Intestinal bacterial flora in normal adults in the U.K.

S. PEACH

St. Bartholomew's Hospital, London

Interest in anaerobes as normal flora began early in the present century [1–3] and during the last 15 years it has been established that non-sporing anaerobes are present as normal flora at various sites in the body [4, 5] and are by far the most numerous of the indigenous flora of man. The human mouth and intestinal tract are of particular interest to bacteriologists studying anaerobic bacteria because they provide very rich sources of isolates.

The mouth

Because of easy access to atmospheric oxygen the mouth is not generally considered to be an anaerobic environment; however, deep in the gingival crevices facultative bacteria lower the oxygen potential and produce local anaerobic conditions.

The indigenous flora of the oral cavity is complex and anaerobic bacteria are numerous (Table 1). Many original studies concentrated on the microbial flora of saliva, since samples were easily obtainable. *Streptococcus salivarius* was found to be the predominant organism (Table 2). For several years now, however, it has been recognised that certain bacterial types preferentially colonize different sites in the mouth and the sampling of these sites has brought to light microbial flora which is present. In addition to *S. salivarius*, the soft tissues of the mouth such as the surface of the gums, tongue and cheeks are preferentially colonized by *S. sanguis*, while *S. mutans* colonized the hard surfaces of the teeth. Possible differences in inherent abilities to attach to different sources may account for these observations [6, 7]. Bifidobacteria, fusobacteria and spirochaetes may be isolated from salivary samples [8 9,] and both *Bacteroides melaninogenicus* and *B. oralis* have been isolated from gingival cavities. It should be noted that strains of *B. malaninogenicus* isolated from the mouth have been shown to be bio-

chemically different from strains isolated from other areas of the gastrointestinal tract [10]. Dental plaque contains a relatively high proportion of Gram positive rods, especially *Actinomyces* species, and this is often accompanied by high levels of veillonellae [11] and anaerobic Gram negative rods.

TABLE 1

Oral flora

SITE	Typical bacterial counts per ml	
	Aerobes	Anaerobes
Saliva	10^7-10^8	10^8-10^9
Tooth surfaces	10^6	10^6
Gingival scrapings	10^7	10^7

From: Hoeprich, P.D. [29].

TABLE 2

Oral flora

Common bacteria	SITE
S. salivarius	Saliva, soft tissues
S. sanguis	Soft tissues
S. mutans	Teeth
Bifidobacteria, Fusobacteria Spirochaetes	Saliva
Bacteroides melaninogenicus	Gingival cavities
Bacteroides oralis	Gingival cavities
Veillonellae	Dental plaque
Actinomyces	Dental plaque
Bacteroides	Dental plaque

The stomach and the small bowel

The stomach provides an important barrier to microbial overgrowth of the small bowel [12]. Acid stomach contents are normally sterile [9] as the pH of the stomach content falls the number of bacteria per millilitre of contents falls correspondingly. The bactericidal action is thought to be due to free hydrochloric acid. Drasar and Coll. [9] have shown that immediately after a meal a count of 10^5–10^7 bacteria/ml of gastric juice can be recorded and includes Streprococci, Enterobacteria, Bacteroides and Bifidobacteria, derived from the mouth and from the meal. As the pH falls the bacterial count declines and relatively few bacteria are found when the stomach contents have fallen below about pH 3.

Data obtained using intubation techniques [9, 13–15] have shown that the upper regions of the small bowel (duodenum, jejunum and upper ileum) have a sparse microbial flora consisting predominantly of Gram–positive facultative organisms. In the lower small intestine anaerobes, including Bacteroides, occur and there is a richer more permanent flora. The lower ileum, however, still maintains a relatively low total concentration of organisms (10^5–10^9/ml) and represents a transitional zone between the sparse flora of the upper gastrointestinal tract and the rich flora of the large bowel [16].

Bacterial flora of the large bowel

Distal to the ileocaecal valve a striking change in the number and types of bacteria can be seen [14, 15]. There is an increase in anaerobic populations, Bacteroides and Bifidobacteria become the dominat micro–organisms, outnumbering the aerobic and facultative flora by 1,000 to 10,000 : 1. The intraabdominal colon is obviously difficult to sample, but intubation studies [14, 15] together with studies of the contents of healthy appendices obtained at operation [17] suggest that within broad limits, the faecal flora is representative of the bacterial flora of the large bowel.

The adult faecal flora

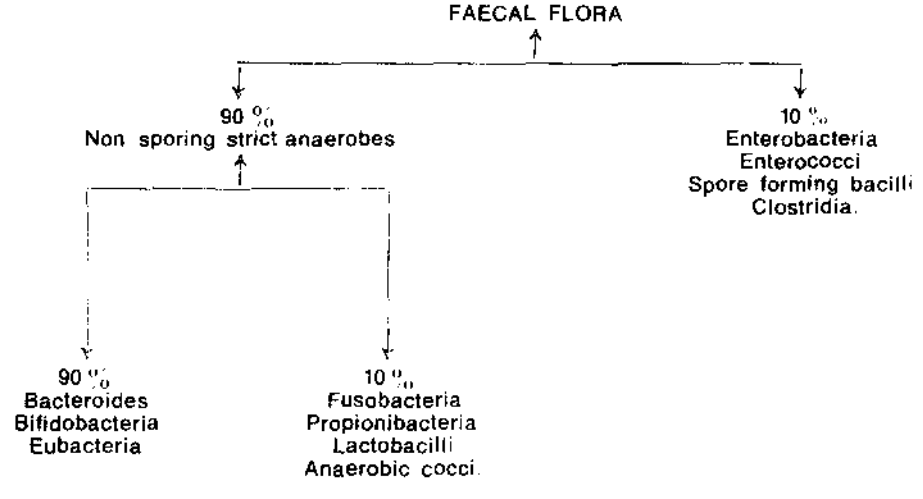
Scheme 1 is a diagrammatic representation of the faecal flora of European adults. The numerical dominance of the non–sporing anaerobes in faeces is well established [2, 18, 19] . Bacteria commonly regarded as faecal isolates, *e.g.* the Enterobacteria, the Enterococci and the Clostridia, in fact constitute only a small proportion of the total bacterial flora. Various authors using differing bacteriological techniques generally agree that 10^{10}–10^{11} viable bacteria can be recovered from 1 g (wet–weight) of faeces. In the U.K. only about 10^6 of these are facultative bacteria and sporing anaerobes.

A study in 1971 by Hill and Coll. [20] at St. Mary's Hospital in London showed that the faeces of persons living in the U.K. and America generally contained higher numbers of Bacteroides and fewer Enterococci than did the faeces of groups of people living on high carbohydrate diets in Uganda, India and Japan. Thus, the ratio of the number of anaerobes to the number of aerobes differed in these two populations. In addition Sarcinae (Gram positive sporing cocci) were isolated only from groups of people consuming diets rich in vegetable matter. In Britain, such people were strict vegetarians [21].

In a continuation of the study at St. Mary's Hospital, comparing the faecal flora of persons in the U.K. with persons in other countries, 196 strains of non-sporing anaerobes were isolated from the faeces of 34 people living on a mixed Western diet in the U.K. and in America. These isolates were compared with 215 strains isolated from the faeces of 45 people consuming carbohydrate rich diets in Uganda, India and Japan [22]. All the isolates could be assigned to genera. The predominant groups of non-sporing strict anaerobes in faeces were found to be similar for all the populations studied: they fell into three main genera: Bacteroides, Bifidobacteria and Eubacterium. Minor components of the anaerobic flora consisted of Fusobacteria and Propionibacteria. Although similar groups of organisms were isolated from all the faecal samples, with Bacteroides the most common isolates, there were differences in the distribution and relative proportions of these organisms. Faeces from Britain and America appeared to have a

SCHEME 1

Main genera of bacteria in faeces



larger proportion of Gram negati veorganisms (Bacteroides spp. and Fusobacteria) than did those from people from India, Uganda and Japan. The consequently larger proportion of Gram positive organisms found in faeces from these countries seemed to be due mainly to the contribution of the Eubacteria (Table 3). These differences were shown to exist when the populations were taken as a whole. On close examination of individuals within a population four types of faecal flora appeared to exist, *i.e.* persons with (1) mainly Bacteroides, (2) mainly Bifidobacteria, (3) mainly Eubacteria, (4) mixed flora. As a whole, the populations reflected the most common type of individual flora. Almost all the Bacteroides isolates could be shown to belong to the *B. fragilis* group of organisms (Table 4). Two subspecies of *B. fragilis* are common in faeces — vulgates and thetaiotamicron. These subspecies of *B. fragilis*, amongst others, have now been afforded full

TABLE 3

Percentage frequency of genera of non-sporing anaerobes among isolates from faeces of populations consuming different diets

POPULATION AND DIET	Number of isolates identified	Percentage frequency, among isolates from the stated populations, of genus					
		Bacteroides	Fusobacterium	Bifidobacterium	Eubacterium	Propionibacterium	Lactobacillus
Mixed Western (U.S.A., England, Scotland)	224	61.6	0.9	25.0	6.7	3.1	0
High Carbohydrate (Uganda, India, Japan)	215	37.7	1.4	27.0	23.2	5.1	1.1

TABLE. 4

Major species of non- sporing anaerobes found in faeces

Gram–negative rods:

Bacteroides thetaiotamicron
Bacteroides vulgatus
} « Fragilis group »

Gram–positive rods:

Bifidobacterium adolescentis
Bifidobacterium bifidum
Bifidobacterium eriksoni
Bifidobacterium aerofaciens

species status and are shown as such in Table 4 among the Bifidobacteria. *B. adolescentis* was the commonest isolate; *B. bifidum* and *B. eriksoni* were also common. Most of the eubacteria appeared to be *E. aerofaciens*.

In this paper discussion has been confined to the bacterial flora of the lumen of the gastrointestinal tract. However, in addition there appears to exist a microbial flora which is closely associated with the intestinal mucosa, probably colonizing the layer of mucus which overlies the cells [23, 24]. In a study at St. Bartholomew's Hospital in London [25] a microbial flora was found to be associated with tissue from various parts of the human gastrointestinal tract. Large bowel tissue yielded greater numbers of bacteria (10^7/g of tissue) than did tissue from the small bowel (10^4/g of tissue). From all sites, about half of the isolates were aerobic or facultative bacteria and half were strictly anaerobic. Mucosal flora, therefore, unlike the feacal flora, does not appear to be dominated by anaerobic bacteria. Within broad limits the bacterial flora of the intestine appears to be stable and self regulating. Several factors may be involved in its regulation and these are shown in Table 5.

The influence of diet on the faecal flora of various populations has already been mentioned. Different dietary groups may have different proportions of various bacteria in their faeces. In addition it has been known for many years [26], that infants fed wholly on breast milk have a particularly characteristic faecal flora dominated by large numbers of Bifidobacteria. In 1974, Willis and Coll. [27] showed that the composition of breast milk is important in determining this flora and Bullen, Rogers and Leigh [28] showed that certain factors in human breast milk may protect the infant against colonization by enteropathogenic strains of *E. coli*.

As previously discussed, gastric acid provides an important barrier to bacterial overgrowth in the small bowel. This action may be modified to some extent by the neutralizing effect of food when the stomach is full. As in any mixed flora, bacterial interactions play a large part in determining the flora of the gatrointestinal tract. These interactions may be either synergistic (*e.g.* the production of anaerobic conditions through the meta-

TABLE 5

Factors influencing the intestinal flora

Diet	Gastric acid
Bacterial interactions	Bile
Gastrointestinal mobility	Specific antibody
Lysozyme	

bolism of oxygen by aerobic bacteria) or competitive (*e.g.* the production of colicines by coliform bacteria). Direct competition for nutrient sources may also play a role in determining the flora of the colon and faeces.

Tolerance to bile is used by bacteriologists in culture media to select for intestinal organisms and will obviously exert a selective effect *in vivo*, particularly in the small bowel. Similarly, the gastrointestinal mobility associated with the small bowel will limit bacterial populations in this area.

Finally, lysozyme and specific antibody — particularly IgA, may play a role in confining gut bacteria to the gastrointestinal tract.

In conclusion, the gastrointestinal tract support a rich and varied bacterial flora, particularly as far as the non-sporing anaerobic bacteria are concerned. Under normal circumstances the host lives happily in association with these organisms, however if this flora is disturbed or displaced, serious consequences result and often become the problems of the clinical bacteriologist.

Summary (*Intestinal bacterial flora in normal adults in U.K.*). — The A. reports on the composition of the bacterial flora being present all through the intestine of normal adults in the United Kingdom, listing the number and families of the aerobes and anaerobes occupying the various portions of the alimentary tract.

Emphasis is then laid on a comparison between the normal « British » flora in the colon and rectum, where the ratio of anaerobes to aerobes is 100 to 1, and the flora found in normal adults in Africa, India and Japan, stressing the major differences in the numbers of clostridia and non-sporing anaerobes.

The A. finally highlights the importance of the factors governing the intestinal bacterial ecology, as well as the serious consequences likely to arise from the alteration of such flora.

Riassunto. (*Flora batterica intestinale in adulti sani in U. K.*). — L'A. riferisce la composizione della flora batterica presente lungo tutto l'intestino di adulti normali nel Regno Unito, elencando il numero e le famiglie dei germi anaerobi ed aerobi che occupano i vari tratti dell'apparato digerente.

Successivamente si sofferma a confrontare la flora normale presente nel colon e nel retto, dove gli anaerobi sono in una proporzione di 100 a 1 rispetto agli aerobi, con quella presente in adulti normali in Africa, India e Giappone, mettendo in risalto la differenza che si rileva tra numero di clostridi e di anaerobi asporigeni.

Conclude infine sottolineando l'importanza dei fattori che regolano la ecologia batterica intestinale e le serie conseguenze che possono derivare da una modificazione della flora stessa.

REFERENCES

1. Lewkowicz, X. 1901. Recherches sur la flore microbienne de la bouche des nourrissons. *Arch. Med. Exp. Anat. Pathol.* **13**: 633.

2. Sanborne, A. G. 1931. The faecal flora of adults with particular attention to individual differences and their relationship to the effects of various diets. I. Individual differences on normal diet. *J. Infect. Dis.* **48**: 541–569.

3. Thomas, C. G. A. & Hare, R. 1954. The classification of anaerobic cocci and their isolation in normal human beings and pathological processes. *J. Clin. Pathol.* **7**: 300.

4. Rosebury, T. 1962. *Microorganisms Indigenous to Man.* New York, U.S.A.

5. Smith, L. D. & Holdeman, L. V. 1968. *The Pathogenic Anaerobic Bacteria.* Blacksberg., Virginia, U.S.A.

6. Van Houte, J., Gibbons, R. J. & Banghost, S. B. 1970. Adherence as a determinant of the presence of *Streptococcus salivarius* and *Streptococcus sanguis* on the tooth surface. *Archs. Oral Biol.* **15**: 1025.

7. Gibbons, R. J. & van Houte, J. 1971. Selective bacterial adherence to oral epithelial surface and its role as an ecological determinant. *Infect. Immun.* **3**: 567–571.

8. Richardson, R. L. & Jones, M. 1958. A bacteriological census of human saliva. *J. Dent. Res.* **37**: 697–700.

9. Drasar, B. S., Shiner, H. & Mcleod, E. M. 1969. Studies on the intestinal flora of the gastrointestinal tract in healthy and achlorhydric persons. *Gastroenterology.* **56**: 71–79.

10. Macfarlane, T. W. 1974. Dentistry and Bacteroides. *Symposium on Bacteroides Infections.* The West of Scotland Clinical Microbiology Group. Glasgow.

11. Hardie, J. M. & Bowden, G. H. 1973. The normal microbial flora of the mouth. In: *The Normal Microbial Flora of Man.* F. A. Skinner and J. G. Carr (Eds.) Academic Press, London.

12. Gray, J. D. A. & Shiner, M. 1967. Influence of gastric pH on gastric and jejunal flora. *Gut.* **8**: 574–581.

13. Shiner, M., Waters, T. E. & Gray, J. D. A. 1963. Culture studies of the gastrointestinal tract with a newly devised capsule. *Gastroenterology.* **45**: 625–632.

14. Gorbach, S. L., Nahas, L. Lerner, P. I. & Weistein, L. 1967. Studies of intestinal microflora. I. Effects of diet, age and periodic sampling on numbers of faecal microorganisms in man. *Gastroenterology.* **53**: 845–855.

15. Gorbach, S. L., Plant, A. G., Naha, L. & Weinstein, L. 1967. Studies of intestinal microflora. II. Microorganisms of the small intestine and their relationship to oral and faecal flora. *Gastroenterology.* **53**: 856–867.

16. Gorbach, S. L. 1971. Intestinal microflora. Progress in gastroenterology. *Gastroenterology.* **60**: 1110–1122.

17. Seeliger, H. & Werner, H. 1963. Recherches qualitatives et quantitatives sur la flore intestinale de l'homme. *Ann. Inst. Pasteur.* **105**: 911–6936.

18. Eggerth, A. H. & Gagnon, C. H. 1933. The bacteroides of human faeces. *J. Bacteriol.* **25**: 389–413.

19. Lewis, K. H. & Rettger, L. F. 1940. Non-sporing anaerobic bacteria of the intestinal tract. I. Occurrence and taxonomic relationships. *J. Bacteriol.* **40**: 287–307.

20. Hill, M. J., Crowther, J. S., Drasar, B. S., Hawksworth, Gabrielle, Aries, Vivienne & Williams, R. E. O. 1971. Bacteria and the aetiology of cancer of the large bowel. *Lancet*, i: 95–100.

21. Crowther, J. S. 1971. *Sarcina ventriculi* in human faeces. *J. Med. Microbiol.* **4**: 343–350.

22. Peach, S., Fernandez, F. Johnson, K. & Drasar, B. S. 1974. The non-sporing anaerobic bacteria in human faeces. *J. Med. Microbiol.* **7**: 213–221.

23. Nelson, D. P. & Mata, L. J. 1970. Bacterial flora associated with the human gastrointestinal mucosa. *Gastroenterology*. **58**: 56–61.

24. Plaut, A. E., Gorbach, S. L., Nahas, L., Weinstein, L., Spanknebel, G. & Levitan, R. 1967. The microbial flora of human small intestinal mucosa and fluids. *Gastroenterology*. **53**: 868–873.

25. Peach, S., Lock, R., Katz, D., Todd, I. & Tabaqchali, S. 1978. Mucosal associated bacterial flora of the intestine in patients with Crohn's disease and in a control group. *Gut*. **19**: 1034–1042.

26. Tissier, H. 1900. *Recherches sur la flore intestinale des nourissons*. Theses, Paris.

27. Willis, A. T., Bullen, J. J. & Williams, K. 1974. Breast milk substitute, a bacteriological study. *Brit. Med. J.* **3**: 67.

28. Bullen, J. J., Rogers, H. J. & Leigh, H. 1973. Iron-binding proteins in milk and resistance to *Escherichia coli* infection in infants. *Br. Med. J.* **1**: 69–72.

29. Hoeprich, P. D. 1970. Etiological diagnosis of lower respiratory tract infection. *Calif. Med.* **112**: 1.

Flora batterica intestinale in adulti normali

P. GIANFRILLI MASTRANTONIO (*a*), I. LUZZI (*a*), M. OCCHIONERO (*a*),
F. BELSITO (*a*), G. PANICHI (*b*), E. MORERA (*b*),
F. ERCOLANI (*b*), L. PIAZZAI (*b*), A. L. PANTOSTI (*b*),

(*a*) *Istituto Superiore di Sanità, Roma*
(*b*) III *Clinica Medica, Università di Roma*

Negli ultimi anni l'interesse per la flora batterica intestinale è notevolmente aumentato perché l'uso di nuove metodiche ha permesso di coltivare con più facilità i germi anaerobi così da dimostrare che questi rappresentano la maggior parte dei batteri presenti nell'intestino, e da permettere studi più completi sulle possibili variazioni quantitative e qualitative della flora intestinale per valutare il ruolo svolto da certi batteri nel determinismo sia di varie forme patologiche quali ad es. il cancro del colon, sia di variazioni metaboliche importanti nella economica del corpo umano [1–3].

Con il nostro studio ci siamo posti come obiettivi la rilevazione della flora *predominante* aerobica ed anaerobica nelle feci di soggetti sani, la valutazione del rapporto quantitativo tra germi aerobi, anaerobi non formanti spore e clostridi e l'identificazione del genere degli aerobi e degli anaerobi così da poter individuare nell'ambito di queste due componenti batteriche quelle maggiormente rappresentate. I risultati ottenuti con questo lavoro rappresentano dunque i dati di una flora batterica predominante del basso intestino di soggetti sani non sottoposti a diete particolari o a trattamenti con chemioantibiotici o antiacidi e possono fornire una indicazione qualitativa e quantitativa dei microorganismi che albergano nel colon in condizioni normali.

A questo fine sono stati esaminati 12 individui sani di età tra i 20 e i 60 anni, che presentavano regolari funzioni dell'alvo intestinale. Per ogni soggetto sono stati prelevati tre campioni di feci in giorni successivi. Ogni campione veniva in parte immediatamente insemenzato in un *container* contenente terreno di trasporto precedentemente tarato, in modo da ricavare dopo l'insemenzamento il peso reale delle feci ed in parte posto in un *container* per sottoporlo ad un esame microscopico diretto. Per il nostro lavoro venivano accettati solo quei campioni che risultavano negativi al precedente

esame. Il terreno di trasporto contenente le feci in esame veniva introdotto nella cappa in atmosfera di CO_2, H_2 e N_2, omogeneizzato e diluito in provette contenenti terreno di Ringer preridotto a diluizioni scalari —0.1 ml delle diluizioni 10^{-5}, 10^{-6}, 10^{-7} veniva piastrato su terreno Reinforced Clostridial Agar (RCA) preridotto. Queste piastre venivano poste in Gas Pack, estratte dalla cappa ed incubate a 37 °C × 3 gg. 0.1 ml delle diluizioni 10^{-3}, 10^{-4}, 10^{-5} veniva utilizzato per seminare piastre di agar sangue necessarie per la ricerca di germi aerobi. Le provette con le diluizioni 10^{-1}, 10^{-2} venivano invece sottoposte a shock termico a 80 °C × 20 min e poi seminate su piastre di RCA per la ricerca dei germi sporigeni, incubate dentro contenitori Gas Pack a 37° × 3 gg.

10 colonie sviluppatesi in condizioni di aerobiosi venivano prelevate seguendo una linea casuale segnata precedentemente sul retro della capsula Petri, scegliendo le piastre con sviluppo tra 20 e 200 colonie e seminate su *containers* di Trypticase Say Agar (TSA) la cui crescita veniva utilizzata per le prove biochimiche necessarie alla identificazione della specie batterica. Le piastre all'RCA su cui erano state seminate le diluizioni trattate con lo shock termico di 80 °C × 20 min per la ricerca dei clostridi venivano lette e contate dopo 72 h, così come le piastre di RCA chiuse in Gas Pack a 37 °C per la crescita dei germi anaerobi non sporigeni, dalle quali si prelevavano casualmente secondo una linea tracciata nel diametro della piastra, come per gli aerobi. 10 colonie asportando anche una parte dell'agar sangue sottostante, si seminavano in *containers* di Robertson Cooked Meat (RCM) incubandoli a 37 °C × 2 gg. Dopo 48 h i *containers* venivano dunque aperti e seminati su piastre di RCA + dischi di vancomicina, necessaria per la discriminazione tra Gram — resistenti e Gram + sensibili, su piastre di agar sangue da incubare in condizioni di aerobiosi per ulteriore controllo ed in *containers* di glucosio brodo necessario per gli esami gas–cromatografici.

Le colonie cresciute solo in condizioni di anaerobiosi su RCA venivavano dunque osservate per il loro comportamento verso la vancomicina, esaminate al microscopio dopo la colorazione con il Gram ed i relativi *containers* di glucosio brodo venivano quindi trattati con acido solforico 50 % ed etere, centrifugati, il supernatante prelevato ed iniettato nel gas–cromatografo. I profili relativi agli acidi grassi volatili, insieme al Gram e alla morfologia rilevata al microscopio erano nella maggior parte dei casi sufficienti ad indicare il genere del germe mentre lì dove vi potevano essere dei dubbi si eseguivano delle prove biochimiche. Ad es. per differenziare i Lattobacilli dagli Eubatteri e dai Bifido nel caso in cui questi ultimi non fossero già chiaramente riconoscibili all'esame microscopico per il loro particolare aspetto, si eseguiva la crescita a 45 °C positiva solo per i Lattobacilli, o per distinguere i Peptostreptococchi dai Peptococchi si eseguiva la fermentazione del glucosio, positiva per Peptostreptococchi tranne per il solo *P.*

micros facilmente però distinguibile all'esame microscopico per le sue esigue dimensioni. Non mi addentro ora nei dettagli delle prove biochimiche e gas–cromatografiche perché ciò è compito di altri relatori.

Dopo questa serie di esami, si avevano completi i protocolli per ogni campione e si potevano quindi ottenere le percentuali relative ai generi riscontrati, costruendo delle $\bar{M}$ tra i risultati ottenuti nei tre campioni successivi relativi ad ogni singolo individuo esaminato. Da questi dati risulta (Tab. 1) che la concentrazione degli aerobi era di 10^8/g di feci mentre quella degli anaerobi non sporigeni era di 10^{10}/g di feci. I clostridi invece sono risultati molto variabili da soggetto a soggetto, infatti mentre in alcuni individui le concentrazioni erano 10^5–10^6/g di feci in altri la loro presenza era talmente massiva da impedirne la conta.

Sulle 720 colonie esaminate predominanti tra i germi aerobi sono risultati i Coli e gli Enterococchi mentre tra gli anaerobi i Bacteroides ed i Bifido. (Tab. 2 e Tab. 3). Questi 4 generi sono gli unici ad essere risultati presenti costantemente in tutti i campioni di feci esaminati.

Invece è da sottolineare una certa variabilità nella presenza degli altri generi batterici sia aerobi che anaerobi non solo tra i vari individui ma anche nei tre campioni ottenuti in giorni successivi dallo stesso individuo. Mentre le differenze osservate nella composizione della flora in individui diversi, secondo una analisi della varianza compiuta da Moore e Coll., [4] sembra riflettano reali differenze esistenti tra individuo ed individuo, per quelle osservate nei tre campioni successivi dello stesso soggetto abbiamo ipotizzato si trattasse di differenze dovute a prelievi effettuati in punti diversi della massa fecale.

Inoltre abbiamo potuto rilevare dalla analisi dei risultati una flora batterica intestinale simile nelle uniche due coppie di soggetti appartenenti allo stesso nucleo famigliare. Infatti 2 individui (moglie–marito) presentavano entrambi una massa di enterococchi predominante tra tutti gli aerobi e altri 2 individui (madre e figlia) evavano entrambi una presenza massiva di clostridi. Considerando i limiti dovuti al numero di soggetti esaminati, non abbiamo fatto ipotesi ma ci siamo proposti di approfondire il problema con analisi successive su soggetti conviventi.

I risultati di questo lavoro, seppure preliminare, confermerebbero dunque i dati ottenuti da altri AA., in altri Paesi, fornendo l'indicazione della sovracolonizzazione degli anaerobi rispetto agli aerobi nel colon, con un rapporto di 1 : 100 e la predominanza dei Bacteroides rispetto a tutti gli altri generi presenti [5–9].

Dalla Tab. 4 risulta inoltre evidente che tra gli anaerobi i generi maggiormente rappresentati oltre ai Bacteroides ed i Bifido sono gli Eubatteri ed i Lattobacilli. Secondo lavori di Peach e Coll. [10], popolazioni che consumano diete di tipo occidentale ad elevato contenuto di grassi (Inghil-

TABELLA I

Concentrazioni degli aerobi, anaerobi asporigeni e clostridi in 12 soggetti normali
(medie ottenute da 3 campioni)

CAMPIONI	1	2	3	4	5	6	7
Aerobi totali	$1,8.10^8$	2.10^9	1.10^8	$2,4.10^8$	$8,5.10^8$	$4,2.10^7$	$2,2.10^8$
Anaerobi asporigeni totali	$1,3.10^{10}$	$2,2.10^{10}$	$3,8.10^{10}$	$2,1.10^{10}$	$4,6.10^{11}$	$4,3.10^{10}$	$2,1.10^{10}$
Clostridi	$5,9.10^5$	$3,6.10^6$	$5,4.10^4$	$8,4.10^5$	4.10^6	incont.	incont.

Concentrazioni degli aerobi, anaerobi asporigeni e clostridi in 12 soggetti normali
(medie contenute da 3 campioni)

CAMPIONI	8	9	10	11	12	$\bar{M}$
Aerobi totali	$1,8.10^8$	$5,4.10^7$	$1,2.10^8$	$1,3.10^8$	$5,1.10^8$	$3,8.10^8$
Anaerobi asporigeni totali . . .	$1,6.10^{10}$	$4,7.10^9$	$1,6.10^{10}$	$7,5.10^{10}$	6.10^{10}	$6,5.10^{10}$
Clostridi	2.10^3	incont.	$2,1.10^5$	$7,1.10^5$	incont.	—

TABELLA 2

Distribuzione in percentuale degli aerobi predominanti nelle feci di 12 soggetti sani

CAMPIONI	1	2	3	4	5	6	7	8	9	10	11	12	M
E. coli	3,8	—	54	63	50	73	48	27	89	62	50	42	46,8
Enterococco	92,3	100	4	10	36	3	11	50	3	30	28	28	33
Klebsiella	—	—	23	—	—	—	—	3	—	—	—	5	2,5
S. viridans	3,8	—	15	—	—	3	14	7	3	4	2	3	4,5
Staf. coag +	—	—	4	—	3,3	—	11	—	—	—	—	—	1,5
Staf. coag −	—	—	—	27	3,3	13	—	—	3	—	16	15	6,4
Salmonella		—	—	—	3,3	—	—	—	—	—	—	—	0,2
Strep. γ		—	—	—	—	3	7	—	—	—	—	—	0,8
Pneumococco	—	—	—	—	—	—	3	—	—	—	—	—	0,2
Citrobacter	—	—	—	—	—	—	3	—	—	—	—	—	0,2
Proteus morganii	—	—	—	—	—	—	—	10	—	3	3	7	1,9

TABELLA 3

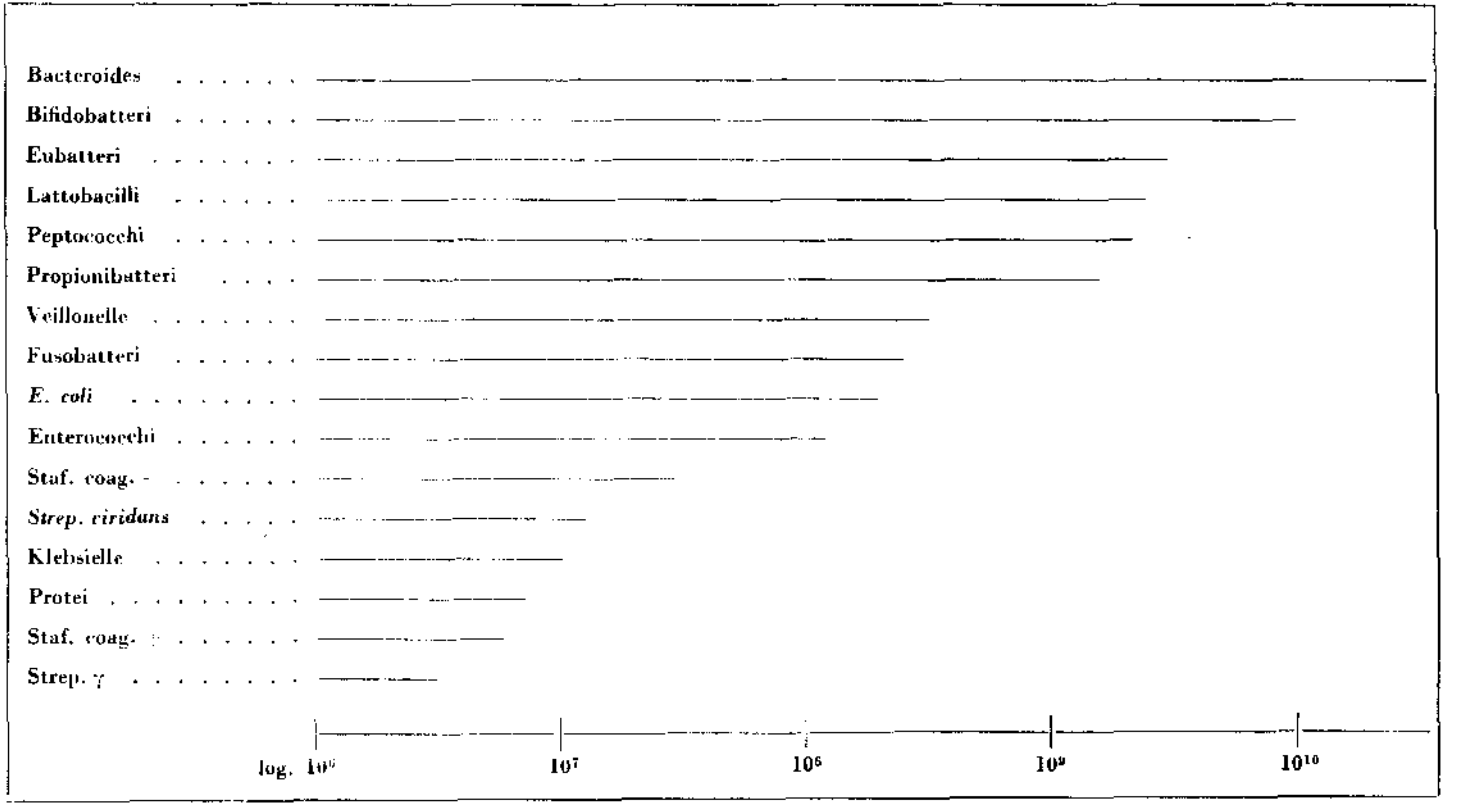
Distribuzione in percentuale degli anaerobi asporigeni predominanti nelle feci di 12 soggetti sani

CAMPIONI	1	2	3	4	5	6	7	8	9	10	11	12	M
Bacteroides	40,9	51,8	35	70,8	24,8	23	20	40	13	45	43,3	10	31,8
Bifido	4,5	—	20	8,3	33,3	3	5	30	13	22	23,3	26,6	15,7
Eubatteri	—	—	5	—	—	23	—	—	3	15	3,3	—	4,1
Lattobacilli	—	7,4	—	—	—	17	—	3	10	3	—	3,3	3,6
Peptococchi	—	—	5	—	9,2	—	20	3	—	5	—	—	3,5
Propionibatteri	—	—	5	16,6	—	—	—	7	3	—	—	—	2,6
Veillonelle	—	—	5	—	—	—	—	—	3	—	—	—	0,66
Fusobatteri	—	—	—	—	—	—	5	—	—	—	—	—	0,41
Anaerobi facoltativi	54,5	33	5	—	9,2	7	5	—	7	10	—	—	10,9
Non cresciuti o non tipizzabili	—	7,4	20	8,5	4,6	20	35	17	37	—	30	23,3	16,9

TABELLA 4

Concentrazione per grammo di feci degli aerobi e degli anaerobi non sporigeni

(espressa in logaritmi)



terra, USA, Scozia) presentano una flora fecale ricca di anaerobi Gram negativi (Bacteroides, Fusobatteri) mentre quelle popolazioni che consumano diete ricche di carboidrati (India, Giappone) presentano un maggior numero di anaerobi Gram positivi.

I nostri dati sembrerebbero dunque indicare nelle feci esaminate una predominanza di anaerobi Gram negativi come quella riscontrata in popolazioni con diete ricche di grassi, anche se il valore elevato di Lattobacilli ed Eubatteri sembrerebbe collocarci in una posizione intermedia, che in effetti bene si addice ad una popolazione quale la nostra che consuma una dieta ricca sia di grassi che di carboidrati.

Per i Clostridi inoltre si sono ottenuti dei valori che si discostano dai risultati di altri AA. dal momento che 4 soggetti sui 12 esaminati avevano una presenza di clostridi massiva tanto da impedirne la conta. Questo dato può essere molto interessante e quindi degno di approfondimento data la importanza dei Clostridi quali degradatori degli steroidi nell'intestino. Infatti studi recenti hanno dimostrato che il cancro del colon può essere il risultato dell'azione di carcinogeni prodotti da steroidi intestinali e dal momento che questi possono essere degradati da molti Clostridi, il più rappresentativo di tutti nella produzione di steroido-deidrogenasi è il *Cl. paraputrificum*, è ovvia la loro importanza in questo tipo di ricerche [1, 2, 11].

L'evidenza del rapporto quantitativo tra germi aerobi ed anaerobi nell'intestino di soggetti normali può essere utile inoltre negli studi sul metabolismo umano. Infatti è stato accertato (Gall e Coll. [3]) che gli anaerobi sono implicati in molti dei processi associati alla digestione, compreso il metabolismo di certi carboidrati, grassi e proteine e nella produzione di vitamina B_2, B_{12}, acido pantotenico, folico, o in produzioni di sostanze particolari quali ad es. il colesterolo o la formazione di amine tossiche dovute alla decarbossilazione di certi aminoacidi.

È quindi necessario tener conto nell'ecologia della flora intestinale delle relazioni tra i vari microorganismi anaerobi ed aerobi e delle interazioni tra flora intestinale ed ospite nel mantenimento di uno stato di equilibrio.

Infine i dati ottenuti potranno servire quale base per la valutazione e l'interpretazione di cambiamenti in questa microflora in situazioni patologiche.

Riassunto. — Al fine di valutare il rapporto quantitativo tra germi aerobi, anaerobi non formanti spore e clostridi nelle feci di soggetti sani sono stati esaminati in quasta fase preliminare del nostro lavoro 12 individui sani di età compresa tra i 20 ed i 60 anni, che presentavano regolari funzioni dell'alvo, mediante prelievo di tre campioni di feci ottenute in giorni succes-

sivi. L'identificazione del genere batterico veniva eseguita con prove biochimiche e gas–cromatografiche.

I risultati hanno dimostrato che la concentrazione dei germi aerobi è di 10^8/g di feci mentre quella dei germi anaerobi non sporigeni è di 10^{10}/g di feci. I Clostridi sono risultati variabili da soggetto a soggetto, infatti mentre in alcuni individui le concentrazioni sono 10^5–10^6/g di feci, in altri la loro presenza è talmente massiva da impedirne la conta. Tra i germi aerobi predominanti sono risultati essere i Coli e gli Enterococchi, mentre tra gli anaerobi i Bacteroides ed i Bifido.

I risultati di questo lavoro confermerebbero dunque i dati ottenuti da altri AA. in altri Paesi, fornendo l'indicazione della sovracolonizzazione degli anaerobi rispetto agli aerobi nel colon con un rapporto di 1 : 100 e la predominanza dei Bacteroides rispetto a tutti gli altri generi presenti.

Summary (*Intestinal bacterial flora in normal adults*). — In this preliminary study we have examined 12 healthy men aged between 20 – 60 years, by three specimens each. All the isolated could be assigned to genera by biochemical tests and gas–liquid chromatography. The results showed a 10^8/g concentration of aerobe bacteria in faeces and a 10^{10}/g concentration of anaerobe non sporing bacteria.

The results of the count of Clostridia showed a variability among the examined individuals. The predominant groups of aerobes in the faeces were found to be *E. coli* and *S. faecalis* spp. and the predominant group of non sporing anaerobes were Bacteroides and Bifidobacteria. These results were similar to the data obtained from foreign AA., showing the predominance of anaerobes in the faeces and particularly of Bacteroides.

BIBLIOGRAFIA

1. Hill, M. J. 1974. Colon cancer: a disease of fibre depletion of or dietary excess? *Digestion*, **11**. 289–306.

2. Hill, M. J. 1974. Bacteria and the etiology of colonic cancer. *Cancer*. **34**: 815–818.

3. Gall, L. S. 1970. Normal fecal flora of man. *Am. J. Clin. Nutr.* **23**: 1457–1465.

4. Holdman, L., Good, J. & Moore, W. 1976. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. *Appl. Environm. Microbiol.* **31**: 359–375.

5. Gorbarch. S. L. 1971. Intestinal microflora. *Gastroenterology*. **60**: 1110–1122.

6. Hill, M. J. & Drasar, B. S. 1975. The normal colonic bacterial flora. *Gut*. **8**: 318–323.

7. Drasar, B. S., Shiner, M. & Mcleod, G. M. 1969. Studies in the intestinal flora. *Gastroenterology*. **56**: 71–79.

8. Gorbach, S. L., Plaut, A. G., Nahas, L., Weinstein, L., Spanknebel, G. & Levitan, R. 1967. Studies on intestinal microflora. II. Microorganism of the small intestine and their relations to oral and faecal flora. *Gastroenterology*. **53**: 856–867.

9. Moore, W. & Holdeman L. 1974. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl. Microbiol.* **27**.: 961–979.

10. Peach, S., Fernandez, F. Johnson, K. & Drasar B. S. 1974. The non-sporing anaerobic bacteria in human faeces *J. Med. Microbiol.* **7**: 213–221.

11. Drasar, B. S., Goddard, P., Heaton, S. Peach, S. & West, B. 1976. Clostridia isolated from faeces. *J. Med. Microbiol.* **9**: 63–71.

Abnormal intestinal flora

S. TABAQCHALI

Department of Medical Microbiology, St. Bartholomew's Hospital, London.

Conditions associated with abnormal bacterial overgrowth

Three main types of gastrointestinal abnormality encourage bacterial overgrowth. The first group consists of gastric abnormalities such as achlorhydria and partial gastrectomy and the second group consists of conditions which cause stasis, such as surgical enteroanastomosis; strictures, congenital or infective; small intestinal diverticulosis, multiple or single; and conditions with abnormal motility as in scleroderma. The third group consists of conditions in which there is free communication between the small and large bowel as in gastrocolic or enterocolic fistulas or following massive intestinal resection with the removal of the ileocaecal valve [1, 2].

The microflora colonising the small intestine under abnormal conditions

Recent studies have emphasized the importance of investigating the whole of the small intestine from duodenum to ileum in order to understand the relationship between the microflora and the host. Furthermore, improved anaerobic techniques have been used which have demonstrated that the small intestinal flora under these conditions consists predominantly of anaerobic bacteria.

The micro-organisms colonising the small intestine in the above conditions are different from the normal flora; they are 'faecal' in type. The bacteria isolated are the aerobic Enterococci, Enterobacteria, aerobic Lactobacilli and yeasts, and the anaerobic micro-organisms such as Bacteroides, Bifidobacteria, Clostrida and Veillonella. The concentrations and distributions are complex and differ from patient, depending on the site and extent of the causative lesion. The concentrations range from 10^5–10^9 organisms per ml of intestinal fluid [1].

TABLE I

Conditions associated with an abnormal bacterial flora

1. – Abnormalities of gastric function:
   Polya partial gastrectomy – Afferent loop syndrome;
   Malfunctioning gastrojejunostomy;
   Pernicious anaemia.

2. – Conditions causing stasis:
   a) Surgical blind loops:
      Entero–anastomosis;
   b) Strictures:
      Congenital:
      Crohn's disease;
      Tuberculous;
   c) Adhesions:
      X-ray irradiation;
   d) Small intestinal diverticulosis;
   e) Abnormal motility:
      Scleroderma;
      Tropical sprue;
      Diabetic neuropathy;
      Vagotomy;
      Ganglion blocking agents;
      Intestinal pseudo–obstruction;
   f) Partial biliary obstruction with cholangitis.

3. – Free communications between large and small bowel:
      Gastrocolic fistula;
      Enterocolic fistula;
      Massive intestinal resection;

The effect of gastric acid on the intestinal flora

The presence of acid appears to limit the growth of colonic microorganisms in both stomach and duodenum in most cases, but does not always exclude it. In the absence of gastric acid however, the bacterial counts can go up as high as 10^7–10^8 organisms/ml.

The effect of the anatomical lesion on the bacterial flora

The distribution of two types of organisms, coliforms and Bacteroides in four conditions is shown in order to illustrate how the site and extent of the lesion determine the microbial flora of the small intestine (Fig. 1 and 2).

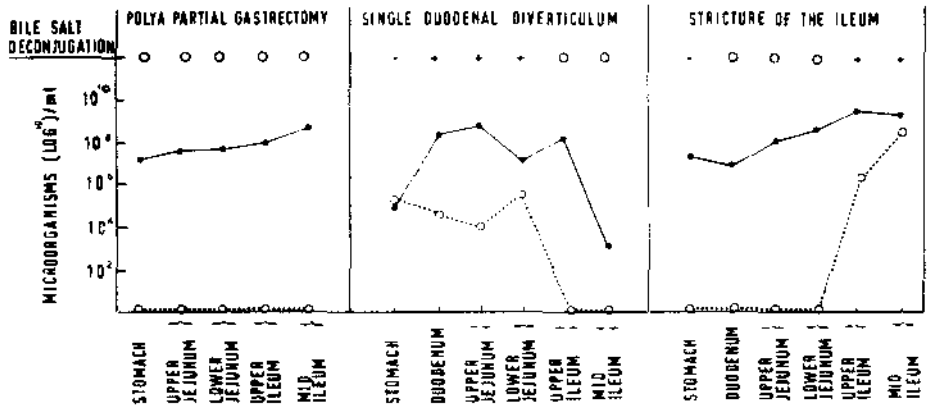


Fig. 1. — The concentration and distribution of two microorganisms, the aerobic coliforms ●——● and the anaerobic Bacteroides ○ ····· ○ at different sites of the gastro-intestinal tract of 3 patients (polya partial gastrectomy, single duodenal diverticulum and stricture of the ileum) together with the presence or absence of bile salt deconjugation in each sample.

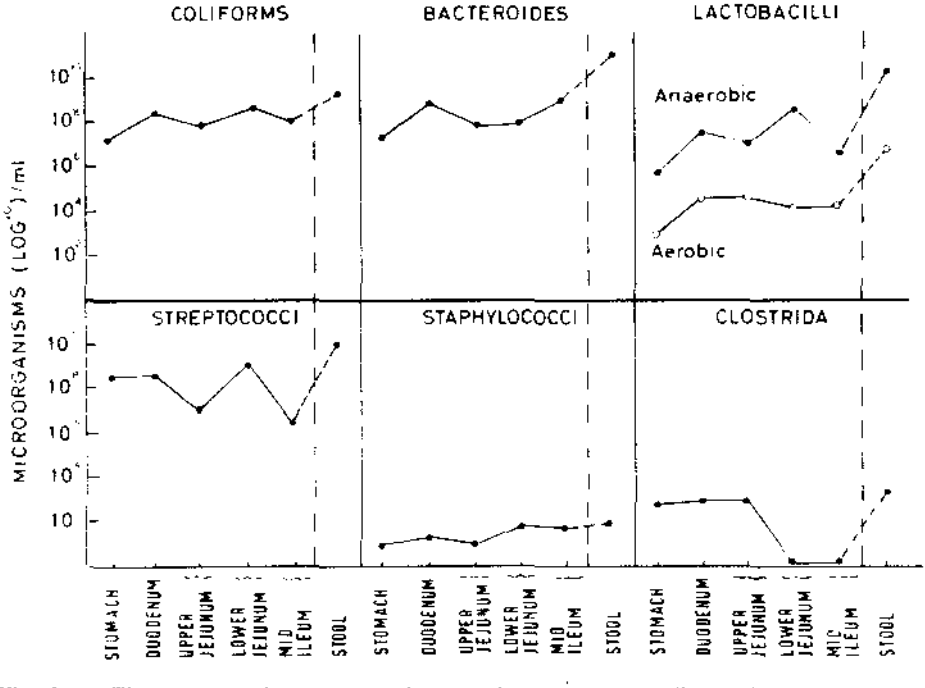


Fig. 2. — The concentration and distribution of organisms at different levels of the gastro-intestinal tract in a patient with multiple duodenal and jejunal diverticulosis.

In uncomplicated polya partial gastrectomy where there is no stasis coliforms may be present throughout the small intestine, increasing in concentration in the ileum, but no Bacteroides were isolated.

In the patient with a single duodenal diverticulum both coliforms and Bacteroides are present in the proximal part of the small intestine where the lesion is, but these micro-organisms are absent lower down the intestine. In contrast, however, in a patient with distal stasis coliforms were present throughout the small intestine, but the anaerobic flora such as Bacteroides only appeared at the area of stasis, distally in the ileum.

The distribution of bacteria in a patient with extensive proximal stasis as may be caused by multiple duodenal and jejunal diverticulosis is shown in Fig. 2. Both aerobic and anaerobic microflora are present in high concentrations throughout the small intestine from stomach to lower ileum.

The aerobic flora therefore, and in particular the coliforms, can colonise the small intestine in a variety of conditions, but the anaerobic flora represented by Bacteroides only appear in areas of stagnation [1].

THE CLINICAL CONSEQUENCES OF ABNORMAL BACTERIAL COLONISATION

There are numerous metabolic activities associated with the intestinal bacteria colonising the small intestine in patients with small intestinal bacterial overgrowth and these have been reviewed previously [2]. In this paper I would like to consider the effects of bacteria on the bile acid metabolism, vitamin B_{12} and protein metabolism and the clinical consequences which may develop in the host.

The metabolism of bile acids

Primary bile acids, cholic acids (3α, 7α 12α) and chenodeoxycholic acid (3α, 7α) are synthesised in the liver from cholesterol and conjugated with glycine or taurine and secreted in the bile acid(*) deoxycholic acid (3α, 12α) which is formed by bacterial action on cholic acid. These conjugated bile acids contribute to fat digestion and absorption in the proximal small bowel and are largely reabsorbed by active transport mechanism in the ileum to return to the liver via the portal vein, thus forming the enterohepatic circulation. A small amount of unabsorbed bile acids pass into the caecum and colon where they are metabolised by bacteria.

(*) As conjugated bile acids. Another constituent of the normal bile is the secondary bile acid.

The main reactions carried out by bacteria are:

i) *Hydrolysis*: Bacteria hydrolyse the amide bond to release the free bile acids from their taurine or glycine conjugates. The bacterial enzyme responsible is cholylglycine peptide hydrolase.

ii) 7α *dehdroxylation*: Bacteria dehydroxylate by removing the OH–group at C7–position to form the secondary bile acids. The enzymes are 7α dehydroxylases.

iii) *Oxido–reduction and dehydrogenation*: Bacteria oxidise and further reduce the bile acid molecule to form ketoacids and α and β epimers. This can occur at the C_3, C_7 and C_{12} position, the enzymes responsible being the dehydrogenases.

These bacterial metabolites of bile acids have been identified in the faeces.

Micro–organisms associated with bile acid metabolism

Various aerobic and anaerobic bacteria have been shown to be capable of carrying out these metabolic activities *in vitro* [3, 4] but due to their relative high concentrations, it is the anaerobic bacteria which are by far the most important. Similarly *in vivo* studies of patients with bacterial overgrowth, there was a good correlation between the presence of free bile acids and the Bacteroides sp. in samples obtained from different levels of the small intestine [1].

Intestinal bile acids

In patients with bacterial overgrowth in the small intestine, bile salt degradation may occur, leading to an abnormal bile acid pattern.

Fig. 3 compares the bile acid concentration in fasting jejunal fluid from 5 control subjects (open columns) and in 7 patients with the stagnant loop syndrome (hatched columns). The mean and range of the bile acids are expressed as mmoles/l of intestinal aspirates. The conjugated bile acids are presented as total and sub–divided into glycine and taurine conjugates. The free bile acids detected were cholic, chenodeoxy–cholic and deoxycholic acids. In the jejunum of control subjects there are only conjugated bile acids detected. In the patients with the bacterical overgrowth (stagnant loop syndrome) the pattern is different. Firstly, there are large amounts of free bile acids present (cholic, chenodeoxycholic and deoxycholic acids). Secondly, the taurine conjugates are much more reduced than the glycine, and, thirdly, as a result of bacterial formation of these free bile acids, the level of the conjugated bile salts falls, all being under 5 mmoles/l.

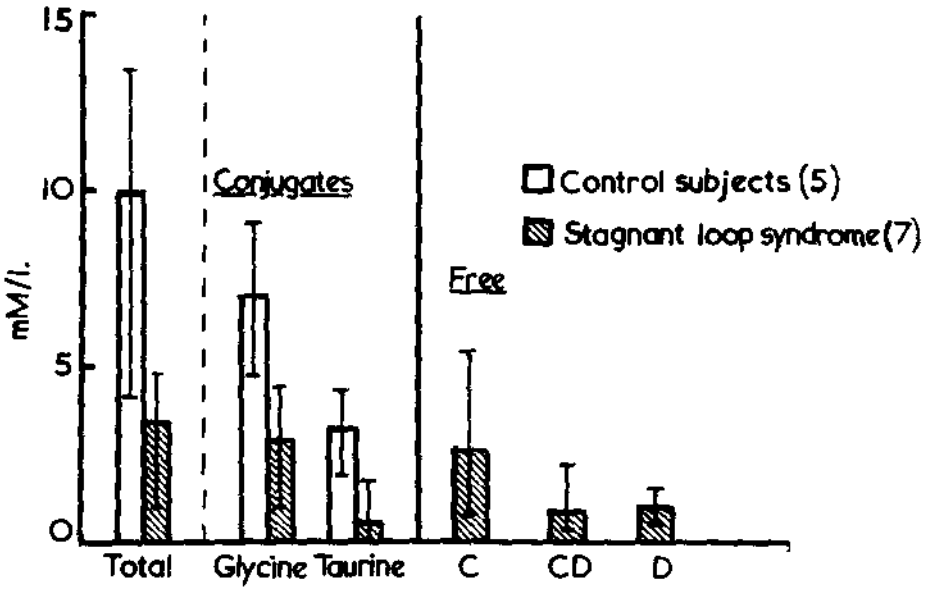


Fig. 3. — The concentration of bile acids in the fasting upper jejunal fluid (mean and range in mmoles/litre) from 5 control subjects (open areas) and 7 patients with the stagnant loop syndrome (hatched areas). Total concentration of conjugated bile acids and their subdivision into glycine and taurine conjugates are represented. The free bile acids are represented by C=cholic acid, CD=chenodeoxycholic acid and D=deoxycholic acid.

Consequences of bacterial degradation of bile acids

1) *Steatorrhoea.* The small intestine normally contains conjugated bile salts in sufficient concentrations to promote the dispersion and absorption of lipids through the formation of mixed micelles. Under abnormal conditions where there is colonisation of the small intestine, bile salt deconjugation and dehydroxylation may occur and thus lead to a reduction in the level of the conjugated bile salts sufficiently to impaire micelle formation, thereby causing malabsorption of fat [5, 6]. The free bile acids, on the other hand, at the pH of intestinal contents, appear to be either absorbed by non-ionic diffusion or precipitated. However, the mere presence of abnormal bacterial overgrowth or the presence of free bile acids in the small intestine does not necessarily lead to steatorrhoea. Limited lesions of the jejunum or ileum may not be associated with steatorrhoea if there is insufficient degradation of bile salts [1]. This subject has been reviewed in detail elsewhere [2].

Reduction in the concentration of intraluminal conjugated bile salts may also lead to malabsorption of fat soluble vitamins (A, D, K) with clinical consequences.

The effect of antibiotics on the faecal fat excretion and the jejunal bile acids are shown in Fig. 4. Before antibiotics, the faecal fat was 22 g/day and there were low levels of conjugated bile acids and high levels of free bile acids. On antibiotics not only did the free bile acids disappear bu

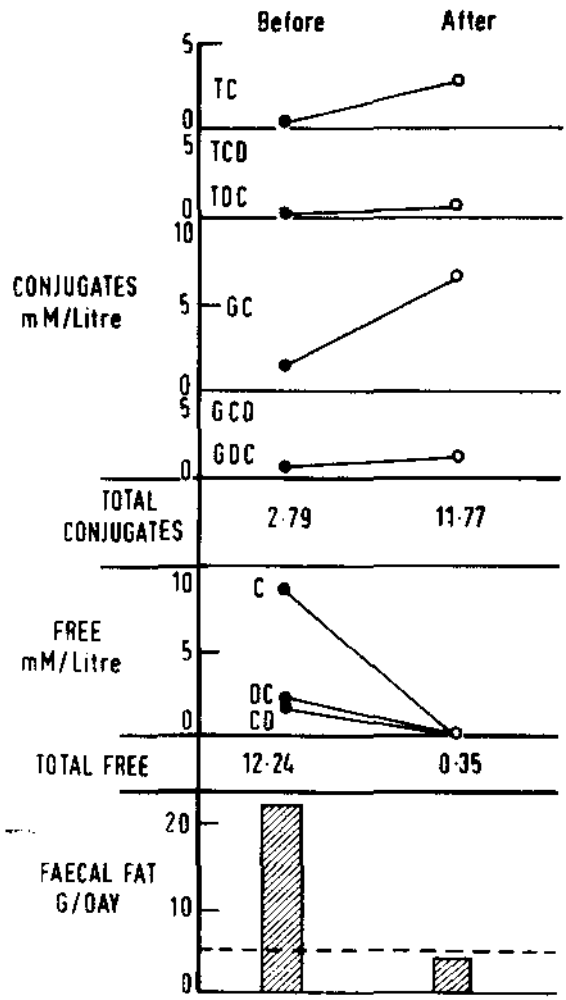


Fig. 4. — Effect of antibiotic on bile salt concentration in upper jejunum and faecal fat excretion. TC: taurocholate, TCD: taurochenodeoxycholate, TDC: taurodeoxycholate, GC: glycocholate, GCD: glycohendeoxycholate, GDC: glycodeoxycholate, C: cholic acid. CD: chenodeoxycholic acid, DC: deoxycholic acid.

also the conjugated bile acids increased and the faecal fat excretion returned to normal.

2) *Diarrhoea*. The mechanism of the diarrhoea associated with bacterial overgrowth in the small intestine and after intestinal resection is not well understood. The absorption of free bile acids have been shown to lead to a disturbance of water and electrolyte absorption in patients [7] and in experimental animals [8].

3) *Monosaccharide malabsorption*. Glucose transport by jejunal mucosa was impaired by the presence of deconjugated bile acids *in vitro* [9] and in experimental animals *in vivo* [8, 10–12] demonstrated reversible inhibition of active intestinal sugar transport by deconjugated bile salts and suggested that this may explain the temporary monosaccharide malabsorption in infancy.

Detection of bile acid deconjugation.

The presence of bile acid deconjugation in the small intestine can be detected without intestinal intubation by two methods:

1) The Breath Test: The measurement of $^{14}CO_2$ in expired air following on oral dose of ^{14}C–glycocholic acid. Bacteria deconjugate the bile acids and further metabolise the glycine, liberating $^{14}CO_2$ [13].

2) The Demonstration of Free Bile Acids in the Serum: The serum bile acid pattern reflects the changes which occur in the small intestine [14, 15].

The tests, however, are not specific for patients with bacterial overgrowth in the small intestine but are also positive in conditions with bile salt malabsorption or billiary tract infections.

Vitamin B_{12} absorption.

It is well established that bacteria can take up free vitamin B_{12} *in vitro*, but binding of vitamin B_{12} to intrinsic factor (IF), protects against its uptake [16]. *In vivo*, however, there is malabsorpiton of IF–bound vitamin B_{12} in patients with the stagnant loop syndrome [17]. This discrepancy was investigated by *in vitro* and *in vivo* experiments.

i) In vitro *'uptake' of vitamin B_{12}.*

Various intestinal bacteria isolated from patients with the stagnant loop syndrome were incubated with ^{57}Co–vitamin B_{12}, free and IF–bound

(Fig. 5). The bacteria used were Enterobacteria, Bacteroides, Bifidobacteria and Clostridia. All the bacteria ested were capable of taking up free vitamin B_{12} whereas only certain strains of the anaerobic Bacteroides were capable of taking up IF–bound vitamin B_{12} *in vitro* [18].

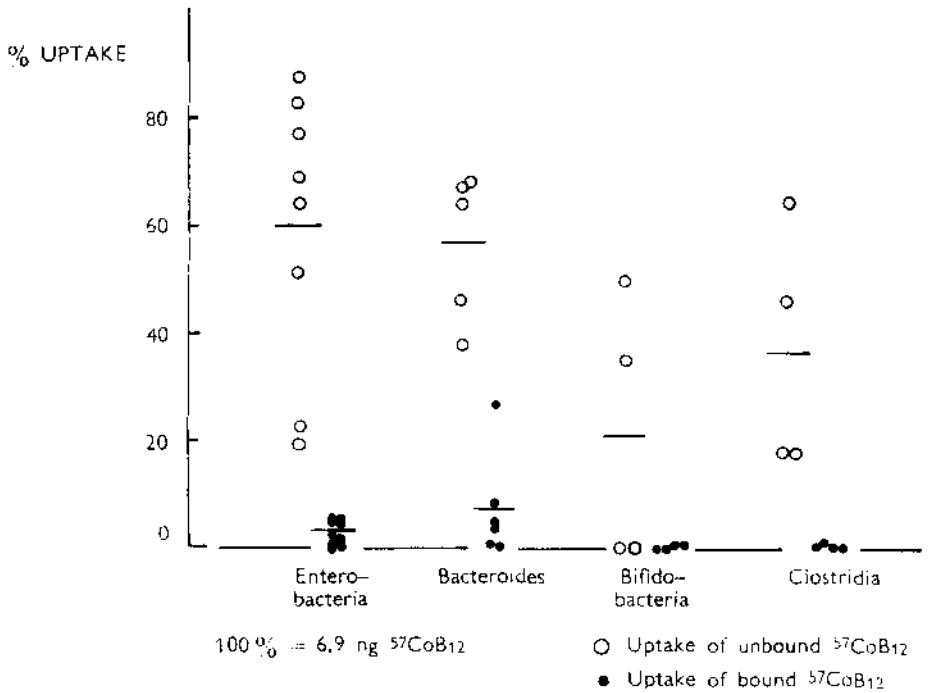


Fig. 5. — Uptake of $^{57}CoB_{12}$ by bacteria subcultured from small intestine aspirates.

ii) In vivo '*uptake*' *of vitamin* B_{12}.

Patients with the stagnant loop syndrome in whom there was vitamin B_{12} malabsorption, patients with bacterial overgrowth with normal vitamin B_{12} absorption, and control subjects, were intubated to the ileum; after an overnight fast a test meal was administered containing 1 microgram of ^{57}Co–vitamin B_{12} bound to human IF. Ileal aspirates were obtained at different times for 5 hours and centrifuged. The percentage radioactivity in the deposit was measured and found to be on average from 46 to 74 % in the patients with the stagnant loop syndrome in whom there was vitamin B_{12} malabsorption, whereas it was less than 10 % in the other two groups (Fig. 6) [19].

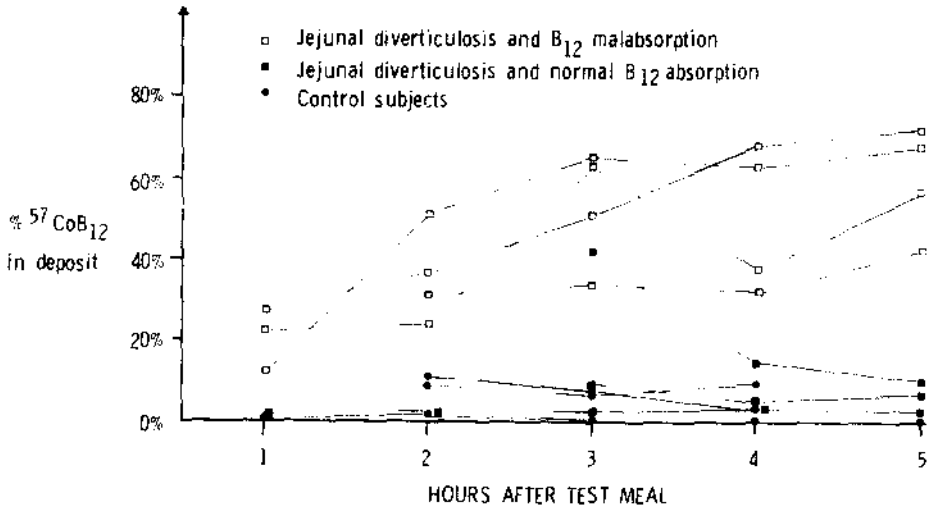


Fig. 6. — Amount of $^{57}CoB_{12}$ in centrifuged deposits of upper ileal aspirates after feeding a test-meal with μg $^{57}CoB_{12}$ bound to human gastric juice.

Thus, the evidence so far suggests that bacteria interfere with vitamin B_{12} absorption in the stagnant loop syndrome by competitive uptake of the vitamin within the lumen of the small intestine, even when the vitamin is bound to IF.

Malabsorption of vitamin B_{12} can lead to vitamin B_{12} deficiency and megaloblastic anaemia in the host.

METABOLISM OF AMINOACIDS

Intestinal bacteria metabolise various amino acids and in particular L-tryptophan. L-trytophan is degraded by bacteria to form indoles which are absorbed, conjugated in the liver and excreted as indoxyl sulphate (indican), in the urine. The normal range is < 10 mg/24 hour urine.

The urinary indican excretion is usually raised in the stagnant loop syndrome but this is not specific as it may also be raised in other conditions where there is protein malabsorption.

How much of the dietary trytophan is degraded by bacteria is uncertain; in some patients the urinary indican excretion may be as high as 500 mg per day, suggesting that a major proportion of the dietary tryptophan, is metabolised by bacteria. Other amino acids are metabolised by bacteria such as tryrosine, phenylalanine and their metabolites can be detected in the urine of patients with bacterial overgrowth and in experi-

mental animals. The metabolites include hippuric acid and volatile and non–volatile phenols [20, 21].

The degradation of aminoacids by bacteria may deprive the host of essential aminoacids, which in turn may lead to protein–calorie malnutrition. If this occurs in adults, a situation resembling kwashiorkor may develop [22].

In children, on the other hand, the long continued caloric under–nutrition can lead to intestinal infantilism, where there may be failure of growth and sexual development [23].

CONCLUSION

There are numerous conditions which can lead to abnormal bacterial over–growth in the small intestine. The bacteria which colonise the small bowel are qualitatively similar to the faecal flora and these bacteria can lead to malabsorption of fat, fat soluble vitamins, vitamin B_{12} and disturb protein metabolism, and if the malabsorption is severe, it can lead to multiple nutritional deficiencies in the host.

Summary. — The normal small intestine in man usually harbours a sparse microflora consisting of Gram–positive microorganims derived from the oro–pharynx.

The concentrations in the upper jejunum are approximately 10^3–10^4 organisms/ml, whereas in the ileum the concentrations may be higher and faecal type organisms may also be present, such as Enterobacteria, Bifidobacteria, and Bacteroides in concentrations of 10^5–10^6 organisms per ml of intestinal aspirate [24, 25]. This situation is well maintained unless the integrity of the small intestine is deranged. There are however, certain conditions in which bacterial proliferation in the lumen of the small intestine may occur and give rise to various metabolic abnormalities [2]. In this paper I would like to list some of these conditions, then describe the type of microflora which colonizes the small intestine, and finally discuss a few aspects of the metabolic consequences of these bacteria.

Riassunto (*Flora intestinale anormale*). — L'intestino tenue normalmente è colonizzato da batteri Gram–positivi derivanti dall'orofaringe. Le concentrazioni nel tratto superiore del digiuno sono approssimativamente 10^3–10^4 organismi/ml, mentre nell'ileo queste concentrazioni sono più elevate e possono essere presenti anche organismi di tipo fecale come Enterobatteri, Bifidobatteri e Bacteroides in concentrazioni di 10^5–10^6 organismi/ml

di aspirato intestinale [24, 25]. Questa situazione si mantiene normalmente costante senza alterare l'integrità dell'intestino tenue. Tuttavia vi sono delle condizioni in cui può avvenire una moltiplicazione batterica nel lume intestinale che di consequenza determinano alterazioni metaboliche [2].

In questo lavoro ho voluto citare alcune di queste condizioni descrivendo il tipo di microflora che colonizza l'intestino tenue ed infine discutere qualche aspetto delle consequenze metaboliche di questi batteri.

REFERENCES

1. Gorbach, S. L. & Tabaqchali, S. 1969. Bacteria, bile and the small intestine. *Gut.* **10**: 963–972.

2. Tabaqchali, S. 1970. The pathophysiological role of small intestinal bacterial flora. *Scand. J. Gastroenterol.* Suppl. **6**: 139.

3. Midvedt, T. & Norman, S. 1967. Bile acid transformations by microbial strains belonging to genera found in intestinal contents. *Acta Pathol. Microbiol. Scand.* **71**: 629–638.

4. Hill M. J. & Drasar B. S. Degradation of bile salts by human intestinal bacteteria. *Gut.* **9**: 22–27.

5. Kimm, Y. S., Srits, N., Blum, M., Terz, J. & Sherlock, P. 1966. The role of the altered bile acid metabolism in the steatorrhoea of experimental blind loop. *J. Clin. Invest.* **45**: 956–962.

6. Tabaqchali, S., Hatziaonnou, J. & Booth, C. C. 1968. Bile salt deconjugation and steatorrhoea in patients with the stagnant loop syndrome. *Lancet.* ii: 12–16.

7. Mekhjian, H. S., Phillips, S. F. & Hofman, A. F. 1971. Colonic secretion of water and electrolytes induced by bile acids: perfusion studies in man. *J. Clin. Invest.* **50**: 1569–1577.

8. Harries, J. T. & Sladen, C. E. 1972. The effects of different bile salts on the absorption of fluid, electrolytes and mono-saccharides in the small intestine of the rat *in vivo*. *Gut.* **13**: 596–603.

9. Pope, J. L., Parkinson, T. M. & Olson, J. A. 1966. Action of bile salts on the metabolism and transport of water-soluble nutrients by perfused rat jejunum *in vitro* . *Biochim. Biophys. Acta.* **130**: 218–232.

10. Tabaqchali, S. & Booth, C. C. 1966. Alteration of bile salt metabolism in the stagnant loop syndrome. *Gut.* **7**: 712.

11. Gracey, M., Burke, V. & Anderson, C. M. 1969. Association of mono-saccharide malabsorption with abnormal small intestinal flora. *Lancet.* ii: 384–385.

12. Gracey, M., Burke, V. & Oshin, A. 1971. Reversible inhibition of intestinal active sugar transport by deconjugated bile salts *in vitro*. *Biochim. Biophys. Acta.* **225**: 308–314.

13. FROMM, H. & HOFMANN, A. F. 1971. Breath test for altered bile acid metabolism. *Lancet*, ii,: 621–625.

14. LEWIS, B., PANVELLIWELLA, D., TABAQCHALI, S. & WOOTTON, I. D. P. 1969. Serum bile acids in the stagnant loop syndrome. *Lancet*. i: 219–220.

15. PANVELLIWELLA, D., TABAQCHALI, S., WOOTTON, I. D. P. & LEWIS, B. 1969. Bile acid metabolism in the stagnant loop syndrome. In: *Proc. 8th International Congress on Nutrition*, Prague. Amsterdam, Excerpta Medica (Int. Congress Series No. 213, 448.)

16. DONALDSON, R. M. JR. 1962. Malabsorbption of Co^{60} labeled cyanocobam in rats with intestinal diversicula: I. Evaluation of possible mechanisms. *Gastroenterology*, **43**: 271–281.

17. MOLLIN, D. L., BOOTH, C. C. & BAKER, S. J. 1957. The absorption of vitamin B_{12} in control subjects in Addisonian Pernicious Anaemia and in the malabsorption syndrome. *Brit. J. Haematol.* **3**: 412–428.

18. SCHJONSBY, H. & TABAQCHALI, S. 1971. Uptake of rat gastric-juice–bound vitamin B_{12} by intestinal brush borders isolated from blind-loop rats. Effect of small intestina] bacterial on intrinsic factor and the vitamin B_{12}– intrinsic factor complex. *Scand. J. Gastroenterol.* **6**: 515–521 and 707–713.

19 SCHJONSBY, H., DRASAR, B. S., TABAQCHALI, S. & BOOTH, C. C. 1973. *Scand. J. Gastroenterol.* **8**: 41–47. Uptake of vitamine B_{12} by intestinal bacteria in the stagnant loop syndrome.

20. MILLER, B., MITCHISON, R., TABAQCHALI, S. & NEALE, G. 1971. The effect of excessive bacterial proliferation on protein metabolism in rats with self filling jejunal sacs. *Europ. J. Clin. Invest.* **2**: 23–31.

21. BONE, E., TAMM, A. & HILL, M. J., 1976. The production of urinary phenols by gut bacteria and their possible role in the causation of large bowel cancer. *Amer. J. Clin. Nutr.* **29**: 1448–1454.

22. NEALE, G., ANTCLIFFE, A. C., WELBOURNE, R. G., MOLLIN, D. L. & BOOTH, C. C. 1967. *Quant. J. Med.* **36**: 469.

23. NEALE, G. 1968. Protein deficiency in temperate zones. *Proc. Roy. Soc. Med.* **60**: 1069–1071.

24. GORBACH, S. L., PLAUT, A. G., NAHAS, L. & WEINSTEIN, L. 1967. Studies of intestinal microflora. II. Microorganisms of the small intestine and their relations to oral and faecal flora. *Gastroenterology*. **53**: 856–867.

25. DRASAR, B. S., SHJINER, M. & MCLEOD, G. M. 1967. Studies on intestinal flora. I. Effects of diet, age and periodic sampling on numbers of faecal micro-organisms in man. *Gastroenterology*. **53**: 845–855.