Instability in the faecal flora of a patient suffering from food-related irritable bowel syndrome

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Summary. The faecal microbial flora of a patient with severe irritable bowel syndrome related to multiple food intolerances was very variable and contained a high proportion of facultative bacteria and an unusual incidence of *Clostridium* species.

Introduction

It is recognised that intolerance to specific foods or food components is important in some patients suffering from irritable bowel syndrome (IBS) and diet management has been successful in controlling symptoms (Hunter and Alun-Jones, 1985; Hunter *et al.*, 1985). Several factors suggest involvement of the intestinal flora and patients may have variable faecal floras, with higher total counts of facultative bacteria (Bayliss *et al.*, 1984) or higher proportions of gram-negative facultative bacteria (Bradley *et al.*, 1985) than controls.

Some patients exhibit extreme symptoms with multiple intolerances and are restricted to very limited diets. It has been recommended that these patients return to management by drug treatment rather than attempt complex exclusion diets (Hunter *et al.*, 1985). In view of the established value of antibiotic treatment in diverticulitis (Larson *et al.*, 1976) and pseudomembranous colitis (Tedesco *et al.*, 1978), together with the possible involvement of the intestinal flora and previous work on gut sterilisation and sterile diets in IBS (Kane and Neale, 1976), courses of antibiotics were prescribed. The patient in this study had not responded to pharmacological management. Her multiple food intolerances resulted in a severely restricted diet and, at times, total parenteral nutrition was required to sustain her. To assess whether the composition of the intestinal flora was abnormal, microbial analyses were performed on samples of faeces collected during a period of 18 months.

Patients and methods

Case history of patient P2

A 42-year old woman presented 6 weeks after hysterectomy, with abdominal pain, diarrhoea, severe headaches and muscular weakness. These symptoms have been shown to be associated with food intolerance developing after hysterectomy (Alun-Jones *et al.*, 1984). The initial response of patient P2 to an elimination diet was good, but many foods provoked symptoms when re-introduced. Subsequently, on a limited diet, her weight was maintained between 45 and 50 kg by dietary supplementation with proprietary medium-chain-triglyceride feeds (Trisorban). Indices of nutrition including serum albumin, pre-albumin and transferrin have been maintained at normal levels.

Courses of antibiotics and total parenteral nutrition (TPN) with no oral intake other than sterile water were employed in an attempt to allow her to eat a wider variety of foods. TPN was used on three occasions in 1981, 1982 and 1983 for periods of 4–12 weeks. On the first occasion, immediately after TPN, she was able to eat a wider range of foods for several months, but the number of food intolerances eventually rose again. On subsequent occasions the improvement was of much shorter duration.

Faecal bacterial counts

Freshly voided stools were collected in a plastic bag and immediately placed in an anaerobic cabinet (Forma Scientific) containing an atmosphere of N₂ 85%, H₂ 10% and CO₂ 5%. After kneading by hand, 1g was transferred to a pre-weighed bottle containing glass beads and 10 ml of anaerobic dilution solution (ADS; Bryant and Burkey, 1953). After reweighing, the contents were mixed to form a slurry by vortex mixing. A tenfold dilution series was prepared in ADS and 0.1-ml volumes were plated on pre-reduced VLhlf agar, a complex medium on which a high proportion of faecal anaerobic bacteria can be isolated (Croucher *et al.*, 1983), and incubated in the cabinet for 7 days at 37°C for determination of the viable

anaerobic count. Outside the cabinet, 0.03-ml volumes were plated on CLED Agar (Oxoid) and incubated aerobically for 2 days at 37°C to determine the viable count of facultative bacteria.

A sample of the faeces was dried to constant weight at 100°C for determination of moisture content.

Similar counts were performed on single faecal samples from two daughters of the patient (P2A and P2B) who showed no symptoms of IBS.

Isolation and characterisation of organisms

Twenty colonies from the highest dilution showing growth on VLhlf plates were purified by streaking on to VLhlf agar. Single colonies were selected after incubation for up to 5 days at 37°C, inoculated into tubes of SM10 agar (Barnes and Impey, 1974), a complex medium suitable for use in oxygen-free CO₂ (Hungate, 1969). All subsequent manipulations were performed by this method unless otherwise stated. After checking isolates for aerobic growth, obligate anaerobes were identified to genus level by the methods of Holdeman *et al.* (1977). Preliminary identification of aerobic isolates was made on CLED agar and further characterisation of organisms isolated from the highest dilutions was by the API 20E system (API Laboratory Products, Basingstoke, Hants) or by the methods of Cowan (1974). Isolates were maintained by freezing at -70°C.

Results

Faecal bacterial counts

The total viable bacterial counts in faeces from patient P2 during an 18-month period varied between 1.3×10^{10} and 5.9×10^{11} /g (dry weight) of faeces (table I). The proportions of facultative

organisms in these counts were from 72% (P2/7) to 0.7% (P2/5). The moisture content of faeces varied by more than 25%.

Similar total counts were found in specimens from P2A and P2B but the proportions of facultative bacteria were $\leq 0.01\%$ of the total counts.

Bacteria isolated

The rank order of species in samples from P2 varied considerably from count to count (table II); in several samples species of *Clostridium* formed a considerable proportion of the obligate anaerobes and in one sample (P2/2) all obligate anaerobes isolated were *Clostridium* spp. The dominant facultative organisms in P2 samples were *Streptococcus* spp, *Escherichia coli* and *Proteus* spp.

In samples from P2A and P2B, the principal obligate anaerobes were *Bacteroides* and *Bifidobacterium* spp, with *E. coli* dominating the facultative flora.

Discussion

The faecal flora of the patient in this study contained a high proportion of facultative organisms, in contrast to the findings of Moore and Holdeman (1974) that the dominant facultative species, *E. coli* and *Str. salivarius*, were each present at a mean of 0.4% of the total count. As in the study of Finegold *et al.* (1974), *E. coli*, *Str. faecalis* and *Str. faecium* were the dominant facultative bacteria. Moore and Holdeman (1974), Holdeman *et al.* (1976) and Moore *et al.* (1981) found that the most

Table I. Viable counts and moisture contents of faecal samples from patient P2 and daughters P2A and P2B.

Count no.	Date	Viable count log ₁₀ /g (dry weight)		Ratio of counts, total: facultative	Moisture content(%)
		total	facultative		
P2/0	18 Jun. 83	ND	11.15	—	77.3
P2/1	22 Jul. 83	10.11	9.72	2.6:1	58.1
P2/2	4 Aug. 83	10.53	9.56	9.4:1	84.2
P2/3	8 Aug. 83	10.75	10.15	4.0:1	86.6
P2/4	6 Sep. 83	11.77	10.89	7.6:1	62.5
P2/5	7 Nov. 83	10.23	8.08	138:1	66.1
P2/6	17 Jan. 84	11.26	9.49	57:1	81.8
P2/7	5 Jan. 85	10.48	10.34	1.4:1	71.4
P2A/1	5 Jan. 85	11.40	6.38	104,000:1	62.4
P2B/1	5 Jan. 85	11.86	7.26	41,000:1	60.0

ND = not done.

Table II. Principal organisms ($\geq 10\%$ of isolates) in faecal samples from P2 and her daughters

Count no.	Date	Anaerobes		Facultative bacteria	
		Genus	Percentage of total isolates	Genus (or species)	Percentage of total isolates
P2/0	18 Jun. 83	Not tested	—	<i>Streptococcus</i>	45
				<i>Proteus</i>	40
P2/1	22 Jul. 83	<i>Bacteroides</i>	50	<i>Str. faecalis</i>	60
		<i>Clostridium</i>	30	<i>E. coli</i>	30
P2/2	4 Aug. 83	<i>Clostridium</i>	100	<i>Str. faecalis</i>	99
P2/3	8 Aug. 83	<i>Clostridium</i>	60	<i>Streptococcus</i>	40
		<i>Lactobacillus</i>	30	<i>P. mirabilis</i>	35
		<i>Bifidobacterium</i>	10	<i>E. coli</i>	25
P2/4	6 Sep. 83	<i>Bacteroides</i>	30	<i>E. coli</i>	90
		<i>Clostridium</i>	20	<i>Str. faecium</i>	10
		<i>Ruminococcus</i>	10		
		<i>Lactobacillus</i>	10		
		<i>Bifidobacterium</i>	10		
P2/5	7 Nov. 83	<i>Lactobacillus</i>	26	<i>E. coli</i>	55
		<i>Bacteroides</i>	25	<i>Streptococcus</i>	32
		<i>Clostridium</i>	22	<i>P. mirabilis</i>	12
P2/6	17 Jan. 84	<i>Ruminococcus</i>	40	<i>Proteus</i>	50
		<i>Bacteroides</i>	25	<i>E. coli</i>	25
		<i>Clostridium</i>	10	<i>Streptococcus</i>	25
P2/7	5 Jan. 85	Predominantly facultative		<i>Str. faecium</i>	85
P2A/1	5 Jan. 85	<i>Bacteroides</i>	50	<i>E. coli</i>	99
		<i>Bifidobacterium</i>	30		
		<i>Eubacterium</i>	10		
P2B/1	5 Jan. 85	<i>Bifidobacterium</i>	85	<i>E. coli</i>	95
		<i>Bacteroides</i>	15		

abundant obligate anaerobes in faeces from normal individuals were *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Peptostreptococcus*, *Fusobacterium* and *Ruminococcus* spp. and the authors concluded that each individual tends to maintain a characteristic pattern of species, in marked contrast to the variation shown in this patient. Moore and Holde- man (1974) also found that in 20 healthy individuals, *Clostridium* spp. represented $< 5\%$ of the flora, again in contrast to P2.

In a study of the microbial populations of patients with IBS, but not specifically associated with food intolerance, Balsari *et al.* (1982) noted the appearance, in 6 out of 20 patients, of facultative species (*Pseudomonas* and enterobacteria) which were not present in control subjects. Furthermore, the numbers of *E. coli* and *Bifidobacterium* spp. were lower in patients, but the numbers of clostridia did not differ between patients and controls. The authors suggested that the differences were related to the peristaltic disturbances found in IBS patients but queried whether the flora was influenced by the repeated antibiotic therapy which these patients

undergo. Before taking the sample for count P2/1 the patient was treated at intervals with oxytetracycline; a high level of resistance to this antibiotic was found amongst isolates from sample P2/1; this declined in subsequent counts (data not presented). Between counts P2/1 and P2/2 the patient was treated with colistin sulphate, nalidixic acid, trimethoprim and vancomycin, and strains of two genera present in count P2/1 (*Bacteroides* and *E. coli*) were not detected in P2/2. It seems likely that antibiotic therapy could account for the temporary disappearance of *E. coli* (which reappeared in count P2/3) but it is more difficult to attribute the simultaneous loss of *Bacteroides* directly to the antibiotic treatment used.

In a healthy individual, the composition of the gut flora is affected by complex regulatory forces (Savage, 1982) exerted partly by the host's physiological and immunological status and partly by the microbial community itself. Although the rank order of species in the flora is usually stable, the characteristics of the population may change with diet (Wyatt *et al.*, 1986). It has been established that

the symptoms of food-related IBS connect specific foodstuffs and specific individuals (Hunter and Alun-Jones, 1985); the variable faecal flora seen in patient P2 and in some other patients (Bayliss *et al.*, 1984; Bradley *et al.*, 1985) may be a further factor,

but the underlying mechanisms involved are not yet known.

We thank Anne Smyth for skilled technical assistance with part of this work.

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