

CROSS REFERENCE

FOLDER(S) TITLED: U.S. Tobacco + Health Research Program
SEE: Cigarettes - Nicotine
Report March 1968
RE: "Factors That Influence
Nicotine Level in
Tobacco"

**THE TOBACCO AND HEALTH RESEARCH PROGRAM
AT THE UNIVERSITY OF KENTUCKY**

Presented by Dr. Raymond C. Bard, University of Kentucky, at
the Seventeenth Annual Convention of the Burley and Dark Leaf
Tobacco Export Association, Inc., in French Lick, Indiana, on
October 3, 1966.

First of all, let me thank you for the opportunity to be with you and to describe briefly the development of our Tobacco and Health Research Program at the University of Kentucky. Tobacco research has been going on in Lexington for many years, as it has in many other locations, but I believe it is proper to state that our program is the first, integrated effort to relate smoking and human health by a coordinated group of scientists, together, in the same place, at the same time.

During the summer of 1964, it became general knowledge that the United States Department of Agriculture had been authorized to support a program of research relating the usage of tobacco to human health, such research to be financed by an appropriation of one and one-half million dollars, allocated for expenditure in Kentucky.

In October of 1964, negotiations with the Department of Agriculture were initiated by the University of Kentucky and, at that time, the University submitted a two-year program of research. Intimate liaison was established with three divisions of the Agricultural Research Service, resulting in a vigorous exchange of visits and views between the Department's and the University's scientists and administrators. It soon became apparent that, like the University, the Department had established certain of its own research objectives and, to be perfectly frank, these objectives were not always shared mutually, especially in the area of biomedical research. Accordingly, a program has evolved which is appropriately to be considered the combined efforts of the Department of Agriculture and the University of Kentucky, not a program which can be rightly considered uniquely and solely as that of the University.

That such a development occurred is, in hindsight, readily understandable. The vast area of tobacco research is characterized by complexity, as any individual or group competent in this area will attest. Many research objectives were reviewed and, as a consequence, research agreements took time to prepare, and were not terminated officially until June of 1965. These agreements then became effective on a two year contract basis, funded from the original appropriation, starting in July 1965. Thus, actual program research preparation and activity have been in effect for only the past fifteen months, placing me in no position today to report any significant scientific results that will permit us to draw any important conclusions. With the availability of the new appropriation for 1966-67, we again negotiated contracts for the years 1966-68, covering additional new areas of research.

Before attempting to outline the specific components of the collaborative research program being described, let me tell you something about how we coordinate the several projects being conducted within the program. Internally, the University is guided by a Technical Committee composed of two representatives from each of the three major University units involved in the program: the Agricultural Science Center, the Medical Center, and the College of Arts and Sciences. This committee meets often, and its ac-

tions provide general guidance to the project directors in charge of specific research activities. Aside from the typical informal liaison so important among scientists, all project directors receive copies of the semiannual progress reports prepared by each project director. By these means, program participants are aware of what is going on in all projects making it easy for all concerned to be kept informed, to exchange ideas, and generally to contribute to the overall endeavor. Fortunately, the three major physical facilities in which our program is housed are within easy walking distance of each other, so that physical separation of research activities is not a problem.

Stated in general terms, the program is intended to develop tobacco plants with known genetic composition, grown under controlled environmental and nutritional conditions, which will then be treated under known circumstances in such ways as to yield cigarettes whose histories are relatively well understood. These cigarettes will be smoked mechanically, and the smoke and other combustion products analyzed in a variety of ways for a number of components. Smoke products will then be studied biologically to determine effects, if any, upon tissues and organs -- providing thereby the final link in our quest to evaluate the relationships between tobacco and health.

Let me sketch for you, in broad outline, the individual major projects now underway in our program.

Starting as we must with the tobacco plant, a team headed by Dr. G. W. Stokes (Departments of Agronomy and Plant Pathology) is determining the genetic control of the chemical constituents of the plant, over several successive generations, that might be related to smoking and health, including substances such as nitrogen bases, sterols, phenolic compounds, isoprenoids, etc. These plants are being grown in the field as well as in laboratory growth chambers, so that conditions are well controlled and subject to analysis.

A biochemical group, under the direction of Dr. Andrew J. Hiatt (Department of Agronomy), is investigating the metabolic and regulatory processes of the tobacco plants reared by Dr. Stokes, from the green stage through post-harvest treatments, in order to determine what happens to chemical constituents that may be related to smoking and health.

Two groups, headed by Doctors John L. Ragland and John L. Sims (Department of Agronomy), are conducting investigations of experimental tobacco grown on extremes of soil types which differ with regard to nitrogen content, moisture, fertilizer use, and other environmental conditions, such as light and temperature. Tobacco grown under these defined conditions and cured in a variety of ways are being analyzed for chemical composition, to include such constituents as alkaloids, nitrates, amino acids, proteins, amines (especially nitrosamines), and other nitrogenous substances whose concentrations in the plant may be affected

by nitrogen nutrition, including agricultural chemicals containing amines.

Doctors Charles Yang and Malcolm R. Siegel (Department of Plant Pathology) are determining the changes in the chemical composition of tobacco leaf, including the formation of carcinogens, that occur during growth, curing, and storage of tobacco as a result of the metabolic activities of microorganisms, both the natural flora and the flora causing disease, to include viruses, bacteria, and fungi.

Dr. Herbert F. Massey (Department of Agronomy) and his associates are developing methods for separating, identifying, and quantitatively determining in cigarette smoke, derived from the tobacco grown in the program, such constituents as polynuclear aromatic hydrocarbons and heterocyclic compounds. The objective of this project involving analytical chemistry is largely the development of optimum methods, with the shortest performance time, to be used throughout our program.

The efforts of Dr. Ellis V. Brown (Department of Chemistry) are dedicated to the isolation of heterocyclic bases in cigarette smoke and to the determination of their structures and quantities, placing emphasis on individual compounds not presently known to occur in cigarette smoke. In addition, he is launching a special study of the nitrosamines found in cigarette smoke condensates. He will employ such advanced methodology as required to attain his objective, including ultraviolet, infrared, fluorometric, and nuclear magnetic resonance spectrometry.

Doctors John M. Patterson and Walter T. Smith, Jr. (Department of Chemistry) are conducting investigations on the pyrolysis of pure amino acids, (phenylalanine, tryptophan, leucine, lysine) alone and in combination, in a nitrogen atmosphere at certain temperatures in the range of 450-950C to obtain pyrolytic products which will then be isolated and identified by standard and advanced methods.

Under the direction of Dr. Daniel L. Weiss (Department of Pathology), a team is investigating the physiological activities of tobacco extracts and components, and of cigarette smoke condensates by determining the biological responses of test animals. These responses will be studied in a variety of ways in order to establish evaluation criteria. Thus, the epithelial tissues of rodents will be employed as test sites, whereupon cytological analysis of these tissues will be performed utilizing suitable techniques, including electron microscopy. The objective of this project is the development of new and rapid bioassay procedures for cigarette smoke.

Dr. Weiss and his associates are also studying the physiological effects of smoke and fractions thereof in the intact respiratory tract of rodents. An associated project involves the joint exposure of the respiratory tract of experimental animals to myxovirus infection (mouse adapted human influenza virus strain) and tobacco smoke.

Doctors Irene E. Roeckel and Louis D. Dubliler (Department of Pathology) are also investigating the respiratory epithelial tissue of mice, including tracheal, bronchial, and alveolar cells, in order to determine the effects of cigarette smoke and fractions thereof upon such tissue. Cell turnover rates and chromosomal patterns will be measured as a means of quantitating the effects of tobacco products upon the tissues of the respiratory tree.

Dr. Jerome E. Cohn (Department of Medicine) is determining the effect of tobacco products and fractions thereof on rat tracheal rings grown in tissue culture. The effects upon such epithelial structures as base, brush, and goblet cells are being studied histologically and histochemically.

Dr. Rudolph J. Muelling (Department of Pathology) is conducting investigations designed to determine the effect of trace metals in tobacco products on animal tissue utilizing, among other techniques, atomic absorption and X-ray analytical procedures. The objective of this project is the determination of interrelationships between the trace metal content of tobacco products and exposed tissue, especially as tissue changes occur.

Under the direction of Doctors Paul Courant and Ernest Lalonde of the College of Dentistry, investigations are underway to determine the effect of tobacco, tobacco smoke, and fractions thereof on their absorption by oral tissues, on the secretion of saliva, and on certain oral bacteria. Syrian hamsters are being employed for the absorption studies in which tissues will be examined histologically and cytochemically. Human subjects will be studied for the effect of smoking on salivary secretion, especially as this practice relates to salivary levels of thiocyanate and peroxidase.

Doctors Paul Mandelstam and Douglas Rees (Department of Medicine) are studying the intestinal absorption of C^{14} or tritium labeled polynuclear aromatic hydrocarbon carcinogens in rodents, using this information for comparison of the similar absorption of tobacco products. This project, like other biomedical projects, seeks to develop more meaningful tests to determine the biological effects of tobacco utilization.

Dr. James Flesher (Department of Pharmacology), using radioisotope technics, is measuring the distribution and metabolism of known carcinogens in the bodies of rodents, including the stomach, mammary gland, liver, fat, kidney, skin, and adrenal glands. This project will provide background for similar distribution studies using tobacco products.

Finally, let me note the fact that the specially grown tobacco being studied in this program is processed under well-defined conditions and then manufactured into the cigarettes to be analyzed. A special manufacturing laboratory as well as a cigarette smoking

laboratory are being activated to provide materials to project directors who are analyzing tobacco products by the chemical and biological approaches described.

You will note that our program begins with biology, and swings the full circle back to biology. This strategy is not surprising, for two major reasons. We are interested in learning about the effects of tobacco, a plant whose composition is much affected by what happens to it while it is living and how it is treated thereafter, and we are interested in the effects of tobacco products, mainly smoke, upon the living consumer, namely, the human being.

It is fortunate that our research is going on now, during what can be properly called the Age of Biology. Within the past two decades, there has been great progress in biological research, and a vast amount of new knowledge is being accumulated dealing with life at all levels, from the master molecules that control life and reproduction, through an increasing insight into the biochemical mechanisms of cell behavior, toward the ultimate understanding of man as a complex living entity. Where and how the interaction between tobacco and man occurs is what we want to learn much more about, and this search deals, in the final sense, with biology.

Summarizing these activities in terms of the number of individuals participating in the program thus far, let me tell you that 34 senior scientists and about twice that many assistants are involved at this time.

Included in this program are four senior Agricultural Research Service scientists. Dr. Donald Burdick is engaged in smoke chemistry research, Dr. Paul Legg is pursuing genetics research, while Doctors Amber Chang and Robert Anderson are involved in several biochemical projects. As has been the tradition of collaborative research with ARS personnel, we welcome these individuals to our program, and we express gratitude to them for their participation.

This sketchy outline of our program will not satisfy the technical experts seeking details. I invite such experts to direct their inquiries to our project directors or to me personally, and we shall be happy to provide details. For the proper implementation of our projects, the University has altered space for laboratories and animal quarters to accommodate project needs, representing a major capital investment on our part. Thus, we are underway. Progress will be slow as is true of any form of progress of lasting significance.

I have been serving as the director of this program. It has been an exciting experience. We are still looking for an individual to fill this role, not only to coordinate the entire program internally, but to serve as the focus of information collection, from all sources,

regardless of location. In the latter capacity, with our scientists, we wish to maintain close liaison with centers for tobacco research throughout the world, in order to synthesize all data available into a meaningful pattern of understanding.

Since we have more ideas for research than we can activate into specific projects, due to financial limitations, we seek public and private means to expand our program. We view our current efforts as the core of a much enlarged program, having as a dream the creation of a center for tobacco research, located in Lexington, unmatched anywhere in the world for scope, capability, achievement, and coordination so that the total objective of the program will be attained.

Let me point out that our current efforts will not approach the critical mass for quite a long time. I have borrowed the concept of the critical mass from the nuclear physicists who had to learn how much uranium or plutonium was needed to be put together in the same place at the same time before the desired chain reaction would occur, and which led finally to the atomic explosion. This analogy is pertinent in the case being discussed. Until a large enough, coordinated effort is brought together, in the same place at the same time, the knowledge explosion needed will not occur. Splintered research efforts in many locations, as has been the situation in the past, will continue to lead to minor and ineffective sputterings, encouraging at times, but doomed to failure because the needed chain of facts and evidence will not be forthcoming. Half-vast plans for research will not yield the power of truth needed to guide the actions of responsible leadership, with the end result known to all of you.

In closing, let me say that it is obvious that we are aware of the many difficulties facing us in this program. At times, we may seem naive, but such a posture is better than one exhibiting cynicism and depression from past defeats. We are enthusiastic, and we are vigorous. Only time will tell how successful we shall be. But we are aware that you and many others can assist us, and I solicit such aid, advice, and understanding from any quarter. We do not wish to work in isolation. We do wish to collaborate with any individual or group whose assistance and guidance will, in our judgment, facilitate our voyage toward the truth.

While reading a report from the Texas A & M Research Foundation, I spotted a statement by Walter Lippmann which can be considered the motto of our program. Let me quote: "To bring to light the hidden facts, to set them into relation with each other, and to make them a picture of reality on which men can act." I think these words describe our objectives well.

Let me thank you again for the opportunity of talking with you. More important, let me thank you in advance for any help and understanding you will extend to us.