SMALL INTESTINAL BACTERIAL OVERGROWTH

IN ACUTE AND PERSISTENT INFANTILE DIARRHOEA

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ABSTRACT

INTRODUCTION

Small intestinal bacterial overgrowth refers to the proliferation of abnormal numbers and types of microorganisms in the lumen of the proximal bowel. Bacterial overgrowth has been implicated as a possible factor in prolonging some episodes of infantile gastroenteritis.

This thesis examines 2 different aspects of the duodenal flora of infants with gastroenteritis, and has therefore been divided into 2 separate studies.

CARBOHYDRATE STUDY

Objective

To test the hypothesis that during a diarrhoeal episode the presence of malabsorbed carbohydrate in the duodenal lumen acts as a factor promoting bacterial proliferation.

Patients and methods

Infants admitted to the rehydration ward with acute gastroenteritis were selected for study if they fulfilled various criteria in terms of age, nutritional status, previous diarrhoeal episodes and antibiotic administration. They were admitted to the research ward. Weights were measured and if they had severe diarrhoea ($\geq$ 30g/kg) were included in the study. Twenty patients were entered into the study. On admission into the trial the first duodenal intubation was done to measure the duodenal flora quantitatively and qualitatively. Thereafter the patients were assigned on an alternate basis to one of 2 groups. One group (carbohydrate-containing group) received a soy-based infant formula containing carbohydrates (Isomil, Ross). The other group (carbohydrate-free group) received an identical milk but from which all carbohydrate had been omitted (Ross CHO-free). To

these infants carbohydrate was given intravenously. Stool output was measured daily. After 3 days of the respective diets the duodenal flora was re-examined.

Results

Longitudinal analysis of the duodenal flora of the carbohydrate-containing group showed a small decrease in the number of bacterial isolates and in their magnitude. The duodenal flora of the carbohydrate-free group was virtually unchanged. Comparing the duodenal bacteriology of the groups the only significant difference was that the number of isolates and the magnitude of *Haemophilus* was greater in the carbohydrate-free group ($p < 0.05$). The diarrhoea resolved in 5 patients: 2 in the carbohydrate-containing and 3 in the carbohydrate-free group.

Conclusions

The lack of difference in the response of the duodenal flora between the two groups studied suggests that the presence of carbohydrates in the lumen is not important in encouraging the growth of bacteria in that site. The possible causes for an increase in *Haemophilus* numbers in the carbohydrate-free group are discussed.

BOWEL COCKTAIL STUDY

Objective

Small intestinal bacterial overgrowth has been proposed as a cause of progression of acute diarrhoeal episodes to persistence. The "bowel cocktail", a combination of oral gentamicin and cholestyramine, has been shown to be effective in terminating episodes of persistent diarrhoea. It has been postulated to work by eradicating small intestinal bacterial overgrowth, but its mode of action is not known. The objective of this study was to examine the changes in the duodenal flora associated with administration of the bowel cocktail in order to elucidate its possible mechanism or mechanisms of action.

Patients and methods

The study group comprised 15 patients. Fourteen were infants from the carbohydrate study who had ongoing diarrhoea. The remaining infant (the "late entry") was selected from the rehydration ward. Severe diarrhoea, as defined by a stool output equal to or greater than 30g/kg/day, was a pre-requisite for entry into the study. The investigation involved 2 duodenal intubations for microbiological analysis of the duodenal fluid. After the first intubation (which was the second intubation for the 14 infants who had been in the carbohydrate study) the bowel cocktail was administered. This comprised a 3 day course of oral gentamicin and 5 days of oral cholestyramine. Forty eight hours after the start of therapy the duodenal bacteriology was repeated. The patient management was the same as during the carbohydrate study and the feeding regimen of the infants was not altered. The study ended immediately after completion of the bowel cocktail course.

Results

Administration of the bowel cocktail was associated with a decreased stool output in all patients. Bacteriological analysis of the duodenal flora after this treatment showed a statistically significant decrease in the total microbial count, the aerobic microbial fraction and the Enterobacteriaceal fraction. On analysis of the bacterial genera a significant decrease was noted in *Neisseria*, *Haemophilus*, and aerobic lactobacilli.

Analysis of individual patients' duodenal fluid bacteriology in conjunction with the stool bacteriology results before administration of the bowel cocktail often provided an explanation as to the possible aetiology of the diarrhoea and its resolution by therapy.

Conclusions

Small intestinal bacterial overgrowth, in the accepted sense of a luxuriant flora teeming with faecal organisms, did not appear to be a feature of the patients in this study. The total bacterial count was only slightly above the accepted upper limit of normal. Although the decrease in the number of *Enterobaceriaceae* could possibly be interpreted in the context of

bacterial overgrowth, a study of the individual patients' duodenal flora shows that these microorganisms were more likely to be acting as specific enteric pathogens.

It is concluded that small intestinal bacterial overgrowth, as currently defined, is not an important cause of persistent diarrhoea. The efficacy of the bowel cocktail is more likely to reside in its ability to eradicate specific enteric pathogens. The author ends by questioning the validity of the whole concept of small intestinal bacterial overgrowth.

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CHAPTER 1

INTRODUCTION

DIARRHOEAL DISEASE IN A GLOBAL PERSPECTIVE

It is estimated that every year there are between 500 million and two billion episodes of diarrhoea in children less than five years old. At least 4.5 million of these children die. Diarrhoea is probably the greatest single cause of infant mortality in the developing world[288].

The majority of diarrhoeal episodes are self-limiting and last only a few days. About 50% of deaths occurring during the course of diarrhoeal illness occur in the early stages, and are mainly due to dehydration. In a minority the diarrhoeal episode is prolonged and the mortality in these is disproportionately high. In a study from India only 5% of diarrhoeal episodes lasted longer than 14 days, but carried a mortality rate of 14% compared with 0.7% for acute episodes[34]. Other studies from developing countries have shown that between 36 - 56% of deaths occurring during an episode of diarrhoea in children are related to episodes of prolonged diarrhoea[8].

In terms of morbidity the major problems in the acute stage of diarrhoea are those of dehydration and electrolyte imbalance. Normally their correction with oral rehydration solution is all that is needed. If the diarrhoeal episode becomes prolonged, although the above problems persist, the nutritional factors assume increasing importance. The diminished caloric and protein intake because of anorexia or traditional feeding restriction[195,222], together with the endogenous protein loss which may accompany this condition[36], frequently leads the already nutritionally compromised child into a downward spiral of malnutrition, sepsis, and death.

It has been shown that intensive education in the home use of oral rehydration solution can dramatically reduce the mortality related to acute diarrhoeal disease[104]. As the

effectiveness of home and community treatment of acute diarrhoea increases, death occurring during the course of persistent diarrhoea will form a higher proportion of diarrhoea-associated mortality. The effective management of persistent diarrhoea can be expected to become a major priority in health care in the future.

THE PROBLEMS OF DEFINITION OF PERSISTENT DIARRHOEA

Many terms such as chronic[154a], intractable[12], protracted[208], delayed recovery[322] and persistent have been used to describe prolonged diarrhoea. Despite this plethora of descriptions there is general agreement that a diarrhoeal episode lasting longer than two or three weeks is prolonged. At a recent WHO meeting a consensus was reached that persistent diarrhoea should be defined as the passage of three or more liquid stools daily for at least 14 days following a diarrhoeal episode that begins acutely[8]. This definition is in agreement with most community-based studies published, and has a simplicity which makes it well suited to field studies. Despite its limitations it is likely that this definition, because of its clarity and WHO sanction, will become enshrined as the definition of persistent diarrhoea.

PROBLEMS WITH THE WHO DEFINITION

If one of the future aims of health care is to provide effective management and prevention of persistent diarrhoea, the previously mentioned definition is unsatisfactory. There are two main objections to its use:

1. No mention is made of the severity of diarrhoea. A period of increased stool frequency with little or no increased fluid loss, due to an irritable bowel syndrome often follows infectious diarrhoea. This can in no way be compared with a situation of ongoing watery diarrhoea with potentially life-threatening dehydration

and diminished absorption of nutrients. Both these episodes would be defined as persistent diarrhoea using the WHO criteria.

2. If the aim is prevention of persistent diarrhoea, then one should not wait until 14 days have elapsed. The factors at work in prolonging the diarrhoeal episode are likely to be present before then and it would be sensible to treat them in the early stages. Inevitably by throwing the net wider one would include cases where the diarrhoea would resolve spontaneously[225], but it might be a price worth paying.

TOWARDS A PRACTICAL DEFINITION OF PERSISTENT DIARRHOEA

Because of the problems with the current definition of persistent diarrhoea, and because of the success in the author's institution in treating prolonged episodes of diarrhoea, a different definition of persistent diarrhoea has evolved.

Persistent diarrhoea has been defined as a diarrhoeal episode which is severe enough to require additional fluids to maintain hydration, and has lasted a week or more following the initial hospital treatment[52].

This definition encompasses all children with significant diarrhoea that has progressed beyond the acute phase. The effective treatment of diarrhoea in this group would prevent the progression towards a more prolonged period of disease with all its attendant risks. The shortened duration of diarrhoea can only be to the nutritional advantage of these already vulnerable children.

THE CAUSES OF PERSISTENT DIARRHOEA

There is no single cause of persistent diarrhoea. There are probably many causes with one common theme - prolonged small intestinal damage with ineffective villous repair[209]. It is

unlikely that the agent responsible for the acute episode is also responsible for prolonging it (although some organisms have been found more frequently in persistent episodes). It is more probable that the offending agent has paved the way and created a suitable environment for some other factor or factors which cause the persistence of diarrhoea.

Groups at risk

Diarrhoeal episodes are more likely to become persistent in two groups of infants: the very young and the malnourished.

THE VERY YOUNG

Most studies addressing the relation of persistent diarrhoea to age have shown that a higher proportion of diarrhoeal episodes become prolonged in children less than two years old than in older children. Huttly found that in infants less than six months old 25% of diarrhoeal episodes became persistent (> 14 days)[180]. Bhan, using the same definition of persistence, found an even higher incidence in this age group: acute diarrhoea in infants 3 - 5 months old was associated with a 37% chance of persistence[35]. Househam in his hospital-based survey found that diarrhoea in infants was less likely to be self-limiting (< 4 days after hospital admission) if they were less than six months old[177].

THE MALNOURISHED

Although controversy rages as to whether malnutrition predisposes to acute diarrhoea, few would dispute its link with persistent diarrhoea. Studies from disparate developing countries have shown this association. Tomkins in Nigeria found that in underweight children (< 75% weight/age) diarrhoeal episodes lasted 33% longer than in their well-nourished peers[309]. If in addition the children were wasted (< 80% weight/height), suggesting recent malnutrition, the diarrhoea lasted 79% longer than in well-nourished controls. Two studies from Bangladesh support this link between pre-existing malnutrition and prolongation of diarrhoeal episodes[39,45].

The above are no more than associations. It is likely that many factors are at work. Infancy and malnutrition are themselves associated with overcrowding and its attendant increase in infections. This environment is heavily contaminated with bacteria and the water supply may be drawn from faecally contaminated streams or wells. The malnourished host frequently suffers from impaired immunity and diminished intestinal mucosal repair. To dissect out which of these frequently coexisting socioeconomic and pathological factors is the most important in promoting persistent diarrhoea would be impossible.

Proposed causes of persistent diarrhoea

Many different causes have been incriminated in the aetiology of persistent diarrhoea. The most frequently quoted is intolerance to ingested carbohydrates, mainly lactose[65,213], but with extensive intestinal mucosal damage even glucose intolerance has been implicated[214]. With regards to lactose intolerance confusion reigns, despite the enormous amount of research in this field. In how many cases does lactose actually cause the diarrhoea, and in how many is it malabsorbed non-specifically because of the mucosal damage and attendant disaccharidase depression which occurs as a result of the agent causing the diarrhoea? The answer to this question is not resolved. In recent years the incidence of lactose intolerance in diarrhoeal disease seems to have decreased. This has been observed both in developed[9] and developing countries (Bowie, unpublished data).

Cow's milk protein intolerance[183,323] and soy protein intolerance[184] have also been incriminated as causes of persistent diarrhoea. Mucosal disruption during the course of acute gastroenteritis is thought to facilitate the entry of whole dietary protein antigen into the enterocytes. The host subsequently becomes sensitised to the antigen and, by a variety of ill-understood mechanisms, persistent mucosal damage results. Walker-Smith has hypothesised an interrelationship between cow's milk protein and lactose intolerance[321].

He speculates that malabsorption of lactose is mainly a secondary phenomenon which occurs as a result of intestinal mucosal damage. This in turn may be the result of the offending agent causing the acute diarrhoeal episode or cow's milk protein intolerance. This interesting hypothesis would explain the apparent recent decline in lactose intolerance following acute gastroenteritis. The new low-solute modified cow's milk formulae are thought to provide less antigen sensitisation than the old preparations[9], and hence less mucosal damage, during the course of gastroenteritis .

Yet another cause of persistent diarrhoea has been proposed by Walker-Smith's group[250]. In a study of 69 infants with delayed recovery from acute gastroenteritis, 21 were found to have a new enteropathogen during the course of their illness. On the basis of their findings they put forward the theory that a significant proportion of patients who have persistent diarrhoea following an episode of acute gastroenteritis are in fact suffering from a new infection with a different enteric pathogen. These two illnesses merge into one and give the clinical impression of a prolonged episode. It is only by careful serial examination of the stools that the true nature of the problem can be explained. Theirs was a hospital-based study, but there is no reason to suppose that this does not also happen in the community, particularly in a Third World setting, with its overcrowded and dirty living conditions.

There is another proposed cause of persistent diarrhoea, always quoted, but almost never acted upon: bacterial overgrowth of the small intestine. This is the subject of the present thesis. This dissertation examines two aspects of bacterial overgrowth in infantile gastroenteritis. The study was divided into two parts. The first was designed to investigate one factor that has been postulated to promote small intestinal bacterial overgrowth in gastroenteritis, namely the presence of malabsorbed carbohydrate in the small bowel. The second part examines the effect of an antibiotic/cholestyramine combination - the "bowel cocktail"[54] - on the small intestinal flora. Treatment with the bowel cocktail has been found to be very effective in terminating persistent diarrhoea but its mode of action is uncertain. In an attempt to cast some light on the mechanism of

action of the bowel cocktail the duodenal flora was examined bacteriologically before and after treatment with this agent.

CHAPTER 2

MILESTONES IN THE STUDY OF BACTERIAL OVERGROWTH

The idea that bacteria could cause disease was a product of the second half of the nineteenth century. This concept was a prerequisite for the field of intestinal bacterial overgrowth. The first mention that the proliferation of bacteria in the small intestine could cause illness dates from the closing decade of that century. It was in the form of a case report by Faber, initially written in Danish in 1895, but subsequently published in the German literature in 1897[108]. Faber described the case of a 27 year old woman who presented with abdominal distension and diarrhoea. She was cachectic and had a marked macrocytic anaemia. At post mortem two strictures about a metre apart were found in the small bowel. The portion of intestine immediately above the strictures was markedly dilated. Faber speculated that toxins accumulated in the dilated portion of gut, perhaps as a result of local infection, and that they in some way destroyed the red blood corpuscles.

In subsequent years the bacteriology of the gastrointestinal tract began to be studied in more detail. Hewetson in 1904 published a series of investigations in which he looked at humans with a variety of surgical abdominal conditions and sampled intestinal contents at laparotomy[160]. He also studied animals, and even himself. He found that just under half of the jejunal specimens were sterile.

Progress was hindered by the inaccessibility of the small intestine, making the bacteriological study of its contents in living subjects very difficult. Only the stomach and very proximal duodenum could be sampled with any ease. For the rest of the intestine studies could only be performed by intestinal puncture at laparotomy. The advent of rubber tubes, which could be passed relatively easily and the positioning of which could be checked radiologically, changed this. The earliest rubber tube for insertion into the intestine was constructed by Scheltema in 1908 (quoted in[230]), but it was Buckstein in 1920 who suggested its use for bacteriological sampling[60]. The invention of the Miller-Abbott tube in 1934[230], the first really practical intubation device, marked a new era in the

study of the intestinal flora. After this bacteriological analysis of small intestinal contents was done with increasing frequency.

The next advance in the methods of study of small intestinal bacterial overgrowth was the creation of an animal model by Cameron in 1949[66]. He surgically constructed blind pouches in the jejunum of rats: these were filled by the normal process of peristalsis but did not empty (self-filling blind loops, SFBL). Intestinal contents stagnated resulting in bacterial overgrowth of the luminal contents. Cameron showed that the creation of an artificial blind loop in rats led to pernicious anaemia, in an analogous manner to humans with small intestinal stasis. Animal experiments have extensively used this technique, often using self-emptying blind loops (SEBL) in other rats as controls. SEBL are fashioned in a way that the lumen is always empty and stagnation of bowel contents does not occur. Much of our knowledge on the pathophysiology of bacterial overgrowth has been based on studies using this animal model.

At about the same time in the late 1940's the bacteriology of the ruminal contents of herbivores was the subject of intense study by veterinary scientists. Much of the derived expertise, particularly in anaerobic methods[179], was adapted for use in humans. During the following two decades with improved and simplified techniques in anaerobic microbiology the bacteriological study of small intestinal contents in humans became commonplace in research laboratories.

The studies by Gorbach in the 1960's led to an understanding of the normal microbiology of the various levels of the human gastrointestinal tract, and validated the use of polyethylene tubes for the collection of specimens[134,251].

As a result of these advances the decade of the 1970's was one of intense activity. Many studies were done in humans to evaluate the importance of small intestinal bacterial

overgrowth in causing gastrointestinal disease. Much experimental work was done in animals to elucidate its pathogenesis and pathophysiology.

In the last ten years the pace of research has somewhat slackened. Although much progress has been made into developing non-invasive diagnostic techniques such as the xylose breath test some of the momentum into basic research seems to have been lost. The great effort put into the study of specific intestinal pathogens such as *E.coli*, and the explosion in molecular biology research, seems to have diverted efforts of researchers away from the somewhat less glamorous field of bacterial overgrowth.

The current status at the threshold of the 1990's is that despite the great amount of work done in this field there is much uncertainty on all fundamental aspects of bacterial overgrowth. What causes it? What are the mechanisms by which it causes disease? In which clinical settings is it important? How should it be treated? No clear answers can be given to any of these questions.

THE CONCEPT OF A NORMAL FLORA

Each part of the gastrointestinal tract, from mouth to colon, has its own resident bacterial flora. It is of a type unique to the particular anatomical niche which it inhabits, and is remarkably constant over long periods of time. The flora of the mouth and colon have been the most intensively studied. For a long time it was thought that the proximal bowel did not have a resident flora, any microorganisms found there being merely ingested bacteria in caudalad transit. It is now known that this is incorrect. The proximal small bowel does indeed have a resident bacterial population, albeit a sparse one.

The duodenal or upper jejunal luminal fluid of the normal inhabitant of a developed country is sterile, or contains at the most 4 $\log_{10}$ organisms/ml. When organisms are

present they are mainly of the type that colonise the mouth and pharynx, such as streptococci, staphylococci and *Haemophilus*. Gram negative enteric bacteria e.g. *E.coli* or *Klebsiella* are found very rarely, and then only in numbers less than 3 $\log_{10}$ organisms/ml. Anaerobes are conspicuous by their absence. *Post cibum* there is a transient rise in bacterial numbers, presumably representing displaced oropharyngeal inhabitants. Those bacteria that have escaped the bactericidal action of gastric juice are quickly removed by peristalsis[96]. On moving down the small intestine there is a progressive rise in the bacterial count. Enteric bacteria are found more often and in greater numbers, but anaerobes are still scant. The intact ileocaecal valve acts as a trapdoor: the bacterial count distal to it being up to 5 log units greater than in adjacent ileal fluid, even though the distance between sampling sites may be as short as 4 inches[304].

Beyond the ileocaecal valve the luminal fluid assumes all the bacteriological characteristics of faeces: the organism count rises progressively to about 11 - 12 $\log_{10}$ organisms/ml and there is a marked predominance of anaerobic organisms, particularly *Bacteroides*.

This normal state of affairs may be disrupted, and colonisation of the small bowel with large numbers of microorganisms results. This proliferation may be "silent", or may cause symptoms.

THE PROBLEMS OF DEFINING SMALL INTESTINAL BACTERIAL OVERGROWTH

One confusing aspect of the subject of small intestinal bacterial overgrowth is the lack of an adequate definition. The topic is made even more muddled by the frequent use of terms loaded with aetiological or clinical implications, such as "stagnant bowel syndrome" or "blind loop syndrome".

In the majority of publications dealing with the subject, the definition is dealt with by avoidance. King and Toskes, in their very authoritative review, merely state what constitutes a normal small intestinal flora[199]. Simon and Gorbach define overgrowth in relation to a constellation of clinical and biochemical features, and by implication, the mere presence of a luxuriant bacterial flora in the small intestine in the absence of these abnormal features does not constitute bacterial overgrowth[285]. They associate bacterial overgrowth with the presence of profuse numbers of microorganisms, at least 7 $\log_{10}$ organisms/ml, in the lumen of the proximal small intestine. The flora is complex, resembles that of the colon, and contains anaerobic bacteria. The authors separate this entity from other conditions associated with an abnormal small intestinal flora, such as tropical sprue or infectious diarrhoea. Bernhardt's definition is based on the bacteriological findings alone[31]. He defines the normal upper small intestinal flora as a state of eubiosis. A frankly abnormal flora constitutes dysbiosis, and a borderline picture is one of eubiosis/dysbiosis. The dysbiotic flora is further classified numerically according to whether a generalised overgrowth is found, or if there is a predominance of one particular type of organism. Bernhardt's rather Germanic terminology is the most serious attempt to date of a definition.

Despite the previously mentioned lack of a definition an attentive study of the literature shows that there is a degree of tacit acceptance that to constitute small intestinal bacterial overgrowth the proximal bowel flora should show the following features:

1. The total bacterial numbers should be in excess of that found in "normal" individuals.

2. The flora should be complex, consisting of a wide variety of organisms.

3. The type of microorganisms found should be of a predominantly faecal type i.e. *Enterobacteriaceae*, faecal streptococci or colonic anaerobes, such as *Bacteroides*.

4. The bacteria found should be of a type not normally thought to be pathogenic.

THE PATHOPHYSIOLOGY OF SMALL INTESTINAL BACTERIAL OVERGROWTH

Most work investigating the pathophysiology of this condition has been done in animals with surgically constructed blind loops. It must therefore be interpreted with circumspection when dealing with the situation in humans. Small intestinal bacterial overgrowth can produce a wide variety of changes in the bowel with harmful effects to the host.

Initially the noxious effects of bacterial proliferation were thought to be confined to the lumen of the small intestine. It is now known that bacterial overgrowth produces a more profound disturbance in the gut. Structural damage to the lining of the small intestine is often found. This can be seen both at ultramicroscopic level in the brush border, and at lower magnification in the mucosa[311]. The end result of bacterial overgrowth is a disturbance of absorption of a wide range of nutrients and vitamins.

Vitamin B12 malabsorption

It was in the context of macrocytic anaemia due to vitamin B12 deficiency that the small intestinal bacterial overgrowth syndrome was first described, and for the first forty years of this century it was thought to be the only disturbance found in this condition. The malabsorption of this vitamin is not primarily due to its consumption by bacteria. Certain

microorganisms bind vitamin B12 preventing its uptake and utilisation by the host[97,99]. This binding effect is particularly strong in *Bacteroides* species, and can be reversed by the administration of antibiotics directed against these bacteria[325].

Fat malabsorption

Steatorrhoea is the hallmark of small intestinal bacterial overgrowth syndrome. It is regarded as the main feature of this condition and even in the absence of symptoms increased stool fat loss can frequently be detected by biochemical methods. Dawson in 1960 was the first to speculate that steatorrhoea in the blind loop syndrome might be the result of bacterial action on conjugated bile acids[92]. Bacteria hydrolyse conjugated bile acids to deconjugated (free) bile acids. Tabaqchali found a high concentration of free bile acids and diminished concentration of conjugated ones in the small intestine of patients with small intestinal bacterial overgrowth[298]. In a subsequent study the same author cautiously implicated *Bacteroides* as being the principal bacterial culprit in the deconjugation process[136]. Since then a wide range of bacteria have been found to have the capacity to deconjugate bile acids *in vitro*[212,284]. The classical explanation on the mechanism of steatorrhoea in bacterial overgrowth is that the bacteria deconjugate the bile salts. There is a lower concentration of conjugated bile salts in the lumen leading to inadequate micellar formation[298]. Evidence has subsequently accumulated that deconjugated bile acids or salts may have more far-reaching effects not necessarily confined to the bowel lumen. Gracey in a series of rat experiments demonstrated that deconjugated bile salts caused inhibition of sugar uptake across the intestinal mucosa, both *in vitro* and *in vivo*[140,141]. The effect was reversible, suggesting that the deconjugated bile salts were in some way competing in the transport process. There is some evidence that free bile acids may damage the enterocyte, and *in vitro* that they loosen the tight junctions, thereby increasing jejunal mucosal permeability[114]. Intestinal mucosal damage might explain Gracey's findings of diminished maltase and disaccharidase levels in the small intestine of rats who had been fed sodium deoxycholate[145]. Direct contact of free bile

acids with the mucosa may not even be necessary. Ingenious experiments by Berant have revealed yet another effect of deconjugated bile acids, quite independent of their physical presence in the lumen or the mucosa[27]. In a series of experiments on dogs he investigated the effects of serum free bile acids. These were used in concentrations similar to those that are found in the serum of patients with bacterial overgrowth. Infusion of these bile acids into the superior mesenteric artery, which supplies the proximal small intestine, showed inhibition of water and sodium transport across the bowel wall and mild ultrastructural changes in the enterocytes. Both these effects were reversed after stopping the infusion.

CLINICAL CONDITIONS ASSOCIATED WITH SMALL INTESTINAL BACTERIAL OVERGROWTH

BACTERIAL OVERGROWTH IN ADULTS

Bacterial overgrowth of the small intestine in adults can be divided into two broad groups: classical small intestinal bacterial overgrowth and tropical sprue. The only common link between these groups is the presence of large numbers of bacteria in the lumen of the proximal small bowel. From a bacteriological, pathological and clinical viewpoint they are totally different.

"Classical" overgrowth

A luxuriant growth of bacteria, predominantly of faecal type, is found. Faecal anaerobes, notably *Bacteroides*, are most numerous. Enteric facultative anaerobes, *E.coli* in particular, are also frequently present. The generally accepted view is that the offending organisms are the obligate anaerobes, particularly *Bacteroides*[181,285].

Histological damage of the small bowel mucosa, as assessed by light microscopy, is not a prominent feature. Sometimes biopsies at many sites have to be taken before any enteropathy can be detected[4].

In the early years small intestinal bacterial overgrowth was described in the context of macrocytic anaemia with anatomical abnormalities such as postgrastrectomy jejunal stumps, and intestinal diverticuli and strictures. By 1939 Barker in his review of the world literature was able to report 51 cases[17]. Because of its connection with localised intestinal abnormalities this condition was initially named the blind loop syndrome. This terminology still survives, but with the subsequent realisation that it occurs in many other clinical settings it has also been called the stagnant bowel syndrome, and more aptly the contaminated small bowel syndrome. In adults there seem to be two broad predisposing factors, and in addition a variety of less clearly defined ones.

ACHLORHYDRIA

Patients with diminished gastric acid production often have been found to harbour abnormally large numbers of bacteria in their upper small intestine[101]. On the basis of dog experiments Greenlee has postulated that achlorhydria alone is not sufficient to produce small intestinal bacterial overgrowth[149]. Some other factor such as diminished upper intestinal motility (e.g. vagotomy or a surgically produced anatomical abnormality) also has to be present. More recently this view has been questioned with the observation that diarrhoea and bacterial overgrowth may occur with cimetidine therapy, and that these resolve when gastric acidity returns to normal after stopping the drug[272].

Achlorhydria may be implicated in the abnormal small intestinal flora which is often found in patients with generalised (but not selective) immunoglobulin deficiency[58] Achlorhydria is a common feature in immunoglobulin deficiency and the relative importance of these two factors in the production of intestinal overgrowth is impossible to assess.

Elderly, otherwise healthy patients, are prone to malabsorption because of contaminated small bowel syndrome and have gained relief with antibiotic therapy[260]. The ageing body is prone to achlorhydria (documented in some of the patients) and to decreased intestinal motility. Either or both these factors may be implicated.

Decreased small intestinal motility

It is in this setting that bacterial overgrowth in adults is most frequently found. The impaired motility may be localised, as in the case of stagnation that results from a single diverticulum[124], or it may be more generalised. Bacterial overgrowth has commonly been demonstrated when surgery has disrupted the normal anatomy and created areas of stasis, as in partial gastrectomy with drainage, or following jejunoileal bypass operations for obesity[80]. Procedures in which the anatomy is less disturbed are associated with a lower incidence of bacterial proliferation[254]. Although diarrhoea can be due to several possible mechanisms in these patients, antibiotic therapy gives dramatic relief in some. This suggests a cause and effect relationship.

The association of bacterial overgrowth with Crohn's disease[26] and intestinal tuberculosis[300] is probably also by virtue of an abnormality in small intestinal motility and the presence of strictures. An abnormal flora is also common in more generalised disorders of small intestinal motility such as occurs in scleroderma[191], diabetes mellitus[126], and the obscure entity of intestinal pseudo-obstruction[219].

Miscellaneous

The creation of an ileostomy regularly leads to a luxuriant bacterial flora in that part of the small intestine[132], and in the case of continent ileostomies may actually cause symptoms[277]. The situation of an ileostomy stands alone in the context of this discussion. Its resident flora is probably the result of a part of bowel being put in direct contact with the external environment as Nature never intended, with none of the protective mechanisms at work.

Tropical sprue

An abnormal proliferation of bacteria in the small intestine is thought to play a central role in the pathogenesis of this condition[200]. The situation is totally different to that of the classic contaminated bowel syndrome. Studies in Indians, Haitians, and British expatriates have shown a proliferation of coliform bacteria, mainly *Klebsiella* and *E.coli*, in the small intestinal lumen and mucosa[131,203,310]. The presence of anaerobic bacteria is not a feature of this condition. In marked contrast with the classic contaminated bowel syndrome histological damage of the small intestinal mucosa is easily seen. On light microscopy there is partial or complete villous atrophy. Nutrient and folate malabsorption and marked loss of body weight is a regular feature of this condition. Although therapy with folic acid diminishes malabsorption and improves well-being, the enteropathy persists. It is only by eradication of the coliform bacteria with tetracycline or sulphonamides that a complete cure is obtained.

Klipstein believes that toxins produced by *Enterobacteriaceae* living in the small bowel are responsible for causing the diarrhoea of tropical sprue. He investigated the toxin-producing capacity of *Klebsiella*, *E.coli*, and *Enterobacter cloacae* isolated from patients with this condition[201]. The ability of these toxins to induce net water secretion was tested *in vivo* by perfusion tests in segments of rat jejunum. This was compared to the capacity of toxin preparations of *Enterobacteriaceae* isolated from the jejunum of patients with acute diarrhoea and others with blind loop syndrome. Strains of *E.coli* isolated from the stools of healthy children were also studied. Fourteen of the 16 strains of bacteria from the sprue patients produced at least one toxin that induced net water secretion at low toxin concentrations. The 7 strains of *Enterobacteriaceae* from the patients with acute diarrhoea also produced toxin which was effective at low concentrations. In contrast none of the 9 blind loop strains showed any ability to produce a fluid secreting state. Of the 10 strains

isolated from stools of normal subjects, half produced toxins which induced net water secretion. This only occurred at high, probably supraphysiological concentrations.

Klipstein's theory that toxigenic bacteria cause diarrhoea does not explain the extensive enteropathy that is found in tropical sprue.

Dissenting voices exist. The Vellore group have consistently failed to show increased coliform colonisation in the small bowels of their tropical sprue subjects, when compared with controls (who interestingly had much higher coliform counts than their Northern Indian counterparts)[41]. They naturally do not believe in the bacterial hypothesis. The possibility exists that tropical sprue may indeed have different aetiologies which vary on a geographical basis.

SMALL INTESTINAL BACTERIAL OVERGROWTH IN CHILDREN

The literature dealing with this subject is much smaller than is the case for adults. The main *corpus* of work is in connection with gastroenteritis and will be dealt with in chapter 3.

In the late 1950's Anderson et al in Melbourne examined the small intestinal flora of healthy children and of others suffering from gastrointestinal disorders[5,42]. They looked at patients with coeliac disease, cystic fibrosis, and took specimens of small intestinal fluid at operation from children with acute small intestinal obstruction. They found that a minority of children in the control groups as well as in the groups with coeliac disease and cystic fibrosis had a resident faecal-type flora in the jejunum. They also did a bacteriological study of small intestinal juice, both above and below the level of intestinal obstruction, in infants and children with surgical obstructive lesions. They made the important observation that an abnormal flora, even of a faecal type, is established from above

downwards. This is contrary to the previously held belief that bacteria travelled from the lower reaches of the bowel and became resident in the jejunum and duodenum.

More recently, bacterial overgrowth of the small intestine has been implicated as a cause of chronic non-infectious diarrhoea or of abdominal pain[90]. In the developed world the small intestinal flora has been most often studied in infants suffering from surgical conditions. It has been thought to play a role in causing post-operative carbohydrate intolerance[62], and has been incriminated in causing intestinal obstruction[279] and gastrointestinal haemorrhage[137]. In underdeveloped countries attention has been predominantly on the association of an abnormal small intestinal flora with malnutrition[147,161,223].

CHAPTER 3

THE SMALL INTESTINAL FLORA IN INFANTS AND CHILDREN WITH DIARRHOEAL DISEASE

A BRIEF HISTORICAL SURVEY

Moro in 1916 was the first to examine bacteriologically the small intestinal contents of infants with gastroenteritis (quoted in[32]). Post-mortem examinations of patients dying of this condition showed a heavy growth of coliform bacteria. He was of the opinion that gastroenteritis was an endogenous infection of the small intestine. Davison in 1925 looked at duodenal contents, post-mortem in babies dying of gastroenteritis, and by means of a duodenal tube in others who had recently suffered from an acute diarrhoeal illness[91]. He found a heavy growth of *Bacterium coli (E.coli)* in nearly all the post-mortem specimens, and noted a "colonic flora" in many of the convalescent infants. These findings were confirmed by Blacklock, who also found a high incidence of middle ear infection with coliform bacteria in children dying of diarrhoeal disease[46]. He proposed that infection ascended from the small intestine to the ear by way of the lymphatic system. In the mid 1950's Thomson used a tube to obtain duodenal specimens from infants suffering from gastroenteritis associated with excretion of *E.coli* serotypes O11, O55 or O26 in the stools[306]. These patients had a luxuriant growth of the same organisms in the duodenum as were found in the stool. Babies convalescing from these infections but still excreting the organisms in the stool no longer harboured them in the duodenum. Babies with non-specific gastroenteritis were also examined, and their proximal small intestine was nearly always sterile.

Dammin in 1965 described how his interest in the proximal small bowel was stimulated by experiments which showed that *Shigella* infection could not be induced experimentally in the presence of normal motility of the upper small bowel[89]. This led him to examine the jejunum of malnourished infants dying of non-specific diarrhoeal illness. He often found the proximal small intestine to be dilated and to contain large numbers of bacteria. He

postulated that malnutrition impaired small intestinal motility. This in turn led to bacterial proliferation in the upper small bowel resulting in diarrhoea, either by toxin production or by some other mechanism. He recommended more intensive study of the host rather than of specific infectious agents. Although the methods Dammin used are now obsolete his paper is one of great importance. His imaginative ideas on the role of normally non-pathogenic bacteria in the small intestine in causing diarrhoea, and on the pathogen-host response, are relevant and thought-provoking to this day.

PROBLEMS IN INTERPRETATION

The interpretation of clinical studies is bedevilled both by factors inherent to the studies themselves, and by others outside the control of the individual investigators.

In most studies the patients are poorly characterised and show a wide age spread. The severity of diarrhoea is not well defined in terms of stool output. Widely different methods of intestinal juice collection are used and in some cases the juice is frozen before bacteriological analysis. The reporting of the bacteriological findings is also often woefully inadequate in the following ways. The number of organisms found in a group of patients is usually reported as a mean number, with no ranges given. As a result if in a group there is a single heavily contaminated specimen among many clean ones the mean value will be high, and this is misleading. The median value gives a much better idea of the level of bacterial contamination of the group as a whole. When a specific organism predominates usually no information is given on the magnitude of predominance. Also in most publications little idea can be gained on whether there is a general increase in bacterial flora, or if there is a pure growth of one particular organism.

To make matters more difficult the investigations come from scattered parts of the world, with marked differences in the types of pathogens isolated in the stool, and possibly differences in the level of bacterial contamination of the environment.

THE CAPE TOWN STUDIES

Bowie and his associates have carefully studied the duodenal flora of infants during various stages of gastroenteritis. The patient details were well defined, severity of diarrhoea was assessed by measurement of stool output, and anaerobic methods were used. In the first of these investigations Hill et al studied 3 different groups of infants with acute diarrhoea[162]. The first group was studied the day after admission to hospital, the second after 4 days, and the third after 7 days. They found that most infants had increased total bacterial counts in their duodenum when compared to normal controls reported from developed countries. This increase was noted in all types of bacteria: upper respiratory, faecal and anaerobic. There was no difference between the first two patient groups. Those patients who still had ongoing diarrhoea one week after admission to hospital had a median total bacterial count of about 7 $\log_{10}$ organisms/ml, 100 times greater than that of the other two groups. Although this increase was found in all individual bacterial types it was statistically significant only for *E.coli*.

In the second study Househam et al looked at a group of infants one day after admission to hospital[178]. They confirmed the findings of a luxuriant duodenal flora, with a total median count of about 5.4 $\log_{10}$ organisms/ml of juice. This increase was "across the board" in all bacterial types. He could not predict from the initial duodenal flora which patients would progress to persistent diarrhoea and which would have a self-limiting illness (although there was a tendency to higher total bacterial counts in those infants in whom the diarrhoea persisted, and lower in those in whom it resolved). Administration of the bowel cocktail resulted in prompt reduction of the diarrhoea in nearly all Househam's and Hill's patients (Hill ID, personal communication). On the basis of these findings the authors postulate that at some stage during the acute diarrhoeal episode a change occurs in the duodenal flora of some infants with the possibility of *E.coli* playing an important role, and that this leads to persistence of diarrhoea.

OTHER STUDIES

Total bacterial count

The studies of Fagundes Neto in Buenos Aires and Sao Paulo[110,111] and Albert in Vellore[2] support the finding of increased numbers of jejunal bacteria in well nourished infants with acute diarrhoea. These investigators used local control infants. In the case of persistent diarrhoea Gracey found a high level of bacterial contamination in the small intestine of 18 Australian Aboriginal children[146]. The age of the patients, their nutritional status, and the duration and severity of diarrhoea were not stated. Well nourished Australian whites were used as controls.

Rowland from the Gambia in his series of 37 children with a wide age scatter suffering from persistent diarrhoea again found a high total duodenal bacterial count compared to reported First World controls[271]. The younger infants had a higher bacterial count than the older ones. Rowland placed these findings in the context of his "weanling's dilemma" hypothesis, whereby the infant's gut is subjected to an onslaught of microorganisms from contaminated food at the time of weaning. Of interest in supporting his hypothesis is the low level of stool pathogens isolated in acute diarrhoea in the Gambia, suggesting that perhaps the proliferation of bacteria in the small bowel may have been important.

Bhan in New Delhi found that of 54 malnourished patients with diarrhoea of longer than 3 weeks' duration 52% were harbouring more than 5 $\log_{10}$ organisms/ml in their duodenal juice[38]. The duodenal flora consisted of a wide range of organisms. This overgrowth was not found in 10 age-matched malnourished controls.

Not everyone agrees. In an early study from Jamaica, James found no difference between 5 malnourished infants with acute diarrhoea and 6 equally malnourished children without

diarrhoea[186]. This study can be criticised in that few patients were studied and the specimens were sent to the U.K. on dry ice. More recently Omoike in Nigeria found that the total bacterial count in 11 well nourished children with acute diarrhoea did not differ from that of 11 well nourished controls, although in some diarrhoeal patients recognised enteropathogens were isolated in the small intestinal juice[244]. He also cultured the duodenal juice of malnourished children with and without acute diarrhoea. He found a much higher number of bacteria when compared with the well nourished patients, but there was no difference between the two malnourished groups. Omoike is of the opinion that the principal association of small intestinal bacterial overgrowth is with malnutrition and not with diarrhoea, although he agrees that the small intestinal flora may not be totally innocent in the context of diarrhoea. Yet more conflicting results come from Penny's study of 151 children from a poor area of Lima, Peru[248]. 37 were controls, 38 had acute diarrhoea, and 81 had persistent diarrhoea (>2 weeks). The patients were carefully selected and stool output was measured in most of the diarrhoeal infants (the only study apart from those of Hill & Househam to do this). She found that most infants had high duodenal bacterial counts, irrespective of the presence of diarrhoea. There was no association with malnutrition and no difference between the three groups. The children with diarrhoea who were intubated more than 24 hours after admission to hospital had a lower total bacterial count and lower count of *Enterobacteriaceae* than those who were studied shortly after admission. Penny conjectures that it is probably the environmental contamination so often associated with malnutrition rather than malnutrition *per se* that is the cause of small intestinal bacterial overgrowth. She does not believe that an abnormal duodenal flora is important in causing diarrhoea. No mention is made of the treatment or outcome of those children with persistent diarrhoea; it would have been interesting to see their response to the bowel cocktail.

Upper respiratory flora

This comprises a wide range of gram positive and gram negative microorganisms, mostly aerobic or facultatively anaerobic. The most representative members are various staphylococci, streptococci, *Haemophilus*, and *Neisseria*. These types of bacteria are quite frequently found in the duodenal juice, but have not until recently been implicated in causing diarrhoea.

McNeish found that children with acute diarrhoea due to Rotavirus harboured a greater number of upper respiratory types of organisms in their duodenum when compared to children with acute diarrhoea in whom no pathogens were found[228]. He in no way incriminated this flora in causing the diarrhoea, and merely speculated that perhaps diarrhoea caused by Rotavirus predisposed to an abnormal growth of upper respiratory types of organisms in the small intestine. These findings were not confirmed by a subsequent more detailed study from the same group of investigators[246].

Dahlström in Sweden studied 11 children with what he called chronic non-specific diarrhoea of childhood[87]. On closer scrutiny it seems likely that these patients in fact had persistent diarrhoea following gastroenteritis. The duodenal juice was collected by means of a string test. 10 of the children had a heavy growth of oropharyngeal type of bacteria, mostly α haemolytic *Streptococcus*. Some of these children were treated with co-trimoxazole and their diarrhoea promptly resolved. Those who were treated with a low-lactose milk did not improve until they in turn received the antibiotic, which was associated with termination of the diarrhoea. The results of this study must be treated with caution. The string can easily become contaminated with pharyngeal organisms as it is withdrawn, and one is not completely reassured by the statement that only 2 children had the same bacteria in the throat as in the duodenum. No mention is made of any other bacteria that were found, in particular *Enterobacteriaceae*, which might have been sensitive to co-trimoxazole. It is not stated whether the investigators observing the clinical response had

any knowledge of the treatment given. In the absence of actual measurement of stool output this makes one very wary of any statement regarding the efficacy of treatment.

Enterobacteriaceae

The *Enterobacteriaceae* are a large family of gram negative rods and comprise the most numerous portion of the faecal flora that are not obligatory anaerobes. It is the *Enterobacteriaceae* that have been most frequently isolated in the small intestine of infants and children with diarrhoea but to incriminate them in causing diarrhoea is more difficult. They are hardy organisms and comprise the main part of the faecal flora to survive outside the gastrointestinal tract for any length of time (in Public Health studies the coliform count of the drinking water is used as an index of faecal contamination). Their presence in the upper small bowel of children with diarrhoea may simply be no more than an indication of living in unsanitary conditions.

In one of the early studies pertaining to acute diarrhoea Fagundes Neto found *E.coli* in either the upper or middle small intestine of five well nourished infants with acute diarrhoea[111]. It is not clear whether the sampling tube remained *in situ* overnight for collection of juice from the middle small intestine, a factor known to increase the coliform count[70,110].

Stintzing working in Ethiopia in the late 1970's looked at 27 infants with acute diarrhoea[294]. They were a heterogeneous group with acute or chronic diarrhoea and often with coexisting malnutrition or systemic illness. The study was nevertheless valuable because the *E.coli* were serotyped and tested for toxin production. The results were also clearly set out. Enteropathogenic *E.coli* (EPEC) were found in the "jejunum" (the sampling tube was not checked radiologically) of 7 patients with a median count of 7 $\log_{10}$ organisms/ml and in the stools of 9 patients. All the EPEC found in the jejunum were also detected in the stool. Enterotoxigenic *E.coli* (ETEC) were found in the jejunum of 3

patients (with a median count of approximately 7 $\log_{10}$ organisms/ml) and in the stools of 6. In one case the ETEC found in the jejunum was not seen in the stool. Although most control patients harboured *Enterobacteriaceae* in their small intestine, the diarrhoeal patients with EPEC or ETEC in their proximal small intestine had much larger numbers. When the patients with acute and chronic diarrhoea were analysed separately they were found to have similar bacterial counts. Five patients in the EPEC and one in the ETEC groups were treated with antibiotics: in all there was clinical improvement, and in all the EPEC cases these strains disappeared from the upper small intestine. Stintzing was also involved in a later study from New Delhi[33]. Twenty six infants with acute diarrhoea were compared to 10 local controls. A total of 7 patients had EPEC in the upper small intestine, all in numbers greater than 5 $\log_{10}$ organisms/ml, the same serotype also being found in the stool. No EPEC were missed by looking only at the small intestine. No ETEC were found, compared to two isolates in the stool. No EPEC or ETEC were found in the small bowel of the control infants. *Klebsiella* was found in the small intestine of 4 patients compared to only one in the controls. This study is of interest because the control patients were so "clean" compared to the diarrhoeal ones making it tempting to incriminate any *Enterobacteriaceae* present.

Other studies have looked at persistent diarrhoea, sometimes comparing it to acute episodes. Early publications from Australia[146] and the UK[72] highlighted the large proportion of children with chronic diarrhoea found to be harbouring *Enterobacteriaceae* in their upper small bowel. Unfortunately their patients were poorly defined, and what was meant by chronic diarrhoea is not clear. More recently McNeish's group in the U.K. compared 40 infants with acute diarrhoea (of less than 7 days' duration) with 15 infants in whom it persisted beyond 14 days[246]. In the acute group 9 had faecal organisms isolated in their duodenum. It is likely that these were *Enterobacteriaceae* or faecal streptococci since anaerobic cultures were not done. Seven patients had EPEC isolated in the stool, in 3 the same serotype also being found in the duodenum. Of the 15 patients with persistent diarrhoea 12 had faecal organisms present in the duodenum, a significantly higher

proportion than in those with acute diarrhoea. They were also present in greater numbers, a finding in agreement with those of Hill[162]. In 3 patients EPEC were cultured from the duodenum. Two of these infants had been intubated during the acute phase, and the same EPEC serotypes was also found at that time. Analysing the group with persistent diarrhoea, it was found that the patients who were excreting EPEC during the acute phase were more likely to have a faecal-type duodenal flora during the phase of persistent diarrhoea. Unfortunately no mention is made of the stool bacteriology at the time of persistent diarrhoea. Bhan in New Delhi recently examined the duodenal juice of 54 malnourished infants and children with diarrhoea of longer than three weeks' duration[38]. *Enterobacteriaceae*, alone or in combination with other microorganisms were found in 27 patients. The *E.coli* which were isolated were fully studied and it is of interest that whereas the duodenal ETEC and EPEC isolation rate correlated well with the stool findings, this was not the case for enteroaggregative *E.coli* (EA-Agg EC), a controversial group of *E.coli* which has recently been associated with persistent diarrhoea[37,82]. EA-Agg EC was found in the duodenum of two patients but in the stools of nine. *Klebsiella* was found in the duodenum of 4 patients. All the strains isolated were tested for adherence using Hep-2 cells, and for toxin production in the rabbit ileal loop assay, with negative results for both tests.

E.coli in the small bowel has been more directly incriminated as a cause of diarrhoea by Rothbaum[266]. He described 15 infants 3 to 28 months old with dehydrating diarrhoea of acute onset which failed to resolve. All infants eventually received parenteral hyperalimentation. *E.coli* O119 was recovered from the stools and jejunum of all the patients. When jejunal biopsies were done they usually showed villous stunting and bacterial clustering on the surface of the mucosa. All infants except one (who died) showed improvement in the diarrhoea after treatment with neomycin for 5 to 7 days, and could resume enteral feeds. A further 12 infants had *E.coli* O119 in the stools but not in the jejunal fluid. They were not treated with antibiotics and their diarrhoeal illness followed a less protracted course, although the exact duration is not stated. Independently

from Rothbaum, Clausen described 2 infants with very similar clinical profiles, in whom *E.coli* O111 was isolated from the stools and small intestinal fluid[75]. The response of these patients to intravenous gentamicin was gratifying.

James does not think that *Enterobacteriaceae* are important. In his early study he did not find them in greater numbers or in a higher proportion of patients with diarrhoea compared to controls[186]. He also tested some strains of coliforms for toxin production in the rabbit ileal loop model, with invariably negative results. Bishop in Melbourne investigated 39 infants with acute diarrhoea[44]. No stool pathogens were found in 38 (this study immediately preceded her discovery of Rotavirus in humans). *Enterobacteriaceae* were not found in the duodenum in a greater proportion of the diarrhoeal infants compared to controls from surgical wards, and EPEC were not isolated. Omoike also did not find a difference in the rate of isolation of *Enterobacteriaceae* between diarrhoeal and non-diarrhoeal patients[244]. Penny's findings were similar with respect to the presence or absence of diarrhoea[248]. She did however find that diarrhoeal infants with *E.coli* in the duodenum had a greater stool output on days 3 to 5.

The anaerobic bacteria

By this large and heterogeneous group is meant the obligate anaerobes, which cannot survive in a normal oxygen-containing atmosphere. Their degree of sensitivity to oxygen varies widely: strict anaerobes are not able to survive in an atmosphere of more than 0.5% oxygen, whereas moderate anaerobes can grow in an environment containing up to 3% oxygen[215]. Unlike aerobic and coliform bacteria which are hardy and can be isolated easily with unsophisticated bacteriological techniques, the isolation of anaerobic bacteria is critically dependent on careful handling at the time of collection, swift delivery to the laboratory, and meticulous technique in the laboratory itself. In the case of strict anaerobes isolation is even more difficult and beyond the reach of studies done in a clinical setting. Anaerobes inhabit the whole length of the gastrointestinal tract, each area having

its own unique resident flora. *Fusobacterium* and *Veillonella* are found in the saliva and *Bacteroides* is a normal inhabitant of the large bowel. *Lactobacillus* has been included in the anaerobic group of bacteria, but several species and strains can actually tolerate aerobic conditions. *Lactobacillus* can normally be found throughout the gastrointestinal tract.

Few paediatric studies have used anaerobic methods, and some are of dubious quality. For these reasons it is difficult to find studies in which anaerobes have been implicated in acute or persistent diarrhoea.

In terms of total anaerobic microorganisms, no studies that have included their own control patients have shown that anaerobic bacteria are found significantly more frequently or in greater numbers in diarrhoeal patients. A wide variety of anaerobic bacteria were found in Household's and Hill's studies, although it is not stated what proportion of patients were harbouring anaerobes. There were no control patients. Bishop found no anaerobes in the duodenum of her diarrhoeal infants, a rather surprising finding which somehow casts a shadow on her methods[44].

Bacteroides is par excellence the bacterial culprit of the adult blind loop syndrome. Evidence linking this microorganism to paediatric diarrhoea is less firm. In Household's study of 17 infants with acute diarrhoea there were 11 isolates of *Bacteroides*. The presence of this microorganism in the duodenal fluid was not associated with progression to persistent diarrhoea. Of the 6 diarrhoeal infants who were examined by Hill one week after admission to hospital (i.e. with persistent diarrhoea) *Bacteroides* was found in 5 patients. This compared with 6 isolates from 12 patients studied during the acute episode, and in whom the diarrhoea resolved. Albert found *Bacteroides* in the jejunum of 6 of 28 infants with acute diarrhoea, compared to no isolates in 10 control infants. In contrast James, Omoike and Penny did not find a difference in the *Bacteroides* isolation rate between their diarrhoeal and non-diarrhoeal patients.

A single publication from Italy suggests a possible role of *Clostridium*[121]. The authors found this organism in the duodenum of 6 out of a rather motley group of 19 infants with chronic diarrhoea. Ten much older patients were used as controls and in these no *Clostridium* was found. It is difficult to interpret these findings as *Clostridium* is a normal inhabitant of the large bowel in small infants but not in older children[290]. *Clostridium* is not routinely looked for in anaerobic methods.

A single publication possibly implicates *Lactobacillus*. Albert found that the jejunal *Lactobacillus* count was significantly higher in patients with acute diarrhoea than in controls, but the proportion of infants actually harbouring these bacteria did not differ significantly[2].

Candida

Three studies have implicated this fungus. Bishop found *Candida* in the duodenum in 12 of the 39 infants with acute gastroenteritis compared to two of 20 control[44]. This finding was not influenced by antibiotic administration prior to sampling. In his 1980 publication Fagundes Neto isolated the fungus in the duodenal juice of 6 of 17 patients with acute gastroenteritis, with a median count of about 4 $\log_{10}$ organisms/ml[110]. This compared with two isolates with a count of 2 $\log_{10}$ organisms/ml from 8 control patients. In the case of chronic diarrhoea Gracey found that of the 13 children in his group who had secondary sugar intolerance 10 were harbouring *Candida*[146].

Summary

Acute diarrhoea in infants is often associated with an abnormally high number of bacteria in the upper small intestine when compared to asymptomatic controls. The flora assumes more faecal characteristics, with a predominance of coliform bacteria, and sometimes these coliform bacteria are found to be specific enteric pathogens. Coliforms have been most

often implicated in the pathogenesis of diarrhoea but other organisms have also been blamed, such as the upper respiratory organisms, *Bacteroides*, *Clostridium*, and *Candida*. Those children whose diarrhoea is more prolonged tend to have higher bacterial counts, with an even greater preponderance of coliform organisms. To each of these statements a contradictory one can be found in the literature. Some studies have shown no difference in the small bowel flora between patients with diarrhoea and controls, placing the role of a contaminated small bowel in causing diarrhoea into question.

IS BACTERIAL OVERGROWTH IMPORTANT IN CAUSING DIARRHOEA?

Despite the well described association of small intestinal bacterial overgrowth with infantile gastroenteritis little work has been done to implicate specific microorganisms or groups of microorganisms in the pathogenesis of diarrhoea. This is in marked contrast with the case of the blind loop syndrome, in which the experimental literature is vast. The studies can be divided into two groups: those which have looked at affected patients, and animal experiments.

HUMAN STUDIES

These have been confined to looking for deconjugated bile salts in the small intestine of the patients. By analogy with the situation in the blind loop syndrome, their presence implicates the presence in the small bowel of microorganisms with the ability to convert primary bile salts to secondary (deconjugated) bile salts. The most common bacteria in this group are *Bacteroides*, but the list is long[212,284].

Gracey et al found deconjugated bile salts in all three patients described with chronic diarrhoea and monosaccharide intolerance[139]. They gave no details as to the cause of the infants' carbohydrate intolerance, the exact sampling site in the small intestine, or the

methods used to measure the bile salts. Anaerobic cultures were not done but the authors postulated that organisms possessing the ability to deconjugate bile salts were probably present. Challacombe looked for deconjugated bile salts by means of thin layer chromatography in the duodenum of his 7 infants with chronic diarrhoea[71]. In only one patient were they present. This was an infant with secondary lactose intolerance of unstated cause. *Bacteroides* in concentration of 6 $\log_{10}$ organisms/ml were found in the duodenal fluid. In Rowland's series of 37 infants and children with persistent diarrhoea the total duodenal bile salt concentration was measured, as were the conjugated and unconjugated levels, by means of thin layer chromatography[270]. The total bile salt concentration was found to be abnormally low in half the patients and the concentration of deconjugated bile salts was not increased. These findings were in keeping with impaired ileal function rather than bacterial overgrowth. Ten percent of his patients had *Bacteroides* present in the duodenal juice but it was not mentioned what the bile salt pattern of this particular group of patients was. There was no correlation between total bacterial count and total bile salt concentration.

Few conclusions can be drawn from the above studies on whether bacteria capable of deconjugating bile salts are present in the small bowel of infants with persistent diarrhoea. The patients themselves may not have been truly representative of this group and, with the exception of Rowland's series, were too few in number. Moreover, the duodenum or upper jejunum may be an inappropriate sampling site, since the process of bile salt deconjugation may occur in a more distal part of the small intestine.

ANIMAL EXPERIMENTS

A different approach has been to study the effects on animals of bacteria which have been isolated from the small intestine of patients. The literature is replete with this type of investigation, but the only studies that have used bacteria from children with persistent diarrhoea have been from Gracey's group in Perth. In a series of experiments individual

bacteria or their cell-free supernatants were instilled into jejunal loops of Wistar rats[64,143,305]. The intestinal absorption of various substances was measured by means of perfusion studies. Using a non-absorbable marker many bacteria and their cell-free preparations were found to inhibit the absorption of various sugars and fatty acids. Frequently there was failure of sodium and water absorption from the bowel lumen and in some cases there was net fluid secretion into the lumen. These observations were found with a wide range of microorganisms and with *Candida*. Even a species such as *Staphylococcus aureus*, a microorganism not thought to be of any pathogenic significance in the small bowel, inhibited monosaccharide absorption. These experiments must be treated with great circumspection. Although attempts were made to mimic the clinical setting by keeping the bacterial inoculum at the same concentration as was found in the patients, the bacteria were instilled in monoculture form. An animal species with its own resident intestinal microflora was used. Further, rats are coprophagic, and even show diminished growth when coprophagy is prevented[19]. To extrapolate the findings of these rat experiments to the setting of human infants with diarrhoea is a huge step.

THE BOWEL COCKTAIL

THE USE OF ANTIBIOTICS IN PERSISTENT DIARRHOEA

Bacterial overgrowth of the small intestine is frequently quoted as one of the causes of persistent diarrhoea following acute gastroenteritis. Yet, there is steadfast reluctance to proceed to the logical step of treating it with antibiotics. In Lebenthal's influential book, Gracey, the doyen of bacterial overgrowth in paediatrics, glosses over antibiotic therapy in persistent diarrhoea, and then only in the context of specific enteric infections[138]. The recommendation in the recent WHO memorandum is that antimicrobials should be given only when a specific enteric pathogen is isolated, or in the presence of dysentery[8]. In a recent review by Claeson no mention whatsoever is made of antibiotic treatment for persistent diarrhoea[74]. Coetzer in his review emanating from a country which has used

antibiotics successfully in persistent diarrhoea again only advocates their use in specific enteric infections[78].

The reason for this reluctance to use antibiotics is understandable and probably lies in the generally accepted belief that they do not shorten an acute diarrhoeal episode, and may actually prolong excretion of certain pathogens, such as *Salmonella*. A degree of courage and iconoclastic spirit is needed to break away from this concept and use antibiotics in the setting of prolonged diarrhoea.

THE DEVELOPMENT OF THE BOWEL COCKTAIL

At the author's institution persistent diarrhoea has long been a major clinical problem. Following encouraging results obtained with the use of oral gentamicin[77], a routine evolved in which patients with persistent diarrhoea were treated successively with gentamicin, cholestyramine, and metronidazole. If one agent failed the next was tried.

Following the serendipitous observation that the three agents together were more successful than when they were used separately a combined regime known as the "bowel cocktail" was introduced. This was administered on the seventh day following hospital admission if the patient had significant ongoing diarrhoea despite dietary manipulation with low lactose or soya formulae.

CLINICAL EXPERIENCE WITH THE BOWEL COCKTAIL

In an initial report from the same institution the effect of the bowel cocktail on stool output was investigated[163]. Gentamicin was given orally 4 hourly in a dose of 50mg/kg/day for 3 days, together with metronidazole 100mg 8 hourly and cholestyramine 1g 6 hourly for five days. The patients were infants less than one year old with diarrhoea persisting at least 8 days after admission to hospital. Five were on a soya-based milk, 2 of the remaining

infants were still receiving a high solute non-modified powdered milk. The patients were nursed on a balance bed and the stool output was carefully measured. Before administration of the antibiotic and cholestyramine combination all the infants had significant diarrhoea, with stool weights ranging from 42 to 163g/kg/day. Within 24 hours of giving the bowel cocktail a marked drop in stool output was observed. Intravenous medication was stopped in all patients within 48 hours of starting treatment. By the end of the 5 day course of treatment the diarrhoea had resolved and all infants were successfully challenged with a normal infant formula.

These gratifying findings led to a subsequent controlled trial[164]. The study was designed to assess the efficacy of the bowel cocktail in reducing stool output compared to when it was not given. It also explored the individual constituents of the drug combination to find which were the effective ones, and if there were any synergistic effects leading to a greater diminution in stool output. In order to keep the number of infants studied at a realistic level a 2 x 2 x 2 factorial method of analysis was chosen. This well-accepted statistical technique allows one to assess the efficacy of a drug by comparing all the patients receiving it with those who are not. 40 infants were studied and they were divided into 8 groups of 5 infants each, allowing every possible permutation of the combination. The patients were all between 6 weeks and one year old. All had diarrhoea in excess of 30g/kg/day on day 7 following hospital admission and had received a low-lactose soya based formula on day 4 with no apparent improvement. The drug combination was given as already described with the obvious exception that in some infants one, two, or all the constituents were omitted in accordance with the various groups to which they had been assigned. Stool output was measured, as were nitrogen balance and fat absorption. For logistical reasons bacteriological analysis of small intestinal contents was not carried out. These were the main findings:

1. During the first 24 hours cholestyramine administration resulted in diminution of stool volume compared to when it was not given. During the same period the administration of gentamicin did not significantly reduce stool volume.
2. The patients who received a combination of gentamicin and cholestyramine showed a dramatic decrease in stool volumes in the first 24 hours. This was far greater than when either drug was used alone.
3. By the second day of treatment the effect of gentamicin was significant.
4. The administration of metronidazole had no effect, when used either alone or in combination.
5. There was a very significant decrease in stool output when the gentamicin and cholestyramine combination was given, compared to when no drugs were used.

One can draw the following conclusions from these results: during the first 24 hours cholestyramine seems to be the dominant agent. One can postulate that it acts by absorbing toxins elaborated by bacteria, or by binding bile acids which have been deconjugated mainly by predominantly anaerobic bacteria. That killing of bacteria is important is suggested by the beneficial action of gentamicin which becomes apparent by day 9 when used alone, but is already noticeable by the first day because of its synergistic effect with cholestyramine. Metronidazole, which was used to eliminate the anaerobic component of the flora, so often cited as the cause of diarrhoea in bacterial overgrowth, was ineffective. Lastly and most importantly the bowel cocktail works. This has also been the experience of others[281,302].

HOW DOES THE BOWEL COCKTAIL WORK?

There seems little doubt that the bowel cocktail is effective, but how does it work? The above study does not explain which are the components of the supposedly abnormal flora responsible for causing diarrhoea.

1. Is it by reducing the overall bacterial load, or by more selectively eliminating one or more components of the small intestinal bacterial flora?
2. Is it by decreasing the numbers of coliform bacteria, as is suggested by the action of gentamicin?
3. Is it the anaerobic fraction that is important, a view supported by the beneficial effect of cholestyramine?
4. Why is metronidazole not effective? Is it because anaerobes are not important, or could gentamicin have an effect on the anaerobic fraction?
5. Is there a unifying bacterial theme or are there different causes with the common link of response to the bowel cocktail?

One of the aims of the present thesis is to attempt to answer the above questions by means of an antibiotic/cholestyramine probe. It is hoped thereby to cast some light on the very confusing (and confused) subject of bacterial overgrowth in persistent diarrhoea. The question has been formulated as follows:

"Is the diminution in stool volume that occurs after administration of a gentamicin/cholestyramine combination associated with a change in the duodenal flora? If so, what are the changes?"

ANTIBIOTICS AND THE SMALL INTESTINE

THE AIM OF ANTIBIOTICS

Antimicrobial agents have been used against bacteria in the small intestine with two totally different objectives in mind: to eradicate a specific enteric pathogen, or to attempt to reduce the overall bacterial load.

The eradication of enteric pathogens

With the exception of neonates, the immunocompromised, and bacteraemic patients, antibiotics have little place in the management of gastrointestinal infections. The majority of these infections are self-limiting making chemotherapy superfluous. Their short lived nature also makes antibiotic trials notoriously difficult to interpret. Antimicrobial agents are certainly indicated in the treatment of cholera and *Shigella* enteritis, and are helpful in traveller's diarrhoea[129]. Their effect in *Campylobacter* infection is more controversial. There is some evidence to suggest that antibiotics are effective against *E.coli*. In a well conducted double blind trial from Addis Abbaba Thorén et al[307] showed that administration of mecillinam or co-trimoxazole was associated with more rapid abatement of diarrhoea and elimination of pathogens from the small and large intestine of treated patients, when compared with controls. This effect was noticeable within 3 days of starting treatment. The results of this trial support the clinical impression of previous investigators that antibiotic treatment is effective against of *E.coli* gastroenteritis[30,67,77,168,326]. The duration of therapy in Thorén's patients was 5 days but this may be longer than is necessary. A study by Nelson using oral neomycin showed that a 3 day course was as effective as a longer duration of treatment[240]. Antibiotics have also been used in the treatment of persistent diarrhoea associated with *E.coli* excretion[75,167,266]. Although no control patients were used the clinical course of the patients subsequent to the administration of antibiotics was highly suggestive that these agents were indeed beneficial (see later section).

The reduction of overall bacterial load

As long ago as 1958 Smythe treated his kwashiorkor patients on admission to hospital with a 3 day course of oral antibiotics followed by yoghurt feeds[287]. He noticed a marked improvement in diarrhoeal symptoms (present in almost all) by the third day, the stools assuming a jelly-like consistency. The stools became firm shortly after commencement of

yoghurt. He was of the opinion that an abnormal proliferation of bacteria in the small intestine played a part in the production of diarrhoea. He also believed that the abnormal flora might produce the intolerance to certain foods that is often found in kwashiorkor. He was probably incorrect on the last count since it is now known that lactose intolerance is common in kwashiorkor[51]; this would explain the beneficial effects of yoghurt in his patients, since yoghurt contains its own endogenous source of lactase[204].

A different approach has been advocated, one which takes animal husbandry practice as its example. It is well known that feeding small quantities of antibiotics to animals on a regular basis improves their growth, and hence meat yield (quoted in[265]). This is only found in animals living in unsanitary conditions, and it is suggested that one possible mode of action is by reducing the bacterial load in the small intestine. This bovine and porcine situation has by a rather large leap been translated to that of the puny infant living in a contaminated environment and suffering from repeated gastrointestinal infections. It has been suggested that administering to such an infant continuous low-dose antimicrobial agents might break the vicious circle of malnutrition, intestinal bacterial overgrowth, and diarrhoeal disease[169,265]. The results of studies have been conflicting. An early investigation from Kenya in malnourished hospital patients, showed that the chlortetracycline-treated children showed a definite improvement in growth parameters compared to the placebo group[217]. A recent study from Burma, in a more realistic outpatient setting, showed no clear benefit from the use of amoxycillin or metronidazole[196]. Antibiotics have also been given continuously in the less ambitious attempt of preventing acute diarrhoeal episodes, again with no effect[170].

PAEDIATRIC STUDIES

The paediatric literature dealing with antibiotics in relation to small intestinal bacterial overgrowth is very scanty. The studies that have looked at the microflora before and after antibiotic treatment are even fewer in number and none to the reviewer's knowledge has

systematically looked at the effects of antibiotics on diarrhoeal disease. The literature is best looked at in terms of overgrowth in the context of gastroenteritis, and in other disorders.

Gastroenteritis

Some studies have included a cross-section analysis of the flora of those children receiving antibiotics and of others who had not. Gracey's investigation of Australian Aboriginal infants with chronic diarrhoea did not exclude patients receiving antibiotics[146]. Of his 18 patients 10 were on antibiotics and 11 were not (3 patients were studied before and during drug therapy). He could find no significant difference between the two groups. The total bacterial counts were similar, as were the types of bacteria, both in terms of frequency of isolation and of magnitude. There was also no significant difference in the rate of *Candida* isolation or its colony counts. Unfortunately he did not document the changes in the bacterial flora of the three patients who had bacteriological studies before and during antibiotic treatment. The type, mode of administration or duration of antibiotic treatment of the ten patients were not mentioned, nor were the indications for treatment stated. Bishop's group of 39 infants with acute gastroenteritis included 16 who had received antibiotics in the week before duodenal intubation[44]. In the 10 patients these were of the "broad spectrum" variety (not penicillin), and in only one were antibiotics given parenterally. The results for the two groups were not tabulated separately, and the only microorganisms to be mentioned in connexion with antibiotics were *E.coli* and *Candida*. There was no significant difference in the rate of isolation of *E.coli* when the sampling sites of mouth, stomach, or duodenum were taken together. Surprisingly the rate of recovery of *Candida* in the duodenum was higher in the patients who had not received antibiotics, but this did not reach statistical significance.

Penny's large study of diarrhoeal and non-diarrhoeal Peruvian children included patients who had received antibiotics in the week prior to study. Of 151 subjects 37 had received

antibiotics[248]. The type of antibiotic given, or the duration of treatment was not mentioned. In setting out the results Penny did not separate the two groups. She merely stated that when the results were analysed with the antibiotic group excluded the results were unchanged, so she decided to include the antibiotic group in the final analysis.

One can draw almost no conclusions from the results of these studies. One cannot imply that antibiotics have no effect on the small bowel flora. The patient groups were always cross-sectional and the clinical and pharmacological information given is minimal. It is possible that the abnormal small bowel flora became established subsequent to the start of antibiotic therapy, in which case one might not find a difference between an antibiotic and non-antibiotic group.

Lifshitz's group of 23 infants with persistent diarrhoea and monosaccharide intolerance were treated with antibiotics[214]. Most improved and were eventually able to tolerate dietary carbohydrates. This was associated with eradication of bacteria from the duodenum. Two infants who initially improved and had sterile duodenal fluid on the second intubation subsequently relapsed. Repeat culture of small intestinal contents showed bacterial contamination. After treatment with antibiotics the diarrhoea abated and the patients regained carbohydrate tolerance. In 3 infants who died with ongoing diarrhoea the *ante mortem* duodenal fluid was still contaminated with coliform bacteria. Unfortunately in this study the details concerning antibiotic therapy and the bacteriology results are woefully inadequate. Aerobic methods only were used. The evidence for a definite dose-response effect is lacking since the infants spent a considerable portion of their time with diarrhoea and the improvement may simply have been coincidental.

Stintzing's investigation of Ethiopian infants included 5 patients in whom jejunal cultures were done before and after antibiotics[294]. These were all infants less than one year old and had EPEC in the jejunal fluid, in counts ranging from about 4 $\log_{10}$ to 8 $\log_{10}$ organisms/ml. In 2 patients EPEC alone was isolated, in a third it was the predominant

microorganism. The patients were treated with a five day course of mecillinam or co-trimoxazole parenterally and all improved. The jejunal bacteriology done after treatment showed that the EPEC had completely disappeared in all 5 infants. One aspirate was sterile, the other 4 still contained organisms in counts ranging between about 3 $\log_{10}$ to 6 $\log_{10}$ organisms/ml. In 3 of these different types of organisms were found on the second intubation not seen initially. The last patient contained organisms of the same group in both aspirates. This study shows that the diarrhoeal pathogen does not always predominate in the small bowel lumen. The organism population may be quite small. Antibiotics can completely eradicate it. It also shows very clearly that different bacteria can colonise the small bowel in the wake of antibiotic treatment (the time of repeat bacteriology is not stated), and yet not interfere with the patient's recovery.

Of the 15 infants in Rothbaum's series given oral neomycin, four had repeat bacteriology of the jejunal fluid[266]. This was about two weeks after completion of therapy. The initial culture showed a pure growth of *E.coli* O119 in numbers greater than 4.7 $\log_{10}$ organisms/ml. The jejunal contents after treatment did not contain *E.coli* but other organisms were present in concentrations less than 3 $\log_{10}$ organisms/ml. Three patients improved, one died of septicaemia. Clausen's 2 infants with secretory diarrhoea had *E.coli* O111 in the proximal bowel fluid[75]. Both improved after intravenous gentamicin was given and repeat culture of the same site showed complete eradication of the *E.coli*. In both Rothbaum's and Clausen's publications the details given about the small intestinal fluid analysis are minimal. Their investigations were mainly concerned with the histological features of EPEC enteric infection; the bacteriological aspect of the small bowel fluid was a peripheral part of their studies.

In the series from Walker-Smith's group of 6 infants with persistent diarrhoea associated with excretion of EPEC in the stool, three patients received intravenous gentamicin[167]. In one it was the only antimicrobial agent given, in the other 2 patients antibiotics of the penicillin or cephalosporin group were also administered. All 3 infants had severe

secretory diarrhoea of at least one month's duration. Administration of antibiotics was associated with a dramatic relief of symptoms. In 2 infants EPEC adherent to the jejunal mucosa were observed before antibiotics were given, while no patient had these organisms detected on repeat jejunal biopsy during or after antibiotic treatment. Interestingly, EPEC excretion in the stools continued for weeks after the diarrhoea had stopped.

The objection can be made that antibiotics played no part in eradicating *E.coli* from the small intestine, the natural course of an enteric infection being the eventual clearing of the offending organisms from the bowel. This argument is difficult to maintain in the case of the patients studied by Rothbaum, Clausen, and Walker-Smith. All the infants had longstanding diarrhoea, far beyond the usual time course for a self-limiting gastrointestinal infection. There was a definite temporal relationship between giving antibiotics and marked symptomatic improvement. One can more easily criticise Stintzing's results. In three of his five infants the duration of diarrhoea was less than 10 days. When, however, his data is interpreted in conjunction with his publication from the same hospital, in which favourable results are reported in the antibiotic therapy of *E.coli*-associated gastroenteritis, his findings seem more convincing.

Other conditions

There have been other publications that have looked at small intestinal fluid before and after antibiotics, but none have done so in a systematic fashion. These papers are not relevant to this dissertation. The patients did not have acute diarrhoea or post-enteritis persistent diarrhoea. They were patients with intestinal haemorrhage[137] or obstruction[279]. In Schwöbel's series small intestinal contents were not looked at, gastric juice being used as a "poor man's" substitute. Davidson's paper, interesting in other aspects, is weak from a bacteriological viewpoint[90]. In only two patients was the small intestinal fluid cultured after antibiotics, and then after several months. Metoclopramide was given to facilitate passage of the sampling tube through the pylorus: this would increase the risk of

contamination of small intestinal fluid with gastric contents, and potentially invalidate the results.

STUDIES IN ADULTS WITH BLIND LOOP SYNDROME

All the publications describing the bacteriology of the small bowel contents before and after antibiotic therapy take the form of case reports or very small series. The first report dates from 1961[127]. In it Goldstein described two patients who were treated with antibiotics from a series of seven with postgastrectomy blind loop syndrome and steatorrhoea. The first had a very heavy growth of *Proteus* at 7.9 $\log_{10}$ organisms/ml with "smaller number of streptococci and coliforms" near the region of the gastrojejunostomy anastomosis. Tetracycline was given followed by neomycin, with no effect. Repeat bacteriology showed the *Proteus* numbers to have risen by one log unit and to be sensitive to chloramphenicol, administration of which resulted in clinical improvement and sterilisation of small bowel juice. The second patient had *Paracolon bacillus* (an obsolete term denoting poorly defined *Enterobacteriaceae*-like microorganisms) in concentration of 7.9 $\log_{10}$ organisms/ml in the small intestinal juice. Tetracycline was given for six days and he improved. Repeat bacteriology on the day of stopping tetracycline showed the total bacterial count to be 5.3 $\log_{10}$ organisms/ml, mainly streptococci. The author speculated that the gram negative enteric bacteria were in some way responsible for the steatorrhoea, but no anaerobic cultures were done. Paulk in 1964 described the case of an 81 year old man with macrocytic anaemia, steatorrhoea, and multiple jejunal diverticula[245]. Jejunal culture showed a predominance of *E.coli* at 7.5 $\log_{10}$ organism/ml, with a smaller number of enterococci. Administration of tetracycline resulted in improvement of vitamin B12 and fat absorption but the numbers of *E.coli* were unchanged. On closer scrutiny it was found that the *E.coli* were of a totally different strain, presumably innocuous to the small bowel. This report is important because it shows that simply looking at bacterial species and numbers may not be informative. Sometimes it is only by looking more closely at the bacteria involved that a possible explanation for antibiotic success or failure can be found.

The first study to include anaerobic bacteriology was by Polter, working in Finegold's laboratory, with its impeccable anaerobic credentials[253]. It took the form of a single case report, but the patient in question was studied in considerable detail. He was a 76 year old man with blind loop syndrome. He possessed two possible causes for this: a subtotal gastrectomy, and multiple jejunal diverticula distal to the gastrojejunostomy. Various antibiotics were given and bacterial culture of afferent loop contents was done before and after administration of the antibiotics. Initial culture showed a heavy growth of about 8.9 $\log_{10}$ organisms/ml of *Enterobacteriaceae*, mainly *E.coli*, and anaerobes at about one log higher concentration, consisting predominantly of *Bacteroides*. The only antibiotic to produce improvement in clinical and laboratory parameters was lincomycin. This was not associated with a reduction in the aerobic bacterial count, but there was complete eradication of the anaerobic flora. Tetracycline produced an improvement in fat absorption, but not in the absorption of vitamin B12. Tetracycline did not change any of the components of the small intestinal flora. Neomycin administration was not associated with a response, although there was a drop in the aerobic count by about one log unit. The anaerobes were not affected. Likewise chloramphenicol and nitrofurantoin were equally ineffective and were associated with very little change in the flora. The study can be criticised for the lack of a "washout" period between antibiotics. Only the afferent loop fluid was cultured, and it is possible that the bacteriological changes in the jejunal diverticula may not have paralleled those occurring in the more proximal site. This investigation coincided with the extensive work of others on bacterial deconjugation of bile salts. It signalled the turn of the tide towards incriminating anaerobic bacteria as the main culprits in causing the blind loop syndrome. The author has excluded any investigation subsequent to this that did not include anaerobic methods. In 1969 Gorbach published his observations on the effect of giving lincomycin for one week to healthy volunteers and to ileostomists[135]. The antibiotic had very little effect on the bacteria at various levels of the small intestine, but none of the subjects had high numbers of bacteria at any of these sites initially. The main effects of lincomycin were on the distal ileum of the ileostomists and

the colon (stools) of all the subjects studied. There was a marked decrease in the anaerobic component of the flora and also a decrease in the number of *Enterobacteriaceae*. The emergence of large numbers of lincomycin-resistant *Enterobacteriaceae* was also noted in some patients.

Farrar in 1972 gave a detailed report on four patients with the blind loop syndrome secondary to either gastrectomy or jejunal diverticula[113]. Antibiotics were given and "before and after" bacterial cultures were done. All the patients harboured large numbers of bacteria, ranging from counts of about 7 to 9 $\log_{10}$ organisms/ml, in the small bowel. In all *E.coli* predominated, but *Bacteroides* were also present in 3, and faecal streptococci in the remaining patient. The administration of tetracycline was associated with improvement in 2 patients. There was no change in the total bacterial count or the number of *E.coli* present, but in one patient the *Bacteroides* completely disappeared, and in the other there was eradication of anaerobic lactobacilli with persistence of *Bacteroides*. In the 2 other patients tetracycline did not produce an improvement in their symptoms. Ampicillin was then tried. In one patient this was associated with a clinical remission and complete eradication of *Bacteroides* from the upper small intestinal juice. The numbers of *E.coli* were not affected. The fourth patient failed to improve with antibiotics. His small intestinal juice contained high numbers of *E.coli* (7 $\log_{10}$ organisms/ml), but numerous faecal streptococci were also present at 6 $\log_{10}$ organisms/ml. The authors' discussion focused on the importance of anaerobes in the causation of symptoms, by virtue of their bile-splitting properties. In the case of the fourth patient who did not have anaerobes, the faecal streptococci (potential deconjugating agents of bile salts) were incriminated. The deconjugated bile salt levels of the specimens were measured. It is of interest that the patient in question was the only one to have no detectable levels present, but this finding was not discussed. This study is rich in implications. It highlights the lack of importance of total bacterial load in causing symptoms. Closely related to this it shows that the predominant organism is not necessarily the one to be blamed for causing symptoms. It shows that the theme of response to a particular antibiotic can be due to effects on different

components of the flora (*Bacteroides* in one, anaerobic lactobacilli in the other). It also makes the point that there may be no correlation between the in-vitro effects of an antibiotic and what actually occurs in the small intestine of a human. Neither tetracycline or ampicillin have anaerobicidal properties, yet their administration was associated with the complete eradication of some anaerobic species.

Ament's 3 patients with blind loop syndrome all had *E.coli* in their duodenal juice ranging from about 6 to 7 $\log_{10}$ organisms/ml[4]. The first two patients also had *Bacteroides* (about 5 x 10^9 and 5 $\log_{10}$ organisms/ml) and enterococci (3 $\log_{10}$ and 7 $\log_{10}$ organisms/ml). These were not found in the last patient. The first subject showed a marked improvement in symptoms and laboratory parameters of intestinal absorption after treatment with tetracycline. The second received the same treatment with no effect, but showed an incomplete response to a 2 week course of ampicillin. The last patient showed no benefit at all from treatment with ampicillin followed by tetracycline. Repeat duodenal cultures at an unspecified time after antibiotic treatment showed a drop in *E.coli* by two to three log units in all patients, and complete elimination of the other species. All subjects showed an increase in total duodenal bile salt concentration (initially low). Deconjugated bile salts were present in the duodenum of the first 2 patients before treatment. These actually rose in the first patient after antibiotics and fell in the second. In the last subject they were never present in large quantities. Duodenal histology after treatment showed no improvement in the patchy enteropathy which was found before, but on electron microscopy all patients showed changes suggestive of improved fat transport out of the absorptive cells. Despite Ament's efforts to blame deconjugated bile salts (and by inference bile-splitting bacteria) there are many incongruous findings in his small series. The last patient in particular cannot be neatly dovetailed into such a theory.

Barry in 1977 described an antibiotic trial on 4 patients for the treatment of intestinal pseudo-obstruction following jejunoileal bypass operations[20]. This motility disorder is a well-recognised late complication of the procedure. The treatment consisted of a 5 - 7 day

course of oral metronidazole or kanamycin. These antibiotics were then crossed over and the patients then received either a placebo or both antibiotics together, these were then crossed over for the last treatment period. The study was double blinded and the effectiveness of treatment was judged by decrease in abdominal girth. The bacteriological sampling site was in the region of the anastomosis. It was found that metronidazole was the most effective treatment. Kanamycin was much less effective, and the combination of kanamycin and metronidazole was no more effective than metronidazole alone. The mean counts of aerobic and anaerobic organisms were in the order of 8 and 9 $\log_{10}$ organisms/ml respectively. The administration of metronidazole was associated with minimal change in the aerobic portion but a marked drop in anaerobes by about eight log units. Kanamycin had little effect on the numbers of aerobes isolated, but the aerobic microorganisms found after its administration were resistant to it. There was also an increase by about one log unit in the anaerobic fraction. By the time of giving the combination treatment all the patients had already received both antibiotics separately, and although the aerobic part of the flora numbered about 8 $\log_{10}$ organisms/ml the anaerobic portion was 2 $\log_{10}$ organisms/ml. The administration of both antibiotics together resulted in the virtual elimination of the anaerobes, while the aerobic part of the flora was unaltered. The author concluded that the anaerobic fraction of the flora was the important one in causing the obstructive symptoms. No attempt is made to explain why kanamycin produced some symptomatic relief, in contrast to its observed effects on the flora. It should also be said that the bacteria and mechanisms by which obstruction is caused may not be the same ones that cause diarrhoea in the blind loop syndrome.

ANIMAL EXPERIMENTS

Rats with experimental blind loop syndrome do not provide a good model when dealing with antibiotic therapy. They are coprophagic animals and their normal small intestine contains large numbers of microorganisms of a faecal type[98,194]. Moreover the situation

of a rat with an artificially created self-filling blind loop is not to be compared with an intact human small intestine in the throes of infective enteritis.

The animal model that most closely approximates the human situation is that of a natural affliction of German Shepherd dogs. Normally their upper small intestine is "clean", containing less than 4 $\log_{10}$ organisms/ml, but proliferation in their proximal small bowel of an abnormal quantity and type of bacteria is associated with chronic diarrhoea and weight loss[24]. This condition has been studied in detail by Batt and his colleagues in Liverpool[21,22]. They found that the abnormal flora was usually predominantly aerobic, but sometimes obligate anaerobes predominated. These two patterns of bacterial colonisation were associated with different and distinct biochemical changes in the small bowel mucosa. Eight dogs were treated with oxytetracycline for 28 days and all improved[23]. In 7 dogs the jejunal fluid bacteriology was repeated after treatment and showed widely different results. In one canine patient the bacterial count was reduced by 3 log units, but in most the change was less dramatic, and in one there was even a small increase. The most noticeable change was the disappearance of *Clostridium* spp. from all 3 dogs in whom anaerobes predominated. Most biochemical parameters showed reversal towards normal when repeated after antibiotic treatment. These dog studies show quite clearly that widely differing patterns of bacterial proliferation can respond to the same antibiotic. They also strongly implicate bacteria as the cause of symptoms, rather than simply playing the role of innocent bystanders.

CHOLESTYRAMINE

It is not the aim of this thesis to elucidate the mechanisms of action of cholestyramine, but since it is an active constituent of the bowel cocktail a mention of its possible effects is of some relevance to the reader.

CHEMICAL PROPERTIES AND POSSIBLE MODES OF ACTION

Cholestyramine is an ion exchange resin[171]. It consists of a polymer bristling with positively charged ions. These cations are counteracted by chloride anions, so maintaining electrical neutrality. These chloride anions are exchanged in the intestinal lumen for other anions. Any available anions are potentially exchangeable, but cholestyramine has a predilection for those that have large hydrophobic regions. Bile acids and fatty acids are such substances. Even among bile acids there is an order of preference, dihydroxy bile acids (deconjugated) being bound with greater avidity than trihydroxy acids[187]. Anions normally present in the lumen of the intestine, such as chloride and bicarbonate, can compete with bile acids, decreasing their adsorption onto cholestyramine[187]. Binding of deconjugated bile acids will inhibit their secretagogue effect on the bowel, which is a possible mechanisms by which deconjugated acids may cause diarrhoea. Another way in which cholestyramine might be effective is by binding toxins produced by bacteria. In animal studies it has been found that it diminishes the harmful effects of *E.coli*-elaborated endotoxin when both substances together were administered into the peritoneum of rats, compared to when the endotoxin alone was injected[241]. Further evidence suggestive of toxin binding action comes from its efficacy in the treatment of *Clostridium difficile*-associated colitis in adults and children[207, 258].

The effect of cholestyramine on bile acids and bacterial toxins are only two possible mechanisms of action in decreasing diarrhoea. There is no reason why it should not bind other noxious substances produced during a diarrhoeal illness such as fatty acids and other products of bacterial metabolism or of the host, and thus diminish their harmful effects. This is as yet an unexplored territory and could provide rich hunting ground for the potential investigator.

ITS USE IN INFANTILE DIARRHOEAL DISEASE

Cholestyramine is not effective in the treatment of acute gastroenteritis. Although it seems to shorten the duration of watery diarrhoea[182,319] the stool output is not diminished[182]. This apparent contradiction can be explained by the fact that it produces firmer stools, presumably by absorbing water and acting as a bulking agent[182]. This misleading effect should be borne in mind when evaluating its therapeutic efficacy.

Cholestyramine has been used with some success in the treatment of persistent diarrhoea. In Tamer's study it was given to 7 infants, of whom 6 had diarrhoea of 14 days' duration or longer[299]. It was associated with abatement of diarrhoeal symptoms within two days, and these did not recur when the drug was stopped.

In a subsequent report from Israel it was given with good effect to 20 infants with persistent diarrhoea[28]. In some patients there was a relapse in symptoms after cholestyramine withdrawal. Both publications can be criticised on the grounds that no control patients were used and the stool output was not measured. In the case of the Israeli study where treatment was prolonged, the diarrhoea may well have resolved spontaneously. Hill's study on the bowel cocktail is the only one which clearly documents the efficacy of cholestyramine[164]. It has already been discussed.

CHAPTER 4

ON THE PATHOGENESIS OF SMALL INTESTINAL BACTERIAL OVERGROWTH

The two most common predisposing factors leading to small intestinal bacterial overgrowth in adults are claimed to be diminished gastric acidity and impaired motility of the small intestine.

DIMINISHED GASTRIC ACIDITY

There is good evidence that the acid concentration of the stomach serves as a barrier against bacterial colonisation of the stomach if the pH is maintained below 4[122,249].

It is not known whether the factors responsible for small intestinal bacterial colonisation in infants with diarrhoea are the same as for adults. Malnourished infants, a group with a high incidence of small intestinal bacterial contamination, have been shown by Gracey et al to have impaired gastric acid production[144]. In their report it is unclear if this diminished gastric acidity was of a level sufficient to inhibit bactericidal activity. Breast fed babies have a higher fasting gastric pH compared to bottle fed babies, yet the microbial flora of their stomach (and presumably of the small intestine) is not significantly different[218]. Clearly other factors besides pH must also be at work.

Rowland et al in their study from a Gambian village found a high level of bacterial contamination in the environment[269]. Foodstuffs, water used to reconstitute milk feeds, and kitchen utensils, showed a heavy bacterial growth, particularly of *Enterobacteriaceae*. In developing countries gastroenteritis is very common at the time of weaning ("weanling diarrhoea"). Rowland and his colleagues believe that ingestion of large numbers of bacteria in foodstuffs might play an important role in causing weanling diarrhoea. Subsequent studies from other developing countries have confirmed the massive bacterial contamination of food and of water used for the reconstitution of powdered milk[105,296]. It

is likely that ingestion of a large number of bacteria in food and drink overwhelms the "gastric acidity barrier" and causes bacterial colonisation of the small bowel. Penny et al found that poor living conditions (and presumably an accompanying contaminated environment) were the common link with intestinal overgrowth in her study of Peruvian children, which included control subjects without diarrhoea but living in similar conditions[248].

IMPAIRED SMALL INTESTINAL MOTILITY

Most cases of small intestinal bacterial overgrowth in adults are associated with diminished small bowel motility, be it local or diffuse (see chapter 2).

The motility of the small intestine between meals consists of many different phases, each associated with a particular pattern of smooth muscle contraction[316]. This so-called interdigestive motor complex has long been thought to play a role in preventing local proliferation of bacteria - hence the term "intestinal housekeeper", coined by Code.

Small intestinal motility studies in adults have shown that absence of the normal interdigestive motor complex is associated with bacterial colonisation of the small intestine[317]. In rats experimental abolition of the interdigestive motor complex leads to abnormal proliferation of bacteria in the small intestine[280], and a similar situation may exist in humans.

The importance of altered intestinal motility in promoting the bacterial overgrowth found in infantile gastroenteritis is not known. Acute infectious diarrhoea is associated with a diminished intestinal transit time[233], sometimes as short as 20 minutes (Mann MD, personal communication). This increased motility should tend to prevent bacterial colonisation of the small intestine. But it is possible that localised areas of the small bowel have diminished motility during an acute diarrhoeal episode. Vomiting, an antiperistaltic

event, is common during an episode of acute gastroenteritis and can occur even in the absence of gastric involvement by the specific infectious agent e.g. Rotavirus. It is conceivable that a proliferation of bacteria in the small intestine might occur in the wake of diminished motility in a small segment of the bowel.

The evidence currently available from animal experiments supports the view that bacterial colonisation of the small intestine alters intestinal motility, rather than altered motility causing bacterial colonisation. Injection of specific enteric pathogens and their toxins into rabbit ileal loops has been shown to alter the normal myoelectric complex in the small intestine[224]. The same effect has been demonstrated in the course of experimentally induced *E.coli* infection in rabbits[286]. The abnormal motility was observed even before the onset of diarrhoea. Further evidence suggesting that an abnormal flora leads to abnormal motility comes from a study by Justus et al using rats with experimental blind loops[190]. The bacterial flora produced a motility pattern that would tend to produce diarrhoea. This pattern was reversed by antibiotic treatment. We are still far from a true understanding of the interrelationship between bacterial colonisation of the small intestine and abnormal motility.

It is clear that the usually quoted predisposing factors for small intestinal bacterial overgrowth play an uncertain role in the small intestinal bacterial proliferation found in infantile gastroenteritis. It is pertinent to examine other theories that have been proposed to explain this phenomenon. One such theory is the concept that malabsorbed food in the small bowel acts as a substrate for bacterial growth.

THE RELATIONSHIP OF CARBOHYDRATE MALDIGESTION AND BACTERIAL OVERGROWTH

CLINICAL STUDIES

Historical

The idea that malabsorbed food can encourage bacterial proliferation in the bowel is almost as old as the discovery of small intestinal bacterial overgrowth itself. Moro in 1905 was the first to suggest this possible promoting factor[236]. Davison in his 1925 semi-quantitative study of the bacteriology of duodenal contents in infants re-states Moro's idea[91]. He investigated the effect of changing the protein, sugar, and acid content of milk on the duodenal flora of normal infants. There were no qualitative changes. The milk rich in protein tended to increase bacterial numbers, but the quantitative bacteriological techniques of this time must be viewed with caution. Blacklock in his 1937 study of the duodenal flora in paediatric patients again quotes Moro's hypothesis[46]. He states that malabsorbed food acts as "a rich pabulum" for coliform bacteria in the proximal small bowel. Subsequent to this, no publication mentions this theory for over three decades.

The postulate that the carbohydrate portion of the diet might promote the growth of certain bacteria in the intestine and give rise to symptoms also dates from the first decade of this century. Herter in 1907 proposed that anaerobic gas-producing bacilli in the lower reaches of the small bowel and in the large intestine thrive on carbohydrate and produce large quantities of gas ("saccharobutyric putrefaction")[159]. He claimed that saccharobutyric putrefaction could have far-reaching effects, ranging from diarrhoea to "neurasthaenia" and premature senility. In the 1920's Kendall refined the concept of carbohydrate putrefaction, and as a result of his investigations concluded that in some individuals ingestion of carbohydrate leads to the proliferation of *Bacillus welchii* in the intestinal tract[193]. This organism could then give rise to a variety of symptoms, including flatulence, abdominal

pain, and lethargy. He speculated that the absence of lactobacilli in the bowel might predispose to the initial proliferation of these gas producing bacilli. He suggested therapy with a diet low in carbohydrate and rich in soured milk. In their very thorough clinical and bacteriological study on carbohydrate intolerance Althausen and his colleagues could not find a correlation between the numbers of *Bacillus welchii* in the stools and symptoms[3,151]. They thought that symptoms were due to the presence of large numbers of bacteria in the small intestine. These early studies on carbohydrate intolerance concentrated on the interrelationship between the presence of carbohydrate in the intestine, bacterial growth, and carbohydrate intolerance. Later in the century research in the field of carbohydrate (particularly lactose) intolerance focused on the small intestinal mucosa and its enzymes. The wheel has now turned full circle and bacteria are again the subject of study. It is now recognised that the fermentation of carbohydrates in the colon is to the mutual advantage of the host in terms of nutrient absorption ("colonic salvage") and metabolism of colonic epithelial cells[85]. The presence in the intestine of carbohydrates is again being implicated as a cause of bacterial proliferation.

The Coello-Ramirez Hypothesis

Coello-Ramirez et al in 1972 looked at the duodenal flora of 50 Mexican infants with infectious diarrhoea[76]. Nine infants suffering from infectious illnesses but without diarrhoea were used as controls. Of the infants with diarrhoea 16 could tolerate all dietary carbohydrates, 23 were intolerant of lactose, 8 were combined lactose and sucrose intolerant, and 3 were monosaccharide intolerant. All the patients were studied at the time of the acute illness and at the time of carbohydrate intolerance, if present.

The duodenal flora of the diarrhoeal patients who could tolerate carbohydrate was not significantly different from that of the control patients. With increasing degrees of carbohydrate intolerance - lactose, sucrose and lactose, monosaccharide - there was a corresponding stepwise increase in duodenal bacterial numbers, mainly of

Enterobacteriaceae. All infants with monosaccharide intolerance had total bacterial counts of 7 $\log_{10}$ organisms/ml or greater. The difference between the groups was highly significant. There was no correlation between the duration of diarrhoea before intubation and the bacterial count, nor between the degree of malnutrition and the duodenal flora. The only relationship was between the severity of carbohydrate intolerance and the luxuriance of the duodenal flora: the greater the degree of intolerance the greater the bacterial numbers.

This publication can be faulted in several ways. There is almost no information on the dietary manipulations carried out, and in particular how combined lactose and sucrose intolerance was diagnosed. This is a major omission for a study in which subdivisions of carbohydrate intolerance form the central theme. Only aerobic culture techniques were used. It is conceivable that the anaerobic portion of the flora may not have mirrored the aerobic component. These criticisms apart, the study was well conducted and shows clear cut results.

In his discussion Coello-Ramirez speculates on the significance of his findings. He states that the proliferation of bacteria in the small intestine may cause carbohydrate intolerance by disrupting the normal absorptive mechanisms. He also puts forward an alternative hypothesis that the malabsorption of carbohydrate may lead to bacterial multiplication and massive overgrowth. This then exacerbates the diarrhoea. A vicious circle is created of diarrhoea, malabsorption, and bacterial overgrowth. An accompanying diagram clearly shows the proposed relationship between these factors.

This hypothesis of carbohydrate intolerance leading to bacterial multiplication has subsequently been quoted as one of the causes of bacterial overgrowth in infantile diarrhoeal illness. In Lebenthal's book it is shown in diagrammatic form[210]. It is also stated in Brown's review of the nutritional aspects of diarrhoea[57].

No further studies have been done that specifically address the ideas put forward by Coello-Ramirez. One of the aims of the present thesis is to test the hypothesis that it is the presence of malabsorbed carbohydrate that leads to bacterial proliferation in the small intestine in infants with diarrhoea. Since one cannot actively induce carbohydrate intolerance in infants the problem has been approached in a different way. If the presence of malabsorbed carbohydrates is important in promoting bacterial growth, one would expect to see a difference in the duodenal flora between a group of diarrhoeal infants that is fed carbohydrate, and one in whom dietary carbohydrate is withdrawn, all other factors being kept constant. In other words:

"Does withdrawal of carbohydrate from the diet lead to a change in the duodenal flora, when compared with a group that is fed carbohydrate?".

Other paediatric studies

Burke and Anderson in 1966 reported their findings in a group of 18 neonates with diarrhoea and carbohydrate intolerance following intestinal operations for correction of congenital anomalies[62]. The neonates with large bowel pathology (Hirschprung's disease or imperforate anus) invariably suffered from lactose intolerance only, whereas some of those who had undergone procedures on the small intestine also had sucrose or monosaccharide intolerance. In 10 patients aerobic bacteriology of the duodenal fluid was done. The cultures were sterile in the 4 neonates with large bowel pathology. In contrast the 6 patients with small intestinal problems showed a heavy growth of microorganisms, mainly *Enterobacteriaceae*, in their duodenal fluid. Antimicrobial agents were given, but it was only after several dietary manipulations that the diarrhoea resolved. No attempt was made to correlate the severity of carbohydrate intolerance with the degree of bacterial contamination, and the cultures were semiquantitative only. The authors' aim went no further than to describe the association of carbohydrate intolerance with a contaminated small bowel. They recommended the use of antibiotics, but with the main purpose of preventing cholangitis and septicaemia rather than to diminish the sugar intolerance. The

same authors were involved in a later short report describing 3 non-surgical infants with persistent diarrhoea and carbohydrate intolerance[139]. All patients had duodenal fluid heavily contaminated with *Enterobacteriaceae*. This small study gives some support to the work of Coello-Ramirez as the bacterial count parallelled the degree of carbohydrate intolerance. Anaerobic methods were not used but deconjugated bile acids were measured. These were present in all patients, suggesting that anaerobic bile-splitting microorganisms were present, since *Enterobacteriaceae* cannot split bile acids. The authors believe that establishment of bacterial overgrowth precedes the carbohydrate intolerance. As a supporting argument they state that the presence of excess monosaccharides in the lumen, as is found in monosaccharide intolerance, should not lead to greater bacterial proliferation than if disaccharides are present (as in lactose or sucrose intolerance), as monosaccharides or disaccharides can be utilised with equal ease by the bacteria that they isolated in the duodenal juice. In response to this it can be said that the inability to absorb monosaccharides is usually associated with severe damage to the absorptive surface of the gut. This would lead to greater malabsorption of other nutrients. These would then be available for the bacteria to metabolise.

Lifshitz examined the aerobic bacteria in the duodenal fluid of 20 patients with monosaccharide intolerance[214]. These were malnourished infants with persistent diarrhoea. 18 showed a heavy bacterial growth, in most cases of *Klebsiella* or *E.coli*. Antibiotic treatment sterilised the duodenal fluid of 13 patients, 12 of whom showed renewed tolerance of dietary monosaccharides. Two patients who bacteriologically relapsed showed a concomitant clinical relapse of glucose intolerance and diarrhoea. Antibiotic treatment again normalised both parameters. The bacteriology was very scantily reported and no quantitative results were given. This study implicated the bacteria as causing monosaccharide intolerance.

Barnes et al's investigation of infants with acute diarrhoea included duodenal biopsies[18]. The duodenal fluid bacteriology of those infants with diminished mucosal disaccharidase

levels was done. A significant association was found between lactase depression and the numbers of *Candida albicans*. No such relationship could be found for *E.coli*. The authors believed that the yeast caused lactase depression rather than *vice versa* as lactose cannot be used by Candida for its metabolic needs. No mention is made of the clinical course of the patients, or if they were clinically intolerant of lactose. The level of mucosal disaccharidase has since been shown to bear little correlation with the patient's ability to tolerate disaccharides[156]. In the absence of relevant clinical data this publication must be viewed with circumspection.

Not all studies show a close correlation between carbohydrate intolerance and bacterial overgrowth of the small intestine. Challacombe's publication on the duodenal bacteriology of infants with persistent diarrhoea included 3 patients with carbohydrate intolerance[72]. Two had monosaccharide intolerance; their total bacterial counts were respectively 7 $\log_{10}$ organisms/ml and less than 3 $\log_{10}$ organisms/ml. The other patient had lactose intolerance and his total bacterial count was 7 $\log_{10}$ organisms/ml. Unfortunately the number of patients is too small for any meaningful interpretation.

Kilby et al studied ten infants with acute or persistent diarrhoea and monosaccharide intolerance[198]. Seven had duodenal bacteriology at the time of maximal symptoms. In only 2 was the total bacterial count greater than 4 $\log_{10}$ organisms/ml. In 3 the cultures were completely sterile. They conclude that bacterial proliferation is unlikely to be a cause of monosaccharide intolerance. It is possible, however, to reconcile these findings with the apparently contradictory ones of Coello-Ramirez. Rotavirus gastroenteritis has been shown to be strongly associated with monosaccharide intolerance[221]. Kilby's patients were from an area where rotavirus infection is common. In only 2 of the 7 infants was rotavirus actually sought and was present in both cases. In contrast, Coello-Ramirez' patients were more typical of a group with diarrhoeal disease in a developing country. They were malnourished, had persistent diarrhoea, and their small intestinal mucosa had probably suffered from repeated infectious insults. Monosaccharide intolerance in these

circumstances is the expression of a severely damaged gut. The intestinal epithelium has a limited repertoire of response to injury and almost certainly different mechanisms are at work in the two groups of patients.

In summary it can be said that there appears to be an association between carbohydrate intolerance and intestinal bacterial overgrowth, but it is not invariably found. When this association is present it is unclear whether it is merely an association or if there is a causal link between the two factors. The interpretation of all the studies, as is the case with most publications on carbohydrate intolerance, is bedevilled by the question of definition. True carbohydrate intolerance is diarrhoea caused by a specific sugar or sugars, which resolves on withdrawing the offending substance. This is often confused with the maldigestion of carbohydrates which may often accompany diarrhoea, in which removal of dietary sugars does not terminate the diarrhoea. There is insufficient information given to adequately distinguish the two entities.

EXPERIMENTAL AND ANIMAL WORK

There is considerable experimental work, mainly done in animals, to suggest that bacteria in the small bowel can lead to malabsorption of carbohydrates, either directly or indirectly.

Monosaccharide malabsorption

Deconjugated bile acids have been often blamed for the monosaccharide malabsorption associated with bacterial overgrowth. In 1971 Gracey et al did a series of experiments using rats with surgically constructed blind loops and control animals[142]. They examined the blind loops themselves, and the adjacent afferent and efferent jejunum. Aerobic and anaerobic bacteriology of all the areas was done. Conjugated and deconjugated bile acids were measured by thin layer chromatography. *In vivo* perfusion experiments were done on the areas adjacent to the blind loops, but for technical reasons the blind loop itself was not

tested. The monosaccharides used were arbutin, a non-metabolisable analogue of glucose, sharing the same active transport pathway, and D-fructose, which has a different transport mechanism. It was found that the rate of transport of arbutin across the mucosa was diminished in rats with blind loops compared with the controls, and that this was particularly marked in the efferent jejunum. A similar effect was seen with D-fructose but there were too few observations for statistical analysis. *In vitro* experiments were also done in which the uptake of the same sugars was measured on everted pieces of gut tissue of the experimental animals. Similar results were obtained, with the added observation that the transport of sugars across the gut mucosa was most impaired in the region of the blind loop itself. In both the *in vivo* and *in vitro* experiments the severity of derangement in sugar transport found in a particular segment of jejunum closely parallelled the total bacterial count, in particular the *Bacteroides* fraction, and the concentration of deconjugated bile acids. The authors speculated that the presence of large numbers of anaerobic bacteria in blind loops leads to the deconjugation of bile salts. These deconjugated bile salts are then responsible for the decrease in monosaccharide absorption.

The same group of investigators set out to test this hypothesis in further studies. They examined the absorption of arbutin *in vitro* after incubation in an everted segment of rat small intestine[141]. The absorption was measured with the incubation of arbutin alone, after addition of a conjugated bile salts (sodium taurocholate), and after the addition of a deconjugated bile salt (sodium deoxycholate). In the presence of the deconjugated bile salt there was a marked decrease in arbutin absorption, whereas the conjugated bile salt had no effect on arbutin absorption. The action of deoxycholate was reversible, since after washing the gut segment the arbutin absorption returned to normal. This reversibility implied that the effect of deconjugated bile salts was not due to non-specific damage to the gut mucosa, as had previously been speculated[95], but in some way specifically interfered with the normal active transport of monosaccharides. The *in vitro* inhibition of monosaccharide transport by deoxycholate was confirmed in live animals using perfusion techniques[140]. Harries and Sladen did similar perfusion experiments on rats, but using a

wider range of bile salts[155]. They confirmed the findings of Gracey's team and made the added observation that not all deconjugated bile salts share the property of inhibition of monosaccharide transport.

It is likely that other factors besides deconjugated bile salts are implicated in the monosaccharide malabsorption. In 1975 Gracey et al tested the effect of bacteria independently from bile salts on intestinal monosaccharide absorption[143]. They used aerobic microorganisms that had been isolated in the duodenal juice of Indonesian infants and children with diarrhoea and duodenal bacterial overgrowth (total count > 4 $\log_{10}$ organisms/ml). These bacteria and yeasts were grown in pure cultures. Broth cultures were prepared and the supernatant was used in the experiments. For the perfusion studies jejunal segments with entry and exit cannulae were prepared in rats *in vivo*. These segments were not in contact with the normal bile flow. Arbutin and supernatant broth from the pure cultures of bacteria were then instilled in the jejunal segments. It was found that nearly all the bacterial and yeast supernatants caused a diminished absorption of arbutin. This effect was seen with even seemingly innocuous bacteria such as lactobacilli.

Disaccharide malabsorption

Giannella et al found that rats with self-filling blind loops had diminished levels of all mucosal disaccharidases[123]. The decrease was greatest in the blind loop itself, the area of greatest bacterial proliferation, and smaller in the less contaminated adjacent areas. Treatment with antibiotics restored the maltase and sucrase levels to normal within 6 days, but that of lactase lagged somewhat. In a series of experiments Jonas and her associates found that experimentally induced overgrowth in rats causes a decrease of disaccharidase levels[188]. Lactase was the most severely affected and maltase the least. Using radioactive tracers and by measuring other enzymes specific for the brush border they established that the damage was specific to disaccharidases themselves, and was not a result of generalised injury to the brush border. Sherman et al confirmed the reversibility of disaccharidase

depression after antibiotic treatment[282]. In addition they noted the additive effect of malnutrition in depressing disaccharidase levels in rats with bacterial overgrowth.

Jonas et al attempted to discover which were the species important in causing the decrease in disaccharidases[189]. They used an *in vitro* preparation of rat microvillus membrane vesicles, and the ability of various bacteria to leach maltase from the membrane was tested. The bacteria used in the experiments were all isolated from rats with self-filling blind loops. These microorganisms were grown in pure cultures. A wide range of species and strains of aerobic and anaerobic bacteria were used. They were applied on the microvillus preparation either as a broth preparation of intact bacteria or after disruption of their cell by sonication and centrifugation, effectively producing a suspension of bacterial enzymes. It was found that only 3 bacterial species - *Bacteroides fragilis*, *Clostridium perfringens*, and *Streptococcus faecalis* - were able to leach maltase from the microvillus membrane; moreover this was the case with only some strains of the above species. The maltase-releasing effect was much more marked with the disrupted bacterial preparations, suggesting that intracellular bacterial enzymes were responsible. The 3 bacterial species, particularly *Bacteroides*, have been implicated in causing symptoms in the blind loop syndrome. Although these experiments were thorough and elegant they differ from the real-life setting in at least four major ways. A rat microvillus membrane on a bench is very different to intact living human small intestinal mucosa. Secondly, the most clear cut results were seen with disrupted bacterial cells. Thirdly the bacteria were in pure culture form. Lastly, the microorganisms used in the experiments were taken from the blind loops of rats, and not from humans.

Candida is another microorganism that has been implicated in disaccharidase depression. Bishop and Barnes' study of diarrhoeic infants associated the presence of this yeast with a decrease of disaccharidase activity in the duodenal mucosa[18] and led them to further investigate this phenomenon[43]. They used *Candida* which had been isolated in the duodenum of infants with diarrhoea. A large inoculum was injected in the lumen of ligated

small intestinal loops of rabbits *in vivo*. Different loops in the same animals were used as their own controls. It was found that the mucosa in the test loops had a slightly (but statistically significant) lower lactase activity than the control loops. There was no histological evidence of mucosal invasion by the yeast. The authors concluded that *Candida* does produce disaccharidase depression and by a luminal effect rather than as a result of mucosal invasion. Burke and Gracey's experiments on gastrointestinal candidiasis did not confirm these findings[63]. The two studies are however difficult to compare. Burke and Gracey used rats rather than rabbits. The yeast was given intragastrically rather than injected in the bowel lumen. The numbers of *Candida* found in the lumen in Burke's study much more closely approximate the populations found in diarrhoeal infants whereas in Bishop and Barnes' experiments they far exceeded these. Although Burke and Gracey did not find diminished disaccharidase levels they did note by the use of perfusion methods that the *Candida*-infected rats showed a significant decrease in arbutin absorption.

A novel approach to investigating the effects of bacteria on disaccharidases was devised by Bampoe et al[16]. The substrate for study was a solution of lactase of yeast origin. In this solution were incubated pure cultures of bacteria or fungi which had been isolated from the duodenal lumen or mucosa of children with diarrhoea. These microorganisms were mainly staphylococci or α haemolytic streptococci. Almost all the bacterial and fungal suspensions produced a decrease in lactase activity, as did their cell-free supernatants.

The relevance of this study to the clinical setting is very uncertain. The lactase used was in solution whereas in humans it is bound to the intestinal brush border membrane. Lactase of yeast origin was used, and it is not known whether human lactase behaves in the same way.

There is also some evidence that deconjugated bile salts can cause disaccharidase depression. Gracey et al found that feeding deoxycholate to rats for 4 days significantly decreased small intestinal mucosal disaccharidases[145]. The quantity of deoxycholate given

if extrapolated to a human setting would be equivalent to about 20 grams per day. This is a level far higher than would ever be found in humans.

Summary

There is considerable experimental evidence that the action of bacteria on the small intestine can produce profound disruption in the absorption of monosaccharides and can lead to a decrease in the brush border disaccharidases. However, all the studies that have shown these effects have been conducted in a highly rarefied setting. They have used pure bacterial cultures, often in supraphysiological doses. The effects of these bacteria have been tested in rodents, not humans. Many of the experiments have been done using *in vitro* tissue preparations.

On the basis of these studies it is very difficult to surmise whether the presence of bacteria in the small intestine of infants with diarrhoea can cause carbohydrate malabsorption.

BACTERIAL METABOLISM OF CARBOHYDRATES

GENERAL CONSIDERATIONS

Major pathways

The growth of microorganisms as with every living organism, is dependent on a supply of nutrients. The availability of this nutrient supply is the major factor limiting the growth of bacteria. It has been calculated that if allowed to grow unchecked a single organism of *E.coli* with a doubling time of 20 minutes, within three days would produce a weight of bacteria amounting to 1000 times the earth's mass[303].

Carbon is an essential element for all living organisms. All need it for incorporation into cell components (anabolism). In the majority it is also required for a variety of metabolic pathways, releasing energy which is needed for continued existence (catabolism). With the exception of photosynthetic organisms, which utilise the carbon in carbon dioxide, all obtain this element from the carbon skeletons of inorganic or organic compounds[278]. In the case of bacteria inhabiting the gastrointestinal tract the carbon is derived entirely from organic compounds. The majority of these are carbohydrates. These may be complex, such as cellulose or polysaccharides, or can be in the form of more simple sugars. Regardless of their initial structure, in nearly all cases they are broken down or converted to glucose which is the starting point for most metabolic pathways[232]. The conditions prevailing in the small intestine are anaerobic or nearly so[29] and the metabolic pathways of the bacteria inhabiting this region must correspondingly be of the anaerobic type. The most common energy producing pathway is the Emden-Meyerhof cycle[232]. The main end products are short chain fatty acids with the particular fatty acid generated being dependent on the particular bacterial species. Other less common pathways exist, sometimes unique to one particular bacterial species[255,274].

The breakdown of carbohydrates to monosaccharides in the small intestine can often be achieved by the host. Humans possess the enzymatic *armamentarium* needed to break down starch and disaccharides into monosaccharides[148]. These can then be utilised by the bacteria. Non-digestible complex carbohydrates such as cellulose and pectin can be used only by the bacteria, many of which possess the enzymes that break down these compounds. In ruminant herbivores the stomach, also called rumen, is the major site of this process. The bacteria break down cellulose, utilise the carbon skeletons and produce short chain fatty acids which serve as a major source of nutrition for the animal host[329]. In healthy humans the overwhelming mass of the intestinal flora inhabits the colon. This can be viewed as a human rumen. The non-digestible vegetable polysaccharides such as dietary fibre, and the small quantity of more simple carbohydrates which have escaped digestion, enter the colon. The bacteria break down and utilise these carbohydrates exactly

as in the rumen. The short chain fatty acids are the main source of energy of the colonic epithelial cells in humans[261]. There is also growing evidence that they provide a small, but not insignificant contribution to the human's total calorific needs[226]. The bacterial population of the colon and its human host can be thought to exist therefore in a state of mutual benefit.

This brief overview of intestinal bacterial metabolism has purposefully been an oversimplification of the true state of affairs. Bacteria, phylogenetically the oldest living organisms on Earth, are metabolically very flexible. They are able to repress or induce a wide variety of metabolic enzymes in accordance with the nutritional milieu in which they live, be it "famine or feast"[154]. This enables them to survive or even thrive in sometimes quite hostile conditions.

Bacterial interdependence

The study of bacterial metabolism in the gut is made more complicated by the phenomenon of bacterial interdependence. The normal human possesses as many as 400 different bacterial species in the large bowel[234]. Whilst there is considerable variation between individuals, the bacterial flora remains remarkably constant in the same subject over long periods of time[133]. The myriad of bacterial species live in a state of balance, each occupying its own ecological niche. A state of microbial harmony exists in which the different bacteria live in a state of interdependence. One particular species not possessing the necessary enzymes may utilise substrates broken down by another bacterial species. The end products of bacterial metabolic pathways may also be used by other bacteria, as is the case with methanogenic bacteria, which use hydrogen as the starting point for their own metabolic needs[330].

CARBOHYDRATE SOURCES TO COLONIC BACTERIA

As has been already mentioned carbohydrates are a source of carbon skeletons for the bacteria inhabiting the intestine. These carbohydrates are either of dietary origin (exogenous) or are produced by the human host (endogenous). As may be expected from the functions of the gastrointestinal tract, the relative quantities of these carbohydrates will vary according to the part of the intestine examined. The work done has been almost exclusively on the colonic microflora and its utilisation of carbohydrate. This will be reviewed first.

Exogenous

DIGESTIBLE

Between 2 and 20% of potentially digestible carbohydrate escapes digestion in the small bowel and enters the colon[292]. The evidence for this is strong. Small intestinal malabsorption of carbohydrate has been shown using different experimental approaches. It has been demonstrated by the use of non-invasive breath tests, which detect the end product of bacterial metabolism[6]. Starch malabsorption has also been shown directly by its measurement in ileostomists[106] and by ileal intubation in human volunteers[292]. It has been shown that small amounts of sucrose escape digestion by the small intestinal enzymes[47]. Larger quantities of more complex digestible starch, such as is found in bananas and potatoes, are also malabsorbed by the small intestine[106,107]. Simple carbohydrates malabsorbed in the human small intestine are utilised by the colonic bacteria for their own metabolic needs[48]. This is doubly advantageous to the human host: not only can the products of bacterial metabolism provide a source of energy, but diarrhoea, which would otherwise result from the presence of osmotically active carbohydrates in the colon, is prevented[47,211]. In the last decade our perception of the colon has changed. Originally it was viewed as no more than a holding area for faeces. Now, the concept of "colonic salvage" is widely accepted. This is the process whereby potential nutrients that would

otherwise be lost to that host can be utilised as a result of the action of bacteria resident in the colon.

NON-DIGESTIBLE

Many different complex polysaccharides such as cellulose, hemicellulose, and pectin form this group of carbohydrates. They are broadly termed dietary fibre. The human small intestine does not possess the enzymes necessary to digest these compounds. Consequently ingested dietary fibre passes through the small bowel unchanged and enters the colon. This provides a ready source of nutrients for the bacterial population. There is ample evidence both *in vitro*[275] and in humans[318] that the colonic microflora can utilise this source of carbohydrate. Not all the dietary fibre is used by microorganisms. Residual fibre constitutes about 17% of the dry weight of faeces in subjects consuming a Western diet[291].

Endogenous

This comes from at least two potential sources, mucin and sloughed intestinal epithelial cells.

MUCIN

Mucus is produced by the goblet cells lining the small and large intestine. In addition the intestine contains some mucus that has been produced in the respiratory tract and salivary glands and subsequently been swallowed. Mucus consists of many different substances, loosely named mucin. They are all glycoproteins of large molecular weight. There is a peptide core to which are attached oligosaccharide chains[118]. Small intestinal and pancreatic enzymes are able to degrade mucin but some is also broken down by bacteria in the colon. It has been known for many years that germ-free rats excrete more mucus than their conventional counterparts[176]. Subsequently some bacteria inhabiting the human colon have been shown to have the ability to degrade mucin[175]. They form a distinct subgroup, making up about 1% of the total bacterial population[231]. They produce glycosidases which

split the oligosaccharide chains of the glycoproteins. The glycosidases are extracellular[175] enabling other bacteria not possessing the necessary enzymes to utilise the carbohydrate chains which have been cleaved.

INTESTINAL EPITHELIAL CELLS

It is estimated that about 290g of epithelial cells are shed daily from the intestine, 90% of this from the small bowel[83]. Their carbohydrate moiety consists of glycoproteins, which as has previously been stated are potentially degradable by the bacteria. It is reasonable to assume that epithelial cells which are not digested in the small intestine, or those sloughed in the large intestine may provide a source of nutrition to the bacterial population of the colon. This hypothesis is quoted in several publications[174,275,318], but to date no studies have been done to address this issue.

CARBOHYDRATES AND SMALL INTESTINAL BACTERIA

The resident microflora of the small bowel is very scanty and carbohydrate is readily available. The bacteria are in intimate contact with simple sugars, some of dietary origin, and others the by-product of digestion by brush border and luminal enzymes[148]. These sugars could serve as nutrients to the bacteria. Despite the large quantity of nutrients available the microflora is kept at very low levels by the normal defence and clearing mechanisms. With the greater bacterial numbers present in a contaminated small bowel one might expect that the availability of nutrients might be an important factor limiting the bacterial population. This may not be a correct assumption. The quantity of bacteria inhabiting the colon is much higher than that found in even the most heavily contaminated small intestine. This enormous bacterial population is maintained despite a much lower nutrient availability. The available evidence suggests that the bacteria present in small intestinal overgrowth behave in a similar manner to those that inhabit the colon.

Studies addressing the nutritional issue in small intestinal bacterial overgrowth are few, and almost entirely confined to animals. Goldstein[125] studied xylose absorption in humans with bacterial overgrowth syndrome. He used conventional xylose absorption tests, but also did perfusion studies and *in vitro* incubation experiments using various bacteria. He found that bacteria in the small intestine consumed large quantities of xylose, in the order of grams, and attributed this property to the *Enterobacteriaceae*. The study can be criticised on the grounds that the xylose malabsorption could be blamed on mucosal damage resulting from bacterial overgrowth. This argument cannot be levelled against Toskes' study in rats with experimental blind loops[312]. Using xylose breath tests he proved that the intestinal bacteria metabolised the xylose, radiolabelled xylose being converted to carbon dioxide.

There is evidence that bacteria in small intestinal overgrowth also utilise endogenous nutrients. Chernov et al in 1972 found increased quantities of short chain fatty acids in the jejunum in patients with contaminated small bowels[73]. In a series of careful rat experiments Prizont et al confirmed this[257]. They also found that the levels of these acids were unchanged, irrespective of whether the rats were starving or fed, suggesting that endogenous nutrients were being used. Prizont in a later study showed that bacteria present in experimental blind loops of rats possess glycosidases, enabling them to break down and utilise the sugars of glycoproteins[256].

DIET AND THE INTESTINAL FLORA

The studies of the effects of diet and nutrients on the intestinal flora have dealt almost entirely on the flora of the mouth and teeth, and that of the colon. Because the flora in small intestinal bacterial overgrowth much more closely resembles that of the colon than the dental flora, the literature survey deals exclusively with studies connected with the faecal (colonic) and small intestinal flora.

THE FAECAL FLORA

Problems associated with the study of the colonic microflora

All the studies in humans looking at the effects of diet on the colonic microflora have invariably looked at the faecal bacteriology. This is because of the obvious difficulties in obtaining colonic contents, particularly in healthy volunteers. It has been shown that the stool microflora is very similar to that of the lumen of the distal colon, but does not resemble closely that of the ascending colon and caecum[234]. No inferences can be made however on the mucosal bacterial flora.

Few subjects in microbiology are as complex as the study of the faecal flora. Numerous bacterial species must be identified and quantitated. This is enormously labour-intensive. In Holdeman's laboratory (the "gold standard") one faecal specimen alone provided full-time work for a technologist for one week. Five specimens generated 22,000 analytical tests[234]. Inevitably, short cuts must be taken and these may affect the validity of the results.

Attempts have been made to mimic the colonic bacterial environment *in vitro* by the use of continuous flow (CF) cultures. In this method an *inoculum* of faeces or colonic contents is injected into fermentation vats, into which at regular intervals a supply of nutrients is added and some effluent is removed. After some days a steady-state bacterial population is reached. Continuous flow cultures have been shown to approximate, albeit crudely, the colonic bacterial environment in rats[119] and humans[103]. Using CF cultures a tentative start has been made in our understanding of the complex interrelationships of the bacterial species of the colon, and of the effects of nutrients on their growth[327]. This *in vitro* model also makes possible dietary manipulations which would otherwise be very difficult in humans, for ethical or practical reasons.

Diet and the faecal flora in infancy

It has long been known that there is a striking difference in the faecal flora between babies who are entirely breast fed and those who receive formula milk. In the early years of this century Tissier[308] and Moro[235] remarked on the preponderance of bifidobacteria in the stools of breast fed babies. This they contrasted with the very mixed flora of their bottle-fed counterparts.

Several modern studies, with few exceptions[216,264], have confirmed and refined these early observations[15,289,331]. Their findings are all very similar. They show that there are striking differences between breast and bottle-fed babies, and these differences are maintained even when modern "humanised" formulae are given[15]. At birth the neonate's stools are sterile. In the first week of life they contain predominantly *E.coli* and enterococci. After this the stool bacteriology diverges. In entirely breast-fed infants the flora consists overwhelmingly of bifidobacteria. These overshadow the *Enterobacteriaceae* and enterococci by about 5 log units. This is in marked contrast to the pattern in formula-fed infants. They also have bifidobacteria in the faeces, but the predominant bacterial types are the *Enterobacteriaceae*. *Bacteroides* spp. are sometimes recovered which are not a feature of the breast-fed neonate. The bacterial pattern of the stool flora in breast-fed babies changes at the time of introduction of solids. The flora of the breast-fed infant then assumes all the features of the formula fed coetaneous baby. No further change is noted in babies already receiving infant formula. Later in infancy *Bacteroides* spp. increase in number and by one year of age the faecal flora becomes very similar to that of the adult. What has been shown convincingly from Stark and Lee's very careful investigation is that the change in pattern is determined by diet and not by age. All studies so far have involved small numbers of infants. No one has looked at the effect of changing from bottle to breast milk nor has the changeover from breast to formula feeds been methodically studied.

The cause for this marked difference in stool microflora between breast-fed and formula-fed infants has led to a search for the "*Bifidus* factor": a substance or a property inherent to breast milk that would promote the growth of bifidobacteria. This "*Bifidus* factor" has proved elusive.

Initially attention was focused on carbohydrate present in breast milk that might act as a bacterial substrate. György in 1953[153] found that *in vitro* N-acetylglucosamine from human milk stimulated the growth of one variety of *Bifidobacterium*. This variety has since been shown not to be an important part of the normal bifidobacterial population. Braun[55] quotes another theory on the substrate theme. He states that "mutarotation" - the process by which ß lactose is converted to α lactose - occurs more slowly in human milk. Because ß lactose is digested less efficiently more reaches the colon. Fermentation of this substance by bacteria would then render the environment more acidic encouraging the growth of bifidobacteria. The higher lactose content of human milk would also enhance this effect. More recently French workers claim to have identified a group of oligosaccharides in human milk that stimulate the growth of bifidobacteria (quoted in[320]).

An interesting hypothesis by Bullen centres on the lack of buffering capacity of human milk[61]. Bifidobacteria require an acidic environment to thrive. He speculated that the *E.coli* and enterococci that are found in the first two days of life provide this environment by the production of short chain fatty acids. The acidic anaerobic conditions would then "kick start" the bifidobacteria. This would only occur in breast fed babies because formula milk has a high buffering capacity. Bullen provides support for this theory by consistently showing the presence of acetate buffer in the stools of breast-fed infants as opposed to those fed formula milk. He also showed that the stool pH was always acidic in breast fed babies and when supplementary feeds were started the pH rose. Despite its elegance this hypothesis does not explain how bifidobacteria can survive in large if lesser numbers in the large bowel of bottle-fed infants (about one log unit less than in breast-fed babies).

A recent study from Birmingham compared the faecal flora of neonates fed human milk with those fed a casein based formula or a whey-based formula[14]. They found that stool flora of infants fed the whey formula more closely approximated that of breast fed infants than did the casein group. Bifidobacteria were present in substantial numbers in the whey-formula group but other differences remained. They ascribed the similarity between the whey and breast milk group to the fact that breast milk is whey-predominant. They attempted to make the whey formula even more closely resemble breast milk by the addition of lactoferrin (albeit of the bovine variety). This did not produce any change in the stool flora.

Despite the plethora of hypotheses no one has convincingly explained the cause or causes of the difference in faecal flora. The various theories try to show why the growth of bifidobacteria is encouraged in breast-fed babies but they make no attempt to explain why those bacteria so markedly overshadow the other components of the stool flora. It is also surprising that no studies have been done to explain why the introduction of solids so dramatically changes the stool bacteriology despite the continuing ingestion of breast milk.

What is clear is that a dietary factor, perhaps a small one, can make a profound difference to the faecal flora of neonates and small infants.

Diet and intestinal flora in adult life

A review of the literature dealing with the effects of diet on the adult's intestinal flora is fraught with difficulties. Most studies have been prompted by the proposed link between diet and cancer of the large bowel. They are primarily concerned with the effects of dietary patterns such as vegetarian or high-fibre diets, rather than specific constituents. They are almost all concerned with the faecal flora.

In dealing with the subject of the effect of carbohydrates on the intestinal flora it is almost always necessary to extrapolate the findings from investigations that were not primarily designed to investigate this topic.

General diets

In Japan there is a low incidence of cancer of the colon; "Westernised" Japanese living in America and consuming a Western diet have the same incidence of large bowel cancer as other Americans. Finegold et al compared the stool flora of Japanese subjects on a Western diet with those on a traditional Japanese diet (rich in rice, noodles, and raw and dried fish)[115]. Both groups lived in the U.S. Some differences were found but these were minor, especially when one considers the large number of bacterial species isolated. Moreover the subjects on the traditional diet were older and the faecal microflora has been shown to alter with age[133]. Finegold et al also studied the effect of a meat diet on Seventh Day Adventists[117]. They compared strictly vegetarian volunteers with those on a normal meat-containing diet. Significant numerical differences in the flora were only found for 5 bacterial species. An investigation of different design looked at the effect of a high or low fat diet on student volunteers[86]. They were put on these diets successively for 4 weeks each with no demonstrable effect on the faecal flora.

Effects of carbohydrates

Decreasing carbohydrate intake.

Elemental diets have been available for the almost two decades. Initially they were used for astronauts to provide a compact source of nutrients and to minimise the production of solid excreta in cramped spaceships. They have since been used extensively in the treatment of gastrointestinal disorders. They contain all the necessary amino acids and fats. The carbohydrate is in the form of mono or disaccharides. There is no complex starch or fibre. The administration of an elemental diet can be viewed as removal of fibre intake. Consequently less carbohydrate substrate would be available for the colonic

bacteria to metabolise. It must be borne in mind that the protein and fat fractions also differ from those of a normal diet.

Winitz et al in 1970 did a series of studies examining the faecal flora of human volunteers after feeding them an elemental diet[328]. They found that after 6 days of a glucose-based elemental diet a decrease in the faecal microbial count was evident. This effect was seen by 4 days if the bowel had been emptied by enemas before commencing the diet.

Within 13 days the faecal flora was confined to three bacterial types: *Bacteroides*, coliforms, and enterococci. There was a drastic decrease in bacterial concentration to about 3 $\log_{10}$ organisms/gram of wet faeces.

In a separate experiment the effect of altering the type of carbohydrate in the feed was investigated. Eight subjects received a glucose-based elemental diet and 8 consumed a sucrose-based one. The glucose-based feed was associated with a far greater and more uniform decrease in bacterial types and numbers than was the sucrose-based diet. After the sucrose diet *Bacteroides* tended to remain in high concentration whereas the enterococci numbers decreased.

In the last experiment a single volunteer was studied in detail for 43 days. After 16 days on a glucose-based elemental diet the faeces were sterile. Ingestion of one glass of beer resulted in a dramatic rise in the stool microflora within 24 hours. After the volunteer was fed an elemental diet containing glucose and fructose the faeces contained large numbers of bacteria, with a preponderance of coliforms. Within 24 hours of resuming a glucose-only based diet the coliform count had dropped from 6 to 2 $\log_{10}$ organisms/gram of wet faeces.

Winitz's study shows that an elemental diet decreases the bacterial concentration in the stool. The bacterial concentration and type can vary greatly, according to the type of

carbohydrate used. The change in bacterial numbers can occur rapidly - within 24 hours - and markedly, by 4 log units, in response to changing the feed.

The findings of studies subsequent to those of Winitz have been much less dramatic. Attebery et al fed a glucose-based elemental diet to three volunteers[11]. The only significant change in the stool flora was the complete disappearance of extremely oxygen-labile bacteria. No other investigators have used methods able to detect those bacteria so these findings have not been confirmed.

Crowther et al in their study examined the effect of an elemental diet of unspecified carbohydrate content on the faecal flora of three volunteers[84]. Bounous et al used a sucrose-based diet on 14 volunteers[50]. In both investigations the only significant bacteriological finding was of a reduced *Enterococcus* count.

Two studies, those of Bornside et al[49] and Axelsson et al[13] were unable to detect any change in the stool flora after the feeding of elemental diets. Bornside used a dextrin-containing preparation and fed it to ten volunteers. It was not a true elemental diet since it contained no protein. Axelsson was the only investigator not to use healthy volunteers. All his patients were suffering from gastrointestinal disorders.

An almost invariable finding in all the studies was a great diminution in total stool weight and a prolonged transit time. The one exception was Axelsson's study. All his patients had gastrointestinal illnesses and their daily stool volumes were vastly in excess of normal values. The consumption of coffee was allowed and this may also have contributed to the increased faecal output.

The greatly diminished stool volume is important from the bacteriological viewpoint. Bacteria comprise about 75% of the wet stool weight. The drastic reduction in stool weight associated with elemental diets means that the total number of bacteria excreted is

diminished. One possible explanation is that less nutrients enter the colon. Increased transit time has been associated with a diminished faecal microbial output[293]. This is another possible mechanism by which elemental diets reduce the quantity of bacteria excreted.

In interpreting the results of administration of elemental diets on the stool flora it is important to bear in mind the implication of a greatly reduced stool volume. Although the concentration of bacteria per gram of faeces may not be decreased, the total number of bacteria multiplying in the colonic lumen may be greatly diminished.

Increasing carbohydrate intake.

There is evidence to suggest that changing the carbohydrate content of the diet produces a change in the faecal microflora. Drasar et al fed a diet rich in fibre but unchanged in other constituents to 4 human volunteers[100]. They could not find a change in the qualitative faecal microflora. They observed a three-fold increase in daily stool weight and a diminished transit time. The greater stool weight could not be accounted for by the extra fibre content or water-binding capacity of the fibre alone. It could only be explained by a greater bacterial mass. It is not possible to discuss the relative contributions of substrate availability and diminished transit time. In contrast the study of Fuchs et al in which the dietary fibre was increased did show qualitative changes[120]. There was a significant increase in the ratio of anaerobes to aerobes although no individual bacterial species could be implicated. Fuchs also attempted to quantify the faecal bacterial output using calculations based on faecal wet weights. He could only find an increase in the excretion of anaerobic microorganisms.

Supporting evidence for the role of carbohydrate acting as substrate comes from the investigation of Shetty et al[283]. He fed to healthy volunteers a diet rich in cornstarch gruel, a digestible carbohydrate. No bacteriology was done but the stools were carefully analysed biochemically to ascertain the microbial contribution to the faecal weight. They

found that daily stool output rose significantly and there was no change in transit time. There was a significant increase in nitrogen content of the faeces. Starch was never detected in the stools. From these observations they concluded that digestible starch produces a rise in the microbial faecal content, and this increase is not related to a change in colonic transit time. It is likely that some starch escaped digestion in the small intestine and served as a pabulum for the colonic bacteria.

In vitro experiments manipulating carbohydrate intake.

As part of a series of experiments using continuous flow cultures, Edwards et al looked at the effects of carbohydrate manipulation on bacterial growth[103]. For the carbohydrate experiments they used 3 cultures seeded by faecal bacteria from human volunteers. The carbohydrate source was a mixture of soluble starch, glucose, and maltose. Doubling the concentration of carbohydrate resulted in an increase in *E.coli* in all 3 systems. *E.coli* was present in all 3 cultures at the beginning of the experiment. *Klebsiella*, initially present in all cultures increased in 2 and decreased in one. *Streptococcus faecalis*, initially present in 2, increased in two and decreased in one. Omitting carbohydrate reduced the count of the various bacteria, but in most cases not below the original level. The continuous flow system is liquid, with a constant amount of effluent so the bacterial concentrations can also be viewed as absolute quantities.

THE SMALL INTESTINAL FLORA

Only two studies in humans investigating the effect of diet on the small intestinal flora could be found in a literature search. Both deal with elemental diets and in neither study were the patients healthy.

Dickman in 1975 described a patient with ulcerative colitis and an ileo-rectal anastomosis[94]. He had severe diarrhoea. Culture of jejunal juice showed bacterial overgrowth by a large variety of microorganisms including upper respiratory bacteria,

Enterobacteriaceae, and anaerobes. No *Bacteroides* spp. were present. After 11 days of elemental diet the diarrhoea lessened although sigmoidoscopic signs of inflammation persisted. Jejunal bacteriology at this time showed a 100-fold decrease in the total bacterial count to about 5 $\log_{10}$ organisms/ml. This decrease was not selective and was proportionate in all the bacterial genera. A normal diet was resumed but because of persistent symptoms an ileostomy was done. Post-ileostomy cultures showed an almost sterile fluid. No meaningful conclusions can be drawn on the effect of elemental diet on the small intestinal flora from this case report. Elemental diets are known to be associated with symptomatic improvement during relapses of ulcerative colitis. Also, it has been shown in patients with ulcerative colitis and pouch-anal anastomosis that the jejunal flora is scanty in those with disease quiescence, while there is bacterial overgrowth in those with ongoing inflammation[242]. The diminished flora may simply reflect decreased disease activity. The very striking improvement in jejunal bacteriology following a "cure" by means of an ileostomy in Dickman's patient supports this.

Axelsson investigated 18 patients, of whom 17 had chronic intestinal conditions[13]. Jejunal cultures were done in 17 before the start of elemental diet and at the end of treatment. The duration of treatment varied between one and seven weeks. Six patients were found to have an abnormal flora before treatment (4 $\log_{10}$ organisms/ml or more of faecal-type bacteria). In two of these subjects the flora remained abnormal, in the other four it reverted to acceptable values. There was a trend towards the isolation of fewer species and in lower numbers in the other 11 patients. None of the changes seen in the jejunal flora were statistically significant. No clinical details were given of the response to the elemental diet, but the stool weights remained high.

CONCLUSIONS

In summary it may be stated that beyond infancy different dietary lifestyles e.g. vegetarian, do not produce major changes in the faecal microflora.

Increasing the amount of carbohydrates reaching the colon increases the number of bacteria passed in the faeces. Decreasing the amount of carbohydrate, as in the case of elemental diets, decreases the bacterial numbers. The qualitative changes in the stool flora have been much less predictable.

It is likely that the effects on total microbial output are largely due to substrate availability in the colon; *in vitro* experiments suggest that the supply of carbohydrate *per se* is important. An indirect effect on colonic motility cannot however be ruled out in the case of altering the quantity of fibre. Limiting the amount of fibre entering the colon as is the case with elemental diets decreases the microbial faecal output. The relative contribution of increased transit time is difficult to estimate, but it may be a major one.

No meaningful comments can be made on the response of the small intestinal flora to dietary changes, because of the limited data available.

CHAPTER 5

PATIENTS AND METHODS

PATIENTS AND METHODS

PATIENTS

INTRODUCTION

This investigation took place between October 1988 and May 1990 at the Red Cross War Memorial Children's Hospital. The study was designed in two parts (see scheme of study at end of this section).

Carbohydrate study. This was designed to compare the effects of a carbohydrate free diet on the duodenal flora. There were 20 patients selected for this part.

Bowel Cocktail study. This was designed to investigate the effect of a combination of gentamicin and cholestyramine on the duodenal flora. It directly followed the carbohydrate part. Fifteen patients were enroled: 14 of these comprised the infants in the carbohydrate part of the study who had ongoing diarrhoea. The additional infant was the "late entry" group. He was selected for study at a later stage than the patients who had been in the carbohydrate part of the study.

Patients studied were selected from infants in the rehydration ward at Red Cross War Memorial Children's Hospital, to which all infants and children with dehydrating diarrhoea are initially admitted. The routine management is the same for all patients. The day of admission to the rehydration ward is designated day 0. Routine initial care consists of correction of shock if present, and intravenous or nasogastric rehydration with half strength Darrow's solution in 5% dextrose. Feeds are withheld for 6 hours, thereafter a clear feed is given and if this is tolerated full strength milk feeds are then started at 12 hours.

CARBOHYDRATE STUDY: To investigate the effects of a carbohydrate-free feed on the duodenal flora

Selection of patients

On the third day following admission to hospital (i.e. day 2) infants requiring additional oral intravenous fluids to maintain hydration were selected for further study if they fulfilled the following criteria:

1. Were males (for ease of separation of stool from urine).
2. Were between 6 weeks and 18 months old.
3. Had a history of diarrhoea of 96 hours' duration or less before admission to hospital.

Any of the following were exclusion criteria from the study:

1. Severe protein-energy malnutrition: kwashiorkor or marasmus or any skin changes of specific nutrient deficiencies, such as pellagra.
2. Any systemic illness e.g. pneumonia, urinary tract infection, tuberculosis.
3. A history of diarrhoea in the month prior to admission.
4. A history of antibiotic administration in the 2 weeks preceding hospital admission. The administration of antifungal or vermifugal agents were not considered exclusion criteria.
5. A history of antidiarrhoeal medication in the 2 weeks preceding hospital admission.

Informed written consent was obtained in each case, and the patients were then transferred to the research ward. They were nursed on a balance bed. By means of a glass bulb attached to the penis complete separation of urine from stool was obtained, ensuring an accurate stool collection. The plastic bag into which the stool passed was emptied several

times daily into a collection pot, which was immediately sealed and refrigerated for later weighing (see data collection section).

Feeding regime

Patients were fed a modified cow's milk formula; S26 (Wyeth) if they were less than 3 months old, Lactogen (Nestlé) if older than 3 months. The feed volume was 150ml/kg/day, calculated on the estimated rehydrated weight of the patient. This volume was not altered for the duration of the study.

Feeds were given in 8 equally divided amounts. If the patients were reluctant to take the feed, or did not complete it, it was given by means of a nasogastric tube, which was removed immediately on completion of the feed.

Additional fluids

For the duration of the study any fluids which the patients required to maintain hydration were given intravenously. The standard rehydration solution was half strength Darrow's solution in 5% dextrose. Hyponatraemia was corrected with half normal or normal saline in 5% dextrose. Hypernatraemia was managed by increasing the infusion rate of half strength Darrow's dextrose solution.

Medications

If patients required paracetamol for fever it was given as a suspension of crushed tablets in water, specially prepared by the pharmacy.

Motility-enhancing drugs such as metoclopramide were never given to facilitate intubation, lest the duodenal fluid become contaminated with gastric juice.

Data collection

For the purpose of the collection of data the day began at 11:00 and ended at 11:00 the following day. The infants' body weight was recorded daily. For analysis of results the rehydrated weight was defined as the weight of the patient immediately after recovery from the diarrhoeal episode and while not receiving additional fluids. All stools passed were collected and weighed daily by an independent observer, unaware of any clinical details.

Inclusion into the study

If the stool output in the 24 hours following admission into the research ward was equal to or greater then 30g/kg of the estimated rehydrated weight the infants were finally included into the study.

On day 3 the infants underwent the first of two duodenal intubations to obtain duodenal juice for determining the flora qualitatively and quantitatively. On the same day of the intubation and all subsequent intubations a fresh stool specimen was also collected for microscopy, bacterial culture and virological analysis. After the first intubation the patients were alternatively allocated to one of two groups:

Carbohydrate-containing group

The full cream milk feeds were replaced by a soya-based feed containing as carbohydrate source a mixture of glucose polymers and sucrose (Isomil, Ross). The volume given was not changed.

Carbohydrate-free group

The full cream milk feeds were replaced by a soya based feed without carbohydrate (Ross CHO-free), which is identical to Isomil except for the carbohydrate content. The volume of feed was not altered. Since the milk did not include carbohydrate, this was given intravenously as dextrose added to the intravenous fluids. The amount of dextrose was calculated to provide 30% of the caloric intake. In practice this would involve raising the concentration of the intravenous solution to 10 or 15% dextrose. Blood glucose was checked at 6 hourly intervals by means of dextrostix.

Both groups received additional fluids as already described.

On day 6 all infants underwent the second duodenal intubation to obtain juice for determination of the flora. The carbohydrate part of the study ended on day 6.

BOWEL COCKTAIL STUDY: To investigate the effects of oral gentamicin and cholestyramine on the duodenal flora

Patient selection

Patients for this part of the study were obtained from two sources:

a) Any patient from the carbohydrate part of the study with a stool weight equal to or greater than 30g/kg for the 24 hours prior to the second intubation (i.e. day 5). These infants were maintained on the same feeds as for carbohydrate part, i.e. Isomil or Ross CHO-free.

b) Infants from the rehydration unit, not initially included in the first part of the study, who fulfilled the previously mentioned general selection criteria, who had been started on Isomil feeds on day 3 and who still required additional fluids

(intravenous or oral) to maintain hydration on day 5. There was one such "late entry" infant. He was admitted to the research ward on day 5 and nursed on the balance bed. Feeds were given three hourly as Isomil at 150ml/kg/d based on his estimated rehydrated weight and additional fluids were given intravenously as half strength Darrow's solution in 5% dextrose.

His stool weight for the 24 hours following admission to the research ward was greater than 30g/kg of the estimated rehydrated weight and he was admitted into the study.

Further management

On day 6 the patients underwent the first duodenal intubation of the bowel cocktail part of the study (which was the second intubation of the carbohydrate part of the study). Immediately after the intubation all the infants were started on a combination of antibiotic and cholestyramine in the following regimen:

i) Gentamicin at a dose of 50mg/kg/day p.o. (max dose 360mg/day) given 4 hourly for 3 days.

ii) Cholestyramine at a dose of 4g/day p.o. given 6 hourly, for 5 days.

On day 8 the final duodenal intubation was carried out.

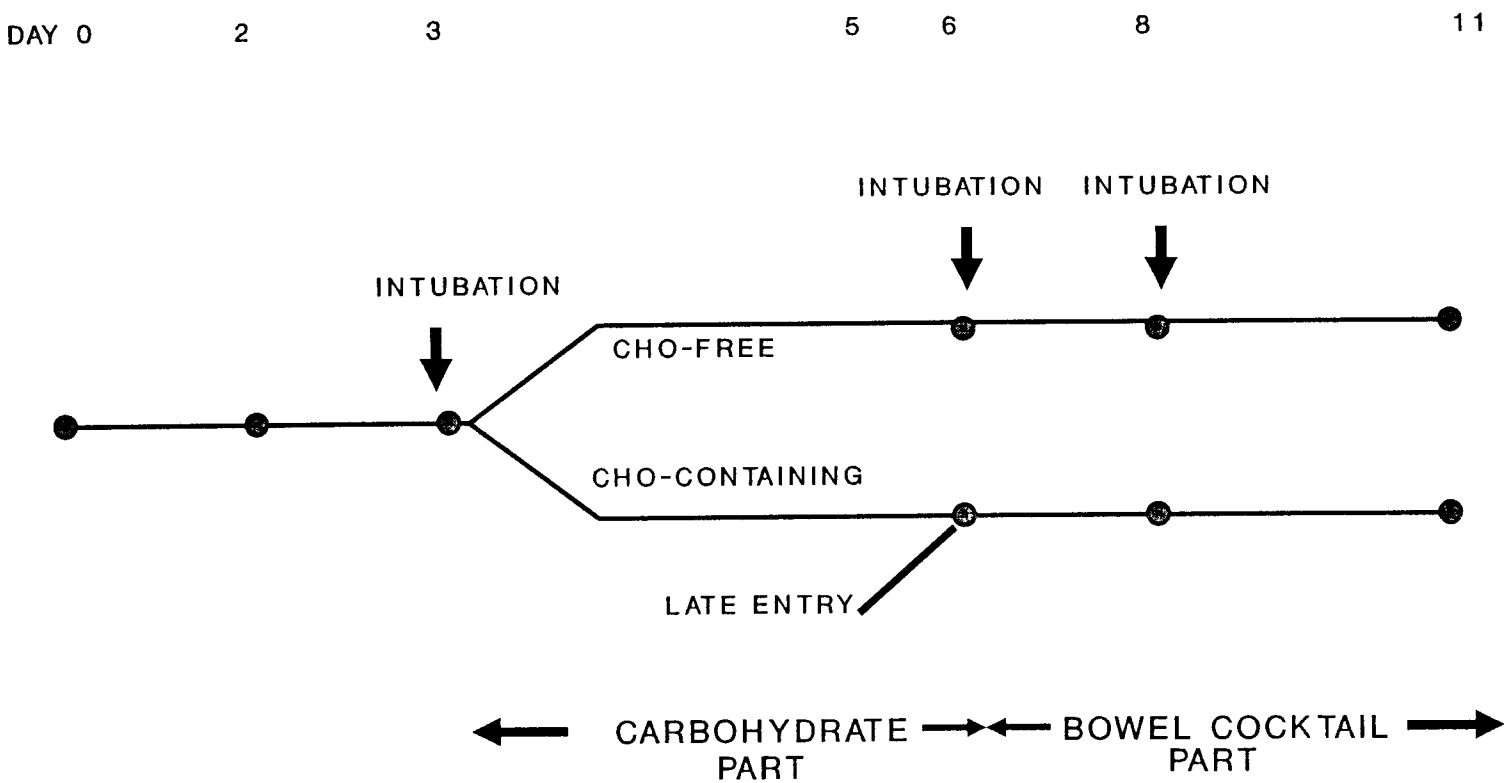
The feeding regimen and patient management was not altered for the duration of the study.

The study ended on day 11. At this stage the infants in the Isomil group and in whom the diarrhoea had resolved were discharged from hospital.

In those infants receiving Ross CHO-free milk in whom the diarrhoea resolved, the milk was changed to Isomil and they were observed in hospital for 24 hours for relapse of diarrhoea.

Those infants who had ongoing diarrhoea on completion of the study were managed according to the protocol in use at the Red Cross War Memorial Children's Hospital. This consists in changing the feeds to a casein hydrolysate formula (Nutramigen, Mead Johnson) on day 12.

SCHEME OF STUDY



METHODS

COLLECTION OF DUODENAL JUICE

After the patient was fasted for 4 - 6 hours a sterile F10 Replogle tube (Sherwood Industries) was inserted via the oral route. Under fluoroscopic guidance it was manipulated until the most proximal port hole lay in the third part of the duodenum. Duodenal juice was then aspirated. The first 3 - 5 ml were set aside and 1 - 2 ml of juice was then collected in a different sterile syringe and immediately placed in a preweighed rich broth transport medium (see appendix). At the end of collection the fluoroscopic examination was repeated, to ensure that displacement of the tube had not occurred. The specimen was without delay taken by the investigator to the laboratory where it was immediately processed.

PREPARATION OF DUODENAL JUICE FOR BACTERIOLOGY

The initial preparation was done by the investigator. This included the weighing and plating out procedures and placing the plates in the incubator.

Weighing

Before the specimen was processed the Bijou bottle containing the duodenal juice was weighed. This weight, when subtracted from the initial one of the bottle containing one ml of rich broth transport medium, gave the weight of the sample.

Serial dilutions and plating

Dilutions of the sample were made by vigorously shaking the bottle by hand and then inoculating 0.1ml of the sample into a sterile Bijou bottle containing 2ml of rich broth, and

0.01ml of sample into another sterile bottle containing 10ml of rich broth (see diagram). From these three bottles aliquots of either 0.1ml or 0.01ml of juice were evenly spread to dryness onto a series of culture plates, to give serial dilutions of 10^{-1} to 10^{-5}.

CULTURE TECHNIQUES

The microbiology was always performed by the same experienced bacteriological technician (L.M.) who was unaware of the clinical details of the patient.

Identification of microorganisms

A comprehensive range of aerobic and anaerobic culture media was used. Organisms were identified on the basis of colonial morphology, growth on selective media, gram stain, and biochemical tests. Identification of aerobic microorganisms was done according to Cowan and Steele's manual[81]. Anaerobic identification was done according to Wadsworth's manual and Bergey's textbook[59].

Expression of bacterial counts

The number of viable bacteria was found by counting the number of colonies that were found on the highest dilution plate. By multiplying this number by the dilution factor the number of organisms/ml was calculated. This was then expressed as the $\log_{10}$ of organisms/ml of duodenal juice.

Culture of anaerobic organisms

The anaerobic plates were processed first. Plating was done on the work bench and as soon as the plates of a particular dilution were completed they were placed in an anaerobic

cabinet which served as a holding area. The time from specimen collection to incubation never exceeded 45 minutes.

The following pre-reduced non-selective media were used:

4% blood agar

Wilkins Chalgren blood agar

Brain-heart infusion with vancomycin

Selective media were used to identify the following organisms:

Rogosa V agar for *Veillonella*

Ethyl violet agar for *Fusobacterium*

Rogosa SL for anaerobic lactobacilli

The plates were incubated under anaerobic conditions in GasPak (BBL, Cockeysville) jars at 37°C for 5 days before being examined.

Culture of aerobic organisms

The following non-selective media were used:

2 % blood agar

Brain-heart infusion

Selective media were used to culture the following organisms:

McConkey's agar for *Enterobacteriaceae*

Salmonella-Shigella (SS) agar for *Salmonella* and *Shigella*

Boiled blood agar (BBA) for *Haemophilus* spp.

Mannitol salt agar for staphylococci

Sabouraud's dextrose agar for yeasts

The plates were placed in an incubator at 37°C for 24 hours before being examined.

The Boiled blood agar plates were placed in a separate incubator containing an atmosphere of 10% CO_2 at 37°C for 24 hours.

Identification

The organisms were identified according to Cowan and Steele's manual. The *Enterobacteriaceae* were identified using the API 20E system.

Culture of microaerophilic organisms

Tryptose boiled blood agar (TBBA) with antibiotics was used for the isolation of *Campylobacter* spp. The plates were incubated in a Gas Pak jar adapted for microaerophilic conditions at 37°C for 96 hours before being examined.

E.coli

EPEC serotypes were determined for any *E.coli* which were isolated. A loopful of *E.coli* was prepared in 2ml of trypticase soy broth containing 20% glycerol. These preparations were then maintained as stock cultures at -70°C for batch analysis at the end of the study.

After confirming the identity of the *E.coli* by API 10E or 20E, EPEC serotypes were determined by means of the slide agglutination tests using a commercial kit (Wellcome Diagnostics). The following common serotypes were tested for: O26:K60(B6); O44:K74(L); O55:K59(B5); O86:K61(B7); O111:K58(B4); O112:K66(B11); O114:K90(B); O119:K69(B14); O124:K72(B17); O125:K70(B15); O126:K71(B16); O127:K63(B8); O128:K67(B12); O142:K86(B); O18c:K77(B21). Those positive for slide agglutination were confirmed by tube agglutination using boiled cultures.

Microscopy of duodenal juice

The duodenal juice used for microscopy was taken from the initial 3 - 5 ml aliquot obtained at intubation.

It was spun in a centrifuge at 2000 x g for two minutes. The sediment was then examined for the following:

1. Giardia intestinalis

   The specimen was examined under high-power microscopy for giardia trophozoites and cysts by an experienced technologist in the routine laboratory. The microscopist was not aware of the clinical details. The findings were only divulged at the end of the individual patient's study.

2. Cryptosporidium

   The sediment was spread onto a microscope slide and allowed to dry. A modified Ziehl-Nielsen preparation was made as used by Casemore[69]. This was examined by oil immersion microscopy by the investigator.

ANALYSIS OF STOOL SPECIMENS

On the same day as the duodenal intubations a faecal specimen was also collected and divided into 3 parts.

Microscopy

The microscopy and routine culture was done by the Microbiology Laboratory at the Red Cross Children's Hospital.

1. Giardia intestinalis

   A fresh specimen was examined under high-power light microscopy for giardia cysts and trophozoites.

2. Cryptosporidium

   A microscope slide was lightly smeared with faeces and then stained by the previously described a modified Ziehl-Nielsen method. It was then examined for cryptosporidium by oil-immersion microscopy.

Routine culture

Commonly occurring pathogens - *Salmonella*, *Shigella*, and *Campylobacter* - were looked for. Isolation and serotyping of *E.coli* was not attempted by the routine laboratory.

The following media were used:

Salmonella-Shigella (SS) agar

Tetrathionate broth enrichment

Tryptose blood agar supplemented with horse blood and antibiotics.

Isolates were confirmed biochemically and serologically.

E.coli

The tests for *E.coli* were done in the investigator's laboratory by the same technologist who performed the bacteriological analysis.

This was initially processed by the investigator immediately after completion of the duodenal fluid procedures. A loopful of fresh stool was spread onto McConkey's agar and incubated for 24 hours. The following day a loopful of lactose-fermenting colonies (in excess of 10) morphologically resembling *E.coli* was prepared in 2ml of trypticase soy broth. Thereafter the procedures were as described for *E.coli* in the duodenal juice.

Virology

The Rotavirus analysis was performed by the Virology Department at the Medical School of the University of Cape Town. Rotavirus only was looked for, Adenovirus not being associated with pathogenicity in the area studied (Coltman D, PhD in preparation). For reasons of economy only the sample taken on the day of the first intubation was examined. The specimen was stored at -70°C until completion of the whole study. All samples were tested in one batch. The method used consisted of extracting RNA from the stool and subjecting this to polyacrylamide gel electrophoresis[158].

STATISTICAL ANALYSIS

CARBOHYDRATE STUDY

Non parametric statistical methods were used for all cross-sectional comparisons i.e. in comparing the clinical details, blood tests, stool weights, stool pathogens and duodenal flora between the carbohydrate-containing and carbohydrate-free groups the Mann Whitney U test was used.

In analysing the changes in the duodenal flora occurring between day 3 and 6 within each group the Wilcoxon matched pairs signed-ranks test was used.

BOWEL COCKTAIL STUDY

This compared longitudinal changes in one group alone. In analysing changes in the stool output and in the duodenal flora the Wilcoxon matched pairs signed-ranks test was used.

All calculations were done on a PC computer, using Statgraphics version 3.0 software.

CHAPTER 6

CARBOHYDRATE STUDY

CLINICAL DETAILS ON ADMISSION TO STUDY

20 patients were enrolled for this study. Ten were fed on carbohydrate-containing milk (Isomil), and 10 on carbohydrate-free formula (Ross CHO-free).

Table 1 gives relevant clinical details for the two groups on day 2.

TABLE 1

Carbohydrate group

	Mean	**Median**	**Range**
Age (months)	5.79	5.55	1.45-9.89
% of expected weight	91.4	90	63-122
Weight for length %	96.2	92	84-124
Duration of diarrhoea (hrs) preceding hospital admission	53	60 (Mode = 24)	24-96
Dehydration (%) on admission to trial	3	2 (Mode = 0)	0-8

Carbohydrate-free group

	Mean	**Median**	**Range**
Age (months)	6.85	6.64	2.96-13.2
% of expected weight	87.5	87	66-104
Weight for length %	93	89	73-138
Duration of diarrhoea (hrs) preceding hospital admission	67	72 (Mode = 72)	48-96
Dehydration (%) on admission to trial	3.3	4 (Mode = 4)	0-6

Although the patients in the carbohydrate-containing group tended to be younger, have a shorter duration of diarrhoea preceding admission, and to be less dehydrated, these differences did not reach statistical significance.

HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

Table 2 outlines the results of blood tests done on day 2 on admission to the research ward. Blood tests were only repeated when they were clinically indicated.

TABLE 2

Carbohydrate group

	Mean	Median	S.D.	Range
Haemoglobin g/dl	10.8	10.6	1.22	9.2-12.3
Leucocytes $x10^9$/l	13.3	13.2	2.65	9.3-17.2
Platelets $x10^9$/l	677	688	138	418-859
Sodium mmol/l	138	138	3.3	133-143
Potassium mmol/l	4.2	3.9	0.77	3.3-5.6
Albumin g/l (n = 9)	38	39	6.2	24-46
pH	7.28	7.3	0.19	7.07-7.43
Base deficit mmol/l	11.1	8.8	6.5	2.6-19.7

Carbohydrate-free group

	Mean	Median	S.D.	Range
Haemoglobin g/dl	10.6	10.6	0.73	9.5-11.8
Leucocytes $x10^9$/l	13.0	13.0	1.83	10.7-17.0
Platelets $x10^9$/l	625	608	139	394-911
Sodium mmol/l	135	136	7.3	126-146
Potassium mmol/l	3.4	3.2	0.95	2.1-5.1
Albumin g/l	33	32	7.6	17-45
pH	7.32	7.33	0.11	7.06-7.47
Base deficit mmol/l	8.2	7	5.8	1.8-22.3

There were no significant differences between the groups in any of the haematological or biochemical indices.

STOOL OUTPUT

Table 3 shows the stool weights in g/kg of rehydrated weight of the patients subdivided into carbohydrate-containing and carbohydrate-free groups.

TABLE 3

Carbohydrate-containing

	Mean	Median	S.D.	Range
Day 2	81	65.2	40.5	45.8-171.1
Day 3	91.5	69.9	65.2	13.5-206.6
Day 4	96.4	77	77.5	5.1-210.9
Day 5	95.3	84.9	73.4	0.4-204.2

Carbohydrate-free

	Mean	Median	S.D.	Range
Day 2	87.8	50.9	63.6	30.9-187.8
Day 3	79.9	56.7	58.6	18.1-187.6
Day 4	80.1	66.3	54.5	12.8-189.9
Day 5	68.1	55.8	54.7	4.2-151.1

The daily median stool weights were higher in the carbohydrate-containing groups, but this did not reach statistical significance.

CLINICAL COURSE

The diarrhoea resolved in five patients. Two (nos. 9 and 10) were in the carbohydrate-containing group, and three (nos. 14, 15, 19) in the carbohydrate-free group. In all 5 the stool output was less than 30g/kg/day on day 5 and the stools were soft. These patients left the trial on day 6 and were discharged from hospital. One infant (no. 16) in the carbohydrate-free group developed a fever and became unwell on day 6. His clinical and

radiological findings were consistent with pneumonia. Because of systemic illness he was excluded from the bowel cocktail part of the study despite continuing diarrhoea.

No difficulties were encountered with patient management. No patients in the carbohydrate-free group developed hypoglycaemia. There were no complications associated with intravenous lines.

STOOL PATHOGENS

Table 4 lists the pathogens isolated in the stools of the 20 patients included in this part of the study. The following *E.coli* serotypes are recognised enteric pathogens in Cape Town: O111, O119, O126, O127 (Coltman D, PhD thesis in preparation). For reasons of economy Rotavirus was sought only on day 3. In the analysis of multiple pathogens, and whether the same pathogen was present on both day 3 and 6 this must be taken into account.

A total of 39 stool specimens was analysed. The day 6 stool sample from patient no.12 was lost in transit to the routine microbiology laboratory.

TABLE 4

	Day 3	**Day 6**
Carbohydrate-containing group		
Patient no.		
1	*Campylobacter jejuni*	*Campylobacter jejuni*
2		*E.coli* O111
3	*E.coli* O127	*E.coli* O127
4	Rotavirus	*Cryptosporidium*
5	Rotavirus	
6	Rotavirus	
10	Rotavirus	

Carbohydrate-free group

11	*E.coli* O126	
12	*Shigella flexneri*	NO SPECIMEN
13	*Cryptosporidium*	*Cryptosporidium*
		Salmonella group B
14	Rotavirus	
15	Rotavirus	
18	*Campylobacter jejuni*	*Campylobacter jejuni*
19		*Salmonella* group B
20	*Campylobacter jejuni*	*Campylobacter jejuni*
	E.coli O126	*E.coli* O126

Enteric pathogens were isolated in the stools of 15 patients at some stage of this study. 7 were in the carbohydrate-containing group, and 8 in the carbohydrate-free group.

On day 3, 14 enteric pathogens were found in 13 patients. 6 of the patients were in the carbohydrate-containing group and 7 in the carbohydrate-free group.

On day 6, 10 enteric pathogens were isolated in 8 patients: 4 in the carbohydrate-containing and 4 in the carbohydrate-free group. Of the 10 pathogens found on day 6, 6 were the same as had been isolated on day 3. Of the 4 new isolates 2 were detected in patients in whom no pathogens were found on day 3.

Multiple pathogens were found in 2 patients on 3 occasions: *Cryptosporidium* and *Salmonella* group B on day 6 in patient no. 13; *Campylobacter jejuni* and *E.coli* O126 on days 3 and 6 in patient no. 20.

The same pathogen was isolated on both day 3 and day 6 in 5 patients. Multiple pathogens were found in 2 patients in a total of 3 isolates.

Table 5 lists the frequency of occurrence of individual stool pathogens.

TABLE 5

	no. of patients	no. of isolates
EPEC	4	6
Campylobacter jejuni	3	6
Salmonella group B	2	2
Shigella flexneri	1	1
Rotavirus	6	6
Cryptosporidium	2	3
Giardia intestinalis	0	0

DUODENAL FLORA

Microscopy

Giardia intestinalis trophozoites were seen on day 3 and day 6 in patient no.12 (carbohydrate-free group).

No other pathogens were detected.

Bacteriology

Tables 6 and 7 show the bacteriological findings for day 3 and day 6 in the carbohydrate-containing group. Tables 8 and 9 show the findings for day 3 and day 6 for the carbohydrate-free group.

Figures 1 and 2 give a visual presentation of these bacteriological findings for the carbohydrate(CHO)-containing group, and figures 3 and 4 for the carbohydrate(CHO)-free group.

TABLE 6

DUODENAL FLORA OF CARBOHYDATE-CONTAINING GROUP ON DAY 3

	Number of Isolates	Organisms in log_{10}/ml Median	Min	Max	LQ*	UQ*
Streptococcus viridans	6	2.52	0.00	7.09	0.00	4.33
Beta haemolytic streptococci	1	0.00	0.00	4.39	0.00	0.00
Other streptococci	1	0.00	0.00	1.44	0.00	0.00
Staphylococcus epidermidis	4	0.00	0.00	2.88	0.00	1.75
Staphylococcus aureus	5	0.84	0.00	3.58	0.00	2.23
Coagulase neg staphylococci	1	0.00	0.00	1.66	0.00	0.00
Pneumococcus	0					
Haemophilus	6	2.84	0.00	5.79	0.00	4.85
Diphtheroids	2	0.00	0.00	2.06	0.00	0.00
Neisseria	2	0.00	0.00	5.00	0.00	0.00
Corynebacteria	1	0.00	0.00	5.00	0.00	0.00
E.coli	4	0.00	0.00	6.88	0.00	3.74
Klebsiella	3	0.00	0.00	5.32	0.00	1.23
Citrobacter	0					
Enterobacter	1	0.00	0.00	5.14	0.00	0.00
Providencia	0					
Proteus	0					
Streptococcus faecalis	1	0.00	0.00	3.53	0.00	0.00
Aerobic lactobacilli	6	2.41	0.00	5.39	0.00	4.93
Micrococcus	1	0.00	0.00	4.19	0.00	0.00
Acinetobacter	0					
Anaerobic streptococci	0					
Anaerobic lactobacilli	1	0.00	0.00	3.00	0.00	0.00
Propionibacterium	2	0.00	0.00	5.53	0.00	0.00
Bacteroides	6	2.47	0.00	6.97	0.00	4.28
Fusobacterium	5	0.61	0.00	4.18	0.00	3.41
Veillonella	6	2.02	0.00	6.76	0.00	4.56
Actinomyces	0					
Candida	6	2.56	0.00	6.77	0.00	3.99
Upper respiratory microorganisms	29	3.53	0.00	7.11	2.47	4.85
Enterobacteriaceae	8	3.01	0.00	6.88	0.00	4.01
Aerobic microorganisms	51	4.73	2.07	7.13	3.01	6.43
Anaerobic microorganisms	20	3.97	0.00	7.19	2.52	4.78
TOTAL	**71**	**4.99**	**2.22**	**7.26**	**3.63**	**6.14**

* LQ = Lower quartile, UQ = Upper quartile

TABLE 7

DUODENAL FLORA OF CARBOHYDRATE-CONTAINING GROUP ON DAY 6

	Number of Isolates	Organisms in log_{10}/ml Median	Min	Max	LQ*	UQ*
Streptococcus viridans	6	1.61	0.00	7.14	0.00	4.48
Beta haemolytic streptococci	1	0.00	0.00	2.51	0.00	0.00
Other streptococci	0					
Staphylococcus epidermidis	5	1.27	0.00	3.36	0.00	3.26
Staphylococcus aureus	4	0.00	0.00	3.83	0.00	2.96
Coagulase neg staphylococci	0					
Pneumococcus	0					
Haemophilus	5	0.97	0.00	5.57	0.00	4.08
Diphtheroids	1	0.00	0.00	2.35	0.00	0.00
Neisseria	3	0.00	0.00	4.57	0.00	3.38
Corynebacteria	0					
E.coli	3	0.00	0.00	4.41	0.00	1.92
Klebsiella	4	0.00	0.00	4.79	0.00	3.87
Citrobacter	0					
Enterobacter	1	0.00	0.00	3.18	0.00	0.00
Providencia	1	0.00	0.00	3.26	0.00	0.00
Proteus	0					
Streptococcus faecalis	0					
Aerobic lactobacilli	7	2.89	0.00	4.88	0.00	3.59
Micrococcus	1	0.00	0.00	1.49	0.00	0.00
Acinetobacter	0					
Anaerobic streptococci	0					
Anaerobic lactobacilli	2	0.00	0.00	5.24	0.00	0.00
Propionibacterium	1	0.00	0.00	1.23	0.00	0.00
Bacteroides	2	0.00	0.00	6.44	0.00	0.00
Fusobacterium	4	0.00	0.00	3.88	0.00	2.94
Veillonella	7	3.04	0.00	6.69	0.00	4.28
Actinomyces	1	0.00	0.00	5.65	0.00	0.00
Candida	7	2.31	0.00	3.48	0.00	2.70
Upper respiratory microorganisms	25	3.12	0.00	7.20	1.53	4.54
Enterobacteriaceae	9	1.59	0.00	4.81	0.00	4.11
Aerobic microorganisms	49	4.43	1.53	7.20	2.94	5.16
Anaerobic microorganisms	17	3.10	0.00	6.69	1.23	5.82
TOTAL	**66**	**4.51**	**1.70**	**7.32**	**3.33**	**5.84**

* LQ = Lower quartile, UQ = Upper quartile

TABLE 8

DUODENAL FLORA OF CARBOHYDRATE-FREE GROUP ON DAY 3

	Number of Isolates	Organisms in log_{10}/ml Median	Min	Max	LQ*	UQ*
Streptococcus viridans	7	3.83	0.00	6.28	0.00	5.57
Beta haemolytic streptococci	1	0.00	0.00	5.18	0.00	0.00
Other streptococci	2	0.00	0.00	5.46	0.00	0.00
Staphylococcus epidermidis	6	1.98	0.00	3.37	0.00	3.05
Staphylococcus aureus	3	0.00	0.00	3.32	0.00	1.88
Coagulase neg staphylococci	1	0.00	0.00	2.85	0.00	0.00
Pneumococcus	0					
Haemophilus	8	3.06	0.00	5.51	1.27	4.17
Diphtheroids	1	0.00	0.00	5.85	0.00	0.00
Neisseria	3	0.00	0.00	3.68	0.00	1.17
Corynebacteria	0					
E.coli	5	1.07	0.00	7.80	0.00	2.99
Klebsiella	5	0.80	0.00	3.74	0.00	1.73
Citrobacter	2	0.00	0.00	5.39	0.00	0.00
Enterobacter	0					
Providencia	0					
Proteus	1	0.00	0.00	1.87	0.00	0.00
Streptococcus faecalis	1	0.00	0.00	2.47	0.00	0.00
Aerobic lactobacilli	7	1.98	0.00	4.40	0.00	4.34
Micrococcus	1	0.00	0.00	2.70	0.00	0.00
Acinetobacter	1	0.00	0.00	2.78	0.00	0.00
Anaerobic streptococci	0					
Anaerobic lactobacilli	5	1.33	0.00	5.45	0.00	4.00
Propionibacterium	1	0.00	0.00	3.17	0.00	0.00
Bacteroides	1	0.00	0.00	4.14	0.00	0.00
Fusobacterium	6	1.61	0.00	5.18	0.00	2.58
Veillonella	10	3.99	1.93	6.14	2.88	4.11
Actinomyces	0					
Candida	6	1.77	0.00	5.50	0.00	3.75
Upper respiratory microorganisms	32	4.08	0.00	6.44	3.51	5.58
Enterobacteriaceae	13	2.74	0.00	7.80	1.93	4.09
Aerobic microorganisms	61	5.03	3.40	7.82	3.96	5.59
Anaerobic microorganisms	23	4.05	2.88	6.19	3.14	5.27
TOTAL	**84**	**5.07**	**3.58**	**7.82**	**4.50**	**6.26**

* LQ = Lower quartile, UQ = Upper quartile

TABLE 9

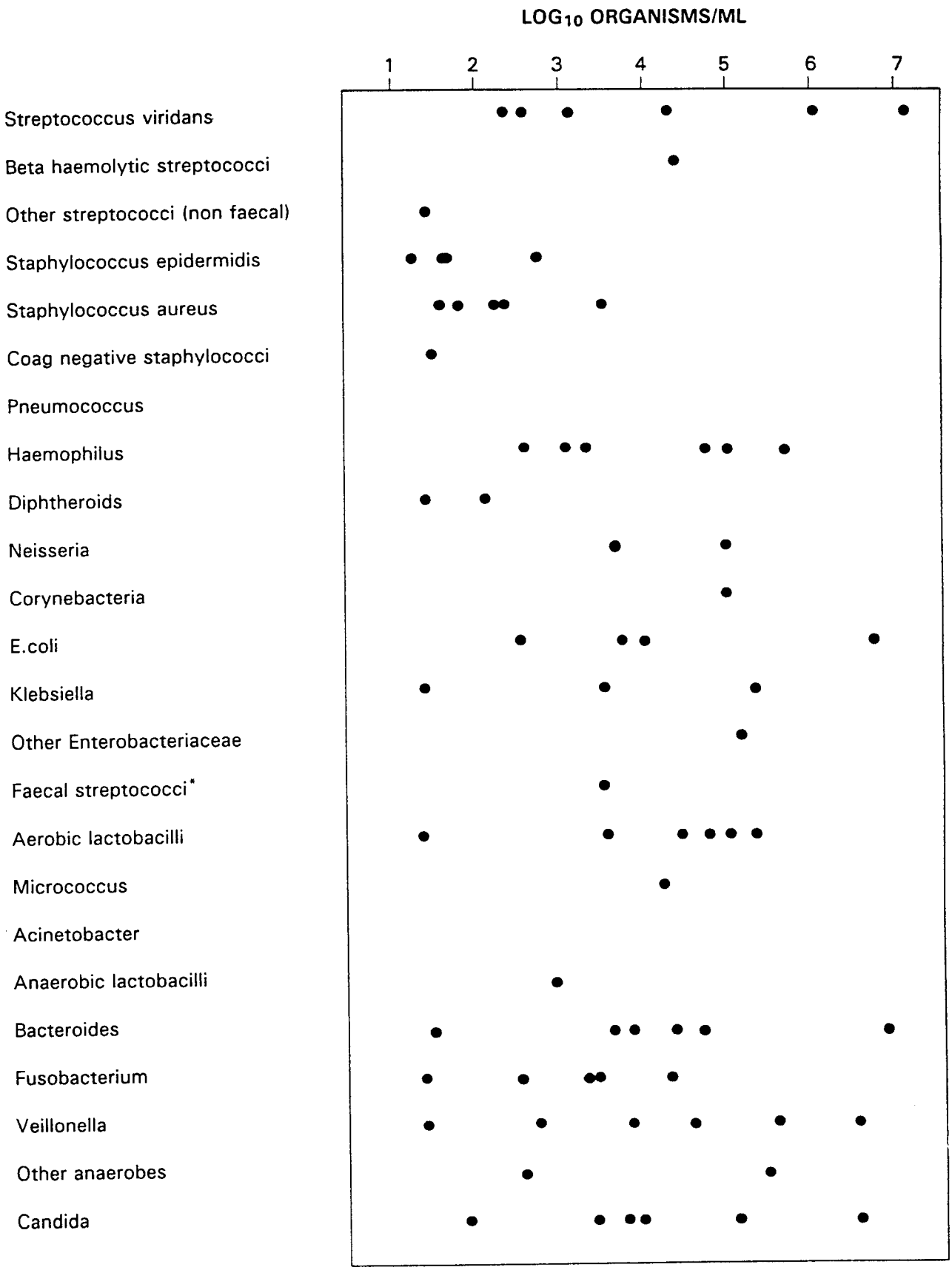
DUODENAL FLORA OF CARBOHYDRATE-FREE GROUP ON DAY 6

	Number of Isolates	Organisms in log_{10}/ml Median	Min	Max	LQ*	UQ*
Streptococcus viridans	9	4.22	0.00	6.99	3.23	5.12
Beta haemolytic streptococci	3	0.00	0.00	5.22	0.00	3.56
Other streptococci	1	0.00	0.00	3.14	0.00	0.00
Staphylococcus epidermidis	5	0.57	0.00	3.51	0.00	1.59
Staphylococcus aureus	5	0.53	0.00	3.53	0.00	1.59
Coagulase neg staphylococci	0					
Pneumococcus	2	0.00	0.00	6.44	0.00	0.00
Haemophilus	10	4.46	2.36	6.90	3.78	6.23
Diphtheroids	3	0.00	0.00	6.36	0.00	5.74
Neisseria	7	3.21	0.00	6.21	0.00	5.08
Corynebacteria	0					
E.coli	2	0.00	0.00	5.96	0.00	0.00
Klebsiella	4	0.00	0.00	6.37	0.00	2.22
Citrobacter	1	0.00	0.00	2.82	0.00	0.00
Enterobacter	0					
Providencia	0					
Proteus	0					
Streptococcus faecalis	0					
Aerobic lactobacilli	5	1.57	0.00	6.76	0.00	3.44
Micrococcus	2	0.00	0.00	4.93	0.00	0.00
Acinetobacter	0					
Anaerobic streptococci	1	0.00	0.00	3.76	0.00	0.00
Anaerobic lactobacilli	2	0.00	0.00	4.06	0.00	0.00
Propionibacterium	0					
Bacteroides	5	1.64	0.00	6.62	0.00	3.66
Fusobacterium	7	2.70	0.00	5.04	0.00	3.76
Veillonella	7	3.63	0.00	5.65	0.00	4.88
Actinomyces	0					
Candida	5	0.94	0.00	6.04	0.00	3.26
Upper respiratory microorganisms	45	4.82	3.66	7.20	4.18	6.76
Enterobacteriaceae	7	1.59	0.00	4.81	0.00	4.11
Aerobic microorganisms	64	5.06	3.66	7.40	4.21	6.76
Anaerobic microorganisms	22	4.13	0.00	6.68	2.64	5.01
TOTAL	**86**	**5.07**	**4.06**	**7.47**	**4.66**	**6.78**

* LQ = Lower quartile, UQ = Upper quartile

FIGURE 1

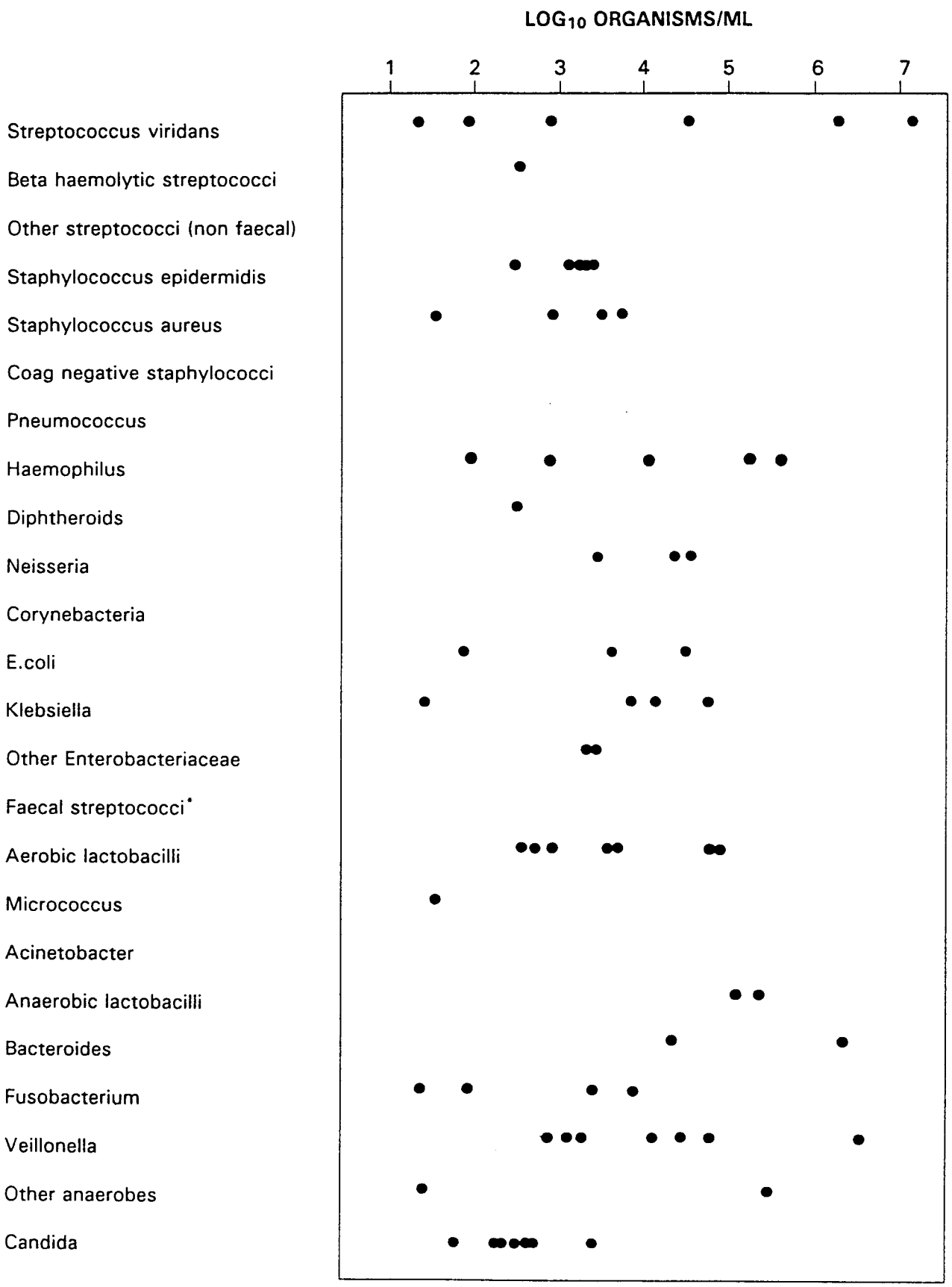
DAY 3 DUODENAL MICROFLORA OF CHO-CONTAINING GROUP



* Streptococcus faecalis, anaerobic streptococci

FIGURE 2

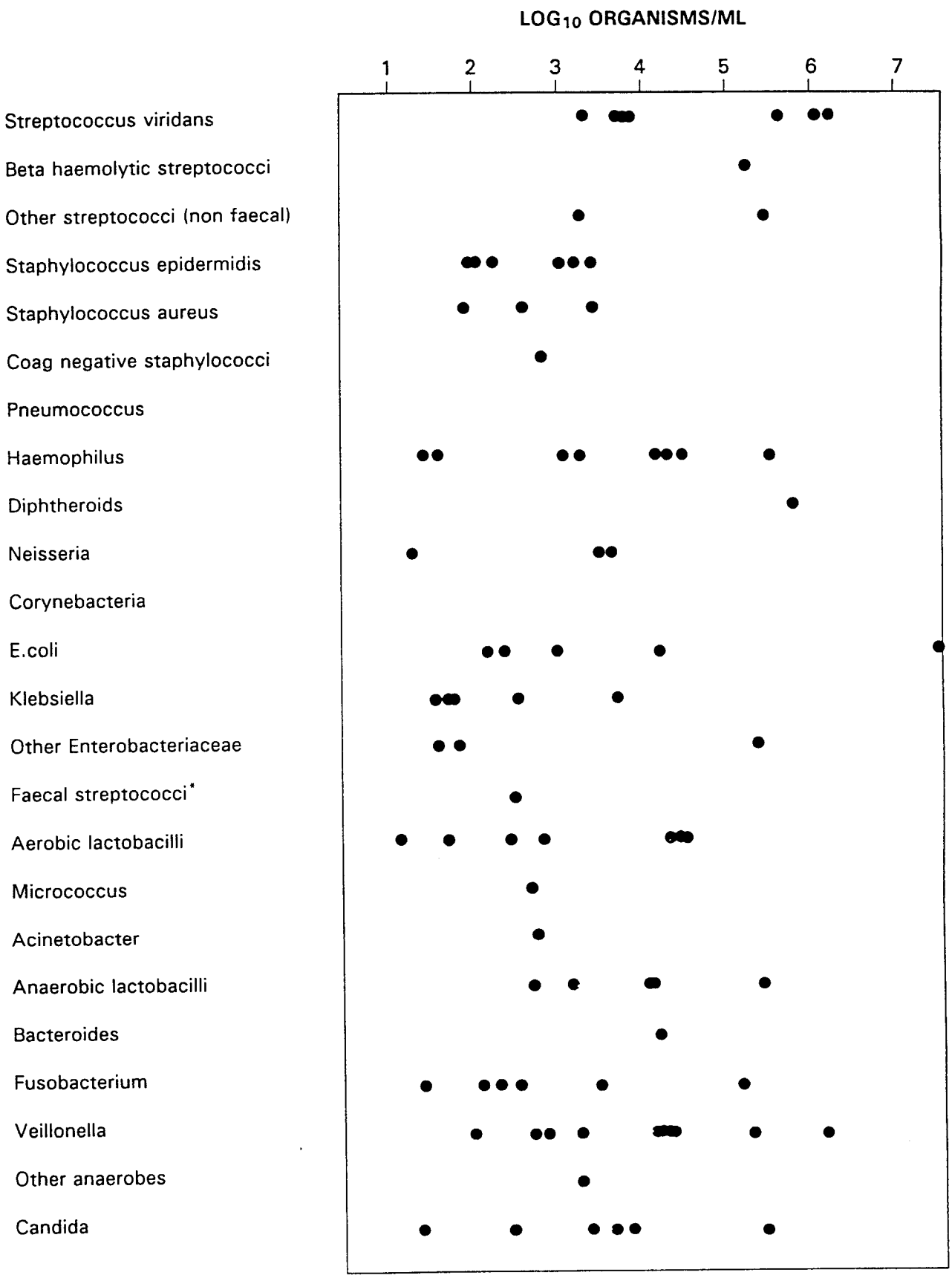
DAY 6 DUODENAL MICROFLORA OF CHO-CONTAINING GROUP



* Streptococcus faecalis, anaerobic streptococci

FIGURE 3

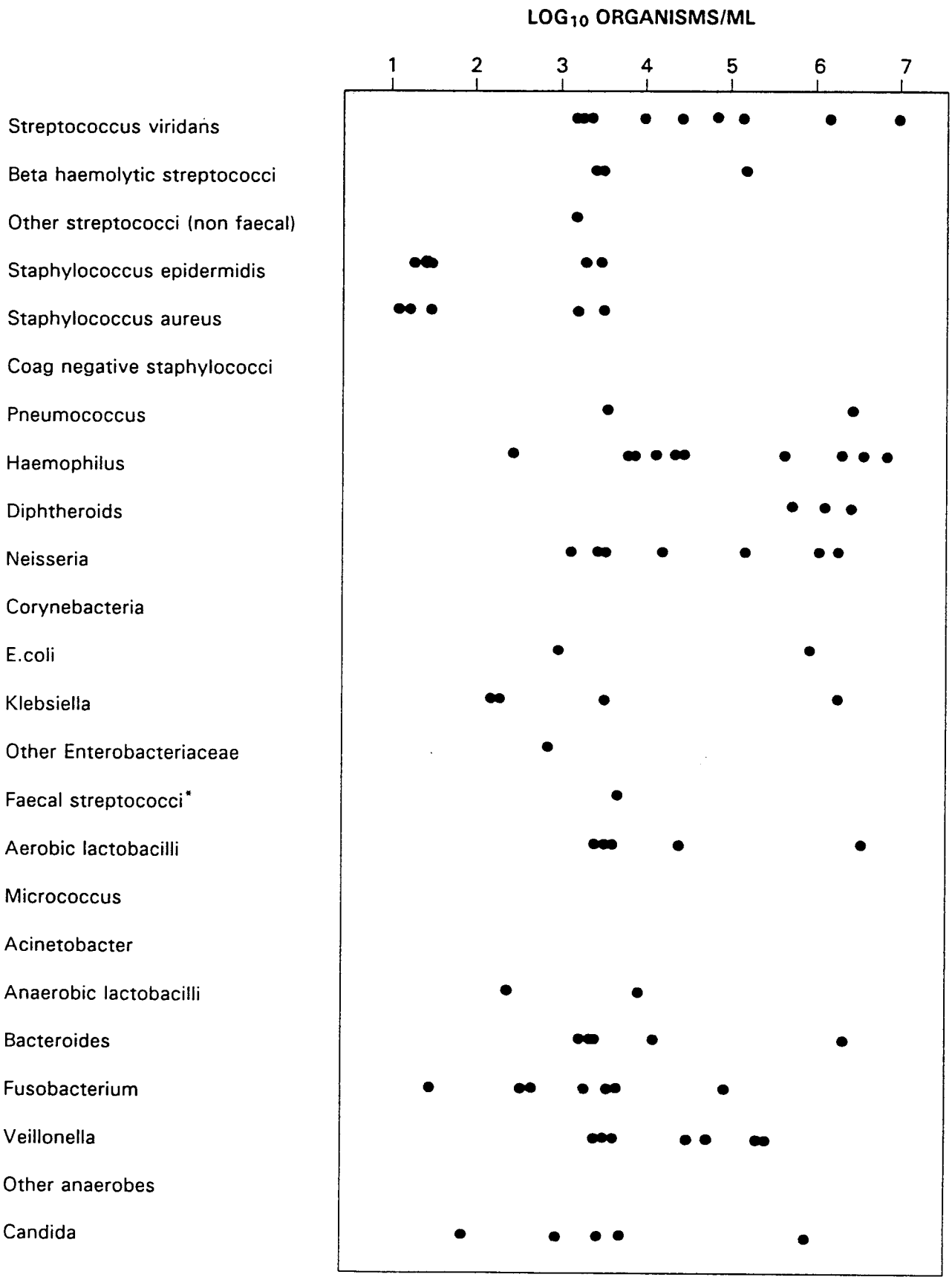
DAY 3 DUODENAL MICROFLORA OF CHO-FREE GROUP



* Streptococcus faecalis, anaerobic streptococci

FIGURE 4

DAY 6 DUODENAL MICROFLORA OF CHO-FREE GROUP



* Streptococcus faecalis, anaerobic streptococci

DAY 3

There was little difference between the two groups. The only statistically significant differences were that *Bacteroides* spp. were found in greater numbers in the carbohydrate-containing group ($p = 0.029$), and anaerobic lactobacilli were more numerous in the carbohydrate-free group ($p = 0.05$).

DAY 6

There was a tendency for a greater number of bacterial isolates and higher bacterial numbers in the carbohydrate-free group. This reached statistically significant levels only for *Haemophilus* ($p = 0.01$). When the upper respiratory organisms were analysed together the number was greater in the carbohydrate-free group ($p = 0.045$). This was accounted for by the *Haemophilus* fraction.

BETWEEN DAY 3 AND DAY 6

Within groups:

There was a tendency for the number of bacterial isolates and their magnitude to fall in the carbohydrate-containing group. This did not reach statistical significance. There was virtually no change in the carbohydrate-free group.

BOWEL COCKTAIL STUDY

CLINICAL DETAILS ON ADMISSION TO STUDY

The study group comprised 15 patients. Fourteen were infants from the carbohydrate part of the study with ongoing diarrhoea and stool weights greater than 30g/kg/day on day 5. Eight of these patients were from the carbohydrate-containing group, and 6 from the carbohydrate-free group. The infant formulae were not altered for the duration of the study. The remaining patient (no. 21) was selected for study on day 5 and comprised the

"late entry" group. His stool output for day 5 was greater than 30g/kg and he was admitted into the study. He received Isomil feeds.

Table 10 outlines relevant clinical details on day 2 (day 5 for the late entry infant).

TABLE 14

	Mean	**Median**	**Range**
Age (months)	6.13	6.01	1.45-13.2
% of expected weight	87.2	87	636-111
Weight for length %	94.2	92	73-138
Duration of diarrhoea (hrs)	59	72	24-96
preceding hospital admission		(Mode = 72)	
Dehydration (%)	2.5	2	0-6
on admission to trial		(Mode = 0)	

HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

TABLE 11

	Mean	Median	S.D.	Range
Haemoglobin g/dl	10.6	10.5	0.95	9.2-12.3
Leucocytes $x10^9/l$	12.4	11.9	2.06	9.3-17.2
Platelets $x10^9/l$	674	685	131	394-911
Sodium mmol/l	137	137	3.2	130-141
Potassium mmol/l	4.5	4.1	1.08	3.2-7.1
Albumin g/l	36	37	5.4	24-46
pH	7.31	7.32	0.02	7.18-7.47
Base deficit mmol/l	6.8	7.6	2.2	19.7-+17.4

STOOL OUTPUT

Table 12 shows the stool weights for this study group. The day 2 to day 4 values apply to 14 patients only, since the "late entry" infant was not studied during those days.

TABLE 12

Stool output in g/kg

	Mean	Median	S.D.	Range
n = 14				
Day 2	81.6	65.2	47.6	30.9-171.1
Day 3	98.2	71.6	63.5	28.6-206.6
Day 4	108.1	92.9	64.8	21.4-210.9
n = 15				
Day 5	99.7	86.7	55.6	37-204.2
Day 6	54.1	36.1	43.3	16.9-157.3
Day 7	21.7	20.5	14.1	7.1-65.7
Day 8	20.3	18.4	11.8	2.5-39.1
Day 9	19.6	18.8	13.1	3.7-50.3
Day 10	17.5	15.2	12.9	0-46.9

The stool output tended to increase from the day of admission until administration of the bowel cocktail on day 6 (the day 6 stool output was the first to be potentially affected by this therapy). This difference did not reach statistically significant levels.

Administration of the bowel cocktail was associated with a marked fall in stool output. This was highly significant beginning on the first day of treatment (D5 vs D6, $p = 0.009$). Statistical analysis for the other days is as follows:

D5 vs D7, $p = 0.003$

D5 vs D8, $p = 0.003$

D5 vs D9, $p = 0.001$

D5 vs D10, $p = 0.003$

CLINICAL COURSE

Two patients (nos. 12 and 21) had a stool output in excess of 30g/kg at the end of the study. In patient no. 21 this was associated with soft stools and he was discharged from

hospital. Patient no.12 had ongoing diarrhoea. In this patient *Giardia intestinalis* trophozoites had been present in all the duodenal aspirates. At the end of the study period he received a three day course of metronidazole with no improvement (stool weight of 41g/kg at the end of treatment). His formula was then changed to Nutramigen. This was associated with resolution of his diarrhoea and he was subsequently discharged from hospital.

Patient no. 2 developed a new episode of profuse watery diarrhoea and fever the day after completion of the study. *Klebsiella pneumoniae* grew in the blood culture. No bacterial pathogens were isolated in the stool. He was treated with parenteral antibiotics and his milk was changed to nutramigen. He rapidly improved and the diarrhoea resolved.

On completion of the study 4 of the 6 patients receiving the carbohydrate-free milk were successfully changed to Isomil. The two exceptions were patient no. 12 (already mentioned) and patient no. 17. This was an infant with loose stools at the end of treatment, despite a stool output of only 21g/kg/day. His milk was changed to nutramigen and his stools became firmer.

No complications of any type were encountered during this part of the study.

STOOL PATHOGENS

There were 15 patients in this part of the study. Stool specimens were taken on day 6 and day 8. Rotavirus was only looked for on day 6 only in patient no.21, the "late entry" infant. A total of 29 stools were analysed.

Table 13 lists the patients and their stool pathogens.

TABLE 13

Patient no.	Day 3	Day 6	Day 8
1	*Campylobacter jejuni*	*Campylobacter jejuni*	
2		*E.coli* O111	
3	*E.coli* O127	*E.coli* O127	
4	Rotavirus	*Cryptosporidium*	
5	Rotavirus		
6	Rotavirus		
7			
8			
11	*E.coli* O126		
12	*Shigella flexneri*	NO SPECIMEN	
13	*Cryptosporidium*	*Cryptosporidium*	
		Salmonella group B	
17			
18	*Campylobacter jejuni*	*Campylobacter jejuni*	
20	*Campylobacter jejuni*	*Campylobacter jejuni*	
	E.coli O126	*E.coli* O126	
21			

Eight patients had stool pathogens on day 6. If one includes the stool samples on day 3 a total of 11 patients were excreting enteric pathogens in the stool at some stage of the study.

No stool pathogens were detected in any patient on day 8.

DUODENAL FLORA

Microscopy

Giardia intestinalis trophozoites were seen on day 6 and day 8 in patient no.12. *Cryptosporidium* cysts were seen on day 8 in patient no.13.

Bacteriology

Tables 14 and 15 show the bacteriological findings on day 6 and day 8. Figures 5 and 6 provide a more visual representation of the bacteriology.

TABLE 14

DUODENAL FLORA OF BOWEL COCKTAIL GROUP ON DAY 6

	Number of Isolates	Organisms in log_{10}/ml Median	Min	Max	LQ*	UQ*
Streptococcus viridans	9	2.90	0.00	7.14	0.00	4.45
Beta haemolytic streptococci	3	0.00	0.00	5.22	0.00	0.00
Other streptococci	1	0.00	0.00	3.14	0.00	0.00
Staphylococcus epidermidis	7	0.00	0.00	3.36	0.00	3.05
Staphylococcus aureus	6	0.00	0.00	3.83	0.00	1.59
Coagulase neg staphylococci	0					
Pneumococcus	2	0.00	0.00	6.44	0.00	0.00
Haemophilus	11	3.78	0.00	6.90	0.00	5.22
Diphtheroids	2	0.00	0.00	5.74	0.00	0.00
Neisseria	8	2.88	0.00	6.14	0.00	4.29
Corynebacteria	0					
E.coli	5	0.00	0.00	5.96	0.00	2.99
Klebsiella	6	0.00	0.00	4.79	0.00	3.87
Citrobacter	1	0.00	0.00	2.82	0.00	0.00
Enterobacter	1	0.00	0.00	3.18	0.00	0.00
Providencia	1	0.00	0.00	3.26	0.00	0.00
Proteus	0					
Streptococcus faecalis	0					
Aerobic lactobacilli	9	2.84	0.00	5.00	0.00	4.44
Micrococcus	3	0.00	0.00	4.93	0.00	0.00
Acinetobacter	0					
Anaerobic streptococci	1	0.00	0.00	3.76	0.00	0.00
Anaerobic lactobacilli	3	0.00	0.00	5.06	0.00	0.00
Propionibacterium	1	0.00	0.00	1.23	0.00	0.00
Bacteroides	5	0.00	0.00	6.44	0.00	3.59
Fusobacterium	9	1.66	0.00	5.17	0.00	3.39
Veillonella	11	3.09	0.00	6.69	0.00	4.61
Actinomyces	0					
Candida	9	1.89	0.00	3.72	0.00	2.84
Upper respiratory microorganisms	49	4.18	0.00	7.20	2.72	5.86
Enterobacteriaceae	14	2.82	0.00	5.96	0.00	4.11
Aerobic microorganisms	83	4.44	1.53	7.20	4.06	5.86
Anaerobic microorganisms	30	3.71	0.00	6.69	2.64	5.01
TOTAL	**113**	**4.77**	**1.70**	**7.32**	**4.06**	**5.91**

* LQ = Lower quartile, UQ = Upper quartile

TABLE 15

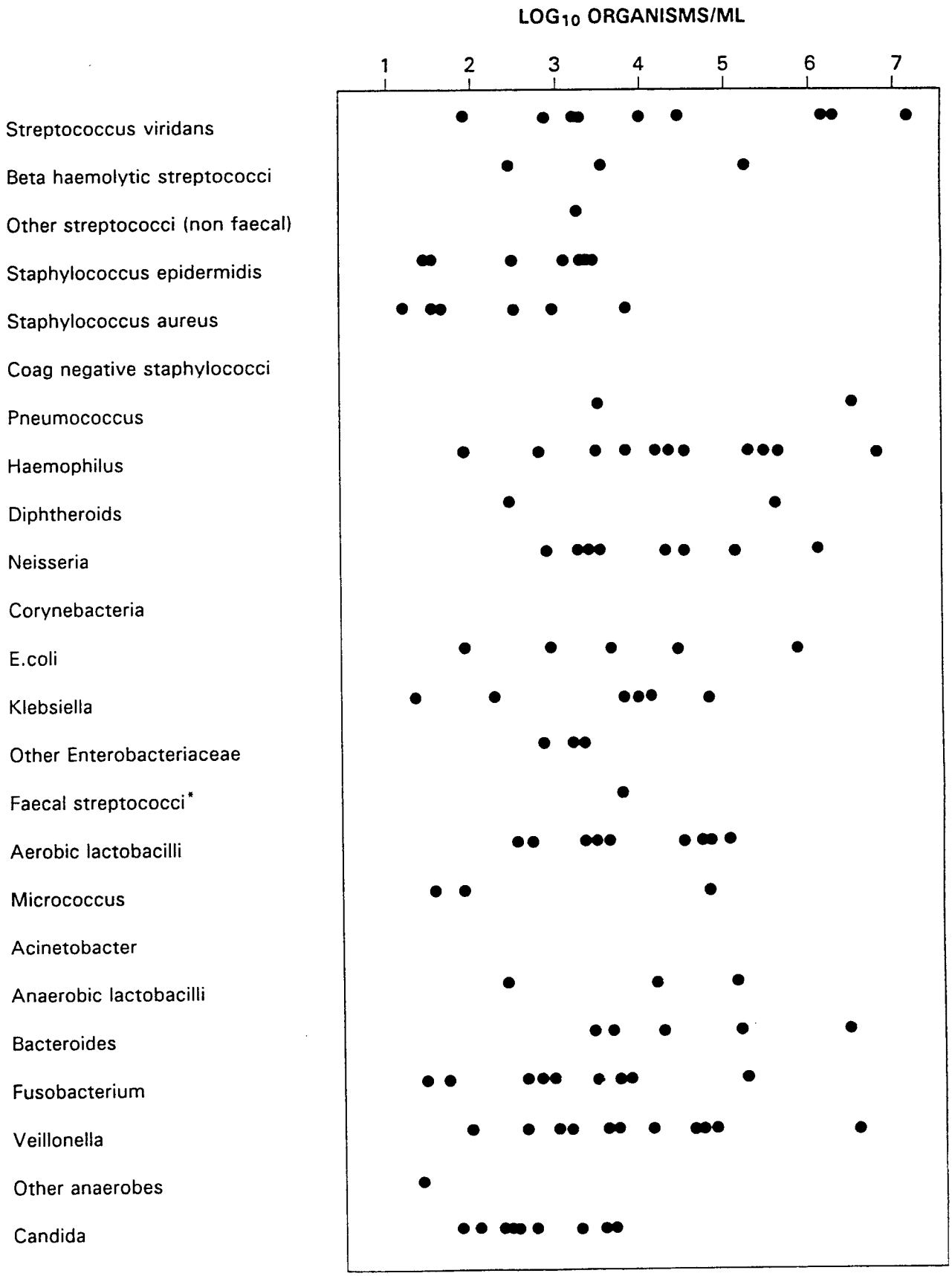
DUODENAL FLORA OF BOWEL COCKTAIL GROUP ON DAY 8

	Number of Isolates	Organisms in log_{10}/ml Median	Min	Max	LQ*	UQ*
Streptococcus viridans	6	0.00	0.00	6.51	0.00	3.10
Beta haemolytic streptococci	1	0.00	0.00	1.69	0.00	0.00
Other streptococci	0					
Staphylococcus epidermidis	8	1.54	0.00	2.84	0.00	2.10
Staphylococcus aureus	5	0.00	0.00	3.71	0.00	1.69
Coagulase neg staphylococci	2	0.00	0.00	3.02	0.00	0.00
Pneumococcus	2	0.00	0.00	3.81	0.00	0.00
Haemophilus	1	0.00	0.00	3.75	0.00	0.00
Diphtheroids	5	0.00	0.00	4.41	0.00	2.14
Neisseria	0					
Corynebacteria	0					
E.coli	2	0.00	0.00	2.28	0.00	0.00
Klebsiella	6	0.00	0.00	3.53	0.00	1.73
Citrobacter	0					
Enterobacter	0					
Providencia	0					
Proteus	0					
Streptococcus faecalis	0					
Aerobic lactobacilli	6	0.00	0.00	4.43	0.00	2.83
Micrococcus	0					
Acinetobacter	0					
Anaerobic streptococci	1	0.00	0.00	4.67	0.00	0.00
Anaerobic lactobacilli	0					
Propionibacterium	1	0.00	0.00	2.24	0.00	0.00
Bacteroides	3	0.00	0.00	5.30	0.00	0.00
Fusobacterium	3	0.00	0.00	5.30	0.00	0.00
Veillonella	3	0.00	0.00	5.30	0.00	0.00
Actinomyces	0					
Candida	10	2.24	0.00	5.90	0.00	3.72
Upper respiratory microorganisms	30	3.01	0.00	6.51	2.15	4.31
Enterobacteriaceae	8	0.00	0.00	3.53	0.00	2.18
Aerobic microorganisms	54	3.40	0.00	6.51	3.13	4.92
Anaerobic microorganisms	11	0.00	0.00	5.77	0.00	2.67
TOTAL	**65**	**3.59**	**0.00**	**6.51**	**3.26**	**5.72**

* LQ = Lower quartile, UQ = Upper quartile

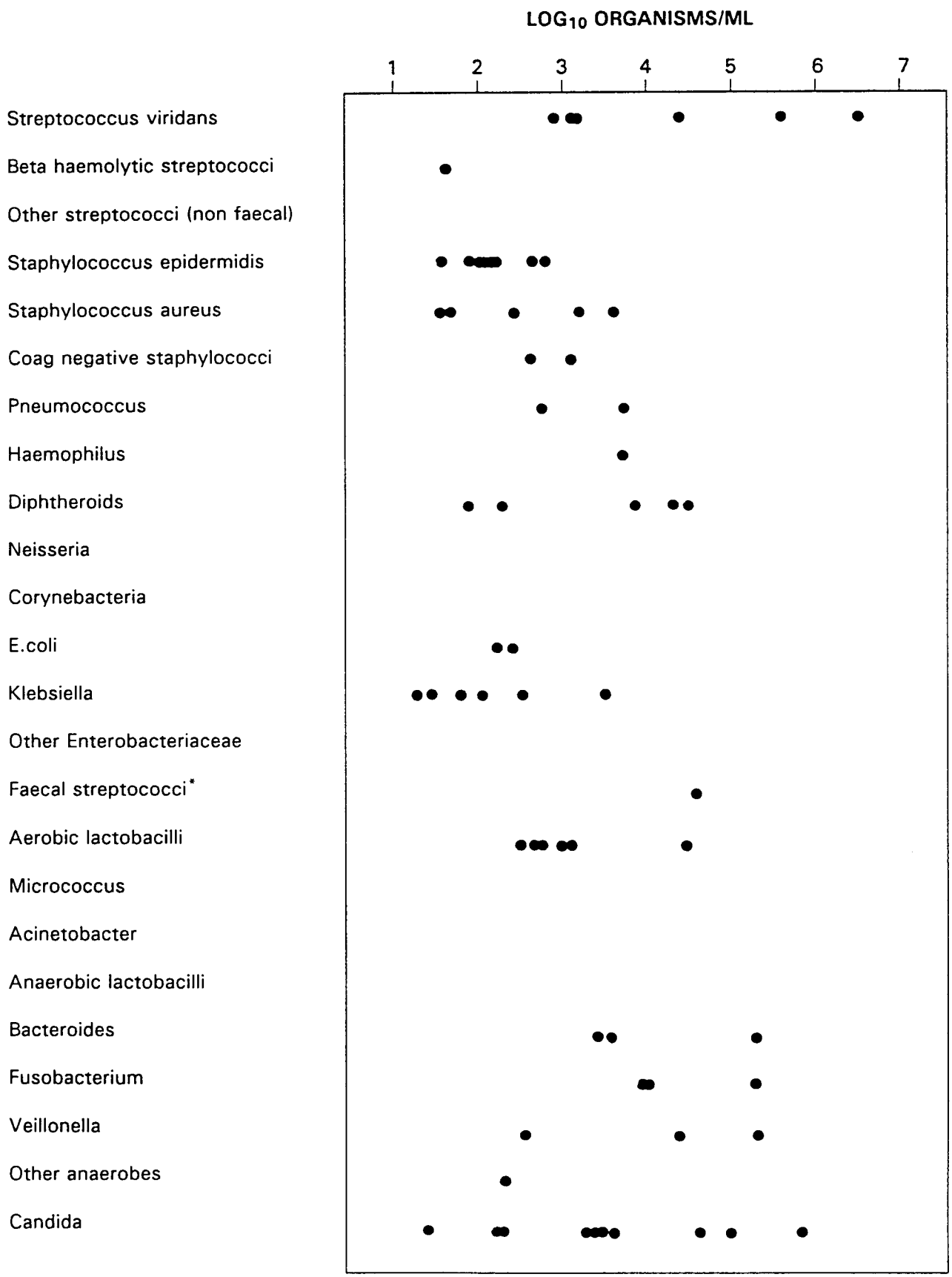
FIGURE 5

DAY 6 DUODENAL MICROFLORA OF BOWEL COCKTAIL GROUP



* Streptococcus faecalis, anaerobic streptococci

FIGURE 6 DAY 8 DUODENAL MICROFLORA OF BOWEL COCKTAIL GROUP



* Streptococcus faecalis, anaerobic streptococci

COMPARISON OF DAY 6 WITH DAY 8

Microorganisms were isolated less frequently and in smaller numbers on day 8. These differences reached statistically significant levels for the following groups:

Total microorganism count	$p = 0.038$
Aerobic microorganisms	$p = 0.038$
Enterobacteriaceae	$p = 0.043$
Microorganisms sensitive *in vitro* to gentamicin*	$p = 0.009$.

* The sum of staphylococci, *Haemophilus*, diphtheroids, *Neisseria*, *Corynebacterium*, *Enterobacteriaceae*, *Micrococcus*, *Acinetobacter*.

A decrease was noted in the number of isolates and magnitude of anaerobic bacteria, but this did not reach statistical significance.

When the microorganisms were analysed individually the following were found in significantly smaller numbers on day 8.

Neisseria	$p = 0.013$
Haemophilus	$p = 0.015$
Aerobic lactobacilli	$p = 0.045$

CHAPTER 7

DISCUSSION

DISCUSSION

A. CARBOHYDRATE AND THE DUODENAL FLORA

In investigating the effect of diet on the intestinal flora two entirely different strategies may be used. The oldest and best established is that of conventional taxonomic bacteriology, by means of which microorganisms are classified in terms of numbers and types. An extensive *corpus* of work exists on diet and the intestinal flora using conventional bacteriological methods. The alternative approach observes the changes occurring in the metabolic activities of the bacterial population[165]. These changes can be detected by measuring selected enzymes produced by the bacteria. The metabolic activities do not necessarily reflect the quantity or type of bacteria; widely different microorganisms (by taxonomic criteria) may share the same metabolic properties. This functional approach has been shown to be a more sensitive indicator of dietary-induced changes than merely counting and classifying the various microorganisms. Profound changes can occur in the metabolic activities of bacteria, with little or no change in their numbers or Latin and Greek names. These metabolic changes have been implicated in the link between diet and cancer of the large bowel[166].

For the purpose of this thesis it was decided to observe the effect of diet on the duodenal flora by conventional bacteriological methods. This was for the following reasons: Coello-Ramirez, whose hypothesis is being tested, used standard bacteriological techniques. The theories which he proposed were based on bacterial numbers, not on bacterial metabolic activities as measured by enzymes. It is logical that to test his hypothesis a similar yardstick should be used. Secondly, the day 6 duodenal intubation of the carbohydrate study also formed the first of the intubations of the bowel cocktail section of the thesis. This investigated the effects of a gentamicin/cholestyramine combination on the duodenal flora and it was essential that the microorganisms should be analysed in terms of accepted taxonomic groups. Finally, the department in which the author worked has over the last

decade acquired extensive expertise in the conventional bacteriological analysis of small intestinal fluid[162,178]. It was sensible to continue using the same, well-proven methods.

In this investigation omitting carbohydrate from the diet produced no effect on duodenal flora. The number or types of bacteria isolated remained virtually unchanged.

These results show that the bacterial population of the duodenum is maintained, and can even thrive, in the absence of dietary carbohydrate. This is in sharp contrast with the observed response of the colonic flora to dietary carbohydrate limitation. When the amount of carbohydrate entering the colon is decreased by the use of an elemental diet or by diminishing dietary fibre a marked fall in bacterial numbers results. The most extreme example of this was the study by Winitz in which the faeces became almost sterile[328]. But this decrease usually manifests as a dramatic drop in stool weight rather than as a fall in the faecal bacterial concentration, as expressed in number of microorganisms/g of faeces.

The interesting difference between the duodenal and colonic flora in their response to carbohydrate limitation may be explained by the size of their respective resident bacterial population. The number of bacteria inhabiting the large bowel is enormous. It is likely that the maintenance of this colonic flora is largely dependent on the bacteria using as nutrient sources undigestible fibre or carbohydrates that have escaped digestion in the small bowel. From the viewpoint of the host this process is termed colonic salvage, from a bacterial perspective it is probably necessary for survival. When the exogenous nutrient source is removed the endogenous pabulum in the form of colonic mucus and shed epithelial cells is probably insufficient for the needs of the resident flora. A fall in bacterial numbers results.

In contrast, the resident bacterial population of the small intestine is much smaller than that of the colon (even in the case of small intestinal bacterial overgrowth). It is likely that the endogenous nutrient supply in the form of epithelial cells, mucus, and possibly the small

amount of glucose present in normal small intestinal secretions and swallowed saliva, may be quite sufficient to maintain bacterial numbers. In addition, although in this study dietary carbohydrate was omitted, other nutrients in the form of protein and fat were not. It is plausible that sufficient carbon skeletons were present in the diet for the nutrient and metabolic needs of the bacteria.

Further, the administration of a carbohydrate-free milk was associated with no demonstrable changes in the qualitative bacterial flora of the duodenal juice. The number of different types of microorganisms remained virtually unchanged. Any differences observed in the bacterial types were slight and no discernible pattern was seen.

The above findings of virtually no qualitative changes are in agreement with some of the studies dealing with the effects of elemental diets on the faecal flora[13,49] but other investigators have shown qualitative changes. Attebery et al found a decrease in the ratio of anaerobes to aerobes, explainable by a rise in some aerobic microorganisms, mainly *Enterobacteriaceae*[11].

No such altered anaerobe to aerobe ratio was seen in this study. The rise in the number of *Enterobacteriaceae* observed by Attebery, and Crowther et al[84] was not found in the current investigation. Several authors agree that the faecal population of enterococci (faecal streptococci) diminished after giving an elemental diet[50,84]. Enterococci are rarely found in the duodenal lumen - in this study only one patient had enterococci in the duodenal juice before administration of the carbohydrate-free milk. On day 6 no enterococci were isolated.

When the duodenal flora of the carbohydrate and carbohydrate-free groups is compared on day 6 after three days of their respective diets, the only significant difference to emerge is for *Haemophilus* ($p=0.01$). All ten samples of duodenal juice from the carbohydrate-free group contained organisms of the *Haemophilus* genus, compared with five from the

carbohydrate group. The median *Haemophilus* count of the carbohydrate-free group was over 1000 times higher at 4.46 $\log_{10}$ organisms/ml, compared with 0.97 $\log_{10}$ organisms/ml for the carbohydrate group. This interesting finding cannot be explained simply in terms of greater substrate availability. One would have expected greater *Haemophilus* numbers in the carbohydrate-containing group if this was the case. The most likely explanation is that this was a chance finding. This is always a problem when interpreting studies on intestinal microbiology, in which so many results are being compared.

Another possible explanation for the rise in *Haemophilus* numbers can be provided in terms of competition for bacterial attachment sites. It is accepted that bacteria bind to epithelial surfaces by means of receptors[25]. Lectins on the surface of the microorganisms adhere to specific receptor sites on the epithelial cell surface. These receptors are usually sugars, such as glycoproteins or glycolipids[243] and it is possible to competitively inhibit bacterial attachment to mucosal surfaces in the presence of alternative binding sites. If an exogenous source of carbohydrate identical to the receptor carbohydrate is given it can act as a false receptor, with the bacterial lectins (and hence the bacterium) binding to the false receptor rather than to the epithelial cell surface.

There is evidence that small intestinal mucus contains receptors for certain enteric pathogens[324] and that this may prevent diarrhoea by acting as a false receptor. At present it is unclear whether the bacteria which attach to the mucus layer are able to produce diarrhoea in the host.

In vitro experiments have shown diminished adherence of *E.coli* and other microorganisms to intestinal cells, in the presence of breast milk[10,172]. It has been postulated that carbohydrate present in human milk may prevent enteric infection by acting as false receptors, thereby preventing attachment of the pathogen to the intestinal mucosa. This

may provide yet another mechanism by which breast feeding exerts a protective effect against gastroenteritis.

The attachment of *Haemophilus influenzae* to cell surfaces is also by means of binding to specific carbohydrate receptors. The situation is complex, different receptors existing at different sites[315]. It is not known whether the other species of the *Haemophilus* genus share the same receptor. *In vitro* experiments by Andersson et al[7] have shown that the high molecular weight fraction of human milk prevents attachment of *Haemophilus influenzae* to epithelial cells, but modified cow's milk formula, cow's milk and buffalo milk had the opposite effect. They increased adherence.

There is no information available on the relative *Haemophilus* population of lumen and mucosa, or on the dynamics between them. Any attempt to explain the observed increase in the luminal *Haemophilus* numbers in terms of displacement from binding sites must therefore remain highly speculative.

The key question to emerge from the carbohydrate part of the study is: what do the results mean in relation to the publication of Coello-Ramirez? He interpreted the findings of an increasingly luxuriant duodenal flora *pari passu* with severity of carbohydrate intolerance in one of two ways. Put in the simplest terms either the abnormal flora produced carbohydrate intolerance, or the carbohydrate intolerance encouraged bacterial growth. The present study found that elimination of carbohydrate from the diet did not decrease the duodenal flora. This strongly suggests that the availability of carbohydrate in the duodenal lumen is not an important factor in the dynamics of the bacterial population of that region. The logical conclusion is that in the patients of Coello-Ramirez it was the abnormal duodenal flora that produced carbohydrate intolerance. This interpretation fits in well with the animal and *in vitro* experiments, presented in chapter 4, which showed that various bacterial preparations can produce carbohydrate maldigestion.

These conclusions must be tempered by the *caveat* that the two investigations differ greatly in several respects. Although few details are given about the duration of diarrhoea in the series of Coello-Ramirez, many infants had persistent diarrhoea, whereas all the patients in the present study had a history of diarrhoea of one week or less at the time of starting the special feeds. Most patients in the Mexican study were malnourished, and some had kwashiorkor. This contrasts with the Cape Town patients, who had a median expected weight approaching 90%. The most striking aspect of the Mexican study was the high incidence of true carbohydrate intolerance, as defined by the presence of reducing substances in the stool and prompt resolution of the diarrhoea on elimination of the offending carbohydrate from the diet. Sixty eight percent of the patients had intolerance to either lactose, sucrose, or all dietary carbohydrates.

In the present study of the ten infants in the carbohydrate-containing (sucrose and glucose polymers) group two were possibly lactose intolerant, in so much as the diarrhoea resolved on removal of lactose from the feed. In the remaining eight infants no change was noted on removal of lactose and the diarrhoea resolved without further dietary manipulation on being given the bowel cocktail. Of the ten infants in the group in whom all dietary carbohydrates were excluded the diarrhoea stopped in three: in those patients lactose, sucrose, or glucose intolerance could possible be incriminated. In summary at most 25% (5 of 20) infants could be described as being carbohydrate intolerant.

Malnutrition and carbohydrate intolerance are linked. There is evidence that in animals and humans malnutrition is associated with diminished intestinal disaccharidase levels[227,262] and that malnutrition may actually cause clinical carbohydrate intolerance[185]. In addition it has been shown *in vitro* that bacteria leach disaccharidases to a greater extent from malnourished small intestinal cells than from well-nourished enterocytes[282].

It is clear therefore that host factors, quite apart from duodenal bacterial numbers, may have contributed to the carbohydrate intolerance observed in the infants of Coello-Ramirez.

As already mentioned an interesting observation in this investigation was the small number of patients who responded to withdrawal of lactose from the infant formula. The proportion of presumably lactose intolerant infants was lower than that found in the author's institution one decade ago: in a Cape Town publication describing infants studied in 1981 and 1982 about 50% of patients with severe diarrhoea responded to lactose withdrawal[53]. The clinical experience with numerous infants was in agreement with this figure. The decline in lactose intolerance in recent years has also been substantiated in a contemporaneous study: in a group of infants with severe diarrhoea the illness resolved with equal frequency in those who were given Soya milk with lactose as the carbohydrate source compared with the patients who received Soya with glucose polymers and sucrose (Bowie MD, unpublished).

This trend of decreasing incidence of lactose intolerance during acute diarrhoea mirrors the experience of Western countries.[9,313] Walker-Smith has hypothesised that lactose intolerance may be a consequence of cow's milk protein intolerance[321]. He explains the decline in lactose intolerance to the widespread use of new low-solute humanised cow's milk formulae. The preparation of these new formulae denatures the milk proteins more than was previously the case and hence renders the proteins less allergenic. By sensitising the intestinal mucosa to cow's milk protein less than the old high-solute milks the newer preparations are thought to decrease the incidence of cow's milk protein intolerance, and hence of lactose intolerance.

Walker-Smith's hypothesis fits in neatly with the Cape Town experience of lactose intolerance. The declining incidence of this phenomenon coincides with the widespread use of low-solute infant formulae in this city. The theory also explains the lag period compared with Western countries, where the decline was already noticeable by 1980. Whereas in developed countries the newer infant formulations were popular by the late 1970's, in Cape Town it is only in the 1980's that they were widely adopted.

Of the ten infants in whom all carbohydrates were withdrawn from the diet the diarrhoea resolved in three. Comparing this number with that of the two "responders" in the group in whom only lactose was excluded suggests that intolerance to other carbohydrates - sucrose, glucose polymers, glucose - was not an important feature of the patients in this investigation. It is interesting that two of the three responders in the carbohydrate-free group were excreting Rotavirus in the stools. Monosaccharide intolerance is associated with Rotavirus gastroenteritis[221], and one can speculate that these infants were suffering from glucose intolerance.

What conclusions can be drawn from the pattern of response to dietary carbohydrate manipulations in the study patients? Lactose intolerance was not an important factor in causing diarrhoea and withdrawal of lactose from the diet is unlikely to be a useful measure. Similarly, withdrawal of other carbohydrates is not likely to play an important part in the management of acute gastroenteritis.

It must be stated that the patients were not severely malnourished and that the dietary manipulations were done at a relatively acute stage of gastroenteritis - in all patients the diarrhoea was of one week's duration or less. Manipulation of dietary carbohydrates may play a more important part in treating malnourished infants with acute gastroenteritis, or those who have persistent (longer than two week's duration) diarrhoea. Malnourished children with acute diarrhoea have a high incidence of intolerance to many dietary carbohydrates[112]. Infants and children with persistent diarrhoea frequently have severe malabsorption of lactose[247], sucrose[76], glucose polymers[112], and monosaccharides[214]. Malnutrition and persistent diarrhoea frequently coexist and in interpreting the studies that have been done it is impossible to dissect out their relative contribution to carbohydrate intolerance.

One might question the validity of testing the effect of nutrient excess by means of nutrient deprivation. Bacteria, although the simplest independent living organisms, possess a remarkable degree of adaptability allowing survival in very hostile environments. They have great metabolic flexibility, with the ability to induce or repress metabolic enzymes, depending on substrate availability. They can even change their principal metabolic pathways to suit the prevailing nutrient conditions[154]. It is therefore conceivable that nutrient limitation may not affect the duodenal bacterial population, while the same bacterial flora might respond to nutrient excess by an increase in numbers. Although no relevant studies could be found dealing with the flora of the small intestine the available examples from the mouth and colon suggest that limiting the amount of carbohydrate entering these regions does lead to a decrease in bacterial numbers. Completely omitting carbohydrate from the diet leads to a drastic fall in the numbers of the cariogenic species *Streptococcus mutans* in the mouth[93]. As has been reviewed in chapter 4 the colonic bacteria also respond to a limitation in carbohydrate intake by a fall in numbers. There is no reason to suppose that the responses of the metabolic enzymes of the small intestine bacteria to carbohydrate limitation should be different to those of the bacterial ecosystems of the mouth and colon.

One may circumvent the previously mentioned criticism by postulating that it is the carbohydrate-containing groups which could be viewed as the study group in whom the effect of carbohydrate on the bacterial flora is being investigated. There is evidence from stool balance studies that carbohydrate malabsorption (as distinct from intolerance) is a universal finding in infants with severe gastroenteritis[53,220]. All the infants admitted in this study had severe diarrhoea and the infants who received the carbohydrate-containing milk would be expected to be malabsorbing some of the carbohydrate, which would then be available to the bacteria. In support of this is their higher stool output compared with the patients receiving no carbohydrate, although it must be stated that their stool volume was greater even before the start of the trial.

On examining the effect of a carbohydrate-containing milk on the duodenal flora no major differences were observed when compared with the flora of a group that received no carbohydrate. There was no increase in the total number of microorganisms or in any individual genera. The carbohydrate present in the feed and readily available to the duodenal bacterial population did not act as a pabulum. The conclusions which had been reached when observing the flora of the carbohydrate-free group were still valid even when the carbohydrate-containing group was examined.

CONCLUSIONS

In the context of whether malabsorption of carbohydrates leads to duodenal bacterial proliferation or *vice versa*, it is very unlikely that the presence of maldigested carbohydrates in the duodenal lumen leads to an increase in bacterial numbers. One may interpret the findings of Coello-Ramirez by postulating that it was the bacteria which were the cause of the carbohydrate intolerance. This explanation is in keeping with the experience of numerous *in vitro* and animal experiments.

The theory that during gastroenteritis the presence of malabsorbed carbohydrate in the small bowel is a factor promoting small intestinal bacterial overgrowth has often been stated as fact. The findings of this investigation do not support this and suggest that malabsorbed carbohydrate is not important in encouraging bacterial proliferation. One can presume that the endogenous carbohydrate supply in the form of mucus glycoproteins or shed epithelial cells is sufficient for the bacterial needs. In addition, maldigested protein and fat, plentiful in severe gastroenteritis[220], might be expected to supply additional nutrient substrate for the bacteria.

B. BOWEL COCKTAIL ADMINISTRATION AND THE DUODENAL FLORA

DISCUSSION OF DAY 6 FINDINGS

In discussing the duodenal flora prior to the administration of the bowel cocktail, in an attempt to explore the mechanisms of action of this agent and also to set the author's findings in context with those of other workers, the duodenal bacteriology was approached in two ways. Firstly, what was the general pattern of the flora? Was there bacterial overgrowth in the accepted sense of the term? Secondly, were there any specific enteric pathogens present, possibly causing diarrhoea and on which the gentamicin/cholestyramine combination might be acting?

General features of the duodenal bacteriology

The striking feature of the duodenal flora on day 6 was the lack of a consistent pattern among the patients. The total microorganism count varied from almost sterile to very high. In three patients (nos. 3, 4, 5) the total count was below 4 $\log_{10}$ organisms/ml - the usually quoted, though highly arbitrary, upper limit of normal. Five further patients (nos. 2, 7, 13, 18, 20) had total counts between 4 and 5 $\log_{10}$ organisms/ml. The remaining 7 had counts exceeding 5 $\log_{10}$ organisms/ml. The number of different microorganisms isolated in each duodenal aspirate was likewise highly variable, ranging from 2 to 12. Usually no single isolate predominated (greater than 1 $\log_{10}$ organisms/ml) over the other bacteria which were cultured: in only 3 patients (nos 1, 7, 8) was there a predominant isolate.

The group of patients with whom the day 6 findings can be most closely compared are the 6 infants comprising group 3 of the study by Hill and colleagues from the same institution

as the author[162]. These infants were studied at a similar stage of the illness and the inclusion selection criteria were also very similar.

The duodenal flora on day 6 of the current investigation was altogether "cleaner" than in Hill's patients. The median total microorganism count was about 100 times smaller: 4.77 $\log_{10}$ organisms/ml compared with 7.1 $\log_{10}$ organisms/ml. There were also fewer different microorganisms isolated in the individual duodenal aspirates: each patient harboured a mean of 7.5 different organisms (113 isolates in 15 patients) compared with 11 in Hill's group 3 infants (66 isolates in 6 patients). Not only were there fewer different bacteria isolated, but their numbers were also smaller. Sixty six percent (75 of 113 isolates) were found in concentration of less than 4 $\log_{10}$ organisms/ml compared to 30% (20 of 66 isolates).

There is evidence that the quantity of duodenal flora (as measured by the total bacterial count) relates to the contamination of the environment[248]. It is tempting to explain the "cleaner" day 6 duodenal fluid in the present investigation to the improvement in public health which has taken place in Cape Town and its hinterland in the decade since Hill's study. Clean tap water is now readily available in the squalid shanty towns surrounding the city, where many of the infants admitted with diarrhoea to the author's hospital reside. In support of a less contaminated environment is a recent survey from the same area which shows a changing trend in the pattern of faecal enteric pathogens (Coltman D, PhD thesis in preparation). In the last decade there has been a decrease in the incidence of bacterial isolates such as *Shigella* and *Salmonella* in the stools of both diarrhoeal patients and controls while the incidence of Rotavirus isolation has remained unchanged. But the hypothesis cannot explain the findings of this study: on comparing the duodenal flora of 14 patients (one infant was a "late entry") in the bowel cocktail group examined on day 3 with Hill's group 2 patients, studied at a similar stage of the illness, no major differences emerge. The median total microorganism count was 5.5 $\log_{10}$ organisms/ml compared to 5.2 $\log_{10}$ organisms/ml in Hill's patients and the mean number of isolates per patient was

7.6 to 8 respectively. The proportion of isolates at concentrations less than 4 $\log_{10}$ organisms/ml in this study was 61% (66 of 108 isolates) compared with 58% (28 of 48 isolates in Hill's study). If one speculates that the duodenal fluid in the author's patients is "cleaner" on day 6 because of improved hygienic conditions in the community then even more would one expect the day 3 specimens to be less contaminated than in Hill's study which is clearly not so. One cannot attempt to explain the more profuse duodenal growth in the group 3 patients of Hill by arguing that they came straight from the community and its attendant contaminated environment, whereas the group 2 patients had already been subjected to four days of "clean" hospital conditions. The group 3 patients were admitted to hospital at an early stage of their diarrhoeal illness and they were hospitalised one week before the duodenal fluid was sampled.

The argument for a cleaner environment, although attractive, cannot therefore be proffered to explain the less luxuriant flora on day 6. This intriguing finding remains unexplained.

Comparing the author's duodenal culture results with those of other workers is difficult because of many possible confounding factors. In other studies the patients came from areas with widely different levels of environmental contamination with its possible effects on the small bowel flora. Many patients were malnourished, again possibly influencing the bacterial population of the small intestine[244]. The stage of diarrhoea differed in studies from other units with small intestinal sampling either during an acute episode (usually less than one week) or when the diarrhoea followed a more prolonged course (usually longer than two weeks). In the current investigation the pre-bowel cocktail duodenal intubation on day 6 (by which time the patients would have had diarrhoea for about 9 days) lies between these time scales of acute and prolonged. Some investigations have not included anaerobic methods, ignoring a potentially important part of the small bowel flora. The results are frequently reported in an unclear way making it impossible to assess the bacterial profiles of individual patients, or sometimes even the total bacterial count.

ACUTE DIARRHOEA

For obvious reasons a valid comparison of total microorganism count can only be made with studies that have used anaerobic methods. In the context of acute diarrhoea an interesting feature emerges: the total bacterial count seen in this investigation is similar to that observed by other workers in developing countries and higher than that observed in the UK. The median total microorganism count on day 6 for the bowel cocktail group was 4.77 $\log_{10}$ organisms/ml and the mean count 4.92 $\log_{10}$ organisms/ml. This is very close to what was seen observed by Penny et al in Peru (mean of 4.7 $\log_{10}$ organisms/ml)[248], and Omoike in Nigeria in well-nourished infants and children (mean of 4.5 $\log_{10}$ organisms/ml)[244]. The total count is lower than that found by Household et al working in the author's institution (median of 5.4 $\log_{10}$ organisms/ml)[178], and by Albert in India (mean of 5.6 $\log_{10}$ organisms/ml)[2]. Penny's et al study from the UK contrasts with these findings[246]: no mention is made of the total microorganism count, but only 16 of 40 infants had total duodenal microorganism counts exceeding 4 $\log_{10}$ organisms/ml. From the figure provided the median count can be estimated at about 3.5 $\log_{10}$ organisms/ml.

The closeness in the total bacterial count between this investigation and other Third World studies can be most plausibly explained by their common link with contaminated living conditions, in contrast with the clean environment of Developed Countries. In support of this theory is the high proportion of patients found to be harbouring faecal organisms in the small intestine in Third World studies, including the author's own (13 of 14 patients on day 3). This contrasts with Penny's UK findings of 9 of 40 patients with faecal bacteria in the duodenum[246] and those of Bishop et al in Melbourne (14 of 39 patients)[44]. The implication is that the total number of microorganisms found in the proximal small bowel during the course of acute diarrhoea is probably an epiphenomenon in which the connecting thread is the contaminated environment. This statement is supported by the findings of the only publication in which the control patients were closely matched for living conditions: no difference was seen between diarrhoea patients and controls[248].

Looking more closely at the small intestinal flora two different patterns have been described in acute diarrhoea: an increased number of upper respiratory type of organisms in Rotavirus diarrhoea, and a faecal-type flora in EPEC gastroenteritis.

McNeish et al in Birmingham observed that infants with acute diarrhoea who excreted Rotavirus in the stools had higher numbers of upper respiratory bacteria in the duodenum compared to patients excreting no recognised enteropathogens[228]. No explanation was given for this finding and in a later study by the same team these observations were not confirmed[246]. In the current investigation the 3 patients in the bowel cocktail group who were excreting Rotavirus on day 3 (nos. 4, 5, 6) did not have an increased upper respiratory type flora on that day when compared to other patients. Their total upper respiratory counts on day 3 were 2.5, 3 and 3.3 $\log_{10}$ organisms/ml respectively. It is likely that McNeish's initial observation was a chance finding.

A more widely reported phenomenon has been the isolation of greater numbers of faecal-type bacteria in the proximal bowel of infants who excreted EPEC in the stools. This has been found in both Developed and Developing countries. McNeish et al found *Enterobacteriaceae* in greater numbers in EPEC-associated gastroenteritis[228]. Penny et al working in the same unit observed that a greater proportion of patients with EPEC diarrhoea had faecal-type organisms in the duodenum[246]. Studies from India and Ethiopia have confirmed this association[2,295]. When the studies are more closely scrutinised it becomes apparent that the increase in faecal bacteria in the small intestine can be accounted for almost entirely by the EPEC component. These EPEC were presumably acting as pathogens. When these EPEC were excluded from the analysis the small intestinal juice of patients excreting EPEC was no different to that of other diarrhoeal patients. McNeish's study, published as an abstract, is the only exception to this.

Three patients in the bowel cocktail groups were excreting diarrhoeagenic EPEC serotypes on day 6 (nos. 2, 3, 20). Their day 6 duodenal flora did not show large numbers of faecal-

type microorganisms when compared to the other patients: in patient no. 3 there were no faecal organisms, no. 20 had less than 1000 nontypable *E.coli*/ml, no. 2 had 4.05 $\log_{10}$ *Klebsiella*. The results of this investigation therefore agree with the previously discussed studies although the patient numbers are too few to draw firm conclusions.

In summary, the duodenal flora during acute diarrhoea found by the author is similar to that of most other Third World studies. The total bacterial numbers are close to those observed by other workers in developing countries and may well be associated with environmental contamination rather than the diarrhoeal episode. The general features of the duodenal flora did not vary in accordance with specific enteropathogen excretion in the stool.

One very important point needs to be made at this stage. On examining the duodenal bacterial profiles of each patient the author has been struck by the uniqueness of each patient. Innumerable permutations of various microorganisms and their numbers are possible, and may actually exist judging by the patients in the present series. Grouping the results together and subjecting the results to statistical analysis may completely obscure the pathogenic importance of the small intestinal flora as it applies to the individual patient. This key concept will be more fully discussed in a later section.

PERSISTENT DIARRHOEA

The median total microorganism count of nearly 4.8 $\log_{10}$ organisms/ml on day 6 was lower than has been found in other studies of persistent diarrhoea. Penny's Peruvian patients had a mean duodenal count of 5.4 $\log_{10}$ organisms/ml. Gracey's Aboriginal children with "chronic diarrhoea" (the definition was not given) had mean total counts of approximately 6 $\log_{10}$ organisms/ml[146]. The comparison with First World experience is startling: Challacombe's infants with persistent diarrhoea following gastroenteritis had a mean total count of 7.3 $\log_{10}$ organisms/ml[72]. This was only 0.5 $\log_{10}$ organisms/ml higher than the control patients with non-sterile cultures, but no diarrhoeal patient had a

sterile duodenal culture, compared with just over half of the controls[70]. Penny's 15 UK infants with persistent diarrhoea also had a mean count of about 7.1 $\log_{10}$ organisms/ml This marked difference between studies from the Developed and Developing countries is striking, especially since in acute diarrhoea the duodenal flora is relatively scanty judging from the limited information available. An explanation for this phenomenon cannot easily be given. To blame a contaminated home environment for these changes is very difficult in the case of First World patients. Possibly hospital-related factors may have played a part, such as the presence of nasogastric tubes, allowing bacteria to gain access into the small intestine. But Challocombe's report the "control" infants - hospitalised infants with failure to thrive - were all fed by nasogastric tube and yet 7 of 13 had a sterile duodenal juice[70]. This interesting and paradoxical difference between a duodenal juice teeming with bacteria in studies from the "hygienic" First World and a less luxuriant flora from the "contaminated" Third World remains unexplained.

The proportion of patients harbouring faecal-type bacteria in their duodenal juice (12 of 15 infants, of whom 11 had *Enterobacteriaceae*) is the same as in Penny's UK study (12 of 15 patients), and very similar to that found by Challacombe (6 of 7 patients). A smaller proportion of Penny's Peruvian patients with persistent diarrhoea harboured *Enterobacteriaceae* than in the author's series (47.6% vs 73.3%, i.e. 11 of 15 infants). It is not possible from Gracey's data to gauge in how many patients faecal organisms were found.

Enteric pathogens

On day 6 only 2 infants were harbouring enteric pathogens in the duodenal fluid. Patient no. 12 had trophozoites of *Giardia intestinalis* on microscopy. No stool specimen was analysed by the bacteriology laboratory for this patient. Patient no. 11 had *E.coli* O126, which was not isolated in the stool.

In contrast 7 of 14 patients (no stool specimen was analysed for patient no. 12) were excreting enteropathogens in the stool. In 3 *Campylobacter jejuni* was isolated; in 3 infants *E.coli* - one each of EPEC serotype O111, O126, O127; in 2 *Cryptosporidium* was detected on microscopy; one had *Salmonella* group B. Two of the 7 patients were excreting multiple pathogens in the stool (*Campylobacter* and *E.coli* O126, *Cryptosporidium* and *Salmonella* group B).

The low incidence of enteric pathogens in the duodenum is consistent with the findings of other workers. Echeverria in Thailand found no enteric pathogens, if one excludes Rotavirus and *Aeromonas*, in the duodenal fluid of 100 infants and children with acute diarrhoea[102]. One may attempt to explain this absence of pathogens by the fact that a string capsule was used to obtain small intestinal juice. Possibly only the most proximal duodenum was sampled, missing enteropathogens residing more distally in the duodenum. The enteric pathogen isolation rate in the duodenum of the group of patients with acute diarrhoea in Penny's et al UK study was also low[246]. In only 3 of 40 infants were any pathogens isolated. Somewhat higher pick-up rates in acute diarrhoea have been found by Bhan (4 of 26 patients)[33] and Omoike (4 of 22 patients)[244]. The highest rate of pathogen detection was by Stintzing et al in Ethiopia[294]: 10 of 25 infants with acute diarrhoea had enteropathogens in the duodenum, all *E.coli*. The experience in persistent diarrhoea is similar to that of acute diarrhoea. Penny et al found enteropathogens in the duodenum of 3 of 15 infants[246]. A similar rate (5/29) was observed by Fagundes Neto[109]. The highest rate of isolation was seen by Bhan and associates[38]. Enteropathogens were found in the duodenum of 12 of 54 infants with persistent diarrhoea. This number is higher possibly because the *E.coli* were more fully characterised than in other studies. If one only includes EPEC from the *E.coli* isolated in the small intestinal fluid, the only type normally tested for in most other small intestinal flora studies, the positive duodenal enteropathogen rate drops to four of 54 infants.

Another striking feature of the day 6 results was the much higher pick-up rate of enteric pathogens in the stool when compared with the duodenal fluid. This has also been a notable feature of other investigations. The isolation rate of *Salmonella* in the small bowel has been low in almost all paediatric studies. Of the 17 patients with positive stool cultures in Echeverria's series none harboured the organism in the duodenum[102]. No *Salmonella* was found by Gracey in the duodenum compared to five positive stool cultures[146]. Bhan found one duodenal isolate and four positive stool cultures, a patient with *Salmonella* in the duodenum also excreting it in the stool. Fagundes Neto has been the only worker to find *Salmonella* in the small bowel in a relatively high number of patients. In his 1976 study[111] in 2 of 3 patients who were excreting *Salmonella* in the stools it was also cultured from the duodenal fluid. In addition the pathogen was found in the jejunum of one patient (in whom the duodenum was not sampled) with a negative stool culture. A more recent publication by the same author after his move to S. Paulo again shows a quite high pick-up rate of *Salmonella*[109]: it was found in the jejunum of 3 patients and in the stools of 8. However, there was little agreement between the jejunal and stool findings: of the 3 jejunal positives in only one was the pathogen found in the stool, and of the 8 stool isolates only one was positive in the jejunum. The reason for this high rate of isolation of *Salmonella* in the South American infants is unclear. It cannot be explained on the assumption of a high rate of biliary excretors of this organism: a recent survey carried out in the same city and at the same time as the latest publication by Fagundes Neto does not show a high rate of asymptomatic stool excretion in infants[128]. To make matters even more confusing yet another study done by Mostaco in collaboration with Fagundes Neto has not shown a high incidence of *Salmonella* isolates in the jejunum: of 67 infants and children less than 2 years old with acute or persistent diarrhoea 2 had positive jejunal isolates and in 17 *Salmonella* was found in the stool[237]. Any concordance between small bowel and stool was not mentioned.

Shigella, the other enteric pathogen usually described in *tandem* with *Salmonella*, was not isolated in the duodenum in the current investigation. *Shigella* has almost never been

found in the small bowel of infants and children. The only report of a positive isolate was by Fagundes Neto[111]: it was found in the jejunum but not the duodenum of an infant with a positive stool culture.

The explanation for the low pick-up rate of *Salmonella* and *Shigella* in the proximal small bowel probably lies in their site of action. Both these microorganisms are known to exert their pathogenic effects in the more distal parts of the small intestine: it is likely that if one sampled the distal jejunal and ileal contents the isolation rate for these pathogens would be higher.

The failure to culture *Campylobacter jejuni* from the duodenum of the three infants who were excreting it in the stools is at first sight surprising, the upper small bowel being the site of action of this enteric pathogen. But an attentive study of the relevant paediatric literature has revealed no instance of *Campylobacter* isolation in the small bowel. In most studies the number of patients excreting this agent in the stool was very low. In Penny's publication from the U.K. 8 infants were excreting *Salmonella*, *Shigella* or *Campylobacter* in the stools but in no patient were these pathogens found in the duodenum[246]. It is likely that *Campylobacter* was the most common pathogen in the stool but no details were given. In the Peruvian study by the same author 20 infants were excreting *Campylobacter* in the stools but unfortunately it is not stated if it was found in the duodenum[248]. Various explanations can be put forward to explain the failure to isolate *Campylobacter*. It is a very delicate microorganism: if only small numbers are present in the small intestinal fluid it is conceivable that the bacteria may die in transit or during the plating procedures, whereas if much greater numbers are found in the stool it is likely that enough bacteria would survive to permit a positive stool culture. Alternative explanations are that the *Campylobacter* might reside distal to the third part of the duodenum, or may only be found adherent to the mucosa.

On day 6 one duodenal culture was positive for *E.coli* of the locally enteropathogenic EPEC variety while 3 stool cultures were positive. There was no concordance between duodenal and stool isolates. The lower rate of positive small intestinal culture compared to the stool has also been found by most other workers, but the isolation rate for EPEC has been higher than for the other previously mentioned enteric pathogens (*Salmonella*, *Shigella*, *Campylobacter*). This is not surprising, since it is known to have a pathological effect on the proximal small bowel[75,167,266,267]. Mostaco et al found that of 31 infants with acute diarrhoea 9 were excreting EPEC in the stools but in only 3 was it found in the jejunum[237]. No mention is made of concordance between jejunum and stool. In the same investigation 36 patients with persistent diarrhoea were studied, with fairly similar results: 14 EPEC were found in the stool and 4 in the jejunum. In a later publication from the same group, of 29 infants with persistent diarrhoea 10 were excreting EPEC in the stool and in 3 it was cultured in the jejunal fluid[109]. The same 3 patients were also excreting EPEC in the stool: in 2 it was an identical serotype to what was found in the jejunum, in the other patient a different EPEC serotype was isolated. In Penny's UK study of 40 infants with acute diarrhoea EPEC was found in the stools of seven and the duodenal fluid of three[246]. There was complete concordance between duodenal and stool serotypes. No EPEC was cultured in the duodenal juice of the remaining 4 patients excreting EPEC in the stool. Of the 15 infants with persistent diarrhoea 3 had EPEC in the duodenum; the same serotype had been present in the stools of these patients during the acute stage of diarrhoea. Unfortunately no stool cultures were done at the time of duodenal intubation, which would have provided a more valid comparison. In Stintzing's group of 27 Ethiopian infants with acute diarrhoea EPEC was isolated in the stools of 9 patients and the jejunal juice of 7[294]. An identical EPEC serotype was found in the stool of the 7 positive jejunal isolates. Bhan found a perfect correlation between jejunum and stool and an identical isolation rate: of 26 infants with acute diarrhoea 4 had EPEC in the stool and the same patients harboured identical serotypes in the jejunum[33]. Albert has been the only worker to show a higher isolation rate in the small intestine: of 28 infants with acute diarrhoea 5 were excreting

EPEC in the stool; these patients had identical serotypes in the jejunum. Four additional infants had EPEC isolated in the jejunum in whom none were found in the stool[2].

In summary, the author's experience of enteric pathogen isolation in the duodenum has been similar to that of other investigators: enteropathogens were found very infrequently when compared to the stool. It highlights that with the possible exception of EPEC, sampling the proximal small bowel gives poor results compared to the stool. It strongly implies that one is sampling proximally to where the enteropathogen is exerting its pathological effect. The author is not implying that the enteric pathogens isolated in the stool on day 6 were necessarily the cause of the infants' diarrhoea, but the discrepancy between the duodenal and stool findings suggests that examination of the duodenal fluid may miss potentially important pathogenic microorganisms.

DISCUSSION OF FINDINGS ON DAY 8 (post-bowel cocktail)

The duodenal bacteriology on day 8

Administration of the bowel cocktail was associated with changes in the duodenal flora. The duodenal fluid contained less microorganisms and fewer isolates. The median total microorganism count decreased about fifteen-fold when compared to day 6: 3.59 $\log_{10}$ organisms/ml compared to 4.77 $\log_{10}$ organisms/ml ($p < 0.05$). Most duodenal samples harboured fewer different microorganisms: 4.3 per patient (65 isolates in 15 patients) compared to 7.5 per patient on day 6 (113 isolates in 15 patients).

Comparing groups of organisms, a significant decrease occurred in aerobic microorganisms ($p < 0.05$) and in the *Enterobacteriaceae* ($p < 0.05$). A very marked fall ($p<0.01$)was observed in the bacteria normally considered to be sensitive *in vitro* to gentamicin (staphylococci, *Haemophilus*, diptheroids, *Neisseria*, *Corynebacterium*,

Enterobacteriaceae, *Micrococcus*, *Acinetobacter*). Their median total count dropped nearly one hundred-fold, from 4.41 to 2.64 $\log_{10}$ organisms/ml.

Analyzing individual genera there was a significant decrease for *Haemophilus* ($p < 0.05$), *Neisseria* ($p < 0.05$), and aerobic lactobacilli ($p < 0.05$).

A fall was also observed in the anaerobic component of the duodenal flora and in the bacteria normally resistant to gentamicin. These changes did not reach statistical significance.

These results show that administration of the bowel cocktail generally produced predictable changes in the duodenal flora: a very marked fall occurred in the bacteria normally sensitive to gentamicin. The decrease in the total bacterial numbers and in the number of aerobic bacteria was largely by virtue of the gentamicin-sensitive component of the flora. *Enterobacteriaceae*, which are par excellence gentamicin-sensitive, also decreased in number. The decrease in *Haemophilus* and *Neisseria* can likewise be explained by their susceptibility to aminoglycosides. A statistically significant drop in the individual bacterial genera comprising the *Enterobacteriaceae* family was not observed. The reason for this is that probably there were insufficient individual isolates for valid interpretation in only 15 patients.

The observed fall in aerobic lactobacilli cannot be explained by the predictable bactericidal effects of gentamicin, since this organism is resistant to aminoglycosides. A fall in lactobacilli has also been observed in studies that have investigated the effects of aminoglycosides on the faecal flora[88,273]. Possibly it may be the result of a previously existing interrelationship between lactobacilli and gentamicin-sensitive bacteria, which has been disrupted by antibiotic administration, thereby decreasing lactobacilli numbers.

The failure to find a significant decrease in anaerobic bacteria is important. It has been hypothesised that in anatomical sites containing bacterial populations one may indirectly eradicate the anaerobic flora by killing the aerobic component. The environment would then become aerobic (since the aerobic organisms previously consumed the oxygen) and the anaerobes would die[295]. This argument may hold true for the colonic microflora - aminoglycoside therapy greatly decreases colonic anaerobic bacteria such as clostridia[88], or even completely eliminates the anaerobic flora (Finegold 1965, quoted in[116]). This study shows that this situation does not appear to apply to the duodenal lumen.

Stool output

There was a dramatic fall in stool volume comparing the 24 hours preceding day 6 with day 8. The median stool output decreased about four-fold: on the day before administration of the bowel cocktail the median stool volume was 86.7 g/kg. Within 24 hours it had dropped to less than half this volume (36.1 g/kg). By 48 hours it was 20.5 g/kg.

This drastic fall in stool output following administration of the gentamicin/cholestyramine combination is of a similar magnitude to that observed in the controlled trial done in the same institution, in which the therapeutic efficacy of this treatment was demonstrated[164]. It contrasts with the increase in median stool volume which had taken place in the two days before (from 62.5 to 86.5 g/kg/day). It can be best described as a "switching off" in stool output: there can be little doubt that the bowel cocktail was responsible for the marked decrease in diarrhoeal output.

Did examination of the duodenal flora explain the fall in stool output?

When the patients comprising the bowel cocktail group are analysed as a group what clearly emerges is that their undoubted response to the gentamicin/cholestyramine

combination cannot be explained on the basis of an effect on duodenal bacterial overgrowth, in the generally accepted meaning of the term. Although the median total bacterial count before administration of the bowel cocktail was above the quite arbitrarily defined upper limit of normal (4 $\log_{10}$ organisms/ml), it certainly cannot be described as luxuriant. Paediatric publications which have stressed bacterial overgrowth as a cause of symptoms have described patients with total bacterial counts in excess of 6 $\log_{10}$ organisms/ml[72,146,246]. Likewise, in no adult series have patients with total bacterial numbers below 5 $\log_{10}$ organisms/ml ever been classified as having overgrowth. Adults with the blind loop syndrome usually have total counts in excess of 7 $\log_{10}$ organisms/ml.

Also, on examining the duodenal flora 2 days after starting the bowel cocktail it can be seen that although changes have taken place, the duodenal fluid was far from sterile. The total bacterial population was diminished by just over one log unit but still contained many bacteria. Further, the average number of different duodenal microorganisms harboured by each patient only decreased from 7.5 to 4.3 There are no publications describing enough patients with which one can adequately compare the results of this study. But these findings are very different to those observed after antibiotic administration against bacterial pathogens at other sites. For example the bacterial count in urinary infections and meningitis decreases by many log units after antimicrobial therapy. The two situations are not enitrely comparable, because the urine and cerebrospinal fluid are normally sterile whereas the duodenal lumen often contains a small number of organisms. Nevertheless, it must be admitted that when the bowel cocktail group as a whole is examined the changes which would normally be expected to occur after antibiotic administration for bacterial infections were not observed.

The argument against bacterial overgrowth being the cause of persistent diarrhoea in the patients studied becomes even stronger when one analyses the changes in the duodenal flora which took place between day 3 and day 6 in the 14 patients who were studied on both days (patient no. 21, the "late entry", is therefore excluded from the following

analysis). The duodenal flora showed a tendency to become "cleaner" between days 3 and 6, a period during which the stool output of the patients increased. The median total count decreased over six-fold from 5.53 to 4.72 $\log_{10}$ organisms/ml. The number of patients harbouring faecal-type bacteria (*Enterobacteriaceae*, *Streptococcus faecalis*, anaerobic streptococci, *Bacteroides*) also decreased, from 13 of 14 patients on day 3 to 12 of the same 14 patients on day 6. Six of 14 patients harboured *E.coli* in the duodenum on day 3, compared to 5 of 14 on day 6: in two patients these were present in the same numbers as on day 3, in 4 the number had decreased, and in one patient the *E.coli* disappeared altogether.

These findings differ greatly from those of Hill[162]. In a cross sectional study he found that the duodenal flora of infants examined on day 7 was much more luxuriant than that of infants studied on day 4: the total bacterial counts were almost 100 times greater, with an across-the-board increase in bacterial types, significantly so for *E.coli*. He hypothesised that in infants with continuing diarrhoea an increase in the bacterial population, particularly of *E.coli*, becomes established between days 4 to 7 and this was responsible for the persistence of diarrhoea. This concept is supported by Penny's UK study, also cross sectional, which showed similar findings[246]. The current study is the only one to have systematically studied infants longitudinally during the course of a diarrhoeal episode, and does not support Hill's theory. In attempting to explain the difference between Hill's findings and the author's one can postulate that Hill's patients already had a profuse flora on admission to hospital. But the investigation by Household from the same institution weakens this argument somewhat: he studied diarrhoeal infants the day after hospital admission and found that the infants who went on to develop persistent diarrhoea on day 7 had a similar duodenal juice bacteriology to the infants who recovered spontaneously[178]. Perhaps subtle differences in the hospital management between Hill's patients and those of the author may have contributed to the different findings. For instance, nasogastric feeding tubes were used in Hill's patients (Hill ID, personal communication) whereas they were never left *in situ* in the author's patients.

The findings will now be discussed in terms of groups of bacteria which have been implicated by previous workers in the pathogenesis of diarrhoea associated with bacterial overgrowth. The discussion will pertain to the whole group of infants studied, rather than to individual patients.

UPPER RESPIRATORY FLORA

Upper respiratory microorganisms have been blamed for causing persistent diarrhoea by Dahlström et al[87]. They found *Streptococcus viridans* in high numbers and to be predominant in the duodenum of a group of infants and children with persistent diarrhoea. *Haemophilus* and *Neisseria* were found very infrequently and no details were given of other aspects of the flora apart from the upper respiratory component. Their patients were labelled as having chronic non-specific diarrhoea of infancy but closer inspection of the clinical details shows that most were probably suffering from post-enteritis diarrhoea. The response of these patients to co-trimoxazole was dramatic, and although the duodenal culture was not repeated after antibiotic therapy the authors speculated that the duodenal upper respiratory flora, *Streptococcus viridans* in particular, was responsible for the diarrhoea symptoms.

The current findings are against this hypothesis. The duodenal flora of 6 of the 15 patients did not contain *Streptococcus viridans* before administration of the bowel cocktail. One would have difficulty implicating this organism in small intestinal bacterial overgrowth as it was not present in fully 40% of patients.

Haemophilus and *Neisseria* showed a significant fall in numbers in response to the gentamicin/cholestyramine combination. *Haemophilus* was found in 11 patients on day 6, at a median count of 3.78 $\log_{10}$ organisms/ml. On day 8 it was only isolated in one patient (in whom it was also present on day 6) at a count of 3.75 $\log_{10}$ organisms/ml. *Neisseria* was isolated in 8 samples on day 6, at a median count of 2.88 $\log_{10}$ organisms/ml. No

patients were harbouring *Neisseria* in their duodenum on day 8. The author cannot exclude *Haemophilus* or *Neisseria* as potential pathogens, but thinks that probably they were innocent bystanders killed by gentamicin. A search of the literature revealed no publications incriminating these bacteria with gastrointestinal symptoms, with the exception of one report from France, which tentatively linked *Haemophilus* with acute appendicitis[229].

ENTEROBACTERIACEAE

This bacterial family is commonly cited as being potentially pathogenic in the case of blind loop syndrome, tropical sprue, and small intestinal bacterial overgrowth in children. Examining the group as a whole a reasonable argument can be made in incriminating *Enterobacteriaceae*. Organisms of this family were found in 11 of the 15 patients before administration of the bowel cocktail. The median count was 2.82 $\log_{10}$ organisms/ml. On day 8 most patients had no *Enterobacteriaceae* (median count was therefore 0 organisms/ml). Closer analysis shows that in 3 of these patients (nos. 1, 2, 18) the bacteria were found in similar numbers as on day 6, making them unlikely candidates in causing the diarrhoea. So in 8 of the 15 patients *Enterobacteriaceae* could possibly be blamed. But the next section, which examines individual patients findings shows that in those infants an alternative explanation can be given for the *Enterobacteriaceae* in causing diarrhoea: namely, by their acting as single pathogens. This is a more likely event than that of a general Enterobacteriaceal *motif* in the context of bacterial overgrowth.

BACTEROIDES

This microorganism is almost universally quoted as being the most important pathogen in the blind loop syndrome. It has also been mentioned in the context of small intestinal bacterial overgrowth in paediatric patients. The results of this investigation do not support a role for *Bacteroides* in most patients with persistent diarrhoea. It was isolated in only 5 patients on day 6, and 3 patients on day 8.

These findings agree with the results of the controlled trial in which the various constituents of the bowel cocktail were individually tested: metronidazole, which has excellent activity against *Bacteroides*, gave no extra benefit. The undoubted efficacy of cholestyramine is therefore more likely to reside in an indirect effect on bacteria, such as a toxin-binding effect, rather than by binding bile salts which may have been deconjugated by *Bacteroides*.

To explore if several different mechanisms were at work that could explain the efficacy of the bowel cocktail in terminating persistent diarrhoea the duodenal flora of the individual patients will be examined in conjunction with the stool culture.

Individual patient analysis

SPONTANEOUS RECOVERY

Patients nos. 2 and 12

Although both infants had a high stool output on day 5 which had dropped dramatically by day 7, it is difficult to implicate the bowel cocktail for this decrease. In both patients the stool volume had been diminishing at an equal rate between days 2 and 4 as between days 5 and 7.

Patient no. 20

This infant's faecal losses on day 5 were not high when compared to the other patients in the bowel cocktail study. They decreased after administration of the antibiotic/cholestyramine combination, but not by a very convincing amount.

Patient no. 21

This, the only "late entry" patient, possibly recovered spontaneously: his stool output before day 5 is not known, and his faecal weight for day 5 was lower than for most patients who received the bowel cocktail.

E.COLI

Patient no. 7

On day 6 *E.coli* of EPEC serotypes O86 and O142 was found in the duodenal juice at a count of 4.41 $\log_{10}$ organisms/ml. The only other microorganism isolated in the duodenal juice was *Candida*, in numbers about 2 log units smaller. The stool culture on day 6 showed non typable *E.coli*. No enteric pathogens were isolated in this patient prior to day 6. After the bowel cocktail was given the diarrhoea literally "switched off", with a drop in stool weight from 204g to 10 g/kg/day for days 5 and 7 respectively. The duodenal juice was sterile for *E.coli* on day 8.

EPEC can be strongly implicated as being the cause of this patient's continuing diarrhoea on day 6, even though EPEC serotypes O86 and O142 are not recognised enteropathogens in Cape Town. These *E.coli* were found in almost pure culture as has been the experience in other clinical studies and volunteer experiments[205,266]. EPEC were not isolated in the stool on days 3 or 6; in this patient examination of the duodenal fluid was more informative. Of interest, EPEC O86 was found in the stool on day 8. The continued excretion of EPEC in the stool after successful antibiotic therapy has also been noted by Walker-Smith's group[167].

Patient no. 3

The duodenal juice on day 6 was almost sterile and contained no *E.coli*. Stool culture on days 3 and 6 showed EPEC O127, a recognised Cape Town enteropathogen. No other stool pathogens were detected. The response to the bowel cocktail was very impressive.

It is very likely that EPEC O127 was the cause of this patient's diarrhoea. This organism was probably residing distal to the duodenal sampling site or possibly was tightly adherent to the duodenal mucosa.

Patient no. 11

E.coli of EPEC serotype O126 was found in high numbers in the duodenal juice on day 6. It was not the predominant microorganism; the duodenal flora on day 6 contained many different bacteria and the total count was very high, being 7.18 $\log_{10}$ organisms/ml. No stool pathogens were isolated on day 6 but EPEC O126 was found in the stool on day 3. There was a very good response to the bowel cocktail. The duodenal flora on day 8 did not contain *E.coli* but the total count was still high, at 5.72 $\log_{10}$ organisms/ml.

EPEC O126, an accepted pathogen EPEC in Cape Town, was probably the cause of this patient's diarrhoea. It was not the predominant microorganism but this does not exclude pathogenicity[295]. Duodenal culture was superior to examination of the stool in explaining this infant's continuing diarrhoea.

Patient no. 20

Non typable *E.coli* were isolated in the duodenal juice on day 6. They were not the predominant microorganisms. Stool culture on day 6 showed EPEC O126 and *Campylobacter jejuni*. The response to the bowel cocktail was not impressive. The duodenal culture on day 8 showed only *Candida* and the stool showed no enteropathogens.

The bowel cocktail may have worked by an effect on EPEC, or by a bactericidal effect on nontypable *E.coli*. In this investigation nontypable *E.coli* would include all the organisms of this species with the exception of a battery of EPEC serotypes: Varieties such as ETEC and certain groups of adherent *E.coli* would therefore belong to this category.

KLEBSIELLA

Patient no. 8

Klebsiella was isolated in the duodenal juice on day 6. It was not the predominant organism, and the duodenal juice showed a luxuriant growth of many different bacteria. The stool was negative for enteric pathogens. Therapy with the bowel cocktail was

associated in a great diminution in faecal output. Post-cocktail duodenal culture showed no *Klebsiella*.

Patient no. 6

Klebsiella was found at a count of 4.79 $\log_{10}$ organisms/ml in the day 6 duodenal juice. The stools did not contain enteropathogens on the same day. The response to the bowel cocktail was good. *Klebsiella* was found in much lower numbers on day 8.

One may tentatively attribute this patients diarrhoea to *Klebsiella*.

Patient no. 4

Klebsiella was isolated in very small numbers (1.3 $\log_{10}$ organisms/ml) in the day 6 duodenal culture. The total bacterial count was low. The stool contained *Cryptosporidium* on day 6. Rotavirus had been isolated in the day 3 stool. Administration of the bowel cocktail was associated with an abrupt fall in stool output, from 200 to 7 g/kg/day. The day 8 duodenal fluid contained no *Klebsiella* and was otherwise little changed.

Possibly the very small numbers of *Klebsiella* found in the duodenal lumen provide a clue that greater numbers might be present elsewhere (more distally or adherent to the mucosa). The response to the bowel cocktail was the most impressive of all the patients studied. It suggests that perhaps toxin-producing organisms were responsible for the diarrhoea, and these were eradicated by the antibiotic. *Klebsiella* could explain this infant's diarrhoea.

Klebsiella, a member of the *Enterobacteriaceae* family, has been incriminated in the pathogenesis of tropical sprue[200]. This microorganism is a known secretory toxin-producer and this is one way in which it might produce symptoms in this condition[201,202]. No proof exists that it causes gastroenteritis but there is suggestive evidence from case reports and epidemiological surveys that it might on occasions be an enteric pathogen[56,259]. The elaboration of a secretory toxin has been proposed as the mechanism

of diarrhoea also in the case of *Klebsiella*-associated gastroenteritis[150]. One would expect *Klebsiella* organisms to respond to the bowel cocktail, since aminoglycosides have good bactericidal activity against the *Enterobacteriaceae*.

PROVIDENCIA

Patient no. 6

In this previously described patient *Providencia* was found in the duodenal fluid on day 6. It was in lower numbers (3.26 $\log_{10}$ organisms/ml) than the *Klebsiella*, which was also isolated. The *Providencia* was not present on day 3, suggesting that perhaps it was a superinfecting organism, possibly causing the ongoing diarrhoeal symptoms.

Providencia, another member of the *Enterobacteriaceae* family, has been implicated in diarrhoeal disease. It may be one cause of traveller's diarrhoea[157]. An Indian study has also tentatively incriminated it as an enteric pathogen in childhood gastroenteritis[40].

CITROBACTER

Patient no. 17

Citrobacter was found in the duodenal juice on day 6 at a count of 2.82 $\log_{10}$ organisms/ml. It was not the predominant organism. No enteropathogens were isolated in the stool at any stage. There was a good response to the bowel cocktail. The day 8 duodenal culture was sterile for *Citrobacter*.

To the author's knowledge *Citrobacter* has not been incriminated in causing diarrhoea. But it belongs to the *Enterobacteriaceae*, of which several members are putative enteric pathogens, quite apart from *E.coli* - the commonest and most protean of all bacterial enteropathogens. No other causes could be found for this infant's diarrhoea. It is reasonable to assume that *Citrobacter* may have been responsible.

SALMONELLA

Patient no. 13

The duodenal flora on day 6 was unremarkable, showing mainly a upper respiratory type flora. *Salmonella* group B and *Cryptosporidium* were isolated in the stool on the same day. The stool output halved after gentamicin/cholestyramine therapy. The repeat duodenal bacteriology showed only anaerobic bacteria. The day 8 stool specimen contained no enteric pathogens.

Possibly in this patient the bowel cocktail was effective against *Salmonella*, which was possibly residing in the lower reaches of the small intestine[268]. The accepted view is that antibiotics are ineffective in non-invasive diarrhoea caused by *Salmonella*, and that they merely encourage a carrier state. But a recent uncontrolled study from India suggests that antibiotics are indeed beneficial in terminating persistent diarrhoea associated with excretion of *Salmonella* in the stools[197].

UPPER RESPIRATORY ORGANISMS

Patient no. 13

An alternative explanation for this patient's diarrhoea is that upper respiratory bacteria may be responsible. One cannot specifically incriminate any particular bacterial isolate since all the upper respiratory organisms were present in almost equal numbers and all were absent in the day 8 duodenal bacteriology.

NO EXPLANATION EVIDENT

Patients nos. 1, 5, 18

No convincing explanation can be given for these patients. It would be tempting to incriminate classical bacterial overgrowth for patient no. 1 because of his luxuriant flora, but his duodenal fluid contained almost no faecal type organisms and no *Bacteroides* and has been included in the unexplained category.

TOWARDS A UNIFYING HYPOTHESIS

It can be seen that examination of the duodenal flora of the individual patients, in conjunction with the stool bacteriology, gives an incomplete but tantalizing glimpse of the possible mechanisms of action of the bowel cocktail. By implication it provides some important clues on the possible causes of diarrhoea in these patients. What emerges clearly is that there are probably different causes in different patients. The connecting link is response to the bowel cocktail.

The presence of *Enterobacteriaceae* and their subsequent reduction by the bowel cocktail was the only feature present which was compatible with bacterial overgrowth. But analysis of the individual patients' flora shows that these bacteria were probably acting as specific enteric pathogens rather than by a "group effect".

Duodenal bacterial overgrowth, in the accepted sense of the term, did not explain the patients' persistent diarrhoea on day 6, nor its resolution after administration of the bowel cocktail. In the author's opinion the criteria which constitute small intestinal bacterial overgrowth are not acceptable and for the following reasons.

Reliance on the total bacterial count (the cornerstone of the definition) may be very misleading. From a bacteriological viewpoint the small intestinal juice is a poor man's guide to the mucosal state of affairs. A tightly adherent bacterial species may only spill over in small numbers into the luminal fluid and yet be of pathogenic importance.

Likewise the insistence on finding many different microorganisms is also unjustified, as is the pre-requisite for the flora not to be of a type normally thought to be pathogenic. Cholera and *E.coli* gastroenteritis are both associated with a luxuriant pure growth of these organisms in the small intestinal fluid[130,205]. Surely in such an event there is "bacterial overgrowth" by these agents?

On studying the literature dealing with small intestinal bacterial overgrowth in connexion with the blind loop syndrome one immediately notes the great diversity between individual patients in the reponse of the proximal bowel flora to antimicrobial therapy. In some patients *Enterobacteriaceae* were greatly reduced with a resultant improvement in symptoms[127]. In others *Bacteroides* were deemed responsible for symptoms[4,113,253]. In others still anaerobic lactobacilli have been blamed[113]. In the canine counterpart of small intestinal bacterial overgrowth syndrome the symptomatic improvement that followed antibiotic therapy was not associated with one uniform pattern of response in the jejunal flora[23].

The only common link which can be observed in these cases is the presence of bacteria in the small bowel fluid and improvement in symptoms following treatment with antibiotics. The author believes that attempts to define bacterial overgrowth in terms of general properties of the flora are mistaken. Rather, the flora should be viewed in terms of the individual bacteria that comprise it, and their properties.

The same argument can also be made for the duodenal flora in infantile gastroenteritis. On this subject the author disagrees with Penny. In her Peruvian study, having found little difference between the duodenal flora of infants with acute diarrhoea, persistent diarrhoea, and controls, she probably correctly ascribes this to the contaminated living conditions common to all 3 groups[248]. She then makes the possibly incorrect assumption that because there was little difference in the duodenal flora between infants with acute and persistent diarrhoea that this implies that the bacteria found in the duodenum of the children with persistent diarrhoea were not culpable in prolonging the diarrhoeal episodes. On the contrary, it is plausible that some microorganisms in the duodenum may well have been responsible, but these could not be identified as culprits among a myriad of other innocent bacteria. Also, the analysis of patients as groups would tend to further obscure analysis.

The author believes that the term bacterial overgrowth should be abandoned. It has served the original purpose of describing the abnormal finding of bacteria in the proximal small bowel and in linking this with various symptoms. The concept of small intestinal bacterial overgrowth has now outgrown its usefulness and is actually hindering progress by potentially diverting attention away from the study of individual bacteria in relation to small intestinal pathophysiology.

Suggestions for future research

The results of this investigation are rich in implication for future work. First, intensive study of individual patients is recommended. This individual approach is particularly suited to conditions in which many patients would be needed for statistically significant results to emerge. The collection of a suitably large number of patients for statistical comparison would present almost insurmountable logistical difficulties. The careful study of few patients has been successful in discovering aetiological agents which only rarely cause diarrhoea[239]. It was used to good effect by Ament in his classic investigation of the blind loop syndrome[4]. He exhaustively studied only 7 patients, but was able to cast more light on this condition than other workers who had studied many more patients but in less detail.

Conventional taxonomic bacteriology of the small intestinal lumen has provided valuable information but has been taken to the limit of its potential. Future work will need to focus much more closely on the individual microorganisms. Bacterial identification should be at species level. Furthermore one should examine properties such as toxin production, which may vary between strains of the same species. The pathogenic potential of any microorganisms isolated should also be explored. One possible approach is to feed the putative pathogen to animals and observe if diarrhoea results. Properties such as the ability to cause small intestinal fluid production or enteropathy could also be tested *in vivo* and *in vitro* by using small intestinal loops.

In addition future investigators might attempt to sample the lower reaches of the small intestine. Examination of the distal duodenum and proximal jejunum has been useful, but in most patients the main theatre of action in diarrhoeal disease is probably the mid-jejunum and beyond. Unfortunately, sampling these regions would be technically arduous and in a paediatric setting very difficult (if not impossible) to justify ethically. Researchers should not be content with one sampling site but should examine multiple areas of the small intestine.

Attachment to the wall of the bowel is a necessary prerequisite for all bacterial pathogens, whatever their mode of action, be it by toxin elaboration, mucosal damage, or direct invasion of epithelial cells[25]. Although examination of the luminal fluid may reveal the offending organism it is logical that attention should concentrate on where the pathophysiological effects are taking place - the mucosa. Quite apart from standard bacteriological analysis, in recent years studies have emerged to suggest that scanning electron microscopy (SEM) has much to offer. Poley has looked at the duodenojejunal mucosa of 56 infants and children using SEM[252]. These patients had chronic diarrhoea of different aetiologies, mainly non-specific diarrhoea of infancy. He found that nearly all the mucosal specimens yielded microorganisms. These were adherent to the mucosa or trapped in the overlying mucus layer. Many of these organisms resembled *Mycoplasma*. A more recent Scandinavian investigation using SEM has confirmed the presence of a seemingly distinct mucosal bacterial population, and its possible association with damage to the villi[173]. It is conceivable that a detailed SEM examination of the mucosa of infants with persistent diarrhoea will reveal previously unrecognised pathogenic microorganisms which would normally fail to grow using accepted culture media. This should not come as a total surprise: SEM has shown that the mucosal population of bacteria inhabiting the stomach and colon of mice is quite distinct from that of the lumen[276,301]. The same situation may also apply to humans.

Finally, future workers should enter this field of study with a completely open mind about the potential of bacteria to cause diarrhoea. For too long we have been constrained in the mental straitjacket of considering only a handful of different bacteria as being enteric pathogens. Bacteria previously considered harmless are increasingly being incriminated as enteropathogens by both medical and veterinary researchers (who are often a step ahead of their medical counterparts)[1,152,192,206,238,239,314]. Moreover, previously undescribed bacteria are also being blamed for causing diarrhoea[263]. Almost certainly new pathogens will continue to be discovered. It is likely that in future the recognition of new enteric pathogens will decrease the proportion of gastroenteritis episodes for which no cause can be found.

CONCLUSIONS

Acute infectious diarrhoea is not one disease. It is many illnesses, caused by different agents and with different pathological and pathophysiological mechanisms. The one common link is that diarrhoea is the end result.

Just as with acute diarrhoea it is likely that the causes of persistent diarrhoea are also manifold. Yet, in its management there lies a paradox. If one excludes host factors, such as malnutrition or systemic illness, the bowel cocktail is remarkably effective in terminating the persistent diarrhoea which sometimes follows acute gastroenteritis. This effect is readily apparent even when the therapeutic group comprises few patients - a very unusual event in the study of diarrhoeal disease, when often many cases must be analysed for an obvious pattern to emerge.

This study has shown that the gentamicin/cholestyramine combination did not appear to work by an effect on bacterial overgrowth in the duodenum in the generally accepted sense of the term. It is the author's belief that the bowel cocktail is a therapy which may work on differing aetiologies in different individuals. In some patients it may eradicate

recognised enteric pathogens, such as the diarrhoeagenic *E.coli*. In others it may possibly be effective against other bacteria which may occasionally cause diarrhoea, such as *Klebsiella* or other *Enterobacteriaceae*. In other patients the bowel cocktail may be effective against some as yet undiscovered microorganisms closely adherent to the intestinal mucosa. The enteropathogens eradicated by this treatment may not always be the same ones responsible for the acute diarrhoeal episode, but may follow in their wake and cause diarrhoeal persistence.

The results of this thesis show exciting glimpses into the possible causes of persistent diarrhoea but the duodenal lumen gives an incomplete picture because it is a site twice removed from where the pathophysiological events are taking place - the mucosa, and the more distal parts of the small intestine. This site has provided useful information into the causes of diarrhoea but is not recommended for future research.

In the future individual patients should be studied in considerable detail. Every effort should be made to sample more distal parts of the small intestine. An intensive study of the mucosa should be made, including the use of special techniques such as SEM. Any microorganisms isolated should be exhaustively analysed, not only by a full bacteriological profile but also by testing any functional properties such as toxin production, adherence to mucosa, and ability to produce diarrhoea in animals.

Finally the author believes that small intestinal bacterial overgrowth is a term which should now be laid to rest, at least in the context of paediatric infectious diarrhoea. Although in the past it has stimulated the study of the effects of bacteria on the small intestine and provided many insights into their noxious effects, this concept is now more likely to hinder progress. The future lies in the study of individual bacteria and their effect on the host.

APPENDIX

PATIENT 1

Age (months) 9.89

Duration of diarrhoea (hrs) 96

Admission wt. (kg) 7.55 **Rehydrated wt. (kg)** 7.58

Total protein (g/l) 68

Albumin (g/l) 39

Stool wt. g/kg

Day				
2	3	4	5	
56.5	56.2	39.5	54.1	
6	7	8	9	10
23.5	18.3	18.4	13.6	26.8

	Day 3	Day 6	Day 8
Stool pathogens	Campylobacter jejuni	Campylobacter jejuni	-
Stool E.coli serotypes	non typable	non typable	non typable
Microscopy of juice	-	-	-

Clinical course Discharged on day 11

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 1	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans	7.09	7.14	3.1
Beta haemolytic streptococci	4.39		
Other streptococci			
Staphylococcus epidermidis		3.05	1.86
Staphylococcus aureus		2.96	
Coagulase-negative staphylococci	1.66		
Pneumococcus			
Haemophilus	5.79	5.22	
Diphtheroids		2.35	3.94
Neisseria	3.69	4.29	
Corynebacteria			
E.coli (serotypes)	4.01(non)	1.92(non)	2.11(non)
Klebsiella			1.38
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli	5.39	3.51	
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium			
Bacteroides			
Fusobacterium	3.57	3.88	
Veillonella	5.75	6.69	
Actinomyces			
Candida		2.84	2.11
TOTAL	**7.14**	**7.32**	**4.02**

PATIENT 2

Age (months) 5.09

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 5.84 **Rehydrated wt. (kg)** 5.83

Total protein (g/l) 53

Albumin (g/l) 33

Stool wt. g/kg

Day				
2	3	4	5	
171.1	206.6	210.9	110.5	
6	7	8	9	10
157.3	27.3	25.9	18.8	46.9

	Day 3	Day 6	Day 8
Stool pathogens	-	E.coli O111	-
Stool E.coli serotypes	non typable	O111	non typable
Microscopy of juice	-	-	-

Clinical course Developed watery stools and fever on day 10. Klebsiella in blood culture. Treated with antibiotics and changed to nutramigen milk. Discharged from hospital on day 20.

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 2	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans	6.04	2.9	4.3
Beta haemolytic streptococci		2.51	
Other streptococci			
Staphylococcus epidermidis			
Staphylococcus aureus			
Coagulase-negative staphylococci			2.7
Pneumococcus			
Haemophilus	5.0	4.08	
Diphtheroids			
Neisseria	5.0	3.38	
Corynebacteria	5.0		
E.coli (serotypes)			
Klebsiella	5.32	4.05	3.53
Enterobacter	5.14		
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli	4.93	2.84	4.43
Micrococcus			
Acinetobacter			
Anaerobic streptococci			4.67
Anaerobic lactobacilli			
Propionibacterium	5.53		
Bacteroides	6.97		3.82
Fusobacterium	4.18	3.39	3.98
Veillonella	6.76	4.03	4.45
Actinomyces			
Candida	5.08	2.51	5.9
TOTAL	**7.26**	**4.61**	**5.97**

PATIENT 3

Age (months) 8.41

Duration of diarrhoea (hrs) 24

Admission wt. (kg) 9.44 **Rehydrated wt. (kg)** 9.9

Total protein (g/l) 74

Albumin (g/l) 46

Stool wt. g/kg

Day				
2	3	4	5	
99.8	76.7	68.4	116.9	
6	7	8	9	10
24.3	23.5	8.1	5.4	7.7

	Day 3	Day 6	Day 8
Stool pathogens	E.coli O127	E.coli O127	-
Stool E.coli serotypes	O114, O127	O114,O127	O114
Microscopy of juice	-	-	-

Clinical course Discharged on day 11

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 3	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans			
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis			
Staphylococcus aureus	1.92	1.53	
Coagulase-negative staphylococci			
Pneumococcus			
Haemophilus			S
Diphtheroids	1.23		
Neisseria			T
Corynebacteria			
E.coli (serotypes)			E
Klebsiella			
Enterobacter			R
Citrobacter			
Providencia			I
Proteus			
Streptococcus faecalis			L
Aerobic lactobacilli	1.23		
Micrococcus			E
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium		1.23	
Bacteroides	1.27		
Fusobacterium	1.23		
Veillonella	1.23		
Actinomyces			
Candida			
TOTAL	**2.22**	**1.7**	**0**

PATIENT 4

Age (months) 6.01

Duration of diarrhoea (hrs) 24

Admission wt. (kg) 7.52 **Rehydrated wt. (kg)** 7.6

Total protein (g/l) 64

Albumin (g/l) 39

Stool wt. g/kg

Day				
2	3	4	5	
58.5	130.6	192.9	199.9	
6	7	8	9	10
30.4	7.1	8.8	3.7	2.8

	Day 3	Day 6	Day 8
Stool pathogens	Rotavirus	Cryptosporidium	-
Stool E.coli serotypes	non typable	non typable	No E.coli
Microscopy of juice	-	-	-

Clinical course Discharged on day 11

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 4	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans	2.38	1.94	
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis	1.77	2.55	2.64
Staphylococcus aureus			
Coagulase-negative staphylococci			
Pneumococcus			2.77
Haemophilus		1.94	
Diphtheroids			
Neisseria			
Corynebacteria			
E.coli (serotypes)			
Klebsiella	1.23	1.3	
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli		2.5	2.49
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium	2.52		
Bacteroides			
Fusobacterium		2.94	
Veillonella		2.61	2.67
Actinomyces			
Candida			
TOTAL	**2.81**	**3.33**	**3.26**

PATIENT 5

Age (months) 9.03

Duration of diarrhoea (hrs) 24

Admission wt. (kg) 9.09 **Rehydrated wt. (kg)** 9.28

Total protein (g/l) 65

Albumin (g/l) 36

Stool wt. g/kg

Day	2	3	4	5	
	78.2	63	85.5	55.2	
Day	6	7	8	9	10
	21.1	7.5	2.5	5.2	0

	Day 3	Day 6	Day 8
Stool pathogens	Rotavirus	-	-
Stool E.coli serotypes	no E.coli	no E.coli	no E.coli
Microscopy of juice	-	-	-

Clinical course Discharged on day 11

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 5	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans	2.66		6.51
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis	1.2		2.84
Staphylococcus aureus			3.14
Coagulase-negative staphylococci			
Pneumococcus			
Haemophilus	2.65	2.88	3.75
Diphtheroids	2.06		
Neisseria			
Corynebacteria			
E.coli (serotypes)			
Klebsiella			1.2
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli			
Micrococcus		1.49	
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium			
Bacteroides			
Fusobacterium	3.41	1.2	
Veillonella	2.81	2.98	
Actinomyces			
Candida			3.52
TOTAL	**3.63**	**3.25**	**6.51**

PATIENT 6

Age (months) 8.25

Duration of diarrhoea (hrs) 48

Admission wt. (kg) 6.93 **Rehydrated wt. (kg)** 7.33

Total protein (g/l) 53

Albumin (g/l) 24

Stool wt. g/kg

	Day				
	2	3	4	5	
	50.4	53.1	57.9	59.2	
	6	7	8	9	10
	16.9	8.5	9.2	4.5	3.9

	Day 3	**Day 6**	**Day 8**
Stool pathogens	Rotavirus	-	-
Stool E.coli serotypes	non typable	non typable	No E.coli
Microscopy of juice	-	-	-

Clinical course Discharged on day 11

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 6	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans			
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis		3.36	
Staphylococcus aureus	1.68		
Coagulase-negative staphylococci			3.02
Pneumococcus			
Haemophilus	3.2		
Diphtheroids			1.78
Neisseria			
Corynebacteria			
E.coli (serotypes)			
Klebsiella	3.53	4.79	1.73
Enterobacter		3.18	
Citrobacter			
Providencia		3.26	
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli	5.05	4.88	3.07
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium			
Bacteroides	4.28		
Fusobacterium			
Veillonella		3.09	
Actinomyces			
Candida	3.9	3.48	2.24
TOTAL	**5.16**	**5.17**	**3.4**

PATIENT 7

Age (months) 1.45

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 3.58 **Rehydrated wt. (kg)** 3.68

Total protein (g/l) 56

Albumin (g/l) 37

Stool wt. g/kg

Day	2	3	4	5	
	126.2	193.6	100.3	204.2	
Day	6	7	8	9	10
	46.5	10.8	10.5	22.2	15.6

	Day 3	Day 6	Day 8
Stool pathogens	-	-	-
Stool E.coli serotypes	non typable	non typable	O86
Microscopy of juice	-	-	-

Clinical course Discharged on day 11

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 7	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans			
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis			2.01
Staphylococcus aureus			1.61
Coagulase-negative staphylococci			
Pneumococcus			
Haemophilus			
Diphtheroids			
Neisseria			
Corynebacteria			
E.coli (serotypes)	6.88(O128)	4.41(O86,O142)	
Klebsiella			
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli			
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium			
Bacteroides			
Fusobacterium			
Veillonella			
Actinomyces			
Candida	6.77	2.38	3.72
TOTAL	**7.13**	**4.42**	**3.74**

PATIENT 8

Age (months) 4.17

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 4.23 **Rehydrated wt. (kg)** 4.26

Total protein (g/l) 68

Albumin (g/l) 39

Stool wt. g/kg

	Day				
	2	3	4	5	
	71.8	86	195.4	149.3	
	6	7	8	9	10
	125.2	28.5	30.4	23.3	14.8

	Day 3	Day 6	Day 8
Stool pathogens	-	-	-
Stool E.coli serotypes	O86	non typable	non typable
Microscopy of juice	-	-	-

Clinical course Discharged on day 11

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 8	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans	3.09	6.26	3.1
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis	2.88	3.26	1.54
Staphylococcus aureus	3.58	3.83	2.39
Coagulase-negative staphylococci			
Pneumococcus			
Haemophilus	3.03	5.57	
Diphtheroids			
Neisseria		4.57	
Corynebacteria			
E.coli (serotypes)	3.74(non)	3.74(non)	2.28(non)
Klebsiella		3.87	
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis	3.53		
Aerobic lactobacilli	3.59	4.88	
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli		5.06	
Propionibacterium			2.24
Bacteroides	3.66	6.44	
Fusobacterium			
Veillonella		4.74	
Actinomyces			
Candida	1.79	1.74	
TOTAL	**4.37**	**6.72**	**3.28**

PATIENT 9

Age (months) 1.68

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 5.5 **Rehydrated wt. (kg)** 6

Total protein (g/l)

Albumin (g/l) No specimen

Stool wt. g/kg

	Day				
	2	3	4	5	
	51.3	35.2	8	0.4	
	6	7	8	9	10

	Day 3	Day 6
Stool pathogens	-	-
Stool E.coli serotypes	non typable	non typable
Microscopy of juice	-	-

Clinical course Discharged on day 6

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 9	**Day 3**	**Day 6**
Streptococcus viridans		1.27
Beta haemolytic streptococci		
Other streptococci	1.44	
Staphylococcus epidermidis	1.75	
Staphylococcus aureus	2.23	
Coagulase-negative staphylococci		
Pneumococcus		
Haemophilus	4.85	
Diphtheroids		
Neisseria		
Corynebacteria		
E.coli (serotypes)	2.49(non)	
Klebsiella		
Enterobacter		
Citrobacter		
Providencia		
Proteus		
Streptococcus faecalis		
Aerobic lactobacilli	4.42	3.59
Micrococcus	4.19	
Acinetobacter		
Anaerobic streptococci		
Anaerobic lactobacilli	3.0	
Propionibacterium		
Bacteroides	4.7	
Fusobacterium	2.62	
Veillonella	3.93	
Actinomyces		
Candida	3.99	2.24
TOTAL	**5.26**	**3.61**

PATIENT 10

Age (months) 4.01

Duration of diarrhoea (hrs) 24

Admission wt. (kg) 6.2 **Rehydrated wt. (kg)** 6.68

Total protein (g/l) 61

Albumin (g/l) 44

Stool wt. g/kg

	Day			
2	3	4	5	
51.3	35.2	8	0.4	
6	7	8	9	10

	Day 3	**Day 6**
Stool pathogens	Rotavirus	-
Stool E.coli serotypes	non typable	non typable
Microscopy of juice	-	-

Clinical course Discharged on day 6

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 10	**Day 3**	**Day 6**
Streptococcus viridans	4.33	4.48
Beta haemolytic streptococci		
Other streptococci		
Staphylococcus epidermidis		3.28
Staphylococcus aureus	2.41	3.41
Coagulase-negative staphylococci		
Pneumococcus		
Haemophilus		
Diphtheroids		
Neisseria		
Corynebacteria		
E.coli (serotypes)		
Klebsiella		
Enterobacter		
Citrobacter		
Providencia		
Proteus		
Streptococcus faecalis		
Aerobic lactobacilli		2.94
Micrococcus		
Acinetobacter		
Anaerobic streptococci		
Anaerobic lactobacilli		5.24
Propionibacterium		
Bacteroides	3.89	4.24
Fusobacterium		
Veillonella	4.56	4.28
Actinomyces		5.65
Candida	3.33	2.7
TOTAL	**4.83**	**5.84**

PATIENT 11

Age (months) 2.96

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 5.5 **Rehydrated wt. (kg)** 5.6

Total protein (g/l) 76

Albumin (g/l) 42

Stool wt. g/kg

Day	2	3	4	5	
	119.8	155.1	189.9	151.1	
Day	6	7	8	9	10
	93.8	65.7	38.5	27.2	19.7

	Day 3	Day 6	Day 8
Stool pathogens	E.coli O126	-	-
Stool E.coli serotypes	O126	non typable	non typable
Microscopy of juice	-	-	-

Clinical course Discharged on Isomil on day 12

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 11	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans	6.28	6.12	5.59
Beta haemolytic streptococci	5.18	5.22	
Other streptococci			
Staphylococcus epidermidis			
Staphylococcus aureus			
Coagulase-negative staphylococci			
Pneumococcus		6.44	3.81
Haemophilus		6.9	
Diphtheroids	5.85	5.74	4.41
Neisseria		6.14	
Corynebacteria			
E.coli (serotypes)	7.8(O126)	5.96(O126)	
Klebsiella			
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli	4.34	4.44	
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium			
Bacteroides			3.59
Fusobacterium	2.11	2.64	4.02
Veillonella	4.11		
Actinomyces			
Candida	3.95	3.72	4.96
TOTAL	**7.82**	**7.18**	**5.72**

PATIENT 12

Age (months) 7

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 7 **Rehydrated wt. (kg)** 7.26

Total protein (g/l) 66

Albumin (g/l) 32

Stool wt. g/kg

Day				
2	3	4	5	
165.2	187.6	126.5	111.2	
6	7	8	9	10
96.7	26.4	39.1	39.9	26.3

	Day 3	**Day 6**	**Day 8**
Stool pathogens	Shigella flexneri	No specimen	-
Stool E.coli serotypes	O128	No E.coli	No E.coli
Microscopy of juice	Giardia	Giardia	Giardia

Clinical course Given 3 day course of metronidazole at end of trial. Stool wt 41.3 g/kg at end of course. Changed to Nutramigen. Stool wt 16.1g/kg/d one day after formula changed. Discharged on Nutramigen.

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 12	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans	5.57	4.45	
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis		1.57	2.1
Staphylococcus aureus			
Coagulase-negative staphylococci			
Pneumococcus			
Haemophilus	4.09	5.76	
Diphtheroids			2.14
Neisseria		5.08	
Corynebacteria			
E.coli (serotypes)	2.15(O55,O128)		
Klebsiella			
Enterobacter			
Citrobacter			
Providencia			
Proteus	1.87		
Streptococcus faecalis			
Aerobic lactobacilli			
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli	5.45	4.06	
Propionibacterium			
Bacteroides		4.12	
Fusobacterium		2.76	
Veillonella	5.09	4.88	
Actinomyces			
Candida	3.75	3.26	3.18
TOTAL	**5.9**	**5.91**	**3.25**

PATIENT 13

Age (months)	13.2		
Duration of diarrhoea (hrs)	72		
Admission wt. (kg)	9.3	**Rehydrated wt. (kg)**	9.08
Total protein (g/l)	63		
Albumin (g/l)	39		

Stool wt. g/kg

Day				
2	3	4	5	
30.9	28.6	47.7	42.2	
6	7	8	9	10
36.1	20.5	13.7	24.6	10.2

	Day 3	**Day 6**	**Day 8**
Stool pathogens	Cryptosporidium	Cryptosporidium Salmonella B	-
Stool E.coli serotypes	O86	O86	No E.coli
Microscopy of juice	-	-	Cryptosporidium

Clinical course Discharged on day 12

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 13	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans	3.83	3.18	
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis	2.17	3.36	
Staphylococcus aureus			
Coagulase-negative staphylococci			
Pneumococcus			
Haemophilus	1.47	3.78	
Diphtheroids			
Neisseria	1.17	3.18	
Corynebacteria			
E.coli (serotypes)			
Klebsiella			
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis	2.47		
Aerobic lactobacilli	2.95		
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium	3.17		
Bacteroides			5.3
Fusobacterium		1.66	5.3
Veillonella	3.12		5.3
Actinomyces			
Candida			
TOTAL	**4.04**	**4.06**	**5.77**

PATIENT 14

Age (months)	7.16		
Duration of diarrhoea (hrs)	96		
Admission wt. (kg)	7.98	**Rehydrated wt. (kg)**	8.45
Total protein (g/l)	72		
Albumin (g/l)	45		

Stool wt. g/kg

Day				
2	3	4	5	
58.4	18.1	12.8	9.6	
6	7	8	9	10
2.2				

	Day 3	**Day 6**
Stool pathogens	Rotavirus	-
Stool E.coli serotypes	non typable	non typable
Microscopy of juice	-	-

Clinical course Discharged on Isomil on day 7

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 14	**Day 3**	**Day 6**
Streptococcus viridans		6.99
Beta haemolytic streptococci		3.56
Other streptococci		
Staphylococcus epidermidis		
Staphylococcus aureus		3.1
Coagulase-negative staphylococci		
Pneumococcus		
Haemophilus		6.69
Diphtheroids		6.04
Neisseria		
Corynebacteria		
E.coli (serotypes)		
Klebsiella	3.74	6.37
Enterobacter		
Citrobacter		
Providencia		
Proteus		
Streptococcus faecalis		
Aerobic lactobacilli	1.59	6.76
Micrococcus		
Acinetobacter		
Anaerobic streptococci		
Anaerobic lactobacilli		
Propionibacterium		
Bacteroides		6.62
Fusobacterium		5.04
Veillonella	3.99	5.65
Actinomyces		
Candida	5.5	6.04
TOTAL	**5.52**	**7.47**

PATIENT 15

Age (months) 8.11

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 7.88 **Rehydrated wt. (kg)** 8.25

Total protein (g/l) 59

Albumin (g/l) 32

Stool wt. g/kg

Day				
2	3	4	5	
40.8	47	47.1	4.2	
6	7	8	9	10
6.4				

	Day 3	Day 6
Stool pathogens	Rotavirus	-
Stool E.coli serotypes	non typable	non typable
Microscopy of juice	-	-

Clinical course Discharged on Isomil on day 7

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 15	**Day 3**	**Day 6**
Streptococcus viridans	3.84	5.12
Beta haemolytic streptococci		
Other streptococci		
Staphylococcus epidermidis	3.37	3.51
Staphylococcus aureus		3.53
Coagulase-negative staphylococci		
Pneumococcus		
Haemophilus	4.41	6.23
Diphtheroids		6.36
Neisseria	3.68	6.21
Corynebacteria		
E.coli (serotypes)		
Klebsiella	1.73	3.61
Enterobacter		
Citrobacter	1.54	
Providencia		
Proteus		
Streptococcus faecalis		
Aerobic lactobacilli		
Micrococcus		
Acinetobacter		
Anaerobic streptococci		
Anaerobic lactobacilli	3.08	
Propionibacterium		
Bacteroides		
Fusobacterium	2.01	3.76
Veillonella	1.93	5.51
Actinomyces		
Candida		3.02
TOTAL	**4.62**	**6.78**

PATIENT 16

Age (months) 9.36

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 7.7 **Rehydrated wt. (kg)** 8.02

Total protein (g/l) 38

Albumin (g/l) 17

Stool wt. g/kg

	Day				
	2	3	4	5	
	161.7	111.9	115	148.7	
	6	7	8	9	10
	100.4	39.6			

	Day 3	Day 6
Stool pathogens	-	-
Stool E.coli serotypes	non typable	non typable
Microscopy of juice	-	-

Clinical course Developed fever on day 6. CXR showed pneumonia. Left trial on day 6. Treated with antibiotics.

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 16	**Day 3**	**Day 6**
Streptococcus viridans	3.28	4.84
Beta haemolytic streptococci		
Other streptococci		
Staphylococcus epidermidis	1.98	1.14
Staphylococcus aureus	2.51	1.14
Coagulase-negative staphylococci		
Pneumococcus		
Haemophilus	2.98	4.14
Diphtheroids		
Neisseria		4.14
Corynebacteria		
E.coli (serotypes)	4.09(non)	
Klebsiella		2.17
Enterobacter		
Citrobacter		
Providencia		
Proteus		
Streptococcus faecalis		
Aerobic lactobacilli	4.37	3.44
Micrococcus		
Acinetobacter		
Anaerobic streptococci		
Anaerobic lactobacilli		
Propionibacterium		
Bacteroides		
Fusobacterium		
Veillonella	2.88	
Actinomyces		
Candida	2.28	
TOTAL	**4.6**	**5.0**

PATIENT 17

Age (months) 4.57

Duration of diarrhoea (hrs) 48

Admission wt. (kg) 6.93 **Rehydrated wt. (kg)** 7.33

Total protein (g/l) 55

Albumin (g/l) 37

Stool wt. g/kg

Day	2	3	4	5	
	36.3	66.4	108.4	86.7	
Day	6	7	8	9	10
	48.2	21.2	30.6	22.5	21

	Day 3	Day 6	Day 8
Stool pathogens	-	-	-
Stool E.coli serotypes	O26	O26	non typable
Microscopy of juice	-	-	-

Clinical course Changed to Nutramigen on day 11 because stools loose. Discharged on day 12.

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 17	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans		3.23	2.95
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis	3.09		2.07
Staphylococcus aureus			
Coagulase-negative staphylococci			
Pneumococcus			
Haemophilus	4.17	4.63	
Diphtheroids			
Neisseria		2.88	
Corynebacteria			
E.coli (serotypes)			
Klebsiella			
Enterobacter			
Citrobacter	5.39	2.82	
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli	4.4	3.26	3.0
Micrococcus		4.93	
Acinetobacter	2.78		
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium			
Bacteroides	4.14		
Fusobacterium	5.18		
Veillonella	6.14	3.71	
Actinomyces			
Candida			
TOTAL	**6.26**	**5.14**	**3.3**

PATIENT 18

Age (months) 6.31

Duration of diarrhoea (hrs) 48

Admission wt. (kg) 6.1 **Rehydrated wt. (kg)** 6.5

Total protein (g/l) 53

Albumin (g/l) 29

Stool wt. g/kg

	Day				
	2	3	4	5	
	33.7	39.8	68.5	69.4	
	6	7	8	9	10
	19	18.8	33	17.3	15.2

	Day 3	**Day 6**	**Day 8**
Stool pathogens	Campylobacter jejuni	Campylobacter jejuni	-
Stool E.coli serotypes	non typable	non typable	No E.coli
Microscopy of juice	-	-	-

Clinical course Discharged on Isomil

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 18	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans			
Beta haemolytic streptococci		3.56	1.69
Other streptococci	3.18		
Staphylococcus epidermidis	1.98	1.59	2.17
Staphylococcus aureus	1.88	1.07	1.69
Coagulase-negative staphylococci			
Pneumococcus		3.36	
Haemophilus	1.27	4.29	
Diphtheroids			
Neisseria			
Corynebacteria			
E.coli (serotypes)			
Klebsiella	1.61	2.22	1.97
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli	2.38	3.14	2.83
Micrococcus	2.7	1.98	
Acinetobacter			
Anaerobic streptococci		3.76	
Anaerobic lactobacilli	2.67	2.27	
Propionibacterium			
Bacteroides		3.59	
Fusobacterium	2.58	3.76	
Veillonella	2.64	3.56	
Actinomyces			
Candida	1.27		1.23
TOTAL	**3.58**	**4.66**	**3.02**

PATIENT 19

Age (months) 5.62

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 6 **Rehydrated wt. (kg)** 5.9

Total protein (g/l) 64

Albumin (g/l) 31

Stool wt. g/kg

Day	2	3	4	5	6	7	8	9	10
	187.8	111.9	64.1	21.1	11.7				

	Day 3	Day 6
Stool pathogens	-	Salmonella B
Stool E.coli serotypes	non typable	non typable
Microscopy of juice	-	-

Clinical course Discharged on Isomil

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 19	**Day 3**	**Day 6**
Streptococcus viridans	3.82	3.36
Beta haemolytic streptococci		
Other streptococci		
Staphylococcus epidermidis		
Staphylococcus aureus		
Coagulase-negative staphylococci	2.85	
Pneumococcus		
Haemophilus	3.15	2.36
Diphtheroids		
Neisseria		
Corynebacteria		
E.coli (serotypes)	2.3(non)	
Klebsiella	1.72	
Enterobacter		
Citrobacter		
Providencia		
Proteus		
Streptococcus faecalis		
Aerobic lactobacilli	1.0	
Micrococcus		
Acinetobacter		
Anaerobic streptococci		
Anaerobic lactobacilli	4.0	
Propionibacterium		
Bacteroides		3.66
Fusobacterium	3.52	3.36
Veillonella	4.07	3.44
Actinomyces		
Candida		
TOTAL	**4.53**	**4.15**

PATIENT 20

Age (months) 4.27

Duration of diarrhoea (hrs) 48

Admission wt. (kg) 5.02 **Rehydrated wt. (kg)** 4.55

Total protein (g/l) 54

Albumin (g/l) 34

Stool wt. g/kg

Day	2	3	4	5	
	43.3	32.1	21.4	37	
Day	6	7	8	9	10
	41.3	23.7	17.6	15	14.1

	Day 3	Day 6	Day 8
Stool pathogens	Campylobacter Jejuni	Campylobacter Jejuni	-
	E.coliO126	E.coli O126	-
Stool E.coli serotypes	O126	O126	non typable
Microscopy of juice	-	-	-

Clinical course Discharged on Isomil day 12

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 20	**Day 3**	**Day 6**	**Day 8**
Streptococcus viridans	6.05	3.99	
Beta haemolytic streptococci			
Other streptococci	5.46	3.14	
Staphylococcus epidermidis	3.05		
Staphylococcus aureus	3.32	1.59	
Coagulase-negative staphylococci			
Pneumococcus			
Haemophilus	5.51	3.37	
Diphtheroids			
Neisseria	3.51	3.25	
Corynebacteria			
E.coli (serotypes)	2.99(non)	2.99(non)	
Klebsiella	2.4		
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli			
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli	4.0		
Propionibacterium			
Bacteroides		3.29	
Fusobacterium	1.2		
Veillonella	3.99	4.61	
Actinomyces			
Candida	3.32	1.89	3.59
TOTAL	**6.29**	**4.77**	**3.59**

PATIENT 21

Age (months) 1.46

Duration of diarrhoea (hrs) 72

Admission wt. (kg) 4.3 **Rehydrated wt. (kg)** 4.56

Total protein (g/l) 57

Albumin (g/l) 37

Stool wt. g/kg

	Day				
	2	3	4	5	
				48.9	
	6	7	8	9	10
	31.6	17.7	18.8	50.3	37.6

	Day 6	**Day 8**
Stool pathogens	-	-
Stool E.coli serotypes	non typable	non typable
Microscopy of juice	-	-

Clinical course Fed Isomil. Discharged on day 11, stools soft

On the opposite page are the duodenal bacteriology results. Microorganism counts are expressed in $\log_{10}$ organisms/ml.

Patient 21		**Day 6**	**Day 8**
Streptococcus viridans			
Beta haemolytic streptococci			
Other streptococci			
Staphylococcus epidermidis			
Staphylococcus aureus		2.65	3.71
Coagulase-negative staphylococci			
Pneumococcus			
Haemophilus			
Diphtheroids			4.37
Neisseria			
Corynebacteria			
E.coli (serotypes)			
Klebsiella		4.01	2.68
Enterobacter			
Citrobacter			
Providencia			
Proteus			
Streptococcus faecalis			
Aerobic lactobacilli		5.0	2.76
Micrococcus			
Acinetobacter			
Anaerobic streptococci			
Anaerobic lactobacilli			
Propionibacterium			
Bacteroides		5.17	
Fusobacterium		5.17	
Veillonella		1.95	
Actinomyces			
Candida		2.48	4.72
TOTAL		**5.61**	**4.92**

LABORATORY PROCEDURES

A. PRIOR TO INTUBATION

The following is done the day before duodenal intubation.

The anaerobic culture plates are pre-reduced. They are placed in a Brewer Gas-Pak jar and the following are added:

a. 3 wire-mesh containers, which screw into the lid of the jar. These contain aluminium pellets coated with palladium catalyst. (They are regularly rejuvenated by placing in an incubator AT 37°C for at least 2 days).

b. 3 gas-generating sachets (Oxoid, Basingstoke UK) containing hydrogen-producing sodium borohydride, and carbon dioxide-producing citric acid and sodium bicarbonate.

c. A wet "Anaerotest" (Merck, Darmstadt) methylene-blue indicator, to check for anaerobiosis.

10ml of water are added to each sachet and the Gas-Pak jar is immediately sealed.

The following are carried out on the morning of the intubation.

(i) Preparation of rich broth transport medium

Under aseptic conditions the following are added to 20ml of rich broth, using a Gilson pipette:

0.2ml of 10% dextrose

0.2ml of 5% cysteine hydrochloride

(ii) **Preparation of broth bottles**

Three empty sterile McCartney bottles are weighed on a Mettler H 10 W balance. The bottles are then numbered.

To bottle 1 1ml of broth is added

To bottle 2 2ml of broth are added

To bottle 3 10ml of broth are added

The bottles are then reweighed to ensure accuracy.

Bottle 1 is used for collection of the duodenal fluid.

B. PREPARATION OF THE DUODENAL SPECIMEN FOR PLATING

This is done immediately after collection of the duodenal fluid.

(i) **Quantitation of the juice**

Bottle 1, containing transport medium and duodenal juice specimen, is reweighed. From this figure the weight of the duodenal juice specimen can be calculated.

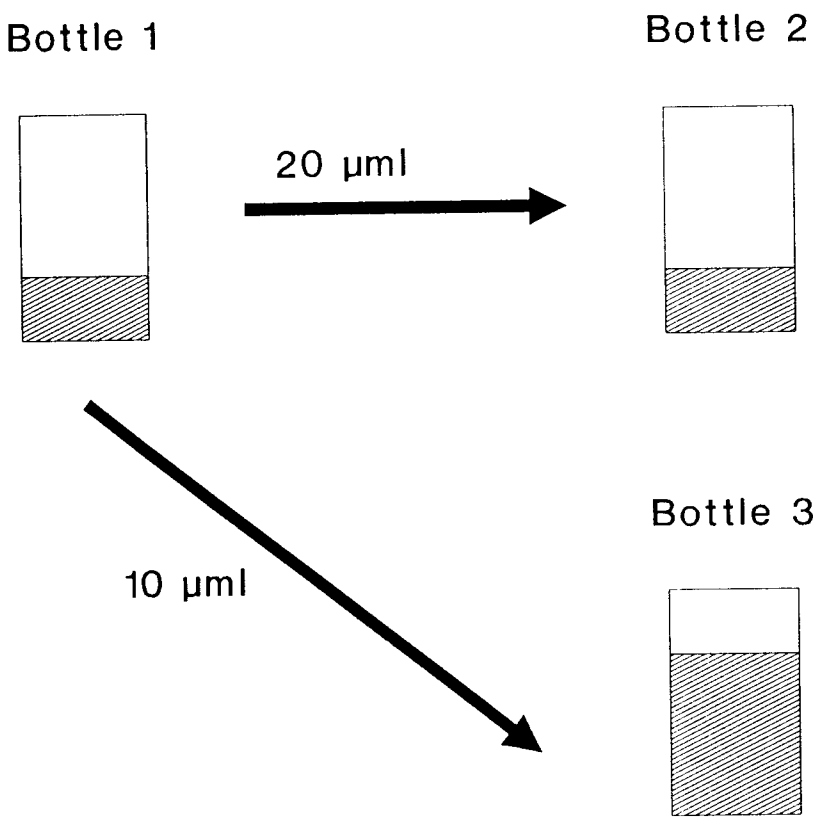
(ii) **Dilution procedure**

Bottle 1 is shaken vigorously for at least 30 seconds to thoroughly mix the contents. Dilutions are then prepared in the following manner:

Using a Gilson pipette 20μml of fluid are transferred to bottle 2, and 10μml to bottle 3 (see figure 1). The bottles are vigorously shaken after the procedure.

The bottles will now give dilutions from 10^{-1} to 10^{-5} when the quantity of fluid shown in figure 2 is plated onto the culture media plates.

Figure 1



C. PLATING OUT PROCEDURE

Glass rods flamed to give a right angle bend ("hockey sticks") are used for plating. After every procedure they are sterilised by dipping in ethyl alcohol, placing in a bunsen burner flame, and allowing them to cool.

There are 5 Petri dish plates for each culture medium, each representing a dilution from 10^{-1} to 10^{-5}.

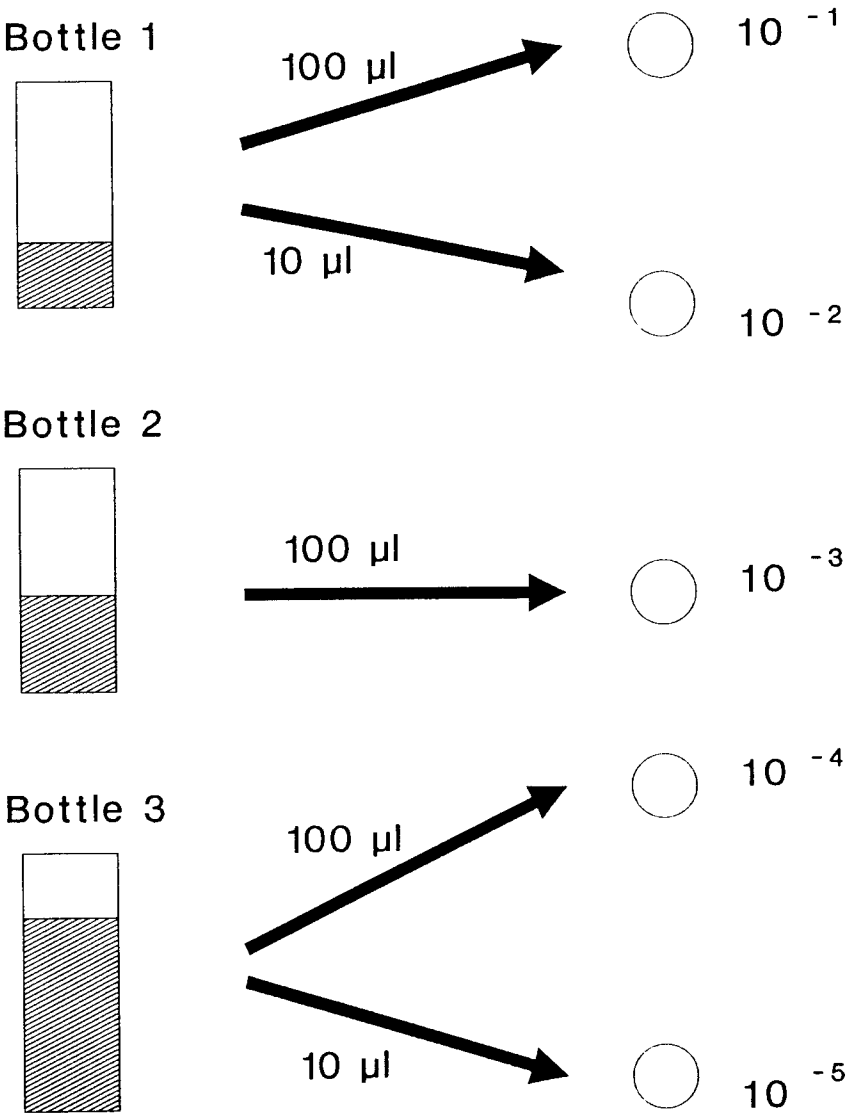
The 10^{-1} dilution of every different culture media is plated out first. Prior to this procedure all the plates for this dilution are placed in a row. Using a Gilson pipette with a sterile tip 100μml of fluid are taken from bottle 1 which has just been vigorously shaken, and placed onto each plate, the lid of which is immediately replaced. When all the plates for this dilution have been inoculated the plating procedure is begun.

Using a "hockey stick" the fluid is evenly distributed on the plate in a circular motion, until the plate is dry. This procedure is repeated for each plate.

The 10^{-2} dilution is then plated out in an identical manner. Succeeding dilutions are prepared as shown in figure 2.

The anaerobic plates are processed first. After the anaerobic specimens have been placed in the incubator the aerobic plates are prepared.

Figure 2


Bottle 1
100 µl
10 µl
10 -1
10 -2
Bottle 2
100 µl
10 -3
Bottle 3
100 µl
10 µl
10 -4
10 -5

D. ANAEROBIC SPECIMENS

As already mentioned these are the first to be prepared.

(i) Plating out

The plating procedure is carried out as described in the preceding section. For ease and speed it is done on the open bench, but as soon as the plates for a given dilution have been prepared they are placed in an anaerobic holding area, consisting of a sealed glove box through which nitrogen from a cylinder is flowing at 5 litres/minute.

(ii) Preparation for incubation

When all the dilutions have been prepared the plates are placed in a Brewer Gas-Pak jar, and fresh wire mesh containers with catalyst, 3 gas-generating sachets and an "Anaerotest" indicator are added as previously described. The jar is then immediately sealed and placed in an incubator at 37°C.

E. AEROBIC SPECIMENS

On completion of the plating procedure all the plates with the exception of those containing the BBA and TBBA culture media are placed in an incubator at 37°C.

The BBA plates are placed in an incubator at 37°C containing an atmosphere of 10% CO_2.

The TBBA plates are placed in a Brewer Gas-Pak containing the following:

a. 3 palladium catalyst meshes (already described)

b. 3 BBL Campy Pak microaerophilic sachets (Beckton Dickinson, Cockeysville MD) containing sodium borohydride, sodium bicarbonate and citric acid. 10ml of water are added to each sachet. The jar is sealed and put in the incubator at 37°C.

F. CALCULATION OF THE DILUTION FACTOR

The formula is as follows:

$$\text{Dilution factor} = \frac{\text{wt of rich broth transport medium + wt of duodenal specimen}}{\text{wt of duodenal specimen}}$$

G. REPRODUCIBILITY OF BACTERIOLOGY

The author's department has considerable experience in the bacteriological analysis of duodenal contents[162,178]. To confirm that reproducibility of duplicate aliquots was still satisfactory collection of duodenal fluid was done in duplicate on the first intubation of patients no. 18. The duodenal fluid was collected in two bottles which were then bacteriologically analysed separately. The results are expressed as $\log_{10}$ microorganisms/ml.

	Original specimen	**Duplicate specimen**
Other streptococci	3.18	3.36
Staphylococcus epidermidis	1.98	0
Staphylococcus aureus	1.88	2.36
Haemophilus	1.27	1.3
Klebsiella	1.61	0
Aerobic lactobacilli	2.38	0
Micrococcus	2.7	2.3
Anaerobic lactobacilli	2.67	3.44
Fusobacterium	2.58	3.14
Veillonella	2.64	2.67
Candida	1.27	0
TOTAL	**3.58**	**3.87**

PREPARATION OF CULTURE MEDIA (in alphabetical order)

Addresses for ordering stock:

Difco Laboratories. Detroit, Michigan 48232. USA.

Oxoid Ltd. Basingstoke. UK.

2% Blood agar

Blood agar base (Oxoid code CM55)	20g
Distilled water	1 litre

Suspend and bring to the boil, dissolve completely.

Autoclave at 121°C for 15 mins.

Cool to 50°C.

Add 70ml of fresh horse blood.

Mix gently and pour onto plates.

4% Blood agar

Blood agar base (Oxoid code CM55)	40g
Distilled water	1 litre

Suspend and bring to the boil, dissolve completely.

Autoclave at 121°C for 15 mins.

Cool to 50°C.

Add 70ml of fresh horse blood.

Mix gently and pour onto plates.

Boiled blood agar (BBA)

To prepare stock:

Blood agar base (Oxoid code CM55)	40g
Distilled water	800ml

Mix well and autoclave at 104°C for 15 minutes.

Brain-heart infusion (BHI) agar

To prepare stock:

Brain-heart infusion (Difco code 0140-01)	14.8g
Bacto agar (Difco code 0037-01-6)	6g
Distilled water	400ml

Dissolve and autoclave at 104°C for 15 minutes.

BHI agar (aerobic medium)

Melt down contents of stock bottle and cool to 56°C.

Add: Vitamin K-haemin solution 4ml

Horse blood 25ml

Mix well and pour onto plates.

BHI with Vancomycin (anaerobic medium)

Melt down contents of stock bottle.

Cool to 56°C.

Add: Vitamin K-haemin solution 4ml

Horse blood 25ml

Vancomycin (400μg/ml) 1ml

Mix well and pour onto plates.

Ethyl violet agar (E.V.A.)

Protease peptone	10g
Yeast extract (Oxoid code L21)	1g
Lab lemco	3g
Soluble starch	2g
Sodium nitrate	1g
Sodium permanganate	5g
Distilled water	1 litre

Dissolve, by steaming if necessary. Cool to approximately 56°C.

Adjust pH to 7.6.

Weigh out 6g Bacto-Agar into each 500ml size autoclave bottle.

Pour out 400ml of E.V.A. broth into each bottle.

Autoclave at 104°C for 20 minutes.

Melt down contents of EVA stock bottle. Cool to 56°C.

Then add to each 400ml:

Foetal calf serum	20ml
10% Glucose	20ml
5% Cysteine hydrochloride	4ml
1:150 Ethyl violet	4ml
Streptomycin (20μg/ml)	1ml

Mix well between additions.

Pour onto plates.

Mannitol Salt agar

Mannitol salt agar (Oxoid code CM85)	111g
Distilled water	1 litre

Suspend and bring to boil, dissolve completely.

Autoclave at 121°C for 15 minutes.

Cool to abou 50°C and pour onto plates.

McConkey's agar

MacConkey's agar (Oxoid code CM17)	52g
Distilled water	1 litre

Suspend and bring to boil, dissolve completely.

Autoclave at 121°C for 15 minutes.

Cool to about 50°C and pour onto plates.

Rich broth transport medium

1% Tryptone	10g
1% Soya peptone	10g
1% Yeast autolysate	10g
1% Liver digest	10g
1% Potassium permanganate	1g
Distilled water	1 litre

Dissolve ingredients in water. Heat on magnetic stirrer and while still hot dispense into aliquots of 20ml into sterile McConkey bottles. Autoclave at 104°C for 15 minutes.

Rogosa SL agar

Rogosa SL agar (Difco code 0480-01-8)	30g
Distilled water	400ml

Mix well.

Heat to boiling and dissolve.

Add 53ml of glacial acetic acid. Mix thoroughly.

Continue boiling for 2-3 minutes.

Cool to 56°C and pour onto plates.

Rogosa V Agar

To prepare stock:

Trypticase soy broth	5g
Yeast extract (Difco code O127-01)	3g
Sodium thioglycholate (Difco code O233-13)	0.75g
Basic fuscein	0.002g
Tween 80	1g
50% Sodium lactate	25ml
Distilled water	1 litre

Dissolve, by steaming for about 10 minutes.

Cool to about 50°C.

Adjust pH to 7.5.

Weigh out 6g Bact-agar to each 500ml size autoclave bottle.

Pour out 400ml at Rogosa broth into each bottle.

Autoclave at 104°C for 20 minutes.

Melt down contents of stock bottle. Cool to 56°C.

Add aseptically 0.4mg of Cidomycin. Pour onto plates.

Sabouraud's Dextrose Agar

To prepare stock:

Sabouraud's dextrose agar (Difco code O109-01)	26g
Distilled water	1 litre

Mix well.

Autoclave at 104°C for 15 minutes.

To pour plates:

Melt down contents of stock bottle.

Cool to 56°C.

Add aseptically 0.4mg of cidomycin.

Salmonella-Shigella (SS) agar

SS Agar (modified) (Oxoid code CM533)	57g
Distilled water	1 litre

Bring to boil and simmer.

Do not autoclave.

Cool to about 50% and our onto plates.

Tryptose blood agar

Tryptose blood agar (Oxoid code CM233)	30g
Distilled water	1 litre

Suspend and bring to boil, dissolve completely.

Autoclave at 121°C for 15 minutes.

Cool to about 50°C.

Add 70g of sterile horse blood.

Mix thoroughly and pour onto plates.

Wilkins Chalgren blood agar

To prepare stock:

Wilkins Chalgren agar (Oxoid code CM619)	43g
Distilled water	1 litre

Suspend and bring to the boil, dissolve completely.

Autoclave at 121°C for 15 minutes.

Cool to 50°C and add 25ml defibrinated blood.

Mix gently and pour.

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