be carried out on the distal colon and rectum and some factors which influence the pd have been examined. The pd is of significance both because it considerably influences the flows of charged particles across the epithelium and also because it is a measurable variable which reflects something of the functional state of the tissue. Since, however, it is affected by a variety of factors, these must be defined and considered if any meaningful interpretation is to be made.

References


The normal colonic bacterial flora

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Interest in the human intestinal bacterial flora has increased greatly in recent years. To a large extent this is because techniques have now been developed which permit the study of the dominant members of the gut flora, the non-sporing strictly anaerobic bacteria.

There are two major approaches to the study of the gut flora. The first is the classical bacteriological approach, the identification and the enumeration of the major groups of bacteria. The second is the study of the biochemical activities of the flora. These two aspects of the flora will be discussed in turn.

The Normal Colonic Flora

The normal colonic flora is usually inferred from the composition of the normal faecal flora, since suitable techniques for sampling various levels of the colon have yet to be developed. Data from animal studies would support the assumption that the flora does not alter during defaecation, indicating that the faecal flora adequately represent that of the recto-sigmoid. We know that the flora of the recto-sigmoid differs from that at the ileocecal junction (table 1) and can infer, but no more, that the change takes
place in the upper rather than the lower parts of the colon.

Non-sporing anaerobic, rod-shaped organisms overwhelmingly predominate in the human faecal flora, accounting for more than 99% of the total organisms. The major species present are Bacteroides fragilis, Bifidobacterium adolescentis, and Eubacterium aerofaciens (Peach, Fernandez, Johnson, and Drasar, 1974). The remainder are mainly Escherichia coli, Streptococcus viridans, and Streptococcus salivarius and lactobacilli, but there are in addition a host of minor components of the flora including veillonellae, baccili, peptococci and peptostreptococci, clostridia, Strep faecalis, and coliform organisms other than Esch. coli (e.g., Klebsiella spp, Proteus spp). It must be stressed that, although these are minor components of the average normal flora, they may be of major importance in an individual person.

There are difficulties in interpreting the data on the normal flora. For example, the enumeration of clostridia is very difficult due to the lack of a suitable selective or diagnostic medium. Consequently, although the numbers of clostridia producing lecinthinase can readily be determined there is no means of counting vegetative clostridia which do not produce this enzyme. In the case of the latter organisms only the numbers of spores can be counted and this may be totally unrelated to the vegetative count; it is possible, therefore, that clostridia may be far more numerous in the colonic flora than is realized at present. Within the genus Bacteroides there are more than 20 species. More than 95% of faecal bacteroides belong to the species B. fragilis and consequently the enumeration of the minor species is extremely time consuming. It is important to recognize, therefore, that a species of non-sporing anaerobe that represents only 0.1% of the organisms of that genus would nevertheless be present in numbers comparable to those of the coliforms, streptococci, or lactobacilli. Until good selective media are available for the individual species of non-sporing anaerobes it will be necessary to pick 1000 colonies in order to be able to detect an organism present in considerable numbers. Thus there are still many problems remaining to be solved before we can characterize the normal faecal flora.

It must be noted, too, that the faecal flora represents only the luminal flora of the recto-sigmoid region and that the mucusal flora (the bacteria growing in close association with the mucosal surface and in the villus crypts) may differ markedly from this. This criticism also applies to the data on the small intestinal flora. Determination of the mucosal flora presents great difficulties which will not readily be overcome; however, it is suspected that the mucusal flora is more important than the luminal flora in, for example, urea metabolism.

The Effect of Diet on the Flora

People living in Uganda on a diet of matoke (boiled mashed banana) have a faecal flora which differs markedly from that of English people living on a normal western diet (Aries, Crowther, Drasar, Hill, and Williams, 1969) and this difference has been assumed to be due to the difference in diet. Alteration of the flora of adults by modifying their diet has proved to be extremely difficult to demonstrate, however. The flora of people on a low-fat diet for four weeks was not detectably different from that of the same people on a control diet; a diet rich in bagasse (fibre from sugar cane) had no detectable effect even in 12 weeks. However, differences in the flora of three dietary groups living in Hong Kong could be demonstrated. Our conclusions from these data are that (a) the effects of dietary alteration are only slowly manifest, and in controlled studies prolonged periods on a diet (more than a year) may be necessary before the effects can be demonstrated using the classical bacteriological techniques; (b) classical bacteriological techniques are unsuitable for the study of faecal micro-ecology. If total faecal β-glucuronidase is measured then the effects of diet on the flora can be demonstrated readily in four weeks (Wynder and Reddy, 1974). Using a battery of such tests, together with the results of screening studies to determine the mean enzymic activity of strains of each genus, such finer alterations in the flora might be detected.

Metabolic Activity of the Flora

In the colon there is approximately 1.5 kg wet weight of bacteria and therefore it would be amazing if their metabolic activity did not play a significant role in the overall economy of the human body. There has
been considerable interest in this subject in recent years and it would be impossible to cover this subject adequately. In the subsequent section three selected topics will be discussed in some depth rather than an attempt to cover the full range of the subject.

HYDROLYSIS OF GLYCOSIDES AND GLUCURONIDIES

A high proportion of taxonomic tests in bacteriology are fermentation reactions that involve an initial hydrolysis of a glycosidic bond followed by fermentation of the released monosaccharide. Examples of such reactions are the fermentation of aesculin, salicin, and amygdalin. All three examples given are of β-glucosides and such compounds are poorly metabolized by intestinal enzymes. Many plant products are glucosides of toxic compounds and it is the ability of the colonic flora to hydrolyse these compounds to release the toxic aglycone that renders an item of food dangerous. Thus, untreated cassava contains amygdalin which is hydrolysed by the flora to release cyanide. Untreated cassava is highly toxic and in areas where cassava is a staple source of starch the raw product is extracted exhaustively to remove the amygdalin before converting the ‘purified’ cassava into flour.

In other parts of the world cycad nuts are a staple source of starch but, again, untreated cycads are extremely hepatotoxic because they contain the glucoside cycasin, which is hydrolysed by the gut flora to release methylazoxy-methanol. In germ-free rats cycasin administered orally is harmless at a dose which would kill a conventional rat in 48 hours (Spatz, Smith, McDaniel, and Laqueur, 1967). To render them harmless the cycads are extracted repeatedly with water to remove the cycasin.

The ability of the flora to hydrolyse plant glucosides has been used extensively in medicine. The glucoside cathartics senna and cascara are hydrolysed to release their active agents. It has been demonstrated (Hardcastle and Wilkins, 1970) that whereas cascara sagrada has no effect on the colon the aglycone increases colic motility of rabbits. If the free aglycone is fed to the animals it has no cathartic action, indicating that it is sensitive to either gastric or small intestinal secretions. Thus the glucose moiety protects the aglycone during its transit through the upper digestive tract allowing it to be released in an active form in the colon.

The ability of the bacterial flora to hydrolyse glucuronides is important in the enterohepatic circulation of compounds (Smith, 1966). This has both beneficial and harmful effects. For example, chloramphenicol, which undergoes extensive enterohepatic circulation, is retained within the body for a prolonged period of time and therefore has a better opportunity to exert its antibacterial effect. Similarly phenolphthalein and morphine are also retained thereby improving their therapeutic effect. However, retention within the enterohepatic circulation also allows more time for bacterial degradative enzymes to be induced. If we use chloramphenicol as the example there is a wide range of bacterial modifications that can occur (Smith, 1966), all of which result in loss of antibacterial activity and some of which may result in the formation of toxic products.

Lactose is readily metabolized by bacterial β-galactosidase but this is normally of no consequence in western human adults since the intestinal β-galactosidase degrades any dietary lactose long before it reaches the large bowel. In most parts of the world, however, intestinal β-galactosidase is lost during childhood and ingestion of lactose results in fermentative diarrhoea. This has been reviewed by Neale (1971). Bacterial β-galactosidase is utilized in lactulose therapy. Lactulose is the ketoisomer of lactose; it is not hydrolysed by intestinal β-galactosidase but is readily degraded by the bacterial enzyme. By carefully controlling the dose of lactulose the amount of acid produced in the colon, and therefore the colonic pH, can be controlled.

Cellulose and hemicelluloses are complex glycosides which are susceptible to bacterial attack; it has been shown that normally 20% of a dose of hemicellulose is degraded during transit through the gut. This degradation will result in the formation of short-chain fatty acids which by their osmotic effect might well explain the bulking effect of fibre.

AMMONIA METABOLISM

Intestinal bacteria produce ammonia by deamination of amino acids but the principal route is by urea hydrolysis. Urea undergoes an enterohepatic circulation, being produced in the liver then entering the colon by passive diffusion, where it is hydrolysed to ammonia and carbon dioxide. The ammonia re-enters the portal blood, again by diffusion, and returns to the liver to be converted back to urea or to be incorporated into protein. Approximately 15-30% of the total urea pool, i.e., about 7 g, is hydrolysed each day (Walser and Bodenlos, 1959; Jones, Smallwood, Craigie, and Rosenoer, 1969). The major source of colonic urease is the gut bacterial flora. Treatment of patients with neomycin or other antibiotics reduces the faecal urease activity (Evans, Aoyagi, and Summerskill, 1966) and germ-free animals fail to catabolize urea to any significant extent (Levenson, Crowley, Horowitz, and Malm, 1959). Interestingly, it appears that it is the mucosal flora that is important in urea metabolism rather than the luminal flora. This conclusion was reached by...
Wolpert, Phillips, and Summerskill (1971) following the observation that urea delivered luminaly was much less efficiently hydrolysed by the flora than that administered systematically. Hardly any urea is detectable in faeces so that virtually all of the urea entering the gut is hydrolysed to ammonia.

In the normal healthy person, the ammonia generated in the gut is restricted to the portal blood system, but in hepatic disease the liver fails to detoxify the ammonia and it enters the general circulation (White, Phear, Summerskill, and Sherlock, 1955). This is said to result in the chronic or recurrent neuropsychiatric disorders related to protein intolerance, ultimately resulting in hepatic coma due to the direct cerebrotoxic action of ammonia (Schenker, McCandless, Brophy, and Lewis, 1967). Similar symptoms arise, for the same reasons, in protein intolerance due to portal systemic anastomosis (Riddell, 1955). Hyperammonaemia has been reviewed by Summerskill (1970).

The major course of therapy of hepatic coma is the control of intestinal ammonia production and nitrogen metabolism. This has been attempted either by (a) reducing the supply of nitrogen compounds to the gut, (b) reducing the number of ammonia-producing bacteria, (c) reducing the time available for bacteria to produce ammonia, and (d) preventing the escape of ammonia from the gut. Attempts to reduce the numbers of ammonia-producing bacteria take two forms: antibiotic therapy and replacement therapy. A number of antibiotics have been shown to be effective for a short time but these have the usual side effects which accompany disturbance of the gut flora, namely, diarrhoea, steatorrhoea, and staphylococcal enterocolitis (Walker, Emlyn-Williams, Craigie, Rosonoer, Agnew, and Sherlock, 1965). Replacement therapy is based on the concept that, since some bacteria are ureolytic while others are not, replacement of the normal (ureolytic) flora by one which fails to hydrolyse urea (e.g., lactobacilli) will be beneficial. It might well be so if such a replacement were possible; as it is this method of therapy has little success, and only serves to emphasize the remarkable stability of the gut flora. The amount of ammonia produced should be reduced if the time available for its production is reduced, and this is the rationale behind the use of enemas and purgatives. There is still some question regarding the amount of agent to be used, and the ultimate effectiveness of this treatment which would inevitably appear to be a short-term measure.

Prevention of the escape of ammonia from the gut is the basis of lactulose therapy. Ammonia is readily absorbed from the gut in its free form. When the colonic pH is reduced the ammonia will be in an ionized form and will be on a pH gradient; under these conditions the ammonia should theoretically move from the blood to the colon rather than the reverse, resulting in reduced blood ammonia levels and increased faecal excretion of ammonia. This is the rationalization of the use of lactulose, the dose of which is carefully controlled to give a faecal pH of 4 to 5 (Bircher, Müller, Guggenheim, and Haemmerli, 1966; Elkington, Floch, and Conn, 1969). Unfortunately for the theory, under conditions in which lactulose was being used successfully, i.e., when the blood ammonia level was reduced, there was no increase in faecal ammonia levels. It is apparent that some rethinking is necessary.

In hepatic failure the production of ammonia by the gut flora is detrimental to the patient; in renal failure the reverse is true. Here the blood urea increases to toxic levels unless the nitrogen intake is controlled. As the blood urea level increases so does the faecal urease (Brown, Hill, and Richards, 1971), resulting in the release of ammonia which returns via the portal system to the liver where a proportion is incorporated into protein. The increased faecal urease could be the result either of an increased proportion of organisms producing urease, or to an increased output of enzyme from a constant number of active organisms. In fact, the former is true, with the proportion of urease-positive organisms in the faecal flora increasing with the blood urea level. There is no apparent reason for this; the production of urease is not known to confer any ecological advantage in the presence of high urea levels.

**Metabolism of bile acids**

The metabolism of bile acids is of interest from two points of view: these are (1) the major metabolic reactions which give rise to the secondary bile acids in bile and faeces; (2) the minor reactions which may give rise to carcinogens and be implicated in the aetiology of colon cancer.

The major metabolic reactions carried out by the gut bacteria are (1) hydrolysis of bile conjugates to release the free bile acids; (2) hydroxyl oxidation to a keto group, followed under certain circumstances by reduction to a β-hydroxyl group; (3) removal of the 7α-hydroxyl group to yield the major secondary bile acids deoxycholic acid (from cholic acid) and lithocholic acid (from chenodeoxycholic acid).

Although the deconjugation reaction occurs extensively in the ileum the other reactions take place mainly in the caecum and colon. The dehydroxylation reaction yields products which are less well absorbed than their substrates and consequently contributes to the total faecal loss of bile acids. Although the products of these major reac-
tions may play a role in small intestinal disease, they do not contribute in any large measure to large intestinal disorders.

The minor reactions of bacteria upon bile acids are of more interest to us because we have produced the hypothesis that they are important in the aetiology of large bowel cancer. We have postulated (Aries et al, 1969) that colon cancer is caused by a carcinogen or cocarcinogen produced in situ in the colon from some otherwise benign substrate; we have also postulated that the substrate is the bile acid moiety. We have shown (Hill, Drasar, Aries, Crowther, Hawksworth, and Williams, 1971; Hill, 1974) that in comparisons between a number of countries, some with high and some with low incidences of colon cancer, the incidence of colon cancer correlates with the total faecal bile acid concentration and with the faecal concentration of dihydroxycholanic acids. The latter correlation was remarkably good.

The question which then arises is, What is the nature of the carcinogen or cocarcinogen produced from the bile acids? We have postulated that the carcinogen is a polyunsaturated bile acid, and have suggested a pathway by which the bacteria could theoretically produce a polycyclic aromatic compound from the bile acids using only four types of nuclear dehydrogenation reaction (Hill, 1971a). These have all been demonstrated in vitro using human gut bacteria and we have produced in vitro a bile acid with rings A and B of the steroid nucleus fully aromatic. One of the four reactions is the dehydroxylation which proceeds via an unsaturated intermediate (Samuelsson, 1960; Goddard and Hill, 1973); this reaction is carried out by a wide range of anaerobic organisms, the proportion of active strains being much higher in areas with a high incidence of colon cancer than in those with a low incidence. The other three reactions are only carried out by certain lecinthinase-negative clostridia, principally *Cl. paraputrificum*, which will subsequently be referred to as nuclear-dehydrogenating (or NDH) clostridia. These organisms are rare in the faeces of people living in areas with a low incidence of colon cancer but much more common in faeces from people living in high incidence areas. Thus in support of our hypothesis that the gut flora produce an unsaturated carcinogen from the bile acids and that this is important in the aetiology of colon cancer, we find that in areas with a high incidence of colon cancer the concentration of the postulated substrate is high and the relevant bacteria are common, whilst in areas of low incidence the substrate concentration is low and the relevant bacteria are rare.

During the last year we have been testing our hypothesis in individuals, since those with a high faecal bile acid concentration and large numbers of NDH clostridia should be at high risk whilst those with low levels of only one or neither of these two requirements should be at low risk. In the study, which is being conducted in collaboration with workers at Northwick Park Hospital, Harrow, and at St Mark's Hospital, London, we have examined the stools of patients with newly diagnosed large bowel cancer and patients admitted to the same ward with diseases other than colon cancer. Only the first stool on entry to the ward was acceptable, since later stools would have been affected by the hospital diet, which is low in fat and would result in a low faecal bile acid level (Hill, 1971b). The study is being carried out blind with the code being broken after each 50 patients. The latest analysis (Hill, Drasar, Goddard, Peach, Williams, Meade, Cox, Simpson, and Morson, 1974) shows that in the large bowel cancer patients 22 of 29 belonged to the high-risk group compared with one of 60 of the control group (table II).

<table>
<thead>
<tr>
<th>Faecal Bile Acid Concentration (mg/l dry wt)</th>
<th>Nos. of NDH Clostridia (per g wet wt)</th>
<th>Large Bowel Cancer Patients</th>
<th>Control Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;6</td>
<td>&gt;10⁴</td>
<td>22 (76%)</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>&lt;6</td>
<td>&gt;10⁴</td>
<td>4 (14%)</td>
<td>25 (42%)</td>
</tr>
<tr>
<td>&gt;6</td>
<td>&lt;10⁴</td>
<td>2 (7%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>&lt;6</td>
<td>&lt;10⁴</td>
<td>1 (3%)</td>
<td>28 (47%)</td>
</tr>
</tbody>
</table>

Table II Bile acid concentration and numbers of NDH clostridia in faeces from patients with large bowel cancer and controls admitted to the same gastroenterology ward with diseases other than large bowel cancer

These findings support those obtained in the international study. The next steps are (a) to see whether the faecal bile acid concentrations together with the numbers of NDH clostridia can be used to predict colon cancers in a prospective study; (b) to see whether any of the products of nuclear dehydrogenation of the bile acids obtained in vitro is carcinogenic in animal tests; (c) to see which of these unsaturated bile acids are produced in vivo in man; (d) to see the effect of dietary changes on the faecal bile acid concentration and on the numbers of NDH clostridia produced.

Conclusions

Although our knowledge of the gut flora both in terms of its composition and of its function has increased dramatically in recent years, the unanswered questions still greatly outnumber those
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answered. In this communication we have been able merely to outline the problems involved and the progress made towards solving some of them. It is evident that this is still a new field of study which can make a contribution to the understanding not only of gastroenterological but also of general medical and pharmacological problems.

References


Absorption and secretion by the colon

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Various aspects of colonic absorptive function in man have been dealt with in a number of reviews (Phillips, 1969; Turnberg, 1970; Wrong, 1971; Shields, 1972; Sladen, 1972; Edmonds and Pilcher, 1972).

Electrolytes and Water

The role which the colon plays in conserving water and electrolytes has been known for some time, deduced largely from a comparison of the volume and composition of ileostomy effluent with that of faeces and confirmed by perfusion of the colon in vivo. The quantity of these substances absorbed daily by the colon has, however, to be revised, since it has been shown that the flow of ileal contents into the colon each day is three times the normal volume of ileostomy effluent (Kanaghinis, Lubran, and Coghill, 1963; Phillips and Giller, 1973). Fasting ileal flow rates, measured by slow intestinal
The normal colonic bacterial flora.

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