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The faecal flora of children in the United Kingdom

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SUMMARY

The faecal flora of 55 children (aged 8 days to 8 years) and 16 adults was determined. All the children were artificially fed from birth. The faecal flora of the youngest age group was generally less complex and less predictable than that of adults. Some bacterial groups commonly found in adult stools, for example bacilli, lactobacilli and yeasts, were rarely found in the youngest infants. Most of the changes towards the adult pattern took place between 4 and 12 months. The faecal flora of children aged 1–4 years generally resembled that of adults, although lactobacilli were still infrequently isolated.

INTRODUCTION

Renewed interest in human faecal flora owes much of its impetus to continuing advances in the techniques of anaerobic bacteriology (Drasar, 1967; Holdeman & Moore, 1973). The aerobic and anaerobic faecal flora of infants and children in industrialized countries has not yet been studied using these newer techniques, although earlier reports were published by Smith & Crabb (1961) and Haenel (1961). More recently the faecal flora of breast-fed children from rural Guatemala has been extensively recorded (Mata & Urrutia, 1971).

This paper details the aerobic and anaerobic faecal flora of a group of infants and young children from South London, England. All were bottle fed from birth. This reflects local custom, since breast feeding is now uncommon in our population.

MATERIALS AND METHODS

Subjects

Stools from 16 adults (range 12–63 years, mean age 32 years) and from 55 infants and children (range 8 days–8 years) were collected from healthy persons outside the hospital environment, consisting of staff engaged on the project and their children, or from hospital inpatients within three days of admission. The children were divided into four age ranges; 16 infants (range 8 days to < 4 months, mean age 5·5 weeks), 17 infants (4–12 months, mean 6·3 months), 12 children (13–48 months, mean 25·3 months) and 12 children (49 months to 8 years, mean 77·2 months). None had a history of abnormal bowel function or of antibiotic therapy within the preceding month.

No infants in the study had been breast-fed. Most were fed on diets which

included a wide range of commercial milk food preparations as the predominant component.

Bacteriology

Adult stools were cultured within 24 hr. of being passed, after storage at 4° C. (Crowther, 1971). Infant stools were not stored, and culture was initiated within 10 min.

One gram of solid faeces (wet weight) was homogenized for not longer than 2 min. in 9 ml. of distilled water in an M.S.E. microemulsifier, to give a homogeneous suspension, and 10-fold dilutions in distilled water were made from this suspension. Volumes of 0·1 ml. were pipetted from selected dilutions onto the surface of the defined bacteriological media and spread with bent glass rods before incubation in appropriate atmospheres.

Media

For aerobic culture. Blood agar (Oxoid CM55 with 7% Wellcome defibrinated horse blood) and MacConkey agar without salt (Oxoid CM 7. b) were incubated for 18–24 hr. at 37° C. Manitol-salt agar (Oxoid CM 85) and Sabouraud's dextrose agar (Oxoid CM 41) with sodium benzyl penicillin 200 iu/ml. and neomycin base 40 μg/ml. (as neomycin hydrochloride) were incubated for 48 hr. at 37 °C.

For growth of organisms requiring CO_2, Rogosa agar (Oxoid PM 221) was incubated for 5 days in an atmosphere of increased CO_2 concentration produced by the action of 3 N-HCl on marble chips.

For anaerobic culture. Blood agar with added filtered sterile L-cysteine hydrochloride to a final concentration of 0·05%, the same medium with neomycin base 70 μg./ml. (as neomycin hydrochloride), tomato juice agar (Oxoid CM 113) and Bacto-Veillonella agar (Difco Laboratories 0·917.02) with vancomycin base 7·5 μg./ml. (as vancomycin hydrochloride) were all incubated anaerobically for 5 days at 37 °C. in 95% H_2 and 5% CO_2.

All media for anaerobic incubation were reduced for 24–28 hr. immediately before use. Anaerobic incubation was carried out in McIntosh and Fildes jars (or specially adapted milk churns) with 3 extra catalysts (palladiumized alumina catalyst 'D') after evacuation to a pressure of 60 mmHg and replacement with the anaerobic gas mixture (two cycles). The whole process from homogenization until anaerobiosis was complete took 10–20 min. on an open bench.

Identification of organisms

Broad definitions of both aerobic and anaerobic species were similar to those of Drasar (1967). Aerobic organisms were identified according to Cowan & Steel (1970). The following groups were recognized: enterobacteria, staphylococci, streptococci, lactobacilli, yeasts, bifidobacteria, bacteroides, clostridia and veillonella. All strains isolated from anaerobic incubation were subcultured to determine obligate anaerobiosis. Continuing taxonomic advances (Holdeman & Moore, 1973) show that these broad groups of anaerobes include many different genera which this study did not further define.

Colony counts

At suitable dilutions on the various media the colonies were counted. The colony counts were corrected for dilution, and the concentrations of viable organisms were expressed as $\log_{10}$ organisms/g. of faeces (wet weight). The lower limit of detection is 100 organisms/g. Failure to isolate a bacterial group does not always imply a concentration of < 100 organisms/g. because no selective media are perfect, and specific organisms may occasionally have been masked by overgrowth. Most of these groups grew well on several media, and the aerobes were facultatively anaerobic. The defined groups were thus always counted on the same media: viz. enterobacteria on MacConkey agar, streptococci on blood agar, staphylococci and bacilli on mannitol-salt agar, lactobacilli on Rogosa L agar, yeasts on Sabouraud's dextrose agar, bacteroides and clostridia on neomycin blood agar, bifidobacteria on tomato juice agar and veillonella on Bacto-Veillonella agar.

Statistics

Plots of the bacterial concentrations are given in Fig. 1, and the numbers of subjects from whom bacterial groups were not isolated are given in the left hand column of each diagram ('-ve'). Where no numerical value is obtainable the measurement can reasonably be taken as $< d$, where d is the lowest 10-fold dilution on which there were no organisms. Thus, while the mean cannot be calculated, we usually determine the median of a group as a measure of location. The medians are also shown in Fig. 1.

As a guide to interpreting these results we have used tests of statistical significance. Each bacterial group is considered separately, and the median test (Mood & Graybill, 1963) is used for difference in location, comparing two age groups at a time. Nominal 5% significance levels are used throughout.

RESULTS

In adult stools the ratio of total anaerobes to total aerobes was 200:1 (Table 1). In contrast, this ratio in babies less than 4 months old was approximately 1·5:1 reflecting the significantly higher concentrations of enterobacteria and streptococci (Fig. 1). All anaerobic bacterial groups were present in similar concentrations in the faeces of these infants. This also contrasts with the findings in adult faeces in which clostria and veillonella have significantly lower concentrations than bifidobacteria and bacteroides. Lactobacilli and bacilli were rarely isolated, although yeasts were present in half the specimens.

Changes in the direction of adult findings are already evident in the stools of infants aged 4–12 months. Bifidobacteria were fully established as an integral and numerically important component of the faecal flora. Bacteroides too, sometimes not detected in the stools of the youngest infants (5/16 negative), were now present in all specimens. Bacilli tend to be isolated more frequently (7/17 positive) than in the previous age group (1/16 positive), and the concentrations of staphylococci show a clear tendency to diminish with ageing. The ratio total anaerobes: total

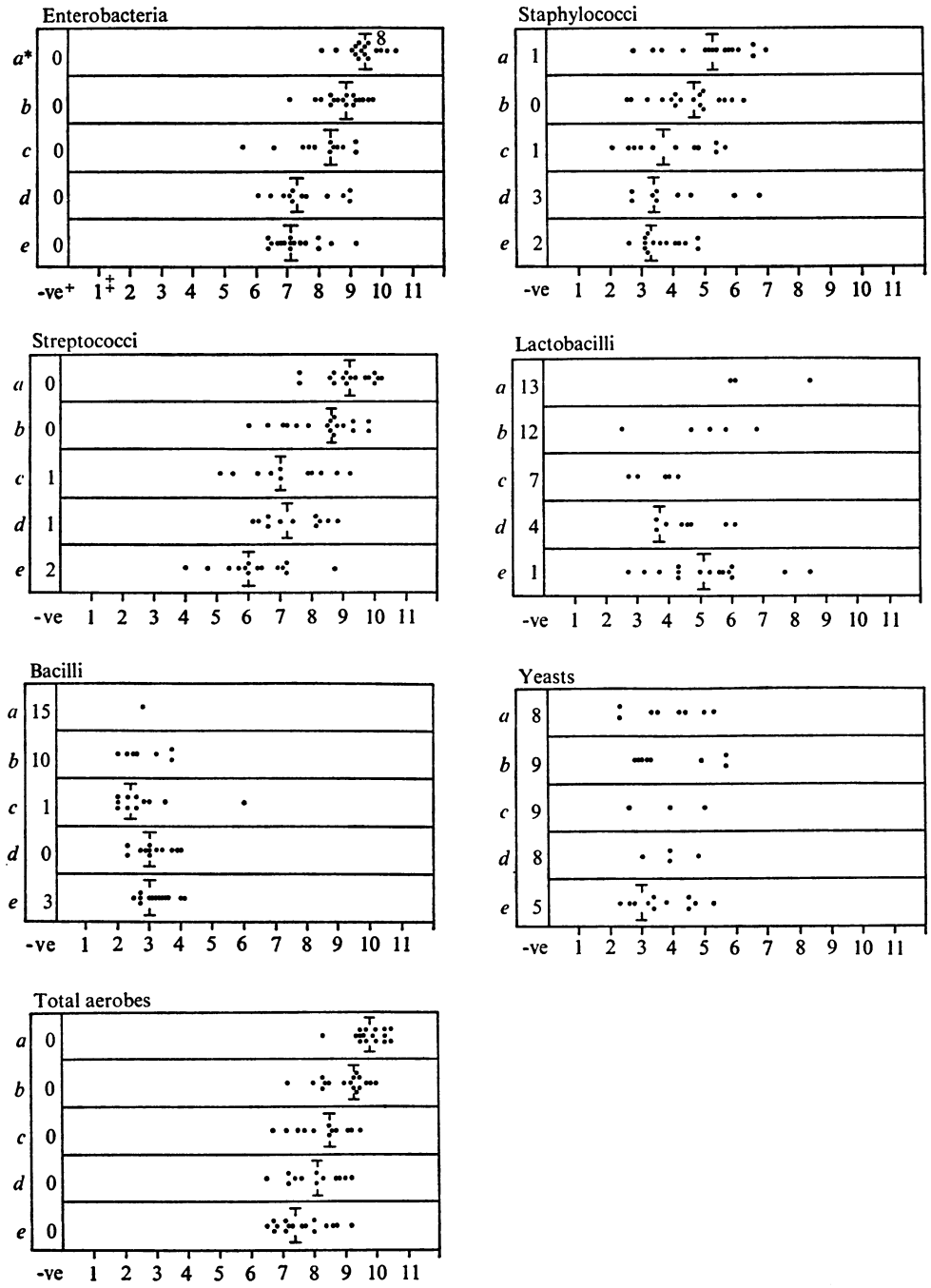

Fig. 1. For legend see opposite.

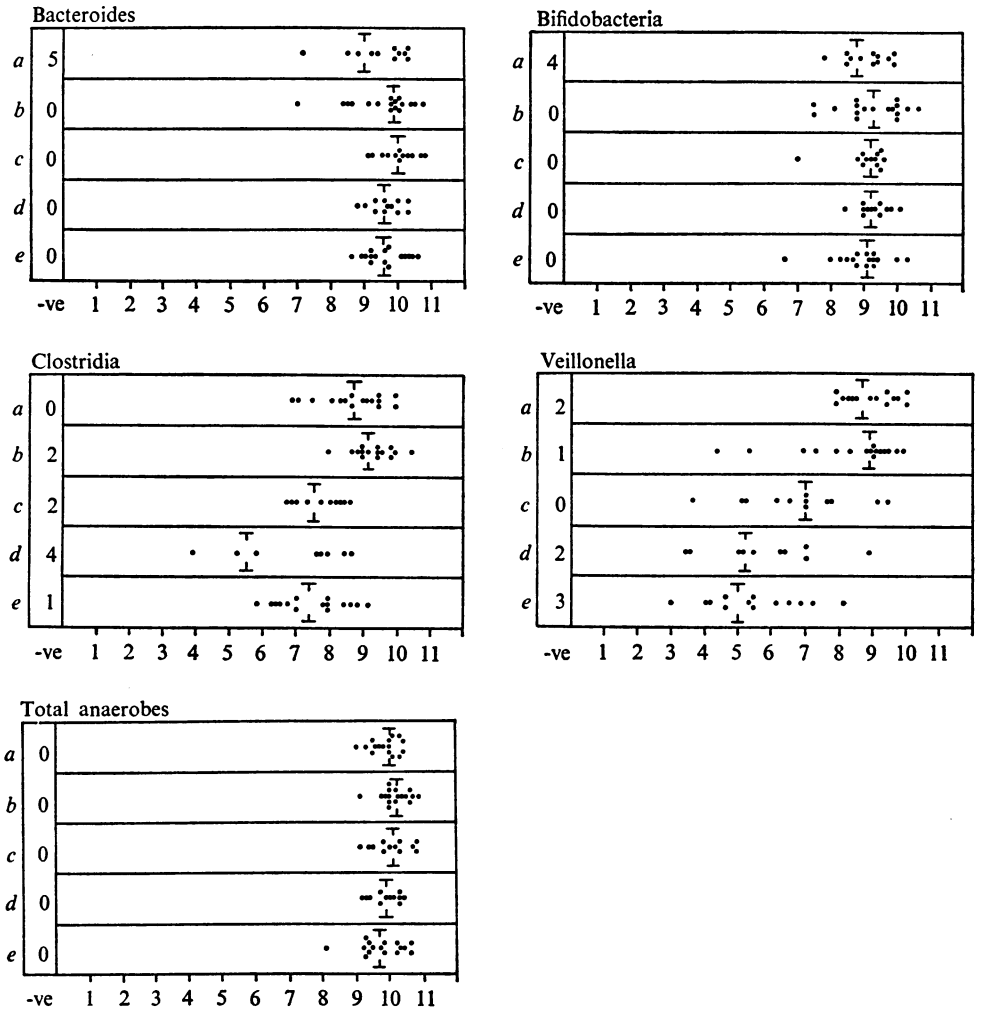

Fig. 1. Concentrations of 10 groups of organisms in the faeces of healthy humans of five different age groups. Concentrations are expressed as $\log_{10}$ organisms/g. of faeces (wet weight). $\overset{\top}{\perp}$ represents the median organism concentration. -ve refers to the number of occasions when organisms of each bacterial group were not isolated. It does not necessarily imply concentrations of < 100 organisms/g. of faeces. *a*, 16 infants aged < 4 months (mean age 5·5 weeks); *b*, 17 infants aged 4–12 months (mean 6·3 months); *c*, 12 children aged 13–48 months (mean 25·3 months); *d*, 12 children aged 49 months–8 years (mean 77·2 months); *e*, 16 adults aged 12–63 years (mean 32 years).

aerobes was now approximately 10:1, and this change reflects almost entirely the now falling concentrations of enterobacteria and streptococci.

This ratio, again dominated by these two bacterial groups, is about 50:1 in children aged 13–48 months. The more numerous faecal organisms are now present in concentrations not significantly different from their adult concentrations, although some, e.g. veillonella, still appear at concentrations which are clearly part of a delayed trend towards adult values. Alone of all the bacterial groups

Table 1. *Ratio of total anaerobes to total aerobes in faeces*

	Age groups				
	< 4 months	4–12 months	13–48 months	49 m.–8 years	12–63 years
Mean age ...	5·5 wk	6·3 m.	25·3 m.	77·2 m.	32 yr
No. of subjects ...	16	17	12	12	16
Total anaerobes	10·0*	10·2	10·2	9·9	9·7
Total aerobes	9·8	9·3	8·5	8·1	7·4
Ratio	1·6:1	7·9:1	50:1	63:1	200:1

* Concentrations are expressed as $\log_{10}$ organisms/g. of faeces (wet weight).

aerobic lactobacilli still remained at significantly lower concentrations than in adult stools, and these organisms subsequently reached adult concentrations in children aged 49 months to 8 years.

DISCUSSION

The concentrations of organisms in adult faeces are in broad agreement with the findings of others published from this (Drasar, Shiner & McLeod, 1969), and other industrialized countries (Gorbach, Nahas, Lerner & Weinstein, 1967) using reasonably comparable techniques. The preponderance of anaerobic over aerobic species is again demonstrated.

Tissier (1899, cited by Rosebury, 1962) first reported that the predominant bacterium in the faeces of nursing infants was a gram-positive strictly anaerobic bacillus. He also noted that in bottle-fed infants this organism persisted but was more commonly accompanied by others, e.g. coliforms, yeasts, staphylococci and aerobic lactobacilli, but with much variation from infant to infant. Gyllenberg & Roine (1957) showed that counts of *Actinomyces bifidus* were often higher in bottle fed than breast fed babies. In infants fed on cow's milk the concentrations of coliforms and enterococci increased. Braun *et al.* (1965) stated that bifidus bacteria were constantly present in bottle-fed babies, but that the types were to some extent different from those of the breast-fed infant. Our study shows that the organisms now classified as bifidobacteria are occasionally absent from the stools of very young artificially fed infants. By 4–12 months, however, they are always present, and persist in similar concentrations until adult life.

Putrefactive anaerobic bacteria (bacteroides and clostridia) are known to increase in concentration or in frequency of isolation with weaning, although the actual mechanisms responsible for this are unknown. These bacteria, often isolated within hours or days of birth, generally disappear again within days in the breast fed infant (Smith & Crabb, 1961; Haenel, 1970), but persist in children fed on artificial formula feeds from birth. This would explain the high concentrations and isolation rates of those particular organisms in this study, in which the infants never received breast milk. Isolation rates and concentrations of veillonellas as high as this have been previously described only in breast-fed Guatemalan infants from about 5 weeks of age (Mata & Urrutia, 1971).

Bullen & Willis (1971) have provided experimental evidence which suggests that the inter-relationships between *E. coli* and anaerobic lactobacilli are governed by the relative lactose, phosphate and protein concentrations and relative buffering capacities of breast and artificial milks. Differences in the concentrations and metabolic activities of human faecal bacteria have been described in adults from different geographical areas and these have been related to the effects of differing diets (Hill *et al.* 1971).

Most faecal bacteria reached adult concentrations within the age group 13–48 months, although there were exceptions both before and after this age range. In particular, aerobic lactobacilli reached adult concentrations only within the age group 49 months–8 years, and were not always isolated then. Although diet seems to play a fundamental role in determining the development of the faecal flora, the reasons why particular organisms appear in particular concentrations and at these particular ages are not clear. The continuing accumulation of knowledge about faecal organisms and their complex inter-relationships should provide more precise answers to some of these questions.

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