On the Etiology and Metabolic Epidemiology of the Main Human Cancers

J. H. Weisburger, L. A. Cohen and E. L. Wynder

Naylor Dana Institute for Disease Prevention
American Health Foundation, Valhalla, New York 10595

It has been stated in scientific as well as in lay circles that the majority of human cancers (70–90%) are due to environmental causes (Higginson and Muir 1973; Higginson 1976; Wynder 1976). By environmental causes, it is generally assumed that one means chemical causes and, more specifically, those due to our modern technology and industrial development. In support of this understanding, scientific papers and also reports in the public press indicate that a number of food additives, pesticides, insecticides, and industrial chemicals introduced commercially in the last 40 years have exhibited carcinogenic properties in animal models (Weisburger 1976). Historically, human cancers have also been shown to be related to chemical exposure in an occupational environment, to the intake of specific drugs, or to exposure to specific chemicals (Saffiotti and Wagoner 1976). Thus the association in the public mind between our chemical environment and cancer causation is easily understood. Regrettably, current evidence suggests that the main human cancers, discussed in detail below, do not stem from intentional or even inadvertent chemical contaminants in our environment. We say regrettably because if such an association could actually be demonstrated, prevention of the main human cancers through removal of the offending substances would be a rather readily achievable goal.

To acquire insight into the causes of cancer, it is necessary to analyze the conditions inherent in the occurrence of each specific type of cancer. From worldwide statistics illustrating the incidence of diverse cancers, the altered risk of migrants from areas of high to low incidence over several generations, and the corresponding analysis of data obtained under controlled conditions in animal models, a picture emerges permitting delineation of the multiple causative factors involved in each of the main human cancers (Wynder and Mabuchi 1972; Fraumeni 1975). According to current ideas, these causal factors are basically unrelated to food additives, insecticides, pesticides, or synthetic contaminants in drinking water. The exception, of course, is the occurrence of specific cancers due to exposure in an occupational setting or to the consumption of drugs known to be carcinogenic. It would appear that at most 5% of human cancers are due to such exposures (Weisburger 1976; Higginson 1976).

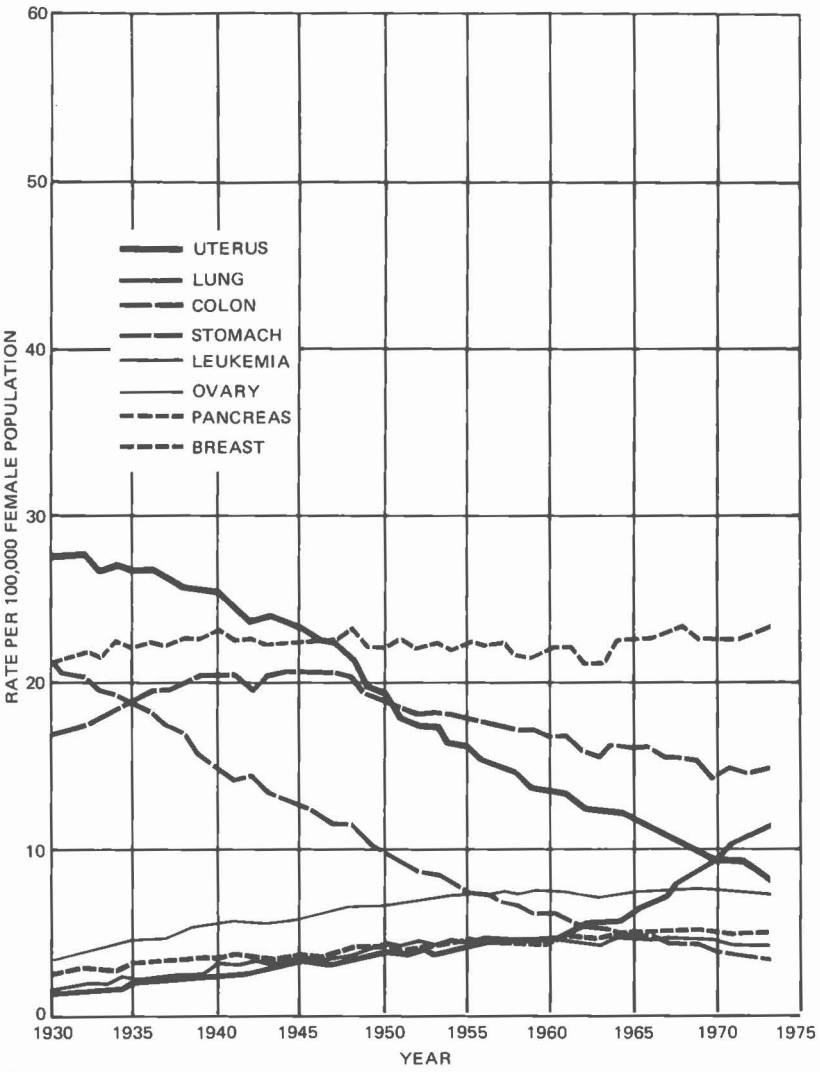

Figure 1
Age-adjusted cancer death rates for selected sites, females (U. S., 1930–1973); standardized on the age distribution of the 1940 U. S. Census Population. Sources: U. S. National Center for Health Statistics and U. S. Bureau of the Census. (Reprinted, with permission, from Seidman et al. 1976.)

The one cogent argument we can marshal for these views depends on two facts: The introduction of a carcinogen to the general public, i.e., cigarette smoking, mostly in U.S. males during and after World War I and in females during and after World War II, has resulted 15–25 years later in an impressive rise in the incidence of lung cancer as well as in an increase in other specific cancers, such as those of the renal excretory pathway (Wynder and Hecht 1976; Wynder et al. 1976a) (Figs. 1 and 2). On the other hand, the introduction of insecticides, pesticides, and food additives in the last 40 years appears to have had no detectable effect on the incidence of the main human

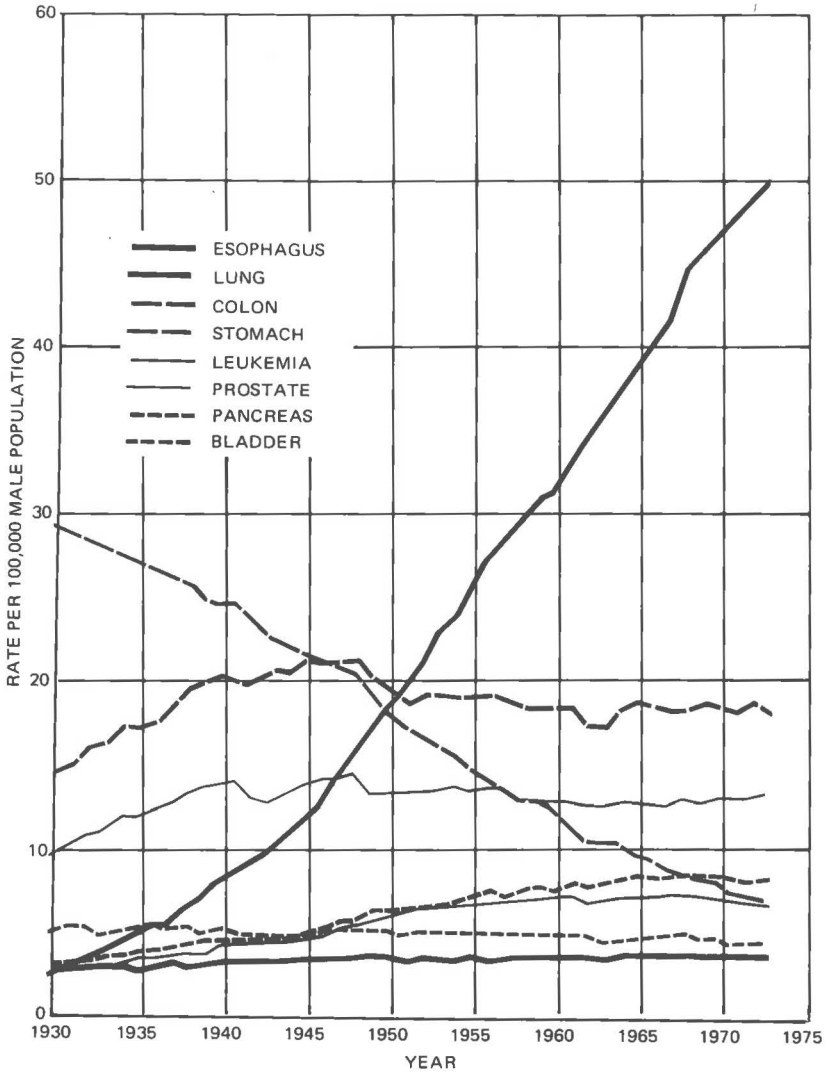

Figure 2
Age-adjusted cancer death rates for selected sites, males (U. S., 1930–1973); standardized on the age distribution of the 1940 U. S. Census Population. Sources: U. S. National Center for Health Statistics and U. S. Bureau of the Census. (Reprinted, with permission, from Reddy and Wynder 1973.)

cancers in the United States, such as those of the breast, prostate, and colon (Seidman et al. 1976). Breast and colon cancer have exhibited a slightly increased incidence, almost paralleling the increased daily intake of dietary fat in the United States (Gortner 1975). For reasons that are as yet unclear, the incidence of pancreatic cancer also appears to have risen in the last few years, although one risk factor is smoking and another is dietary fat (Wynder 1975; Seidman et al. 1976). On the other hand, stomach cancer has decreased appreciably, as has primary cancer of the liver.

In this paper, we present summary statements of the factors contributing to

the etiology of the main human cancers in the United States, specifically, cancers of the lung, breast, prostate, stomach, and colon.

CANCER OF THE LUNG AND OTHER TOBACCO-RELATED DISEASES

Prior to 1930, lung cancer was a relatively rare disease. Since then, however, its incidence has increased considerably in the United States for men and since about 1950 also for women (DHEW 1964, 1967, 1968, 1969, 1971, 1972, 1973, 1974; Wynder and Hecht 1976; Wynder et al. 1976a; Seidman et al. 1976; Hammond et al., this volume) (Figs. 1 and 2).

The death rate from lung cancer (standardized for age distribution of the U.S. population in 1940) rose from about 4 per 100,000 in 1930 to 52 per 100,000 in 1975, and the age-adjusted death rate for women climbed from 2 per 100,000 to 11 per 100,000 in the same time period (Seidman et al. 1976). It is well established that the primary reason for this dramatic increase in lung cancer incidence and deaths in the United States is the smoking of manufactured cigarettes, a practice which men began around the time of World War I and which became fashionable for women just prior to World War II. Data on lung cancer trends in other countries with accurate statistics for lung cancer mortality, such as England, the Scandinavian countries, and Japan, also bear out the direct relationship between lung cancer and smoking habits in men and women, besides reflecting variations due to lessened availability of cigarettes at certain times (during wars) (Doll and Hill 1964; Kreyberg 1969; Segi and Kurihara 1972).

The strong statistical association of cigarette smoking and lung cancer incidence, developed chiefly by Wynder, Doll, and Hammond, have, since 1953, prompted laboratory investigations on the carcinogenic potential of tobacco smoke (Wynder et al. 1953). The biological evaluation of tobacco smoke and its condensate, together with the chemical identification of the major components involved in tobacco carcinogenesis, has not only contributed much information but has also led to product modifications aimed at less harmful cigarettes (Gori 1976; Hammond et al. 1976; Hammond et al., this volume). In the United States, major laboratory work was initiated in 1953 by Wynder, and contributions have been made and continue to emanate from work by E. L. Wynder and D. Hoffmann, F. Bock, B. Van Duuren, F. Homburger, and G. Gori. These and worldwide contributions are reflected in a book, *Experimental Tobacco Carcinogenesis* (Wynder and Hoffman 1967), more recently in the *Proceedings of the Third World Conference on Smoking and Health* (Wynder et al. 1976a), and also in the monograph *Lung Cancer* (Wynder and Hecht 1976).

Tobacco smoke is an aerosol consisting of a gaseous and a particulate phase with a distribution equilibrium of certain smoke constituents in both phases. As a respiratory carcinogen, with a median particle size of 0.4 μ, the aerosol exerts multiple effects of gaseous as well as particulate components. Smoke constituents play a role as ciliatoxins, as initiators of the carcinogenic process, and as cocarcinogens and tumor promoters (Hoffmann et al. 1976). Tobacco carcinogenesis has been studied in a variety of species and sites in animal

models. Tumors have been induced with smoke particulates on mouse skin, rabbit ear, and mouse cervix, in newborn mice, and also in the connective tissue, trachea, and lungs of rats, and in the trachea of dogs. In the upper respiratory tracts of hamsters, mice, and rats, tumors have been elicited as a response to spraying of the smoke condensates as aerosols (Hoffmann et al. 1976). Tobacco smoke particulates and fractions thereof have also been found to have mutagenic effects in several strains of *Salmonella,* as was demonstrated by Ames and his group (Kier et al. 1974) and by Sugimura et al. (this volume).

The gaseous phase of cigarette smoke condensate has not induced tumors in animal models but it has been shown to contain trace amounts of carcinogens such as the volatile nitrosamines, tumor promoters such as formaldehyde, and major ciliatoxic agents such as hydrogen cyanide, formaldehyde, acrolein, and acetaldehyde (Table 1).

Although animal models and passive inhalation techniques for a study of respiratory carcinogens have now been developed to a greater degree, they do not appear to circumvent one chief obstacle in tobacco smoke carcinogenesis, the natural defense of obligatory nosebreathers against particles in inhaled aerosols. Much of the particulate is filtered from the smoke and fails to reach the lungs. Nonetheless, neoplastic lesions in the larnyx have been obtained by

Table 1
Tumorigenic Agents in the Gas Phase of Tobacco Smoke

Toxic agents	*Concentration in smoke of one cigarette*
Carcinogens[a]	
H_3C N—NO	5–180 ng
H_3C R N—NO (6 compounds)	2–200 ng
R^1	
$H_2N—NH_2$	24–43 ng
$H_2C=CHCl$	10–40 ng
Tumor promoters	
HCHO	20–90 μg
Ciliatoxic agents	
HCN	100–700 μg
HCHO	20–90 μg
$H_2C=CH—CHO$	45–140 μg
$H_3C—CHO$	18–1440 μg

List is based only on publications with unambiguous identifications of toxic agents. (Data from Wynder et al. 1976a.)

[a] Tobacco smoke is suspected of also containing H_3As, $Ni(CO)_4$, and possibly volatile chlorinated olefins and nitroolefins.

chronic exposure of hamsters to cigarette smoke. More direct introduction of tobacco smoke into the trachea of dogs by a tracheotomy has led to early neoplastic lesions in the lung in as short a time as 3 years (Auerbach et al. 1967, 1970).

The complexity of the particulate phase of tobacco smoke was unraveled by fractionation techniques, which have led to the identification of the major tumorigenic components and their relative roles in bioassays. These compounds are listed in Tables 2–4.

It was shown that the major carcinogenic activity on mouse skin was due to the neutral components (mainly polynuclear aromatic hydrocarbons), although cocarcinogens in this fraction, as well as tumor promoters in the weakly acidic portion and in the basic portion of the smoke condensate, further contribute to the total carcinogenic effects. The structural elucidation of tobacco smoke constituents with biological activities has recently led to the finding of tobacco-specific nitrosamines which are derived from tobacco alkaloids. Testing of synthetic alkaloid nitrosamines has shown carcinogenicity

Table 2
Tumor-initiating Agents in the Particulate Phase of Tobacco Smoke

Compound	*Relative activity as complete carcinogen*[a]	*Concentration ng/ cigarette*[b]
Benzo(a)pyrene	+++	10–50
5-Methylchrysene	+++	0.6
Dibenz(a,h)anthracene	++	40
Benzo(b)fluoranthene	++	30
Benzo(j)fluoranthene	++	60
Dibenzo(a,h)pyrene	++	pr
Dibenzo(a,i)pyrene	++	pr
Dibenzo(a,j)acridine	++	3–10
Indeno(1,2,3-cd)pyrene	+	4
Benzo(c)phenanthrene	+	pr
Benzo(a)anthracene	+	40–70
Chrysene	+	40–60
Benzo(e)pyrene	+(?)	5–40
2-,3-Methylchrysene	+	7
1-,6-Methylchrysene	–	10
2-Methylfluoranthene	+	34
3-Methylfluoranthene	?	40
Dibenz(a,c)anthracene	(+)	pr
Dibenz(a,h)acridine	(+)	0.1
Dibenzo(c,g)carbazole	(+)	0.7

Incomplete list; all mentioned compounds are active as tumor initiators on mouse skin. (Data from Hoffmann et al. 1976.)

[a] Relative carcinogenic activity on mouse skin as measured in our own laboratory on Swiss albino (ha/ICR/Mil) mice. ? = carcinogenicity unknown; (+) = not tested in our own laboratory.

[b] pr = present, but no quantitative data given.

Table 3
Cocarcinogenic Agents in the Particulate Matter of Tobacco Smoke

Compound[a]	*Cocarcinogenic activity*[b]	*Concentration ng/cigarette*[c]
Neutral fraction		
pyrene (−)	+	50–200
methylpyrenes(?)	?	50–300
fluoranthene (−)	+	100–260
methylfluoranthene (+;?)	?	180
benzo(ghi)perylene (−)	+	60
benzo(e)pyrene (+)	+	30
other PAH (+)	?	?
naphthalenes (−)	+	360–6300
1-methylindoles (−)	+	830
9-methylcarbazoles (−)	+	140
4,4′-dichlorostilbene (−)	+	1500
other neutral compounds (?)	?	?
Acidic fraction		
catechol (−)	+	200,000–500,000
4-alkylcatechols (?)	?	>10,000
other phenols (?)	?	?
other acidic agents (?)	?	?

Incomplete list. (Data from Hoffmann et al. 1976.)
[a] Items in parentheses indicate carcinogenic activity on mouse skin; ? indicates unknown.
[b] + = active; ? = unknown.
[c] Value from 1968 U.S. cigarette; today's values would be lower because DDT and DDD decreased in U.S. tobaccos.

in rats and in the upper respiratory tracts of the Syrian golden hamster for *N′*-nitrosonornicotine (Hilfrich et al. 1977). Since compounds of this type are found in chewing tobacco in concentrations up to 90 ppm (Hoffmann et al. 1974), a further evaluation of their role in human carcinogenesis appears warranted. The possible in vivo nitrosation of tobacco constituents which would lead to the formation of organ-specific carcinogenic nitrosamines is an area of investigation that requires further attention.

The presence in tobacco smoke particulates of aromatic amines and other bladder carcinogens and the statistical association of bladder and kidney cancer mortality with cigarette smoking (Hammond 1975) demand a structured and systematic approach toward the elucidation and quantitation of biologically active compounds.

Although the association of tobacco smoking and lung cancer is a major one and is therefore the one most extensively studied, the risk factors for cancer of the kidney and urinary bladder, cancer of the pancreas, and cancer of the upper alimentary tracts, as well as for emphysema and coronary heart disease in smokers, must be more thoroughly identified (Hammond 1975). The study of such risk factors and of the pathogenesis of the associated

Table 4
Organ-specific Carcinogens in Tobacco Smoke Particulates

Carcinogen	*Concentration/ cigarette*	*Carcino-genicity*[a]
Esophagus		
N'-nitrosonornicotine	137 ng	+
nitrosopiperidine	0–9 ng	
unknown unsymmetrical nitrosamines	?	+
Lung		
polonium-210	0.03–1.3 pCi	+
nickel compounds	0–600 ng	+
cadmium	9–70 ng	
unknowns	?	?
Pancreas		
nitrosamines	?	+
unknowns	?	?
Kidney and bladder		
β-naphthylamine	22 ng	+
x-aminofluorene	+	+
x-aminostilbene	+	+
o-toluidine	+	+
unknown aromatic amines	?	?
o-nitrotoluene	21 ng	?
unknown nitro compounds	?	?
di-*n*-butylnitrosamine	0–3 ng	+
other nitrosamines	?	+

List is incomplete and based on experimental data only. (Data from Hoffmann et al. 1976.)
[a] Animal data on carcinogenicity.

diseases is a prerequisite to preventive intervention. It has been estimated that 40% of male premature deaths are due to diseases associated with the habit of smoking cigarettes (Hammond 1975). In view of the less than totally successful attempts to eliminate smoking, the modification of tobacco products toward less harmful cigarettes was a commendable step. Cigarettes characterized by low-tar and low-nicotine contents are now available and are becoming increasingly popular due to efforts in educating the public through statements such as the Surgeon General's Report and educational campaigns by the American Cancer Society and other national organizations (Wynder and Stellman 1977).

Human evidence appears to indicate a trend toward reduced cancer incidence and mortality rates (Wynder 1972; Wynder and Hecht 1976; Wynder et al. 1976b) in line with the reduced exposure to cigarette smoke constituents since educational campaigns and product modifications were begun two decades ago. Laboratory data reflect a reduction of harmful constituents in cigarette smoke, and smoke particulates exhibit lesser degrees of tumorigenicity in bioassays than the corresponding products did one or two decades ago (Gori 1976; Hammond et al., this volume).

However, a cigarette engineered with all technical refinements known today is not as effective in diminishing the risk of disease as is total cessation of smoking. Thus the efforts of smoke withdrawal clinics, such as those associated with the American Health Foundation and the American Cancer Society, must be continued. More importantly, education of the young people must be stressed so as to present the risk factors clearly and to motivate them never to smoke at all.

Current data show that a heavy smoker who stops his habit will decrease his risk of cancer progressively, and, after 15 years, his risk factor is considered to be nearly that of someone who never smoked (Hammond 1975; Wynder and Hecht 1976). The underlying reason for this risk reduction is thought to relate to the fact that tobacco carcinogenesis is due mainly to cocarcinogenic and tumor-promoting factors which exert their effects in the long-term smoker only because of their continuous presence. In this context, it is important to stress tobacco smoking, as well as other life-style factors, as potentiating occupational cancer risks (Hoffmann and Wynder 1976).

In association with heavy drinking of alcoholic beverages, smoking also leads to cancer of the oral cavity and the esophagus (Schottenfeld et al. 1974). In the United States, this appears to be more prevalent in lower socioeconomic groups. In areas of western Europe, such as in France, the disease is also regionalized and appears to be highest in smokers who drink certain concentrated alcoholic beverages, such as calvados (Day 1975). Preliminary experiments by Dr. G. D. McCoy in our Institute suggest that the malnutrition resulting from consumption of alcoholic beverages may alter the target organ for respiratory carcinogens through one or more mechanisms such as: (1) induction of carcinogen-activating enzymes; (2) nutritional deficiencies, particularly of vitamins, and other factors leading to shifts in cell energetics and oxidative mechanisms; or (3) a promoting or cocarcinogenic effect of metabolites of alcohol.

Thus the study of tobacco carcinogenesis still has to resolve many underlying questions with regard to the total adverse health effects associated with tobacco smoking.

CANCERS ASSOCIATED WITH DIET

Several lines of evidence suggest that cancers of the digestive tract, and, in particular, cancers of the stomach, colon, and pancreas, are associated with dietary components. Furthermore, cancers of the endocrine-related organs, such as breast, prostate, ovary, and endometrium, are also linked to dietary factors. With the exception of lung cancer, discussed above, these are the human cancers with the highest incidence in the United States. A recent symposium dealt in detail with the relation between nutrition and cancer (Wynder et al. 1975).

Knowledge in these areas stems from observations of cancer incidence in various parts of the world. For example, cancer of the stomach is prevalent in Japan, the western part of South America, parts of Central America, and northern and eastern Europe, but it has a low incidence in the United States. In addition, the lower socioeconomic groups have a higher incidence of

stomach cancer than more affluent groups. On the other hand, the pattern of cancer of the colon, breast, prostate, pancreas, ovary, and endometrium exhibits almost the opposite picture, being high in the United States, western Europe, Australia, New Zealand, and Argentina. This cancer distribution has been shown to have origins in the environment rather than in genetic differences between populations through a study of the changes in incidence found in first- and second-generation migrant groups. For example, Japanese migrants have a decreasing risk for gastric cancer and an increasing risk for colon and breast cancer. In light of the high degree of industrialization in Japan, it is probably true that industrial activity per se is not responsible for the etiology of the main human cancers.

People from northern or eastern Europe or Japan, who would have a high risk for gastric cancer, in successive generations in the United States do not present this risk but instead show cancer in the colon, breast, or prostate. It is sometimes stated, with fatalistic resignation, that man is destined to have cancer of one type or another. It will be shown that the etiologic factors for gastric cancer are of a totally different nature than those for colon or breast cancer and thus can be controlled independently of those factors leading to the latter types of cancers. With developing knowledge in this field, it can be hoped and expected that the risk for gastric cancer can be reduced without necessarily incurring a simultaneous risk for the other cancers.

The key factors currently considered to be responsible for the cancers discussed below are dietary in nature. They are specific micronutrients and components (gastric cancer, perhaps also liver and esophageal cancer in certain populations) or macronutrients (colon, breast, prostate, ovary, endometrium, and perhaps pancreas cancer). Further research efforts are required to pinpoint the underlying mechanism and secure data on interactions between macro- and micronutrients which might modulate and even control the overall effect.

Gastric Cancer

In many countries of the world, gastric cancer is or was once a major neoplastic disease of humans. In the United States, there has been a pronounced decrease in the incidence of this disease in both men and women during the last 40 years.

Epidemiologic data show that gastric cancer incidence is high in Japan and eastern Europe, compared to the United States (Wynder et al. 1963; Haenszel and Correa 1975). The theory that gastric cancer could be attributable to dietary or environmental factors is supported by findings from the study of Japanese migrants to Hawaii. Although the first generation of Japanese residing in Hawaii showed almost the same gastric cancer incidence as native Japanese, the second generation showed a pronounced trend to the low incidence rates of the Hawaiians (Haenszel 1975).

Numerous hypotheses have been proposed, mostly concerning diet, to account for the etiology of this important cancer. Thus the consumption of foods high in oxidized fats or the intake of smoked fish have been incriminated in the past (Higginson 1967; Haenszel and Correa 1975), but experimental support for these suggestions is unconvincing.

Until recently, there were no reliable animal models for the study of this cancer (Bralow and Weisburger 1976). Sugimura, however, demonstrated that chemicals of the group of alkylnitrosoureas and particularly alkyl-*N'*-nitro or *N'*-acyl *N*-nitrosoureas induced gastric cancer in many species (Sugimura and Kawachi 1973). Sander and Schweinsberg (1972) observed that such chemicals could be formed from suitable substrates and nitrites at the pH of the stomach. Mirvish (1975) reported on the kinetics of this reaction which can undergo positive catalysis by agents such as thiocyanate (Fan and Tannenbaum 1973; Boyland and Walker 1974). On the other hand, vitamin C powerfully inhibited the nitrosation reaction (Mirvish et al. 1972; Raineri and Weisburger 1975).

We have asked whether the endogenous formation of such chemicals may be responsible for human gastric cancer (Weisburger and Raineri 1975). In the United States, sizable amounts of nitrate and salt were used as food preservatives, especially for fish and meat, prior to the advent of refrigeration. This is no longer necessary since such foods are now preserved through routine storage at low temperatures. Also, federal regulations currently limit the amounts of nitrate and nitrite used for this purpose. In fact, there has been a tendency toward eliminating nitrates altogether. Thus, in the United States, there has been a sizable decrease in dietary nitrate intake. On the other hand, in some regions of the world, such as Central and South America, the soil, and hence the agricultural products grown on such soil, contains large amounts of nitrate. Some well waters likewise are high in nitrate. Such nitrate is reduced to nitrite during storage. Thus, in various parts of the world (e.g., South America), there are now or have been appreciable amounts of nitrite in the dietary environment. In their migrant studies, Haenszel (1975), Haenszel and Correa (1975) and Correa et al. (1975) noted that the risk for gastric cancer is lower in those people eating more fruits and lettuce, both sources of vitamin C. This vitamin has been shown experimentally to inhibit nitrosation reactions and hence the production of potential carcinogens of the type described.

In the search for the etiologic factors responsible for gastric cancer, we have examined extracts of nitrite-treated foods for mutagenic activity in different test strains of *Salmonella typhimurium* (Ames et al. 1973). Foods tested were those consumed predominantly in regions with high stomach cancer incidence. Extracts of fish, borscht, and beans, each of which is a dietary staple in Japan, eastern Europe, or Latin America, showed mutagenic activity upon treatment with nitrite. In contrast, typical American foods such as hot dogs and beef failed to develop mutagenic activity with nitrite, perhaps because nitrite reacts preferentially with myoglobin (Marquardt et al. 1977a,b) (Table 5). The formation of the mutagen was maximal at pH 3. Storage under highly acidic conditions, as well as under alkaline conditions, destroyed the mutagen(s). In a dose–response study, incubation with 5000 ppm of sodium nitrite yielded the highest amount of mutagenic activity, but activity was also observed with 1000 ppm of nitrite. The mutagenic material did not require metabolic activation. Moreover, and importantly, ascorbic acid prevented the formation of the mutagen(s) in nitrite-treated foods. These data suggest that the mutagen(s) may be of the alkylnitrosamide type.

In 1975, Endo et al. showed that nitrosation of methylguanidine, under

Table 5
Mutagenic Effects of Extract of Nitrite-treated Food in *S. typhimurium* TA1535 Plate Incorporation Assay as a Function of Dose per Plate

Extract	*Doses/plate* (μl)	*Number of his+ revertant colonies*
Fish[a]	1	70 ± 12.7
	5	184 ± 19.8
	10	252 ± 29.0
	20	212 ± 5.7
	40	killing
Beans[b]	1	26 ± 1.4
	10	59 ± 5.7
	25	73 ± 0
Borscht[b]	1	26 ± 7.1
	10	29 ± 3.5
	25	65 ± 0

Mutagenicity assays were performed after incubation for 48 hr at 37°C, mean values/plate ± S.D., using 4 plates per assay. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, which does not require metabolic activation, was used as a positive control. An average of 1535 colonies per plate was observed at a concentration of 20 μg/plate. (Data from Marquardt et al. 1977b.)

[a] Spontaneous revertants 8 ± 2.8.

[b] Spontaneous revertants 6 ± 1.4.

simulated gastric conditions, generated a mutagenic principle identified as nitrosocyanamide (Endo et al. 1974, 1975). However, it has been found recently that fish and other foods probably do not contain significant amounts of methylguanidine (Fujinaka et al. 1976). Also, although highly mutagenic, nitrosocyanamide induced mainly forestomach tumors in rats and thus fails to exhibit the specificity of inducing cancer in the glandular stomach, as seen in man, which chemicals like *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine do produce reliably.

Thus identification of mutagenic agents in nitrosated foods requires further study. Also, the direct relevance of our findings for human gastric carcinogenesis remains to be demonstrated. Animal experiments investigating the in vivo carcinogenic properties of the mutagenic principle found in the extracts of fish, borscht, and beans are under way.

If the concept that gastric cancer stems from an in situ nitrosation of endogenous substrates is borne out experimentally, it would appear that one relatively minor change in the human diet in high-risk groups would prevent this important cancer. The required alteration would make available fruits, vegetables, and salads as sources of vitamin C on a continuous rather than intermittent seasonal basis, as is presently the custom in many countries. It is necessary to emphasize the need for a continuous dietary intake of foods with

vitamin C to prevent even an intermittent exposure to carcinogens, inasmuch as in animal models gastric cancer can be induced by relatively infrequent application of alkynitrosamides. Also, epidemiologic data indicate that first-generation migrants from high-risk countries like Japan, Poland, and Scandinavia maintain the risk for gastric cancer in their adopted country, suggesting that, once initiated, the reaction proceeds. Hence there is a need to avoid formation of gastric carcinogens early in life and continue this practice by minimizing the intake of actual or potential nitrite and optimizing the intake of foods containing ascorbate. On the basis of our evidence that meats in the presence of nitrite do not generate mutagens but that nitrite and fish or beans do, we can conclude that addition of small amounts of nitrite to meats as a preservative measure, as now practiced, is probably not hazardous. On the other hand, populations eating mainly fish need to minimize environmental nitrite and ensure the regular presence of ascorbate in their daily diet.

Colon Cancer

On the basis of variations in incidence for different regions of the world and in view of the altered risk of migrant populations, it has been accepted that diet is a major etiologic factor in colon cancer (Correa 1975). Further epidemiologic evaluation has implicated a high intake of dietary fat (Wynder and Shigematsu 1967; Wynder et al. 1969; Wynder and Reddy 1975), protein (Armstrong and Doll 1975), beef (Haenszel et al. 1973), and possibly dietary fiber deficiency (Burkitt 1975a,b) as strongly associated with large-bowel carcinogenesis. A major portion of the dietary fat in high-risk areas is derived from meat, in particular, beef. Also, diets high in fat are often low in fiber. On the basis of epidemiologic and laboratory evidence, we have emphasized dietary fat, but new efforts are under way to round out knowledge on other dietary components suspected of playing a role in large-bowel carcinogenesis.

The typical American diet contains 40–45% of calories as fat, direct and hidden (in meats). In Japan, fat, more of it unsaturated, accounts for only 15–20% of daily calories, which in turn is about 10% lower than in the United States.

Rectal Cancer

In the past, rectal cancer was often considered together with colon cancer as "colo-rectal" or "large-bowel" cancer. There begins to be a realization that these may be two separate diseases. A better distinction is afforded by the more precise anatomic localization, with rectal cancer involving tissue within 8 cm of the anus. Thus defined, colon cancer has exhibited a small increase in rate over the last 30–40 years, paralleling an increased intake of dietary fat (Gortner 1975), but rectal cancer has decreased. Furthermore, the male/female ratio for colon cancer is about 1/1, for rectal cancer 1.4/1. Also, the incidence in a high-risk country versus a low-risk country, like the United States versus Japan, is about 5 times higher for colon cancer but only 1.5 times higher for rectal cancer. Etiologic factors for rectal cancer remain to be defined.

Etiologic Factors

Most of the research has been concerned with the possible etiologic role of fats, especially since they affect cholesterol and bile salt metabolism and excretion as well as colonic bacterial flora. To account for the relationship between dietary fat and colon cancer, it has been postulated that (1) the amount of dietary fat determines both the concentration of acid and neutral sterol substrates in the large bowel and also the composition of the microflora acting on such substrates, and (2) the gut microflora metabolize acid and neutral sterols to carcinogens or cocarcinogens active in the large bowel (Hill 1975; Wynder and Reddy 1973). Such microflora-mediated reactions may also yield products which may be metabolized further to a carcinogen by the intestinal mucosa itself.

Burkitt (1975b) and Painter and Burkitt (1975) have emphasized dietary fiber and consequent volume of intestinal content and size of stools in relation to several diseases of the intestine and other conditions. Although diverticulitis, for example, may stem in part from a lack of dietary fiber, the current view is that colon cancer is unrelated to fiber. Wynder and Shigematsu (1967) failed to find an association between constipation and colon cancer, and experimental models have so far not provided positive data (Ward et al. 1973), but we are currently exploring the role of fiber. However, as discussed below, fat levels do influence colon carcinogenesis.

Metabolic Studies in Man

Hill et al. (1971) observed a correlation between the death rate due to colon cancer in various populations and the fecal excretion of metabolites of cholesterol and bile acids as well as their products of degradation by bacterial flora. Reddy and Wynder (1973) similarly found that populations on a mixed

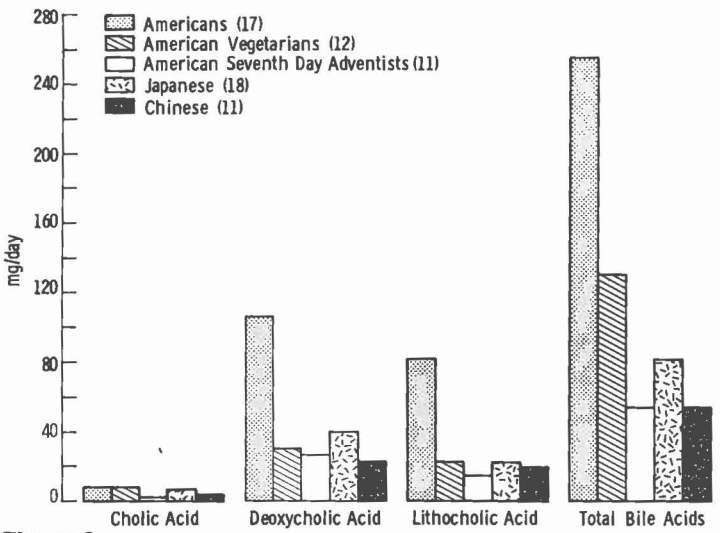

Figure 3

Excretion of fecal bile acids in populations with different risks for colon cancer. (Reprinted, with permission, from Reddy and Wynder 1973.)

western diet, among whom the rate of large-bowel cancer is high, degraded and excreted acid and neutral sterol metabolites to a greater degree than a similar population with a comparatively low rate of colon cancer (Fig. 3). The fecal bacteria of groups consuming a mixed western diet also had higher β-glucuronidase activity. These differences are associated with dietary composition, mainly with a higher content of animal fat and protein in the high-risk group. Controlled studies comparing a high-meat, high-fat diet with a meatless, low-fat diet showed that the former resulted in an elevated level of fecal bile acid and cholesterol metabolites, increased bacterial β-glucuronidase activity, and more total microflora (Reddy et al. 1974, 1975).

Comparisons of fecal constituents in terms of bacteria, cholesterol, bile acid metabolites, and bacterial enzymes were performed on patients with colon cancer. In a case-control study, Hill et al. (1975) found that patients with colon cancer had increased levels of fecal bile acids and nuclear dehydrogenating *Clostridia* compared to controls. Reddy and Wynder (1977) and Mastromarino et al. (1976) showed that the concentration of bile acids and cholesterol metabolites in colon cancer patients was also higher than in controls, as was the fecal bacterial 7α-dehydroxylation of primary bile acids (Fig. 4).

It is generally agreed that patients with familial polyposis, ulcerative colitis, and adenomatous polyps have an increased risk of developing carcinoma of the colon.

Based on studies conducted thus far in our laboratories (Reddy et al. 1976c; Reddy and Wynder 1977), those patients at high risk for colon cancer fall into three distinct groups with regard to their fecal bile acid and cholesterol metabolite profiles. Patients with adenomatous polyps excreted

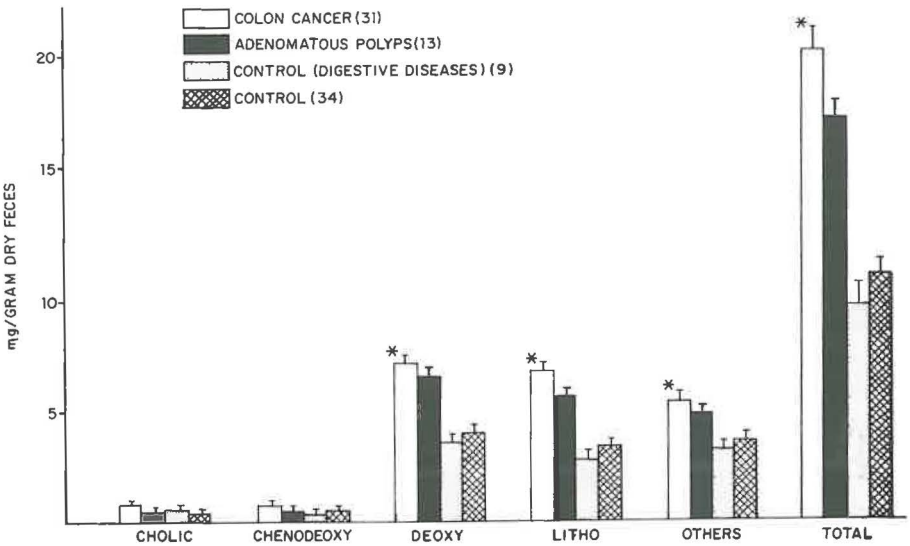

Figure 4
Excretion of bile acids in patients with colon cancer, adenomatous polyps, or other digestive diseases, and controls. (*) $P < 0.05$. (Reprinted, with permission, from Reddy and Wynder 1977.)

high levels of both cholesterol metabolites and bile acids compared to controls; patients with familial polyposis excreted similar levels of total neutral sterol and bile acids but significantly more unchanged cholesterol compared to controls; and ulcerative colitis patients excreted high levels of neutral sterols but similar levels of bile acids compared to controls. It is evident from these studies that the common denominator in all cases is cholesterol metabolites.

Animal Models for Colon Cancer

Research on the mechanisms of cancer causation in the large bowel has been assisted by the discovery, during the last 20 years, of several animal models which mirror relatively faithfully the type of lesions seen in man. These models involve the induction of large-bowel cancer by (1) chemicals of the types 3-methyl-4-aminobiphenyl and 3-methyl-2-naphthylamine, tested so far only in rats and hamsters (less effective); (2) derivatives and precursors of cycasin and methylazoxymethanol, such as azoxymethane and 1,2-dimethylhydrazine, which work well in rats, mice of select strains, and hamsters; (3) intrarectal administration of direct-acting carcinogens such as methylazoxymethanol acetate, or alkylnitrosoureas such as *N*-methyl-nitrosourea and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, which induce cancer of the descending large bowel in every species tested thus far; and (4) the oral administration of large doses of 3-methylcholanthrene which induces large-bowel cancer in select strains of hamsters (see Bralow and Weisburger 1976).

The modes of action of these various carcinogens, which may also bear on human cancer causation, have received only limited study, and further efforts in this area are being pursued in a number of laboratories, including our own (Zedeck et al. 1970; Spjut and Noall 1971; Hawks and Magee 1974; Laqueur and Spatz 1975; Fiala et al. 1976; Fiala 1977). Specifically, we are investigating why *ortho*-methylarylamines appear to exhibit organotropism for the colon in rodents, whether such chemicals are present in our environment, and whether they are responsible, in part, for large bowel cancer in man. We are further studying the metabolism and mode of action of 1,2-dimethylhydrazine, and the derived azoxymethane and methylazoxymethanol, by the use of radioisotope and specific separation and analytical techniques. Only small amounts of a given dose are secreted in the bile, and the metabolites secreted may bear on the development of duodenal tumors but probably not on tumors in the large intestine. The latter seems to relate to specific metabolites brought to the colon by the blood. There is preliminary information that the colon mucosa does not metabolize 1,2-dimethylhydrazine or azoxymethane well, if at all, whereas the liver does (Fiala 1977; Fiala et al. 1977). The problem, then, would be why methylazoxymethanol, produced in the liver, exhibits a specific action on the colon.

Modifying Effect of Dietary Fat in Colon Carcinogenesis

The possible role of dietary fat in the induction of human cancer of the large bowel has received some support from experimental studies. Rats fed either 20% lard or 20% corn oil were more susceptible to colon tumor induction by 1,2-dimethylhydrazine and also excreted higher levels of fecal bile acids and cholesterol metabolites compared to those fed 5% lard or 5% corn oil

Table 6
Tumor Incidence in Rats Treated with DMH and Fed Two Levels of Dietary Corn Oil or Lard

Diet	Animals with tumors (%)				Animals with multiple colonic tumors (%)	Total colon tumors (per rat)
	ear canal	kidney	small intestine	colon		
Corn oil, 5%	32	4	27	36	14	0.77
Corn oil, 20%	59	14	36	64	32	1.55
Lard, 5%	13	0	4	17	4	0.22
Lard, 20%	67	0	50	67	29	1.50
Purina Lab Chow	15	0	20	25	0	0.25

Number of animals per group ranged from 20 to 24. Animals received weekly subcutaneous injections of 10 mg/kg body weight for 20 weeks and were autopsied 10 weeks after the last injection. (Data from Reddy et al. 1976d.)

(Reddy et al. 1976d) (Table 6). Recent data by Reddy et al. (1976a) indicate that a high-meat, high-fat diet or a high-soy-protein, high-fat diet led to more colonic tumors in rats given 1,2-dimethylhydrazine than did control diets. On the other hand, fiber in the form of alphacel failed to affect colon carcinogenesis (Ward et al. 1973), but we have studies under way with other forms of fiber. Selenium has inhibited colon cancer induction by as yet unknown mechanisms (Jacobs et al. 1977).

Role of Bile Metabolites in Colon Carcinogenesis in Animal Models

There is evidence that bile acids can promote colon tumor development. Narisawa et al. (1974) have reported that both taurodeoxycholic acid and lithocholic acid are promoters in conventional rats, and Reddy et al. (1976b) have shown that in germ-free and conventional rats the secondary bile acids, deoxycholate and lithocholic acid, act as colon tumor promoters. The effect of the primary bile acids, cholic acids and chenodeoxycholic acid, as tumor promoters was more pronounced in conventional rats compared to germ-free rats. Thus, in this animal model, these secondary bile acids, present in high concentrations in human stools, serve as promoters. Likewise, Nigro et al. (1973) and Chomchai et al. (1974) observed that an increase of bile salts in the colon of rats, induced either by feeding cholestyramine or by surgically diverting bile to the middle of the small intestine, enhanced colon tumor formation.

Further research is required to elucidate the mechanism by which a high-fat diet might translate into a high risk for colon cancer. A search for the carcinogens involved, such as in the laboratories of Heddle and Bruce (this volume) or Mower et al. (1977), and in our laboratories, may provide a basis for blocking the carcinogenic process at the onset by eliminating or at least decreasing the level of the agents involved. The recent findings by Sugimura et al. (this volume) of certain complex heterocyclic aromatic amines after pyrolysis of proteins and amino acids provide interesting and suggestive leads.

With increasingly detailed knowledge of causative factors and the mechanisms involved, it is hoped that the requirements for preventing this cancer, which now has such a high incidence, can be obtained through multidisciplinary research approaches.

Breast Cancer

Epidemiologic studies show that premenopausal American and Japanese women have comparable rates of breast cancer as a function of age, although Japanese women exhibit a somewhat lower incidence compared to American women (Fig. 5). Furthermore, the incidence in Japanese women reaches a plateau around 45 years of age and then decreases (Berg 1975). In contrast, the incidence in Americans increases sharply during and following menopause,

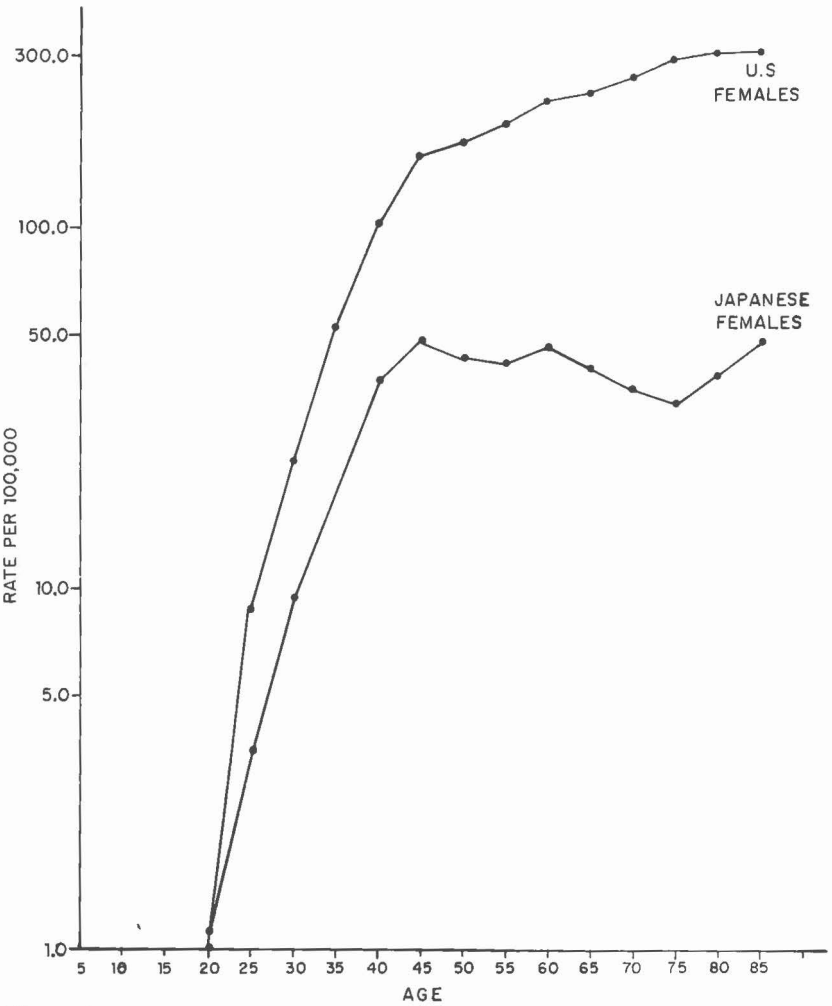

Figure 5
Age-specific incidence rates for female breast cancer, U.S. (1971) and Japan (1973). (Reprinted, with permission, from Wynder and Hirayama 1977.)

Table 7
Comparison of Dietary Components in Japan and Hawaii

Nutrient	*Japan*	*Hawaii*
Calories	2132	2274
Total protein (g)	76	94
animal protein (g)	40	71
vegetable protein (g)	37	24
Total fat (g)	36	85
principally saturated (g)	16	59
principally unsaturated (g)	21	26
Total carbohydrate (g)	335	260
simple (g)	61	92
complex (g)	278	169
Alcohol (g)	28	13
Cholesterol (mg)	457	545

Mean diet values, 24-hr recall. (Data from Stemmermann and Hayashi 1977.)

the curve showing a characteristic "hook." A distinct change in the incidence rate of breast cancer after 48 years of age has also been reported in Scandinavian populations (Hakama 1969). This biphasic incidence of breast cancer in pre- and postmenopausal women possibly reflects two independent disease factors (de Waard 1975).

The key difference in environmental factors between Japan and western countries such as the United States (Hawaii) appears to be dietary in origin, with the main variable being the quantity of dietary fat (Table 7). High-risk populations consume a higher proportion of dietary fat, a percentage which has been increasing over the past 50 years (Gortner 1975). This change may be related to the slight but definite increased incidence of breast cancer in western women over the same time span. de Waard (1975) reported that obesity was a promotional factor in Dutch women, but this apparently is not seen in epidemiologic studies on North American Caucasian women (Wynder et al. 1977).

Migrant studies with respect to colon cancer have demonstrated that the first-generation migrants from a low-risk region have an appreciable increase in risk when transferred to a high-incidence area such as the United States. However, with regard to breast cancer, first-generation migrants show only a slight increase and it is not until the second generation that a risk similar to that of long-term residents in the high-incidence region is attained. This aspect deserves further documentation since it suggests that, for breast cancer, residence in a high-risk area at the time of puberty and breast development is critical. In a recent study on Japanese A-bomb survivors (C. Hand, National Cancer Institute, pers. comm.), it was found that irradiation between the ages of 10 and 19 resulted in an increased breast cancer incidence (15 years later) of approximately 5½% per rad exposure above that of unexposed women of the same age group. The increased incidence for women exposed to radiation in their twenties, however, was only 1½% per rad above that of un-

exposed women of the same age group. For colon cancer, where the cell kinetics are quite different, the factor of exposure at a young age evidently is not as critical. An analogous situation may be seen in the Huggins rat model. Female Sprague-Dawley rats are more sensitive to a single dose of hydrocarbon carcinogen at or around puberty than are older or younger animals, and this susceptibility seems to be correlated with the rate of cell division and serum prolactin levels (Yanai and Nagasawa 1976). The curve denoting sensitivity may be broader with the newly developed model of Gullino, which involves methylnitrosourea as a carcinogen (Gullino et al. 1975).

There have been a number of studies attempting to relate differences in breast cancer incidence between high- and low-risk populations to hormonal profiles in urine, serum, and plasma. Most studies have involved data on the levels of urinary steroid hormones: estrogen and androgens (Hellman et al. 1971; MacMahon et al. 1973; Bulbrook et al. 1976). However, the etiologic significance of abnormal or discriminating estrogen and androgen secretion patterns is a subject of controversy at present.

To investigate the relationship between diet and the circulating hormone profile, we have compared a variety of ovarian and adrenal steroids in a low-risk population (Japanese) and a high-risk population (North American Caucasian) (Table 8). In premenopausal women, the main difference found was a higher level of estradiol in Japanese women as compared to Caucasian women; however, in postmenopausal women, although estradiol levels were similar, the androstenedione and testosterone levels in Japanese women were significantly lower than in their Caucasian counterparts. Although plasma steroid concentrations may serve as discriminant functions in Caucasian or Japanese populations, it remains to be determined whether a change in ovarian function in adrenal metabolism is causally associated with breast cancer. On the basis of our studies in animal models, we believe that, although steroids are certainly important and relevant to the question of breast cancer growth and development, pituitary hormones and perhaps thyroid hormones merit greater attention than they have received in the past.

Recently, emphasis has been placed on the role of prolactin in breast cancer (Smithline et al. 1975; Horrobin 1976). Although evidence in the rat model is convincing, evidence in humans is controversial and requires further substantiation. Elevated prolactin levels have been noted by Henderson et al. (1975) in the daughters of women with breast cancer as compared to controls. Kwa et al. (1974) also reported a familial relationship between high serum prolactin levels and breast cancer. More recently, however, Kwa et al. (1976) reported no relation between a variety of risk factors (age at first pregnancy, age at menarche) and circulating prolactin concentrations.

Since hypothalamopituitary (Wurtman and Fernstrom 1975), adrenal (Savage et al. 1975), and ovarian activity (Fishman et al. 1975) can be altered by dietary factors, we have attempted to modify this activity by transferring women from a standard western diet to a low-fat vegetarian diet. Initial data suggest not only that levels of hormones such as testosterone and prolactin are altered, but also that the menstrual cycle may be shortened by 1 to 2 days by dietary modification (Hill et al. 1976a,b) (Fig. 6).

In the same context, Hill and Wynder (1976) demonstrated that volunteers on a western diet have higher prolactin levels than a similar group on a

Table 8
Estrogen and Androgen Levels in Healthy Pre- and Postmenopausal Caucasian and Japanese Women

	Estrone	*Estradiol*	*Andro-stenedione*	*DHEA*	*Testosterone*
Premenopausal					
Caucasian (21)	24.6 ± 2.5	23.8 ± 1.0	285 ± 21.6	352 ± 25.4	45.4 ± 3.5
Japanese (12)	30.4 ± 3.3	29.8 ± 1.9[a]	358 ± 44.3	294 ± 41.9	49.0 ± 5.1
Postmenopausal					
Caucasian (27)	24.4 ± 3.5	7.7 ± 0.6[b]	219 ± 15.5	230 ± 26.5[b]	40.5 ± 1.8
Japanese (16)	22.3 ± 2.3	5.9 ± 0.8[b]	90 ± 6.1[a]	301 ± 34.1	28.0 ± 1.5[a, b]

Number per group indicated in parentheses. (Data from Hill et al. 1976a.)
[a] $P < 0.01$ significantly different from Caucasian women.
[b] $P < 0.01$ significantly lower in postmenopausal women.

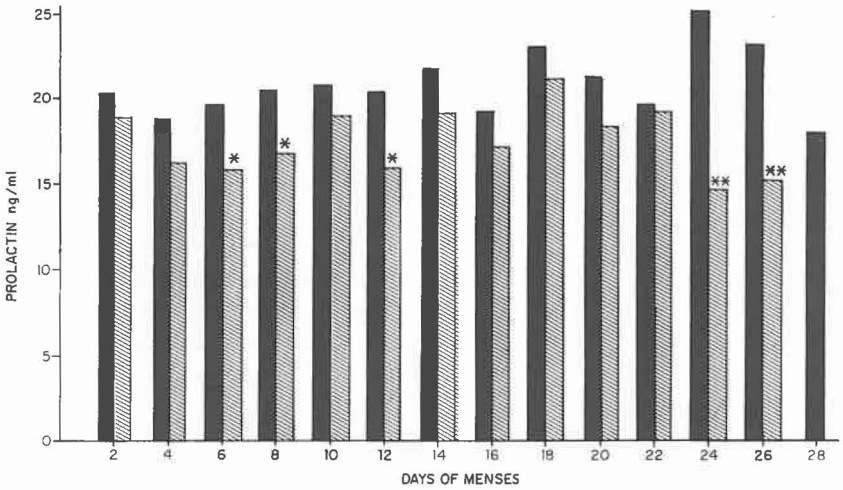

Figure 6
Prolactin levels taken at 9:00 a.m. on alternate days of the menstrual cycle in seven nurses. The nurses were maintained on a western diet (solid bars) and transferred for 2 months to a vegetarian diet (hatched bars). Blood samples were taken while they were on the western diet and during the second month of the vegetarian diet. Differences between the prolactin levels were determined by a paired t = test. (*)$P < 0.05$; (**)$P < 0.01$. (Reprinted, with permission, from Wynder et al. 1976c.)

vegetarian diet, with the difference between the levels being most pronounced during deep sleep (Fig. 7). Accordingly, additional studies of 24-hour hormonal patterns in high- and low-risk populations are indicated in order to understand what specific hormonal changes may serve to stimulate breast carcinogenesis. The data obtained may also have diagnostic value for distinguishing high-risk individuals within a given population.

Animal Models of Breast Cancer

Experimental work conducted in our laboratory (Chan and Cohen 1974), as well as that in others (Tannenbaum 1942; Carroll 1975), has shown unequivocally that a high-fat diet promotes the development of both spontaneous and chemically induced mammary tumors in rodents. The enhancing effect has been observed with many different types of fats and oils and is related more to the quantity of fat ingested than to the type of fat (Carroll and Khor 1971). Nonetheless, at low (0.5%) fat levels, unsaturated oils appear to give a definitely higher tumor incidence on a per animal basis than saturated fats. At high fat levels (20%), the effect with both types is similarly high, with the notable exception of coconut oil, which is unique in its low levels of linoleic and other C-18-20 fatty acids (Carroll 1975). In contrast, the levels of protein or carbohydrate consumption did not significantly affect breast cancer development provided there was no dietary restriction (Tannenbaum 1945).

By paired feeding of isocaloric high- and low-fat diets, Tannenbaum and Silverstone (1957) demonstrated that the high-fat effect was independent of caloric intake. In addition, Carroll and Khor (1970) have shown that the high-fat effect occurs only during the promotional phase of carcinogenesis,

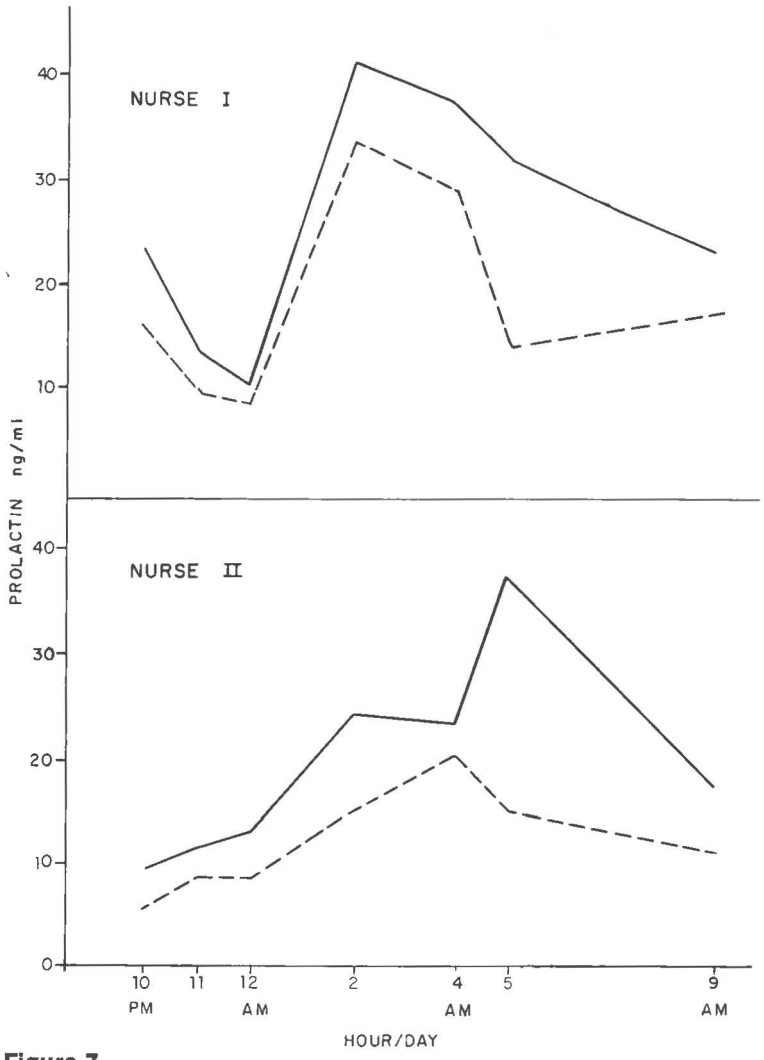

Figure 7
Nocturnal release of prolactin in two nurses maintained on a western diet (——) and then transferred for 2 weeks to a vegetarian diet (– – –). Blood samples were taken by an indwelling catheter. Sleep period began at 11 p.m. (Reprinted, with permission, from Hill and Wynder 1976.)

the most critical period being the first 2 weeks after initiation by a chemical carcinogen.

Thus both epidemiologic and experimental evidence suggest that the quantity of dietary fat is associated with breast cancer development.

Diet and nutrition apparently act as modifiers rather than initiators of tumor development. The modifying effect of diet may be exerted either directly or indirectly. Direct mechanisms involve specific alterations in cell-membrane structure and functions; indirect mechanisms, on the other hand, involve changes in on-going physiological processes. For example, diet can affect the endocrine system, colon bacteria and the substrates which colon bacteria

metabolize, and possibly other systems, such as the mixed-function oxidase system and the immune system. Recent reviews by Hopkins and West (1976) and Alcantara and Speckmann (1976) discuss in detail the current knowledge in this area.

In our laboratory, we have focused on two hormones, prolactin and estrogen. Both of these have been shown to play a critical role in the maintenance and continued development of breast cancer, prolactin possibly being the more important of the two (Pearson 1973). Using the Huggins model, Chan and Cohen (1975) demonstrated that administration of the antiprolactin drug 2-bromo-α-ergocryptine (Sandoz) to rats abolishes the tumor-promoting effect of a high-fat diet, whereas administration of the antiestrogen drug U11,100A (Upjohn) did not eliminate the high-fat effect. Further studies have shown that high-fat intake elevates serum prolactin levels, particularly during the proestrous-estrous stage of the estrous cycle (Chan et al. 1975) (Fig. 8).

This study focused attention on two important methodological aspects of prolactin assays often neglected in human studies, namely, that there is a distinct periodicity in circulating prolactin concentrations governed by (1) stage in the estrous (menstrual) cycle and (2) time of day (Horrobin 1976). The importance of distinguishing between basal and peak periods is exemplified in the finding of Hill and Wynder (1976) that the most striking difference in prolactin levels in women on vegetarian or western diets occurred during the late-night prolactin peak and not during the day.

Since prolactin is a well-known promoting agent in breast cancer (Furth

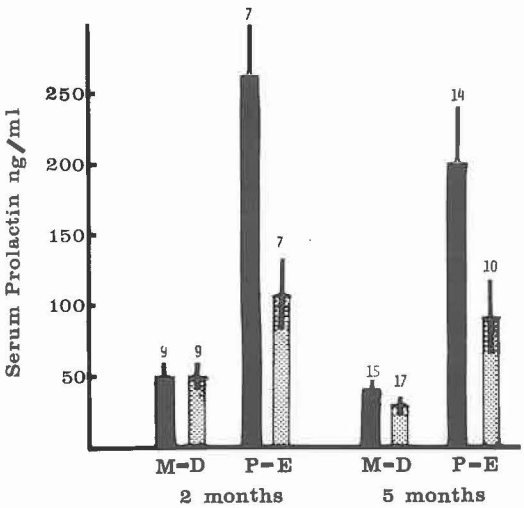

Figure 8

Serum prolactin titers of rats fed high-fat (solid bars) and low-fat (stippled bars) diets. Vertical line above each bar indicates ±S.E.M. Numbers above lines designate the number of individual rats assayed at M–D (metestrus–diestrus) and P–E (proestrus–estrus). Difference between means at P–E was significant ($P < 0.05$); that at M–D was not. (Reprinted, with permission, from Chan et al. 1975.)

1972; Meites 1972; Pearson 1972), Chan and Cohen (1975) proposed that the high-fat effect was mediated by increased synthesis and/or secretion of prolactin by the pituitary gland. The resulting periodic hyperprolactinemia induced by high fat intake was then envisaged to stimulate mammary tumor growth.

Results of in vitro studies on long-term cultures of normal and neoplastic mammary epithelium (Cohen et al. 1974; Chan et al. 1976b) have added another element to this hypothesis. Estradiol above 1 μg/ml was toxic to cultured mammary adenocarcinoma. Surprisingly, prolactin at concentration ratios above 5–10 times that of estradiol counteracted the inhibitory effect of estradiol (Fig. 9). Though it remains to be seen whether this phenomenon can be explained within the present context of receptor theory (McGuire et al. 1974), it suggests that estrogen and prolactin act in opposition to each other at the level of the mammary tumor cell and that the actual quantitative relations between the two hormones may determine the rate of mammary cell metabolism and growth. No doubt this is an oversimplification, and other hormones, such as progesterone, insulin, and hydrocortisone, will eventually have to be integrated into this conceptual scheme (McGuire et al. 1974).

Precisely how prolactin acts at the subcellular level in either normal or neoplastic mammary epithelium is not yet known. There is good evidence that cellular receptors for prolactin exist and are partially controlled by circulating prolactin concentrations (Kelly et al. 1974). Estrogens apparently can

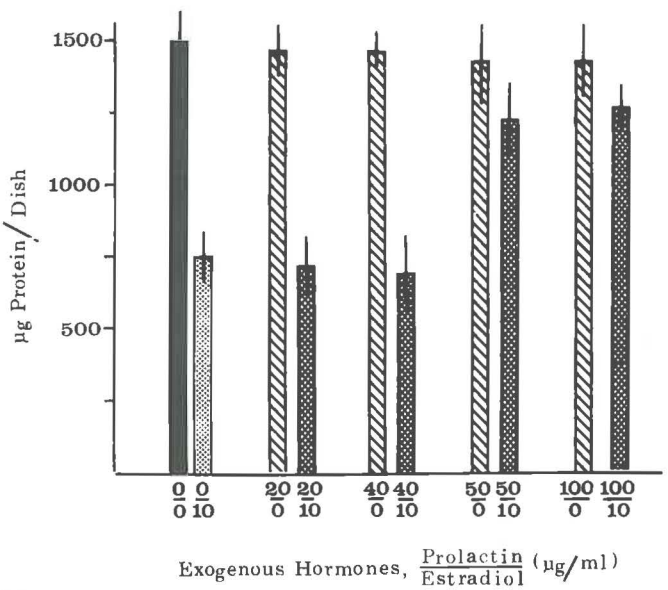

Figure 9

Effects of estradiol and prolactin alone (stippled and hatched bars, respectively) and in combination (cross-hatched bars) on growth of rat mammary tumor cells. Solid bar represents growth in standard MEM + 10% fetal calf serum. Hormones were added at medium change on days 1, 4, and 7. Total protein was determined after 10 days of growth. Lines above bars represent ±S.E.M. (Reprinted, with permission, from Chan et al. 1976b.)

Table 9
Dietary Fat Influence on Serum Hormonal Profiles and Mammary Tumor Incidence in NMU-treated Fischer Rats (Group 1)

Diet (tumor incidence)	*Estrous stage*	*Prolactin (ng/ml)*	*Total estrogen (ng/100 ml)*	*Prolactin estrogen (× 10²)*
High fat (90%)	P–E[a]	237 ± 98 (8)[b]	23.7 ± 1.7 (8)	11.1 ± 4.8
	M–D[a]	100 ± 20 (7)[c]	22.2 ± 2.0 (7)	4.5 ± 0.9[d]
Low fat (40%)	P–E	140 ± 79 (4)	23.2 ± 2.2 (4)	6.4 ± 3.9
	M–D	38 ± 3 (12)	18.9 ± 1.1 (12)	2.0 ± 0.1

The median latency periods were 83 days and 103 days for the high- and low-fat groups, respectively; $N = 20$. (Data from P. C. Chan, J. F. Head, L. A. Cohen and E. L. Wynder, unpubl.)

[a] P–E: proestrus–estrus; M–D: metestrus–diestrus.

[b] Mean ± S.E.M.; number of animals assayed indicated in parentheses.

[c] $P < 0.05$, high fat vs. low fat.

[d] $P < 0.01$, high fat vs. low fat.

regulate prolactin receptor capacity as well (Vignon and Rochefort 1974). Moreover, prolactin has been shown to induce changes in the cyclic-AMP system principally at the level of the cAMP-activated protein kinase (Turkington 1973) in normal mammary glands. We are currently studying the role of protein kinases in the action of prolactin on the growth and development of neoplastic mammary cells (Cohen et al. 1976).

Recently, Gullino et al. (1975) described a method of inducing mammary adenocarcinoma in rats by multiple injections of the carcinogen methylnitrosomethylurea. The resultant mammary tumors exhibited many properties in common with the human disease: they exhibited metastasis, were hormone-dependent, and were associated with hypercalcemia. Using this model, we have shown, as did Carroll (1975) in the DMBA model, that a high-fat diet ingested by rats after a single injection of NMU increased tumor incidence and decreased the latent period when compared to a similar population of rats fed a low-fat diet (Chan et al. 1976a). In addition, assay of serum prolactin and estrogen levels in these animals revealed a significant increase in both prolactin and prolactin/estrogen ratios (Table 9). Parallel studies in high- and low-risk human populations (Hill et al. 1976a,b) have shown that Caucasian breast cancer patients have significantly higher mean plasma prolactin/estrogen ratios as compared to their Japanese and Bantu counterparts.

In summary, the study of animal models has provided new insights into the possible role of diet in the development of human breast cancer. A hypothesis based on the evidence from the rat model and partially verified in humans has been proposed which suggests that the tumor-enhancing effect of a high-fat diet may be mediated by an increased serum prolactin concentration, which ultimately is reflected in an increased prolactin-to-estrogen ratio in the

circulation. Confirmation of this hypothesis in humans awaits analysis of the 24-hour profiles of prolactin in individuals from populations at high or low risk for breast cancer.

Prostate Cancer

Epidemiology has provided data on the incidence of prostate cancer as a function of a number of factors such as race, age, area of residence, and diet (Wynder et al. 1971). Thus Caucasians in the western world have a higher incidence of prostatic cancer than Japanese. First-generation Japanese migrants to the United States retain their low risk, but later generations exhibit a higher risk (Akazaki and Stemmermann 1973). However, native Japanese (Akazaki 1973) have in situ lesions, and it has been suggested that the difference in proliferative types of prostatic carcinoma as seen in western men is due to promotion by environmental factors, among which diet deserves primary consideration.

American blacks, particularly in the south, have a lower risk for both colon and breast cancer (and these diseases are increasing at the present time) presumably because of sizable alterations in dietary habits in the south. However, the risk for prostate cancer is higher in American blacks than in American whites. This factual observation has not yet evoked any explanation or even a rational hypothesis. Nonetheless, it is clear that, as a rule, population groups who have a high risk of breast, colon, and endometrial cancer also have a high risk of prostate cancer (Armstrong and Doll 1975). Since, for the former types, an association with diet has been documented not only in epidemiologic studies, but also in metabolic and physiological approaches, it is probable that dietary fat plays a role in the etiology of prostate cancer. For breast and colon cancer, the dietary fat hypothesis is further supported by detailed studies in animal models as described above. Unfortunately, at present, there is no reliable and realistic animal model for prostate cancer amenable to mechanistic studies.

Dietary factors may become operative during puberty or at some later time. In a study of boys from different socioeconomic groups in Hong Kong, physiological development was greater in the higher socioeconomic groups (Lee et al. 1963). Trends toward increased stature in Polish boys over the last 80 years further suggest that dietary factors are involved (Wolanski 1966). The effect of diet on growth, height, and weight is also evident in Japanese boys (Frisch and Reveille 1969). From 1948 to 1963, the total protein intake increased 12%, from 63 to 70.6 g/day, but the animal protein rose 113%, from 11 to 27.9 g/day while the age of maximum height increment decreased from 14.5 to 12.5. The animal protein component also included animal fat. Comparable data for American-born Japanese boys for the maximum increase in height is 10.5 years. Although the data clearly implicated nutritional factors in stimulating puberty and growth, the effect of nutritional factors, including protein, fat, and micronutrients, on the endocrine patterns in boys and men in relation to prostate cancer risk remains to be clarified.

Since 1890, it has been known that castration produces prostatic atrophy. The relationship of castration and sex hormones to histological changes in

prostatic tissue was established by Brandes (1966), who demonstrated that testosterone promoted the growth of prostatic epithelium, whereas estrogen and castration reversed or inhibited epithelial cell growth.

Although prostatic cancer occurs predominantly in men over 50 years of age, there is little decrease in the plasma testosterone level until late in life (Kent and Acone 1966), nor is there any significant decrease in prostatic cancer patients (Sciarra et al. 1973).

We are studying dietary relationships in urban and rural Polish, Bantu and white South African, and American white and black men at various ages and examining several gonadal, adrenal, and pituitary hormones.

CONCLUSIONS

We have presented an overview of the concepts that maintain that the etiologies of the main human cancers stem largely from our life-styles. The underlying mechanisms and supporting research developments have been discussed. Thus most cancers of the respiratory tract are due to excessive smoking. Prevention of these cancers hinges on educating the public about the hazards connected with this habit, not only with respect to the development of cancer of the lung, pancreas, kidneys, and bladder, but also with respect to myocardial infarction. In addition, those who cannot be induced to stop their cigarette habit should be convinced to smoke the lower tar cigarettes now available, since evidence is accumulating that these indeed have a lower risk. Furthermore, through managerial approaches to prevention, industry and government should collaborate further toward the production and preferential marketing of such lower risk cigarettes.

Cancers of the endocrine-controlled organs—breast, prostate, ovary, and endometrium—and of the colon are largely associated with diet and, more specifically, with the high fat content of the diet. These concepts are underwritten not only by human data for colon and breast cancer but also by detailed studies with animal models in which approaches to the underlying mechanisms could be formulated. In addition, cancer of the pancreas is also associated, in part, with such dietary customs.

Prevention of these cancers hinges on the development of altered dietary customs to lower fat consumption from the now prevailing level of 45% of calories. One step in the right direction is the adoption of the "prudent diet," with about 33% of all calories as fat and less than 100 mg of cholesterol, recommended for the prevention of heart disease (Bennett and Simon 1973). However, to reduce the cancer risk, it may be necessary to lower the fat content further to 25–27% of all calories. Connor and Connor (1972) recommended a level of only 20% of calories. With respect to intestinal cancer especially, but perhaps to the other diet-related cancers as well, the questions of dietary fiber, of micronutrients such as vitamins A and E, or of minerals such as selenium require consideration and further research.

The approach involving managerial prevention would have industry, in cooperation with agricultural and governmental agencies, produce and market foodstuffs that would permit the consumer to adopt the prudent diet, or even the lower fat diet, more readily and, in fact, automatically.

Cancer of the stomach, already on the decline in the United States, is still the major cancer in western Latin America, the Orient, and northern and eastern Europe. This cancer may be prevented by increasing the consumption of foods high in vitamin C, by low-temperature food storage to prevent nitrite accumulation, and by controlling the use of nitrite as a preservative.

If these measures are taken and if, in addition, the readily preventable occupational cancers are eliminated, we would enter an era where cancers of all types would no longer represent a major cause of death in man.

Note Added in Proof

New developments in the study of tobacco carcinogenesis warrant note. It was already clear that polycyclic aromatic hydrocarbons alone fail to account for the total carcinogenic activity of tobacco tar (Hoffmann et al. 1976; Wynder and Hecht 1976; Wynder and Gori 1977). The classic bioassay for tobacco tar activity has been a test on mouse skin. In this test, the basic fraction of tar has low activity. Recently, however, attention has been called to this basic fraction because it contains materials with appreciable mutagenic activity (Kier et al. 1974; Matsumoto et al. 1977; Mizusaki et al. 1977; McCann and Ames, this volume; Sugimura et al., this volume) and has the capability to effect cell transformation (Benedict et al. 1975). Select quinolines are mutagenic (Nagao et al. 1977), and this sort of compound is not expected to cause induction of tumors on mouse skin because of the lack of enzymic activation there but may do so in lung, or even pancreas or urinary bladder, target organs in human smokers. Since the basic fraction of tobacco smoke stems largely from the protein component of tobacco, lowering carcinogenicity of tobacco products may be quite feasible if it can be shown that this fraction does indeed contain carcinogens.

Acknowledgments

The authors acknowledge the assistance of Drs. D. Hoffmann, P. Hill, P. C. Chan, B. Reddy, H. Marquardt, and Mrs. I. Hoffmann, and thank Mrs. A. Skowronski for editing and typing the manuscript. This work was supported by National Cancer Institute Grants CA-12376, CA-14298, CA-15400, and CA-17613; National Institute of Occupational Safety and Health Grant OH-00611; and Contracts No. 1-CP-33208 (National Cancer Institute) and No. 1-ES-6-2130 (National Institute of Environmental Health Sciences).

REFERENCES

Akazaki, K. 1973. Comparative histologic studies on the latent carcinoma of the prostate under different environmental circumstances. In *Host environment interactions in the etiology of cancer in man* (ed. R. Doll and I. Vodopija), p. 89. International Agency for Research on Cancer, Lyon, France.

Akazaki, K. and G.N. Stemmermann. 1973. Comparative study of latent carcinoma of the prostate among Japanese in Japan and Hawaii. *J. Natl. Cancer Inst.* **50:**1137.

Alcantara, E.N. and E.W. Speckmann. 1976. Diet, nutrition, and cancer. *Am. J. Clin. Nutr.* **29:**1035.

Ames, B.N., J. McCann and E. Yamasaki. 1973. Methods for detecting carcinogens and mutagens with the *Salmonella* mammalian microsome mutagenicity test. *Mutat. Res.* **31:**347.

Armstrong, B. and R. Doll. 1975. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int. J. Cancer* **15:**617.

Auerbach, O., E.C. Hammond, D. Kirman and L. Garfinkle. 1970. Effects of cigarette smoking on dogs. *Arch. Environ. Health* **21:**754.

Auerbach, O., E.C. Hammond, D. Kirman, L. Garfinkle and A.P. Stout. 1967. Histologic changes in bronchial tubes of cigarette smoking dogs. *Cancer* **20:**2055.

Benedict, W.F., N. Rucker, J. Faust and R.E. Kouri. 1975. Transformation of mouse cells by cigarette smoke condensate. *Cancer Res.* **35:**857.

Bennett, I. and M. Simon. 1973. *The prudent diet.* D. White, New York.

Berg, J.W. 1975. Can nutrition explain the pattern of international epidemiology of hormone-dependent cancers? *Cancer Res.* **35:**3345.

Boyland, E. and S.A. Walker. 1974. Catalysis of the reaction of aminopyrine and nitrite by thiocyanate. *Arzneim.-Forsch.* **24:**1181.

Bralow, S.P. and J.H. Weisburger. 1976. Experimental carcinogenesis. *Clinics Gastroenterol.* **5:**527.

Brandes, D. 1966. The fine structure and histochemistry of prostatic glands in relation to sex hormones. *Int. Rev. Cytol.* **20:**207.

Bulbrook, R.D., M.C. Swain, D.Y. Wang, J.L. Hayward, S. Kumaoka, O. Takatani, O. Abe and T. Utsunomiya. 1976. Breast cancer in Britain and Japan: Plasma-oestradiol-17β, oestrone and progesterone, and their urinary metabolites in normal British and Japanese women. *Eur. J. Cancer* **12:**725.

Burkitt, D.P. 1975a. Large bowel cancer: An epidemiological jigsaw puzzle. *J. Natl. Cancer Inst.* **54:**3.

Burkitt, D. 1975b. Benign and malignant tumours of large bowel. In *Refined carbohydrate foods and disease* (ed. D.P. Burkitt and H.C. Trowell), p. 117. Academic Press, New York.

Carroll, K.K. 1975. Experimental evidence of dietary factors and hormone-dependent cancers. *Cancer Res.* **35:**3374.

Carroll, K.K. and H.T. Khor. 1970. Effects of dietary fat and dose level of 7,12-dimethylbenz(a)anthracene on mammary tumor incidence in rats. *Cancer Res.* **30:**2260.

———. 1971. Effects of level and type of dietary fat on incidence of mammary tumors induced in female Sprague-Dawley rats by 7,12-dimethylbenz(a)-anthracene. *Lipids* **6:**415.

Chan, P.C. and L.A. Cohen. 1974. Effect of dietary fat, antiestrogen, and anti-prolactin on the development of mammary tumors in rats. *J. Natl. Cancer Inst.* **52:**25.

———. 1975. Dietary fat and growth promotion of rat mammary tumors. *Cancer Res.* **35:**3384.

Chan, P.C., L.A. Cohen and E.L. Wynder. 1976a. Increased yield of N-nitrosomethylurea-induced mammary tumors by dietary fat. *Proc. Am. Assoc. Cancer Res.* **17:**133.

Chan, P.C., F. Didato and L.A. Cohen. 1975. High dietary fat, elevation of rat serum prolactin and mammary cancer. *Proc. Soc. Exp. Biol. Med.* **149:**133.

Chan, P.C., J. Tsuang, J. Head and L.A. Cohen. 1976b. Effects of estradiol and prolactin on growth of rat mammary adenocarcinoma cells in monolayer cultures. *Proc. Soc. Exp. Biol. Med.* **151:**362.

Chomchai, C.C., N. Bhadrachari and N.D. Nigro. 1974. The effect of bile on the induction of experimental intestinal tumors in rats. *Dis. Colon Rectum* **17**:310.

Cohen, L.A., E. Tovbis and P.C. Chan. 1976. Cyclic nucleotide-dependent protein kinases from cultured normal and neoplastic rat mammary epithelial cells. *Fed. Proc.* **35**:296.

Cohen, L.A., J. Tsuang and P.C. Chan. 1974. Characteristics of rat normal mammary epithelial cells and DMBA-induced mammary adenocarcinoma cells grown in monolayer culture. *In Vitro* **10**:51.

Connor, W.E. and S.L. Connor. 1972. The key role of nutritional factors in the prevention of coronary heart disease. *Prev. Med.* **1**:49.

Correa, P. 1975. Comments on the epidemiology of large bowel cancer. *Cancer Res.* **35**:3395.

Correa, P., W. Haenszel, C. Cuello, S. Tannenbaum and M. Archer. 1975. A model for gastric cancer epidemiology. *Lancet* **ii**:58.

Day, N.E. 1975. Some aspects of the epidemiology of esophageal cancer. *Cancer Res.* **35**:3304.

Department of Health, Education, and Welfare (DHEW). 1964. *Smoking and health.* Public Health Service Publication No. 1103, p. 387. DHEW, Washington, D.C.

———. 1967, 1968, 1969. *The health consequences of smoking.* Public Health Service Publication No. 1696, p. 227; Suppl. 1968, p. 117; Suppl. 1969, p. 98. DHEW, Washington, D.C.

———. 1971. *The health consequences of smoking.* Publication No. (HSM) 71-7513, p. 458. DHEW, Washington, D.C.

———. 1972. *The health consequences of smoking.* Publication No. (HSM) 75-7516, p. 158. DHEW, Washington, D.C.

———. 1973. *The health consequences of smoking.* Publication No. (HSM) 73–8704, p. 249. DHEW, Washington, D.C.

———. 1974. *The health consequences of smoking.* Publication No. (CDC) 74-8704, p. 124. DHEW, Washington, D.C.

de Waard, F. 1975. Breast cancer incidence and nutritional status with particular reference to body weight and height. *Cancer Res.* **35**:3351.

Doll, R. and A.B. Hill. 1964. Mortality in relation to smoking: Ten years observation of British doctors. *Br. Med. J.* **1**:1399.

Endo, H., K. Takahashi, N. Kinoshita and T. Baba. 1974. Production of gastric and esophageal tumors in rats by methylnitrosocyanamide, a possible candidate of etiological factors for human gastric cancer. *Proc. Jpn. Acad.* **50**:497.

Endo, H., K. Takahashi, N. Kinoshita, T. Utsunomiya and T. Baba. 1975. An approach to the detection of possible etiologic factors in human gastric cancer. *Gann Monogr.* **17**:17.

Fan, F.Y. and S.R. Tannenbaum. 1973. Factors influencing the rate of formation of nitrosomorpholine from morpholine and nitrite: Acceleration by thiocyanate and other anions. *J. Agric. Food Chem.* **21**:237.

Fiala, E.S. 1977. Investigations into the metabolism and mode of action of the colon carcinogens 1,2-dimethylhydrazine and azoxymethane. *Cancer* (in press).

Fiala, E.S., G. Bobotas, C. Kulakis and J.H. Weisburger. 1976. Separation of 1,2-dimethylhydrazine metabolites by high-pressure liquid chromatography. *J. Chromatogr.* **117**:181.

Fiala, E.S., G. Bobotas, C. Kulakis, L.W. Wattenberg and J.H. Weisburger. 1977. The effects of disulfiram and related compounds on the *in vivo* metabolism of the colon carcinogen 1,2-dimethylhydrazine. *Biochem. Pharmacol.* (in press).

Fishman, J., R.M. Boyar and L. Hellman. 1975. Influence of body weight on estradiol metabolism in young women. *J. Clin. Endocrinol. Metab.* **41:**989.

Fraumeni, J.F., Jr., ed. 1975. *Persons at high risk of cancer.* Academic Press, New York.

Frisch, R. and R. Reveille. 1969. Variation in body weights and the age of adolescent growth spurts among Latin American and Asian populations in relation to calorie supplies. *Hum. Biol.* **41:**185.

Fujinaka, N., Y. Masuda and M. Kuratsune. 1976. Methylguanidine content in food. *Gann* **67:**679.

Furth, J. 1972. Prolactin and carcinogenesis. In *Prolactin and carcinogenesis* (ed. A.R. Boyns and K. Griffiths), p. 137. Alpha Omega Alpha, Cardiff, Wales.

Gori, G.B. 1976. Low-risk cigarettes—A prescription. *Science* **194:**1243.

Gortner, W.A. 1975. Nutrition in the United States. *Cancer Res.* **35:**1900.

Gullino, P.M., H.M. Pettigrew and F.H. Grantham. 1975. N-nitrosomethylurea as mammary gland carcinogen in rats. *J. Natl. Cancer Inst.* **54:**401.

Haenszel, W. 1975. Migrant studies. In *Persons at high risk of cancer* (ed. J.F. Fraumeni, Jr.), p. 361. Academic Press, New York.

Haenszel, W. and P. Correa. 1975. Developments in the epidemiology of stomach cancer over the past decade. *Cancer Res.* **35:**3452.

Haenszel, W., J.W. Berg, M. Segi, M. Kurihara and F.B. Locke. 1973. Large bowel cancer in Hawaiian Japanese. *J. Natl. Cancer Inst.* **51:**1765.

Hakama, M. 1969. The peculiar age specific incidence curve for cancer of the breast—Clemmesen's Hook. *Acta Pathol. Microbiol. Scand.* **75:**370.

Hammond, E.C. 1975. Tobacco. In *Persons at high risk of Cancer* (ed. J.F. Fraumeni, Jr.), p. 131. Academic Press, New York.

Hammond, E.C., L. Garfinkel, H. Seidman and E.A. Lew. 1976. "Tar" and nicotine content of cigarette smoke in relation to death rates. *Environ. Res.* **12:**263.

Hawks, A. and P.N. Magee. 1974. The alkylation of nucleic acids of rat and mouse *in vivo* by the carcinogen 1,2-dimethylhydrazine. *Br. J. Cancer* **30:**440.

Hellman, L., B. Zumhoff, J. Fishman and T.F. Gallagher. 1971. Peripheral metabolism of ^{3}H-estradiol and the excretion of endogenous estrone and estriol glucosiduronate in women with breast cancer. *J. Clin. Endocrinol.* **33:**138.

Henderson, B.E., V. Gerkins, I. Rosario, J. Casagrande and M.C. Pike. 1975. Elevated serum levels of estrogen and prolactin in daughters of patients with breast cancer. *N. Engl. J. Med.* **293:**790.

Higginson, J. 1967. Etiology of gastrointestinal cancer in man. *Natl. Cancer Inst. Monogr.* **25:**191.

———. 1976. Importance of environmental factors in cancer. *INSERM* **52:**15.

Higginson, J. and C.S. Muir. 1973. Epidemiology. In *Cancer medicine* (ed. J.F. Holland and E. Frei), p. 241. Lea and Febiger, Philadelphia, Pennsylvania.

Hilfrich, J., S.S. Hecht and D. Hoffman. 1977. A study of tobacco carcinogenesis. IV. Effect of N-nitrosonornicotine and N-nitrosoanabasine in Syrian golden hamsters. *Cancer Lett.* **2:**169.

Hill, M.J. 1975. Metabolic epidemiology of dietary factors in large bowel cancer. *Cancer Res.* **35:**3398.

Hill, M.J., J.S. Crowther, B.S. Drasar, G. Hawksworth, V. Aries and B.E.O. Williams. 1971. Bacteria and etiology of cancer of the large bowel. *Lancet* **i:**95.

Hill, M.J., B.S. Drasar, B.E.O. Williams, T.W. Meade, A.G. Cox, J.E.P. Simpson and B.C. Morson. 1975. Fecal bile acids and clostridia in patients with cancer of the large bowel. *Lancet* **ii:**535.

Hill, P. and E. Wynder. 1976. Diet and prolactin release. *Lancet* **ii:**806.

Hill, P., E.L. Wynder, P. Helman, R. Hickman and G. Rona. 1976a. Plasma

hormone profiles in populations at different risk for breast cancer. *Cancer Res.* **36:**1883.

Hill, P., E.L. Wynder, H. Kumar, P. Helman, G. Rona and K. Kumo. 1976b. Prolactin levels in populations at risk for breast cancer. *Cancer Res.* **36:**4102.

Hoffmann, D. and E.L. Wynder. 1976. Smoking and occupational cancers. *Prev. Med.* **5:**245.

Hoffmann, D., S.S. Hecht, R.M. Ornaf and E.L. Wynder. 1974. N′-nitrosonornicotine in tobacco. *Science* **186:**265.

Hoffmann, D., I. Schmeltz, S.S. Hecht and E.L. Wynder. 1976. Chemical studies on tobacco smoke. XXXIX. On the identification of carcinogens, promoters, and cocarcinogens. In *Proceedings, 3rd World Conference on Smoking and Health. I. Modifying the risk for the smoker,* p. 125. Department of Health, Education, and Welfare, Washington, D.C.

Hopkins, G.J. and C.E. West. 1976. Possible roles of dietary fats in carcinogenesis (Minireview). *Life Sci.* **19:**1103.

Horrobin, D.F. 1976. *Prolactin 1976,* p. 104. Eden Press, Montreal, Canada.

Jacobs, M.M., B. Jansson and A.C. Griffin. 1977. Inhibitory effects of selenium on 1,2-dimethylhydrazine and methylazoxymethanol acetate induction of colon tumors. *Cancer Lett.* **2:**133.

Kelly, P.A., C. Bradley, R.P.C. Shiu, T. Meites and H.G. Freisan. 1974. Prolactin binding to rat mammary tumor tissue. *Proc. Soc. Exp. Biol. Med.* **146:**816.

Kent, J.R. and A.B. Acone. 1966. Plasma testosterone levels and aging in males. *Excerpta Med. Int. Congr. Ser.* **101:**31.

Kier, L.D., E. Yamasaki and B.N. Ames. 1974. Detection of mutagenic activity in cigarette smoke condensates. *Proc. Natl. Acad. Sci.* **71:**4159.

Kreyberg, L. 1969. *An etiology of lung cancer. A morphological, epidemiological and experimental analysis,* p. 99. Universitetsforlaget, Oslo, Norway.

Kwa, H.G., M. DeJong-Bakker, E. Engelsman and F.J. Cleton. 1974. Plasma-prolactin in human breast cancer. *Lancet* **i:**433.

Kwa, H.G., M. Cleton, M. DeJong-Bakker, R.D. Bulbrook, J.L. Hayward and D.Y. Wang. 1976. Plasma prolactin and its relationship to risk factors in human breast cancer. *Int. J. Cancer* **17:**441.

Laqueur, G.L. and M. Spatz. 1975. Oncogenicity of cycasin and methylazoxymethanol. *Gann Monogr. Cancer Res.* **17:**189.

Lee, M.M.C., K.S.F. Chang and M.M. Chan. 1963. Sexual maturation of Chinese girls in Hong Kong. *Pediatrics* **34:**389.

MacMahon, B., P. Cole and J. Brown. 1973. Etiology of human breast cancer: A review. *J. Natl. Cancer Inst.* **50:**21.

Marquardt, H., F. Rufino and J.H. Weisburger. 1977a. Mutagenic activity of nitrite-treated foods: Human stomach cancer may be related to dietary factors. *Science* **196:**1000.

———. 1977b. On the aetiology of gastric cancer: Mutagenicity of food extracts after incubation with nitrite. *Food Cosmet. Toxicol.* **15:**97.

Mastromarino, A., B.S. Reddy and E.L. Wynder. 1976. Metabolic epidemiology of colon cancer: Enzymic activity of fecal flora. *Am. J. Clin. Nutr.* **29:**1445.

Matsumoto, T., D. Yoshida, S. Mizusaki and H. Okamoto. 1977. Mutagenic activity of amino acid pyrolyzates in *Salmonella typhimurium* TA 98. *Mutat. Res.* **48:**279.

McGuire, W.L., G.C. Chamness, M.E. Costlow and R.E. Shepherd. 1974. Progress in endocrinology and metabolism: Hormone dependence in breast cancer. *Metabolism* **23:**75.

Meites, J. 1972. Relation of prolactin and estrogen to mammary tumorigenesis in the rat. *J. Natl. Cancer Inst.* **48:**1217.

Mirvish, S.S. 1975. Formation of N-nitroso compounds: Chemistry, kinetics, and *in vivo* occurrence. *Toxicol. Appl. Pharmacol.* **31**:325.

Mirvish, S.S., L. Wallcave, M. Eagen and P. Shubik. 1972. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitrosocompounds. *Science* **177**:65.

Mizusaki, S., H. Okamoto, A. Akiyama and Y. Fukuhara. 1977. Relation between chemical constituents of tobacco and mutagenic activity of cigarette smoke condensate. *Mutat. Res.* **48**:319.

Mower, H.F., J. Setliff, M. Mandel, G. Stemmermann, A. Nomura, S. Kamiyama and A. Shimado. 1977. Detection of mutagens in human feces of populations at high and low risk for large bowel cancer. *Lancet* (in press).

Nagao, M., T. Yahagi, Y. Seino, T. Sugimura and N. Ito. 1977. Mutagenicities of quinoline and its derivatives. *Mutat. Res.* **42**:335.

Narisawa, T., N.E. Magadia, J.H. Weisburger and E.L. Wynder. 1974. Promoting effect of bile acid on colon carcinogenesis after intrarectal instillation of N-methyl-N′-nitro-N-nitrosoguanidine in rats. *J. Natl. Cancer Inst.* **55**:1093.

Nigro, N.D., N. Bhadrachari and C. Chomchai. 1973. A rat model for studying colonic cancer: Effect of cholestyramine on induced tumors. *Dis. Colon Rectum* **16**:438.

Painter, N. and D. Burkitt. 1975. Diverticular disease of the colon. In *Refined carbohydrate foods and disease* (ed. D.P. Burkitt and H.C. Trowell), p. 99. Academic Press, New York.

Pearson, O.H. 1972. Endocrine aspects of breast cancer. In *Current research in oncology* (ed. C.B. Anfinsen et al.), p. 125. Academic Press, New York.

———. 1973. Animal models of endocrine-related cancer. In Publication No. 7, p. 137. International Agency for Research on Cancer, Lyon, France.

Raineri, R. and J.H. Weisburger. 1975. Dietary factors and the etiology of gastric cancer. *Cancer Res.* **35**:3469.

Reddy, B.S. and E.L. Wynder. 1973. Large bowel carcinogenesis: Fecal constituents of populations with diverse incidence rates of colon cancer. *J. Natl. Cancer Inst.* **50**:1437.

———. 1977. Metabolic epidemiology of colon cancer: Fecal bile acids and neutral sterols in colon cancer patients and patients with adenomatous polyps. *Cancer* **39**:2533.

Reddy, B.S., T. Narisawa and J.H. Weisburger. 1976a. Effect of a diet with high levels of protein and fat on colon carcinogenesis in F344 rats treated with 1,2-dimethylhydrazine. *J. Natl. Cancer Inst.* **57**:567.

Reddy, B.S., J.H. Weisburger and E.L. Wynder. 1974. Fecal bacterial β-glucuronidase: Control by diet. *Science* **183**:416.

———. 1975. Effect of high risk and low risk diets for colon carcinogenesis on fecal microflora and steroids in man. *J. Nutr.* **105**:878.

Reddy, B.S., T. Narisawa, J.H. Weisburger and E.L. Wynder. 1976b. Promoting effect of sodium deoxycholate on colon adenocarcinoma in germfree rats. *J. Natl. Cancer Inst.* **56**:441.

Reddy, B.S., A. Mastromarino, C. Gustafson, M. Lipkin and E.L. Wynder. 1976c. Fecal bile acids and neutral sterols in patients with familial polyposis. *Cancer* **38**:1694.

Reddy, B.S., T. Narisawa, D. Vukusich, J.H. Weisburger and E.L. Wynder. 1976d. Effect of quality and quantity of dietary fat and dimethylhydrazine in colon carcinogenesis in rats. *Proc. Soc. Exp. Biol. Med.* **151**:237.

Saffiotti, U. and J.K. Wagoner. 1976. Occupational carcinogenesis. *Ann. N.Y. Acad. Sci.* **271**:1.

Sander, J. and F. Schweinsberg. 1972. *In vivo* and *in vitro* experiments on the formation of N-nitroso compounds from amines and amides and nitrate or

nitrite. In *N-nitroso compounds, analysis and formation.* Publication No. 3, p. 97. International Agency for Research on Cancer, Lyon, France.

Savage, D.C.L., C.C. Forsyth and J. Carxeron. 1975. Excretion of individual adrenocortical steroids in obese children. *Arch. Dis. Child.* **49:**946.

Schottenfeld, D., R.C. Gantt and E.L. Wynder. 1974. The role of alcohol and tobacco in multiple primary cancers of the upper digestive system, larynx and lung. A prospective study. *Prev. Med.* **3:**277.

Sciarra, F., G. Sorcini, F. DiSilverio and V. Gagliardi. 1973. Plasma testosterone and androsteredione after orchidectomy in prostatic adenocarcinoma. *Clin. Endocrinol.* **1:**101.

Segi, M. and M. Kurihara. 1972. *Cancer mortality for selected sites in 24 countries 1966–1967,* No. 6. Japan Cancer Society, Tokyo, Japan.

Seidman, H., D. Silverberg and A. Holleb. 1976. *American Cancer Society cancer facts and figures 1976.* American Cancer Society, New York.

Smithline, F., L. Sherman and H.D. Kolodny. 1975. Prolactin and breast carcinoma. *N. Engl. J. Med.* **292:**784.

Spjut, H.J. and M.W. Noall. 1971. Experimental induction of tumors of the large bowel of rats: A review of the experience with 3,2′-dimethyl-4-aminobiphenyl. *Cancer* **28:**21.

Stemmermann, G.N. and T. Hayashi. 1977. The enteric microenvironment and its effect upon the morbid anatomy of the colon: A study of colon cancer, its precursors and companions in Hawaii Japanese. In *Pathology annual* (ed. S. Sommers). Lenox Hill, New York. (In press.)

Sugimura, T. and T. Kawachi. 1973. Experimental stomach cancer. In *Methods in cancer research* (ed. H. Busch), p. 245. Academic Press, New York.

Tannenbaum, A. 1942. The genesis and growth of tumors. III. Effects of a high fat diet. *Cancer Res.* **2:**468.

———. 1945. The dependence of tumor formation on the composition of the calorie restricted diet as well as on the degree of restriction. *Cancer Res.* **5:**616.

Tannenbaum, A. and H. Silverstone. 1957. Nutrition and the genesis of tumors. In *Cancer* (ed. R.W. Raven), vol. 1, p. 306. Butterworth, London.

Turkington, R.W. 1973. Hormonal regulation of gene expression in mammary cells. In *Recent progress in hormone research* (ed. R.O. Greep), vol. 29, p. 417. Academic Press, New York.

Vignon, F. and H. Rochefort. 1974. Regulations des "recepteurs" des oestrogens dans les tumeurs mammaires: Effect de la prolactine *in vivo. Compt. Rend. Acad. Sci.* **278:**103.

Ward, J.M., R.S. Yamamoto and J.H. Weisburger. 1973. Cellulose dietary bulk and azoxymethane-induced intestinal cancer. *J. Natl. Cancer Inst.* **51:**713.

Weisburger, J.H. 1976. Environmental cancer. *J. Occup. Med.* **18:**245.

Weisburger, J.H. and R. Raineri. 1975. Assessment of human exposure and response to N-nitroso compounds. A new view on the etiology of digestive tract cancers. *Toxicol. Appl. Pharmacol.* **31:**169.

Wolanski, N. 1966. Secular trends. Microevolution, physiological adaptation and migration and their causative factors. In *Problems of world nutrition. Proceedings of the 7th International Congress on Nutrition,* vol. 4, p. 96. Pergamon Press, Oxford.

Wurtman, R.J. and J.D. Fernstrom. 1975. Control of brain monamine synthesis by diet and plasma amino acids. *Am. J. Clin. Nutr.* **28:**638.

Wynder, E.L. 1972. Etiology of lung cancer. Reflections on two decades of research. *Cancer* **30:**1332.

———. 1975. An epidemiological evaluation of the causes of cancer of the pancreas. *Cancer Res.* **35:**2228.

———. 1976. Nutrition and cancer. *Fed. Proc.* **35:**1309.

Wynder, E.L. and G.B. Gori. 1977. Contribution of the environment to cancer incidence: An epidemiologic exercise. *J. Natl. Cancer Inst.* **58:**825.

Wynder, E.L. and S.S. Hecht, eds. 1976. *Lung cancer.* UICC Technical Report Series 25. Union Internationale contre le Cancer, Geneva, Switzerland.

Wynder, E.L. and T. Hirayama. 1977. A comparative epidemiologic study of the United States and Japan with special reference to cancer. *J. Natl. Cancer Inst.* (in press).

Wynder, E.L. and D. Hoffmann. 1967. *Tobacco and tobacco smoke, studies in experimental tobacco carcinogenesis.* Academic Press, New York.

Wynder, E.L. and K. Mabuchi. 1972. Etiological and preventive aspects of human cancer. *Prev. Med.* **1:**300.

Wynder, E.L. and B.S. Reddy. 1973. Studies of large bowel cancer: Human leads to experimental application. *J. Natl. Cancer Inst.* **50:**1099.

———. 1975. Dietary fat and colon cancer. *J. Natl. Cancer Inst.* **54:**7.

Wynder, E.L. and T. Hirayama. 1977. A comparative epidemiologic study of the colon and rectum. *Cancer* **20:**1520.

Wynder, E.L. and S.D. Stellman. 1977. Epidemiology of tobacco-related cancers with special emphasis on the effect of lower tar cigarette. *Cancer Res.* (in press).

Wynder, E.L., G.B. Gori and D. Hoffmann, eds. 1976a. *Proceedings, 3rd World Conference on Smoking and Health. I. Modifying the risk for the smoker.* Department of Health, Education, and Welfare, Washington, D.C.

Wynder, E.L., E.A. Graham and A.B. Groninger. 1953. Experimental production of carcinoma with cigarette tar. *Cancer Res.* **13:**855.

Wynder, E.L., K. Mabuchi and W.F. Whitmore. 1971. Epidemiology of cancer of the prostate. *Cancer* **28:**344.

Wynder, E.L., M. Mushinski and L. Stellman. 1976b. The epidemiology of the less harmful cigarette. In *Proceedings, 3rd World Conference on Smoking and Health. I. Modifying the risk for the smoker,* p. 1. Department of Health, Education, and Welfare, Washington, D.C.

Wynder, E.L., J.A. Peters and S. Vivona. 1975. Nutrition in the causation of cancer. *Cancer Res.* **35:**3231.

Wynder, E.L., J. Kmet, N. Dungal and M. Segi. 1963. An epidemiological investigation of gastric cancer. *Cancer* **16:**1461.

Wynder, E.L., L.A. Cohen, P.C. Chan, E. MacCornack and P. Hill. 1977. Etiology and prevention of breast cancer. In *Recent results in cancer research* (ed. E. Grundman), vol. 54. Springer-Verlag, Berlin. (In press.)

Wynder, E.L., T. Kajitani, S. Ishikawa, H. Dodo and A. Takano. 1969. Environmental factors of cancer of the colon and rectum. II. Japanese epidemiological data. *Cancer* **23:**1210.

Yanai, R. and H. Nagasawa. 1976. Effects of pituitary graft and 2-bromo-α-ergocryptine on mammary DNA synthesis in mice in relation to mammary tumorigenesis. *J. Natl. Cancer Inst.* **56:**1055.

Zedeck, M.S., S.S. Sternberg, R.W. Poynter and J. McGowen. 1970. Biochemical and pathological effects of methylazoxymethanol acetate, a potent carcinogen. *Cancer Res.* **30:**801.

Origins of Human Cancer

BOOK A
Incidence of Cancer in Humans

edited by

H. H. Hiatt
Harvard School of Public Health

J. D. Watson
Cold Spring Harbor Laboratory

J. A. Winsten
Harvard School of Public Health

COLD SPRING HARBOR CONFERENCES ON CELL PROLIFERATION
VOLUME 4

Cold Spring Harbor Laboratory
1977

Origins of Human Cancer

International Standard Book Number 0-87969-119-0
Library of Congress Catalog Card Number 76-57915

Printed in the United States of America

Cover and book design by Emily Harste

This publication was supported in part by
grants from the Rita Allen Foundation and
the Charles E. Merrill Trust.