Intestinal bacteria and cancer

B. S. Drasar, Ph.D., and M. J. Hill, Ph.D.

Cancer research has approached the human disease through two major avenues. The study of carcinogenic agents in laboratory animals and epidemiological studies on the incidence of human cancers have both proved fruitful, but for many cancers a discontinuity exists between laboratory studies and explanations about the human disease. Environmental factors are thought to be involved in the etiology of 80 to 90% of human cancer. The intestinal flora constitutes the most integral portion of the human environment and for this reason it has been invoked to explain some human cancers. In this paper, we consider briefly the ways in which the flora may contribute to carcinogenesis and examine the evidence for the involvement of the flora in the etiology of cancers of the colon, breast, and stomach.

Intestinal bacteria can influence the action of known carcinogens; thus, germfree, C3H mice are comparatively resistant to the action of 7,12 dimethylbenz(A)anthracene (1). The naturally occurring carcinogen cycasin requires activation by the intestinal flora. Cycasin, methylazoxymethanol-glycoside, is synthesized by plants of the genus Cycas as the glycoside, which is noncarcinogenic. Intestinal bacteria hydrolyze the glycoside to release the active aglycone (2). Bacteria are able to modify a very wide range of environmental chemicals and in particular intestinal bacteria can modify food additives such as cyclamate (3), digestive secretions, e.g., bile salts (4, 5), and hormones, e.g., testosterone (17β hydroxyandrost-4-ene-3 one) (6). Ethionine, a carcinogen, is synthesized by Escherichia coli (7). The relevance of these and many other observations to human disease is uncertain but it is indisputable that intestinal bacteria do modify man’s intimate chemical environment.

Intestinal bacteria: cancers of specific sites

Cancer of the colon. Cancer of the colon is primarily a disease of the developed nations. The disease is much more common in North America and Northwest Europe than in most of Africa, Asia, and South America (8–10). Japan provides the most notable exception to this general pattern, having by far the lowest colon cancer incidence among the developed nations. These geographical differences are not explicable on racial grounds. Although Japanese who migrate to California may retain their low incidence, this retention is dependent upon maintenance of their original cultural habits (11). “Westernization” in either California or Japan is associated with a trend to a higher incidence of colon cancer (12, 13). Changes in dietary habit have been suggested as explaining the effects of westernization. Fat (14), protein (15), fiber, and refined sugar (16, 17) have each been suggested to be of primary importance in the etiology of cancer of the colon. Consideration of a number of dietary and socioeconomic indicators derived from United Nations Organization statistics demonstrated that cancer of the colon was significantly correlated with a number of these indicators, including fat and protein intakes, per capita income, and the number of motor vehicles per capita of population. When regression analysis was applied, animal protein intake and combined fat intake were shown to be the most important factors (B. S. Drasar and D. Irving, unpublished observations). The search for dietary carcinogens has not proved fruitful.

To account for these observations, we (18, 19) advanced a hypothesis involving the action of intestinal bacteria: a) That cancer of the colon is caused by the production of carcinogens and/or co-carcinogens from dietary components or from intestinal secretions produced in response to diet. b) That

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the nature of the diet affects the composition of the intestinal flora and the substrates available for bacterial metabolism.

If diet controls the intestinal flora and the substrates available for production of carcinogens and also, partly as a result of these, the conditions within the colon under which the reactions occur, this would explain the correlation between diet and incidence of colon cancer.

Our studies have been an attempt to test this hypothesis (18–20). In view of the known association between steroids and fats and considering the metabolic significance of many steroids, our main effort with respect to substrates for bacterial action has been directed toward the bile acids (acid steroids) and the neutral steroids. This orientation was reinforced by the knowledge that a number of steroids have been shown to be carcinogenic in animal studies; these include deoxycholic acid (21–23), bis-nor Δ⁸ cholic acid (24), apochoic acid (25), and estradiol (26–28). The amount of bile acids in feces is dependent on the amount of fat in the diet (29).

We have examined the bacterial flora and steroid content of feces of people living in areas with both low and high risks of colon cancer. Bacteria were cultivated using an anaerobic cabinet and various selective media (30). Examination of the flora was restricted to the enumeration of the major bacterial groups. Fecal steroids were analyzed by solvent extraction followed by gas chromatography (31). Our initial studies compared Scotland, England, and the United States with India, Japan, and Uganda and some striking differences were demonstrated (Table 1). The numbers of Bacteroides isolated were fewer and the number of enterococci isolated greater from fecal specimens originating in areas of low cancer incidence than those of high cancer incidence. Furthermore, the amount of fecal steroid (neutral and acid) was less in specimens from Uganda, India, and Japan than in those of the other countries. Not only was less steroid present in these specimens but the percentage of that available that had been degraded by bacterial action was much lower. This finding was paralleled by the decreased steroid degrading ability of bacterial strains isolated from feces of inhabitants of countries with low colon cancer incidence. More recently, studies in Hong Kong have revealed similar

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Cancer incidence, diet, bacterial flora, and steroid metabolism in various countries</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Countries studied</strong></td>
<td>Scotland</td>
</tr>
<tr>
<td><strong>Incidence colon cancer/100,000 population</strong></td>
<td>31.2</td>
</tr>
<tr>
<td><strong>Incidence breast cancer/100,000 population</strong></td>
<td>111</td>
</tr>
<tr>
<td><strong>Diet (grams per person per day)</strong></td>
<td></td>
</tr>
<tr>
<td>Animal protein</td>
<td>?</td>
</tr>
<tr>
<td>Combined fat</td>
<td>?</td>
</tr>
<tr>
<td>Sugar and sweets</td>
<td>?</td>
</tr>
<tr>
<td>Fiber</td>
<td>?</td>
</tr>
<tr>
<td><strong>Selected bacteria log₁₀/gram feces</strong></td>
<td></td>
</tr>
<tr>
<td>Bacteroides</td>
<td>9.8</td>
</tr>
<tr>
<td>Enterococci</td>
<td>5.3</td>
</tr>
<tr>
<td><strong>Fecal steroids (milligram gram dry weight)</strong></td>
<td></td>
</tr>
<tr>
<td>Neutral steroids</td>
<td></td>
</tr>
<tr>
<td>% Degraded</td>
<td>71</td>
</tr>
<tr>
<td>Acid steroids</td>
<td>6.18</td>
</tr>
<tr>
<td>% Degraded</td>
<td>49</td>
</tr>
</tbody>
</table>
differences between low and high income groups.

Considering these results in the light of our initial hypothesis, the amount of substrate available for carcinogen production is greater in the high risk groups and the degree of bacterial action upon it is also greater; furthermore, the bacteria isolated were more active in modifying it. In addition to the known steroid carcinogens mentioned previously, it is possible that bacteria might produce a polycyclic aromatic hydrocarbon from steroids. A scheme for the production of a 17-substituted cyclopentaphenanthrene from bile acids utilizing bacterial enzyme systems has been discussed previously (32).

In addition to the steroid metabolites, a number of products of bacterial protein metabolism have been shown to be co-carcinogenic (33); these include the phenolic products of tyrosine metabolism and some tryptophan metabolites (34). These findings may be very significant when the close correlation between colon cancer and protein intake is considered.

**Cancer of the breast**

The geographical variations in the incidence of cancer of the breast are similar to those of cancer of the colon (8–10). The incidence of this cancer is high in North America and Northwest Europe and low in Africa, Asia, and South America. Studies on Japanese migrants indicate that the retention of a low incidence of cancer of the breast within a group is dependent upon the retention of cultural habits (35, 36). Wynder (35) postulated a relation between fat intake and breast cancer. This relationship was born out by our studies (Drasar and Irving, unpublished observations). A high fat diet has long been known to increase the incidence of spontaneous breast tumors in experimental animals (37). Breast tumors can be induced and stimulated by estrogens (38, 39). The demonstration that intestinal bacteria could produce estradiol, estrone, and 17-methoxyestradiol from androstenedione (40), cholesterol, and 3-oxo-chol-4-en-24oic acid (P. Goddard and M. J. Hill, unpublished observations) led us to consider that bacteria might be involved in the causation of breast cancer (41). Cholestenone is present in feces and is a bacterial metabolite of cholesterol; gut bacteria can produce 3oxo-chol-4en-24oic acid from bile acids (42).

These observations can be explained by the hypothesis that: a) Cancer of the breast is dependent upon bacterial production of estrogens that displace hormonal homeostasis. b) Diet controls the composition of the intestinal flora and the amount of steroid available for modification.

Studies of fecal specimens, initially considered with respect to colon cancer, show that there are differences between areas of low and high breast cancer incidence with respect to both steroid and bacterial flora (Table 1).

**Cancer of the stomach**

Elsewhere we have examined the possibility that stomach cancer results from the production of nitrosamines in the bladder from dietary nitrate and endogenous secondary amine (43, and G. M. Hawksworth, unpublished observations). However, an alternative hypothesis that might explain some cancers involves production of carcinogens in the stomach (44).

This might postulate: a) That achlorhydria precedes development of a cancer. b) That achlorhydria (either surgical or natural) leads to colonization of the stomach. c) That the gastric flora is able to produce carcinogens, probably nitrosamines, from dietary substances, probably nitrate and secondary amines.

Stomach cancer seems to be associated with intestinal metaplasia and atrophy of the gastric mucosa (45, 46). Such metaplasia is rarely associated with high acidity. Atrophy of the gastric mucosa associated with pernicious anemia and achlorhydria carries an increased risk of gastric carcinoma (47–49). Recent work suggests that gastric surgery for benign gastric ulcer increases the risk of gastric carcinoma (50).

Achlorhydria associated with pernicious anemia often leads to colonization of the stomach (51, 52). Gastric surgery and most especially Billroth II (Polya) partial gastrectomy can lead to colonization of the stomach (53–57) with a mixed bile salt resistant flora
qualitatively similar to that found in feces. Thus there is some degree of association between bacterial colonization and cancer of the stomach.

If this association is accepted, we must consider the possible production of carcinogens. Production of nitrosamines by bacteria in the stomach is the most likely source of carcinogen but others should not be discounted. Gastric cancer is found associated with a high level of nitrate in the drinking water in Colombia (G. Gordillo, personal communication). Nitrosamine synthesis from nitrate or nitrite and secondary amine is performed by *Escherichia coli* (43, 58), and in the achlorhydric stomach would utilize dietary secondary amine and nitrate.

**Intestinal bacteria: general considerations**

A prima facie case for believing that the flora is involved in carcinogenesis can be established with the cancers discussed above. However, unless the methodology for the examination of intestinal specimens, both with respect to their bacterial content and chemical composition, can be simplified, the hypotheses discussed here cannot be used predictively because the cost of screening 100,000 specimens would be prohibitive. Tests for bacterial metabolism that do not involve the cultivation of the bacteria or the complex chemical analysis of their products must be devised. A test for the metabolism of bile acids, in patients thought to be suffering from the blind loop syndrome, has been developed (59), and similar tests for the in vivo and in vitro production of carcinogens and their precursors could be devised.

Implicit in this discussion is the assumption that the role of the flora in carcinogenesis involves the production of carcinogens. However, other mechanisms may be involved. The possibilities are wide-ranging and Table 2 lists some of them. The flora is most important for the development of immune systems (e.g., (60)), and bacteria are probably involved in the development of self-/not self-recognition systems such as ABO blood groups (61). Considering the diversity of bacterial antigens, it is at least conceivable that some bacteria have antigens similar to the antigenic determinants of cancer cells.

A further assumption in these hypotheses is that bacteria colonization of areas of the intestine leads to an increased incidence of cancer at that site. In view of this, it would be of value to obtain evidence as to the incidence of cancer of the lower ileum following ileostomy, as the bacterial colonization of the ileum is modified in such patients (62, 63).

Finally, it must be realized that although a link between the bacterial flora and cancer, especially of the colon, can be postulated and suggestive evidence put forward, much more work is required to establish the actual causal mechanisms.

**References**

1. **Roe, F. J. C., and G. A. Grant.** Inhibition by germ-free status of development of liver and lung tumours in mice exposed neonatally to 7,12-dimethylbenz(A)anthracene: implications


