

Leading article

Bile, bacteria and bowel cancer

The large bowel is one of the commonest sites of carcinogenesis in western populations; it is the commonest site in the United States, West Germany, Australia, and New Zealand and is the second commonest in England and Wales, Sweden, Denmark and many other west European countries. It is, however, rare in Africa, Asia and South America and studies of migrants, religious subgroups and the like, indicated that the disease has an environmental aetiology. Most epidemiologists agree that diet is important in the causation of the disease although there is little agreement concerning which specific dietary item is most strongly implicated. This lack of agreement probably indicates that the relation between diet and colorectal carcinogenesis is not simple and is probably mediated by some factor, the concentration of which is in turn determined by diet. In 1969 Aries *et al*¹ postulated that colorectal cancer is caused by metabolites produced in the colon from some benign substrate by the bacterial flora. As the diet would control both the concentration of substrate and the composition and metabolic activity of the flora, it would determine the amount of metabolite; this would explain both the fact that the risk of colorectal cancer is related to diet and that the relationship is far from simple.

In following up this hypothesis most investigators have pursued the suggestion^{2 3} that the substrates are the bile acids. The liver synthesises two bile acids (cholic and chenodeoxycholic acids) and they are secreted as conjugates with glycine, or taurine. These are extensively metabolised by the colonic bacterial flora.⁴ The major metabolites are the products of deconjugation and 7-dehydroxylation, namely deoxycholic acid and lithocholic acid. In addition there are the numerous products of the hydroxyl oxidoreductases active at the 3, 7 and 12 positions and producing oxo bile acids and β -hydroxyl groups; there are 25 possible major keto bile acids: all of them have been detected in human faeces. There is also a host of other metabolites such as sulphate esters (principally of the 3-hydroxyl group), the methyl and ethyl esters of the carboxylic acid group (released by deconjugation) various unsaturated bile acids and the allo bile acids (formed by inversion at the 5 positions of the steroid nucleus). Quantitative analysis of most of these metabolites is difficult and so there is little evidence which strongly incriminates any of them in large bowel carcinogenesis.

The evidence that the bile acids may act as tumour promoters in colorectal cancer is derived mainly from animal studies.^{5 6} Suspension of deoxycholic acid in croton oil, repeatedly painted on the skin of a mouse causes many more skin tumours^{7 8} than the croton oil alone. As the croton oil may have contained a tumour initiator, this indicates that deoxycholic acid is a tumour promoter in this model. When rodents are treated with azoxymethane or dimethylhydrazine they develop colorectal tumours (as

well as tumours of the small intestine, ear duct and many other sites). In such animals the instillation of solutions of deoxycholic and lithocholic acids into the rectum causes an increase in the number of colorectal tumours, while instillation of other bile acids does not. Surgical procedures which divert the faecal stream cause a decrease in the number of tumours in the isolated segment of the bowel, while procedures which increase the faecal bile acid concentration (such as diverting the bile duct into the caecum, jejunioileal bypass, or feeding cholestyramine, or other bile acid sequestering agents) increase the number of colorectal tumours. The problem with this model of colorectal carcinogenesis is that 'spontaneous' colorectal cancers are rare in laboratory animals, especially rodents. In a study of 142 000 neocropsied mice only 19 primary malignancies of the intestine were found⁹ indicating a lifetime risk of 13 per 100 000 compared with 3000 per 100 000 in humans. Consequently powerful organ specific carcinogens must be used to initiate the disease. The carcinogens used – for example, azoxymethane or dimethylhydrazine – are not likely to be experienced by humans and so it is necessary to assume that the colorectal cancers arising in rodents treated with these agents behave similarly to those arising in humans. Reddy¹⁰ has shown that when the carcinogen used was azoxymethane, citrus pectin protected against colon carcinogenesis, but when 3,2'-dimethyl-4-aminobiphenyl was used citrus pectin had no effect to either on the proportion of animals with tumours, or on the number of tumours per animal. Similarly, germ-free status offers some protection against the colon carcinogenesis initiated by dimethylhydrazine, but not against that initiated by MNNG. Thus the behaviour of the disease in this model apparently depends on the initiator used. In addition, the histopathology depends on the strain of rodent, as well as on the initiators and so whereas Deschner and her coworkers are clearly working with a model which mimics human colorectal cancer in showing an adenoma carcinoma sequence,¹¹ others are using a model in which carcinomas arise *de novo* with no benign precursors.¹² In these circumstances, interpretation of the results obtained using this animal model must be treated with considerable caution.

Because of the reservations that many workers have concerning the animal data and because of the relative cheapness of *in vitro* systems, many have turned to microbial mutagenicity tests such as the Ames test.¹³ In such *in vitro* tests bile acids have been shown to be non-mutagenic, but deoxycholic and lithocholic acids have been shown to be co-mutagenic; for example, mixtures of bile acids with known mutagens give more mutations than recorded with the mutagen alone.^{14 15} In addition, lithocholic acid has been shown to be mutagenic in the mouse embryo mutation test.¹⁶ It is important to remember, however, that for a mutagen to be a carcinogen it must first be able to reach the target cell and in so doing overcome the various host defence mechanisms. It must then react in the target cell as it does in the mutagenesis assay and the transformed cell must then escape removal by any immune defence mechanism and fail to be repaired by any of the DNA repair mechanisms of the host. For this reason there is a tendency to treat the results of *in vitro* test systems with considerable reservation. Indeed, one of the virtues of these systems is that their distance from the human situation is sufficiently obvious to make it easier to resist excessive interpretation of the results.

Research in humans is very much more difficult to carry out than is work on animal models, or using *in vitro* systems. Furthermore, superficially it might appear that human experiments are more difficult to interpret (involving for example genetically heterogeneous populations). They are also controlled by different ethical considerations which make, for example, carcinogenicity testing impossible. Studies of aetiology in humans, however, have clear advantages over animal, or *in vitro* studies which more than outweigh the disadvantages. These have been listed many times elsewhere in the context of the study of carcinogenesis or other disease states. While animal models are invaluable in – for example – studies of treatment, there is no substitute for human study in determining the causation of disease. For this reason it is gratifying to note that there has been a recent increase in the number of studies of human colorectal carcinogenesis.

Among the most important studies of human colorectal carcinogenesis are the histopathological observations which established the existence of a polyp cancer sequence, then refined it to the dysplasia carcinoma sequence.¹⁷ These observations not only gave an insight into the causation of colorectal cancer, but should also make us examine critically many of the animal models in which carcinomas clearly arise *de novo*. In the dysplasia carcinoma sequence all colorectal cancers arise in preformed adenomas, or other areas of mild dysplasia. There is thus a multistage process in carcinogenesis involving formation of areas of mild dysplasia (usually an adenoma), followed by increases in the area and in the severity of dysplasia and ultimately in neoplasia. The growth in the area of dysplasia and the increase in its severity are different processes, but an increase in size makes an increase in the severity of dysplasia more likely.

Early studies of the role of bile acids in large bowel cancer in humans were limited to epidemiological studies comparing populations at various levels of risk. Almost all of these reports supports a role for bile acids in bowel carcinogenesis (Table). Case control studies comparing the faecal

Table Studies of populations relating large bowel cancer (LBC) incidence to the mean faecal bile acid (FBA) concentration (for details and references see ref 6)

Authors	Type of study	Observation
Hill <i>et al</i> (1971)	Comparison of 6 populations from 4 continents	LBC incidence well correlated with FBA concentration
Hill and Drasar (1973)	Extension of the above study to 9 populations	LBC incidence well correlated with FBA concentration
Reddy and Wynder (1973)	Comparison of a number of racial groups in New York	LBC incidence well correlated with FBA concentration
Antonis and Bersohn (1962)	Comparison of black and white South Africans	LBC incidence well correlated with FBA concentration
Turjman <i>et al</i> (1982)	Comparison of religious and dietary subgroups in California	LBC incidence well correlated with FBA concentration
Reddy <i>et al</i> (1978)	Comparison of Finns and Americans	LBC incidence well correlated with FBA concentration
IARC Working Party (1977)	Comparison of Finns and Danes	Weak correlation between LBC incidence and FBA concentration
IARC Working Party (1982)	Extension of the above study	LBC incidence well correlated with FBA concentration
Shurpelekar <i>et al</i> (1971)	Study of South Indians	Population with a very low LBC incidence has very low FBA concentration
Crowther <i>et al</i> (1978)	Comparison of 3 income groups in Hong Kong	LBC incidence well correlated with FBA concentration

bile acid concentration in bowel cancer cases and controls have failed to show such a clear relationship, but this could be because of a number of factors (discussed in reference 18) including the choice of controls: patients with non-malignant gastrointestinal disease would be more suitable as they might have suffered a similar loss of appetite and diet is an important factor controlling faecal bile acid loss. The choice of patients is also important: those with early disease are clearly better for aetiological studies, while those with liver metastases would be unsuitable, as the metastases might affect hepatic synthesis of bile acids. In addition, genetic factors may also be important, possibly in determining the sensitivity to factors causing the formation of the precursor benign adenoma.

Studies of the benign adenoma have indicated more clearly a relation with bile acids, possibly because there have been fewer such studies. Reddy *et al*¹⁹ showed that the faecal bile acid concentration in adenoma patients was higher than in controls and a relation between adenoma size and faecal bile acid concentration has also been shown.⁶ Van der Werf *et al*²⁰ showed increased bile acid turnover and colonic absorption of deoxycholic acid in adenoma patients, compared with age-matched controls. In this issue (pp 876–880) the same group have expanded their study and have shown that not only is the colonic exposure to bile acids greater than normal in adenoma patients, but also the ratio of anaerobic to aerobic bacteria in the faeces of adenoma patients was higher.

No single study of this type is going to provide the final answer to the cause of colorectal cancer. Nevertheless, such studies on humans make an important contribution to our knowledge of colorectal carcinogenesis. They are building a picture which strongly indicates a role for bile acids and bacteria in the causation of colorectal cancer, possibly through their effect in stimulating the growth of small size benign adenomas with a low risk of malignancy, to a large size with a correspondingly high risk of malignancy. At present there is no evidence that bile acids can cause the formation of the precursor adenoma, but they may also be implicated in causing an increase in the severity of dysplasia.

This is a much clearer picture of colorectal carcinogenesis and helps to explain the lack of agreement in the case control studies. Unfortunately, it also indicated that simple solutions to the prevention of the disease are not likely to be very effective.

M J HILL

*Bacterial Metabolism Research Laboratory
PHLS Centre for Applied Microbiology and Research
Porton Down
Salisbury, Wilts SP4 0JG*

References

- 1 Aries VC, Crowther JS, Drasar BS, Hill MJ, Williams REO. Bacteria and the aetiology of cancer of the large bowel. *Gut* 1969; **10**: 334–5.
- 2 Aries VC, Crowther JS, Drasar BS, Hill MJ. Degradation of bile salts by human intestinal bacteria. *Gut* 1969; **10**: 575–7.
- 3 Hill MJ, Drasar BS, Aries VC, Crowther JS, Hawksworth GM, Williams REO. Bacteria and aetiology of cancer and large bowel. *Lancet* 1971; **1**: 95–100.
- 4 Hill MJ. The role of colon anaerobes in the metabolism of bile acids and steroids, and its relation to colon cancer. *Cancer* 1975; **36**: 2387–400.

- 5 Hill MJ. Influence of nutrition on intestinal flora. In: Goebbel H, Kaspar H, eds. *Colon and nutrition*. Lancaster: MTP, 1982: 37–46.
- 6 Hill MJ. Metabolic epidemiology of large bowel cancer. In: De Cosse J, Sherlock P, eds. *Gastrointestinal cancer*. The Hague: Martinus Nijhoff, 1981: 187–226.
- 7 Cook JW, Kennaway EL and Kennaway NM. Production of tumours in mice by deoxycholic acid. *Nature* 1940; **145**: 627.
- 8 Salaman MH, Roe FJC. Further tests for tumour initiating ability: N, N-di-(2-chloroethyl)-p.amino phenylbutyric acid (CB 1348) as initiator of skin tumour formation in the mouse. *Br J Cancer* 1956; **10**: 363–78.
- 9 Wells HG, Slye M, Holmes HF. Comparative pathology of cancer of the alimentary canal, with report of cases in mice. *Am J Cancer* 1938; **33**: 223.
- 10 Reddy BS. Dietary fibre and colon carcinogenesis: a critical review. In: Vahouny G, Kritchevsky D, eds. *Dietary fibre in health and disease*. New York: Plenum, 1982: 265–86.
- 11 Deschner EE. Relationship of altered cell proliferation to colonic neoplasia. In: Malt R, Williamson R, eds. *Colonic Carcinogenesis*. Lancaster: MTP, 1982: 25–30.
- 12 Maskens AP, Dujardin-Loits RM. Experimental adenomas and carcinomas of the large intestine behave as distinct entities, most carcinomas arise *de novo* in rat mucosa. *Cancer* 1981; **47**: 81–90.
- 13 Ames B, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. *Mutation Res* 1975; **31**: 347–57.
- 14 Silverman SJ, Andrews AW. Bile acids: co-mutagenic activity in the *Salmonella*-mammalian microsome mutagenicity test. *J Nat Cancer Inst* 1977; **59**: 1557–9.
- 15 Wilpart M, Mainguet P, Maskens A, Roberfroid M. Mutagenicity of 1,2-dimethylhydrazine towards *Salmonella typhimurium*, co-mutagenic effect of secondary bile acids. *Carcinogenesis* 1983; **4**: 45–8.
- 16 Kelsey MI, Pienta RJ. Transformation of hamster embryo cells by cholesterol- α -epoxide and lithocholic acid. *Cancer Letters* 1979; **6**: 143–9.
- 17 Morson BC, Konishi F. Dysplasia in the colorectum. In: Wright R, ed. *Recent advances in gastrointestinal pathology*. London: Saunders, 1981: 331–44.
- 18 Hill MJ. Genetic and environmental factors in human colorectal cancer. In: Malt R, Williamson R, eds. *Colon carcinogenesis*. Lancaster: MTP, 1982: 73–82.
- 19 Reddy NS, Wynder EL. Metabolic epidemiology of colon cancer: fecal bile acids and neutral steroids in colon cancer patients with adenomatous polyps. *Cancer* 1977; **39**: 2533–9.
- 20 Werf SDJ van der, Nagengast FM, Berge-Henegouwan GP van *et al*. Colonic absorption of secondary bile acids in patients with adenomatous polyps and matched controls. *Lancet* 1982; **1**: 759–62.