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Diet and faecal flora in three dietary groups in rural northern Nigeria

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SUMMARY

Quantitative bacterial counts, concentrations of bile acids and bacterial enzyme profiles were measured in faecal samples from three tribal groups with distinctive dietary patterns in a rural area of northern Nigeria. Samples were obtained from Maguzawa (with a predominantly cereal diet), Hausa (cereal with regular meat consumption) and Fulani (cereal with frequent consumption of cows' milk). Numbers of bacteroides, clostridia and concentrations of bile acids were lower in the Maguzawa than the other dietary groups but these differences were not statistically significant. Bacterial enzyme profiles in each group were similar. The results are discussed with respect to possible influences of diet on the human intestinal microflora.

INTRODUCTION

Millions of bacteria become established as part of the autochthonous flora of the human intestine within a few hours of birth. In a previous comparative study of infants and children in the UK and rural Nigeria we emphasized the importance of differing dietary patterns in determining the differences in intestinal microflora that we observed (Tomkins *et al.* 1981). The introduction of cows' milk to the diet rapidly decreases numbers of bifidobacteria and increases the pH and numbers of bacteroides (Bullen, Tearle & Stewart, 1977).

The autochthonous flora has an important role in the protection against establishment of enteropathogens such as *Vibrio cholerae*, *Clostridium difficile*, *Shigella* sp., *Salmonella* sp. and *Campylobacter* sp. (Floch, Gorbach & Luckey, 1970). This is of considerable benefit to children in the contaminated environment of rural northern Nigeria (Tomkins *et al.* 1978). However, in recent years the metabolic activity of the intestinal flora, particularly its effects on bile acids has also been implicated in the development of colon cancer (Drasar & Hill, 1974). International studies have compared the striking differences in dietary fibre, protein and fat between rural African populations and UK populations inferring that their dietary differences might account for the differences in numbers of intestinal bacteria between these populations (Aries *et al.* 1969).

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Relatively few studies have examined the faecal flora between subjects in a similar environment but eating different diets. A study of vegetarian subjects in UK showed similar flora to meat-eating compatriots (Aries *et al.* 1971) and the addition of fibre or decrease of fat appears to have relatively little effect on the faecal flora of volunteers (Drasar, Jenkins & Cummings, 1976).

It might be argued that changes in diet can only influence the type of faecal flora if they occur during infancy or early childhood. Indeed our previous data suggests that the flora is relatively 'flexible' up to the age of weaning on to solid food. Thereafter it becomes more 'rigid' (Tomkins *et al.* 1981).

The Savannah area of northern Nigeria gives an ideal opportunity for the study of populations living close to one another yet having different lifestyles and dietary patterns. Our objective was to compare and contrast the faecal flora of a relatively underprivileged group of rural villagers who ate predominantly cereal (the Maguzawa) with better-off villagers who frequently took meat in addition to cereal (the Hausa) and a group of pastoralists (the Fulani) who regularly drank considerable quantities of cows' milk.

METHODS

Our original study was performed as part of an investigation of diet, nutritional status and intestinal microflora among Hausa adults and children in Malumfashi district, a rural area in Kaduna state, northern Nigeria (Tomkins *et al.* 1978, 1981). This study also describes findings among two other groups, each with characteristic dietary patterns.

The Maguzawa live in single compounds at some distance from the villages and are predominantly farmers of sorghum, maize or millet which constitutes their staple diet. Meat is eaten less frequently than once a month and cows' milk is taken infrequently.

The Hausa are of similar ethnic stock to the Maguzawa but live in compounds within villages where access to markets is greater. They are also farmers of sorghum, maize or millet but many had other occupations, especially trading, in addition. For a variety of reasons the Hausa appeared, in this area at any rate, to be more prosperous than the Maguzawa and meat was taken, on average, twice weekly. In addition both adults and children drank milk, which was purchased from the pastoralist Fulani, quite regularly.

The Fulani are a pastoralist group of different ethnic origin to either of the above groups who usually migrate in order to enable their cattle to forage. During the time of this study (the rainy season of 1979) they were living adjacent to the Hausa villages and their herds of cattle provided a regular supply of milk which was consumed by themselves and, in lesser quantities, by the Hausa.

This study was approved by the Ethics Committee of the Faculty of Medicine, Ahmadu Bello University, Zaria.

Collection and storage of specimens

Faecal samples were collected by a field worker who transported them rapidly in sterile containers to a portable liquid nitrogen refrigerator. 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 serial dilutions were prepared in pre-reduced anaerobically sterilized (PRAS) diluents and 1 ml of each dilution was transferred under continuous anaerobiosis into tubes of molten selective and non-selective PRAS agars. The inoculated tubes of agar were spun to form roll tubes (Drasar, 1974; Holdeman, Cato & Moore, 1977). After incubation for 48 h at 37 °C, colonies were counted and the number of bacteria in the specimen calculated on the basis of those dilutions yielding 50–200 colonies/tube.

All media used for the PRAS cultures contained 0·05% cysteine and 0·03% sodium formaldehyde sulphoxalate as an Eh buffer, 1 μg/ml of resazurin as an Eh indicator; haemin 10 μg/ml and 1 μg/ml menadione. The pH buffer was 0·5% (w/v) $NaHCO_3$ with a 100% carbon dioxide gas phase (oxygen-free carbon dioxide, British Oxygen Special Gases Division) except for the bifidobacterium medium.

Brain–heart infusion agar and Columbia agar (Oxoid) were used as non-selective media. Bacteroides were isolated on the bile–kanamycin agar of Drasar, Jenkins & Cummings (1976) without blood.

Bifidobacteria were isolated on a maltose, kanamycin, naladix acid agar similar to the medium described by Sutter, Vargo & Finegold (1975). The medium was prepared from reinforced clostridial agar (Oxoid) pH 5 with added maltose, 0·1%, kanamycin, 75 μg/ml; and naladixic acid, 100 μg/ml. Enterobacteria were counted on McConkey's agar incubated aerobically. Clostridia were detected in reinforced clostridial agar made selective by the addition of 400 μg/ml of D-cycloserine and incubated for 2 days at 37 °C in an atmosphere of 90% hydrogen and 10% carbon dioxide. These media were prepared as plates and spread inoculated with 0·1 ml of the dilution series prepared in PRAS dilutent. Bacteria were assigned to broad groups on the basis of Gram reaction and growth characteristics on the various media. The lower level of detection of bacteroides and bifidobacteria was 10000/g for 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.

Faecal bile acids

Faecal steroids were extracted from faeces using glacial acetic acid. The acid and neutral steroids were separated by the method of Evrard & Janssen (1968). Solvent extractions were performed in plastic bags. Homogenization of solvents with other materials was performed in a Stomacher 80 (Colworth Laboratory Equipment, Suffolk, UK), the acid steroid fraction was dissolved in 3 ml ethanol and treated with 5 mg sodium borohydride for 1 h, after careful acidification with hydrochloric acid the reduced acid steroids were extracted with ether and quantified by the hydroxysteroid dehydrogenase method of Iwata & Yamasaki (1964).

Table 1. *Bacteria in faeces from three tribal groups in northern Nigeria*

(Viable organisms mean ± S.D. $\log_{10}$/g faeces. Number of subjects with organism in parentheses.)

	Maguzawa		Hausa		Fulani	
Tribal group...	Children	Adults	Children	Adults	Children	Adults
No. of subjects...	16	9	30	19	15	16
Anaerobic bacteria	9·7 ± 0·5 (16)	10·2 ± 0·6 (9)	10·9 ± 0·3 (30)	10·0 ± 0·2 (19)	11·5 ± 0·6 (15)	10·3 ± 0·4 (16)
Bacteroides	7·4 ± 0·6 (14)	8·1 ± 0·4 (7)	8·7 ± 0·2 (28)	8·5 ± 0·4 (19)	10·3 ± 0·9 (15)	9·0 ± 0·7 (16)
Bifidobacteria	9·3 ± 0·5 (16)	9·5 ± 0·8 (9)	9·5 ± 0·4 (30)	9·3 ± 0·2 (19)	10·2 ± 0·5 (15)	9·9 ± 0·8 (16)
Clostridia	2·7 ± 0·6 (10)	6·0 ± 0·4 (8)	1·0 ± 1·5 (5)	5·4 ± 1·3 (19)	6·1 ± 1·2 (6)	7·0 ± 1·7 (16)
Enterobacteria	7·7 ± 1·7 (16)	7·4 ± 0·4 (9)	7·4 ± 1·1 (30)	7·3 ± 0·4 (19)	8·7 ± 1·5 (15)	7·0 ± 1·6 (16)

Table 2. *Faecal bile acids mg/g dry weight faeces* (± S.D.) *in faeces from three tribal groups in northern Nigeria*

	Maguzawa		Hausa		Fulani	
Tribal group...	Children	Adults	Children	Adults	Children	Adults
No. of subjects...	9	6	11	10	10	15
Faecal bile acids	2·8 ± 2·1	3·4 ± 1·8	3·5 ± 1·4	5·4 ± 1·9	3·7 ± 1·5	4·2 ± 1·7

Bacterial enzymes in faeces

Semi-quantitative data was obtained on a range of enzymes using a commercially developed system (APIzyme, API Laboratory Products Ltd, Basingstoke, Hampshire, UK). The enzymes assayed were alkaline phosphatase, acid phosphatase, phosphoamidase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, chymotrypsin, trypsin α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The system consists of a series of preformed plastic cupules each, save one, of which contains a colorimetric substrate for a particular enzyme. Cupules were filled with a 10-fold, 100-fold, or 1000-fold dilution of faeces in PRAS diluent. Strips of inoculated cupules were incubated at 37 °C for 18 h. After incubation the colour developed from the enzyme substrate was compared with the colour in the cupule without any substrate and with a standard colour chart. Results were scored on arbitrary scale 0–4.

RESULTS

The bacterial numbers of faecal samples from children and adults in the three tribal groups are shown in Table 1. Total numbers of anaerobic bacteria, bifidobacteria and enterobacteria were similar in each group. There were fewer bacteroides and clostridia in the Maguzawa than in the Hausa or Fulani, however

Table 3. *Enzyme activities in faeces: averages score on API colour scale of* 1–4

	Magazawa			Hausa			Fulani	
Tribal group...	Infants	Children	Adults	Infants	Children	Adults	Children	Adults
No. of subjects	1	6	2	3	12	4	3	4
Phosphatase alkaline	3	4	3·5	4	4·08	4	4	3·75
Esterase (C4)	—	1·25	—	1	1	—	1	—
Esterase lipase (C8)	3	2·5	2·5	3·6	3·09	2·5	2·3	2·25
Lipase (C14)	—	1	—	—	1·5	—	1	—
Leucine arylamidase	3	1·75	1	2·3	1	1·5	1·63	1
Valine arylamidase	3	3	—	1·6	1	—	2	—
Cystine arylamidase	3	—	—	—	—	—	—	—
Trypsin	—	1	1	—	—	—	—	—
Phosphatase acid	4	3·66	2·5	3·3	1·3	3·65	3·3	3·5
Phosphoamidase	4	3·5	3	4	3·16	3·65	3·63	3·25
L-galactosidase	1	2	2·5	—	3·08	3·65	1·63	3·75
β-galactosidase	3	3·5	3	1·3	3·5	3·5	3	3·75
β-glucuronidase	1	3·66	2·5	3	3·5	3·65	2·63	4·25
L-glucosidase	4	3·16	2·5	4	3·58	3·65	3·63	3·5
β-glucosidase	2	2·6	2	—	3·25	3·65	2	3·5
N-acetyl-β-glucosaminidase	3	2·84	2·5	3	3·3	3·65	2·63	3·25
L-mannosidase	—	—	—	1	—	—	—	—
L-fucosidase	—	—	—	—	1	2	1	2

these differences were not statistically significant. The concentrations of faecal bile acids are shown in Table 2. Again there were lower concentrations of faecal bile acids in the Maguzawa compared with the Fulani or Hausa but these were not statistically significant.

The results of bacterial enzyme analysis are shown in Table 3. There were similar profiles in each dietary group.

DISCUSSION

Studies of the faecal microflora have, for the most part, concentrated on the isolation and identification of bacteria. Improvements in the methods for the isolation and identification of the non-sporing anaerobes now make it possible to identify 300–400 species of bacteria within faecal samples (Holdeman, Good & Moore, 1976). Thus the labour involved for full bacteriological examination is impractical for population-based studies. For this reason we limited our isolation and identification of bacteria to major groups.

In previous studies there have been considerable differences in the counts of bacteroides between populations, those eating greater quantities of meat having more bacteroides (Koornhoff *et al.* 1979). Our sample size was of necessity rather small to achieve results capable of detecting statistically significant differences between the populations in this community. Nevertheless there was an obvious trend in the three populations we studied (i.e. those who ate meat or milk regularly had higher bacteroides counts than those who were predominantly cereal eaters).

Similarly, previous studies have shown that greater concentrations of bile acids

are excreted by populations who habitually consume more meat (Drasar & Hill, 1974). We noted greater concentrations of bile acids in samples from the meat-eating and milk-drinking Hausa and Fulani populations compared with the cereal-eating Maguzawa.

More recent studies on indirect approaches to assessment of bacterial metabolism have examined the use of selected microbial enzymes as markers of bactericidal activity (Goldin, Duyer & Gorbach, 1978). In view of its important role in drug metabolism and its susceptibility to dietary manipulation, β-glucuronidase was of particular interest. However we could not detect any consistent pattern of enzyme profiles which could be regarded as characteristic for any of the dietary groups.

There is now strong epidemiological and experimental evidence for the role of dietary factors in the development of colon cancer and considerable support for a role for diet in the development of an autochthonous flora and subsequent response to enteric pathogens. Such evidence has been accompanied by studies of intestinal metabolism in small numbers of individuals within one community (Drasar, Jenkins & Cummings, 1976) or by international comparisons of communities with very different life styles and dietary patterns (Aries *et al.* 1969).

Our study has tried to limit some of the variables by investigating a single community with a common basic cereal diet but in whom some consume much more meat and milk than others. While the numbers of subjects examined have not been sufficient to detect differences which are statistically significant, we did detect trends in numbers of bacteroides and quantities of bile acids excreted in the different dietary groups. The failure to detect microbial enzyme profiles that were characteristic of the three groups may merely reflect the rather limited range of enzymes that we measured. In view of the need for indirect markers of intestinal metabolism, we suggest that this approach should receive further attention. Particularly valuable would be studies of individuals with different dietary intakes within a similar environment. This might overcome the difficulties in interpreting studies of human populations which have hitherto relied on measurement of intestinal numbers alone (Holdeman, Good & Moore, 1976). Our study has demonstrated that such approaches using reliable techniques for sample storage are suitable in quite remote areas of Nigeria.

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