 2.5	2.2 - 66.0	0.09 - 9.10	1.3 - 60.0	0.05 - 21.00
Chewing tobacco	1	nd	0.6	0.03	0.2	0.01
Dry snuff	3	nd	1.2 - 4.5	0.14 - 0.46	3.0 - 8.1	0.05 - 0.21
Sweden						
Moist snuff	8	0.01 - 0.68	1.0 - 3.3	0.05 - 0.23	0.63 - 8.30	0.04 - 0.11
United Kingdom						
Moist snuff	7	0.03 - 1.10	1.4 - 19.0	0.06 - 8.00	0.33 - 5.00	nd
Nasal snuff	5	ND - 0.04	1.0 - 2.8	0.10 - 0.28	2.7 - 8.7	nd
Germany						
Nasal snuff	7	ND - 0.09	0.49 - 4.30	0.08 - 0.41	0.77 - 7.50	nd

[a] *Adapted from Djordjevic et al., 1989; Hoffmann et al., 1991; Tricker and Preussmann, 1989.*
[b] *NSAR,* N*-nitrososarcosine; MNPA, 3-(methylnitrosamino)propionic acid; MNBA, 4-(methylnitrosamino)butyric acid; NPRO,* N*-nitrosoproline; iso-NNAC, 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid; ND, not detected; nd, not determined.*

in environmental tobacco smoke. Of the seven TSNA identified in ST (Figure 3), *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are the only known carcinogens in tobacco that induce oral tumors in laboratory animals. *N'*-nitrosoanabasine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, and 4-(methylnitrosamino)-4-(3-pyridyl)butanol are carcinogenic in mice or rats (Hoffmann et al., this volume). The high carcinogenic potency and high levels of TSNA have prompted in-depth investigations on the formation and concentration of the alkaloid-derived nitrosamines in the various tobacco products (Table 4). As for the other nitrosamines, the nitrate or nitrite content and the various steps of processing are the determining factors for the yields of carcinogenic TSNA in ST products. According to analytical studies, NNN, nitrosoanabasine, and nitrosoanatabine are formed primarily from the corresponding secondary amines at the early stages of the tobacco processing, whereas TSNA such as NNK are formed from the tertiary amine nicotine (Figure 3) and occur at the later stage of tobacco curing and fermentation (Spiegelhalder and Fisher, 1991). This observation provides a partial explanation of the abundance of TSNA in snuff.

The carcinogenic risk associated with oral ST use and the major contributions of TSNA to this risk are underscored by a number of analytical data. In 1981, the National Research Council estimated the daily exposure of U.S. residents to carcinogenic nitrosamines and found the average nonsmoker is exposed to about 1 μg and the smoker of 20 cigarettes per day to about 11 to 12 μg of carcinogenic nitrosamines (U.S. National Research Council, 1981).

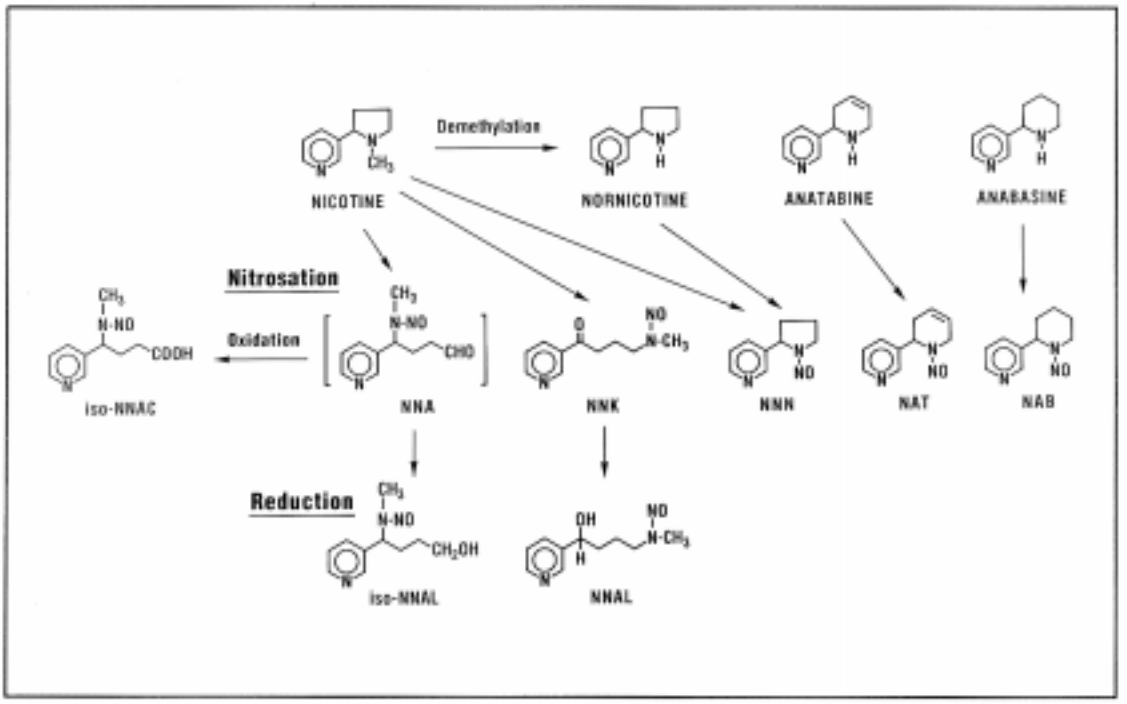
Table 4
TSNA in smokeless tobacco, 1981 to 1989

Country and Tobacco Type	Samples (n)	NNN[a] (μg/g)	NAT[a] (μg/g)	NAB[a] (μg/g)	NNK[a] (μg/g)
United States					
Moist snuff	16	0.83 - 64.00	0.24 - 215.00	0.01 - 6.70	0.08 - 8.30
Chewing tobacco	2	0.67 - 1.50	0.7 - 2.4[b]		0.11 - 0.38
Dry snuff	6	9.4 - 55.0	11 - 40	0.5 - 1.2	0.88 - 14.00
Sweden					
Moist snuff	8	2.0 - 6.1	0.9 - 2.4	0.04 - 0.14	0.61 - 1.70
Canada					
Moist snuff	2	50 - 79	152 - 170	4.0 - 4.8	3.2 - 5.8
Plug	1	2.1	1.7[b]		0.24
Germany					
Plug	2	1.4 - 2.1	0.36 - 0.55[b]		0.03 - 0.04
Nasal snuff	7	2.8 - 19	1.0 - 5.8[a]		0.58 - 6.40
India					
Chewing tobacco	4	0.47 - 0.85	0.40 - 0.50[b]		0.13 - 0.23
Zarda	11	0.40 - 79.00	0.78 - 99[b]		0.22 - 24.00
USSR					
Nass	4	0.12 - 0.52	0.04 - 0.33[b]		0.02 - 0.11
United Kingdom					
Moist snuff	7	1.1 - 52.0	2.0 - 65.0[b]		0.4 - 13.0
Nasal snuff	5	3.0 - 16.0	1.8 - 2.5[b]		0.97 - 4.30

[a] *NNN*, N-*nitrosonornicotine; NAT*, N-*nitrosoanatabine; NAB*, N-*nitrosoanabasine; NNK*, *4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.*
[b] *Contains NAB.*

On the basis of 1986 data for the two leading U.S. snuff brands, which had about 90 percent of the market share, the average snuff dipper, who consumes snuff at 10 g/d, is exposed to an additional 270 to 280 μg of carcinogenic nitrosamines (Hoffmann et al., 1987). Most of the nitrosamines are extracted from the tobacco during snuff dipping, as is reflected in data from saliva analysis (Hoffmann and Adams, 1981; Nair et al., 1985; Oesterdahl and Slorach, 1988; Paladino et al., 1986; Sipahimalani et al., 1984). In addition, it is strongly indicated that additional amounts of TSNA are endogenously formed during chewing (Nair et al., 1985). Recently we estimated that the average snuff dipper has a lifetime exposure to about 0.70 mmol/kg body weight of NNN and 0.03 mmol/kg body weight of NNK. These levels compare with 1.6 mmol/kg body weight of a mixture of NNN and NNK that induced tumors in the mouths of rats after oral swabbing (Hecht et al., 1986; Hoffmann et al., 1990).

Figure 3
Formation of tobacco-specific *N*-nitrosamines



CONTROL OF CARCINOGENS IN ST

The chemical-analytic data and the results from bioassays and epidemiological studies (Hoffmann et al., 1992; Preston-Martin, 1991; Winn et al., 1981) strongly support the World Health Organization's recommendation that, short of getting people to cease using tobacco, the harmful agents in chewing tobacco and snuff must be reduced (WHO, 1988). The history of the snuff analyses in the United States and Sweden has shown that a drastic reduction of the major carcinogens in ST products is feasible.

In 1981, the U.S. Environmental Protection Agency mandated a ban of maleic hydrazide diethanolamine (MH-30) for use on tobacco (US EPA, 1981). The diethanolamine part of this sucker growth inhibitor gives rise to the carcinogen NDELA (Brunnemann and Hoffmann, 1981). Following the ban of MH-30, the NDELA concentrations in smokeless tobacco declined, as shown by our monitoring of leading brands of snuff and chewing tobaccos. The reduction of NDELA values occurred gradually between 1981 and 1990, from 6,840 ppb to 94 ppb in snuff and from 224 ppb to 74 ppbin chewing tobacco (Brunnemann and Hoffmann, 1991). The concentration of the strongly carcinogenic NMOR in a snuff brand fell from 690 ppb in 1981 to a nondetectable level (< 2 ppb) in 1990 with the elimination of traces of morpholine in the packaging (Brunnemann et al., 1982; Brunnemann and Hoffmann, 1991).

While the reduction or disappearance of NDELA and NMOR was possible through the elimination of their precursors, this approach is not feasible for the reduction of nitrosamino acid and TSNA levels, because proteins and alkaloids, the precursors for these carcinogens, are integral parts of the tobacco. Nevertheless, elimination of nitrate-rich ribs and stems of certain tobacco varieties and changes in ST processing, especially of snuff, can lead to a major reduction of nitrosamines. Using NNN as an indicator for levels of TSNA, we have confirmed its gradual decrease in the two U.S. moist snuff brands that account for more than 85 percent of the current market share. In 1980, we reported 26.5 ppm and 39 ppm of NNN for brands A and B, respectively; in 1990, these levels had decreased to 10.4 and 9.6 ppm, respectively. In Sweden, the average NNN value for the leading five snuff brands in 1980 amounted to 11.4 ppm and in 1990 for three leading brands to 5.4 ppm. Two new snuff brands introduced in 1989 and 1990 on the U.S. market had NNN values of 4.1 and 3.2 ppm, respectively. Because the volatile nitrosamines and the nitrosamino acids are formed during the preparation of snuff by mechanisms similar to those leading to TSNA, their concentrations also have been reduced.

These observations strongly support the concept that product modifications can lead to a significant reduction of nitrosamines in smokeless tobacco (Table 5). Therefore, it was rather surprising that another snuff brand introduced in the United States in 1989 and 1990 contained extremely high concentrations of TSNA and other carcinogenic nitrosamines, in fact the highest ever reported (Table 5; see brand D). The increased pH of this snuff (7.7 to 8.2), compared with other U.S. brands (5.6 to 7.3), suggests that changes in manufacturing were possibly intended to facilitate the absorption of nicotine through the oral mucosa. Unprotonated nicotine, which increases steadily with increased pH above 6.2, is absorbed more rapidly than protonated nicotine (Brunnemann and Hoffmann, 1974; US DHHS, 1988).

The latter finding underscores the WHO recommendation to have the harmful substances in ST subject to governmental control (WHO, 1988), at least as it concerns the United States. Regulating agencies should be encouraged also to evaluate the flavor components and other chemical additives that are used in the manufacture of smokeless tobacco products. Any agents that are teratogenic or genotoxic should be banned.

Table 5
Alkaloids and *N*-nitroso compounds in moist snuff brands, 1990 to 1991[a]

	United States					Sweden
	Brand A	Brand B	Brand C	Brand D[b]	Brand E[c]	Three Brands
Moisture, %	56.0	57.8	51.8	50.00 - 57.8	51.9	46.6 - 54.2
pH	7.11	7.30	5.61	7.72 - 8.17	7.36	7.67 - 7.90
Nicotine, %	2.04	2.17	2.15	1.22 - 2.21	1.47	1.13 - 1.25
Total Alkaloids, %	2.18	2.32	2.32	1.32 - 2.38	1.59	1.24 - 1.41
Volatile Nitrosamines,[d] ng/g						
NDMA	ND[g]	ND[g]	ND[g]	147 - 265	ND[g]	51 - 63
NPYR	44	59	120	245 - 757	ND[g]	ND[g] - 155
Nitrosamino Acids,[e] µg/g						
NSAR	0.06	0.06	ND[h]	0.4 - 2.5	0.10	0.03 - 0.68
MNPA	5.13	3.62	2.72	8.9 - 65.7	2.20	3.10 - 3.28
MNBA	0.47	0.26	0.09	1.9 - 9.1	0.20	0.19 - 0.23
Total	5.70	3.90	2.80	11.2 - 77.3	2.50	3.30 - 4.20
TSNA,[f] µg/g						
NNN	10.40	9.57	4.14	21 - 147	3.20	5.24 - 5.67
NNK	2.19	3.14	1.24	6 - 18	0.70	1.37 - 2.08
NAT + NAB	9.76	7.90	2.97	22 - 115	2.00	2.58 - 3.47
Total	22.30	20.60	8.30	48 - 280	5.90	9.20 - 11.20

[a] *All values are based on dry weight. Total alkaloids include nicotine, nornicotine, mysomine, anatabine, anabasine, 2,3'-dipyridyl, and cotinine.*
[b] *Range of five samples bought in different stores in Texas.*
[c] *Snuff in sachets imported from Sweden.*
[d] *NDMA,* N-*nitrosodimethylamine; NPYR,* N-*nitrosopyrrolidine.*
[e] *ND, not detected < 0.005 µg/g.*
[f] *NSAR,* N-*nitrososarcosine; MNPA, 3-(methylnitrosamino)propionic acid; MNBA, 4-(methylnitrosamino)butyric acid.*
[g] *ND, not detected < 0.01 µg/g.*
[h] *TSNA, tobacco-specific* N-*nitrosamines; NNN,* N'-*nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT,* N'-*nitrosoanatabine; NAB,* N'-*nitrosoanabasine.*

ACKNOWLEDGMENT We thank Ilse Hoffmann and Jennifer Johnting for their editorial assistance.

REFERENCES

Mookherjee, B.D., Wilson, R.A. Tobacco constituents: Their importance in

Andersen, R.A., Burton, H.R., Fleming, P.D., et al. Effect of storage conditions on nitrosated, acetylated and oxidized pyridine alkaloid derivatives in smokeless tobacco products. *Cancer Research* 49: 5895-5900, 1989.

Brückner, H. *Die Biochemie des Tabaks und der Tabakverarbeitung*. Berlin: Verlagsbuchhandlung Paul Parey, 1936.

Brunnemann, K.D., Genoble, L., Hoffmann, D. *N*-nitrosamines in chewing tobacco: An international comparison. *Journal of Agricultural and Food Chemistry* 33: 1178-1181, 1985.

Brunnemann, K.D., Hoffmann, D. The pH of tobacco smoke. *Food and Cosmetics Toxicology* 12: 115-124, 1974.

Brunnemann, K.D., Hoffmann, D. Assessment of the carcinogenic *N*-nitrosodiethanolamine in tobacco products and tobacco smoke. *Carcinogenesis* 2: 1123-1127, 1981.

Brunnemann, K.D., Hoffmann, D. Decreased concentrations of *N*-nitrosodiethanolamine and *N*-nitrosomorpholine in commercial tobacco products. *Journal of Agricultural and Food Chemistry* 39: 207-208, 1991.

Brunnemann, K.D., Scott, J.C., Hoffmann, D. *N*-Nitrosomorpholine and other volatile *N*-nitrosamines in snuff tobacco. *Carcinogenesis* 3: 693-696, 1982.

Brunnemann, K.D., Scott, J.C., Hoffmann, D. *N*-Nitrosoproline, an indicator for *N*-nitrosation of amines in tobacco. *Journal of Agricultural and Food Chemistry* 31: 905-909, 1983.

Campbell, J.M., Lindsey, A.J. Polycyclic hydrocarbons extracted from tobacco: The effect upon total quantities found in smoking. *British Journal of Cancer* 10: 649-652, 1956.

Campbell, J.M., Lindsey, A.J. Polycyclic aromatic hydrocarbons in snuff. *Chemical Industry* July 6, 1957, p. 951.

Chamberlain, W.J., Schlotzhauer, W.S., Chortyk, O.T. Chemical composition of commercial tobacco products. *Journal of Agricultural and Food Chemistry* 36: 48-50, 1988.

Djordjevic, M.V., Brunnemann, K.D., Hoffmann, D. Identification and analysis of a nicotine-derived *N*-nitrosamino acid and other nitrosamino acids in tobacco. *Carcinogenesis* 10: 1725-1731, 1989.

Hecht, S.S., Rivenson, A., Braley, J., et al. Induction of oral cavity tumors in F-344 rats by tobacco-specific nitrosamines in snuff. *Cancer Research* 49: 3063-3069, 1986.

Hoffmann, D., Adams, J.D. Carcinogenic tobacco-specific *N*-nitrosamines in snuff and in the saliva of snuff dippers. *Cancer Research* 41: 4305-4308, 1981.

Hoffmann, D., Adams, J.D., Lisk, D., et al. Toxic and carcinogenic agents in dry and moist snuff. *Journal of the National Cancer Institute* 79: 1281-1286, 1987.

Hoffmann, D., Brunnemann, K.D., Venitt, S. Carcinogenic nitrosamines in oral snuff. *Lancet* 1988, p. 1232.

Hoffmann, D., Djordjevic, M.V., Brunnemann, K.D. On the control of toxic substances in smokeless tobacco. *Journal of Smoking-Related Disease* 2: 165-172, 1991.

Hoffmann, D., Rivenson, A., Chung, F.L., et al. Relevance of nicotine-derived *N*-nitrosamines in tobacco carcinogenesis. In: *Effects of Nicotine on Biological Systems*, F. Adlkofer and K. Thurau (Editors). Boston: Birkhäuser, 1990, pp. 89-101.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985.

LaVoie, E.J., Tucciarone, P., Kagan, M., et al. Quantitative analysis of steam distillates and aqueous extracts of smokeless tobacco. *Journal of Agricultural and Food Chemistry* 37: 154-157, 1989.

Liu, Y.Y., Schmeltz, I., Hoffmann, D. Quantitative analysis of hydrazine in tobacco and cigarette smoke. *Analytical Chemistry* 46: 885-889, 1974.

Martell, E.A. Radioactivity of tobacco trichromes and insoluble cigarette smoke particles. *Nature* 249: 215-217, 1974.

McMurtrey, J.E., Jr., Bacon, C.W., Ready, D. Growing tobacco as a source of nicotine. *USDA Technical Bulletin* 820: 1-38, 1942.

Mookherjee, B.D., Wilson, R.A. Tobacco constituents: Their importance in flavor and fragrance chemistry. *Recent Advances in Tobacco Science* 14: 114-168, 1988.

Nair, J., Ohshima, H., Friesen, M., et al. Tobacco-specific and betel nut-specific *N*-nitroso compounds: Occurrence in saliva and urine of betel quid chewers and formation in vitro by nitrosation of betel quid. *Carcinogenesis* 6: 295-303, 1985

Oesterdahl, B.G., Slorach S. Tobacco-specific *N*-nitrosamines in the saliva of habitual snuff dippers. *Food Additives and Contamination* 57: 581-586, 1988.

Ohshima, H., Nair, J., Bourgarde, M.-C., et al. Identification and occurrence of two new *N*-nitrosamino acids in tobacco products: 3-(*N*-nitroso-N-methylamino)propionic acid and 4-(*N*-nitroso-N-methylamino)butyric acid. *Cancer Letters* 26: 153-162, 1985.

Ough, C.S. Ethyl carbamate in fermented beverages and foods: I. Naturally occurring ethyl carbamate. *Journal of Agricultural and Food Chemistry* 24: 323-328, 1976.

Palladino, G., Adams, J.D., Brunnemann, K.D., et al. Snuff dipping in college students: A clinical profile. *Military Medicine* 151: 342-346, 1986.

Preston-Martin, S. Evaluation of the evidence that tobacco-specific nitrosamines (TSNA) cause cancer in humans. *Critical Review of Toxicology* 21: 295-298, 1991.

Robert, N.L. Natural tobacco flavor. *Recent Advances in Tobacco Science* 14: 49-81, 1988.

Schmeltz, I., Chiang, K.G., Hoffmann, D. Formation and determination of ethyl carbamate in tobacco and tobacco smoke. *Journal of Analytical Toxicology* 2: 265-268, 1978.

Sharma, A.K., Prokopczyk, B., Hoffmann, D. Supercritical fluid extraction of moist snuff. *Journal of Agricultural and Food Chemistry* 39: 508-510, 1991.

Sharma, P.K., Lal, N., Nagpaul, K.K. Study of trace amounts of U in snuff. *Health Physics* 48: 811-812, 1985.

Sipahimalani, A.T., Chadka, M.S., Bhide, S.V., et al. Detection of *N*-nitrosamines in the saliva of habitual chewers of tobacco. *Food Chemistry and Toxicology* 22: 261-264, 1984.

Smyth, J.H. Moist snuff sales gain. *Tobacco Reporter* 116 (8): 30-31, 1989.

Spiegelhalder, B., Fischer, S. Formation of tobacco-specific nitrosamines. *Critical Reviews of Toxicology* 21: 241, 1991.

Tricker, A.R., Preussmann, R. Preformed nitrosamines in smokeless tobacco. In: *Tobacco and Cancer: Perspectives in Preventive Research*, A.P. Maskens, R. Molimard, R. Preussmann, and J.W. Wilmer (Editors). Amsterdam: Excerpta Medica, 1989, pp. 35-47.

Tricker, A.R., Preussmann, R. Exposure to nicotine-derived *N*-nitrosamines from smokeless tobacco and evidence against endogenous formation. In: *Effects of Nicotine on Biological Systems*, F. Adlkofer and K. Thurau (Editors). Boston: Birkhäuser, 1990, pp. 109-113.

Tso, T.C., Harley, N., Alexander, L.T. Source of lead-210 and polonium-210 in tobacco. *Science* 153: 880-882, 1966.

U.S. Department of Agriculture. *Tobacco Situation and Outlook* 213: 18, 1990.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. 86-2874, 1986.

U.S. Department of Health and Human Services. *The Health Consequences of Smoking: Nicotine Addiction. A Report of the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Health Promotion and Education, Office on Smoking and Health. DHHS Publication No. (CDC) 88-8406, 1988.

U.S. Environmental Protection Agency. Maleic hydrazine: Notification of issuance of intent to suspend pesticide registrations. *Federal Register* 46(179): 45999-46000, 1981.

U.S. National Research Council. The Health Effects of Nitrate, Nitrite and *N*-nitroso Compounds. Washington, DC: U.S. National Research Council, 1981, p. 51.

Weybrew, J.A., Stephens, R.L. Survey of the carbonyl contents of tobacco. *Tobacco Science* 6: 53-57, 1962.

Winn, D.M., Blot, W.J., Shy, C.M.,Picke, L.W., Toledo, A., Fraumeni, J.F. Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

World Health Organization. Smokeless Tobacco Control. Technical Report Series 773. Geneva: WHO, 1988.

Wynder, E.L., Hoffmann, D. *Tobacco and Tobacco Smoke: Studies in Experimental Carcinogenesis*. New York: Academic Press, 1967.

Carcinogenesis of Smokeless Tobacco[1]

Dietrich Hoffmann, Abraham Rivenson, and Stephen S. Hecht

ABSTRACT Smokeless tobacco induces tumors in the oral cavity of rats. Of the 28 known carcinogens in tobacco, the major contributors to the carcinogenic activity of ST are the nitrosamines, especially the tobacco-specific nitrosamines (TSNA). Among seven TSNA that have been identified in ST, *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are the most potent carcinogens. A total dose of 450 μg NNK is sufficient to induce tumors in rats, and 420 μg NNK suffice to elicit tumors in mice. A mixture of NNN and NNK causes oral tumors in rats at a dose comparable to that ingested by a lifelong snuff dipper. In accordance with the recommendations of the World Health Organization, harmful substances in ST should be reduced and should be subject to governmental control and regulations.

INTRODUCTION Epidemiological investigations have revealed that tobacco chewers and snuff dippers face an increased risk for cancer of the oral cavity and pharynx. Chewing of smokeless tobacco has also been linked with cancer of the nasal cavity, esophagus, pancreas, and urinary bladder (International Agency for Research on Cancer, 1985; Kabat et al., 1986; Winn, 1992). Topical application of extracts from tobacco and from snuff has induced tumors in mouse skin. Such extracts have also exhibited cocar-cinogenic activity (IARC, 1985; US DHHS, 1986). In some bioassays, ST products were tested for tumorigenicity by painting the oral cavity with extracts, by implantation, or by repeated insertion of chewing tobacco or snuff into the cheek pouch (Table 1) (Gothoskar et al., 1975; Hecht et al., 1986; Homburger, 1971; Homburger et al., 1976; Kandarkar et al., 1981; Peacock et al., 1960). Although most of these bioassays have led to epithelial hyperplasia in the mouth or cheek pouches, none of them actually induced oral tumors.

Hirsch and Thilander (1981) developed a new method for the bioassay of ST in the oral cavity of laboratory animals. A canal is created in the lower lip of rats by surgery, and snuff is inserted and exchanged twice daily (Hirsch and Thilander, 1981). The first assays with this technique led to hyperplasia, dysplasia, and other lesions in the lip canal and oral cavity but not to oral tumors (Hirsch and Johansson, 1983; Hirsch and Thilander, 1981). Subsequently, other investigators modified the lip canal methodology and assayed commercial snuff. In one case, 2 of 32 rats treated with snuff developed epithelial tumors in the lip canal and 1 rat had a papilloma of the palate (Hecht et al., 1986). Johansson and colleagues (1989) induced five oral tumors and two nasal tumors in a group of 29 rats in a long-term assay with snuff. Together with experimentally induced *herpes simplex* virus type 1 (HSV-1) infection, snuff treatment caused squamous cell carcinoma of the oral cavity in two of seven rats (Hirsch et al., 1984). Park and associates (1986) repeatedly infected the buccal pouches of hamsters with either

[1] Supported by National Cancer Institute grant no. CA-29580.

Table 1
Bioassays for the induction of oral tumors with ST

Material[a]	Animal[b]	Method	Result	Reference
CT	Hamster	Implantation into cheek pouch	No oral tumors	Peacock et al., 1960
CT	Hamster	Thrice weekly insertion of CT with lime into cheek pouch	No neoplastic changes	Kandarkar et al., 1981
CT	Rat	Painting of oral cavity with extract twice weekly	No oral tumors	Gothoskar et al., 1975
S	Hamster, M	Feeding of S with diet (20%)	No oral tumors	Homburger et al., 1976
S	Hamster	Insertion in cheek pouch	No tumors	Peacock et al., 1960
S	Hamster	Daily attachment of cartridge containing 0.5 g S to lower incisors for 30 min; 1 yr	No oral tumors	Homburger, 1971
S	Rat, M	Twice daily painting of oral cavity and lips with concentrated S extract	No oral tumors	Hecht et al., 1986
S	Rat	Lip canal; twice daily insertion of 0.2 g S; up to 18 to 22 mo	Epithelial hyperplasia and dysplasia, no oral tumors	Hirsch and Thilander, 1981
S	Rat, M	Lip canal; one daily insertion of 0.05 g S; up to 25 mo	3/32 oral tumors	Hecht et al., 1986
S	Rat	Lip canal; twice daily insertion of 0.2 g S; up to 18 mo	0/10 oral tumors	Hirsch et al., 1984
		As above plus HSV-1 infection	2/7 oral tumors	
S	Rat	Lip canal; twice daily insertion of 0.1 g; up to 20 mo	5/29 oral and lip tumors 2/29 nasal tumors	Johansson et al., 1989
S	Hamster	Insertion twice daily of 0.15 g S into buccal pouch; up to 6 mo	0/15 oral tumors	Park et al., 1986
		As above plus HSV-1 infection	10/20 oral carcinoma	
		As above plus HSV-2 infection	11/20 oral carcinoma	

[a] *CT, chewing tobacco; S, snuff.*
[b] *M, males.*

HSV-1 or HSV-2 and inserted 150 mg of commercial snuff into the infected pouches twice daily. After 6 mo, 10 of 20 hamsters inoculated with HSV-1 and exposed to snuff developed invasive squamous cell carcinoma in the buccal pouch; in the group treated with HSV-2 and snuff, 11 of 20 hamsters developed carcinoma in the buccal pouch. None of the animals treated with either virus type or with snuff alone developed oral tumors within 6 mo (Park et al., 1986).

Thus, bioassays support the epidemiological observation that the long-term use of ST leads to cancer in the oral cavity. The next goal in explaining the causes of oral cancer by tobacco is the identification of those agents among the more than 3,050 known tobacco constituents (Roberts, 1988) that make smokeless tobacco carcinogenic. Until now, 28 known carcinogens have been reported in processed tobacco (Table 2) (Brunnemann and Hoffmann, 1992; Hoffmann et al., 1991; IARC, 1987 and 1988).

CARCINOGENS IN SMOKELESS TOBACCO

The contamination of processed tobacco with benzo[*a*]pyrene and other carcinogenic polynuclear aromatic hydrocarbons (PAH) stems mostly from polluted air (Campbell and Lindsey, 1956 and 1957; Wynder and Hoffmann, 1967). However, the levels of PAH appear to be too low to make a significant contribution to the carcinogenicity of snuff in the oral cavity (IARC, 1973). The possible carcinogenic effect of α- and β-angelica lactones cannot be evaluated at this time, because these tobacco constituents have not yet been assayed by oral application. Coumarin applied to rats as a dietary component induced tumors in the bile duct but not in the upper digestive tract (IARC, 1976). Urethan (ethyl carbamate), when given in the drinking water to mice, induces primarily lung tumors; in rats, urethan causes tumors at multiple sites, but predominantly hepatomas. In hamsters, urethan causes tumors of the forestomach and melanotic tumors of the skin (IARC, 1975).

Three carcinogenic volatile aldehydes have been detected in smokeless tobacco; their concentrations in oral snuff are lower than in other processed tobacco types and products (Brunnemann and Hoffmann, 1992; Wynder and Hoffmann, 1967). Acetaldehyde, for example, is found in the major tobacco types in amounts up to 270,000 ng/g (270 ppm) (Weybrew and Stephens, 1962), yet the data reported for U.S. commercial snuff showed about 1/10 of those concentrations (Table 2). The International Agency for Research on Cancer (1987) regards formaldehyde as an animal carcinogen and as probably carcinogenic to humans. After inhalation of formaldehyde (14.3 ppm), rats developed squamous cell carcinoma of the nasal cavity (Albert et al., 1982; IARC, 1982 and 1987). Inhalation of acetaldehyde produced adenocarcinoma and squamous cell carcinoma of the nasal mucosa in rats and laryngeal carcinoma in hamsters (IARC, 1987). Acetaldehyde is also known to inhibit the repair of DNA lesions (Grafström et al., 1986). Croton aldehyde, fed to rats in the drinking water (0.6 mM), induced benign and malignant tumors of the liver (Chung et al., 1986). Data are lacking for the carcinogenicity of volatile aldehydes in the upper digestive tract, but one would assume that these components contribute to the carcinogenicity of ST.

Table 2
Carcinogenic agents in tobacco

	Tobacco Type[b]	Concentration, ng/g[c]	IARC Evaluation of Evidence of Carcinogenicity[a]	
			In Laboratory Animals	In Humans
Benzo[*a*]pyrene	NT, S	> 0.1 - 90.0	Sufficient	Probable
α-Angelica Lactone	NT	Present		
β-Angelica Lactone	NT	Present		
Coumarin	NT	600	Limited	
Ethyl Carbamate	CT	310 - 375	Sufficient	
Volatile Aldehydes				
Formaldehyde	NT, S	1,600 - 7,400	Sufficient	Probable
Acetaldehyde	NT, S	1,400 - 27,400	Sufficient	
Crotonaldehyde	S	200 - 2,400		
Nitrosamines				
Nitrosodimethylamine	CT, S	ND - 270	Sufficient	Probable
Nitrosopyrrolidine	CT, S	ND - 760	Sufficient	
Nitrosopiperidine	CT, S	ND - 110	Sufficient	
Nitrosomorpholine	CT, S	ND - 690	Sufficient	
Nitrosodiethanolamine	CT, S	40 - 6,800	Sufficient	
Nitrosamino Acids				
Nitrososarcosine	S	ND - 2,500	Sufficient	
3-(Methylnitrosamino)-propionic acid	CT, S	200 - 65,700		
4-(Methylnitrosamino)-butyric acid	CT, S	ND - 9,100		
Nitrosoazetadine-2-carboxylic acid	CT	4 - 140		
Tobacco-Specific Nitrosamines				
N'-Nitrosonornicotine	CT, S	400 - 147,000	Sufficient	
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	CT, S	ND - 18,000	Sufficient	
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol	S	Present		
N'-Nitrosoanabasine	SM, S	Present - 560	Limited	
Inorganic compounds				
Hydrazine	SM	14 - 51	Sufficient	Inadequate
Arsenic	NT	500 - 900	Inadequate	Sufficient
Nickel	SM, S	180 - 2,700	Sufficient	Sufficient
Cadmium	SM	700 - 790	Sufficient	Probable
		p Ci/g		
Polonium-210	NT, S	0.16 - 1.22	Sufficient	Sufficient
Uranium-235 and -238	S	2.4, 1.91		

[a] *Absence of a designation indicates that IARC has not evaluated.*
[b] *NT, natural tobacco; SM, smoking tobacco; S, snuff; CT, chewing tobacco.*
[c] *ND, not determined.*

There is no information on the possible contribution of inorganic carcinogenic ST constituents to the increased oral cancer risk of chewers and snuff dippers. However, tobacco chewers have a higher level of trace amounts of some metals in the oral mucosa than do nontobacco users (Robertson and Bray, 1988). Of special concern is the human carcinogen polonium-210, which is a decay product of radon (IARC, 1988). Data on the polonium-210 content of oral tissues are needed before one can consider the carcinogenic effect of polonium-210 on the oral cavity of chewers.

_N_-NITROSAMINES Processing of tobacco to chewing tobacco or snuff yields several types of nitrosamines. Precursors to such carcinogens are nitrate or nitrite, the amino acids and proteins of the tobacco, and the *Nicotiana* alkaloids. These constituents form volatile nitrosamines (VNA), nitrosamino acids (NNA), and tobacco-specific nitrosamines (TSNA), respectively. In addition, residues of morpholine and diethanolamine from tobacco contaminants can serve as precursors for the corresponding nitrosamines.

Nitrosamines are generally organ-specific carcinogens: they induce benign and malignant tumors in specific organs, independent of site and mode of application (Table 3). The VNA are powerful animal carcinogens. For example, a daily dose of 40 μg of nitrosodimethylamine in the drinking water during the lifetime of rats induces liver tumors in 50 percent of the animals (Peto et al., 1984). None of the nitrosamines listed in Table 3, except nitrosodiethanolamine, are known to induce oral cavity tumors. Nitrosodiethanolamine causes tumors of the upper aerodigestive tract in hamsters in addition to tracheal tumors after swabbing of the oral cavity with an aqueous solution (Hoffmann et al., 1983).

So far, 10 nitrosamino acids have been identified in ST (Brunnemann and Hoffmann, 1991). Only six of these have been assayed for carcinogenicity. Nitrosoproline and nitrosothioproline are inactive in mice and rats. Nitrososarcosine and 3-(methylnitrosamino)propionic acid induce liver tumors in mice or rats, whereas 4-(methylnitrosamino)butyric acid induces bladder cancer in rats (Preussmann and Stewart, 1984; Rivenson et al., 1989). None of the nitrosamino acids have been assayed by topical application to the oral cavity, although their relatively high concentrations in snuff (up to 65 ppm) make such tests highly desirable.

TSNA The most important carcinogens in ST are TSNA. They are formed by nitrosation of the *Nicotiana* alkaloids during curing, fermenting, and aging of the leaves. Seven TSNA have been identified in ST (Brunnemann and Hoffmann, this volume). Two of these, *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridine)-1-butanol (NNAL), are powerful carcinogens in mice, rats, and hamsters, inducing tumors in the lung, upper aerodigestive tract, or pancreas (Table 4). *N'*-Nitrosoanabasine is a weak esophageal carcinogen in rats. *N'*-Nitrosoanatabine (NAT) and 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid (iso-NNAC) are not carcinogenic and 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (iso-NNAL) has not been bioassayed.

Table 3
Organ-specific carcinogenicity of nitrosamines in mice, rats, and hamsters

	Major Target Organs for Carcinogenicity		
	Mice	Rats	Syrian Golden Hamsters
Nitrosodimethylamine	Liver, lung	Liver, kidney	Liver
Nitrosopyrrolidine	Lung	Liver	Lung, trachea
Nitrosopiperidine	Lung, forestomach	Esophagus, liver	Trachea, nasal cavity
Nitrosomorpholine	Liver, lung	Liver, nasal cavity	Trachea, nasal cavity
Nitrosodiethanolamine		Liver	Trachea, nasal cavity

Table 4
Carcinogenicity of TSNA

	Animal (Strain)	Route of Application	Principal Target Organ	Dose, mmol/Animal
TSNA[a]				
NNN	Mouse	Topical (TI)[b]	None	0.028
	Mouse (A/J)	Intraperitoneal	Lung	0.1
	Rat (F-344)	Subcutaneous	Nasal cavity, esophagus	0.2 - 3.4
	Rat (F-344)	Oral	Esophagus, nasal cavity	1.0 - 3.6
	Rat (Sprague-Dawley)	Oral	Nasal cavity	8.8
	Syrian golden hamster	Subcutaneous	Trachea, nasal cavity	0.9 - 2.1
NNAL	Mouse (A/J)	Intraperitoneal	Lung	0.12
	Rat (F-344)	Subcutaneous	Lung, pancreas	0.32
NAB	Rat (F-344)	Oral	Esophagus	3 - 12
	Syrian golden hamster	Subcutaneous	None	2
NAT	Rat (F-344)	Subcutaneous	None	2.8
iso-NNAC	Mouse (A/J)	Intraperitoneal	None	0.2

Source: Hecht and Hoffmann (1989), except iso-NNAC, Rivenson et al. (1989).
[a] For data on NNK, see Table 20-5.
[b] TI, tumor-initiating assay with TPA as promoter; NNN, N′-nitrosonornicotine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NAB, N′-nitrosoanabasine; NAT, N′-nitrosoanatabine; iso-NNAC, 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid.

The most powerful carcinogen in ST is 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Table 5). NNK induces lung cancer and tumors of the nasal cavity and liver in rats. When NNK is administered in drinking water to rats, it also induces malignant tumors of the exocrine pancreas. In fact, NNK and its enzymatic reduction product NNAL are the only pancreas carcinogens known to occur in ST and in tobacco smoke. This finding is significant because both tobacco smokers and ST users have an increased risk for cancer of the exocrine pancreas (IARC, 1985; US DHHS, 1986 and 1989). NNK is also a weak transplacental carcinogen in mice and hamsters, resulting in lung tumors in offspring (Anderson et al., 1989; Anderson and Rice, this volume; Correa et al., 1990). Perhaps the most important aspect of NNK is its high carcinogenic potency; 450 μg induce tumors in rats (Belinsky et al., 1990).

NNN and NNK as an admixture have induced oral tumors in laboratory animals, when an aqueous solution of these TSNA was used to swab the oral cavity of rats twice daily for up to 131 wk (Hecht et al., 1986). The total dose applied amounted to about 97 mg of NNN (1,400 μmol/kg) and 19 mg of NNK (240 μmol/kg). Based on the analytical data for the best-selling U.S. snuff brand in 1980 (Hoffmann and Adams, 1981), consumption of 10 g of snuff per day exposes the oral cavity of a snuff dipper during a lifetime to about 5,700 mg of NNN (460 μmol/kg) and to about 360 mg of NNK (25 μmol/kg). This comparison supports the concept that the TSNA greatly contribute to the increased risk of snuff dippers for cancer of the oral cavity. The estimate of exposure of snuff dippers to the carcinogenic NNN and NNK did not consider the likely event that additional amounts of TSNA are formed endogenously during chewing (Tsuda and Kurasima, 1991).

DISCUSSION The bioassay data strongly support the epidemiological observation that ST is carcinogenic to humans. Twenty-eight carcinogens have been identified in chewing tobacco and snuff. The high concentrations of *N*-nitrosamines in ST, and especially the high levels of TSNA, are of great concern. The TSNA derive exclusively from the tobacco alkaloids, predominantly from the pharmacoactive nicotine, and are formed during tobacco processing. A bioassay has shown that a mixture of NNN and NNK induces oral tumors in rats. The orally applied amounts of NNN and NNK are comparable to the cumulative doses to which a snuff dipper is exposed during a lifetime.

Emphasis should be placed on educating the public to the hazards of tobacco chewing and snuff dipping. Because of the millions of ST users throughout the world, urgent support for the recommendations of the World Health Organization to regulate the harmful substances in ST is needed (WHO, 1988). As discussed in Brunnemann and Hoffmann (1992), significant reductions in the unacceptably high concentrations of carcinogens in tobacco, especially those of the nitrosamines, are feasible.

Table 5
Carcinogenicity of NNK in laboratory animals

	Route of Application	Principal Target Organ	Dose, mmol/Animal	Reference
Mouse (Sencar)	Topical (TI)[a]	Skin	0.028	Hecht and Hoffmann, 1989
	Oral	Lung, nasal cavity, liver	0.6 - 1.2	Prokopczyk et al., 1992
Mouse (A/J)	Intraperitoneal	Lung	0.01 - 0.12	Hecht and Hoffmann, 1989 Morse et al., 1991
Rat (F-344)	Subcutaneous	Lung, nasal cavity, liver	0.2 - 2.8	Hecht and Hoffmann, 1989
		Lung, nasal cavity	0.0025 - 3.6	Belinsky et al., 1990
	Oral (in drinking water)	Lung, liver, pancreas	0.075 - 0.31	Hecht and Hoffmann, 1989
	Oral (by gavage)	Liver, lung	1.3	Lijinsky et al., 1990
	Intravesical	Lung, liver	1.5	Lijinsky et al., 1990
Syrian Golden Hamsters	Subcutaneous	Trachea, lung, nasal cavity	0.005 - 0.9	Hoffmann et al., 1991

[a] *TI = tumor-initiating assay with TPA as a promoter.*

REFERENCES

Albert, R.E., Sellakumar, A.R., Laskin, S., et al. Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in the rat. *Journal of the National Cancer Institute* 68: 597-603, 1982.

Anderson, L.M., Hecht, S.S., Dixon, D.E., et al. Evaluation of the transplacental tumorigenicity of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mice. *Cancer Research* 49: 3770-3775, 1989.

Belinsky, S.A., Foley, J.F., White, C.M., et al. Dose-response relationship between O^6-methylguanine formation in Clara cells and induction of pulmonary neoplasia in the rat by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Research* 50: 3772-3780, 1990.

Brunnemann, K.D., Hoffmann, D. Analytical studies on tobacco-specific *N*-nitrosamines in tobacco and tobacco smoke. *Critical Reviews of Toxicology* 2: 235-240, 1991.

Campbell, J.M., Lindsey, A.J. Polycyclic hydrocarbons extracted from tobacco: The effect upon total quantities found in smoking. *British Journal of Cancer* 10: 649-652, 1956.

Campbell, J.M., Lindsey, A.J. Polycyclic hydrocarbons in cigar smoke. *British Journal of Cancer* 11: 192-195, 1957.

Chung, F.-L., Tanaka, T., Hecht, S.S. Induction of liver tumors in F344 rats by crotonaldehyde. *Cancer Research* 46: 1285-1289, 1986.

Correa, A., Joshi, P.A., Castonguay, A., et al. The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is an active transplacental carcinogen in Syrian golden hamsters. *Cancer Research* 50: 3435-3438, 1990.

Gothoskar, S.V., Sant, S.M., Ranadive, K.J. Effect of tobacco and lime on oral mucosa of rats fed on vitamin B deficient diet. *Indian Journal of Cancer* 12: 424-429, 1975.

Grafström, R.C., Curren, R.D., Yang, L.L. Acetaldehyde-induced inhibition of DNA repair and potentiation of *N*-nitroso compounds-induced mutagenesis in cultured human cells. *Progress in Clinical and Biological Research* 209A: 255-264, 1986.

Hecht, S.S., Hoffmann, D. The relevance of tobacco-specific nitrosamines to human cancer. *Cancer Surveys* 8: 273-294, 1989.

Hecht, S.S., Rivenson, A., Braley, J., et al. Induction of oral cavity tumors in F344 rats by tobacco-specific nitrosamines and snuff. *Cancer Research* 46: 4162-4166, 1986.

Hirsch, J.-M., Johansson, S.L. Effect of long-term application of snuff on the oral mucosa: An experimental study in the rat. *Journal of Oral Pathology* 12: 187-198, 1983.

Hirsch, J.-M., Johansson, S.L., Vahlne, A. Effect of snuff and *herpes simplex* virus-1 on rat oral mucosa: Possible associations with the development of squamous cell carcinoma. *Journal of Oral Pathology* 13: 52-62, 1984.

Hirsch, J.-M., Thilander, H. Snuff-induced lesions of the oral mucosa: An experimental model in the rat. *Journal of Oral Pathology* 10: 342-353, 1981.

Hoffmann, D., Adams, J.D. Carcinogenic tobacco-specific *N*-nitrosamines in snuff and in the saliva of snuff dippers. *Cancer Research* 41: 4305-4308, 1981.

Hoffmann, D., Castonguay, A., Rivenson, A., et al. Comparative carcinogenicity and metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N'*-nitrosonornicotine in Syrian golden hamsters. *Cancer Research* 41: 2386-2393, 1981.

Hoffmann, D., Djordjevic, M.V., Brunnemann, K.D. On the control of toxic substances in smokeless tobacco. *Journal of Smoking-Related Disease* 3: 165-172, 1991.

Hoffmann, D., Rivenson, A., Adams, J.D. Effects of route of administration and dose on the carcinogenicity of *N*-nitrosodiethanolamine in the Syrian golden hamster. *Cancer Research* 43: 2521-2524, 1983.

Homburger, F. Mechanical irritation, polycyclic hydrocarbons, and snuff. Effects on facial skin, cheek pouch, and oral mucosa in Syrian hamsters. *Archives of Pathology* 91: 411-417, 1971.

Homburger, F., Hsueh, S.-S., Russfield, A.B., et al. Absence of carcinogenic effects of chronic feeding of snuff in inbred Syrian hamsters. *Toxicology and Applied Pharmacology* 35: 515-521, 1976.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans: Benzo[a]pyrene* (volume 3). Lyon: IARC, 1973, pp. 91-136.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans: Urethane* (volume 7). Lyon: IARC, 1975, pp. 111-140.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans: Coumarin* (volume 10). Lyon: IARC, 1976, pp. 113-119.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans: Formaldehyde* (volume 29). Lyon: IARC, 1982, pp. 345-389.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans: Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs* (volume 1 to 42, supplement 7). Lyon: IARC, 1987.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans: Man-Made Mineral Fibres and Radon* (volume 43). Lyon: IARC, 1988.

Johansson, S.L., Hirsch, J.-M., Larsson, P.-A., et al. Snuff-induced carcinogenesis: Effect of snuff in rats initiated with 4-nitroquinoline-N-oxide. *Cancer Research* 49: 3063-3069, 1989.

Kabat, G.C., Dieck, G.S., Wynder, E.L. Bladder cancer in nonsmokers. *Cancer* 57: 362-367, 1986.

Kandarkar, S.V., Hasgekar, N.N., Sirsat, S.M. Optical and ultrastructural pathology of vitamin A pretreated hamster cheek pouch exposed to lime [$Ca(OH)_2$] and tobacco over total lifespan. *Neoplasma* 28: 729-737, 1981.

Lijinsky, W., Farnsworth, D., Farrelly, J., et al. Tumors of the bladder and other organs induced by intravesical administration of *N*-nitroso compounds to rats. Annual Report, Basic Research in Progress NCI, Frederick Cancer Research and Development Center, September 1990, pp. 76-78.

Morse, M.A., Eklind, K.I., Hecht, S.S., et al. Structure-activity relationship for inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone lung tumorigenesis by arylalkyl isothiocyanates in A/J mice. *Cancer Research* 51: 1846-1850, 1991.

Park, N.H., Sapp, J.P., Herbosa, E.G. Oral cancer induced in hamsters with *herpes simplex* infection and simulated snuff dipping. *Oral Surgery, Oral Medicine, Oral Pathology* 62: 164-168, 1986.

Peacock, E.D., Jr., Greenberg, B.G., Brawley, B.W. The effect of snuff and tobacco on the production of oral carcinoma: An experimental and epidemiological study. *Annals of Surgery* 151: 542-550, 1960.

Peto, R., Gray, R., Brantom, P., et al. Nitrosamine carcinogenesis in 5120 rodents: Chronic administration of sixteen different concentrations of NDEA, NDMA, NPYR and NPIP in the water of 4,440 inbred rats, with parallel studies on NDEA alone of the effect of age of starting (3, 6 or 20 weeks) and of species (rats, mice or hamsters). *IARC Scientific Publications* 57: 627-665, 1984.

Preussmann, R., Stewart, B.W. *N*-Nitroso carcinogens. In: *Chemical Carcinogens* (volume 132), E.C. Searle (Editor). Washington, DC: American Chemical Society, 1984, pp. 643-828. (American Chemical Society Monographs)

Prokopczyk, B., Rivenson, A., Hoffmann, D. Comparative carcinogneicity of 3-(methylnitrosamino)propionitrile, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone upon local application to mouse skin and rat oral mucosa. *Cancer Letters* 60: 153-157, 1991.

Rivenson, A., Djordjevic, M.V., Amin, S., et al. Bioassay in A/J mice of some *N*-nitrosamines. *Cancer Letters* 47: 111-114, 1989.

Roberts, D.L. Natural tobacco flavor. *Recent Advances in Tobacco Science* 14: 49-81, 1988.

Robertson, J.B., Bray, J.T. Development of validation test for self-reported abstinence from smokeless tobacco products: Preliminary results. *Preventive Medicine* 17: 496-502, 1988.

Tsuda, M., Kurashima, Y. Tobacco smoking, chewing and snuff dipping: Factors contributing to the endogenous formation of *N*-nitroso compounds. *Critical Reviews of Toxicology* 21: 243-253, 1991.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

U.S. Department of Health and Human Services. *Reducing the Health Consequences of Smoking: 25 Years of Progress. A Report of the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. DHHS Publication No. (CDC) 89-8411, 1989.

Weybrew, J.A., Stephens, R.L. Survey of carbonyl contents of tobacco. *Tobacco Science* 6: 53-57, 1962.

World Health Organization. *Smokeless Tobacco Control. Report of a WHO Study Group*. Geneva: WHO, 1988. World Health Organization Technical Report 773.

Wynder, E.L., Hoffmann, D. *Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis*. New York: Academic Press, 1967.

Oncogenes in Head and Neck Cancer[1]

Kenneth D. Somers, Suzanne E. Glickman, Suzanne L. Cartwright, Bryan G. Stoll, Stella L. Maurizio, and Gary L. Schechter

ABSTRACT Squamous cell carcinomas of the head and neck (SCCHN) were analyzed for activated oncogenes by DNA transfection assay and Southern blot hybridization. Transforming activity was not detected in SCCHN DNAs (n=31) by two DNA transfection assays. A cluster of proto-oncogenes (*int*-2, *hst*-1, *bcl*-1) localized to chromosome 11 band q13 was amplified two- to eightfold in approximately 30 percent of SCCHN (n=45); c-*erb*B-1 was amplified in 10 percent of the same tumors. Adjacent clinically and histologically normal tissue from the same patients had single proto-oncogene copy number. No amplification or rearrangement of c-*erb*B-2/HER2, c-*myc*, N-*myc*, N-*ras,* H-*ras*-1, or K-*ras*-2 was detected in any of the SCCHN. The 11q13 amplicon in SCCHN consisted of *int*-2, *hst*-1, and *bcl*-1, but did not include c-*sea*, also on 11q13, or extend to the collagenase gene locus (11q21-22), c-*ets*-1 (11q23), or H-*ras*-1 (11p15.5). The data implicate amplification of *int*-2/*hst*-1/*bcl*-1 as one of the genetic alterations underlying head and neck tumor formation.

INTRODUCTION Proto-oncogenes are normal cellular genes known to function in the control of cell growth and differentiation. When mutated or abnormally expressed, proto-oncogenes can be activated to oncogenic potential resulting in aberrant cell signaling and unrestrained cell proliferation (Bishop, 1991). Increasingly, activated oncogenes are being identified as one of the genetic alterations underlying human tumor pathogenesis. The most informative studies derive from the genetic analysis of colorectal cancer in which the stepwise accumulation of genetic damage in the form of activation of proto-oncogenes and inactivation of tumor suppressor genes leads to tumor development and progression (Fearon and Vogelstein, 1990).

There are few reports of activated oncogenes in head and neck tumors. Activated *ras* genes have been detected in < 10 percent of head and neck tumors (Howell et al., 1990; Sheng et al., 1990). We reported amplification of the *int*-2 proto-oncogene in squamous cell carcinomas of the head and neck (SCCHN) (Somers et al., 1990) and recently demonstrated that *hst*-1 is frequently co-amplified with *int*-2 in SCCHN; *int*-2 and *hst*-1 are in the fibroblast growth factor family (Dickson et al., 1990), whose members function in angiogenesis and as mitogenic growth factors (reviewed in Burgess and Maciag, 1989). Although the expression and function of *int*-2 and *hst*-1 in SCCHN is uncertain, amplification of these genes in head and neck tumors implicates their role in tumor formation. In this study, we analyzed SCCHN for biologically active oncogenes by DNA transfection and extended our analysis of the *int*-2 gene amplification unit in SCCHN.

[1] Supported by Smokeless Tobacco Research Council grant no. 0112 and the Medical College of Hampton Roads Foundation.

MATERIALS AND METHODS

Tissue Specimens and DNA Extraction

Surgical specimens were obtained from patients with histologically identified SCCHN. Adjacent normal tissue was obtained whenever possible. Tissue specimens were stored at -70 °C for subsequent DNA extraction. Human laryngeal SCC cell lines UMSCC10A, UMSCC10B, and UMSCC16 were provided by T.E. Carey, and Hep 2 was obtained from the American Type Culture Collection (ATCC). Cell lines were grown in Eagle's minimum essential medium supplemented with 10 percent fetal bovine serum. High molecular weight DNA was prepared as described by Somers and coworkers (1990).

Transfection Assays

DNA transfection was performed by the calcium phosphate precipitation technique (Graham and van der Eb, 1973) using NIH3T3 mouse fibroblasts as recipients. Foci of morphologically transformed cells were counted after 14 to 21 days. The nude mouse tumorigenicity assay was performed as described (Fasano et al., 1984) using $pSV2_{neo}$ as a dominant selectable marker, thereby permitting G418 antibiotic selection of stable transfectants. Positive control DNA for transfection assays was extracted from H-*ras*-transformed NIH3T3 cells, 44-9. DNA extracted from human diploid fibroblasts or placenta served as the negative control. DNA extracted from primary transfectants and tumors was used in a second cycle transfection and examined for human DNA sequences by Southern blot analysis with the human *Alu* repetitive DNA probe BLUR 8.

Southern Blot Analysis

DNA (10 µg) was digested with *Eco*RI or *Bam*HI, separated by electrophoresis in 0.8 percent agarose gels, and blotted to nylon filters (Biotrans, ICN). Filters were baked for 2 h at 80 °C. Filters were prehybridized for 1 h at 42 °C in 50 percent formamide, 5X SSC (1X SSC is 0.15 M NaCl and 0.015 M sodium citrate), 5X Denhardt's solution, 0.05 M sodium phosphate (pH 6.5), 0.1 percent sodium dodecyl sulfate (SDS), and 250 µg/mL denatured salmon sperm DNA. Hybridization was performed with $1x10^6$ cpm of heat-denatured ^{32}P-labeled oncogene DNA probes per milliliter of prehybridization solution overnight at 42 °C. Filters were washed for 20 min at room temperature in 2X SSC and 0.1 percent SDS and at 50 °C for 30 min in 0.1X SSC and 0.1 percent SDS and then exposed at -70 °C to Kodak XAR-5 film with an intensifying screen. DNA probes used for hybridization included the following: *int*-2 probe SS6 (Casey et al., 1986) provided by C. Dickson; *hst*-1 probe pORF1 (Taira et al., 1987) provided by M. Terada; *bcl*-1 probe b (Tsujimoto et al., 1985) provided by Y. Tsujimoto; c-*sea* probe p6.2 (Williams et al., 1988) provided by M. Hayman; and human collagenase (CLG) probe pCllase 1 (Whitman et al., 1986) obtained from ATCC. Probes for c-*ets*-1, H-*ras*-1, K-*ras*-2, N-*ras*, N-*myc*, c-*myc*, c-*erb*B-1, and c-*erb*B-2 were described previously (Somers et al., 1990). Cloned insert DNA (50 ng) was labeled with [^{32}P]dCTP by the random primer method to a specific activity of $5x10^7$ cpm/µg of DNA.

RESULTS

Transforming Activity of SCCHN DNA

We used two general oncogene detection techniques, the NIH3T3 focus assay and the nude mouse tumorigenicity assay, to analyze proto-oncogene activation in 31 primary or metastatic SCCHN and four human cell lines derived from SCC of the larynx (Hep 2, UMSCC10A, UMSCC10B, and UMSCC16). Provisional evidence for

the transfer of oncogenes by DNA transfection could not be confirmed by analysis of transformant or nude mouse tumor DNAs for the presence of human *Alu* repetitive DNA sequences (data not shown). Control transfections of NIH3T3 cells with 44-9 DNA containing activated H-*ras* produced foci of morphologically transformed cells and induced tumors in nude mice with a latency of 1 to 2 wk.

Southern Blot Analysis of Activated Oncogenes

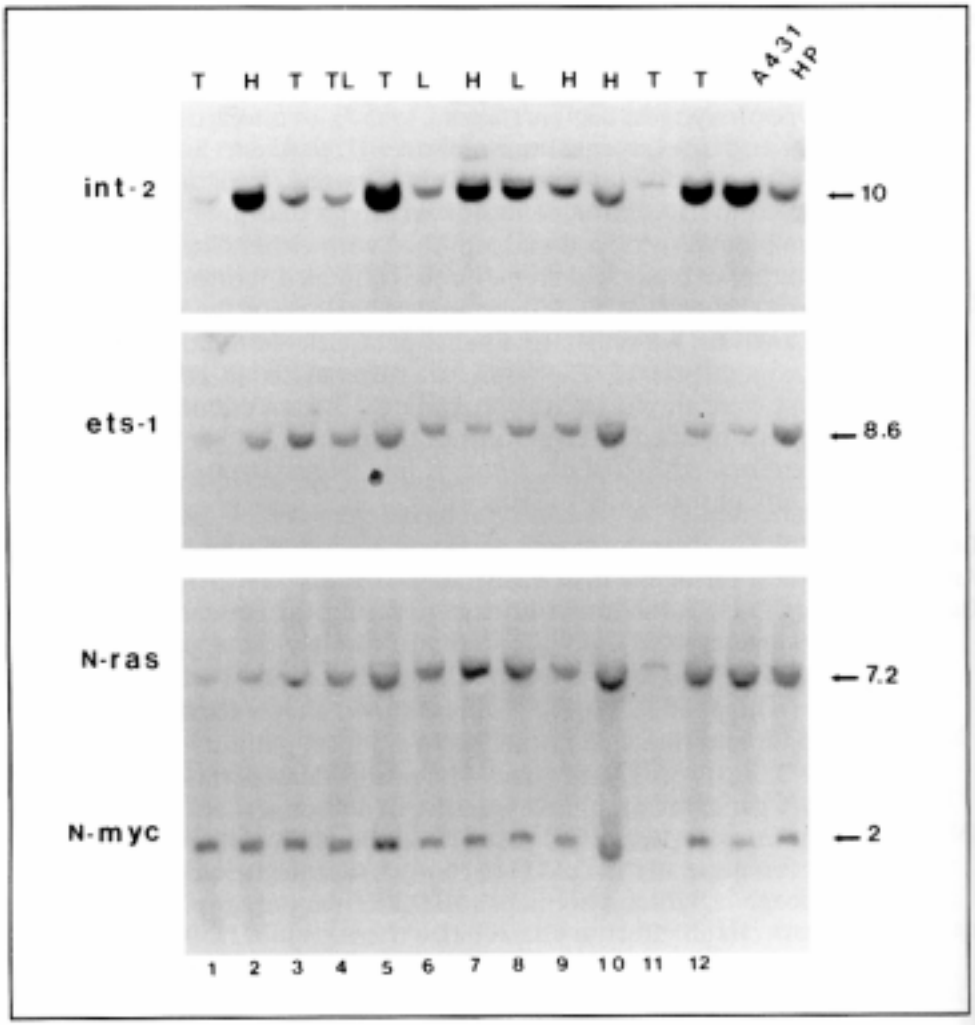
In an alternative approach, DNA extracted from SCCHN and tumor-derived cell lines was analyzed for amplified or rearranged proto-oncogenes by Southern blot hybridization. An example of such an analysis using probes corresponding to *int*-2, c-*ets*-1, N-*ras*, and N-*myc* is shown in Figure 1. *int*-2 was amplified two- to fivefold in two hypopharyngeal tumors (lanes 2 and 7), two tongue tumors (lanes 5 and 12), and one laryngeal tumor (lane 8), relative to human placenta DNA used as a single copy *int*-2 gene control (Figure 1, top). *int*-2 was amplified threefold in A431 epidermoid carcinoma cells used as a positive control. The blot was stripped and reprobed with either c-*ets*-1 (Figure 1, middle) or a mixture of N-*ras* and N-*myc* probes (Figure 1, bottom). No alterations of c-*ets*-1, N-*myc*, or N-*ras* were detected in any of the tumors. Using this type of analysis, we subjected a panel of SCCHN to Southern blot analysis using oncogene probes. The results are summarized in Table 1. *int*-2, *hst*-1, and *bcl*-1 were amplified in approximately 30 percent of SCCHN, whereas c-*erb*B-1 was amplified in 10 percent of the tumors. No amplification or rearrangement of c-*erb*B-2/HER2, c-*myc*, N-*myc*, N-*ras*, H-*ras*-1, or K-*ras*-2 was detected in any of the SCCHN (Table 1).

Gene Amplification on 11q13

We demonstrated previously amplification of the *int*-2 proto-oncogene in SCCHN (Somers et al., 1990). As shown in Table 1, *int*-2, *hst*-1, and *bcl*-1 were the most frequently amplified proto-oncogenes in SCCHN. To define accurately the region of DNA amplification on 11q13, we analyzed two other gene loci, *bcl*-1 and c-*sea*, which have also been mapped to 11q13. Figure 2 shows a representative *Bam*HI digest of tumor and matched normal DNA from six patients serially probed with *int*-2, *hst*-1, *bcl*-1, and c-*sea*. The results demonstrate that the *int*-2, *hst*-1, and *bcl*-1 genes are amplified together in a laryngeal and pharyngeal tumor but not in adjacent normal tissue from the same patients. The degree of amplification generally ranged between two- and eightfold. The c-*sea* proto-oncogene, which also maps to 11q13, was not amplified in any of the tumors. In an effort to characterize the region of DNA amplification, filters were reprobed with the collagenase gene (CLG) located at segment 11q21-22 (Figure 2). No amplification of the CLG gene was detected in tumors where *int*-2, *hst*-1, and *bcl*-1 were clearly amplified.

A map of chromosome 11 and the region of amplification is shown in Figure 3. The 11q13 amplicon contains four previously recognized oncogenes (*int*-2, *hst*-1, *bcl*-1, c-*sea*). Their location and map distances are shown. *hst*-1 is located 35 kb from *int*-2, and *bcl*-1 is approximately 1,000 kb proximal to the centromere from *int*-2 (Nguyen et al., 1988). The precise location of c-*sea* relative to the other loci in 11q13 is unknown. We examined a group of *int*-2 amplified head and neck tumors for amplification of *hst*-1, *bcl*-1, and c-*sea*. The results of this analysis are depicted in Figure 3.

Figure 1
Amplification of *int*-2 in SCCHN. DNA (10 μg) was digested with *Eco*RI and analyzed by Southern blot hybridization sequentially with the *int*-2 probe (top), c-*ets*-1 probe (middle), and mixed N-*ras* and N-*myc* probes (bottom). Lanes 1 to 12: DNA from 12 separate tumors derived from the tongue (T), hypopharynx (H), tonsil (TL), and larynx (L); A431: DNA from A431 cells; HP, human placenta DNA. Numbers on the right indicate DNA fragment sizes in kb detected by the probes.



Eleven of 12 tumors contained DNA coamplified for the *int*-2 and *hst*-1 gene; one tumor of the larynx exhibited *int*-2, but not *hst*-1, amplification. Eight of the *int*-2 amplified tumors were evaluated for *bc*l-1 and c-*sea* DNA amplification. All eight contained amplified *bcl*-1 DNA, whereas none of eight had amplified c-*sea* DNA.

Table 1
Frequency of oncogene amplification in head and neck squamous cell carcinoma

	Chromosome Location	Total	Amplification
Oncogene			
int-2	11 q13	45	14 (31%)
hst-1	11 q13	39	11 (28%)
bcl-1	11 q13	28	8 (29%)
c-*sea*	11 q13	29	0
c-*ets*-1	11 q23	24	0
H-*ras*-1	11 p15.5	33	0
K-*ras*-2	12 p12.1	21	0
N-*ras*	1 p22	24	0
N-*myc*	2 p24	24	0
c-*myc*	8 q24	21	0
c-*erb*B-1	7 p12-13	21	2 (10%)
c-*erb*B-2	17 q11-12	21	0

DISCUSSION In this study we examined a series of SCCHN for activated oncogenes. We were unable to detect transforming activity in 31 SCCHN DNAs by transfection assays. Detection of activated oncogenes by the biological transformation assay may underestimate the frequency of activated oncogenes (Barbacid, 1987). Few studies have examined head and neck cancer for activated *ras* genes. Tadokoro and associates (1989) detected activated H-*ras*-1 by DNA transfection in two cell lines established from metastatic lymph nodes of patients with palatal or floor-of-mouth SCC. Activated K-*ras*-2 was detected in 1 gingival SCC of 11 tumor DNAs tested by DNA transfection (Howell et al., 1990). Recently, the use of allele-specific oligonucleotide hybridization assays or RNase A mismatch cleavage analysis has significantly increased the detection of mutated *ras* genes in human malignancies (Bos, 1989). Using polymerase chain reaction and oligonucleotide hybridization to detect *ras* mutations at codons 12, 13, and 61, Sheng and associates (1990) detected a mutation at codon 12 of the H-*ras*-1 gene in 2 of 54 head and neck tumors. No mutations were detected at positions 12, 13, and 61 of the K-*ras*-2 gene or at positions 12 and 61 of the N-*ras* gene (Sheng et al., 1990). Although further studies are needed to confirm these findings, the combined data indicate that *ras* mutations are an uncommon genetic alteration in SCCHN.

Proto-oncogenes can be activated to oncogenic potential by mutational alterations or overexpression (Bishop, 1991). Overexpression of oncogenes at the level of gene amplification is a frequent observation in human tumors (Schwab and Amler, 1990). In certain tumors, oncogene amplification is associated with a more aggressive tumor phenotype and a poor clinical prognosis. Notable examples of oncogene amplification correlated with decreased survival include N-*myc* amplification in neuroblastomas (Brodeur et al., 1984; Seeger et al., 1985) and c-*erb*B-2/HER2 amplification in breast and ovarian cancers (Slamon et al., 1989).

Figure 2

Coamplification of *int*-2, *hst*-1, and *bcl*-1 in SCCHN. DNA (10 μg) was digested with *Bam*HI and the filter probed sequentially with *int*-2, *hst*-1, *bcl*-1, c-*sea*, and CLG probes. Tumor (T) and adjacent normal tissue (N) from individual patients with tumors of the larynx (L), floor of mouth (FOM), and pharynx (PX). A431 and HP are the same as in Figure 1. Numbers on the right indicate DNA fragment sizes in kb detected by the probes.

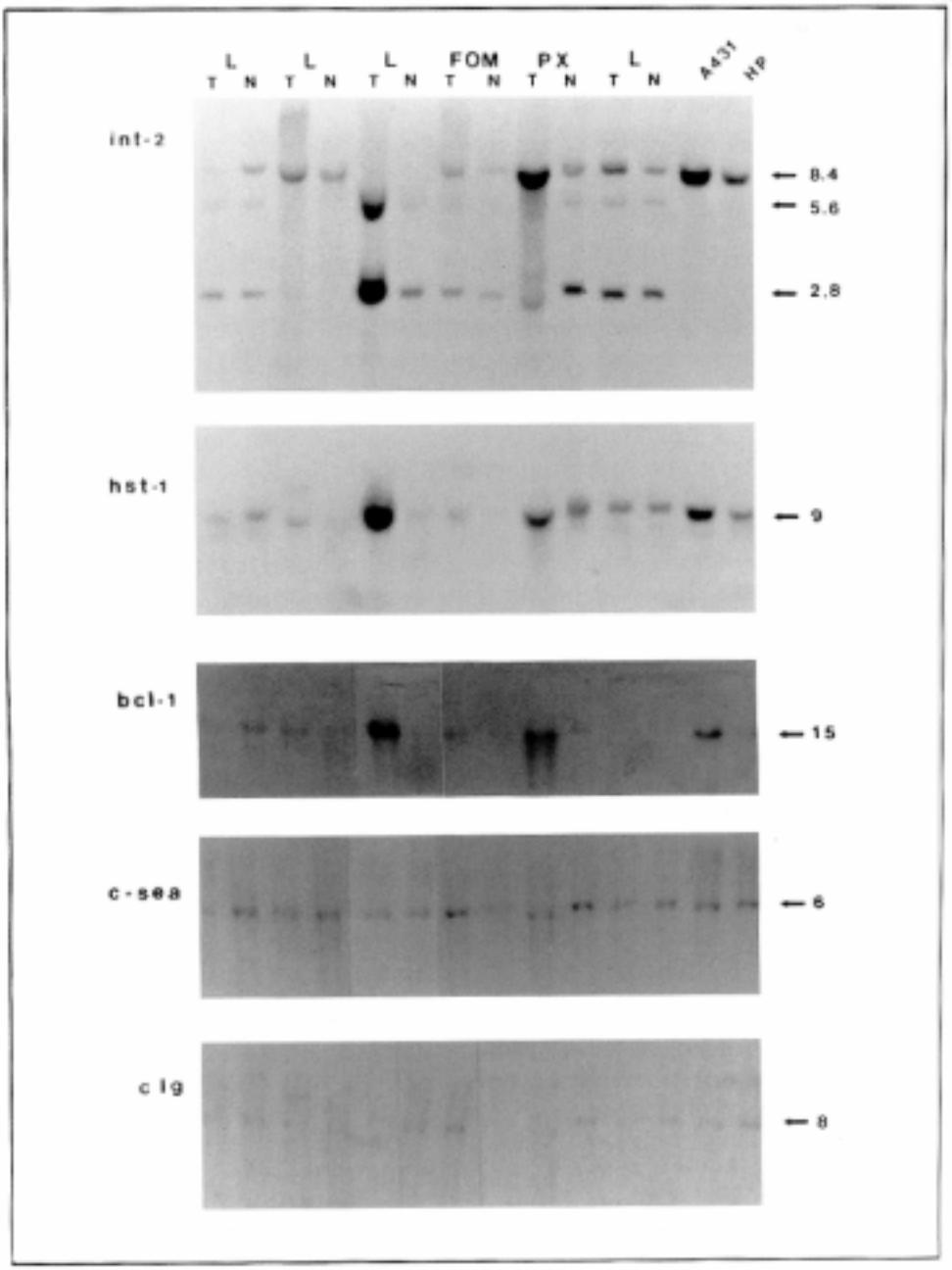
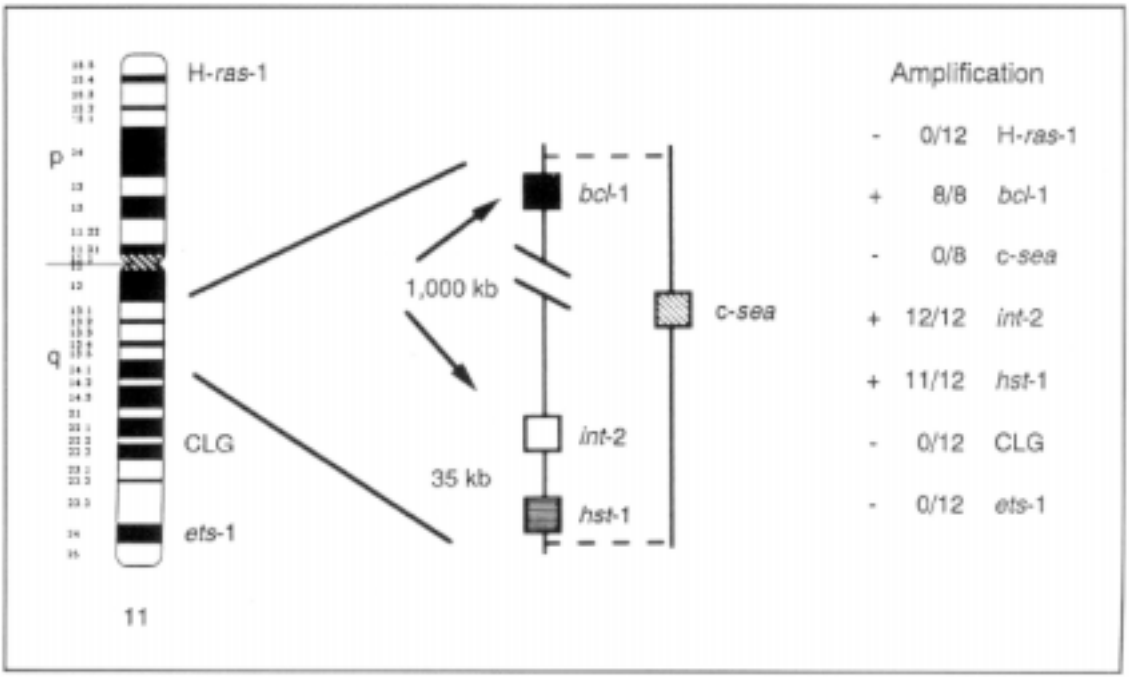


Figure 3
Map of chromosome 11 with the region of gene amplification in band q13 expanded. The table on the right indicates the presence (+) or absence (-) and frequency of amplification of specific gene loci on chromosome 11 in a panel of SCCHN.



There are few studies that report oncogene amplification and overexpression in head and neck cancer. Amplification of N-*myc* and N-*ras* was observed in approximately 40 percent of 23 cases of oral cavity SCC (Saranath et al., 1989). We were unable to confirm these findings. Increased transcription of c-*myc* was detected in 14 SCCHN (Field and Spandidos, 1987) and in 8 of 9 oral and laryngeal SCC (Riviere et al., 1990), but the number of cases is too small to make any meaningful clinical correlations. Results of the present study reveal the coamplification of *int*-2, *hst*-1, and *bcl*-1 in about 30 percent of SCCHN. The three genes comprise a rather large amplicon on 11q13. We have broadly defined the limits of the 11q13 amplicon in SCCHN to include *int*-2, *hst*-1, and *bcl*-1, but not c-*sea*, which also maps to 11q13. The amplicon does not extend to the collagenase locus 11q21-22, the c-*ets*-1 locus 11q23, or the pepsinogen cluster mapped to 11p11-q13 (Somers et al., 1990). Further work is needed to map more accurately the size of the 11q13 amplicon and to identify additional genes that reside in this region. It is also important to define the selective mechanisms that drive and maintain the 11q13 amplicon in SCCHN. Because *int*-2 and *hst*-1 are members of the FGF gene family, one model would predict that overexpression of *int*-2 or *hst*-1 might confer a growth advantage on the tumor cell. Studies of *int*-2/*hst*-1 gene expression in SCCHN are needed to address this possibility. However, data from other studies have shown low or undetectable levels of *int*-2 or *hst*-1 transcripts in

human breast cancer, regardless of any amplification of the gene (Fantl et al., 1990). Alternatively, maintenance of the 11q13 amplicon would be driven by the expression of an unidentified gene on 11q13. Ultimately, it will be necessary to express *int*-2 and *hst*-1cDNAs in squamous epithelial cells of the upper aerodigestive tract and examine these cells for properties that distinguish the malignant tumor phenotype.

Finally, it is important that amplification of *int*-2, *hst*-1, and *bcl*-1 on 11q13 may be involved in different human tumors. One or more of the three proto-oncogenes within the amplicon have been reported to be amplified in breast carcinoma (Ali et al., 1989; Theillet et al., 1990), melanoma (Adelaide et al., 1988), esophageal carcinoma (Tsuda et al., 1989), stomach cancer (Yoshida et al., 1988), and SCCHN (Berenson et al., 1989; Merritt et al., 1990; Somers et al., 1990; Zhou et al., 1988). Thus, the elucidation of the role of the 11q13 amplicon in SCCHN might provide clues in understanding the development and progression of other human malignancies. Clearly, this marker in SCCHN should encourage future studies to determine if 11q13 gene amplification might be useful to predict tumor behavior and disease progression.

REFERENCES

Adelaide, J., Mattei, M-G., Marics, I., Raybaud, F., Planche, J., de Lapeyriere, O., Birnbaum, D. Chromosomal localization of the *hst* oncogene and its co-amplification with the *int*-2 oncogene in human melanoma. *Oncogene* 2: 413-416, 1988.

Ali, I.U., Merlo, G., Callahan, R., Lidereau, R. The amplification unit on chromosome 11q13 in aggressive primary human breast tumors entails the *bcl*-1, *int*-2 and *hst* loci. *Oncogene* 4: 89-92, 1989.

Barbacid, M. *ras* genes. *Annual Review of Biochemistry* 56: 79-82, 1987.

Berenson, J.R., Yang, J., Mickel, R.A. Frequent amplification of the *bcl*-1 locus in head and neck squamous cell carcinomas. *Oncogene* 4: 1111-1116, 1989.

Bishop, J.M. Molecular themes in oncogenesis. *Cell* 64: 235-248, 1991.

Bos, J.L. *ras* oncogenes in human cancer: A review. *Cancer Research* 49: 4682-4689, 1989.

Brodeur, G.M., Seeger, R.C., Schwab, M., Varmus, H.E., Bishop, J.M. Amplification of N-*myc* in untreated human neuroblastomas correlates with advanced disease stage. *Science* 224: 1121-1124, 1984.

Burgess, W.H., Maciag, T. The heparin binding (fibroblast) growth factor family proteins. *Annual Review of Biochemistry* 58: 575-606, 1989.

Casey, G., Smith, R., McGillivray, D., Peters, G., Dickson, C. Characterization and chromosome assignment of the human homolog of *int*-2: A potential proto-oncogene. *Molecular and Cellular Biology* 6: 502-510, 1986.

Dickson, C., Acland, P., Smith, R., Dixon, M., Deed, R., MacAllan, D., Walther, W., Fuller-Pace, F., Kiefer, P., Peters, G. Characterization of *int*-2: A member of the fibroblast growth factor family. *Journal of Cell Science* (Suppl.) 13: 87-96, 1990.

Fantl, V., Richards, M.A., Smith, R., Lammie, G.A., Johnstone, G., Allen, D., Gregory, W., Peters, G., Dickson, C., Barnes, D.M. Gene amplification on chromosome band 11q13 and oestrogen receptor status in breast cancer. *European Journal of Cancer* 26: 423-429, 1990.

Fasano, O., Birnbaum, D., Edlund, L., Fogh, J., Wigler, M. New human transforming genes detected by a tumorigenicity assay. *Molecular and Cellular Biology* 4: 1695-1705, 1984.

Fearon, E.R., Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell* 61: 759-767, 1990.

Field, J.K., Spandidos, D.A. Expression of oncogenes in human tumors with special reference to the head and neck region. *Journal of Oral Pathology* 16: 97-197, 1987.

Graham, F.L., van der Eb, A.J. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52: 456-467, 1973.

Howell, R.E., Wong, F.S.H., Fenwick, R.G. A transforming Kirsten *ras* oncogene in an oral squamous carcinoma. *Journal of Oral Pathology and Medicine* 19: 301-305, 1990.

Merritt, W.D., Weissler, M.C., Turk, B.F., Gilmer, T.M. Oncogene amplification in squamous cell carcinoma of the head and neck. *Archives of Otolaryngology—Head and Neck Surgery* 116: 1394-1398, 1990.

Nguyen, C., Roux, D., Mattei, M-G., de Lapeyriere, O., Goldfarb, M., Birnbaum, D., Jordan, B.R. The FGF-related oncogenes *hst* and *int*-2, and the *bcl*-1 locus are contained within one megabase in band q13 of chromosome 11, while the *fgf*-5 oncogene maps to 4q21. *Oncogene* 3: 703-708, 1988.

Riviere, A., Wilckens, C., Loning, T. Expression of c-*erb*B2 and c-*myc* in squamous epithelial and squamous cell carcinomas of the head and neck and the lower female genital tract. *Journal of Oral Pathology and Medicine* 19: 408-413, 1990.

Saranath, D., Panchal, R.G., Nair, R., Mehta, A.R., Sanghavi, V., Sumegi, J., Klein, G., Deo, M.G. Oncogene amplification in squamous cell carcinoma of the oral cavity. *Japanese Journal of Cancer Research* 80: 430-437, 1989.

Schwab, M., Amler, L.C. Amplification of cellular oncogenes: A predictor of clinical outcome in human cancer. *Genes Chromosomes Cancer* 1: 181-193, 1990.

Seeger, R.C., Brodeur, G.M., Sather, H., Dalton, A., Siegel, S.E., Wong, K.Y., Hammond, D. Association of multiple copies of N-*myc* oncogene with rapid progression of neuroblastomas. *New England Journal of Medicine* 313: 1111-1116, 1985.

Sheng, Z.M., Barrois, M., Klijanienko, J., Micheau, C., Richard, J.M., Riou, G. Analysis of the c-Ha-*ras*-1 gene for deletion, mutation, amplification and expression in lymph node metastases of human head and neck carcinomas. *British Journal of Cancer* 62: 398-404, 1990.

Slamon, D.J., Godolphin, W., Jones, L.A., Holt, J.A., Wong, S.G., Keith, D.E., Levin, W.J., Stuart, S.G., Udove, J., Ullrich, A., Press, M.F. Studies of the HER2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244: 707-712, 1989.

Somers, K.D., Cartwright, S.L., Schechter, G.L. Amplification of the *int*-2 gene in human head and neck squamous cell carcinomas. *Oncogene* 5: 915-920, 1990.

Tadokoro, K., Ueda, M., Ohshima, T., Fujita, K., Rikimaru, K., Takahashi, N., Enomoto, S., Tsuchida, N. Activation of oncogenes in human oral cancer cells: A novel codon 13 mutation of c-H-*ras*-1 and concurrent amplification of c-*erb*B-1 and c-*myc*. *Oncogene* 4: 499-505, 1989.

Taira, M., Yoshida, T., Miyagawa, K., Sakamoto, H., Terada, M., Sugimura, T. DNA sequence of human transforming gene *hst* and identification of the coding sequence required for transforming activity. *Proceedings of the National Academy of Sciences* 84: 2980-2984, 1987.

Theillet, C., Adnane, J., Szepetowski, P., Simon, M-P., Jeanteur, P., Birnbaum, D., Gaudray, P. BCL-1 participates in the 11q13 amplification found in breast cancer. *Oncogene* 5: 147-149, 1990.

Tsuda, T., Tahara, E., Kajiyama, G., Sakamoto, H., Terada, M., Sugimura, T. High incidence of coamplification of *hst*-1 and *int*-2 genes in human esophageal carcinomas. *Cancer Research* 49: 5505-5508, 1989.

Tsujimoto, Y., Jaffe, E., Cossman, J., Gorham, J., Nowell, P.C., Croce, C.M. Clustering of breakpoints on chromosome 11 in human B cell neoplasms with the t(11;14) chromosome translocation. *Nature* 315: 340-343, 1985.

Whitham, S.E., Murphy, G., Angel, P., Rahmsdorf, H-J., Smith, B.J., Lyons, A., Harris, T.J.R., Reynolds, J.J., Herrlich, P., Docherty, J.P. Comparison of human stromelysin and collagenase by cloning and sequence analysis. *Biochemistry Journal* 240: 913-916, 1986.

Williams, B.P., Shipley, J.M., Spurr, N.K., Smith, D.R., Hayman, M.J., Goodfellow, P.N. A human sequence homologous to v-*sea* maps to chromosome 11, band q13. *Oncogene* 3: 345-348, 1988.

Yoshida, M.C., Wada, M., Satoh, H., Yoshida, T., Sakamoto, H., Miyagawa, K., Yokota, J., Koda, T., Kakinuma, M., Sugimura, T., Terada, M. Human HST1 (HSTF1) gene maps to chromosome band 11q13 and coamplifies with the INT2 gene in human cancer. *Proceedings of the National Academy of Sciences* 85: 4861-4864, 1988.

Zhou, D.J., Casey, G., Cline, M.J. Amplification of human *int*-2 in breast cancers and squamous cell carcinomas. *Oncogene* 2: 279-282, 1988.

Oncogenes in Tobacco-Induced Oral Cancer

Madhav G. Deo and Dhananjaya Saranath

ABSTRACT This paper reviews the status and implications of oncogenes in oral cancer caused by smokeless tobacco (chewing), which accounts for 40 percent of malignancies in India. A variety of abnormalities consisting of amplification, overexpression, point mutation, deletion, and rearrangement of oncogenes, particularly of *myc* and *ras* families, are seen in more than 90 percent of the cancers in India. A longitudinal study of oncogenes at various stages of oral carcinogenesis, particularly in leukoplakias and early cancers, will not only throw light on the molecular biology of malignant transformation but also provide clues to the early diagnosis of this cancer through molecular biology techniques.

INTRODUCTION Oral cancer, which is among the 10 most prevalent cancers in the world, is a common cancer in South and Southeast Asia, where the habit of tobacco chewing is widely prevalent. In India, tobacco chewing is a major cause of cancer mortality, accounting for about 40 percent of the total malignancies. Clinical, epidemiological, and laboratory studies indicate a causal relationship between prolonged tobacco chewing and oral cancer, smokeless tobacco being the sine qua non (Gupta et al., 1987; Jussawalla and Deshpande, 1971). A latent period of 5 to 15 yr is common, and almost every tobacco-related oral cancer is preceded by a phase of leukoplakia.

Several constituents of tobacco, such as tobacco-specific *N*-nitrosamines, polycyclic aromatic hydrocarbons, and polonium-210 (a radioactive alpha emitter), are capable of inducing preneoplastic and neoplastic changes in the oral cavity (IARC, 1985; Mattson and Winn, 1989). Although much information is available about their chemistry, the critical link between the tobacco-specific carcinogens and the cellular macromolecules leading to the malignant phenotype is an enigma. Tobacco-specific nitrosamines are known to form DNA adducts, resulting in mutations that could profoundly affect cellular genes (Hecht et al., 1988).

The importance of proto-oncogenes in cellular growth and differentiation is well documented (Bishop, 1987; Klein and Klein, 1985). One of the mechanisms involved in the process of carcinogenesis is the activation of proto-oncogenes (oncogenes), which has been observed in a number of human and experimental cancers (Klein, 1988). Illegitimate activation of proto-oncogenes may occur by various mechanisms, including gene amplification, chromosomal translocation, point mutation, and retroviral insertion, that in turn contribute to tumor development and progression in several systems (Klein, 1988).

This review elucidates the various molecular lesions that involve oncogenes in oral cancers. Such studies can be grouped into four categories: (1) examination of genomic DNA of the primary tumor for oncogene

amplification; (2) aberrant oncogene expression as seen by an increase in mRNA transcript level or alteration in the transcript size or an increase in the protein product; (3) allelic loss, rearrangement, and point mutation; and (4) detection, isolation, and cloning of a functional oncogene in oral cancers in a transfection assay. The methodology used in our studies is outlined in Figure 1.

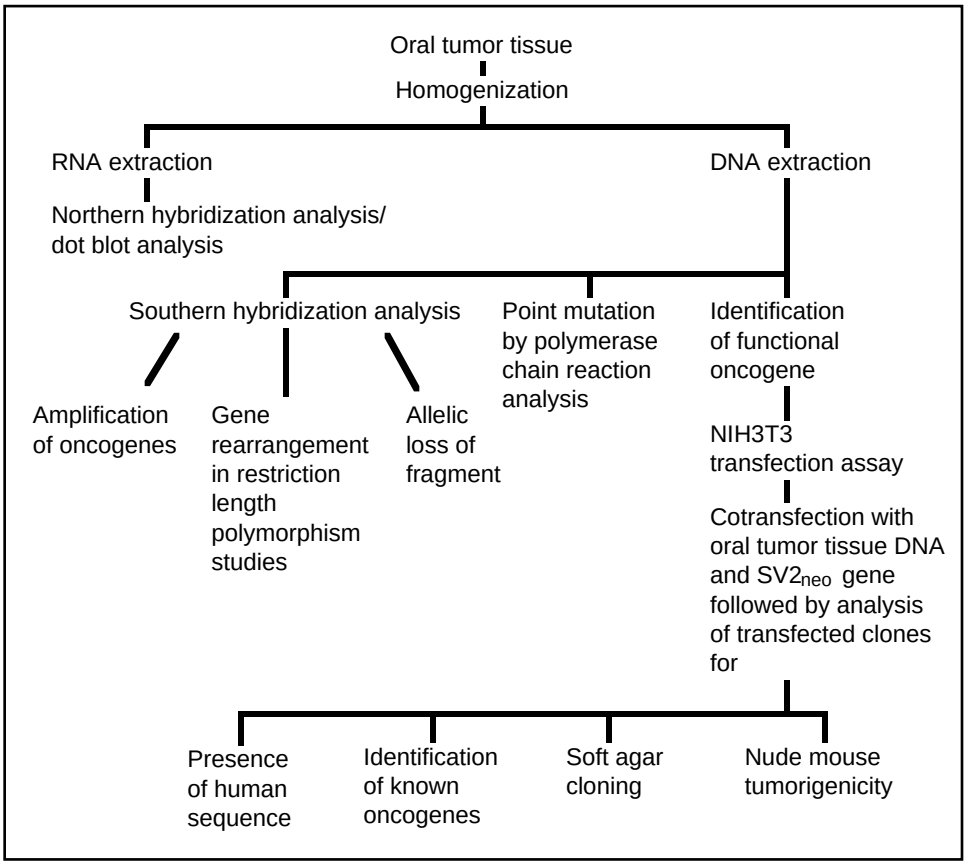
ONCOGENE AMPLIFICATION

We investigated the status of the commonly amplified oncogenes of the *myc* and *ras* families in 102 primary oral tumor tissues (Saranath et al., 1989). All tumors were squamous cell carcinomas (SCCs) involving various regions: buccal mucosa (46 patients), lower alveolus (29 patients), tongue (23 patients), and floor of the mouth (4 patients). All patients had chewed tobacco regularly for 10 to 15 yr. Although histological grading varied from well to poorly differentiated, the majority of the tumors were large and in an advanced stage (TNM III/IV). Lymph node metastases were frequent, particularly in the T3 and T4 lesions. However, none of the patients showed distant metastasis. Peripheral blood cells (PBCs) from the patients were analyzed for oncogene involvement. PBCs from healthy volunteers and human placental DNA were included in the study as controls. Vimentin, a single copy gene, was used as an internal control gene.

Southern hybridization analysis of the *Eco*RI-digested DNA samples revealed a 3-fold to 10-fold amplification of c-*myc*, N-*myc*, K-*ras*, or N-*ras* in 49 of the 102 (48 percent) primary oral tumor tissues screened (Table 1). H-*ras* and L-*myc* oncogenes were not amplified in any of the samples. The controls—PBC DNA from the patients and healthy volunteers and normal human placental DNA—showed the presence of a single copy of the oncogenes.

Amplification of *myc* oncogenes was observed in 37 of the 102 patients. N-*myc* and c-*myc* were amplified in 26 percent (27 of 102) and 21 percent (21 of 102) of the patients, respectively (Table 1). An unusual finding was the coamplification of c-*myc* and N-*myc* in 11 of 37 (30 percent) of the patients. It has been hypothesized that the products of *myc* family oncogenes, c-*myc*, N-*myc*, and L-*myc*, down-regulate each other (Alt et al., 1986). As a consequence, it is predicted that tumor cells would have amplification or increased expression of only one of the *myc* oncogenes. Indeed, this appears to be the rule. However, oral cancer seems to be unique in this respect, with the two *myc* oncogenes coamplified in a number of patients. However, it is not possible from these studies to know if the coamplification represents the existence of two different clones that each contain a single amplified *myc*. Alternatively, the two *myc* oncogenes could be amplified in the same cell. Coexpression of c-*myc* and N-*myc* has been reported in the early undifferentiated pre-β cells in mice (Zimmerman et al., 1986), implying coexpression during a particular developmental stage or in a particular differentiation window of the cell. In our studies, amplification bore no correlation with clinicopathological features such as degree of differentiation, tumor size, TNM staging, nodal metastasis, or recurrence.

Figure 1
Flow chart of methods used to study oncogene aberrations in oral cancers



Opinion is divided on the status of c-*myc* amplification or its increased expression in oral cancer. Although Berenson and colleagues (1989) found no amplification, Yokota and coworkers (1986) reported a fivefold to eightfold amplification of the oncogene in SCCs of the head and neck. They also found that, compared with the primary tumor, the metastatic tumor tissue showed higher *myc* amplification. Field et al. (1986) observed an elevated c-*myc* mRNA expression correlating well with the stage of the disease. In another study, tumors with poor prognosis exhibited increased c-*myc* oncoprotein (Field et al., 1989). Oncogenes from the *myc* family have been involved in regulating cellular proliferation, and their amplification is associated with certain aggressive tumors (Alitalo et al., 1985).

Our data also demonstrate amplification of K-*ras* or N-*ras* in 33 percent (34 of 102) of the total samples analyzed. K-*ras* showed a threefold to eightfold amplification in 18 percent of the samples, whereas N-*ras* was amplified in 28 percent (Table 1). Amplification of *ras* oncogene did not correlate with any clinical parameters, including nodal metastasis. H-*ras* was not amplified. Other investigators also have observed no amplification of H-*ras* in oral cancer (Sheng et al., 1990; Sommers et al., 1990).

Table 1
Oncogene amplification in oral cancers[a]

Oncogenes	Range of Amplification	Cellular DNA Fragment (kb)	Samples Amplified n (%)
c-*myc*	3-6	12.7	21 (21)
N-*myc*	3-10	3.8	27 (26)
L-*myc*	1	10.0, 6.6	0
K-*ras*	3-8	2.7	18 (18)
N-*ras*	3-9	7.2	28 (28)
H-*ras*	1	23-30	0
EGF-R	3-8	7.8, 7.0, 6.1, 5.6, 3.7, 2.5, 2.1, 1.9, 1.6, and 1.3	19 (29)

[a] *Total number of samples analyzed was 102 for* myc *and* ras *and 66 for EGF-R gene.*

In our study, coamplification of multiple oncogenes was an important feature, being observed in 24 of the 49 samples showing amplification (Table 2). Two oncogenes were coamplified in 10 tumor tissues and 3 in an additional 10 samples; 5 patients showed concurrent amplification of the four oncogenes—c-*myc*, N-*myc*, K-*ras*, and N-*ras*. Multiple oncogene amplification indicates the complex, multistage process of oral carcinogenesis, and it implies alternate or simultaneous activation of the different oncogenes in oral carcinogenesis.

There are very few studies on the status of other oncogenes in oral cancer. In the studies of Sommers and colleagues (1990), amplification of *int*-2 oncogene correlated well with frequency of recurrence. However, no correlation was observed with clinical staging or histological grading of the malignancy. Berenson and coworkers (1989) have reported a 2-fold to 10-fold amplification of the *bcl*-1 oncogene in poorly differentiated tumors, but no correlation was seen with the TNM staging.

The epidermal growth factor-receptor (EGF-R) gene is considered to be the proto-oncogene of the *erb*-B gene of avian erythroblastosis virus, which is involved in transformation of chicken cells (Downward et al., 1984). Its ligand EGF is a potent mitogen for a variety of cells. EGF-R is commonly elevated in squamous cell carcinomas. In our study, in which all tumors were SCCs, the EGF-R gene was amplified in 29 percent (19 of 66) of the samples screened (Table 1). Preliminary results, from mRNA dot blot hybridization, indicate that amplification is associated with increased expression of the gene. Amplification and overexpression of the EGF-R gene, through interaction with the ligand, could be an additional step in oral carcinogenesis.

Table 22-2
Amplification of oncogenes c-*myc*, N-*myc*, N-*ras*, and K-*ras*

	Number of Patients[a]				
	Total	c-*myc*	N-*myc*	N-*ras*	K-*ras*
Number of Patients	102				
Patients Showing Oncogene Amplification	49[b]	21	27	28	18
One Oncogene	24	9	5	7	3
Two Oncogenes	10				
myc/*myc*	1				
ras/*ras*	7	(c-*myc*/K-*ras* 1; N-*myc*/K-*ras* 1; N-*myc*/K-*ras* 5)			
ras/*ras*	2				
Three Oncogenes	10				
myc/*myc*/*ras*	5	(N-*myc*/c-*myc*/K-*ras* 1; N-*myc*/c-*myc*/N-*ras* 4)			
myc/*ras*/*ras*	5	(N-*myc*/N-*ras*/K-*ras* 5)			
Four Oncogenes	5				

[a] *In many patients, more than one oncogene was amplified. The number derived by addition of the individual figures is therefore higher than the total number of positive samples.*

EXPRESSION OF CELLULAR ONCOGENES

Expression of cellular oncogenes in oral cancers has been investigated through a variety of techniques, such as Northern hybridization/dot blot analysis for mRNA transcript in tumor tissue, in situ hybridization for detecting mRNA specific to oncogenes, and immunohistochemical demonstration of oncoproteins using monoclonal antibodies. Using dot blot hybridization, Spandidos and coworkers (1985) reported multiple transcriptional activation of H-*ras*, K-*ras*, and c-*myc* in oral cancers compared with normal tissue and premalignant pleomorphic salivary adenomas. The authors also observed a significant increase in c-*myc* oncogene expression in advanced stages III and IV compared with stages I and II. Recently, the authors also demonstrated correlation of increased c-*myc* expression with poor prognosis. No correlation was seen with the patient age, sex, TNM staging, site of tumor, histopathological grading, lymph node metastasis, or encapsular rupture of the tumor (Field et al., 1989).

Using immunohistochemical techniques and oncoprotein-specific monoclonals, Azuma and colleagues (1987) studied *ras* p21 protein in oral cancers. Although 59 of 121 specimens reacted positively to Y13-259-*ras* p21-specific Mab, 44 oral leukoplakias and 58 normal mucosa showed a negative staining. Only 43 patients with oral cancer were followed up.

In these persons, *ras* p21 expression correlated well with poor prognosis and only weakly with regional lymph node metastasis. Furthermore, *ras* p21 expression was higher in the patients with a tobacco habit.

RFLP ANALYSIS OF L-*myc* AND H-*ras* ONCOGENES

Researchers have examined restriction fragment length polymorphism (RFLP) in a number of human cancers to establish a specific association of a particular allele with an increased incidence of cancer, suggesting genetic predisposition to the cancer, or as an indicator of clinical behavior, in particular, metastatic potential or prognosis. In our studies, we used Southern hybridization analysis of 76 oral cancer primary tumor tissues and PBCs from the corresponding patients, and PBCs from 101 healthy volunteers, to classify the Indian population into three genotypes—L-L, S-L, S-S—according to the polymorphic patterns defined by the two L-*myc* alleles (Saranath et al., 1990). The pattern of the alleles in the PBC DNA of the patients was identical to the corresponding tumor DNA. The relative ratios of the three genotypes in the oral cancer patients were not significantly different from those seen in the healthy Indian population (χ^2=3.06, df=2, $p > 0.25$), implying no predisposition to oral cancer by the presence of either allele. The L and S alleles were equally distributed in the population, with the frequency of each allele being 0.50, consistent with the Hardy-Weinberg law.

L-*myc*

A striking correlation was observed between the RFLP pattern and the stage of differentiation, as well as the size of the tumors (Saranath et al., 1990). Thus, a preponderance of the S-fragment was observed in the moderate to poorly differentiated tumors (χ^2=4.97, df=1, $p < 0.05$) and the larger sized (> 4 cm) tumors (χ^2=5.65, df=1, $p < 0.025$). These observations suggest that the S allele product may be arresting the cells in a particular differentiation window, providing a further proliferative advantage to the cells, resulting in the larger sized tumors. There is also the possibility for involvement of another gene in proximity to the S-allele, or for a partially different S-fragment-coded L-*myc* protein, or a crucial role for the regulatory region of the S-fragment protein in the tumors. Lung and kidney tumors associated with S-fragment show aggressive behavior and higher tendency for metastasis (Kakehi and Yoshida, 1989; Kawashima et al., 1987). However, this is not the case with malignancies of other organs (Ikeda et al., 1988).

H-*ras*

The H-*ras* locus includes a hypervariable region, designated as variable tandem repetition region (VTR), consisting of a series of 28 base pair repeats 3′ to the gene (Capon et al., 1983). The VTR has been implicated in the regulation of the H-*ras* oncogene (Spandidos and Holmes, 1987). RFLP of the human H-*ras* oncogene has been ascribed to changes in the size of the VTR and can be defined by several restriction enzymes, including *Bam*HI, *Pvu*II, and *Taq*I (Capon et al., 1983; Pierotti et al., 1986).

In our studies, the status of H-*ras* locus was investigated in 62 patients with oral cancers (Saranath et al., 1991a). Southern blot analysis on *Bam*HI digestion of the tumor tissue DNA revealed 23 patients with H-*ras* heterozygosity. *Bam*HI digestion identified restriction fragments ranging from 6.6 kb to 8.6 kb. The allelic heterozygosity was better resolved by *Pvu*II and *Taq*I digestion. The former yielded an invariable fragment of 2.6 kb and variable

fragments ranging from 2.7 kb to 5.3 kb. *Taq*I digestion of the samples and hybridization with the VTR region probe (1 kb *Msp*I-digested, VTR-specific H-*ras* probe) resolved four variable fragments of 2.4 kb, 3.0 kb, 3.6 kb, and 5.1 kb. In three samples, *Taq*I restriction analysis also demonstrated presence of a unique VTR rearrangement as indicated by 2.1 kb, 0.9 kb, and 0.6 kb fragments, substituting a 3.6 kb fragment, implying additional *Taq*I sites. Such a variant VTR fragment could be generated by either mutational events creating the *Taq*I sites or reiteration of the existing *Taq*I site present 18 bp from the 3′ end of the VTR region during its amplification/duplication. This rearrangement, which suggests altered function of the VTR, could be one of the molecular lesions in tobacco-induced oral cancer.

Analysis of matched tumor tissue and PBC DNA from the same patient demonstrated tumor-associated loss of one of the allelic fragments in 7 of 23 patients (30 percent) with H-*ras* heterozygosity. Similar observations have been made by Howell and colleagues (1989) in oral SCCs. H-*ras* allelic loss has been reported in a number of human cancers (Ali et al., 1987); however, its implication in carcinogenesis is not yet clear. Tumor suppressor genes or anti-oncogenes have been implicated in the pathogenesis of some human tumors (Klein, 1988). Ali and coworkers (1987) identified a putative suppressor gene on chromosome 11, in the vicinity of the H-*ras* locus, localized between the β-globin and parathyroid hormone loci. The loss of H-*ras* allele observed in our patients also may encompass functional loss of the potent tumor suppressor gene on chromosome 11, further influencing the process of oral carcinogenesis in the patients. Alternatively, the loss could involve the normal H-*ras* gene, giving a selective functional advantage to the mutated H-*ras* allele involved in cell transformation and a consequent influence in oral carcinogenesis. In this respect, it is significant that 6 of 7 patients showing H-*ras* mutation at codon 12.2 also exhibited associated loss of the wild type gene. Such a loss may not be evident in RFLP studies or if the alleles are in a homozygous state.

H-*ras* POINT MUTATIONS

Point mutations of *ras* oncogenes are observed in a variety of human cancers (Bos, 1989). We employed the polymerase chain reaction (PCR) technique for in vitro amplification of specific sequences followed by allele-specific oligonucleotide hybridization to examine *ras* activation by point mutations in 57 primary oral tumors (Saranath et al., 1991b). The mutational activation was studied in all three *ras* family members (K-*ras*, N-*ras*, and H-*ras*) at codons 12, 13, and 61, the codons affected in human cancers (Bos, 1989). Suitable primers were used to amplify sequences of 111 bp and 178 bp for the regions flanking codons 12, 13, and 61, respectively. The amplified sequences were initially screened with sets of mixed probes, each set covering one nucleotide position of a particular codon. Probes used to identify mutations in H-*ras* have been described elsewhere (Saranath et al., 1991b). On indication of a mutation in the sample DNA, a duplicate PCR of the genomic DNA was performed, and the two independently amplified DNAs were screened simultaneously with a set of single oligonucleotide probes specific for the nucleotide position. The presence of wild type codons was screened for in every set.

Mutations were detected in 20 to 57 (35 percent) of the samples and were restricted exclusively to the H-*ras* oncogene at codons 12, 13, and 61 (Table 3). None of the samples showed mutations in the K-*ras* or N-*ras* oncogenes. The mutations demonstrated an equal number (11 each) of nucleotide transitions and transversions. The mutations were seen primarily in codons 61.2 and 12.2. Nine samples showed A —> G transition in codon 61.2 resulting in glutamine to arginine substitution. G —> T transversion was observed in seven samples at codon 12.2 with glycine to valine substitution. Two patients concurrently carried mutations at codons 61.2 and 12.2. Six samples with a point mutation at codon 12.2 and two samples at codon 61.2 also demonstrated loss of the corresponding wild type codons, as judged by the absence of signals on allele-specific oligonucleotide hybridization. In addition, three novel mutations, not yet reported in human malignancies, were seen. These include the G —> A (glycine to serine) substitution at codon 12.2, G —> A (glycine to aspartate) at codon 13.2, and G —> T (glutamine to histidine) (three cases) at codon 61.3.

In contrast to the very high frequency (35 percent) of H-*ras* mutations we observed, Sheng and coworkers (1990) detected mutations in only 3.7 percent (2 of 54) of their samples. Although mutations in H-*ras* occurred in the studies by Sheng and colleagues, the mutations were restricted to codon 12. Similarly, Johnson and coworkers (personal communication, 1991) and Rumsby and colleagues (1990) very rarely encountered mutations in oral SCCs from patients in the United Kingdom. Thus, it is clear that mutational frequencies in oral cancers in the West are infrequent, and perhaps only codon 12 is affected. The differences in oral cancers in India and the West may be attributed to differing tobacco habits. Whereas tobacco chewing appears to be the prime factor in the development of oral cancer in India, alcohol consumption contributes substantially in the West, where smoking also is a confounding factor in the pathogenesis of oral cancer. The mode of tobacco usage, strain or species of tobacco used, and curing process (which differs between East and West) also may contribute to these differences.

There appears to be, to a certain extent, organ specificity in activation of *ras* family oncogenes (Bos, 1989). Thus, N-*ras* point mutations are seen primarily in hematological malignancy, whereas in cancers of the lung, colon, and pancreas, K-*ras* frequently is affected. H-*ras* mutations are observed in bladder cancers (Bos, 1989) and experimentally induced skin tumors (Quintanilla et al., 1986). No explanation is readily available for this phenomenon, but it could be attributable to relative expression of different *ras* oncogenes in various organs (Leon et al., 1987). In lung cancer, another important tobacco-related cancer, mutation is observed primarily in codon 12 of K-*ras* (Slebos et al., 1990). However, in tobacco-related oral cancer, both codons 12 and 61 of H-*ras* are affected. In experimentally induced animal tumors, carcinogens show positional preference for *ras* mutations. Nitrosamines and DMBA preferentially affect codons 12 and 61, respectively (Quintanilla et al., 1986; Zarbl et al., 1985).

Table 3
H-*ras* mutations

Codon Position of Nucleotide	Number of Patients	Number of Patients With Wild Type Signal Missing	Nucleotide Change	Amino Acid Change	Number of Patients With Amplification of Oncogenes[a]			
					L-*myc*	N-*myc*	K-*ras*	N-*ras*
12.1	1	0	GGC—>AGC	Gly—>Ser	1	N	N	N
12.2[b]	7	6	GGC—>GTC	Gly—>Val	N	N	N	1
13.2	1	0	GGC—>GAC	Gly—>Asp	N	N	N	N
61.2[b]	9	2	CAG—>CGG	Gln—>Arg	3	2	N	2
61.2	1	0	CAG—>CTG	Gln—>Leu	N	N	N	N
61.3	3	0	CAG—>CAT	Gln—>His	N	2	2	2

[a] *N = nil.*
[b] *Two patients showed mutations at both codon 12.2 and 61.2.*

As mentioned earlier, tobacco contains several potent mutagens and carcinogens such as *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Nitrosamines are known to produce metabolites capable of binding DNA, resulting in products such as O^6-methylguanine, which can lead to miscoding during DNA replication (Topal, 1988). In India, tobacco is chewed generally as betel quid, which contains areca nut, slaked lime, catechu, and flavoring agents in addition to tobacco. Whereas mutation of codon 12 could be attributable to tobacco-specific nitrosamines, other chemical constituents may be responsible for mutation at codon 61. This aspect needs further elucidation.

IDENTIFICATION OF A FUNCTIONAL ONCOGENE

NIH3T3 mouse fibroblast transfection combined with the nude mice tumorigenicity assay is widely used to identify activated oncogenes (Shih and Weinberg, 1982). Using this system, Friedman and colleagues (1983) reported the presence of transforming genes in DNA from head and neck SCCs. However, the oncogene has yet to be characterized. Recently, Howell and coworkers (1990) identified the K-*ras* oncogene in head and neck SCCs from primary and secondary NIH-transformed cells. The oncogene was isolated from only 1 of the 11 tumors studied by the investigators.

The NIH3T3 transfection assay shows a bias toward detection of *ras* oncogenes. However, with the use of selectable markers such as $SV2_{neo}$ gene, in cotransfection followed by tumorigenicity assays, several novel genes, besides the *ras* family oncogenes, also have been detected in human cancers (Fasano et al., 1984).

We recently carried out the NIH3T3 cotransfection assay, using oral tumor tissue DNA and $SV2_{neo}$ selectable marker gene, followed by nude mouse tumorigenicity assay. Calf thymus DNA and H-*ras* pEJ6.6 clone were used as negative and positive controls, respectively. The H-*ras* oncogene-transfected cells induced tumors in the nude mice within 3 to 5 weeks.

These tumors and the cell lines established from them showed the presence of H-*ras* DNA in Southern hybridization analysis as well as a 1.2 kb H-*ras* RNA transcript in Northern hybridization analysis, validating the absence of technology errors in our studies.

Transfection assay was carried out with oral tumor DNA from three patients. The samples from the three patients induced transformation of NIH-3T3 cells, forming colonies in soft agar. The transfected cells induced tumors in nude mice within 5 to 10 wk. Further, DNA isolated from the nude mice tumors hybridized with human Alu Blur-2 probe in Southern analysis and cell lines established from the tumors retained the human Alu sequences. However, DNA isolated from oral tumor DNA transfected nude mice tumors, as well as their established tumor cell lines, revealed no hybridization with any of the *ras* or *myc* oncogenes. This was surprising because N-*ras* was amplified in two of the original primary tumors and one of them also showed H-*ras* point mutation at codon 12.2; the third tumor did not show any aberration of *ras* or *myc* oncogenes. As mentioned earlier, positive results were obtained in three of three oral tumor samples. Generally, a maximum of 30 percent transformation rate has been observed in NIH3T3 transfection/nude mouse tumorigenicity assays, using DNA from solid tumors (Krontiris and Cooper, 1981). Very high transfection and nude mice tumorigenicity frequency, in our studies, indicate the possibility of a highly potent, tobacco-induced activated oncogene, different from the *myc* or ras oncogenes. The situation may be similar to the presence of N-*myc* oncogene in neuroblastomas or L-*myc* in small cell lung carcinomas. We are currently in the process of cloning this gene from the nude mouse tumors to isolate, sequence, and identify the gene.

CONCLUSIONS AND FUTURE PROSPECTS

The observation that more than 90 percent of the oral cancers caused by smokeless tobacco (chewing) show some sort of oncogene aberration (Table 4) indicates that tobacco-induced oncogene changes are closely linked to the pathogenesis of these cancers. The occurrence of multiple oncogene aberrations suggests that the process of malignant transformation results, probably, as a consequence of diverse molecule alterations. In our studies, the functional lesions in oncogenes could be grouped into (1) overproduction of oncogene effector molecules through amplification or overexpression of the genes, (2) production of abnormal oncogene products through point mutations, and (3) deletion of normal oncogene allele or putative tumor suppressor, as evidenced by allelic loss. These perturbations may act independently or in concert, resulting in deregulation of cellular growth.

A distinct difference emerges in the mode of activation of oncogenes in oral cancers in the Indian population and Western countries. Low levels of amplification of *myc* and *ras* oncogenes and low incidence of point mutations are observed in the West. Comparative studies of molecular biology of oral cancers vis-a-vis the varied tobacco habits and the associated confounding factors, such as alcohol in the West and different constituents of betel quid in India, may throw light on the pathogenic mechanisms responsible for these differences. Studies in suitable laboratory models using individual agents may be equally fruitful.

Table 4
Oncogenes in oral cancers

Summary Analysis	Percentage	Comments
Amplification c-*myc*, N-*myc*, K-*ras*, N-*ras* (n=102)	48.0%	
Point Mutations H-*ras* (n=57)	23.0	Excluding 12% samples showing, in addition, amplification/allelic loss/ gene rearrangement
H-*ras* Allelic Loss or VTR Rearrangement (n=62; heterozygous=23)	14.5	Excluding 21.5% samples showing other oncogene aberrations
EGF-R Amplification (n=66)	9.0	Excluding 20% samples showing other oncogene aberrations
Samples Showing at Least One Oncogene Aberration	94.5	

Transfection assays, in our studies, indicate involvement of a highly potent activated oncogene, outside the *ras* and *myc* families, in tobacco-induced oral carcinogenesis. The oncogene must be fully characterized. A high incidence of point mutations is observed in Indian patients at codons 12 and 61 of the H-*ras* oncogene. Some of the tobacco-specific carcinogens, such as nitrosamines, which form DNA adducts, are known to have positional preference for inducing point mutation in *ras* oncogenes. The precise role of point mutation, which is an early event in certain human malignancies, in the pathogenesis of oral cancer needs further elucidation.

Some tobacco chewers develop oral cancer at a relatively young age, a situation reminiscent of smoking and lung cancer. There is evidence that an autosomal gene, showing Mendelian codominant inheritance, is responsible for early-onset lung cancers (Bonney, 1990; Sellers et al., 1990). Interaction of the gene, which has yet to be characterized, with smoking accounts for 69 percent and 47 percent of lung cancers occurring at ages 50 and 60, respectively. Although this in no way negates the crucial role of smoking and should not be a deterrent to antitobacco efforts, similar studies should be conducted with tobacco chewers.

Our study comprised primarily large and advanced tumors. To get an idea of the absolute and relative importance of these perturbations, it is essential to investigate the status of oncogenes in a longitudinal study encompassing different stages of tobacco carcinogenesis, including leukoplakia. Such a study is also required to define the molecular counterpart of

precancerous lesions to identify leukoplakias that run a high risk of malignant transformation. In this respect, a retrospective study, using PCR techniques to examine paraffin blocks of leukoplakias and oral cancers in different stages of evolution, would be fruitful. Such studies are currently in progress in our laboratory.

REFERENCES

Ali, I.U., Liderau, R., Theillet, C., Callahan, R. Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. *Science* 238: 185-188, 1987.

Alitalo, K., Koskinen, P., Makela, T.P., Saksela, K., Sistonen, L., Winqvist, R. *myc* oncogene: Activation and amplification. *Biochimica et Biophysica Acta* 907: 1-32, 1985.

Alt, F.W., DePinho, R., Zimmerman, K., Legouy, E., Hatton, K., Ferrier, P., Tesfaye, A., Yancopoulos, G., Nisen, P. The human *myc* gene family. *Cold Spring Harbor Symposia on Quantitative Biology* LI: 931-941, 1986.

Azuma, M., Furumoto, N., Kawamata, H., Yoshida, H., Yanagawa, T., Yura, Y., Hayashi, Y., Takegawa, Y., Sato, M. The relation of *ras* oncogene product p21 expression to clinicopathological status criteria and clinical outcome in squamous cell head and neck cancer. *Cancer Journal* 1: 375-380, 1987.

Berenson, J.R., Yang, J., Mickel, R.A. Frequent amplification of the *bcl*-1 locus in head and neck squamous cell carcinomas. *Oncogene* 4: 1111-1116, 1989.

Bishop, J.M. The molecular genetics of cancer. *Science* 235: 311, 1987.

Bonney, G.E. Interactions of genes, environment and life-style in lung cancer development. *Journal of the National Cancer Institute* 82: 1236-1237, 1990.

Bos, J.L. *ras* oncogenes in human cancer: A review. *Cancer Research* 49: 4682-4689, 1989.

Capon, D., Chen, E., Levinson, A., Seeburg, P., Goeddel, D. Complete nucleotide sequences of the t24 human bladder carcinoma oncogene and its normal homologue. *Nature* 302: 33-37, 1983.

Downward, J., Yarden, Y., Mayeot, S. Close similarity of epidermal growth factor receptor and v-*erb*B oncogene protein sequences. *Nature* 37: 521-528, 1984.

Fasano, O., Birnbaum, D., Edlund, L., Fogh, J., Wigler, M. New human transforming genes detected by a tumorigenicity assay. *Molecular and Cellular Biology* 4: 1695-1705, 1984.

Field, J.K., Lamothe, A., Spandidos, D.A. Clinical relevance of oncogene expression in head and neck tumors. *Anticancer Research* 6: 595-600, 1986.

Field, J.K., Spandidos, D.A., Stell, P.M., Vaughan, E.D., Evan, J.I., Moore, J.P. Elevated expression of the c-*myc* oncoprotein correlates with poor prognosis in head and neck squamous cell carcinoma. *Oncogene* 4: 1463-1468, 1989.

Friedman, W.H., Rosenblum, B., Lowenstein, P., Thornton, H., Katsantonis, G., Green, M. Oncogenes: Preliminary studies in head and neck cancer. *Laryngoscope* 93: 1441-1444, 1983.

Gupta, P.C., Mehta, F.S., Pindborg, J.J., Aghi, M.B., Bhonsle, R.B., Murti, P.R. An educational intervention study for tobacco chewing and smoking habits among Indian villagers. In: *Smoking and Health,* A.M. Hisamichi and S. Tominaga (Editors). Amsterdam: Excerpta Medica, 1987, pp. 623-627.

Hecht, S., Spratt, T.E., Trushin, N. Evidence for 4-(3-pyridyl)-4-oxobutylation of DNA in F344 rats treated with the tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N'*-nitrosonornicotine. *Carcinogenesis* 9: 161-165, 1988.

Howell, R.E., Wong, F.S.H., Fenwick, R.G. Loss of Harvey *ras* heterozygosity in oral squamous carcinoma. *Journal of Oral Pathology and Medicine* 18: 79-83, 1989.

Howell, R.E., Wong, F.S.H., Fenwick, R.G. A transforming Kirsten *ras* oncogene in an oral squamous carcinoma. *Journal of Oral Pathology and Medicine* 19: 301-305, 1990.

Ikeda, I., Ishizaka, Y., Ochiai, M., Sakai, R., Itabashi, M., Onda, U., Sugimura, T., Nagao, M. No correlation between l-*myc* restriction fragment length polymorphism and malignancy of human colorectal cancers. *Japanese Journal of Cancer Research* 79: 674-676, 1988.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985.

Jussawalla, D.J., Deshpande, V.A. Evaluation of cancer risk in tobacco chewers and smokers: An epidemiologic assessment. *Cancer* 28: 469-476, 1971.

Kakehi, Y., Yoshida, O. Restriction fragment length polymorphism of the L-*myc* gene and susceptibility to metastasis in renal cancer patients. *International Journal of Cancer* 43: 391-394, 1989.

Kawashima, K., Shikama, H., Imoto, K., Izawa, M., Naruke, T., Okahayashi, K., Nishimura, S. Close correlation between restriction fragment length polymorphism of the L-*myc* gene and metastasis of human lung cancer to the lymph nodes and other organs. *Proceedings of the National Academy of Sciences* 85: 2353-2356, 1987.

Klein, G. Oncogenes and tumor suppressor genes. Reviews in Oncology 1. *Acta Oncologica* 27: 427-437, 1988.

Klein, G., Klein, E. Evolution of tumors and the impact of molecular oncology. *Nature* 315: 190-195, 1985.

Krontiris, T.G., Cooper, G.M. Transforming activity of human tumor DNA. *Proceedings of the National Academy of Sciences* 78: 1181-1184, 1981.

Mattson, M.E., Winn, D.M. Smokeless tobacco: Association with increased cancer risk. *National Cancer Institute Monographs* 8: 13-16, 1989.

Pierotti, M., Radice, P., Biunno, I., Borrello, M., Cattadori, M., Porta, G. Detection of two *Taq*I polymorphisms in the VTR region of the human H-*ras*-1 oncogene. *Cytogenetics and Cell Genetics* 43: 174-180, 1986.

Quintanilla, M., Brown, K., Ramsden, D., Balmain, A. Carcinogen-specific mutation and amplification of Ha-*ras* during mouse skin carcinogenesis. *Nature* 322: 78-82, 1986.

Rumsby, G., Carter, R.L., Gusterson, B.A. Low incidence of *ras* oncogene activation in human squamous cell carcinomas. *British Journal of Cancer* 61: 365-368, 1990.

Saranath, D., Panchal, R.G., Nair, R., Mehta, A.R., Sanghavi, V., Sumegi, J., Klein, G., Deo, M.G. Oncogene amplification in squamous cell carcinoma of the oral cavity. *Japanese Journal of Cancer Research* 80: 430-437, 1989.

Saranath, D., Panchal, R.G., Nair, R., Mehta, A.R., Sanghavi, V., Deo, M.G. Restriction fragment length polymorphism of the L-*myc* gene in oral cancer patients. *British Journal of Cancer* 61: 530-533, 1990.

Saranath, D., Bhoite, L.T., Mehta, A.R., Sanghavi, V., Deo, M.G. Loss of allelic heterozygosity at the Harvey *ras* locus in human oral carcinomas. *Journal of Cancer Research and Clinical Oncology* 117: 484-488, 1991a.

Saranath, D., Chang, S.E., Bhoite, L.T., Panchal, R.G., Kerr, I.B., Mehta, A.R., Johnson, N.W., Deo, M.G. High frequency mutation in codons 12 and 61 of H-*ras* oncogene in chewing tobacco-related human oral carcinoma in India. *British Journal of Cancer* 63: 573-578, 1991b.

Sellers, T.A., Bailey-Wilson, J.E., Elston, R.C., Wilson, A.F., Elston, G.Z., Ooi, W.L., Rothschild, H. Evidence for Mendelian inheritance in the pathogenesis of lung cancer. *Journal of the National Cancer Institute* 82: 1272-1279, 1990.

Sheng, Z.M., Barrois, M., Klijanienko, J., Micheau, C., Richard, J.M., Riou, G. Analysis of the c-Ha-*ras*-1 gene for deletion, mutation, amplification and expression in lymph node metastasis of human head and neck carcinomas. *British Journal of Cancer* 62: 398-404, 1990.

Shih, C., Weinberg, R.A. Isolation of a transforming sequence from a human bladder carcinoma cell line. *Cell* 29: 161-169, 1982.

Slebos, R.J.C., Kibbelaar, R.E., Dalesio, O., Kooistra, A., Stam, J., Meijer, C.J.L.M., Wagenaar, S.S., Vanderschueren, R.G., Zandwijk, N., Mooi, W.J., Bos, J.L., Rodenhuis, S. H-*ras* oncogene activation as a prognostic marker in adenocarcinoma of the lung. *New England Journal of Medicine* 323: 561-565, 1990.

Sommers, K.D., Cartwright, S.L., Schechter, G.L. Amplification of the *int*-2 gene in human head and neck squamous cell carcinoma. *Oncogene* 5: 915-920, 1990.

Spandidos, D.A., Holmes, L. Transcriptional enhancer activity in the variable tandem repeat DNA sequence downstream of the human Ha-*ras*-1 gene. *FEBS Letters* 218: 41-46, 1987.

Spandidos, D.A., Lamothe, A., Field, J. Multiple transcriptional activation of cellular oncogenes in human head and neck solid tumours. *Anticancer Research* 5: 221-224, 1985.

Topal, M.D. DNA repair, oncogenes and carcinogenesis. *Carcinogenesis* 9: 691-696, 1988.

Yokota, J., Tsunetsugu-Yokota, Y., Baltitora, H., Leferre, C., Cline, M.J. Alterations of *myc*, *myb* and *ras*-ha protooncogene in cancers are frequent and show clinical correlation. *Science* 231: 261-265, 1986.

Zarbl, H., Sukumar, S., Arthur, A.V., Martin-Zanca, D., Barbacid, M. Direct mutagenesis of Ha-*ras*-1 oncogenes by n-nitroso-n-methylurea during initiation of mammary carcinogenesis in rats. *Nature* 315: 382-385, 1985.

Zimmerman, K.A., Yancopoulos, G.D., Collum, R.G., Russell, K.S., Kohl, N.E., Denis, K.A., Nau, M.M., Witte, O.N., Toran-Allerand, D., Gee, C.E., Minna, J.D., Alt, F.W. Differential expression of *myc* family genes during murine development. *Nature* 319: 780-783, 1986.

Metabolism and Macromolecular Binding of NNK and NNN, Important Carcinogens in Smokeless Tobacco[1]

Stephen S. Hecht, Steven G. Carmella, and Sharon E. Murphy

ABSTRACT This paper describes the pathways of metabolic activation and macromolecular binding of two tobacco-specific nitrosamines, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine (NNN). NNK and NNN are important because of their tumorigenicity in the oral cavity and their presence in significant quantities in snuff and the saliva of snuff dippers. Studies in cultured rat oral tissue have shown that both compounds are metabolically activated by α-hydroxylation. Methylation of DNA has been detected in rat oral tissue incubated with NNK. Human oral tissue also can metabolize these nitrosamines. Analysis of hemoglobin isolated from snuff dippers has demonstrated the presence of adducts resulting from the metabolic activation of NNK and NNN. Together, these results support the hypothesis that NNK and NNN are involved in the induction of oral cancer by snuff dipping.

INTRODUCTION Epidemiological studies have established that snuff dipping is a cause of oral cancer (IARC, 1985; Winn et al., 1981). Two tobacco-specific nitrosamines—4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine (NNN)—are the most prevalent strong carcinogens in oral snuff and the saliva of snuff dippers (Hoffman and Adams, 1981; Hoffman et al., 1987). A mixture of NNK and NNN swabbed daily for life in the oral cavities of rats induced a significant incidence of oral tumors (Hecht et al., 1986). The total dose was similar to that encountered by snuff dippers (Hecht and Hoffman, 1989). NNK and NNN are the only tobacco constituents known to induce oral tumors in animals. These data support the hypothesis that NNK and NNN are causative factors in human oral cancer induced by snuff dipping (Hecht and Hoffman, 1989).

In this paper, we summarize our current understanding of the mechanisms by which NNK and NNN are metabolized to intermediates that bind to cellular macromolecules such as DNA and hemoglobin. We focus on data that relate to oral cavity carcinogenesis. The metabolism of carcinogens to DNA-reactive compounds is called metabolic activation. Its hallmark is the generation of electrophilic intermediates that can bind to nucleophilic centers in DNA, RNA, or protein. Binding to DNA with formation of covalently attached residues called "DNA adducts" is important in carcinogenesis. DNA adducts have miscoding properties and can interfere with the normal processes of replication. The presence of DNA adducts in proto-oncogenes can lead to their activation; the presence of DNA adducts in tumor suppressor genes can lead to their inactivation. Both processes are

[1] These studies were supported by National Cancer Institute grants no. CA-29580 and no. CA-44377.

involved in the transformation of normal cells to neoplastic cells and the eventual development of tumors. Although hemoglobin adducts, in contrast to DNA adducts, are not believed to play any part in tumor development, they can serve as surrogates for estimating levels of DNA adducts in human tissues.

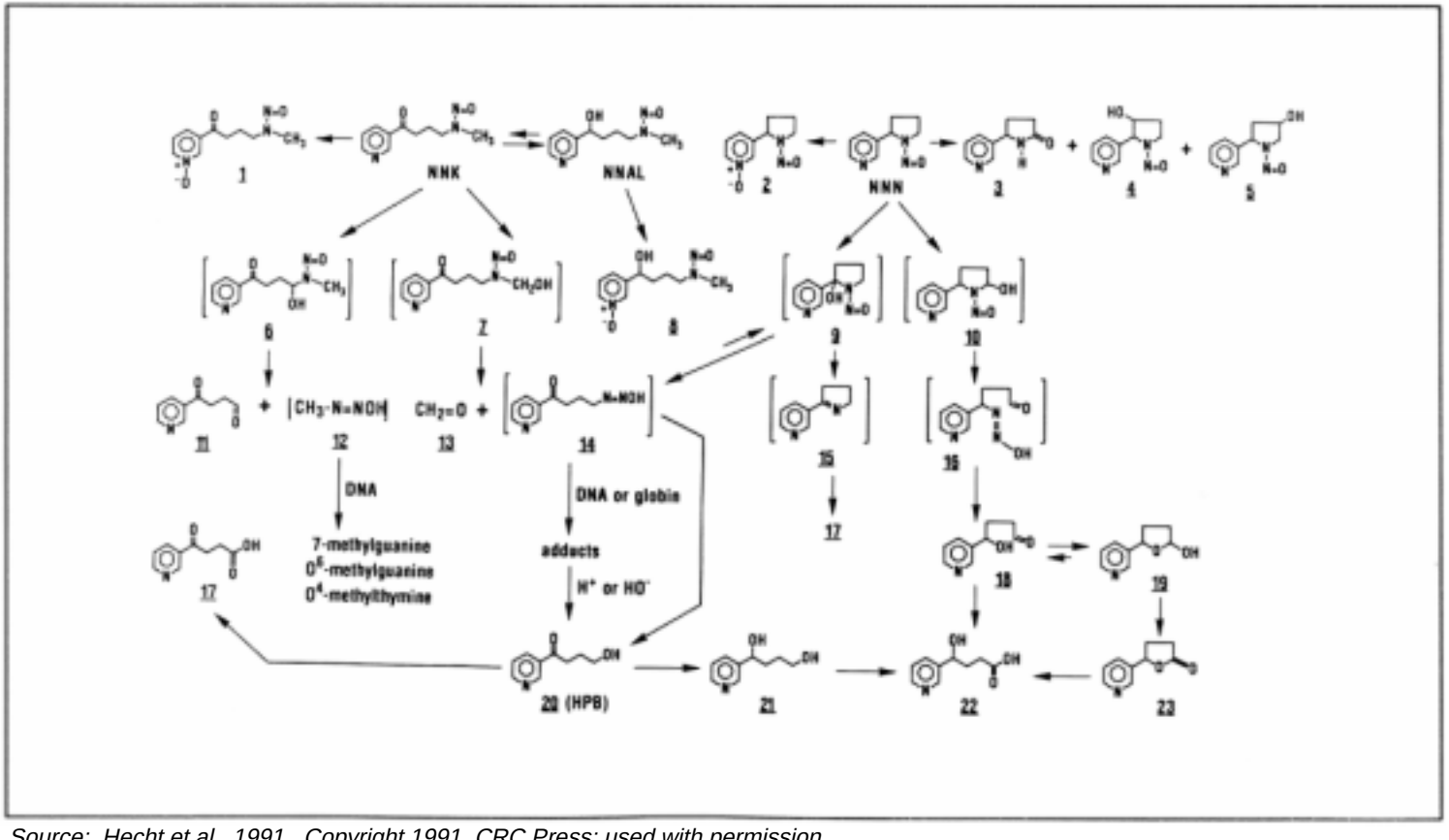
METABOLISM OF NNK AND NNN

Figure 1 summarizes metabolic pathways of NNK and NNN. Metabolites have been identified in the urine of rats, mice, and hamsters and in in vitro experiments with cultured tissues or subcellular fractions (Hecht and Hoffman, 1988; Hecht et al., 1983). There are three major metabolism pathways for NNK—pyridine N-oxidation to give NNK-1-N-oxide (marked 1 in Figure 1), carbonyl reduction to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and α-hydroxylation to the unstable α-hydroxy-NNK derivatives 6 and 7. α-Hydroxylation is the major metabolic activation pathway of NNK because compounds 6 and 7 spontaneously decompose to the electrophiles methyl diazohydroxide (12) and 4-(3-pyridyl)-4-oxobutyl diazohydroxide (14) with simultaneous formation of the keto aldehyde (11) and formaldehyde (13). Methyl diazohydroxide reacts with DNA, producing a mixture of methylated DNA bases among which 7-methylguanine, O^6-methylguanine, and O^4-methylthymidine have been identified in the tissues of animals treated with NNK. The diazohy-droxide (14) reacts with DNA, producing adducts of unknown structure that release 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB, 20) upon acid hydrolysis. This process is referred to as pyridyloxobutylation. Both diazohydroxides also react with hemoglobin, producing globin adducts (Carmella and Hecht, 1987). The adduct formed from reaction of 14 with globin releases HPB upon mild base hydrolysis. The aldehyde 11 and HPB, which are initial products of α-hydroxylation of NNK, undergo further oxidation and reduction in vivo and in certain in vitro systems producing keto acid 17, diol 21, and hydroxy acid 22.

NNAL is formed from NNK in most tissues and also methylates and pyridyloxobutylates DNA (Hecht and Trushin, 1988). These results occur either by reconversion to NNK or by α-hydroxylation. The latter pathways are not illustrated in Figure 1, but give rise to diol 21 and hydroxy acid 22. NNAL also undergoes pyridine N-oxidation producing NNAL-N-oxide 8. Another pathway of NNAL metabolism not shown in Figure 1 is formation of its *O*-glucuronide, which is a major metabolite in rodent urine (Morse et al., 1990).

Metabolism of NNN occurs by pyridine-N-oxidation to NNN-1-N-oxide 2, by denitrosation and oxidation yielding norcotinine 3, and by hydroxylation of the four positions of its pyrrolidine ring. The hydroxylation products 4 and 5 are minor metabolites and appear to be stable. The α-hydroxylation products 9 and 10 are major metabolites and are unstable. Compound 9 spontaneously decomposes to the same diazohydroxide 14 that is formed from methyl hydroxylation of NNK. Therefore, NNN also pyridyloxobutylates DNA and globin to give adducts that release HPB upon hydrolysis (Carmella and Hecht, 1987). Compound 10 generates the hydroxy aldehyde 18, which exists in the cyclic form as lactol 19. The initial

Figure 1
Metabolic pathways of NNK and NNN in rodents



Source: Hecht et al., 1991. Copyright 1991, CRC Press; used with permission.

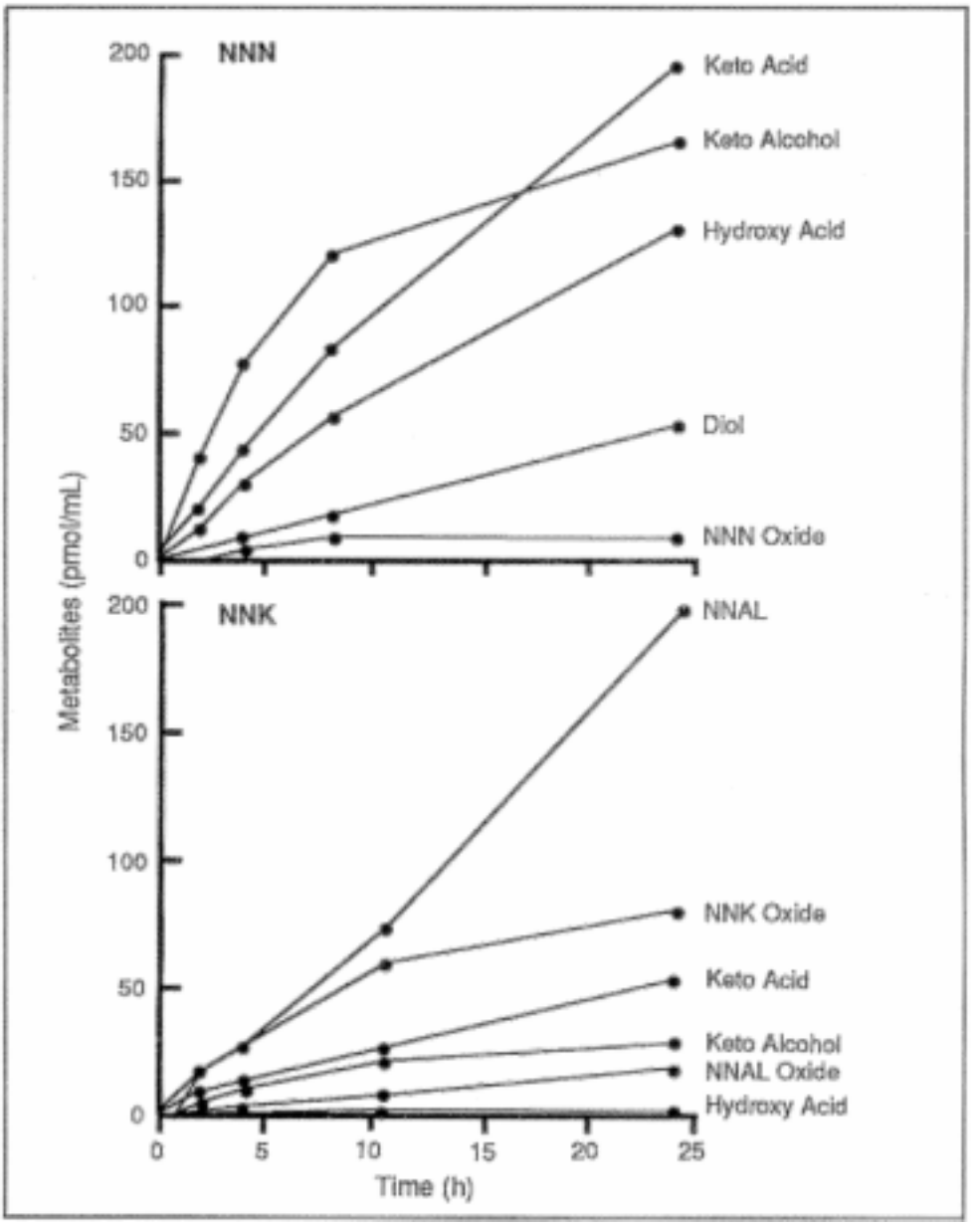
products of NNN α-hydroxylation, HPB and lactol 19, undergo further oxidation, producing keto acid 17 and hydroxy acid 22. These two acids are good indicators of the extent of α-hydroxylation of NNN by the two pathways illustrated in Figure 1.

Methylation and pyridyloxobutylation of DNA, via 12 and 14, are the two most likely pathways by which NNK would initiate tumorigenesis. The importance of the DNA methylation pathway in NNK tumorigenesis has been demonstrated in certain systems, whereas the role of the pyridyloxobutylation pathway is less clear (Bebinsky et al., 1989); Hecht et al., 1990. For NNN, which cannot methylate, DNA pyridyloxobutylation is likely to be important in tumorigenesis, but the mechanism is unclear at present.

In Rat Oral Tissue The metabolism of NNK and NNN can be conveniently studied by incubation of the tritium-labeled compounds with rat oral tissue (Murphy et al., 1990). Oral tissue is obtained from the insides of both cheeks, the roof of the mouth, and the inside of the lips. It is trimmed of muscle and connective tissue. Pieces of tissue are placed epithelial side up on a dry filter in a 60-mm culture dish and are then covered with 5 mL of Williams' medium with additives. They are incubated with [5-^{3}H]NNK or [5-^{3}H]NNN, which have tritium at the 5 position of the pyridine ring. Incubations are carried out for various periods up to 24 h; tissue viability is excellent under these conditions. At the end of the incubation, the medium is analyzed for metabolites by high-performance liquid chromatography (HPLC) with radioflow detection. Figure 2 illustrates the time course of metabolite formation from 1 μM NNK or NNN in rat oral tissue. The major products of NNK metabolism were NNAL and NNK-1-N-oxide (1 in Figure 1). α-Hydroxylation produced keto acid (17) and HPB. Metabolism experiments with HPB demonstrated that the keto acid was formed from both keto aldehyde (11) and HPB, indicating that both α-hydroxylation pathways of NNK were operative. α-Hydroxylation of NNN was six times greater than α-hydroxylation of NNK. Keto acid (17) and HPB were the major α-hydroxylation products. In contrast to pyridine-N-oxidation of NNK, pyridine-N-oxidation of NNN was barely detectable. These results indicate that metabolic activation of NNN to intermediates capable of binding to DNA exceeded metabolic activation of NNK in cultured rat oral tissue.

DNA binding was examined in these cultured tissues, and 7-methylguanine was detected in oral tissue incubated with NNK labeled with tritium in the methyl group. This is consistent with the metabolic activation pathway that produces keto aldehyde (11) and keto acid (17). Although *O*^{6}-methylguanine is known to be formed by the same pathway, it was not detectable under these conditions. It was also not possible to detect HPB in acid hydrolysates of DNA isolated from rat oral tissue incubated with [5-^{3}H]NNK or [5-^{3}H]NNN. Surprisingly, in rat esophagus, which metabolizes NNN to a similar extent by α-hydroxylation as oral tissue, HPB was detected in acid hydrolysates of isolated DNA (Murphy et al., 1990b). These results suggest that cellular constituents of oral tissue other than DNA may have a particular affinity for reaction with diazohydroxide (14).

Figure 2
Formation of NNK and NNN metabolites by cultured rat oral tissue as a function of time



Source: Murphy et al., 1990b. Copyright 1990, Cancer Research*; used with permission.*

The enzymology of NNK and NNN activation in oral tissue also requires further study. Cytochrome P_{450} isozymes are believed to be responsible for the α-hydroxylation of these nitrosamines, but the forms present in oral tissue have not been characterized. Comparative studies of NNK and NNN

metabolism in rat oral tissue indicated that at least two different enzymes were involved in α-hydroxylation (Murphy et al., 1990b).

In Human Oral Tissue

Limited information is available on the metabolism of NNK and NNN by human oral tissue. In one study, buccal mucosa obtained at immediate autopsy was cultured with labeled NNK or NNN (Cantonguay et al., 1983). NNK was extensively converted to NNAL, as in all human tissues examined to date. Hydroxy acid (22 in Figure 21-1) produced by α-hydroxylation of NNAL, was also detected. Similar results have been obtained in ongoing experiments with oral tissue obtained surgically. NNN was metabolized to NNN-1-N-oxide and to hydroxy acid (22). α-Hydroxylation of both NNK and NNN was far less extensive in human oral tissue than in rat oral tissue. However, it is not clear whether this is attributable to differences in the conditions under which the rat and human tissues were obtained and cultured.

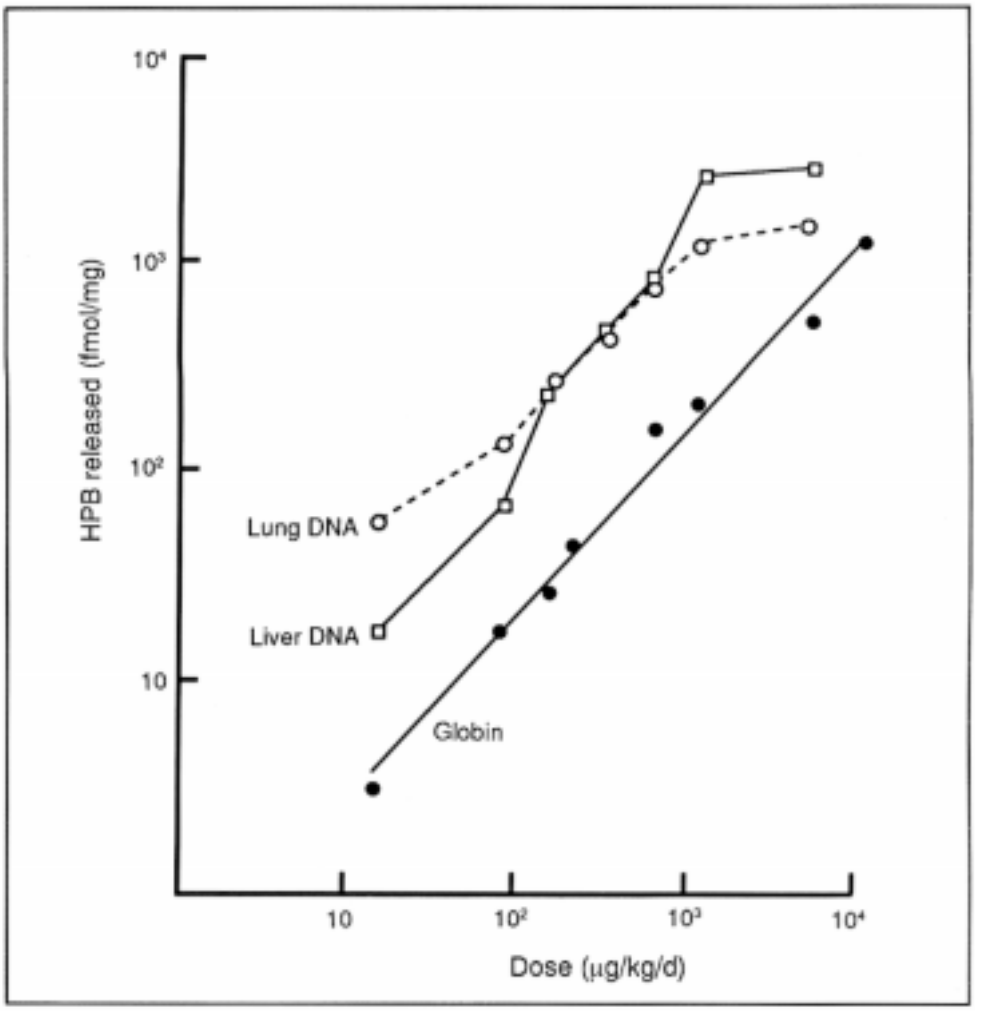
No information is available on oncogene activation by NNK or NNN in oral cavity tumors. This information could be useful in assessing their role in human oral cancer. Amplification of several oncogenes including c-*myc*, N-*myc*, N-*ras*, K-*ras*, and *int*-2 have been observed in carcinomas of the oral cavity (Saranath et al., 1989; Somers et al., 1990).

DETECTION OF NNK/NNN-HEMOGLOBIN ADDUCTS

As illustrated in Figure 1, the diazohydroxide 14, which is formed from both NNK and NNN, forms adducts in globin that release HPB upon mild-base hydrolysis. This was established by experiments in which labeled NNK or NNN was administered to rats (Carmella and Hecht, 1987). For NNK, approximately 0.1 percent of the dose was bound to hemoglobin, and about 20.0 percent of this could be released as HPB. The half-life of the adduct in rats is approximately 9 d. As shown in Figure 3, the formation of the HPB-releasing adduct was linear over doses ranging from 15 to 10,000 μg/kg/d, administered i.p. for 4 d (Murphy et al., 1990a). The release of HPB from lung and liver DNA by acid hydrolysis also increased over this dose range, but the increase was not linear. No data are available for comparison of oral tissue DNA adduct formation to hemoglobin adducts.

Measurement of hemoglobin adducts in humans could serve as a biomarker of the internal dose of a given electrophile (Ehrenberg and Osterman-Golkar, 1976). In this case, the electrophile of interest is the diazohydroxide 14. Its formation would be specifically related to uptake and metabolic activation of NNN and NNK, in contrast to methyl diazohydroxide 12, which could have many environmental sources. Advantages of hemoglobin adducts as surrogates for DNA adducts include the ready availability of hemoglobin in quantities sufficient for accurate analysis and the long lifetime of the erythrocyte in humans (120 d), which permits integration of dose (Ehrenberg and Osterman-Golkar, 1976). Because HPB is easily released from hemoglobin upon base hydrolysis and can be separated from the protein by extraction, it appeared to be a suitable compound for analysis.

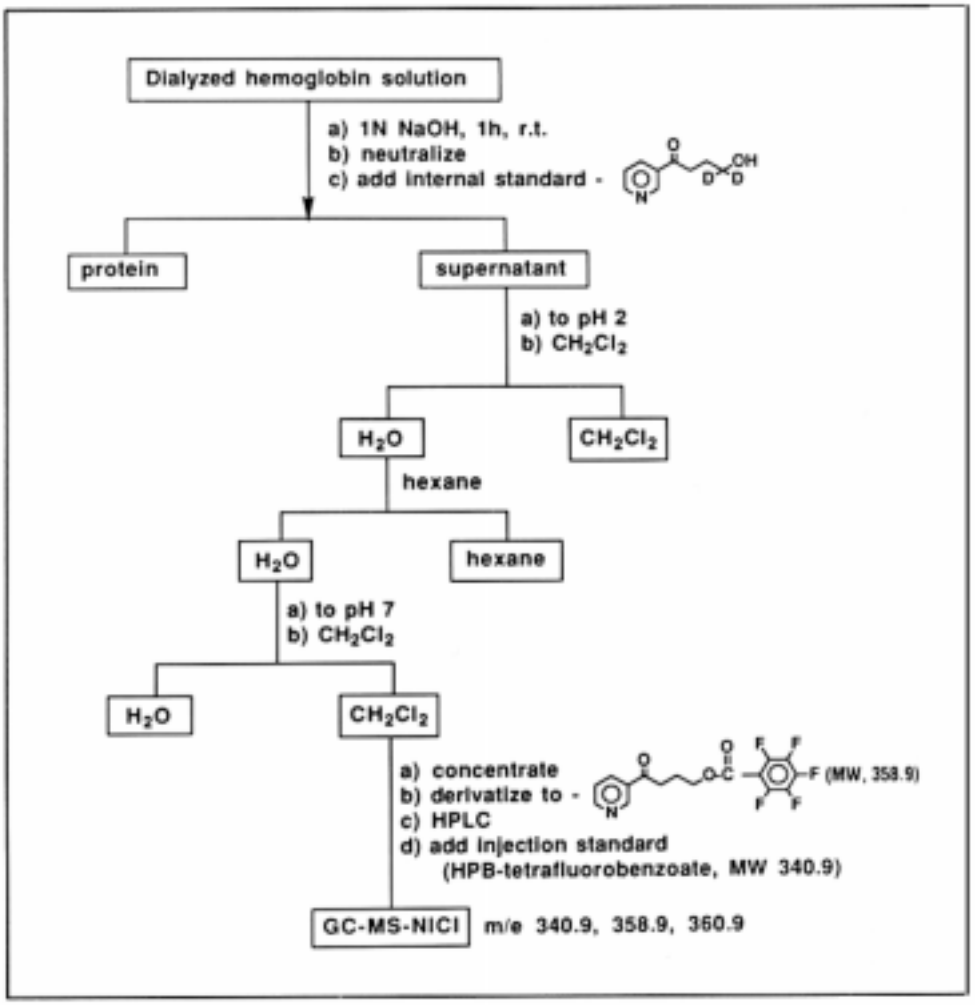
Figure 3
Log log plot of HPB released from globin, after base hydrolysis, and from lung and liver DNA, after hydrolysis. Globin and DNA were obtained from rats treated with [5-^{3}H]NNK (15 to 10,000 μg/kg/d, for 4 d).



Source: Murphy et al., 1990b. Copyright 1990, Cancer Research*; used with permission.*

Figure 4 summarizes the scheme used for analysis of HPB released from human hemoglobin (Carmella et al., 1990). The key step is derivati-zation to its pentafluorobenzoate, which can be analyzed with high sensitivity (detection limit, approximately 1 fmol) by gas chromatography-negative ion chemical ionization mass spectrometry (GC-NICI-MS) with selective ion monitoring. With this method, HPB can readily be detected in samples of human hemoglobin that have been dialyzed and treated with mild base.

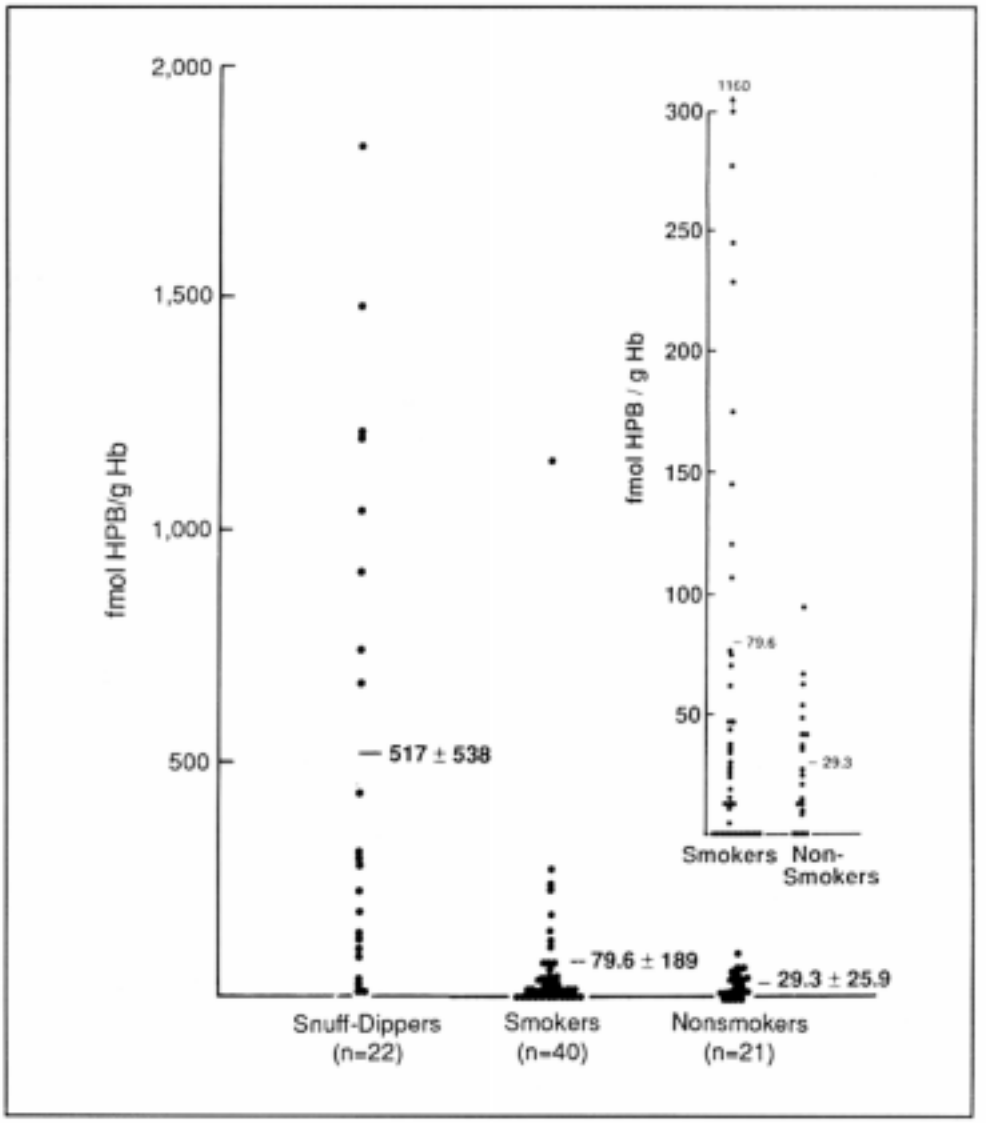
Figure 4
Scheme for analysis of HPB in hydrolysates of human hemoglobin



Source: Carmella et al., 1990. Copyright 1990, Cancer Research*; used with permission.*

Figure 5 illustrates results from analysis of hemoglobin of snuff dippers, smokers, and nonsmokers (Carmella et al., 1990). In this group, snuff dippers had the highest adduct levels; it is not known whether this will be a general phenomenon. The mean adduct level observed in snuff dippers was higher than expectations based on studies with rats. The effective daily dose of NNK and NNN producing HPB in these snuff dippers could be estimated as approximately 0.07 µg/kg/d. Injection of rats i.p. with 1 µg/kg NNK for 5 wk resulted in 517±3.2 fmol/g HPB. The higher levels of HPB-releasing adducts in snuff dippers than in rats could result from the endogenous formation of NNN and NNK. NNN may be formed by nitrosa-tion of nor-nicotine, which is present in tobacco or produced in metabolism of nico-tine. NNK could be formed by nitrosation of nicotine. The wide variation

Figure 5
Levels of HPB released from hemoglobin of snuff dippers, smokers, and nonsmokers



Source: Carmella et al., 1990. Copyright 1990, Cancer Research*; used with permission.*

in levels of hemoglobin adducts among the snuff dippers suggests that there are differences in individual capacities for metabolic activation of NNK and NNN and, possibly, related differences in risk.

The presence of HPB-releasing adducts in snuff dippers' hemoglobin supports our hypothesis that NNK and NNN are causative agents for oral cancer in snuff dippers. The data demonstrate that snuff dippers can metabolically activate NNK and NNN to diazohydroxide (14 in Figure 1).

It will be important to investigate the presence of HPB-releasing adducts in oral DNA from snuff dippers and to determine whether their levels correlate with levels of hemoglobin adducts. A method for the analysis of HPB released by acid hydrolysis of human DNA has recently been developed (Foiles et al., 1991).

GAPS IN ASSESSING THE ROLE OF NNK AND NNN

Although the metabolic activation pathways of NNK and NNN have been characterized in a number of tissues, there are relatively limited data for the oral cavity. It would be important to determine which P_{450} isozymes are involved in NNK and NNN metabolism in the rat oral cavity as well as in human oral tissues. This would allow one to more accurately assess human risk by determining an individual's level of expression of these particular isozymes in oral tissues. The DNA adducts that result from the metabolic activation of NNK and NNN in oral tissue have not been thoroughly examined. More sensitive methods are needed to detect these adducts. It would be useful to understand the spectrum of adducts formed from these nitrosamines in oral tissue; the presence of particular adducts could perhaps be related to oncogene activation, tumor suppressor gene inactivation, and risk for oral cancer.

The characteristics of the model in which a mixture of NNK and NNN induces oral tumors upon lifetime swabbing in the rat oral cavity should be more clearly defined. Is NNK or NNN responsible for the tumorigenic effect, or are both compounds necessary? Does irritation caused by continuous swabbing contribute to the tumorigenic effect? Could the protocol be varied in some way to decrease the latent period and improve tumor yield in order to provide a more practical model? It is not clear how the route of administration affects tumor incidence in this model. Lung tumors were observed, consistent with the organospecificity of NNK, but no tumors of the esophagus and nasal cavity were observed. They might have been expected, given the known target tissues of NNK and NNN.

The potent pulmonary tumorigenicity of NNK suggests that snuff dippers may be at risk for lung cancer. This should be investigated.

The role of cofactors in tumorigenesis by NNK and NNN, as components of a complex mixture like snuff, is not clear. Snuff extract inhibited the tumorigenicity of NNK and NNN (Hecht et al., 1986). This could be attributable to the inhibitory effect of nicotine on their metabolic activation or to other compounds in the extract. Snuff is tumorigenic when instilled in the rat lip canal; the precise role of NNK and NNN in this model is not clear (Hecht et al., 1986). It seems likely that the irritant properties of snuff would enhance NNK and NNN tumorigenesis by increasing cell replication. This should be tested. Viruses may also play a role as cofactors in NNK and NNN tumorigenesis (Park et al., 1986).

Hemoglobin adducts appear to provide a method for assessing the metabolic activation of NNK and NNN in humans. What contributes to a high adduct level and what are its consequences? Does endogenous nitrosation increase the dose of NNK and NNN beyond what is found in snuff? Or are high adduct levels strictly a function of an individual's ability

to metabolically activate the compounds? What are the separate roles of NNK and NNN in contributing to adduct levels? These questions must be answered before the hemoglobin adduct data can be fully interpreted.

SUMMARY The evidence that NNK and NNN play a role in human oral cancer induced by snuff is strong. Both compounds are present in significant amounts in snuff and in the saliva of snuff dippers. They are metabolically activated in snuff dippers to intermediates that bind to hemoglobin. They cause oral tumors in rats and are metabolically activated by rat and human oral tissue. Although there are many questions about the mechanisms by which snuff causes oral tumors in rats and humans, there is no doubt that the presence of NNK and NNN in snuff is an unacceptable risk to people who choose to use these products.

REFERENCES

Belinsky, S.A., Devereux, T.R., Maronpot, R.R., et al. Relationship between the formation of promutagenic adducts and the activation of the K-*ras* proto-oncogene in lung tumors from A/J mice treated with nitrosamines. *Cancer Research* 49: 5305-5311, 1989.

Carmella, S.G., Hecht, S.S. Formation of hemoglobin adducts upon treatment of F344 rats with the tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N'*-nitrosonornicotine. *Cancer Research* 47: 2626-2630, 1987.

Carmella, S.G., Kagan, S.S., Kagan, M., et al. Mass spectrometric analysis of tobacco-specific nitrosamine hemoglobin adducts in snuff dippers, smokers, and nonsmokers. *Cancer Research* 50: 5438-5445, 1990.

Castonguay, A., Stoner, G.D., Schut, H.A.J., et al. Metabolism of tobacco-specific *N'*-nitrosamines by cultured human tissues. *Proceedings of the National Academy of Sciences* 80: 6694-6697, 1983.

Ehrenberg, L., Osterman-Golkar, S. Alkylation of macromolecules for detecting mutagenic agents. *Teratogenesis, Carcinogenesis, and Mutagenesis* 1: 105-127, 1976.

Foiles, P.G., Akerkar, S.A., Carmella, S.G., et al. Mass spectrometric analysis of tobacco-specific nitrosamine DNA adducts in smokers and nonsmokers. *Chemical Research in Toxicology*, 4: 364, 1991.

Hecht, S.S., Castonguay, A., Rivenson, A., et al. Tobacco specific nitrosamines: Carcinogenicity, metabolism, and possible role in human cancer. *Journal of Environmental Science and Health. Part C.* (CI) 1: 1-54, 1983a.

Hecht, S.S., Haley, N.J., and Hoffmann, D. Monitoring exposure to tobacco products by measurement of nicotine metabolites and derived carcinogens. In: *Molecular Dosimetry and Human Cancer: Analytical, Epidemiological, and Social Considerations*, J.D. Groopman and P.L. Skipper (Editors). Boca Raton, FL: CRC Press, 1991, pp. 325-361.

Hecht, S.S., Hoffmann, D. Tobacco-specific nitrosamines: An important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis* 9: 875-884, 1988.

Hecht, S.S., Hoffmann, D. The relevance of tobacco-specific nitrosamines to human cancer. *Cancer Surveys* 8: 273-294, 1989.

Hecht, S.S., Jordan, K.G., Choi, C.-I., et al. Effects of deuterium substitution on the tumorigenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in A/J mice. *Carcinogenesis* 11: 1017-1020, 1990.

Hecht, S.S., Rivenson, A., Braley, J., et al. Induction of oral cavity tumors in F344 rats by tobacco-specific nitrosamines and snuff. *Cancer Research* 46: 4162-4166, 1986.

Hecht, S.S., Trushin, N. DNA and hemoglobin alkylation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in F344 rats. *Carcinogenesis* 9: 1665-1668, 1988.

Hoffmann, D., Adams, J.D. Carcinogenic tobacco-specific N-nitrosamines in snuff and saliva of snuff dippers. *Cancer Research* 41: 4305-4308, 1981.

Hoffmann, D., Adams, J.D., Lisk, D., Fiseune, I., Brunnemann, K. Toxic and carcinogenic agents in dry and moist snuff. *Journal of the National Cancer Institute* 79: 1281-1286, 1987.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985, pp. 113-116.

Morse, M.A., Eklind, K.I., Toussaint, M., et al. Characterization of a glucuronide metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and its dose dependent excretion in the urine of mice and rats. *Carcinogenesis* 11: 1819-1823, 1990.

Murphy, S.E., Heiblum, R., Trushin, N. Comparative metabolism of *N'*-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by cultured rat oral tissue and esophagus. *Cancer Research* 50: 4685-4691, 1990b.

Murphy, S.E., Palomino, A., Hecht, S.S., et al. Dose-response study of DNA and hemoglobin adduct formation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in F344 rats. *Cancer Research* 50: 5446-5452, 1990a.

Park, N.H., Sapp, J.P., Herbosa, E.G. Oral cancer induced in hamsters with *herpes simplex* infection and simulated snuff-dipping. *Oral Surgery, Oral Medicine, Oral Pathology* 62: 164-168, 1986.

Saranath, D., Panchal, R.G., Nair, R., et al. Oncogene amplification in squamous cell carcinoma of the oral cavity. *Japanese Journal of Cancer Research* 80: 430-437, 1989.

Somers, K.D., Cartwright, S.L., Schechter, G.L. Amplification of the *int*-2 gene in human head and neck squamous cell carcinomas. *Oncogene* 5: 915-920, 1990.

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Frameni, J.F., Jr. Snuff dipping and oral cancer among women in southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

Role of Nicotine as a Cofactor In Smokeless Tobacco Carcinogenesis[1]

Christopher A. Squier and Georgia K. Johnson

ABSTRACT Nicotine is a constituent of all tobacco but is present at higher concentrations in smokeless tobacco than in other forms. The ST user is likely to have nicotine come into contact more frequently with the oral lining than is the smoker. Studies with animals suggest that the presence of nicotine enhances the effect of known carcinogens in the development of oral carcinoma. In vitro studies show that 2 percent and 6 percent nicotine applied topically to oral mucosa causes epithelial damage and an increase in permeability. We suggest that the method by which nicotine contributes to tumor development involves initial tissue damage, followed by a reparative response, accompanied by increased permeability to carcinogens. Continued exposure will lead to malignant transformation of the tissue.

INTRODUCTION Nicotine is the component of tobacco that causes the psychoactive effects that lead to addiction as well as to a variety of physiological changes (Benowitz, 1986 and 1988). The concentration of nicotine in tobacco ranges from 1 to 2 percent of the dry weight of the processed leaf in cigarette tobacco (Gritz et al., 1981), between 1.45 and 8.00 percent in smokeless tobacco (snuff and plug tobacco) in the United States (Gritz et al., 1981), and between 0.5 and 3.0 percent in snuff worldwide (Hoffmann and Adams, 1981). However, unlike smoking tobacco where only 15 to 20 percent of nicotine is transferred into mainstream smoke (Schmeltz et al., 1979), which is present intermittently in the oral cavity, all of the nicotine in ST is present in the mouth for extended periods of time. The possibility that the nicotine in smokeless tobacco may be a precursor of the tobacco-associated nitrosamines, some of which are known carcinogens (Hoffmann and Adams, 1981), has been thoroughly explored (Hoffmann et al., 1985 and 1986). However, little attention has been given to the effect that high levels of topical nicotine might have on the oral mucosa.

IN VIVO STUDIES WITH NICOTINE In a series of studies using skin, Bock (1980) was able to show that carcinogenesis by benzo[*a*]pyrene was enhanced by nicotine, although nicotine did not initiate or promote tumors; nicotine acted as a cofactor but was not a carcinogen by itself. We were able to demonstrate a similar effect in oral mucosa (Chen and Squier, 1990) by painting hamster cheek pouches with either 7,12-dimethylbenz[*a*]anthracene (DMBA) alone or in combination with 6 percent nicotine for 12 wk. Animals treated with DMBA and nicotine showed significantly ($p < 0.001$) more tumors and a significantly ($p < 0.05$) greater than expected proportion of large tumors (> 3 mm diameter) in the cheek pouch than hamsters treated with DMBA alone. Animals treated with nicotine alone did not develop tumors. In a similar experiment, using 6 percent nicotine and either *N'*-nitrosonornicotine (NNN) or 4-(methylnitrosamino)-1-(3 pyridyl)-1-butanone (NNK),

[1] Supported in part by National Institute of Dental Research grants no. R01-DE07930 and R29-DE/AI10153-01.

we have observed greater epithelial hyperkeratosis and dysplasia in the oral mucosa and the presence of papillomas in gastric mucosa as compared with treatment with nicotine alone (Chen and Squier, unpublished results).

Studies in which snuff was applied topically to the rat periodontium have indicated decreased gingival blood flow (Schroeder and Milo, 1989) and bone loss (Hill et al., 1990). However, other workers have reported increased gingival blood flow in dogs after topical application of nicotine (Johnson et al., 1989). These differences may represent a paradoxical vasoactive effect of nicotine at various dose levels, but the studies do show that nicotine can exert a local tissue effect.

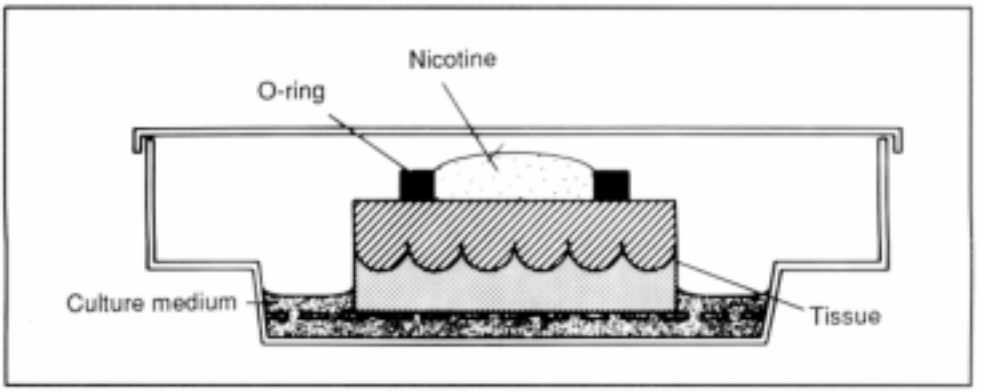
IN VITRO STUDIES WITH NICOTINE

To examine the local acute effects of nicotine applied topically to oral mucosa, we have developed an in vitro model system (Figure 1). Nicotine is prepared in phosphate-buffered saliva (PBS) at concentrations of 2 or 6 percent and placed in a circular rubber gasket attached to the surface of specimens of oral mucosa with cyanoacrylate. The tissue is maintained in culture medium and incubated at 37 °C for 1 or 2 h; controls are incubated with PBS alone at the same pH (7.5) and osmolarity (450 mosm) as the nicotine solution. The tissue consists of pig oral mucosa taken at death from the gingiva, floor of the mouth, and the buccal mucosa, which have a structure very similar to that of the corresponding human tissue (Lesch et al., 1989). Specimens either are processed for morphological examination by light and electron microscopy, or the functional capacity of the tissue is assessed by measure of the permeability. We accomplish this by clamping specimens between the halves of a glass perfusion chamber and determining the amount of tritiated water that passes across per unit time. From these data, a permeability constant (K_p) can be calculated (Siegal et al., 1971).

Morphologically, in all mucosal regions there was more damage after treatment with 6 percent than with 2 percent nicotine and greater damage after 1 h incubation with 6 percent nicotine than after 2 h with 2 percent nicotine. Changes in controls were minimal. The epithelium of gingiva (Figure 2) and floor-of-mouth mucosa (Figure 3) showed greater destruction than buccal mucosa (Figure 4). Changes were usually more evident in the deeper cellular layers than at the surface, and this type of damage was particularly striking for gingiva. The changes represented acantholysis and separation of epithelial strata, cell shrinkage, and nuclear pyknosis. It is notable that the in vitro appearances frequently resemble those illustrated for biopsies from oral mucosa of chronic snuff users (Andersson, 1991).

Those tissues treated with 2 percent nicotine that showed only slight alterations at the light microscope level (Figures 3 and 5) revealed considerable ultrastructural changes when examined with the transmission electron microscope. Changes included peripheral condensation of chromatin in cell nuclei and the presence of frequent intracellular vacuoles (Figure 5, left) that appear to represent damaged organelles such as mitochondria (Figure 5, right) as well as discontinuities in the basal lamina. Such changes seem to represent the initial cellular responses to nicotine and are of particular importance because 2 percent nicotine is found in many types of snuff worldwide.

Figure 1
The model used to expose oral mucosa to topical nicotine



Source: Squier and Lesch, 1988; used with permission.

The pattern of damage may reflect the chemical action of the nicotine molecule, as controls did not suggest any effect caused by pH or osmolarity. Nicotine may disrupt phospholipid-containing membranes, such as those of the deeper cell layers, whereas the superficial layers contain greater amounts of glycolipid or pure lipid rather than phospholipid (Squier et al., 1991).

Measurement of permeability to tritiated water of tissues treated with 6 percent nicotine for 2 h (Table 1) revealed significant ($p > 0.05$) increases in K_p values in all regions compared with controls incubated in PBS or normal, untreated mucosa (values from Lesch and coworkers, 1989). Because the putative carcinogens in ST (the tobacco-associated nitrosamines) are present in an aqueous environment and can be extracted by saliva (Hecht et al., 1974), our results indicate that nicotine-induced damage could increase the access of such carcinogens to the deeper, proliferative compartment of the oral epithelium.

CLINICAL IMPLICATIONS OF STUDIES WITH NICOTINE

One of the characteristic features of chronic ST use is the presence of a hyperkeratotic oral mucosal lesion at the site of regular tobacco placement. Characterized clinically as a white lesion, leukoplakia, or snuff-dipper's lesion, these lesions show an increased likelihood of malignant transformation such that 4 percent of lesions may become carcinoma (Schafer et al., 1983). The pathogenesis of these lesions may reflect the acute epithelial damage brought about by the presence of nicotine in tobacco that is applied to an area of oral mucosa. The initial damage would evoke a hyperplastic reparative response and would be associated with an increased permeability to any carcinogens. Chronic irritation, as a result of continued placement of tobacco at the same site, would lead to hyperkeratosis that appears as a white patch. Hyperplasia and hyperkeratosis are protective responses of skin and oral mucosa to mechanical or chemical irritation. However, hyperkeratotic regions of skin (Grice, 1980) and oral mucosa (Squier et al., 1985) tend to show increased permeability so that tobacco-associated carcinogens will continue to enter the lesional tissue. Thus, the tissue represents a site of elevated cell proliferation that is exposed to higher levels of carcinogens than the adjacent normal mucosa. This will increase the likelihood of development of carcinoma.

Figure 2
Sections of porcine gingiva after exposure for 2 h with PBS (left), 2 percent nicotine (center), and 6 percent nicotine (right). Note the gradation of damage from left to right, all of which occurs in the deeper cell layers (magnification x200).

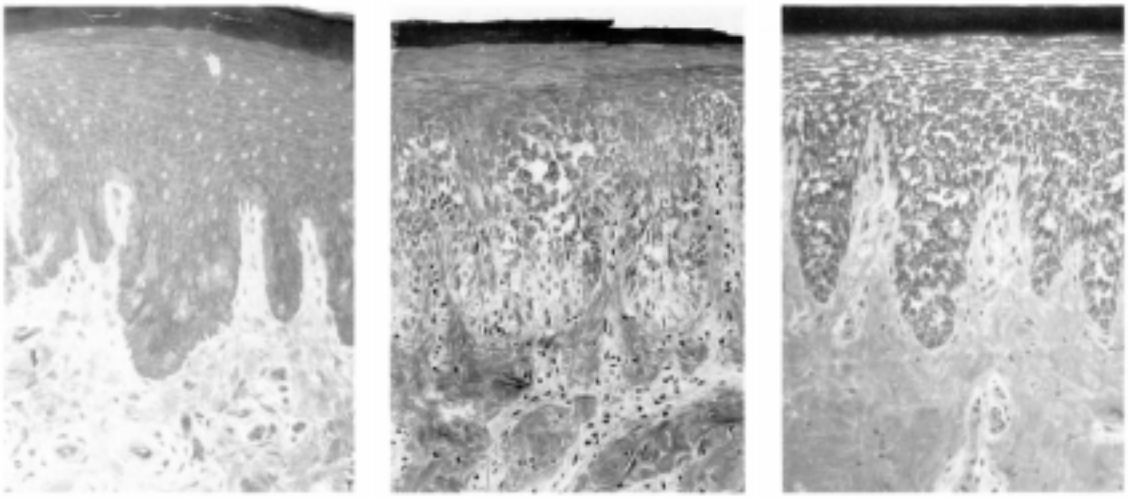
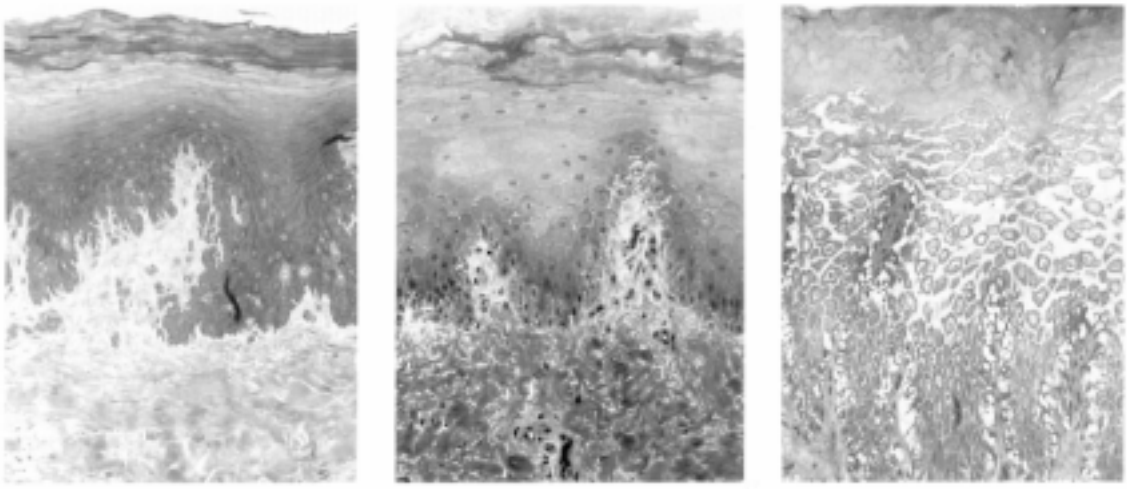
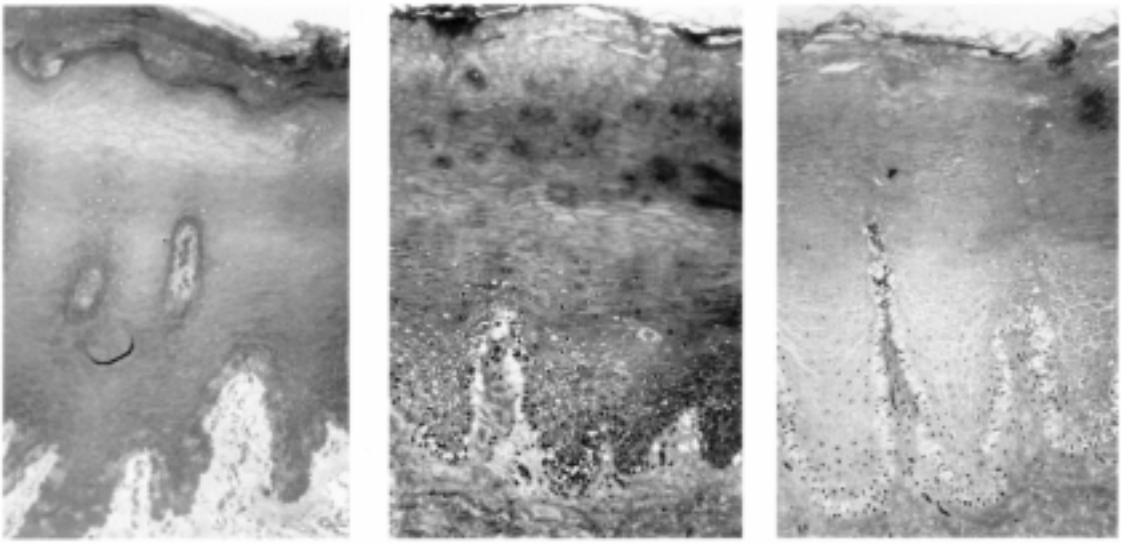


Figure 3
Sections of porcine floor-of-mouth mucosa after exposure for 2 h with PBS (left), 2 percent nicotine (center), and 6 percent nicotine (right). There is greater intercellular disruption after treatment with 6 percent (right) than with 2 percent nicotine (center) (magnification x200).



Although this sequence of events is conceptual, it is consistent with clinical evidence. When smokeless tobacco is first placed on the oral mucosa, there is erythema and, histologically, evidence of acute inflammation (G.K. Johnson, personal communication). If a habitual tobacco user places tobacco at a new site in the mouth, the original lesion usually resolves clinically and histologically (Andersson, 1991), but another develops at the

Figure 4
Sections of porcine buccal mucosa after exposure for 2 h with PBS (left), 2 percent nicotine (center), and 6 percent nicotine (right). There is marked intercellular disruption after treatment with 6 percent nicotine, but the deepest cells show nuclear shrinkage and separation with 2 percent nicotine (magnification x125).



new site of placement. Such events clearly implicate tobacco as the causative agent in pathological changes in the oral mucosa and lead to the hypothesis that nicotine may be the comp onent that initiates the morphological changes described. The events proposed above would also be consistent with suggestions that ST carcinogenesis involves factors other than a carcinogen (Park et al., 1988) and that the development of oral carcinoma requires extended exposure to tobacco (Winn et al., 1981).

If nicotine is involved in the production of the snuff-induced lesion, then the local effect of nicotine gum, often prescribed during smoking cessation, might be of importance. In a study that evaluated gingival response to nicotine chewing gum (2 mg or 4 mg per portion), Silver and coworkers (1989) reported transient gingival blanching in all subjects. When the gum was repeatedly held in the same location, vesicle formation occurred that resolved when the gum was placed in different positions. The absence of severe, prolonged changes in nicotine gum chewers (Christen et al., 1985; Silver et al., 1989), in contrast to the tissue changes described in our experiments, can probably be explained by the levels of nicotine exposure. Although bioavailability of nicotine may vary, nicotine concentration in chewing gum (0.2 percent by weight) is almost an order less than that found in typical ST products (0.5 to 3.0 percent nicotine) (Hoffmann and Adams, 1981). Furthermore, the gum is typically used for only 5 to 6 h daily. This represents a much lower nicotine exposure than that of individuals with an established ST habit who may continually use the product, with its higher concentrations of nicotine, for more than 12 h/d (Andersson, 1991).

Figure 5

Transmission electron micrographs of floor-of-mouth mucosa exposed for 2 h to 2 percent nicotine. Epithelial cells show condensation of nuclear chromatin (top, magnification x17,000) and numerous vacuoles that appear to represent damaged cell organelles, including mitochrondria (bottom, x28,500).

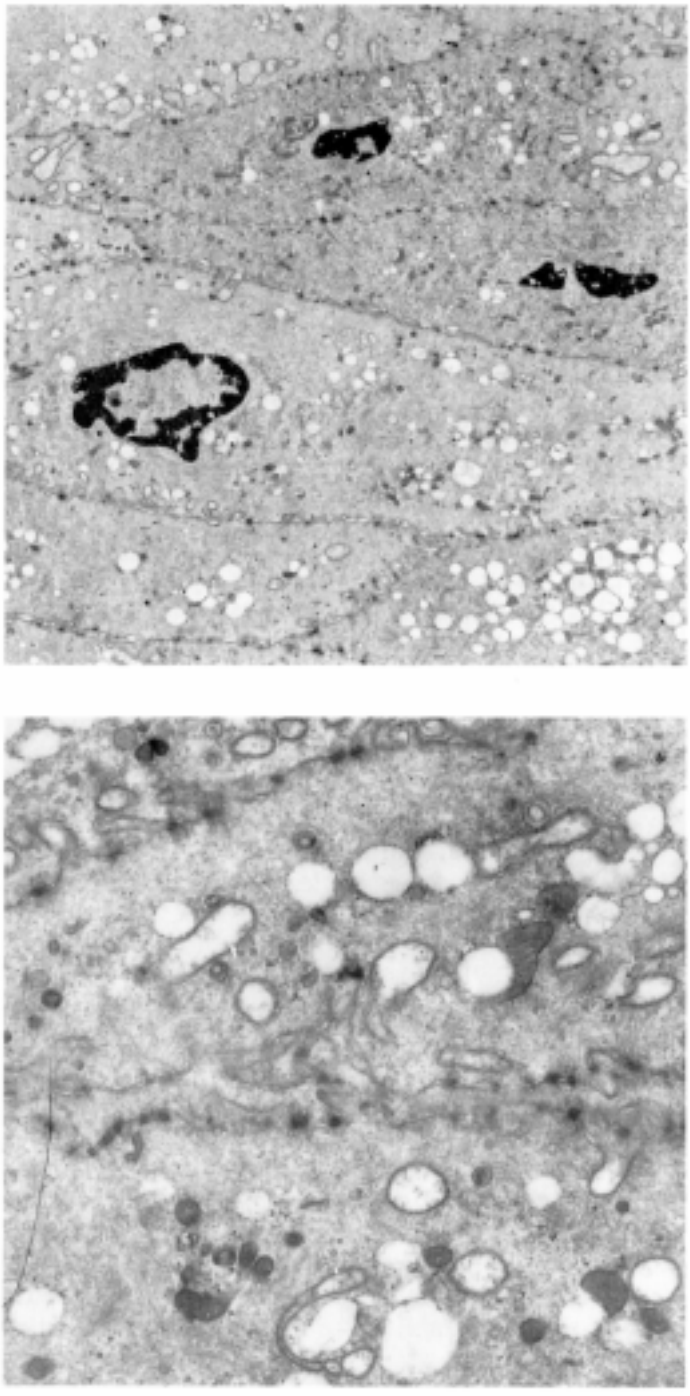


Table 1
Effect of nicotine on mucosal permeability to tritiated water[a]

	Untreated Control	PBS Control	Nicotine Treated[b]
Tissue Region			
Gingiva	364 ± 18	351 ± 54	667 ±77
Buccal mucosa	634 ± 19	692 ± 49	781 ± 46
Floor of mouth	808 ± 23	814 ± 46	971 ± 94

[a] K_p *SEM x* 10^{-7} *cm/min.*
[b] *Values significantly greater than controls (p > 0.05).*

FUTURE DIRECTIONS A logical consequence of the implication of nicotine in ST carcinogenesis would be to reduce its concentration to levels that do not cause tissue damage. As this threshold is unknown, it is not possible to predict whether such a strategy would still provide adequate nicotine levels to offer the psychoactive effect that is the major reason for ST use while limiting the pathological effects on the oral mucosa.

ACKNOWLEDGMENTS We thank Mary Kremer and Chuck Lesch for technical assistance.

REFERENCES

Andersson, G. Snuff-induced changes associated with the use of loose and portion-bag-packed Swedish moist snuff: A clinical, histological, and followup study. *Swedish Dental Journal* Suppl. 75: 1-88, 1991.

Benowitz, N.L. Clinical pharmacology of nicotine. *American Review of Medicine* 37: 21-32, 1986.

Benowitz, N.W. Nicotine and smokeless tobacco. *CA—A Cancer Journal for Clinicians* 38: 224-256, 1988.

Bock, F.G. Cocarcinogenic properties of nicotine. *Banbury Report* 3: 129-139, 1980.

Chen, Y.-P., Squier, C.A. Effect of nicotine on 7,12-dimethylbenz(*a*)anthracene carcinogenesis in hamster cheek pouch. *Journal of the National Cancer Institute* 82: 861-864, 1990.

Christen, A.G., Beiswanger, B.B., Mallatt, M.E., et al. Effects of nicotine-containing chewing gum on oral soft and hard tissues: A clinical study. *Oral Surgery, Oral Medicine, Oral Pathology* 59: 37-42, 1985.

Grice, K.A. Transepidermal water loss in pathological skin. In: *The Pathophysiology of Skin*, A. Jarrett (Editor). London: Academic Press, 1980, p. 2147.

Gritz, E.R., Baer-Weiss, V., Benowitz, N.L., Yunakis, H.V., Jarvik, M.E. Plasma nicotine and cotinine concentrations in habitual smokeless tobacco users. *Clinical Pharmacology Therapy* 30: 201-209, 1981.

Hecht, S.S., Ornaf, R.M., Hoffmann, D. Chemical studies of tobacco smoke XXXIII. *N'*-nitrosonornicotine in tobacco: Analysis of possible contributing factors and biologic implications. *Journal of the National Cancer Institute* 54: 1237-1244, 1974.

Hill, L.N., Milo, A.G., Schroeder, K.L. Bone loss relative to gingival blood flow in snuff-treated versus control rats (abstract 707). *Journal of Dental Research* 69: 197, 1990.

Hoffmann, D., Adams, J.D. Carcinogenic tobacco-specific N-nitrosamines in snuff and in the saliva of snuff dippers. *Cancer Research* 41: 4305-4308, 1981.

Hoffmann, D., Hecht, S.S., Melikian, A.A., Haley, N.J., Brunnemann, K.D., Adams, J.D., Wynder, E.L. Tumorigenic agents in tobacco products and their uptake by chewers, smokers and nonsmokers. In: *Biochemical and Molecular Epidemiology of Cancer*. New York: Alan R. Liss, 1986, pp. 191-204.

Hoffmann, D., Lavoic, E.J., Hecht, S.S. Nicotine: A precursor for carcinogens. *Cancer Letters* 26: 67-75, 1985.

Johnson, G.K., Todd, G.L., Fung, E.Y.K. Effects of nicotine on gingival blood flow (abstract 834). *Journal of Dental Research* 68: 285, 1989.

Lesch, C.A., Squier, C.A., Cruckley, A., Williams, D.M., Speight, P. The permeability of human oral mucosa and skin to water. *Journal of Dental Research* 68: 1345-1349, 1989.

Park, N.H., Akoto-Amanfu, E., Paik, D.I. Smokeless tobacco carcinogenesis: The role of viral and other factors. *CA—A Cancer Journal for Clinicians* 38: 224-233, 1988.

Schafer, W.G., Hine, M.K., Levy, B.M. *A Textbook of Oral Pathology* (4th edition). Philadelphia: W.B. Saunders, 1983, p. 104.

Schmeltz, I., Wenger, A., Hoffmann, D., Tso, T.C. On the fate of nicotine during pyrolysis and in a burning cigarette. *Agricultural and Food Chemistry* 27: 602-608, 1979.

Schroeder, K.L., Milo, A.G. Topically applied smokeless tobacco effects on rat gingival blood flow (abstract 879). *Journal of Dental Research* 68: 291, 1989.

Siegal, I.A., Hall, S.H., Stambaugh, R. Permeability of the oral mucosa. In: *Current Concepts of the Histology of Oral Mucosa*, C.A. Squier and J. Meyer (Editors). Springfield, IL: Charles C. Thomas, 1971, pp. 274-186.

Silver, K.J., Sachs, D.P.L, Hottel, T.L. Gingival response to nicotine polacrilex. *Journal of the American Dental Association* 118: 53-56, 1989.

Squier, C.A., Hall, B.K. The permeability of hyperplastic oral epithelium. *Journal of Oral Pathology* 15: 357-362, 1985.

Squier, C.A., Wertz, P.W., Cox, P. Thin-layer chromatographic analysis of lipids in different layers of porcine epidermis and oral epithelium. *Archives of Oral Biology* 36: 647-653, 1991.

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Fraumeni, J.F., Jr. Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

Perinatal Carcinogenesis by Constituents Of Smokeless Tobacco: Animal Models And Potential Human Risk

Lucy M. Anderson and Jerry M. Rice

ABSTRACT Carcinogenic risk for human perinates from parental use of smokeless tobacco would be implied if (1) increased risk in organs distant from the oral site of exposure were demonstrated in adults; (2) increased cancer risk from parental cigarette smoking were found; and (3) carcinogens in ST were active perinatal carcinogens in animal models. Epidemiological evidence for the first two points is limited but increasing. In regard to the third point, in animal models, the most potent carcinogen present in significant quantity in ST, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), has been demonstrated to be a relatively potent carcinogen in fetal hamsters, especially in the respiratory tract, and in newborn mice (lung and liver). NNK was significantly but weakly positive in lungs and livers of fetal mice. Interpreted with other studies on perinatal effects of nitrosamines and other carcinogens, the results with NNK are consistent with metabolic activation in perinatal tissues being a limiting factor in risk for the tobacco-specific nitrosamines and other smokeless tobacco carcinogens, such as benzo[*a*]pyrene and *N*-nitrosodimethylamine. Investigation of this biochemistry with rodent tissue and human or primate perinatal tissues or both is therefore of high priority in evaluation of human risk, along with analysis of amniotic fluid and breast milk of ST users. The possibility of preconception effects, especially in males, predisposing to cancer in the offspring may be studied with animal models. In addition, epidemiological studies of childhood and adult cancers as related to the amount and kind of ST used by both parents are indicated.

INTRODUCTION Increasing use of smokeless tobacco by women of child-bearing age (Orlandi and Boyd, 1989) presents the possibility of adverse effects, including cancer, for their children. Such a possibility exists if effective doses of the toxic constituents of these topically applied products reach the fetus transplacentally via blood circulation or the suckling infant through milk. This possibility is confirmed by epidemiology linking ST with increased cancer at sites other than the head and neck. Available evidence suggests an association with cancers of the bladder, pancreas, and kidney (Bjalke and Schuman, 1982; Goodman et al., 1986; Heuch et al., 1983; Kabat et al., 1986). Both transplacental (Alaoui-Jamali et al., 1989; Anderson et al., 1989a; Castonguay et al., 1984; Correa et al., 1990; Rossignol et al., 1989) and transmammary (Diaz Gomez et al., 1986; LaVoie et al., 1987) passage of tobacco product carcinogens occurs in rodents. In humans, an increase in 4-aminobiphenyl hemoglobin adducts in fetuses of smoking mothers was recently demonstrated (Coghlin et al., 1991). It is therefore necessary to consider risk of tumorigenesis in the fetuses and neonates of ST users.

SENSITIVITY AND SUSCEPTIBILITY FACTORS A wide variety of chemicals cause tumors when delivered transplacentally to rodent fetuses or administered directly to newborns (Rice, 1981; Toth, 1968). The tumors that result are mostly adult rather than embryonal type and present in tissues characteristic of the chemical and the species. Fetal sensitivity varies from minimal at toxic adult doses to much greater than that observed in the adult (see Anderson et al., 1985a). The most effective transplacental carcinogens are

the rapidly penetrating, direct-acting alkylating agents, especially *N*-ethylnitrosourea (ENU), to which the nervous systems of rat fetuses are approximately fiftyfold more sensitive than those of the adult animal. In mice, transplacental ENU not only initiated more lung and liver tumors in fetuses than in adults at the same dose, but the prenatally initiated neoplasms became larger and more malignant (Branstetter et al., 1988 and 1989). ENU was also found to be a transplacental carcinogen in two nonhuman primates, patas and rhesus monkeys, causing, in addition to neoplasms of the vasculature, tumors that were not seen in the mothers or after treatment of other adult monkeys (Rice et al., 1989), including hepatocellular carcinoma, leukemia, lung adenoma, nephroblastoma, and an assortment of tumors of the brain.

These results indicate that animal fetuses, both rodent and primate, have high innate sensitivity to tumor initiation by genotoxic carcinogens that are in an activated state and an effective dose. The reasons for this special sensitivity probably include high rates of cell division, which permits fixation of lesions before repair can occur, and multiple divisions after initiation to give rise to a large clone of initiated cells. Other factors, such as limited ability to repair DNA damage, high proportion of undifferentiated cells, and immaturity of immune surveillance, hormonal, and growth control systems, have also been suggested to play a role, although convincing evidence is lacking.

More variable results are obtained for transplacental carcinogens that are activated or detoxified or both by enzymes in the mother, the placenta, or the fetus. Nitrosamines, in contrast to nitrosoureas, require metabolic activation and are generally ineffective transplacental carcinogens; they seem to elicit tumors in direct proportion to the capacity of fetal tissues to activate them metabolically. Thus, *N*-nitrosodiethylamine (NDEA) causes respiratory tumors in mice and hamsters with steadily increasing efficiency as the end of gestation nears, correlated with the acquisition of an unusually high ability to metabolize NDEA by fetal hamster lungs at term (Lofberg and Tjalve, 1984).

Larger metabolism-dependent carcinogens such as polycyclic aromatic hydrocarbons (PAHs) present an even more complex picture, because their effectiveness may be limited by poor placental penetration (Neubert and Tapken, 1988) or detoxification in the placenta (Pelkonen, 1985; Remmer, 1987). Nevertheless, certain PAHs, including 3-methylcholanthrene (MC) and 7,12-dimethylbenz[*a*]anthracene (DMBA), are more active transplacentally than in adult rodents (Anderson et al., 1985b; Rice et al., 1978). In mice, fetal risk of lung and liver carcinogenesis by MC is influenced strongly by both maternal and fetal ability to respond to induction of a cytochrome P_{450} (1A1) that metabolizes MC; maternal induction protected against and fetal induction potentiated MC's effect (Anderson et al., 1985b and 1989c). Biochemical analysis of this model suggests that extensive induction in maternal liver reduces the effective dose of unchanged carcinogen reaching the fetuses, but in the fetus, the more limited induction in liver does not outweigh the increased level of activation to ultimate carcinogen in the lung (Chauhan et al., 1991; Miller et al., 1989, 1990a, and 1990b).

The newborn infant is a particularly vulnerable target for carcinogens, often being more sensitive to carcinogens than adults (Toth, 1968) or than late fetuses after an equivalent dose to the mother. The reasons for this include high rates of cell division, as for fetuses, and long half-life of chemicals, because the newborn's detoxifying enzymes are generally inadequate. For the carcinogens in ST, newborn sensitivity would be of greatest concern if these were secreted in milk in significant quantities. This would seem to be a possibility, because tumorigenesis after transmammary delivery has been demonstrated for a chemically diverse assortment of carcinogens (Maekawa and Odashima, 1975; Mohr and Althoff, 1971; Nomura, 1973; Nomura et al., 1974; Vesselinovitch et al., 1979).

Activated oncogenes have been found in transplacentally induced tumors and show tissue specificity, but the type and incidence of activated oncogenes were similar to those in tumors induced in the same tissues in adults by these agents (Loktionov et al., 1990; Perantoni et al., 1987; Sukumar and Barbacid, 1990; Yamasaki et al., 1987). Special fetal susceptibility does not appear to relate to unique features of oncogene activation.

In some cases the appearance of prenatally initiated tumors may be dependent on or hastened by postnatal exposure to tumor promoters. This has been demonstrated for a variety of tumors in epithelial tissue and several promoters (Anderson et al., 1985a; Diwan et al., 1989; Napalkov et al., 1987; Suganuma et al., 1987). Hormones and tissue regeneration also provided a promotional stimulus (Ogawa et al., 1982; Rice, 1981). On the other hand, postnatal exposures to such diverse agents as hormones, barbiturates, growth factors, or nicotine sometimes suppressed tumor development (Alexandrov et al., 1989; Anderson et al., 1985a; Beniashvilli and Zedginidze, 1989; Berger et al., 1987; Naito et al., 1985).

In sum, the perinate can present a high intrinsic sensitivity to tumorigenesis, which may be counterbalanced by maternal or placental detoxification, poor transplacental or transmammary delivery, limitations in metabolic activation, other poorly understood species- and organ-specific constraints, lack of necessary postnatal stimulation, or postnatal inhibitory influences. No doubt other factors influencing the process in both directions remain to be discovered. All of these must be considered in evaluation of potential human perinatal risk from ST.

TOBACCO-SPECIFIC NITROSAMINES IN ANIMAL MODELS

Cigarette smoke condensate administered to pregnant Syrian hamsters caused a significant increase in tumors, including neoplasms of the adrenal gland, pancreas, liver, respiratory system, ovary, and connective tissue (Nicolov and Chernozemsky, 1979), primarily in the female offspring. In short-term studies with mice, exposure of mothers during pregnancy to mainstream or sidestream cigarette smoke resulted in increased sister chromatid exchanges in fetal livers (Karube et al., 1989), and extract of ST administered before and during gestation resulted in reduced fetal weight, decreased ossification, and increased resorptions (Paulson et al., 1991).

Other efforts have focused on the chemicals present in tobacco products. The carcinogens of greatest concern in ST are the tobacco-specific

nitrosamines, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N'*-nitrosonornicotine (NNN), *N'*-nitrosoanabasine (NAB), and *N'*-nitrosoanatabine (NAT); all occur in ST in the parts-per-million range, being especially high in snuff (Hecht and Hoffmann, 1988). Thus far, perinatal carcinogenicity studies have been carried out only with NNK, the most potent in adult rodents. NNK administered to pregnant Syrian golden hamsters was detected in placenta, along with a twentyfold greater quantity of its metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Both were present, at about one-half the placental level in the fetal lung, and were found in amniotic fluid (Rossignol et al., 1989). Explants of exposed fetal lung and trachea showed a high incidence of chromosomal aberrations.

These findings predicted the positive outcome of the bioassay in hamsters (Correa et al., 1990); transplacental NNK caused a significant incidence of respiratory tract tumors at doses of 50 to 300 mg/kg, with the greatest effect shown in 61 percent of females after a single dose of 200 mg/kg on the last day of gestation. There was also a significant number of pheochromocytomas of the adrenal glands and a few hepatocellular carcinomas and pancreatic ductular adenomas. Thus NNK joins other nitrosamines as an effective transplacental carcinogen of the respiratory system in hamsters, again correlated with development of capacity for metabolic activation. The fetal hamster lung and tracheal tissues were capable of metabolizing NNK in vitro to a markedly increased extent over the last 4 d of gestation; the amount of most metabolites doubled between days 14 and 15 (Rossignol et al., 1989). In a comparison of methylated bases in DNA of lung explants from fetuses and adults, fetal DNA contained slightly more O^6- and N^7-methylguanines. These findings are consistent with another report, that fetal hamster lung metabolized NDEA at a threefold greater rate than adult lung (Lofberg and Tjalve, 1984).

Metabolism of [^{14}C]NNK was similarly examined in fetal C57BL/6 mice (Castonguay et al., 1984). The compound and its metabolites were detected in all fetal tissues and amniotic fluid. Macromolecule-bound label was found in fetal nose and liver and to a lesser extent in lung and kidney. Fetal liver, lung, and nasal tissue, incubated in vitro with NNK, formed the keto acid product of αC-hydroxylation (carcinogen activation), but only at levels 3 to 4 percent those of the corresponding maternal tissue. Nasal tissue was most active.

These biochemical findings suggested that NNK would be a positive but weak transplacental carcinogen in the mouse, and bioassay confirmed this prediction (Anderson et al., 1989b). Pregnant females of three mouse strains of differing characteristics were given three NNK doses of 100 mg/kg. Transplacentally exposed offspring of strain A mice, highly susceptible to lung tumor initiation, showed a tenfold increase in incidence of lung tumor bearers among females and a twofold change in males, with a small effect on multiplicity. Although of statistical significance, the degree of carcinogenicity was much less than that in the mothers, who exhibited a tumor multiplicity of > 20. It is of interest that a greater effect was seen in the

female offspring, as was noted above for both cigarette smoke condensate and NNK in hamsters.

Progeny of C3H/He x C57BL/6 mice ($C3B6F_1$) and of outbred Swiss [Cr:NIH(s)] mice, exposed transplacentally to NNK, were held for 18 mo and included groups treated postnatally with barbital or polychlorinated biphenyls (PCBs) as tumor promoters. Prenatal NNK treatment of male $C3B6F_1$ mice caused a significant increase in incidence of hepatocellular tumors (40 vs. 17 percent in controls), with no further effect of barbital and PCBs. Male offspring of Swiss mice had a low incidence of liver tumors (5 percent) after transplacental NNK; this increased to 19 percent in those receiving PCBs postnatally, a significant tumor-promotive effect. Although the mothers again developed lung tumors in high multiplicity, there was no increase in these in the $C3B6F_1$ or Swiss mice offspring.

The results from the three strains together show convincingly that NNK is a relatively weak transplacental carcinogen in the mouse, affecting primarily fetal organs of high sensitivity. The postnatal effects of the PCBs in Swiss mice are of special interest, indicating that, in certain genetic situations, tumors initiated prenatally by NNK could be promoted to expression postnatally by exposure to other environmental agents.

NNK was also tested in neonatal Swiss mice, with 50 mg/kg doses given five times between days 1 and 14. NNK was much more effective with neonatal treatment than after transplacental exposure, causing liver tumors in 57 percent of males and 14 percent of females (none in controls) and lung tumors in 57 percent of males and 37 percent of females (21 to 22 percent in controls) (Anderson et al., 1991a). Here, the males were more susceptible to lung tumorigenesis than were females. Although neonates appear to be more sensitive than fetuses, the actual dose received may be greater, because maternal and placental metabolism and the physical placental barrier probably reduce the amount of NNK reaching the fetus.

Overall the studies of perinatal carcinogenesis by NNK in hamsters and mice show that this chemical is a weak (transplacentally in mice) to moderate (in neonatal mice and transplacentally in hamsters) perinatal tumorigen, that its efficacy correlates with ability of target tissue to activate it metabolically, and that the tumors caused were those characteristic of the species and strain.

OTHER ST CONSTITUENTS

Other carcinogens are present in ST at lower levels than tobacco-specific nitrosamines (Hecht and Hoffmann, 1988) but could contribute nonetheless to a perinatal carcinogenic effect. Of those found at levels > 1 ppb, *N*-nitrosodimethylamine (NDMA) and benzo[*a*]pyrene (B[*a*]P) have been tested for perinatal carcinogenicity. In rats, hamsters, and mice, NDMA was a weak transplacental carcinogen, giving a significant but low yield of tumors (Anderson et al., 1985a and 1989a). NDMA is quite fetotoxic, so only relatively low doses (5 to 30 mg/kg) could be tolerated for testing. The limited prenatal effectiveness of NDMA as either a teratogen or carcinogen may relate to lack of capacity for metabolic activation, because a spontaneously reacting derivative, acetoxymethyl-methylnitrosamine, was active as a teratogen (Platzek et al., 1983) and as a transplacental carcinogen in rats (J.M. Rice, unpublished observations).

In contrast, NDMA is highly carcinogenic in neonatal mice (Frei, 1970; Toth et al., 1964), resulting in many lung and liver tumors. Coccia and coworkers (1988) found that NDMA demethylase activity in neonatal Swiss mouse liver, compared to adult liver, was two-thirds less, the rate of cell division twenty-fivefold greater, the amount of O^6-methylguanine adduct in DNA 2 and 4 h after a carcinogenic NDMA dose fourfold greater, and the level of O^6-methylguanine transferase repair enzyme fivefold less. Thus the factors suggested as predisposing to tumor initiation in the neonate were all confirmed.

B[*a*]P is a moderate transplacental carcinogen in mice, even though transplacental passage of B[*a*]P may be limited (Neubert and Tapken, 1988). Experiments in which B[*a*]P or its metabolites were injected directly into mouse fetuses demonstrated that B[*a*]P itself caused lung tumors, but the diol-epoxide metabolite, the ultimate carcinogenic form, was more effective, suggesting that ability to activate B[*a*]P is limiting in the fetus (Rossi et al., 1983). B[*a*]P, like the nitrosamines, is a potent carcinogen in neonatal mice (Truhaut et al., 1966). As a highly soluble lipid compound, B[*a*]P would be expected to partition readily into breast milk and has been quantified there (LaVoie et al., 1987).

In contrast to B[*a*]P, DMBA is a potent transplacental carcinogen in the rat (Rice et al., 1978). The possibility thus exists that other naturally occurring PAHs, including some that occur in tobacco products, may be significantly potent transplacentally. Most such substances are not readily available and have not been tested.

POTENTIAL RISK AND FUTURE RESEARCH

A conclusion from the assays of perinatal carcinogenesis by NNK in animal models is that this chemical and similar ones in ST may contribute to the risk of human perinatal carcinogenesis, depending on factors about which we have little or no information. Some of these information gaps are as follows:

- Presence, ontogeny, and variability of cytochromes P_{450} activating NNK, NDMA, and PAHs in human fetuses and placentas, especially cytochrome P_{450} 1A1/2, 2A3, 2D6, and 3A7
  - Activation of PAH diols by cytochrome P_{450} 3A7
  - DNA and hemoglobin adducts as markers of activation
  - Capacity of primate placenta to detoxify NNK, and influence of other factors such as smoking and coexposure to other xenobiotics; induction and competitive inhibition;
- Occurrence of aromatic amine carcinogens in ST;
- Toxicokinetics of NNK during pregnancy and lactation and modulating effects of other chemicals in nonhuman primate studies;
- Concentration of NNK and metabolites, other ST carcinogens in amniotic fluid and breast milk of users;
- Preconception effects of NNK on cancer in animal models; and

- Relations of childhood or adult cancers to use of ST by parents as revealed by epidemiological studies.

These gaps might be bridged by application of current technologies, as discussed below.

Biochemistry: Carcinogen Metabolism and DNA Adducts

By extrapolation of conclusions reached with rodent models, human perinatal risk as a result of carcinogens present in ST is probably determined in part by the levels of activating cytochrome P_{450} enzymes in the target tissues. Of cytochromes P_{450} in human fetal liver, 40 to 85 percent is the fetus-specific P_{450} 3A7, related but not identical to the predominant isoform of this family in adult liver, 3A4 (Cresteil et al., 1985; Kitada et al., 1985a; Komori et al., 1989a and 1989b; Wrighton and Vandenbranden, 1989). Which P_{450}'s constitute the remaining 15 to 60 percent is an important unresolved question. P_{450} 2D6 is detectable in human fetal liver by both immunoblot and Northern blot, although at low levels compared with adults (Treluyer et al., 1991). Low levels of P_{450} 1A1 gene expression were detected by use of the polymerase chain reaction in the majority of a small sample of fetal adrenals, livers, and lung, but not kidneys (Omiecinski et al., 1990). P_{450} 1A2 mRNA was reported to be absent (Cresteil, reported in Anderson et al., 1991b); however, a protein cross-reacting with an antibody to 1A2 was partially purified (Kitada et al., 1990), and a 1A isoform, suggested to be 1A2, was recognized by a monoclonal antibody to rat P_{450} 1A1 in human fetal hepatocytes (Murray et al., 1992). P_{450} 2E1 was not expressed in fetal (Komori et al., 1989b) or newborn (Morel et al., 1990) livers.

To relate these observations to carcinogens in ST, activation of NNK to a mutagen in cell expression systems is supported by human cytochromes P_{450} 1A2, 2A3, 2D6, and 2E1 (Crespi et al., 1991). As noted above, in human perinatal liver 2E1 is probably absent, 2D6 is present at low levels, and a P_{450} related to 1A2 may occur. Perinatal expression of 2A3 should be investigated, because it acts not only on NNK but also on NDMA (Crespi et al., 1990). P_{450} 3A7, the predominant human fetal form, also exhibits considerable NDMA demethylase activity (Kitada et al., 1985a).

With regard to PAHs such as B[*a*]P, B[*a*]P hydroxylase activity, generally considered a detoxification step, correlated with levels of 3A7 and was inhibitable by anti-3A7 antibody in human fetal livers (Kitada et al., 1985b and 1987); phenols but not diols were formed (Blanck et al., 1983). It is not certain which human P_{450} provides the initial activation of B[*a*]P to a diol; both 2C8,9 (Cresteil et al., 1985) and 1A1 (Shimada et al., 1989b) have been suggested. P_{450} 1A1 may be effectively induced in human placentas by smoking (Pasanen et al., 1990; Pasanen and Pelkonen, 1990), and placental microsomes induced by smoking do produce B[*a*]P-7,8-diol (Blanck et al., 1983). Whether ST brings about this induction is not known and should be determined.

P_{450} 3A4 carries out the further activation of B[*a*]P-7,8-diol to the ultimate diol-epoxide carcinogen (McManus et al., 1990; Shimada et al., 1989a). Whether the fetal form, 3A7, also has this capacity should be determined. If

so, PAH-diols formed by induced placenta might cross to the fetus where they could be further activated by the P_{450} 3A7 present in some abundance in the fetal tissues.

In sum, for carcinogens in ST, there seems reason for concern about tumor initiation in human fetuses by NDMA, as a result of its metabolism by P_{450} 3A7, and by PAHs, because P_{450} 1A1 in fetal tissues and especially in induced placenta could provide the initial activation, followed by possible ultimate activation by the substantial levels of 3A7 in the fetus. Potential effects of the more abundant NNK catalyzed by the low level of P_{450} 2D6 present is more problematic; it is particularly important to discover whether there is a 1A2-like protein present with activity toward NNK.

DNA and hemoglobin adducts have been a popular exposure biomarker. Thus far, of the tobacco-related carcinogens, only 4-aminobiphenyl adducts on hemoglobin have been detected in fetuses in association with maternal cigarette smoking (Coghlin et al., 1991). The occurrence of aromatic amines in ST has not been reported and should be investigated. O^6-methylguanine adducts of DNA from NNK or NDMA correlate with tumorigenesis in fetal hamster and newborn mouse and are consistent with the known oncogene mutations in the resultant tumors. Sensitive methods for detection of this adduct in human tissues are becoming available (Shields et al., 1990) and might profitably be applied to human fetuses and newborns, as related to tobacco use and other parameters. O^6-methyldeoxyguanosine has been detected in human placental DNA (Foiles et al., 1988). The pyridyl-oxobutyl adduct could be an indicator of exposure specifically to NNK. Repair and persistence of adducts after perinatal exposure will be difficult to evaluate with human material, but this question might be addressed with nonhuman primates.

Distribution and Toxicokinetics

As noted above, NNK and a metabolite were present in the amniotic fluid of hamsters and mice. Fetuses both breathe and ingest amniotic fluid. Human amniotic fluid has the capacity to activate carcinogens (Daubeze et al., 1986) and contains Ames-test mutagens, with a significantly higher level for heavy smokers (Rivrud et al., 1986). It might be worthwhile to analyze human amniotic fluid for tobacco-specific nitrosamines and their metabolites.

The dose of NNK incurred by the fetus or nursing infant will be influenced significantly by metabolic clearance in maternal liver and possibly in placenta, and both enzyme induction and competitive inhibition are possible. Are there NNK-metabolizing enzymes in placenta and, if so, are they induced by cigarette smoking, snuff dipping, or both? This important question is addressable now, by use of placentas from normal pregnancies to search for relevant P_{450} isoforms (discussed above) and for measurement of total NNK substrate conversion. This in vitro system could also be used to study competitive inhibition of metabolism by chemicals such as hormones, ethanol, pharmaceutical drugs, and industrial and household solvents.

The question of induction and inhibition in maternal liver, with an influence on clearance, will be more difficult for researchers to address using human tissue. A reasonable start may be made with nonhuman primates.

Epidemiology No epidemiological studies of cancer associated with parental use of ST have been reported. Suggestions may be sought from the epidemiology of cigarette smoking, which entails the same set of chemical carcinogens, and from the animal model work described above. Reviews of the epidemiological evidence have been given by Preston-Martin (1989) and John et al. (1991). Relative risks of 1.1 to 3.0 were frequently associated with maternal or paternal cigarette smoking or both, with positive effects of statistical significance in three studies: childhood leukemia associated with numbers of cigarettes smoked daily by the mothers (Stjernfeldt et al., 1986); in a prospective study, a relationship between all cancers and maternal smoking (Golding et al., 1990); and increased adult lung cancers among children of smoking mothers (Correa et al., 1983). Increased risk of hematopoietic cancers in adulthood from maternal or paternal smoking (Sandler et al., 1985) had a particularly strong association if both parents smoked. In studies implicating fathers, there was a positive association between mothers living with a smoker and brain tumors (Preston-Martin et al., 1982) and between paternal smoking and rhabdomyosarcomas (Grufferman et al., 1982). An effect of paternal smoking could imply (1) enhanced placental penetration of the smoke constituents in nonsmoking mothers with uninduced detoxification enzymes; (2) transmammary exposure of the infant; (3) direct respiratory exposure of the infant; or (4) a germ cell effect on the father.

There are corresponding possibilities for ST, especially snuff, that epidemiologists should pursue, and both childhood and adult cancers should be included. Populations in India and southeast Asia, where use of ST by women is common, could be especially fruitful to study. In addition to use of ST during pregnancy, use during lactation should receive attention, in light of the high sensitivity of rodent neonates. Paternal use should also be examined because preconception paternal exposures are becoming increasingly implicated in various childhood cancers (Bunin et al., 1992), with germ cell effects being the most likely mechanism. This possibility is supported by the finding of increased tumors in the offspring of male rodents exposed to carcinogens before conception (Mohr et al., 1989; Turusov et al., 1989). Considering the high concentration of mutagens present in some smokeless tobacco, epidemiological investigation of the children of male as well as female users seems warranted.

REFERENCES

Alaoui-Jamali, M.A., Rossignol, G., Schuller, H.M. Transplacental genotoxicity of a tobacco-specific *N*-nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in Syrian golden hamster. *Mutation Research* 223: 65-72, 1989.

Alexandrov, V.A., Popovich, I.G., Anisimov, V.N., Napalkov, N.P. Influence of hormonal disturbances on transplacental and multigeneration carcino-genesis in rats. *IARC Scientific Publication* 96: 35-49, 1989.

Anderson, L.M., Donovan, P.J., Rice, J.M. Risk assessment for transplacental carcinogenesis. In: *New Approaches in Toxicity Testing and Their Applications in Human Risk Assessment*, A.P. Li (Editor). New York: Raven Press, 1985a, pp. 179-202.

Anderson, L.M., Hagiwara, A., Kovatch, R.M., Rehm, S., Rice, J.M. Transplacental initiation of liver, lung, neurogenic, and connective tissue tumors by *N*-nitroso compounds in mice. *Fundamental and Applied Toxicology* 12: 604-620, 1989a.

Anderson, L.M., Hecht, S.S., Dixon, D.E., Dove, L.F., Kovatch, R.M., Amin, S., Hoffmann, D., Rice, J.M. Evaluation of the transplacental tumorigenicity of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mice. *Cancer Research* 49: 3770-3775, 1989b.

Anderson, L.M., Hecht, S.S., Kovatch, R.M., Amin, S., Hoffmann, D., Rice, J.M. Tumorigenicity of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in infant mice. *Cancer Letters* 58: 177-181, 1991a.

Anderson, L.M., Jones, A.B., Rice, J.M. Perinatal carcinogenesis: Current directions (meeting report). *British Journal of Cancer* 63: 1025-1028, 1991b.

Anderson, L.M., Jones, A.B., Riggs, C.W., Kovatch, R.M. Modification of transplacental tumorigenesis by 3-methylcholanthrene in mice by genotype at the *Ah* locus and pretreatment with β-naphthoflavone. *Cancer Research* 49: 1676-1681, 1989c.

Anderson, L.M., Jones, A.B., Riggs, C.W., Ohshima, M. Fetal mouse susceptibility to transplacental lung and liver carcinogenesis by 3-methylcholanthrene: Positive correlation with responsiveness to inducers of aromatic hydrocarbon metabolism. *Carcinogenesis* 6: 1389-1393, 1985b.

Berger, M.R., Petru, E., Habs, M., Schmahl, D. Influence of perinatal nicotine administration on transplacental carcinogenesis in Sprague Dawley rats by N-methylnitrosourea. *British Journal of Cancer* 55: 37-40, 1987.

Beniashvili, D.S., Zedginidze, T.G. Significance of modifying factors in the development of transplacental renal tumors in rabbits. *IARC Scientific Publication* 96: 51-55, 1989.

Bjalke, E., Schuman, L.M. Chewing of tobacco and use of snuff. Relationship to cancer of the pancreas and other sites in two prospective studies. In: *Proceedings of the 13th International Cancer Congress*. Seattle: 13th International Congress, 1982, p. 207.

Blanck, A., Rane, A., Toftgard, R., Gustafsson, J. Biotransformation of benzo[*a*]pyrene and 7-ethoxyresorufin and heme-staining proteins in microsomes from human fetal liver and placenta. *Biochemical Pharmacology* 32: 1547-1552, 1983.

Branstetter, D.G., Stoner, G.D., Budd, C., Conran, P.B., Goldblatt, P.J. Effect of gestational development on lung tumor size and morphology in the mouse. *Cancer Research* 48: 379-386, 1988.

Branstetter, D.G., Stoner, G.D., Budd, C., Conran, P.B., Goldblatt, P.J. Relationship between in utero development of the mouse liver and tumor development following transplacental exposure to ethylnitrosourea. *Cancer Research* 49: 3620-3626, 1989.

Bunin, G., Noller, K., Rose, P., Smith, E. Carcinogenesis. In: *Occupational and Environmental Reproductive Hazards: A Guide for Clinicians*, M. Paul (Editor). Baltimore: Williams and Wilkins, 1992.

Castonguay, A., Tjalve, H., Trushin, N., Hecht, S.S. Perinatal metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in C57BL mice. *Journal of the National Cancer Institute* 72: 1117-1126, 1984.

Chauhan, D.P., Miller, M.S., Owens, I.S., Anderson, L.M. Gene expression, ontogeny and transplacental induction of hepatic UDP-glucuronosyl transferase activity in mice. *Developmental Pharmacology and Therapeutics* 16: 139-149, 1991.

Coccia, P., Salmona, M., Diomede, L., Citti, L., Marioni, L., Romano, M. Liver DNA alkylation after a single carcinogenic dose of dimethylnitrosamine to newborn and adult CFW Swiss mice. *Chemico-Biological Interactions* 68: 259-271, 1988.

Coghlin, J., Gann, P.H., Hammond, S.K., Skipper, P.L., Taghizadeh, K., Paul, M., Tannenbaum, S.R. 4-Aminobiphenyl hemoglobin adducts in fetuses exposed to the tobacco smoke carcinogen in utero. *Journal of the National Cancer Institute* 83: 274-280, 1991.

Correa, E., Joshi, P.A., Castonguay, A., Schuller, H.M. The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is an active transplacental carcinogen in Syrian golden hamsters. *Cancer Research* 50: 3435-3438, 1990.

Correa, P., Pickle, L.W., Fontham, E., Lin, Y., Haenszel, W. Passive smoking and lung cancer. *Lancet* 2: 595-597, 1983.

Crespi, C.L., Penman, B.W., Gelboin, H.V., Gonzalez, F.J. A tobacco smoke-derived nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, is activated by multiple human cytochrome P_{450}s including the polymorphic human cytochrome P_{450} 2D6. *Carcinogenesis* 12: 1197-1201, 1991.

Crespi, C.L., Penman, B.W., Leakey, J.A.E., Arlotto, M.P., Stark, A., Parkinson, A., Turner, T., Steimel, D., Rudo, K., Davies, R.L., Langenbach, R. Human cytochrome P_{450} IIA3: cDNA sequence, role of the enzyme in the metabolic activation of promutagens, comparison to nitrosamine activation by human cytochrome P_{450} IIE1. *Carcinogenesis* 11: 1293-1300, 1990.

Cresteil, T., Beaune, P., Kremers, P., Celier, C., Guengerich, F.P., Leroux, J. Immunoquantification of epoxide hydrolase and cytochrome P-450 isozymes in fetal and adult human liver microsomes. *European Journal of Biochemistry* 151: 345-350, 1985.

Daubeze, M., Cassand, P., Migaud, M.L., Garrigue, P., Leng, J.J., Narbonne, J.F. Metabolic activation of benzo[*a*]pyrene by human amniotic fluid. *Comptes Rendus de l'Acadamie des Sciences* 302: 625-628, 1986.

Diaz Gomez, M.I., Tamayo, D., Castro, J.A. Administration of *N*-nitrosodimethylamine, *N*-nitrosopyrrolidine, or *N*-nitrosonornicotine to nursing rats: Their interactions with liver and kidney nucleic acids from sucklings. *Journal of the National Cancer Institute* 76: 1133-1136, 1986.

Diwan, B.A., Ohshima, M., Rice, J.M. Effects of postnatal administration of tumour-promoting barbiturates on the development of tumours initiated by prenatal exposure of fetal rats and mice to *N*-alkylnitrosoureas. *IARC Scientific Publication* 96: 75-80, 1989.

Foiles, P.G., Miglietta, L.M., Akerkar, S.A., Everson, R.B., Hecht, S.S. Detection of O^6-methyldeoxyguanosine in human placental DNA. *Cancer Research* 48: 4184-4188, 1988.

Frei, J.V. Toxicity, tissue changes, and tumor induction in inbred Swiss mice by methylnitrosoamine and -amide compounds. *Cancer Research* 30: 11-17, 1970.

Golding, J., Paterson, M., Kinlen, L.J. Factors associated with childhood cancer in a national cohort study. *British Journal of Cancer* 62: 304-308, 1990.

Goodman, M.T., Morgenstern, H., Wynder, E.L. A case-control study of factors affecting the development of renal cell cancer. *American Journal of Epidemiology* 124: 926-941, 1986.

Grufferman, S., Wang, H.H., DeLong, E.R., Kimm, S.Y.S., Delzell, E.S., Falletta, J.M. Environmental factors in the etiology of rhabdomyosarcoma in childhood. *Journal of the National Cancer Institute* 68: 107-113, 1982.

Hecht, S.S., Hoffmann, D. Tobacco-specific *N*-nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis* 9: 875-884, 1988.

Heuch, I., Kvale, G., Jacobsen, B.K., Bjelke, E. Use of alcohol, tobacco and coffee, and risk of pancreatic cancer. *British Journal of Cancer* 48: 637-643, 1983.

John, E.M., Savitz, D.A., Sandler, D.P. Prenatal exposure to parents' smoking and childhood cancer. *American Journal of Epidemiology* 133: 123-132, 1991.

Kabat, G.C., Dieck, G.S., Wynder, E.L. Bladder cancer in nonsmokers. *Cancer* 57: 362-367, 1986.

Karube, T., Odagirai, Y., Takemoto, K., Watanabe, S. Analyses of transplacentally induced sister chromatid exchanges and micronuclei in mouse fetal liver cells following maternal exposure to cigarette smoke. *Cancer Research* 49: 3550-3552, 1989.

Kitada, M., Kamataki, T., Itahashi, K., Rikihisa, T., Kanukobo, Y. Significance of cytochrome P-450 (P-450HFLa) of human fetal livers in the steroid and drug oxidations. *Biochemical Pharmacology* 36: 453-456, 1987.

Kitada, M., Kamataki, T., Itahashi, K., Rikihisa, T., Kato, R., Kanakubo, Y. Purification and properties of cytochrome P-450 from homogenates of human fetal livers. *Archives of Biochemistry and Biophysics* 241: 275-280, 1985a.

Kitada, M., Kamataki, T., Itahashi, K., Rikihisa, T., Kato, R., Kanakubo, Y. Immunochemical examinations of cytochrome P-450 in various tissues of human fetuses using antibodies to human fetal cytochrome P-450, P-450 HFLa. *Biochemical and Biophysical Research Communications* 131: 1154-1159, 1985b.

Kitada, M., Taneda, M., Ohta, K., Nagashima, K., Itahashi, K., Kamataki, T. Metabolic activation of aflatoxin B_1 and 2-amino-3-methylimidazo[4,5-f]-quinoline by human adult and fetal livers. *Cancer Research* 50: 2641-2645, 1990.

Komori, M., Nisho, K., Ohi, H., Kitada, M., Kamataki, T. Molecular cloning and sequence analysis of cDNA containing the entire coding region for human fetal liver cytochrome P-450. *Journal of Biochemistry* 105: 161-163, 1989a.

Komori, M., Nishio, K., Fujitani, T., Ohi, H., Kitada, M., Mima, S., Itahashi, K., Kamataki, T. Isolation of a new human fetal liver cytochrome P450 cDNA clone: Evidence for expression of a limited number of forms of cytochrome P450 in human fetal livers. *Archives of Biochemistry and Biophysics* 272: 219-225, 1989b.

LaVoie, E.J., Stern, S.L., Choi, C., Reinhardt, J., Adams, J.D. Transfer of the tobacco-specific carcinogens *N'*-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and benzo[*a*]pyrene in the milk of lactating rats. *Carcinogenesis* 8: 433-437, 1987.

Lofberg, B., Tjalve, H. The disposition and metabolism of *N*-nitrosodiethylamine in adult, infant, and foetal tissues of the Syrian golden hamster. *Acta Pharmacological et Toxicologica* 54: 104-114, 1984.

Loktionov, A., Hollstein, M., Martel, M., Galendo, D., Cabral, R.P., Tomatis, L., Yamasaki, Y. Tissue-specific activating mutations of Ha- and Ki-*ras* oncogenes in skin, lung, and liver tumors induced in mice following transplacental exposure to DMBA. *Molecular Carcinogenesis* 3: 134-140, 1990.

Maekawa, A., Odashima, S. Induction of tumors of the nervous system in ACI/N rat with 1-butyl-1-nitrosourea administered transplacentally, neonatally, or via maternal milk. *Gann* 66: 175-183, 1975.

McManus, M.E., Burgess, W.M., Veronese, M.E., Huggett, A., Quattrochi, L.C., Tukey, R.H. Metabolism of 2-acetylaminofluorene and benzo[*a*]pyrene and activation of food-derived heterocyclic amine mutagens by human cytochromes P-450. *Cancer Research* 50: 3367-3376, 1990.

Miller, M.S., Jones, A.B., Anderson, L.M. The formation of 3-methylcholanthrene-initiated lung tumors correlates with induction of cytochrome P_{450} IA1 by the carcinogen in fetal but not adult mice. *Toxicology and Applied Pharmacology* 104: 235-245, 1990a.

Miller, M.S., Jones, A.B., Chauhan, D.P., Anderson, L.M. Role of the maternal environment in determining susceptibility to transplacentally induced chemical carcinogenesis in mouse fetuses. *Carcinogenesis* 11: 1979-1984, 1990b.

Miller, M.S., Jones, A.B., Chauhan, D.P., Park, S.S., Anderson, L.M. Differential induction of fetal mouse liver and lung cytochromes P-450 by β-naphthoflavone and 3-methylcholanthrene. *Carcinogenesis* 10: 875-883, 1989.

Mohr, U., Althoff, J. Carcinogenic activity of aliphatic nitrosamines via the mother's milk in the offspring of Syrian golden hamsters. *Proceedings of the Society for Experimental Biology and Medicine* 136: 1007-1009, 1971.

Mohr, U., Emura, M., Aufderheide, M., Riebe, M., Ernst, H. Possible role of genetic predisposition in multigeneration carcinogenesis. *IARC Scientific Publication* 89: 93-103, 1989.

Morel, F., Beaune, P.H., Ratanasavanh, D., Flinois, J., Yang, C.S., Guengerich, F.P., Guillouzo, A. Expression of cytochrome P-450 enzymes in cultured human hepatocytes. *European Journal of Biochemistry* 191: 437-444, 1990.

Murray, G.I., Foster, C.O., Barnes, T.S., Weaver, R.J., Snyder, C.P., Ewen, S.W.B., Melvin, W.T., Burke, M.D. Cytochrome P_{450} IA expression in adult and human fetal liver. *Carcinogenesis* 13: 165-169, 1992.

Naito, M., Aoyama, H., Ito, A. Inhibitory effect of phenobarbital on the development of gliomas in WF rats treated neonatally with *N*-ethyl-*N*-nitrosourea. *Journal of the National Cancer Institute* 74: 725-728, 1985.

Napalkov, N.P., Likhachev, A., Anisimov, V., Loktionov, A., Zabezhinski, M., Ovsyannikov, A., Wahrendorf, J., Becher, H., Tomatis, L. Promotion of skin tumours by TPA in the progeny of mice exposed pre-natally to DMBA. *Carcinogenesis* 8: 381-385, 1987.

Neubert, D., Tapken, S. Transfer of benzo[*a*]pyrene into mouse embryos and fetuses. *Archives of Toxicology* 62: 236-239, 1988.

Nicolov, I.G., Chernozemsky, I.N. Tumors and hyperplastic lesions in Syrian hamsters following transplacental and neonatal treatment with cigarette smoke condensate. *Journal of Cancer Research and Clinical Oncology* 94: 249-256, 1979.

Nomura, T. Carcinogenesis by urethan via mother's milk and its enhancement of transplacental carcinogenesis in mice. *Cancer Research* 33: 1677-1683, 1973.

Nomura, T., Okamoto, E., Tateishi, N., Kimura, S., Isa, Y., Manabe, H., Sakamoto, Y. Tumor induction in the progeny of mice receiving 4-nitroquinoline 1-oxide and *N*-methyl-*N*-nitrosourethan during pregnancy or lactation. *Cancer Research* 34: 3373-3378, 1974.

Ogawa, K., Yokokawa K., Tomoyori, T., Onoe, T. Induction of α-glutamyltranspeptidase-positive altered hepatocyte lesions by combination of transplacental-initiation and postnatal-selection. *International Journal of Cancer* 29: 333-336, 1982.

Omiecinski, C.H., Redlich, C.A., Costa, P. Induction and developmental expression of cytochrome P_{450} IA1 messenger RNA in rat and human tissues: Detection by the polymerase chain reaction. *Cancer Research* 50: 4315-4321, 1990.

Orlandi, M.A., Boyd, G. Smokeless tobacco use among adolescents: A theoretical overview. *National Cancer Institute Monographs* 8: 5-12, 1989.

Pasanen, M., Haaparanta, T., Sundin, M., Sivonen, P., Vakakangas, K., Raunio, H., Hines, R., Gustafsson, J., Pelkonen, L. Immunochemical and molecular biological studies on human placental cigarette smoke-inducible cytochrome P_{450}-dependent monooxygenase activities. *Toxicology* 62: 175-187, 1990.

Pasanen, M., Pelkonen, O. Human placental xenobiotic and steroid biotransformations catalyzed by cytochrome P450, epoxide hydrolase, and glutathione S-transferase activities and their relationships to maternal cigarette smoking. *Drug Metabolism Reviews* 21: 427-461, 1990.

Paulson, R.M., Shanfel, J., Prause, L., Iranpour, S., Paulson, J.O. Pre-conceptional and post-conceptional tobacco effects on the CD-1 mouse fetus. *Journal of Craniofacial Genetics and Developmental Biology* 11: 48-58, 1991.

Pelkonen, O. Fetoplacental biochemistry—xenobiotic metabolism and pharmacokinetics. In: *Occupational Hazards and Reproduction*, K. Hemminki, M. Sorsa, and V. Vainio (Editors). Washington: Hemisphere Publishing, 1985, pp. 113-126.

Perantoni, A.O., Rice, J.M., Reed, C.D., Watatani, M., Wenk, M.F. Activated *neu* oncogene sequences in primary tumors of the peripheral nervous system induced in rats by transplacental exposure to ethylnitrosourea. *Proceedings of the National Academy of Sciences* 84: 6317-6321, 1987.

Platzek, T., Bochert, G., Rahm, U. Embryotoxicity induced by alkylating agents. Teratogenicity of acetoxymethyl-methylnitrosamine: Dose-response relationship, application route dependency and phase specificity. *Archives of Toxicology* 52: 45-69, 1983.

Preston-Martin, S. Epidemiological studies of perinatal carcinogenesis. *IARC Scientific Publication* 89: 289-313, 1989.

Preston-Martin, S., Yu, M.C., Benton, B., Henderson, B.E. *N*-Nitroso compounds and childhood brain tumors: A case-control study. *Cancer Research* 42: 5240-5245, 1982.

Remmer, H. Passively inhaled tobacco smoke: A challenge to toxicology and preventive medicine. *Archives of Toxicology* 61: 89-104, 1987.

Rice, J.M. Effects of prenatal exposure to chemical carcinogens and methods for their detection. In: *Developmental Toxicology*, C.A. Kimmel and J. Buelke-Sam (Editors). New York: Raven Press, 1981, pp. 191-212.

Rice, J.M., Joshi, S.R., Shenefelt, R.E., Wenk, M.L. Transplacental carcinogenic activity of 7,12-dimethylbenz[*a*]anthracene. In: *Carcinogenesis, Polynuclear Aromatic Hydrocarbons* (volume 3), P.W. Jones and R.I. Freudenthal (Editors). New York: Raven Press, 1978, pp. 413-422.

Rice, J.M., Rehm, S., Donovan, P.J., Perantoni, A.O. Comparative transplacental carcinogenesis by directly acting and metabolism dependent alkylating agents in rodents and nonhuman primates. *IARC Scientific Publication* 96: 17-34, 1989.

Rivrud, G.N., Berg, K., Anderson, D., Blowers, S., Bjro, K. Mutagenic effect of amniotic fluid from smoking women at term. *Mutation Research* 171: 71-77, 1986.

Rossi, L., Barbieri, O., Sanguineti, M., Staccione, A., Santi, L.F., Santi, L. Carcinogenic activity of benzo[*a*]pyrene and some of its synthetic derivatives by direct injection into the mouse fetus. *Carcinogenesis* 4: 153-156, 1983.

Rossignol, G., Alaoui-Jamali, M.A., Castonguay, A., Schuller, H.M. Metabolism and DNA damage induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in fetal tissues of the Syrian golden hamster. *Cancer Research* 49: 5671-5676, 1989.

Sandler, D.P., Everson, R.B., Wilcox, A.J., Browder, J.P. Cancer risk in adulthood from early life exposure to parents' smoking. *American Journal of Public Health* 75: 487-492, 1985.

Shields, P.G., Povey, A.C., Wilson, V.L., Weston, A., Harris, C.C. Combined high-performance liquid chromatography/^{32}P-postlabeling assay of N^7-methyldeoxyguanosine. *Cancer Research* 50: 6580-6584, 1990.

Shimada, T., Iwasaki, M., Martin, M.V., Guengerich, F.G. Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by *umu* gene response in *Salmonella typhimurium* TA 1535/pSK1002. *Cancer Research* 49: 3218-3228, 1989a.

Shimada, T., Martin, M.V., Pruess-Schwartz, D., Marnett, L.J., Guengerich, F.P. Role of individual human cytochrome P-450 enzymes in the bioactivation of benzo[*a*]pyrene, 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene, and other dihydrodiol derivatives of polycyclic aromatic hydrocarbons. *Cancer Research* 49: 6304-6312, 1989b.

Stjernfeldt, M., Berglund, K., Lindsten, J., Ludvigsson, J. Maternal smoking during pregnancy and risk of childhood cancer. *Lancet* 1: 1350-1352, 1986.

Suganuma, M., Fujiki, H., Morino, K., Takayama, S., Sugimura, T. Tumor promoting activity of teleocidin in skin and forestomach of mice initiated transplacentally with 7,12-dimethylbenz[*a*]anthracene. *Journal of Cancer Research and Clinical Oncology* 113: 123-125, 1987.

Sukumar, S., Barbacid, M. Specific patterns of oncogene activation in transplacentally induced tumors. *Proceedings of the National Academy of Sciences* 87: 718-722, 1990.

Toth, B. Critical review of experiments in chemical carcinogenesis using newborn animals. *Cancer Research* 28: 727-738, 1968.

Toth, B., Magee, P.N., Shubik, P. Carcinogenesis study with dimethylnitrosamine administered orally to adult and subcutaneously to newborn BALB/c mice. *Cancer Research* 24: 1712-1721, 1964.

Treluyer, J.M., Jacqx-Aigrain, E., Alvarez, F., Cresteil, T. Expression of CYP2D6 in developing human liver. *European Journal of Biochemistry* 202: 583-588, 1991.

Truhaut, R., Lesca, P., Dechambre, R.P., Gerard-Marchant, P. Sur les modalites de manifestation du pouvoir cancerogene du benzo-3-4-pyrene chez la souris nouveau-nee ou traitee immediatement apres le sevrage. *Pathologie et Biologie (Paris)* 14: 955-959, 1966.

Turusov, V.S., Cardis, E. Review of experiments of multigeneration carcinogenicity: Discussion of design, experimental models and analyses. *IARC Scientific Publication* 89: 105-120, 1989.

Vesselinovitch, S.D., Rao, K.V.N., Mihailovich, N. Transplacental and lactational carcinogenesis by safrole. *Cancer Research* 39: 4378-4380, 1979.

Wrighton, S.A., Vandenbranden, M. Isolation and characterization of human fetal liver cytochrome P_{450} HLp2: A third member of the P_{450} III gene family. *Archives of Biochemistry and Biophysics* 268: 144-151, 1989.

Yamasaki, H., Hollstein, M., Martel, N., Galenda, D., Cabral, R.P., Tomatis, L. Transplacental induction of a specific mutation in fetal Ha-*ras* and its critical role in postnatal carcinogenesis. *International Journal of Cancer* 40: 818-822, 1987.

Interaction Between Smokeless Tobacco-Related Carcinogens and Human Papillomaviruses in the Pathogenesis of Oral Dysplasia and Cancer[1]

Joel M. Palefsky, John S. Greenspan, Troy E. Daniels, Jennifer Berline, Deborah G. Grady, and Virginia L. Ernster

ABSTRACT Several lines of evidence point to a role for smokeless tobacco-related carcinogens in the pathogenesis of oral cancer. Previous studies of oral cancer suggest that other factors may play a role as well, including use of alcohol and infection with human papillomavirus (HPV). To examine the role of HPV in the pathogenesis of ST-related oral cancer, we sought HPV DNA in three different sets of formalin-fixed biopsy tissues, using the polymerase chain reaction (PCR) technique: (1) nondysplastic oral lesions associated with short-term ST use, (2) oral cancers and dysplasias associated with long-term use of ST, and (3) oral cancers and dysplasias not related to the use of ST. In these studies HPV was not detected in early, nondysplastic lesions associated with short-term use. However, HPV was detectable in approximately 30 percent of the oral cancers—whether or not they were associated with ST use. These results suggested that HPV infection may be one of several factors contributing to the pathogenesis of ST-related oral cancer, and that HPV infection does not occur at the earliest stages of ST lesion development.

INTRODUCTION It has been estimated that 30 percent of all cancers in the United States are associated with the use of tobacco in its various forms (Doll and Peto, 1981; Wynder and Gori, 1977). Approximately 53 million Americans are believed to smoke, and approximately 12 million to use smokeless tobacco (Hecht and Hoffmann, 1988). Smoking is strongly associated with malignancy of the respiratory tract, upper digestive tract, bladder, kidney, and pancreas (Doll and Peto, 1981; Wynder and Gori, 1977). There is also a large body of evidence linking smoking to oral cancer (Doll and Peto, 1981; Wynder and Gori, 1977), and death rates from oral cancer are four times higher among smokers than nonsmokers (Doll and Peto, 1976; Hammond, 1966; Rogot and Murray, 1980). Oral cancer is also strongly associated with the use of smokeless tobacco, especially snuff (Consensus Conference, 1986; IARC, 1985; US DHHS, 1986; Vogler et al., 1962; Winn, 1988); the incidence of cancer of the cheek and gums has been shown to be as much as 50 times higher among long-term ST users than among age-matched controls (Winn, 1986).

The mechanisms by which smoking and ST contribute to the development of oral cancer or its precursors are not yet known. One potential mechanism is the direct effect of carcinogens in cigarette smoke or ST on the oral epithelium. Tobacco products are known to contain a number of substances that may give rise to carcinogens (Hecht and Hoffmann, 1988).

[1] This study was supported by National Institute of Dental Research grant no. PO1 DE08547.

The most important of these are nicotine, a tertiary amine, and nornicotine, anabasine, and anatabine, which are secondary amines. These compounds may react with nitrosating agents to form nitrosamines (NNN, NNK, NNA, NAB, and NAT), which have been shown to be carcinogenic in mice, rats, and hamsters (Hecht and Hoffmann, 1988; Hoffmann and Hecht, 1985). Other potential carcinogens found in smoked tobacco products include formaldehyde, acetaldehyde, croton aldehyde, and benzopyrene. The mechanism by which nitrosamines and other potential carcinogens act to promote malignancy is not yet known. One possibility is modification of cellular DNA by nitrosamines. There are several mechanisms by which this could occur; for example, nitrosamines may be α-hydroxylated by specific cytochrome P_{450} isozymes to yield methyldiazohydroxide, which is capable of methylating DNA to produce O^6-methylguanine and other point mutations.

A second mechanism may involve induction of chromosomal DNA breakage. It has been shown that tobacco-related products may induce single-stranded DNA breaks because of the reduction of oxygen to superoxide radicals (Borish et al., 1987), and the resulting repair process may increase the likelihood of a mutation (Richter et al., 1988). These mutations may then contribute to carcinogenesis by altering the levels or functions of genes involved in the control of cell growth.

In addition to the use of tobacco products, viral infection is increasingly implicated in the pathogenesis of oral cancer. Oral cancer bears significant resemblance to other mucosal epithelial cancers, such as cancer of the cervix and anus, both of which have been associated with infection with human papillomavirus (HPV) (Beaudenon et al., 1986; Boshart et al., 1984; Crum et al., 1985; Lorincz et al., 1986 and 1987; Reid et al., 1987). The association between HPV infection and mucosal cancer has been characterized best for anogenital cancer. Of the more than 60 known HPV types, infection with HPV types 16 and 18 is most closely associated with anogenital cancer and high-grade intraepithelial neoplasia (grades 2-3); types 31, 33, and 35 are associated with an intermediate risk of cancer; and types 6, 11, 42, 43, and 44 are associated with low-grade intraepithelial neoplasia (grade 1) and condyloma, both of which carry a low risk of progression to invasive disease.

The relationship between HPV infection and oral cancer was first described in 1983, when cytopathic changes of HPV infection (koilocytosis) similar to those seen in cervical HPV infection were noted in oral lesions (Syrjanen et al., 1983). Subsequent to that report, several investigators have looked for HPV DNA in oral lesions (Adler-Storthz et al., 1986; Dekmezian et al., 1987; Kashima et al., 1990; Loning et al., 1985 and 1987; Lookingbill et al., 1987; Maitland et al., 1987; Milde and Loning, 1986; Ostrow et al., 1987; Scully et al., 1985; Syrjanen et al., 1988; de Villiers et al., 1985 and 1986), using a wide variety of HPV DNA hybridization techniques with differing sensitivities and specificities. In one study, involving dot blot hybridization, HPV DNA was detected in 8 of 22 oral cancers (36 percent): type 16/18 in 3 cancers, type 6/11 in 1, and other types in 4 other cancers (Loning et al., 1987). Using in situ hybridization, one group reported detection of HPV

DNA in 6 of 51 (12 percent) oral cancer specimens, and 6 of 21 (29 percent) oral dysplasia specimens (Syrjanen et al., 1988). Using Southern blot hybridization, another group detected HPV sequences in almost 50 percent of invasive cancers, but also detected HPV in a similar proportion of normal tissues (Maitland et al., 1987). Using a similar technique, Kashima et al. reported that 7 of 74 (9 percent) were positive for HPV DNA. In this series of tests, a wide range of types were identified, including HPV-16, -6, -57, -3, and -13 (Kashima et al., 1990). Similar to the report by Maitland et al. (1987), HPV DNA was detectable in 3 of 33 (9 percent) clinically normal tissues obtained from the mucosal surface contralateral to the lesion. Taken together, these studies suggest that HPV DNA is detectable in fewer than half of the specimens of oral cancer, and that a wide range of HPV types may be present. Moreover, detection of HPV DNA in clinically normal oral tissues in proportions similar to those of the cancerous lesions in some studies raises the question of the nature of the contribution of HPV to the pathogenesis of oral lesions.

The techniques used in the studies described above, that is, Southern blot hybridization, dot blot hybridization, and in situ hybridization, all have limited sensitivity. Thus, the inability to detect HPV DNA in a higher proportion of oral cancers and precancerous lesions may reflect a level of HPV infection below the limit of the sensitivity of these tests or infection with HPV types other than would be normally detected with currently available probes. To address the question of the prevalence of HPV in oral dysplasias and cancers, using DNA detection techniques that are both highly sensitive and capable of detecting a large number of HPV types, we used the PCR technique with HPV L1 consensus primers.

Studies of the role of HPV in ST-associated lesions provide a unique opportunity to determine the stage at which HPV infection may begin to play a role in the pathogenesis of oral cancer for two reasons: (1) the use of ST products is clearly associated with low-grade lesions such as hyperkeratosis and hyperorthokeratosis, which may represent precursors to dysplasia, given sufficient exposure; and (2) as described previously, use of ST products is also clearly a risk factor for development of oral cancer. Using in situ hybridization, Greer et al. (1990) studied 50 leukoplakias associated with the use of ST, and found 2 (4 percent) containing HPV-6 and 3 (6 percent) containing HPV-2. Therefore, to address the question of the stage of precancerous disease at which HPV may first be detected through more sensitive techniques, we used PCR also to study ST-associated lesions ranging from histologically normal to invasive cancer.

METHODS One hundred eight oral biopsies ranging from histologically normal to invasive cancer were obtained, fixed in formalin, and embedded in paraffin. To perform PCR, tissue sections 7 μm in thickness were cut from each paraffin block, and the paraffin was removed by suspending the section in 500 μL of xylene in its original Eppendorf tube. The tissue was dried, resuspended in 100 μL of water containing proteinase K at a concentration of 100 μg/mL, and digested overnight at 37 °C. Consensus primers for the detection of the L1 region were employed as described elsewhere (Ting et al., 1990). Positive

controls consisted of the amplification of human beta-hemoglobin DNA from each tissue. Negative controls were used to minimize the possibility of false-positives attributable to contamination from one specimen to another in the laboratory, and consisted of amplification of each mixture with all components of the reaction except target DNA. Fifty cycles of amplification were performed with 800.0 μM dNTPs (United States Biochemical Corp., Cleveland, Ohio), 1.0 μM of each primer, 2.5 mM MgCl2, 0.5% Tween-20, 0.5% NP-40, and 50 units/mL AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Connecticut). Five microliters of 100 μL amplification product were applied to a nylon membrane and the presence of HPV DNA was sought with ^{32}P-labeled consensus probes. Positive samples identified positive with the consensus probes were then studied with probes specific for HPV-6, -11, -16, -18, -31, and -33.

RESULTS To begin to understand the stages of oral disease at which HPV infection may occur, we examined 52 biopsies obtained from professional baseball players who were short-term users of ST. The tissues represented 26 biopsies with histologically diagnosed lesions ranging from hyperparakeratosis to basal cell hyperplasia (Daniels et al., this volume; Grady et al., 1991) and 26 histologically normal biopsies adjacent to the lesion, which served as controls. None of the tissues demonstrated dysplastic changes. In these studies HPV-33 could be detected in only one hyperparakeratotic lesion, and HPV type 6/11 in the normal tissue adjacent to another hyperparakeratotic lesion, which was itself HPV-negative.

To study the prevalence of HPV in high-grade lesions associated with long-term use of ST, 10 high-grade dysplasia/oral cancer lesions and 4 histologically normal control tissues were obtained from the laboratory of Dr. George Kaugars of the University of Virginia. In contrast to the low-grade lesions obtained from the baseball players, 3 of 10 high-grade dysplasia/oral cancer lesions (30 percent) associated with long-term use of ST were found to be HPV-16 DNA-positive; none of the normal control tissues were positive for HPV DNA.

Thirty-two oral cancer and dysplasia tissues not associated with use of ST were studied, along with 10 negative controls. The results indicated that 8 of 25 of the oral cancer tissues (32 percent) were HPV DNA-positive and 3 of 7 (43 percent) of high-grade oral dysplasia were HPV DNA-positive (Palefsky et al., unpublished data). The HPV types detected were found to be heterogeneous; fewer than half of the HPV types detected were represented by the common anogenital HPV types. In contrast to the oral dysplasia and cancer tissues, none of seven fibromas and none of three normal tissues were positive for HPV.

DISCUSSION Studies of the prevalence of HPV in ST-associated lesions in short-term and long-term users are not strictly comparable, since the latter represented an older population from a specific geographic area of the United States. Nevertheless, results of these studies suggest that the low-grade non-dysplastic lesions associated with short-term ST use (< 5 yr) are not associated with HPV, whereas high-grade ST-associated lesions contain HPV DNA in a proportion similar to that seen in oral dysplasia and cancers that are not

associated with ST use. These results suggest that HPV does not play a significant role in the pathogenesis of ST-associated disease at its earliest stages, but instead begins to be detectable in association with dysplasia. While dysplastic and cancerous tissues not associated with use of ST were also found to be associated with HPV, a large number of cases of oral cancer and dysplasia were HPV-negative on PCR examination, consistent with studies using less sensitive techniques. Oral cancer therefore appears to be a more heterogeneous disease than cervical cancer with respect to etiology; while HPV may play a significant role in the pathogenesis of oral cancer, it is clear that it need not necessarily be present, and that other factors are likely to be important as well.

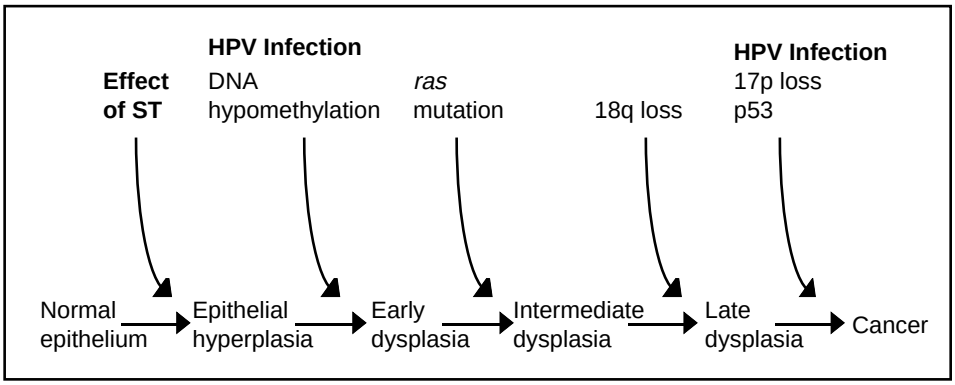
Among other factors that may play a role in the pathogenesis of oral cancer are genetic mutations. Reports have been published that document the sequence of cellular changes at the molecular level that occur as a benign colonic adenoma progresses to invasive colorectal cancer (Baker et al., 1990; Fearon et al., 1990). In these studies, a series of genetic mutations accumulated as the lesion progressed, and the accumulation of further abnormalities was necessary for progression to the next stage of disease. These included mutations in the *ras* oncogene, translocations of the short arm of chromosome 5, and breakage of the long arm of chromosome 11. A model such as this may also be applicable to the progressive changes that occur in the oral mucosa as a lesion develops from benign to increasing grades of dysplasia and, ultimately, to invasive cancer.

On the basis of the data described above, we propose a model for the development of ST-associated cancer (Figure 1). In this model, ST-related products are responsible for the development of low-grade nondysplastic lesions, including hyperparakeratosis, hyperorthokeratosis, and basal cell hyperplasia, that may progress to dysplasia with continued exposure. It is not yet known if these low-grade lesions are associated with chromosomal abnormalities or oncogenic mutations. With continued exposure to these products, as well as other cofactors such as alcohol, further genetic damage may accumulate, resulting in dysplasia. ST products may induce these chromosomal changes via DNA adduct formation or chromosomal breakage. The result of these changes may include activation of oncogenes such as *ras* or inactivation of a member of the anti-oncogene family, such as the RB or p53 proteins. We also propose that infection with HPV may represent one of the cofactors that play a role, in conjunction with ST products, in the pathogenesis of ST-related dysplasia and cancer. At this time, it is not known if dysplasia occurs as a result of HPV infection or if HPV infects a preestablished dysplastic lesion and potentiates its progression. However, recent studies of HPV-16-transfected cells with the floating raft model of epithelial differentiation have been conducted, in which transfection of HPV-16 DNA has been shown to result in epithelial changes consistent with dysplasia (McCance et al., 1988). This supports the hypothesis that dysplasia results from HPV infection.

Once established, HPV infection may contribute to the development and/or progression of dysplasia in a number of ways. Among these are the binding and inactivation of p53 gene product by HPV E6 protein, as well as

Figure 1

Proposed schema for the development of ST-related oral cancer. In this schema, several different etiologic factors may play a significant role. ST appears to play an important role and may continue to do so at any subsequent stage. HPV does not appear to play an important role at the earliest stages but may facilitate development and progression of dysplasia in some ST-related lesions. Like ST, HPV may also play a role in development of invasive cancer. A variety of other factors such as *ras* mutations or loss of chromosomal arms may also play a role at different stages of disease. The alterations depicted in this figure were adapted from studies of the development of colon cancer (Fearon and Vogelstein, 1990); similar studies of ST-related cancer have not yet been performed.



the inactivation of the retinoblastoma gene product by the HPV E7 protein (Munger et al., 1989; Werness et al., 1990). In addition, other mechanisms by which HPV contributes to the development of dysplasia almost certainly exist and remain to be characterized.

In summary, ST-related oral cancer may result from cumulative genetic damage induced by ST, with or without other cofactors. Among possible significant cofactors is HPV infection, which does not appear to play a role in pathogenesis of the earliest ST-related lesions, but may instead play a role at later stages, beginning with dysplasia.

This model suggests that the development of ST-related cancers may represent the end product of cooperation between a number of different factors, including HPV infection. Prevention of cancer development must therefore focus on interference with this process at a number of levels. Cessation of exposure to ST-related products remains the most important intervention, but interference with potential cofactors, including abstinence from alcohol, may play an important role as well. Currently, no therapies are available to interfere with HPV gene products, and the only method of preventing the consequences of HPV infection is the removal of HPV-infected lesional tissue. Nevertheless, if and when therapies directed against HPV do become available, they may constitute useful adjunctive measures for those cases in which HPV infection can be demonstrated.

Much remains to be learned about the pathogenesis of ST-related cancer at the molecular level. The evidence for an important role for HPV in the pathogenesis of oral cancer in general, and ST-related oral cancer in particular, remains incomplete; studies of larger numbers of dysplastic and cancerous oral tissues obtained from populations with well-matched controls are needed. Such studies should include analysis of histologically normal control tissues from matched control subjects, as well as of normal tissue from the individuals with oral lesions. Standardized methods of HPV detection are needed, as well as standardization of histopathologic criteria for grading the lesions. Adequate in vitro models for studying the interaction between ST products, HPV, and alcohol are needed; the recently developed floating raft system may be very useful for this purpose. Further research is needed to define which of the ST-related products are most toxic to epithelial cells; whether and how HPV gene products interact with ST products to induce dysplastic changes; characterization of chromosomal changes induced by ST; characterization of the role of oncogenic activators, or inactivation of anti-oncogenes in these cells; characterization of the HPV types present in ST-related cancer; and characterization of the risk factors for acquisition of HPV infection. Knowledge of cofactors that play a role along with ST products will permit a more effective approach to the prevention of ST-related dysplasia and cancer. Knowledge of the molecular mechanisms of the pathogenesis of these diseases will also be of great value in preventing progression or inducing regression of ST-related dysplasia, and in preventing progression to cancer among those in whom dysplasia has already occurred.

REFERENCES

Adler-Storthz, K., Newland, J.R., Tessin, B.A., et al. Identification of human papillomavirus types in oral verruca vulgaris. *Journal of Oral Pathology and Medicine* 15: 230-233, 1986.

Baker, S.J., Preisinger, A.C., Jessup, J.M., et al. p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Research* 50: 7717-7722, 1990.

Beaudenon, S., Kremsdorf, D., Croissant, O., et al. A novel type of human papillomavirus associated with genital neoplasias. *Nature* (London) 321: 246-249, 1986.

Borish, E.T., Pryor, W.A., Venugopal, S., et al. DNA synthesis is blocked by cigarette tar-induced DNA single-strand breaks. *Carcinogenesis* 8: 1517-1520, 1987.

Boshart, M., Gissmann, L., Ikenberg, H., et al. A new type of papillomavirus: Its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO Journal* 3: 1151-1157, 1984.

Consensus Conference. Health implications of smokeless tobacco use. *Journal of the American Medical Association* 255: 1045-1048, 1986.

Crum, C., Mitao, M.R., Levine, R., et al. Cervical papillomaviruses segregate within morphologically distinct precancerous lesions. *Journal of Virology* 54: 675-681, 1985.

Dekmezian, R.H., Batsakis, J.G., Goepfert, H. In situ hybridization of papillomavirus DNA in head and neck squamous cell carcinomas. *Archives of Otolaryngology and Head and Neck Surgery* 113: 819-821, 1987.

Doll, R., Peto, R. Mortality in relation to smoking: 20 years' observations on male British doctors. *British Medical Journal* 2: 1525-1536, 1976.

Doll, R., Peto, R. The causes of cancer: Quantitative estimate of avoidable risks of cancer in the United States today. *Journal of the National Cancer Institute* 66: 1191-1308, 1981.

Fearon, E.R., Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell* 61: 759-767, 1990.

Grady, D., Greene, J., Ernster, V.L., et al. Short-term changes a surprise with smokeless tobacco oral lesions. *Journal of the American Dental Association* 122: 62-64, 1991.

Greer, R.O., Eversole, L.R., Crosby, L.K. Detection of human papillomavirus-genomic DNA in oral epithelial dysplasias, oral smokeless tobacco-associated leukoplakias and epithelial malignancies. *Journal of Oral and Maxillofacial Surgery* 48: 1201-1205, 1990.

Hammond, E.C. Smoking in relation to the death rates of one million men and women. *National Cancer Institute Monograph* 19: 127-204, 1966.

Hecht, S.S., Hoffmann, D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis* 9: 875-884, 1988.

Hoffmann, D., Hecht, S.S. Nicotine-derived *N*-nitrosamines and tobacco-related cancer: Current status and future questions. *Cancer Research* 45: 935-944, 1985.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*: *Tobacco Habits Other than Smoking: Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985, pp. 113-116.

Kashima, H.K., Kutcher, M., Kessis, T., et al. Human papillomavirus in squamous cell carcinoma, leukoplakia, lichen planus, and clinically normal epithelium of the oral cavity. *Annals of Otology, Rhinology and Laryngology* 99: 55-61, 1990.

Loning, T., Ikenberg, H., Becker, J., et al. Analysis of oral papillomas, leukoplakia and invasive carcinomas for human papillomavirus type related DNA. *Journal of Investigative Dermatology* 84: 417-420, 1985.

Lookingbill, D.P., Kreider, J.W., Howitt, M.K., et al. Human papillomavirus type 16 in bowenoid papulosis, intraoral papillomas, and squamous cell carcinoma of the tongue. *Archives of Dermatology* 123: 363-368, 1987.

Lorincz, A.T., Lancaster, W., Temple, G. Cloning and characterization of a new human papillomavirus from a woman with dysplasia of the uterine cervix. *Journal of Virology* 58: 225-229, 1986.

Lorincz, A.T., Quinn, A., Lancaster, W., et al. A new type of papillomavirus associated with cancer of the uterine cervix. *Virology* 159: 187-190, 1987.

Maitland, N.J., Cox, M.F., Lynas, C., et al. Detection of human papillomavirus DNA in biopsies of human oral tissue. *British Journal of Cancer* 56: 245-250, 1987.

McCance, D.J., Kopan, R., Fuchs, E., et al. Human papillomavirus type 16 alters human epithelial cell differentiation in vitro. *Proceedings of the National Academy of Sciences* 85: 7169-7173, 1988.

Milde, K., Loning, T. The detection of papillomavirus DNA in oral papillomas and carcinomas: Application of in situ hybridization with biotinylated HPV 16 probes. *Journal of Oral Pathology* 15: 292-296, 1986.

Munger, K., Werness, B.A., Dyson, N., et al. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. *EMBO Journal* 8: 4099-4105, 1989.

Ostrow, R.S., Manias, D.A., Fong, W.J., et al. A survey of human cancers for human papillomavirus DNA by filter hybridization. *Cancer* 59: 429-434, 1987.

Reid, R., Greenberg, M., Jenson, A.B., et al. Sexually transmitted papillomaviral infections. I. The anatomic distribution and pathologic grade of neoplastic lesions associated with different viral types. *American Journal of Obstetrics and Gynecology* 156: 212-222, 1987.

Richter, C., Park, J-W., Ames, B.N. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proceedings of the National Academy of Sciences* 85: 6465-6467, 1988.

Rogot, E., Murray, J.L. Smoking and the causes of death among U.S. veterans: 16 years of observation. *Public Health Reports* 95: 213-222, 1980.

Scully, C., Prime, S., Maitland, N. Papillomaviruses: Their role in oral disease. *Oral Surgery, Oral Medicine, Oral Pathology* 60: 166-174, 1985.

Syrjanen, K., Syrjanen, S.M., Lamberg, M., et al. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *International Journal of Oral Surgery* 12: 418-424, 1983.

Syrjanen, S.M., Syrjanen, K.J., Happonen, R-P. Human papillomavirus (HPV) DNA sequences in oral precancerous lesions and squamous cell carcinoma demonstrated by in situ hybridization. *Journal of Oral Pathology* 17: 273-278, 1988.

Ting, Y., Manos, M.M. In: *PCR Protocols and Applications*. New York: Academic Press, 1990, pp. 356-367.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHSS Publication No. (NIH) 86-2874, 1986, pp. 33-58.

de Villiers, E-M., Neumann, C., Le, J-Y., et al. Infection of the oral mucosa with defined types of human papillomavirus. *Medical Microbiology and Immunology* 174: 287, 1986.

de Villiers, E-M., Wiedauer, H., Otto, H., et al. Papillomavirus in human tongue carcinomas. *International Journal of Cancer* 36: 575, 1985.

Vogler, W.R., Lloyd, J.W., Milmore, B.K. A retrospective study of etiologic factors in cancer of the mouth, pharynx and larynx. *Cancer* 15: 246-258, 1962.

Werness, B.A., Levine, A.J., Howley, P.M. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 248: 76-79, 1990.

Winn, D.M. Smokeless tobacco and oral/pharynx cancer: The role of cofactors. *Banbury Report* 23: 361-371, 1986.

Winn, D.M. Smokeless tobacco and cancer: The epidemiologic evidence. *CA—A Cancer Journal for Clinicians* 38: 236-243, 1988.

Wynder, E.L., Gori, G.B. Contribution of the environment to cancer incidence: An epidemiologic exercise. *Journal of the National Cancer Institute* 58: 825-832, 1977.

Identification of Human Papillomavirus DNA in Smokeless Tobacco Keratoses And Premalignant and Malignant Oral Lesions, by PCR Amplification With Consensus Sequence Primers[1]

Robert O. Greer, Jr., Kenneth R. Shroyer, and Louise Crosby

ABSTRACT Human papillomaviruses (HPVs), trophic for cutaneous and mucosal epithelium, have been reported in association with benign, dysplastic, and malignant proliferations of the oral mucous membrane. More than 60 HPV types have been recognized on the basis of significant sequence divergence following molecular cloning as recombinant DNAs. To evaluate the role of HPV in oral neoplasia, completion of large-scale studies is needed to determine the diversity of papillomaviruses involved and studies implemented to identify patterns of transcription of transforming sequences in high-risk HPV types with a trophism for the oral cavity. The purpose of the current investigation was to test for the presence of HPVs in biopsies of oral epithelial dysplasia, verrucous hyperplasia, smokeless tobacco keratoses, and squamous cell carcinoma by examining lesional tissue by polymerase chain reaction (PCR) amplification techniques using L1 consensus sequence primers. HPV sequences were detected in all four categories of lesions and in no control samples. PCR amplification allows rapid and specific detection of HPV in oral biopsy specimens and may enhance our ability to evaluate large numbers of clinical samples and demonstrate a broad variety of HPV sequences and novel HPV types in oral cancer and precancer.

INTRODUCTION Papillomaviruses are tenacious, persistent viruses capable of residing latent within host cells for extended periods. HPV is an epitheliotrophic infectious agent with a genome consisting of approximately 7,900 base pairs (bp) of double-stranded circular DNA encapsulated within an icosahedral protein shell. HPV has been increasingly associated with mucosal cancers, particularly carcinoma of the cervix (Howley, 1987; Vousden, 1989; zur Hansen and Schneider, 1987). The cloned DNAs of a significant number of human and animal papillomavirus DNAs have been sequenced completely, and they are notable for their overall similarity in genetic organization, in spite of substantial sequence variation and diversity in host and tissue specificity, histological presentation, and malignant potential (Koutsky et al., 1989; zur Hansen and Schneider, 1987). Although human papillomaviruses are characterized by a low degree of pathogenicity, their synergistic effect with other carcinogens, such as tobacco and alcohol, enhances their carcinogenic potential.

[1] This investigation was supported in part by funding from Smokeless Tobacco Research Council grant no. 0278, National Cancer Institute grant no. CA-21098-14, and a grant from the Sands House Foundation.

In previous studies using in situ hybridization methods and dot blot hybridization, we have demonstrated human papillomavirus in ST keratoses, oral epithelial dysplasia, verrucous hyperplasia, verrucous carcinoma, and squamous cell carcinoma (Greer et al., 1987, 1990a, and 1990b).

Recently we used a PCR DNA amplification system for the identification of HPV type 16 E6 DNA sequence in formalin-fixed, paraffin-embedded biopsy material from 61 oral precancers and cancers, including squamous cell carcinoma, epithelial dysplasia, ST keratoses, verrucous hyperplasia, and verrucous carcinoma (Shroyer and Greer, 1991). This PCR amplification procedure allowed rapid and specific detection of low-abundance viruses and single-copy genes in biopsy specimens. The PCR amplification technique was judged simple and highly sensitive for analyzing specific HPV DNA sequences from archival material. The test can be correlated easily with the histological appearance seen in adjacent sections.

Several investigators (Bauer et al., 1991; Manos et al., 1990; Resnick et al., 1990) have developed a PCR DNA amplification system using two distinct consensus oligonucleotide primer sets for the improved detection and typing of a broad spectrum of human genital papillomavirus sequences including those of novel viruses. Their systems incorporate one consensus primer set designed to amplify the L1 domain from a wide variety of HPV types and a second consensus primer set designed to amplify a domain within the E6 open reading frame sequence of HPV. Resnick and associates (1990) have demonstrated that when consensus and type-specific oligonucleotide probes are used in hybridization analysis of amplified products, as few as 10 copies of HPV can be detected. The investigators have been able also to amplify many other known genital HPV types including 6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 45, and 51 to 59, as well as a large number of other HPVs that have not yet been assigned a type.

In the current investigation, we analyzed paraffin-embedded tissue sections from squamous cell cancers, oral epithelial dysplasias, verrucous hyperplasias, and oral ST keratoses for the presence of HPV DNA by a PCR-based system, using consensus sequence primers that amplify an approximately 450-bp region of the L1 open reading frame. We also evaluated 18 PCR product bands in the four categories of lesions, using dot blot hybridization to confirm the presence of HPV-16 DNA.

MATERIALS AND METHODS

Sections from 44 paraffin-embedded tissue blocks representing 8 squamous cell carcinomas, 11 oral epithelial dysplasias, 9 verrucous hyperplasias, and 16 ST keratoses were examined from the pathology archives of Western States Regional Oral Pathology Laboratory and the University of Colorado Oral Pathology Laboratory. All tissue samples were obtained during 1990. Sections 5 to 10 μm in thickness were cut from blocks and placed in 0.6-mL capped centrifuge tubes and prepared for PCR amplification as described by Wright and Manos (1990).

PCR

Aliquots (10 μL) of the prepared samples were subjected to 40 cycles of amplification, with HPV L1 consensus sequence primers MY11 and MY09 with β-globin primers PC04 and GH20 (Perkin-Elmer Cetus, Norwalk, Connecticut), as previously described (Resnick et al., 1990). Recombinant

plasmid-containing HPV-16 DNA (Oncor, Gaithersburg, Maryland) and sections from cervical squamous cell carcinoma were used as positive controls. Great care was taken to avoid the possibility of sample contamination during preparation. Amplification was evaluated by agarose gel electrophoresis (10 μL from each reaction), ethidium bromide staining, and visualization under ultraviolet light. Samples that failed to yield the 268-bp β-globin amplification product were excluded from the study.

HPV negative controls included tissue samples that had been previously characterized as negative for HPV DNA. In addition, multiple aqueous controls were included with each experiment to monitor for the possibility of reagent contamination.

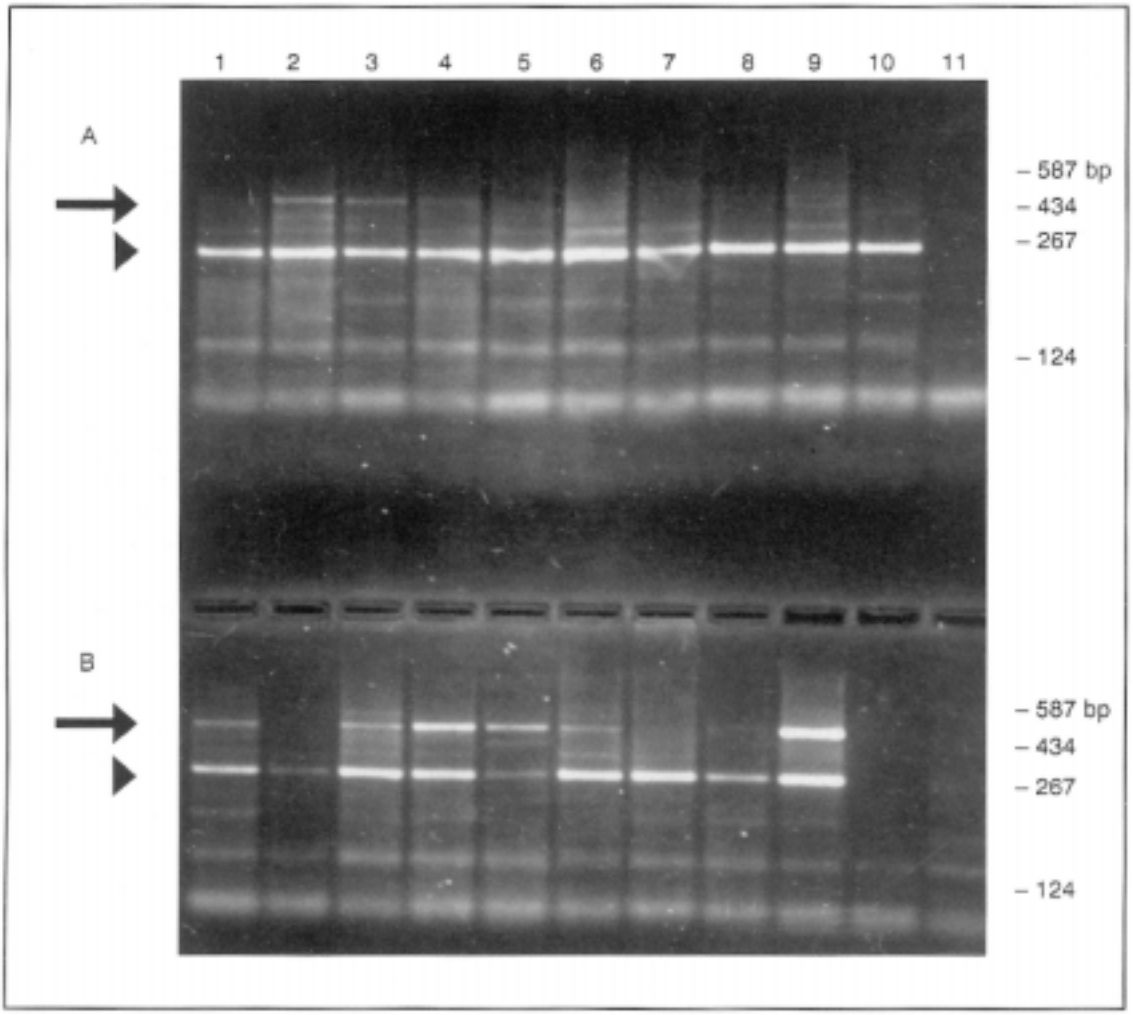
Dot blot hybridization for HPV-16 was carried out on 18 cases with BioRad's Bio-Dot minifold apparatus for six replicate membranes (Sambrook et al., 1989). DNA oligomer probes were end-labeled with γ-[^{32}P]ATP with hybridization carried out for 3 h at 56 °C in hybridization solution with each of the radio-labeled probes. Subsequent autoradiographic exposure was at -70 °C for 12 to 48 h.

RESULTS Biopsy specimens and positive controls were amplified with HPV L1 consensus primers (Figure 1). HPV amplification products were observed in smokeless tobacco keratoses, squamous cell carcinomas, oral epithelial dysplasia, and verrucous hyperplasias. From the total of 30 amplifiable specimens, 8 showed evidence of infection with HPV. The data reported in Table 1 represent the specific lesions analyzed and the determination of whether or not HPV was present. Subsequently, 18 cases representing each of the four categories of lesions were probed for HPV-16 using residual PCR product via dot blot hybridization. Of 18 cases, 8 were positive for HPV-16: 3 ST keratoses, 2 squamous cell carcinomas, 2 epithelial dysplasias, and 1 verrucous hyperplasia. Two cases positive by generic probe analysis were negative for HPV-16 and are currently being analyzed by dot blot for HPV-2, -4, -6, -11, -18, -31, -33, and -35. The eight HPV-16-positive cases are shown in Figure 2 in lanes A1, 2, and 3, B5, and C1 through 4.

DISCUSSION A distinct advantage of PCR lies in its ability to make large numbers of copies of DNA sequences from targets that are present in minute quantities in the original sample. As a result, the improved L1 consensus sequence amplification method is significantly more sensitive in detecting amplified types of HPV in oral tissue samples than in situ hybridization. Although the PCR method applied here allows a demonstration of only generic HPV, it is anticipated that we will subsequently be able to determine specific HPV types through the use of type-specific DNA probes in hybridization analysis of the PCR products. The increased sensitivity of the PCR method seems to be critical in detecting HPV in small tissue fragments from the oral cavity and in suboptimal tissue samples where low-level infections may be present. One disadvantage of the PCR technique is the potential for obtaining false positive results because of sample-to-sample contamination, or more importantly, because of the carryover of DNA from previous DNA amplifications (Bauer et al., 1991). To minimize this risk, we used only single-designated-use pipettes and separated pre- and post-PCR samples and reagents throughout all stages of the investigation.

Figure 1

PCR amplification with HPV L1 consensus sequence primers, representative cases. HPV L1 DNA was amplified over 40 cycles. β-Globin primers were included as an amplification control. Amplified products were analyzed by 4 percent (wt/vol) agarose gel electrophoresis, stained with ethidium bromide, and photographed under UV light. Molecular weight markers (Hae III cut pBR322) are shown in the right margin. HPV L1 positive cases (A2-4, B3-6) show a band at about 450 bp (arrow). β-Globin amplification products show a band at about 268 bp (arrowhead). HPV-16 plasmid DNA positive control (B9) and reagent controls (A11, B10, 11) are also shown.



Although the procedure may improve the likelihood of detecting a wide variety of HPV sequences, including novel types, it still does not answer the basic question of whether HPV assumes the function of a tumor promoter, as suggested by Amtmann (1987), or a causal agent, as suggested by Chow and associates (1987) and McCance (1988). To accomplish that, investigators must correlate expression of transforming sequences and lesional

Table 1
Identification of HPV in oral biopsy specimens through PCR analysis

Pathological Diagnosis	HPV[a]
Squamous Cell Carcinoma	2/8
Oral Epithelial Dysplasia	1/11
Verrucous Hyperplasia	2/9
ST Keratosis	3/16

[a] *Positive cases over total number of amplifiable cases tested.*

presentation with patient historical data, including specific etiologic influences such as alcohol consumption, smoking habits, wart history, racial origin, age, and sex. While PCR is a highly sensitive technique for the demonstration of HPV DNA, it provides no information on the functional state of the viral genome. Investigation of the possible role of HPV in the neoplastic transformation of the oral mucosa will require an understanding of the pattern of gene expression in HPV DNA-positive cases. Such an analysis in the past has been encumbered by considerable problems with inadequate degrees of sensitivity in the detection of mRNA.

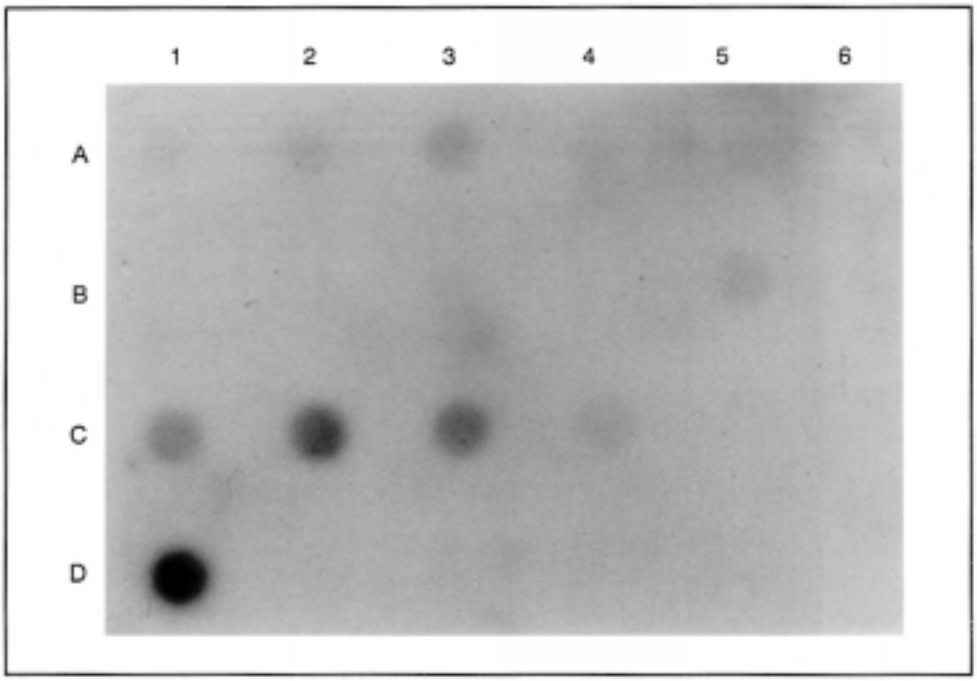
Another question to be answered is whether there is clinical significance in detecting HPV in the oral precancerous and cancerous lesion. Nuovo and associates (1990), in a study of HPV DNA in penile lesions histologically negative for condyloma, suggest that lesions with low copy numbers of the virus may in fact be either exceedingly early or regressing. They question whether these lesions, which are associated with small copy numbers of the virus, are truly infectious. The same questions apply to the oral cavity.

There is little doubt that HPV plays a significant role in the development of mucosal cancer. Accumulated evidence over the last several years links specific HPV types to the various manifestations of anogenital infection (Bartholoma et al., 1991). The seven most prevalent anogenital HPV types are HPV-6, -11, -16, -18, -31, -33, and -35. It is reasonable to suggest that some of these same viruses play a significant role in the development of oral cancer.

Because it is unlikely that all latent affected sites are eradicated with treatment, such as incisional biopsy, complete surgical excision, or stripping, it is possible that once someone is infected with HPV, the virus is harbored for life. It is also likely that HPV infections of the oral mucous membranes are not completely curable by surgical excision.

Bauer and associates (1991) have suggested that there may be an age-specific prevalence for cervical HPV infection. The same postulate may be made for HPV-infected oral mucous membranes. Although other genetic, environmental, and exogenous variables may be implicated in the progression to oral dysplasia or neoplasia, infection with specific HPVs may very well still be predictive of disease in certain subsets of patients. To determine

Figure 2
Dot blot hybridization for HPV-16 with residual PCR product. Eight positive cases are seen in lanes A1, 2, and 3, B5, and C1 through 4. A1, B5, and C4 are weakly positive. A2, 3, and C1, 2, and 3 are strongly positive. HPV-16 plasmid DNA positive control is D1.



whether HPV is responsible for preneoplastic or neoplastic mucosal changes, long-term prospective studies are needed to aid understanding of the cause of HPV oral infections in asymptomatic patients, patients who have immune disorders, and patients who are extensive users of exogenous agents such as tobacco and alcohol.

Clearly, more work is needed to obtain a complete catalog of oral HPV types. From an epidemiological standpoint, investigators need to determine the prevalence of infections with multiple HPV types in the oral cavity. Also, Bauer and associates (1991), in their evaluation of genital HPV infections, have shown that infections with multiple HPV types were underestimated both when a commercially available dot blot kit system was used and when they used the PCR method. These investigators are currently developing a system that utilizes restriction endonuclease digestion of amplification products to detect multiple infections.

There is little question that PCR will play a significant role in long-term epidemiological studies of HPV. PCR offers the advantage of type detection and high sensitivity in assessing the incidence and prevalence of HPV infection. Further analysis via PCR is needed to determine the demographic and behavioral risk factors for HPV infection in subsets of high-risk patients,

such as tobacco and alcohol abusers, patients with immune deficits, and perhaps even patients with long-term chronic oral infections, including periodontal disease. Such prospective studies should allow us to more adequately understand the natural history of HPV infection, its role in mucous membrane disease, and potential clinical applications of HPV detection by PCR methods.

Experimental data strongly suggest that, besides the presence of HPV DNA in cervical epithelium, other events are required for full malignant conversion of cells infected by so-called oncogenic HPVs. Bosch and co-workers (1990) have suggested that a change in the state of the viral DNA may be one factor in progression toward malignancy. Lehn and associates (1988) have suggested that in premalignant lesions of the cervix, viral DNA is present as an episome, with a tendency toward integration in high-grade cervical interepithelial neoplasias. Several studies have provided evidence that HPV-16 and HPV-18 E6 and E7 genes can profoundly influence proliferation and differentiation capacities of rodent and human cells and tissue culture. It is completely unknown, however, which cellular regulatory mechanisms are responsible for either downregulation of HPV-18 E6 and E7 genes or growth inhibition of the nontumorigenic hybrid cells in vivo, and whether these mechanisms are partially or completely different or identical (Bosch et al., 1990). Future studies are needed to analyze whether there is a causal relationship between the downregulation of the HPV-18 E6 and E7 gene expression and in vivo growth inhibition of nontumorigenic cells. Identification of cellular genes that interfere with the expression of HPV transforming genes will be required. Bosch and others suggest that this will contribute to a better understanding of the cellular growth-regulatory networks affected in HPV-associated carcinogenesis (Bosch et al., 1990).

REFERENCES

Amtmann, E., Randeria, J., Wayss, K. Interactions of papillomaviruses with carcinogens and tumor promoters. In: *Cancer Cells 5, Papillomaviruses*, B.M. Steinberg, J.L. Brandsma, and L.B. Taichman (Editors). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1987, pp. 259-265.

Bartholoma, N.Y., Adelson, M.D., Forbes, B.A. Evaluation of two commercially available nucleic acid hybridization assays for detection and typing of human papillomavirus in clinical specimens. *American Journal of Clinical Pathology* 95: 21-29, 1991.

Bauer, H.M., Ting, Y., Greer, C., et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. *Journal of the American Medical Association* 265: 472-477, 1991.

Bosch, F.X., Schwarz, E., Boukamp, P., et al. Suppression in vivo of human papillomavirus type 18 E6-E7 gene expression in nontumorigenic hela fibroblast hybrid cells. *Journal of Virology* 64: 4743-4754, 1990.

Bouck, N.P., Good, D.L., Polverini, P.J., et al. Suppressor control of an inhibitor of angiogenesis. In: *Recessive Oncogenes and Tumor Suppression*, W. Cavenee, N. Hastie, and E. Stanbridge (Editors). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989, pp. 179-185.

Chow, L.T., Hirochika, H., Nasseri, M., et al. Human papillomavirus gene expression. In: *Cancer Cells 5, Papillomaviruses*, B.M. Steinberg, J.L. Brandsma, and L.B. Taichman (Editors). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1987, pp. 55-72.

Elovic, A., Galli, S.J., Weller, P.F., et al. Production of transforming growth factor-alpha by hamster eosinophils. *American Journal of Pathology* 137: 1425-1434, 1990.

Greer, R.O., Douglas, J.M., Breese, L., et al. Evaluation of oral and laryngeal specimens for human papillomavirus (HPV) DNA by dot blot hybridization. *Journal of Oral Pathology and Medicine* 19: 35-38, 1990.

Greer, R.O., Eversole, L.R., Crosby, L.K. Detection of papillomavirus-genomic DNA in oral epithelial dysplasias, oral smokeless tobacco-associated leukoplakias, and epithelial malignancies. *Journal of Oral Maxillofacial Surgery* 48: 1201-1205, 1990.

Greer, R.O., Eversole, L.R., Poulson, T.C., et al. Identification of human papillomavirus DNA in smokeless tobacco-associated keratoses from juveniles, adults and older adults using immunocytochemical and in situ DNA hybridization techniques. *Gerodontics* 3: 201-208, 1987.

Howley, P.M. The role of papillomaviruses in human cancer. In: *Important Advances in Oncology*, V.T. DeVita, Jr., S. Hellman, and J.A. Rosenberg (Editors). Philadelphia: Lippincott, 1987, pp. 55-73.

Koutsky, L.A., Galloway, D.A., Holmes, K.K. Epidemiology of genital human papillomavirus infection. *Epidemiology Review* 10: 122-163, 1989.

Lehn, H., Villa, F., Marziona, M., et al. Physical state and biologic activity of human papillomavirus genomes in precancerous lesions of the female genital tract. *Journal of General Virology* 69: 187-196, 1988.

Manos, M.M., Ting, Y., Wright, D.K., et al. Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. In: *Cancer Cells 7, Molecular Diagnostics of Human Cancer*, M. Furth and M. Greaves (Editors). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1990, pp. 209-214.

McCance, D.J. Human papilloma virus (HPV) infections in the aetiology of cervical cancer. *Cancer Survey* 7: 499-506, 1988.

Nuovo, G., Hochman, H., Yehuda, D., et al. Detection of human papillomavirus DNA in penile lesions histologically negative for condyloma. *American Journal of Surgical Pathology* 14: 829-836, 1990.

Resnick, R.M., Cornelissen, M.T., Wright, D.K., et al. Detection and typing of human papillomavirus in archival cervical cancer specimens by DNA amplification with consensus primers. *Journal of the National Cancer Institute* 82: 1477-1484, 1990.

Sambrook, J., Fritsch, E.F., Maniafis, T. (Editors) *Molecular Cloning: A Laboratory Manual* (2nd edition). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989, pp. 1051-1957.

Shroyer, K.R., Greer, R.O. Detection of human papillomavirus DNA by in situ DNA hybridization and polymerase chain reaction in premalignant and malignant oral lesions. *Oral Surgery, Oral Medicine, Oral Pathology* 71: 708-713, 1991.

Ting, Y., Manos, M.M. Detection and typing of genital human papillomaviruses. In: *PCR Protocols: A Guide to Methods and Applications*, M. Innis, D. Gelfand, J. Sninsky, and T. White (Editors). San Diego: Academic Press, 1990, pp. 356-367.

Vousden, K.H. Human papillomaviruses and cervical carcinoma. *Cancer Cells*. 1: 43-50, 1989.

Wright, D.K., Manos, M.M. Sample preparation for paraffin-embedded tissues. In: *PCR Protocols: A Guide to Methods and Applications*. M. Innis, D. Gelfand, J. Sninsky, and T. White (Editors). San Diego: Academic Press, 1990, pp. 153-158.

zur Hansen, H., Schneider, A. The role of papillomaviruses in human anogenital cancer. In: *Papoviridae: The Papillomaviruses*, volume 2, N.P. Salzman and P.M. Howley (Editors). New York: Plenum Press, 1987, pp. 245-264.

Lipids as Factors in the Cell Response to Tobacco Components[1]

George S. Schuster, Scott Lubas, Thomas R. Dirksen, and John F. Erbland

ABSTRACT Lipids are recognized as major components of or act in concert with components of the intracellular signaling systems that regulate cell growth and behavior. Extracellular modulators, by altering signaling-associated lipids, can affect cell responses. The current study examines the effects of *N'*-nitrosonornicotine (NNN), a tobacco-associated nitrosamine, and a tumor promoter, 12-*0*-tetradecanoylphorbol-13-acetate (TPA), on cell lipids and on protein kinase C (PKC) activity in oral epithelial cells. Cells were exposed to NNN or TPA for 30 min and homogenized, and then their PKC and non-PKC histone phosphotransferase activity was assessed. NNN significantly increased PKC activity in the cells, especially in the particulate fraction, whereas TPA produced a slight decrease in activity compared with dimethyl sulfoxide (DMSO) solvent-treated cells. Both modulators produced increased non-PKC phosphotransferase activity compared with control cells. Lipid synthesis by cells during and after exposure to the modulators was determined through use of [^{14}C]acetate. Cells treated concurrently with modulator and [^{14}C]acetate showed varied responses, depending on the lipid class and dose of modulator. Generally both modulators stimulated labeling of phospholipids, whereas NNN increased and TPA decreased diglyceride labeling. Fatty acid labeling was decreased by both modulators, whereas triglyceride labeling was enhanced by TPA. Posttreatment labeling showed that modulator-induced changes were transient. The results indicate that NNN can alter some components of the signaling pathway, notably PKC, and may affect de novo synthesis of diglycerides, which are putative endogenous promoters.

INTRODUCTION Lipids are recognized as important participants in the responses of cells to a variety of modulators, that is, substances that alter cell behavior. Lipids function as components of cell membranes to which the modulators bind, specifically or nonspecifically; they are components of specific receptors for tumor promoters, and they act as part of or in concert with components of the intracellular signaling systems that are activated after binding of agents to specific receptors. These signaling pathways regulate cell growth and behavior. For example, studies have demonstrated that tumor promoters such as 12-*0*-tetradecanoylphorbol-13-acetate specifically bind to receptors. In the case of TPA, these receptors are composed of Ca^{2+}, phospholipid, and protein kinase C (PKC) (Konig et al., 1985). Receptor binding then results in activation of the intracellular signaling pathways.

Intracellular signaling involves the closely interconnecting activities of the inositol phosphate-Ca^{2+} and diacylglycerol (DAG)-PKC pathways, which have been implicated in the control of cell proliferation. In these pathways there is activation of certain proteins through phosphorylation of tyrosine and hydrolysis of phosphatidylinositol-4,5-bisphosphate (PlP_2) with formation of 1,2-diacylglycerol and inositol-1,4,5-triphosphate (IP_3). The diglyceride activates PKC, and IP_3 promotes increased cytosolic free calcium. These latter two activities initiate a cascade of reactions that bring about

[1] Funded by Smokeless Tobacco Research Council grant no. 0056 and National Institutes of Health grant nos. DE-07143 and RR-05795.

various cellular responses (Berridge, 1987). Thus any stimuli or cell reactions that affect components of these pathways can in turn alter cell behavior. In addition there are a variety of associated reactions, such as prostaglandin production, that may contribute to the signaling-induced responses (Parker, 1987).

Nitrosamines from smokeless tobacco are carcinogens that may act independently or in synergy with other agents to induce esophageal, nasal, and oral tumors (Hecht and Hoffmann, 1988). Indeed, synergy or promotion should be considered critical factors in the activities of some of these nitrosamines, especially some of the weaker ones (Hecht and Hoffmann, 1988).

Previous studies in our laboratories have shown that modulators of cell behavior such as retinoic acid (RA), TPA, and the nitrosamine *N'*-nitrosonornicotine may affect cell lipids and other components of the cell signaling system, either directly or indirectly. Ringler and associates (1984) demonstrated that RA decreased formation of cholesterol, sphingomyelin, phosphatidylinositol, and phosphatidylserine and increased formation of triglycerides of normal and transformed hamster fibroblast cell types. In hamster oral epithelial cells, RA also affected lipid synthesis from acetate, decreasing synthesis of cholesterol and fatty acids but increasing that of triglycerides and phosphatidylcholine. In these same cells, NNN enhanced acetate incorporation into phosphatidylcholine and decreased its incorporation into cholesterol (Schuster et al., 1988). TPA also has been shown to alter cell lipids in a variety of cell types. For example, TPA altered incorporation and release of arachidonic acid, especially that associated with the phospholipids of U937 cells. The pattern of change was to a great extent dependent on the state of cell differentiation (Wiederhold, 1988).

Additional studies have demonstrated that pretreatment of cells with one modulator can alter the subsequent cell surface binding of another modulator. For example, RA decreased binding of phorbol esters to oral epithelial cells, whereas NNN enhanced binding significantly (Table 1). These changes occurred at times that corresponded to the altered lipid synthesis induced by RA (Schuster et al., 1986 and 1988). The opposite has been shown to occur as well, that is, pretreatment of oral epithelial cells with TPA enhanced binding of NNN to the cells by 89 percent. These studies also demonstrated that, although TPA bound to receptors on the cell, NNN binding did not appear to involve receptors.

The results of these various studies suggested a relation between cell lipid metabolism and the extent and nature of cell behavior in the presence of these modulators. They also indicated that nitrosamines and promoters can interact to alter the cell responses to each other and that these may be related to their effects on cell lipids. Thus, various cell responses and perhaps tobacco-related carcinogenesis may be dependent on one agent altering the response to others. The current studies describe the effects of NNN and TPA on some components of the signaling system that may be related to transformation-associated responses.

Table 1
Specific binding of PDB to oral epithelial cells after pretreatment with RA or NNN (percent control)[a]

	RA	NNN
Time of Pretreatment,		
4	68[b]	-
24	94	100
48	100	-
72	-	218[b]
168	-	170[b]

Source: Schuster et al., 1988.

[a] Specific binding of [^{3}H]phorbol dibutyrate (PDB) to hamster buccal pouch cells (HCP) was assayed after pretreatment of the cells for various periods with 10 μM NNN, 10 μM retinoic acid (RA), or 0.1 percent dimethyl sulfoxide solvent (control). Percentage of control was calculated based on pmol [^{3}H]PDB specifically bound/10^6 viable cells. Results were based on three samples per time point in three to five experiments.

[b] Different from control (based on pmols/10^6 cells), $p < 0.05$.

METHODS

Cells

The cells used were a line of cloned normal hamster buccal pouch (HCP) cells, the characteristics of which are described elsewhere (Schuster et al., 1990). The cells were maintained in Dulbecco's modification of minimal essential medium (DMEM) supplemented with 5 percent fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The same lot of FBS (GIBCO) was used for all studies.

Protein Kinase C Assay

HCP cell suspensions ($2x10^6$ cells) were exposed to 10 μM NNN or 0.1 μM TPA in DMSO such that the final concentration of the solvent was 0.1 percent. Control cultures contained the same amount of DMSO. Cells were incubated 30 min at 37 °C in an atmosphere of 95 percent air-5 percent CO_2 and then harvested by centrifugation. PKC was assayed by the methods described by Chida and associates (1988) and Ways and associates (1987). Cells were suspended in buffer consisting of 20 mM Tris-HCL (pH 7.4), 2 mM EDTA, 0.5 mM EGTA, 0.25 M sucrose, and 50 mg/mL phenylsulfonyl-methyl chloride (PMSF). They were disrupted by 50 strokes in a glass homogenizer, then centrifuged at 100,000 *g* for 1 h at 4 °C. The supernatant was separated, and the pellet resuspended in the same buffer as described above except that it lacked sucrose but contained 0.1 percent Triton X-100.

The final assay mixture consisted of 20 mM Tris-HCL (pH 7.4), 5 mM $MgS0_4$, 200 μg/mL histone (type V-S, Sigma), 10 μM ATP (including 1 μCi γ-[^{32}P]ATP), and 200 μL of enzyme preparation. Activators consisted of 64 μM phosphatidylserine, diglyceride (1.3 μM 1,2-sn-diolein), and 1 mM $CaCl_2$. The samples were incubated for 3 min at 30 °C. The reaction was stopped by the addition of 1 mL of ice-cold 25 percent trichloroacetic acid (TCA). Samples were filtered through membrane filters (Millipore type HA) and then washed three times with the TCA. They were then counted in a

liquid scintillation counter. Protein content of aliquots was measured by the Bio-Rad assay. We determined specific activity for PKC by subtracting ^{32}P incorporation into histone in the absence of activators from that in the presence of activators. The basal histone phosphotransferase activity (non-PKC) is the ^{32}P incorporated into histone in the absence of activators. Activity was calculated on the amount of protein in duplicate aliquots of cell supernatant or pellet.

Lipid Assays HCP cells were plated in 100-mm dishes at $5x10^6$ cells/dish. After overnight attachment, the medium was removed by aspiration, and the plates refed with medium containing TPA (0.1 μM, 0.2 μM, or 0.5 μM), NNN (10 μM, 20 μM, or 50 μM), or DMSO (0.1 percent). The medium also contained 10 μCi/mL [^{14}C]acetate. At the end of 30 min, the media were removed, and the cells were harvested. An identical set of plates was pretreated for 30 min with 0.1 μM TPA or 10 μM NNN or 0.1 percent DMSO alone, but no isotope. After 30 min, the plates were refed with medium containing 1 μCi/mL of isotope but no modulator. These were incubated for an additional 4 h and then harvested.

At the end of the respective labeling periods, the cells were washed three times with saline, dislodged with Teflon scrapers, and harvested by centrifugation. Cell pellets were washed twice with saline, and the lipids extracted by the method of Bligh and Dyer (1959). Lipids were separated on SG-81 paper by use of the solvent system described by Marinetti (1965) for neutral lipids and the solvents of Rouser and coworkers (1970) for polar lipids. Radioactive lipids were located by autoradiography, and the spots cut from the paper and counted in a liquid scintillation spectrometer. Aliquots of the cells were assayed for protein as described above, and the number of cells counted in a hemocytometer. Results were compared by a t test based on three to five experiments.

PKC RESULTS PKC activity was assayed after 30 min of exposure to the modulator, when maximum NNN uptake was previously shown (Schuster et al., 1990). The total PKC activity in cells exposed to NNN was about 1.5 times that of control cells, 826±67 (SD) cpm/μg protein for NNN-exposed cells vs. 555±84 (SD) cpm/μg protein for control (DMSO-treated) cells (n=12). This difference was significant ($p < 0.05$). PKC activity was present in both the cytosol and particulate fractions, and NNN produced an apparent change in the distribution of this activity (Table 2). The majority of activity was present in the particulate fraction, and PKC activity in this fraction was enhanced in response to NNN; TPA had a minimal effect on distribution at this point.

Figure 1 shows the specific activity of PKC in the cell fractions after 30 min of modulator exposure. PKC activity was significantly reduced in the cytosol after treatment with NNN compared with DMSO exposure, whereas it was significantly increased in the particulate fraction. The increase in overall as well as percent of activity in the particulate fraction suggests that the major response to NNN occurs here, not only as de novo synthesis but also as a shift from one site to the other. After 30 min of incubation, TPA produced a slight overall decrease in total activity that was not statistically significant.

Table 2
Enzyme activity in cell fractions after modulator exposure[a]

	Cell Fraction	
	Cytosol	Solubilized particulate
	PKC activity[b]	
Modulator		
DMSO	24.8%	75.2%
TPA	16.8	83.2
NNN	2.3	97.7
	Non-PKC histone phosphotransferase activity[b]	
DMSO	51.6	48.4
TPA	41.9	58.1
NNN	48.4	51.6

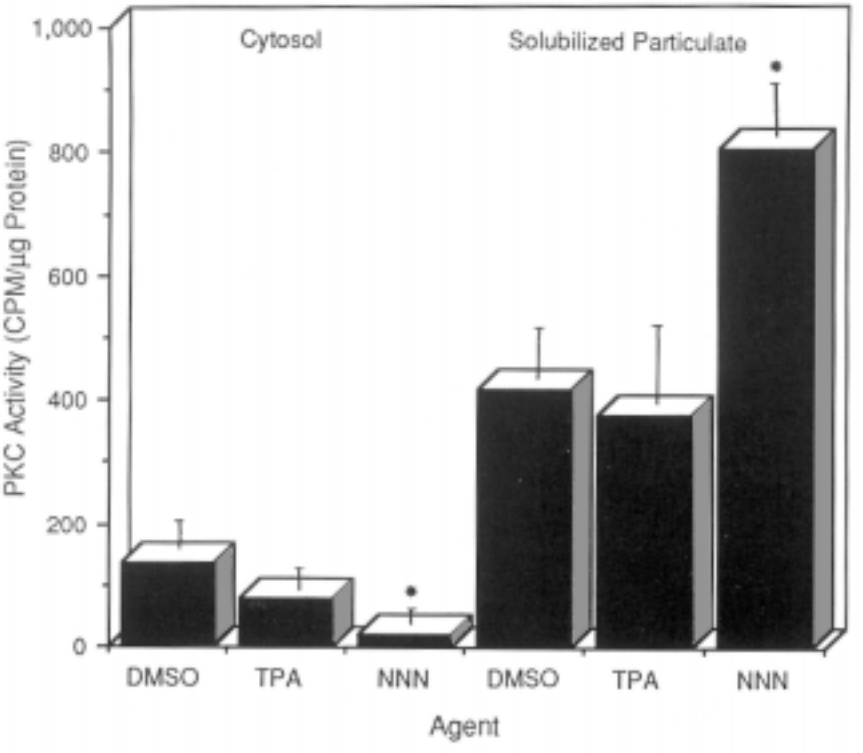
[a] *Percentage of protein kinase C (PKC) activity and non-PKC histone phosphotransferase activity was assayed in cytosolic and solubilized particulate fractions of hamster buccal pouch cells (HCP) following exposure of the cells to 10 μM N'-nitrosonornicotine (NNN), 0.l μM 12-0-tetradecanoylphorbol-13-acetate (TPA), or 0.1 percent dimethyl sulfoxide solvent for 30 min.*

[b] *Mean of six experiments.*

Non-PKC Phospho-transferase Both NNN and TPA did increase the levels of total non-PKC phosphotransferase activity somewhat. This was most notable in cells treated with NNN, where total activity was increased to 1.5 times that of DMSO-exposed cells (1,941±98 [SD] cpm/μg protein vs. 1,282±95 [SD] cpm/μg protein) (n=12). The increase was significant ($p < 0.05$). This increase in activity occurred in both cytosol and particulate fractions (Figure 2). The TPA-produced increase in enzymic activity was less; 1,425±139 (SD) cpm/μg protein for TPA-treated cells vs. 1,282±95 (SD) cpm/μg protein for DMSO-treated cells (n=12), but this change was evident in only the particulate fraction (Figure 2). Non-PKC phosphotransferase activity generally was about evenly distributed between the cytosol and particulate fractions. NNN did not appear to affect this distribution, whereas TPA caused a modest shift of activity from cytosol to particulate fraction (Table 2).

Diglyceride Formation In addition to PKC, diglycerides are major components of cell signaling pathways and may serve as endogenous promoters. Diglycerides may be formed from phospholipids via the activities of phospholipases or synthesized de novo by cells. The current studies were directed at the latter source. Formation of diglycerides and other lipid classes in direct response to the modulators NNN and TPA was examined through labeling of the cells in the presence of the modulator or through pretreating of the cells, removing the modulator, and then labeling the cells. The latter study was done to permit assessment of the longevity of any responses. NNN increased cell lipid labeling by nearly 15 percent compared with solvent-treated cells, whereas TPA decreased labeling about 20 percent. This was not the result of differences in cell number, because direct cell counts and protein

Figure 1
The effects of 10 μM NNN, 0.1 μM TPA, or 0.1 percent DMSO on the specific activity of protein kinase C were measured in cytosolic and solubilized particulate fractions of hamster buccal pouch cells after 30 min exposure to the modulators. Values are the mean of six experiments (±SD).

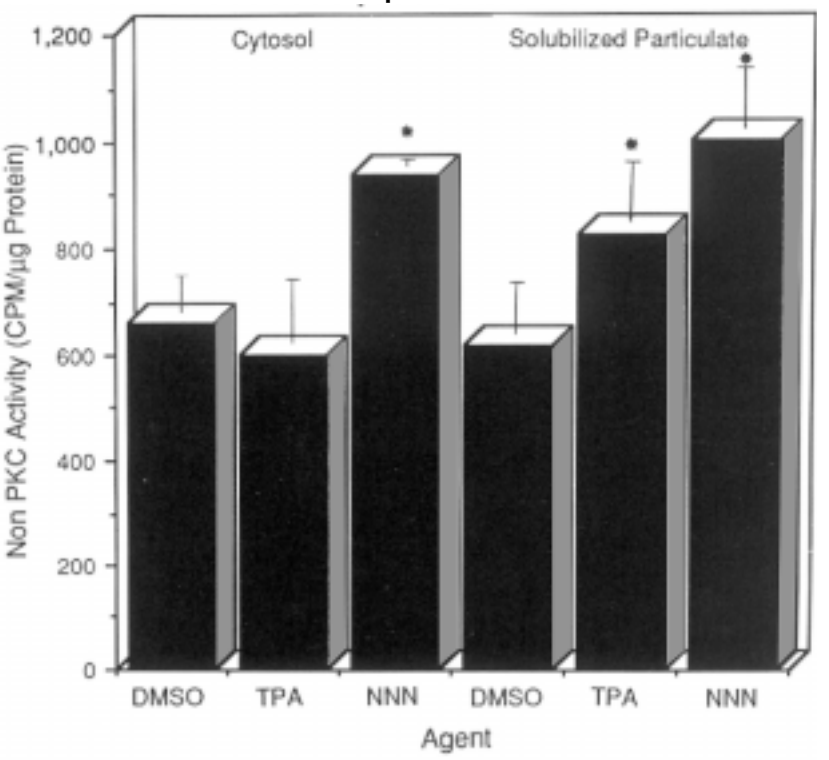


** p < 0.05 compared with DMSO-treated cells.*

determination of aliquots from the samples revealed no significant differences in these parameters among the treatments.

Concurrent treatment and labeling of the cells with modulators and isotope for 30 min produced various responses at modulator concentrations equal to or above those previously shown (Schuster et al., 1986) to produce changes in cell lipids, the response depending on the dose of modulator and lipid class. Total cell lipid labeling and labeling of various classes by acetate differed somewhat between experiments; thus, when responses in various classes are taken as an aggregate of all experiments, some differences are not statistically significant. However, the labeling of lipid classes at a given concentration of modulator, compared with solvent-treated control cultures, was generally very consistent between experiments (Figures 3 and 4). Thus, phospholipid labeling was uniformly increased by both modulators. Diglyceride labeling was decreased by TPA (Figure 4) and stimulated at all except the highest dose of NNN (Figure 3). Cholesterol labeling was

Figure 2
The effects of 10 μM NNN, 0.l μM TPA, or 0.1 percent DMSO on the specific activity of non-PKC histone phosphotransferase were determined in cytosolic and solubilized particulate fractions of HCP cells after 30 min exposure to the modulators. Values are the mean of six experiments (±SD).



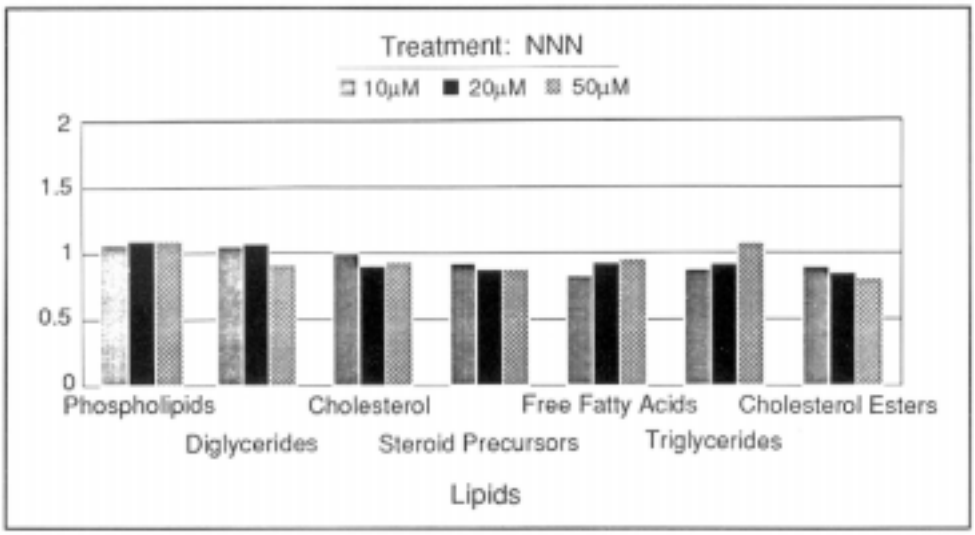
** p < 0.05 compared with DMSO-treated cells.*

moderately but consistently decreased by both modulators, whereas triglyceride labeling was greatly increased and fatty acid labeling decreased by TPA. NNN decreased labeling of cholesterol esters.

Posttreatment labeling of cell lipids indicated that most of the alterations disappeared within 4 h after modulator removal, although labeling of phospholipids in TPA-treated cells remained slightly but significantly elevated as did that of sphingomyelin and glycolipids. At 4 h after NNN exposure, there was significantly elevated labeling only in steroid precursors.

DISCUSSION Some tobacco-derived substances may affect cell behavior only marginally in normal time or concentration exposures, and cofactors may therefore be critical for responses such as altered division or transformation to occur. Many of these cell responses are lipid associated, such as signaling pathways, or involve lipid-containing components, such as the cell membranes (Schuster et al., 1986, 1988, and 1990). Therefore, if lipid metabolism is altered it can affect the cell behavior.

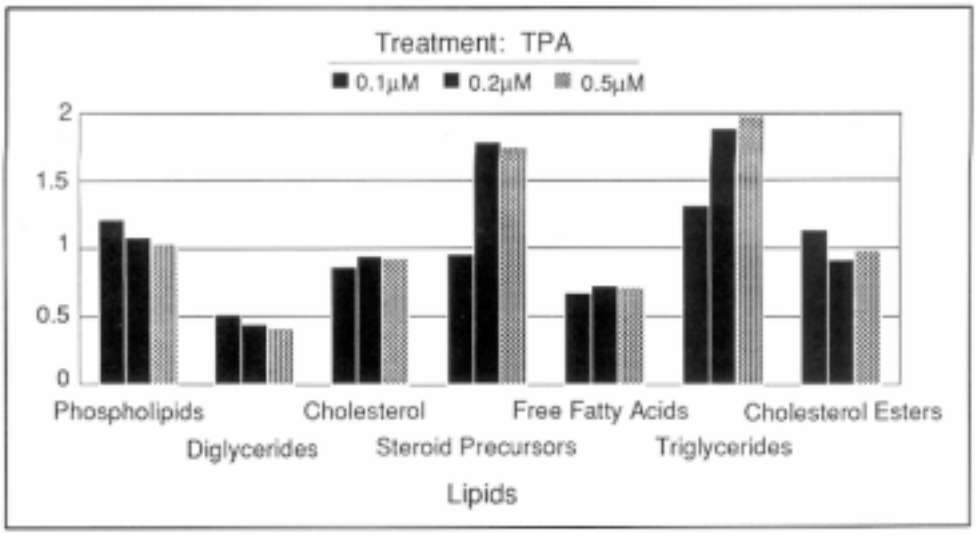
Figure 3
Relative effects of various concentrations of NNN on [^{14}C]acetate incorporation into lipids of HCP cells during 30 min exposure to the modulator plus 10 μCi/mL [^{14}C]acetate. Treated cells are compared with cells exposed to 0.1 percent DMSO, which were given a value of 1.00.



Although signaling pathway components such as PKC are usually activated by binding of agents to specific receptors, NNN binds nonspecifically (Schuster et al., 1990). However, it does significantly affect specific activity of both PKC and non-PKC histone phosphotransferase activity. PKC activity is increased in the particulate fraction, whereas activity in the cytosol decreases (Table 2 and Figure 1), suggesting that there may be a shift in activity to the particulate fraction as well as an increase in activity in this fraction. These results are similar to the shifts seen in MCF-7 cells in response to TPA and the diglyceride 1,2-dioctanoyl-sn-glycerol demonstrated by Issandou and associates (1988). The TPA-associated decrease in PKC activity in HCP cells was not significant, but a similar response in U937 cells, as shown by Ways and coworkers (1991), was more extensive, suggesting differences between cell types. The optimal time and dose of exposure to the NNN and TPA were based on our previous studies with HCP cells as well as studies by others (Issandou et al., 1988; Schuster et al., 1986; Ways et al., 1991). The present results demonstrate that changes in PKC activity can occur in oral epithelial cells in response to NNN, meaning that this nitrosamine and perhaps other modulators that do not bind to receptors are able to activate this component of the intracellular signaling system.

The significance of the NNN-induced increase in non-PKC phosphotransferase activity is not clear at this time, but Ways and coworkers (1987) suggest that such activity may mediate other effects produced by cell modulators.

Figure 4
Relative effects of various concentrations of TPA on [^{14}C]acetate incorporation into cellular lipids during 30 min exposure to the modulator plus 10 μCi/mL [^{14}C]acetate. Treated cells are compared with cells exposed to 0.1 percent DMSO, which were given a value of 1.00.



Cell signaling pathways also involve lipids as direct mediators in the responses to stimuli (Berridge, 1987). Diglycerides may serve as endogenous promoters (Rozengurt, 1984). Although phospholipase activity likely is the major source for diglycerides involved in cell signaling, neosynthesized diglycerides are important in activation and downregulation of PKC and altered mitogenic signaling (Chiarugi, 1989a and 1989b; Peter-Riesch et al., 1988). Those studies showed increased diglyceride synthesis from glucose. In contrast, significantly increased synthesis from acetate was not evident in the HCP cells used in our studies, and indeed, TPA caused a significant decrease in diglycerides from this source. This may be a result of specific metabolic patterns of the HCP cell type; that is, the pathways used for neosynthesis of diglycerides from acetate are not affected by NNN and are downregulated by TPA. HCP cells apparently do not synthesize significant quantities of lipids from radiolabeled glucose, even in media totally lacking nonlabeled glucose (unpublished observations). Therefore, other pathways for lipid production in these cells will have to be examined for their response to the various modulators, because others also have shown cell-specific differences (Chida et al., 1988; Harris et al., 1982; Hecht and Hoffmann, 1988.) The changes in diglyceride formation appear to be transient because prior studies with longer periods of TPA exposure and labeling (Schuster et al., 1990) as well as the current posttreatment labeling showed no differences from control cells.

Altered labeling of lipid classes other than the diglycerides during the short term may relate to cells adjusting to the binding or embedment of the modulators in the cell membrane. Frezard and coworkers (1989) found that,

when TPA binds to cells, it becomes embedded in the cytoplasmic membranes. NNN does not bind to receptors but likely will at least transiently affect membrane organization. Therefore, the changes seen in phospholipids and fatty acids may reflect the cells' adjustment to the presence of the modulators, a result consistent with previous studies showing TPA-associated increases in 18:2, 18:3, 20:3, and 20:4 fatty acids in these cells (Schuster et al., 1990).

Although incorporation of acetate into cell lipids varied between experiments, the patterns of response to modulators are consistent. The variation may be the result of the nature of the HCP cells in culture. The cells were plated so as to be loose monoloyers when used. However, even when thoroughly dispersed for plating, these epithelial cells would tend to stick together and settle to the surface of the dish in patches, resulting in areas where the cells may be locally dense. In such a situation the cells in the center of the clusters may be less active in their growth and synthetic activities, including their responses to the modulators. In general, metabolic or growth rate-associated responses are likely to be affected by culture density, resulting in interexperiment variability. Cell regulatory characteristics that have been shown to be density dependent include cyclic nucleotide levels, availability of various receptors, and lipid metabolic pathways (D'Armiento et al., 1973; Holley et al., 1978; Jetten et al., 1989; Ponec et al., 1987).

The previous and current studies taken together suggest that NNN and TPA can directly or indirectly affect the cell signaling pathways, including some of the lipid components. They also suggest that the combined effects of modulators may be a significant factor in the response of oral epithelial cells to initiators and promoters. Finally, the results suggest that these cells may differ from other cell types in some ways, necessitating the use of oral epithelial cells to fully define responses to tobacco exposure.

REFERENCES

Berridge, M.J. Inositol lipids and cell proliferation. *Biochimica et Biophysica Acta* 907: 33-45, 1987.

Bligh, E.G., Dyer, W.J. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37: 911-917, 1959.

Chiarugi, V., Bruni, P., Pasquali, F., et al. Synthesis of diacylglycerol de novo is responsible for permanent activation and down-regulation of protein kinase C in transformed cells. *Biochemical and Biophysical Research Communications* 164: 816-823, 1989b.

Chiarugi, V.P., Magnelli, L., Pasquali, F., et al. Signal transduction in EF-J-*ras*-transformed cells: de novo synthesis of diacylglycerol and subversion of agonist-stimulated inositol lipid metabolism. *FEBS Letters* 252: 129-134, 1989.

Chida, K., Kato, N., Yamada, S., et al. Protein kinase C activities and bindings of a phorbol ester in 41 cell lines. *Biochemical and Biophysical Research Communications* 157: 1-8, 1988.

D'Armiento, M., Johnson, G.S., Paston, I. Cyclic AMP and growth of fibroblasts: Effect of environmental pH. *Nature New Biology* 242: 78-80, 1973.

Frezard, F., Garnier-Suillerot, A., Ballard, J., et al. Membrane-phorbol ester interaction monitored by circular dichroism. *Biochimica et Biophysica Acta* 979: 316-320, 1989.

Harris, C.C., Trump, B.F., Grafstrom, R., et al. Differences in metabolism of chemical carcinogens in cultured human epithelial cells. *Journal of Cellular Biochemistry* 18: 285-294, 1982.

Hecht, S.S., Hoffmann, D. Tobacco-specific nitrosamines: An important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis* 9: 875-884, 1988.

Holley, R.W., Armour, R., Baldwin, J.H. Density-dependent regulation of growth of BSC-1 cells in cell culture: Growth inhibitors formed by the cells. *Proceedings of the National Academy of Sciences* 75: 1864-1866, 1978.

Issandou, M., Bayard, F., Darbon, J.M. Inhibition of MCF-7 cell growth by 12-*O*-tetradecanoylphorbol-13-acetate and 1,2-dioctanoyl-sn-glycerol: Distinct effects on protein kinase C activity. *Cancer Research* 48: 6943-6950, 1988.

Jetten, A.M., George, M.A., Nervi, C., et al. Increased cholesterol sulfate and cholesterol sulfotransferase activity in relation to the multi-step process of differentiation in human epidermal keratinocytes. *Journal of Investigative Dermatology* 92: 203-209, 1989.

Konig, B., DiNitto, P.A., Blumberg, P.M. Phospholipid and Ca^{2+} dependency of phorbol ester receptors. *Journal of Cellular Biochemistry* 27: 255-265, 1985.

Marinetti, G.V. Chromatography of lipids on commercial silica gel loaded filter paper. *Journal of Lipid Research* 6: 315-317, 1965.

Parker, J., Daniel, L.W., Waite, M. Evidence of protein kinase C involvement in phorbol diester-stimulated arachidonic acid release and prostaglandin synthesis. *Journal of Biological Chemistry* 286: 5385-5393, 1987.

Peter-Riesch, B., Fathi, M., Schlegel, W., et al. Glucose and carbachol generate 1,2-diacyl-glycerol by different mechanisms in pancreatic islets. *Journal of Clinical Investigations* 81: 1154-1161, 1988.

Ponec, M., Kempenaar, J., Boonstra, J. Regulation of lipid synthesis in relation to keratinocyte differentiation capacity. *Biochimica et Biophysica Acta* 921: 512-521, 1987.

Ringler, M.B., Erbland, J.F., Singh, B.B., et al. Effects of retinoic acid on ^{14}C-acetate incorporation into lipids of normal and transformed hamster fibroblasts. *Experimental Cell Research* 154: 171-180, 1984.

Rouser, G., Fleischer, S., Yamamoto, A. Two-dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* 5: 494-496, 1970.

Rozengurt, E., Rodriguez-Pena, A., Coombs, M., et al. Diacylglycerol stimulates DNA synthesis and cell division in mouse 3T3 cells: Role of Ca^{2+}-sensitive phospholipid-dependent protein kinase. *Proceedings of the National Academy of Sciences* 81: 5748-5752, 1984.

Schuster, G.S., Erbland, J.F., Wyrick, S.D., et al. Oral epithelial cell lipid synthesis in the presence of retinoic acid or nitrosonornicotine. *Journal of Oral Pathology* 15: 430-433, 1986.

Schuster, G.S., Erbland, J.F., Wyrick, S.D., et al. Phorbol ester binding to oral epithelial cells in the presence of retinoic acid on *N'*-nitrosonornicotine. *Cytobios* 54: 53-60, 1988.

Schuster, G.S., Lubas, S., Erbland, J.F., et al. Binding and uptake of *N'*-nitrosonornicotine by oral epithelial cells. *Journal of Oral Pathology and Medicine* 19: 114-118, 1990.

Ways, K.D., Dodd, R.C., Earp, H.S. Dissimilar effects of phorbol ester and diacylglycerol derivatives on protein kinase activity in the monoblastoid U937 cell. *Cancer Research* 47: 3344-3350, 1987.

Ways, K., Riddle, R., Ways, M., et al. Effect of phorbol esters on cytosolic protein kinase C: Content and activity in the human monoblastoid U937 cell. *Journal of Biological Chemistry* 266: 1258-1264, 1991.

Wiederhold, M.D., Anderson, K.M., Harris, J.E. Labeling of lipids and phospholipids with ^{3}H-arachidonic acid and the biosynthesis of eicosanoids in U937 cells differentiated by phorbol ester. *Biochimica et Biophysica Acta* 959: 296-304, 1988.

Role of Viruses in Oral Carcinogenesis[1]

No-Hee Park, Byung-Moo Min, Sheng-Lin Li,
Henry M. Cherrick, and Jay Doniger

ABSTRACT The role of herpes simplex virus and human papillomavirus in oral carcinogenesis was studied. Herpes simplex virus showed no carcinogenicity in vivo; repeated viral inoculation of hamster buccal pouch mucosa failed to produce tumors or histopathologic evidence of malignancy in pouches. However, herpes simplex virus demonstrated in vivo cocarcinogenicity; viral inoculation significantly enhanced the oncogenic capacity of benzo[*a*]pyrene, a tobacco-chemical carcinogen, in the oral cavity of hamsters. Human papillomavirus types 16 and 18 demonstrated oncogenicity by transforming normal human oral keratinocytes. While normal cells exhibited a limited lifespan, cells transformed by these viruses showed immortality and altered morphology in comparison with their normal counterparts. The transformed HOK-16A and B and HGK-18 cells contained intact type 16 or 18 human papillomaviral DNA integrated into cellular chromosomes, respectively. Further, these cells expressed several viral-specific poly(A^+)RNAs including viral E6/E7 polyadenylated RNAs. Notably, these cells overexpressed cellular *myc* proto-oncogene compared to their normal counterparts; however, the immortalized cell lines were not able to produce tumors in nude mice, indicating that the cells are only partially transformed.

INTRODUCTION Clearly cigarette, cigar, and pipe smoking are causally associated with oral cancer (US DHHS, 1982) as is snuff dipping (US DHHS, 1986). The constituents of smoked tobacco tar and smokeless tobacco responsible for oral cancer are the tobacco-specific *N'*-nitrosamines (TSNA) and benzo[*a*]pyrene (B[*a*]P). TSNA are formed from nicotine and minor tobacco alkaloids during aging, curing, and fermentation of tobacco (Hecht et al., 1977). Among TSNA, high levels of *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are found in tobacco (Hoffmann et al., 1984). Some studies, however, indicate a lack of linkage between malignant changes and ST use by humans (Offenbacher and Weathers, 1985). Moreover, several laboratory studies report failure to develop oral malignancy with repeated intraoral placement of smokeless tobacco in animals (Park et al., 1985; Shklar et al., 1985). Possible involvement of other factors such as alcohol, caffeine, and viruses has therefore been postulated to be associated with development of tobacco-related oral malignancies in humans. Among these factors, the role of viruses, especially herpes simplex virus (HSV) and human papillomavirus (HPV), in oral carcinogenesis has been studied in our laboratory.

Oral cancers appear to be associated with an increased immune response to HSV-1 (Shillitoe et al., 1982). The expression of HSV-1 genes has been detected in oral cancer tissues (Eglin et al., 1983). HSV infection is extremely prevalent, with up to 90 percent of individuals having antibodies to HSV by age 10 (Overall, 1979). More than one-third of the world's population suffers from recurrent intraoral or orofacial herpetic infections (National Institutes of Health, 1973). Individuals with latent HSV infection in the sensory or autonomic ganglia actively shed infectious virions onto

[1] Supported in part by Smokeless Tobacco Research Council grants no. 0052 and no. 0231.

oral mucosae, yet are without clinical symptoms, providing an opportunity for HSV to interact with water-soluble components of smokeless tobacco in the oral cavity. Tobacco and HSV are synergistic in developing precancerous lesions in mice (Park et al., 1985). Furthermore, repeated HSV infection in combination with simulated snuff dipping leads to oral cancer in animals (Hirsch et al., 1983; Park et al., 1985). Also, HSV-1 infection significantly increases the carcinogenic activity of 7,12-dimethylbenz[*a*]anthracene (DMBA) in hamster buccal pouch mucosa by, in part, accelerating DMBA-induced activation of c-*erb*-B-1 proto-oncogene in the pouch epithelium (Oh et al., 1989).

Human papillomavirus (HPV) is also linked to certain human malignancies. This association is based on the finding that up to 90 percent of cancer tissues from genital lesions contain viral DNA (Durst et al., 1983). Of the more than 60 genotypes of HPV, types 16 (HPV-16) and 18 (HPV-18), as well as recently isolated types 31 (HPV-31) and 33 (HPV-33), are most frequently associated with cervical cancer (Schwarz et al., 1985). In a high percentage of cervical carcinomas and in cell lines derived from these cancers, HPV-16 and -18 DNAs are integrated into cellular chromosomes, whereas the viral DNAs are generally retained as extrachromosomal episomes in premalignant dysplastic lesions (McCance, 1986). As in cervical cancers, HPV is also positively correlated with human oral malignancies, with up to 60 percent of cancer tissues from oral biopsies containing viral DNA (Dekemezian et al., 1987). Since the epithelia of oral and female genital mucosae are histologically similar, and both are continuously challenged by many environmental factors, close association of HPV with the development of oral malignancies is not surprising. Although human oral keratinocytes are undeniably major target cells for HPV infection and HPV-induced tumorigenesis, the in vitro transforming activity of HPV in human oral keratinocytes has never been studied because of the unavailability of a suitable culture system.

In the present study, we demonstrate the in vivo cocarcinogenic effect of HSV in the oral cavity of hamsters, and the carcinogenicity of HPV for one of its target cells, the oral keratinocytes.

METHODS

Viruses, Plasmids, And Primary Culture of Oral Keratinocytes

HSV-1 (F-strain; American Type Culture Collection, Rockville, Maryland) was propagated in Vero cell monolayers with viral titers being adjusted to 10^8 plaque-forming units (PFU) per milliliter. pMHPV-16d (a head-to-tail dimer of HPV-16 DNA inserted into the *Bam*HI cloning site of the plasmid pdMMT$_{neo}$) and pSHPV-18m (recombinant DNA containing single copy of HPV-18 DNA inserted into *Eco*RI cloning site of plasmid PSV2$_{neo}$) were constructed as described elsewhere (Woodworth et al., 1989; Park et al., 1991). The primary normal human oral keratinocytes (NHOK) and normal human gingival keratinocytes (NHGK) were established from the excised tissues of hard palate and retromolar areas of a healthy male volunteer as described elsewhere (Park et al., 1991).

Animals, HSV-1 Inoculation, and Application of Carcinogens To investigate the in vivo carcinogenicity of NNN, NNK, and B[*a*]P, alone or in combination with HSV-1 inoculation, we inoculated the right buccal pouch mucosa with HSV-1 or culture medium (mock inoculation), as described elsewhere (Park et al., 1988), and applied either mineral oil or chemical carcinogens topically as follows: group 1 (control), mock inoculation plus topical application of mineral oil; group 2, HSV-1 inoculation plus topical application of mineral oil; group 3, mock inoculation plus topical application of 1 percent NNK; group 4, HSV-1 inoculation plus topical application of 1 percent NNK; group 5, mock inoculation plus topical application of 1 percent NNN; group 6, HSV-1 inoculation plus topical application of 1 percent NNN; group 7, mock inoculation plus topical application of 1 percent B[*a*]P; group 8, HSV-1 inoculation plus topical application of 1 percent B[*a*]P. Approximately 100 µL of mineral oil, NNK, NNN, or B[*a*]P solution were applied as described in Table 1. One hundred sixty hamsters were divided into eight equal groups, and right pouch mucosae were inoculated with HSV-1 ($1x10^8$ PFU per pouch) or culture medium (mock inoculation). Twenty-four hours after the inoculation, mineral oil, NNK, NNN, or B[*a*]P was topically applied to the inoculated pouch mucosa, three times per week for 15 (for B[*a*]P) or 20 (for mineral oil, NNK, and NNN) consecutive weeks. Left pouches were used as internal controls. Since our preliminary study showed that mild premalignant microscopic changes were developed by 15 wk and 20 wk of treatment of topical B[*a*]P and TSNA (NNN and NNK), respectively, in hamster buccal pouches, the chemical carcinogens were applied for 15 or 20 wk to demonstrate the cocarcinogenicity of HSV-1. The pouches were examined once a week for the appearance of tumors, and the animals were sacrificed at 30 wk after the initiation of topical application of mineral oil or chemical carcinogens. The above data were obtained just before the animals were sacrificed. Of animals receiving both HSV-1 inoculation and topical B[*a*]P, two hamsters died of HSV-1 encephalitis at 2 wk after inoculation. No changes were observed from the left pouch mucosae used as internal controls. At the end of the experimental period, the animals were sacrificed and the buccal pouches were fixed for light microscopic examination. Although NNN and NNK are hydrophilic compounds, they were dissolved in mineral oil to enhance the penetration of the compounds into tissue.

Transformation Of Primary NHOK And NHGK With HPV-16 and HPV-18 Primary NHOK and NHGK were transfected with pMHPV-16d (or $pdMMT_{neo}$) and pSHPV-18m (or $pSV2_{neo}$) using Lipofectin reagent (BRL Life Technologies, Gaithersburg, Maryland). Two G418-resistant cell colonies transfected with pMHPV-16d were isolated, subcultured, and named HOK-16A and HOK-16B lines. One cell colony transfected with pSHPV-18m was isolated and named HGK-18 (Park et al., 1991).

Analysis of Cellular DNA and RNA From HPV-Transformed Oral Keratinocytes High molecular weight cellular DNA from NHOK, NHGK, HOK-16A, HOK-16B, and HGK-18 lines was extracted. To determine the presence of viral DNA and, if present, its copy number per cell, cellular DNA were restricted and electrophoresed in 1 percent agarose, and Southern blot hybridization carried out under stringent condition using ^{32}P-labeled 7.9-kbp total HPV-16 DNA or total HPV-18

Table 1
Effect of tobacco-related chemical carcinogens and HSV-1, alone or in combination, on the development of oral cancer in hamster buccal pouches

	Effect on Right Buccal Pouches					
	Number of Pouches per Group	Number of Pouches With Tumors	Number of Discrete Tumors	Average Number of Tumors per Pouch	Average Tumor Size (mm diameter)[a]	Average Cumulative Tumor Diameter per Pouch(mm)[a]
Experimental Group[b]						
1. Control (mock inoculation + TA of mineral oil)	20	0	0	0	NA	NA
2. HSV-1 inoculation + TA of mineral oil	20	0	0	0	NA	NA
3. Mock inoculation + TA of 1% NNK	20	0	0	0	NA	NA
4. HSV-1 inoculation + TA of 1% NNK	20	0	0	0	NA	NA
5. Mock inoculation + TA of 1% NNN	20	0	0	0	NA	NA
6. HSV-1 inoculation + TA of 1% NNN	20	0	0	0	NA	NA
7. Mock inoculation + TA of 1% B[*a*]P	20	4	6	1.5	0.5	0.75 ± 0.40
8. HSV-1 inoculation + TA of 1% B[*a*]P	18	10[c]	30[c]	3.0[c]	0.8[d]	2.4 ± 0.5[d]

[a] *NA: not applicable.*
[b] *TA: topical application.*
[c] *Significantly different ($p < 0.05$) from group 7 (Fisher's exact test, double tailed).*
[d] *Significantly different ($p < 0.05$) from group 7 (Student's t test, double pair).*

DNA probes. After hybridization, the filter was washed and exposed to X-ray film. The physical state of viral DNA in the HOK-16A, HOK-16B, and HGK-18 lines was determined by Southern blot hybridization analysis as indicated in the figure legends. Northern blot hybridization was performed to determine expression of HPV DNA, c-*myc* proto-oncogene, and β-actin gene in the transformed cells (Park et al., 1991).

In Vivo Tumorigenicity of HPV-Transformed Oral Keratinocytes

NHOK, NHGK, HOK-16A, HOK-16B, and HGK-18 monolayer cultures were trypsinized, resuspended in PBS, and subcutaneously injected into 25 athymic nude mice (*nu/nu*; $1x10^7$ cells/0.1 mL per animal; five animals per cell type) 1 d after mice had been X-irradiated (300R). All mice were injected on the right flank and monitored twice weekly for the appearance of tumors over a period of more than 3 mo.

RESULTS

Cocarcinogenicity Of HSV-1 in B[*a*]P-Induced Oral Cancer

As we reported previously (Park et al., 1988), repeated HSV-1 inoculation induces neither tumors nor histopathologic changes in hamster buccal pouch mucosa. The present data also show that tumors or malignant histopathologic changes do not develop in the buccal pouch mucosa of animals receiving mineral oil, NNN, or NNK, alone or in combination with repeated HSV-1 inoculation. The body weights of animals were not altered by HSV-1 inoculation and/or topical application of chemical carcinogens. However, 20 percent of hamsters receiving topical B[*a*]P along with mock inoculation developed tumors in the right pouches, while 56 percent of animals treated with topical B[*a*]P and HSV-1 inoculation developed tumors in the right pouch mucosa (Table 1). HSV-1 inoculation also significantly hastened the appearance of B[*a*]P-induced tumor formation. The tumors appeared 16 wk after the initiation of topical B[*a*]P in animals receiving B[*a*]P plus mock inoculation, but the tumors occurred 11 to 12 wk after initiation of B[*a*]P treatment in animals treated with both topical B[*a*]P and HSV-1 inoculation. The average size of tumors in animals receiving both HSV-1 and B[*a*]P was significantly greater than in those receiving B[*a*]P treatment with mock inoculation (Table 1).

Microscopic findings also show that HSV-1 inoculation induced more malignant histopathologic changes in pouch mucosa receiving B[*a*]P. A significantly higher number of pouches showed epithelial atypia and cancer invasion in the group receiving both HSV-1 inoculation and topical B[*a*]P, than in the group receiving mock inoculation and topical B[*a*]P (Figure 27-1 and Table 2). These data indicate that NNN and NNK are not carcinogenic in hamster buccal pouches and that HSV-1 does not alter the noncarcinogenicity of these compounds in the pouch epithelium. B[*a*]P, alone, demonstrated weak carcinogenicity in buccal pouches, an effect that was significantly increased by HSV-1. These results also confirm that HSV-1 alone is not carcinogenic, but can selectively enhance the oncogenicity of certain chemical carcinogens in the oral cavity of hamsters.

Table 2
Effect of tobacco-related chemical carcinogens and HSV-1, alone or in combination, on the histopathologic changes of hamster buccal pouch mucosa

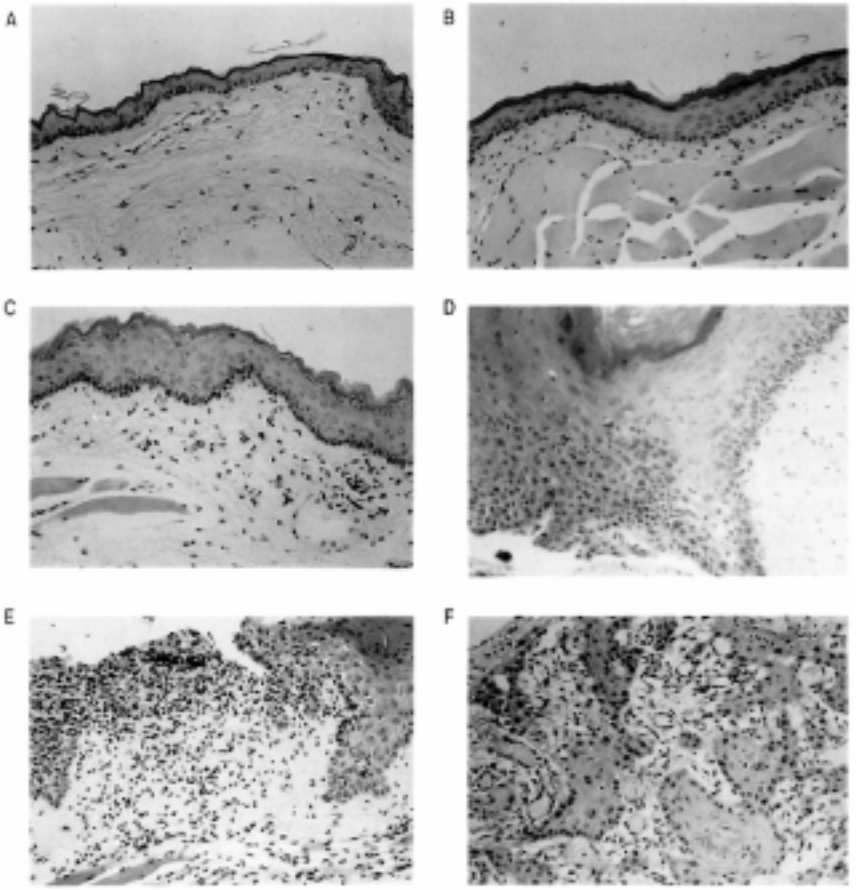
	Histopathologic Changes of Right Pouch Mucosa[a]					
	Hyper-keratosis	Granular Cell Layer Hyperplasia	Acanthosis	Inflammatory Infiltrate in Lamina Propria	Epithelial Atypia	Invasive Squamous Cell Carcinoma
Experimental Group[b]						
1. Control (mock inoculation + TA of mineral oil)	0/20	0/20	0/20	0/20	0/20	0/20
2. HSV-1 inoculation + TA of mineral oil	0/20	0/20	0/20	0/20	0/20	0/20
3. Mock inoculation + TA of 1% NNK	10/20	5/20	5/20	0/20	0/20	0/20
4. HSV-1 inoculation + TA of 1% NNK	9/20	6/20	6/20	0/20	0/20	0/20
5. Mock inoculation + TA of 1% NNN	11/20	5/20	7/20	0/20	0/20	0/20
6. HSV-1 inoculation + TA of 1% NNN	10/20	7/20	6/20	0/20	0/20	0/20
7. Mock inoculation + TA of 1% B[*a*]P	11/20	9/20	9/20	7/20	4/20	1/20
8. HSV-1 inoculation + TA of 1% B[*a*]P	12/18	10/18	12/18	15/18[c]	10/18[c]	4/18[c]

[a] *The excised pouch tissues were fixed in 10% neutral formalin, sectioned in paraffin, and stained with hematoxylin and eosin for light microscopic findings. Numerator is the number of pouches with the described histopathologic changes; denominator is the number of pouches examined.*
[b] *TA: topical application.*
[c] *Significantly different ($p < 0.05$) from group 7 (Fisher's exact test, double tailed).*

Proliferation Pattern and Morphology of Oral Keratinocytes

NHOK, NHGK, and G418-resistant cell colonies transfected with the vector plasmids ($pdMMT_{neo}$ or $pSV2_{neo}$) were similar in their morphology and could not be subcultured beyond the fifth to sixth passage. The G418-resistant cell colonies transfected with recombinant pMHPV-16d or pSHPV-18m plasmids, however, appear to be immortal, these cells have now been maintained through 40 passages over

Figure 1
Microphotographs representing the induction of hyperkeratosis, hyperplasia, acanthosis, carcinoma in situ, and invasive cancer from the hamster buccal pouches. A., control; B., hyperplasia and hyperkeratosis; C. and D., acanthosis and hyperkeratosis; E., carcinoma in situ; F., invasive cancer (original magnification x100).



8 mo. The cells display keratinocyte morphology and are characterized by a lack of stratification. These cells continue to proliferate and to retain an undifferentiated morphology. The transformed cell lines proliferate faster than NHOK or NHGK, have a cobblestone-like morphology, and establish a higher density at confluence in comparison with the normal counterpart.

Viral DNA in HPV-Transformed Oral Keratinocytes

DNA from NHOK and NHGK did not hybridize to HPV-16 DNA and HPV-18 DNA, respectively, indicating that NHOK and NHGK did not contain HPV-16 and HPV-18 DNA, respectively. DNA from HOK-16A, HOK-16B, and HGK-18 cell lines hybridized to the viral probes, suggesting the presence of viral DNA in the immortalized cell lines. Densitometric analysis showed that HOK-16A and HOK-16B cell lines

contain approximately 40 and 25 copies of HPV-16 DNA per cell, respectively, while the HGK-18 cell line harbors about 10 copies of HPV-18 DNA per cell (Figures 2 and 3).

After digestion of HOK-16A and HOK-16B cellular DNA with *Eco*RV, an enzyme that does not cut the pMHPV-16d plasmid, Southern blot analysis showed a single HPV-16-specific band, larger than 30 kbp, suggesting that HPV DNA exists as an integrated form, not an episomal form, in the HOK-16A and -16B cell lines. After *Bam*HI digestion, which releases the HPV-16 DNA sequences from vector DNA, Southern analysis showed that HOK-16A and -16B cell lines contained 7.9-kbp HPV-16 DNA genome, indicating an integration of intact HPV-16 DNA into host chromosomes in these cell lines. In addition to the expected 7.9-kbp complete HPV-16 DNA genome, the cell lines contained rearranged HPV-16 DNA sequences; the hybridization of HOK-16A and -16B DNA digested with *Bam*HI showed multiple HPV-16-specific bands that were bigger or smaller than 7.9-kbp (Figure 27-2). Similar to HOK-16A and -16B lines, HGK-18 DNA also contained intact and integrated HPV-18 DNA; after *Eco*RI digestion, which releases HPV-18 DNA from vector, Southern analysis showed 7.9-kbp band hybridized to ^{32}P-HPV-18 DNA. After digestion with *Sal*I, an enzyme that does not cut the pSHPV-18m plasmid, a single HPV-18-specific band larger than 30 kbp was seen, suggesting that HPV-18 DNA exists as an integrated form in the HGK-18 cell line (Figure 3).

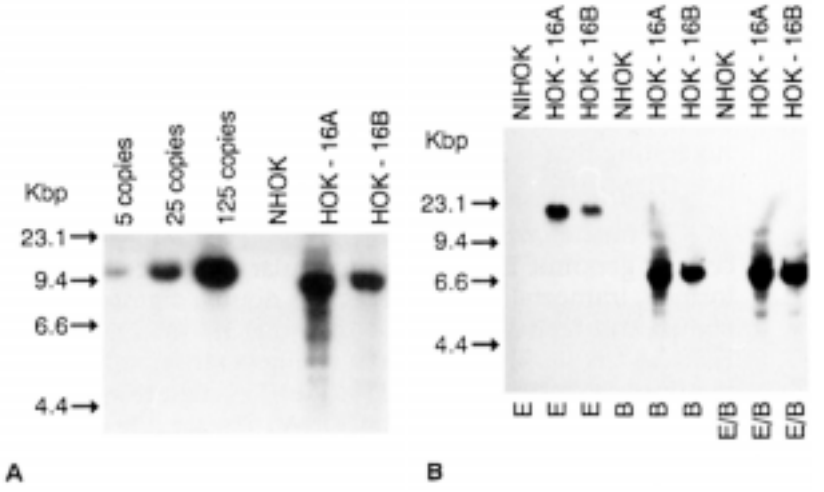
To further assess the integration of rearranged viral sequences into cellular genomic DNA, high molecular weight cellular DNA from the transformed, immortalized cell lines was double digested with two enzymes: *Bam*HI and *Eco*RV (for HOK-16A and HOK-16B DNA) or *Eco*RI and *Sal*I (for HGK-18 DNA). This treatment can generate smaller, viral-specific, rearranged DNA fragment(s) if *Eco*RV or *Sal*I digestion sites exist in cellular DNA covalently linked to viral DNA. The double restriction profiles of HOK-16A and HGK-18 DNA were similar to *Bam*HI and *Eco*RI restriction profiles of the DNA, respectively, indicating no rearranged fragments with junctions between cellular and viral DNA (Figures 2 and 3). This does not rule out integration, because *Eco*RV or *Sal*I restriction sites do not necessarily exist in the rearranged HPV-16-specific *Bam*HI fragments or HPV-18-specific *Eco*RI fragments in HOK-16A DNA and HGK-18 DNA, respectively. However, the double digestion of HOK-16B DNA generated smaller rearranged HPV-16-specific DNA fragments than were observed after the single *Bam*HI digestion (Figure 2 and 3), providing conclusive evidence for integration into this line.

Northern blot hybridization using ^{32}P-HPV-16 DNA probe revealed that multiple HPV-16 poly(A$^+$)RNAs were highly expressed from the HOK-16A and HOK-16B cell lines, whereas HPV-16 poly(A$^+$)RNAs were not expressed from NHOK (Figure 4). The intense 1.6-1.8-kbp band is characteristic of the major HPV-16 E6/E7 message observed in HPV-16 immortalized human keratinocyte and cervical epithelial cell lines (Figure 3). The HGK-18 cell line also abundantly expressed HPV-18 E6/E7 polyadenylated RNAs (Figure 5).

Figure 2
Southern blot hybridization analysis of cellular DNA of NHOK, HOK-16A, and HOK-16B.

A: Determination of the presence and copy numbers of HPV-16 DNA per cell in HOK-16A and HOK-16B cell lines. HPV-16 DNA (corresponding to 5, 25, and 125 copies of viral DNA per cell which were mixed with carrier DNA [*Bam*HI-digested 10 μg of NHOK DNA]) and *Bam*HI-digested cellular DNA (10 μg) extracted from NHOK, HOK-16A, and HOK-16B were electrophoresed in 1% agarose gel. The fragmented DNA was then transferred to a nitrocellulose filter and hybridized to ^{32}P-labeled 7.9-kbp total HPV-16 DNA. The filter was washed and exposed to Kodak SB-5 X-ray film.

B: Determination of physical state of HPV-16 DNA in HOK-16A and HOK-16B cell lines. 10 μg of high molecular weight cellular DNA were digested with *Bam*HI (B) and/or *Eco*RV (E) restriction enzymes. *Bam*HI enzyme separates vector from HPV-16 sequences, while *Eco*RV does not digest pMHPV-16d. The fragmented DNA was then transferred to nitrocellulose filter and hybridized to ^{32}P-labeled 7.9-kbp HPV-16 DNA. The filter was washed and exposed to X-ray film.

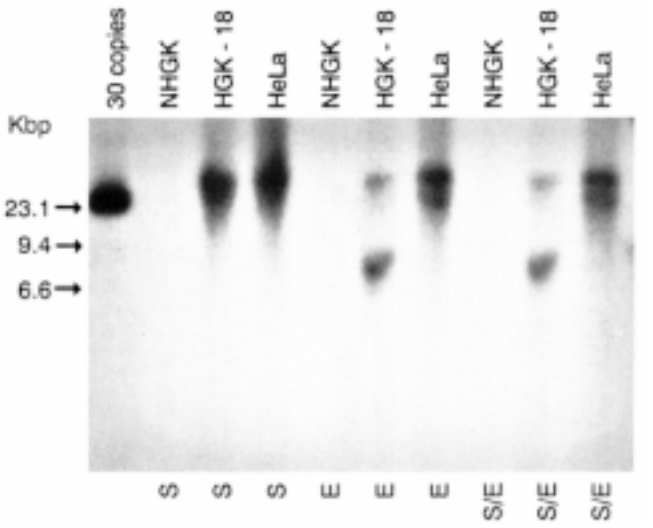


Source: Park et al., 1991; used with permission.

Expression of c-*myc* Proto-oncogene and β-Actin Gene

Figures 4 and 5 show cellular *myc* proto-oncogene and β-actin gene poly(A+)RNAs transcribed from NHOK, NHGK, and the immortalized cell lines. There are three polyadenylated, hybridized c-*myc* RNAs whose sizes are 5.2-kb, 2.4-kb, and 1.1-kb. Expression of the *myc* gene from the HOK-16A, HOK-16B, and HGK-18 cell lines was notably higher than that of the normal counterpart. The expression patterns of β-actin gene from NHOK, NHGK, HOK-16A, HOK-16B, and HGK-18 cell lines were somewhat similar and active. All cells expressed 2.0-kb mRNA in a similar manner, indicating that the cells were metabolically active (Figures 4 and 5).

Figure 3
Determination of the presence and physical state of HPV-18 DNA in HGK-18 and HeLa cell lines. HPV-18 DNA (corresponding to 30 copies of viral DNA per cell which were mixed with carrier DNA [*Eco*RI-digested 10 μg of NHGK DNA]) and *Sal*I (S) and/or *Eco*RI (E)-digested NHGK, HGK-18, HeLa cell DNAs were electrophoresed in 1% agarose gel. *Eco*RI enzyme separates vector from HPV-18 sequences, while *Sal*I does not digest pSHPV-18m. The fragmented DNA was transferred to a nitrocellulose filter and hybridized to ^{32}P-labeled 7.9-kbp total HPV-18 DNA. The filter was washed and exposed to X-ray film.



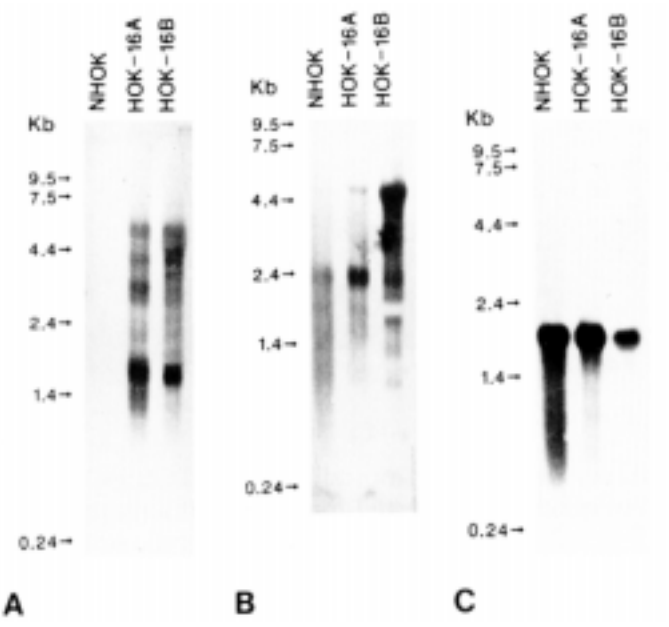
Tumorigenicity of HPV-Transformed Cells The immortalized HOK-16A, HOK-16B, and HGK-18 cell lines were tested for tumorigenicity in nude mice. Mice injected with either the immortalized cells or normal cells did not develop tumors after being monitored for more than 3 mo.

DISCUSSION Present data show that HSV-1 enhances the carcinogenicity of B[*a*]P, a tobacco-chemical carcinogen. The exact mechanism of HSV-1 cocarcinogenicity remains speculative, but HSV-1 has been proposed to stimulate the oncogenicity of chemical carcinogens by impairing the immunologic response of the host, by interfering with cellular chemical detoxification, by altering target cell permeability, or by causing proliferation of latent tumor cells (Casto and DiPaolo, 1973). Since HSV infection has been associated with chromosomal aberrations, mutations, and selective DNA amplification, HSV might alter cellular DNA, making interaction with B[*a*]P more favorable. However, rigorous laboratory investigations must be carried out to elucidate further the mechanism of HSV cocarcinogenicity.

Our results provide evidence that HPV-16 and HPV-18 participate in the carcinogenesis in one of their in vivo target cells, oral keratinocytes. NHOK and NHGK exhibited a limited in vitro lifespan, terminally differentiating after five to six passages. Liposomal transfection of these primary oral keratinocytes with pdMMT$_{neo}$ or pSV2$_{neo}$ plasmid did not extend their

Figure 4
Northern blot hybridization analysis of poly(A$^+$)RNAs of NHOK, HOK-16A, and HOK-16B cell lines

A: Determination of the expression of HPV-16 from HOK-16A and HOK-16B cell lines. Poly(A$^+$)RNAs extracted from NHOK, HOK-16A, and HOK-16B cells were electrophoresed in 1.2% agarose gel containing 2.2 M formaldehyde, transferred to a nylon filter, and hybridized to ^{32}P-labeled 7.9-kbp total HPV-16 DNA. The filter was washed and exposed to X-ray film.
B: The hybridized ^{32}P-HPV-16 DNA was stripped from the nylon filter and rehybridized with ^{32}P-labeled v-*myc* oncogene probe. The filter was washed and exposed to X-ray film.
C: Hybridized ^{32}P-v-*myc* DNA was stripped from the filter and rehybridized to ^{32}P-labeled human β-actin gene. The filter was washed and exposed to X-ray film.

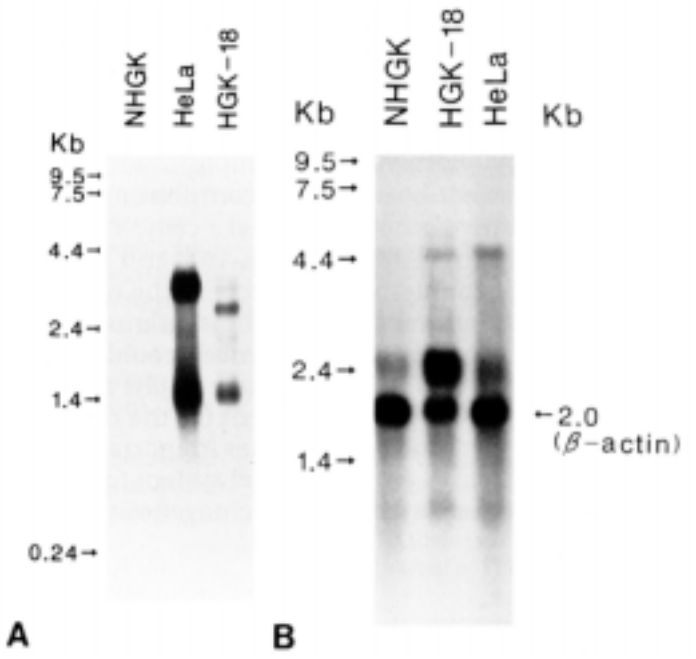


Source: Park et al., 1991; used with permission.

in vitro lifespan, but transfection with pMHPV-16d and pSHPV-18m, cloned HPV-16 and HPV-18 DNA, respectively, recombined into vector plasmid-conferred immortality. These data implicate the presence of HPV-16 or -18 DNA in the cells as a requirement for their immortality. This result supports findings of other reports showing generation of immortalized human keratinocytes by cloned HPV-16 or HPV-18 DNA (Kaur and McDougall, 1988; Woodworth et al., 1989). The transformation mechanism of keratinocytes by HPV-16 DNA transfection is not clear, but expression of HPV-16 E6/E7 gene products has been proposed as being responsible for the transformation (Kaur and McDougall, 1988). Our Northern blot analysis demonstrates that the viral E6 and E7 open reading frames are expressed in

Figure 5
Northern blot hybridization analysis of poly(A$^+$)RNAs of NHGK, HGK-18, and HeLa cell lines

A: Determination of the expression of HPV-18 from HGK-18 and HeLa cell lines. Poly(A$^+$)RNAs extracted from NHGK, HGK-18, and HeLa cells were electrophoresed in 1.2% agarose gel containing 2.2 M formaldehyde, transferred to a nylon filter, and hybridized to ^{32}P-labeled *Eco*RI-*Bam*HI 2.4-kbp fragment of HPV-18 DNA. This 2.4-kbp HPV-18 DNA fragment contains intact HPV-18 E6 and E7 genes which are responsible for cell transformation. The filter was washed and exposed to X-ray film.
B: The hybridized ^{32}P-HPV-18 DNA was stripped from the nylon filter and rehybridized with ^{32}P-labeled v-*myc* oncogene and ^{32}P-labeled human β-actin gene probes. The filter was washed and exposed to X-ray film.



the HOK-16A, HOK-16B, and HGK-18 cell lines, indicating that the transformation mechanism of oral keratinocytes by HPV-16 and HPV-18 could be similar to that of other human cells.

The physical state of the viral DNA in the transformed cell lines in this study is also similar to that reported elsewhere (Kaur and McDougall, 1988; Woodworth et al., 1989). Southern blot hybridization analysis showed that both HPV-16 and HPV-18 DNA are retained in integrated form in the HOK-16B cell line and suggests the same for line HOK-16A and HGK-18 cell lines. Integrated DNA might be absolutely necessary for the morphological transformation and immortalization of the cells, because HPV-16 DNA found in benign lesions is always in the episomal form, whereas it is usually integrated into host chromosome in carcinomas, even though episomal viral DNA can persist concurrently.

Northern blot analysis of the immortalized cell lines shows that the viral DNA, including the early region encoding the E6/E7 genes, is actively transcribed. The E6 and E7 genes of HPV-16 and HPV-18 have been reported to be retained in many cancer cell lines (Schwarz et al., 1985) and may be responsible, in part, for the immortality of the HOK-16A, HOK-16B, and HGK-18 cell lines.

Since HPV-16 and -18 DNAs are integrated in cervical carcinomas and these tumors appear to retain the HPV enhancer region, it is possible that HPV insertion also results in an activation of certain cellular proto-oncogenes (Durst et al., 1987). In HeLa cell chromosomes, HPV-18 DNA has been found to integrate into four specific chromosomes: chromosomes 8, 9, 5, and 22. Of these, chromosomes 8, 9, and 22 correspond to the location of cellular *myc*, *abl*, and *sis* proto-oncogenes, respectively. Lazo and colleagues (1989) reported that the location of the HPV DNA integration site is near the *myc* proto-oncogene, but the *myc* gene is not rearranged or amplified. It may, however, be expressed through a viral regulatory sequence in its proximity. Both viral gene amplification and constitutive c-*myc* gene expression might be important contributing factors to the immortalization and proliferative properties of HeLa cells. Like HeLa cells, the c-*myc* gene is overexpressed in HOK-16A, HOK-16B, and HGK-18 cell lines compared to their normal counterpart. The role of the overexpressed c-*myc* gene products in the immortalized cell lines is unknown, but it, along with the expression of HPV-16 or HPV-18 sequences, could play a crucial role for the immortalization of the cells. These results also suggest that the overexpression of c-*myc* gene is not sufficient for the cells to be tumorigenic. The human oral keratinocyte cell lines immortalized by HPV-16 and HPV-18 provide, therefore, a useful model system for elucidating critical molecular changes associated with oral carcinogenesis.

REFERENCES

Casto, B.C., DiPaolo, J.A. Virus, chemicals, and cancer. *Progress in Medical Virology* 16: 1-47, 1973.

Dekmezian, R.H., Batsakis, J.G., Goepfert, H. In situ hybridization of papillomavirus DNA in head and neck squamous cell carcinomas. *Archives of Otolaryngology—Head and Neck Surgery* 113: 819-821, 1987.

Durst, M., Croce, C.M., Gissmann, L., Schwarz, E., Huebner, K. Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas. *Proceedings of the National Academy of Sciences* 84: 1070-1074, 1987.

Durst, M., Gissmann, L., Ikenburg, H., zur Hausen, H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proceedings of the National Academy of Sciences* 80: 3812-3815, 1983.

Eglin, R.P., Scully, C., Lehner, T., Ward-Booth, P., McGregor, I.A. Detection of RNA complementary to herpes simplex virus in human oral squamous cell carcinoma. *Lancet* 2: 766-768, 1983.

Hecht, S.S., Chen, C.B., Dong, M., Ornaf, R.M., Hoffmann, D., Ts'o, T.C. Chemical studies on tobacco smoke: VI. Studies on nonvolatile nitrosamines in tobacco. *Beitrage Tabakforsch* 9: 1-6, 1977.

Hirsch, J.M., Johansson, S.L., Vahlne, A. Effect of snuff and herpes simplex virus 1 on rat oral mucosa: Possible associations with the development of squamous cell carcinoma. *Journal of Oral Pathology* 12: 187-198, 1983.

Hoffmann, D., Brunnenmann, K.D., Adams, J.D., Hecht, S.S. Formation and analysis of N-nitrosamines in tobacco products and their endogenous formation in consumers. In: *N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer*, I.K. O'Neill, R.C. von Borsteel, C.T. Miller, J. Long, and H. Bartsch (Editors). New York: Oxford University Press, 1984, pp. 743-762.

Kaur, P., McDougall, J.K. Characterization of primary human keratinocytes transformed by human papillomavirus type 18. *Journal of Virology* 62: 1917-1924, 1988.

Lazo, P.A., DiPaolo, J.A., Popescu, N.C. Amplification of the integrated viral transforming genes of human papillomavirus 18 and its 5'-flanking cellular sequence located near the *myc* proto-oncogene in HeLa cells. *Cancer Research* 49: 4305-4310, 1989.

McCance, D. Human papillomaviruses and cancer. *Biochimica et Biophysica Acta* 823: 195-205, 1986.

National Institutes of Health. Workshop on the treatment and prevention of herpes simplex virus infection. *Journal of Infectious Diseases* 127: 117-119, 1973.

Offenbacher, S., Weathers, D.R. Effects of smokeless tobacco on the periodontal, mucosal and caries status of adolescent males. *Journal of Oral Pathology* 14: 162-181, 1985.

Oh, J.S., Paik, D.-I., Christensen, R., Akoto-Amanfu, E., Kim, K., Park, N.-H. Herpes simplex virus enhances the 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced carcinogenesis and amplification and overexpression of c-*erb*-B-1 proto-oncogene in hamster buccal pouch epithelium. *Oral Surgery, Oral Medicine, Oral Pathology* 68: 428-435, 1989.

Overall, J.C., Jr. Dermatologic Diseases. In: *Antiviral Agents and Viral Diseases of Man*, G.J. Galasso, T.C. Merigan, and R.A. Buchanan (Editors). New York: Raven Press, 1979, pp. 305-384.

Park, N.-H., Akoto-Amanfu, E., Paik, D.I. Smokeless tobacco carcinogenesis: The role of viral and other factors. *CA-A Cancer Journal for Clinicians* 38: 248-256, 1988.

Park, N.-H., Min, B.-M., Li, S.-L., Cherrick, H.M., Doniger, J. Immortalization of human oral keratinocytes with type 16 human papillomavirus. *Carcinogenesis* 12: 1627-1631, 1991.

Park N.-H., Niukian, K., Shklar, G. Combined effect of herpes simplex virus and tobacco on the histopathologic changes in lips of mice. *Oral Surgery, Oral Medicine, Oral Pathology* 59: 154-158, 1985.

Schwarz, E., Freese, U.K., Gissmann, L., Mayer, W., Roggenburk, B., Stremlau, A., zur Hausen, H. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. *Nature (London)* 314: 111-114, 1985.

Shillitoe, E.J., Greenspan, D., Greenspan, J.S., Hanson, L.S., Silverman, S., Jr. Neutralizing antibody to herpes simplex virus type 1 in patients with oral cancer. *Cancer* 49: 2315-2320, 1982.

Shklar, G., Niukian, K., Hassan, M., Herbosa, E.G. Effects of smokeless tobacco and snuff on oral mucosa of experimental animals. *Journal of Oral and Maxillofacial Surgery* 43: 80-86, 1985.

U.S. Department of Health and Human Services. *Health Consequences of Smoking: Cancer. A Report of the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, Office on Smoking and Health. DHHS Publication No. (PHS) 82-50179, 1982.

U.S. Department of Health and Human Services. *Health Implications of Smokeless Tobacco Use: National Institutes of Health Consensus Development Conference Statement*. 6, No. 1. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, 1986.

Woodworth, C.D., Doniger, J., DiPaolo, J.A. Immortalization of human foreskin keratinocytes by various human papillomavirus DNAs corresponds to their association with cervical carcinoma. *Journal of Virology* 63: 159-164, 1989.

Chapter 4

Nicotine Effects and Addiction

CONTENTS

Pharmacology of Smokeless Tobacco Use: Nicotine Addiction and Nicotine–Related Health Consequences[1]

Neal L. Benowitz

ABSTRACT Nicotine is easily absorbed from smokeless tobacco and could contribute to adverse health consequences of ST use. Because nicotine is the cause of addiction to cigarettes, ST use may have a similar addiction liability. Cessation of ST use is difficult for many people. The nicotine withdrawal syndrome that follows the cessation of regular ST use supports the idea that some users are addicted to ST. Experimental data in support of this proposition indicate that ST users adjust their tobacco-consuming behavior to regulate levels of nicotine in the body. ST use results in cardiovascular effects that are similar to those of cigarette smoking. Chronic systemic exposure to nicotine from cigarette smoking may contribute to accelerated coronary and peripheral vascular disease, acute cardiac ischemic events, delayed wound healing, reproductive disturbances, peptic ulcer disease, and esophageal reflux. Insofar as nicotine contributes to the adverse health effects of cigarette smoking, the nicotine in ST would be expected to present similar hazards. Illness caused by systemic absorption of nicotine and other toxins from ST should be considered a potential sequel to long-term tobacco use.

INTRODUCTION In addition to causing oral pathology, there is concern that habitual, long-term smokeless tobacco use produces systemic effects that might adversely affect health. Of particular concern is exposure to nicotine, which is present in large amounts in smokeless tobacco (Table 1). This paper reviews the pharmacology of nicotine as related to the use and potential health hazards of smokeless tobacco.

PHARMACOLOGY OF NICOTINE Nicotine is a tertiary amine composed of a pyridine and a pyrrolidine ring. Nicotine binds to acetylcholine receptors at ganglia and neuromuscular junctions and in the brain (Benowitz, 1988). In its non-ionized form, nicotine freely permeates membranes, including the buccal mucosa and the blood-brain barrier. As a weak base, nicotine is less ionized and penetrates membranes more easily in alkaline solutions. Chewing tobacco and snuff, as well as nicotine gum, are buffered to an alkaline pH to facilitate absorption of nicotine.

Nicotine is absorbed more slowly from ST than from tobacco smoke, but peak venous levels are similar (Figure 1). Whereas blood levels of nicotine fall rapidly after smoking, the concentrations plateau during and after ST use, consistent with continued absorption even after the tobacco is removed from the mouth (Benowitz et al., 1988). Possibly, continued absorption of nicotine is attributable to release of nicotine from mucous membranes and/or absorption of nicotine that has been swallowed. The systemic absorption of nicotine per dose is greater with the use of chewing tobacco (average 4.5 mg nicotine from an average dose of 7.9 g chewed for

[1] Supported in part by grants no. CA-32389, DA-02277, DA-01696, and RR-00083 from the National Institutes of Health.

Table 1
Nicotine content of cigarettes and smokeless tobacco

	Concentration of Nicotine (mg/g)	Typical Single Dose (g Tobacco)	Nicotine in Single Dose (mg)	Nicotine in Dose Typically Consumed in 1 Day
Cigarettes (15)[a]	15.7 (13.3-26.9)[b]	0.54	8.4	168 mg per 20 cigarettes
Moist Snuff (8)[a]	10.5 (6.1-16.6)[b]	1.4	14.5	157 mg per 15 g
Chewing Tobacco (2)[a]	16.8 (8.1-24.5)[b]	7.9	133.0	1,176 mg per 70 g

[a] *Number of brands tested.*
[b] *Range.*
Source: Adapted from Benowitz et al., 1990.

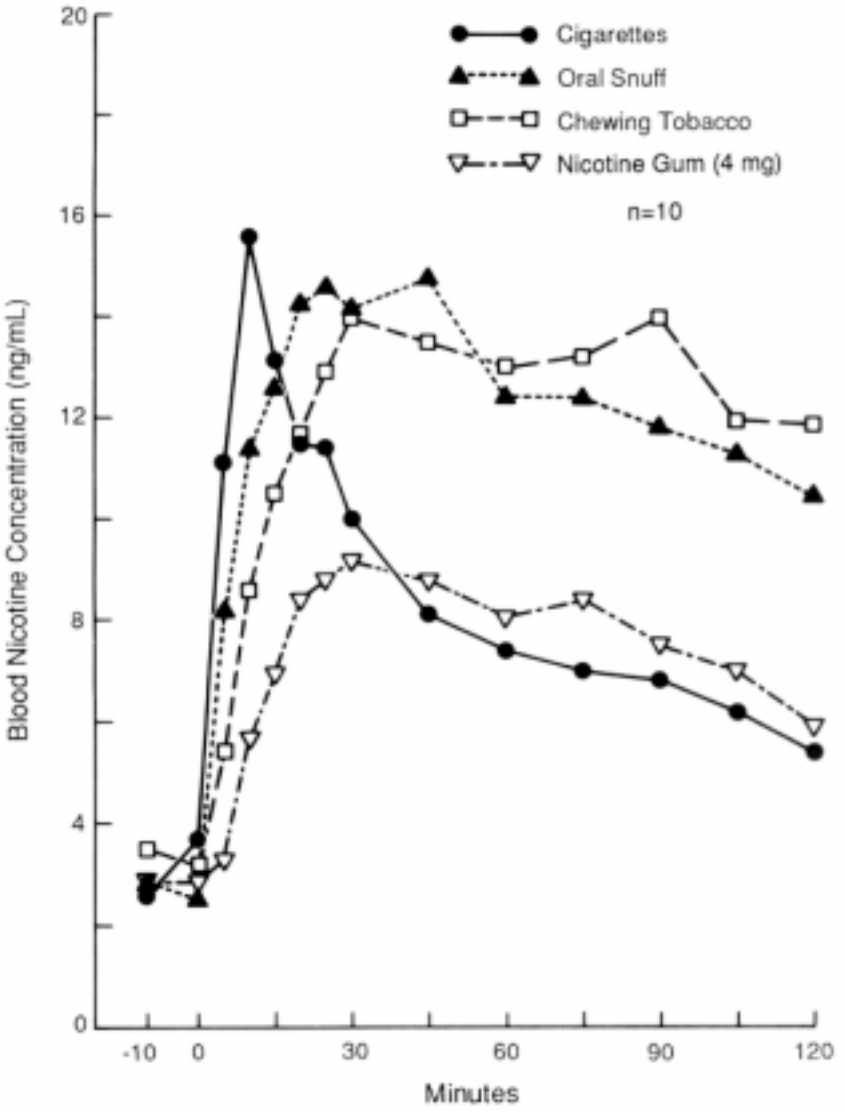
30 min) or snuff (average 3.6 mg nicotine from 2.5 g moist snuff kept in the mouth for 30 min) compared with that from smoking cigarettes (average 1.0 mg nicotine per cigarette). Individuals vary considerably in the amount of nicotine reaching the systemic circulation, even when they use a fixed dose of smokeless tobacco. For example, we found an eightfold range in peak plasma nicotine concentration (range 4 to 33 ng/mL) among 10 subjects who placed 2.5 g snuff in the mouth for 30 min. The average systemic bioavailability of nicotine from cigarettes and smokeless tobacco can be estimated from the average systemic doses of nicotine absorbed, as described above, and the data on the amount of nicotine in tobacco, as shown in Table 1. Systemic bioavailability is estimated to be 12.0 percent, 14.0 percent, and 3.4 percent for cigarette smoke, oral snuff, and chewing tobacco, respectively.

Nicotine is extensively metabolized by the liver (Benowitz, 1988). The major metabolite is cotinine, which has been used as a marker for nicotine intake. The half-life of nicotine averages 2 to 3 h. Consistent with this half-life, nicotine accumulates for 6 to 8 h throughout the day with regular ST use, and the levels of nicotine persist overnight, even while the user sleeps (Figure 2) (Benowitz et al., 1989).

NICOTINE AND COTININE LEVELS IN BLOOD

As Figure 1 shows, peak levels of nicotine after cigarette smoking or single doses of oral snuff or chewing tobacco are similar. Likewise, nicotine levels after the use of ST in sachets (Skoal Bandits) and after nasal insufflation of fine, ground nasal snuff are similar, although in the latter case absorption is very rapid, resembling absorption from cigarette smoke (Russell et al., 1981 and 1985).

Figure 1
Nicotine absorption rates from tobacco smoke and smokeless tobacco

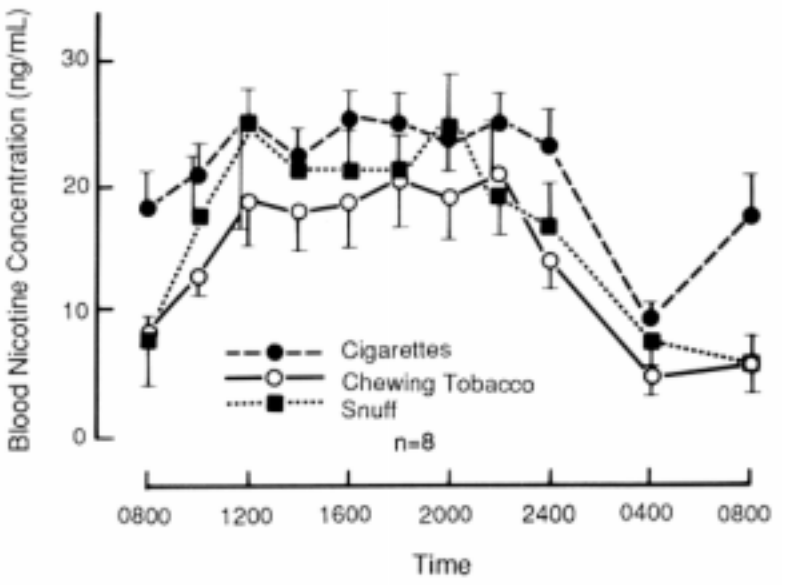


Average blood nicotine levels in 10 men who smoked for 9 min (1.3 cigarettes), placed 2.5 g moist oral snuff in the mouth for 30 min, chewed an average of 7.9 g (range of 0.9 g to 17.8 g) chewing tobacco for 30 min, and chewed 4 mg nicotine gum (two 2-mg pieces of Nicorette) for 30 min. Studies were performed in the morning after overnight abstinence from tobacco.

Source: Adapted from Benowitz et al., 1988.

With regular daily cigarette smoking, blood or plasma levels of nicotine sampled in the afternoon when those levels are at or near steady state generally range from 10 to 50 ng/mL. In a research ward study of eight

Figure 2
Circadian blood nicotine concentration with cigarette smoking, chewing tobacco, and oral snuff



Blood nicotine concentrations in subjects who smoked cigarettes (closed circles), used chewing tobacco (open circles), or used oral snuff (closed squares). Data are shown as mean ± S.E. for eight subjects.

Source: Adapted from Benowitz et al., 1989; used with permission.

men, the levels of nicotine during ad libitum use of oral snuff (averaging 15.6±5.9 g/d) or chewing tobacco (averaging 72.9±21.6 g/d) were similar to those observed with cigarette smokers (average 15.6±5.9 cigarettes/d) (Figure 2) (Benowitz et al., 1989).

Using plasma cotinine as an indicator of daily nicotine consumption from ST, one can compare consumption in different populations (Table 2). Cotinine levels in our research volunteers were somewhat higher than those measured in other groups. The college students and young men studied by Gritz et al. (1981) and Biglan et al. (in press) had cotinine levels averaging 55 to 60 percent of those seen in our research subjects, the latter of whom were, on average, much older. Professional baseball players had, in general, much lower cotinine values, particularly among the chewing tobacco users (Siegel et al., 1992). The lower level of cotinine in the baseball players reflects intermittent use, often in conjunction with playing baseball.

NICOTINE AND ST ADDICTION

People smoke cigarettes for the psychoactive effects of nicotine, and it is presumed that smokeless tobacco is consumed for the same reason. Nicotine may enhance the sense of well-being, produce arousal or relaxation, help maintain vigilance, and reduce anxiety (Benowitz, 1988).

Table 2
Serum cotinine in different populations of ST users

Source	ST Type	Users (n)	Plasma Cotinine (ng/mL)	
			Mean	Range
Gritz, 1981	Snuff	11	197[a]	14–556
Benowitz, 1989	Snuff	8	356[b]	182–868
Benowitz, 1989	Chew	8	354[b]	100–836
Siegel, 1992	Snuff	182	144[c]	–
Siegel, 1992	Chew	48	82[c]	–
Biglan, 1992	Snuff	20	217[d]	–

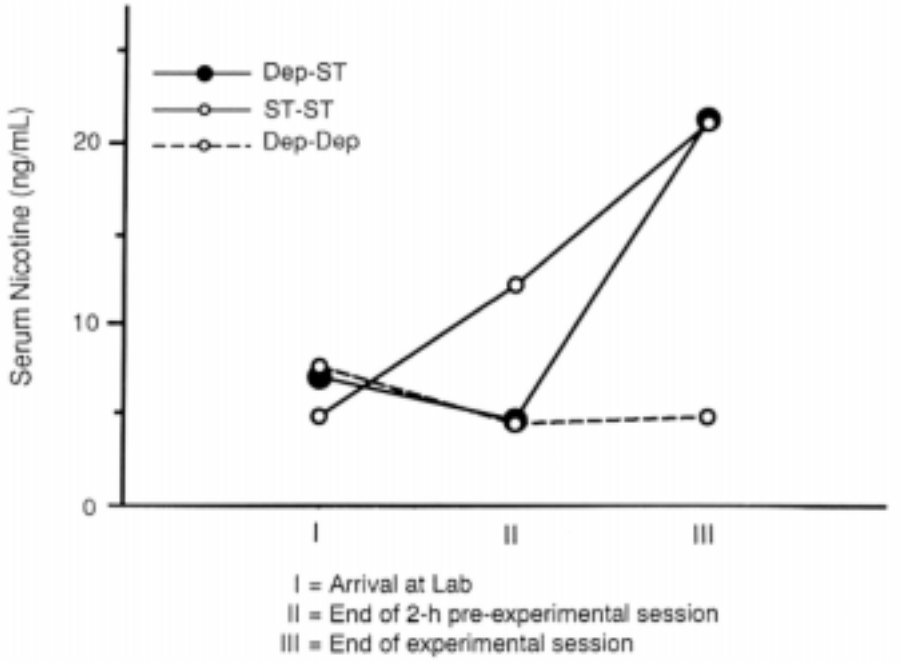
[a] *Afternoon measurement.*
[b] *Mean values throughout 24 h.*
[c] *Different times of day.*
[d] *Morning measurement.*

Whether enhancement of performance and mood is the result of an intrinsic enhancing effect of nicotine, or of relief of symptoms of abstinence in habitual users, is unclear. ST use is especially common among male athletes, some of whom believe that it enhances their athletic performance. Studies of the effects of ST on reaction time in athletes, however, have not confirmed any improvement in performance (Edwards and Glover, 1986).

Addiction to or dependence on smokeless tobacco can be defined as compulsive use despite awareness of substantial reasons not to use it. Formal criteria for establishing drug dependence have been developed in the Surgeon General's Report, *The Health Consequences of Smoking: Nicotine Addiction* (US DHHS, 1988). The primary criteria include highly controlled or compulsive use, psychoactive effects, and drug-reinforced behavior. Additional criteria include physical dependency, among several others. The compulsive use of smokeless tobacco has been reported and is described elsewhere in this volume. Nicotine from ST clearly has psychoactivity, and nicotine withdrawal symptoms develop after sudden cessation of ST use (Hatsukami et al., 1987).

That nicotine reinforces ST use has been shown recently in studies by Biglan and coworkers (in press). In a laboratory setting, depriving young men who regularly use smokeless tobacco from their tobacco for a period of time resulted in a greater consumption of ST than in a condition under which smokeless tobacco was given as a loading condition (Figure 3). The level of ST consumption was such that, whether or not subjects were preloaded with smokeless tobacco, after a period of ad libitum consumption, serum nicotine levels were similar in all conditions. In a second study of young men who regularly used snuff and smoked cigarettes, Biglan and coworkers found that when ST use was restricted, subjects smoked more cigarettes, and when cigarette use was restricted, subjects consumed more smokeless tobacco, compared to when both forms of tobacco were available

Figure 3
Serum nicotine concentration after deprivation then ad libitum oral snuff use in regular users



Condition I was on arrival at the laboratory after overnight tobacco abstinence. Condition II was at the end of a 2-h pre-experimental session when subjects were either deprived of (Dep) or preloaded with smokeless tobacco (ST). Condition III was at the end of the experimental session during which subjects were either deprived or had ad libitum access to smokeless tobacco.

Source: Adapted from Biglan et al., in press.

(Biglan et al., in press). These studies indicate that habitual ST users are titrating a level of nicotine in the body, as has been well described for cigarette smokers, supporting the dependence criterion of drug-reinforced behavior. That habitual ST use increases the likelihood of cigarette smoking when smokeless tobacco is unavailable or undesirable because of social constraints is supported by population survey data (Glover et al., 1989).

Thus, the criteria for addiction to nicotine are met for at least some ST users. The 1986 Surgeon General's Report on smokeless tobacco concluded that ST is an addicting substance (US DHHS, 1986). It is unclear what proportion of all ST users are addicted. Professional baseball players who use smokeless tobacco intermittently, often only in association with playing their game, for the most part do not seem to be addicted to ST.

HEALTH CONSEQUENCES OF NICOTINE EXPOSURE

Nicotine has many actions on the human body (Benowitz, 1988) (Table 3). The role of nicotine in producing these effects has been established by studies of direct administration. In general, the responses are consistent with activation of the sympathetic nervous system. Cardiovascular effects include heart rate acceleration (10 to 20 beats/min) and increased blood pressure (5 to 10 mmHg), similar to the effects of cigarette smoking. Nicotine also increases the circulating levels of catecholamines and free fatty acids, which may contribute to the increased level of total cholesterol and decreased levels of high-density lipoprotein cholesterol that are found in habitual cigarette smokers. Inhibition of prostacyclin synthesis and other effects on platelets may enhance coagulation.

The potential adverse health consequences of nicotine may be summarized as follows:

- Nicotine intoxication;
- Accelerated coronary and peripheral vascular disease;
- Stroke;
- Hypertension (complications);
- Delayed wound healing;
- Reproductive or perinatal disorders (low birth weight, prematurity, spontaneous abortion);
- Peptic ulcer disease; and
- Esophageal reflux.

For more details, see Benowitz (1988, 1991a, and 1991b) and US DHHS (1986 and 1988).

The greatest concern for nicotine–related effects is acceleration or aggravation of cardiovascular disease (Benowitz, 1991a). In a study of the cardiovascular effects of daily ST use, we found that the most prominent effects of nicotine—heart rate acceleration and increased urinary catecholamine excretion—were similar throughout the day in people smoking cigarettes and those using smokeless tobacco (Benowitz et al., 1989). In addition, urine sodium excretion was greater during use of smokeless tobacco than during smoking, probably because of the absorption of sodium, which, in smokeless tobacco, acts as an alkaline buffer to facilitate nicotine absorption. Insofar as nicotine contributes to adverse health effects of cigarette smoking, nicotine in smokeless tobacco would be expected to present similar hazards.

Nicotine could promote atherosclerotic vascular disease by contributing to hyperlipidemia, endothelial injury, or both (Benowitz, 1991a). Although cigarette smoking is not associated with an increased risk of hypertension, complications of hypertension are more severe in people who also smoke cigarettes (Isles et al., 1979). Nicotine may aggravate hypertension by causing vasoconstriction. Case histories of patients with hypertension aggravated by the use of smokeless tobacco have been reported (McPhaul et al., 1984; Wells and Rustick, 1986), and one survey of college students indicated that ST users had elevated blood pressure (Schroeder and Chen,

Table 3
Actions of nicotine in humans

Affected Systems	Effects
CNS	Arousal or relaxation Enhanced concentration, vigilance Appetite suppression Electroencephalographic changes
Cardiovascular	Increased heart rate, cardiac contractility, blood pressure Cutaneous vasoconstriction Systemic venoconstriction Increased muscle blood flow Catecholamine release
Metabolic	Lipolysis with fatty acid release Increased energy expenditure
Endocrine	Increased growth hormone Adrenocorticotrophic hormone/cortisol Vasopressin Beta endorphins Inhibition of prostacyclin synthesis

1985). However, a more recent study of ST use among baseball players revealed no relationship between smokeless tobacco and blood pressure (Ernster et al., 1990).

There is considerable evidence that nicotine may contribute to acute cardiac ischemia, such as aggravating angina pectoris, precipitating unstable angina or acute myocardial infarction, and even sudden death (Benowitz, 1991a). The possible mechanisms of nicotine effect include its systemic hemodynamic effects (which increase myocardial work), enhancement of thrombosis, induction of coronary vasoconstriction, and/or arrhythmogenesis.

Whether habitual use of smokeless tobacco is associated with hyperlipidemia, increased incidence of complications from hypertension, accelerated atherosclerotic vascular disease, and/or increased risk of acute cardiac ischemic events remains to be established in studies of large populations of users. Of interest in this regard is a recent study of users of smokeless tobacco that indicated they had a higher prevalence of hypercholesterolemia (when normalized for age and education) than did nonusers of tobacco (Tucker, 1989). Considering that the levels of nicotine in the blood are similar in smokers and users of smokeless tobacco, it seems likely that people with coronary artery disease are at increased risk from ST use.

Other suspected adverse health effects of nicotine, particularly reproductive disorders and peptic ulcer disease, must also be considered as potential complications of habitual ST use.

CONCLUSIONS The following may be concluded about nicotine and ST use: Systemic absorption and blood levels of nicotine are substantial and are often comparable in ST users and cigarette smokers. Data from a few studies performed to date indicate that ST use has the potential to produce dependency similar to that seen in cigarette smokers. However, it appears that in some populations, such as baseball players who use smokeless tobacco only intermittently, many ST users are not dependent. Smokeless tobacco use by young people does pose a concern for later development of dependence on cigarettes.

The health hazards known to be caused by cigarette smoking, and suspected to be related to acute or chronic nicotine exposure, are expected to be a hazard of habitual ST use as well. The major concerns are acceleration or aggravation of coronary artery disease and reproductive disorders.

REFERENCES

Benowitz, N.L. Pharmacologic aspects of cigarette smoking and nicotine addiction. *New England Journal of Medicine* 319: 1318-1330, 1988.

Benowitz, N.L. Nicotine and coronary heart disease. *Trends in Cardiovascular Medicine* 1: 315-321, 1991a.

Benowitz, N.L. Nicotine replacement therapy during pregnancy. *Journal of the American Medical Association* 266: 3174-3177, 1991b.

Benowitz, N.L., Jacob, P. III, Yu, L. Daily use of smokeless tobacco: Systemic effects. *Annals of Internal Medicine* 111: 112-116, 1989.

Benowitz, N.L., Porchet, H., Jacob, P. III. Pharmacokinetics, metabolism, and pharmacodynamics of nicotine. In: *Nicotine Psychopharmacology: Molecular, Cellular, and Behavioral Aspects*, S. Wonnacott, M.A.H. Russell, and I.P. Stolerman (Editors). Oxford: Oxford University Press, 1990, pp. 112-157.

Benowitz, N.L., Porchet, H., Sheiner, L., Jacob, P. III. Nicotine absorption and cardiovascular effects with smokeless tobacco use: Comparison with cigarettes and nicotine gum. *Clinical Pharmacology and Therapeutics* 44: 23-28, 1988.

Biglan, A., LaChance, P.A., Benowitz, N.L. Experimental analysis of the effects of smokeless tobacco deprivation. *Journal of Abnormal Psychology*, in press, 1992.

Edwards, S.W., Glover, E.D. Snuff and neuromuscular performance (letter). *American Journal of Public Health* 76: 206, 1986.

Ernster, V.L., Grady, D.G., Greene, J.C., Walsh, M., Robertson, P., Daniels, T.E., Benowitz, N., Siegel, D., Gerbert, B., Hauck, W.W. Smokeless tobacco use and health effects among baseball players. *Journal of the American Medical Association* 264: 218-224, 1990.

Glover, E.D., Laflin, M., Edwards, S.W. Age of initiation and switching patterns between smokeless tobacco and cigarettes among college students in the United States. *American Journal of Public Health* 79: 207-208, 1989.

Gritz, E.R., Baer-Weiss, V., Benowitz, N.L., Van Vunakis, H., Jarvik, M.E. Plasma nicotine and cotinine concentration in habitual smokeless tobacco users. *Clinical Pharmacology and Therapeutics* 30: 201-209, 1981.

Hatsukami, D.K., Gust, S.W., Keenan, R.M. Physiologic and subjective changes from smokeless tobacco withdrawal. *Clinical Pharmacology and Therapeutics* 41: 103-107, 1987.

Isles, C., Brown, J.J., Cummings, A.M., et al. Excess smoking in malignant-phase hypertension. *British Medical Journal* 1: 579-581, 1979.

McPhaul, M., Punzi, H.A., Sandy, A., Borganelli, M., Rude, R., Kaplan, N.M. Snuff-induced hypertension in pheochromocytoma. *Journal of the American Medical Association* 252: 2860-2862, 1984.

Russell, M.A.H., Jarvis, M.J., Devitt, G., Feyerabend, C. Nicotine intake by snuff users. *British Medical Journal* 283: 814-817, 1981.

Russell, M.A.H., Jarvis, M.J., West, R.J., Feyerabend, C. Buccal absorption of nicotine from smokeless tobacco. *Lancet* 2: 1370, 1985.

Schroeder, K.L., Chen, M.S.J. Smokeless tobacco and blood pressure. *New England Journal of Medicine* 312: 919, 1985.

Siegel, D., Benowitz, N., Ernster, V.L., Grady, D.G., Hauck, W.W. Smokeless tobacco, cardiovascular risk factors, and nicotine and cotinine levels in professional baseball players. *American Journal of Public Health*, in press, 1992.

Tucker, L.A. Use of smokeless tobacco, cigarette smoking, and hypercholesterolemia. *American Journal of Public Health* 79: 1048-1050, 1989.

U.S. Department of Health and Human Services. *Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

U.S. Department of Health and Human Services. *Health Consequences of Smoking: Nicotine Addiction. A Report of the Surgeon General, 1988*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Health Promotion and Education, Office on Smoking and Health. DHHS Publication No. (CDC) 88-8406, 1988.

Wells, D.G., Rustick, J.M. Hypertension from smokeless tobacco (letter). *Anesthesiology* 65: 339, 1986.

Recent Advances in Understanding The Actions of Nicotine in the Central Nervous System

Paul B.S. Clarke[1]

ABSTRACT There is now a consensus that nicotine is the key factor in habitual tobacco use and that it is the drug's actions on the central nervous system that are particularly important. Knowledge of sites and mechanisms of nicotine's CNS actions is advancing rapidly. Precisely which actions of the drug contribute to its reinforcing properties remain to be identified, but the participation of the mesolimbic dopamine pathway of the brain seems likely. Current pharmacological approaches to cessation of tobacco use have focused on nicotine replacement and have not met with great success. The high relapse rates encountered with this treatment approach (and with other current tobacco cessation techniques) may reflect the persistence of learned associations acquired during tobacco use. These associations could be extinguished (unlearned) through use of a drug that blocks the central actions of nicotine. Nicotine blockade therapy thus represents an attractive but largely untried treatment approach.

INTRODUCTION

Tobacco use is a major cause of preventable cancer. This review is intended primarily for health workers involved in the prevention and treatment of cancer. Its purpose is to provide an overview of recent advances in the understanding of nicotine's actions in the central nervous system and to highlight areas of uncertainty. This survey is selective and does not cover certain fields that may prove extremely important, such as the pharmacogenetic aspects of nicotine (Collins et al., 1990), the effects of nicotine on development (Navarro et al., 1989), and the interactions of nicotine with other drugs.

Two key questions serve as a framework for this review: (1) What does nicotine do in the CNS to account for its central role in tobacco use? and (2) Can pharmacologists help the tobacco user who wishes to quit?

NICOTINE'S ACTION IN THE CNS

There is widespread agreement among scientists and clinicians that most habitual smokers continue to smoke because they are dependent on nicotine. Nicotine dependence is thought to underlie the use of smokeless tobacco as well. The evidence, which has been reviewed at length elsewhere (Jaffe, 1990; US DHHS, 1988), can be summarized as follows:

As the Primary Reinforcer

- Nicotine is consumed not only via tobacco smoke but also via smokeless tobacco, thus avoiding the many pyrolysis products contained in smoke;
- Nicotine-free cigarettes generally are not smoked;

[1] The author receives research funding from Fonds de la Recherche en Santé de Québec and the Medical Research Council of Canada.

- Cigarette smokers regulate their levels of nicotine in response to pharmacological manipulations such as preloading with nicotine or receptor antagonism;
- Nicotine is self-administered intravenously by humans and animals in the laboratory;
- Self-administered doses of nicotine are psychoactive; and
- Habitual smokers experience withdrawal symptoms that can be alleviated by administration of nicotine.

Central vs. Peripheral Effects

Virtually all the known actions of nicotine appear to be mediated by nicotinic receptors (Clarke, 1987; US DHHS, 1988). There are receptors for nicotine in the CNS and in the periphery. Precisely which pharmacological actions of nicotine are important to tobacco smokers is unclear; however, it appears that the primary reinforcing actions of nicotine occur within the CNS.

Most of the evidence for this claim comes from work in animals. Almost all the behavioral effects of nicotine that have been investigated in animals appear to be attributable to direct CNS actions (Clarke, 1987), and recent evidence suggests that nicotine's reinforcing properties are no exception. Several species of laboratory animals have been shown to self-administer intravenous infusions of nicotine voluntarily. Rates of nicotine self-administration in rats were found to be markedly reduced after central administration of the nicotinic receptor blocker chlorisondamine in a low dose that was unlikely to act peripherally (Corrigall et al., 1992). Moreover, as described in a later section of this paper, lesion studies in rats have focused attention on a particular nerve pathway in the brain that seems to be intimately connected with nicotine's rewarding effects.

Central actions of nicotine also appear to be important in regulating cigarette smoking by humans. In one study, acute administration of mecamylamine, a centrally active nicotinic antagonist, altered smoking behavior, whereas the nicotinic antagonist pentolinium, which does not readily pass into the CNS, did not (Stolerman et al., 1973). In this short-term experiment, mecamylamine actually *increased* indices of smoke intake, and it seems likely that subjects smoked more in an attempt to overcome a blockade of nicotine's effects. In contrast, a preliminary report suggests that chronic treatment with mecamylamine can dramatically *reduce* smoking behavior and even permit highly dependent smokers to quit (Tennant et al., 1984). There appear to be no reports of the effects of nicotinic antagonists on consumption of smokeless tobacco.

Actions of nicotine in the peripheral nervous system may also contribute to the maintenance of tobacco use. If the evidence just reviewed suggests that the primary reinforcing effects of nicotine are likely attributable to central actions of the drug, its peripheral actions may act as secondary reinforcers. At doses obtained from cigarette smoke, nicotine appears to exert significant actions at the autonomic ganglia and at certain sensory nerve endings (but not on skeletal muscle). Jarvik and Assil (1988) have

reported that the nicotinic receptor antagonist mecamylamine attenuates the sensation produced by direct application of a nicotine solution to the tongue. This ability of nicotine to stimulate sensory nerve endings is of special interest, since sensory cues that occur in temporal proximity to smoking behavior would most readily tend to acquire the properties of secondary reinforcers. Consistent with this notion, Rose and colleagues (1985) have demonstrated that sensory stimuli produced in the airways by smoke inhalation can affect the immediate satisfaction derived from cigarettes. Although these findings are suggestive, nicotine is but one constituent of tobacco smoke, and the extent to which it contributes to secondary reinforcement remains to be determined.

Subtypes of CNS Nicotinic Receptors

On pharmacological grounds, nicotinic receptors in the periphery have long been subdivided into those occurring at the neuromuscular junction and those found in the autonomic ganglia and adrenal glands. With the advent of powerful immunological and molecular genetic techniques, an extended family of nicotinic receptors has recently been revealed. (See Deneris et al., 1991, for a review.) All known nicotinic receptors comprise a number of subunits. Generally speaking, each subunit is encoded by a different gene. An ever-growing number of such genes have been identified. For example, in the rat, more than a dozen putative subunits have been found; each receptor subtype is made up of a unique permutation of subunits, and the potential for diversity is staggering.

One factor that may limit this diversity is incompatibility between subunits. By injecting species of nicotinic receptor messenger RNA into frog oocytes, it is possible to produce and test the electrophysiological properties of "artificial" nicotinic receptors. The results of such studies show that certain combinations of subunits do not work as nicotinic receptors (Deneris et al., 1991).

In addition, receptor subunits (and, by implication, receptor subtypes) are differentially regulated. Thus, receptor subunits tend to show characteristic anatomical patterns of expression. For example, receptor subunits expressed in muscle are different from those expressed in the brain. The richest diversity of nicotinic receptors probably occurs in the CNS, where overlapping but unique distributions have been elegantly revealed by the technique of in situ hybridization histochemistry (Wada et al., 1989). Not only are the nicotinic receptor subtypes found in different places, but also at different times; receptor subunits manifest characteristic patterns of expression during development, some disappearing before adulthood.

At present, we simply do not know how many receptor subtypes exist. Considerable diversity is suggested not only by the several subunit-encoding mRNA species detected in the brain (Deneris et al., 1991; Wada et al., 1989), but also by findings at the protein level (Clarke et al., 1985; Schulz et al., 1991).

Nicotine's Complex Agonist Actions at the Receptor Level

On a molecular level, the best studied nicotinic receptors are those found in the electric organ of the electric ray and in mammalian muscle. Each of these receptors comprises five subunits, which together form the walls of an ion channel that transverses the cell membrane. Agonists such as nicotine and the neurotransmitter acetylcholine bind to certain of the subunits. Binding of an agonist induces the receptor macromolecule to undergo a conformational change (i.e., a change in shape), which in turn allows the channel to open and results in a net flow of positively charged ions (cations) into the nerve cell. The entry of these cations (primarily Na^+) tends to depolarize the neuron, making firing of the cell more likely. Calcium ions also may enter the cell in significant numbers. Calcium plays an important role in intracellular signaling pathways, and the consequences of calcium entry can be complex. Most nicotinic receptors are believed to control the passage of positively charged ions into the cell, thereby producing excitation. However, two reports in the last 5 yr suggest that some nicotinic receptors in the brain may be inhibitory (de la Garza et al., 1987; Wong and Gallagher, 1989).

Within the receptor macromolecule, certain receptor subunits bind nicotine (and other agonists). Other subunits, sometimes referred to as "structural subunits," do not, but their presence can indirectly influence agonist binding (Deneris et al., 1991). Antagonists can act in several different ways: some tend to compete for the agonist recognition site, others block the ion channel once it is opened, and yet others target additional sites on the molecule (Changeux et al., 1984). The precise mode of action depends on the drug and even on the type of nicotinic receptor in question. The receptor macromolecule also presents a target for endogenous substances, notably certain peptides, exerting a modulatory role (Boksa and Livett, 1984).

It is clear that the diversity of nicotinic receptors and our present state of ignorance about them make generalization difficult. In some respects each nicotinic receptor subtype is unique. In certain other respects there are considerable characteristics in common. Furthermore, the extended family of nicotinic receptors form part of a yet wider *superfamily* of ligand-gated ion channels, and certain common organizing principles are beginning to emerge. Studies of non-nicotinic members of this receptor superfamily suggest that nicotinic receptors may well be modulated by a variety of endogenous and pharmacological factors acting at a number of distinct sites on the macromolecule.

Desensitization Of Receptors

Activation of receptors by nicotine may lead to receptor desensitization. This is seen macroscopically as tachyphylaxis, in which the tissue becomes refractory to further applications of the drug. First described in the periphery, tachyphylaxis also occurs in central actions of nicotine. Central-occurring tachyphylaxis is well established in animals (Clarke, 1987) and also occurs in humans, although there are fewer human data available (US DHHS, 1988).

Neuronal nicotinic receptors (e.g., those located in autonomic ganglia or in the brain) are more sensitive than muscle nicotinic receptors to the

activating and desensitizing actions of nicotine (Paton and Savini, 1968). (Were this not so, tobacco use would be acutely harmful to one's health.) There appear to be considerable differences between putative subtypes of CNS nicotinic receptors in their sensitivity to nicotine-induced activation and desensitization (Couturier et al., 1990; Luetje and Patrick, 1991).

The implication is that doses of nicotine relevant to smoking and ST consumption may selectively activate certain subtypes of CNS receptors, leaving others unactivated and perhaps still other receptor subtypes in a more or less permanent state of desensitization. Evidence for this is discussed below, in the context of central sites of action.

Nicotine's ability to induce desensitization may underlie certain aspects of tobacco use. Habitual cigarette smokers inhale intermittently and space their cigarettes over time. This pattern of intermittent dosing may optimize the tradeoff between receptor activation and desensitization. Habitual smokers report that the first cigarette of the day is generally the most satisfying (US DHHS, 1988); the period of abstinence imposed by sleep may allow a large proportion of receptors to return from a desensitized to an activatable state. In addition, the greater difficulty people have in quitting cigarette smoking compared to giving up other forms of tobacco consumption may be due in part to the pharmacodynamic peculiarities of this form of nicotine administration (Benowitz et al., 1990).

Locating Central Sites of Action

Nicotinic receptors have been visualized most directly by autoradiography. Sections of brain tissue are mounted on microscope slides and incubated with radioactive probes that selectively label nicotinic receptors. The sections are then exposed to film, and the resulting images reveal the neuroanatomical location of the receptors. In this way, nicotinic receptors have been mapped in rat brain (Clarke et al., 1985; Schulz et al., 1991; Swanson et al., 1987), and to some extent in human brain (Adem et al., 1988). Nicotinic receptors are concentrated in a number of brain regions and nuclei, the precise distribution depending on the receptor subtype in question.

In animals, a variety of other approaches have been used to locate central sites of nicotine action. Several of the brain areas that feature prominently in nicotinic receptor autoradiographs have been studied electrophysiologically, and many neurons in these areas respond to the local application of nicotine. (For a review, see Clarke, 1990a.) Through recording the responses of neurons in brain tissue slices maintained in vitro, it has been possible to show that many cells are directly acted upon by nicotine, via receptors located on the cell body, on dendrites, or on both. By contrast, studies of transmitter release, again in vitro, have shown that nicotine can also exert direct effects on nerve terminals (Chesselet, 1984).

It must be stressed that nicotine does not occur naturally in the body. The endogenous agonist for nicotinic receptors is acetylcholine. Thus, nicotine is often employed because it is a convenient cholinergic agonist. In many cases, high doses of the drug are used which are of doubtful relevance to tobacco use. Furthermore, a recent electrophysiological report suggests that there is a prevalent subtype of nicotinic receptor in the brain that has

so far eluded detection because it desensitizes extremely rapidly in the presence of even low concentrations of nicotine (Couturier et al., 1990). This receptor subtype, although physiologically important, may play no role in nicotine dependence.

One way to study the pharmacological effects of "smoking doses" of nicotine would be to expose animals to tobacco smoke. This has rarely been attempted, partly because of practical difficulties, but also because such an approach introduces unwanted chemicals and may be highly stressful. Nevertheless, similar effects of smoke exposure and nicotine have been reported (Fuxe et al., 1986).

Typically, nicotine is administered to conscious animals in single doses which provide steady-state plasma levels similar to those measured in habitual smokers. This sort of approach has been used to measure the effects of nicotine on neuronal activity in the rat brain, as indicated by uptake of radiolabeled 2-deoxyglucose (Grunwald et al., 1988; London et al., 1988). By this index, nicotine activates a number of brain structures, and the most affected structures are, with few exceptions, those that possess the highest density of nicotinic receptors, as measured by autoradiography using radiolabeled nicotine. These functional and receptor mapping studies only roughly approximate actual smoking with the transient peaks that may be attained through individual puffs. Taken together, however, they strongly suggest that only certain nicotinic subtypes are significantly activated by doses of nicotine relevant to tobacco consumption.

Reinforcement Through Mesolimbic Dopaminergic Neurons

The reinforcing effects of nicotine in animals are of central origin (see above). Nicotine activates a large number of brain regions, many of which could conceivably participate in the drug's reinforcing effects. However, one neuronal system calls for particular attention—the mesolimbic dopaminergic pathway. This nerve pathway is one of several in the brain that secrete dopamine. Its cell bodies are located in the ventral tegmental area (VTA) of the midbrain, and it projects to the forebrain, where its major area of termination is the nucleus accumbens. Animals will work hard to increase the release of dopamine from this latter structure. This can be achieved either through electrical stimulation via indwelling electrodes, or through the self-administration of certain drugs, of which amphetamine is a good example (Wise and Rompre, 1989). Crudely, then, this system can be looked upon as a "reward pathway," and the meager evidence available suggests this system serves a similar function in humans.

The neurons that constitute the mesolimbic dopaminergic pathway represent targets for nicotine (reviewed in Clarke 1990b and 1990c). Thus, autoradiography has revealed nicotinic receptors both at the cell body/dendrite level and on terminals in the nucleus accumbens. Electrophysiological experiments have shown that nicotine, applied directly, can increase the firing rate of these neurons, and biochemical studies have shown that nicotine can act directly on terminals to promote the release of dopamine. In conscious rats, nicotine-induced dopamine release has been documented in the nucleus accumbens. Furthermore, near-total destruction

of the mesolimbic dopaminergic system markedly suppresses the rate of nicotine self-administration. The latter observation suggests that nicotine, like amphetamine, exerts its reinforcing effects to a large extent by activating the mesolimbic dopaminergic system.

All these findings are subject to an important caveat. The above studies have firmly established that nicotine, given acutely, can stimulate the release of mesolimbic dopamine. It remains an open question whether nicotine would have the same effect, were it to be administered chronically in such a way as to mimic the pharmacodynamic patterns of cigarette smoking or tobacco chewing.

PHARMACOLOGICAL HELP FOR QUITTING TOBACCO USE

Quitting the tobacco habit is notoriously difficult, and even the best strategies are rather ineffective (US DHHS, 1988). The most effective current methods for quitting are behavior-based, and they place considerable demands on the time of health personnel. For these reasons, it is worthwhile to seek a pharmacological aid for tobacco use cessation.

Limits of Current Pharmacotherapies

The only drug treatment readily available for those who wish to quit using tobacco is nicotine replacement therapy. This approach follows, logically enough, from the widely held view that habitual tobacco use is a form of nicotine dependence. Although considerable success was reported in early studies from cessation clinics using nicotine polacrilex (nicotine gum), the cumulative results are less than dramatic. Lam and coworkers (1987), in reviewing more than a dozen randomized, controlled trials, concluded that nicotine gum was marginally more effective than placebo gum, but in both cases, only a small minority of subjects remained abstinent in the long run (23 percent and 13 percent, respectively, at 12 mo). Beside its modest efficacy, nicotine chewing gum is associated with several undesirable side effects (Hughes, 1988).

As a form of drug delivery, the transdermal patch offers some distinct advantages over nicotine gum. Nevertheless, the problem of efficacy remains. In a double-blind study, Abelin and colleagues (1989) reported a statistically significant but small advantage to using nicotine patches; at the end of 3 mo of treatment, 36 percent of subjects were abstinent or nearly so, compared to 23 percent of controls receiving placebo. Rose and coworkers (1990) reported higher rates of recidivism, with only 18 percent of subjects remaining abstinent after 3 wk (compared to 6 percent of placebo controls). In another recent study (Hurt et al., 1990), high rates of abstinence at 6 wk were followed by substantial relapse, leading the authors to recommend adjunctive behavioral intervention and training to address this problem.

Clearly, results to date with nicotine chewing gum and transdermal patches have been disappointing. In the absence of other cessation strategies, they help only a minority of tobacco smokers achieve lasting abstinence. Can one hope that these treatments will be of more help for those wishing to quit smokeless tobacco products? Nicotine polacrilex and the transdermal patch release nicotine in a gradual manner that fails to mimic the puff-by-puff exposure to nicotine that cigarette smoking affords

(Benowitz et al., 1990). It has been hypothesized that, among many cigarette smokers, those transient boli of nicotine may play an important role in the dependence process (Russell and Feyerabend, 1978). Blood nicotine levels resulting from the use of ST (oral snuff, chewing tobacco), though, are less transient in nature, and thus can be more closely modeled by gum or by transcutaneous delivery (Benowitz et al., 1990). Nicotine gum and skin patches offer complementary features. The former does not fully replace blood nicotine levels, but offers the possibility of obtaining a boost of nicotine when it is wanted; transdermal patches can deliver adequate total amounts of nicotine, but administration is continuous.

Conditioning And Effective Pharmacotherapy

Many people give up the use of tobacco products for a few days or weeks with little difficulty. Some even manage to remain abstinent for a considerable time. Eventually, however, most attempts to quit fail. The addiction can reassert itself by even a minor relapse after years of abstinence. This "priming" effect is not unique to nicotine, but has been reported also, for example, in cocaine abusers (Jaffe et al., 1989).

Why does the abstinent individual remain susceptible to relapse? One reason may be that ex-smokers remain partially tolerant to the nauseating effects of nicotine and hence have less of a barrier to overcome than novice smokers. The persistence of tolerance cannot, however, explain why after the discomfort of quitting, and after experiencing the healthful, gustatory, and economic advantages of cessation, so many tobacco users relapse. Something is drawing them back.

This powerful force appears to be secondary reinforcement. Over the course of their dependence, tobacco users are likely to associate many stimuli with nicotine delivery. Many of these cues will initially be neutral, but through repeated pairing with the primary reinforcer, some are likely to assume reinforcing properties even in the absence of nicotine. The potency of these secondary reinforcers is borne out by verbal reports of smokers. In addition, laboratory studies have formally demonstrated the establishment of secondary reinforcers in monkeys self-administering nicotine intravenously (Goldberg et al., 1981).

Nicotine Blockade Therapy— A Neglected Approach

In my view, most tobacco users relapse because they retain the well-learned connection between tobacco administration and nicotine delivery. For example, an abstinent smoker knows that he or she has only to start smoking a cigarette to experience rapid reinforcement. This association, encoded in some form in the brain, may underlie the phenomenon of craving. It follows that, to abjure the tobacco habit permanently, one should extinguish these long-established associations. Current smoking cessation methods do not provide for this deprogramming (or, in psychological parlance, "extinction").

The most effective extinction procedure would be one in which individuals intending to quit would be administered a drug that would block the reinforcing effects of nicotine. They would then be encouraged to continue to use tobacco products in their customary way for a limited time, under the influence of the medication. It should be possible to achieve such

a blockade by administering a nicotine receptor blocker that is centrally active (Clarke, 1991; Stolerman, 1986). This approach has been largely untried. Results to date, although preliminary, are encouraging (Tennant et al., 1984). However, before nicotinic blockade therapy can be properly tested in human subjects, there is a clear need for a nicotinic receptor antagonist with a selective CNS action (Clarke, 1991).

REFERENCES

Abelin, T., Buehler, A., Muller, P., Vesanen, K., Imhof, P.R. Controlled trial of transdermal nicotine patch in tobacco withdrawal. *Lancet* 1: 7-10, 1989.

Adem, A., Jossan, S.S., d'Argy, R., Brandt, I., Winblad, B., Nordberg, A. Distribution of nicotinic receptors in human thalamus as visualized by ^{3}H-nicotine and ^{3}H-acetylcholine receptor autoradiography. *Journal of Neural Transmission* 73: 77-83, 1988.

Benowitz, N.L., Porchet, H.C., Jacob, P. Pharmacokinetics, metabolism, and pharmacodynamics of nicotine. In: *Nicotine Psychopharmacology: Molecular, Cellular, and Behavioural Aspects*, S. Wonnacott, M.A.H. Russell, and I.P. Stolerman (Editors). Oxford: Oxford University Press, 1990, pp. 112-157.

Boksa, P., Livett, B.G. Substance P protects against desensitization of the nicotinic response in isolated adrenal chromaffin cells. *Journal of Neurochemistry* 42: 618-627, 1984.

Changeux, J.P., Devillers-Thiery, A., Chemouilli, P. Acetylcholine receptor: An allosteric protein. *Science* 225: 1335-1345, 1984.

Chesselet, M.-F. Presynaptic regulation of neurotransmitter release in the brain: Facts and hypothesis. *Neuroscience* 12: 347-375, 1984.

Clarke, P.B.S. The central pharmacology of nicotine: Electrophysiological approaches. In: *Nicotine Psychopharmacology: Molecular, Cellular, and Behavioural Aspects*, S.Wonnacott, M.A.H. Russell, and I.P. Stolerman (Editors). Oxford: Oxford University Press, 1990a, pp. 158-193.

Clarke, P.B.S. Dopaminergic mechanisms in the locomotor stimulant effects of nicotine. *Biochemical Pharmacology* 40: 1427-1432, 1990b.

Clarke, P.B.S. Mesolimbic dopamine activation: The key to nicotine reinforcement? *CIBA Foundation Symposia* 152: 153-168, 1990c.

Clarke, P.B.S. Nicotine and smoking: A perspective from animal studies. *Psychopharmacology* 92: 135-143, 1987.

Clarke, P.B.S. Nicotinic receptor blockade therapy and smoking cessation. *British Journal of Addiction* 86: 501-505, 1991.

Clarke, P.B.S., Schwartz, R.D., Paul, S.M., Pert, C.B., Pert, A. Nicotinic binding in rat brain: Autoradiographic comparison of ^{3}H-acetylcholine, ^{3}H-nicotine, and ^{125}I-alpha-bungarotoxin. *Journal of Neuroscience* 5: 1307-1315, 1985.

Collins, A.C., Bhat, R.V., Pauly, J.R., Marks, M.J. Modulation of nicotine receptors by chronic exposure to nicotinic agonists and antagonists. *CIBA Foundation Symposia* 152: 68-82, 1990.

Corrigall, W.A., Franklin, K.B.J., Coen, K.M., Clarke, P.B.S. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology*, in press.

Couturier, S., Bertrand, D., Matter, J.M., Hernandez, M.C., Bertrand, S., Millar, N., Valera, S., Barkas, T., Ballivet, M. A neuronal nicotinic acetylcholine receptor subunit (alpha 7) is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. *Neuron* 5: 847-856, 1990.

Deneris, E.S., Connolly, J., Rogers, S.W., Duvoisin, R. Pharmacological and functional diversity of neuronal nicotinic acetylcholine receptors. *Trends in Pharmacological Sciences* 12: 34-40, 1991.

Fuxe, K., Andersson, K., Harfstrand, A., Agnati, L.F. Increases in dopamine utilization in certain limbic dopamine terminal populations after a short period of intermittent exposure of male rats to cigarette smoke. *Journal of Neural Transmission* 67: 15-29, 1986.

de la Garza, R., Bickford-Wimer, P.C., Hoffer, B.J., Freedman, R. Heterogeneity of nicotine actions in the rat cerebellum: An in vivo electrophysiologic study. *Journal of Pharmacology and Experimental Therapeutics* 240: 689-695, 1987.

Goldberg, S.R., Spealman, R.D., Goldberg, D.M. Persistent behavior at high rates maintained by intravenous self-administration of nicotine. *Science* 214: 573-575, 1981.

Grunwald, F., Schrock, H., Theilen, H., Biber, A., Kuschinsky, W. Local cerebral glucose utilization of the awake rat during chronic administration of nicotine. *Brain Research* 456: 350-356, 1988.

Hughes, J.R. Dependence potential and abuse liability of nicotine replacement therapies. *Progress in Clinical and Biological Research* 261: 261-277, 1988.

Hurt, R.D., Lauger, G.G., Offord, K.P., Kottke, T.E., Dale, L.C. Nicotine-replacement therapy with use of a transdermal nicotine patch: A randomized double-blind placebo-controlled trial. *Mayo Clinic Proceedings* 65: 1529-1537, 1990.

Jaffe, J., Cascella, N.G., Kumor, K.M., Sherer, M.A. Cocaine-induced cocaine craving. *Psychopharmacology* 97: 59-64, 1989.

Jaffe, J.H. Tobacco smoking and nicotine dependence. In: *Nicotine Psychopharmacology: Molecular, Cellular, and Behavioural Aspects*, S. Wonnacott, M.A.H. Russell, and I.P. Stolerman (Editors). Oxford: Oxford University Press, 1990, pp. 1-37.

Jarvik, M.E., Assil, K.M. Mecamylamine blocks the burning sensation of nicotine on the tongue. *Chemical Senses* 13: 213-217, 1988.

Lam, W., Sze, P.C., Sacks, H.S., Chalmers, T.C. Meta-analysis of randomised controlled trials of nicotine chewing gum. *Lancet* 2: 27-30, 1987.

London, E.D., Connolly, R.J., Szikszay, M., Wamsley, J.K., Dam, M. Effects of nicotine on local cerebral glucose utilization in the rat. *Journal of Neuroscience* 8: 3920-3928, 1988.

Luetje, C.W., Patrick, J. Both alpha- and beta-subunits contribute to the agonist sensitivity of neuronal nicotinic acetylcholine receptors. *Journal of Neuroscience* 11: 837-845, 1991.

Navarro, H.A., Seidler, F.J., Schwartz, R.D., et al. Prenatal exposure to nicotine impairs nervous system development at a dose which does not affect viability or growth. *Brain Research Bulletin* 23: 187-192, 1989.

Paton, W.D.M., Savini, E.C. The action of nicotine on the motor endplate in the cat. *British Journal of Pharmacology* 32: 360-380, 1968.

Rose, J.E., Tashkin, D.P., Ertle, A., Zinser, M.C., Lafer, R. Sensory blockade of smoking satisfaction. *Pharmacology, Biochemistry and Behavior* 23: 289-293, 1985.

Rose, J.E., Levin, E.D., Behm, F.M., Adivi, C., Schur, C. Transdermal nicotine facilitates smoking cessation. *Clinical Pharmacology and Therapeutics* 47: 323-330, 1990.

Russell, M.A.H., Feyerabend, C. Cigarette smoking: A dependence on high-nicotine boli. *Drug Metabolism Reviews* 8: 29-57, 1978.

Schulz, D.W., Loring, R.H., Aizenman, E., Zigmond, R.E. Autoradiographic localization of putative nicotinic receptors in the rat brain using ^{125}I-neuronal bungarotoxin. *Journal of Neuroscience* 11: 287-297, 1991.

Stolerman, I.P., Goldfarb, T., Fink, R., Jarvik, M.E. Influencing cigarette smoking with nicotine antagonists. *Psychopharmacologia* 28: 247-259, 1973.

Stolerman, I.P. Could nicotine antagonists be used in smoking cessation? *British Journal of Addiction* 81: 47-53, 1986.

Swanson, L.W., Simmons, D.M., Whiting, P.J., Lindstrom, J. Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system. *Journal of Neuroscience* 7: 3334-3342, 1987.

Tennant, F.S., Jr., Tarver, A.L., Rawson, R.A. Clinical evaluation of mecamylamine for withdrawal from nicotine dependence. In: *Problems of Drug Dependence, 1983: Proceedings of the 45th Annual Scientific Meeting, the Committee on Problems of Drug Dependence, Inc.*, L.S. Harris (Editor). U.S. Department of Health and Human Services; Public Health Service; Alcohol, Drug Abuse, and Mental Health Administration. NIDA Research Monograph No. 49. DHHS Publication No. 84-1316, 1984, pp. 239-246.

U.S. Department of Health and Human Services. *Health Consequences of Smoking: Nicotine Addiction. A Report of the Surgeon General, 1988*. U.S. Department of Health and Human Services, Public Health Service, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. DHHS Publication No. (CDC) 88-8406, 1988.

Wada, E., Wada, K., Boulter, J., Deneris, E., Heinemann, S., Patrick, J., Swanson, L.W. Distribution of alpha 2, alpha 3, alpha 4, and beta 2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: A hybridization histochemical study in the rat. *Journal of Comparative Neurology* 284: 314-335, 1989.

Wise, R.A., Rompre, P.-P. Brain dopamine and reward. *Annual Review of Psychology* 40: 191-225, 1989.

Wong, L.A., Gallagher, J.P. A direct nicotinic receptor-mediated inhibition recorded intracellularly in vitro. *Nature* 341: 439-442, 1989.

Dependence on Smokeless Tobacco

Martin J. Jarvis[1]

ABSTRACT One of the key issues determining the threat to public health from smokeless tobacco is the degree of dependence experienced by users. Data documenting nicotine absorption and dependence on smokeless tobacco products are reviewed. Single case histories of individuals who either chewed cigarette butts or brushed snuff into their gums provide evidence of compulsive behavioral rituals accompanied by substantial uptake of the drug nicotine. Studies of dependent users of moist oral or dry nasal snuff show rapid absorption of nicotine from a single pinch and blood levels from normal use that parallel those seen in cigarette smokers. Among users of Swedish oral snuff, blood nicotine levels and subjective dependence were similar to those of cigarette smokers. These observations, which are derived from relatively few subjects, indicate that nicotine intake exerts a controlling influence over self-administration of smokeless tobacco products and that subjective dependence may be no less than among cigarette smokers. Available data from short-term cessation have been interpreted as showing that smokeless tobacco users experience less severe withdrawal effects than do cigarette smokers. Further studies on cessation of smokeless tobacco are needed.

INTRODUCTION The potential burden to society from the use of smokeless tobacco depends both on the threat it poses to health and on the users' degree of addiction. With cigarette smoking, we see hundreds of thousands of deaths each year in the United States because tobacco smoke is exceptionally damaging to health and because smokers, despite awareness and acceptance of the risks, find it very difficult to overcome their addiction. If addiction to oral and nasal snuff is less tenacious than addiction to cigarettes, smokeless tobacco users will find it easier to quit. But if dependence on smokeless tobacco turns out to be as strong as it is with tobacco smoke, the trends toward increasing prevalence of use, particularly by the young, may create problems in the future.

In considering the addictiveness of smokeless tobacco, the U.S. Surgeon General drew on three lines of evidence (US DHHS, 1986). The first two related to the presence of nicotine in smokeless tobacco and evidence of its absorption. This led to the following conclusion: "Given the nicotine content of smokeless tobacco, its ability to produce high and sustained blood levels of nicotine, and the well-established data implicating nicotine as an addictive substance, one may deduce that smokeless tobacco is capable of producing addiction in users." Direct evidence of addiction to smokeless tobacco, which was the third line of evidence, was at that time rather scanty, and comprised mainly reports of withdrawal symptoms upon cessation of smokeless tobacco (Hatsukami et al., 1987) or nicotine gum use (Hughes et al., 1986; West and Russell, 1985).

This paper follows a similar course, (1) reviewing our laboratory evidence of patterns of nicotine absorption from smokeless tobacco products and (2) presenting some new data that attempt to link nicotine absorption with the crucial issue of feelings of subjective dependence.

[1] The support of the Medical Research Council and the Imperial Cancer Research Fund is gratefully acknowledged.

NICOTINE UPTAKE FROM UNUSUAL USE OF TOBACCO

Frequently we are approached for advice about chronic psychiatric patients who do not smoke but who use tobacco in unusual ways and whose behavior puzzles those who care for them. Two such cases are presented briefly here.

A woman of Indian origin, diagnosed as schizophrenic, had the habit of brushing her gums with a toothbrush that had been dipped in dry nasal snuff (Edwards, 1987). She spent up to 9 h/d in this activity, could not explain why she did it, and became tense and irritable if deprived of snuff. On a day when she was permitted to apply 1.4 g of snuff in this way for half an hour in the morning, her blood nicotine rose from a baseline of 7.3 ng/mL to peak some 4 h later at 35.5 ng/mL, a value similar to that seen in heavy cigarette smokers.

More recently, we were contacted about a mentally handicapped resident at a long-stay hospital who regularly collected and consumed the butt-ends from used cigarettes. Apparently the patient swallowed the cigarette butts, and the expressed concern was more about the aesthetics of the behavior and disruption of life on the ward than about potential addiction. A blood specimen was taken at 5 p.m. on a day when he had consumed six filter tips. The blood nicotine concentration was found to be 29.2 ng/mL, and the corresponding cotinine was 622.2 ng/mL. Again, the nicotine level is comparable with that seen in dependent smokers, whereas the cotinine concentration, at about double the average in smokers, reflects extensive first-pass metabolism of swallowed nicotine by the liver, preventing nicotine from reaching the systemic circulation.

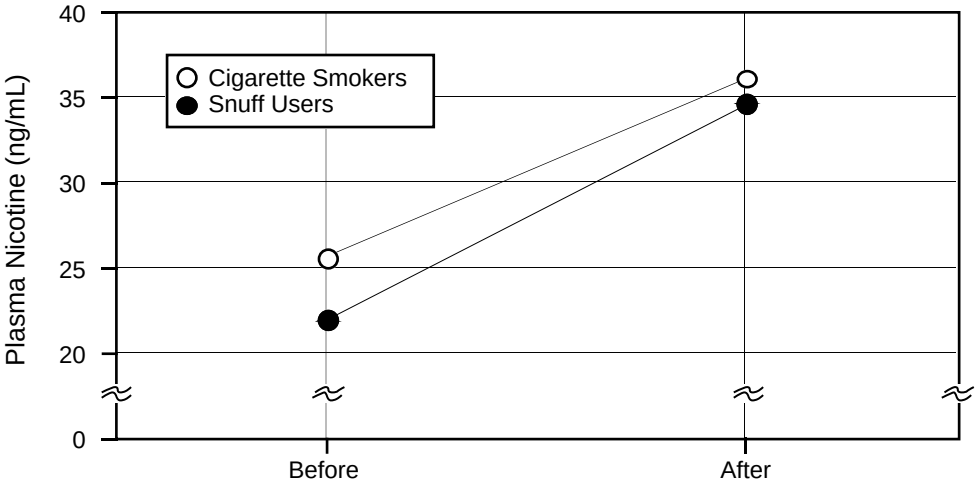
Evidence from single cases such as these has obvious limitations, and explanations couched solely in behavioral terms rather than invoking nicotine dependence cannot be completely ruled out. Nevertheless, that such compulsive rituals should give rise to blood nicotine levels characteristic of cigarette smoking is suggestive.

NICOTINE ABSORPTION FROM DRY NASAL SNUFF

Smokeless tobacco is used in Britain mainly as finely ground tobacco sniffed into the nose. In a habitual user, there was an increase in blood nicotine concentration of 21.1 ng/mL over 5 min from a single pinch (Russell et al., 1980). This evidence of rapid absorption prompted the study of a group of snuff users in the West of England (Russell et al., 1981). Among seven daily users, the average trough blood nicotine was 21.9 ng/mL, rising to 34.5 ng/mL after their taking a pinch of their usual snuff in their usual way. Both the trough and the postdosing peak were close to levels observed in a comparison group of smokers before and after a cigarette (see Figure 1). In interpretation of the results, it was suggested that the similarity was unlikely to be coincidental (Russell et al., 1981):

> To find that one group of people who sniff powdered tobacco into their noses have similar blood nicotine concentrations to those of another group who burn it to inhale its smoke suggests that the concentration of nicotine has some controlling influence. It would be a remarkable coincidence if factors such as flavor, strength of tobacco, social influences, and so on just happened to produce similar blood nicotine

Figure 1.
Average plasma nicotine levels before and after a pinch of snuff (n=7) or a cigarette (n=13). Tobacco use had been normal for both groups up to the time of the test.



Source: Russell et al., 1981; used with permission.

concentrations resulting from two such different behaviors. The most plausible explanation is that the rituals of snuffing and smoking are determined by the nicotine concentrations that they produce.

Several of these snuff users took unusually large multiple doses, as practiced in snuff-taking competitions. The mean increment in plasma nicotine observed was 54 ng/mL. In one subject, who was the current British champion, a boost of 97 ng/mL was obtained over 12.5 min (Russell et al., 1981). This man reported a very high degree of dependence. He said that he "was always on it [snuff]," and even woke several times each night to take a pinch.

NICOTINE INTAKE AND DEPENDENCE IN SWEDEN

The above results paint a convincing picture of the use of smokeless tobacco as a drug-taking behavior. However, subjective aspects of dependence were not systematically studied. We have recently examined nicotine absorption and measures of dependence in users of Swedish moist oral snuff (Holm et al., in press). Snuff has a long tradition in Sweden, and its use is currently on the increase, particularly among the young (Nordgren and Ramström, 1990). About 20 percent of adult Swedish males take snuff.

Two studies were conducted. The first examined absorption from a single dose of 2 g kept in the mouth for 30 min. Among 10 subjects, the peak increment in blood nicotine concentration, which averaged 14.6 ng/mL, was observed at 35 min, shortly after the snuff was discarded. The pattern of absorption showed an average increase of 9.9 ng/mL in the

first 10 min, with a slower rise thereafter, and a relatively flat plateau maintained up to 1 h. The two individuals with the most rapid absorption had increments of 12.8 ng/mL and 16.2 ng/mL in the first 5 min of dosing.

These results, which are similar to findings from the United States (Benowitz et al., 1988; Gritz et al., 1981), showed substantial absorption of nicotine and indicated that a blood specimen taken between 5 and 15 min after the snuff is discarded should adequately capture the peak blood nicotine level. Thus, these results were used to inform the design of the second study, which combined measures of nicotine uptake and subjective dependence in regular snuff takers (n=27) and cigarette smokers (n=35).

On a day of normal snuff use or smoking, blood specimens were taken from snuff users 5 to 15 min after they discarded a pinch of the subjects' usual snuff taken in the usual way, and from cigarette smokers 1 min after their smoking a cigarette of their regular brand. A questionnaire assessed various aspects of dependence. The postdosing nicotine concentrations were similar in the snuff users and smokers (36.6 ng/mL and 36.7 ng/mL, respectively) and were also similar to the earlier findings with British dry snuff users (Russell et al., 1981). On questionnaire measures of dependence, there were no significant differences between the snuff users and smokers in self-assessed addiction, craving for tobacco, or difficulty in giving up. The majority in each case rated themselves as fairly or extremely addicted, they frequently or always craved when without their snuff or cigarettes, and they anticipated that giving up would be very difficult. However, the snuff users found their habit much more enjoyable (16 of 27 rated it as "extremely enjoyable," compared with 3 of 35 smokers, $p < 0.0001$) and rated enforced abstinence for an hour or two as more unpleasant. For their part, the smokers were significantly more likely to have their first cigarette of the day before tea or coffee than were the snuff users ($p < 0.01$).

The picture that emerges from these results is of regular snuff users and cigarette smokers as being remarkably similar in both nicotine intake and dependence. Latency to first tobacco use of the day, which is perhaps the best indicator of dependence among cigarette smokers (Heatherton et al., 1989), was shorter in the smokers, but it seems likely that this disparity reflects the incompatibility between taking snuff and ingesting food or drink rather than any difference in dependence. Of more interest is the apparently greater enjoyment of snuff use than of smoking. Rather than resulting from intrinsic differences in the rewarding qualities of the two habits, it may be that cigarette smokers now carry with them a permanent awareness of the health risk and the increasingly unfavorable climate of social opinion. If snuff users, as seems likely, view their habit as safer than smoking, they are that much freer to indulge without worry or guilt.

Limitations on the generalizability of these findings stem from the small sample size and the possibility that both the snuff users and cigarette smokers might not have been representative of the wider population of users. Nevertheless, there is a strong indication that among groups of snuff users and smokers matched for nicotine levels, subjective dependence is also likely to be similar.

CESSATION OF SMOKELESS TOBACCO USE

The data reviewed so far lead to the conclusion that nicotine exerts a controlling influence on the maintenance of smokeless tobacco use and that regular users experience levels of dependence similar to those of tobacco smokers. But it does not necessarily follow that cessation of smokeless tobacco use will be equally difficult. It could be the same, more difficult, or less difficult. One factor that might contribute to making cessation more difficult is the perception that risks to health from smokeless tobacco are lower. Motivation to quit and level of dependence are not entirely independent in their effects on probability of cessation. At a given level of dependence, a lower level of concern about potential damage to health will reduce the discomfort the person is prepared to tolerate to achieve cessation. On the other hand, it has been suggested that the lack of bolus effects and the lower frequency of reinforcement from smokeless tobacco may make it easier to quit than cigarettes (Hatsukami, 1991; West and Krafona, 1990).

Only limited data are available on acute withdrawal effects from smokeless tobacco. Hatsukami and associates (1987) reported a range of withdrawal symptoms, including craving for tobacco, and raised total scores on self-rated and observer-rated checklists. However, the symptoms were less intense and fewer in number than among a comparison group of cigarette smokers. Decrements in performance measures have also been reported after short-term withdrawal from smokeless tobacco (Keenan et al., 1989). There is an urgent need for further studies in this area.

The scope for successful long-term cessation is even less clear. Among adolescents, 30 percent of smokeless tobacco users believed that quitting their habit would be very difficult, compared with 23 percent of cigarette smokers (Brownson et al., 1990). In the 1986 Adult Use of Tobacco Survey in the United States, 39 percent of current users of smokeless tobacco had attempted to quit, and of these, 47 percent reported experiencing difficulty in doing so (Novotny et al., 1989). The implications of these findings for actual cessation are unclear. Few cessation studies have been undertaken, and those on a small scale (Eakin et al., 1989; Glover, 1986).

FUTURE RESEARCH NEEDS

A number of studies have examined the prevalence of smokeless tobacco use, both in the general population and in subgroups such as male adolescents (US DHHS, 1989). However, dependence, which in conjunction with prevalence of use and health risks will determine the extent of future morbidity and mortality, has received less attention. Available evidence indicates that dependence on smokeless tobacco may be no less tenacious than dependence on cigarettes. This conclusion is based on a limited range of studies on relatively few subjects. A clearer picture will emerge if future surveys include a number of items to assess the degree of subjective dependence. It would also be informative to look not only at whether users of smokeless tobacco see their habit as a threat to health, but also at how hazardous they perceive it to be in comparison with cigarette smoking and the extent to which they see it as a personal risk. These factors have important implications for motivation to quit.

The extent and severity of withdrawal symptoms and ways of facilitating long-term cessation of smokeless tobacco use are important areas for future research. If the analogy with cigarette smoking is valid, the majority of those who wish to quit will do so by themselves, without assistance. This means that motivation derived from public health campaigns will be crucial, and much will depend on epidemiologists' clarifying issues such as the magnitude of the excess mortality attributable to long-term use of smokeless tobacco.

For those who do seek help, there would seem to be a natural affinity between smokeless tobacco use and nicotine replacement methods. Nicotine chewing gum is itself an oral tobacco product, though purified of carcinogens. Nicotine skin patches, which give substantial plateau levels of blood nicotine without rapid absorption and without providing a behavioral ritual, may also have a part to play.

REFERENCES

Benowitz, N.L., Porchet, H., Sheiner, L., Jacob, P. III. Nicotine absorption and cardiovascular effects with smokeless tobacco use: Comparison with cigarettes and nicotine chewing gum. *Clinical Pharmacology and Therapeutics* 44: 23-28, 1988.

Brownson, R.C., DiLorenzo, T.M., Van Tuinen., M., Finger, W.W. Patterns of cigarette and smokeless tobacco use among children and adolescents. *Preventive Medicine* 19: 170-180, 1990.

Eakin, E., Severson, H., Glasgow, R.E. Development and evaluation of a smokeless tobacco cessation program: A pilot study. *National Cancer Institute Monographs* 8: 95-101, 1989.

Edwards, J.G. An unusual case of nicotine dependence. *Psychological Medicine* 7: 779-781, 1987.

Glover, E.D. Conducting smokeless tobacco cessation clinics. *American Journal of Public Health* 76: 207, 1986.

Gritz, E.R., Baer-Weiss, V., Benowitz, N.L., Van Vanakis, H., Jarvik, M.E. Plasma nicotine and cotinine concentrations in habitual smokeless tobacco users. *Clinical Pharmacology and Therapeutics* 30: 201-209, 1981.

Hatsukami, D.K., Gust, S.W., Keenan, R.M. Physiologic and subjective changes from smokeless tobacco withdrawal. *Clinical Pharmacology and Therapeutics* 41: 103-107, 1987.

Hatsukami, D.K., Nelson, R., Jensen, J. Smokeless tobacco: Current status and future directions. *British Journal of Addiction* 86: 559-563, 1991.

Heatherton, T.F., Kozlowski, L.T., Frecker, R.C., Rickert, W.S., Robinson, J. Measuring the heaviness of smoking using self-reported time to the first cigarette of the day and number of cigarettes smoked per day. *British Journal of Addiction* 84: 791-800, 1989.

Holm, H., Jarvis, M.J., Russell, M.A.H., Feyerabend, C. Nicotine intake and dependence in Swedish snuff takers. *Psychopharmacology,* in press.

Hughes, J.R., Hatsukami, D.K., Skoog, K. Physical dependence on nicotine gum: A placebo-substitution trial. *Journal of the American Medical Association* 255: 3277-3279, 1986.

Keenan, R.M., Hatsukami, D.K., Anton, D.J. The effects of short-term smokeless tobacco deprivation on performance. *Psychopharmacology* 98: 126-130, 1989.

Nordgren, P., Ramström, L. Moist snuff in Sweden: Tradition and evolution. *British Journal of Addiction* 85: 1107-1112, 1990.

Novotny, T.E., Pierce, J.P., Fiore, M.C., Davis, R.M. Smokeless tobacco use in the United States: The Adult Use of Tobacco Surveys. *National Cancer Institute Monographs* 8: 25-28, 1989.

Russell, M.A.H., Jarvis, M.J., Devitt, G., Feyerabend, C. Nicotine intake by snuff users. *British Medical Journal* 283: 814-817, 1981.

Russell, M.A.H., Jarvis, M.J., Feyerabend, C. A new age for snuff? *Lancet* 1: 474-475, 1980.

U.S. Department of Health and Human Services. *Smokeless Tobacco Use in the United States: National Cancer Institute Monograph No. 8*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. NIH Publication No. 89-3055, 1989.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

West, R., Krafona, K. Oral tobacco: Prevalence, health risks, dependence potential and public policy. *British Journal of Addiction* 85: 1097-1098, 1990.

West, R.J., Russell, M.A.H. Effects of withdrawal from long-term nicotine gum use. *Psychological Medicine* 15: 891-893, 1985.

Chapter 5
Prevention

CONTENTS

Marketing Smokeless Tobacco: Implications for Preventive Education[1]

Carol N. D'Onofrio

ABSTRACT Efforts to prevent smokeless tobacco use are essentially reactions to a problem created by the tobacco industry. Although the public health community has applied lessons learned from 25 years of progress in preventing cigarette smoking, current preventive efforts do not adequately counteract aggressive product marketing. In spite of fundamental differences between health education and commercial marketing, examination of the industry's success can stimulate new ideas for ST education. Such analysis also reveals some idiosyncrasies of the public health culture that may now limit the effectiveness of interventions to prevent ST use.

INTRODUCTION The marketing of moist snuff in the United States has been profitable for the U.S. Tobacco Company. After snuff was packaged in round tin cans, sales more than doubled between 1974 and 1984 (Bantle, 1980; Negin, 1985). In 1983, the introduction of snuff in small premeasured pouches boosted sales still higher (Dougherty, 1984; O'Connor, 1983; Tobacco Reporter, 1983). The company gained entry to the Fortune 500 in 1985, showing the greatest profit margin of any company its size or larger (Business Week, 1986; Mintz, 1986; U.S. Tobacco, 1985). During the past 6 yr, snuff sales and profits have continued to climb (FTC, 1991; Smith, 1989), moving U.S. Tobacco up 81 positions in rank among the Nation's 500 largest corporations (U.S. Tobacco, 1988 and 1991). Citing its financial strength and proven business strategy, the company confidently forecasts continuing growth (U.S. Tobacco, 1990). Other sources concur, observing that increased bans on cigarette smoking should benefit the ST industry (Deveny, 1990; Ellis, 1989; Smyth, 1989).

Although this record is celebrated in the business world as a spectacular success, health professionals view it with alarm. Tobacco contains known carcinogens, and the link between snuff use and oral cancer has been firmly established (IARC, 1985; Mattson and Winn, 1989; Office of Medical Applications of Research, 1986; US DHHS, 1986; Winn et al., 1981; WHO, 1988). Sharp increases in ST use portend the emergence of a major public health problem.

Fortunately, lessons learned from 25 yr of progress in the prevention and control of cigarette smoking (US DHHS, 1989) have been applied to smokeless tobacco with little delay. Scientific evidence about the health consequences of snuff dipping and tobacco chewing has been consolidated, reviewed, and publicized (IARC, 1985; Office of Medical Applications of Research, 1986; US DHHS, 1986; WHO, 1988). Legislation has banned ST advertising in the broadcast media, required warning labels on product packages and advertisements, restricted the sale of ST to minors in many

[1] Supported in part by National Cancer Institute grant no. CA-41733.

states, and increased taxes on ST products (Connolly et al., 1986; Deveny, 1990; US DHHS, 1989 and 1990a). Research has been funded to develop and test interventions to prevent ST use and encourage cessation (US DHHS, 1990b). In addition, many health agencies and professional groups have produced educational materials for use in schools and community-based tobacco control programs. Work in these areas continues.

Experience from antismoking campaigns indicates that, if these efforts can be sustained, advances in science, policy, and public education will slow the increase in ST prevalence (US DHHS, 1989; Warner, 1982, 1986, and 1989) and eventually turn the tide toward a decrease. However, there will be a time lag, in part because of activities of the ST industry. Research on the carcinogenic properties of ST and the health effects of its use will be complicated by the continual introduction of new products and product variations. Resulting gaps in scientific knowledge will be cited to confound debates about additional policy proposals. Public education will struggle not only against competing priorities in schools, communities, and the media, but also against relentless ST marketing. Moreover, public health workers in each of these areas will compete with each other for scarce resources, while the ST industry will easily finance its expansion through fat profits.

These realities imply that current public health strategies for preventing and controlling ST use must be bolstered by creative new approaches. Because promising directions for basic scientific research, public policy, and cessation programs are discussed elsewhere in this monograph, this paper concentrates on preventive education. First, the need for innovation is emphasized through a brief review of current educational efforts and what they can accomplish. This analysis is then contrasted with ST marketing to identify some specific deficiencies in education, as well as some unconventional ways they might be remedied.

CURRENT PREVENTIVE EDUCATION

At present, education about ST includes public information, educational programs for youth, and some related education of teachers, parents, and health professionals. Most youth education is delivered through schools, often in conjunction with education aimed at smoking prevention. No national data are available on the extent or quality of school-based instruction about ST, but it certainly is less adequate than antismoking education, which itself is highly variable (D'Onofrio, 1989; US DHHS, 1989 and 1990a).

Many schools provide no health education course or regularly scheduled time for health education in the curriculum. In *some* schools, *some* teachers discuss various health topics in science or other courses, but instruction is not systematic and tobacco use may not be covered. Schools with organized health education programs generally teach about tobacco and other substances in a special unit. Alcohol and illicit drugs usually receive the greatest attention, although cigarette smoking causes more deaths annually than all other substances combined (Warner, 1987). Smokeless tobacco receives still shorter shrift; for example, when a single 50-min class period covers tobacco in all forms, it is likely that smoking, not

smokeless tobacco, will be emphasized. In the war on drugs, most of the nation's schools do not identify tobacco as a major enemy.

On the positive side, several state-of-the-art ST prevention programs have been developed and field-tested through research grants from the National Cancer Institute (Boyd and Glover, 1989; US DHHS, 1989 and 1990b). These programs tend to parallel the more effective smoking prevention programs in that they are guided by a social influences model of smoking initiation and are typically directed to children between the ages of 10 and 14. With the exception of a program for 4-H Club members in California and one for Little League baseball teams in Texas, NCI-sponsored prevention programs are conducted in school classrooms by specially trained health educators, college students, or regular teachers, sometimes with the assistance of peer leaders. Their objectives are to provide information about the short-term health and social consequences of ST use; correct misconceptions about the pervasiveness and social acceptability of the practice; reveal how parental modeling, peer pressure, and the media promote ST use; and teach resistance skills. Although evaluation of these programs is still under way, preliminary analyses suggest that, like most theory-based smoking prevention programs (Botvin et al., 1990; Ellickson and Bell, 1990; Evans et al., 1981; Flay, 1985; Flay et al., 1985 and 1989; Gersick et al., 1988; Glynn, 1989; Graham et al., 1990; Hansen et al., 1988a and 1988b; Johnson et al., 1986; Luepker et al., 1983; Murray et al., 1988 and 1989; Pentz et al., 1989a and 1989b; Schinke and Gilchrist, 1984; Telch et al., 1982), they will have positive albeit modest effects on youth least likely to use tobacco.

Although early smoking prevention programs failed to show behavioral effects (Flay, 1985; Thompson, 1978), results from the first generation of ST prevention studies are encouraging. Knowledge gained from efforts to prevent cigarette smoking over the past quarter-century (US DHHS, 1989) has been well applied to the ST problem. A challenge for the future is to promote adoption of NCI-sponsored programs as they become available for widespread use by schools and other organizations serving youth. The first of these projects is now being disseminated (D'Onofrio et al., 1991), and others will soon follow.

As these materials are used and others are developed, a second challenge will be to assure that the principles that led to progress continue to be applied. Thus (1) program design should be guided by behavioral theory, (2) content should extend well beyond the health dangers of ST use, (3) participatory instructional methods should be used, (4) young people should have an opportunity to develop and practice skills for resisting temptations to use ST, (5) teachers should be trained in program delivery, and (6) as appropriate, same-age or older peers should be recruited and trained to assist (Glynn, 1989).

HEALTH EDUCATION vs. MARKETING

Despite early indications that preventive education is on a promising track, current educational efforts pale in comparison to ST marketing. To a certain extent this will always be the case, for health education and marketing differ in several fundamental respects.

The goal of the smokeless tobacco industry is to enlarge the market for its products, while each company aims to improve its market share. Increasing sales by a few percentage points is regarded as a triumph. Public health, on the other hand, tries to protect entire populations. Because preventive education is evaluated against this absolute standard, programs that reduce tobacco use by a few percentage points are viewed as only marginally effective.

Marketing goals are expressed in positive terms like "increase, capture, build, and acquire." These words convey and invite initiative. However, in aiming to "reduce, curtail, decrease, and delay" ST use, the goals of public health carry negative connotations. These differences are reflected in themes and messages used in marketing and health education campaigns. Whereas tobacco advertising urges positive and expansive action ("buy this, try that"), health education warns, "don't do it."

A marketing campaign is initiated by a single corporation after a long period of careful market analysis and product development. The campaign typically is planned to run for several years, with intermediate objectives delineating shorter phases within a well-orchestrated long-term strategy. Resources are assured to execute the plan, which is sufficiently flexible to respond to unanticipated threats or opportunities. Marketing budgets are generous. In 1989, ST manufacturers spent $81 million advertising and promoting their products (Federal Trade Commission, 1991).

In contrast, health education is conducted by multiple agencies, groups, and individuals with a vast array of objectives and priorities. These priorities, the resources available to support them, and organizational leadership shift with economic and political currents. Health care professionals who recognize the need for initiatives to prevent ST use must struggle in this milieu to convince others that a problem exists, but agencies differ in the amount and quality of scientific evidence they require to include ST on their agenda. Once an agency has acknowledged the problem, competition for resources begins.

Agency plans for preventive education frequently are prepared for review by fiscal decisionmakers. Program objectives are shaped not only by the degree to which agency executives and staff understand the ST problem and state-of-the-science approaches to prevention, but also by the agency's mission, commitments, assets, constituent expectations, and time pressures. Optimal proposals for prevention therefore tend to be compromised in the budgetmaking process. Once plans are approved, implementation is expected to begin immediately, often with little regard to what others are doing.

Because agencies tend to plan independently, local, state, and national resources for the prevention of ST use are accumulated sporadically. Educational programs and materials enter the field in an unsystematic and unpredictable flow. These interventions vary in the effectiveness with which they target key issues in prevention, and many of them have not been field-tested or evaluated prior to broad distribution. Although companies that

market ST carefully select and limit their product lines, educational approaches to prevention proliferate with little quality control.

These factors complicate the knitting of fragmented educational elements into a coordinated master plan for prevention. When planning can be accomplished, the result is not streamlined or efficient, but at best a patchwork. In this pluralistic society, institutional autonomy is protected, and enthusiasm for close interorganizational collaboration is frequently lukewarm.

Commercial marketing and health education also differ in the products that they offer. ST products are tangible and cheap; good health education is neither. Moreover, while ST fosters habituation and dependence, health education promotes independence, active decisionmaking, and informed choices. If marketing persuades youth to try ST enough times, nicotine helps to recruit permanent customers (Boyd et al., 1987; US DHHS, 1990b; WHO, 1988). Health education is directed to a more fundamental and continuing change called learning.

Nevertheless, those who market ST are clever in applying learning principles and appealing to the basic needs of children and adolescents. Advertising promotes the fantasy that, with just a can of snuff or a pouch of chew, young people can satisfy their curiosity, demonstrate their maturity and independence, enjoy adventures, belong to an admired group, and enhance their social image. Youth must be highly sophisticated to view the development of resistance skills as an achievement. And of course, learning how to resist temptation, defer gratification, communicate effectively, win friends, and make good decisions is much harder than taking a dip or a chew, especially when the tobacco industry provides free samples and step-by-step instructions for use (Ernster, 1989).

Finally, ST marketing and health education are separated by deep differences in the values and ethics that underlie attempts to influence behavior. Human concern and conscience preclude health educators' promoting products that harm the consumer and from using some strategies that are effective in building business profits.

IMPLICATIONS OF ST MARKETING

Education to prevent ST use is essentially reactive to a problem created by the tobacco industry. For most of this century, the ST market was stagnant or shrinking (Tye et al., 1987; US DHHS, 1986). National surveys conducted between 1964 and 1975 found the prevalence of ST use to be fairly stable at less than 5 percent and use rates to be highest among persons over age 50 (US DHHS, 1986). However, in the early 1970's, the industry extended its product lines and began aggressive marketing to males between ages 18 and 30, with a "substantial emphasis on the 18 to 24 group" (Maxwell, 1980).

The results have been well documented. Dramatic increases in advertising budgets for moist snuff were soon paralleled by sharp increases in production and sales (Rosenthal, 1985; US DHHS, 1986). National surveys of adults conducted between 1985 and 1987 found startling shifts in patterns of ST use, with older adolescents and young adult males using the

products at a higher rate than any other age group (Marcus et al., 1989; Novotny, et al., 1989; Orlandi and Boyd, 1989). Although the ST industry has steadfastly insisted that its products are meant for adults, national and regional surveys also have reported high rates of use among young boys and adolescents (Bauman et al., 1989; Boyd et al., 1987; Glover, 1986; Orlandi and Boyd, 1989; Rouse, 1989). And whereas earlier ST use was largely restricted to rural areas in the South and the West, it is now reported in all regions of the country (Orlandi and Boyd, 1989).

The ST industry, health professionals, and other analysts all recognize clever and aggressive marketing as a major force in these changes (Christen et al., 1982; Connolly et al., 1986; Deveny, 1990; Glover et al., 1981 and 1982; Hunter et al., 1986; Shelton, 1984; Smyth, 1989; US DHHS, 1986; U.S. Tobacco, 1985; WHO, 1988). Sophisticated marketing is also identified as a major obstacle in achieving national health objectives for the prevention and control of tobacco use (US DHHS, 1990a). Detailed descriptions of the creative strategies employed by ST companies can be found in industry publications, trade journals, news magazines, and the health literature (Anderson et al., 1979; Braverman et al., 1989; Christen et al., 1982; Connolly et al., 1986; Deveny, 1990; Dougherty, 1984; Ernster, 1986 and 1989; Feigelson, 1983; Glover et al., 1981 and 1982; Harper, 1980; Maxwell, 1983; Mintz, 1986; Negin, 1985; O'Conner, 1983; Rosenthal, 1985; Shelton, 1982; Tobacco Reporter, 1983; US Tobacco and Candy Journal, 1987).

Those responsible for preventive interventions have used this information to understand the parameters of the problem, to estimate its magnitude, to identify fruitful policy directions, and to acquaint youth with persuasive advertising strategies. To date, however, knowledge about ST marketing has not been systematically applied either in the design of counteractive educational programs or in evaluation of current educational efforts.

Such omissions leave health education continually defending against aggressive ST marketing teams that are committed to keeping the competition under fire. To increase the chances for success, health educators need to study the opposition, strengthen their defense, and develop new offensive moves. As the following examples illustrate, examining secrets of the industry's success can stimulate new ideas for ST education and reveal some idiosyncrasies of the public health culture that may be limiting the effectiveness of current approaches to prevention.

Market Research Commercial marketing invests heavily in research and product testing *before* a campaign is launched. Once a campaign is under way, its effectiveness is evaluated very simply by changes in sales. In contrast, public health provides few resources for market research to guide the design of educational programs. Health education is initiated as soon as possible and then subjected to rigorous evaluation. To do it right in public health, educational interventions must be subjected to longitudinal randomized trials in defined populations with biochemical validation of self-reports and

appropriate units of statistical analysis. The effectiveness of ST use prevention might be increased through a shift of resources from extensive evaluation of educational programs after they are delivered, toward more front-end research to guide initial development and refinement.

Market Definition Although the ST industry has identified males aged 18 to 24 as its primary marketing target (Maxwell, 1980), public health researchers have assumed that prevention will be most effective during initial experimentation with dipping and chewing. Because the onset of cigarette smoking peaks during the transition from elementary to middle or junior high school (Flay et al., 1983), resources for ST education have been concentrated on those aged 10 to 14. The need for preventive programs for younger children is now recognized, but older adolescents and young adults are discussed only as targets for ST cessation.

Nonetheless, among adults who use ST regularly, the median age of initiation for both snuff and chewing tobacco is 19 (Novotny, 1989). Although this figure may drop with the maturing of youth who grew up and began to use ST during intense marketing, it suggests that the initiation of ST use may occur throughout adolescence and into early adulthood. Preliminary data from a California survey (California Department of Health Services, 1990), presented in Table 1, further indicate that many young adults who dip and chew are not yet addicted to daily use. Preventive education, therefore, should be directed not only to children and young adolescents, but also to high school students and to young men making the transition into college or the workplace. Like ST marketing, health education should especially target young men entering blue collar occupations in factories, on farms, or in industries such as lumber, steel, fishing, and firefighting.

Market Segmentation Smokeless tobacco marketing is precisely targeted. According to an executive of U.S. Tobacco:

> We've built our business by identifying the "pockets" of Americans whose lifestyles include smokeless tobacco . . . . Other consumer product companies are beginning to realize what we've understood all along. America is not necessarily a "mass-market"; it is more a collection of "micro-markets" or regional markets defined by a variety of factors (including age, sex, occupation, and hobbies), which may be broadly defined as consumer "lifestyle" (Africk, 1985).

To date, education to prevent ST use has been only modestly adapted to reach different segments of the youth population. Current projects with 4-H, Little League, and Native Americans provide an important start in tailoring prevention programs to the groups at high risk for ST use. However, within these market segments, youth are still treated very much alike. Health education thus needs to seek additional sites and alternative models for preventing ST use among youth at greatest risk. Like U.S. Tobacco, we should learn to "fish where the fishing is good" (Deveny, 1990).

Table 1
Current use of smokeless tobacco as a weighted percentage of California males, aged 18 to 44, who have ever used chewing tobacco or snuff

	Age 18 to 24	Age 25 to 44
Chewing Tobacco		
n	172	393
Daily use	4.5%	6.6%
< daily use	18.9	9.6
Snuff		
n	141	318
Daily use	7.9%	8.5%
< daily use	11.8	9.1

School-based programs treat all students as potential users, but most girls and many boys do not like ST and never intend to use it. Teaching such youngsters skills to resist ST offers probably is not the best use of educational time and resources. The limited effectiveness of school-based programs in preventing high-risk youth from using tobacco raises questions about optimal use of classroom time. With increasing pressures on their curricula, some schools already are reluctant to commit multiple sessions to tobacco education. Youth groups that hold only weekly or monthly meetings also hesitate to devote a high proportion of their program time to prevention of tobacco use. Ironically, this problem will be exacerbated if rates of cigarette smoking and ST use among young people begin to fall.

Maintaining and increasing the support of schools for preventive education thus requires the development of programs that are relevant to all youth served and that advance basic organizational objectives. One possible approach is to emphasize the rights of individuals to a tobacco-free environment and to present tobacco use as a social as well as a personal health issue. Some programs already are teaching children how to ask others not to use tobacco in their presence, to discourage friends from trying tobacco, and to support users who are trying to quit (D'Onofrio, 1991; D'Onofrio et al., 1991). Development of these skills in communication, social relations, and social problem-solving nurtures children's development while simultaneously establishing their social identity as non-users of tobacco.

An extension of this approach is to involve youth as active partners in the planning, delivery, and evaluation of school- and community-based prevention programs. Their creativity should be tapped in the design and production of educational materials and presentations. Young people should testify at city council meetings on proposed tobacco control ordinances. They should appear on radio and television to debate the issues, discuss their efforts, and showcase the songs, poems, skits, and artwork they have produced. They should be recruited to help with surveillance of ST promotions. Youth also can participate effectively in sting operations to identify merchants who illegally sell ST or distribute free samples to minors.

Assuming responsibility for adult tasks satisfies young people's curiosity, fosters their maturity and independence, provides them with new experiences, and enables them to affiliate with admired adults, including doctors, dentists, attorneys, community activists, media and sports personalities, law enforcement officers, and others. Recognition of their capabilities and talents also enhances the self-image of youth, while participation with others in the fight against tobacco deepens their sense of community and creates future leaders. By responding to the developmental needs of children and youth, these activities lead to outcomes that are widely recognized as protective against all forms of substance use (Hawkins et al., 1985).

Product Development

U.S. Tobacco tailors its products to promote their acceptance. Packaging snuff in round tin cans was instrumental in increasing consumer awareness: having a round can of snuff in the back pocket of blue jeans became a status symbol among young males (Negin, 1985). Skoal Bandits were designed to provide new users with a gradual introduction to moist snuff, as well as to overcome some of the messiness associated with ST use and to increase its acceptability in the urban environment (Bantle, 1980; Dougherty, 1984; Maxwell, 1983; Negin, 1985; O'Conner, 1983). Prevention programs might similarly benefit from more creative packaging to attract interest, and from product diversification to encourage easy trial, which leads to more regular use of stronger products. However, to avoid dilution of educational efforts, such initiatives should be part of a larger strategy designed to promote eventual adoption of more potent programs.

Another industry principle that merits consideration is product variation to assure consumer choice. Both ST products and the strategies used to market them are tailored to diverse tastes and customs. The public health community, though, attributes great importance to the generalizability of preventive programs and their potential for widespread acceptance. Possibly, tailoring health education programs to a variety of micro-markets would prove more effective than developing generic programs for mass distribution. An additional principle borrowed from the ST industry indicates that such innovations may win quicker acceptance if they are introduced not as new educational products, but as extensions of programs already known and popular (Smyth, 1989).

Product Distributors

U.S. Tobacco considers its sales force a key component of success. Nationwide in 1986, the company had 436 field representatives who provide retailers and customers with one-on-one attention (U.S. Tobacco, 1986). Specific functions include assuring fresh products, promoting optimal display, demonstrating use, obtaining customer feedback, and identifying potential areas for development (Deveny, 1990; U.S. Tobacco, 1986 and 1990). These salesman also court retailers with recognition, prizes, tickets to sporting events, and invitations to social gatherings.

In addition, representatives of U.S. Tobacco serve as "traveling billboards" for the company (Hawkins et al., 1985). Like chameleons, they adapt to the local culture and provide exciting in-the-flesh advertisements for chewing and dipping. We have encountered a salesman in southern California driving a red Ferrari. In Bakersfield, a sales representative in a

hard hat was observed distributing free samples in the oil fields. And in a rural mountain county, we found an ST representative wearing a cowboy hat and driving a pickup truck with country-and-western music on the radio and a can of Copenhagen on the dash.

Those who market prevention programs rarely display such cultural adaptability or such verve. Too often, these programs are simply delivered to schools and other organizations with the expectation that they will be used. Given numerous barriers in program dissemination (D'Onofrio, 1989; Glynn, 1989), employing field representatives to nurture the adoption process appears worth a try. As far as possible, these distributors of prevention should establish warm relations with program intermediaries and consumers. In addition, their manner and their personage should demonstrate that tobacco-free lifestyles are both accessible and exciting to youth and young adults within the context of unique community cultures.

Development Of the Delivery System

The promotion of smokeless tobacco is pervasive and continuous. However, prevention education typically is presented only at schools and for a limited time. To counter subtle, sophisticated, and omnipresent promotion of tobacco products, adults who spend time with children should be enlisted in the preventive effort. Ideally, they should provide education spontaneously as issues about the sale, promotion, and use of smokeless tobacco arise in the course of daily life (D'Onofrio, 1991). That is, when parents, youth leaders, and others observe ST being used, they need to discuss the practice as dangerous and socially unacceptable. During visits to the corner store, attendance at baseball games and other events, or while watching the Indianapolis 500 on television, adults should point out how ST is marketed. In these conversations, adults also need to help children understand why a bad product is so readily available, why some good people use it, and why the Government does not ban substances that are harmful. Answering children's questions about ST is likely to require explaining history, politics, government, law, economics, and other complex aspects of society.

The educational challenge is formidable, but if young people are to make wise decisions about ST, both as individuals and as community members, adults in all walks of life must rise to the task. Nurturing the positive development of children and youth through education is public health's most powerful approach to prevention.

REFERENCES

Africk, J. The right to choose. *US Tobacco Review* 1(3), Third Quarter, 1985.

Anderson, H., Boerger, G., Leslie, C. A tobacco boom with no smoke. *Newsweek,* August 20, 1979, p. 67.

Bantle, L.F. Smokeless tobacco: A trend to watch. *Tobacco Journal International* 4: 344-346, 1980.

Bauman, K.E., Koch, G.G., Fisher, L.A., Bryan, E.S. Use of smokeless tobacco by age, race, and gender in ten standard metropolitan statistical areas of the southeast United States. *National Cancer Institute Monographs* 8: 35-37, 1989.

Botvin, G.J., Baker, E., Filazzola, A.D., et al. A cognitive-behavioral approach to substance abuse prevention: One-year followup. *Addictive Behaviors* 15: 47-63, 1990.

Boyd, G.M., Ary, D.V., Wirt, R., et al. Use of smokeless tobacco among children and adolescents in the United States. *Preventive Medicine* 16: 402-421, 1987.

Boyd, G.M., Glover, E.D. Smokeless tobacco use by youth in the U.S. *Journal of School Health* 59(5): 189-194, 1989.

Braverman, M.T., D'Onofrio, C.N., Moskowitz, J.M. Marketing smokeless tobacco in California communities: Implications for health education. *National Cancer Institute Monographs* 8: 79-85, 1989.

Business Week. What could burn the king of smokeless tobacco. *Business Week*, March 17, 1986, p. 86.

California Department of Health Services. *Tobacco Use in California 1990: A Preliminary Report Documenting the Decline of Tobacco Use*. San Diego: University of California, 1990.

Christen, A.G., Swanson, B.Z., Glover, E.D., et al. Smokeless tobacco: The folklore and social history of snuffing, sneezing, dipping, and chewing. *Journal of the American Dental Association* 105: 821-829, 1982.

Connolly, G.N., Winn, D.M., Hecht, S.S., et al. The reemergence of smokeless tobacco. *New England Journal of Medicine* 31(16): 1020-1027, 1986.

D'Onofrio, C.N. Making the case for cancer prevention in the schools. *Journal of School Health* 59(5): 225-230, 1989.

D'Onofrio, C.N. *Tobacco Talk: Educating Young Children About Tobacco*. Santa Cruz, CA: Network Publications, 1991.

D'Onofrio, C.N., Braverman, M.T., Moskowitz, J.M., et al. *The Project 4-Health Tobacco Education Program*. School of Public Health, University of California, Berkeley, 1991.

Deveny, K. With help of teens, snuff sales revive. *Wall Street Journal*, May 3, 1990, p. B1.

Dougherty, P.H. Moving smokers to snuff. *New York Times*, January 13, 1984.

Ellickson, P.L., Bell, R.M. Drug prevention in junior high: A multi-site longitudinal test. *Science* 247(16): 1299-1305, 1990.

Ellis, J. Stocks picked by 1988's A-Team smartly outpaced the market's record run. *Money*, October 1989, p. 122.

Ernster, V.L. Advertising and promotion of smokeless tobacco products. *National Cancer Institute Monographs* 8: 87-94, 1989.

Ernster, V.L. Advertising of smokeless tobacco products. Presented at the NIH Consensus Development Conference on Health Implications of Smokeless Tobacco Use, Bethesda, Maryland, January 1986.

Evans, R.I., Rozelle, R.M., Maxwell, S.E., Raines, B.E., Dill C.A., Guthrie, T.J. Social modeling films to deter smoking in adolescents: Results of a three-year field investigation. *Journal of Applied Psychology* 66(4): 399-414, 1981.

Federal Trade Commission. Report to Congress, Pursuant to the Comprehensive Smokeless Tobacco Health Education Act of 1986. FTC, Washington, D.C., 1991.

Feigelson, J. Skoal Bandit blitz kicks off N.Y. entry. *Advertising Age*, August 8, 1983, p. 46.

Flay, B.R. Psychosocial approaches to smoking prevention: A review of findings. *Health Psychology* 4(5): 449-488, 1985.

Flay, B.R., d'Avernas, J.R., Best, J.A., Kersell, M.W., Ryan, K.B. Cigarette smoking: Why young people do it and ways of preventing it. In: *Pediatric and Adolescent Behavioral Medicine*, P. Firestone and P. McGrath (Editors). New York: Springer-Verlag, 1983, pp. 132-183.

Flay, B.R., Koepke, D., Thompson, S.J., Santi, S., Best, J.A., Brown, K.S. Six-year followup of the first Waterloo school smoking prevention trial. *American Journal of Public Health* 79(10): 1371-1376, 1989.

Flay, B.R., Ryan, K.B., Best, J.A., Brown, K.S., Kersell, M.W., d'Arvenas, J.R., Zanna, M.P. Are social-psychological smoking prevention programs effective? The Waterloo Study. *Journal of Behavioral Medicine* 8(1): 37-59, 1985.

Gersick, K.E., Grady, K., Snow, D.L. Social-cognitive skill development with sixth graders and its initial impact on substance use. *Journal of Drug Education* 18(1): 55-67, 1988.

Glover, E.D. Regional prevalence and patterns of smokeless tobacco use. Presented at the NIH Consensus Development Conference on Health Implications of Smokeless Tobacco Use, Bethesda, Maryland, January 1986.

Glover, E.D., Christen, A.G., Henderson, A.H. Just a pinch between the cheek and gum. *Journal of School Health* 51: 415-418, 1981.

Glover, E.D., Christen, A.C., Henderson, A.H. Smokeless tobacco and the adolescent male. *Journal of Early Adolescence* 2(1): 1-13, 1982.

Glynn, T.J. Essential elements of school-based smoking prevention programs: Research results. *Journal of School Health* 59: 181-188, 1989.

Graham, J.W., Johnson, C.A., Hansen, W.B., et al. Drug use prevention programs, gender, and ethnicity: Evaluation of three seventh-grade project SMART cohorts. *Preventive Medicine* 19: 305-313, 1990.

Hansen, W.B., Johnson, C.A., Flay, B.R., Graham, J.W., Sobel, J. Affective and social influences approaches to the prevention of multiple substance abuse among seventh grade students: Results from Project SMART. *Preventive Medicine* 17: 135-154, 1988a.

Hansen, W.B., Malotte, C.K., Fielding, J.E. Evaluation of a tobacco and alcohol abuse prevention curriculum for adolescents. *Health Education Quarterly* 15(1): 93-114, 1988b.

Harper, S. In tobacco, where there's smokeless there's fire. *Advertising Age*, June 23, 1980.

Hawkins, D.J., Lishner, D.M., Catalano, R.F. Childhood predictors of adolescent substance abuse. In: *Etiology of Drug Abuse: Implications for Prevention*, C.L. Jones and R.J. Battjes (Editors). NIDA Research Monograph 56, DHHS Publication (ADM) 85-1335. Washington, DC: Government Printing Office, 1985.

Hunter, S.M., Croft, J.B., Burke, G.L., et al. Longitudinal patterns of cigarette smoking and smokeless tobacco use in youth: The Bogalusa Heart Study. *American Journal of Public Health* 76: 193-195, 1986.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines*. (volume 37). Lyon: IARC, 1985.

Johnson, C.A., Hansen, W.B., Collins, L.M., Graham, J.W. High school smoking prevention: Results of a three-year longitudinal study. *Journal of Behavioral Medicine* 9(1): 439-452, 1986.

Luepker, R.V., Johnson, C.A., Murray, D.M., Pechacek, T.F. Prevention of cigarette smoking: Three-year followup of an education program for youth. *Journal of Behavioral Medicine* 6: 53-62, 1983.

Marcus, A.C., Crane, L.A., Shopland, D.R., Lynn, W.R. Use of smokeless tobacco in the United States: Recent estimates from the Current Population Survey. *National Cancer Institute Monographs* 8: 17-23, 1989.

Mattson, M.E., Winn, D.M. Smokeless tobacco: Association with increased cancer risk. *National Cancer Institute Monographs* 8: 13-16, 1989.

Maxwell, J.C. Chewing, snuff is growth segment. *Advertising Age*, June 23, 1980.

Maxwell, J.C. Smokeless tobacco volume sales drop. *Advertising Age*, June 16, 1986, p. 62.

Maxwell, J.C., Jr. Smokeless keeps growing: Cigars keep declining. *Tobacco International,* July: 90-91, 1983.

Mintz, M. The artful dodgers. *Washington Monthly*, October 1986, pp. 9-16.

Murray, D.M., Davis-Hearn, M., Goldman, A.I., Pirie, P., Luepker, R.V. Four- and five-year followup results from four seventh-grade smoking prevention strategies. *Journal of Behavioral Medicine* 11(4): 395-405, 1988.

Murray, D.M., Pirie, P., Luepker, R.V., Pallonen, V. Five- and six-year followup results from four seventh-grade smoking prevention strategies. *Journal of Behavioral Medicine* 12: 207-218, 1989.

Negin, E. Just a pinch between your cheek and gum. *Public Citizen,* Spring: 28-32, 1985.

Novotny, T.E., Pierce, J.P., Fiore, M.C., Davis, R.M. Smokeless tobacco use in the United States: The Adult Use of Tobacco Surveys. *National Cancer Institute Monographs* 8: 25-28, 1989.

O'Conner, J.J. Bandits out to steal bite from cigarettes. *Advertising Age*, June 1983, pp. 2, 27.

Office of Medical Applications of Research. Health implications of smokeless tobacco use. *Journal of the American Medical Association* 255: 1045-1048, 1986.

Orlandi, M.A., Boyd, G. Smokeless tobacco use among adolescents: A theoretical overview. *National Cancer Institute Monographs* 8: 5-12, 1989.

Pentz, M.A., Dwyer, J.H., MacKinnon, D.P., Flay, B.R., Hansen, W.B., Wang, E.Y.I., Johnson, C.A. A multicommunity trial for primary prevention of adolescent drug abuse: Effects on drug use prevalence. *Journal of the American Medical Association* 261(22): 3259-3266, 1989a.

Pentz, M.A., MacKinnon, D.P., Dwyer, J.H., et al. Longitudinal effects of the midwestern prevention project on regular and experimental smoking in adolescents. *Preventive Medicine* 18: 304-321, 1989b.

Rosenthal, J. Son of the Marlboro Man. *Washington Monthly*, March 1985, p. 51.

Rouse, B.A. Epidemiology of smokeless tobacco use: A national study. *National Cancer Institute Monographs* 8: 29-33, 1989.

Schinke, S.P., Gilchrist, L.D. Preventing cigarette smoking with youth. *Journal of Primary Prevention* 5: 48-56, 1984.

Shelton, A. Moist snuff leads American market. *Tobacco Reporter* 111(7): 30-31, 1984.

Shelton, A. Smokeless sales continue to climb. *Tobacco Reporter* 109(8): 42-44, 1982.

Smyth, J. Moist snuff sales gain. *Tobacco Reporter* 116(8): 30-31, 1989.

Telch, M.J., Killen, J.D., McAlister, A.L., et al. Long-term followup of a pilot project on smoking prevention with adolescents. *Journal of Behavioral Medicine* 5(1): 1-8, 1982.

Thompson, E.L. Smoking education programs, 1960-76. *American Journal of Public Health* 68: 250-257, 1978.

Tobacco Reporter. Smokeless tobacco: The bright star of the U.S. market. *Tobacco Reporter* 110(11): 68-69, 1983.

Tye, J.B., Warner, K.E., Glantz, S.A. Tobacco advertising and consumption: Evidence of a causal relationship. *Journal of Public Health Policy,* Winter: 492-508, 1987.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

U.S. Department of Health and Human Services. *Promoting Health/Preventing Disease: Year 2000 Objectives for the Nation* (conference edition). U.S. Department of Health and Human Services, Public Health Service, 1990a.

U.S. Department of Health and Human Services. *Reducing the Health Consequences of Smoking: 25 Years of Progress. A Report of the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. DHHS Publication No. (CDC) 89-8411, 1989.

U.S. Department of Health and Human Services. *Smoking, Tobacco, and Cancer Program: 1985-1989 Status Report*, D.R. Shopland and M.M. Massey (Editors). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. NIH Publication No. 90-3107, 1990b.

U.S. Tobacco. 75 years of marketing and advertising. *US Tobacco Review* 2(1): 10-12, 1986.

U.S. Tobacco. U.S. Tobacco rises to Fortune 500 Status. *US Tobacco Review* 1(1): 1, 1985

U.S. Tobacco. *UST, 1988 First Quarter Report*. Greenwich, CT: U.S. Tobacco, 1988.

U.S. Tobacco. *UST, 1990 Annual Repor*t. Greenwich, CT: U.S. Tobacco, 1990.

US Tobacco and Candy Journal. Corporate sponsors drive the right messages home. *US Tobacco and Candy Journal*, July 27-August 24, 1987, pp. 46, 84.

Warner, K.E. Effects of the antismoking campaign: An update. *American Journal of Public Health* 79(2): 144-151, 1989.

Warner, K.E. Health and economic implications of a tobacco-free society. *Journal of the American Medical Association* 258(15): 2080-2086, 1987.

Warner, K.E. *Selling Smoke: Cigarette Advertising and Public Health*. Washington, DC: American Public Health Association, 1986.

Warner, K.E., Murt, H.A. Impact of the antismoking campaign on smoking prevalence: A cohort analysis. *Journal of Public Health Policy* 3(4): 374-390, 1982.

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Fraumeni, J.F., Jr. Snuff dipping and oral cancer among women in the Southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

World Health Organization. *Smokeless Tobacco Control: Report of a WHO Study Group*. Geneva, Switzerland: WHO, 1988. WHO Technical Report Series 773.

Applying the Social Inoculation Model To a Smokeless Tobacco Use Prevention Program With Little Leaguers[1]

Richard I. Evans, Bettye E. Raines, and J. Greg Getz

ABSTRACT This paper first considers the question of how research on smoking prevention among adolescents can become the basis for developing programs to prevent the use of smokeless tobacco. Within this context, the evolution of the social inoculation strategy is discussed. The paper encompasses procedures we used in developing, implementing, and evaluating such programs, drawing from our current National Cancer Institute-supported project dealing with prevention of ST use among Little League baseball players. Recommendations for public policy related to prevention of ST use are offered.

INTRODUCTION The invitation to this conference provides an opportunity to describe how health promotion investigators, employing a social influence orientation, can consider the cross-application of well-studied models from cigarette smoking to the emerging problem of smokeless tobacco use. To introduce the discussion, we describe the evolution of the "social inoculation strategy" originated by our research group to prevent cigarette smoking among adolescents (Evans, 1976; Evans et al., 1978 and 1981). Variations of this approach have been extended to the prevention of use of harmful substances such as alcohol and illicit drugs (Botvin and Wills, 1985; Flay, 1985), suggesting that its application to preventing ST use may help meet stated research goals (Chassin et al., 1989; Evans and Raines, 1990). Our current NCI-supported ST use prevention program, which involves a large population of Little League baseball players, is designed to test the efficacy of such a cross-application (Evans and Raines, 1990).

We emphasize both psychosocial and methodological barriers to the successful development, implementation, and evaluation of tobacco use prevention programs, drawing on our broader program of health-related research and on the Little League baseball project specifically for examples. We define methodological problems to include the structural-organizational barriers inherent in research conducted in natural settings.

While we are pleased that our approach has proved valuable in prevention efforts, we are concerned that one technique of this overall prevention strategy—"Just Say No" to resist peer pressure—has been taken out of context and redirected in simplistic form as a formula for preventing all substance abuse. In our original program, "Just Say No" was presented as a resistance response to low-level peer pressure and was only one of a series of

[1] Support provided in part by National Cancer Institute grants no. CA-41722 and no. CA-41471.

responses and strategies designed to address increasing levels of peer pressure as well as other social influences in the young adolescent's environment. Because of the current pervasiveness of the catchphrase, we emphasize that "Just Say No" is not enough! Such generalized use of a single component of prevention strategy is something about which prevention program planners should be cautioned (Evans, 1988).

EVOLUTION OF THE STRATEGY

Our smoking prevention research program evolved from a series of studies during the 1960's, in which we attempted to apply social-psychological theories and strategies to problems in preventive dentistry (i.e., effective toothbrushing and flossing among a population of junior high school students). The results of these studies (Evans et al., 1970 and 1975) were encouraging, including the successful use of a chemical measure, a tooth-staining disclosant that allowed us to compare the effects on oral hygiene of actual vs. reported toothbrushing behavior (Evans et al., 1968). Several findings from these studies proved useful as we entered the next phase of our research program:

- the probability that reported health behavior may not correlate with actual behavior;
- the observation that fear arousal alone in persuasive messages may not be the most effective means of influencing health enhancement behavior;
- the fact that community institutions such as schools may lend themselves as settings for implementing persuasive health messages in a systematic and controlled manner; and
- the importance of tailoring both content and style of health promotion messages to the target audiences in such settings.

In 1973 we began addressing why young adolescents begin smoking cigarettes even when they are fully aware of the related health dangers. An extensive survey of junior high students in the Houston Independent School District (Evans and Raines, 1982) identified several problems related to preventive education about smoking in the school setting. These problems created barriers to effective communication of important health messages to adolescents. A student survey indicated that smoking prevention programs in the curriculum

- focused too heavily and inappropriately on fear arousal;
- emphasized the long-term consequences of cigarette smoking (e.g., heart disease and cancer), failing to recognize that young adolescents are more present- than future-oriented;
- used audiovisuals (e.g., films, videotapes) and other materials in general use that had not been evaluated for their relevance to adolescents; and
- were predicated on the notion that mere awareness of a health threat leads to the desired health enhancement behaviors.

According to this survey, not only were students unresponsive to such programs, but in some cases, it appeared that the health education messages delivered were *counter*productive.

In addition to the student survey findings, the evaluation of concurrent in-place school smoking prevention programs identified two underlying problems that might be addressed in a well-planned intervention study: (1) Other than their reliance on imparting information about the dangers of a potentially harmful behavior, the programs rarely had any guiding theoretical conceptualization; and (2) in the development of interventions, program planners did not seek feedback from their target audiences.

To address such barriers to the understanding and acceptance of important health messages and, more critically, to elicit appropriate behavioral action as a result of exposure to the messages, we undertook a longitudinal investigation using a large population of junior high school students as they entered seventh grade. Because the development of our intervention strategy is described elsewhere (Evans, 1982, 1984, and 1990; Evans et al., 1984a and 1984b), in this paper we only outline the development of this program.

Results from an initial survey that included fifth- through ninth-grade students indicated a significant increase in experimental cigarette smoking at about the time students entered junior high school. At the time, junior high schools in Houston included grades seven through nine. Thirteen junior high schools were selected for assignment to various experimental conditions, and two were chosen as resource schools to be used in the development and pretesting of procedures and materials.

We established priorities in developing our research program, dictating that (1) it was guided by appropriate theoretical considerations and (2) it reflected input from the target population. In addition to drawing on various psychosocial-behavioral theories and models, which are described in more detail below, we instituted a systematic process assessment of the knowledge, experiences, perceptions, beliefs, behaviors, and terminology of the target audience. From the beginning, we attempted to establish and maintain a feedback loop, which we defined as the linkage between the content of persuasive messages created for disease prevention research programs and data from the target audiences (Evans et al., 1984a). We thus relied heavily on formative evaluation as we developed and implemented our intervention strategies (Evans et al., 1989).

We worked from the premise that tobacco use, although an age-related behavior, occurs most often within the context of social interactions; and although it involves the use of cognitive or knowledge structures, social adaptation appears to override intellectual adaptation or knowledge in decisionmaking. Social learning theory (e.g., Bandura, 1977; Evans, 1989) appeared to be particularly relevant. As applied to smoking initiation, the theory suggests that through observation children acquire expectations and learned behaviors *vis-à-vis* smoking. We considered that vicariously learned positive or negative consequences of cigarette smoking might be important factors in the decision to start smoking. Smoking behavior and expressed

smoking-related attitudes of peers, family, and media figures could be expected to affect the adolescent's smoking-related attitudes, beliefs, values, expectations, and learned behaviors. Young adolescents often perceive smoking as glamorous or as a behavior distinctive to adults. Because people tend to imitate the actions of their models (Bandura and Huston, 1961), we expected smokers in the young person's social setting to have an influence out of proportion to their numbers.

Adolescents tend to overestimate the proportion of individuals who smoke. Subjects in our investigations believed that "almost everyone" in their age group smoked, although the data indicated that only a relatively small percentage actually did (Evans et al., 1984b). Perceptions of group norms, of course, can constitute a vicarious peer pressure in influencing the behavior of adolescents (e.g., Ajzen and Fishbein, 1980; Evans et al., 1988; Fishbein and Ajzen, 1975). Recent studies (Graham et al., 1991; MacKinnon et al., in press) demonstrate that modifying subjects' perceptions of group norms, as a component of prevention programs, may sometimes be stronger than a peer pressure resistance training component. We therefore incorporated modifying perceptions to conform to reality into our social inoculation strategy.

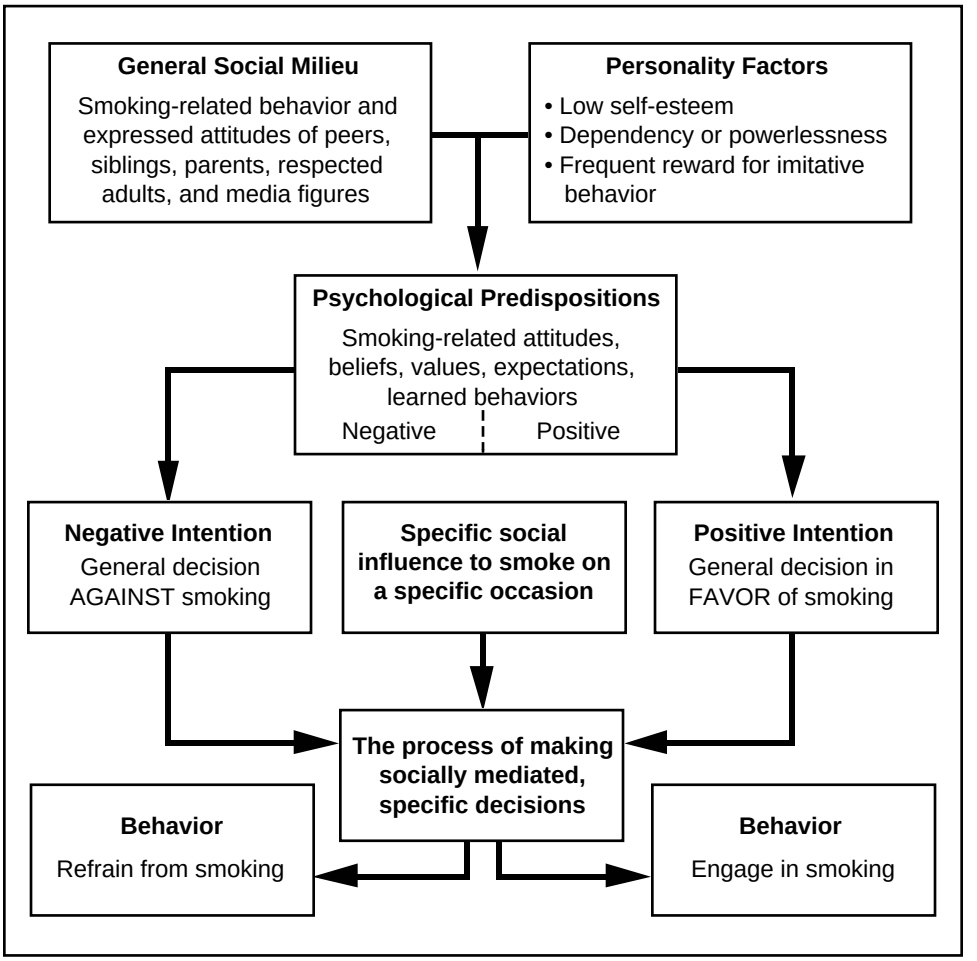
Another concept for addressing the problem of smoking prevention is the theory of reasoned action (Ajzen and Fishbein, 1980; Fishbein and Ajzen, 1975). It proposes a framework for predicting behavioral intentions, which are assumed to mediate and thus predict subsequent overt behavior. This approach suggested empirically testable hypotheses that could reveal important components of the development of smoking behavior.

Personality characteristics of the subject may also interact with social influences to encourage smoking. Bandura (1977) lists three characteristics that appear to facilitate imitative learning: (1) low self-esteem, (2) dependence or powerlessness, and (3) a history of receiving frequent rewards contingent on engaging in an imitative behavior.

The model shown in Figure 1 reflects the possibility that both social environmental and personality determinants contribute to the complex of psychological predispositions producing an intention to smoke or to not smoke. The actual decision on a particular occasion may, of course, depend on the effect of situational social influences. Teaching adolescents to cope with such influences might decrease the probability that they will smoke.

Ideally, a prevention program would incorporate all the components of the model reflected in Figure 1. In the development of our research program, however, we encountered time constraints and barriers to access to potential study populations in the school district in which we were working that precluded programs designed to modify the social environment within the schools. Therefore, inoculation against social influences on the individual to smoke became our primary focus. We described this initial social-influences approach in our 3-yr field investigation (e.g., Evans, 1976; Evans et al., 1978; Evans et al., 1981; Evans et al., 1984a) as the "social inoculation strategy."

Figure 1
A model of smoking-related social psychological processes that have impact on behavior

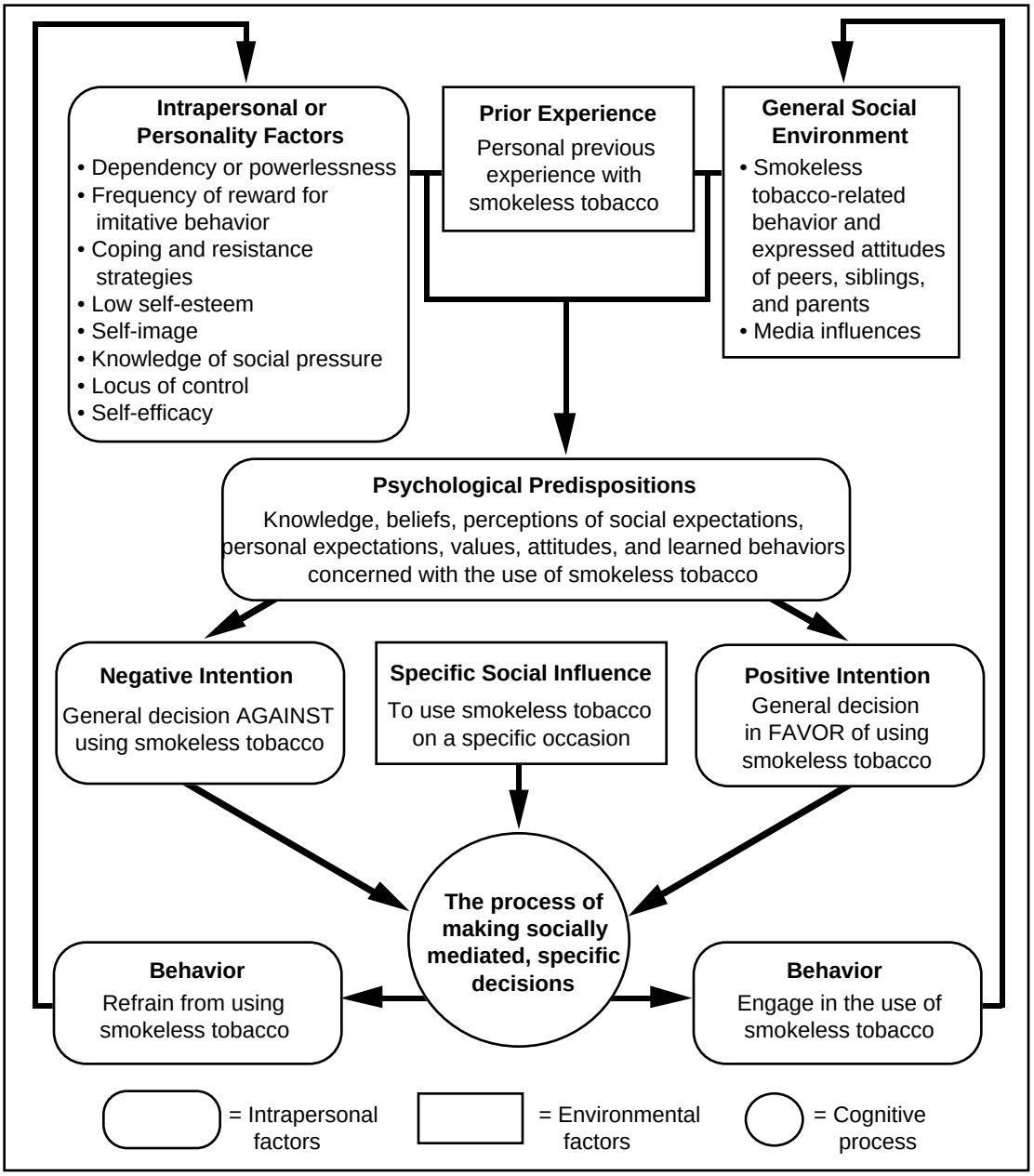


DATA-BASED INTERVENTIONS

In developing our interventions, we tried to create messages to which adolescents would listen and on which they might act. We were guided, to some extent, by Laswell's classic social-communication model (Laswell and Casey, 1946), McGuire's (1969 and 1974) information-processing communication model, and, as previously mentioned, the model of an ongoing feedback loop (Evans et al., 1984a) that allowed specific tailoring of the program to the target audience. (See Figure 2.)

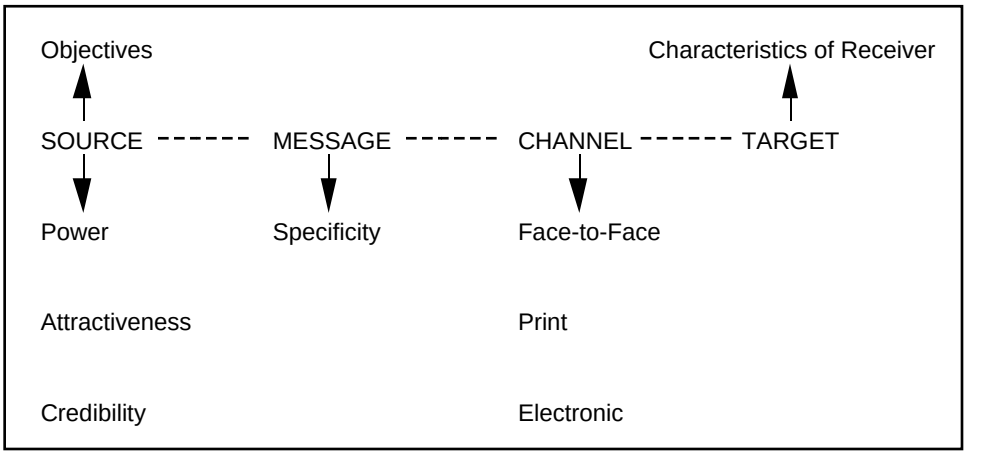
In discussing the development of our interventions, we might first consider the *source* of the communication in terms of Laswell's model. (See Figure 3.) Instead of adults who could be perceived to represent power, which adolescents might reject, our messages were delivered by adolescent narrators, selected for their perceived acceptability by or attractiveness to junior high school students. Although a certain amount of peer credibility

Figure 2
A model of ST-related social psychological processes that have impact on behavior



was inherent in their appearance as actors, in their presentation, the narrators acted as information brokers rather than authority figures. Increased credibility accrued through the narrators' prefacing biopsychosocial information with such phrases as "The researchers have found . . ." or "The researchers have asked me to tell you . . . ."

Figure 3
Synthesis of three social-communication models: external properties



The next component of Laswell's model addresses the *message*. Based on the literature (Janis and Feshbach, 1953; Leventhal et al., 1965; Sutton, 1982) and findings of our previous research (Evans et al., 1970 and 1975; Evans, 1979), that fear arousal does not provide adequate long-term motivation to accept or comply with health-related messages, we relegated fear arousal as only one aspect of our messages. We thus moved away from merely emphasizing the more global approach of the period (e.g., "Smoking is bad for you.") to include messages specific to the adolescent's social and developmental level (Evans, 1979; Evans and Raines, 1982; Leventhal et al., 1965).

McGuire's information-processing communication model (McGuire, 1969 and 1974) was particularly useful in developing our intervention. As indicated by this model, to be effective, messages must be (1) *attended* to and (2) *comprehended*. The content of the message must be (3) *accepted* by the target audience as useful and personally applicable, and it must be (4) *retained* if the individual is to (5) *act* on it, now or later. Obviously, if a persuasive message is to be effective, the audience must attend to it, and it must be couched in language or visual design that can be understood.

Failing to gain attention of the target audience appeared to be a major problem of earlier school-based health education programs. Furthermore, educational materials such as videotapes with a high fear arousal content, as described earlier, emphasized biomedical information (e.g., the physiological effects of smoking on the body or the etiology of cancer) at a level beyond the comprehension of the young adolescent audience. Although the problem is fairly easy to identify, the solution (e.g., effective messages that catch and hold the attention of a seventh-grade student or accurate scientific information at an elementary school reading level) is substantially more difficult. Employing standard educational methodology for reading level assessment, we created persuasive messages and pretested them with students at the resource schools. Their feedback ensured that the language

used was fully understood. We asked students representative of the study population to assist us in writing scripts and acting out role-played situations, using their own words and actions. Our evaluations indicate that target audiences perceive such scenes as realistic.

Although a wide range of media may be effectively employed as channels for persuasive communications with groups (e.g., school classes) (Weiss, 1969), in the early studies described here, pragmatic considerations mitigated for videotape as the primary delivery mode, with printed materials (e.g., posters with scenes from the videotapes) serving as supplements. Videotaped messages could be standardized for presentation across groups and schools, thus averting the problem of differential treatment.

One effect of producing the intervention audiovisuals locally was the target audiences' perceived personalization of the communication. Local scenes on the videotapes (e.g., school campuses, shopping malls) held the attention of the students. Personalization often requires little extra effort by the researcher. In a later multischool, multidistrict study, the students' attention to a standardized sound/slide presentation of health resources in their county was greatly increased when one picture of the specific school was presented as the first slide (Evans et al., 1990).

Following McGuire's model, after attention and comprehension, acceptance and retention of the material are critical. To encourage acceptance, students were urged to become involved in discussions and feedback sessions. Training, rehearsal, and role-playing reinforced personal involvement. A series of "booster" videotapes with supplemental materials such as classroom posters was used over a 2-yr period after the primary treatment series, allowing for the repetition, reinforcement, and reintegration of material required for retention of persuasive messages that might not be acted on immediately.

The messages in the videotapes and discussions were presented so as to reinforce self-attributions and self-determinations of decisions to smoke or not. Throughout the messages, the audiences are told, "You can decide for yourself," or "Here is some information that might help you decide," but they are not given the "correct" decision in a prescribed manner. Process data indicate that students like this low-key, nondirective approach.

In addition to tailoring our intervention as described above, we also employ a concept of *behavioral* inoculation instead of the concept of *cognitive* inoculation that McGuire (1961) directed at restoring or maintaining beliefs and attitudes. In several papers (e.g., Evans, 1982, 1984, 1990), we described this concept as the "social inoculation strategy." The strategy involves increasing resistance to social influences to smoke that children and adolescents encounter by inoculating them with both knowledge and a repertoire of social skills to help them resist such pressures. Included also are such coping responses as "Just say no" to *low* peer pressure to smoke. From the social inoculation strategy orientation, a response to a higher level of peer pressure might be, "I thought you were my friend. Why do you want to give me cancer?" In addition to training adolescents to recognize

and cope with such *overt* social influences to smoke as peer pressure, this approach addresses possible *covert* social influences, such as models who smoke in ads or the individual's perceptions of peer group smoking norms.

THE HOUSTON LITTLE LEAGUE PROJECT

Within the past decade, there has been a significant increase in ST use, particularly among young males (Boyd and Glover, 1989; US DHHS, 1986). As we considered extending our research program into prevention of ST use, a number of factors contributed to the design of our current project, which involves Little League baseball players as the study population. (In fact, current NCI-supported projects are also directed to other special population groups: 4-H Club members and Native Americans.)

- Some evidence indicates that the tendency to use ST relates to the tendency to smoke cigarettes and may involve some of the same psychosocial mechanisms, suggesting that models and methodologies developed for smoking prevention research might be applicable to ST use prevention (Chassin et al., 1989; Evans and Raines, 1990).
- ST use appears to begin at an earlier age than that usually noted for initial experimentation with cigarette smoking (Ary et al., 1987; Bonaguro et al., 1986; Schaefer et al., 1985), suggesting the efficacy of using a target group of preadolescent subjects.
- With the exception of Native American populations, in which females and males report about equal use (Schinke et al., 1986), ST is used primarily by males (US DHHS, 1986), suggesting the selection of a potential study group that is primarily male.
- Heavy ST use appears to be closely associated with the public lifestyle of a significant number of professional baseball players (Connolly et al., 1988), suggesting possible imitative behavior, particularly by sports-minded youth. About 28 percent of our current Little League sample (aged 12 or younger) believes that more than half of professional players use ST. However, this perception is not a strong discriminator between never having used ST and having initiated ST use. It remains to be determined whether perceived use of ST by professional baseball players influences use by older adolescents.
- The widely accepted belief that ST use is a relatively "safe alternative" to smoking cigarettes (Schaefer et al., 1985) has implications at both individual and program levels. Some evidence suggests that such beliefs may increase the likelihood that adolescents who are non-users will take up the practice in the future (Chassin et al., 1985).

  Data from the first wave of our longitudinal investigation (n=1,141) reveal that 23 percent of the Hispanics and 18 percent of the Anglos believe that ST is less habit-forming than smoking. Eighty percent of both Hispanics and Anglos believe that ST use is safer than cigarette use.

  If school administrators and curriculum developers also perceive ST to be safer than cigarette smoking, and program planners perceive ST use

as limited only to males and a lower health risk, there could be less curriculum time and fewer ST prevention research programs (Evans et al., 1990).

- Overt influences to smoke cigarettes appear to be primarily peer influences, actual or perceived (Evans, 1984; Evans et al., 1988). Apparently less important in influencing adolescents to smoke cigarettes, *covert* influences to use ST may come from adult authority figures (Ary et al., 1987), including coaches (Marty et al., 1986), indicating that different resistance strategies may be needed in the setting of athletics.

As a function, in part, of integrating these factors into a longitudinal study design, we proposed a research program that applies our social inoculation strategy to an ST use prevention program for preadolescents (aged 9 to 12) and young adolescents (aged 13 to 15), within the context of baseball-related activities, using as subjects Little League baseball and Senior League baseball players who are, in our area, 95 percent male. Shifting from a school-based setting to the baseball setting allows a concentrated focus on ST use, including correction of misperceptions such as the relative safety of its use, and provides for the involvement of both players and coaches.

The study has the support of Little League baseball at various administrative levels, including endorsement by the national executive director and the Texas state director. At the local level, district administrators and league presidents act as liaisons between our project staff and Little League teams. We also have the cooperation of the local Major League team, the Houston Astros.

The 5-yr project involves three stages:

- An assessment of the psychosocial-behavioral processes involved in the initiation and use of ST among young people, aged 9 to 15, who are members of active Little League and Senior League baseball teams;
- The development of a theory-guided, data-based prevention-deterrence program to be executed within Little League baseball activities; and
- The implementation and evaluation of the prevention program in a 3-yr longitudinal study involving a cohort of Little League players assigned, by team, to program (treatment) or measurement-only (control) condition.

During the initial phase, the cross-sectional survey instrument was developed, pretested, and administered to players in several Little League administrative districts in and around Harris County. Data from the initial cross-sectional survey indicated no significant differences among the participating districts. Therefore, the longitudinal phase involves approximately 180 Little League teams from one large Little League administrative district in Harris County and Galveston County. The geopolitical division of the district allows us to sample teams from both urban and rural areas with a

wide range of socioeconomic markers while maintaining a simplified administrative approach that facilitates cooperation and averts the attrition problems that often plague research studies.

The cross-sectional survey instrument operationalized approximately 20 hypothetical predictors of ST use. With a representative sample of Little League players (n=57) drawn from several administrative districts, the instrument was pretested for comprehensibility by young subjects (< 12 yr) and administration time, including subject attention span and optimal approaches for conducting surveys in field houses or Little League field bleachers. Based on feedback from the pretest, items were modified, combined into a second instrument, and administered, under field conditions, to a second representative sample of Little League players (n=273). In this sample, about 85 percent were male, white, and 12 yr old or younger; 12.9 percent had tried ST at least once; 12.5 percent had tried smoking cigarettes at least once; 2.2 percent had used ST during the previous week; and 1.8 percent had smoked cigarettes during that same period.

The final form of instrument was administered as a cross-sectional survey to a third representative sample of Little League players (n=293; age range, 7 to 15 yr) to help shape the longitudinal measurement questionnaire and to provide content for the intervention program. The overall responses of this sample to four key questions are summarized as follows:

- Players who had tried smokeless tobacco at least once—14 percent.
- Players who answered "maybe" or "yes" to try in future—16 percent.
- Players reporting at least one of their friends uses ST—27 percent.
- Players reporting using ST during past week—5 percent.

It is interesting to note that reported use ". . . during the past week . . .," shown in Table 1, is similar for all three groups, although other substantial differences appear to be age related. It should be noted, of course, that the youngest and oldest groups are fairly small subsamples.

At present, we are carrying out the second year of the Little League longitudinal study with a longitudinal sample (n=1,141) that was identified at its initial measurement as 94.7 percent male with 99.0 percent of the sample falling between the ages of 8 and 12. As for racial distribution, 74.7 percent are white, 11.1 percent Hispanic, 8.5 percent black, 2.5 percent Native American, and 2.5 percent other.

POTENTIAL BARRIERS

The investigator must be sensitive to the issue of relevance in the design and implementation of any risk-behavior prevention program. For example, our Little League study involves a very diverse population, and interventions must be tailored to a wide range of demographic factors. In a discussion of tailoring risk-behavior prevention programs to special populations, Orlandi (1986) listed several specific problems that we have found relevant to our current study. Below is a brief discussion of some barriers and examples of steps we have taken to address or eliminate the particular problem:

Table 1
Summary of findings from cross-sectional study, by age

	Percentage, by Age Group		
	7 to 8 yr (n=71)	9 to 11 yr (n=149)	12 to 15 yr (n=73)
Reported Use of ST			
Tried it	3%	9%	33%
Will try it	10	13	26
Friends use it	10	18	64
Used it past week	6	5	7

- *Use of language that is unfamiliar or uncomfortable for the target population*—For example, it is sometimes difficult for an academic-oriented researcher to communicate at the level required for interaction with 9-yr-old Little League players from varied socioeconomic backgrounds. As described previously, we use a feedback loop involving children similar to the target group to assess the appropriateness of language in terms of attention and comprehension.
- *Use of printed materials that are too sophisticated*—Printed intervention materials are assessed by a member of our staff who is trained in preparing written material for elementary students. Printed material to be distributed to coaches and parents is reviewed by the Little League district administrator, whose responsibilities include distributing effective written materials for Little League.
- *Using individuals who are not well known in the community*—In our Little League program, we have involved professional baseball players who are well known to Little League players and their adult sponsors. We also have used the resources of the University of Houston baseball coach and his staff, who develop training materials for Little League and are highly respected. At all times, we keep outside communicators or interveners within the context of the game of baseball.
- *Using unfamiliar motivational devices*—In the Little League study, we use motivational devices to encourage full participation and continued participation in the study. Motivational devices range from cold drinks, if the program is being implemented during hot weather, to free tickets to an Astros or University of Houston baseball game (tickets were provided by the Astros or Houston Cougars). A major motivator during the critical first year (when at least 75-percent team participation was required for all testing and intervention occasions) was a set of Skills and Drills Training Videotapes produced by Dr. Bragg Stockton, coach of the University of Houston baseball team. The videotapes were a highly significant motivator, and each League that met the 75-percent criterion for participation received a set.

- *Conveying the impression that the program is not intended for long-term adoption or that community leaders are not expected to participate*—From the beginning we have made it clear that the final, evaluated program will be available to Little League baseball for general distribution. Little League district personnel provide continuous feedback to the research staff. Our field coordinator is a Little League district administrator with more than 25 yr of experience. The state director acts as a consultant to the program.

A major barrier to developing ST use prevention programs is the perception that ST is relatively harmless in comparison with other drugs. Although all of the communities where we are working have active drug abuse prevention programs (e.g., Chicken Club, Just Say No Club) to which community members contribute time and financial resources, many individuals do not perceive ST, cigarettes, or alcohol as drugs in the same category as the illicit drugs emphasized by many of the community programs. Most of these issues suggest the importance of developing and maintaining rapport with the organizations participating in the project. To address this issue, we are presently developing a videotape with the theme, "Tobacco is a drug, too!"

A frequent barrier found by health promotion investigators is the sample biasing effects of stringent informed consent measures that often have been reported for both measurement and intervention components (e.g., Evans et al., 1977). In our present school-based projects, we have gained the consent of the school districts involved to include the prevention program as part of the regular Texas Education Agency-approved curriculum. Under these guidelines, measurement and intervention activities can proceed at the discretion of the school superintendent and the advisory school board, given that all procedures and materials meet the guidelines of the Texas Education Agency and the school district. The Little League program involves obtaining parental consent. However, parents of the players attend games and practice sessions and are generally far more available for providing consent than is usual in school- or other community-based programs. To date, we have not encountered serious problems gaining informed consent for Little League players.

A taboo on any behavior (e.g., illicit drug use or, in the case of children and adolescents, the use of alcohol or tobacco) remains a barrier to reliable measurement of that behavior. To address this problem in our studies, we emphasize the strict regulations regarding anonymity and confidentiality and use strategies that have been shown to increase the validity of self-reports. Such strategies include the collection of saliva specimens under "pipeline" conditions, a procedure we had developed earlier (US DHHS, 1986).

Many researchers place an emphasis on summative evaluation and, under constraints of time or funding, ignore the formative evaluation procedures that frequently are valuable in the development of interventions and measurement instruments. Providing for continuous feedback so that appropriate modifications can be made should be a high priority for the

researcher, particularly in the implementation of programs in natural community settings. Our use of formative evaluation has been described in a paper that draws on its use in field settings with smoking prevention and ST prevention research (Evans et al., 1989). Formative evaluation was used, for example, in the development of our measurement instrument for the longitudinal phase of the Little League program, to stringently revise the original instrument and its administrative procedures. The first field draft of the instrument was 45 pages long, 206 questions, and constructed at a third- to fourth-grade reading level. The initial reaction of subjects was so resistant we had to decrease its bulk, both through deleting some scales and reformatting so that the form looked less threatening. The revised instrument now has 145 questions, is 18 pages long, and is scaled down to a reading level of second to third grade to ensure comprehensibility for even less able readers. Measurement time originally averaged 30 to 45 min; the revised questionnaire now takes 20 min or less, well within the attention span of the average Little League player.

CONCLUSION We have addressed the potential for cross-application of well-evaluated smoking prevention programs to the newer issue of ST prevention programs, drawing on our own extended program of research in smoking prevention. We have discussed some of the barriers to successful development, implementation, and evaluation of such programs, including the problems related to tailoring interventions for special populations. We also reviewed some problems inherent in developing valid and reliable measurements in field-based studies, steps that might be taken to maintain a longitudinal study sample over time, and the value of formative evaluation throughout the course of a research program. A final issue of special concern to ST prevention researchers is that, to many young adolescents and their community leaders, tobacco—especially ST—does not fit within their concept of "drug" in the development of drug abuse prevention programs. We recommend that special emphasis be given to promoting the notion that tobacco is a drug, too. With respect to the relative emphasis on smoking in contrast to smokeless tobacco as a health threat, it seems more persuasive to no longer distinguish between smoking cigarettes and using ST. We recommend that a more generic phrase, "tobacco and health," be used.

REFERENCES

Ajzen, I., Fishbein, M. *Understanding Attitudes and Predicting Social Behavior*. Englewood Cliffs, NJ: Prentice-Hall, 1980.

Ary, D.V., Lichtenstein, E., Severson, H.N. Smokeless tobacco use among male adolescents: Patterns, correlates, predictors and the use of the drug. *Preventive Medicine* 16: 385-401, 1987.

Bandura, A. *Social Learning Theory*. Englewood Cliffs, NJ: Prentice-Hall, 1977.

Bandura, A., Huston, A.C. Identification as a process of incidental learning. *Journal of Abnormal and Social Psychology* 63: 311-318, 1961.

Bonaguro, J.A., Pugh, M., Bonaguro, E.W. Multivariate analysis of smokeless tobacco use by adolescents in grades four through twelve. *Health Education* 17: 4-7, 1986.

Botvin, G.J., Wills, J.A. Personal and social skills training: Cognitive behavioral approaches to substance abuse prevention. In: *Prevention Research: Deterring Drug Abuse Among Children and Adolescents*, C.S. Bell and R. Battjes (Editors). U.S. Department of Health and Human Services; Public Health Service; Alcohol, Drug Abuse, and Mental Health Administration; National Institute on Drug Abuse DHHS Pub. No. (ADM) 87-1334. 1985, pp. 8-49. (NIDA Research Mongraph 63)

Boyd, G.M., Glover, E.D. Smokeless tobacco use by youth. *U.S. Journal of School Health* 59: 189-194, 1989.

Chassin, L., Presson, C.C., Sherman, S.J., et al. Adolescent smokeless tobacco use: Future research needs. *National Cancer Institute Monographs* 8: 101-105, 1989.

Chassin, L., Presson, C.C., Sherman, S.J., et al. Psychosocial correlates of adolescent smokeless tobacco use. *Addictive Behaviors* 10: 431-435, 1985.

Connolly, G.N., Orleans, C.T., Kogan, M. Use of smokeless tobacco in major league baseball. *New England Journal of Medicine* 381: 1281-1284, 1988.

Evans, R.I. *Albert Bandura: The Man and His Ideas*. New York: Praeger Press, 1989.

Evans, R.I. Development of the social inoculation strategy to deter smoking and related health threatening behaviors in adolescents: Review and an update. Paper presented at the International Workshop on Prevention of Drug Addictions, Alicante, Spain, December 3-5, 1990.

Evans, R.I. Fear is not enough: Modification of behavior to prevent disease. *Postgraduate Medicine* 65: 195-197, 1979.

Evans, R.I. Health promotion: Science or ideology? *Health Psychology* 7(3): 203-219, 1988.

Evans, R.I. Modifying health lifestyles in children and adolescents: Development and evaluation of a social psychological intervention. In: *Handbook of Psychology and Health: Issues in Child Health and Adolescent Health* (volume 2), A. Baum and J.E. Singer (Editors). Hillsdale, NJ: Lawrence Erlbaum Associates, 1982.

Evans, R.I. A social inoculation strategy to deter smoking in adolescents. In: *Behavioral Health: A Handbook of Health Enhancement and Disease Prevention*, J.D. Matarazzo, S.M. Weiss, J.A. Herd, N.E. Miller, and S.M. Weiss (Editors). New York: John Wiley and Sons, 1984, pp. 765-774.

Evans, R.I. Smoking in children: Developing a social psychological strategy of deterrence. *Journal of Preventive Medicine* 5: 122-127, 1976.

Evans, R.I., Dratt, L.M., Raines, B.E., et al. Social influences on smoking initiation: Importance of distinguishing descriptive versus mediating process variables. *Journal of Applied Social Psychology* 18: 925-943, 1988.

Evans, R.I., Hansen, W.B., Mittelmark, M.B. Increasing the validity of self-reports of smoking behavior in children. *Journal of Applied Psychology* 62: 521-523, 1977.

Evans, R.I., Raines, B.E. Applying a social psychological model across health promotion interventions: Cigarettes to smokeless tobacco. In: *Social Influence: Processes and Prevention*, J. Edwards, R.S. Tindale, L. Heath, et al. (Editors). New York: Plenum Press, 1990, pp. 143-157.

Evans, R.I., Raines, B.E. Control and prevention of smoking in adolescents: A psychosocial perspective. In: *Promoting Adolescent Health: A Dialog on Research and Practice*, T.J. Coates, A.C. Petersen, and C. Perry (Editors). New York: Academic Press, 1982.

Evans, R.I., Raines, B.E., Hanselka, L.L. Developing data-based communications in social psychological research: Adolescent smoking prevention. *Journal of Applied Social Psychology* 14(3): 289-295, 1984a.

Evans, R.I., Raines, B.E., Owen, A.E. Adolescent Health Promotion: The Galveston Project. Final Report to the Bureau of Health Care Delivery and Assistance, Division of Maternal and Child Health. 1990.

Evans, R.I., Raines, B.E., Owen, A.E. Formative evaluation in school-based health promotion investigations. *Preventive Medicine* 18: 229-234, 1989.

Evans, R.I., Rozelle, R.M., Lasater, T.M., et al. Fear arousal, persuasion, and actual versus implied behavior change: New perspective utilizing a real-life dental hygiene program. *Journal of Personality and Social Psychology* 16(2): 220-227, 1970.

Evans, R.I., Rozelle, R.M., Lasater, T.M., et al. New measure of effects of persuasive communications: A chemical indicator of toothbrushing behavior. *Psychological Reports* 23(2): 731-736, 1968.

Evans, R.I., Rozelle, R.M., Maxwell, S.E., et al. Social modeling films to deter smoking in adolescents: Results of a three-year field investigation. *Journal of Applied Psychology* 66: 399-414, 1981.

Evans, R.I., Rozelle, R.M., Mittelmark, M.B., et al. Deterring the onset of smoking in children: Knowledge of immediate physiological effects and coping with peer pressures, media pressure, and parent modeling. *Journal of Applied Social Psychology* 8: 126-135, 1978.

Evans, R.I., Rozelle, R.M., Noblitt, R., et al. Explicit and implicit persuasive communication over time to initiate and maintain behavior change: A new perspective utilizing a real-life dental hygiene program. *Journal of Applied Social Psychology* 5(2): 150-156, 1975.

Evans, R.I., Smith, C.K., Raines, B.E. Deterring cigarette smoking in adolescents: A psychosocial-behavioral analysis of an intervention strategy. In: *Social Psychological Aspects of Health,* A. Baum, J. Singer, and S. Taylor (Editors). Hillsdale, NJ: Lawrence Erlbaum Associates, 1984b.

Fishbein, M., Ajzen, I. *Belief, Attitude, Intention, and Behavior: An Introduction to Theory and Research.* Reading, MA: Addison-Wesley, 1975.

Flay, B.R. Psychosocial approaches to smoking prevention: A review of findings. *Health Psychology* 4: 449-488, 1985.

Graham, J.W., Marks, G., Hansen, W.B. Social influence processes affecting adolescent substance use. *Journal of Applied Psychology* 76(2): 291-298, 1991.

Janis, I.L., Feshbach, S. Effects of fear-arousing communications. *Journal of Abnormal and Social Psychology* 48: 78-92, 1953.

Laswell, H.D., Casey, R.D. *Propaganda, Communication and Public Opinion*. Princeton, NJ: Princeton University, 1946.

Leventhal, H., Singer, R., Jones, S. Effects of fear and specificity of recommendations upon attitudes and behavior. *Journal of Personality and Social Psychology* 2: 20-29, 1965.

MacKinnon, D.P., Johnson, D.A., Pentz, M.A., et al. Mediating mechanisms in a school-based drug prevention program: First year effects of the midwestern prevention project. *Health Psychology*, in press.

Marty, P.J., McDermott, R.J., Williams, T. Patterns of smokeless tobacco use in a population of high school students. *American Journal of Public Health* 76: 190-192, 1986.

McGuire, W.J. Communication-persuasion models for drug education: Experimental findings. In: *Research on Methods and Programs of Drug Education*, M. Goodstadt (Editor). Toronto, Canada: Addiction Research Foundation, 1974, pp. 1-26.

McGuire, W.J. The effectiveness or supportive refutational defenses in immunizing and restoring beliefs against persuasion. *Sociometry* 24: 184-197, 1961.

McGuire, W.J. The nature of attitudes and attitude change. In: *Handbook of Social Psychology: The Individual in a Social Context* (volume 3), G. Lindzey and E. Aronson (Editors). Reading, MA: Addison-Wesley, 1969, pp. 136-314.

Orlandi, M.A. Community-based health promotion: A multicultural perspective. *Journal of School Health* 56: 394-401, 1986.

Schaefer, S.D., Henderson, A.H., Glover, E.D., et al. Pattern of use and incidence of smokeless tobacco consumption in school-age children. *Archives of Otolaryngology–Head and Neck Surgery* 111: 639-642, 1985.

Schinke, S.P., Gilchrist, L.D., Schilling, R.F., et al. Smokeless tobacco use among Native American adolescents. *New England Journal of Medicine* 314: 1051, 1986.

Severson, H.N., Ary, D.V. Sampling bias due to consent procedures with adolescents. *Journal of Addictive Behaviors* 8(4): 433-437, 1983.

Sutton, S.R. Fear-arousing communications: A critical examination of theory and research. In: *Social Psychology and Behavioral Medicine*, J.R. Eiser (Editor). New York: John Wiley and Sons, 1982, pp. 303-337.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

Weiss, W. Effects of the mass media of communication. In: *The Handbook of Social Psychology*: *Applied Social Psychology* (volume 5), G. Lindzey and E. Aronson (Editors). Reading, MA: Addison-Wesley, 1969, pp. 77-195.

Chapter 6
Cessation

CONTENTS

Enough Snuff: ST Cessation From the Behavioral, Clinical, and Public Health Perspectives

Herbert H. Severson

ABSTRACT Despite concerns over the high prevalence and detrimental health effects of regular smokeless tobacco use by American males, there has been little research on assisting users to quit. A review of research shows promising results by adapting smoking cessation procedures. A review of clinical cessation studies and psychosocial studies provides support for development and assessment of ST cessation clinical and self-help programs. Distinctive aspects of ST cessation include the high prevalence of oral lesions, need for an oral substitute during withdrawal, the potential use of nicotine polacrilex as an adjunct, perception of ST as a safe alternative to smoking, frequent use of both cigarettes and ST, and difficulty in using nicotine-fading approaches. Specific components and measures involved in ST cessation are reviewed. Support for public health interventions is provided by a recent study of ST cessation in HMO dental clinics that demonstrated the efficacy of providing ST users advice to quit in the context of health care delivery. Cessation materials have also been developed for special populations, such as Native Americans and baseball players.

INTRODUCTION Over the past 6 yr, there has been a great deal of public interest in and scientific attention to the detrimental health effects of smokeless tobacco use. The publication of the Surgeon General's report on ST use (US DHHS, 1986a) and the publicity surrounding the NIH Consensus Development Conference on Smokeless Tobacco (US DHHS, 1986b) focused attention on these health concerns. These publications identified three major health risks associated with the oral use of tobacco: oral cancer, development of leukoplakia and other oral health problems, and nicotine addiction. These two documents increased public awareness and spurred Congress to pass House Bill 99-252, the Smokeless Tobacco Act of 1986. This act instituted rotating health warnings on ST products and eliminated radio and television ads for ST. Although the focus on ST increased public awareness of health effects, the sales of ST products have increased steadily with only a minor flat spot in the growth curve following the enactment of Public Law 99-252.

Because the majority of new users of ST products are young teen males, recent research programs have focused on preventing young people from taking up the use of snuff or chew through school-based prevention programs (Severson and Zoref, 1991). Despite these educational efforts, the use of ST, especially moist snuff, is increasing, especially among male adolescents and young male adults (US DHHS, 1986a). As of 1986, there were already an estimated 6 million regular users of ST in the United States (US DHHS, 1986a). Unfortunately, there has been little effort toward assisting current users in quitting their habitual use of snuff or chewing tobacco.

Despite the concern for potential negative health consequences, scientific study of ST cessation has lagged behind epidemiological, health, and preventive efforts. To date, there are few published studies on ST cessation, although several are currently in progress.

This paper broadly reviews the field of smokeless tobacco cessation. Published and unpublished studies are reviewed to provide a current assessment of ST cessation and guide future studies. Psychosocial studies that provide information on ST use and can guide development and implementation of effective cessation programs are also considered. The paper concludes with a review of ongoing public health interventions that provide direction on development of broad-based efforts to promote ST cessation.

CLINICAL ST CESSATION STUDIES

Currently, there are only three published cessation studies involving smokeless tobacco. Unfortunately, these studies had relatively small samples and could be best characterized as pilot clinical research. Although they are not well-controlled randomized clinical trials, they are instructive.

The first published study of ST cessation was done by Glover (1986) who adapted the American Cancer Society's FreshStart Adult Smoking Cessation Program for use with 41 adult ST users. He reported a 6-mo abstinence rate of only 2.3 percent. However, these subjects were mandated to attend the program, as they had been found in violation of school rules at a college that prohibited the use of tobacco products. Given the nonvoluntary nature of the subject sample, it is not unexpected to find a low success rate in cessation. Subsequent studies by Eakin and coworkers (1989) and DiLorenzo and coworkers (1991) provide a more optimistic view of the potential cessation rates that can be achieved by formal ST cessation treatment.

Eakin and coworkers (1989) reported an intervention with adolescent daily users, aged 14 to 18, who were recruited by referrals from counselors, coaches, and teachers in Eugene, Oregon, high schools. The study had 25 chronic ST users with a quasi-experimental design in which 11 of the 25 subjects provided a comparison group by receiving delayed treatment (3-wk delay). This behavioral treatment consisted of three 1-h small-group meetings led by counselors. The multiple-component treatment was cognitive-behavioral in nature and focused on encouraging subjects to use coping skills for cessation. Of the 21 subjects completing treatment, 9 were successful in quitting their ST use at the end of treatment. Self-reported quitting was confirmed with saliva cotinine assessment, and subjects were followed up at 6 mo after treatment. Long-term cessation rates were reduced to 12 percent at 6-mo followup; however, subjects not achieving abstinence had a self-reported reduction of 45 percent in their daily use of ST from baseline levels. The participants in this study who quit reported that, in addition to the group sessions, the ongoing telephone calls and support by the counselor were key elements in their success.

DiLorenzo and associates (1991) reported a multiple baseline design intervention on nine adult males recruited for a behavioral ST cessation program. Mean age of the subjects was 32 yr and the average length of use of ST was 9.3 yr. Seven subjects completed the eight 1-h behavioral treatment sessions provided over a period of 7 wk in small groups of three subjects each. Cue extinction, setting a target date for quitting, the use of a buddy system, and relapse prevention were the primary components of the

intervention. Cue extinction involved identifying two or three situations most strongly associated with ST use and breaking these associations by refraining from taking a dip for 30 min. Cessation of ST use was associated with the introduction of the program. Six subjects remained abstinent through the treatment phase and remained abstinent at the 9-mo followup. These data were confirmed by collateral sources. The program appears to be quite successful, although the modest number of subjects dictates caution in interpretation of this study.

The studies to date show that, for subjects who wish to quit, clinical cessation techniques based on smoking cessation are modestly effective. The 6-mo followup by Eakin and coworkers (1989) is discouraging, as there was high level of relapse, although 43 percent of the subjects who completed the treatment were able to quit. DiLorenzo and associates (1991) demonstrated the effectiveness of cue extinction procedures and the importance of getting the user to eliminate ST use first in situations in which the person is usually cued to use ST. Unfortunately, the studies have modest numbers of subjects and lack a comparison, no-treatment group.

There are several unpublished studies in cessation of ST use. Dorothy Hatsukami and colleagues at the University of Minnesota report promising results using nicotine polacrilex gum (Nicorette) as part of a behavioral group treatment program for adult ST users. They found no difference between the use of the gum ad libitum or on a fixed interval schedule, or between the 2 mg and 4 mg doses, in initial or 3-mo followup quit rates. Dr. Hatsukami reports that approximately 75 percent of the adult men had quit ST at the end of treatment (Hatsukami, personal communication, 1991).

Other studies are in progress. These studies involve comparing active and placebo nicotine gum as an adjunctive aid for a multicomponent cessation program for adult ST users, as well as studies of adolescent and adult cessation using ground mint leaf products as a snuff substitute.

From the discussion above of clinical studies to date, ST cessation involves adapting smoking cessation procedures. By and large, ST treatment programs have adapted standard cognitive-behavioral techniques used in smoking cessation programs. Personal communication with counselors indicates that self-help smoking cessation materials can be readily adapted and used by ST users. In our study of adolescent users, we depended heavily on the adaption of smoking cessation materials for each of the group sessions (Eakin et al., 1989). Further research and clinical experience are needed to determine how ST cessation differs from smoking cessation.

IMPLICATIONS FROM OTHER ST STUDIES Studies assessing ST quit efforts, health problems, and use patterns provide additional information that can have direct implications on the design and implementation of a cessation program.

Quit Attempts Studies support the need for ST cessation. Men who use ST appear interested in quitting. Researchers report that recruitment in current ongoing studies has not been a problem. In our current study assessing nicotine gum, 675 men responded to twice-weekly newspaper ads (for 7 wk)

soliciting daily ST users for a cessation program. In interviews, 64 percent of ST users have reported that they would make a serious quit attempt in the next 6 mo (Severson et al., 1990a). Of those trying to quit, 68 percent reported an average of four attempts each, and 77 percent of those quit attempts were "cold turkey" efforts. Additional support comes from a survey of dental patients in which 54 percent of ST users reported that they would make a quit attempt in the next year (Severson et al., 1990b). Ary and coworkers (1989) found that more than one-third of current male adolescent ST users reported unsuccessful quit attempts. Novotny and coworkers (1989) in a national probability sample found a large percentage (39.1 percent) of adult ST users had made unsuccessful attempts to quit. Most studies to date have been convenience samples or subsets of other studies. There is a need for a general population survey of ST users with regard to quit attempts, success rates of self-quitting, interest in cessation assistance, and relapse of self-quitters.

Oral Health Problems We also know that regular users often experience oral health problems that they can easily identify, and these can be used to motivate them to institute a quit attempt. Two-thirds of regular daily ST users reported that they had experienced health problems that they directly attributed to their use of snuff (Severson et al., 1990a). These symptoms included bleeding and sore gums, lesions, receding gums, and upset stomach from swallowing the juice. Although the sample in the study of Severson and associates (1990a) was a self-selected sample of adult daily ST users, there is ample evidence of frequent oral health problems among daily users (Glover et al., 1989; Schroeder, 1989; US DHHS, 1986a). Estimates are that more than 50 percent of regular users have at least a degree 1 (early-stage) leukoplakia lesion (Glover et al., 1989). Our own study of dental patients revealed that 78 percent of ST users had detectable oral lesions, and these were distributed evenly across three levels of severity (Little et al., in press). These lesions, along with bleeding and receding gums (gingivitis), can motivate a user to seek cessation assistance and provide an objective outcome from cessation, because the lesions usually heal quickly after ST cessation.

Nicotine Addiction Studies of nicotine absorption by snuff and chewing tobacco users conclude that venous plasma nicotine levels are similar in users of ST and cigarettes (Benowitz et al., 1988). Gritz and coworkers (1981) and Russell and coworkers (1981) concluded that plasma nicotine and cotinine levels for daily habitual snuff users were comparable to those of a group of heavy smokers. Finally, it appears that physiological dependence develops regardless of whether nicotine is taken in through a cigarette, smokeless tobacco, or nicotine polacrilex (Hughes and Hatsukami, 1986).

Withdrawal Symptoms and Substitutes We also know that symptoms experienced during ST withdrawal appear to be the same as those for smoking cessation (Hatsukami et al., 1987; Severson et al., 1990a). Hatsukami and colleagues reported that ST users experienced cravings, irritability, distractibility, and hunger, but the symptoms were less intense and fewer in number than those of the cigarette smokers. Self-report measures of withdrawal symptoms can be readily adapted from smoking cessation programs.

In response to their withdrawal symptoms, persons going through treatment for ST addiction often request an oral substitute. Chewers report using cinnamon sticks, gum, sunflower seeds, finely ground mint leaves, or other chewed foodstuff substitutes for ST during withdrawal. Unpublished case reports and ongoing cessation studies report success with ad libitum use of a mint snuff-like product as an ST substitute. This product, which contains finely ground mint in tins, is used in the same way as snuff but provides no nicotine.

Cigarette Smoking

We also know that there is a co-morbidity with cigarette smoking. In studies we have done in ST cessation, we have found that 25 to 30 percent of all regular ST users also use cigarettes (Eakin et al., 1989; Little et al., in press; Severson, in press). This is important because we may get people to quit ST but then increase their use of cigarettes. This would result in no net gain in the health risk status of subjects, and they would remain addicted to nicotine. The use of cigarettes by ST users has serious implications for cessation efforts. A dental-based intervention described later in this paper reported significantly lower cessation rates among men who use both tobacco products than those who use ST exclusively (Hollis et al., in press).

Studies of psychosocial factors in ST use and physical dependence on nicotine support the adoption of cognitive-behavioral multicomponent smoking cessation programs for use in ST cessation. There is ample evidence that regular ST users make quit attempts and experience serious withdrawal symptoms when quitting (Hatsukami et al., 1991). The plasma nicotine levels for ST users approximate those of a regular smoker, and the levels of nicotine dependence are similar for both groups. The use of cigarettes is high for ST users, and this factor can be problematic in getting a person to quit ST use. Unfortunately, we also have much to learn. Although the preliminary evidence is that ST cessation rates are similar to smoking cessation rates, the small sample sizes, self-selective nature of subjects, the lack of control groups, and lack of long-term followup make one cautious about any interpretation. Particular areas to focus on include determining how cessation processes differ by level of ST use. Also, we do not know the relapse rates because the followup on studies has been minimal. We know little about the use of nicotine replacement in cessation self-help quitting, effects of a health professional's advice, or the effects of work site restriction in use. In other words, although the field of smoking cessation has a well-established research base, ST cessation lags far behind in answering the same questions.

UNIQUE ASPECTS OF ST CESSATION

Smokeless tobacco use presents some unique issues that need to be considered. First, we have to remember that snuff can be used without other people being aware. This surreptitious behavior provides some difficulty in monitoring the use of snuff by others and allows use in situations where its use is not permitted (e.g., in school classrooms or on the job).

Second, the frequent oral lesions experienced by ST users provide the cessation counselor with a direct proximal measure of a physical problem caused by ST use. For a cigarette smoker, it is often difficult to point out direct evidence of detrimental health effects from smoking.

Third, cigarettes can be used during ST withdrawal. As described above, up to one-third of men also use cigarettes and may smoke during the ST withdrawal process to minimize cravings.

Fourth, we know that ST is perceived as a safe alternative and that perception may result in less motivation to quit. Chewers perceive ST use to be less harmful than smoking, although most nonchewers disagree (Bauman et al., 1989; Lichtenstein et al., 1984). Among youth, 86 percent regard ST as a safe alternative to cigarette smoking (US DHHS, 1986c). There is a higher acceptance of teenage ST use by parents than of smoking (Chassin et al., 1985). Parents' acceptance of their sons' chewing or dipping contributes to the general perception that this behavior is acceptable and encourages continued use.

And fifth, some of the cessation approaches that are used for cigarette smoking are not easily translated to ST because snuff and chewing tobacco products are generally not packaged in individual doses. For example, it is difficult to do nicotine-fading procedures (i.e., gradually reducing the amount of bioavailable nicotine). The lack of a standard dose, as in a cigarette, makes gradual reduction procedures difficult to self-monitor. The known nicotine content of cigarettes, via government reports, makes nicotine exposure relatively easy to compute. However, no comparable data on nicotine content of ST products are available, and individuals vary greatly in what constitutes a single pinch or dip (Severson et al., 1990a). Nicotine reduction procedures call for users to control the amount of nicotine exposure by either changing products or reducing the amount of snuff or chew they put in their mouths; however, in practice these procedures are difficult to implement because the nicotine content of the product is difficult to ascertain and the dip size is subjective.

SUMMARY OF CLINICAL WORK

From clinical and psychosocial studies, we can conclude smoking cessation procedures can be adapted for use with ST users with minor modification. Preliminary information supports ST cessation success rates being similar to smoking cessation, but there are few published studies to date. Oral substitutes appear to be important adjuncts in ST cessation. Users report use of mint snuff or other oral substitutes in quitting programs. Nicotine polacrilex gum has a topography of use that is very similar to the topography of snuff or chew use, and its use may be better received by ST users than by smokers as an aid in cessation. This is supported by Hatsukami and our own experience where we found that ST users report compliant use of nicotine polacrilex gum as a part of cessation. The new transdermal nicotine patches could also be a valuable aid, but to date their use has not been evaluated for ST users. Finally, there is a unique opportunity for self-exam of the mouth to increase motivation and provide feedback on oral health recovery that accompanies cessation.

COMPONENTS OF ST CESSATION

Adaptation of smoking cessation materials has been used in all aspects of ST cessation. A brief review of some of these measures and components may be useful. These include assessing motivation and readiness to quit, oral health measures, and assessing addiction level.

Assessing Addiction Level

The most accurate way to measure addiction level would be to measure nicotine exposure via plasma nicotine levels or urinary cotinine levels. However, collecting blood or urine samples is invasive, and laboratory procedures for measuring nicotine or cotinine in body fluids are expensive. Unfortunately, the product packaging and lack of government testing of ST products for nicotine content preclude easy measurement of nicotine exposure. A parsimonious, but less accurate, procedure for estimating a subject's nicotine exposure level is to take the available nicotine level of their usual product and multiply that times number of tins per week. For example, if Copenhagen is used, which is a very high nicotine product with 30.76 mg/g, and a user reported using one tin of snuff per day or seven tins per week, his total nicotine exposure would be 30.76 mg/g x 34.02 g per tin x seven tins per week = approximately 7,325 mg nicotine. Products such as Skoal and Kodiak are considered medium in nicotine content with 10.7 mg/g and 14.6 mg/g, respectively (Hoffman et al., 1986). The low-nicotine snuff products include Hawken, Bandits, and Happy Days. Hawken, for example, has 5.7 mg/g of nicotine. To date, no data have been collected that correlate this computation of nicotine bioavailability and plasma nicotine levels; however, it appears that identifying the ST product used by the individual can assist in assessing addiction levels. Even if one does not compute the total bioavailable nicotine, the number of tins per week can give a general view of addiction. Schroeder and colleagues (1988) have suggested categorizing ST users as light, moderate, or heavy on the basis of the number of tins used per week. A light user would consume one tin or pouch or less per week, a moderate user would use one and one-half tins or pouches weekly, and a heavy user would be a person who uses more than two tins or pouches per week. This method is compromised by the wide disparity in the level of bioavailable nicotine in the various products as noted above.

An alternative measure of addiction is suggested by Eakin and coworkers (1989), who adapted the Fagerstrom Addiction Scale (Fagerstrom, 1978), which has been used in smoking cessation. Cigarette-based questions were converted to ST items (e.g., "I chew or dip the first thing in the morning within 30 minutes of waking"; "I swallow the juice when I can't spit"; "I chew or dip where it is prohibited"; and "I crave ST even when I'm sick in bed"). These items are scored plus or minus and the total of seven or more positive responses indicates the person is heavily addicted to ST. Although this measure is being used in a number of ongoing ST cessation studies, there are currently no validity data with ST users.

Assessing Readiness To Quit

Another useful measure that can be adapted from smoking research is an assessment of readiness of the individual to quit. This involves using the Prochaska-DiClemente model (DiClemente et al., 1991; Prochaska and DiClemente, 1983) in which they postulate that the process of cessation can be conceptualized as along a continuum from precontemplation to contemplation to action in making an actual quit effort. The person's readiness to quit is assessed by questions such as, "Are you seriously considering quitting your use of chew or snuff in the next 6 months?" An affirmative response indicates the subject is at the contemplation stage. Agreement with statements such as, "I'm seriously going to quit my use of chew or snuff in the

next month," is an indication of readiness to make the commitment to quit. An alternative procedure to a series of questions is a 10-step "contemplation ladder" on which subjects indicate where they are in their readiness to quit by placing themselves on the ladder (Biener and Abrams, 1991).

PUBLIC HEALTH INTERVENTIONS Parallel to individual cessation programs of a clinical nature are public health cessation efforts. These efforts involve more population-based interventions that may be less effective in terms of quit rates but are more likely to affect large numbers of individuals who use ST. In the end, interventions of this type may have a much larger public health impact by getting more people to quit (Severson, 1980). These interventions are diverse and range from getting hygienists and dentists or physicians and nurses to provide ST users direct advice to quit, to policy changes in restrictions of sale and use of ST products. Health care professionals can advise patients to quit ST and provide materials regarding health risks in the context of regular health care. These interventions are minimal in terms of cost and time, but similar programs for smokers have been shown to have a significant impact on tobacco use (Vogt et al., 1989). Public health interventions also include media messages, such as radio or television public health messages, written materials, and information from voluntary groups (e.g., American Cancer Society and American Lung Association), policy changes such as work site restrictions on ST use, or restrictions on ST product sales to minors. Legislative actions such as increased taxation on ST products or putting health warnings on products are also considered public health interventions. Professional groups such as the American Dental Association and Academy of Otolaryngology have been active in publishing materials on the detrimental health effects of ST, and the distribution of these materials could also be considered a public health intervention.

An example of a public health cessation program was a 4-yr randomized trial that used dentists and hygienists as the providers of cessation advice (Little et al., in press). Men (aged 15 to 65) who came into HMO dental clinics for regular hygiene visits and identified themselves as ST users were randomized either to intervention or usual care based on an identification number. The dental office intervention involved first having a dentist or hygienist ask the patient about his tobacco use. Second, the clinician conducted an oral health exam and provided specific feedback on lesions in the mouth. Third, the practitioner provided direct advice to the patient to quit. The key element is for the dentist or hygienist or both to give a clear personal message that they believe ST use is harmful and they want the patient to quit. Fourth, patients were asked to watch a brief 10-min video that was motivational in nature, asked to set a quit date, and given self-help materials. There was also a 1-wk followup phone call by the hygienist to see how they were doing.

The results of this intervention were encouraging. Table 1 shows that the self-reported quit rate for the intervention group was 22 percent at the 3-mo followup. The usual care group had an overall cessation of only 14 percent. However, the cessation rates were very different for people who used only ST and those who used both ST and cigarettes. For men who used

Table 1
Three-month followup of dental intervention for ST users in an HMO

	Baseline Status of Users		Self-Reported Quit Rates at 3-Mo Followup	
	Percentage	n	Percentage	n
Intervention				
ST only	69%	170	26%	45
ST and cigarettes	31	75	12	9
Overall cessation			22	54
Usual Care				
ST only	70	191	17	33
ST and cigarettes	30	81	3	5
Overall cessation			14	38

ST and cigarettes, the cessation rates were only 12 percent in the intervention group and 3 percent in the usual care group. It appears that men who used cigarettes were much less likely to be successful in giving up ST than were subjects who do not smoke cigarettes. The good news is that very few of the ST-only users reported subsequently smoking cigarettes. Only 3 percent of men who reported ST-only use at baseline and reported no ST use at followup reported smoking cigarettes.

There is increased attention being paid to special ST populations such as baseball players and Native Americans, among whom the use of ST by females is similar to males (Schinke et al., 1987 and 1989). Baseball players also represent a special group as ST use has long been associated with the sport, and these professionals are highly visible role models for American boys. Surveys report 34 to 39 percent of professional players report using ST in the past week (Connolly et al., 1988; Ernster, 1989). A recent publication by the National Cancer Institute is specifically targeted to baseball players and provides a self-help cessation manual (Orleans et al., 1991). Materials are needed that target other groups in which ST use is prevalent. Specific materials for cowboys, minority groups, and specific vocational groups (i.e., wood products or other factory workers) are more likely to be effective. Adolescent ST prevention programs have been developed under NCI funding and are being evaluated, but cessation programs for adolescent ST users have been lacking. The recent publication of a self-help quit pamphlet by the American Cancer Society (1991), and the first self-help manual for quitting snuff and chew on your own (Severson, 1992), offer valuable aids to counselors providing cessation advice to young chewers and dippers.

SUMMARY There is evidence emerging that men are interested in quitting their use of ST and that cessation programs can be developed that are modestly successful. The program content and success rates appear similar to multicomponent smoking cessation programs. The need for oral substitutes such as mint snuff or nicotine polacrilex also appears important and suggests adjunctive aids.

There is a need for development and evaluation of cessation programs for ST users. There is a large group of men who are addicted to the nicotine in this tobacco product, estimated at 6 million American men (US DHHS, 1986a), but little has been done to assist them in quitting. There is a need for self-help quitting materials, cessation groups, and public health interventions. Preliminary evidence of successful adaptation of cognitive-behavioral smoking cessation programs is encouraging. That users report trying to quit on their own provides further support for developing cessation materials and providing access to cessation programs. Studies using nicotine replacement (nicotine polacrilex or transdermal nicotine patches) or oral substitutes such as mint snuff are needed to determine whether those adjuncts significantly increase long-term cessation rates. There are unique aspects of ST use that have not received adequate attention in cessation programs, and components (such as an oral self-exam) may provide significant motivation for the ST user to quit.

Public health interventions for ST users also show promise. Early results of a dental office-based intervention provide impetus for developing and implementing public health interventions that have proved successful in getting cigarette smokers to quit (Little et al., 1992). These interventions may involve health professionals providing direct advice to quit in the context of regular health care delivery. Special interventions may be most relevant for high ST user groups such as baseball players or wood product workers or ethnic groups such as Native Americans where prevalence of use is endemic. In sum, there is much to be done in ST cessation, but the groundwork has been laid and the direction and preliminary results are promising.

ACKNOWLEDGMENTS The author thanks Ed Lichtenstein for his helpful editing of this manuscript and personal support for this professional endeavor.

REFERENCES

American Cancer Society. *Smokeless tobacco: Check it out, think it over, take it out, throw it out, snuff it out, keep it out.* Atlanta: American Cancer Society 1991, No. 2090.

Ary, D.V., Lichtenstein, E., Severson, H., et al. An in-depth analysis of male adolescent smokeless tobacco users: Interviews with users and their fathers. *Journal of Behavioral Medicine* 12: 449-467, 1989.

Bauman, K.E., Koch, G.G., Lentz, G.M. Parent characteristics, perceived health risk, and smokeless tobacco use among white adolescent males. *National Cancer Institute Monographs* 8: 43-48, 1989.

Benowitz, N.L., Porchet, H., Sheiner, L., et al. Nicotine absorption and cardiovascular effects with smokeless tobacco use: Comparison with cigarettes and nicotine gum. *Clinical Pharmacology and Therapeutics* 44: 23-28, 1988.

Biener, L., Abrams, D.B. The contemplation ladder: Validation of a measure of readiness to consider smoking cessation. *Health Psychology* 10: 360-365, 1991.

Chassin, L., Presson, C.C., Sherman, C.J., et al. Psychological correlates of adolescent smokeless tobacco use. *Addictive Behavior* 10: 431-435, 1985.

Connolly, G.N., Orleans, C.T., Kogan, M. Use of smokeless tobacco in major-league baseball. *New England Journal of Medicine* 318: 1281-1284, 1988.

DiClemente, C.C., Prochaska, J.O., Fairhurst, S.K., et al. The process of smoking cessation: An analysis of pre-contemplation, contemplation, and preparation stages of change. *Journal of Consulting and Clinical Psychology* 59(2): 295-304, 1991.

DiLorenzo, T.M., Kern, T.G., Pieper, R.M. Treatment of smokeless tobacco use through a formalized cessation program. *Behavior Therapy* 22: 41-46, 1991.

Eakin, E., Severson, H.H., Glasgow, R.E. Development and evaluation of a smokeless tobacco cessation program: A pilot study. *National Cancer Institute Monographs* 8: 95-100, 1989.

Ernster, V.L. Advertising and promotion of smokeless tobacco products. *National Cancer Institute Monographs* 8: 87-94, 1989.

Fagerstrom, K.O. Measuring the degree of dependence in tobacco smoking with reference to individualization of treatment. *Addictive Behaviors* 3: 235-241, 1978.

Glover, E.D. Conducting smokeless tobacco cessation clinics. *American Journal of Public Health* 76: 207, 1986.

Glover, E.D., Schroeder, K.L., Hinningfield, J.E., et al. An interpretive review of smokeless tobacco research in the United States: Part I. *Drug Education* 18(4): 305-330, 1989.

Gritz, E.R., Baer-Weiss, V., Benowitz, N.L., et al. Plasma nicotine and cotinine concentrations in habitual smokeless tobacco users. *Clinical Pharmacology and Therapeutics* 30: 201-209, 1981.

Hatsukami, D.K., Gust, S.W., Keenan, R.M. Physiological and subjective changes from smokeless tobacco withdrawal. *Clinical Pharmacology and Therapeutics* 41: 103-107, 1987.

Hatsukami, D., Nelson, R., Jensen, J. Smokeless tobacco: Current status and future directions. *British Journal of Addiction* 86: 559-563, 1991.

Hoffman, N.D., Harley, N.H., Fisenne, I., et al. Carcinogenic agents in snuff. *Journal of the National Cancer Institute* 76(3): 9, 1986.

Hollis, J.F., Vogt, T.M., Stevens, V., et al. The tobacco reduction and cancer control (TRACC) program: Team approaches to counseling in medical and dental settings. In: *Tobacco and the Clinician: Interventions for Medical and Dental Practice*, D.M. Burns, S. Cohen, E. Gritz, and T. Kottke (Editors). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute, 1992, in press.

Hughes, J.R., Hatsukami, D.K. Signs and symptoms of tobacco withdrawal. *Archives of General Psychiatry* 43: 289-294, 1986.

Lichtenstein, E., Severson, H.H., Friedman, L.S., et al. Chewing tobacco use by adolescents: Prevalence and relation to cigarette smoking. *Addictive Behavior,* 9: 351-355, 1984.

Little, S.J., Stevens, V., LaChance, P., et al. Smokeless tobacco habits and oral mucosal lesions on dental patients. *Journal of Public Health Dentistry*, in press.

Little, S.J., Stevens, V., Severson, H.H., Lichtenstein, E. An effective smokeless tobacco intervention for dental hygiene patients. *Journal of Dental Hygiene* 66(4): 185-190, 1992.

Novotny, T.E., Pierce, J.P., Fiore, M.C., Davis, R.M. Smokeless tobacco use in the United States: The Adult Use of Tobacco Surveys. *National Cancer Institute Monographs* 8: 25-28, 1989.

Orleans, C.T., Connolly, G.N., Workman, S. *Beat the Smokeless Habit: A Game Plan for Success*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. NIH Publication No. 92-3270, 1991.

Prochaska, J.O., DiClemente, C.C. Stages and processes of self-change of smoking: Toward an integrative model of change. *Journal of Consulting and Clinical Psychology* 5: 390-395, 1983.

Russell, M.A.H., Jarvis, M.J., Devitt, G., et al. Nicotine intake by snuff users. *British Medical Journal* 283: 814-817, 1981.

Schinke, S.P., Gilchrist, L.D., Ashby, M.R. Pacific Northwest Native American youth and smokeless tobacco use. *International Journal of the Addictions* 22(9): 881-884, 1987.

Schinke, S.P., Shilling, R.F. II, Gilchrist, L.D., Ashby, M.R., Kitajimar, E. Native youth and smokeless tobacco: Prevalence rates, gender differences, and descriptive characteristics. *National Cancer Institute Monographs* 8: 39-42, 1989.

Schroeder, K.L. Oral and systemic concerns with smokeless tobacco. *Clinical Dentistry* 2(9): 1-27, 1989.

Schroeder, K.L., Chen, M.S., Jr., Iaderosa, G.R., et al. Proposed definition of a smokeless tobacco user based on "potential" nicotine consumption. *Addictive Behaviors* 13: 395-400, 1988.

Severson, H.H. *Enough Snuff: A Manual for Quitting Smokeless Tobacco on Your Own*. Eugene, OR: Rainbow Publications, 1992.

Severson, H.H. Psychosocial factors in the use of smokeless tobacco and their implications for P.L. 99-252. *Journal of Public Health Dentistry* 50(1): 90-97, 1990.

Severson, H.H. Smokeless tobacco: Risks, epidemiology and cessation. In: *Nicotine Addiction: Principles and Management*, C.T. Orleans and J. Slade (Editors). New York: Oxford University Press, in press.

Severson, H.H., Eakin, E.G., Lichtenstein, E., et al. The inside scoop on the stuff called snuff: An interview study of 94 adult male smokeless tobacco users. *Journal of Substance Abuse* 2: 77-85, 1990a.

Severson, H.H., Eakin, E.G., Stevens, V.J., et al. Dental office practices for tobacco users: Independent practice and HMO clinics. *American Journal of Public Health* 80(12): 1503-1505, 1990b.

Severson, H.H., Zoref, L. Prevention and early interventions for addictive behaviors: Health promotion in the schools. In: *Interventions for Achievement and Behavior Problems*, G. Stoner, M. Shinn, and H. Walker (Editors). Silver Spring, MD: National Association of School Psychologists, 1991, pp. 539-557.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report to the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986a.

U.S. Department of Health and Human Services. *Health Implications of Smokeless Tobacco Use: National Institutes of Health Consensus Development Conference*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Office of Medical Applications Research, 1986b.

U.S. Department of Health and Human Services. *Youth Use of Smokeless Tobacco: More Than a Pinch of Trouble*. Washington, DC: Office of the Inspector General, 1986c.

Vogt, T.M., Lichtenstein, E., Ary, D., et al. Integrating tobacco intervention into a health maintenance organization: The TRACC Program. *Health Education Research* 4: 125-135, 1989.

Smokeless Tobacco Cessation and Nicotine Reduction Therapy

Elbert D. Glover and Penny N. Glover

ABSTRACT Given the health and social consequences of smokeless tobacco, strategies to reduce and stop ST consumption and the occurrence of tobacco-related diseases warrant close examination and analysis. This paper examines ST use as an addiction and presents various pharmacological adjuncts for helping smokeless tobacco users with their addiction. Specifically, 2-mg nicotine polacrilex, 4-mg nicotine polacrilex, and transdermal nicotine patches are examined as potential pharmacological adjuncts for ST cessation.

INTRODUCTION For more than 150 years, it has been known that nicotine is absorbed by various organs (Orfila, 1851; Glover et al., 1989). Since 1950 we have known that the brain is the highest among all the organs in its ability to absorb nicotine (Werle and Meyer, 1950). Larson and his colleagues have extensively reviewed the various physiological and behavioral actions of nicotine (Larson and Silvette, 1968 and 1971; Larson et al., 1961). Here we provide the basic knowledge necessary for attempting smokeless tobacco cessation with nicotine therapy.

Since the turn of the century, it has been suspected that the central nervous system is the site of action for the tobacco effects sought by users (Armstrong-Jones, 1929; Russell and Feyerabend, 1981). Nicotine is a euphoriant (Henningfield and Nemeth-Coslett, 1988). In a series of abuse liability studies, Henningfield and Nemeth-Coslett found that nicotine produced dose-related increases in euphoria, as measured by the drug-liking scale. Their data demonstrated that increased euphoriant scale scores exceeded placebo values and were directly related to increased doses of nicotine, which identifies nicotine as a drug that produces dependence. As a psychoactive substance, nicotine exerts a number of effects on the brain and CNS. Once nicotine is absorbed into the blood, it is rapidly distributed and absorbed by the brain and other highly blood-perfused tissues (Henningfield and Nemeth-Coslett, 1988).

Animal experiments have shown that nicotine induces some of the same changes in brain energy utilization that have been observed with drugs like cocaine (Henningfield and Nemeth-Coslett, 1988). From the first use of tobacco, the self-administered dosage gradually escalates. Tolerance, the need to take larger doses to obtain the same physiological effect, begins during the earlier stages of drug dependence and is a major factor in dose escalation (Henningfield and Nemeth-Coslett, 1988). Henningfield and Nemeth-Coslett further report that the self-reported number of cigarettes smoked from day 1 through year 8 reveals that several years of smoking are required before a stable level of one to two packs of cigarettes per day is reached.

Johnston (1942), studying intravenous nicotine delivery in humans, came to the following conclusions:

- Smoking tobacco is essentially a means of administering nicotine, just as smoking opium was a means of administering morphine.
- Nicotine has a psychic action.
- The actions of intravenous nicotine are similar in temporal pattern and quality to those of inhaled tobacco smoke.
- Smokers show the same attitude toward tobacco as do people addicted to other drugs, and their judgment was therefore biased in giving an opinion of its effect on them.

Johnston's conclusions have been largely confirmed by a variety of techniques, including strategies available only in recent years.

Similar conclusions have been published by the National Institute on Drug Abuse, the Public Health Service, and an Advisory Committee to the U.S. Surgeon General (NIDA, 1984; US DHHS, 1983, 1986, and 1987). Drug addiction is most often defined as the behavior of repeatedly ingesting a substance that results in the delivery of a behavior-modifying chemical to the central nervous system (Schuster, 1980). The symptoms associated with nicotine withdrawal—craving, irritability, increasingly frequent waking from sleep, slowed heart rate, anxiety, impaired concentration, restlessness, drowsiness, impatience, confusion, increased hunger, and impaired reaction time—can undermine the tobacco user's motivation to stop (American Psychiatric Association, 1987). The stronger the dependence on nicotine, the lower the likelihood of successful tobacco cessation.

NICOTINE IN SMOKELESS TOBACCO

Plasma nicotine and cotinine levels were measured in 12 ST users by Gritz and colleagues (1981). The subjects were male college students who used approximately one-third of a can of smokeless tobacco per day and did not smoke cigarettes (Gritz et al., 1981). The mean plasma nicotine concentration of the subjects prior to tobacco use was 2.9 ng/mL; this increased to 21.6 ng/mL after using smokeless tobacco throughout the day. The plasma nicotine and cotinine levels found in the ST users were comparable to concentrations found among regular cigarette smokers (Gritz, 1981). One-third of a can of ST per day is considered light use (Schroeder et al., 1988). Similarly, in England, Russell and colleagues (1980 and 1981b) found plasma nicotine levels in nasal snuff users reaching levels similar to those of heavy smokers, as well as exhibiting a more rapid rate of absorption than noninhaling cigar smokers. Furthermore, Russell and coworkers found plasma cotinine levels to average 23 percent higher among daily snuff users than among smokers.

CESSATION PROGRAMS

Currently, there is little reported knowledge about smokeless tobacco cessation. Glover (1986) reported research on two smokeless tobacco cessation programs adapting the American Cancer Society's FreshStart Program for cigarette smokers. A total of 41 subjects initially enrolled in both programs (20 in the first and 21 in the second). Glover reported a

2.3 percent success rate for the quit-ST clinics at 6 mo, compared with his 38.0 percent success rate at 6 mo for cigarette smokers. Of the 41 ST users who attempted abstinence, only 1 of the participants was able to go for more than 4 h during the waking hours without using ST. Interestingly, the success of this one individual who did achieve long-term success (1-yr abstinence) was accomplished through a successive reduction of ST intake with nicotine polacrilex used as an adjunct. Nicotine gum after 6 mo was faded out.

After this experience, Glover concluded that smokeless tobacco appears to be more addictive than cigarette smoking. Given the results of the cessation program in which Glover had adapted the FreshStart Program, in 1986 the American Cancer Society convened an advisory group to provide guidance for a coordinated educational effort to develop an ST cessation manual for high school seniors. After 18 mo of development and revision, the cessation manual was completed and released in 1991. The manual is available through local chapters of the American Cancer Society (American Cancer Society, 1991). The self-help program relies heavily on the theoretical bases of social contracting, peer education, and relapse prevention (Glover et al., in press). Similar programs have been developed by others (Hatsukami, 1991a; Severson, 1987). All three are similar in presentation with one noted exception: Hatsukami's program uses nicotine polacrilex, which may be the most appropriate way to attempt ST cessation (Glover, 1992b). Given that the ST user and the smoker evidence similar nicotine and cotinine concentration levels (Gritz et al., 1981; Russell et al., 1980 and 1981), a similar therapy could be beneficial—specifically, nicotine reduction therapy.

Nicotine Reduction Therapy

Both smokeless and smoking tobacco contain nicotine, which is the basis for addiction (Glover et al., 1989). If we are to assist ST users, nicotine reduction therapy (NRT) should be the basis for cessation programs, just as it is the basis for effective programs for smoking cessation. A decade ago, we used groups, education, counseling, social contracting, and social support to assist with smoking cessation (Glover et al., 1992b). Today, we are using NRT to assist smokers with successful cessation (Hatsukami, 1991b; Russell et al., 1983).

Both nicotine polacrilex and nicotine transdermal systems were developed in part to improve quit rates by helping to diminish withdrawal symptoms (particularly nicotine craving). Both have proven to be effective stop-smoking therapies. Because nicotine plasma and cotinine levels are similar after cigarette or smokeless tobacco use and because the addictive potential of ST can be as great as or greater than that of cigarettes, we need to begin to investigate NRT for ST cessation—specifically, 2-mg and 4-mg nicotine polacrilex and transdermal patches.

Nicotine Polacrilex (2-mg)

Nicotine gum—nicotine polacrilex—has consistently been shown in clinical trials to be more effective than placebo or no intervention in terms of 6-mo and 1-yr abstinence rates (Hatsukami, 1991b). Nicotine polacrilex is successful with smokers when used in conjunction with a behavioral modification program (Russell et al., 1983; US DHHS, 1989 and 1990;

Wilson et al., 1988). Compliance problems are related to the patient's chewing correctly and chewing a sufficient number of pieces per day. Apparently many physicians do not provide proper chewing instructions to patients.

If used correctly, nicotine polacrilex can be an excellent pharmacological adjunct for smokeless tobacco cessation. Moreover, nicotine polacrilex is more like ST than cigarette smoking, so it provides a mimic cue. The method of absorption from the nicotine gum is similar to that of smokeless tobacco. Nicotine blood plasma levels, cotinine levels, placement of polacrilex and ST, and outward appearance (bulge in the gingival area while the material is in use) are identical. If researchers are to investigate nicotine polacrilex as a pharmacological aid for ST cessation, it is critical to avoid errors similar to those made with smoking cessation. Potential methodological errors include the following:

- Not using the proper chewing technique;
- Underdosing—not using sufficient quantities;
- Using nicotine with liquids; and
- Not using nicotine polacrilex in conjunction with some other type of behavior modification program (Cummings et al., 1988).

Nicotine Polacrilex (4-mg)

Nicotine polacrilex in the 4-mg dose is identical to 2-mg nicotine polacrilex except that it has twice the nicotine concentration. Currently, 4-mg nicotine polacrilex is not generally available in the United States; however, clinical trials have been conducted. Glover and colleagues (1992b) enrolled more than 500 highly dependent smokers (Fagerstrom score of 7 or greater) in a double-blind, placebo-controlled study. They found no significant difference in cesssation rates between placebo and 2-mg nicotine polacrilex; however, the 4-mg version was found to be more effective than either placebo or the 2-mg dose with highly dependent smokers. Given the similarity of nicotine and cotinine levels in ST users and highly dependent smokers, it may well be that 4-mg gum is more effective with ST users than is the 2-mg version.

Transdermal Patch

The nicotine transdermal patch has promise for nicotine reduction therapy. A rate-controlling nicotine transdermal system has demonstrated efficacy in smoking cessation, including significant relief from nicotine craving (Transdermal Nicotine Study Group, 1991). The transdermal nicotine patch should afford fewer compliance problems than the nicotine gum because the patch is placed on the upper part of the body only once a day. Three pharmaceutical companies have 24-h patches approved by the Food and Drug Administration for smoking cessation, and a 16-h patch is currently awaiting FDA approval.

The patch delivers a specific amount of nicotine to the central nervous system every hour, allowing for a steady rate of nicotine absorption; consequently, withdrawal symptoms are minimized. This technique has been found to be very promising for cigarette smokers (Transdermal Nicotine

Study Group, 1991). Several transdermal nicotine systems are available, and they vary in nicotine dose, drug delivery design and technology, absorption and efficacy rates, and effects on skin. The patch eliminates the nicotine absorption peaks and troughs associated with nicotine polacrilex use (Hatsukami, 1991b). It is hoped that the new transdermal technology can be applied for ST users with similar success.

Other Pharmacological Adjuncts

Several nicotine products that are in various phases of testing for smoking cessation could one day be used for ST cessation:

- Nicotine nasal spray—a device that delivers nicotine via the nose (Schneider, 1992);
- Oral inhaler—a device that delivers nicotine via the lungs (Glover et al., 1992a); and
- Oral lozenge—a device that delivers nicotine via the oral mucosa.

Validating Self-Reports

One obstacle to the use of nicotine reduction therapy with ST cessation was the inability to validate self-reports. Previously, it was impossible to distinguish chemically the nicotine in smokeless tobacco from the nicotine in polacrilex gum. However, Peyton and Benowitz (1991) have developed a method for validating self-reports using excretion of the alkaloids anabasine and anatabine in the urine of ST users. Their test allows the researcher to validate self-reports for ST users attempting to quit with NRT.

In light of the available literature, NRT has potential benefits for smokeless tobacco cessation and deserves further study. NRT may well be our best hope for ST users.

REFERENCES

American Cancer Society. *Smokeless Tobacco: Check It Out, Think It Out, Take It Out, Throw It Out, Snuff It Out, Keep It Out*. Atlanta: American Cancer Society, 1991 (Publication 91-10M-No. 2090).

American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition, revised. Washington, DC: American Psychiatric Association, 1987.

Armstrong-Jones, S. Tobacco, its use and abuse: From the nervous and mental aspect. *Practitioner*: 6-19, 1929.

Cummings, S.R., Hansen, B., Richard, R.J., Stein, M.J., Coates, T.J. Internist and nicotine gum. *Journal of the American Medical Association* 260: 1565-1569, 1988.

Glover, E.D. Conducting smokeless tobacco cessation clinics. *American Journal of Public Health* 76: 207, 1986.

Glover, E.D., Glover, P.N., Nilsson, F., Sawe, U. To determine the safety and efficacy of the Nicohaler as an aid in smoking cessation. Presented at the Eighth World Conference on Tobacco and Health, Buenos Aires, Argentina, 1992a.

Glover, E.D., Schroeder, K.L., Henningfield, J.E., Severson, H.H., Christen, A.G. Smokeless tobacco research in the United States: Part II. *Journal of Drug Education* 19(1): 1-19, 1989.

Glover, E.D., Wadland, W., Sachs, D.P.L., Rennard, S.I., Daughton, D., Pomerleau, O.F., Huster, W.J., Nowak, R.T., Rolf, C.N. The safety and efficacy of 4 mg Nicorette as compared to 2 mg Nicorette versus placebo in highly dependent smokers. Presented at the Eighth World Conference on Tobacco and Health. Buenos Aires, Argentina, 1992b.

Glover, E.D., Wang, M., Glover, P.H. Development of a high school self-help smokeless tobacco cessation manual. *Health Values*, in press.

Gritz, E.R., Baer-Weiss, V., Benowitz, N.L., Van Vunakis, J., Jarvik, M.E. Plasma nicotine and cotinine concentrations in habitual smokeless tobacco users. *Clinical Pharmacology Therapy* 30: 201-209, 1981.

Hatsukami, D. *Tough Enough to Quit Using Snuff*. Minneapolis: Tobacco Research Laboratory, University of Minnesota, 1991a.

Hatsukami, D. Clinical trials with nicotine replacement therapies. Presented at the Centennial Meeting of the American Psychological Association. San Francisco, 1991b.

Henningfield, J.E., Nemeth-Coslett, R. Nicotine dependence: Interface between tobacco and tobacco-related disease. *Chest* 93(Suppl): 37S-55S, 1988.

Johnston, L.M. Tobacco smoking and nicotine. *Lancet* 2 (742): 743, 1942.

Larson, P.S., Haag, H.B., Silvette, H. *Tobacco Experimental and Clinical Studies: A Comprehensive Account of the World Literature*. Baltimore: Williams and Wilkins, 1961.

Larson, P.S., Silvette, H. *Tobacco Experimental and Clinical Studies: A Comprehensive Account of the World Literature. Supplement I*. Baltimore: Williams and Wilkins, 1968.

Larson, P.S., Silvette, H. *Tobacco Experimental and Clinical Studies: A Comprehensive Account of the World Literature. Supplement II*. Baltimore: Williams and Wilkins, 1971.

National Institute on Drug Abuse. *Drug Abuse and Drug Abuse Research*, the first in a series of triennial reports to Congress from the Secretary, Department of Health and Human Services. Department of Health and Human Services, National Institutes of Health, 1984.

Orfila, B. Memoire sur la nicotine et sur la conicine. *Ann Hygiene Pub* 45: 147-230, 1851.

Peyton, J., Benowitz, N.L. Excretion of the alkaloids anabasine and anatabine in urine of smokeless tobacco users and cigarette smokers: Biochemical markers of smokeless tobacco use. Presented at the First International Conference on Smokeless Tobacco. Columbus, Ohio, April 10-13, 1991a.

Russell, M.A.H., Feyerabend, C. Cigarette smoking: A dependence disorder. *British Journal of Medical Psychiatry* 8(1): 29-37, 1981.

Russell, M.A.H., Jarvis, M.J., Feyerabend, C. A new age for snuff. *Lancet* 1: 474-475, 1980.

Russell, M.A.H., Jarvis, M.J., Dewitt, C., Feyerabend, C. Nicotine intake by snuff users. *British Medical Journal* 283: 814-817, 1981.

Russell, M.A.H., Merriman, R., Stapleton, J., Taylor, W. Effects of nicotine chewing gum as an adjunct to general practitioners' advice against smoking. *British Medical Journal* 287: 1782-1785, 1983.

Schneider, N. The safety and efficacy of the nicotine nasal solution as an aid for smoking cessation. Presented at the Smoking Cessation Scientific Meeting. Thundridge, England, 1992.

Schroeder, K.L., Chen, M.S., Iaderosa, G.R., Glover, E.D., Edmundson, E.W. Proposed definition of a smokeless tobacco user based on "potential" nicotine consumption. *Addictive Behaviors* 13: 395-400, 1988.

Schuster, C.R. Addiction: Report of the Task Force on Smoking. *World Smoking and Health* 5: 22, 1980.

Severson, H.H. *Up to Snuff: A Handbook on Smokeless Tobacco*. Eugene, OR: Independent Video Services, 1987.

Transdermal Nicotine Study Group. Transdermal nicotine for smoking cessation. *Journal of the American Medical Association* 22: 3133-3138, 1991.

U.S. Department of Health and Human Services. *Why People Smoke Cigarettes*. U.S. Department of Health and Human Services, Public Health Service. PHS Publication No. (PHS) 83-50195, 1983.

U.S. Department of Health and Human Services. *Nicotine: Drug Abuse Research*. Second in a series of triennial reports to Congress from the Secretary of Health and Human Services. DHHS Publication No. (ADM) 87-1486, 1987.

U.S. Department of Health and Human Services. *Reducing the Health Consequences of Smoking: 25 Years of Progress. A Report of the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. DHHS Publication No. (CDC) 89-8411, 1989.

U.S. Department of Health and Human Services. *Health Benefits of Smoking Cessation: A Report of the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. DHHS Publication No. (CDC) 90-8416, 1990.

U.S. Department of Health and Human Services. *Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. NIH Publication No. 86-2874, 1986.

Werle, E., Meyer, A. Uber den Abbau von Tabakalkaloiden surch Tierische Gewebe. *Biochemische Zeitschrift* 321: 221-235, 1950.

Wilson, D.M., Taylor, D.W., Gilbert, J.R., Best, J.A., Lindsay, E.A., Willms, D.G., Singer, J. A randomized trial of a family physician intervention for smoking cessation. *Journal of the American Medical Association* 260: 1570-1574, 1988.

Role of Dentists in Cessation Counseling: Survey Findings[1]

Kathleen L. Schroeder and David E. Heisel

ABSTRACT Dental professionals in public health and research have been instrumental in raising the public's awareness of smokeless tobacco, yet recent data indicate that the general dental practitioners' and specialists' role in reducing ST use has been underestimated and underused. A 73-item survey was mailed to 1,064 dentists in Central Ohio to ascertain their knowledge of and receptivity to providing an ST or tobacco use intervention in a clinical setting. Of the 529 dentists responding to the survey, only 9 percent stated that they were effective at getting their patients to stop using tobacco. Relative to ST cessation, 71 percent of the dentists would be willing to provide educational pamphlets on ST to their patients; 6 percent consider prescribing nicotine gum; 3 percent consider delegating prevention responsibility; and 15 percent consider referring to an outside cessation program. Of the dentists who responded, 73 percent felt cessation counseling was frustrating. Dentists considered that barriers to ST cessation counseling included their lack of training (66 percent) and lack of insurance coverage (73 percent). The results indicate the need for further education in tobacco and cessation counseling for dentists.

INTRODUCTION The dental professional's role in reducing smokeless tobacco use has been double-edged. Public health dentists and dental researchers studying the effects of ST (Archard and Tarpley, 1979; Axéll et al., 1976; Christen, 1980; Connolly, 1986; Greer, 1983; Hirsch et al., 1982; Hoge and Kirkham, 1983; Offenbacher and Weathers, 1985; Park et al., 1985; Pindborg and Renstrup, 1963; Poulson, 1983; Roed-Petersen and Pindborg, 1973; Schroeder, 1989; Schroeder et al., 1985, 1988a, 1990; Schroeder and Chen, 1985; Squires, 1984; Van Wyk, 1976) report that dental professionals have been among the most instrumental in scientific and public health awareness of ST. Lobbying efforts by dental professionals have played a major role in having the Federal Trade Commission change its final rules for warnings on snuff and chewing tobacco (*ADA News*, 1986). Yet recent data indicate that the dental practitioners' role in reducing ST use by their patients and in the community has been underestimated and underused (Fried and Rubinstein-DeVore, 1990; Geboy, 1990; Schroeder, 1989 and 1990; Schroeder et al., 1988b and 1990).

In the past 10 yr, we have found the increased use of snuff in countries such as Sweden and increases in dipping and chewing in the United States, particularly in high schools and with young adult males (Christen et al., 1979; Greer and Poulson, 1983; Hoffman et al., 1986; Poulson et al., 1984). Clinicians have noted associated oral problems such as dental caries, abrasion, gingivitis, gingival recession, periodontitis, leukoplakia, and oral cancer (Christen, 1980; Greer and Poulson, 1983; Greer et al., 1986 and 1988; Hoffman et al., 1986; Hoge and Kirkham, 1983; Offenbacher and Weathers, 1985; Pindborg and Renstrup, 1963; Roed-Petersen and Pindborg,

[1] Supported in part by a grant to Dr. Schroeder from the American Heart Association, Central Ohio Chapter.

1973; Schroeder et al., 1985) with the increased use of ST. Often, ST-induced lesions are found to have a hyperkeratotic white patch (leukoplakia) (Greer and Poulson, 1983; Greer et al., 1986 and 1988; Pindborg and Renstrup, 1963; Roed-Petersen and Pindborg, 1973). Leukoplakia is a white patch or plaque that cannot be characterized clinically or pathologically as any other disease (WHO, 1978) and is considered a premalignant lesion that has a transformation rate of more than 6 percent for either dysplasia or carcinoma (US DHHS, 1986).

Furthermore, nicotine concentrations in the blood of ST users have been shown to be as high as those in cigarette smokers, and exposure to nicotine from ST is 8 to 10 times higher than from an average package of cigarettes (Schroeder et al., 1988c and 1990). ST has further been related to increases in heart rate and blood pressure (Edwards et al., 1987; Gritz et al., 1981; Neal et al., 1988; Schroeder and Chen, 1985; Schroeder et al., 1988c and 1990; Squires et al., 1984). Thus, ST is a potential risk factor for coronary and peripheral vascular disease, peptic ulcer, reproductive disorders, and neuromuscular disease (US DHHS, 1986).

Health professionals in the past few years have become aware of the health hazards of ST, following the enactment of P.L. 99-252 aimed at decreasing ST use (Chen and Schroeder, 1990; Chen et al., 1991; Schroeder, 1990). The American Dental Association, American Medical Association, American Cancer Society, American Heart Association, Federal Government, and State dental health departments include ST information in their efforts to control tobacco use (CDC, 1986; Chen and Schroeder, 1987). Recently, the American Academy of Otolaryngology-Head and Neck Surgery began a 1-yr campaign with a comprehensive slide and lecture series (McGuirt, 1990) and videotape (American Academy of Otolaryngology-Head and Neck Surgery, 1990) offered to their members. The American Dental Association now offers several educational brochures and videotapes (ADA, 1990a and 1990b), and even the Office of the Commissioner of Major League Baseball worked with the National Cancer Institute to produce a booklet on ST education (US DHHS, 1991). Despite the availability of information on the health hazards of ST, in one screening only 10 percent of ST users reported that their dentist informed them that they had lesions associated with ST use (Schroeder et al., 1988c). Dental practitioners should actively provide information on ST and initiate early detection and prevention protocols for patients at high risk for ST use. This could include health questions regarding tobacco use, measurement of blood pressure, more thorough identification and detection of lesions, and involvement in tobacco cessation counseling in the office or by referral. To promote these efforts to the dental community, the dental components of two major NCI tobacco use interventions, the Community Intervention Trial for Smoking Cessation (COMMIT, 1988 to 1993) and the American Stop Smoking Intervention Study for Cancer Prevention (ASSIST, 1994 to 1998), provide a framework for the dissemination of oral health and cessation counseling information and the adoption of tobacco-related policies for community organizations (Mecklenburg, 1989).

Because of dentists' prevention-oriented role, exemplified in their effectiveness with using fluorides and reducing dental decay rates, dentists could be an effective force in the prevention and reduction of tobacco use as well. There are more than 140,000 dentists in the United States, who serve most populations and treat more than 70 percent of the total population within any 2-yr interval (e.g., 79 percent of working adults ages 18 to 64, 1985 to 1986) (Mecklenburg, 1989). Dentists have a significant amount of public contact and reinforce other health professional and community activities that are directed toward tobacco prevention and cessation. Dentists also can prescribe nicotine gum or other alternative nicotine delivery therapies for assisting their patients in office-based cessation programs.

For the most part, there has been insufficient education for dental professionals to make them aware of how they can assist in tobacco use prevention and cessation. We do know that, to assist or implement any cessation programs through dental practitioners, the knowledge and receptivity of dental professionals for providing an office intervention for tobacco use must be understood and assessed. Therefore, the current study provides information on the involvement and views of a large population of dentists, representative of Central Ohio, toward ST prevention and cessation activities, as well as the attitudes and barriers among the dental community relative to tobacco knowledge and cessation counseling.

MATERIALS AND METHODS

A 73-item survey and a computer-readable response sheet were mailed to 1,064 dentists who were Ohio Dental Association members within the Central Ohio area between April and June of 1989. Central Ohio provides a representative sampling from urban, suburban, and rural populations (Table 1). The mailing list for area dentists was obtained from the Ohio Dental Association. Dentists who did not respond within 3 wk were sent a second mailing.

The survey contained items on demographics, knowledge of tobacco, cessation counseling practices, and attitudes about counseling, particularly relative to ST. Prior to the mailing, the survey was pilot-tested with 30 dentists and dental students, with a repeat reliability of r=0.73-1.00.

The completed response sheets were scanned by computer, and data were tabulated. Data analysis was performed with the Statistical Analysis System (SAS) computer program.

RESULTS

Responses to the survey were received from 529 dentists (50 percent of the sample). Table 1 provides the demographics of the responding dentists.

Many dentists reported conducting thorough soft tissue exams on all patients (88 percent) and followup visits for those with suspected lesions (82 percent); 82 percent indicated that it was their responsibility to educate the public about ST. However, other questions revealed limited ST counseling practices (Table 2). Although 82 percent believed that it was their responsibility to influence others about ST use, only 44 percent of the dentists discussed the subject of ST cessation during every visit or almost every visit. The most commonly reported method of counseling that dentists would be willing to provide was distribution of pamphlets; very few

Table 1
Characteristics of dentists completing the survey

Characteristic	Percentage (n=529)
Age	
25 to 35 yr	22%
36 to 45	29
46 to 55	19
> 55	29
Gender	
Male	95
Female	5
Race	
White	96
Asian	2
Declined to state	2
Professional Status	
General dentist	85
Specialist	15
Practice Location	
Urban	23
Suburban	53
Rural	24
Smoking Status	
Never smoked	53
Former smoker	39
Current smoker	7
Chewing/Dipping Status	
Never chewed/dipped	91
Former chewer/dipper	7
Current chewer/dipper	2

thought to prescribe nicotine gum, to delegate teaching prevention responsibility, or to refer the patient to an outside cessation program.

Dentists' attitudes (Table 3) may contribute to the current state of reduced counseling practices. Only 9 percent thought themselves effective at getting patients to stop using tobacco, 26 percent forgot to discuss tobacco use, and 23 percent believed they had no time to counsel patients. Many of the responding dentists felt that ST counseling was frustrating and ineffective. Often they believed the tobacco users were not interested, and a few dentists felt that some patients might leave the practice as a result of their counseling efforts. However, the primary inhibitors of the interest in

Table 2
Counseling practices

Statement of Practice	Percentage (n=529)
I bring up quitting or cutting down with patients who chew/dip.	
Every visit/almost every visit	44%
Occasionally	48
Initial visit only	2
Never	3
I have other staff counsel patients about chewing.	
Often or sometimes	36
Never	61
With regard to cessation programs for ST, I would be most willing to:	
Prescribe nicotine-containing gum	6
Provide educational pamphlets on ST	71
Delegate teaching prevention responsibility	3
Refer to outside program	15
None of the above	3
I give pamphlets or educational materials on chewing/dipping.	
Often or sometimes	36
Never	63

ST counseling (Table 3 and Figure 1) were inadequate training in counseling (66 percent) and lack of insurance coverage for cessation counseling (73 percent). However, 68 percent of the dentists stated that they would participate in a continuing education program regarding ST prevention and cessation.

The survey did reveal certain exam-related counseling practices that the dentists offered to their patients (Table 4). A high percentage (82 to 90 percent) of dentists provided thorough soft tissue exams on all patients, conducted followup visits on suspected lesions, and informed their patients about caries, stains, gingivitis, and soft tissue changes caused by ST use. However, only a small percentage of the dentists reported routinely asking questions about tobacco use on health histories (35 percent) or screening for blood pressure (17 percent).

CONCLUSIONS Results from this study of ST cessation counseling among Central Ohio dentists were similar to Gerbert and colleagues' study on dentists' attitudes about smoking cessation (Gerbert et al., 1989). Both studies revealed very limited counseling efforts by dentists with regard to tobacco use. Both studies indicate similar reasons for these disappointing results.

In our study, many of the dentists indicated uncertainty toward their role as counselors. Although 82 percent indicated that it was a dentist's responsibility to educate the public about ST, 26 percent reported they forget to discuss it with their patients. This may be attributable, in part, to

Table 3
Dentists' attitudes about ST cessation counseling

Statement	Percentage Who Agree With Statement (n=529)
Counseling against tobacco is frustrating.	66%
Tobacco users will leave practice.	14
I am not interested.	16
It is not covered by insurance.	73
If health insurance paid for counseling, I would spend more time counseling tobacco users to quit.	52
I forget to bring it up.	26
I feel advice is not effective.	36
I feel tobacco users are not interested.	50
I have no time to counsel.	23
I have no training in counseling.	66
I am quite effective at getting patients to stop using tobacco.	9

the perception that ST does not represent a major health risk, for whatever reason, to their patients. Although 93 percent believed cigarette smoking to be very harmful to a smoker's health, only 74 percent believed ST to be very harmful to a user's health. Additionally, many found counseling on tobacco use to be frustrating. A lack of training in counseling was a prime reason for feeling ineffective as cessation counselors. A smaller percentage believed that tobacco users were not interested.

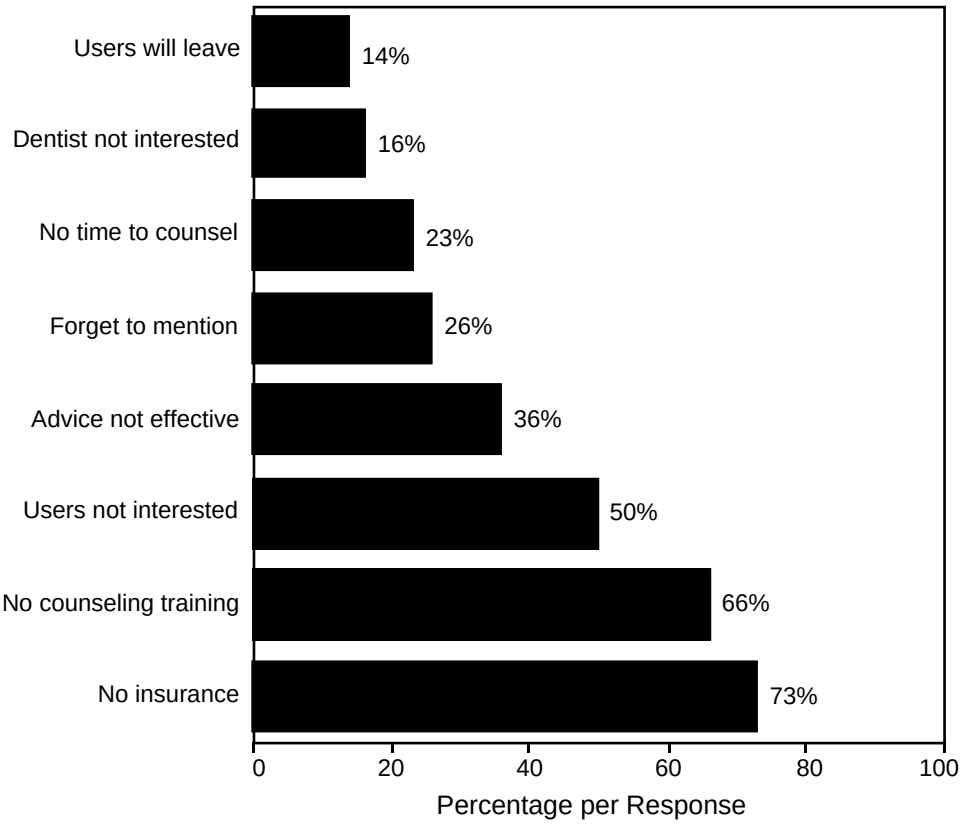
Another major barrier appeared to be a lack of insurance coverage for preventive counseling. Economic incentives through insurance coverage may promote increased counseling and prevention.

Dentists can have a more effective role in counseling patients about ST and smoking. Dentists are "ideally positioned to counsel against the use of cigarettes and smokeless tobacco products." The dental encounter probably constitutes a teachable moment, when the patient is receptive to counseling about lifestyle issues. Past efforts by dentists to prevent oral disease ally them with the preventive health care movement; active counseling in other areas seems an appropriate adjunct. Because many adolescents and adults visit the dentist each year, the dental visit is an excellent opportunity to reach a captive audience (Gerbert et al., 1989; Schroeder, 1989).

The results of this study seem to warrant the following steps:

- *Promotion of counseling courses in dental schools and continuing education*—In our study, 66 percent of the respondents reported inadequate counseling education. Access to counseling courses would enhance the dental practitioners' confidence in their ability to counsel effectively.

Figure 1
Dentists' barriers to ST cessation counseling



- *Promotion of the use of dental staff for cessation counseling*—Dental hygienists and assistants have equal or greater access to the dental patient (Fried and Rubinstein, 1990). Delegation of counseling duties to staff would be cost-effective and result in additional time for other dental procedures by the dentist.
- *Reimbursement for preventive counseling by insurance companies*—Costs for private practitioners continue to escalate because of factors such as infection control and Occupational Safety and Health Administration guidelines. Most practitioners will hesitate to spend time counseling without appropriate remuneration.
- *Marketing all tobacco use as offensive and harmful*—Although education about ST has been increasing, some dentists still view ST as a safe alternative to smoking. This is evident among the participating dentists in the present survey. While 93 percent believed cigarette smoking to be very harmful, fewer believed ST to be very harmful.

Among the indicators of the impact of P.L. 99-252 on decreasing ST use is the presence of curricula on ST. This potential means of evaluation of the impact of the law indicates that the curriculum directors, not only in

Table 4
Exam-related counseling practices

Practice	Percentage Who Perform Practice (n=529)
I ask specific questions regarding my patients' tobacco habits on health history.	35%
I conduct a thorough soft tissue exam on all patients.	88
I conduct followup visits on suspected initiated lesions.	82
I inform about caries, stains, gingivitis, and soft tissue changes due to ST use.	90
I screen for blood pressure on all patients.	17

primary and secondary education (Chen et al., 1991) but also in dental and medical schools (Fried and Rubinstein-DeVore, 1990; Geboy, 1990; Schroeder, 1990), consider smokeless tobacco, tobacco in general, and programs on cessation counseling of sufficient importance that faculty should include these topics in courses.

This study reveals the importance of addressing tobacco use cessation education in dental schools and dental hygiene programs in the future, and the important role the oral health practitioner can play in controlling tobacco use. The potential for dentists as tobacco cessation counselors and for prescribing of alternative nicotine delivery systems, such as nicotine gum, as an adjunct to their counseling should not be overlooked.

ACKNOWLEDGMENTS The authors thank Dr. Steven Margulies and Lawrence Hill for their assistance with the survey and preparation of the manuscript.

REFERENCES

ADA News. FTC issues rules on smokeless. *ADA News* 17(22): 1, 1986.

American Academy of Otolaryngology-Head and Neck Surgery. Smokeless Tobacco: Is It Worth the Risk? (Videotape) 1990.

American Dental Association. Smokeless Tobacco: The Risks. (Videotape) 1990a.

American Dental Association. Smokeless Tobacco: The Problem. (Videotape) 1990b.

Archard, H.O., Tarpley, T.M. Clinicopathologic and histochemical characterization of submucosal deposits in snuff dipper's keratosis. *Journal of Oral Pathology* 1: 3-11, 1979.

Axéll, T., Mörnstad, H., Sundström, B. The relation of the clinical picture to the histopathology of snuff dipper's lesions in a Swedish population. *Journal of Oral Pathology* 5: 229-236, 1976.

Centers for Disease Control. Smokeless Tobacco Education Resources. Atlanta, GA: CDC, 1986.

Chen, M.S., Schroeder, K.L. Analysis of print and audiovisual materials to prevent smokeless tobacco use. *Journal of Cancer Education* 2(4): 239-245, 1987.

Chen, M.S., Schroeder, K.L. An epilogue to evaluating the impact of P.L. 99-252 on decreasing smokeless tobacco use. *Journal of Public Health Dentistry* 50(1): 1-4, 1990.

Chen, M.S., Schroeder, K.L., Glover, E.D., Bonaguro, J., Capwell, E.M. Tobacco use prevention in the national school curricula: Implications of a stratified random sample. *Health Values* 15(2): 3-9, 1991.

Christen, A.G. The case against smokeless tobacco: Five facts for the health professional to consider. *Journal of the American Dental Association* 101(9): 464-469, 1980.

Christen, A.G., McDaniel, R.K., Doran, J.E. Snuff dipping and tobacco chewing in a group of Texas college athletes. *Texas Dental Journal* 97: 6-10, 1979.

Connolly, G.N., Winn, D.M., Hecht, S.S., et al. The reemergence of smokeless tobacco. *New England Journal of Medicine* 314(16): 1020-1027, 1986.

Edwards, S.W., Glover, E.D., Schroeder, K.L. The effects of smokeless tobacco on heart rate and neuromuscular reactivity in athletes and non-athletes. *The Physician and Sportsmedicine* 15(7): 141-147, 1987.

Fried, J.L., Rubinstein, L. Attitudes and behaviors of dental hygienists concerning tobacco use. *Journal of Public Health Dentistry* 50(3): 172-177, 1990.

Fried, J.L., Rubinstein-DeVore, L. Tobacco use cessation curricula in U.S. dental schools and dental hygiene programs. *Journal of Dental Education* 54(12): 730-735, 1990.

Geboy, M.J. Dental school-based continuing education in tobacco use cessation counseling for oral health care providers. *Journal of Dental Education* 54(12): 736-738, 1990.

Gerbert, B., Coates, T., Zahn, E. Dentists as smoking cessation counselors. *Journal of the American Dental Association* 118(1): 29-32, 1989.

Greer, R.O., et al. Smokeless tobacco-associated oral changes in juvenile, adult and geriatric patients: Clinical and histomorphometrical features. *Gerodontics* 2(3): 87-98, 1986.

Greer, R.O., Poulson, T.C. Oral tissue alterations associated with the use of smokeless tobacco by teenagers. *Oral Surgery, Oral Medicine, Oral Pathology* 56(3): 275-284, 1983.

Greer, R.O., Schroeder, K.L., Crosby, L. Morphologic and immunohistochemical evidence of human papillomavirus capsid antigen in smokeless tobacco keratoses from juveniles and adults. *Journal of Oral and Maxillofacial Surgery* 46: 919-929, 1988.

Gritz, E.R., et al. Plasma nicotine and cotinine concentrations in habitual smokeless tobacco users. *Clinical Pharmacology Therapy* 30(2): 201-209, 1981.

Hirsch, J.M., et al. A clinical histomorphological and histochemical study on snuff-induced lesions of varying severity. *Journal of Oral Pathology* 11: 387-398, 1982.

Hoffman, D., et al. Carcinogenic agents in snuff. *Journal of the National Cancer Institute* 76: 435-436, 1986.

Hoge, H.W., Kirkham, D.B. Clinical management and soft tissue reconstruction of periodontal damage resulting from habitual use of snuff. *Journal of the American Dental Association* 107: 744-745, 1983.

McGuirt, W.F. Smokeless tobacco usage: A historical review and discussion of its health implications. Slide Lecture Series, American Academy of Otolaryngology-Head and Neck Surgery Foundation, Alexandria, VA, 1990.

Mecklenburg, R.E. Tobacco in America in the 1990's: The role of dentists, the dental team and organized dentistry. In: S*moking Cessation: Helping Dental Patients To Quit Smoking. Proceedings of the First National Dental Symposium on Smoking Cessation*, A.G. Christen and J.L. McDonald (Editors). Chicago: American Dental Association, 1989.

Neal, C.J., Schroeder, K.L., Wenrick, C.J. Effects of smokeless tobacco on peripheral cardiovascular flow in young male adults. *Journal of Dental Research* 67: 274, 1988.

Offenbacher, S., Weathers, D.R. Effects of smokeless tobacco on the periodontal mucosal and caries status of adolescent males. *Journal of Oral Pathology* 14: 169-181, 1985.

Park, N.-H., Niukian, D., Shklar, G. Combined effects of herpes simplex virus and tobacco on the histopathologic changes in lips of mice. *Oral Surgery, Oral Medicine, Oral Pathology* 59(2): 154-158, 1985.

Pindborg, J.J., Renstrup, G. Studies in oral leukoplakias: Effect of snuff on oral epithelium. *Acta Dermato-Venereologica* (*Stockholm*) 43: 271-276, 1963.

Poulson, T.C., Lindenmuth, J.E., Greer, R.O. A comparison of the use of smokeless tobacco in rural and urban teenagers. *CA—A Cancer Journal for Clinicians* 34: 248-261, 1984.

Roed-Petersen, B., Pindborg, J.J. A study of Danish snuff-induced oral leukoplakias. *Journal of Oral Pathology* 2: 301-313, 1973.

Schroeder, K.L. Oral and systemic concerns with smokeless tobacco. In: *Clark's Clinical Dentistry* (volume 2), J. Hardin (Editor). New York: J.B. Lippincott, 1989.

Schroeder, K.L. P.L. 99-252: Implications for dentists and their clinical practice. *Journal of Public Health Dentistry* 50(1): 1-6, 1990.

Schroeder, K.L., Chen, M.S. Smokeless tobacco and blood pressure. *New England Journal of Medicine* 312(14): 919, 1985.

Schroeder, K.L., Chen, M.S., Jr., Iaderosa, G.B., Glover, E.D., Edmundson, E.W. Proposed definition of a smokeless tobacco user based on potential nicotine consumption. *Addictive Behaviors* 13: 395-400, 1988a.

Schroeder, K.L., Chen, M.S., Kuthy, R.A. Smokeless tobacco: The new thing to chew on. *Ohio Dental Journal* 59(178): 11-14, 1985.

Schroeder, K.L., Glover, E.D., Zebracki, M. Comparison of snuff and cigarettes on cardiovascular hemodynamics in young adults. In: *Tobacco and Health 1990: The Global War. Proceedings of the Seventh World Conference on Tobacco and Health*, B. Durston and K. Jamrozik (Editors). Perth: Cancer Foundation of Western Australia, 1990, pp. 412-415.

Schroeder, K.L., Heisel, D.E., Margulies, S., Williams, D. Dentists and dental students knowledge and practices regarding smoking and smokeless tobacco. *Journal of Dental Research* 69: 1005, 1990.

Schroeder, K.L., Neal, C.J., Chen, M.S. Alteration in cardiovascular hemodynamics from smokeless tobacco use in young adult males. *Journal of Dental Research* 67: 274, 1988b.

Schroeder, K.L., Soller, H.A., Chen, M.S., Neal, C.J., Glover, E.D. Screening for smokeless tobacco-associated lesions: Recommendations for the dental practitioner. *Journal of the American Dental Association* 116: 37-42, 1988c.

Squier, C.A. Smokeless tobacco and oral cancer: A cause for concern? *CA—A Cancer Journal for Clinicians* 34(5): 242-247, 1984.

Squires, W.G., et al. Hemodynamic effects of oral smokeless tobacco in dogs and young adults. *Preventive Medicine* 13: 195-206, 1984.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986.

U.S. Department of Health and Human Services. *Beat the Smokeless Habit: Game Plan for Success*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 92-3270, 1991.

Van Wyk, C.W. Oral lesions caused by habits. *Forensic Science* 7: 41-49, 1976.

World Health Organization Collaborating Center for Oral Precancerous Lesions. Definition of leukoplakia and related lesions: An aid to studies on oral precancer. *Oral Surgery* 46(4): 518-539, 1978.

An Intervention Study of Tobacco Habits Among Rural Indian Villagers[1]

Mira B. Aghi, P.C. Gupta, F.S. Mehta, and J.J. Pindborg

ABSTRACT In a house-to-house screening survey, 36,000 tobacco chewers and smokers were selected from the rural population in three districts of three States in India—Kerala, Andhra, and Gujarat. These individuals were interviewed about their tobacco habits and examined for the presence of precancerous lesions in a baseline survey and then annually over 9 yr. They were educated through personal communication as well as mass media to give up their tobacco habits. The results have indicated consistently that it is possible to induce changes in the tobacco habits of rural populations through educational efforts. This is further substantiated by a significant decrease in the incidence of precancerous lesions. As most cancers in India are reported to be preceded by precancer, this study demonstrates that the primary prevention of oral cancer is feasible, effective, and practicable.

INTRODUCTION India is afflicted with the problem of oral cancer. The incidence of oral cancer in India is higher than the incidence of most other cancers (National Cancer Registry, 1988). The relationship between oral cancer and tobacco habits is established as much beyond doubt as the relationship between cigarette smoking and lung cancer, although it may not be as much publicized as the latter. Thus, it appears logical that one way oral cancer can be controlled is through intervention in the tobacco habits of people. The disease can be arrested and cured by treatment, but an epidemic would be impossible to control if tobacco habits were not checked.

As in the case of lung cancer, it is yet not known how much tobacco use would actually result in oral cancer. It seems improbable that such a guideline will be available in the near future. Therefore, it appears that our best bet would be to help people give up the tobacco habit. The primary health care delivery system ought to include such education.

The question then arises: How do we design such a system? Not all forms of health care delivery meet with success. Unless a health care delivery system is designed in consideration of the target population, their habits, beliefs, lifestyles, and knowledge, the system may be ineffective.

The Tata Institute of Fundamental Research has specifically designed delivery of health care services to help people stop their tobacco habits. The communication strategies were developed with the full involvement of the target population. The project study went through two phases before it undertook to intervene in the people's tobacco habits. Phase I was a series of cross-sectional field surveys undertaken to determine the prevalence rates of oral precancerous lesions and their association with tobacco habits. The total population surveyed was more than 155,000 (Bhonsle et al., 1976; Mehta et al., 1969 and 1972). Phase II of the project was a 10-yr followup

[1] This research was supported solely by funds from the National Institutes of Health under PL-480 research agreement no. 01-022-N.

study of 30,000 of the original study population. The results obtained in Phases I and II established that (1) oral cancer and precancerous lesions occurred almost solely among those who smoked or chewed tobacco and (2) oral cancer was almost always preceded by some kind of precancerous lesions (Gupta et al., 1980).

On the basis of these results, an intervention study was launched. The objectives of the study were to make people give up the tobacco habit and to investigate any effect this might have on the incidence and regression rates of precancerous lesions and thus on controlling oral cancer.

In the present study, the intervention efforts have been implemented through a carefully designed comprehensive program in a specifically delineated target population. Every individual in the target population was examined annually to measure changes in the incidence and regression patterns of the disease. The intervention program was carried out by a team of professional dental surgeons and social scientists who were given special training in conducting in-depth interviews, approaching and interacting with the tobacco users in a considerate, sympathetic, warm, and positive manner without condescending in any way.

MATERIALS AND METHODS

Study Population

The study population was selected from Ernakulam district in the State of Kerala, Bhavnagar district in the State of Gujarat, and Srikakulam district in the State of Andhra Pradesh. A large number of individuals were screened, and about 12,000 tobacco users aged 15 and over were selected in each district. The tobacco habits practiced in the study included smoking cigarettes, smoking *bidis*, smoking *chutta* in conventional and reverse manner, and chewing tobacco with or without betel quid (Aghi, 1989).

Intervention Methods

A program of intervention was developed after appropriate pilot and pretesting surveys. In-depth interviews of the participants were conducted to investigate (1) the reasons for their starting and continuing the tobacco habit (such as when and how the habit started, who encouraged it, was it a peer or a parent?); (2) the perceived implications of the habit in its social, economic, and health aspects; and (3) possible reasons they would give up their habits (Aghi, 1987).

In addition, pilot surveys were conducted to assess the communication media facilities available to the target population that could be used to convey the intervention message. The intervention program outlined a timetable for employing different media of communication and regulating the flow of information so as not to overwhelm the target population. Pilot studies also helped to modify the messages whenever necessary, with a view to making them easily understandable. All this was an ongoing process for every followup.

This paper comprises the results of 10 yr. Each followup used strategies of communicating messages to the target population that assisted and enabled them to give up the tobacco habit. The unique feature of this project has been its user-based philosophy, which is characterized by appropriate input, provided according to the stage of the subject, as follows:

- Knowledge regarding association of tobacco habits and oral cancer was imparted.
- This knowledge was further strengthened by visual reinforcement through flipcharts, posters, films, etc., and a small amount of fear was deliberately induced.
- Knowledge of the health benefits and other advantages of quitting the habit were conveyed; for example, regression of lesions and saving of money.
- Various possible methods of discontinuing the tobacco habit, such as cold turkey, gradual reduction, postponing the first smoke or quid, etc., were described and the most appropriate one for the subject was suggested, depending on the psychological profile of the subject and characteristics of the habit.
- Withdrawal symptoms were explained and emphasis placed on their temporary nature.
- Appropriate praise and reinforcement were given and leadership roles suggested for successful quitters.

Modes of Communication

Personal communication involved one-to-one contact with the target population with a view to helping them sort out doubts and learn behaviors that would result in abstaining from tobacco. The steps in personal communication were worked out in a logical order, recognizing the dynamics of human psychology and what it takes for people to make decisions and change their attitudes and behavior. Certain visual aids were used in the personal communication.

Personal Communication

Films

Two films were produced specifically for this phase of the study. The objective of the first film was to give information and create awareness of the relationship between tobacco habits and oral cancer. The objective of the second film was to point out how habits are taken up and developed. In addition, this film presents ways the habits can be given up. Film is a very powerful medium for creating awareness and, when effectively utilized, works to motivate behavior changes.

Posters

Posters were displayed to remind the target population that they ought to be reconsidering their tobacco habits. Two kinds of posters were used, one with a written message only and the other with a visual display along with a written message. Slides were prepared from the posters and were projected in movie theaters in the vicinity to serve the same objective. In addition, handwritten posters summarizing the findings about the particular village were left behind to remind subjects that they had been examined for oral cancer.

Newspaper Articles

Articles were published in newspapers to inform and educate people about oral cancer. It was known that many members of the target population do not read; nevertheless, the articles could be read by some, including schoolchildren, who have been found to be important agents of change in rural India.

Other Media There were folk drama enactments with the objective of talking about the prevalent superstitions and misconceptions about tobacco. In addition, radio programs were designed to motivate people to give up their tobacco habits in light of the fact that they might lead to oral cancer. The programs were used to create an environment to help the target population think about their tobacco habits.

Cessation Camps In the last two followups, we held cessation camps to address the demand of our target population, who told us that they believed what we said about tobacco being harmful and that they would like to give it up; they wanted to know how to do it. In the cessation camps, detailed discussions were held to suggest the solutions to the problems encountered in discontinuing the tobacco habit. Regular daily followup was maintained for a few days after the camps were held.

Other Modes In addition to the above methods, clinics were run to address dental complaints and thereby try to eliminate at least some of the reasons given by users for practicing the habit. Also, exhibits and group meetings were conducted to show common lesions and hold discussions that might help the target population to ask questions and express their feelings and doubts.

Assessment of Methods The various methods of communication were assessed individually through carefully designed questionnaire-based sample studies for their impact relative to their objectives. Since all of the communication methods could impinge on an individual's decision to quit or reduce tobacco use, it is hard to ascertain which actually led to the decision. A rank ordering of the various inputs by the target population revealed that subjects have been helped the most by one-to-one interaction; cessation camps had also aided them immensely.

DISCUSSION

The results of the study have been consistently positive (Table 1). Considering that the target population was not innately motivated to give up tobacco use, it is quite remarkable that such successful results were obtained.

A detailed assessment of the project was made after 1 yr and after 5 yr. After 1 yr, we found that the regression of lesions was significantly higher among those who had stopped or reduced their tobacco use than among individuals who did not do so (Mehta et al., 1982). After 5 yr, it was found that a significantly higher percentage of individuals had stopped and reduced their tobacco use than in the control cohort. As a consequence of this increased stoppage and reduction in tobacco use, the incidence of oral precancerous lesions decreased significantly and substantially in two of the three study areas (Gupta et al., 1986b). Through a multiple logistic regression analysis, it was shown that the intervention efforts definitely helped people stop their tobacco habits (Gupta et al., 1986a).

Some new questions now arise: Where do we go from here? How can this project be used as a takeoff point for intervention at the national level in India?

Table 1
The cessation of tobacco use in successive followups

	Percentage Stopping Tobacco Use		
	Bhavnagar	Ernakulam	Srikakulam
First Followup	4.1%	2.0%	4.9%
Second	7.6	4.4	10.9
Third	10.5	6.4	14.2
Fourth	11.6	7.6	15.4
Fifth	12.5	9.4	16.6
Sixth	14.1	11.2	17.0
Seventh	11.5	11.6	17.3
Eighth	12.2	12.3	17.4
Ninth	12.7	13.1	18.1
Tenth	13.6	14.0	18.8

We have examined the feasibility of training basic health care workers to examine mouths for early detection of both precancerous and cancerous lesions, and we have found this to be possible (Mehta et al., 1986). It would be indeed worthwhile to assess the possibility of training these health personnel to intervene in tobacco habits. If it is possible, the potential is great. Most of the strategies of communication that were utilized effectively in this project are those that involve the mass media or an approach that can be used on a large scale. Undoubtedly, this needs to be validated.

REFERENCES

Aghi, M.B. Psychosocial aspects of acquisition and cessation of tobacco habits in India. *World Smoking and Health Journal* 12(2): 4-7, 1987.

Aghi, M.B. The tobacco tradition in India. *World Health*, January-February, 1989.

Bhonsle, R.B., Murti, P.R., Gupta, P.C., et al. Reverse dhumti smoking in Goa: An epidemiologic study of 5,449 villagers for oral precancerous lesions. *Indian Journal of Cancer* 13: 301-305, 1976.

Gupta, P.C., Mehta, F.S., Daftary, D.K., et al. Incidence of oral cancer and natural history of oral precancerous lesions in a 10-year followup study of Indian villagers. *Community and Oral Epidemiology* 8: 287-333, 1980.

Gupta, P.C., Mehta, F.S., Pindborg, J.J., et al. Intervention of tobacco chewing and smoking habits (letter). *American Journal of Public Health* 76: 709, 1986.

Gupta, P.C., Mehta, F.S., Pindborg, J.J., et al. Intervention study for primary prevention of oral cancer among 36,000 Indian tobacco users. *Lancet* 1(8492): 1235-1239, 1986.

Mehta, F.S., Aghi, M.B., Gupta, P.C., et al. An intervention study of oral cancer and precancer in rural Indian populations: A preliminary report. *Bulletin of the World Health Organization* 60: 441-446, 1982.

Mehta, F.S., Gupta, P.C., Bhonsle, R.B., et al. Detection of oral cancer using basic health workers in an area of high oral cancer incidence in India. *Cancer Detection and Prevention* 9: 219-225, 1986.

Mehta, F.S., Gupta, P.C., Daftary, D.K., et al. An epidemiologic study of oral cancer and precancerous conditions among 101,761 villagers in Maharashtra, India. *International Journal of Cancer* 10: 134-141, 1972.

Mehta, F.S., Pindborg, J.J., Gupta, P.C., et al. Epidemiologic and histologic study of oral cancer and leukoplakia among 50,915 villagers in India. *Cancer* 24: 832-849, 1969.

National Cancer Registry. *Annual Report* 1985. New Delhi: Indian Council of Medical Research, 1988.

Chapter 7

Policy

CONTENTS

WHO Strategies To Curb Smokeless Tobacco: A Global Perspective

Roberto Masironi

ABSTRACT Smokeless tobacco is a leading cause of disease in many developing countries where its use is common, e.g., in the Indian subcontinent. Among the industrialized nations, Sweden and the United States show increasing consumption trends, particularly among the young. Unless its market expansion is stopped, smokeless tobacco may become a problem also in countries where it is still practically unknown. Tobacco manufacturers have launched marketing programs to promote the use of smokeless tobacco. The World Health Organization recommends that a preemptive ban on manufacture, import, promotion, advertising, and sale of smokeless tobacco be introduced in countries where the product is not yet known. In countries where smokeless tobacco is already widely used, various types of prohibition and control are urged, in keeping with local circumstances. Australia, Hong Kong, Ireland, Israel, New Zealand, Norway, Saudi Arabia, Singapore, and the United Kingdom have banned smokeless tobacco products. The European Economic Community countries are about to adopt similar policies.

INTRODUCTION The use of smokeless tobacco has become a leading cause of disease in some countries and may become so in others unless urgent preventive action is taken. Smokeless tobacco is widely used in the Indian-Pakistani subcontinent, where it is estimated that 100 million people are using it (International Union Against Cancer, 1989; WHO, 1988). Under various names and consisting of various combinations of tobacco and other ingredients, smokeless tobacco is also widely used in Afghanistan, Bangladesh, Malaysia, the Islamic Republic of Iran, the Central Asian republics, Sri Lanka, and Thailand (WHO, 1986 and 1988), as well as in Bhutan (Dr. Yok Heng Tamang, personal communication, 1991). It is used, although to a lesser extent than a few decades ago, by the native circumpolar populations of Alaska and the Canadian arctic, but not Greenland (Hart Hansen et al., 1990). Among the industrialized countries, Sweden has the highest per capita consumption of smokeless tobacco, with 17 percent of its population using it at least occasionally and almost 30 percent of young adult males using it daily (Nordgren and Ramström, 1990). In the United States, more than 12 million people use smokeless tobacco, and 12 percent of young adult males are daily users (Connolly, 1991; WHO, 1988).

In the rest of the world, however, smokeless tobacco is almost unknown. In central and eastern Europe, nasal snuff was used in past centuries but now very little of it is marketed. In Australia, smokeless tobacco represents a minuscule 0.01 percent of all tobacco products consumed, and its use is estimated to be limited to between 3,000 and 5,000 people, mainly miners, oil riggers, farmers, and aborigines (Trade Practices Commission of Australia, 1989). In Japan, no smokeless tobacco is produced, and only a small amount is imported (Dr. M. Chiba and S. Watanabe, letters, 1991). In China, it is also practically unknown; very few people chew tobacco, and nasal snuff is used only by some local inhabitants of Tibet (Prof. Weng Xin-zhi, letter, 1991). Unlike tobacco smoking, which is widespread in all countries of the world, the use of smokeless tobacco is extremely uneven, being used extensively in some countries but virtually unknown in many others.

Tobacco manufacturers have launched well-orchestrated marketing programs to promote the use of smokeless tobacco in countries where the product is unknown. For instance, a tobacco company opened a factory in Scotland in 1985 to produce moist snuff for sale to other countries of Europe, Africa, and the Middle East. The factory was later closed as a result of pressure from health groups (WHO, 1988). The same company had established a regional office in Hong Kong to promote sales in the Pacific area and in Asia; this office also closed in 1987 as a result of a ban on smokeless tobacco by the Hong Kong government that year (Dr. J. Mackay, letter, 1991).

The recent manufacture and promotion of new forms of smokeless tobacco by transnational tobacco companies has increased the need for urgent action to prevent the spread of the ST habit to areas where it is currently unknown. Of particular concern is the fact that some new forms of smokeless tobacco are being marketed in ways that appeal to children and young people. In Denmark, for instance, 60 percent of children know what smokeless tobacco is, and 11 percent of 15-yr-olds, particularly boys, have tried it (Commission of the European Communities, 1991). While decreasing among adults, ST use is rising among young males in some developed countries (see Tables 1 and 2).

In Finland, snuff use had almost disappeared by the mid-1970's. After enhanced marketing efforts by national tobacco companies, smokeless tobacco consumption increased from 43,000 kg in 1981 to 105,000 kg in 1989. Users are mainly young people, particularly 16- to 18-yr-old boys. In a national survey done in 1987, about 5 percent of boys reported occasional or daily use (WHO [EURO], 1991).

WHO ACTION In the face of an impending new epidemic of tobacco-related diseases, and following reports on the health hazards of smokeless tobacco (IARC, 1986; US DHHS, 1986), the World Health Organization called together an international group of experts to review the evidence and propose strategies for national and international control of smokeless tobacco. The recommendations of that group are summarized here. According to the WHO expert group, the major objectives of any national smokeless tobacco control program should be the following (WHO, 1988):

- In countries where smokeless tobacco is not known yet, prevent its introduction, with special emphasis on preventing its use by children.
- In countries where smokeless tobacco is already in use, prevent any increase, and reduce the prevalence of its use in the population.
- In all countries, establish and maintain a social climate unfavorable to smokeless tobacco use.

The most effective means of preventing the emergence of new tobacco-related problems in any country is to prevent the introduction of new tobacco products rather than to allow them to be introduced and take action only after the resultant health problems have become apparent.

Table 1
U.S. smokeless tobacco use

	Percentage of Users	
	Teenaged Males	50-Yr-Old Males and Females
1970	0.3%	2.7%
1980	2.7	1.3
1985	6.0	—

Table 2
ST use in Sweden

	Percentage of Users			
	Teenaged		55-Yr-Old	
	Males	Females	Males	Females
1955	0%	0%	21%	0%
1986	38	5	13	1

Health education and public information are important components of any national tobacco control strategy, but the most important preventive measure is *legislation*. Voluntary agreements are ineffective, because tobacco companies are likely to circumvent them. Because of the relatively large variety of smokeless tobacco products and different historical patterns of use, laws are enacted differently in different countries. Certain types of ST products are banned in some countries, while other types are only restricted, and still others remain unaffected. Table 3 contains a summary of the situation in various countries, to the extent we could ascertain it from a review of available legislative texts (WHO, 1991).

AREAS OF LEGISLATION

The same arguments that apply to legislation for the control of smoking also apply to smokeless tobacco. The possibility of adopting legislation to control smokeless tobacco is actually more favorable than that for the control of smoking. Indeed, while it is not possible to ban cigarette smoking completely, since the use of cigarettes is too deeply ingrained in the socioeconomic structure of most countries, there is an opportunity to legislate against the introduction of smokeless tobacco products in the many nations where they are not yet on the market.

Smokeless tobacco is not only a health problem of individual countries, but an international problem as well. Fortunately, the experience gained in more than 30 years of smoking control activities worldwide can be drawn upon for planning and implementing smokeless tobacco control programs. Some nations have already benefited from the experience of other nations in dealing with smokeless tobacco. For instance, the governments of Hong Kong, Ireland, Israel, New Zealand, and some Australian states enacted

Table 3
Legislative action to control smokeless tobacco

Country	Year	Banned Import, Manufacture, Sale, and/or Promotion
Ireland	1985	Moist snuff only
United Kingdom	1986	All smokeless tobacco sale to minors
Israel	1986	All smokeless tobacco
Hong Kong	1987	All smokeless tobacco (except nasal snuff)
New Zealand	1987	All smokeless tobacco
Singapore	1987	All smokeless tobacco (except nasal snuff)
Tasmania	1986	All smokeless tobacco (except nasal snuff)
S. Australia	1986	"Sucking tobacco" (no other types)
Victoria	1987	All smokeless tobacco (except nasal snuff)
W. Australia	1987	All smokeless tobacco (except nasal snuff)
Australia (nationwide)	1989	Oral snuff
United States	1987	Sale only of all smokeless tobacco to minors
Norway	1989	"New" types of tobacco products (no ban on "traditional" chewing and snuff)
United Kingdom	1990	Moist snuff (no ban on nasal snuff and chewing tobacco) (overturned in December 1990, awaiting new ruling)
Saudi Arabia	1990	Chewing and moist snuff
China (Taiwan)	1990	All smokeless tobacco
E.E.C.	1990	Moist snuff (to be enforced beginning in 1992)

legislation from 1985 through 1987 to prohibit the importation, manufacture, advertising, promotion, and sale of smokeless tobacco products (WHO, 1988). Other countries have learned from this experience: Norway, Saudi Arabia, Singapore, the United Kingdom, other Australian states, and Taiwan have recently banned smokeless tobacco products (BASP, 1990; Connolly, 1991; Trade Practices Commission of Australia, 1989). E.E.C. countries other than the United Kingdom are about to adopt the same policy (BASP, 1990).

Mongolia and China are in the process of adopting legislation to ban or restrict ST use (Dr. J. Mackay, letter, 1991). A new law on the control of tobacco hazards in the People's Republic of China has been drafted to read, "All importation and production of chewing tobacco is banned" (Prof. Weng Xin-zhi, letter, 1991).

According to the WHO expert group, legislation to control smokeless tobacco is most effective in the following areas: ban or control of manufacture, import, promotion, and sale; taxation and other economic disincentives; restriction on use in public places and places of work; and health warnings. These are described in more detail below.

Ban or Control Of ST Commerce In countries where smokeless tobacco is not yet used, steps should be taken to prohibit by law the manufacture, import, promotion, and sale of ST products. In these countries, there is an opportunity, which may never be repeated, to prevent such products from coming into use. This approach, called a preemptive ban, has been widely publicized by the World Health Organization through a worldwide press release (WHO, 1987).

In countries where smokeless tobacco use is already too well established for a comprehensive ban of this nature to be feasible, legislation should at least prohibit promotion of smokeless tobacco products through

- Direct and indirect advertising in all its forms;
- Sponsorship of sporting, artistic, and other events and media programs;
- Distribution of free samples of ST products;
- Use of sports and other popular personalities;
- Promotion of other products of the same name, packaging, and design, including items like T-shirts and toys; and
- Any other form of promotion, including those introduced from outside the country.

If advertising of smokeless tobacco products cannot be completely prohibited, it should at least be restricted by law to ensure the following:

- The impression is not given that ST is a safe alternative to cigarette smoking;
- Misleading links do not appear established between ST and positive values like success, youth, sports, open air, and the like;
- Advertising is restricted as to the type of media used, the size and layout of pictorial material, and so forth; and
- Health warnings appear prominently on ST packaging and on any advertising and promotional material.

In countries where smokeless tobacco products are sold, sales to minors should be prohibited by law. Vending machines should be banned or allowed only in areas where unaccompanied minors have no access.

In many developing countries, smokeless tobacco is a cottage industry and products are generally prepared by small local stores. In these countries, it is particularly important that major manufacturing industries are not permitted to develop. Priority should, at the same time, be given to identifying and promoting replacement crops that will provide profitable employment and economic substitutes for tobacco.

Taxation and Other Economic Disincentives

The World Health Organization has always advocated the use of increased taxation as a part of comprehensive programs for the control of both smoking and ST products. There is sound evidence that taxation can be used to discourage young people from starting to use tobacco, and to encourage users to discontinue the habit, without decreasing government revenue. Ideally, a proportion of the tax increase should be used to finance health education programs. If it cannot be prohibited altogether, the importing of ST products can be discouraged through high import duties.

No government subsidies should be provided for any form of tobacco growing, manufacturing, sale, or export. If this is not possible, the World Health Organization recommends that governments ensure that no new form of tobacco becomes eligible for subsidies.

Restrictions On ST Use Although not because of the same health criteria as those that relate to passive smoking, the use of smokeless tobacco may nevertheless be unpleasant to nearby non-users. Restrictions on ST use may be justified in light of health and cleanliness problems associated with spitting and the disposal of chewed tobacco.

Health Warnings In countries where the sale and promotion of smokeless tobacco products are still allowed, health warnings should be mandatory on packages of such products as well as on any related advertising and promotional material. Similar to the rotating health warnings that appear on cigarette packages, ST products should carry health warnings about the harmful health effects associated with use of the products. Examples are shown in Table 4.

Table 4
Examples of health warnings on ST packages and advertisements

Country	Warning
France	Dangerous if abused.
Iceland	Snuff and chewing tobacco may damage the mucous membranes.
Ireland	This product may cause oral cancer.
Portugal	Tobacco damages health and, in particular, causes cancer.
Greece	Tobacco damages your health.
Sweden	Warning: Snuff and chewing tobacco contain nicotine. Therefore, snuff produces just as strong a dependence as tobacco smoking. The buccal cavity, mucous membranes and gums can be damaged and may require treatment.
United States	Warning: This product may cause mouth cancer.
	Warning: This product may cause gum disease and tooth loss.
	Warning: This product is not a safe alternative to cigarettes.

PRESENT ACTION In addition to the measures recommended by the WHO group of experts (WHO, 1988), further action is now under way, particularly in Europe. At the First European Conference on Tobacco Policy, held in Madrid under the auspices of both the World Health Organization and the E.E.C., 10 strategies for a tobacco-free Europe have been recommended (WHO [EURO], 1989). One of these reads, "To prohibit new methods of nicotine delivery, and to block future tobacco industry marketing strategies." This is a clear mandate to stop the development of a new hazardous addiction in Europe.

The countries in eastern Europe represent a special case, as they are now facing a new tobacco-related danger. These nations are emerging under new sociopolitical systems involving free-market economies. The transnational

tobacco companies are already exerting marketing and promotional pressure on these countries, as was revealed at the conference, "A Tobacco-Free New Europe," held in Kazimierz, Poland, November 21 through 23, 1990, under the auspices of WHO and the International Union Against Cancer. One recommendation of the conference was that governments of eastern European countries be requested to ban the introduction of smokeless tobacco, which is practically unknown in these nations (International Union Against Cancer, 1991). The E.E.C. is also taking action. The Commission of the European Communities has issued a directive that, if approved, would require member states to ban the import, manufacture, and sale of moist snuff starting in March 1992. Moist snuff is already prohibited from import into Switzerland, which is not an E.E.C. country (Swiss Federal Office of Public Health, 1987; Dr. B. Meili, letter, 1991).

In conclusion, it can be said that action is now being taken, both nationally and internationally, to stem the spread of ST use. Until several years ago, there was no legislation specifically to restrict smokeless tobacco; now an increasing number of countries have adopted legislation for that purpose.

REFERENCES

BASP, European Bureau for Action on Smoking Prevention. *A New Form of Smokeless Tobacco: Moist Snuff*. Commission of the European Communities, Europe Against Cancer Programme, Brussels, 1990.

Commission of the European Communities, Europe Against Cancer Programme. "Skoal Bandits" et autres "Catch" au Danemark. *Information Circular*, 12 March 1991, Brussels.

Connolly, G.N. Health effects and regulations of smokeless tobacco products. In: *A Tobacco-Free New Europe: Proceedings of an International Union Against Cancer Conference, Kazimierz, Poland, 21-23 November 1990,* M.Wood and W. Zatonski (Editors). Belfast: Ulster Cancer Foundation, 1991.

International Agency for Research on Cancer. *Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985.

International Agency for Research on Cancer. *Tobacco, A Major International Health Hazard*, D. Zaridze and R. Peto (Editors). IARC Scientific Publication No. 74. Lyon, France: IARC, 1986.

International Union Against Cancer. *Tobacco and Health: Proceedings of a Workshop, Bombay, 15-16 April 1987*. L.D. Sanghvi and P. Notani (Editors). Geneva: International Union Against Cancer, 1989.

International Union Against Cancer. *A Tobacco-Free New Europe. Proceedings of an International Union Against Cancer Conference, Kazimierz, Poland, 21-23 November 1990*, M.Wood and W. Zatonski (Editors). Belfast: Ulster Cancer Foundation, 1991.

Nordgren, P., Ranström, M. Moist snuff in Sweden: Tradition and evolution. *British Journal of Addiction* 85: 1107-1112, 1990.

Peterson, J.S., et al. Smokeless tobacco. In: *Tobacco and Health Proceedings of the International Union of Health Education/World Health Organization Circumpolar Conference on Tobacco and Health, Yellowknife, NWT, Canada, April 4-6 1989*, J.P. Hart Hansen, B. Harvald, and E.M. Nuutinen (Editors). *Arctic Medical Research* 49 (Suppl. 2): 32-38, 1990.

Swiss Federal Office of Public Health. *Ordonnance sur les Denrées Alimentaires*. Bern: Chancellerie Fédérale, 1987.

Trade Practices Commission of Australia. *Smokeless Tobacco Products*. Trade Practices Act 1974, Trade Practices Commission, Canberra, September 1989.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Insitutes of Health, National Cancer Institute. NIH Publication No. 86-2874, 1986.

World Health Organization. *WHO Study Group Calls for "Pre-Emptive Ban" on Smokeless Tobacco*. Press Release WHO/17. Geneva: WHO, June 9, 1987.

World Health Organization. *Smokeless Tobacco Control*. Technical Report Series No. 773. Geneva: WHO, 1988.

World Health Organization (EURO). *It Can Be Done: A Smoke-Free Europe. Report of the First European Conference on Tobacco Policy, Madrid, 7-11 November 1988*. Copenhagen: WHO Regional Office for Europe, 1989.

World Health Organization. *Legislative Responses to Tobacco Use*. Geneva: WHO, 1991.

World Health Organization (EURO). *Smokeless Tobacco in Finland*. Country Files, Tobacco or Health Programme. Copenhagen: WHO Regional Office for Europe, 1991.

Legal and Administrative Strategies for Control and Prevention of the Use of Smokeless Tobacco

Seamus O'Hickey

ABSTRACT The Irish government considers that the public health is more important than the economic effects of a fall in tobacco consumption over a period of years. For the past 25 years, the Department of Health has operated a strategy, based on education and legislation, to reduce demand for and supply of tobacco. Public support for the strategy has facilitated implementation of strong legal controls and prohibitions on tobacco sales and use. The importation, manufacture, and distribution of oral smokeless tobacco are totally banned by law in Ireland. The laws banning oral smokeless tobacco have twice been challenged in the courts by U.S. Tobacco International, Inc., with success in one case. Currently, the ban is in place but awaits final judgment. National laws of European Community Member States are subject to E.C. legislation, which is based on the Treaty of Rome. Irish laws and decisions of Irish courts therefore have a wider, European influence. The E.C. and some of its Member States have adopted Irish antitobacco legislation as a model.

INTRODUCTION

The legal prohibition of commerce in oral smokeless tobacco in Ireland arose initially from official action by the U.S. Public Health Service in the early 1980's, which was a response to rising public concern in the United States about the increasing use of the product there. Through the agency of organizations such as the International Chief Dental Officers' Conference of the International Dental Federation, supported by the World Health Organization's Oral Health Unit, public health dental officers around the world were alerted by their American colleagues to the potential and serious consequences to the health of the public if the American experience were to be exported to and repeated in other countries.

Accordingly, in 1985 the Irish Minister for Health made a decision to ban oral smokeless tobacco in the moist snuff form. This decision received support shortly thereafter in the U.S. publication of *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General* (US DHHS, 1986).

HISTORY

For a quarter of a century, the Department of Health in Ireland has been engaged in developing and implementing programs designed to reduce the supply of tobacco products and the demand for them.

Tobacco use is the single most significant cause of death in middle age in Ireland. Illnesses resulting from tobacco impose an enormous strain on the health services. Among a total population of 3.5 million people, about 0.5 million days per year are spent in hospital as a result of tobacco-related illnesses, at a cost of more than £15 ($25) per year for every person in the country (Lyons, 1988). Other health care costs, disability costs, and costs of time lost from work place an equally high burden on the economy.

The Irish government, having examined and considered all aspects and implications of the tobacco issue, decided that public health considerations outweigh by far the economic impact that a reduction in tobacco consumption would cause over a number of years.

STRATEGY The Department of Health has implemented its antitobacco policy by means of a double-pronged strategy. From the start, an education and information program has been conducted in various ways through the mass media, the educational system, and official and voluntary agencies. The second part of the strategy is the legislative program by which legal controls and/or prohibitions are in place with respect to tobacco advertising, sponsorship, promotion, labeling, smoking in public buildings and offices, and selling tobacco products to children and young people.

Advertising of tobacco on television was prohibited in 1971 and on radio in 1975. The education and information program increased the public's knowledge about the hazards associated with tobacco, and this in turn led to widespread public support for measures to curtail tobacco use even further in the interests of users and non-users alike.

Public opinion polls had shown such a high level of support for the Department's strategies that a new Tobacco Act (1988) was brought forward to further curtail and restrict tobacco consumption. The 1988 Act is crucial insofar as it contains the ban on importing, making, and distributing oral smokeless tobacco.

LEGISLATION Ireland's ban on oral smokeless tobacco is contained in the Tobacco (Health Promotion and Protection) Act, 1988. The preamble to the Act summarizes its provisions: (a) prohibition and restriction on the consumption of tobacco and restriction on the consumption of tobacco products in designated areas and facilities; (b) restriction on the sale of tobacco products to persons under 16 years of age; (c) prohibition on the importation, manufacture, and sale of certain tobacco products; (d) amendment of previous legislation; and (e) provision for other connected matters.

Specifically, the ban on oral smokeless tobacco is contained in Section 6 of the 1988 Tobacco Act, as follows:

> (1) Any person who imports, manufactures, sells or otherwise disposes of, or offers for sale or other disposal, or advertises, an oral smokeless tobacco product shall be guilty of an offense and shall be liable—
>
> (a) on summary conviction, to a fine not exceeding £1,000, or
>
> (b) on conviction on indictment to a fine not exceeding £10,000.
>
> (2) In this section "oral smokeless tobacco product" means any product or substance, made wholly or partly from tobacco, which is intended for use, unlit, by being placed in the mouth and kept there for a period, or by being placed in the mouth and sucked or chewed.

This is a comprehensive ban, which is feasible to implement in Ireland where there has never been an oral snuff habit.

As already mentioned, a somewhat similar type of ban was previously introduced, in 1985. The first ban did not involve new legislation at that time, as it was introduced under previous legislation (Health Act, 1947). The 1947 Health Act, by virtue of its Section 66, allowed the Minister for Health to prohibit the import, manufacture, sale, or other disposal of a restricted article or substance when it was likely, when accessible to the general public, to be used for purposes involving risks of serious injury to health or body. The 1985 ban (Health [Restricted Article] Order, 1985) was confined to the Skoal Bandit form of oral smokeless tobacco—that is, sachet-type products.

In addition to the legislation mentioned above (i.e., the 1985 Regulations and the 1988 Act), there are a couple of other important pieces of relevant legislation. The first of these is the Tobacco Products Act (1978), which gives the Minister for Health extensive powers to control all aspects of advertising, sponsorship, and promotion of tobacco products. The second is the Tobacco (Number 2) Regulations (1986), which greatly extend the Minister's powers in implementing the 1978 Act.

LITIGATION The 1985 ban on oral smokeless tobacco, under the 1947 Health Act, was challenged in the Irish High Court by U.S. Tobacco (Ireland) Limited and U.S. Tobacco International, Inc. U.S. Tobacco won the case, on the grounds that, when framing the Act, the legislature did not intend that it be used for this particular type of purpose. The Court held that the Minister for Health had exceeded his powers, and so the first ban was nullified.

Unfortunately, the lawyers had decided to fight the case without reference to the health risks of smokeless tobacco use and to defend the ban on purely legal technical grounds. With hindsight, this was seen as a mistake. After the defeat in the High Court, a decision was made in the Department of Health not to appeal to the Supreme Court, as the second ban, in the new proposed Tobacco Act (1988), was well on its way toward implementation as Section 6.

The second ban also was challenged in the Irish High Court by U.S. Tobacco. The case was heard late in 1990, and the decision, which upheld the ban, was given on February 22, 1991 (High Court, 1990, 1991).

It is instructive to study this second court case: U.S. Tobacco claimed that Section 6 was invalid, as it breached the Treaty of Rome (1957), by which the European Community was formed, at Article 30, and that it was unenforceable because of noncompliance with E.C. Council Directives 83/189 as amended by 88/182 (E.C., 1983 and 1988), and 89/622 (E.C., 1989)—the "Notification" and "Labelling" Directives, respectively. Article 30 states: "Quantitative restrictions on imports and all measures having equivalent effect shall, without prejudice to the following provisions, be prohibited between Member States."

U.S. Tobacco contended that Section 6 offends the principle of proportionality in that the same objective could be achieved by a less repressive measure than the ban. The corporation contended also that the current legal restrictions in Ireland on smoking tobacco could be applied to

smokeless tobacco and that they would be adequate to protect the health and lives of humans in Ireland.

The law involved is European Community law. It covers three topics: (a) Articles 30 and 36 of the Treaty of Rome, (b) the principle of proportionality, and (c) the rights of Member States when there are uncertainties in current research. Article 30 of the Treaty is stated above. Article 36 is as follows:

> The provisions of Articles 30 to 34 shall not preclude prohibitions or restrictions on imports, exports or goods in transit justified on grounds of public morality, public policy or public security; the protection of health and life of humans, animals or plants; the protection of national treasures possessing artistic, historic or archeological value; or the protection of industrial and commercial property. Such prohibitions or restrictions shall not, however, constitute a means of arbitrary discrimination or a disguised restriction on trade between Member States.

The State agreed that Article 30 was breached but contended that Article 36 gives an exception from it. Under Article 36, the case law is clear that exceptions are justified on grounds of necessity for "protection of health and life of humans." Exceptions are not allowed, under the principle of proportionality, if the health and life of humans can be as effectively protected by less strict measures. Insofar as there are uncertainties in the present state of research, Member States can decide the degree of protection they wish to assure for the health and life of humans.

The evidence fell into two categories: first, the conclusions of authoritative bodies, and second, opinions of expert witnesses.

Conclusions of Authoritative Bodies

According to volume 37, page 116, of the International Agency for Cancer Research Monographs (IARC, 1985), "There is sufficient evidence that oral use of snuffs . . . is carcinogenic to humans."

From the preface (page vii) to the report of the Advisory Committee to the U.S. Surgeon General (US DHHS, 1986), "The scientific evidence is strong that the use of snuff can cause cancer in humans."

The World Health Organization Study Group (WHO, 1988b) stated, "There is conclusive scientific evidence that the use of smokeless tobacco causes cancer in humans" (page 18).

According to the First European Conference on Tobacco Policy (WHO, 1988a), "These products are associated with increased risk of contracting oral cancer and other diseases of the mouth."

Testimony of Expert Witnesses

The expert testimony was extremely detailed and went on for 6 days. All of the State's witnesses were of the opinion that there is an association between oral smokeless tobacco and oral cancer. The judge had no hesitation in accepting their evidence.

The evidence of U.S. Tobacco's expert witnesses did not convince the judge, as can be seen from his assessment, which took the form of answers to three questions:

(a) Was the ban justified when introduced in July 1988?

(b) If so, is it still justified?

(c) If the answers to (a) and (b) are "Yes," could the objectives of the ban be achieved by less restrictive means?

The judge's answers were as follows:

(a) Yes, there was enough evidence that smokeless tobacco could cause oral cancer. People had to be protected by ensuring it was not available by means of a total ban.

(b) Yes, it was not proven there is a scientific controversy. The plaintiff's witnesses disagreed with authoritative bodies, but no responsible body agrees with them—the evidence is the other way. To impose the ban it was sufficient that there was evidence that cancer might be caused or that the risk was increased. As the State is obliged to protect its citizens, it had enough evidence to take action. It would fail in its duty if it were to act otherwise.

(c) It is submitted that existing restrictions (on smoking tobacco) would be adequate, but it is not submitted that they would be as effective as the ban. They are not adequate, not sufficient, not as effective, and are "a second best."

E.C. Council Directives The Notification Directives (E.C., 1983 and 1988) require that draft technical regulations be communicated to the E.C. Commission. Section 6, however, is not a technical regulation, specification, or standard. Agricultural produce is excluded from the 1983 Directive, and the 1989 Directive did not come into force until January 1, 1989, and was not operable when the 1988 Act was passed in July 1988.

The Labelling Directive (E.C., 1989) is concerned only with harmonizing laws, etc., regarding health warnings on packages of tobacco products. It does not affect the right of a Member State to lay down its own rules concerning the import, sale, and consumption of tobacco products, for example, a ban, which is not imposed for reasons of labelling but for the public health.

Judgment In conclusion, and in the judge's own words, "the plaintiff's claim fails in toto and must be dismissed" (High Court, 1990, 1991).

INTERNATIONAL MEASURES Irish legislation in relation to restrictions on sales, promotion, and advertising of tobacco has served in the past as a model for subsequent European Community laws, regulations, and directives. The introduction to the E.C. of a similar legal ban on oral smokeless tobacco is under consideration at present. It is, therefore, possible that before long there will be an E.C. ban similar to the Irish one.

A number of other countries have already adopted measures aimed at banning or controlling the importation, manufacture, and sale of smokeless tobacco products.

Within the E.C. and following the Irish initiative, in February 1988 the United Kingdom announced its intention to ban moist snuff. Since March 1990 in the United Kingdom, "no person shall supply, offer to supply, agree to supply, expose for supply or possess for supply any oral snuff" (excluding nasal dry snuff) (Consumer Protection, 1989).

Belgium adopted a Royal Decree, effective December 31, 1990, which lays down that "it is forbidden to market moist snuff tobacco in pouches aimed at being placed as such in the mouth."

Luxembourg and France impose restrictions on the advertising of smokeless tobacco products. In France (under the Law Concerning Measure Against Tobacco, 1976) and Belgium (under the 1990 Royal Decree), health warnings are required on smokeless tobacco packages. France is currently examining a draft law (Law Concerning Measure Against Alcohol and Tobacco, 1990) aimed at banning all forms of direct or indirect advertising of tobacco products.

Outside the European Community, Hong Kong, New Zealand, and Israel have banned all forms of smokeless tobacco (Public Health and Municipal Services Ordinance, 1987; Toxic Substances Act [amendment], 1985; and Ministry of Trade Industry Codes, 1986, respectively), as have Japan and Taiwan (European Bureau, 1990). Singapore adopted measures banning the importation and sale of chewing tobacco and moist snuff, and Saudi Arabia has banned moist snuff and chewing tobacco (European Bureau, 1990). The Isle of Man prohibits the importation of moist snuff under the Custom and Excise Acts Orders (Amendment No. 2) of 1986. In the United States, "Masterpiece Tobacs" was banned because it did not comply with the Food and Drug Administration rules (European Bureau, 1990).

Iceland's Act on Prevention of Use of Tobacco (1984) bans all tobacco advertising and promotion. India, Canada, Sweden, and the United States have enacted legislation that makes the inclusion of health warnings on smokeless tobacco packages compulsory. Massachusetts was the first U.S. state to declare moist snuff a hazardous substance, and a ban was considered but not enacted because of the large consumer demand for the product (European Bureau, 1990).

FUTURE STRATEGY

In 1988, the First European Conference on Tobacco Policy (WHO, 1988b), organized jointly by WHO and the European Community, produced a 10-point strategy for a "Europe without tobacco." Point 8 of the strategy concerns the banning of all new tobacco products containing nicotine. The conference adopted the following recommendation for strategy on this issue:

> The participants endorsed the recommendations of the WHO Study Group on Smokeless Tobacco Control and urged Member States to use these recommendations as a basis for action. Particularly important are the Study Group's recommendations for a smokeless tobacco program:
>
> - Where smokeless tobacco is not used, prevent its introduction, with special emphasis on preventing its use by children;

- Where smokeless tobacco is already used, act to reduce the prevalence of use in the population; and
- Establish or maintain a social climate unfavorable to smokeless tobacco use.

ADDENDUM In March 1991, U.S. Tobacco lodged an appeal to the Supreme Court in Ireland against the High Court judgment (1990). The basis of the appeal is the contention that the judge in the High Court misdirected himself in law and in fact. In the Supreme Court the appeal will be heard by the full court of five judges sitting together. The outcome of this most important case is awaited with great interest.

REFERENCES

Consumer Protection, the Oral Snuff (Safety) Regulations, 1989. London: Her Majesty's Stationery Office, 1989.

European Bureau for Action on Smoking Prevention. *Report on a New Form of Smokeless Tobacco: Moist Snuff*. Brussels: European Bureau, December 1990.

European Community. Council Directive 83/189, March 28, 1983 (laying down a procedure for the provision of information in the field of technical standards and regulations). E.C. Official Journal No. L109, 26/04/83: 0008, 1983.

European Community. Council Directive 88/182, March 22, 1988 (amending Directive 83/189). E.C. Official Journal No. L081, 26/03/88: 0075, 1988.

European Community. Council Directive 89/622, November 13, 1989 (on the approximation of the laws, regulations, and administrative provisions of the Member States concerning the labeling of tobacco products). E.C. Official Journal No. L359, 08/12/89: 0001, 1989.

Health Act. Dublin: The Stationery Office, 1947.

Health (Restricted Article) Order. (S.I. No. 429 of 1985). Dublin: The Stationery Office, 1985.

High Court, 1990. No. 871P: Judgment of Mr. Justice Blayney delivered the 22nd day of February, 1991. Dublin: Irish Law Reports, 1991.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosomines* (volume 37). Lyon: IARC, 1985.

Lyons, M. Paper presented at joint meeting of World Health Organization and USSR Institute of Preventive Medicine, Moscow, September 1988.

Tobacco (Control of Advertising, Sponsorship and Sales Promotion, No. 2) Regulations. (S.I. No. 107 of 1986). Dublin: The Stationery Office, 1986.

Tobacco (Health Promotion and Protection) Act. Dublin: The Stationery Office, 1988.

Tobacco Products (Control of Advertising, Sponsorship and Sales Promotion) Act. Dublin: The Stationery Office, 1978.

Treaty of Rome. Treaty establishing the European Economic Community, signed in Rome, Italy, on March 25, 1957. Luxembourg: Office for Official Publications of the European Communities, 1957.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. Publication No. DHHS (NIH) 86-2874, 1986.

World Health Organization. *First European Conference on Tobacco Policy: It Can Be Done—A Smoke-Free Europe*. Regional Publications. European Series; No. 30. Copenhagen: WHO, 1988a.

World Health Organization. *Smokeless Tobacco Control: Report of a WHO Study Group*. Technical Report Series 773. Geneva: WHO, 1988b.

National Cancer Institute's Role In Reducing Tobacco Use

Robert Mecklenburg

ABSTRACT The tobacco industry exploits the public good—trading human lives, economic well-being, and the environment for profit. The industry knows that its earnings are built on consumer addiction, loss of health, and death. Waking the public to the needless and despicable consequences of a morally bankrupt industry requires determined action by a wide variety of private and public sector organizations. Each organization, doing what it can do best, can help turn public opinion from tolerance to outrage, and public behavior from use to abstinence. The National Cancer Institute, through its research and dissemination of knowledge, is a vital partner in this quest for a healthy society. This paper provides an overview of NCI's mission and current activities related to the control of smoking and smokeless tobacco use. Major research and demonstration projects, health professional roles, and public and private-sector NCI partnerships are described.

NCI MISSION AND MANDATE NCI aims to provide knowledge and guidance for reducing cancer incidence and mortality in the United States and around the world. Evidence strongly links tobacco use, primarily smoking, with many types of cancers (US DHHS, 1982). About 30 percent of all cancers are attributed to tobacco use. Both smoking and ST use increase the risk for cancer of the mouth and pharynx about fourfold, with the relative risk for both tobacco forms related to the duration and intensity of exposure (US DHHS, 1990a; Winn, 1981).

Goals set by the U.S. Department of Health and Human Services include an objective of reducing cigarette smoking to no more than 15 percent among people aged 20 and older (US DHHS, 1991). The smokeless tobacco objective is to reduce prevalence of use among males aged 12 through 24 to no more than 4 percent (US DHHS, 1991). These two goals are also objectives for NCI's Smoking and Tobacco Control Program.

One study suggests that 25 to 30 percent of all regular ST users also smoke (Eakin et al., 1989). Children and youth may become addicted to ST, then later become smokers. Smokers may perceive ST as a safe alternative to smoking and change to ST for maintaining their levels of nicotine. Both forms of tobacco are addictive, produce health problems, and create intervention challenges (US DHHS, 1986a). To be successful, strategies to meet national health objectives must address both smoking and smokeless means of nicotine administration.

These objectives call for action, but just any action will not do. It is essential that major tobacco use interventions be supported by scientifically sound, established methods. Thus, NCI developed an 18-yr plan to develop hypotheses, define evaluation criteria, examine scientific evidence to date, support well-designed studies in areas where evidence seemed insufficient, and build large-scale intervention trials (US DHHS, 1990b).

TOBACCO USE INTERVENTION PROGRAM

Since 1983, specific NCI research efforts in the Division of Cancer Prevention and Control have been directed toward the development of scientifically sound and practical tobacco use interventions. Through fiscal year 1990, nearly $250 million had been invested in tobacco use research and control. Sixty studies on smoking intervention have been completed or are in progress, and an additional eight studies focus on ST (US DHHS, 1990b).

Of the eight ST studies, four are directed to students, one is limited to dental clinic patients, one targets Little League youth, one targets Native American youth, and one targets 4-H members. Preliminary results suggest that methods found to be effective in helping smokers quit can achieve similar results among ST users.

The Community Intervention Trial for Smoking Cessation (COMMIT) is a large, controlled study of randomly assigned subjects. COMMIT involves, directly or indirectly, more than 6 million people in 22 paired study and control communities to test intervention methods that were found effective during smaller studies (US DHHS, 1990b). COMMIT is a university-based research project that includes schools; worksites; professional, religious, civic, and government organizations; and the media. The COMMIT trials began in 1989 and are scheduled to run until 1993. Although intended to focus on heavy smokers, the study suggests approaches to the psychological and social patterns and physical dependencies of ST users. Already invaluable baseline and operational information has been obtained, but future results hold high promise.

Findings from COMMIT should significantly contribute to a larger initiative, the America Stop Smoking Intervention Study for Cancer Prevention (ASSIST) (US DHHS, 1990b). ASSIST will have an impact on patterns of tobacco use nationwide. States will administer contracts for organizing and operating coalitions that promote a multifaceted approach to influencing people to avoid and discontinue tobacco use. From 50 to 100 million people are expected to be included in this project. An organizational phase for ASSIST will run from 1991 to 1993, and an operational phase from 1993 to 1998.

ST was specifically identified in the original Request for Proposals that was sent to the states for ASSIST. These guidelines defined smokers as tobacco users rather than just individuals who smoke tobacco (cigarettes, cigars, pipes). For purposes of ASSIST, ST users are included in the definition of "smoker" (ASSIST Program Guidelines, October 1991, Glossary of Terms, unpublished).

PROFESSIONAL ROLES

The medical profession has great incentive to become involved in smoking control. Physicians often see patients sicken and die from tobacco-related diseases. Indeed, surveys of patients and physicians suggest that from one-third to one-half of medical practitioners provide at least some smoking intervention services during encounters with patients (Frank et al., 1991; Gerbert et al., 1989; US DHHS, 1976). Surveys also indicate that dentists are involved to a lesser degree (Geboy, 1989; Gerbert et al., 1989;

Secker-Walker et al., 1989) but may not consistently use the best intervention methods (Secker-Walker et al., 1987).

NCI recognized that both the medical and dental professions are strategically situated to provide effective intervention. About 70 percent of Americans see a physician at least once a year (US DHHS, 1986b). About 63 percent of the population see a dentist each year, including 75 percent of children and youth (Hayward et al., 1989). Studies suggest that selected tobacco use interventions are effective when provided in the dental clinic environment (Cohen et al., 1989) and are as effective as physician intervention services (Cohen et al., 1987).

The time that physicians and dentists spend with patients offers an excellent opportunity to influence behavior. Medical and dental visits provide many opportunities for one-to-one discussion about tobacco use and health consequences and methods for quitting. Medical visits for prenatal care, child health, and upper respiratory or cardiovascular conditions provide special opportunities to discuss reasons for quitting. Dental checkups are usually performed at regular intervals and often are prevention oriented. Dental visits also provide opportunities for effective interventions because tobacco effects are commonly found in the mouth. Oral lesions that are visible to patients lead to teachable moments that help motivate patients to stop. Both physicians and dentists can prescribe nicotine replacement therapy, when indicated. Followup visits for many routine dental services can also be used for followup of tobacco use interventions.

NCI has developed and conducted training programs for medical and dental clinicians, focusing on train-the-trainer programs for medical and oral health teams who are willing to teach colleagues. Dental education institutions are encouraged to integrate key issues into their curricula and to establish behavioral outcome objectives. The NCI manual, *How To Help Your Patients Stop Smoking*, designed for physicians, does not specifically address smokeless tobacco, but the clinical intervention methods can apply to ST intervention (Glynn and Manley, 1990). The NCI manual, *How To Help Your Patients Stop Using Tobacco*, is used with the oral health team and educator training programs (Mecklenburg et al., 1990). The title and contents of this manual specifically refer to "tobacco use" rather than "smoking," reflecting the importance of influencing patients to avoid and discontinue the use of ST as well as smoking tobacco.

PUBLIC AND PRIVATE PARTNERSHIPS

NCI recognizes that intervention successes are the product of a partnership between the research community and educators, community leaders, policymakers, and program administrators. NCI promotes the reduction of tobacco use by collaborating with many private and public organizations. Examples of NCI partnerships include the following:

- NCI partnerships with the academic community are important to its extramural research program. Research and education institutions continually strengthen the scientific basis for rational patient care

and public health action. COMMIT is an example of a university-based tobacco use intervention research project.

- NCI works with public and private-sector organizations to form effective community contacts. Private volunteer, business, and community service government organizations have access to large segments of the public. These organizations want assurances that their efforts will be effective. ASSIST is an NCI collaboration with the American Cancer Society, state health departments, and many other groups with special skills in working with the public (for example, business, religion, education and public service organizations, and the media). NCI's National Dental Tobacco-Free Steering Committee is an alliance between NCI and 11 national dental organizations that are committed to influencing the public to avoid and discontinue tobacco use.
- Within the National Institutes of Health, NCI and the National Institute of Dental Research are jointly planning and identifying several cooperative ventures against ST and in support of the prevention and early detection of oral cancer, with each institute building on its special strengths.
- NCI collaborates with other U.S. Public Health Service agencies, such as the Office of Disease Prevention and Health Promotion for tobacco, cancer, and oral health objectives for the year 2000; with the Centers for Disease Control, especially its Office on Smoking and Health; the National Center for Health Statistics; and more recently, the Health Resources and Services Administration and Indian Health Service for training of Public Health Service clinical personnel in tobacco use interventions.
- NCI collaborates in tobacco control activities by other government agencies, such as the Department of Veterans Affairs and the Department of Defense. These departments are working with many of the same private and public-sector organizations noted above.
- Finally, NCI provides tobacco use intervention support to the World Health Organization and to other countries. The Basic Dental Research Unit of the Tata Institute of Fundamental Research in India deserves special recognition for its 25-yr effort to develop oral cancer control methods, including pioneering work in developing tobacco use interventions.

SUMMARY The NCI program, in collaboration with numerous organizations, is influencing the public to reduce its use of tobacco. ST control is a significant element of NCI's commitment. The mission of NCI with regard to tobacco control is twofold: First, to strengthen the science base for intervention services through its epidemiological and biomedical research programs, and second, to ensure that sound scientific methods reach the right people, at the right time, and in ways that are most likely to create a united effort for reducing cancer incidence and mortality and other tobacco-induced health problems.

REFERENCES

Cohen, S.J., Christen, A.G., Katz, B.P., Drook, C.A., Davis, B.J., Smith, D.M., Stookey, G.K. Counseling medical and dental patients about cigarette smoking: The impact of nicotine gum and chart reminders. *American Journal of Public Health* 77: 313-316, 1987.

Cohen, S.J., Stookey, G.K., Katz, B.P., Drook, C.A., Christen, A.G. Helping smokers quit: A randomized controlled trial with private practice dentists. *Journal of the American Dental Association* 118: 41-45, 1989.

Eakin, E., Severson, H., Glasgow, R.E. Development and evaluation of a smokeless tobacco cessation program: A pilot study. *National Cancer Institute Monographs* 8: 95-100, 1989.

Frank, E., Winkleby, M.A., Altman, D.G., Rockhill, B., Fortman, S.P. Predictors of physicians' smoking cessation advice. *Journal of the American Medical Association* 266(22): 3139-3144, 1991.

Geboy, M.J. Dentists' involvement in smoking cessation counseling: A review and analysis. *Journal of the American Dental Association* 118: 79-83, 1989.

Gerbert, B., Coates, T., Zahnd, E., Richard, R.J., Cummings, S.R. Dentists as smoking cessation counselors. *Journal of the American Dental Association* 118: 29-32, 1989.

Glynn, T.G., Manley, M.W. *How To Help Your Patients Stop Smoking: A National Cancer Institute Manual for Physicians*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH Publication No. 90-3064, 1990.

Hayward, R.A., Meetz, H.K., Shapiro, M.F., Freeman, H.E. Utilization of dental services: 1986 patterns and trends. *Journal of Public Health Dentistry* 49(3): 147-152, 1989.

Mecklenburg, R.E., Christen, A.G., Gerbert, B., Gift, H.C., Glynn, T.J., Jones, R.B., Lindsay, E., Manley, M.W., Severson H. *How To Help Your Patients Stop Using Tobacco: A National Cancer Institute Manual for the Oral Health Team*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH Publication No. 91-3191, 1990.

Secker-Walker, R.H., Hill, H.C., Solomon, L.J., Flynn, B.S. Smoking cessation practices in dental offices. *Journal of Public Health Dentistry* 47: 10-15, 1987.

Secker-Walker, R.H., Solomon, L.J., Hill, H.C. A statewide survey of dentists' smoking cessation advice. *Journal of the American Dental Association* 118: 37-40, 1989.

U.S. Department of Health, Education, and Welfare. *Adult Use of Tobacco, 1975*. Washington, DC: U.S. Government Printing Office, 1976.

U.S. Department of Health and Human Services. *The Health Consequences of Smoking: Cancer. A Report of the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, Office on Smoking and Health. DHHS Publication No. (PHS) 82-50179, 1982.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. DHHS Publication No. (NIH) 86-2874, 1986a.

U.S. Department of Health and Human Services. *Clinical Opportunities for Smoking Intervention*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH Publication No. 86-2178, 1986b.

U.S. Department of Health and Human Services. *Healthy People 2000: National Health Promotion and Disease Prevention Objectives*. U.S. Department of Health and Human Services, Public Health Service. DHHS Publication No. (PHS) 91-50212, 1991.

U.S. Department of Health and Human Services. *The Health Benefits of Smoking Cessation: A Report of the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. DHHS Publication No. (CDC) 90-8416, 1990a.

U.S. Department of Health and Human Services. *Smoking, Tobacco, and Cancer Program: 1985-1989 Status Report*. U.S. Department of Health and Human Services, Public Health Service, National Insitututes of Health. NCI Publication No. 90-3107, 1990b.

Winn, D.M., Blot, W.J., Shy, C.M., Pickle, L.W., Toledo, A., Fraumeni, J.F. Snuff dipping and oral cancer among women in the southern United States. *New England Journal of Medicine* 304: 745-749, 1981.

Chapter 8

Recommendations

CONTENTS

Recommendations for the Control of Smokeless Tobacco

Robert Mecklenburg and R. Craig Stotts

BACKGROUND In 1990, Dr. Joseph Cullen, former Deputy Director, Division of Cancer Prevention and Control, National Cancer Institute, and many others who spoke at the Seventh World Congress on Tobacco and Health, appealed for immediate and widespread action against tobacco use. These speakers said that, although the research base needs strengthening, it is sufficient to promote change now. In 1991, Dr. Louis Sullivan, Secretary of Health and Human Services, when addressing the First International Conference on Smokeless Tobacco, also called for public education, new policies, and responsible action in the private and public sectors.

The recent rise in the use of ST in the United States, particularly the use of moist snuff, is cause for alarm. We know that dreadful health consequences followed the increase in smoking, and we know the toll of ST use in southeast Asia. It would be irresponsible to wait, for perhaps a quarter century, until oral cancer and other health consequences associated with ST use increase significantly. Nor can a wait-and-see attitude be justified when oral cancer incidence already exceeds the incidence of leukemia, cervical cancer, and other well-recognized cancers (American Cancer Society, 1992).

In January 1991, the National Cancer Institute and the National Institute of Dental Research convened a workshop of smokeless tobacco experts (see Appendix) to plan a response to the rise in ST use and the concomitant increase in risks for deaths from oral and other cancers in the United States and many other countries. The group assessed research conducted since the 1986 NIH Consensus Development Conference and the 1986 report of the Surgeon General's Advisory Committee, reexamined earlier conclusions, and proposed action based on current knowledge.

The NCI/NIDR workshop participants concluded that smokeless tobacco is addictive and that it causes, or is strongly associated with, adverse effects on oral and systemic health. Participants found that research findings have reinforced conclusions of other expert committees about the health effects of ST use. As a result of workshop deliberations, a document describing objectives and strategies for smokeless tobacco control and specific recommendations for action during the 1990's was prepared for use in the United States. Copies of the first draft were distributed to participants of the First International Conference on Smokeless Tobacco to facilitate consideration of issues raised during the conference and development of globally oriented conclusions, objectives, and strategies. Scientific presentations and discussions during the conference broadened, clarified, and strengthened the conclusions and recommendations provided by the U.S. workshop. As with the U.S. document, the document from the international conference is not applicable to all countries or to all situations.

Rather, it should serve as a menu from which individuals, groups, governments, or international health organizations may choose to address their tobacco-related problems.

Many ST interventions should be conducted as part of smoking control initiatives. Although smoking and smokeless tobacco differ in many ways, they also share many characteristics: (1) both are methods for nicotine administration; (2) they are often used sequentially or concurrently by the same individual; (3) both adversely affect health and are potentially lethal; and (4) they often are susceptible to similar prevention and cessation interventions.

The STCP monograph on smokeless tobacco and, hence, these recommendations are a synthesis of the work of the U.S. workshop committee and the participants of the First International Conference on Smokeless Tobacco. This volume is based on the latest scientific research in the field. Decision-makers and health care professionals are invited to read it carefully and urged to implement as many of the recommendations as possible.

INTRODUCTION A workshop on the control of smokeless tobacco was sponsored by the National Cancer Institute and the National Institute of Dental Research on January 24, 1991, in Rockville, Maryland, USA. Participants at the NCI/NIDR workshop included biomedical, epidemiological, and behavioral researchers; public health professionals; health educators; and representatives from government offices and research institutes within the U.S. Public Health Service. The workshop was convened to plan a response to the increase in ST use in the United States and the association of ST use with oral cancer and other health effects in the United States and other countries.

In the United States, during most of this century, the use of chewing tobacco and oral snuff, collectively referred to as smokeless tobacco, was seen primarily in rural areas and among particular occupational groups such as miners and agricultural workers, and the prevalence of use was highest among people over the age of 50 (US DHHS, 1986). In the 1970's, production and promotion of ST products increased, and new product types were introduced. Anecdotal reports and local and regional surveys show high rates of use among children and adolescents in some areas, and national surveys of adults indicate the highest prevalence now is among young adult males (Orlandi and Boyd, 1989; Schoenborn and Boyd, 1989; US DHHS, 1986). Government data (U.S. Department of the Treasury, 1991) indicate that sales of moist snuff are again increasing, after a brief period of decline in 1986 and 1987, reaching 45 million pounds in 1989 and 52 million pounds in 1990.

The workshop agreed that the conclusions of the 1986 NIH Consensus Development Conference on Smokeless Tobacco Use—that is, that smokeless tobacco use is addictive and that it causes or is strongly associated with many adverse effects on oral and systemic health—remain valid.

The findings of the 1986 Report of the Advisory Committee to the Surgeon General, *The Health Consequences of Using Smokeless Tobacco*, were summarized as follows (US DHHS, 1986):

> After a careful examination of the relevant epidemiologic, experimental, and clinical data, the committee concludes that the oral use of smokeless tobacco represents a significant health risk. It is not a safe substitute for smoking cigarettes. It can cause cancer and a number of noncancerous oral conditions and can lead to nicotine addiction and dependence.

The above conclusion was supported by the following specific determinations:

- The use of smokeless tobacco can cause cancer in humans;
- Use of smokeless tobacco can result in noncancerous and precancerous oral pathologies including leukoplakias, gingival recession, and possibly gingivitis, dental caries, abrasion, and staining; and
- Smokeless tobacco contains nicotine and its use can lead to nicotine addiction or dependence.

Similar conclusions were reached by the NIH Consensus Development Conference, "The Health Implications of Smokeless Tobacco Use," and, with respect to carcinogenicity, by the International Agency for Research on Cancer (Consensus Conference, 1986; IARC, 1985). The conclusions reached by the Advisory Committee to the Surgeon General have been supported by subsequent research and serve as the bases for the recommendations that follow.

Research is in progress to delineate the mechanisms of carcinogenesis and other biological effects, to describe the time course of tobacco-related oral cancer and other pathologies, to quantify risk, to estimate attributable morbidity and mortality, to understand patterns of use, and to develop appropriate means of intervening with current or potential ST users. Because of the nature of biological, epidemiological, and behavioral research, some questions addressed by this research will not be fully answered for many years.

The workshop participants also recognize and commend the work that has already been carried out by health organizations and educators; advocacy groups; government agencies; biomedical, epidemiological, and behavioral researchers; individual activists; and members of the U.S. Congress. These groups and individuals are responsible for slowing the acceleration in ST use observed in the 1970's and early 1980's and have provided current information for understanding and combating smokeless tobacco use. Future efforts at smokeless tobacco control will build on this foundation.

CONCLUSIONS The following conclusions and recommendations summarize the consensus of the participants in the NCI/NIDR workshop [with subsequent organization and refinement of relevant information by the above-named authors]:

Whereas,

- Smokeless tobacco products (in the form of oral snuff) currently are the only types of tobacco with an increase in use in the United States, particularly among young persons (U.S. Department of the Treasury, 1991);
- ST use begins at a young age;
- The addictive substance in both smokeless tobacco and cigarette smoke is nicotine, and there is a clear potential for ST to serve as a gateway to cigarette smoking (or to reinforce previously established nicotine use) as well as leading to the use of other addictive substances (e.g., alcohol and illicit drugs);
- The prevalence of ST use is high among vulnerable populations (e.g., blue collar workers, rural residents, youth, Native Americans);
- Smokeless tobacco products are marketed aggressively with special targeting of vulnerable populations in the United States and abroad;
- The increase in ST use in the United States over the past decade, especially among young people, has not abated;
- Users of smokeless tobacco are at increased risk for oral cancer, noncancerous oral pathologies, and nicotine addiction;
- The biological effects and epidemiology of ST use are a recent focus of research, and it is possible and probable that continued investigation will identify further health risks and harmful effects;
- The epidemic of cigarette smoking and resulting disease and death that has taken place during this century demonstrates the severe, long-term impact on world health that can occur when use of an addictive product gains wide public acceptance before the full extent of its harmful effects is discovered;

Therefore,

This working group endorses the objectives and strategies described below to reduce the use of smokeless tobacco throughout the world. The following recommendations include endorsements, extensions, and modifications of objectives and recommendations

formulated by the Public Health Service (US DHHS, 1991), the Tata International Symposium on the Control of Tobacco-Related Cancers and Other Diseases (1990), and the World Health Organization (1988).

Public Health Objectives

Introduction

The Nation's objectives for health for the year 2000 include specific objectives for control of all tobacco, including smokeless tobacco (US DHHS, 1991). The risk reduction objective for smokeless tobacco is to "reduce smokeless tobacco use by males aged 12 through 24 to a prevalence of no more than 4 percent."

This working group strongly recommends that ST control activities make use of existing channels and structures that currently focus on control of cigarette smoking for the following reasons:

- Resources for public health activities are limited.
- Cigarette smoking remains the number one preventable cause of death and disability in most countries, and there is no intent or reason for ST control to compete for limited resources with smoking control activities.
- Cigarette smoking control has a long and successful history, and it is most efficient for ST control activities to function within these existing channels, structures, and techniques whenever possible.
- Cigarette smoking and smokeless tobacco use are two methods of self-administering the same addictive substance obtained from different preparations of the same plant. The tobacco industry's marketing strategies for these products are complementary. Hence, the public health response should also show a coordinated approach. The clear and unambiguous message that should be disseminated to the public is **there is no safe form of tobacco**.

A number of general considerations will influence the course of activities recommended to achieve the objectives for controlling ST use. Prominent among them are the following:

- Prevention of initiation of smokeless tobacco use by the young and cessation by current users are both necessary to achieve a significant reduction in prevalence.
- Campaigns or programs discouraging ST use should convey the message that all forms of tobacco are unhealthy. Care must always be taken that users of one tobacco product are not inadvertently persuaded to switch to another tobacco product that is perceived as a safe alternative.
- All tobacco control efforts should include smokeless tobacco. Because prevalence of ST use varies according to region, urbanicity, age, ethnicity, and type of employment, the level and nature of the effort devoted to ST control will depend on the target population.
- Although the recent increase in use of smokeless tobacco in the United States and some other countries has occurred primarily among adolescents and young adults, intervention efforts should not overlook older users and regions where there is a long tradition of tobacco use.
- Organizations and agencies that conduct ST intervention programs should collaborate with other private and public organizations worldwide, to coordinate priorities and strategies and to maximize impact for the resources available.

Recommendations for specific target audiences and locations are listed below.

Public Awareness

- Increase public awareness of the hazards associated with smokeless tobacco, especially among those segments of the population at greatest risk for ST use.
- Increase awareness by parents of the hazards and signs of ST use.
- Promote a shift in public opinion and social norms among predisposed groups so that smokeless tobacco is viewed as socially unacceptable, undesirable, and an unhealthy alternative to smoking.
- Develop public support for policies and legislation that discourage the use of smokeless tobacco.
- Involve professional athletes, celebrities, and other influential role models in public education campaigns.
- Develop effective counteradvertising materials and make them widely available to individuals, agencies, and organizations interested in promoting an antitobacco message.
- Provide education and training to individuals willing to become spokespersons for ST control.
- Provide materials and programs for influential organizations, such as parent and teacher associations and other organizations that reach youth, especially high-risk youth (e.g., Little League and Babe Ruth Baseball, other sports leagues, the Scouts, Future Farmers of America).

Schools

- Provide broad tobacco-related curricula in multiple grades to reduce the initiation of tobacco use, including smokeless tobacco.
- Include cessation support in school ST curricula for students and provide cessation support for school employees.
- Adopt policies that prohibit the use of any form of tobacco by students, faculty, staff, and visitors on school property at any time and at school-sponsored events in other locations.
- Provide prevention and cessation interventions in colleges and trade schools.

Health Services Professionals

- Ascertain tobacco use status of all patients and routinely provide cessation advice, support, and followup.
- Be alert to the physical signs of ST use and use clinical observations, particularly by providers of oral health services, as an opportunity to encourage cessation.
- Adopt tobacco-free policies in health care facilities to prohibit the use of any form of tobacco.
- Acquire continuing education training regarding ST use and strengthen tobacco intervention skills.

	• Provide students with training and require them to demonstrate competency in tobacco use counseling.
	• Disseminate established training programs and materials for health professionals to strengthen tobacco use prevention skills (for example, materials presented in Glynn and Manley, 1989; Glynn et al., 1990; Mecklenburg et al., 1990).
Employers and Worksites	• Implement formal tobacco policies to restrict or ban the sale and use of all tobacco at the workplace.
	• Provide information, encouragement, support, and incentives for employees and their families to quit using smokeless tobacco.
	• Involve labor unions in the planning and implementation of worksite intervention programs.
	• Use labor unions to educate their constituencies about the hazards of tobacco use, including ST, and encourage cessation.
	• Give special attention to schools and health care facilities as worksites with prominence and influence that extend beyond their own employees.
	• Emphasize efforts at worksites with populations at high risk for ST use.
States, Counties, and Municipalities	• Assess extent of smokeless tobacco use and identify populations at risk.
	• Identify currently available resources and intervention needs.
	• Develop a formal state tobacco control plan that includes smokeless tobacco (as recommended by the Association of State and Territorial Health Officials, 1989).
	• Encourage schools to adopt tobacco-free policies and curricula that address all forms of tobacco use.
Federal Government	• Provide appropriate resources for agencies that have responsibilities for smokeless tobacco surveillance and control.
	• Support research and disseminate findings.
	• Provide leadership in identifying research needs.
	• Provide a clearinghouse for ST research, policy, and control activities.
	• Promote transfer of scientific findings to public health educators, communications experts, and activists for dissemination to the public.
	• Coordinate smoking and smokeless tobacco control activities carried out by PHS agencies and offices and promote communication among all Government agencies involved in tobacco surveillance and control.
	• Provide clear and prominent communications to the public about the hazards of ST use.

	• Provide technical support to States, including surveillance data, resource materials and listings, and current information on national activities.
	• Encourage antismoking activists to extend their activities to include smokeless tobacco.
	• Assist in recruitment of prominent spokespersons by endorsing activities, providing thank you's, or awarding special recognition.
	• Provide resources for public education campaigns in the form of media materials (public service announcements or news kits), resource listings, and background information.
	• Provide resources for intervention delivery through resource listings, guidelines for materials development, and copies of materials produced by PHS agencies.
	• Assemble information and arguments needed to support legislative initiatives and make such information available to activists.
Health Educators	• Include smokeless tobacco as part of tobacco education programs, emphasizing that ST is not a safe alternative to cigarettes.
	• When possible, make use of available tobacco education materials. Develop new materials or adapt older ones, as needed.
	• Work with departments of education, school districts, teachers, administrators, and parent associations to elicit support for tobacco education. Encourage national organizations of these groups to pass formal position statements.
	• Promote tobacco-free policies in schools as an essential component of health education programs and interventions.
	• Direct educational efforts to high-risk youth both in school and in outside settings, such as trade schools, shop classes, 4-H Clubs, Future Farmers of America, YMCA, Little League, and other youth organizations and clubs.
	• Extend tobacco education to colleges, universities, and other postsecondary educational institutions.
Health Professional Organizations	• Work for inclusion of interventions in smokeless tobacco as part of professional education.
	• Support continuing education programs to train professionals to intervene with their patients.
	• Promote inclusion of tobacco counseling skills in professional licensing requirements.
	• Adopt formal tobacco control positions that specifically include smokeless tobacco. Strengthen current position statements in light of the increase in ST use that has occurred since 1986.
	• Sponsor, support, or promote national- and community-level smokeless tobacco control programs and public education campaigns.

- Solicit advice from experts in the field to ensure scientific accuracy of all statements regarding ST.
- Provide tobacco cessation services to employees and students.

Activists

- Enlist support of prominent individuals to serve as spokespersons.
- Provide training in advocacy skills to volunteers from the community.
- Work with local media to secure news coverage and adequate placement of public service announcements.
- Sponsor ST control events.

All

- Provide multiple and sustained messages to the public about the dangers of smokeless tobacco and cigarettes.
- Direct public education materials and campaigns to groups at highest risk; for example, televised broadcasts of sporting events provide access to potential users.
- Use schools as a valuable channel of access to youth, but efforts to reach youth should not be confined to schools.
- Enlist support of major sports associations to refuse involuntary promotion (accepting free samples, using tobacco products in public and while on camera, display of logos and tobacco product names at sporting events, and acceptance of tobacco company sponsorships).
- Provide education and cessation counseling to professional athletes.
- Encourage organizations and groups with an interest in reducing ST use to extend their resources by coordinating their activities whenever possible.
- Participate in and support networks, newsletters, and other mechanisms to increase communication among individuals and organizations involved in tobacco control.

Research Priorities

Research, Intervention, and Surveillance Needs

- Additional case control studies should be carried out in geographic regions with high prevalence of use to quantify the risk for oral cancer and to identify other cancers, such as lung cancer, for which smokeless tobacco could be a risk factor.
- On the basis of additional epidemiological research, investigators should develop estimates of the attributable cancer risk from ST use.
- Researchers should develop biological markers to identify and quantify ST use for application in epidemiological, clinical, and behavioral research.
- Data are needed to assess patterns of smokeless tobacco use and monitor trends. All national tobacco surveys, especially surveys of youth, should include questions on ST use. Smokeless tobacco should not be omitted from surveys of smaller populations without clear evidence that it is not used.

- Standardized questions about tobacco use, knowledge, and attitudes should be developed and circulated to all agencies and organizations involved in collecting survey data. Brand names of ST products should be included to prevent confusion between snuff and chewing tobacco.
- Because youth are at special risk, national surveys of youth should include questions on perceived risks of ST use, amount of ST used, age of initiation, quit attempts, and use of cigarettes.
- More information is needed on the relationship of smokeless tobacco use to cigarette smoking, especially among young users.
- Researchers should identify motivations for ST use and barriers to cessation within various populations to develop relevant intervention strategies.
- Investigators should determine the ST knowledge and attitudes of key persons (parents, teachers, coaches, youth group leaders, professional athletes) who can function as intermediaries to bring health messages to youth.
- Researchers should develop prevention and cessation materials appropriate for target audiences, including youth, young adults, athletes, people in high-risk occupations such as logging, people with low literacy, Native Americans, and rural populations.
- Assessing the efficacy of prevention and cessation interventions for ST control should continue.
- A centralized clearinghouse is needed to monitor the literature on smokeless tobacco research, policy, media coverage, and intervention activities and to aid in research dissemination.
- New surveillance sources for clinical data, such as professional organizations and associations, should be established.
- Research on warning labels is needed to determine how they can be made more effective.

Strategies

The following strategies are not endorsed by the U.S. Government or its agencies. These recommendations were developed by smokeless tobacco control experts from the United States and other countries, convened during the U.S. workshop and the First International Conference.

The recommendation of the World Health Organization Study Group on Smokeless Tobacco Control—that countries with no established practice of smokeless tobacco use should ban manufacture, importation, sale, and promotion of ST products—is supported. In countries where smokeless tobacco use is already established, such as in the United States, it is recommended that the following legislative, regulatory, and policy objectives be instituted as *interim* measures to help achieve the long-term goal of a tobacco-free society.

All Levels of Government

- A significant proportion of public revenues derived from the sale of tobacco products should be used for tobacco intervention-related public education, research, and services.
- Taxes on smokeless tobacco should be made commensurate with those on cigarettes, and future tax increases should be applied equally to all tobacco products. The taxes should be indexed to the rate of inflation.
- States should enact legislation to prevent the sale or distribution of smokeless tobacco to minors, including sales through vending machines. Such legislation should include provisions for vigorous enforcement.
- Relevant coalitions should develop and disseminate model State and local legislation for tobacco control.
- Governments should eliminate or severely restrict all forms of tobacco product advertising and promotion to which minors are likely to be exposed, including the following:
  - image-based ads with special appeal to young people (allow only tombstone ads, i.e., black lettering against white background, until a total advertising ban is achieved);
  - placement of advertising in periodicals with large youth readerships and in those with large minority readership;
  - the use of ST product names, logos, or likenesses on other products or packaging, especially on products such as toys, bubble gum, and other child-oriented items;
  - placement of outdoor advertising near schools, recreation areas, or other places where young people congregate;
  - display of smokeless tobacco at point of sale or adjacent to items that typically appeal to children, such as candy, toys, and comic books;
  - distribution of ST samples through the mail;
  - distribution of free ST samples in public places;
  - smokeless tobacco advertising and promotion through sponsorship of athletic, sporting, cultural, or entertainment events;
  - display of promotional ads, company logos, product names, or product representation at sporting and entertainment events, especially when these events are televised.

Federal Government

- Recognizing that there is no safe level of *N*-nitrosamines in tobacco products and that it may not be technically feasible to produce an ST product free of *N*-nitrosamines, the Government should ban smokeless tobacco in the United States.

- Until a total ban is achieved, ST manufacturers should be required to seek approval of all additives used in their products from an appropriate Government agency. Furthermore, manufacturers should reduce the amount of nicotine, nitrosamines, polonium, and other known hazardous substances in their products to the lowest levels technically possible. Smokeless tobacco manufacturers should be required to list the additives in large, easily legible type on each ST container, on billboard advertisements, and in print media.
- The set of mandatory rotating health warnings on smokeless tobacco products and billboards should be expanded to include addiction as a health risk and to indicate that health benefits accrue to users when they quit. Such warnings should be prominently printed on each individually packaged product, on billboards, and in print media.
- The Federal Government should not support, or give the appearance of supporting, the production, manufacture, or sale of tobacco products.

Other Channels

- Activists should monitor the level and nature of ST promotion, especially noting violations of the industry's voluntary code prohibiting promotion to minors.
- All health-related organizations and associations should issue policy statements condemning the use of tobacco products, including ST, and ensure that these statements are well publicized to their members and the public. Such organizations should encourage their members to become active in tobacco control activities and provide, through their contacts with patients or clients, training, technical support, or referral as needed.
- Health and life insurance companies should determine policy applicants' use of smokeless tobacco, as well as cigarettes, to estimate risk and determine insurability.
- Tobacco-control coalitions should assemble information and arguments needed to support legislative initiatives.

REFERENCES

American Cancer Society. *Cancer Facts & Figures—1992*. Atlanta, GA: American Cancer Society, 1992, p. 5.

Association of State and Territorial Health Officials. *Guide to Public Health Practice: State Health Agency Tobacco Prevention and Control Plans*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute. NIH Publication No. 90-1577, October 1989.

Consensus Conference. Health applications [implications] of smokeless tobacco use. *Journal of the American Medical Association* 255(8): 1045-1048, 1986.

International Agency for Research on Cancer. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Habits Other Than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines* (volume 37). Lyon: IARC, 1985.

International Symposium on the Control of Tobacco-Related Cancers and Other Diseases. Tata Institute of Fundamental Research, Bombay, India, January 15-19, 1990.

Orlandi, M.A., Boyd, G.M. Smokeless tobacco use among adolescents: A theoretical overview. *National Cancer Institute Monographs* 8: 87-94, 1989.

Schoenborn, C.A., Boyd, G.M. *Smoking and Other Tobacco Use: United States, 1987*. Vital and Health Statistics, Series 10, No. 169. Hyattsville, MD: U.S. Department of Health and Human Services, 1989.

U.S. Department of Health and Human Services. *The Health Consequences of Using Smokeless Tobacco: A Report of the Advisory Committee to the Surgeon General*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH Publication No. 86-2874, 1986.

U.S. Department of Health and Human Services. *Healthy People 2000: National Health Promotion and Disease Prevention Objectives*. U.S. Department of Health and Human Services, Public Health Service. DHHS Publication No. (PHS) 91-50212, 1991.

U.S. Department of the Treasury. *Monthly Statistical Release: Tobacco Products*. Bureau of Alcohol, Tobacco, and Firearms, Department of the Treasury, 1986-1991, compiled by authors.

World Health Organization. *Smokeless Tobacco Control*. WHO Technical Report Series 773. Geneva: WHO, 1988.

APPENDIX

U.S. Workshop Participants

Myron Allukian, Jr., D.D.S., M.P.H.
Gayle M. Boyd, Ph.D., M.P.H.
Greg Connolly, Ph.D.
Stephen Corbin, D.D.S., M.P.H.
Mary Daum
Carol D'Onofrio, Ph.D., Dr.P.H. (Chair)
Virginia Ernster, Ph.D.
Thomas F. Glynn, Ph.D.
Jerome Goldstein, Ph.D., M.D.
Dietrich Hoffmann, Ph.D.
John Horm, M.Sc.
Susan Johnson
Matthew Kinnard, Ph.D.
Dushanka Kleinman, D.D.S., M.Sc.
Steven Marcus, Ph.D.
Jeffrey McKenna
Robert Mecklenburg, D.D.S., M.P.H.
C. Tracy Orleans, Ph.D.
Kathleen L. Schroeder, D.D.S., M.Sc.
Herbert Severson, Ph.D.
R. Craig Stotts, Dr.P.H., R.N.
Phillip Swango, Ph.D.

Index