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Enterobacteriaceae suppression by three different oral doses of polymyxin E in human volunteers

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SUMMARY

Polymyxin E is frequently used as an oral drug for flora suppression of the gastrointestinal canal. The suppression effect is dose dependent because polymyxin E is moderately inactivated by faecal and food compounds. Three oral polymyxin E doses (150, 300, 600 mg daily) were given to six volunteers for 6 days. The Enterobacteriaceae suppression effect was compared by means of the suppression index i.e. ratio of total number of faecal samples free of Enterobacteriaceae to the total number of faecal samples. The impact on the indigenous (mostly anaerobic) flora was measured in four ways: (i) beta-aspartylglycine content; (ii) volatile fatty acid pattern; (iii) yeast overgrowth and (iv) *Streptococcus faecalis* decrease. Enterobacteriaceae suppression was most successful during 600 mg oral polymyxin E (suppression indices during 150, 300 and 600 mg were 0·32, 0·55 and 0·89 respectively). None of the four markers of indigenous flora alterations were positive. However, using this dosage half of the volunteers suffered rather severe gastrointestinal side-effects. Oral polymyxin E in a dosage of minimum 600 mg daily seems to possess the ideal properties of a flora suppression agent, if the gastrointestinal side-effects could be mitigated.

INTRODUCTION

Polymyxin E is widely used as an intestinal flora suppression agent (Pulaski *et al.* 1949; Urban, 1960; Schmöger, 1961; de Gast & van Saene, 1979; Grylack, Neugebauer & Scanton, 1982). The two major reasons for the peroral use of non-absorbable polymyxin E are the potent activity of the agent against Enterobacteriaceae (except *Proteus* species, naturally insensitive), and the infrequent development of resistance of originally susceptible bacterial species.

Pulaski and co-workers (1949) were the first to report a dosage-dependent flora suppression effect of polymyxin E in patients receiving preoperative bowel preparation. Gotoff & Lepper (1965) observed a 50% failure rate during oral

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polymyxin B, in an attempt to eradicate salmonella from the stool of carriers in a geriatric institution. They stated that 'inactivation of antibiotics in the gastrointestinal tract by a number of mechanisms is probably the explanation of many treatment failures'. Recently, similar suppression failures by oral colistin have been reported in ICU babies by Lambert-Zechovsky and co-workers (1981); inactivation of polymyxin E by food was suggested as an explanation. These various observations prompted us to study the Enterobacteriaceae suppression effect during three different dose regimes of oral polymyxin E and to answer the questions whether a successful Enterobacteriaceae suppression required a certain dosage and was 'selective', i.e. left the indigenous faecal flora intact.

MATERIALS AND METHODS

Tablets

A batch of polymyxin E tablets was made up as follows:

Polymyxin E sulphate (Dumex, Copenhagen, Denmark)	65 g
Microcrystallin cellulose (Avicel pH 101, FMC Corporation, Pennsylvania, USA)	200 g
Lactose monohydrate pulv. 100 mesh (Melkindustrie, Veghel, The Netherlands)	365 g
Sodium starch glycolate (Primojel, Avebe, Veendam, The Netherlands)	40 g
Magnesium stearate (Lamers & Indemans, s'Hertogenbosch, The Netherlands)	5 g

A preblend of polymyxin E sulphate, microcrystalline cellulose, lactose monohydrate pulv. and sodium starch glycolate was prepared by mixing for 15 min in a Turbula mixer (model 2P, WA Bachofen, Basle, Switzerland) at 90 r.p.m. Magnesium stearate as lubricant was subsequently added to the preblend and the mixing was continued for 2 min.

Tablets (6 mm diameter, biconvex) were prepared by direct compression using an excentric tablet press (Hoko KO, Rijswijk, The Netherlands).

The polymyxin E sulphate tablets had a mean weight ($n = 20$) of 150 mg (S.D. 6·06), containing 15 mg polymyxin E sulphate. The mean disintegration time for six tablets was measured using the United States Pharmacopeia (USP) XXI apparatus and was found to be 42 s. The mean crushing strength of ten individual tablets was measured using a Schleuninger E_2 hardness tester and amounted to 3·7 kgf (S.D. 0·50).

Volunteers

Six volunteers (4 males, 2 females, mean age 25 years, range 21–39) participated in the investigation after giving informed consent. None of them had taken antibacterial agents 6 weeks before the start of the trial nor had experienced any illness. All had a normal defaecation pattern (once a day).

Design of the study

The volunteers received 150, 300 or 600 mg respectively as tablets every 8 h for 6 days. Between each of the three treatment periods there was a washout period of

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at least 4 weeks. The total sampling pattern was standardized as follows: from each volunteer a faecal sample (minimally 2 g) was obtained, before treatment began and daily during polymyxin E administration; the follow-up period was 2 weeks: faecal samples were collected the first 2 days after stopping oral polymyxin E and thereafter three times weekly. Thus, each volunteer was asked to produce three series of 14 faecal specimens.

Microbiological procedures

Aerobic flora

Faecal concentrations of Enterobacteriaceae, yeasts and *Streptococcus faecalis* were determined as follows: 1 g of faeces was homogenized in 9 ml of brain heart infusion (BHI) broth (Oxoid, CM 225) serially diluted (1:10) and incubated for 18 h at 37 °C; thereafter all dilutions showing bacterial growth were inoculated onto MacConkey agar (Oxoid, CM 7), yeast isolation agar (Merck, Darmstadt, FRG, Art 13849) and kanamycin aesculin azide agar (Oxoid, CM 481). Morphologically distinct colonies were cultured pure. Identification and typing were performed by means of standard bacteriological techniques (API 20E, API-Benelux, s'Hertogenbosch, The Netherlands). All results were expressed per gram faeces.

Anaerobic flora

The effect of polymyxin E on the anaerobic faecal flora was determined in two ways: (i) by the detection of the dipeptide of beta-aspartylglycine and (ii) by the volatile fatty acids patterns. Both techniques indirectly reveal alterations of the indigenous anaerobic flora.

The dipeptide beta-aspartylglycine undetectable in normal faecal specimens appears in individuals whose faecal flora is altered by antibiotics. The semi-quantitative techniques for beta-aspartylglycine detection were previously described in detail (Welling, 1982). In brief, a 25% w/v faecal suspension was centrifugated for 15 min at 15000 r.p.m. Then 80 μl of the supernatant was subjected to high-voltage paper electrophoresis at pH 3·5. After staining with ninhydrin and drying at 150 °C the paper was examined for the presence of a clear blue spot of beta-aspartylglycine.

Volatile fatty acids are known to be intermediate and end products in fermentation processes of bacteria, the anaerobes in particular. The techniques for volatile fatty acid detection were previously described in detail (van den Bogaard, Hazen & van Boven, 1986). In brief, a 2 ml sample was acidified with 200 μl of 0·1% formic acid, and one drop of antifoaming agent 33151 (BDH Chemicals Ltd, Poole, UK) was added. The specimen flask was connected with a receiver tube, cooled in liquid nitrogen. This receiver tube was then evacuated with a waterjet pump and kept evacuated by continuous suction, while the contents of the specimen flask were mixed with a magnetic stirrer and slowly heated over a period of 20 min from room temperature to 120 °C in an oil bath. After this the specimen was reduced to complete dryness, and after the distillate was thawed at room temperature, it was directly injected into a gas chromatograph (GC). The GC analyses were done by using Carbowax as the stationary phase and the temperature program described in the article previously cited.

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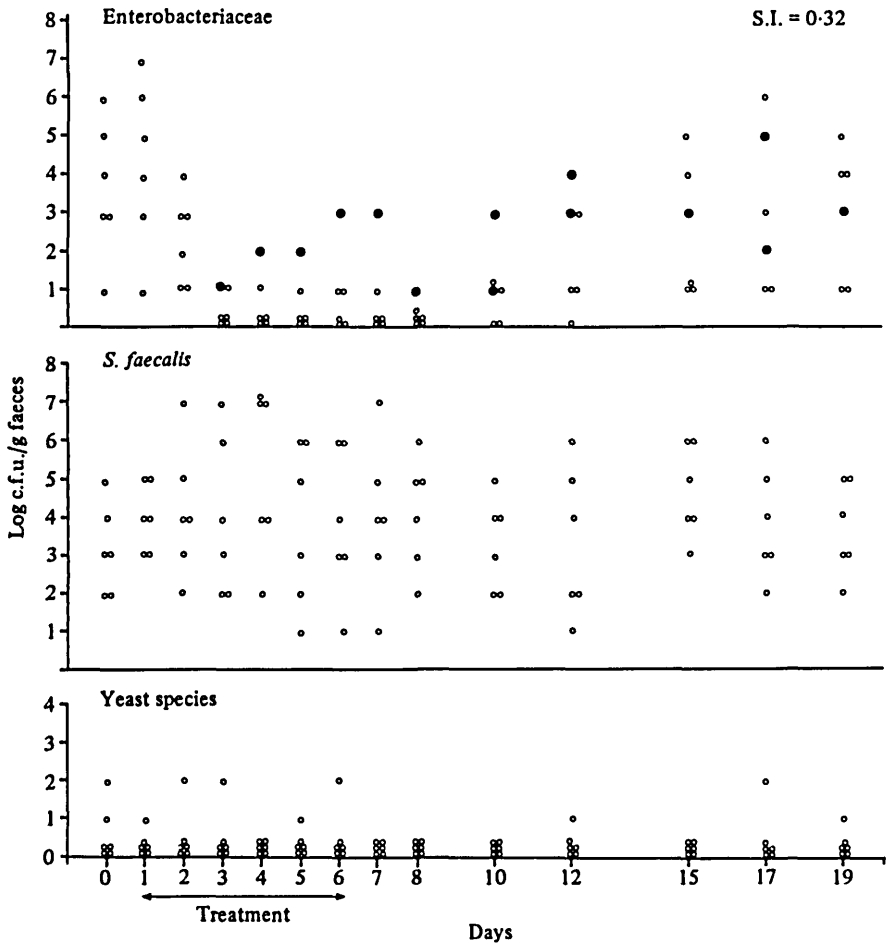

Fig. 1. Intestinal colonization pattern during low dose oral polymyxin E (150 mg daily in six volunteers). In the upper panel *Proteus* species (●) and other Enterobacteriaceae species (○) are shown separately.

Suppression index

The suppression effect of oral polymyxin E was evaluated by the suppression index (S.I.) for the three different regimens. The suppression index is defined as the ratio of total number of faecal samples free of Enterobacteriaceae to the number of faecal samples.

RESULTS

This study in six human volunteers yielded 251 faecal samples. A total of 112 samples (45%) was positive for Enterobacteriaceae: *Escherichia coli* was the main colonizer (71%), followed by *Proteus* species, *Enterobacter* species, *Klebsiella* species and *Citrobacter* species (10, 10, 5 and 4% respectively).

All six volunteers were colonized by *Escherichia coli* and *Streptococcus faecalis*. Two were yeasts carriers.

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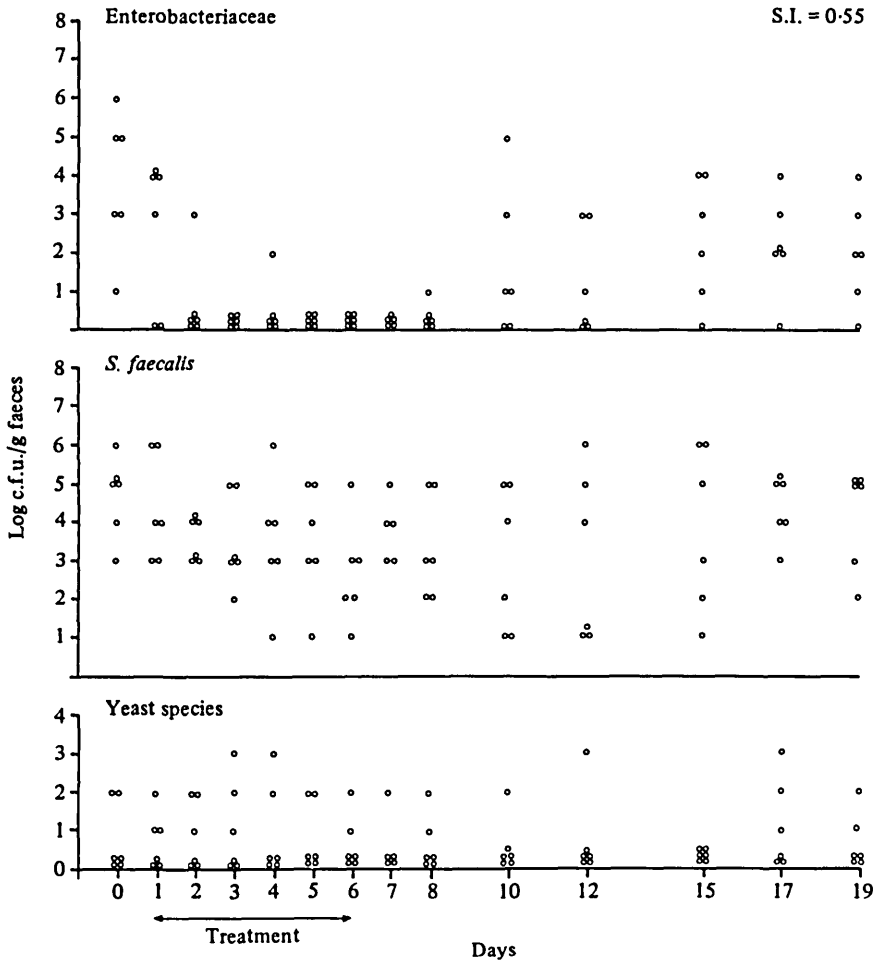

Fig. 2. Intestinal colonization pattern during medium dose oral polymyxin E (300 mg daily in six volunteers).

Suppression effect

The suppression indices for Enterobacteriaceae during the three different oral polymyxin E doses (150, 300 and 600 mg) were found to be 0·32, 0·55 and 0·89 respectively. These indices show that the higher the polymyxin E doses given the better the suppression established. Figure 1 represents the colonization pattern of six volunteers under the lower dosage of 150 mg of polymyxin E. The intestines of four volunteers became free of Enterobacteriaceae within 3 days. Two volunteers were colonized by *Proteus* species. In half of the volunteers the suppression effect disappeared after a few days. With 300 mg polymyxin daily Enterobacteriaceae were successfully suppressed in all six volunteers within 3 days, the effect lasting for 1 week. *Proteus* species did not colonize any volunteer under this dosage (Fig. 2). With 600 mg polymyxin E a day all volunteers were free of Enterobacteriaceae within 2 days, and 9 days after stopping of polymyxin E half of the volunteers were recolonized with *Escherichia coli* (*Fig.* 3). *Proteus* species did not colonize any volunteer in this part of the study.

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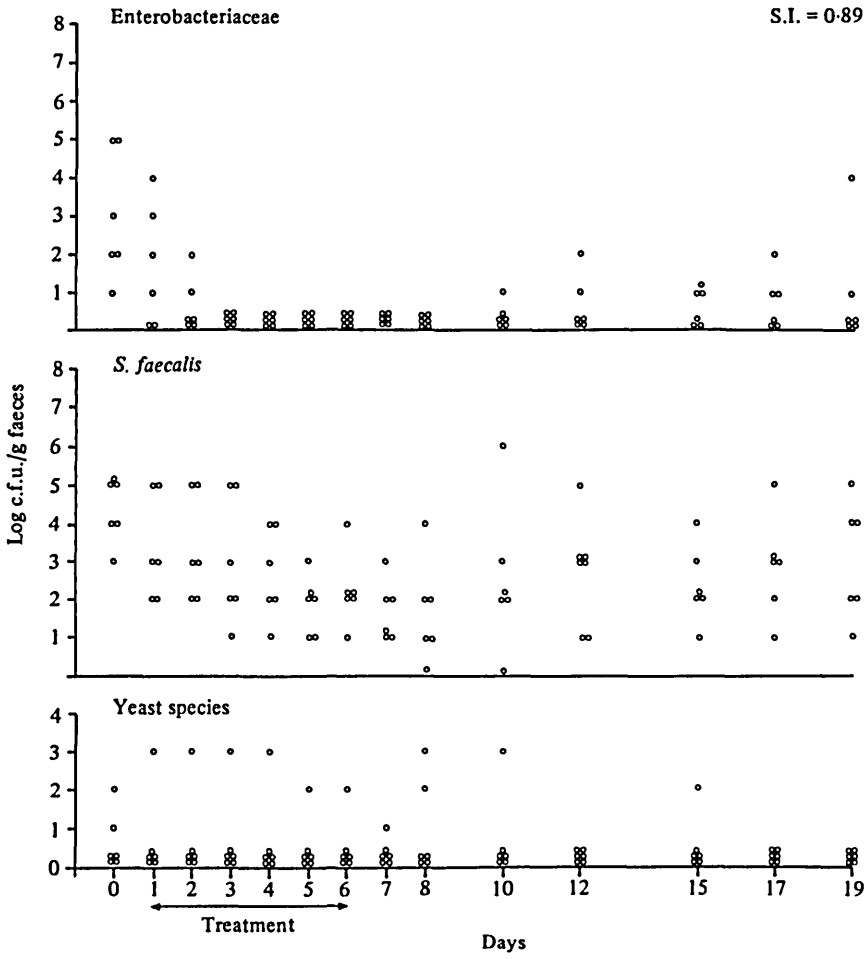

Fig. 3. Intestinal colonization pattern during high dose oral polymyxin E (600 mg daily in six volunteers).

The highest dose (600 mg) of polymyxin E tested was associated with a slight drop of *S. faecalis* faecal concentrations and on the other hand, one volunteer became free of *S. faecalis*.

Yeast species colonization patterns did not show any changes (Figs 1–3)

Influence on anaerobic flora

The interaction of oral polymyxin E with the anaerobic intestinal flora was evaluated in this study by two indirect parameters: (i) emergence of the dipeptide beta-aspartylglycine and, (ii) the patterns of volatile fatty acids. Chemical analyses were performed only on the faecal samples of volunteers given the highest polymyxin E dose (600 mg/day). All faecal samples were found to be negative for beta-aspartylglycine (data not shown), and no major alterations were seen in the volatile fatty acid pattern (Fig. 4).

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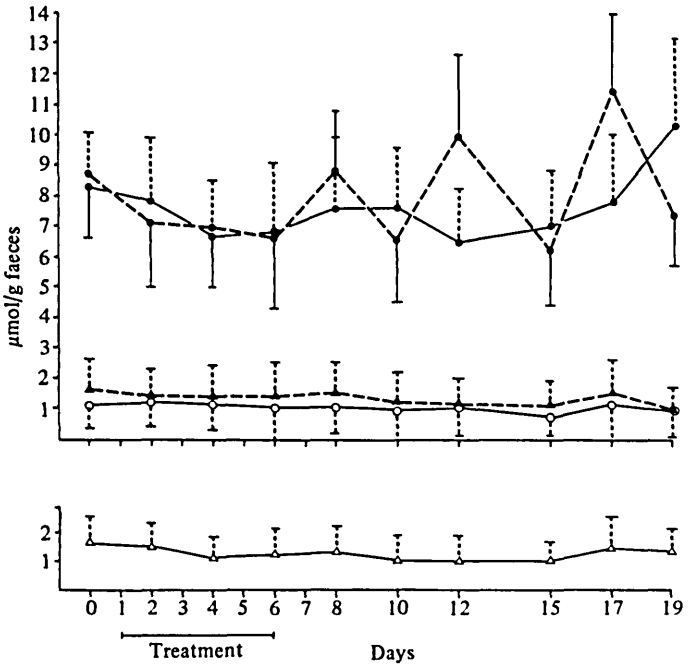

Fig. 4. Volatile fatty acid patterns of faecal specimens during high dose oral polymyxin E (600 mg daily in six volunteers). ●———●, propionic acid; ○———○, isobutyric acid; ●---●, n-butyric acid; ▲---▲, isovaleric acid; △———△, n-valeric acid.

Table 1. *Side-effects of oral polymyxin E occurred in three volunteers only during the highest dose of* 600 *mg; treatment did not necessitate interruption of medication*

	Side effects		
Volunteers	150 mg	300 mg	600 mg
1. C.P.S.	None	None	'Nausea' like complaints (only during intake)
2. A.E.	None	None	'Nausea' like complaints (last half of oral intake)
3. J.V.S.	None	None	Heavy feeling in stomach (stopped after discontinuation)
4. N.S.	None	None	None
5. G.B.	None	None	None
6. H.V.S.	None	None	None

Side-effects

Half of the volunteers suffered from severe gastrointestinal side-effects such as nausea and vomiting on polymyxin E doses of 600 mg/day, whereas no such effects with the lower doses were recorded (Table 1).

DISCUSSION

As far as is known, no report has been published on the effect of polymyxin E on the faecal flora in human volunteers. This study shows that daily oral

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administration of 300 mg and in particular 600 mg of polymyxin E rapidly and successfully suppressed Enterobacteriaceae in a rather 'selective' way. The 150 mg dose was minimally effective. All Enterobacteriaceae were suppressed except *Proteus* species (in two subjects).

All reports on oral polymyxin E published so far deal with patients, both children and adults suffering from a variety of underlying diseases. Polymyxin E is given as monoprophylaxis in preparation of the bowel for surgery (Pulaski *et al.* 1949), in neonates at risk for developing necrotizing enterocolitis (Grylack, Neugebauer & Scanton, 1982) and in patients receiving cytostatic agents (Moriyama *et al.* 1979). Neonates and children suffering from diarrhoea (Klupsch, 1961; Marsden & Hyde, 1962; Kienitz, 1963) and other severe alimentary derangements (Schönenberg, Seeliger & Werner, 1963; Lambert-Zechovsky *et al.* 1981) as well as salmonella carriers (Gotoff & Lepper, 1965; de Gast & van Saene, 1979) have been treated with oral colistin sulphate. High failure rates due to a lack of, or only temporary suppression as well as *Proteus* species colonization were reported. The two main differences between our study group (successful suppression) and the patient populations described in the literature (poor suppression) is the absence of underlying disease as well as of hospitalization in our group.

Underlying disease (Stoutenbeek *et al.* 1984) and old age (Schneider, 1983) are known to be associated with a higher rate of carriage of Enterobacteriaceae other than the indigenous *Escherichia coli* (e.g. *Proteus* species), more particular during hospitalizations where the exposure is thought to be higher (Le Frock, Ellis & Weinstein, 1979). Oral monotherapy with polymyxin E will select *Proteus* species often resulting in *Proteus* species carriage in this population (Pulaski *et al.* 1949). This gap in the spectrum of polymyxin E necessitates combination with, for example, aminoglycosides such as tobramycin when faecal flora suppression regimes are used as infection prevention techniques in high risk patients (van Saene & Stoutenbeek, 1987). We observed *Proteus* species colonization in two healthy volunteers during the lowest dose of the experiment. Presumably, these two volunteers were exposed to *Proteus* species (supplied via food or beverages). They were not able to clear the *Proteus* species although they were young adults and not known to have any underlying disease. Their faecal samples positive for *Proteus* species were all free of *E. coli*. This observation may suggest the existence of an ecological niche normally filled by the hosts' own *E. coli*, but now replaced by *Proteus* species microorganisms naturally insensitive to polymyxin E that completely eliminates the indegenous *E. coli*. These two volunteers did not show any colonization with *Proteus* species in the 300 mg and 600 mg polymyxin E experiments.

Apart from general host factors that may impair the defence mechanisms against colonization with *Proteus* species, local factors can interfere with the suppression outcome as well. Mucositis due to diarrhoeagenic microorganisms, cytostatic agents and/or irradiation represents anatomical and functional abnormalities promoting adherence and invasion. Certain microorganisms, e.g. *Salmonella* species may grow intracellarly, in the plaques of Peyer. These conditions may make the microorganisms unreachable by the non-absorbable

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polymyxin E. For flora suppression in patients having an inflamed mucosa and for treatment of *Salmonella* species carriage, a systemic antimicrobial agent secreted into intestinal mucus and/or lymphoid tissues may be required to complete the intraluminal action of polymyxin E. The older agent cotrimoxazole (Brodie, Macqveen & Livingstone, 1970) and the newer quinolones such as ofloxacin (Löffler & Graf von Westphalen, 1986) are thought to possess these propensities.

Apart from host factors and poor effect on *Proteus* species, a second drug-associated factor, the oral dose of polymyxin E given will influence flora suppression. Dosage of < 10 mg/kg/day are in general associated with high flora suppression failure rates (Pulaski *et al.* 1949; Schmöger, 1961; Schönenberg, Seeliger & Werner, 1963; Lambert-Zechovsky *et al.* 1981; Grylack, Neugebauer & Scanton, 1982). Gotoff & Lepper (1965) suggested another possible explanation for the polymyxin E failures, namely, that polymyxin E is inactivated by faecal compounds. This hypothesis, presented more than 20 years ago, was recently confirmed by three different groups (Hazenberg *et al.* 1984; Veringa & van der Waaij, 1984; van Saene *et al.* 1985). It was further supported by our *in vivo* findings that the higher the oral dosage, the more successful the suppression achieved. Dosages of 300 mg and in particular 600 mg of polymyxin E daily apparently resulted in enough polymyxin E excess in the colon to achieve complete suppression of the aerobic Gram-negative flora. It may be concluded that a successful aerobic flora suppression by polymyxin E is based on microbiologically active faecal concentrations depending on two factors: (i) the intrinsic activity against relevant Gram-negative organisms (minimal bactericidal activity) and (ii) sufficiently high concentration in the intestinal lumen to overcome inactivation of the drug by faeces.

Although the indicators used in this volunteer study to evaluate the 'selectivity' of polymyxin E suppression are not very sensitive ones (van den Bogaard *et al.* 1986), our results (little effect on *S. faecalis*, absence of yeast overgrowth, of beta-aspartylglycine and of volatile fatty acid pattern changes) suggest that faecal flora suppression by polymyxin E is more or less selective i.e. leaves the indigenous flora relatively intact. The clinical significance of 'selectivity' is still subject of debate. Combining successful elimination of aerobic potentially pathogenic micro-organisms from the intestine with preservation of indigenous flora is probably a contradiction in terms, for two reasons. Destruction of aerobes lowers the rate of molecular oxygen consumption permitting an increase in p_{O_2} of the lumen contents from 5 to 60 mmHg; under such conditions strictly anaerobic microorganisms can no longer survive, even though they may not themselves be sensitive to the antimicrobial agents used (Poth, 1982). Secondly, elimination of aerobes from intestine generally requires much higher concentrations of antimicrobials so that agents that are only active against aerobes in systemic concentrations may become active against the indigenous flora as well when 'topical' doses are used (King, 1980). 'Selective' should be interpreted as 'as selective as possible', the efficacy of complete aerobic elimination being more important than complete selectivity.

A major disavantage of the most effective suppression dosage of oral polymyxin E (600 mg daily) was the frequent and rather severe gastrointestinal side-effects. These prompted us to start a comparative study into the suppression effect of

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colonic-coated polymyxin E. At present, 300 mg polymyxin E daily appears to be the best compromise resulting in reasonable flora suppression and little or no side-effects.

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