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BACTERIAL CONTENT OF SMALL INTESTINE OF CHILDREN IN HEALTH, IN COELIAC DISEASE, AND IN FIBROCYSTIC DISEASE OF PANCREAS

BY

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Previous workers (Cregan and Hayward, 1953) have shown that when the adult human intestinal tract is healthy the small intestine does not contain a resident flora, any organisms found there being so few in number that they must be regarded as transient contaminants passing through with the ingesta.

It is thought that in certain disease states the small intestine may be colonized by bacteria of a faecal type. Frazer (1949a) suggested that in the sprue syndrome, including coeliac disease, intestinal bacteria normally confined to the lower intestine invade the upper small intestine in large numbers, and that this may result in competition between bacteria and the host for common essential nutrients, particularly B-complex vitamins. The presence of this abnormal small-bowel flora has been repeatedly referred to as a factor in the development of malabsorption and vitamin deficiencies in coeliac disease and adult idiopathic steatorrhoea (Frazer, 1949b, 1952; Frazer *et al.*, 1949). The published literature contains no adequate proof of the existence of this abnormal flora.

The present study was undertaken to determine the extent and nature of the bacterial flora of the small intestine in children with coeliac disease and with steatorrhoea from other causes, as compared with a group of children of similar age distribution but with no gastro-intestinal symptoms at the time of the examination.

Material and Methods

Four groups of patients were studied, two acting as control groups.

Group 1 consisted of seven patients who had been admitted for elective abdominal surgical operations such as laparotomy, operations for renal anomalies, or interval

appendicectomy. These patients had no gastro-intestinal symptoms at the time of operation. Specimens were obtained by injecting 2 ml. of sterile Ringer's solution into the lumen of the small intestine at several levels and then withdrawing some of this. The technique followed was exactly that described by Cregan and Hayward (1953). This group of children was chosen to parallel these workers' findings in "normal adults."

Group 2 consisted of 12 children with no gastro-intestinal symptoms at the time of investigation. Nine of these showed a fat absorption of over 90% as determined by fat balance. In the remaining three fat balance was not carried out, but the stools were normal both macroscopically and microscopically. Specimens were taken from different levels of the bowel by intubation. These patients acted as a control group to compare intubation results with those obtained by direct needling, and also to compare intubation results from "normal" children with those from children with steatorrhoea.

Group 3 consisted of 15 consecutive patients with coeliac disease. The diagnosis was well established from the clinical history and examination, the demonstration of steatorrhoea by fat balance, the presence of normal pancreatic enzymes, and the subsequent response to treatment with a diet free of wheat gluten. Specimens were obtained by intubation, and all patients were examined at a time when they showed steatorrhoea.

Group 4 consisted of seven patients with fibrocystic disease of the pancreas. The diagnosis was well established again from the history and examination and the laboratory data. Pancreatic enzymes were absent, and all the patients showed gross steatorrhoea. Specimens were taken by intubation.

Collection of Specimens

A tube with a double lumen was used for the intubation. This was constructed by passing a length of fine "polythene" tubing down the inside of a Levin radio-opaque rubber duodenal tube. The polythene was brought out at the tip of the rubber tube. A thin rubber balloon was tied over the tip so that the balloon communicated only with the polythene. The upper end of the polythene was brought out through the side of the rubber tube, and the balloon inflated by attaching a needle and syringe to the end of the polythene. Specimens were aspirated by attaching a syringe to the end of the rubber tube. This tube was used in preference to a Miller-Abbott tube, as it could be passed through the nose in small children and readily visualized under the screen. The tube was sterilized by soaking for 12 to 18 hours in a solution of biniodide of mercury 1/1,000, or by exposure for 24 hours to formalin vapour.

The patient was intubated in the morning after an eight-hour fasting period. A specimen from the stomach was aspirated and the tube was then manoeuvred into the duodenum with the aid of fluoroscopy. A duodenal specimen was aspirated, part of this being taken for the estimation of pancreatic enzymes. The balloon was inflated and the child allowed to take fluids, milk, and soft food. This stage was usually reached about mid-morning. The child was screened again about mid-afternoon to ascertain whether the tube had travelled into the jejunum. If so, a further specimen was aspirated. The position of the end of the tube in the bowel could not be precisely determined, but in all cases it was considered to be probably in the lower jejunum. All jejunal specimens were aspirated within eight hours of passing the tube, and some were obtained within four hours. In all cases before the specimen for bacteriological examination was aspirated the tube was washed through with sterile saline and the first few millilitres withdrawn were discarded. This was done to ensure that the specimen examined was from the bowel lumen and not composed of fluid trapped within the tube. A sterile syringe was used for each aspiration.

Saliva specimens were taken with a sterile syringe before intubation was begun.

Bacteriological Examinations

The method for bacteriological examination of specimens was the same as described by Cregan and Hayward (1953), and is discussed only briefly here. The range of media and conditions of incubation were chosen so that all bacteria likely to occur in the intestinal tract could be cultured. Plain desoxycholate agar and beerwort agar were the only selective media used, as detection of organisms present in very small quantities was not desired. A standard plating procedure was followed to allow both quantitative and qualitative assessment of results. The degree of growth was graded from $\pm$ to $+++$, and all species of bacteria isolated were identified as fully as possible.

Cregan and Hayward (1953) assessed that $+$ or $\pm$ growth indicates a transient flora and that $++$ or $+++$ growth indicates a resident flora in specimens taken at operation. However, these specimens were of diluted bowel contents, and intubation specimens are undiluted. Hence it was necessary to determine whether the quantitative criteria would need adjustment for intubation specimens. To this end four cases were sampled both by intubation and by direct needling. The two methods gave identical quantitative results in all four cases, and it was concluded that the same criteria could be applied to both methods.

Occasionally, particularly in stomach and duodenal specimens obtained at intubation, a quantitatively resident ($++$ or $+++$) flora consisted entirely of organisms of oral type. Further, it was found that two specimens taken by intubation within a short time from the same level of intestine both contained organisms of oral type, but varied widely quantitatively. Since salivary secretion is stimulated by intubation it was concluded that the finding of a profuse flora of oral type could mean that the specimen was swallowed saliva that had escaped the stomach's antibacterial mechanism because its amount was in excess of normal. Of course, the finding of a profuse flora of oral type in the small intestine also could mean that it was resident there, but the intubation technique could not distinguish between these two alternative interpretations of the results. This shortcoming of intubation fortunately was not a great disadvantage in the present investigation, because oral-type organisms were rarely present in profuse amounts. However, care was taken to designate organisms as oral or faecal in type, and only $+++$ and $++$ amounts of faecal type organisms were interpreted as a resident flora at the site sampled.

Results

Table I shows the qualitative and quantitative results from the children with normal gastro-intestinal tracts sampled at operation (group 1). In most of them the washings from the small intestine were sterile. None showed more than a

TABLE I.—*Flora of Healthy Small Intestine of Children*

Site of Sampling	No. of Subjects	Quantitative Results			Qualitative Results	
		Sterile	Transient Flora	Resident Flora	Gram-positive Species	Gram-negative Species
Upper jejunum	7	5	2	0	<i>Neisseria sicca</i> (1) <i>Staph. aureus</i> (1) <i>Candida albicans</i> (1) <i>Streptococcus sp.</i> (1)	<i>Alcaligenes metalcaligenes</i> (1) <i>Proteus morganii</i> (1)
Mid-gut	7	5	2	0	<i>Str. MG</i> (1) <i>N. sicca</i> (1) <i>Str. s.b.e.</i> (1) <i>Corynebacterium xerose</i> (1) <i>Staph. aureus</i> (1)	<i>A. metalcaligenes</i> (1)
Lower ileum	7	3	3	1	<i>Str. s.b.e.</i> (1) <i>Str. mitis</i> (1) <i>N. sicca</i> (1) <i>Staph. aureus</i> (1) <i>Str. faecalis</i> (1) <i>Can. albicans</i> (1)	<i>A. metalcaligenes</i> (1) <i>Bact. coli</i> Type 1 (2) <i>Pr. morganii</i> (1) <i>Veillonella gazogenes</i> (1)

transient flora at the mid-gut level, and only one a resident flora in the lower ileum. These results agree with those of Cregan and Hayward (1953), whose specimens were obtained by the same method from the small intestine of adults without gastro-intestinal disease.

Tables II and III show the quantitative and qualitative results obtained from the children who were examined by intubation (groups 2, 3, and 4). Results obtained from

TABLE II.—*Bacterial Flora of the Small Intestine. Quantitative Results from Intubation Specimens*

	Duodenum			Jejunum		
	Sterile	Transient Flora	Resident Flora (Faecal Type)	Sterile	Transient Flora	Resident Flora (Faecal Type)
Controls (12 cases)	3	9	0	0	8	4
Coeliac disease (15 cases)	4	10	1	2	10	3
Fibrocystic disease of pancreas (7 cases)	1	6	0	0	6	1

TABLE III.—*Qualitative Results from Jejunal Specimens*

Condition and No. of Subjects	Oral-type Bacteria	No. of Isolations	Faecal-type Bacteria	No. of Isolations
Group 2: Controls (12)	"Viridans" <i>Streptococcus sp.</i> <i>H. parainfluenzae</i> Non-pathogenic <i>Staphylococcus sp.</i> <i>V. gazogenes</i> <i>Staph. aureus</i> <i>C. xerose</i> <i>Lactobacillus sp.</i> <i>N. sicca</i> <i>Can. albicans</i>	12 3 3 2 1 1 1 1 1 1	<i>Bact. coli</i> Type 1 " " irregular <i>Bact. aerogenes</i> Type 1 <i>Paracolon sp.</i> <i>Str. faecalis</i> <i>A. faecalis</i> <i>Bacillus subtilis</i> <i>Flavobacterium sp.</i>	2 3 1 3 1 1 1 1
Group 3: Coeliac disease (15)	"Viridans" <i>Streptococcus sp.</i> Non-pathogenic <i>Neisseria sp.</i> Non-pathogenic <i>Staphylococcus sp.</i> <i>Staph. aureus</i> <i>H. parainfluenzae</i> <i>C. xerose</i> <i>V. gazogenes</i> <i>Str. agalactiae</i> <i>Can. albicans</i>	6 6 6 5 4 3 2 2 1 1 1	<i>Bact. coli</i> Type 1 " " intermediate Type 1 <i>Bact. coli</i> irregular type <i>Bact. aerogenes</i> Type 1 <i>Paracolon sp.</i> <i>Ps. pyocyanea</i> <i>Pr. mirabilis</i> <i>Str. faecalis</i>	3 1 1 2 1 1 1 1
Group 4: Fibrocystic disease of pancreas (7)	<i>Staph. aureus</i> "Viridans" <i>Streptococcus sp.</i> Non-pathogenic <i>Neisseria sp.</i> <i>Can. albicans</i> Non-pathogenic <i>Staphylococcus sp.</i> <i>V. gazogenes</i>	4 3 3 3 2 2 1	<i>Bact. coli</i> Type 1 " " irregular <i>Pr. mirabilis</i> <i>Pr. morganii</i> Non-pathogenic <i>Bacillus sp.</i>	1 2 2 2 2

examination of saliva and stomach specimens are not shown in tabular form. Saliva specimens from all groups always yielded a profuse ($+++$) growth of oral-type flora, faecal-type organisms occasionally being present in small amounts. The predominant organism was usually a "viridans" streptococcus. Stomach specimens were usually either sterile or contained a transient flora resembling that of the saliva. A profuse oral flora, presumed to be due to contamination with saliva, was more common with stomach contents than with duodenal or jejunal specimens, where oral-type organisms were usually present in small amounts only.

Among the control series four jejunal specimens showed a resident faecal flora. All duodenal specimens were considered normal. Three patients with coeliac disease had a resident faecal flora in the jejunum. In only one of these was a resident flora present in the duodenum, and in the same child faecal-type organisms were also isolated in resident amounts from the stomach and saliva. The faecal organisms isolated were *Bact. aerogenes* Type 1, *Bact. coli* intermediate Type 1, and *Paracolon sp.*, all being present at the four intestinal levels sampled. In all patients with fibrocystic disease of the pancreas the duodenum was normal, whilst the jejunum of one showed a resident faecal flora. In all the above groups, when a faecal flora was present in

resident numbers in the jejunal specimen the earlier duodenal specimen had usually contained the same faecal bacteria in transient numbers.

Table III lists the organisms isolated from jejunal specimens of groups 2, 3, and 4. In order to simplify this table the generic names *Streptococcus* sp., *Neisseria* sp., and *Staphylococcus* sp., have been used instead of listing separately the species identified within each genus. There was no significant qualitative difference between the three groups.

Discussion

As early as 1908 Herter suggested that the bacterial flora of the intestine in cases of "infantilism" or coeliac disease was different from normal, and this was supported by Kendall (1909) and Brown *et al.* (1925). However, all bacteriological studies carried out by these workers were on stool specimens or colonic washings. The flora of the small intestine was never studied, although it had been suggested (Schiff and Kochmann, 1922) that bacterial fermentation may be occurring there abnormally. From results of duodenal bacterial counts the intestinal flora has again been incriminated as a factor in the aetiology of the sprue syndrome (Frazer, 1949a).

Our investigations have shown that in all but three patients with coeliac disease examined the small intestine was bacteriologically normal—that is, it did not contain a resident flora. The results in the control group and in the group with fibrocystic disease of the pancreas were very similar to this. It is therefore apparent from this study that the small intestine of patients with these diseases does not differ bacteriologically from the normal small intestine, and there is no basis for a hypothesis that the development of malabsorption is related to the bacterial flora of the small intestine.

The accuracy of intubation as a technique for studying the bacteriology of the intestine has been questioned by Cregan and Hayward (1953). There are certainly a number of objections to this method. Firstly, determination of the precise intestinal level of the tube is difficult; secondly, contamination of specimens with organisms from a higher level can occur; thirdly, it is possible that the bacteriological result may be inaccurate owing to multiplication of organisms within the tube lumen, if the tube is left in position for any length of time; and, fourthly, alteration of intestinal conditions, such as motility, during the continuing presence of the tube within the intestine may occur. These objections were all considered in the present work in relation to the experimental findings, and are now discussed in more detail.

Fluoroscopy was used as a guide to the level which the tube had reached, but this could do little more than indicate that the tube had progressed from the duodenum, through a number of coils of the small bowel. Consequently the position was considered to be just "jejunal," although the exact level varied from case to case.

During the passage of the tube through the nose and pharynx increase of salivation was often noted. Therefore numbers of oral organisms could be swallowed and increase the amount of bacteria in the stomach. Again, during manipulation of the tube into the duodenum these oral organisms might pass more quickly from the stomach to the duodenum and possibly to the upper small intestine. It was decided that no interpretation would be given to a profuse or resident growth of oral-type organisms in the duodenum or small intestine because of the impossibility of distinguishing between organisms from swallowed saliva and resident oral-type organisms. Thus, by the technique used, the presence of faecal-type organisms only was regarded as of significance at any site sampled.

In the present study an open tube was used and specimens were aspirated from different levels as the tube progressed down the intestine. Martini *et al.* (1957), when investigating the bacterial content of the small intestine in normal and cirrhotic subjects, preferred to use a closed tube, which was

blown open after having reached the desired intestinal level, specimens then being aspirated from different levels as the tube was withdrawn. This method eliminates the possibility of bacterial multiplication within the tube, although the other objections to intubation remain. However, the use of a closed tube in small children is technically difficult. The possibility of false results due to multiplication of organisms within the open tube has been given recognition in the interpretation of our results. Since stomach and duodenal specimens were usually aspirated no later than one hour after the start of intubation, this multiplication can be discounted in assessing these specimens. Jejunal specimens were often collected six to eight hours after the tube was first introduced, and hence multiplication, particularly of the rapidly growing faecal-type organisms, might have occurred.

It can be seen from Table II that in most cases the jejunal specimens showed no quantitative difference from the earlier duodenal specimen. However, in each group, including normals of group I, there occurred one or more cases having a profuse faecal flora in the jejunum and only a scanty flora in the stomach and duodenum. In these cases the faecal organisms isolated from the jejunum were identical with those found in the duodenum several hours earlier. At present these cases are regarded as having had an abnormal flora resident in the jejunum, although contamination of specimens by multiplication of bacteria within the tube lumen may be the explanation for these abnormal findings.

Changes in motility of the gut may occur during intubation, particularly antiperistaltic movements or creeping up of the bowel over the tube. This may, of course, alter the actual level from which the specimen is taken or bring organisms up from a lower level. Further experiments, which will be discussed in a subsequent communication, revealed that specimens aspirated after 24 hours' intubation showed a gross change in the bacterial flora. The reason for this could be alteration of motility patterns or multiplication of bacteria within the tube. Therefore it is considered that, to obtain the most accurate results by intubation, specimens for bacteriological analysis should be aspirated as quickly as possible, at least within eight hours of the introduction of the tube into the intestine.

Intubation is therefore of limited value as a method for studying the intestinal flora, at least in children. However, it remains the only method of obtaining specimens from patients who cannot be subjected to operation. If intubation results are interpreted with caution they can be valuable. The results presented here show that in the majority of patients studied the areas of small intestine sampled were bacteriologically normal, and it is felt that "negative" results such as this can be accepted, but "positive" results or the presence of a resident faecal-type flora must be viewed with caution using this technique.

Summary

The extent and nature of the bacterial flora of the small intestine have been studied in healthy children, children with coeliac disease, and those with fibrocystic disease of the pancreas.

In healthy children the small intestine was shown to be free from a resident faecal-type flora in all those studied by direct sampling and in the majority studied by intubation methods.

In the majority of patients with coeliac disease and with fibrocystic disease of the pancreas the small intestine was also free from a resident faecal-type flora.

In these patients no association between steatorrhoea and an abnormal small intestinal flora could therefore be demonstrated.

The intubation technique has severe limitations as a means of studying small intestinal bacterial flora, at

least in children. However, results of a "negative" type are of value, but results of a "positive" type must be interpreted with caution.

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A MURMUR FROM THE DUCTUS ARTERIOSUS IN THE NEWBORN BABY

BY

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In the newborn lamb Dawes and his collaborators have shown that the ductus arteriosus closes by muscular contraction (Dawes *et al.*, 1955b; Born *et al.*, 1956b). After a rapid reduction in diameter by about half within a few minutes of birth, it remains as a channel through which a considerable amount of blood passes from aorta to pulmonary artery for 24 hours or longer. During this time a loud continuous murmur is heard through the chest wall, a phenomenon found also in the newborn of other mammalian species, such as the calf and foal (Amoroso *et al.*, 1958).

In the newborn baby there are wide differences in the incidence of murmurs estimated by different observers, from 1.9% (Lyon *et al.*, 1940) to 25% (Siemsen, 1938). Their disappearance after weeks or months has been noted (Richards *et al.*, 1955), together with their faintness and blowing or humming quality. The emphasis in descriptive accounts has been on their benign nature rather than their origin.

Recent workers investigating the state of the human neonatal circulation have not found a murmur, though they concluded that the ductus was open (Eldridge and Hultgren, 1955; Rowe and James, 1957; Adams and Lind, 1957). The animal studies that have been cited suggested none the less that a renewed scrutiny of babies in the first hours of life might reveal comparable findings.

In one-third of 100 healthy infants examined at birth or soon after a rough murmur which was heard in late systole seemed in some to run through the second sound and in others to stop there. In phonocardiographic tracings the murmur displayed a crescendo to the second sound and then in some instances a diminuendo. A high-pitched murmur accentuated in late systole and audible after the second sound is a sign of the patent ductus that was described by Gibson (1898, 1900). He deduced that it signifies blood flow from aorta to pulmonary artery. Bonham Carter and Lovel (1953) have established that in early childhood

the murmur may be confined to systole. In babies a systolic murmur might therefore mean that the ductus is open if it could be distinguished from other systolic murmurs that are heard at this age.

In the present paper a crescendo systolic murmur, whether or not there is a following diminuendo, is referred to as the ductus murmur. Its characteristics are described and two factors that seem to affect its detection discussed.

There is general agreement, summed up in the work of Richards *et al.* (1955), that murmurs in the neonatal period have very little connexion with congenital heart disease. The ductus murmur is usually faint, transient, and not easy to distinguish by ear from other murmurs in the early hours of life. Absence of clinical reports of the murmur is therefore not remarkable.

Material and Methods

The babies were all healthy. Five were premature, 5–5½ lb. (2,270–2,500 g.) in weight. Deliveries took place in a labour room at 75–80° F. The cord was clamped at birth. Oxygen was sometimes given for a brief period at the midwife's discretion. If there was delay in breathing, likely to be due to administration of pethidine to the mother, 1 mg. of N-allyl-morphine was given intramuscularly. After the mother had held the baby for a few minutes it was taken to the nursery (70–75° F. (21–24° C.)), where in the course of the next hour it was bathed. Examinations were carried out in a quiet room next door. An adult-sized bell chest-piece was used for auscultation. Although some faint murmurs were better heard with a diaphragm it was unsuitable for routine use because of friction sounds from the skin if a baby was restless.

The phonocardiograph was a portable direct-writing instrument, manufactured by Elema, of Stockholm. In this apparatus the five frequency ranges are 12 to 50, 25 to 100, 50 to 200, 100 to 400, and 200 to 800 cycles per second. These are denoted by the frequency recorded to the highest amplitude within each range, giving five "standard frequency" bands (Mannheimer, 1955) of 25, 50, 100, 200, and 400 c./s. The 400 c./s. "standard frequency" band is nearest to the auscultatory findings and displayed fairly satisfactorily what was heard through the stethoscope. The microphone, 4 cm. in diameter, was held with light pressure on the infant's chest. Tracings were taken at the point of greatest intensity of a murmur when it had been detected with the stethoscope. The speed was 100 mm. per second. The E.C.G. was omitted if attaching the electrodes disturbed the baby. Interference from breath sounds and bodily movements was always a problem.

A uniform plan of examination had not been settled at the start of the study, and the first 20 babies in the series on which the report is based, in whom examination was later judged to have been sufficiently frequent, were selected from a larger number. The remaining 80 were taken consecutively, provided their hour of birth allowed time for convenient observation, and were examined at intervals of one to two hours. 44 babies were seen within 15 minutes of birth.

Rectal temperature was measured at intervals of two to four hours to give a rough indication of metabolic activity over the period of study.

Ductus Murmur

The murmur was best heard in the region of the third and fourth left costal cartilages. It was usually faint but sometimes fairly loud, intensity varying between grades 1 and 3 (Levine and Harvey, 1949). The louder murmurs were audible at the second left interspace, at the lower left costal cartilages and xiphisternum, and slightly across the midline to the vicinity of the second and third right chondrosternal junctions. The murmur was not conducted to the