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Diet and the faecal microflora of infants, children and adults in rural Nigeria and urban U.K.

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SUMMARY

The faecal microflora of breast-fed infants, weaned children and adults has been examined in rural Nigeria and urban U.K. Breast-fed infants had a similar anaerobic flora dominated by bifidobacteria but bacteroides were isolated in less than a quarter of either community. Weaned children in both communities had greater numbers of bacteroides and clostridia than breast-fed infants. Even higher numbers of bacteroides and clostridia were present in U.K. adults but not in Nigerian adults. Numbers of bacteroides and clostridia were greater in a group of Nigerian infants drinking cow's milk than those receiving breast milk alone and lower in a group of weaned children with diarrhoea compared with uninfected subjects.

INTRODUCTION

Soon after birth the intestinal tract of most mammalian species becomes colonized by a microflora derived from the environment, the so-called autochthonous flora (Schaedler, Dubos & Costello, 1965) which in many species appears to control the replication and colonization of organisms that are ingested subsequently (Floch, Gorbach & Luckey, 1970). This is not always efficient in man for diseases related to intestinal bacteria are major public health problems. In the developing world, diarrhoeal disease, much of which is caused by pathogenic coliform bacteria (Ellis-Pegler, Higgs & Lambert, 1979) continues to be an important cause of morbidity and malnutrition (Mata *et al.* 1976). In more developed countries, colonic cancer, putatively related to bacterial metabolism, is one of the most common malignancies (Aries *et al.* 1969).

There are considerable differences in the intestinal flora between adults from developing and more developed countries (Drasar & Hill, 1974). These have been attributed to the obvious differences in diet but the effect of manipulation of dietary fibre (Drasar, Jenkins & Cummings, 1976) and fat (Cummings *et al.* 1978) in adults on their faecal microflora is minimal. By contrast, the faecal microflora of babies is very sensitive to changes in feeding patterns; the introduction of cow's

milk rapidly decreases numbers of bifidobacteria and increases pH and numbers of bacteroides (Bullen, Tearle & Stewart, 1977). Information on the transition from infant to adult patterns is scarce. A study of 12 children in a village in Guatemala showed a shift towards the adult pattern as the children were weaned on to solid foods (Mata & Urrutia ,1971). A study of infants, children and adults in U.K. showed a different pattern of development (Ellis-Pegler, Crabtree & Lambert, 1975) but because these children were bottle-fed and because of methodological differences it is not possible to compare these countries directly.

In view of the stability of the flora of the adult intestine, we proposed that the differences in microflora between developing and more developed communities become established as a result of differences in diet during infancy and childhood. We therefore examined quantitative faecal bacteriology of groups of subjects on different diets in Nigeria and the U.K. to determine the stage at which the 'adult' flora becomes established.

MATERIALS AND METHODS

Subjects

Nigeria. All the subjects were in villages in Malumfashi district, a rural area in northern Nigeria. Subjects were selected from a list prepared by enumeration of adults and children as part of the demographic studies of the Endemic Diseases Research Unit. Neonates were recruited during the course of the present study. The nutritional and environmental characteristics of this population are described elsewhere (Tomkins *et al.* 1978). Faecal samples were collected by a field worker who transported them rapidly to a portable liquid nitrogen refrigerator.

U.K. The subjects were children and mothers living in north London, U.K., who belonged to the National Childbirth Trust (an organization which among other activities promotes the concept of breast feeding). Faecal samples were collected by a laboratory worker and rapidly transported to a portable liquid nitrogen refrigerator.

Group A. Nigerian babies between one and three months of age who had been breast fed solely without any supplement

Group B. U.K. babies between one and three months of age who had been breast fed solely without any supplement

Group C. Nigerian infants, aged four to six months, receiving cereal diet (guinea corn, millet or maize pap) in addition to breast milk

Group D. Nigerian infants, aged four to six months, receiving cereal diet and breast milk together with tinned cow's milk supplements

Group E. Nigerian children, aged two to four years, fully weaned off the breast, receiving a diet of solids including cereal preparations, beans, cow's milk and occasional meat or eggs

Group F. U.K. children, aged two to four years, fully weaned off the breast, in whom there was more frequent intake of meat and dairy products than children in Group E

Group G. Nigerian mothers of children in Groups A, C, D and E

Group H. U.K. subjects who were asymptomatic apparently healthy laboratory or medical staff personnel

Group I. A group of weaned children taking a similar diet to children in Group E in whom acute diarrhoea, passing three or more loose, watery stools daily, had been present for 1–4 days

Storage of specimens

After collection, specimens were diluted 1 in 10 in 10% glycerol broth and immediately frozen in liquid nitrogen where they were stored until cultured. This procedure minimizes the losses of bacteria during transport (Crowther, 1971).

Culture techniques

The samples were thawed rapidly to minimize losses due to phase changes. Tenfold dilutions were prepared in molten agar and 0·1 ml aliquots of the suspension dispensed and allowed to solidify. A separate dilution series was prepared for each medium used. The procedure was described and evaluated by Sharpe & Kilsby (1971). The Colworth droplette combined dispenser/diluter/viewer obtained from Seward Laboratory (UAC House, London) was used throughout the procedure. On this viewer the agar drop acts as a lens projecting an image on to ground glass screen, this enables some 200 colonies to be counted.

Enterobacteria were detected on McConkey's agar, faecal streptococci on methylene blue sodium azide medium, bifidobacteria on the pH 5 agar of Willis *et al.* (1973) and bacteroides on the bile-kanamycin agar of Drasar, Jenkins & Cummins (1976) without blood. Clostridia were detected in reinforced clostridial agar made selective by the addition of 400 μg/ml of *D*-cycloserine.

Drops of pH 5 agar, bile-kanamycin and cycloserine agar were incubated for two days at 37 °C in an atmosphere of 90% hydrogen and 10% carbon dioxide. Other media were incubated aerobically overnight. Bacteria were assigned to broad groups on the basis of Gram reaction and growth characteristics on the various media.

The droplette technique was adopted as a means of minimising the exposure of anaerobic bacteria to atmosphere oxygen without the use of complex apparatus or pre-reduced anaerobically sterilized media. For this purpose the technique proved admirable, however, the subculture of micro-colonies viewed on the ground glass screen using an inoculating needle needs to be practised.

The lower level of detection of bacteroides and bifidobacteria was 10000/g of faeces; that of other organisms was 100/g of faeces.

Bacterial counts are presented in this paper in terms of the logarithmic means of those samples from which bacteria were isolated.

RESULTS (Table 1)

Weaned children versus breast-fed infants (Fig. 1)

Bacteroides were excreted by more Nigerian (87%) and U.K. (66%) weaned children than breast-fed infants in Nigeria (16%) or U.K. (25%). Numbers of

Table 1. *Concentrations of selected bacterial groups in faeces from Nigerian and English infants, children and adults*

	Number of subjects	Log_{10} viable bacteria per gram of faeces: mean, (range), % of subjects in which bacteria were detected				
		Enterobacteria	Faecal Streptococci	Bifidobacteria	Bacteroides	Clostridia
A Nigerian babies (breast fed)	37	7·4 (4·2–9·6) 97 %	6·7 (2·0–10·1) 76 %	10·2 (8·5–11·0) 95 %	6·9 (6·1–8·0) 16 %	2·7 (1·5–4·6) 85 %
B English babies (breast fed)	12	7·3 (4·5–9·0) 100 %	7·1 (4·3–9·7) 83 %	10·6 (8·6–12·0) 100 %	6·6 (5·0–7·8) 25 %	4·1 (2·1–7·1) 85 %
C Nigerian infants (supplemented - cereals)	26	7·6 (3·7–9·3) 100 %	7·6 (5·0–9·6) 92 %	10·0 (9·6–10·5) 88 %	7·7 (5·3–10·5) 46 %	4·6 (3·0–6·5) 70 %
D Nigerian infants (supplemented – milk)	7	8·1 (6·7–9·6) 100 %	7·0 (2·0–9·5) 100 %	10·3 (10·0–10·7) 100 %	8·0 (7·2–8·6) 71 %	5·2 (3·7–5·6) 71 %
E Nigerian children (weaned)	30	7·5 (3·7–9·6) 100 %	7·5 (5·4–10·3) 97 %	9·8 (7·7–11·0) 83 %	7·4 (6·2–9·0) 87 %	5·2 (2·6–7·6) 100 %
F English children (weaned)	12	6·9 (3·0–8·8) 100 %	5·7 (3·0 -8·6) 92 %	10·4 (9·4–11·2) 100 %	7·8 (5·3–10·0) 66 %	4·6 (2·9–6·6) 100 %
G Nigerian mothers of children in groups A, C, D and E	24	8·3 (5·8–10·8) 100 %	8·0 (3·6–9·6) 100 %	10·0 (8·6–11·2) 100 %	6·8 (4·2–9·5) 15 %	5·9 (3·0–7·8) 94 %
H U.K. subjects who were asymptomatic apparently healthy laboratory or medical staff	10	7·2 (5·0–8·9) 100 %	6·7 (3·9- 9·8) 100 %	10·0 (8·9–10·3) 100 %	10·2 (9·7–10·7) 100 %	5·8 (4·4–6·9) 100 %
I Weaned Nigerian children with acute gastroenteritis	6	7·5 (7·2–8·5) 100 %	8·6 (7·7–9·5) 83 %	8·8 (7·0–9·5) 100 %	5·0 (5·0) 16 %	4·2 (3·0–5·0) 67 %

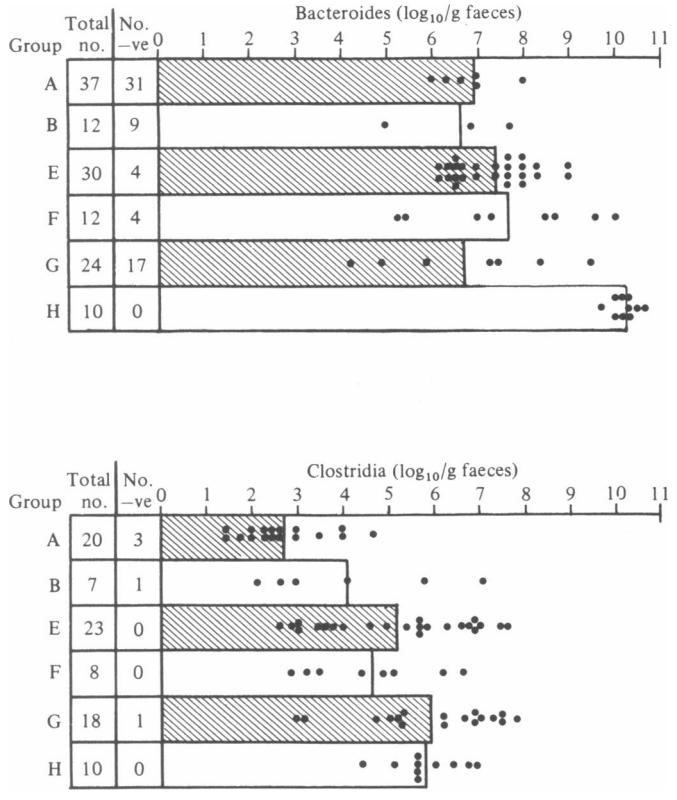

Fig. 1. Concentrations of bacteroides (upper) and of clostridia (lower) in faeces of breast-fed infants, weaned children and adults in rural Nigeria (hatched columns) and urban U.K. (open columns). (In groups A, B, E, F and G some unsatisfactory culture conditions for clostridia limited the number of specimens available for analysis.)

bacteroides were higher in weaned children in Nigeria (7·4 log_{10}/g faeces) and U.K. (7·8 log_{10}/g faeces) than in breast-fed infants in Nigeria (6·9 log_{10}/g faeces) or U.K. (6·6 log_{10}/g faeces). Clostridia were excreted by more U.K. weaned children (100 %) than breast-fed infants (85 %) and by a similar proportion (100 % and 85 %) of weaned and breast-fed Nigerian infants. Numbers of clostridia were greater in weaned children in Nigeria (5·2 log_{10}/g faeces) and U.K. (4·6 log_{10}/g faeces) than in breast-fed infants in Nigeria (2·7 log_{10}/g faeces) or U.K. (4·1 log_{10}/g faeces).

The proportions of subjects excreting bifidobacteria, faecal streptococci and enterobacteria and numbers of these bacterial species isolated were similar in breast-fed infants and weaned children in both communities.

Weaned children versus adults (*Fig.* 1)

Bacteroides were present in a greater proportion (100 % *v.* 66 %) and in greater numbers (10·2 *v.* 7·8 log_{10}/g faeces) in U.K. adults than in weaned children. However, a low proportion of Nigerian adults compared with weaned children

excreted bacteroides (15% *v.* 87%). Bacterial numbers were similar (6·8 *v.* 7·4 $\log_{10}$/faeces) in Nigerian adults and weaned children; both were lower than the numbers in U.K. adults (10·2 $\log_{10}$/g faeces). Clostridia were present in all the adults and weaned children in U.K. They were present in all the Nigerian weaned children and most of the Nigerian adults (94%). There were greater numbers of clostridia in U.K. adults compared with U.K. weaned children (5·8 *v.* 4·6 $\log_{10}$/g faeces) and in Nigerian adults compared with weaned children (5·9 *v.* 5·2 $\log_{10}$/g faeces). The proportion of subjects excreting bifidobacteria, faecal streptococci and enterobacteria were similar in weaned children and adults in both communities. Numbers of these bacterial species were similar in adults and weaned children.

Effect of weaning pattern on Nigerian faecal flora (*Fig.* 2)

Supplementation with cereal paps: Bacteroides were present in a higher proportion (46% *v.* 16%) and in greater number (7·7 *v.* 6·9 $\log_{10}$/g faeces) in Nigerian infants receiving cereal supplements in addition to breast milk compared with those receiving breast milk alone. Clostridia were present in similar proportions, 85% and 70% respectively, in those babies who were breast-fed alone or receiving cereal supplements but greater numbers of clostridia (4·6 *v.* 2·7 $\log_{10}$/g faeces) were isolated in the cereal-supplemented group. The proportion of subjects excreting bifidobacteria, faecal streptococci and enterobacteria were similar in both groups, as were the numbers of their bacterial species isolated.

Supplementation with cow's milk: Bacteroides were present in even higher proportion of those infants receiving cow's milk (71%) than in those receiving breast milk alone (16%). Similarly, the numbers of bacteroides were higher in infants receiving cow's milk (8·0 $\log_{10}$/g faeces) than those receiving cereal (7·7 $\log_{10}$/g faeces) or breast milk alone (6·9 $\log_{10}$/g faeces). The numbers of clostridia were higher in infants receiving cow's milk (5·2 $\log_{10}$/g faeces) compared with those on breast milk alone (2·7 $\log_{10}$/g faeces). Similar proportions of children receiving cow's milk excreted bifidobacteria, faecal streptococci and enterobacteria but numbers of the latter were higher (8·1 $\log_{10}$/g faeces) in those receiving cow's milk than breast milk alone (7·4 $\log_{10}$/g faeces).

Effect of diarrhoea (*Fig.* 2)

Fewer weaned children with diarrhoea (16%) excreted bacteroides compared with uninfected children (87%) and numbers were lower in the diarrhoea group (5·0 $\log_{10}$/g faeces) than in the uninfected (7·4 $\log_{10}$/g faeces). Fewer children with diarrhoea excreted clostridia (67%) compared with uninfected children (100%) and lower numbers of clostridia were isolated in children with diarrhoea (4·2 $\log_{10}$/g faeces) than in those without (5·2 $\log_{10}$/g faeces). Similar proportions of bifidobacteria, faecal streptococci and enterobacteria were present in children with and without diarrhoea and bacterial numbers were similar.

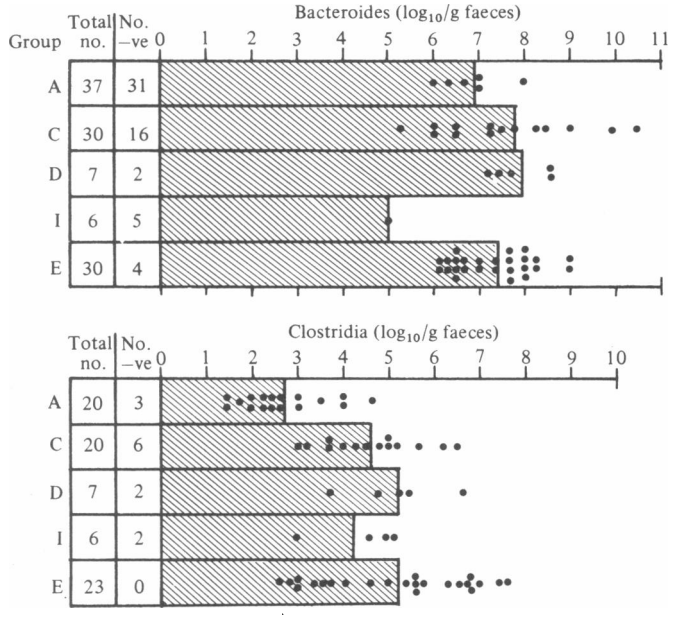

Fig. 2. Concentrations of bacteroides (upper) and of clostridia (lower) in faeces of Nigerian breast-fed infants (A) infants receiving cereal supplement (C) or cow's milk supplement (D), healthy weaned children (E) and weaned children with diarrhoea (I). (In groups A, C, and E some unsatisfactory culture conditions for clostridia limited the number of specimens available for analysis.)

DISCUSSION

Although there are characteristic differences between the faecal flora of adult subjects in developing and more developed countries the factors which control intestinal colonization are largely undefined. The demonstration of low numbers of bacteroides in breast-fed infants in Nigeria and the U.K. is in agreement with previous studies in Guatemala (Mata & Urrutia, 1971) and in the U.K. (Bullen *et al.* 1977) which show a predominantly fermentative microflora with a low pH. The addition of cereals to our breast-fed Nigerian children was associated with greater numbers of bacteroides in a similar manner to that observed in rural Guatemala (Mata & Urrutia, 1971), but they were not as numerous as in our U.K. children weaned on to a more varied diet. The most striking effect was in Nigerian infants who received cow's milk; many more clostridia and bacteroides were isolated and we found similar numbers to those described by Bullen *et al.* (1977) in bottle-fed infants in U.K. and in the bottle-fed infants in Jamaica described by Ellis-Pegler *et al.* (1979).

There were noticeable differences in the faecal flora of the adults in our study. Greater numbers of bacteroides and clostridia were present in U.K. than Nigerian adults whose flora was not very different from that of weaned Nigerian children (Group E). This similarity in faecal flora between weaned children and adults is in agreement with the only other comparative study in a developing country (Mata &

Urrutia, 1971). The greater numbers of clostridia in U.K. adults than weaned children is similar to that described by Ellis-Pegler *et al.* (1975). They found that high numbers of bacteroides were present in their U.K. children in similar concentrations to those in their adult subjects. However, we found fewer bacteroides in the children. We do not have accurate dietary data on our children but the consumption of high fibre breakfast cereals and bread was a characteristic of this U.K. study group. The decrease in numbers of anaerobic organisms during acute diarrhoea as described in previous studies (Gorbach *et al.* 1970) emphasises that during early childhood the faecal flora is susceptible to factors other than diet. However, these changes during diarrhoea are transient (Ellis-Pegler *et al.* 1979) and the greatest influence on the microflora is likely to be dietary intake.

It would be interesting to know the effect of a vegetarian diet taken since childhood in U.K. subjects. A previous study of the faecal flora of adult vegetarians (Aries *et al.* 1971) showed little difference from meat-eating subjects in U.K. but the majority of the vegetarians had changed to this dietary pattern after childhood; perhaps their faecal flora had already become fixed by childhood eating practices. It would also be interesting to know the effect of a change in diet from cereals to a more varied diet, after immigration to a more developed country for example.

There is considerable attention currently towards manipulation of the diet to prevent disease. Most studies suggest that the effect of change in dietary fibre, fat and protein on the faecal flora of adults is very minimal. Our findings, together with those of others, suggest that the diet of infancy and childhood may determine the faecal flora of adulthood. We suggest that for all age groups, informed advice about the potential benefits of diet can only come after a greater understanding of the intestinal metabolism of the developing child.

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