NUTRITION AND ITS RELATIONSHIP TO CANCER

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I. Introduction

During the past two decades, epidemiologic studies have elaborated the influence of the environment on the development of certain forms of cancer and have led to the conclusion that cancer may not be an inevitable consequence of aging (Wynder and Gori, 1977). This is an encouraging observation that suggests that as we identify the ways in which environmental factors increase the risk of cancer we may be in a position to manipulate the environment and reduce to a minimum the risk of cancer in future generations.

Doll (1967) and Higginson (1975) suggest that the lowest reported cancer rates for a given site should be considered the baseline or natural rate of occurrence. Increases in rates, therefore, can be ascribed to environmental factors, or factors that originate almost totally outside the host's body. Based on comparisons of high and low rate areas, Higginson (1975) noted that approximately 90% of human cancers are associated with environmental factors.

Epidemiologically, the effect of environmental factors on cancer incidence has been investigated by studies of incidence patterns between and among population groups, differences in the rates of the disease between the sexes, changes in disease rates over time, demographic and socioeconomic distributions of diseases, effects of migration, and the dietary habits of different populations. Studies of mortality rates also indicate a relationship between disease development and environmental factors. The proportion of deaths for both sexes that have been related to environmental factors are summarized in Tables I and II. Although the accuracy of mortality and incidence data may be suspect in some areas of the world, reliable data obtained from select and scientifically rigorous cancer registries indicate large differences between high-risk and low-risk populations (Doll *et al.*, 1970). The consistency of the findings suggest that environmental rather than racial or genetic factors play a predominant role in the etiology of cancer in man.

Although the concept that diet and nutrition might influence cancer is not new (Tannenbaum, 1940), this relationship has received surprisingly little detailed attention. During the 1930s, a number of laboratories were interested in the possible influence exerted by nutritional factors on susceptibility to cancer, but the question soon lost the interest of both the scientific and lay community. Even though the pioneering studies indicated that dietary restriction reduced the incidence of mammary as well as lung tumors in mice (Tannenbaum, 1942) and that underfeeding of rats led to a lower spontaneous tumor incidence than *ad libitum* feeding

TABLE I
AGE-ADJUSTED MALE MORTALITY RATIOS FOR HIGH- AND LOW-RISK POPULATIONS AND FOR U.S. WHITES BY CANCER SITE[a]

Cancer risk	Low-risk country	High-risk country	Mortality ratios: U.S. White[b]	Mortality ratios: High-risk and low-risk country[c]
Trachea, bronchus lung	Portugal	Scotland	3.6	7.1
Stomach	U.S. (for whites)	Japan	—	7.8
Prostate	Japan	U.S. (for nonwhites)	6.9	12.4
Intestine except rectum	Japan	Scotland	3.9	4.3
Esophagus	Norway	France	1.3	5.9
Larynx	Sweden	France	3.8	19.8
Rectum	Chile	Denmark	2.4	4.8
Buccal cavity, pharynx	Israel	France	3.1	7.2
Pancreas	Italy	U.S. (for nonwhites)	1.9	2.3
Bladder	Japan	S. Africa	2.1	3.3
Skin	Japan	Australia	3.4	5.5
Liver, biliary passage	Norway	Japan	1.6	5.3
Thyroid	New Zealand	Switzerland	1.4	5.5
Leukemia	Japan	Denmark	2.0	2.1

[a] From Wynder and Gori (1977).
[b] U.S. mortality rate:low-risk country mortality rate.
[c] High-risk country mortality rate:low risk country mortality rate.

(Ross and Bras, 1965), it is apparent that additional study of the relationship between dietary factors and human carcinogenesis is in order.

Following up on leads advanced by epidemiologists, experimentalists have found that nutrition, in general, is related to the development of cancer in three ways: (1) Food additives or contaminants may act as carcinogens, cocarcinogens, or both. (2) Nutrient deficiencies may lead to biochemical alterations that promote neoplastic processes. (3) Changes in the intake of selected macronutrients may produce metabolic and biochemical abnormalities, either directly or indirectly, which increase the risk for cancer.

Although the relationship of nutrition and cancer is complex and sometimes perplexing to those who visualize carcinogenesis in terms of a specific carcinogen, it is important to understand that specific carcinogens play only a minor role as initiators in the relationship between nutrition and the development of cancer.

TABLE II
AGE-ADJUSTED FEMALE MORTALITY RATIOS FOR HIGH- AND LOW-RISK POPULATIONS AND FOR U.S. WHITES BY CANCER SITE, 1966–1970[a]

Cancer risk	Low-risk country	High-risk country	Mortality ratios: U.S. White[b]	Mortality ratios: High-risk and low-risk country[c]
Stomach	U.S. (for whites)	Japan	—	7.8
Breast	Japan	Netherlands	5.5	6.6
Uterus (all parts)	Israel	U.S. (for nonwhites)	1.9	4.7
Intestine (except rectum)	Japan	Scotland	3.7	4.3
Trachea, bronchus, lung	Portugal	Scotland	2.4	4.2
Rectum	Chile	Denmark	1.6	3.3
Pancreas	Italy	U.S. (for nonwhites)	1.9	2.4
Esophagus	Austria	Chile	1.0	6.9
Bladder	Japan	U.S. (for nonwhites)	1.5	2.4
Skin	Japan	Australia	2.5	4.2
Buccal cavity, pharynx	Fed. Republ. of Ger.	N. Ireland	2.4	3.8
Larynx	Norway	Ireland	4.4	16.8
Liver, biliary passage	New Zealand, Fed. Repub. of Germany	Federal Republic of Germany	1.6	4.1
Ovary, fallopian tube, broad ligament	Japan	Denmark	3.9	5.9
Thyroid	Australia	Austria	1.1	4.0
Leukemia	Japan	Israel	1.6	1.9

[a] From Wynder and Gori (1977).
[b] U.S. mortality rate:low-risk country mortality rate.
[c] High-risk country mortality rate:low-risk country mortality rate.

Several examples suggest that diet, rather than industrialization and contaminants, is the significant factor in the etiology of certain types of cancer. For instance, colon cancer has shown only a slight upward trend in incidence in the United States in the last 40 years (Cutler and Young, 1975). Cancer of the breast and cancer of the prostate have exhibited similar slow increases in rate in the United States. The logical conclusion is that the pronounced alterations in our environment, such as industrial pollution, intentional and inadvertant food additives, and food contaminants, are not directly or indirectly associated with the development of

these three types of cancer in man. In Japan, a country with a high degree of industrialization, these three types of cancer have a low incidence. Recently, however, an increasing trend has appeared, associated with the progressive Westernization of the Japanese dietary intake since 1945. This also provides some evidence that the dietary pattern, rather than industrial activity is one of the important factors in relation to causative mechanisms for these types of human cancer. However, it must be recognized that the correlation between diet and certain forms of cancer does not prove causation. Many factors may be necessary for cancer causation, but the modification of only one of the contributing factors, such as diet, may be sufficient to retard the chain of causative events.

This review covers six types of cancer. In four of these—breast, large bowel, stomach, and head and neck—the epidemiologic evidence is overwhelming that nutritional factors have a major etiological role. Indeed, the epidemiologic data on diet and nutrition in these four cancers provided the leads for metabolic and animal model studies that now fully support their epidemiologic inspiration.

Dietary factors are also implicated in the etiologies of the two remaining types of cancer surveyed in this article—pancreas and prostate—but the epidemiologic evidence is presently not overwhelming. As a result, more attention is devoted in this article to the four types of cancer that we know most about with respect to variables of diet and nutrition. However, future research will undoubtedly produce abundant evidence for the etiological role of nutrition and diet in *all* of the types of cancer included in this article.

This article also presents an evaluation of the current status of the relationship between nutrition and cancer in man, the use of animal models to determine if the etiological factors established for man can be modified in an experimental setting, and we will question the inconsistencies and make recommendations for additional research and possible preventive measures.

II. Dietary Factors and Cancer of the Large Bowel

A. Epidemiology

Cancer of the large bowel has been the subject of several epidemiologic reviews (Wynder and Shigematsu, 1967; Wynder *et al.*, 1969; Bjelke, 1974; Correa and Haenszel, 1978; Burkitt, 1971; Wynder, 1975b; Weisburger *et al.*, 1975). In addition, the major features of inter- and intra-

country distribution of cancer of the colon and rectum have been detailed in recent publications (Waterhouse *et al.*, 1976; Fraumeni, 1975; Weisburger *et al.*, 1975; Correa and Haenszel, 1978). The highest incidence rates are found in North America, New Zealand, and Western Europe, with the exception of Finland (Fig. 1). Intermediate rates are found in Eastern Europe and the Balkans, whereas the lowest incidences are found in Africa, Asia, and Latin America, with the exception of Uruguay and Argentina, where mortality rates are similar to those found in North America. The mortality data for most of the countries appear to be consistent with the incidence data.

In general, the more economically developed a society and its agricultural industries, the greater the incidence of colon cancer, although not necessarily cancer of the rectum (Wynder, 1975b; Waterhouse *et al.*, 1976). In Israel, colorectal cancer incidence in Jews from Yemen and North Africa is half that of Jews from Europe or North America (Doll *et al.*, 1966). The large difference in colon cancer incidence between Israelis

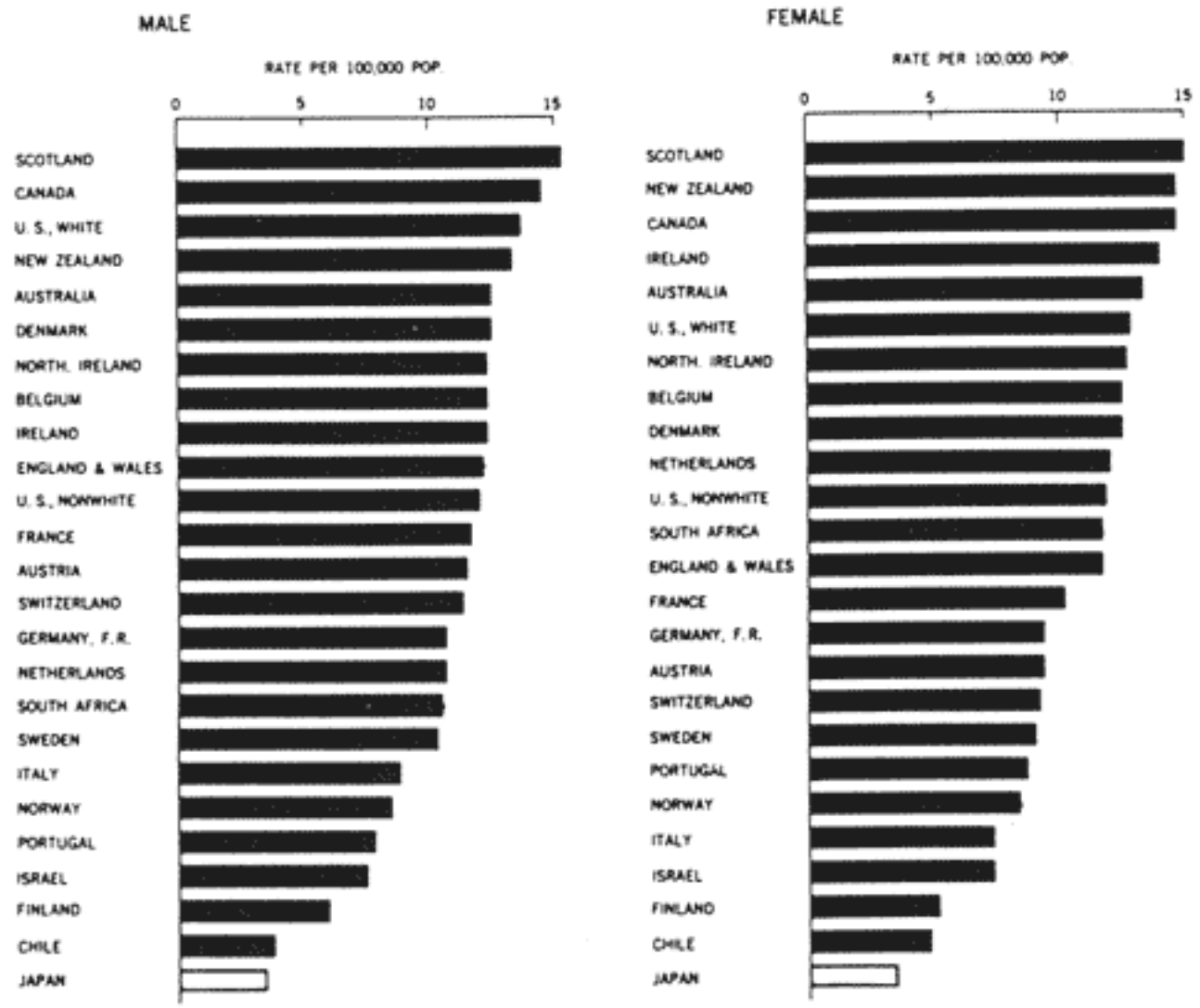

FIG. 1. Age-adjusted death rates for malignant neoplasms of intestine, except rectum, in different countries, 1966–1967. (From Segi and Kurihara, 1972.)

born in Asia or North Africa and those born in Europe or North America probably reflects the influence of traditional dietary habits more than current lifestyles.

Although some geographic and ethnic differences may be due to the well-known artifacts of inaccurate diagnosis and incomplete reporting, these artifacts can account for only a small proportion of the international variations. In Sweden, the methods of diagnosing colon cancer deaths are similar to those used in Denmark and Finland, and thus the differences in colon cancer mortality rates among these regions appear to be valid (Jensen *et al.*, 1974). In Sweden, there is a north–south gradient, with higher incidence in the south. Gastric cancer exhibits an opposite trend (Jensen *et al.*, 1974). However, the difference in colon cancer mortality and incidence rates between the United States or Western Europe and Japan are also real, because the quality of Japanese medical facilities and vital statistics parallel those in the United States and Western Europe (Wynder *et al.*, 1969).

Urban populations generally have higher risks for colon cancer than rural populations (Levin *et al.*, 1960; Teppo *et al.*, 1975; Doll *et al.*, 1970; Staszewski, 1976; Clemmesen, 1974). Among American blacks, the rates are somewhat lower in certain rural parts of the South than the rates for blacks living in industrialized Northern cities (Haenszel and Dawson, 1965) who migrated from rural South to Northern cities. In recent years, the difference had tended to fade, corresponding to the equalization of standard of living, including diet. This is a strong argument for diet as a key element in etiology.

1. *Colon–Rectum Differences*

Generally, lower colon–rectum incidence ratios prevail in Asian and African populations, whereas the results for Japan, India, and Nigeria represent the prevailing pattern in that part of the world (Correa and Haenszel, 1978). The Eastern European registries report distinctly lower colon–rectum ratios, often below unity, and higher colon–rectum ratios are reported in Western Europe and North America than in low-risk countries.

Rectal cancer is relatively more common than colon cancer in males (Wynder, 1975b). It is suggested that cancers of the rectum and colon may have different origins because rectal cancer does not vary as much in incidence in high- and low-risk populations, and it also exhibits a more pronounced sex-linked effect. The male/female ratio is about 1.4 for rectal cancer but is near unity for colon cancer.

It can be postulated that the typical absence of fecal material in the

lower rectum or the difference in histologic structure of mucosa between rectum and colon contributes to this variation in incidence between colon and rectum.

2. *Migrant Studies*

Further evidence for the importance of environmental factors in determining the geographic differences in large-bowel cancer incidence is provided by studies of migrants to the United States and Australia from equally industrialized Japan (Haenszel and Kurihara, 1968; Haenszel *et al.*, 1973; Staszewski *et al.*, 1971). Cancer incidence is higher in the first and second generation Japanese immigrants to Hawaii and California than in the native Japanese in Japan (Haenszel and Kurihara, 1968). A similar upward trend in colon cancer mortality has also been observed for Polish immigrants to Australia (Staszewski *et al.*, 1971). Further support for environmental dietary influence and/or lifestyle in the development of large-bowel cancer is derived from a study of southern Asian migrants to Kenya (Chopra *et al.*, 1975). Of equal interest is the fact that colon cancer seems to be increasing in Japan itself, a finding consistent with the increasing Westernization of the Japanese diet (Oiso, 1975; Hirayama, 1978).

3. *Socioeconomic Status*

With the exception of Japan and Finland, large-bowel cancer is a disease of economically developed countries. Yet, no socioeconomic gradient in risk has been found by intrapopulation comparisons in high-risk countries such as North America and Western Europe. However, the situation in low-risk populations is quite different. The recent findings from Cali, Colombia, where the overall incidence of large-bowel cancer is one-fifth that reported by U.S. registries, indicate a 4-fold excess risk in the upper socioeconomic classes of both sexes in two different age groups (Haenszel *et al.*, 1975). Collateral studies in Cali reveal that the rate of adenomatous polyps of the colon is minimal in the poorest socioeconomic class (Haenszel *et al.*, 1975; Correa, 1975). Nutritional studies have shown a very large socioeconomic difference in meat consumption in Cali (Aragon, 1964). The absence of a socioeconomic gradient for the development of large-bowel cancer in the United States may be due to minimal differences in dietary intake of fat, protein, and carbohydrate.

4. *Religious Differences*

Comparative studies of religious groups have been motivated by the search for factors that link the lifestyle of individual groups within a small

geographical area to their site-specific cancer risks. Studies by Lemon and Walden (1966) indicated the total cancer mortality of California Seventh-Day Adventists to be about 60% of a comparable general population sample. The Seventh-Day Adventists who consume less meat and adhere to a lactoovo-vegetarian diet are reported to have 60–70% of the colon cancer death rate for total California (Lemon *et al.*, 1964; Phillips, 1975). It can be postulated that a lactoovo-vegetarian diet protects against large-bowel cancer (Phillips, 1975).

The incidence of large-bowel cancer in Mormons (members of The Church of Jesus Christ of Latter-Day Saints) also is lower than other U.S. white populations (Enstrom, 1975b; Lyon *et al.*, 1977). Traditionally, Mormons eat more whole grain breads, cereals, fruits, and vegetables.

Jussawalla and Jain (1976) compared colon and breast cancer incidence rates in the Parsi community in Bombay, India, with that of the Hindu population of Bombay and other Indian communities and found that the Parsis exhibited a higher risk for colon and breast cancer, but not for rectal cancer, than the other religious groups of Bombay. Since the Parsis possess distinctive dietary habits, which lean more toward a Western diet, the difference in dietary habits between these two Indian populations could account for the differences in colon cancer rates.

B. Etiology

1. *Correlation Analysis*

Since the risk of large-bowel cancer closely parallels the economic development of a given country, cross-national correlations between colon cancer frequency and diet have been used to select from possible hypotheses for testing in case-control and cohort studies. These studies have shown food preferences that appear to be associated with high- and low-risk populations. Such correlations may be spurious, but when a correlation such as that between fat intake and colon cancer mortality is supported by experimental evidence from animal models, and underlying mechanisms can be described, it seems worthy to consider the possible relevance (Reddy *et al.*, 1975a).

Wynder *et al.* (1969) and Wynder and Reddy (1973) proposed that colon cancer incidence is mainly associated with total dietary fat. They further suggested that dietary fat influences the metabolic activity of the fecal microflora and thus may be involved in the pathogenesis of cancer of the colon. A worldwide correlation between colon cancer incidence and total fat consumption has been established (Fig. 2).

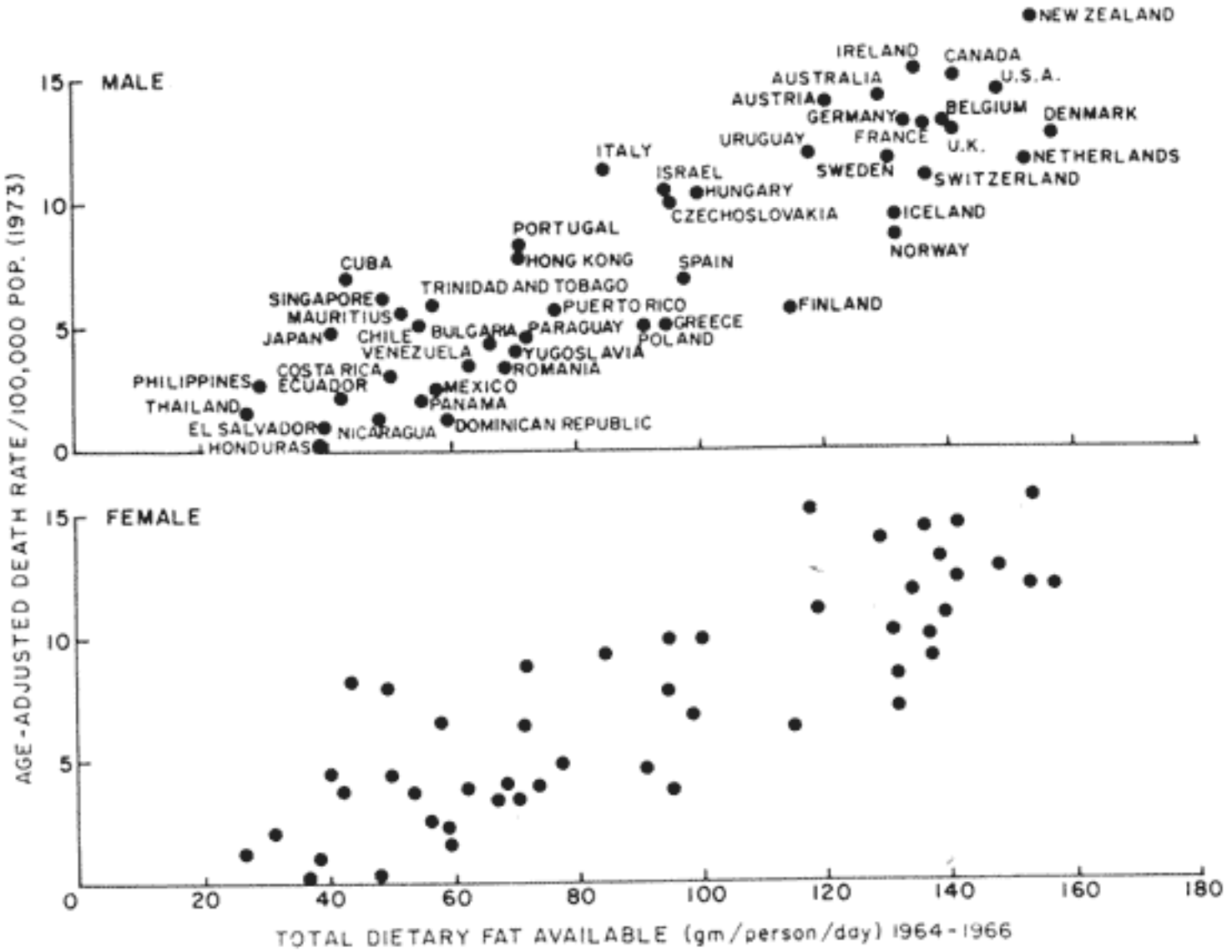

FIG. 2. Correlation between age-adjusted mortality from colon cancer and per capita consumption of fat. (From Carroll and Khor, 1975.)

Gregor *et al.* (1969) analyzed cancer mortality and food consumption data in 28 countries and found a high correlation ($r = 0.81$) between intestinal cancer and animal protein consumption. Dietary fat and fiber were not analyzed. They concluded that their data would be more compatible with a promoter rather than an initiator role for diet during development of the disease. Drasar and Irving (1973) analyzed United Nations Food and Agriculture Organization (FAO) diet data and colon cancer incidence data from 37 countries and showed that the incidence of colon cancer was highly correlated with dietary animal protein and bound fat. These two dietary items are themselves closely associated since much of the bound fat is of animal origin. Enig *et al.* (1978) examined the fat–cancer relationship in the United States and found an equally strong, significant positive correlation of colon cancer with total fat and vegetable fat; but no correlation was found between animal fat

and colon cancer mortality and incidence. These results support a role for total dietary fat in the incidence of colon cancer.

Several investigators have systematically examined correlations between per capita consumption of food items based on FAO data and the incidence and/or mortality of colon cancer (Armstrong and Doll, 1975; Howell, 1975). Armstrong and Doll (1975) have shown that the dietary variables chiefly associated with large-bowel cancer rates were meat and other animal protein. Total fat, meat, and other animal protein are highly correlated. Howell (1975) pointed out that beef consumption was more closely related to colon cancer rates than was pork, poultry, or fish.

Burkitt (1971, 1975) recognized the rarity of large-bowel cancer in most African populations and suggested that countries consuming a natural diet rich in fiber have a low incidence of large-bowel cancer, whereas those eating refined carbohydrates with little fiber have a higher incidence of the disease. It has been argued that large-bowel tumors are related to factors characteristic of modern Western society in which intestinal transit time is decreased, small firm stools produced, and the fecal bacteria flora altered. Slower transit was postulated to allow more time for gut bacteria to degrade intraluminal components and to produce carcinogens, allowing time for such carcinogens to act (Burkitt, 1971, 1975). There is, however, no support for the suggestion that longer transit time results in an increase in the degradation of substrates by gut bacteria (Walters *et al.*, 1975). A recent study comparing populations in Kuopio, Finland (low-risk) and in Copenhagen, Denmark (high-risk) (IARC Microecology Group, 1977), indicated that transit time and stool weight had few significant correlations with diet and defecation habits, but stool weights were higher in the population from Kuopio. Recent data also suggest that one of the factors contributing to the low-risk of large-bowel cancer in Kuopio appears to be that high dietary fiber intake leads to increased stool bulk, in effect diluting tumorigenic compounds in the colon (Reddy *et al.*, 1978a). From these data, we can postulate a possible protective role of dietary fiber in the pathogenesis of large-bowel cancer in man.

The question also arises of whether inhibitors present in the environment affect the response of humans to tumorigenic compounds. Several epidemiologic studies indicate an increased incidence of large-bowel cancer in man in geographic regions where selenium is deficient (Jansson *et al.*, 1975; Shamberger and Willis, 1971). Shamberger and Willis (1971) also associated the amount of selenium in soil and forage crops and cancer mortality rates in U.S. and Canada and noted an inverse relationship between blood selenium levels and cancer mortality rates. Recently,

Schrauzer *et al.* (1977) found that selenium intake at naturally occurring levels varied inversely with breast and colon cancer incidence in many countries. From the apparent dietary selenium intake, estimates of mortality from breast and colon cancer in various countries were calculated. A close fit was observed between the calculated and observed values for these countries.

2. *Case-Control Studies*

Studies attempting to explain the frequency of large-bowel cancer have used both correlation and case-control studies. Wynder *et al.* (1969) conducted a large-scale retrospective study on large-bowel cancer patients in Japan, which suggested a correlation between the Westernization of the Japanese diet and colon cancer. In an earlier large-scale retrospective study on large-bowel cancer patients in the United States, it was concluded that except for the established high risk for patients with ulcerative colitis and familial polyposis, no environmental dietary factors could be identified that differed significantly between the control and study populations (Wynder and Shigematsu, 1967). Based on these two studies, it is reasonable to assume that differences in dietary intake, especially those involving Westernization of the diet, could be determined by retrospective techniques such as a study in Japan (Wynder and Shigematsu, 1967). Such data gathering techniques do not, however, appear applicable to the United States where the total intake of various nutrients is now quite similar, except in special religious groups (Wynder, 1975b).

Haenszel *et al.* (1973) demonstrated an association between colon cancer and dietary beef in 179 Hawaiian Japanese cases and 357 Hawaiian Japanese controls. Meat provided a striking example of a change in food practices between Japan and Hawaii; the rise in beef consumption paralleled the increase in colon cancer risk among Japanese migrants.

However, Enstrom (1975a), in matching time trends and socioeconomic, urban–rural, and regional gradients in beef and fat consumption with the corresponding information on bowel cancer, argued that the data with respect to beef and colon cancer were incompatible. Enstrom (1975a,b) also pointed to a somewhat lower risk of colon cancer among Mormons who have no religious proscription against the use of beef. Some of these inconsistencies may be explained by the fact that (1) beef will differ in fat content depending on cattle age, on how long the animals were range-fed, and whether they were grain-fed in a feed lot, and (2) fiber intake may differ between population groups.

In case-control studies in Israel, Modan *et al.* (1975) found that among a large variety of dietary constituents investigated, those that were lowest

in the diets of patients with colon cancer as compared with controls were those containing fiber. Recently, Dales *et al.* (1979) conducted a case-control study of relationships of diet to colon cancer in American blacks and found that significantly more colon cancer patients than controls reported a high saturated fat-low fibrous foods eating pattern, as opposed to a low saturated fat–high fibrous foods diet. Bjelke (1974), who conducted diet interview study of hospitalized cases and controls in Minnesota and in Norway, found less frequent use of vegetables among colorectal cancer patients; and, in Minnesota, particularly less frequent use of cabbage. Another study on a series of cases and controls from Roswell Park Memorial Institute at Buffalo, New York, showed a lower risk of colon cancer for individuals ingesting vegetables such as cabbage, broccoli, and Brussels sprouts (Graham and Mettlin, 1979).

The studies cited lead us to accept diet as a major etiologic factor in large bowel cancer. Diets high in total fat, low in fiber, and high in beef, are associated with an increased incidence of large-bowel cancer in man.

C. Metabolic Epidemiology

Current evidence indicates that colon cancer may stem from the combined action of currently unidentified carcinogens, of cocarcinogens, and of promoting agents (Wynder and Reddy, 1973; Aries *et al.*, 1969; Reddy *et al.*, 1978b).

To explain the relationship between dietary fat and colorectal cancer, it has been hypothesized 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 active in the large bowel (Aries *et al.*, 1969).

Attention has been focused on the possible role of bacteria in altering the structure of colonic steroids. Investigators (Cook *et al.*, 1940; Coombs *et al.*, 1973; Haddow, 1970; Lacassagne *et al.*, 1966; Hill, 1974) have examined the potential carcinogenic activity of certain bile acids because: (1) their overall structure is similar to carcinogenic polycyclic aromatic hydrocarbons (PAH); (2) they may be converted chemically to 3-methylcholanthrene; (3) full aromatization of the bile acid nucleus would yield a carcinogen metabolite based on cyclopentaphenathrene; (4) human gut flora have been shown to achieve partial aromatization of the sterol ring system; and (5) several bile acids induced sarcomas at the site of injection in experimental animals. It may be noted that such microflora-mediated reactions are unlikely to yield polycyclic aromatic hydrocarbons from

bile salts, but are much more likely to yield products that act as colon tumor-promoters or cocarcinogens rather than as complete carcinogens (Reddy *et al.*, 1978b). Thus, a high-fat diet may not only change the composition of bile acids but also modify the activity of gut microflora, which may in turn produce tumor-promoting substances from bile acids in the lumen of the colon (Hill *et al.*, 1971; Reddy and Wynder, 1973; Reddy *et al.*, 1978b).

High intake of dietary fiber of a certain type leads to an increase in stool bulk, thereby diluting carcinogens and promoters (Burkitt, 1975; Reddy *et al.*, 1978a).

Thus, we are concerned with two aspects: the search for carcinogens, and the search for modifying and, in particular, enhancing factors. Until very recently, the question of carcinogens affecting the large bowel could not be approached experimentally. Now, utilizing metabolic techniques and mutagenicity tests, it is experimentally feasible to test whether carcinogens affecting the colon can be isolated, identified, and quantitated.

Investigations in man have also been carried out in several laboratories to determine (1) whether changes in the diet would alter the concentration of fecal bile acids and cholesterol metabolites, and the activity of fecal microflora, and (2) whether fecal constituents differ between high-risk and low-risk populations for colon cancer and between patients with colon cancer, familial polyposis, adenomatous polyps, and ulcerative colitis, and patients with no known large-bowel disorders.

1. *Fecal Constituents of Populations with Diverse Dietary Habits*

Aries *et al.* (1969) assayed fecal samples for anaerobic and aerobic microflora from British and Ugandan subjects, who constituted high- and low-risk groups for large-bowel cancer, respectively. They found that the British had a higher concentration of anaerobes and lower levels of aerobes than the Ugandans. Hill *et al.* (1971) confirmed these results and further observed a correlation between the death rate due to colon cancer and fecal anaerobes and fecal excretion of cholesterol and bile acid metabolites as well as their degradation by the gut flora. In addition, the feces from U.S. and English subjects contained higher levels of nuclear dehydrogenating clostridia (NDC) than subjects from African and Asian countries (Hill, 1974). The implication is that NDC is involved in the production of unsaturated steroids from the bile acid nucleus (Hill, 1974; Hill *et al.*, 1975). Neither Moore and Holdeman (1974, 1975) nor Finegold *et al.* (1975) have seen significant differences in the composition of fecal flora of high-risk Hawaiian Japanese from whom adenomatous polyps had been removed, high-risk healthy Hawaiian Japanese, high-risk North

Americans, and low-risk rural Japanese. Most of the species of bacteria encountered in North American polyp patients were also seen in the feces of Japanese and Africans, and there was little difference in the overall composite distribution of number and type of species in the two types of populations. Finegold *et al.* (1975) studied subjects with colonic polyps and Japanese–American groups with distinctive Japanese and Western diets, and found no significant differences in the distribution of fecal anaerobes.

A recent comparison of vegetarian Seventh-Day Adventists with non-vegetarian Adventists and non-Adventists failed to show impressive differences among the populations, although there were 21 organisms or groups of organisms present with a significantly different frequency in the high-risk group (non-vegetarian) than in the low-risk group (Finegold and Sutter, 1978). Certainly these organisms should be studied for their capacity to produce carcinogens or cocarcinogens from appropriate substrates. [Some of the differences in fecal flora and discrepancies described by various investigators may be an artifact of procedures used to transport specimens from the several study areas to the laboratory for analysis (Moore and Holdeman, 1975).]

Most diets in man exert little effect on the distribution of microflora until the host's intestinal physiology changes in response to diet. The effect of dietary manipulations on fecal microflora profile has been studied by several investigators. Moore and Holdeman (1975) have shown that Americans consuming nothing but rice and tea or coffee for 3 days had relatively minor changes in the composition of fecal bacteria. Similar results were obtained when leafy vegetables replaced the rice, and, again, when only lean beef was consumed (Moore and Holdeman, 1975). In a study by Hentges *et al.* (1977) in which the meat content was altered without modifying the fat content, there was no great change in the fecal bacterial flora. Reddy *et al.* (1975b) compared the effect of a high-fat, mixed Western diet with a low-fat, nonmeat diet. Although differences in bacterial counts between the two diets were not striking, total, as well as some individual anaerobic counts were greater during the mixed Western diet than during the low-fat, nonmeat diet.

The effect of dietary fiber supplementation on fecal flora was studied by Fuchs *et al.* (1976) who showed that total anaerobic counts were increased significantly while the subjects were on the high fiber diet. Draser *et al.* (1976) obtained no significant changes in fecal bacterial flora in subjects consuming a variety of fibers.

It is evident that the identification and quantitation of bacterial species may be unproductive. We are more interested in metabolic and functional differences than in specific identification of fecal bacteria. Of primary

importance in investigating the etiology of large-bowel cancer is an understanding of the influence of dietary constituents on the enzymic (metabolic) activities of gut bacteria, irrespective of species. Various fecal bacterial enzymes, such as β-glucuronidase, 7α-dehydroxylase, cholesterol dehydrogenase, 7α-hydroxysteroid dehydrogenase, and nuclear dehydrogenase reflect not only the metabolic activity of the colonic bacteria but also the functional capabilities of colonic bacteria to produce putative carcinogens in the gut.

Reddy and Wynder (1973) investigated fecal bile acids and neutral sterols, as well as the microbial β-glucuronidase activity, in the feces to assess the degree of microbial activity for the enzymic hydrolysis of various complete conjugates in the large-bowel of various population groups. (Americans on a high-fat mixed Western diet; Seventh-Day Adventists on a mixed Western diet without meat and less fat; Japanese; Chinese Americans consuming Chinese diet; strict vegetarians.) A significant increase in the excretion of total bile acid (deoxycholic acid, lithocholic acid, 12-ketolithocholic acid, 3β,12α-dihydroxy-5β-cholanic acid, 12α-hydroxy-3-keto-5β-cholanic acid, and 3-keto-5β-cholanic acid) and cholesterol metabolites (coprostanol and coprostanone) were found in Americans consuming a high-fat, mixed Western diet compared with other groups (Table III). The fecal bacteria of groups consuming a mixed Western diet also had a higher β-glucuronidase activity. Since many exogenous and endogenous substances, including tumorigenic metabolites, are excreted via bile as glucuronide conjugates, the data might imply that colonic bacteria of high-risk groups are more active in hydrolyzing these conjugates. These differences are related to dietary composition, mainly a high content of total fat in the high-risk group.

Macdonald *et al.* (1978) studied the fecal NAD- and NADP-dependent 7α-hydroxysteroid dehydrogenase (7α-HSDH), which converts hydroxy-bile salts to keto-bile salts, in vegetarian Seventh-Day Adventists and non-Seventh-Day Adventists consuming a mixed Western diet. The activity of fecal 7α-HSDH was lower in Seventh-Day Adventists compared to non-Adventists, suggesting that increased NAD- and NADP-dependent 7α-HSDH are associated with risk of large-bowel cancer. Thus, evidently a high-risk diet can alter the metabolic activity of gut microflora, and this effect may play an active role in the etiology of large-bowel cancer.

Controlled studies comparing a high-meat, high-fat diet with a nonmeat, low-fat diet showed that the former resulted in elevated levels of fecal bile acid and cholesterol metabolites and increased bacterial β-glucuronidase activity (Reddy *et al.*, 1974, 1975b). Our explanation for this difference is that the fecal bile acids and cholesterol metabolites and the activity of fecal microflora are obviously related to dietary composition,

TABLE III

DAILY FECAL EXCRETION OF NEUTRAL STEROLS AND BILE ACIDS IN DIFFERENT POPULATIONS WITH VARIED RISK FOR COLON CANCER[a]

	North Americans on a mixed Western diet (40)[c]	North American vegetarians (12)	North American Seventh-Day Adventists (25)	Japanese (25)	Chinese (25)
Neutral sterols					
Cholesterol	45 ± 10	67 ± 17	60 ± 20	90 ± 20	60 ± 18
Coprostanol	520 ± 75	231 ± 49[b]	201 ± 26[b]	140 ± 25[b]	129 ± 25[b]
Coprostanone	140 ± 79	20 ± 6[b]	20 ± 3[b]	24 ± 6[b]	25 ± 6[b]
Total neutral sterols	705 ± 104	318 ± 53[b]	281 ± 34[b]	254 ± 35[b]	214 ± 36[b]
Bile acids					
Cholic acid	12 ± 4	7 ± 6	8 ± 1	5 ± 2	10 ± 3
Chenodeoxycholic acid	10 ± 3	6 ± 2	6 ± 1	6 ± 2	12 ± 2
Deoxycholic acid	115 ± 18	32 ± 6[b]	30 ± 5[b]	45 ± 5[b]	40 ± 6[b]
Lithocholic acid	90 ± 10	23 ± 5[b]	29 ± 3[b]	32 ± 3[b]	38 ± 4[b]
Other bile acids	48 ± 9	65 ± 10	17 ± 3	10 ± 2	2 ± 1
Total bile acids	275	133 ± 15[b]	90 ± 6[b]	98 ± 6[b]	102 ± 10[b]

[a] Averages ± SEM. Daily fecal excretion expressed in mg/day.

[b] Significantly different from North Americans on a mixed Western diet ($P < 0.5$).

[c] Number of samples.

mainly to a high dietary fat diet. Hentges *et al.* (1977) compared a diet series consisting of a control diet, a high-beef diet, and a meatless diet. There was no increase in the concentrations of bile acids and neutral sterols in the feces of subjects during the high-meat diet. Fat and fiber were essentially the same in these diets. These data indicate that high animal protein consumption produces no major effect on steroid composition of feces. Hill (1977), after review of several studies, also concluded that it is the fat, not the protein composition, in the meat that determines the effects on the fecal steroids.

Recently, Reddy *et al.* (1978a) have undertaken a study of healthy controls in Kuopio (Finland), a low-risk population for the development of colon cancer. The dietary histories indicate that the total fat consumption is quite similar to the United States populations, but the major part of fat comes from milk and other dairy products, whereas in the U.S. the major source of fat is meat. The voluntary intake of meat in Kuopio is low, and complex cereal consumption is very high in comparison to the United States (Table IV). The daily output of feces is 3-fold higher in healthy controls from Kuopio than in the New York Metropolitan area. The concentration of fecal secondary bile acids, mainly deoxycholic acid and lithocholic acid, as well as β-glucuronidase activity is decreased in Kuopio, but the daily output remained the same in the two groups because of a 3-fold increase in the daily output of feces in Kuopio. The concentration of secondary bile acids was lower, indicating that fecal

TABLE IV

DIETARY INTAKE OF VARIOUS NUTRIENTS AND FECAL EXCRETION OF VARIOUS CONSTITUENTS IN MIDDLE-AGED MALE VOLUNTEERS FROM KUOPIO (FINLAND) AND NEW YORK METROPOLITAN AREA[a]

	Kuopio (15)[b]	New York (40)
Dietary constituents		
Total protein	93 ± 4	89 ± 2
Total fat	110 ± 4	115 ± 3
Saturated fat	59 ± 3	49 ± 2
Other fats	51 ± 3	66 ± 2
Carbohydrates	320 ± 4	285 ± 4
Total fiber	32 ± 3	14 ± 2
Fecal constituents		
Fresh feces excreted	277 ± 20	76 ± 12
Fiber	26 ± 2	9 ± 1
Fecal dry matter	61 ± 8	22 ± 1

[a] Averages ± SEM. Units: gm/day.

[b] Number of samples.

TABLE V
FECAL BILE ACIDS OF HEALTHY MALE SUBJECTS FROM KUOPIO (FINLAND) AND NEW YORK METROPOLITAN AREA

Bile acids	Bile acids: fecal material (mg/gm)		Daily excretion (mg/day)	
	Kuopio (15)[c]	New York (20)	Kuopio (15)	New York (20)
Cholic acid	0.20 ± 0.06[a]	0.24 ± 0.04	12 ± 2.9	6 ± 1.4
Chenodeoxycholic acid	0.13 ± 0.03	0.23 ± 0.03	8 ± 1.3	5 ± 1.1
Deoxycholic acid	1.72 ± 0.16[b]	3.74 ± 0.26	104 ± 12	88 ± 5.1
Lithocholic acid	1.40 ± 0.16[b]	3.27 ± 0.15	84 ± 5	77 ± 4.5
Ursodeoxycholic acid	0.08 ± 0.02[b]	0.13 ± 0.01	5 ± 1.1	3 ± 0.3
3α,β,12α-Trihydroxy-5β-cholanic acid	0.04 ± 0.01[b]	9.12 ± 0.01	2 ± 0.8	3 ± 0.3
12-Ketolithocholic acid	0.06 ± 0.02[b]	0.13 ± 0.01	4 ± 1.0	3 ± 0.2
Other bile acids	0.93 ± 0.08[b]	3.8 ± 0.26	56 ± 5.0[b]	89 ± 6.0
Total bile acids	4.59 ± 0.42[b]	11.7 ± 0.54	277 ± 22	275 ± 14

[a] Averages ± SEM.
[b] Significantly different from New York: $P < 0.05$, or better.
[c] Number of samples.

bulk diluted the fecal secondary bile acids (Table V) and fecal bacterial β-glucuronidase activity. This suggests that increased fecal bulk would dilute any suspected carcinogens or promoters in direct contact with the large bowel mucosa.

2. *Fecal Constituents of Patients with Colon Cancer and Adenomatous Polyps*

In a case-control study, 80% of large-bowel cancer patients had fecal bile acid levels above an arbitrary cut-off level compared with only 17% of the controls; 80% of the cancer patients had fecal NDC compared with 43% of the comparable controls; whereas 70% of the cancer patients had a combination of high NDC and fecal bile acid levels compared with only 9% of the controls (Hill *et al.*, 1975). These findings require confirmation in prospective studies, since the presence of cancer may have altered bacterial metabolism in the gut.

We have carried out a study of fecal constituents of patients with colon cancer and nonhereditary adenomatous polyps (Reddy and Wynder, 1977). The fecal excretion of cholesterol metabolites and secondary bile acids (deoxycholic acid, lithocholic acid, and other microbially modified bile acids) was higher in patients with colon cancer or adenomatous polyps compared to healthy controls (Table VI). The total bile acid and

TABLE VI
FECAL NEUTRAL STEROLS AND BILE ACIDS EXCRETION IN PATIENTS AND IN HEALTHY CONTROLS CONSUMING A MIXED WESTERN DIET

	Patients with colon cancer (50)[c]	Patients with adenomatous polyps (15)	Healthy controls (65)
Neutral sterols			
Cholesterol	10.4 ± 1.4[a,b]	6.4 ± 0.8[b]	3.0 ± 0.2
Coprostanol	22.8 ± 2.3[b]	19.6 ± 3.2[b]	12.8 ± 1.1
Coprostanone	3.9 ± 0.3[b]	4.0 ± 1.0[b]	2.2 ± 0.2
Total	37.1 ± 2.9[b]	30.0 ± 3.1[b]	18.0 ± 0.9
Bile acids			
Cholic acid	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Chenodeoxycholic acid	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
Deoxycholic acid	7.2 ± 0.5[b]	6.1 ± 0.7[b]	3.3 ± 0.3
Lithocholic acid	6.9 ± 0.4[b]	5.4 ± 0.5[b]	3.0 ± 0.2
3α,β,12α-Trihydroxy-5β-cholanic acid	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
12-Ketolithocholic acid	0.8 ± 0.1	0.9 ± 0.1	0.2 ± 0.1
Ursodeoxycholic acid	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Other bile acids	2.2 ± 0.2	2.4 ± 0.3	2.3 ± 0.2
Total bile acids	18.6 ± 0.9[b]	16.3 ± 0.7[b]	10.3 ± 0.8

[a] Averages ± SEM.
[b] Significantly different from healthy controls: $P < 0.05$.
[c] Number of samples.

neutral sterol excretion was also higher in the above patients compared to controls.

Our results also indicate that the activity of fecal bacterial 7α-dehydroxylase, which converts primary bile acids to secondary bile acids, was higher in patients with colon cancer or polyps than controls (Mastromarino *et al.*, 1978). These data support the concept that patients with colon cancer or polyps are more able to convert primary bile acids into secondary bile acids in colonic contents than are the controls. The fecal bacteria of patients with colon cancer or polyps also contained higher cholesterol dehydrogenase activity, which converts cholesterol to coprostanol in colonic contents. Macdonald *et al.* (1978) have demonstrated that the fecal bacterial NAD- and NADP-dependent 7α-hydroxysteroid dehydrogenase activities are higher in large-bowel cancer patients as compared to controls.

An association has been established linking colon cancer to higher dietary fat/meat and low dietary fiber, the metabolic activity of fecal flora, and fecal neutral sterol and bile acid metabolites. The extent to

which findings on bacterial enzymes may be used as indicators or diagnostic markers for high- and low-risk populations, as well as patients with colon cancer, requires further study—studies that should concentrate on identifying and validating such bacterial and/or biochemical indicators.

D. Experimental Studies

Research on the mechanisms of cancer causation in the large bowel has been assisted by the discovery over the last 20 years of several animal models that mirror the type of lesions seen in man. These models are: (1) induction of large-bowel cancer in rats through chemicals such as 3-methyl-4-aminobiphenyl, or 3-methyl-2-naphthylamine; (2) derivatives and analogs of cycasin and methylazoxymethanol (MAM) such as azoxymethane (AOM) and 1,2-dimethylhydrazine (DMH), which work well in rats and mice of select strains; (3) intrarectal administration of direct-acting carcinogens of the type of alkynitrosoureas, such as methylnitrosourea (MNU) or *N*-methyl-*N'*-nitrosoguanidine (MNNG), which lead to cancer of the descending large bowel in every species tested so far; and (4) the oral administration of large doses of 3-methylcholanthrene, which leads to large-bowel cancer in select strains of hamsters (Bralow and Weisburger, 1976).

1. *Effect of Dietary Fat in Colon Carcinogenesis*

Experimental studies have lent some additional support to the possible role of dietary fat in the induction of large-bowel cancer in man. Nigro *et al.* (1975) induced intestinal tumors in rats by AOM and compared animals fed a high beef fat diet and those fed a normal diet. Animals fed a high-fat diet developed more intestinal tumors and more metastasis than the rats fed a low-fat diet.

Inasmuch as humans in various populations usually follow comparable dietary regimens over generations, Reddy *et al.*, (1976a) designed experiments in which animals were exposed to a given regimen for two generations prior to treatment with a carcinogen. Virgin female rats fed diets containing 5% corn oil, 20% corn oil, 5% lard, or 20% lard were bred, and the litters were weaned to the same diet consumed by the mothers. At 7 weeks of age, all second generation animals, except controls received 20 weekly subcutaneous (sc) doses of DMH (10 mg/kg body weight). Animals fed 20% lard or 20% corn oil were more susceptible to colon tumor induction by DMH than those in other groups (Table VII).

TABLE VII
COLON TUMOR INCIDENCE IN RATS FED DIETS HIGH IN FAT AND TREATED WITH CARCINOGENS

Diet fat	Percentage in diet	Protein	Percentage in diet	Carcinogen	Percentage of rats with colon tumors
Lard	5	Casein	25	DMH[a]	17
Lard	20		25	DMH[a]	67
Corn oil	5		25	DMH[a]	36
Corn oil	20		25	DMH[a]	64
Beef fat	24	Beef protein	40	DMH[a]	57
Beef fat	6	Beef protein	19	DMH[a]	35
Corn oil	24	Soybean protein	40	DMH[a]	54
Corn oil	6	Soybean protein	19	DMH[a]	35
Beef fat	20	Casein	22	DMH[b]	60
Beef fat	5		22	DMH[b]	27
Beef fat	20		22	MNU[c]	73
Beef fat	5		22	MNU[c]	33
Beef fat	20		22	MAM acetate[d]	80
Beef fat	5		22	MAM acetate[d]	45

[a] Female F344 rats, at 7 weeks of age, were given weekly sc DMH at a dose rate of 10 mg/kg body weight for 20 weeks and autopsied 10 weeks later.

[b] Male F344 rats, at 7 weeks of age, were given a single dose of sc DMH, 150 mg/kg body weight and autopsied 30 weeks later.

[c] Male F344 rats, at 7 weeks of age, were given ir MNU, 2.5 mg/rat twice in one week and autopised 30 weeks later.

[d] Male F344 rats, at 7 weeks of age, were given ip MAM acetate, 35 mg/kg body weight once and autopsied 30 weeks later.

The type of fat appears to be immaterial at the 20% level, although at the 5% fat level, there is a suggestion that unsaturated fat (corn oil) predisposes to more DMH-induced colon tumors than saturated fat (lard). Combinations of high beef protein (40%) and high beef fat (20%) or high soybean protein (40%) and high corn oil (20%) led to more DMH-induced colon tumors in F344 rats than control diets of beef protein (20%) and low beef fat (6%), or soybean protein (20%) and low corn oil content (6%) (Table VII; Reddy *et al.*, 1976b).

Further, F344 rats fed a diet containing 20% beef fat and treated intraperitoneally with MAM acetate, subcutaneously with DMH, or intrarectally with MNU, had a greater incidence of colon tumors than did rats fed a diet containing 5% beef fat and treated similarly (Reddy *et al.*, 1977b) (Table VII). W/Fu rats fed a 30% lard diet had an increased number of DMH-induced large-bowel tumors compared to the animals fed the standard diet (Bansal *et al.*, 1978). Broitman *et al.* (1977) showed

that rats fed a 20% safflower oil diet had more DMH-induced large-bowel tumors than those animals fed either the 5% or 20% coconut oil diets. However, these studies provide no evidence that dietary polyunsaturated fat per se is more effective than saturated fat in augmenting tumorigenesis by DMH. Rogers *et al.* (1973) found that a diet, marginally deficient in lipotropes but high in fat, enhanced DMH-induced colon carcinogenesis in Sprague–Dawley rats. These results suggest that the total dietary fat, rather than the type or source of fat, may have a function in the pathogenesis of colon cancer.

In order to understand the specifics of the mechanisms whereby dietary fat influences colon cancer, the effect of high dietary fat on biliary and fecal bile acid pattern was investigated. Biliary excretion of total bile acids, as well as cholic acid, β-muricholic acid, ursodeoxycholic acid, and deoxycholic acid, was higher in rats fed a diet containing 20% corn oil or 20% lard than in rats fed diets containing 5% corn oil or 5% lard. High fat (corn oil or lard at 20% level) intake was associated with an increased excretion of fecal neutral sterols and bile acids. The excretion of deoxycholic acid, lithocholic acid, and 12-ketolithocholic acid was increased in rats fed high-fat diets (Reddy *et al.*, 1977a) (Table VIII).

Recent studies indicate that the enhanced tumorigenesis in the animals

TABLE VIII

EFFECT OF TYPE AND AMOUNT OF DIETARY FAT ON FECAL BILE ACIDS IN RATS[a]

	5% Corn oil control (8)	20% Corn oil control (8)	5% Lard control (8)	20% Lard control (8)
Cholic acid	0.68 ± 0.08[b,1]	0.64 ± 0;07[1]	0.74 ± 0.06[1]	0.86 ± 0.10[1]
β-Muricholic acid	0.82 ± 0.05[1]	0.98 ± 0.08[1]	0.80 ± 0.07[1]	0.88 ± 0.11[1]
3α,β,12α-Trihydroxy-5β-cholanic acid	0.11 ± 0.02[1]	0.10 ± 0.01[1]	0.10 ± 0.01[1]	0.13 ± 0.01[1]
Chenodeoxycholic acid	0.12 ± 0.01[1]	0.15 ± 0.01[1]	0.13 ± 0.02[1]	0.16 ± 0.03[1]
Hyodeoxcholic acid	2.76 ± 0.12[1]	2.73 ± 0.16[1]	3.14 ± 0.18[1]	2.73 ± 0.17[1]
Ursodeoxycholic acid	0.10 ± 0.2[1]	0.10 ± 0.02[1]	0.15 ± 0.09[1]	0.08 ± 0.01[1]
Deoxycholic acid	2.53 ± 0.18[1]	4.80 ± 0.23[2]	2.61 ± 0.20[1]	4.54 ± 0.30[2]
Lithocholic acid	0.83 ± 0.11[1]	1.98 ± 0.16[2]	1.00 ± 0.10[1]	2.84 ± 0.13[2]
12-Ketolithocholic acid	0.44 ± 0.03[1]	0.77 ± 0.07[2]	0.51 ± 0.18[1]	0.77 ± 0.02[2]
7-Ketodeoxycholic acid	0.14 ± 0.02[1]	0.08 ± 0.01[1]	0.16 ± 0.02[1]	0.06 ± 0.01[1]
Other bile acids	1.93 ± 0.10	2.52 ± 0.25	1.92 ± 0.16	2.51 ± 0.19
Total bile acids	10.45 ± 0.20[1]	14.86 ± 0.41[2]	11.24 ± 0.49[1]	14.91 ± 0.62[2]

[a] Mean ± SEM. Units: mg/day/kg body weight.

[b] Mean with a common number superscript between groups in a horizontal row are not significant: $P > 0.05$.

fed the high-fat diet is due to promotional effects rather than alterations in carcinogen metabolism (Bull *et al.*, 1979). While there is no human model for tumor promotion by high dietary fat, the above results in an animal model suggest that colon tumor promotion results through a mechanism involving increased colonic bile acid content.

Since the intestinal bacteria contain many inducible enzymes, experiments were conducted to delineate the effects of various dietary factors on the metabolic activity of intestinal microflora in order to understand the relationship of colon cancer to diet-mediated changes in the intestinal bacteria. Goldin and Gorbach (1977) reported that rats fed a meat diet had higher levels of fecal bacterial β-glucuronidase, azoreductase, and nitroreductase activities than did grain-fed rats. After confirming these studies, Reddy *et al.* (1977b) extended the observation that not only a meat diet, but also a high-fat diet or high-protein high-fat diet, changes the bacterial β-glucuronidase activity in the large intestine.

In an investigation of the effect of *Lactobacillus acidophilus* feeding on rat fecal enzymes, Goldin and Gorbach (1977) found that supplemental feeding of *L. acidophilus* significantly lowered the activity of fecal β-glucuronidase, nitroreductase, and azoreductase in rats consuming a meat diet. The ability of *L. acidophilus* to reduce activities of these enzymes in rats fed a meat diet is of great interest, although its significance to carcinogenesis remains unestablished. Although it is premature to conclude from these studies that factors altering microflora enzymes have an effect on tumor formation in the large bowel, these changes in metabolic activity of microflora might alter the biological activity, toxicity, excretion, and reabsorption of many of the endogenous and exogenous compounds such as carcinogens and/or cocarcinogen metabolites.

2. *Effect of Dietary Protein in Colon Carcinogenesis*

The possible role of dietary protein in DMH-induced colon carcinogenesis in Sprague-Dawley rats was studied by Visek *et al.* (1978). Protein source as a modifying factor in colon carcinogenesis was studied, using semi-purified diets containing 20% protein as freeze-dried raw beef, charcoal-broiled beef or soybean protein, and 20% beef fat. It was concluded that the source of protein was not a factor in the DMH-induced colon carcinogenesis in rats. In another study, rats fed 15% and 22.5% protein had a greater number of DMH-induced intestinal tumors than those fed 7.5% protein (Topping and Visek, 1976). However, the percentage of animals with colon tumors was not significantly different between various dietary groups. Whether the reduced number of tumors in rats fed 7.5% protein diet was due to suboptimal protein intake during the period of

rapid body growth could not be answered from this study, although the evidence seems compatible with the conclusion that the time of appearance of tumors and their size and number was influenced by protein intake (Topping and Visek, 1976).

3. *Effect of Fiber in Colon Carcinogenesis*

It has been postulated that the protective effect of dietary fiber may be due to adsorption, dilution, and/or metabolism of cocarcinogens, promoters, and unidentified carcinogens by the components of the fiber. There is evidence that alfalfa, wheat straw, and some other fibers can bind considerable amount of bile acids (colon tumor promoters) *in vitro* (Kritchevsky and Story, 1974; Story and Kritchevsky, 1978; Kay, 1978). On the other hand, wheat bran, oat hulls, and synthetic fibers only bind negligible amounts of bile acids *in vitro*. This indicates that the different types of nonnutritive fibers possess specific binding properties. Dietary fiber could also affect the enterohepatic circulation of bile salts (Kern *et al.*, 1978). Fiber not only influences bile acid metabolism, thereby reducing the formation of potential tumor promoters in colon carcinogenesis, but also exerts a solvent-like effect in that it dilutes potential carcinogens and/or cocarcinogens by its bulking effect and ability to bind water, sterols, bile acids, and fat. Fiber may also influence the gut flora and cause decreased bacterial degradation of intraluminal carcinogens and/or carcinogens.

Although the concept of fiber involvement in colon carcinogenesis is simple, attractive, and appears firmly based on logic, the data are often contradictory and confusing. The reasons for this discrepancy may be that the fiber terminology has been generally incorrectly used. Also, experimental design has failed to take into account the possible subtle effect of inhibitors, especially in relation to the promoting process. In evaluating the biological function of dietary fiber, information regarding the nature of the fiber often has been incomplete. Dietary fiber has been defined as that part of plant material taken in our diet that is resistent to the digestion by secretions of the gastrointestinal tract and that comprises a heterogeneous group of carbohydrates, including cellulose, hemicellulose, pectin, and a noncarbohydrate substance, lignin.

Fibers can be generally classified into three groups according to Van Soest (1978): (1) vegetable fibers, which are highly fermentable with low indigestible residue; (2) brans, which are less fermentable; and (3) chemically purified fibers such as feed cellulose, which are relatively unfermentable. A class of soluble substances including pectins and gums may not be true fibers, but are considered part of the dietary fiber complex

because of the similar effects they can elicit in the diet. Dietary fiber fractions of wheat bran consist principally of hemicellulose and smaller amounts of lignin and cellulose, whereas the dietary fiber fractions of vegetable and fruit fibers have a different percentage composition of hemicellulose lignin, and cellulose.

The relationship between dietary fiber consumption and colon cancer has been studied in animal models. Sprague-Dawley rats fed a diet containing 20% corn oil or beef fat and 20% wheat bran had fewer DMH-induced colon tumors than those on a control diet containing 20% fat and no bran (Wilson *et al.*, 1977). No differences in the incidence of colon cancer were found between rats fed corn oil and those fed beef fat. Recently, Freeman *et al.* (1978) compared DMH-induced colon tumor incidence in Sprague-Dawley rats fed either a fiber-free diet or a 4.5% purified cellulose diet. Cellulose ingestion was associated with a reduced number of animals with colonic neoplasia, as well as a reduction in the total number of colon tumors. In addition, this protective effect appears to be time dependent and associated with a shift in tumor distribution from the proximal colon to a more distal site (Freeman *et al.*, 1978). Although the precise mechanism for this apparent redistribution of the site of the tumors within the colon remains obscure, some change in the luminal physiochemical environment from proximal to distal colon or some inherent difference between the colonic mucosa itself from these two sites may be responsible for the observed differences.

A recent study of Fleiszer *et al.* (1978) indicates that the incidence of DMH-induced colon tumor in rats reduces as dietary fiber increases. In this study, some reduction in tumor incidence in the high-fiber group might be expected on the basis of reduced caloric intake. However, the data suggest that reduction of caloric intake alone cannot account for the significant protective effect of dietary bran. In another study, Cruse *et al.* (1978) reported that a diet containing 20% wheat bran had no effect on DMH-induced colon carcinogenesis in rats. However, the DMH dose levels in this experiment were so high that any protective effect of bran might have been unobservable. (One important concern in a study of the effect of diet on chemical carcinogenesis is to avoid too high a level of carcinogen for a prolonged period, as this may obscure more subtle changes induced by certain dietary modifications.) In fact, the data presented by Cruse *et al.* (1978) suggest that a high-fiber diet reduces the number of DMH-induced early deaths in rats (Lowenfels, 1979).

The effect of dietary alfalfa, pectin, and wheat bran at a 15% level on colon carcinogenesis by AOM or MNU was studied in F344 rats (Watanabe *et al.*, 1979). The animals fed the alfalfa diet and treated with MNU had a higher incidence of colon tumors than did those fed the control

diet containing only 5% alphacel or diets containing pectin or wheat bran (Table IX). There was no difference in MNU-induced colon carcinogenesis in rats fed diets containing pectin or wheat bran. The AOM-induced colon carcinogenesis in rats fed diets containing pectin or wheat bran was lower than that in rats fed the control diet or alfalfa diet (Table IX).

The effect of alfalfa, wheat bran, and cellulose on AOM-induced intestinal tumor incidence was further studied in Sprague-Dawley rats fed diets containing 10% fiber and 35% beef fat or 20 or 30% fiber and about 6% beef fat (Nigro *et al.*, 1979). The addition of 10% fiber to the high-fat diet did not reduce the intestinal tumor frequency. Apparently, the challenge of AOM with the high dietary fat was too great to be affected by the dietary fiber. The addition of 20% bran or cellulose or 30% of any fiber to the 6% fat diet significantly reduced the intestinal tumor frequency. In the proximal half of the large bowel, all dietary groups except the 20% alfalfa group showed a reduction in tumor frequency compared to the fiber-free groups. The concentration, but not the total daily excretion of fecal steroids, was significantly lowered in the groups with reduced tumor frequencies.

Bauer *et al.* (1979) have indicated that the protective effect of dietary fiber in colon carcinogenesis is probably at the promotional stage rather than at the initiating period. Groups of rats were fed a fiber-free diet or diets containing 20% wheat bran, 20% carrot fiber, or 6.5% citrus pectin from 3 days prior to the first DMH administration until 14 days after the last injection. They were then transferred to a standard rat pellet diet for about 10–12 weeks. There was no difference in the incidence of colorectal tumors among the groups fed a fiber-free diet and diets containing wheat

TABLE IX
COLON TUMOR INCIDENCE IN FEMALE F344 RATS FED DIETS CONTAINING PECTIN, ALFALFA, OR WHEAT BRAN AND TREATED WITH AZOXYMETHANE OR METHYLNITROSOUREA

Diet	Percentage of animals with colon tumors	
	Azoxymethane treated	Methylnitrosourea treated
Control	57	69
Pectin	10[a]	59
Alfalfa	53	83[b]
Wheat bran	33[a]	60

[a] Significantly different from the groups fed the control diet or alfalfa diet by χ^2 test; $P < 0.05$.

[b] Significantly different from the other groups; $P < 0.05$.

bran or carrot fiber. However, it is possible in this study that the high tumor yield resulting from large doses of DMH failed to show any protective effect of dietary fibers. In addition, these results (Bauer *et al.*, 1979) and those of others (Watanabe *et al.*, 1979; Nigro *et al.*, 1979; Wilson *et al.*, 1977; Fleiszer *et al.*, 1978) suggested that continuous feeding of high-fiber diets had a protective effect on colon carcinogenesis, whereas transferring from high-fiber diet to low-fiber diet during postcarcinogen treatment resulted in no observable effect. These observations suggest that dietary fiber exerts a protective effect on tumorigenesis during the promotional phase.

These results also indicate that the protective effect of dietary fiber in colon carcinogenesis depends on the source of fiber and type of carcinogen. The inhibition of tumor formation by dietary fiber may be due to the dilution of promoters in the lumen of the large intestine by the additional bulk. The protective effect of various fibers also depends on their capacity to bind bile acids in the intestinal tract, as well as their effect on colonic mucosa and indirect effects on the metabolism of the carcinogen. Although additional studies are warranted to elucidate the protective effect of various fibers in colon carcinogenesis, the human data and animal experiments suggest that increased intake of cereal fibers would, at least in part, reduce the risk for large-bowel cancer.

4. *Bile Acids in Colon Carcinogenesis*

The role of bile acids in colon carcinogenesis has received some support from studies in animal models. Nigro *et al.* (1973) observed that feeding of nonabsorbable resin, cholestyramine, which increases bile salt excretion, enhanced azoxymethane-induced colon tumors in rats. Although this effect was mediated through an increased fecal bile salt excretion, one could not exclude the possibility of some direct effect of cholestyramine itself in stimulating cell duplication. In another study, the carcinogenic effect of AOM in rats was increased by surgically diverting bile to the middle of the small intestine, which also raised the fecal excretion of bile salts (Chomchai *et al.*, 1974). In an experiment pertinent to this area, B. S. Reddy (unpublished observations) has observed that cholestyramine-bound taurocholic acid or taurochenodeoxycholic acid, when incubated with mixed fecal cultures isolated from human stool, are deconjugated and further modified to free bile acids.

The evidence of the importance of bile acids as colon tumor promoters came from studies by Narisawa *et al.* (1974) and Reddy *et al.* (1976c, 1977d; Reddy and Watanabe, 1979) (Table X). The development of adenomas significantly increased among those conventional rats initiated

TABLE X
COLON TUMOR INCIDENCE IN GERMFREE AND CONVENTIONAL RATS TREATED WITH INTRARECTAL MNNG AND/OR BILE ACIDS

	Animals with tumors (percent)	Tumors per rat		
		Total	Adenocarcinoma	Adenoma
Germfree				
CA (10)[a,b]	0	0	0	0
CDC (10)	0	0	0	0
LC (10)	0	0	0	0
MNNG (22)	27	0.27	0.14	0.13
MNNG + CA (24)	50	0.63	0.29	0.34
MNNG + CDC (24)	54	1.08	0.29	0.79
MNNG + LC (24)	71[c]	1.04	0.33	0.71
Conventional				
CA (12)	0	0	0	0
CDC (12)	0	0	0	0
LC (12)	0	0	0	0
MNNG (30)	37	0.55	0.23	0.32
MNNG + CA (30)	67[c]	0.87	0.24	0.63
MNNG + CDA (30)	70[c]	1.23	0.27	0.96
MNNG + LC (24)	83[c]	1.83	0.33	1.50

[a] CA, cholic acid; CDC, chenodeoxycholic acid; LC, lithocholic acid. Number of rats are shown in parentheses.

[b] CA, CDC, or LC group received intrarectally 20 mg of sodium salt of respective bile acid three times weekly for 48 weeks; MNNG group received intrarectally 2 mg of MNNG twice a week for two weeks followed by vehicle for 46 weeks; MNNG + CA, MNNG + LC, or MNNG + CDC group received intrarectally MNNG for two weeks and bile acid thereafter for 16 weeks.

[c] Significantly different from rats given MNNG alone by χ^2 test, $P < 0.05$.

with limited amounts of intrarectal MNNG to give a definite low yield of colon cancer and administered with intrarectal lithocholic acid or taurodeoxycholic acid as promoters compared to the group that was given only the carcinogen. Deoxycholic acid applied topically to the colon increased MNNG-induced colon adenocarcinomas in germfree rats. The bile acids themselves did not produce any tumors.

A recent study also indicates that the primary bile acids, cholic acid and chenodeoxycholic acid, also produced a MNNG-induced colon tumor promoting activity in rats (Reddy *et al.*, 1977b). Cholic acid and chenodeoxycholic acid given intrarectally to conventional rats are subjected to bacterial 7α-dehydroxylation to deoxycholic acid and lithocholic acid, respectively. Cohen *et al.* (1979) reported that cholic acid in the diet increased MNU-induced colon carcinogenesis in rats. Total fecal bile

acids, particularly deoxycholic acid output was elevated in animals fed cholic acid compared to controls. This increase in fecal deoxycholic acid was due to bacterial 7α-dehydroxylation of cholic acid in the colonic contents. These studies demonstrate that these secondary bile acids have a promoting effect in colon carcinogenesis.

The mechanism of action of bile acids in colon carcinogenesis has not been elucidated. Bile acids have been shown to affect cell kinetics in the intestinal epithelium, although the structural specificity of this effect has not been examined extensively (Bagheri *et al.*, 1978; Ranken *et al.*, 1971; Roy *et al.*, 1975). In the intestine, the data do not permit a critical distinction between a direct effect of bile acids on cell division and an indirect or physiological stimulus secondary to increased cell loss from sloughing or damage (Bagheri *et al.*, 1978). The cell renewal system is dynamic and may be influenced by changes in a number of factors including the composition of gut microflora (Matsuzawa and Wilson, 1965) and bile acids in the intestine (Meslin *et al.*, 1974). It has been shown that, with proper modification of the microenvironment of the intestinal tract, it is possible to alter the cellular kinetics of the mucosa (Mastromarino and Wilson, 1976). Recently, Cohen *et al.* (1979) reported an enhanced colonic cell proliferation in rats fed cholic acid, as well as in animals treated with intrarectal MNU. This increased cell population involved in DNA synthesis induced by cholic acid feeding would favor the expression of damage at a far higher level than with the carcinogen, MNU, alone, bringing about not only a greater overall incidence of MNU-induced colon tumors, but also an enhanced number of tumors in rats fed cholic acid. Lipkin (1975) demonstrated that, during neoplastic transformation of colonic cells, a similar sequence of changes leading to uncontrolled proliferative activity develops in colon cancer in humans and in rodents given a chemical carcinogen that induces colon cancer. Irrespective of the mechanism by which bile acids enhance cell proliferation and/or decrease the generation time of proliferating cells, the phenomenon may have important implications for colon carcinogenesis.

5. *Mutagens (or Presumptive Carcinogens)*

Until recently, the nature of the carcinogens responsible for colon cancer, as well as for other important types of cancer, was obscure. The hypothesis has been put forward that the dietary and body fat acts as a reservoir for environmental contaminants such as polycyclic aromatic hydrocarbons, PCB, PBB, and DDT. None of these contaminants have ever induced cancer in animal models in the colon, breast, or prostate.

An important clue to the nature of carcinogens came from the studies

of Nagao *et al.* (1977a,b) who demonstrated the presence of mutagens (presumptive carcinogens) in the charred surface of beef and fish. They speculated that this activity was the result of pyrolysis of the proteins and subsequently demonstrated mutagenicity of protein pyrolysates. Pyrolysis of individual amino acids was carried out, and the pyrolysate of tryptophan had the greatest mutagenic activity (Matsumoto *et al.*, 1977). The active principle in this pyrolysate was a γ-carboline derivative, a heterocyclic *o*-methylarylamine (Sugimura *et al.*, 1977). Other *o*-methylarylamines (i.e., 3,2′-dimethyl-4-aminobiphenyl) have been shown to produce colon cancer in male and female, and breast cancer in female animals (Reddy and Watanabe, 1978). In view of the well established connection between mutagenic activity and carcinogenic activity, and the known properties of certain *o*-methylarylamines, especially in causing colon cancer in rats, it is possible that broiling and frying of meat may lead to carcinogens responsible for cancer of the colon (Weisburger and Spingarn, 1979). However, it is important to determine specifically whether such derivatives resulting from broiling and frying of meat can induce cancer in epithelial tissues, such as colon or breast.

Recently, Commoner *et al.* (1978) observed the formation of mutagenic activity after frying hamburgers. They also investigated the temperature dependence of this phenomenon and found a sharp rise in mutagen formation between 140 and 180°C (Dolara *et al.*, 1979). Spingarn and Weisburger (1979), however, have shown that substantial mutagenic activity is formed whether the meat is fried, broiled, or boiled, despite the finding that surface temperatures of broiled patties do not exceed 130°C.

The hypothesis that browning reactions, which are desirable for producing the flavor and aroma of cooked foods, are responsible for the mutagenic activity, has been investigated in a model system. The reactions between sugars and amines have long been used to investigate the reaction products in browning (Hodges, 1967), and these reactions have now been shown to produce mutagenic activity as well (Weisburger and Spingarn, 1979). The ubiquitous occurrence of browning reactions in cooking suggests that these mutagens are probably not restricted to cooked meats (Weisburger *et al.*, 1980).

Recently Bruce *et al.* (1977) found in the stools of some individuals mutagenic substances that they thought were *N*-nitroso compounds that might be responsible for colon cancer. It will be important to determine whether this mutagen stems from the metabolism of a mutagen from fried meats or whether it is derived from other precursors such as through a nitroso exchange reaction (Mandel *et al.*, 1977) or from an as yet unknown pathway, including a nitrosation at the pH prevailing in the large intestine.

Bruce *et al.* (1977) also demonstrated that increased dietary fiber and decreased dietary fat and protein reduced fecal mutagen levels. A marked diminution of mutagen concentration could also be brought about by supplementing diets with either ascorbic acid or α-tocopherol. Autrup *et al.* (1978) demonstrated that human colonic mucosa can activate several types of carcinogens such as *N*-nitrosamines into forms that bind to DNA. Mandel *et al.* (1977) demonstrated that the stools of certain people contained a bacterial enzyme that can transfer a nitroso group from a nitrosamine to an amide with consequent production of what is presumably a direct-acting nitrosamide. More research in this area is necessary to determine the relevance of these findings to colon cancer in man.

6. *Effect of Miscellaneous Dietary Factors in Colon Carcinogenesis*

Cruse *et al.* (1979) proposed that prolonged exposure to dietary cholesterol is cocarcinogenic for human colon cancer, since it facilitates the development, growth, and spread of the disease, and since dietary fats promote the action of several experimental carcinogens. Broitman *et al.* (1977) studied the effect of polyunsaturated fat and cholesterol on colon tumorigenesis and demonstrated that the interaction between dietary polyunsaturated fat and dietary cholesterol and/or tissue cholesterol may promote tumorigenesis compared with dietary saturated fat and cholesterol in the animal model. Our recent studies indicate that cholesterol does not act as a colon tumor promoter in the rat model (Reddy and Watanabe, 1979).

There is considerable evidence to suggest that vitamin A, its synthetic and natural analogs such as retinol, and the esters and ethers of retinol, retinoic acid and synthetic analogs, can influence the development of some epithelial tumors (Sporn *et al.*, 1976). Because vitamin A is necessary for the control of proliferation and the direction of differentiation of many epithelial tissues, its ability to act on colon carcinogenesis has received much attention. Rogers and Newberne (1973) found that hypervitaminosis A did not alter aflatoxin B_1-induced colon tumors in rats. Chronic dietary deficiency of vitamin A slightly increased the incidence of colon tumors in rats receiving DMH by repeated intragastric administration (Rogers *et al.*, 1973, 1974). They also found that high levels of vitamin A in the diet provided little or no protective effect on DMH-induced colon carcinogenesis. However, intrarectal injection of the direct-acting carcinogen, MNNG, gave a large-bowel tumor incidence in vitamin A-deficient animals that was one-half that of vitamin A-supplemented animals (Narisawa *et al.*, 1976). These studies do not address the possibility that retinoic acid or its analogs might show some protective

effect. A recent study by Newberne and Suphakarn (1977) indicates that dietary supplementation with 13-*cis*-retinoic acid reduced the incidence of DMH-induced colon carcinogenesis in rats.

Many synthetic antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole, and disulfiram, studied by Wattenberg (1978), Weisburger *et al.* (1977), and Fiala (1977), provided one of the most consistent examples of inhibition of carcinogenesis. This subject has been reviewed recently by Wattenberg (1978).

BHA is of interest because of its minimal toxicity and its extensive use as an additive in food for human consumption. Experimental studies on the inhibition of colon carcinogenesis by disulfiram, diethyldithiocarbamate, and bisethylxanthogen indicate that these compounds, when added to the diet, inhibit DMH-induced colon carcinogenesis (Wattenberg 1975, 1978). Disulfiram has also been found to inhibit AOM-induced colon carcinogenesis but to a considerably lesser extent than DMH (Wattenberg, 1978). Fiala (1977) demonstrated that carbon disulfide, a metabolite of disulfiram, inhibits the oxidation of DMH and AOM *in vivo*. The data suggest that carbon disulfide may be the chemical species responsible for the inhibitory action of disulfiram and related compounds. When incubated with microsomes, several thiono-sulfur-containing compounds, including disulfiram and diethyldithiocarbamate, produce a decrease in cytochrome P-450 (Hunter and Neal, 1975). This raises the possibility that thiono-sulfur-containing compounds as a group may have the capacity to modify cytochrome P-450 so as to alter the microsomal metabolism of DMH, AOM, and other carcinogens in a manner that decreases their carcinogenicity (Wattenberg, 1978).

There is some experimental evidence in animal models for the cancer inhibitory activity of selenite. Jacobs *et al.* (1977a) reported that 4 ppm of selenium in drinking water inhibited DMH- or MAM acetate-induced colon tumors in rats. Selenium also reduced the mutagenic activity of 2-AAF, N-OH-AAF, and N-OH-AF in the Ames test system (Jacobs *et al.*, 1977b).

The mechanisms by which these inhibitors operate is incompletely understood. In those instances in which information is available, the inhibitors may act by altering the metabolism of the carcinogen (Wattenberg, 1978). An evaluation of the current and potential role that these inhibitors may play is dependent on acquisition of further data on the range of compounds having the capacity to inhibit carcinogenesis and on mechanisms of inhibition.

Despite the voluminous literature that exists on vitamin C, no adequate studies are available concerning the effect of ascorbic acid on the neoplastic process. Vitamin C intake may control the recurrence of large-bowel neoplasia or adenomatous polyps in man after surgical interven-

tion. DeCosse *et al.* (1977) demonstrated that vitamin C given to patients with familial polyposis inhibited the formation of colorectal cancer by totally unknown mechanisms. Recently, Reddy *et al.* (1979) showed that the addition of 0.25 or 1.0% sodium ascorbate in the diet reduced the number of rats developing DMH-induced colon tumors from 26 to 0%.

The above studies, in general, stress the importance of micronutrients such as selenium, vitamin A, and vitamin C as inhibitory factors in colon carcinogenesis. Except for the selenium, none of the other micronutrients have been studied in detail in colon carcinogenesis.

E. Conclusions

In recent years, salient advances have taken place in our knowledge of factors in the etiology of large-bowel cancer in man. Through epidemiology and geographic pathology, we have learned that high fat and lack of fiber in the diet are involved in the genesis of this disease. Other factors indicated to be of importance by experimental observations include various micronutrients and antioxidants such as vitamins A and E, and selenium, although there is no significant epidemiologic basis for an influence of these compounds on colon tumor incidence.

We have begun to appreciate the complexities through which diet translates its action in leading to colon cancer, involving mammalian and microbiologic enzymic, metabolic conversion steps of endogenous, and perhaps unidentified exogenous materials. Laboratory studies have shown the importance of the interaction of a high-fat diet and the production of bile acids as potentially relevant in the etiology of colon cancer. Other studies also indicate that a high intake of dietary fiber, in spite of high dietary fat, not only leads to an increase in stool bulk, thus diluting carcinogenic and/or promoters in the intraluminal contents, but also modifies the metabolism of these intraluminal compounds. These studies thus suggest that both high intake of fat and low intake of fiber may be necessary for the full expression of risk to colon cancer.

Specific animals models have been developed that permit the detailed study of mechanisms. Animal model studies also indicate that amount of dietary fat, rather than type of fat, and certain types of dietary fibers play a role in colon carcinogenesis. More research in collateral areas, such as physiology and biology of colon and associated cell systems, has provided the information that the bile acids in the gut enhance cell proliferation and/or decrease the generating time of proliferating cells, the phenomenon of which may have important implications for colon carcinogenesis.

Reports on the mechanisms of carcinogenesis have placed emphasis on carcinogens. Attempts have been made in various laboratories to isolate

and identify the carcinogens affecting the colon. Some investigators claim that broiling and/or frying of meat and fish, and the browning reactions between sugars and amines, yield mutagens (presumptive carcinogens) responsible for cancer of the colon. Others have reported the presence of mutagenic *N*-nitroso compounds in human feces that might be responsible for colon cancer.

Although many substances are tumorigenic in experimental animals and a lesser number are carcinogenic in man, significant modifying factors enhance the effect of low-dose or low-potency carcinogens that by themselves would not suffice to induce cancers. In some instances, the modifying factor can be readily identified and removed, thereby eliminating or reducing the incidence of certain cancers. This may be difficult with initiating carcinogens. In the case of colon cancer, evidence has been presented that dietary fat exerts a promoting effect on tumorigenesis, whereas certain dietary fiber exerts a protective effect. Therefore, rather than concentrating on specific carcinogens, more attention must be given to modifying factors—cocarcinogens, promoters, and factors that influence the formation of endogenous tumorigenic compounds.

With respect to further research, we need to continue and indeed to expand work in metabolic epidemiology and in laboratory studies to pinpoint the specific agents related to colon carcinogenesis. New approaches to prevention are urgently needed.

However, while such studies are in progress, we think the time has come to make dietary recommendations to the public. Cardiovascular disease prevention has led to recommendations for a reduction in total fat as a prudent measure, to reduce the leading cause of death in the Western world. It would now appear that such a diet is also likely to lead to a reduction of colon cancer, particularly if this diet also includes a high intake of fibers. A reduction in the portion of total fat in average caloric intake, from 40% to about 25–30%, and a generous intake of dietary fiber in the form of whole-grain breads and cereals should be considered. Diet should be well balanced, including ample fresh fruits and vegetables to provide adequate vitamins and minerals. This was, in fact, recommended as one of the dietary goals for the United States by the Senate Select Committee on Nutrition and Human Needs.

III. Dietary Factors and Cancer of the Stomach

A. Epidemiology

Gastric cancer shows widely varying cross-national incidence and mortality (Wynder *et al.*, 1963; Hirayama, 1971, 1979; Haenszel and Correa,

1975; Bjelke, 1974; Modan *et al.*, 1974). Areas with high incidence include Japan, Latin America west of the Andes, some parts of the Caribbean, Iceland, and Northern and Eastern Europe (Fig. 3). In contrast, Western Europe, the United States, Canada, Australia, New Zealand, and other Anglo-Saxon countries have low incidence. Age-adjusted death rates for stomach cancer in the United States were about 8/100,000 in white males and 17/100,000 in black males (Segi *et al.*, 1969). However, this low incidence is only a recent development, for gastric cancer was the most common cancer in the United States 40 years ago.

It seems that cancer of the glandular stomach with antecedent intestinalization has a different etiology and needs to be distinguished from diffuse stomach cancer. It may be that the diffuse kind is associated with blood group A, and possibly, pernicious anemia (Haenszel *et al.*, 1976; Muñoz and Asvall, 1971; Muñoz and Connelly, 1971). In addition, recently, gastric stump cancer, noted some 15–30 years after partial gastrectomy for peptic ulcer, has been observed mainly after a Billroth

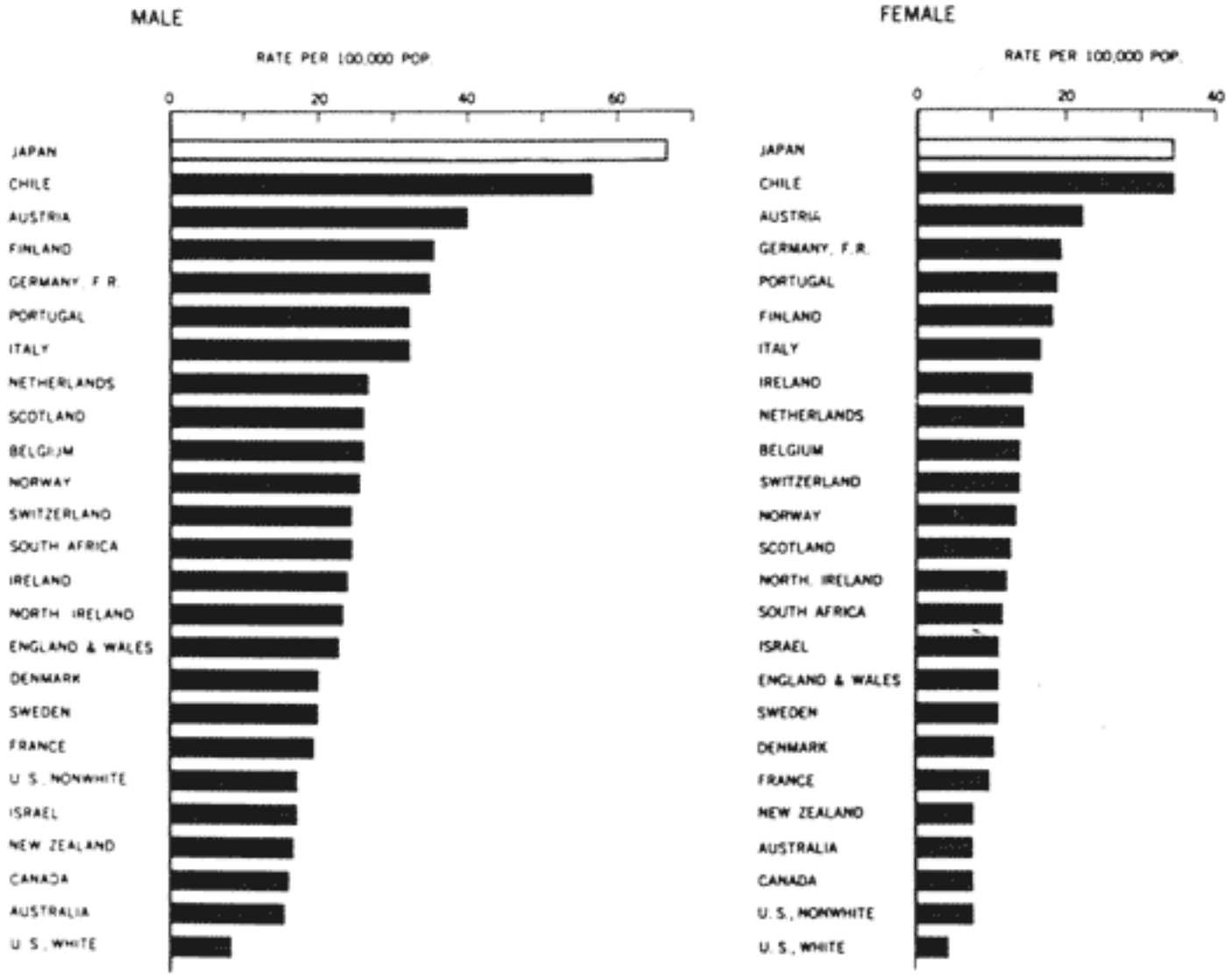

FIG. 3. Age-adjusted death rates for malignant neoplasms of stomach in different countries, 1966–1967. (From Segi and Kurihara, 1972.)

procedure. Domellöf (1979) has reviewed this iatogenic disease entity and has noted that possible alkaline bile reflux may be an important promoting factor. The further discussions of gastric cancer refer mainly to the occurrence and mechanisms relevant to cancer in the glandular stomach and do not relate directly to diffuse stomach cancer.

In addition to the marked international variation in risk, variation within countries is also observed, the general rule being that northern and/or colder regions have higher risks (Haenszel and Correa, 1975). One example is the higher stomach cancer rates in the mountainous region of Croatia, Yugoslavia, as contrasted with the lower risks in the Adriatic coastal zone. Other examples include the lower mortality rates in the Japanese prefectures of southern Kuyushu (Segi *et al.,* 1965) and particularly high mortality in the northwestern part of Iceland (Sigurjonsson, 1966). Certain cities in the tropical zone of Latin America (Bogota, Cali, Guatemala City, and Lima) have elevated risks for stomach cancer; this indicates that populations in the mountainous central Andean region are at high risk, whereas residents of the tropical coastal zones in Latin America are at low risk (Haenszel and Correa, 1975).

The incidence of stomach cancer is higher in males than in females within all populations, the overall female rate being roughly one-half to two-thirds of the corresponding male rate. The sex difference for stomach cancer has been investigated by Griffith (1968) and has been shown to be age-dependent. The male-to-female ratio is close to 1 at ages under 35, reaches a peak of about 2 at around 55, and thereafter declines to about 1.3 to 1.5 at the oldest ages. This pattern, which was peculiar to cancer of the stomach, was observed in mortality statistics from 24 countries, as well as in incidence data from a number of countries. The findings may be explained by histological differences in age- and sex-specific rates (Haenszel and Correa, 1975). The behavior of the sex ratios provides support for the thesis that gastric carcinoma is not a homogenous entity but is composed of at least two distinctive etiologic components (Haenszel and Correa, 1975).

A marked inverse socioeconomic gradient in risk was a prominent characteristic of this disease, the risk for the lower class being roughly 2.5 times that for the higher socioeconomic class (Haenszel and Correa, 1975; Berndt *et al.,* 1968; Graham *et al.,* 1960; Hirayama, 1971).

The major clues for the etiology of stomach cancer have come from utilization of the epidemiologic data of human migration (Haenszel, 1975). The United States foreign-born, migrating from countries with high risks for stomach cancer, continued to experience the risk characteristic of the population of origin. Risk characteristics of the host population did not appear until the succeeding generation was born in the United States

(Haenszel, 1961; Bjelke, 1974). Similar findings have been reported from Australia (Staszewski *et al.*, 1971). A marked excess of stomach cancer cases in Cali, Colombia, has been observed among migrants born in the mountainous region bordering Ecuador (Correa *et al.*, 1970).

The case-control study of Hawaiian Japanese revealed that migrants from the Japanese prefectures with highest stomach cancer risks continued to experience an excess risk in Hawaii, but this effect did not persist among their Nisei (second generation) offspring (Haenszel *et al.*, 1972). Lower risks were suggested for the Nisei, but not Issei (first generation) who adopted Western style diets (Stemmermann, 1977; Haenszel *et al.*, 1972). These distinctions reinforced earlier inferences from migrant study data on the critical nature of exposures in early life (Haenszel, 1975). This decrease in stomach cancer mortality among second generation Hawaiian Japanese is probably not the result of improved diagnostic or treatment methods, but can be explained on the basis of a decreased consumption of traditional Japanese foods.

Investigations of migrant populations benefited from close coordination in the collection of epidemiological and pathological observations (Haenszel and Correa, 1975). Muñoz *et al.* (1971) and Correa *et al.* (1970) classified stomach cancers in Latin American populations at high- and low-risk and reported that the intestinal type predominated among cases in high-risk areas. Typing of stomach cancers in Cali, Colombia, indicated that the intestinal type tumors accounted for most excess incidence in this subpopulation. The pathology studies in Miyagi prefecture and Hawaii also revealed that the incidence for diffuse carcinomas was substantially the same in both localities, but the incidence for intestinal, mixed, and other types was markedly lower in Hawaii (Correa *et al.*, 1973).

Several investigators have correlated national per capita average amounts of selected food items and nutrients available with mortality from cancer of the stomach, and the findings have been reviewed by Bjelke (1974), Haenszel and Correa (1975), Graham (1975), and Tulinius (1979).

Internationally, negative correlations have been found between age-standardized mortality from stomach cancer and the consumption of fats and oils, animal protein, and sugar (Lea, 1967; Gregor *et al.*, 1969) and a positive correlation with cereals (Hakama and Saxen, 1967). Recent data show a negative correlation between incidence and mortality rates of stomach cancer with total fat consumption and a positive association between incidence rates and fish consumption (Armstrong and Doll, 1975). However, the positive association for fish consumption is entirely dependent on the extreme values for Japan and Iceland. Fish consump-

tion is, therefore, unlikely to contribute significantly to international variation in gastric cancer, even though relevant to Iceland and Japan.

In certain countries, high stomach cancer incidence in certain areas has been related to high intake of salted foods (Hirayama, 1971; Joossens, 1979) and certain smoked foods (Dungal and Sigurjonsson, 1967), and has been associated with widespread use of sodium nitrate as a fertilizer and high levels of nitrate in the drinking water (Hill *et al.*, 1973; Zaldivar, 1978).

In Japan, stomach cancer incidence is particularly low in some areas where sweet potatoes are a staple food (Segi *et al.*, 1965). The relative abundance of sweet potatoes and other vegetables in the southernmost prefecture, Kagoshima, which has the lowest mortality from stomach cancer, has been pointed out (Segi *et al.*, 1965). Dungal and Sigurjonsson (1967) observed higher intakes of vitamin C due to higher intakes of potatoes and rutabagas in the low mortality areas of Iceland. The low rates in Yugoslavian areas along the Adriatic Coast may also suggest a protective effect due to the availability of fruits and vegetables.

A survey of dietary habits of the people of Chokai Village in rural Akita Prefecture, Japan (high death rates for stomach cancer) and people in Hawaii indicate that the specific food items that comprise the diet of the Hawaiian Japanese are very different from those that are characteristic of both the Hawaiian Caucasian and the indigenous Japanese (Stemmermann, 1977). The most conspicuous feature of the diet in Akita is a large intake of dried fish and miso soup, both of which have a high salt content. On the other hand, the Hawaiian Japanese have a higher intake of uncooked vegetables such as celery, lettuce, and tomato and fresh fruit juices (Stemmermann, 1977; Hirayama, 1979).

Past case-control studies of diet and stomach cancer have been complicated by problems of informant recall and accuracy of histories and have not been rewarding, but diet inquiries have steadily improved.

A case-control study of gastric cancer in Hawaiian Japanese by Haenszel *et al.* (1972) identified elevated gastric cancer risk with the consumption of pickled vegetables and dried salted fish and a rise in risk with increased frequency of these food items. Low risks were identified for several Western-type vegetables such as lettuce, celery, tomatoes, and corn, and the latter effects appeared to be independent of the associations with Japanese foods, suggesting possible protective effects. Although the companion case-control studies conducted in Hiroshima and Miyagi prefectures of Japan did not reproduce the associations with pickled vegetables and salted dried fish reported for Hawaiian Japanese, the greater use of lettuce and celery reported by controls in Japan rein-

forced the Hawaiian Japanese results on possible protective effects of Western-type vegetables (Haenszel and Correa, 1975).

Case-control studies of stomach cancer were also conducted by Bjelke (1974) in Norway and in Minnesota, USA, for persons mostly of Scandinavian descent. In Norway, (1) recent use of cooked cereals and salted fish was higher among stomach cancer patients than among controls, and (2) more pronounced case-control differences were shown by a number of vegetables and fruits, which were used less frequently by the stomach cancer patients. The greatest deviations from controls were shown for the indices for total vegetables and vitamin C intakes, for which relative deviations were greatest among young patients and women. In Minnesota, (1) recent use of cooked cereals, smoked fish, and canned fruits was higher and intakes of lettuce and tomatoes were lower among stomach cancer patients than among controls, and (2) whereas total intakes of cereal products and fish were only slightly higher than among the controls, the index for total vegetables was considerably lower among the stomach cancer patients. In both Norway and Minnesota, the lower vegetable and vitamin C intakes among stomach cancer patients had persisted over a long period, were more pronounced among women, and, in both sexes were mainly a feature of the diffuse carcinomas (Bjelke, 1974).

Similarly, the diet of gastric cancer patients in the U.S. has been found to include fewer vegetables (Graham, 1975). Israeli case-control studies indicate a higher consumption of starches in gastric cancer patients (Modan *et al.*, 1975).

In Japan, which had the highest death rate due to gastric cancer, a correlation has been reported between the consumption of salted fish and the incidence of gastric cancer. Crude salt used for preserved fish may contain nitrate, which can be reduced to nitrite. Chile and some regions of Colombia and Costa Rica with high death rates due to gastric cancer possess large nitrate deposits and correspondingly elevated levels of nitrate in foods and drinking water (Cuello *et al.*, 1978; Zaldivar and Robinson, 1973). A positive correlation between nitrate levels in drinking water and the incidence of gastric cancer has been established in Worksop, England, by Hill *et al.* (1973). In Worksop, the high nitrate content of the drinking water resulted in a weekly nitrate intake more than double that of people living in Paddington, England, a low-risk area for gastric cancer (Hill *et al.*, 1973). The daily excretion of nitrate in the urine of Worksop people was three times higher than in Paddington. Similar results were obtained by Cuello *et al.* (1978) in Colombia. These factors alone, however, may not be sufficient to account for the complex etiology of gastric cancer.

In summary, the above studies indicate that a high-risk population consumes a diet high in carbohydrates and low in fat and limited protein, limited micronutrients on an annual basis, and of greater relevance, low levels of select micronutrients, especially vitamin C, on a seasonal basis. They also consume limited amounts of fresh fruits and vegetables. Other risk factors also include intake of pickled, highly salted, and smoked foods or foods grown in soils high in nitrate. Studies on migrants from high-risk to low-risk regions have recorded that people with lowered risk include lettuce and other fresh greens as part of their daily diet.

B. Etiology

The fact that gastric cancer has been shown in many studies to be strongly related to such social factors as ethnic background, occupation, and socioeconomic status, suggests that environmental factors play an overriding role in its etiology. The declining incidence and mortality rates observed worldwide in recent years is the most convincing evidence of this assertion. The weight of the evidence that environmental factors are of major importance came from migrant studies. The issue is to elaborate the relationship between these factors and nutrition and diet in the carcinogenic process that produces cancer of the stomach.

The nitrosamines probably constitute the group of carcinogens most intensively studied (Drasar and Hill, 1974). The possible role of nitrosamines in human gastric cancer has excited much interest since nitrate is widely used by the food industry as a preservative, and is, in any case, naturally present in many foodstuffs and in sources of drinking water. Secondary amines are also present in many foodstuffs, although the documentation for this is somewhat scarce, but there is a body of circumstantial evidence compatable with the hypothesis that microflora can produce nitrosamines *in vitro* and that these are relevant to human gastric cancer. Nonetheless, as will be discussed, no nitrosamine is known to induce glandular stomach cancer in animal models, but nitrosamides do so.

C. Metabolic Epidemiology

The question requiring definition is the identification of a carcinogen responsible for the induction of gastric cancer in man, which would also explain the difference in geographical distribution of this disease.

Inasmuch as gastric cancer can be induced by alkylnitrosoureas in

animal models, the question could be asked whether gastric cancer in man might also be caused by exposure to this type of chemical (Druckrey, 1973, 1975; Sugimura and Kawachi, 1973, 1978; Mirvish, 1975; Raineri and Weisburger, 1975; Weisburger and Raineri, 1975). Sander *et al.* (1973) made the important discovery that nitrosamines can be formed *in vivo* by nitrosation by nitrite of suitable substrate amines or amides in the stomachs of animals or man. Later, others established that cancer at various sites could be induced with identical results either by administration of a preformed nitrosamine or nitrosamide or by nitrite together with the corresponding amine or amide (Fan and Tannenbaum, 1973; Mirvish and Chu, 1973).

Sander (1968) first demonstrated that certain strains of enterobacteria were able to *N*-nitrosate diphenylamine at neutral pH using nitrate as the source of the nitrous group. This work was confirmed and extended by Hawksworth and Hill (1971a,b) and by Hill and Hawksworth (1972). The reaction involving nitrate is only carried out using nitrate-reducing bacteria, but some strains of bacteria that do not produce nitrate are able to nitrosate secondary amines using nitrite as the nitrosating agent. The reaction could be brought about by an enzymic reaction, or it could be due to the bacteria producing the reactants and the conditions necessary for the reaction. Since bacteria are able to promote the catalysis of the *N*-nitrosation reaction at physiological pH values, the sites in which nitrosamines might be formed *in vivo* must be extended to include all those in which nitrate, secondary amine, and bacteria might coexist (Drasar and Hill, 1974). Since bacteria are able to reduce nitrate to nitrite, the important factor is no longer the amount of ingested nitrate (Drasar and Hill, 1974). Secondary amines are present in the stomach in small amounts dependent on the nature of the food consumed.

The possible role of salivary nitrate or nitrite is being investigated by Tannenbaum *et al.* (1978a). Nitrite found in normal human saliva appears to be the product of microbial reduction of nitrate that is secreted by the salivary glands upon oral intake and absorption of nitrate. The data support the hypothesis that (1) nitrite formed in the oral cavity contributes to nitrosamine and nitrosamide formation in the normal stomach, (2) nitrosamides can be formed in the weakly acidic or even neutral stomachs, and (3) nitrosamines and nitrosamides are formed at other areas that normally contain bacteria or become infected (Tannenbaum *et al.*, 1978b). Although these possibilities cannot be differentiated on the basis of their relevance to the etiology of gastric cancer in man, Tannenbaum *et al.* (1978a,b) suggest that both microorganisms and acidity are important for controlling nitrosation reactions *in vivo*. A situation in which the pH is not too low to prevent growth of bacteria and not too high to

prevent nitrosation formation would seem to represent the greatest hazard in the presence of catalysts (Tannenbaum *et al.*, 1979). In a recent study in Colombia, Tannenbaum *et al.* (1979) also reported that patients with diagnosed gastric pathology related to a precancerous state had high levels of nitrite in gastric contents with a pH above 5. These studies support, but do not prove, the hypothesis of bacterial reduction of nitrate in the stomach with concomitant formation of carcinogenic *N*-nitroso compounds. The question needs to be asked, however, whether the early events in gastric carcinogenesis would not occur in a high risk region during childhood or in young adults, perhaps before the onset, later in life, of hypochlorhydria. Migrant studies do suggest that the first 20 years of life may be the time of exposure or formation of gastric carcinogens.

D. Experimental Studies

Because of the importance of gastric cancer in man in many parts of the world, serious efforts have been made by experimentalists to develop animal models (Bralow and Weisburger, 1976). Until recently, such efforts were relatively fruitless. While rodents, particularly mice, developed tumors of the forestomach upon treatment with certain of the classic carcinogens such as the polycyclic aromatic hydrocarbons or nitroaryl derivatives analogous to the carcinogenic aromatic amines, this lesion may not be a good model for the human disease, which generally originates in the glandular stomach. Stewart *et al.* (1961) discovered that 2,7-bis-fluorenylacetamide could induce cancer of the glandular stomach in a relatively small, yet reliable, portion of rats at risk, but this procedure is not effective in all species under a variety of conditions. However, Sugimura made the important discovery that MNNG induced cancer of the glandular stomach in rats and other species in high yield (Sugimura and Kawachi, 1973, 1978). A number of investigations have now found this kind of agent to be the most reliable tool for studying model systems of gastric cancer. Druckrey *et al.* (1973) showed that *N*-methyl-*N'*-acetylnitrosourea, nitrosobiuret, and similar substances also have this specific property. Thus, it seems that the molecular features of alkylnitrosamides can cause cancer of the glandular stomach. Salt intake in man (Joossens, 1979) and rats (Tatematsu *et al.*, 1975) has a promoting effect.

Inasmuch as the animal models of gastric cancer induced by alkylnitrosoureas have broad applicability across species lines, the question can be asked whether gastric cancer in man might also be caused by exposure to this type of chemical. Thus, it is important to establish whether such chemicals can enter man's environment and, if so, under what conditions.

The well-established organ-specific carcinogenicity of nitrosamines and nitrosamides in animals (Magee and Barnes, 1956; Magee, 1971) is possibly of relevance to the development of human cancer, since the *in vivo* nitrosation of certain dietary amines and amides under the acidic conditions of the stomach could lead to the formation of these agents in human gastric juices, which have a pH range of 1–5 (Druckrey, 1975; Endo *et al.*, 1974; Sander *et al.*, 1973; Schoental and Bensted, 1969; Sugimura and Kawachi, 1978; Mirvish, 1975; Weisburger *et al.* (1980a,b).

The formation of nitrite from nitrate, the conditions controlling the nitrosation of alkylamides, and the methods of inhibition of the nitrosation reaction have been studied by Weisburger and Raineri (1975). They have demonstrated that under realistic conditions, using foods eaten by populations typical of high-risk situations for gastric cancer, endogenous or added nitrate was converted in substantial amounts to nitrite when this food was stored at room temperature, but not in the refrigerator. Also, less nitrite was formed in the presence of high amounts of ascorbic acid, and in a model study, less carcinogenic methylnitrosourea was formed when nitrite and methylurea were combined in the presence of ascorbic acid. In relation to a working hypothesis that gastric cancer in man may result from the formation in the stomach of a locally active alkylnitrosamide, Weisburger and Raineri (1975) have established a possible source of nitrate (deliberately added or present as a result of geochemistry or agricultural practices) in food stored at room temperature.

In the search for the etiologic factors responsible for gastric cancer, Marquardt *et al.* (1977a,b) examined extracts of nitrite-treated food for mutagenic (presumptive carcinogenic) activity in the Ames *Salmonella typhimurium* system. Extracts of Sanma fish, borscht, and beans, each of which is a dietary staple in Japan, Eastern Europe, or Latin America, areas with a high incidence of gastric cancer, 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. The formation of the mutagen was maximal at pH 3.0. 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 500 ppm. Moreover, and importantly, ascorbic acid prevented the formation of the mutagens in nitrite-treated foods. These data also suggest that the mutagen(s) may be of the alkylnitrosamide type.

Pending the isolation and identification of the active mutagenic principle, Weisburger *et al.* (1980a) reported that the ether extract of the homogenate of Sanma fish treated at pH 3 with nitrite, when fed to Wistar rats, induced tumors mainly in the glandular stomach, and a few

in the pancreas and small intestine (Table XI). None of the animals fed the ether extract of Sanma fish alone showed any tumors. Thus, it would seem that the products obtained from pickling a specific kind of fish can induce glandular stomach cancer identical to that seen in man.

Endo *et al.* (1975) showed that nitrosation of methylguanidine under simulated gastric conditions produced a mutagenic principle identified as nitrosocyanamide. 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 failed to exhibit the specificity of inducing cancers of the glandular stomach, which are seen in man and which chemicals such as MNNG produce reliably.

Thus, identification of mutagenic (presumptive carcinogenic) agents in nitrosated foods requires additional study. A thorough understanding of the conditions governing the formation of nitroso compounds, as well as an understanding of the natural occurrence of potential substrates, allows for a rational approach to the study of the etiology of gastric cancer in areas of high incidence.

TABLE XI
TUMOR INCIDENCE IN RATS GIVEN FISH EXTRACT WITH NITRITE, OR FISH ALONE[a]

Sites of tumors	Number of animals	
	Fish extract diet	Fish extract and $NaNO_2$ diet
Effective number of rats	8	10
Forestomach		
Papilloma	0	0
Squamous cell carcinoma	0	2
Glandular stomach		
Adenoma	0	2
Adenocarcinoma	0	2
Adenosquamouscarcinoma	0	1
Pancreas		
Adenoma	0	2
Adenocarcinoma	0	1
Small intestine		
Adenocarcinoma	0	1

[a] From Weisburger *et al.* (1980).

E. Conclusions

Several investigators are now working on the experimental development of the hypothesis that gastric cancer in man may stem from *in vivo* nitrosation of an alkylamide type of substrate (Endo *et al.*, 1974, 1975; Mirvish, 1975; Correa *et al.*, 1975; Fan and Tannenbaum, 1973; Weisburger and Ranieri, 1975; Weisburger *et al.*, 1980a,b). Current knowledge derived from epidemiology and animal experiments all support this hypothesis.

If this concept could be borne out experimentally, it would appear that one relatively minor change in the human diet in certain high-risk population groups would prevent this important cancer. The required alteration would be to take at each meal fruits, vegetables, and salads as sources of vitamin C on a continuous rather than intermittent seasonal basis, as is presently the custom in many northern regions of several high-risk countries.

It is necessary to emphasize the need for a continuous dietary intake of foods rich in vitamin C to prevent even an intermittent exposure to carcinogens, since in animal models, gastric cancer can be induced by relatively infrequent application of alkylnitrosamides. 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 to continue this practice by minimizing the intake of actual or potential nitrite and optimizing the intake of foods containing ascorbate.

Furthermore, in the light of the demonstrated enhancing effect of salt in experimental gastric cancer, and the known customary high salt intake in areas such as Japan, Iceland, and other high-risk regions, a reduction of dietary salt intake would also seem beneficial (Joossens, 1979).

IV. Dietary Factors and Cancer of the Upper Alimentary and Respiratory Tract

A. Epidemiology

The epidemiology of upper alimentary tract cancer presents many unusual and interesting features. Striking variations in the incidence of esophageal cancer have been found within relatively small areas (Day, 1975). High rates of esophageal cancer have been reported in Central

Asia, namely, the Caspian littoral of Iran, Turkemenia, Kazakhstan, and Uzbekistan of the USSR, and Honan, Hopei, and Shansi Provinces of the Peoples Republic of China (Mahboubi *et al.*, 1973; Tuyns, 1970; Coordinating Group for Research on the Etiology of Esophageal Cancer of North China, 1974). The high-incidence region, called the esophageal cancer belt, begins in northern China, running from the Iranian Caspian littoral into Soviet Central Asia. High incidence of esophageal cancer is found in Singapore Chinese, in general, and particularly those speaking the dialects of Hokkien and Techew, who originate from Fukkien Province or the Swatow region in northern Kwantung Province (Shanmugaratnam and Wee, 1973). A case-control study of esophageal cancer among Chinese in Singapore indicated that those born in the Peoples Republic of China, irrespective of dialect group or sex, had a 3-fold increase in risk, as compared to those born in Singapore (deJong *et al.*, 1970). In contrast, low incidences are found in West Africa.

The incidence is low throughout Europe, with the exception of the northern regions of Brittany and Normandy in France, where esophageal cancer occurs predominantly among males. Both alcohol and tobacco have been shown to be associated factors. These data indicate that consumption of home-distilled apple brandy increases the risk for esophageal cancer. Although an association of alcohol consumption with esophageal cancer in France in the absence of tobacco consumption has been reported, the risk is dramatically increased when tobacco usage is concurrent with alcohol consumption (Tuyns and Masse, 1973; Tuyns *et al.*, 1977).

In the United States, the incidence is higher in both male and female blacks compared to whites (Biometry Branch, National Cancer Institute, 1974). This increase appears most marked among the urban blacks in the north. Puerto Rico has a higher age-adjusted incidence rate of cancer of the mouth, pharynx, and esophagus than in the United States (Martinez *et al.*, 1975) for every age group in both men and women. The predominance of malignant tumors of the upper alimentary tract in Puerto Rico reflects different nutritional and drinking habits from those in the United States survey areas (Martinez *et al.*, 1975). In Utah, where the per capita consumption of alcohol and tobacco is well below the national average, the incidence of esophageal cancer was 55% lower than expected (Lyon *et al.*, 1977).

B. Etiology

Extensive epidemiological evidence has been available for some time now that indicates a strong association of both chronic alcohol and to-

bacco consumption with cancers of the head and neck area, specifically of the oral cavity (Wynder *et al.*, 1957), esophagus (Kamionkowski and Fleshler, 1965), and larynx (Wynder *et al.*, 1956; Hinds *et al.*, 1979) (Figs. 4 and 5). This association is supported further by the low incidences of these types of cancer in populations such as the Seventh-Day Adventists who are known to consume less alcohol than the general population (Phillips, 1975), as well as by the high incidences of these types of cancers in males.

Because of the interaction of the smoking and drinking variables, it is difficult to assess the two independently since, most often, heavy drinkers are also heavy smokers. Even though some increased risk for cancer due solely to alcohol consumption is suggested by the data of Rothman and Keller (1972), a marked synergy is observed when tobacco usage is combined with chronic alcohol consumption. With the possible exception of esophageal cancer in France, the nature of the beverage consumed seems to be important only in terms of its ethanol content (Williams and Horn, 1977; Wynder and Stellman, 1977).

The respective roles that alcohol and tobacco consumption play in the etiology of head and neck cancer have yet to be rigorously defined. However, it seems reasonable to assume that tobacco and/or tobacco

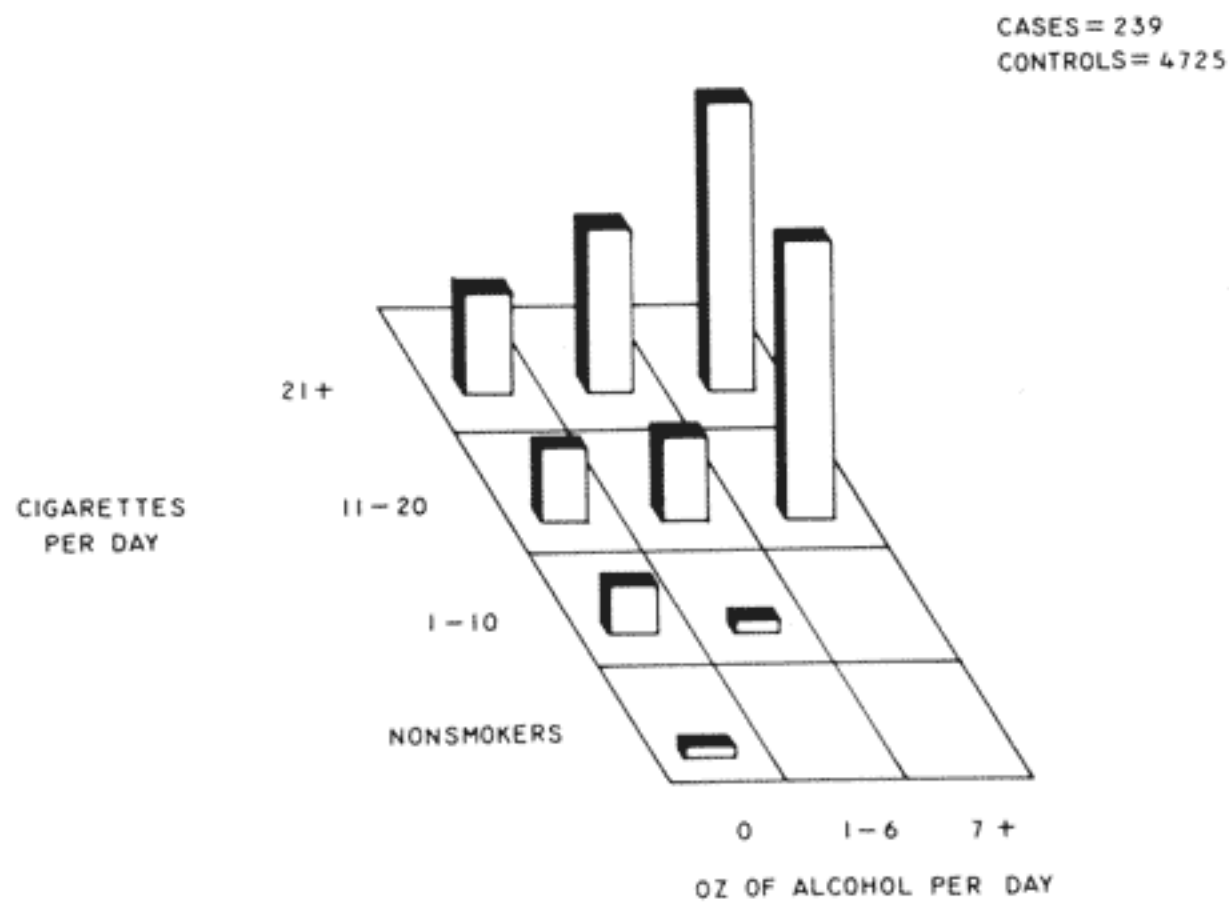

FIG. 4. Relative risk of larynx cancer for daily consumption of alcohol and cigarettes for males. (From McCoy and Wynder, 1979.)

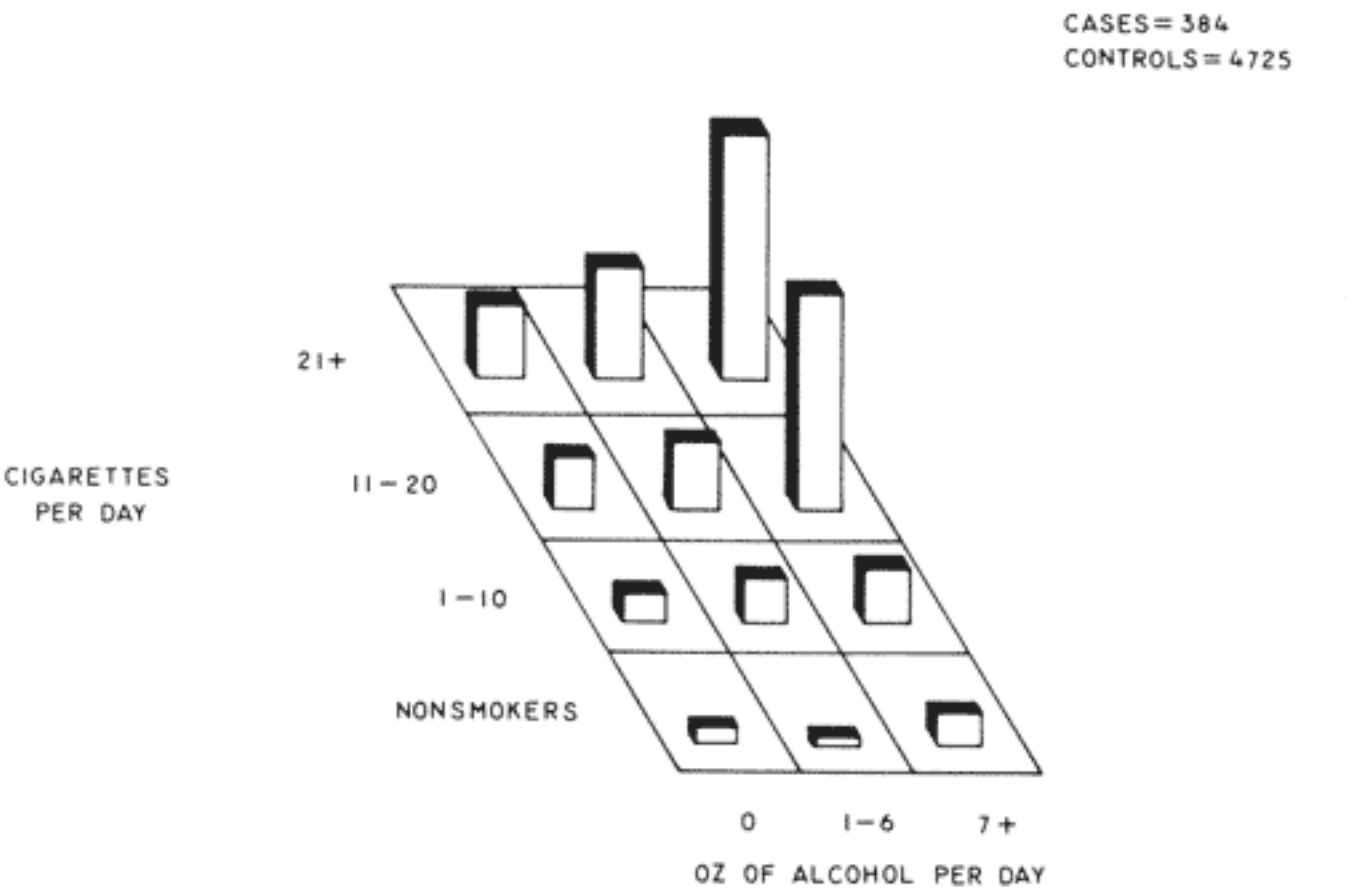

FIG. 5. Relative risk of oral cavity cancer for daily consumption of alcohol and cigarettes for males. (From McCoy and Wynder, 1979.)

smoke are the source of the initiating carcinogenic stimuli, and that ethanol facilitates the reactivity of some tobacco-associated initiator.

As identified above, alcohol and tobacco are the two major risk factors for esophageal cancer in Western Europe and North America, but are of little importance in the esophageal belt of Central Asia. Epidemiologic studies in the Peoples Republic of China indicate a relation between esophageal cancer and wheat-eating, as opposed to rice-eating populations (The Coordinating Group for Research on Etiology of Esophageal Cancer in North China, 1975). A series of epidemiologic studies in the Caspian littoral of Iran indicate that the high risk for esophageal cancer is associated with the severely limited and probably irritant nature of the diet in conjunction with exposure to a carcinogenic agent derived either from opium tars or from wheat contaminants (Joint Iran IARC Study Group, 1977). Factors that have been associated with high risk include the consumption of wheat bread and a low intake of vitamin A, vitamin C, and riboflavin. Subsequent clinical studies have found signs of only vitamin A and riboflavin deficiencies (McClaren and Siasi, 1978).

A case-control study organized to follow up the results of the population investigation in Iran has shown no excess risk among patients for

low intake of animal proteins, but has confirmed the risk attributed to a low intake of fruit and raw vegetables and has shown that even among the generally poor rural communities of northern Iran, it is the lower social strata that are more severely affected by the disease (Cook-Mozaffari, 1979).

A case-control study in Puerto Rico showed that the lower socioeconomic groups were most affected and that there was evidence that these lower socioeconomic groups had a diet deficient in good quality protein, total calories, vitamin A, and riboflavin (Martinez, 1969). Although the relationship between nutrient deficiency and esophageal cancer was not demonstrated on an individual basis, heavy consumption of alcohol and tobacco, together with hot beverages and spices were associated with the disease.

High incidence of esophageal cancer found in Transkei, South Africa, has been linked to molybdenum deficiencies in the soil (Burrell *et al.*, 1966), but no evidence has been produced that associates the possible deficiency with human disease (Rose, 1973; Warwick and Harrington, 1973). Recent studies by Fong *et al.* (1977) in esophageal cancer patients in Hong Kong and in animal model studies show that zinc and copper are important in reducing the incidence of esophageal cancer.

The possibility that nutrition might play a role in the etiology of esophageal cancer is also suggested by consideration of the association of Plummer–Vinson (Paterson–Kelly) syndrome and cancer of the upper alimentary tract. This disease, once prevalent among Swedish women, was shown to be associated with chronic iron and vitamin deficiencies. High rates of upper alimentary tract cancer were observed in the absence of exposure to tobacco or any other obvious source of carcinogen (Wynder and Fryer, 1958). Since the introduction of a national program of iron and B vitamins supplementation in Sweden in the early 1950s, a significant reduction in the number of cases of Plummer–Vinson syndrome with a subsequent reduction of upper alimentary tract cancer has occurred (Larsson *et al.*, 1975).

Thus, one way in which alcohol could increase the risk for cancer would be through the associated nutritional deficiencies commonly associated with alcoholism (Leevy *et al.*, 1965). Since alcoholics often consume 900 or more calories a day from alcohol alone (deLint, 1975), it is not difficult to imagine that the rest of their dietary intake is insufficient for providing necessary nutrients. Cancers of the head and neck also seem to occur most commonly in those individuals who do not eat nutritionally balanced diets. (DHEW Publ. No. 74-124, 1974). Alcohol consumption can lead also to impaired absorption of nutrients and vitamins (Vitale and Coffey, 1971).

C. Metabolic Epidemology and Experimental Studies

Animal studies have provided evidence that suggests that nutrient deficiencies and dietary contamination may interact in esophageal carcinogenesis. Feeding *N*-nitrosodiethylamine to lipotrope-deficient rats increased esophageal carcinogenesis compared to those animals fed a control diet (Rogers *et al.*, 1974). The tumors induced in these investigations were invasive squamous cell carcinomas morphologically similar to those in man.

Feeding mice a riboflavin-deficient diet causes morphological alterations in skin and upper alimentary tract epithelium that are similar to those observed in patients suffering from Plummer–Vinson disease (Wynder and Klein, 1965). As the deficiency progresses, epithelial morphology progressively changes from atrophy to hyperkeratosis to, in several instances, hyperplasia. The experiments of Chan and Wynder (1970) have shown that, following initiation with benzo(*a*)pyrene and promotion with croton oil, riboflavin-deficient mice develop tumors more rapidly than control mice receiving a nutritionally adequate diet. In parallel studies, Chan *et al.* (1972) have shown that basal levels of skin aryl carbon hydroxylase were slightly reduced in riboflavin-deficient mice. However, the skin activity of riboflavin-deficient animals was induced to a much greater extent following a single application of dimethylbenz(*a*)anthracene (DMBA).

The work of Gerson and Meyer (1977) has shown that feeding rats diets deficient in zinc causes morphological changes of the buccal mucosa similar to the riboflavin-deficient condition. [Dietary zinc deficiency has been shown to increase the number of esophageal tumors and to decrease the latent period in rats exposed to methylbenzylnitrosamine (Fong *et al.*, 1978).] Lower levels of zinc have been observed in hair and tissue samples from esophageal cancer patients (Lin *et al.*, 1976).

In animals made deficient for vitamin A, the tracheobronchial epithelium undergoes atrophic degenerative changes (Harris *et al.*, 1972; Salley and Bryson, 1957) that are quite similar to the changes observed in animals exposed to tobacco smoke (Dontenwill *et al.*, 1973; Kobayashi *et al.*, 1974). Vitamin A-deficient animals have been shown to be more susceptible to polycyclic aromatic hydrocarbons or PAH carcinogenesis (Sporn *et al.*, 1976). That ethanol is capable of decreasing Vitamin A levels is noteworthy in view of the participation of this vitamin in the regulation of epithelial cell differentiation (De Luca *et al.*, 1969; Vaughn and Bernstein, 1976).

Thus, currently available evidence suggests that nutritional deficiencies that arise from either undernutrition and/or as a direct consequence of

alcohol intake (impaired absorption or enhanced elimination) could play a role in the etiology of head and neck cancer.

There are at least four possible models for correlating alcohol consumption and cancer: (1) alcohol as a solvent; (2) and (3) alcohol-induced increases or decreases in liver metabolism; or (4) alcohol-induced alterations in target tissue metabolism.

1. The simplest of the four possible models treats alcohol as a solvent. This model is based on the assumption that entry of tobacco-related carcinogens into target tissues is facilitated because of enhanced solubility and easier passage through cellular membranes of the carcinogen. Stenback (1969) demonstrated that administration of the carcinogen DMBA dissolved in ethanol resulted in a reduced latent period and increased skin tumor formation compared to mice treated with DMBA dissolved in acetone. Kuratsune *et al.* (1971) showed that although chronic treatment of mice or rats with various distilled beverages failed to cause tumor formation in either mice or rats, a tumor promoting activity for DMBA similar to that caused by croton oil was found in sake and its distillation residues. This model may apply to both oral and esophageal cancer, but certainly is not sufficient to explain the association of alcohol and cancer of the larynz, since this area does not come into direct contact with alcohol.

2. The extensive body of literature substantiating ethanol-induced alterations in liver metabolism requires that serious attention be given to the second model. Chronic ethanol consumption leads to, among other things, an enhancement in the liver microsomal drug metabolizing capabilities of both humans (Kater *et al.*, 1969; Misra *et al.*, 1971) and experimental animals (Misra *et al.*, 1971; Rubin *et al.*, 1968; Rubin and Lieber, 1968). In the absence of extensive destruction of liver tissue, increased production of a metabolite that is then delivered to the target tissues and further metabolized to its ultimate carcinogenic form could be envisioned. Radike *et al.* (1977) have shown that ethanol decreases the latent period for vinyl chloride carcinogenesis in rats, demonstrating a mechanism by which ethanol could increase the risk for the development of liver cancer.

Recently, we have demonstrated that *in vitro* metabolism of the hepatocarcinogen *N*-nitrosopyrrolidine is increased in microsomal fractions isolated from ethanol-consuming animals and that postmitochondrial supernatants isolated from ethanol-consuming animals are capable of much greater conversion of *N*-nitrosopyrrolidine to a mutagen than control preparations (Fig. 6) (McCoy *et al.*, 1979). Similar ethanol-associated increases in the *in vitro* metabolism of dimethylnitrosamine were reported by Maling *et al.* (1975). This particular model must, of necessity, place

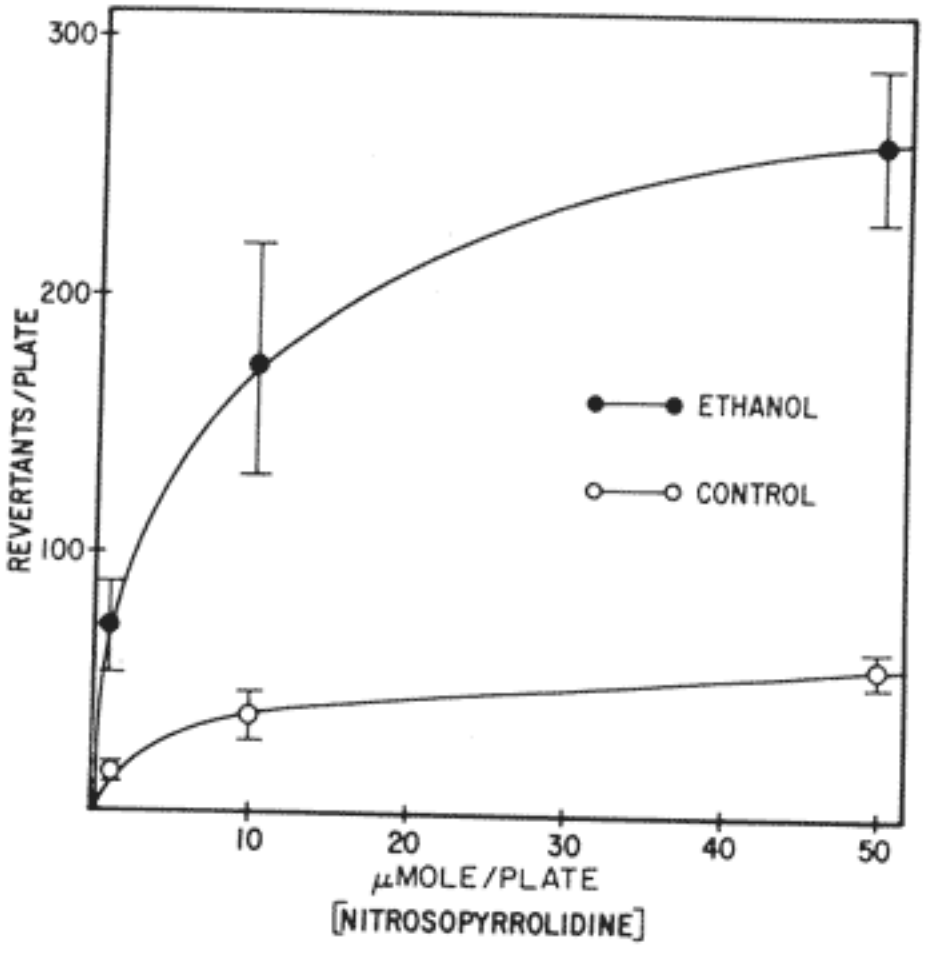

FIG. 6. *N'*-Nitrosopyrrolidine mutagenicity of liver postmitochondrial supernatants from control and ethanol-consuming hamsters. *Salmonella typhimurium* TA 1535 was used as the indicator strain. Final protein concentration for both control and ethanol postmitochondrial supernatants varied from 1.3 to 1.5 mg/ml. Data expressed as mean ± SE of six separate determinations. Data were corrected for spontaneous revertants, which ranged from 4 to 30 per plate. (From McCoy *et al.*, 1979.)

a restriction on the nature of the carcinogen in that the carcinogens and/or their metabolites must have a high degree of site specificity, since all tissues in the body would be exposed to the results of changes in liver metabolism and yet only those in the head and neck area are at increased risk. The major objection to the involvement of enhanced liver metabolic activity in the causation of head and neck cancer is that this model excludes consideration of PAH participation. Though tobacco smoke contains nitrosamines that are site-specific for the upper alimentary tract (Boyland *et al.*, 1964; Hoffmann *et al.*, 1975) and upper respiratory tract (Hilfrich *et al.*, 1977; Lijinsky and Taylor, 1976) in animals, we feel that any model that fails to take into account the carcinogenic potential of PAH in tobacco smoke is a seriously compromised hypothesis. In addition, this model clearly cannot apply in the case of the severely damaged alcoholic liver.

3. An association between cirrhosis and the development of cancer of the liver in man has been observed (Lee, 1966; Leevy *et al.*, 1964). In addition, Keller presented evidence that cancers of the head and neck

area are often found in cirrhotic patients (Keller, 1967, 1977). That this increased risk for liver cancer is due to components other than ethanol can be inferred from the work of Gibel *et al.* (1975). They demonstrated that several of the higher alcohols normally found in distilled beverages can be both hepatotoxic and carcinogenic. For example, they demonstrated that administration either orally or subcutaneously of isoamyl, isobutyl, or *n*-propyl alcohol to rats resulted in the appearance of both benign and malignant tumors of the liver, as well as other sites. Experimental evidence in favor of decreased liver metabolism resulting in increased risk for head and neck cancer was presented by Protzel *et al.* (1964). In their studies, mice were treated with either ethanol or carbon tetrachloride and the buccal mucosa were swabbed with benzo(*a*)pyrene solutions; these mice developed more tumors with a shorter latency period than controls receiving only carcinogen treatment. Falk and Kotin (1963) demonstrated that the rate of bile clearance of benzo(*a*)pyrene was markedly decreased in rats whose livers were damaged by exposure to carbon tetrachloride. In patients suffering from cirrhotic liver disease, the rate of metabolic clearance of a number of drugs known to be metabolized by the microsomal drug metabolizing system was reduced (Chakraborty, 1978). The loss of metabolic capacity in cirrhotic liver would result in a decreased ability to detoxify tobacco-related carcinogens, thus causing a net increase in their systemic concentrations and resulting, in essence, in the exposure of target tissues to more carcinogen than would occur in individuals with uncompromised livers.

4. The fourth model is based on the hypothesis that chronic ethanol consumption alters intracellular metabolism of the epithelial cells at the target sites, resulting in enhanced metabolic activation of tobacco-associated carcinogens. Some of the more attractive features of this model are (1) that the cancer associated with the head and neck are epithelial in origin; (2) that these surface epithelial cells are mostly exposed to tobacco-associated carcinogens; and (3) that site specificity of the carcinogen need not be invoked since all sites, by virtue of their anatomical location, will be exposed to tobacco smoke. This third feature is an important point, because no class of carcinogen need be initially excluded from consideration since we cannot as yet say with any degree of certainty which of the many potential carcinogens in tobacco smoke are actually involved in the initiation of carcinogenesis.

D. Conclusions

The epidemiologic data indicate a strong positive association of both chronic alcohol and tobacco consumption with cancers of the upper

respiratory and upper alimentary tract. One can hope that the increased national interest in both the prevention of alcoholism, as well as smoking cessation programs, will in and of themselves result in a decreased incidence of these cancers. Emphasis should be put on the education of the young as to the health consequences of chronic use of alcohol and tobacco.

In view of the epidemiologic association of vitamin and mineral deficiencies with esophageal cancers, serious consideration must be given to the role that alcohol-related changes in nutritional status have in increasing the risk for cancer. It seems clear that much more experimental work is needed to translate nutrition as a risk factor into an understanding of the cause and effect relationship. Ethanol interacts with tissues in many ways (as a drug, energy source, or solvent) and can influence intracellular metabolism in many ways (enzyme induction, alteration in redox potential, as a metabolite). In addition, ethanol can cause marked changes in cellular and tissue metabolism because of alteration in hormonal status.

It is hoped that evidence for the very strong association of nutritional deficiencies, as well as alcohol and tobacco usage, with cancers of the upper alimentary and upper respiratory tract will lead to more data and further insight into the role each of the known risk factors plays in the etiology of the disease.

V. Dietary Factors and Cancer of the Pancreas

A. Epidemiology

Geographic differences in the incidence of pancreatic cancer are pronounced (Fig. 7; Segi *et al.*, 1972). The highest incidence of this cancer is among male Maoris of New Zealand and female natives of Hawaii. It is noteworthy that two groups of Polynesian descent, the New Zealand Maoris and native Hawaiians, are especially prone to this disease. According to Doll *et al.* (1970), the lowest rates in both sexes are reported for Nigeria and for Bombay, India. Incidence data from the 1969 to 1971 Third National Cancer Survey (Biometry Branch, National Cancer Institute, 1974) reveal that pancreatic cancer predominates in blacks and in males. Factors underlying these racial differences are unclear, but an environmental influence is suggested by the much higher mortality in American Japanese than in native Japanese (Haenszel and Kurihara, 1968).

Pancreatic cancer predominates in males by a ratio of 2:1 before age 50 and declines, although never reaching unity, after that age (Segi *et al.*,

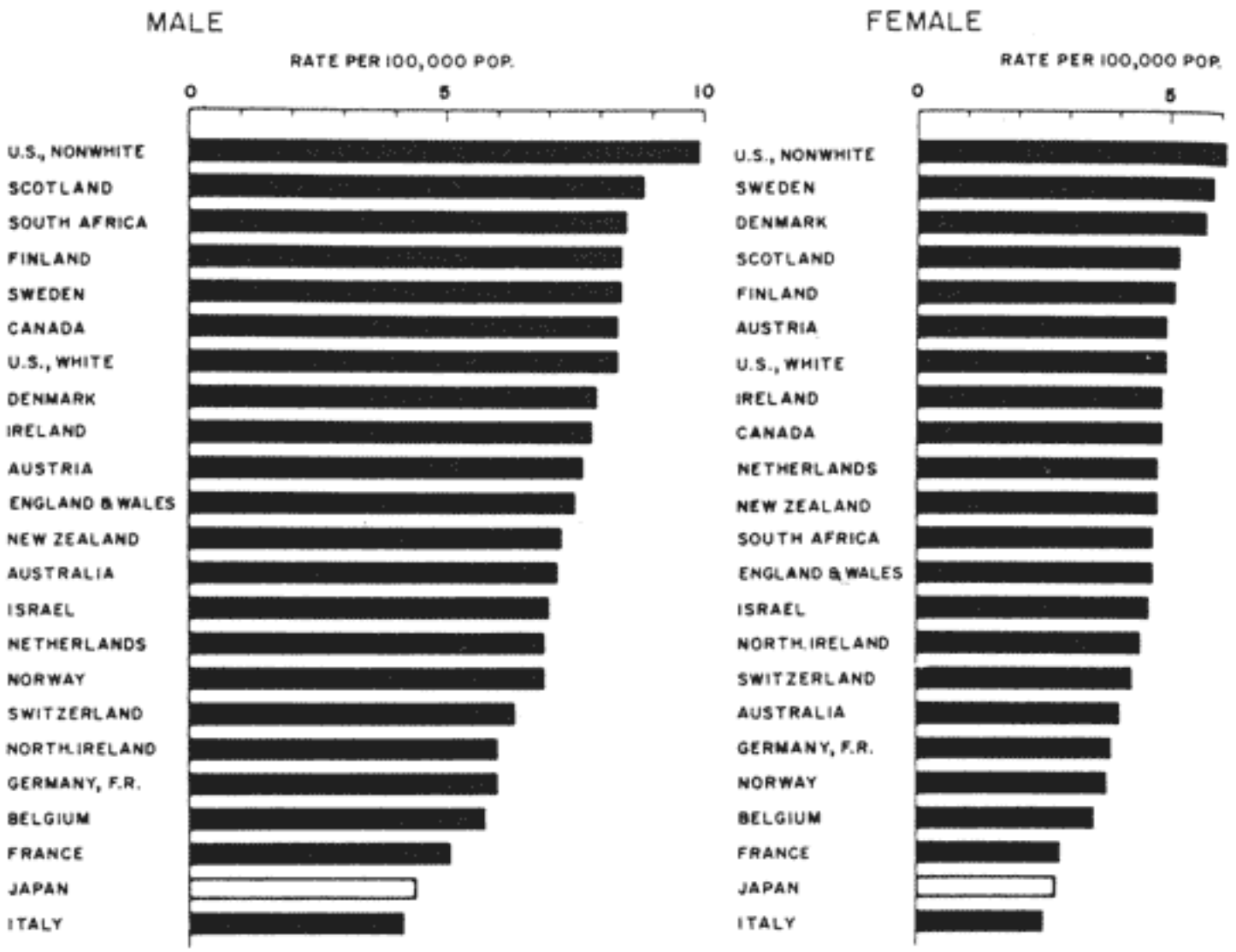

FIG. 7. Age-adjusted death rates for pancreatic cancer in different countries, 1966–1967. (From Segi and Kurihara, 1972.)

1969; Wynder *et al.*, 1973). The peak at ages 25–29 for U.S. whites was found to be related to an increased mortality for males. The data suggest that the different sex ratios for pancreatic cancer by country and age may be influenced by environmental factors.

Studies on migrants have provided valuable information on the influence of environmental factors. A study by Smith (1956) on Japanese immigrants to the United States showed that the standardized mortality rates for pancreatic cancer was higher among Japanese Americans as compared with white Americans. Haenszel and Kurihara (1968) reported a higher mortality from pancreatic cancer for first generation Japanese migrants than for American-born Japanese. A similar effect of migration within the United States is suggested by a cancer mortality survey in Ohio; blacks born in the South have substantially higher rates than Ohio-born blacks (Mancuso, 1974). These findings support the hypothesis that environmental factors (most likely dietary in nature) increase the risk for pancreatic cancer.

Pancreatic cancer rates vary among religious groups. Newill (1961) and Seidman (1970) reported that pancreatic cancer was more frequent among Jews than among other religious groups. The rates among Seventh-Day Adventists are all in the vicinity of 50–75% of general rates (Phillips, 1975). In addition to abstinence from smoking and drinking, the most distinctive feature of the typical Adventist lifestyle is a unique diet: lacto-ovo-vegetarianism. Lyon *et al.* (1977) reported that in Utah the incidence and mortality for pancreatic cancer was 36 and 27% below expected, respectively. Mormon church doctrine prohibits the use of alcohol, tobacco, coffee, and tea.

B. Etiology

Although the epidemiologic studies of pancreatic cancer point to an environmental influence, no environmental factor has been singled out with certainty (Fraumeni, 1975). Although this review is mainly concerned with nutrition and pancreatic cancer, a number of retrospective and prospective studies have shown an increased mortality rate from pancreatic cancer among cigarette smokers (Best, 1966; Weir and Dunn, 1970; Wynder *et al.*, 1973). Cigarette smoking seems to account for the male predominance of pancreatic cancer, since no sex differential exists for this cancer among nonsmokers (Hammond, 1966). We can envision two ways in which tobacco components, or their metabolically activated forms, can reach the pancreas, namely, via reflux from the bile duct into the pancreatic duct or through the blood stream (Hansson, 1967; Wynder, 1975a).

The migrant pattern of pancreatic cancer, the relatively high level of this disease in American Jews (although their cigarette consumption is relatively low as is well reflected in their lower rate of lung cancer), and the association of pancreatic cancer with fat consumption in various countries suggest that diet affects the development of pancreatic cancer (Wynder *et al.*, 1973). Data on pancreatic cancer correlate with a general finding that overnutrition relates to a variety of cancers, including cancer of the colon, breast, and prostate. In Japan, the climbing rates for pancreatic cancer have been linked to consumption of a Western diet (Wynder *et al.*, 1973).

C. Metabolic Epidemiology

As a working hypothesis on the etiology of pancreatic cancer, Wynder *et al.* (1973) proposed that bile may contain carcinogens and/or cocarcin-

ogens and promoters (possibly originating in the diet, tobacco, and occupational environments) and that this bile, refluxed into the pancreatic duct, may cause pancreatic cancer. The effect of dietary fats is on the composition of the biliary bile acids, which have been shown to act as promoters (Reddy *et al.*, 1978b). However, there are no metabolic, epidemiologic, and animal studies to test the relationship of nutrition to pancreatic cancer.

D. Experimental Studies

Animal models have been developed to study pancreatic carcinogenesis. Pancreatic duct neoplasms, biologically and morphobiologically similar to those in man, were induced in Syrian golden hamsters after subcutaneous injection of *N*-nitrosobis(2-oxopropyl)amine (BOP) (Pour *et al.*, 1976). Tumors also developed in the lungs, liver, gallbladder, and kidney. However, oral administration of BOP in drinking water resulted in a few pancreatic neoplasms and in a high incidence of intra- and extrahepatic bile duct neoplasms (Pour and Althoff, 1977). The significance of a bile reflux mechanism in pancreatic carcinogenesis was investigated by Pour and Donnelly (1978) using cholecystoduodenostomized and choledochostomized Syrian golden hamsters. The distribution and patterns of BOP-induced pancreatic neoplasms were not altered by bypassing the bile through the common duct. However, in this study, the possible regurgitation of bile-borne carcinogen from the duodenum into the pancreatic duct is most unlikely because in this species the pancreatic duct enters the common duct well before it opens into the duodenum.

E. Conclusions

The comparison of the incidence of international high-risk groups, as well as intracountry variations and migrant studies, have yielded significant information on risk factors. Cancer of the pancreas, whose relation to dietary factors is less clear than that of cancers of the colon, breast, prostate, and stomach, is worth pursuing from the standpoint of metabolic epidemiology and animal studies. We want to be able to replace our tentative guesses about the risk factors with enough understanding to lower current levels of risk.

VI. Dietary Factors and Cancer of the Breast

A. Epidemiology

Traditionally, epidemiologic studies have generated hypotheses for the etiology of breast cancer through international comparisons of incidence, case-control studies, migration studies, and correlation of selected variables with disease occurrence. Such studies have provided the basis for generalizations concerning the impact of environment and nutrition, in particular, on breast cancer incidence.

1. *International Variation*

Risk of breast cancer has been shown to vary according to geographical area; high incidence rates are found in North America and Western Europe, low rates in Asia, particularly Japan, and intermediate rates prevail in Finland, Southern Europe, and South America (Fig. 8). These differences suggest that environmental factors relate to this disease. It is noteworthy that in Western countries, breast cancer incidence continues to rise with increasing age after menopause, whereas it remains unchanged over a wide age span in postmenopausal Japanese. Recently Moolgavkar *et al.* (1979) reported that incidence and mortality curves for postmenopausal Japanese and Western women increased with age, but the degree of increase was less in the former group than in the latter. These observations suggest that the action of the factors involved in breast cancer is more pronounced in the postmenopausal age group in high-risk Western populations.

2. *Migrant Studies*

The strongest evidence for environmental factors in the etiology of breast cancer is found in the results of migrant studies. Within two to three generations, Japanese migrants to the U.S. experience an increase in cancer incidence rates from those common in Japan to those prevalent in the United States (Fig. 9). In fact, Buell (1973) reported that during the years 1969–1971, the incidence of breast cancer in Japanese–American women had risen to one-half that of white American women and was five times that of age-matched native Japanese women. Staszewski and Haenszel (1965) showed that breast cancer incidence among Polish immigrants to the United States exceeded both urban and rural incidence rates in Poland. Of interest is the short time period in which these rates changed in comparison to those of Japanese immigrants. Possibly, Polish

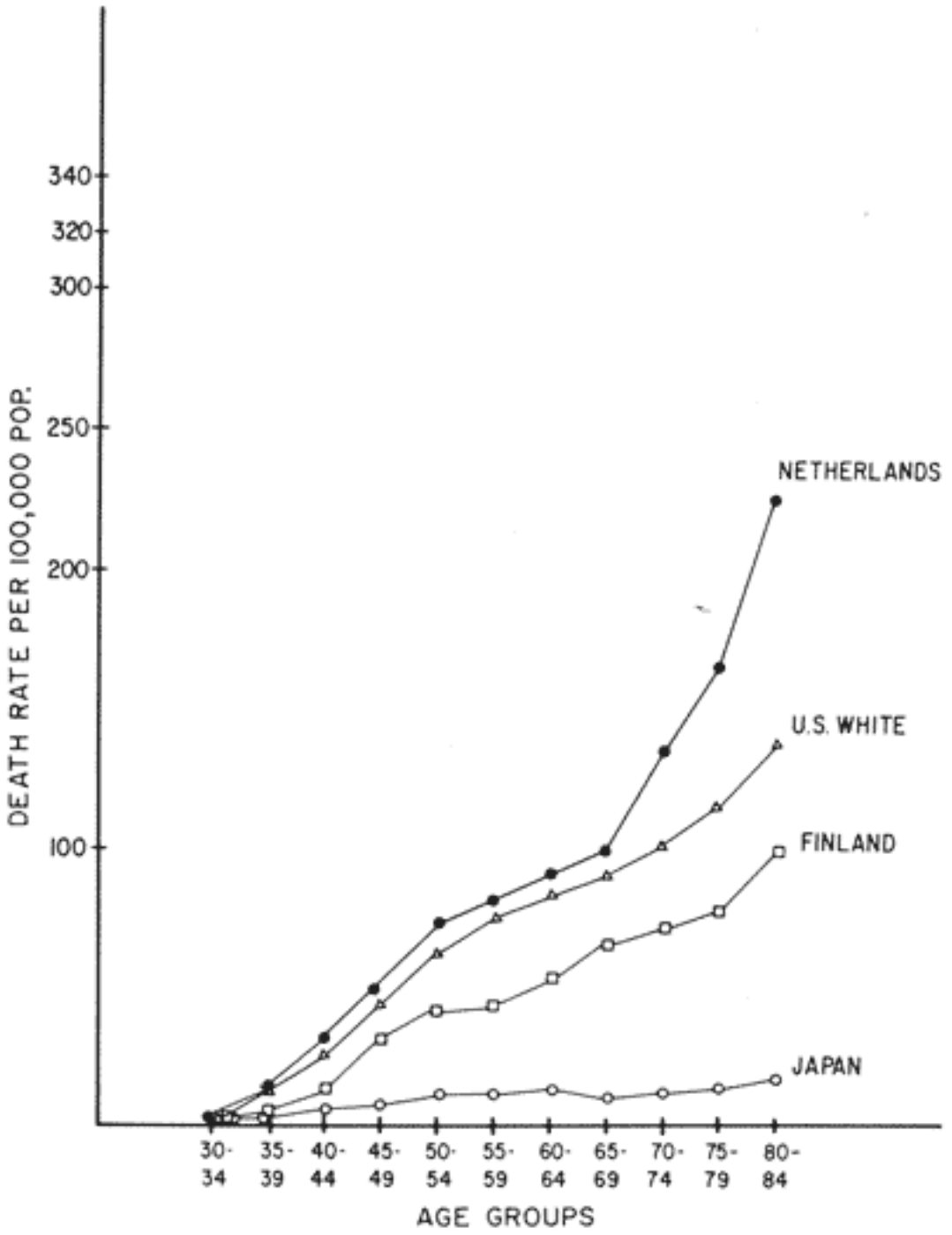

FIG. 8. Female breast cancer death rates by age in four countries, 1966–1967. (From Segi and Kurihara, 1972.)

migrants assimilate more quickly and, therefore, adapt more rapidly than the Japanese.

Although a number of reproductive variables have been associated with differences in rates of breast cancer (Hems, 1970; MacMahon *et al.*, 1973; Hirayama, 1978), not one or any combination of variables can explain the changes in rates seen in migrant populations. Alterations in dietary practices, however, appear to be the environmental factor that best accounts for the increase in risk associated with migration from a low-risk to a high-risk country (*Brit. Med. J.* Editorial, 1974).

3. *Correlation Analyses*

Correlation studies have provided another source of evidence for nutrition's role in the etiology of breast cancer. A positive correlation between breast cancer mortality and daily per capita consumption of fat has been demonstrated by a number of researchers (Lea, 1967; Drasar and Irving, 1973; Carroll, 1975; Armstrong and Doll, 1975) (Fig. 10). Further, Carroll and Khor (1975) and a supporting study by Hems (1978) have shown that in various countries, a strong positive correlation exists between animal fat consumption and breast cancer incidence, but a similar association could not be demonstrated for vegetable fat. In an intracountry investigation, Hirayama (1978) correlated breast cancer incidence in 12 different districts of Japan with specific food consumption patterns. Of the food items studied, the highest positive correlation was found for pork, followed by total animal fat intake.

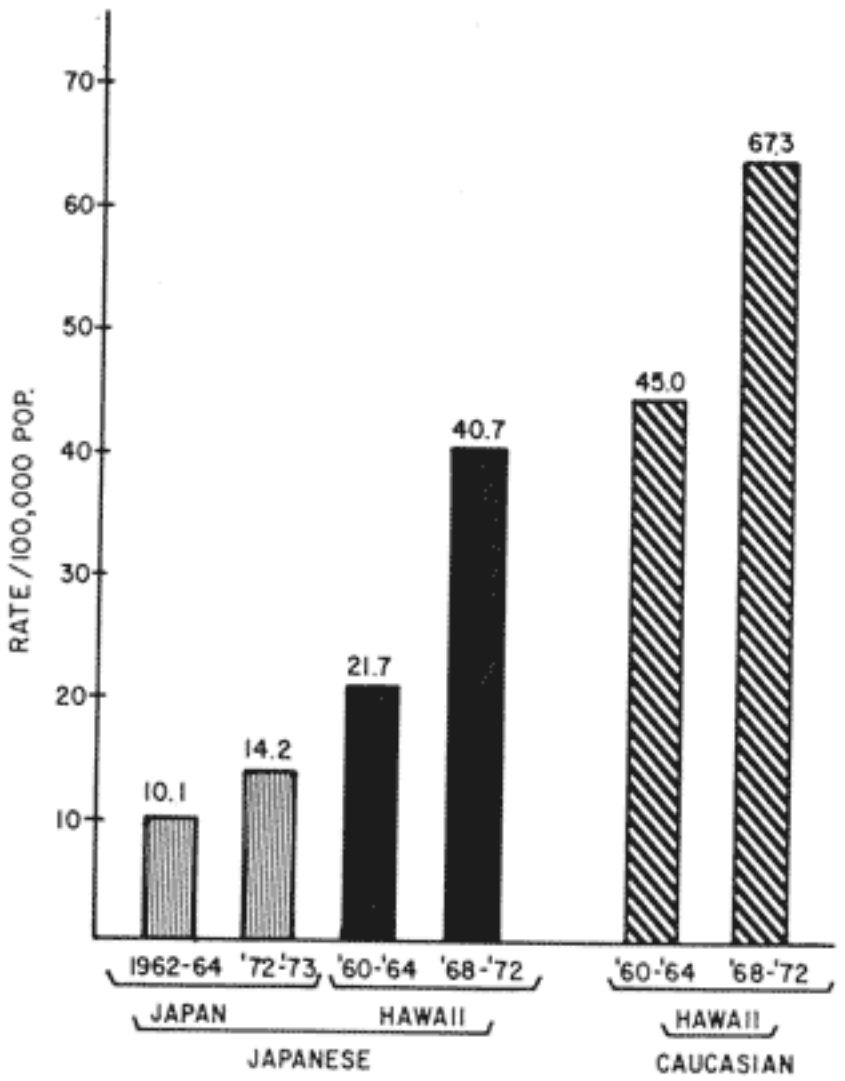

FIG. 9. Age-adjusted breast cancer incidence rates in native Japanese (striped bars), Hawaiian Japanese (solid bars), and white Hawaiians (hatched bars). (From Wynder and Hirayama, 1978.)

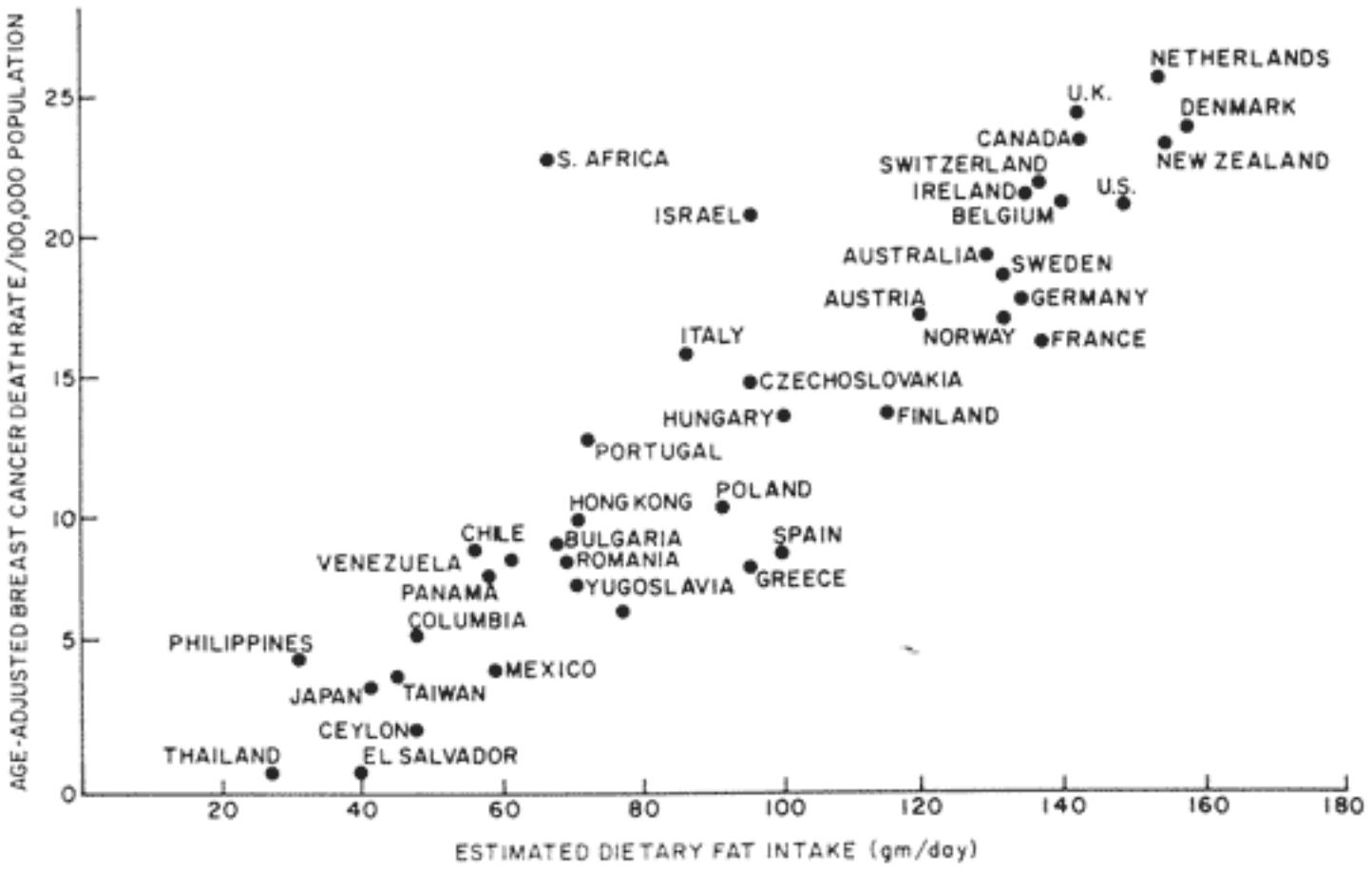

FIG. 10. Correlation between age-adjusted death rates from female breast cancer and per capita consumption of fat. (From Carroll and Khor, 1975.)

4. *Time-Trend Analysis*

Analysis of U.S. food consumption patterns over time provide support for an association between fat disappearance and breast cancer. From 1909 to 1972, the estimated consumption of dietary fat increased from 125 to 156 grams per day per capita (Gortner, 1975). During the same period, breast cancer mortality rates among U.S. whites showed a small but gradual increase (Seidman *et al.*, 1976).

Over the past 40 years, American blacks have experienced a marked increase in breast cancer incidence, from 50/100,000 in 1935 to approximately 65/100,000 in 1970 (Cutler and Young, 1975; Seidman *et al.*, 1976). During this time, large scale black migration from the rural South to the urban North occurred. Accompanying this migration were marked changes in socioeconomic status and lifestyle.

Shifting patterns in breast cancer incidence over time in low-risk countries undergoing Westernization also support an association between high fat intake and increased breast cancer incidence. Hems (1978) found that the temporal changes in breast cancer rates for 20 countries were significantly correlated with total fat and animal protein intake and not obesity,

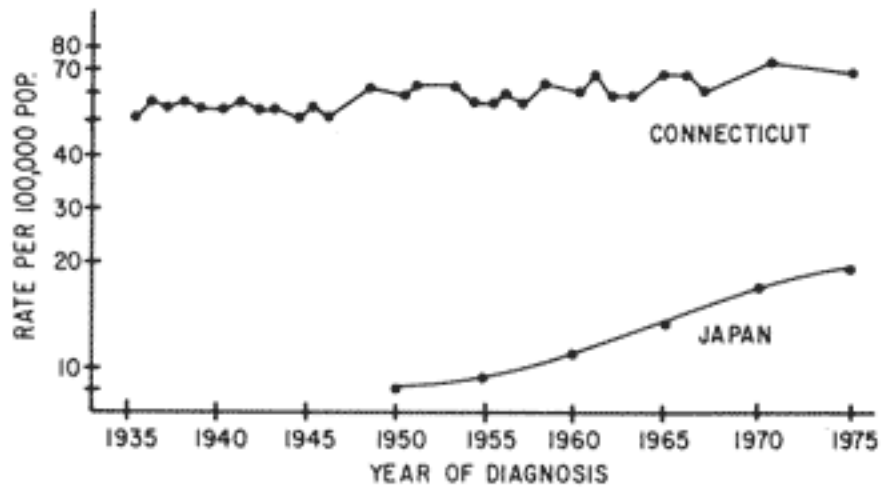

FIG. 11. Changing breast cancer incidence rates in Connecticut (USA) and Japan, 1950–1975. (From Connecticut State Department of Health, 1977, and Hirayama, 1978.)

reinforcing the idea that dietary fat *per se* may serve as a risk factor in breast carcinogenesis.

During the past decade, breast cancer mortality and morbidity rates have sharply increased in Japan (Hirayama, 1978) (Fig. 11). This increase has been associated with a marked shift toward a more Western lifestyle, one in which intake of dietary fat has substantially increased (Fig. 12). For example, in the period 1957 to 1973, the estimated per capita consumption of fat increased from 23 to 52 grams per day. This increasing consumption of fat parallels the increase in the number of annual deaths from breast cancer (1572 in 1955 and 3262 in 1975).

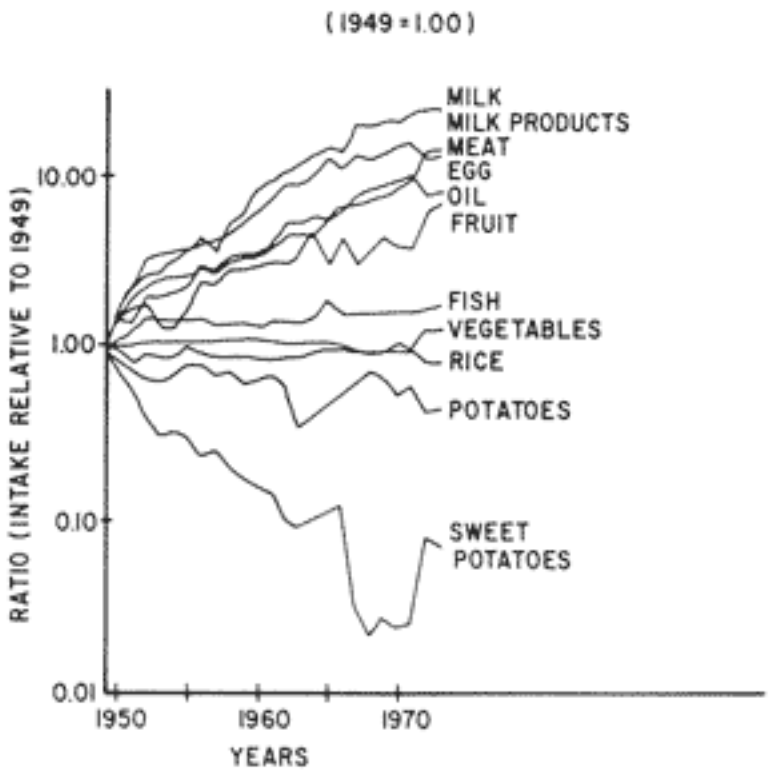

FIG. 12. Change in intake of selected foods in Japan, 1949–1973. (From Hirayama, 1978.)

Although indicative of important trends, correlations involving worldwide fat consumption data must be viewed with caution. Fat consumption data, as compiled by the Food and Agricultural Organization of the United Nations (1971). are only rough approximations of the disappearance of dietary fat in a given population and not the actual per capita consumption. Moreover, they generally tend to correlate contemporary incidence or mortality data with recent food disappearance data, although migration studies suggest that an interval of 30+ years may be more appropriate. Despite these caveats, the correlation between breast cancer mortality and high fat intake is highly consistent both within and between country comparisons.

5. *Special Populations*

Studies of populations that share a common gene pool with the general population but differ significantly in dietary practices, e.g., Seventh-Day Adventists, have indicated that the incidence of breast cancer in this group that eats little or no meat is lower than that found in the general population (Phillips, 1975). Although the difference was not statistically significant, the lower rates are real and this further implicates animal fat ingestion in the etiology of this disease. Moreover, Israeli Jews of Asian origin exhibit lower breast cancer incidence rates in comparison to the Israeli population of European origin (27/100,000 vs 124/100,000) (Gross *et al.*, 1977).

6. *Case-Control Studies*

The value of case-control studies in the investigation of diet and disease is limited because of the difficulties inherent in obtaining accurate historical dietary information. Morgan *et al.* (1978) attempted to refine the methodology of such studies by utilizing three dietary assessment techniques, i.e., 24-hour recall, a four-day diet diary, and a detailed diet questionnaire. Major discrepancies were found among these methods, and it was suggested that the most reliable source of dietary information was obtained by the detailed dietary questionnaire. Recognizing such difficulties, Miller (1977) studied the nutrition/breast cancer relationship in a carefully controlled retrospective study. He demonstrated that saturated fat intake was associated with breast cancer incidence in both pre- and postmenopausal women.

In a recent large-scale prospective study of Hawaiian Japanese, dietary practices of the spouses of women with breast cancer were compared with those of the spouses of women who did not have breast cancer

(Nomura *et al.*, 1975). Again, it was concluded that consumption of a high-fat Western diet contributed to increased breast cancer risk in these migrant Hawaiian Japanese women. The consequence of the findings from these two distinct methods of investigation lends credence to the relationships found.

In a country such as the United States, however, dietary patterns are only partially dictated by custom, and thus accurate dietary histories are more difficult to collect. Some reported inconsistencies in results apparently relate to differences in the selection of the study population and/or the control group. Whole population comparisons, of course, are possible particularly when they reflect widespread differences among populations with regard to food traditions and food availability.

We are in agreement with Miller (1977), Hems (1978), Gray *et al.* (1979), and Hirayama (1978) when we stress that variables reported to affect the risk of breast cancer (onset of menarche, age at menopause, age at first pregnancy, menstrual patterns, family history, fertility, height, or weight) do so at relatively low magnitudes, when compared to the major differences in risk between women living in high- and low-risk countries. We conclude that case-control studies, of the type conducted thus far are not likely to shed much additional light on the etiology of breast cancer.

To summarize epidemiological findings:

1. Inter- and intranational studies, correlation studies, time-trend analyses, and analyses of special populations support the suggestion that an ubiquitous environmental factor may be significantly associated with breast cancer risk. Major dietary factors, particularly the intake of fat, affecting an entire population may represent that basis for the differences in breast cancer rates in various populations.

2. A strong argument for an environmental etiology of breast cancer comes from analysis of population groups migrating from countries of low incidence to countries of high incidence. Characteristically, these groups experience an increase in the incidence of breast cancer in successive generations, eventually approaching the incidence rates of the host population.

3. The concept that dietary fat intake, specifically, is an important determinant of breast cancer is supported by the following observations: (a) a significant positive correlation exists between estimates of dietary fat consumption (both saturated and unsaturated fats) and international mortality rates of breast cancer; (b) results of time-trend analysis in populations undergoing Westernization suggest a positive correlation between breast cancer incidence and consumption of dietary fat; and (c) analysis of special population groups suggest an association between

breast cancer incidence and dietary fat intake. The combined evidence from epidemiological studies, therefore, suggest that dietary fat is an important contributory factor in breast cancer.

4. Since fat and protein consumption parrallel one another in most human populations, on the basis of present information a role for dietary protein intake in breast carcinogenesis cannot be excluded.

B. Experimental Studies

Through the efforts of Tannenbaum (1942), the field of nutritional carcinogenesis experienced a burst of creative activity in the early 1940s. In 1967, interest in this field was regenerated with the publications by Carroll and co-workers of nutritional studies using the 7,12-dimethylbenz(*a*)anthracene mammary tumor model (Carroll, 1975). Today there exists a great deal of knowledge upon which to develop and test mechanistic hypotheses of the relationship between nutrition and breast cancer.

Tannenbaum's early work (1957) brought to light four major facts concerning the relationship of dietary fat to breast cancer, facts which are of compelling interest today: (1) the quantity of dietary fat, holding carbohydrate and protein constant, influenced the subsequent development of mammary cancer; (2) the time of initiation of the diet influenced the course of tumor development; (3) the reproductive history of the animals was an important experimental variable; (4) caloric restriction has a profound retarding effect on the genesis of mammary tumors, as well as tumors at other organ sites. In 1947, he first demonstrated the initiating effect of dietary fat—spontaneous breast tumor incidence rates in female DBA mice were higher in those fed a high-fat diet than in those fed a low-fat diet. Further, tumor incidence was found to be greater in mice when the high-fat diet was initiated at 24 weeks of age than at 38 weeks.

In the ensuing three decades, Tannenbaum's findings have been confirmed by a variety of investigations in both rats and mice. Despite differences in the type or quantity of fat, the nature of the carcinogenic event (spontaneous or chemical), or the strain of animal used, one point stands out clearly: high intake of dietary fat increases the incidence of mammary cancer in rodents (Fig. 13).

1. *Obesity and Caloric Restriction*

Experimentally, obese mice tend to have a higher tumor incidence than normal animals (Tannenbaum and Silverstone, 1957). However, mice

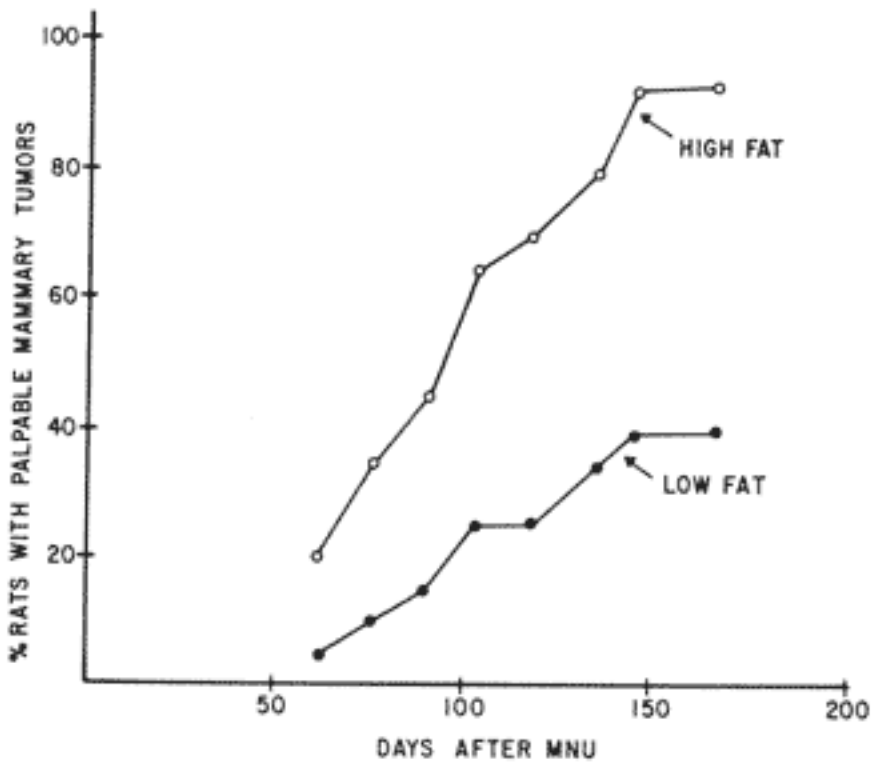

FIG. 13. Effect of high- or low-fat diet on methylnitrosourea(MNU)-induced breast cancer incidence in female F344 rats. MNU was administered intravenously at 55 days of age and the rat was then fed high-fat (20% lard) or low-fat (5% lard) diet. The median latent period (time when 50% of tumor-bearing rats had developed tumors) was 83 days in the high-fat group and 103 days in the low-fat group. The tumor incidence differed significantly from the eightieth day on ($P < 0.01$). (From Chan *et al.*, 1977.)

kept on isocaloric high- and low-fat diets exhibit greater tumor incidence under high-fat conditions, suggesting that the fat effect is separate from the effect of high caloric intake (Tannenbaum, 1942). It has also been reported that rats fed a high-fat diet *ad libitum* have about the same rate of weight gain as rats fed a low-fat diet, but exhibit more tumors with a shorter latency period than animals maintained on a low-fat diet (Chan and Cohen, 1974). This experiment also indicates that the effect of high-fat diets is independent of changes in body weight and caloric intake.

As originally demonstrated by Tannenbaum, caloric restriction inhibits the development of breast cancer in rodents. Because the effects of changing the fat content of the diet were confined to skin and mammary tumors, whereas caloric restriction per se inhibited other tumors as well, Tannenbaum and Silverstone (1957) concluded that such effects were related to the specific action of fat rather than to the general influence of caloric restriction.

2. *Dietary Fat as a Tumor-Promoting Agent*

Since the spontaneous mouse mammary tumor model was used in Tannenbaum studies, it is impossible to discern whether the effect of

and West (1976), Hankin and Rawlings (1978), Alcantara and Speckmann (1976), and Miller (1977). A variety of mechanistic hypotheses have emerged from these reviews. They can be broken down into two broad categories: (a) direct effects at the level of the mammary gland, and (b) indirect effects mediated by host systems remote from the mammary gland.

a. Direct Effects. These models are based on the physical and chemical properties of fat, the formation of lipid peroxides, alterations in membrane structure and/or function, and enhanced prostaglandin synthesis.

The role of adipose tissue in breast carcinogenesis (either acting as a reservoir or depot for carcinogens) has been a regularly reappearing theme over the past 30 years. Since adipose tissue rapidly takes up and slowly releases lipid-soluble agents, it has been proposed that the adipose tissue surrounding ductal cells may contribute to the susceptibility of tissue cells to cancer by prolonged exposure to lipid-soluble carcinogens of endogenous or exogenous origins (Beer and Billingham, 1978; Tannenbaum and Silverstone, 1957). Years ago, Dao *et al.* (1959) suggested that mammary adipose tissue functioned as a storage depot for hydrocarbons. However, this model appears unlikely since tumor incidence is not affected by a high-fat or low-fat diet given prior to treatment with DMBA and then placed on a high-fat diet (Carroll and Khor, 1971). Gammal *et al.* (1968) demonstrated that uptake and clearance of radioactive DMBA in the mammary fat pad was the same, regardless of fat content in the diet.

Since polyunsaturated fatty acids (PUFA) are converted by free radical reactions to lipid peroxides, a model involving breast cancer and lipid peroxidation has been advanced. Lipid peroxidation has been associated with a variety of pathological processes (Dormandy, 1978), including mutagenesis (Mukai and Goldstein, 1976) and carcinogenesis (Shamberger *et al.*, 1974). It is possible that increased peroxidation of membrane lipids results in alterations in the function of transformed mammary cell membranes which, in turn, permit increased rates of growth (Hopkins and West, 1976). However, available evidence suggests that lipid peroxidation and free radical processes accompanying it are primarily associated with the activation of procarcinogens (Bartsch *et al.*, 1976; Floyd *et al.*, 1978) and not with the promotion of tumor development. Little evidence is available to suggest a role for peroxidation processes in the post transformation events, where dietary fat exerts its stimulatory effect.

Rao and Abraham (1975) reported an unstable lipid composition in mouse mammary adenocarcinoma when the fat intake of the host was

altered. Within a relatively short period (8 weeks), changes in dietary fat were reflected in altered tumor lipid profiles. Similar findings were reported by Gammal *et al.* (1967) and Nishizuka *et al.* (1978). Tumor tissue tends to have greater proportions of PUFA than the normal gland (Rao and Abraham, 1975); this observation suggests that tumor growth is related to arachidonic availability through either prostaglandin (PG) synthesis or unknown membrane functions.

Direct changes in the lipid composition of cell membranes induced by diet could have far-reaching structural and functional effects on membrane permeability and the activity of membrane-bound enzyme systems. The relevance of these membrane changes to the stimulatory effect of a high-fat diet remains to be established however.

Prostaglandins apparently act as intracellular regulatory molecules that, together with the cyclic adenosine monophosphate (cAMP) systems, govern the response of cells to hormonal stimulation. In human breast cancer, the capacity of tumor cells to produce prostaglandins increases from normal cells, to benign lesions, to primary tumors, to metastatic cancers. In addition, in animals, DMBA-initiated tumors contain more prostaglandins and a greater capacity to synthesize prostaglandins than normal rat mammary glands (Tan *et al.*, 1974). However, daily injections of 1.5 mg/kg $PGF_{2\alpha}$ have reduced the growth of DMBA-induced mammary tumors over a 10-day period (Jacobson, 1974).

b. Indirect Effects. Indirect mechanisms are those in which dietary fat secondarily stimulates mammary tumor growth by modifying the physiology of the host. Some evidence exists that dietary fat intake alters the function of at least three major systems that regulate the internal environment: (1) immune rejection responses; (2) mixed-function oxidases (MFO); and (3) endocrine control systems. These mechanisms are not necessarily mutually exclusive and may interact with each other. The experimental evidence is as follows: Mice fed a diet containing PUFA are more susceptible to the development of tumors following inoculation with cells from a transplantable tumor than mice fed a diet containing saturated fatty acids (Hopkins and West, 1976). PGE_1 and PGE_2, which are synthesized from PUFA, are present, along with their precursor molecules, in greater amounts in the DMBA tumor than in normal mammary tissue (Tan *et al.*, 1974); PGE_1 and PGE_2 (Plescia *et al.*, 1975) arachidonate and linoleate (Mertin *et al.*, 1973) inhibit cell-mediated immune responses in the lymphocyte test system. On the basis of this evidence, it has been proposed that increased amounts of dietary PUFA may suppress the cell-mediated immune rejection mechanism, either directly or after conversion to prostaglandins and, thereby, enhance the

capacity of transformed cells to proliferate (Hopkins and West, 1976). Quantitative analyses of the immunosuppressive influence of a high-fat diet are needed to confirm this hypothesis.

Evidence suggests that nutritional factors can markedly influence the activity of microsomal MFO (Campbell, 1977). It is well established that mixed function oxidase systems are key in the biotransformation of chemical carcinogens (Weisburger and Williams, 1975) and steroids (Gustafsson *et al.*, 1975). Steroids such as androgens and estrogens have been implicated in breast carcinogenesis, and alterations in steroid metabolizing enzyme systems such as MFO by diet could, in turn, influence breast cancer development. However, although the biotransformation of carcinogens to their active metabolites is a major role of MFO, it is unlikely that this aspect of MFO function is involved in the fat effect, since the influence of fat is exerted days or weeks after carcinogen administration.

The work of Furth (1973), Pearson (1972), and Meites (1977) demonstrated clearly that although a variety of hormones are involved in mammary tumor growth, prolactin is the predominant factor in rodent breast cancer development. In addition, Smith *et al.* (1977) and Costlow and McGuire (1977) demonstrated unequivocally that the DMBA induced tumor and the R3230AC mammary tumor possess large quantities of specific prolactin binding receptors; and Kelly *et al.*(1978) showed that prolactin binding was highest in those tumors that showed the greatest growth response to prolactin injection.

Meites (1977) showed in the DMBA model that experimental procedures that elevate serum prolactin (median eminence lesions, treatment with reserpine, or transplantation of secondary pituitaries under the renal capsule) increase tumor yield. It should be noted that tumor yield is enhanced only if the prolactin elevating procedure is introduced after, not before, DMBA administration—a response strikingly reminiscent of the fat effect on breast carcinogenesis.

The discovery of hypothalamic neurotransmitters capable of regulating prolactin secretion by the anterior pituitary led to the concept that circulating prolactin concentrations may be regulated by environmental factors acting on the central nervous system (CNS). Meites (1977) found that administration of drugs that act by way of the CNS could elevate or depress serum prolactin levels and thereby promote or inhibit mammary tumor growth. In this regard, dietary fat may act via the CNS in a manner similar to that of reserpine, perphenazine, or thyrotropin releasing hormone, namely, by causing metabolic alterations (in the synthesis or action of biogenic amines) that result in the induction of prolactin secretion by the pituitary and ultimately in promotion of mammary tumor development. Early experimental evidence suggesting a CNS mechanism for the

fat effect comes from the studies of Dunning *et al.* (1949). These investigators found that tumor incidence was elevated in rats fed a high-fat diet (40% Crisco) and that the pituitary glands were significantly heavier in high-fat than in low-fat animals. In addition, histological examination of the mammary glands of high-fat animals revealed signs of intense secretory activity. Similar results were reported by Benson *et al.* (1956) and Cutler and Schneider (1974).

Though unknown or ignored by most biomedical researchers, there is considerable literature from comparative physiology suggesting an important role for prolactin, and the regulation of fat metabolism has no doubt been obscured by the emphasis (exemplified by the very name of the hormone) placed on the relationship between prolactin and lactation. However, it is clear that prolactin and growth hormone share amino acid sequence homologies and probably evolved from a common ancestral protein (Niall *et al.*, 1971; Nicoll, 1975). Since growth hormone is intimately involved in the regulation of energy fuels such as fat (Fineberg *et al.*, 1972; Fraser and Blackard, 1977), it is not surprising that prolactin also plays a role in fat metabolism.

Studies concerning the liporegulatory role of prolactin have been conducted principally by Meier (1977) in amphibians, birds, and lower mammals. It is clear from these works that mobilization and deposition of fat is regulated by diurnal (CNS-mediated) variations in serum prolactin concentrations and, furthermore, that these changes are temporarily associated with changes in serum corticosteroid concentrations.

Insight into the mechanism by which prolactin regulates lipid mobilization and deposition is provided by the work of Scow and associates on the enzyme lipoprotein lipase (*Nutrition Review* editorial, 1975; Zinder *et al.*, 1974). Scow found that the activity of this enzyme in the rat was elevated during lactation specifically by prolactin. Since lipoprotein lipase activity represents the major route by which triglyceride fatty acids are cleared from the circulation, induction of this enzyme by prolactin substantiates a liporegulatory function for this hormone.

Based on the foregoing considerations, Chan and Cohen (1974) designed a series of experiments to assess the relationship between high-fat intake, prolactin secretion, and mammary carcinogenesis. Drugs that antagonize estrogen action and block prolactin secretion retarded tumor development as expected. Only the antiprolactin drug abolished the differential in tumor incidence characteristic of animals fed high- and low-fat diets (Table XII). Though indirect, the results suggested that the fat effect was mediated by prolactin.

It was also found that rats fed a high-fat diet exhibited significantly higher serum prolactin levels than rats fed a low-fat diet (Chan *et al.*,

TABLE XII
EFFECT OF HORMONE ANTAGONISTS ON MAMMARY TUMOR INCIDENCE IN DMBA-TREATED RATS FED HIGH-FAT AND LOW-FAT DIETS[a]

		Palpable tumor-bearing rats per total number of rats		Total number of Adenocarcinomas		
	Diet[b]	Number	Percent	Palpable	Nonpalpable	Palpable tumors per rat
Control	HF	18/22	82	39	12	1.77
Control	LF	10/19	52	15	7	0.78
Antiestrogen[c]	HF	7/18	39	7	0	0.38
Antiestrogen	LF	1/13	8	1	2	0.08
Antiprolactin[d]	HF	9/21	42	15	9	0.70
Antiprolactin	LF	8/22	36	12	7	0.54

[a] From Chan and Cohen (1974).
[b] Hf diet, 20% corn oil; LF diet, 5% corn oil.
[c] Nafoxidine hydrochloride (1 mg/kg body weight) administered sc three times.
[d] 2-Bromo-α-ergocryptine (3 mg/kg body weight) administered sc three times.

1975). Moreover, this increase was seen during the proestrus-estrus surge of the estrous cycle; no difference in prolactin levels occurred during the metestrus–diestrus stages, suggesting an influence of fat on the hypothalamic centers controlling circadian rhythms of prolactin secretion.

It is also possible that dietary fat acts either at the ovary or at higher centers controlling ovarian steroidogenesis, thereby stimulating prolactin secretion and breast tumor development. This hypothesis is contradicted by evidence from experiments in which DMBA-treated rats were ovariectomized one month after initiation and then placed on high-fat or low-fat diets (Chan *et al.*, 1977). Ovariectomized animals fed a high-fat diet exhibited a marked increase in both tumor incidence and serum prolactin levels when compared to animals fed a low-fat diet (Fig. 14).

On the other hand, these results do not indicate that a role for estrogens in the fat effect is completely eliminated. Estrogens synthesized by peripheral aromatization of androgens could influence prolactin secretion patterns and, thereby, indirectly influence mammary tumor development (*Nature (London)* Editorial, 1975). Of importance from the standpoint of human breast cancer, this experiment may provide insight into dietary factors operating in postmenopausal women and deserves further detailed study.

In a general sense, these results reinforce the idea that the enhancing effect of dietary fat on breast cancer development is not mediated by an

ovarian mechanism either at the level of the ovary or at higher centers controlling the secretion of estrogens by the ovary.

Direct proof that dietary fat alters circulating prolactin levels, but not estrogen levels, was obtained by simultaneous measurement of prolactin and estrogen levels in the serum of methylnitrosourea (MNU)-treated animals 20 weeks after carcinogen treatment. The high-fat groups exhibited: (a) elevated prolactin levels; (b) unchanged total estrogens; (c) elevated prolactin to estrogen ratios at both proestrus–estrous, and metestrous–diestrous (though significance could be demonstrated only in the latter phases due to the small number of proestrus–estrous samples available) (Table XIII).

These results are in keeping with a small but growing body of knowledge that suggests that the development of both normal rodent mammary gland and mammary tumors are regulated by the relative proportions of circulating prolactin and estrogen (Welsch and Nagasawa, 1977; Wuttke *et al.*, 1976).

Boyns *et al.* (1973) and Hawkins *et al.* (1976) found that in three strains of rats with different genetically determined susceptibilities to the carcinogenic action of DMBA, tumor yield was directly proportional to plasma

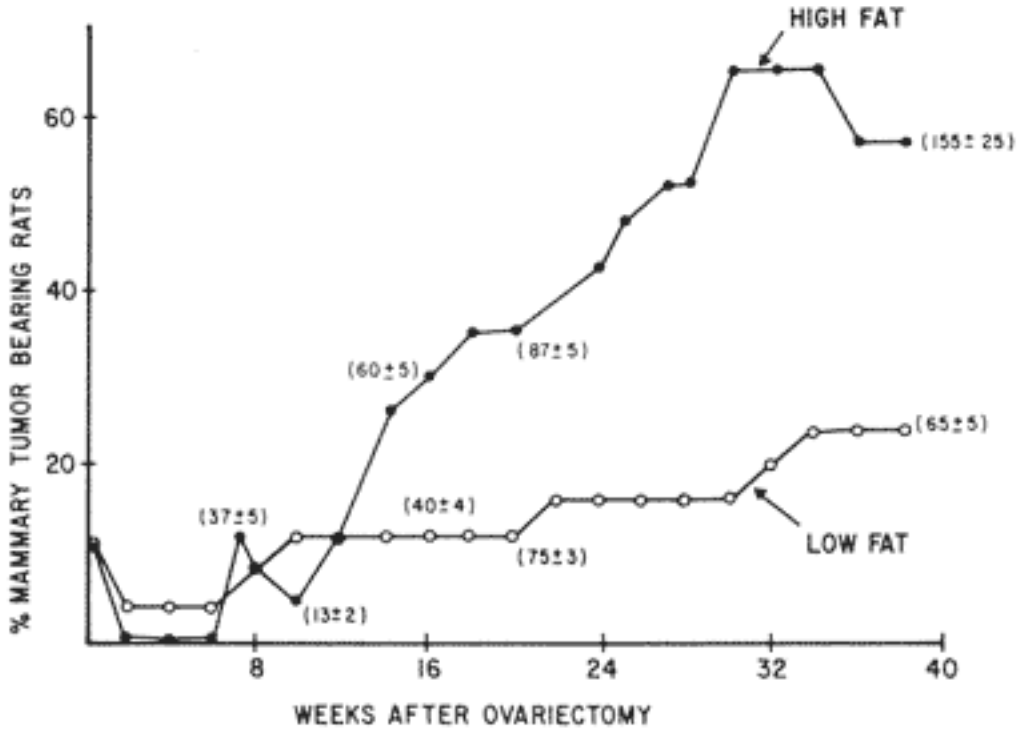

FIG. 14. Effect of high-fat or low-fat diet on mammary tumor incidence in ovariectomized rats. Curves represent cumulative tumor incidence. DMBA (5 mg/kg) was administered on day 50 of age. Ovariectomy was performed on day 130 of age and the animals were then placed on either high-fat (20% lard) or low-fat (5% lard) diet. Experiment was terminated 9 months after ovariectomy. Numbers in parentheses represent serum prolactin concentrations (mean ± SEM). (From Chan *et al.*, 1977.)

TABLE XIII
INFLUENCE OF DIETARY FAT ON SERUM HORMONAL PROFILES AND MAMMARY TUMOR INCIDENCE IN METHYLNITROSOUREA-TREATED F344 RATS[a]

Diet	Tumor incidence (percent)	Estrous stage	Prolactin (ng/ml)	Total estrogen[b] (ng/100 ml)	Prolactin and estrogen ratio
High fat (20% lard)	90	P-E[c]	237 ± 98[d]	23.7 ± 1.7	11.1 ± 4.8
		M-D	100 ± 29	22.2 ± 2.0	4.5 ± 0.9
Low fat (5% lard)	40	P-E	140 ± 79	23.2 ± 2.2	6.4 ± 3.9
		M-D	38 ± 3	18.9 ± 1.1	2.0 ± 0.1

[a] From Chan *et al.* (1977).
[b] Estradiol + estrone.
[c] P-E, Proestrus-Estrus; M-D, metestrus-diestrus.
[d] Mean ± SEM.

prolactin levels, whereas estrogen levels were unchanged—again suggesting that rat mammary tumor development is a function of the relative proportions of circulatory prolactin and estrogen.

The evidence clearly shows that in the rat, dietary fat induced periodic increases in serum prolactin levels; although the mechanism by which this occurs is obscure.

In summary, studies in model systems support the proposition based on epidemiological considerations, that increased dietary fat intake is positively associated with breast cancer risk. Although a variety of models, both direct and indirect, have been proposed to explain the fat effect, available evidence favors a model involving alteration of the neuroendocrine mechanism regulating the secretion of the pituitary hormone, prolactin. Prolactin is proposed to mediate the fat effect by virtue of its dual capacity as a liporegulatory hormone and as a promoter of mammary tumor development. At the level of the mammary tumor cell, the absolute levels of prolactin may be less important than the relative proportions of prolactin and estrogens.

C. METABOLIC EPIDEMIOLOGY

As stated, leads established by epidemiological studies and tested in animal models must be confirmed in the human setting. Therefore, in the following section, the association in humans between hormones, particularly prolactin, and breast cancer risk will be discussed. Emphasis will be placed on nutritional factors as they relate to the endocrine system.

Comprehensive reviews of this area have been published by MacMahon *et al.* (1973), Zumoff *et al.* (1975), Lipsett (1975), and Hayward (1979).

1. *Urinary Androgens and Breast Cancer Risk*

In the early 1960s, Atkins *et al.* (1964) reported that the response of breast cancer patients to adrenalectomy was positively associated with the excretion of two urinary androgen metabolites, the 17-ketosteroids, etiocholanolone, and androsterone. In a large-scale prospective study involving women between the ages of 30–55 years, Bulbrook *et al.* (1962) found that women who eventually developed breast cancer exhibited significantly decreased excretion of urinary 17-ketosteroids when compared to women who did not develop the disease.

On the basis of these early observations, hypothesis was advanced suggesting that breast cancer risk was increased in women who exhibited subnormal excretion of urinary androgen metabolites (Bulbrook *et al.*, 1962, 1971; Bulbrook and Hayward, 1967). However, not all case-control studies confirmed Bulbrook's hypothesis. Urinary androgen metabolites in breast cancer patients were reported to be similar to (Wade *et al.*, 1977; Cameron *et al.*, 1979); and even higher than matched controls (Benard *et al.*, 1962). Case-control comparisons carried out in Japan and Poland also contradicted Bulbrook's hypothesis. Tominaga *et al.* (1975) reported that Japanese breast cancer patients excreted significantly greater amounts of urinary dehydroepiandrosterone sulfate than healthy controls in a population of women (mean age 41). No differences were found in androsterone or etiocholanolone excretion. Sonka *et al.* (1973) reported similar results in a population of postmenopausal Polish women (ages 56–63 years).

When international comparisons were made, the relationship between subnormal androgen metabolite excretion and increased risk for breast cancer did not confirm the Bulbrook hypothesis. Healthy British women (high-risk) exhibited higher, rather than lower, urinary 17-ketosteroid excretion rates than healthy Japanese women (low-risk) (Bulbrook and Hayward, 1967; Hayward *et al.*, 1978). Moreover, in a recent study comparing androgen metabolite excretion in four different ethnic groups with disparate risks for breast cancer, no differences in urinary 11-deoxy-17-ketosteroids were found among the groups studied (Gross *et al.*, 1977).

2. *Plasma Androgens and Breast Cancer Risk*

Evidence has been presented suggesting that plasma levels of DHEA, a major adrenal secretion product, were decreased in postmenopausal breast cancer patients when compared to matched controls (Poortman *et*

al., 1973). However, this relationship could only be demonstrated after mastectomy, and was not shown in an earlier study by Alqvist *et al.* (1968). More recently, Wang *et al.* (1977) reported that no significant difference could be found between mean plasma levels of androstenedione (which is secreted in substantial amounts by the postmenopausal ovary as well as the adrenals) in normal Japanese women compared to breast cancer patients; and as Poortman *et al.* (1973) previously found, subnormal values could be detected after mastectomy, but not before. If subnormal plasma androgens are associated with breast cancer, it is unusual that they cannot be demonstrated prior to surgery. In fact, the surgery itself may be responsible for the altered androgen concentrations noted.

Hill *et al.* (1976b) compared plasma androgen profiles in healthy urban U.S. white (high-risk), Bantu, and Japanese women (low-risk). Among premenopausal women, no significant differences were found in plasma testosterone levels. Mean plasma levels of DHEA, the chief blood precursor of etiocholanolone and androsterone, were significantly higher in Bantu than in U.S. white and Japanese women; plasma androstenedione levels, however, were similar in the three populations. Among postmenopausal women, although mean plasma levels of DHEA were similar in the three populations, plasma androstenedione was markedly elevated in U.S. whites compared to both Japanese and Bantu women. The relevance of the latter finding to cancer risk is unclear at present.

A recent study by Hayward *et al.* (1978) compared several steroid hormones in native Japanese, Hawaiian Japanese, and British women. Plasma androstenedione and urinary 11-deoxy ketosteroids and etiocholanolone levels in Hawaiian Japanese were similar to those in native Japanese women but significantly less than those in British women. Plasma DHEA sulfate levels in Hawaiian Japanese women resembled those of British women and were significantly higher than those in native Japanese women.

In light of the conflicting results obtained from urinary and plasma androgen metabolite studies and the absence of information concerning the physiological role of androgens in breast carcinogenesis, the idea that risk of breast cancer is a direct result of adrenal dysfunction must be regarded with skepticism at present. Nonetheless, since changes in androgen metabolism are commonly found in high-risk groups, one cannot entirely exclude on the basis of present evidence a role for androgens in the etiology of breast cancer.

3. *Urinary Estrogens and Breast Cancer Risk*

The majority of studies in this area have centered around what is commonly known as the estriol hypothesis. Interest in the urinary me-

tabolites of estrogen, estrone (E_1), estradiol (E_2), and estriol (E_3) centered initially on their use as prognostic aids for therapy. In 1966, Lemon *et al.* (1966) reported that premenopausal breast cancer patients exhibited reduced estriol excretion rates compared to matched controls. Later, Cole and MacMahon (1969) suggested that reduced estriol excretion early in reproductive life predisposed to future breast cancer. Stated briefly, the estriol hypothesis holds that the risk for breast cancer is inversely proportional to the ratio of urinary E_3 to E_1 and E_2 (Lemon, 1976).

A number of studies, including case-control comparisons (Lemon *et al.*, 1966), international comparisons (MacMahon *et al.*, 1971), migration studies (Dickinson *et al.*, 1975), within country comparisons (Gross *et al.*, 1977), and animal experiments (Lemon, 1975), support the estriol hypothesis. However, the association between high risk and low urinary estriol ratios was not confirmed in several other case-control studies (Arguelles *et al.*, 1973; Hellman *et al.*, 1971; Marmorston *et al.*, 1965a,b; Tominaga *et al.*, 1975).

The results of international comparisons have also been conflicting. Kumaoka *et al.* (1973) reported, in contrast to MacMahon *et al.* (1971), that differences in urinary estriol ratios in Japanese and British women aged 15 to 19 were small and that there was no difference in women aged 35 to 39. Dickinson *et al.* (1975) reported that Hawaiian Japanese exhibited estriol ratios. Intermediate between native Japanese and white Hawaiians. A recent study by Hayward *et al.* (1978) found no difference in estriol quotients in healthy Hawaiian Japanese compared to either British or native Japanese women.

4. *Plasma Estrogens and Breast Cancer Risk*

The results of case-control studies are mixed. Plasma E_2 levels have been reported to be unchanged (Jones *et al.*, 1977; McFadyen *et al.*, 1976; Hill *et al.*, 1976a), decreased (Hill and Wynder, 1976), and elevated (England *et al.*, 1975; Malarkey *et al.*, 1977a,b) in breast cancer cases when compared to matched controls. It is difficult to make any judgment concerning the relative validity of these studies since each was conducted under different conditions.

Recently, Bulbrook *et al.* (1978) analyzed the plasma estradiol and progesterone levels in pre- and postmenopausal women at varying degrees of risk. The degree at risk was assessed on the basis of family history, age at menarche, and age at first birth. It was found that plasma estradiol values did not vary with risk. Subnormal plasma progesterone levels, however, were associated with increased risks.

In an international comparison, Bulbrook *et al.* (1976) reported that plasma E_2, E_1, and progesterone concentrations in healthy British and

Japanese women were similar at all ages, although Japanese adolescents exhibited higher E_2 levels during the luteal phase. These results confirm an earlier report by Kumaoka *et al.* (1973) that plasma levels of E_2 in British and Japanese women were indistinguishable.

In contrast, Hill *et al.* (1976b) compared plasma E_2 concentrations in three populations with different risks for breast cancer. Premenopausal U.S. white women exhibited significantly lower plasma concentrations of E_2 compared to matched Japanese and Bantu women; postmenopausally, no differences were observed.

In a migrant study, Hayward *et al.* (1978) reported no differences in plasma estrogens in premenopausal native Japanese, Hawaiian Japanese, or British women. In postmenopausal women, however, plasma estradiol concentrations were decreased in Hawaiian Japanese women, compared to both native Japanese and British women.

It is apparent that no consistent pattern can be discerned in reference to urinary or plasma estrogen metabolites and breast cancer risk. If anything, plasma studies suggest that low blood levels of E_2, not high, are associated with increased risk.

Aside from the inconclusive results, the validity of the estriol hypothesis has been questioned for a variety of other reasons. Lipsett (1975) pointed out the limited nature of information obtained from the study of urinary steroid metabolites. The three principal estrogens, for example, have completely different profiles in blood and urine; whereas E_2 is the dominant estrogen in blood, E_3 is the dominant estrogen in urine. Hence, urinary estrogen profiles are not necessarily indicative of blood levels. Cole *et al.* (1978) summed up the current status of the estriol hypothesis by stating that "useful data are sparse and inconsistent, and no conclusion can be drawn regarding the relationship between the estriol ratio and breast cancer risk."

The question of whether a high-fat diet increases the rate of conversion of androgens to estrogens is raised by Nimrod and Ryan (1975). To date, however, no experiments concerning the relationship between dietary fat intake and the rate of peripheral conversion in humans have been reported, nor are there any reported studies on changes in urinary or blood estrogen metabolites in animals fed high- or low-fat diets.

5. *Prolactin and Breast Cancer Risk*

Interest in the role of prolactin in human breast cancer is evidenced by the number of reviews that have appeared recently on the subject (Smithline *et al.*, 1975; Robyn, 1975; Van der Gugten *et al.*, 1973; McGuire *et al.*, 1978; Nagasawa, 1978).

Evidence for the role of prolactin in human breast cancer is largely indirect and no definitive proof of prolactin dependency in human breast cancer has yet been demonstrated. Nonetheless, indirect evidence from comparisons of Japanese and U.S. white women suggest that prolactin plays a role in breast cancer. No significant difference was seen in prolactin levels when single morning prolactin concentrations were averaged over the entire menstrual cycle. However, a significant difference was found when mean plasma prolactin levels taken over 5 consecutive days in healthy whites and healthy Bantu women were compared.

In the migrant study by Hayward *et al.* (1978), no significant differences in mean basal plasma prolactin levels in British, Hawaiian Japanese, and native Japanese women, either premenopausally or postmenopausally, were found.

The inconclusive nature of these studies does not necessarily mean that prolactin concentrations in high- and low-risk populations are indeed identical. As shown by experimental studies in rodent and in several human studies, analysis of nocturnal secretion profiles and/or the use of provocative tests of pituitary reserves may provide more meaningful measures of plasma prolactin in high- and low-risk populations.

Since experimental studies in rodents suggest a link between dietary fat intake, prolactin hypersecretion, and breast cancer risk, Hill and Wynder (1976) conducted a series of dietary intervention experiments to determine whether a similar relationship exists in humans. In the first of three studies, volunteers were placed on a standard Western diet for 2 months and then switched to a strict vegetarian diet for 2 months. After each dietary regimen, overnight prolactin patterns were measured. The data indicate that there was a marked increase in plasma prolactin concentrations during periods of peak secretion (during deep sleep) in women on a high-fat standard Western diet compared to a low-fat vegetarian diet. These results show clearly that the amount and type of fat consumed in the diet can induce marked changes in the nocturnal secretion patterns of prolactin in humans (Fig. 15).

In a second study involving healthy premenopausal women, morning plasma prolactin levels were measured every other day throughout the menstrual cycle after (1) 2 months on a Western diet and (2) 2 months on a vegetarian diet (Hill *et al.*, 1977). It was found that the mean prolactin levels averaged over the entire menstrual cycle was greater after 2 months on a Western diet than after 2 months on a vegetarian diet.

To conclude, although evidence for the role of prolactin in human breast cancer is still largely indirect, there is a growing body of evidence to suggest that the prolactin secretion patterns are associated with increased risk for breast cancer. Moreover, dietary intervention studies

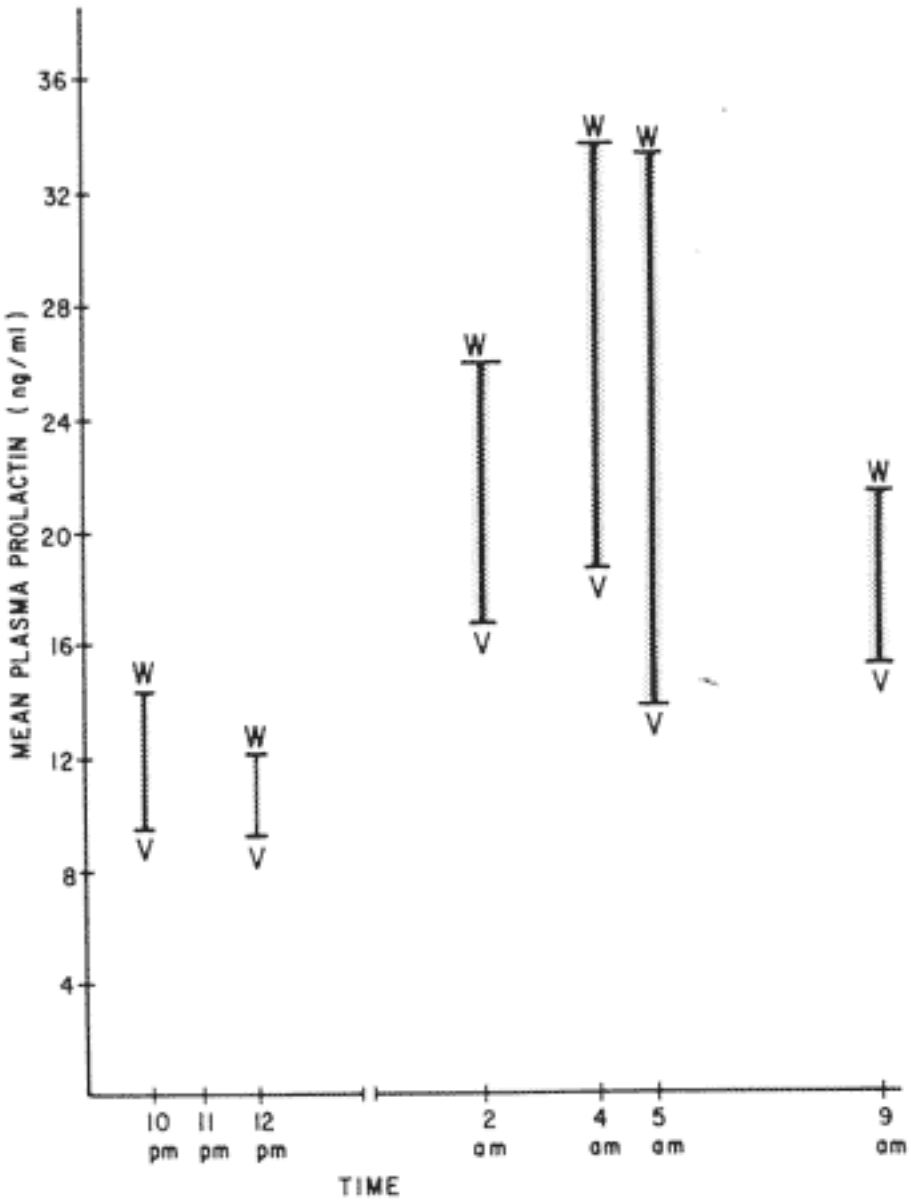

FIG. 15. Twenty-four hour plasma prolactin profiles in four healthy premenopausal women taken at hourly intervals by indwelling catheter. Subjects were maintained on a Western diet (W) for 2 months and then transferred to a vegetarian diet for 2 months. After each 2-month period, nocturnal prolactin concentrations were assayed. Mean prolactin concentrations were significantly higher in the subjects at 2, 4, 5, and 9 AM ($P < 0.01$) and at 10 PM ($P < 0.05$) when on a Western compared to a vegetarian diet. No differences were seen at 12 PM. Bars represent difference between means. (From Hill and Wynder, 1976.)

conducted at our Institute indicate that the regulatory center controlling prolactin secretion is responsive to changes in dietary fat consumption in humans, as well as in rodent. The latter studies also suggest that international differences in breast cancer incidence and in dietary fat intake may be causally related to differences in prolactin secretion patterns.

D. ETIOLOGIC CONSIDERATIONS

In a manner analogous to Berenblum's two-stage mechanism for skin carcinogenesis (1959), breast carcinogenesis can be operationally divided

into two discontinuous stages: initiation and promotion. As will be seen in the following discussion, dietary fat may play a role either directly or indirectly in both aspects of this disease.

Based primarily on the DMBA model, it appears that events that occur prior to or during puberty relate primarily to the initiation phase, while events that occur later in reproductive life increasingly relate to the promotional phase of carcinogenesis.

Viewed from this perspective, there are two points for preventive intervention in breast carcinogenesis: initiation, at or around puberty, and promotion, following puberty. Since initiation is considered to be irreversible, preventive action to block malignant transformation of ductal cells, will require identification of potential carcinogens.

Promotion, on the other hand, is a reversible time-dependent process, and therefore, more accessible to preventive measures. Since preneoplastic lesions will not develop into clinically recognizable tumors in the absence of promoting factors, attentuating effects of promoting agents, whether of endogenous or exogenous origin can serve *de facto* to inhibit the development of breast cancer.

As a general hypothesis, we propose that the promotion of human mammary tumor development is a direct function of the relative proportions of circulating prolactin and estrogen. Agents, whether exogenous or endogenous, that elevate the prolactin/estrogen (P/E) ratio serve to enhance breast tumor development; agents that lower the P/E ratio serve to suppress breast tumor development. A distinction is drawn between E_1 and E_2. Because E_2 is a more potent estrogen, is present in plasma in higher concentrations, and drops more abruptly at menopause, the P/E ratio will be defined for purposes of this discussion as the P/E_2 ratio. Moreover, since ductal fluid concentrates prolactin and probably E_2 as well, the P/E_2 ratio will apply for the present to both plasma and ductal fluid.

Analysis of the histopathogenesis of breast cancer suggests that the promotion phase consists of a series of discontinuous steps rather than a smooth continuous transition from transformed cell to carcinoma (Haslam and Bern, 1977). Although it cannot be said with certainty whether mammary cells transform directly to neoplastic cells or first go through a preneoplastic stage, the weight of evidence suggests the latter. This is an important distinction, since a mechanism involving discrete steps implies that progress from one step to another can be blocked. The P/E_2 ratio, for example, may act at one step in the sequence, and not at other steps. Welsch (1978), found that inhibition of spontaneous mammary tumorigenesis in mice by prolactin suppression was most effective in young nulliparous mice, less in older multiparous mice, and ineffective in old mice. These results imply that whereas early breast lesions in the

mouse are prolactin-dependent, at later stages of development, they lose this dependency. Since preneoplastic lesions in the mouse model do not parallel those in rat or human breast cancer in terms of their histopathogenesis, direct extrapolation of Welsch's studies is not possible. Nonetheless, studies of the mouse model illustrate an important point, namely, that failure to demonstrate prolactin dependence in an advanced mammary carcinoma does not rule out a role for prolactin in the early developmental stages of the disease. Repetition of the study in the DMBA or MNU models would provide valuable insight into the hormone sensitivity of the early atypical lesions in human breast cancer.

There is no direct evidence that intraductal hyperplasia in humans responds to either diet or drug-induced elevations in P/E_2 ratios. However, indirect evidence supporting such a proposition can be found in the study of Nomura *et al.* (1975) referred to previously. In this study, it was found that the percentage of proliferative-type, latent carcinoma in Hawaiian Japanese was twice that of native Japanese. Since breast cancer incidence rates and dietary fat intake have risen in parallel over time among Japanese migrants to Hawaii, these results suggest that environmental factors, and particularly dietary fat intake, may influence the development of latent carcinomas in humans. Measurement of nocturnal P/E_2 ratios in Hawaiian Japanese and native Japanese will be necessary to determine whether or not this effect is mediated by changes in prolactin secretion patterns. In addition, histological analysis of terminal duct hyperplasia in DMBA-treated rats fed high- and low-fat diets, combined with hormone analysis, would provide experimental evidence that a fat-prolactin mechanism is at work during the early phases of breast cancer development.

The possibility that *in situ* lesions can be controlled by exogenous means is of considerable importance from the preventive standpoint. Should the initiation process prove too elusive or difficult to block, then intervention at a second, later, point in the progression from transformed cell to carcinoma may result in the same net effect: reduced tumor incidence. Further understanding of the steps involved in the formation of *in situ* lesions and the hormonal milieu that regulates their development is much needed at present.

The key feature of the hypothesis under consideration is that it provides a plausible explanation for the previously unexplained variations in international incidence patterns noted by epidemiologists. In addition, it eliminates the necessity to postulate a dual etiology for breast cancer: one to account for premenopausal incidence patterns, the other to account for postmenopausal incidence patterns. A single mechanism, involving the relative concentrations of circulating (or ductal fluid) prolactin

and estradiol serves to explain the observed incidence patterns for all age groups. A P/E_2 mechanism can also account for the presence of high-risk groups within a population, and the fact that survival rates in Japanese breast cancer patients are significantly higher than U.S. breast cancer patients, following surgery.

International differences in breast cancer incidence rates are explained as follows: The difference in breast cancer incidence in the U.S. and Japan is 3:1 premenopausally, and 9:1 postmenopausally. In premenopausal women, the ovary actively synthesizes and secretes E_2, throughout the menstrual cycle in a fairly regular cyclical pattern (Korenman *et al.*, 1978; Sherman *et al.*, 1976). While it has not been proven beyond doubt that E_2 stimulates prolactin secretion in humans as it does in rats, it has been demonstrated that E_2 potentiates the effect of centrally acting prolactin-releasing drugs (Carlson *et al.*, 1973; Buckman and Peake, 1973). Hence sleep-related increases in prolactin secretion are centrally or CNS-regulated events, whereas the E_2 effects on prolactin secretion are peripherally regulated events. Within this context, it is proposed that in premenopausal Japanese women dietary fat exerts a small effect on the nocturnal prolactin peak. However, the presence of E_2 tends to potentiate any influence of dietary fat. Accordingly, the net difference in nocturnal P/E_2 ratios in premenopausal Japanese and American women are not expected to be particularly marked. As a result, breast cancer incidence rates, though higher in the U.S., are not dramatically higher in the premenopausal age groups.

At menopause, however, plasma E_2 levels drop by approximately 80% of the premenopausal concentrations (Judd *et al.*, 1974; Chakravarty *et al.*, 1976). Basal plasma prolactin levels, on the other hand, decrease by 40% according to some workers (Vekemann and Robyn, 1975), and according to Hill *et al.* (1977) and Kumaoka *et al.* (1976) may exhibit no decrease following menopause. Although the exact nature and extent of prolactin changes at menopause remain to be clarified, it is clear that sleep-related nocturnal peaks still occur (Rosencweig *et al.*, 1973; Rosen *et al.*, 1977; Malarkey *et al.*, 1977). Hence, as a natural consequence of the menopausal transition, the P/E_2 ratio undergoes an abrupt increase in all postmenopausal women. It is after menopause, then, that central mechanisms (diet/sleep, drugs) controlling prolactin release predominate over peripheral mechanisms (E_2). Since U.S. white women consume three times the amount of fat consumed by Japanese women, the P/E_2 ratio during the postmenopausal years is expected to be significantly higher in U.S. women compared to Japanese women. The subsequent increase in P/E_2 concentrations in U.S. women may then stimulate the growth of *in situ* lesions. Stemmerman's migrant data tends to support

such a prediction. Japanese migrants in the age group 50–59 showed a frequency of proliferative latent carcinoma similar to native Japanese. However, the frequency increased markedly in migrant women between the ages 60–69 and 70–79, compared to native Japanese women in the same age groups. The frequency of proliferative type, *in situ* lesions in premenopausal Japanese women in Hawaii and Japan has not been reported. On the basis of the above considerations, however, one could predict that the frequencies of proliferative type, *in situ* lesions in premenopausal Japanese migrants to Hawaii and native Japanese women would not be dramatically different.

It can be seen from the foregoing that there is no need to postulate a dual etiology for breast cancer. A single mechanism based on the relative concentrations of plasma or ductal fluid prolactin and estradiol may be invoked to explain both pre- and postmenopausal disease patterns. The postmenopausal disease is not the result of a different mechanism of tumor promotion, but of an increase in its magnitude on women on a Western diet.

Since most Western women, by definition, are at high risk, high-risk groups within a Western population can be viewed as extra-high risk individuals. Although most of the evidence is still tentative in nature, it appears that extra-high risk women exhibit disordered mechanisms governing the nycterohemeral secretion of prolactin. The net result of this effect is that women in these populations exhibit both temporal and quantitative changes in prolactin secretion. Analysis of nocturnal plasma and ductal fluid P/E_2 ratios in women from high-risk groups, along with longitudinal studies, should shed further light on this important subject.

Three independent studies have shown that the recurrence rate after breast surgery in postmenopausal breast cancer patients in Japan is markedly lower than in the U.S. Our experimental studies in ovariectomized animals (which partially mimic the postmenopausal condition) suggest a dietary–hormonal basis for these observations. It is proposed that postmenopausal Japanese patients, consuming a low-fat diet, have lower nocturnal ductal or plasma P/E ratios than their U.S. white counterparts. Assuming that some preneoplastic foci are present after surgery, the promoting influence of a high P/E ratio on this lesion in U.S. white women would be considerably greater than in Japanese women. As a result, the period of time elapsing between surgery and breast cancer recurrence would be shortened in U.S. white compared to Japanese women.

Proof that such a diet-based mechanism is indeed occurring would require monitoring of nocturnal prolactin and estrogen levels in postmenopausal Japanese breast cancer patients following surgery. If the P/E_2

hypothesis is corroborated, careful regulation of fat intake in postmenopausal U.S. patients may lower their recurrence rates to those exhibited by Japanese patients.

E. Conclusions

Investigators concerned with the prevention of cardiovascular disease have, for some time, recommended a diet lower in calories, fats (in particular, saturated fats), and cholesterol than is typical of the average American diet. It now appears prudent to suggest a similar diet for the prevention of breast cancer, except that in this case data from animal experiments suggest a total fat reduction, including that of unsaturated fats.

Most Western-conditioned individuals find it restricting to be placed on a low-calorie diet, a low-fat diet of 20% total calories, a low-cholesterol diet intake of 100 mg per day, as suggested by Connor and Connor (1972). Yet, it would appear that this is the type of diet that our sedentary bodies can adequately metabolize without creating an excess of lipids to overwhelm the metabolic capacities of our system.

A prudent diet, even of 30% fat and 300 mg cholesterol per day, is difficult to establish for a sufficient number of women to test the nutritional hypothesis of breast cancer etiology in a prospective survey that would necessarily have to be carried out for at least one decade. While experimental studies need to continue, we propose several metabolic epidemiological studies to test whether prolactin output and particularly its concentration in the breast fluid can be modified by dietary alteration and whether this reduces the occurrence and the progression of breast cancer.

We suggest, for these experiments, that the diet should not exceed 1800 calories, 20% total fat with an equal distribution of polyunsaturated and saturated fats, and cholesterol intake of 100 mg per day. At a time when so many types of clinical trials with a large variety of chemotherapeutic regimens are being carried out on cancer patients, it is time to consider both primary and secondary preventive nutritional trials as well.

We hope that this review of existing evidence relative to the etiology of breast cancer will serve as a constructive stimulus to other engaged in etiological and preventive aspects of breast cancer. Epidemiologic evidence clearly suggests that breast cancer is not an inevitable consequence of aging. What has been done in this field so far, however, can be regarded as only a prologue. Those engaged in attempting to prevent breast cancer will not have succeeded until the incidence of breast cancer

is actually reduced. To realize this possibility requires greater attention and more interdisciplinary activity than we have given to the field of breast cancer etiology so far.

VII. Dietary Factors and Cancer of the Prostate

A. Epidemiology

Epidemiologic data on prostatic cancer have been reviewed by King *et al.* (1963), Wynder *et al.* (1971), Hutchison (1976), and Blair and Fraumeni (1978). Cancer of the prostate is common in western countries, including the United States, and uncommon in Japan and Africa (Doll *et al.*, 1970) (Fig. 16). Southern and Eastern European and Latin American countries have an intermediate incidence of prostatic cancer. The high rates seem to be associated with populations of Northern Europe or of Northern European origin. Among whites in the United States, mortality is elevated in areas with a high percentage of residents of Scandinavian

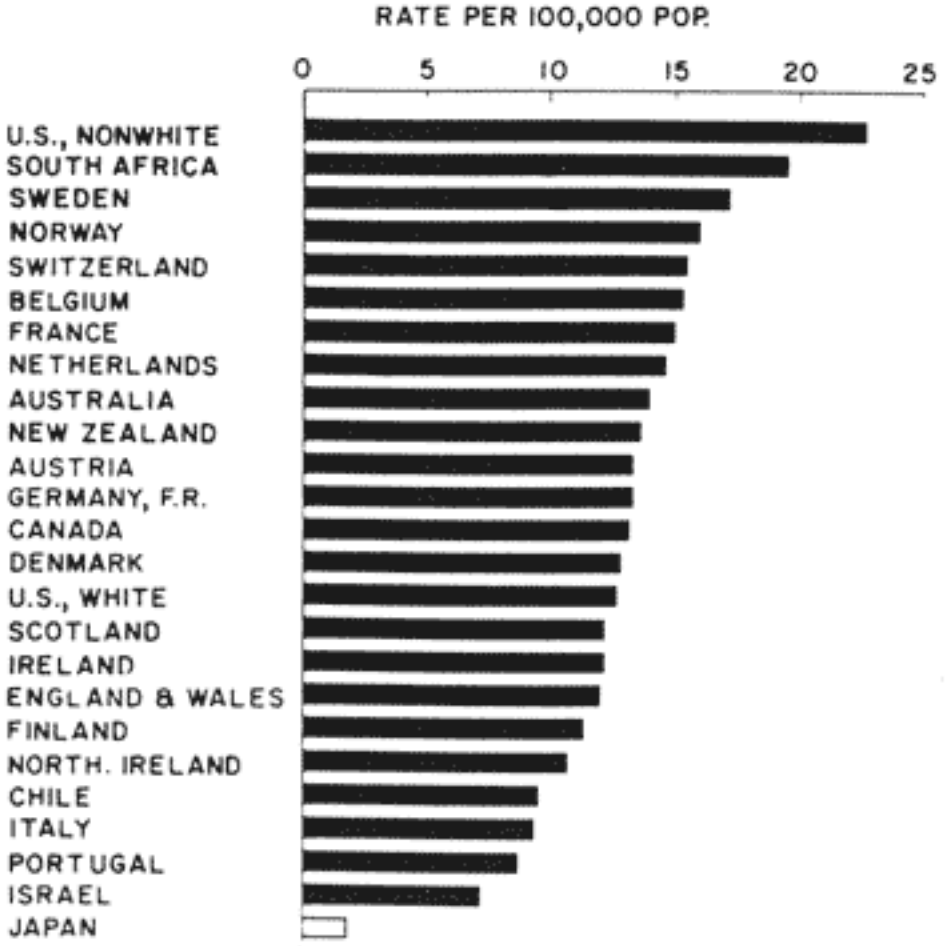

FIG. 16. Age-adjusted death rates for prostate cancer in different countries, 1966–1967. (From Segi and Kurihara, 1972.)

descent (Blair and Fraumeni, 1978), whereas blacks have a higher incidence than whites in the United States.

Compared with U.S. blacks, African blacks seem to have a low rate of cancer of the prostate. The differences suggest that environmental factors contribute to the high incidence of prostatic cancer among U.S. blacks.

Although prostate mortality and morbidity vary by country, the rate appears to be affected by migration of population from low- to high-risk countries. Haenszel and Kurihara (1968) showed that the first generation immigrants in the U.S. from Japan have an increased mortality rate for prostatic cancer. An increased mortality rate was also found in Polish immigrants to the United States and Australia (Staszewski and Haenszel, 1965), and in foreign-born Caucasians compared to native-born (Newill, 1961). Wynder *et al.* (1971) found that an increase in mortality from prostatic cancer in migrants to the U.S. appeared only after 20 or more years residence in the U.S. Studies of migrants suggest that environmental factors, rather than genetic characteristics, account for a substantial part of the international variation in mortality rates for prostatic cancer.

Since association between incidence of prostatic cancer and measures of socioeconomic status are not significant (Seidman, 1970; King *et al.*, 1963), other environmental factors in the high-risk areas would appear to be involved. For example, mortality rates for U.S. blacks rose sharply with increasing population density, whereas the rates for blacks in sparsely populated countries did not (Blair and Fraumeni, 1978). This finding suggests that the special susceptibility of American blacks to prostatic cancer is associated with urban residence (Blair and Fraumeni, 1978).

From comparative studies of prostatic tissue in Japanese and Caucasian men, Akazaki (1973) postulated that environmental factors were responsible for the activation of latent lesions. These small latent lesions, which occur at a constant frequency in all areas, appear to progress to active lesions mainly in Western societies (Barnetson, 1954; Breslow *et al.*, 1977).

The next question is whether or not prostatic cancer has an epidemiology sufficiently similar to the epidemiologies of cancers of the breast in females and the large bowel that we should seek a common cause for them. Internationally, the incidence rates for the endocrine-dependent cancers (breast and prostate) follow fairly closely the rates of large-bowel cancer (Berg, 1975a). Although breast cancer incidence is linked particularly closely with large-bowel cancer, the association between cancer of the large bowel and of the prostate is statistically significant (Berg,

1975a). There is a positive correlation between prostatic cancer and female breast cancer in the United States, suggesting a common etiology for these two endocrine-related cancers (Wynder *et al.*, 1967; Blair and Fraumeni, 1978).

B. Etiology

International differences in prostatic cancer morbidity and mortality are substantial and suggest the influence of environmental factors (Wynder *et al.*, 1967, 1971; Berg, 1975a).

Interestingly, one striking difference between diets in high- and low-risk areas is the fat intake (Fig. 17), which accounts for 40% of the daily calories in high-risk and 20% calories in low-risk areas. Blair and Fraumeni (1978) used national surveys to examine regional differences in the consumption of high-fat foods. The Central United States, including the high-rate North Central and Midwest regions, had a higher consumption of beef and milk products (5.9 and 14.4 lb/week, respectively) per household than did the Northeast or South. For fats and oils, pork, and eggs, the South had a slightly higher consumption than did the Central United States, Northeast, or West. The total consumption of these high-fat foods was highest in the Central United States, intermediate in the Northeast and West, and lowest in the Southeast. This pattern parallels that of mortality for prostatic cancer among whites (Blair and Fraumeni, 1978).

Assuming a positive correlation between dietary factors and incidence of prostatic cancer, what is the connecting link? Clinical studies have clearly shown that this disease is hormonally dependent (Fergusson, 1972) and that changes in hormonal metabolism occur in prostatic cancer patients (Marmorston *et al.*, 1965a,b). Any factor that affects hormonal secretion, retention, and, in particular, the sensitivity of the target organ and/or cells influences the frequency of this cancer (Wynder *et al.*, 1971). Since diet may modify hormonal systems, it has the potential of inhibiting or enhancing tumorigenesis, i.e., diet or nutritional status may function as modifiers of prostatic tumorigenesis.

C. Metabolic Epidemiology

Concerning the relationship of lifestyle and diet to hormonal status, exercise alters androgen metabolism (Kuoppasalmi *et al.*, 1976; Sutton *et al.*, 1973), whereas diet may directly modify hormonal activity (Edozien, 1960; Merimee and Fineberg, 1974) or may act indirectly through

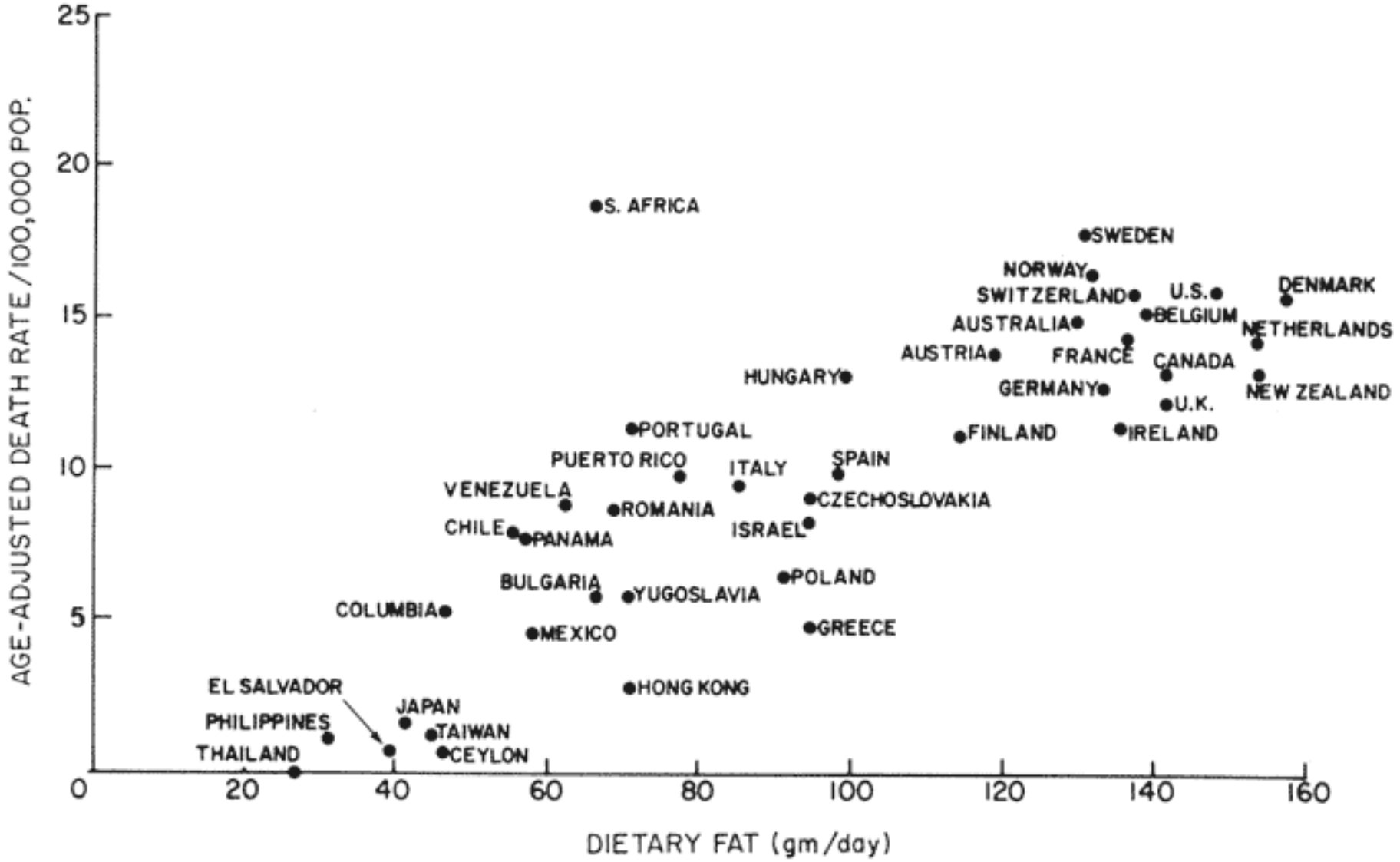

FIG. 17. Correlation between age-adjusted death rates from prostate cancer and per capita consumption of fat.

neurotransmitters, biogenic amines, and alteration in sleep patterns (MacIndoe and Turkington, 1972; Phillips *et al.*, 1975; Boyar *et al.*, 1972).

In a comparative study of the hormonal levels in North American white and black men, high-risk groups, with South African black men, a low-risk group, Hill *et al.* (1978) reported higher plasma estradiol levels in South African black men; testosterone levels were comparable in all three ethnic groups and human chorionic gonadotrophic produced a comparable release of testosterone in the three groups of men. However, in South African black men, the release of prolactin was significantly greater following TRH injection than in North American men. When North American black and South African black men were fed a vegetarian or a Western diet, respectively, urinary androgen and estrogen levels decreased in the South Africans (Hill *et al.*, 1979) (Fig. 18). Hill and Wynder (1979) have also reported that a vegetarian diet decreases the overnight release of prolactin and the plasma level of testosterone. Evidence would

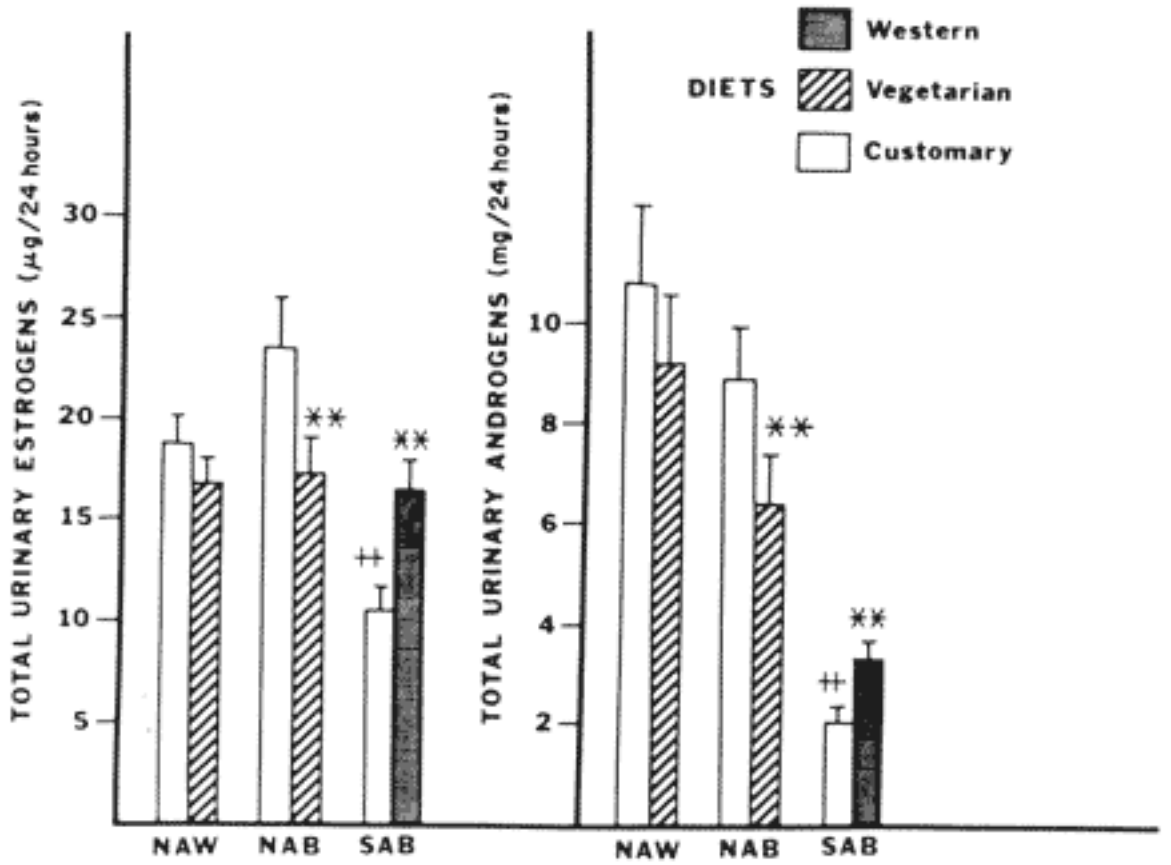

FIG. 18. Effect of vegetarian and Western diets on urinary estrogens and androgens in North American White and Black men and in South African Black men. On their customary diets, South African Black (SAB) men ($n = 21$) had significantly lower ††($P < 0.01$) urinary estrogen and androgen levels than North American Black (NAB) men ($n = 18$). When fed a Western diet, urinary estrogen and androgen levels increased **($P < 0.01$) in SAB men, while a vegetarian diet decreased **($P < 0.01$) the urinary androgen and estrogen levels in NAB men. Levels of urinary androgen and estrogen levels for North American White (NAW) men ($n = 16$) are also given. (From Hill *et al.*, 1979.)

therefore suggest that hormonal metabolism in men can be modified by dietary factors.

Whether similar changes occur in men with prostatic cancer is currently being investigated by Hill and Wynder from our Institute.

D. Experimental Studies

Recently, Fingerhut and Veenema (1977) described an animal model to obtain prostatic animal carcinoma similar to that in man. However, there are as yet no studies to validate the model and to test the relationship of nutrition to prostatic cancer.

E. Conclusions

Based mainly on epidemiologic studies, evidence suggests that nutrition plays an important role in the etiology of this disease. As with breast cancer, this disease is hormonally dependent and is, therefore, subject to manipulation by surgical ablation and by dietary modification. Added support for the importance of environmental factors in the etiology of this disease arises from the findings of Berg (1975a,b) who reported an association between large-bowel and prostatic cancer.

Further study of the effect of specific dietary components on hormonal activity in men at different risk for this disease, complemented with *in vitro* studies, should elucidate the dietary role in the etiology of prostatic cancer.

VIII. Conclusions

At present, we have overwhelming evidence of remarkable variations in the overall cancer incidence and of the incidence of specific types between countries and within countries. None of the risk factors for cancer is probably more significant than diet and nutrition.

The evidence from high- and low-incidence populations, worldwide, and the corresponding correlations with various dietary factors, the even more compelling picture that has emerged and continues to emerge from migrant studies and studies of singular populations (such as Seventh-Day Adventists in the United States), the analysis of parallel trends in incidence and dietary changes in certain well-characterized populations, and evidence arising from case-control and other studies on specific dietary

factors, all seem to provide highly defensible arguments for dietary implications in the causations of certain major human cancers (stomach, breast, and colon), and for corrective dietary recommendations toward prevention.

The evidence is further reinforced by experimental animal studies that provided data strikingly supportive of the epidemiologic studies and that have provided leads to the probable mechanism of action. The various aspects of this article have provided the evidence that justifies the exclusion of environmental, occupational, or genetic factors as significant contributions to the etiology of these cancers.

While the experimental scientist is rightly preoccupied with the need for a mechanistic understanding of the precise epidemiologic and experimental clues on hand, those with interest and responsibility in public health cannot fail to visualize the present opportunities for intervention, even before the detailed mechanistic picture is precisely and totally defined. With certain limits, dietary intervention seems to offer an exceptionally favorable ratio of risk and benefits, a situation where the population would have little to lose and probably much to gain.

Thus, we have the research tools in hand to mount a concerted, effective effort to reduce the risk for the main premature killing diseases. It should be possible to design lifestyles and nutritional habits compatible with local customs and civilizations, not very different from those currently in effect in high-risk countries, for the types of neoplasms discussed in this article. Such modified lifestyles would ultimately serve to reduce the overall risk for these diseases, and hence provide a rational basis for the prevention of types of cancer now affecting literally millions of people around the world.

For some time now, experts in the field of arteriosclerosis have recommended a modification of the typical western diet to reduce the prevalence of hyperlipidemia. To the extent that some aspects of this recommended prudent diet affect the major cause of death in most western countries, it may also relate to the reduction of certain types of cancers. When it was observed that certain types of cancer are prevalent in other western countries, a modification of the western diet along these lines may be necessary. A prudent diet should, therefore, lead to the prevention of nutrition-related cancers, namely, colon, breast, prostate, stomach, pancreas, upper alimentary tract, and the like.

There are a number of studies that suggest that adequate amounts of vitamins A, C, and E and B vitamines, and certain trace minerals such as Se and Zn, in the diet might, in addition, prevent the development of certain cancers such as breast, esophagus, bladder, cervix, and lung.

It is beneficial for general health purposes to have an adequate amount of vitamins and trace minerals in our diet. The reduction of stomach cancer has been suggested to relate, at least in part, to an increased consumption of fresh fruits and vegetables. As we reduce our intake of fat and animal proteins, we need to replace these food categories with certain types of vegetables, including those containing vitamin C. The intake of salt and pickled food should be reduced, and of other foods with unnecessary substances, such as high levels of nitrites and nitrates. Additional recommendations would affect food preparation to avoid or reduce consumption of fried meats and proteins. Inasmuch as we and others have demonstrated that certain types of fibers act as a protective factor in colon carcinogenesis, it would seem that an increase in intake of foods containing fibers (mainly cereal fibers and also vegetable and fruit fibers) and a reduction in the fat calories from 40% to 20% are recommended. Since a high-fat diet has also been associated with cancers of the breast and prostate, the recommended diet may help prevent breast and prostate cancer. Since heavy alcohol consumption is associated with a high risk of cancer of the upper alimentary tract and larynx, reduction of alcohol intake to moderate levels is recommended.

In conclusion, there would seem to be sufficient evidence to propose modifying the diet of Western countries to reduce total dietary fat, animal protein, and cholesterol, increase dietary fiber on the lines of above prudent diet, and increase certain food items, such as fresh vegetables and fruits. Diet should be well balanced to provide adequate amounts of vitamins and minerals. Although further research for more specific preventive measures is required, such measures are unlikely to be hazardous and can be advocated with a strong hope for benefits in the population.

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

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