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The effect of selective decontamination of the digestive tract with the addition of systemic cefotaxime on the aerobic faecal flora of mice

By A. B. J. SPEEKENBRINK, S. R. ALCOCK, J. FORRESTER and D. M. V. PARROTT

University Department of Bacteriology and Immunology, Western Infirmary, Glasgow G11 6NT

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SUMMARY

The administration per-orally to mice of the non-absorbable antibiotics polymyxin E, tobramycin and amphotericin B resulted in the elimination of detectable aerobic gram-negative rods from the faecal flora without affecting the total viable aerobic count. The addition of parental cefotaxime to the regime caused a fall in the number of aerobic lactobacilli and an increase in the number of enterococci. The rise was associated with the translocation of viable enterococci to the mesenteric lymph nodes and the spleen. The changes induced by cefotaxime were reversed when the antibiotic was withdrawn. Following withdrawal of all antibiotics the total aerobic faecal flora increased to above normal levels, but there was no associated diarrhoea. Attempts to implant exogenous enterobacteria into the digestive tract resulted in only low level colonization both in treated mice and in control mice. These results may have implications for the use of this antibiotic regime for selective decontamination of the digestive tract in humans, particularly those who are immunocompromised.

INTRODUCTION

The principle of selective decontamination of the digestive tract (SDD) was first described by van der Waaij and co-workers (1971; 1974). In SDD, potentially pathogenic aerobic gram-negative rods and yeasts in the intestine are eliminated or markedly reduced with only minimal effects on the predominant anaerobic flora. This maintenance of the anaerobic flora prevents overgrowth by drug-resistant aerobes, a phenomenon termed colonization resistance (CR) (van der Waaij, Berghuis-de Vries & Lekkerkerk-van der Wees, 1972). In humans, such techniques have been used with some success to reduce infection of endogenous origin in granulocytopoenic patients (Guiot *et al.* 1983; Hargadon *et al.* 1981; Sleijfer *et al.* 1980). Recently, SDD has been applied to patients in an intensive therapy unit (ITU) and has achieved a striking reduction in infection rates (Stoutenbeek *et al.* 1984). This application is currently the subject of a major clinical trial in a Glasgow ITU (S. R. Alcock and I. McA. Ledingham, personal communication), and entails the oral administration of polymyxin E (Pe), tobramycin

(Tn) and amphotericin B (AmpB). These drugs are thought to have a minimal effect on the anaerobic flora and are not absorbed from the digestive tract. In addition, parenteral cefotaxime (Ctx) is added to the regime for the first 4 days of SDD treatment.

The bacteriological effects of SDD have been extensively studied in mice and to a lesser extent in man. We seek to amplify these studies in areas where information is limited or lacking. A mouse model was employed because of the severe limitations imposed by sampling and other problems in humans. The following questions were asked. Firstly, what is the effect on aerobic intestinal flora of the particular SDD regime employed by Stoutenbeek *et al.* (1984), and what further effects result from the addition of parenteral cefotaxime? Secondly, how effective is CR in the period after stopping the regime? Thirdly, is the translocation of bacteria from the lumen of the intestine to the mesenteric lymph nodes (MLN) and to the spleen affected by the drug regime? In order to investigate these questions, the SDD regime used in humans by Stoutenbeek *et al.* (1984) was simulated as closely as possible in mice.

MATERIALS AND METHODS

Animals

Outbred NIH mice and inbred BALB/c mice aged 6–18 weeks were used. Mice were housed in a standard animal house and received drinking water and standard laboratory chow *ad libitum*. Clean cages were provided twice daily to minimize recolonization by coprophagy.

Antibiotics

Initially, SDD antibiotics and Ctx were used in doses which were exact equivalents, adjusted for body weight, of the human dose. SDD antibiotics were given in the drinking water at a concentration of one equivalent daily dose in 5 ml of water. If used, Ctx was given intraperitoneally. However, these doses failed to maintain SDD, resulting in colonization by *Proteus* species. This was possibly due to the relatively larger intestinal mass in mice. The doses of Pe and Tn were therefore increased threefold, the dose of Ctx was doubled, and the dose of AmpB was not changed. The method of administration of the SDD antibiotics was also changed to direct intragastric instillation twice every 24 h using a feeding tube. This simulated more closely the method of administration to humans, and avoided the risk of inactivation of the antibiotics in the drinking water. The concentrations of antibiotics used is shown in Table 1.

Bacteria used in implantation studies

Facultatively aerobic gram-negative rods sensitive to Pe, Tn, and Ctx, and doubly resistant to nalidixic acid (Na) and rifampicin (Rp) (Table 2) were selected from pure isolates obtained from normal murine faeces. Isolates were first cultured in nutrient broth containing 50 mg l^{-1} Na plus 50 mg l^{-1} Rp (NRB) and were then inoculated onto nutrient agar plates containing the same concentration of Na and Rp (NRA). *Enterobacter aerogenes* 9880–1, a similarly resistant isolate of human

Table 1. *Daily antibiotic dosage for experimental SDD in mice compared to human daily doses*

Antibiotic	Route	Human dose	Equivalent mouse dose*	Revised mouse dose
Polymyxin E	Oral	4 × 100 mg	0·17 mg	0·51 mg
Tobramycin	Oral	4 × 80 mg	0·14 mg	0·42 mg
Amphotericin B	Oral	4 × 500 mg	0·86 mg	0·86 mg
Cefotaxime	Parenteral	50 mg/kg	1·50 mg	3·00 mg

* Mouse dose based on 30 g mouse and 70 kg human.

Table 2. *Experimental recolonization of the intestine 24 h after stopping SDD with nalidixic acid- and rifampicin-resistant mutants of bacteria*

Strain	Source	Dose log (c.f.u.)	Treatment	No. mice colonized / No. mice tested	Mean log (c.f.u./g)*	Duration of colonization
Flavobacterium sp.	Mouse	7·3	None	2/4	3·3	1 day
		7·3	SDD	2/4	3·7	1 day
		—	SDD/C	ND	—	—
Klebsiella oxytoca mS72-2	Mouse	7·9	None	0/8	N	N
		8·2	SDD	0/4	N	N
		8·2	SDD/C	0/4	N	N
Escherichia coli mS73-2	Mouse	8·3	None	1/4	4·9	1 day
		8·3	SDD	4/4	4·1	> 7 days
		8·3	SDD/C	4/4	4/1	> 7 days
Escherichia coli mS76-2	Mouse	6·6	None	1/6	4·7	2 days
		—	SDD	ND	—	—
		6·6	SDD/C	7/8	6·2	> 2 days
Enterobacter aerogenes 9880-1	Human	9·2	None	4/4	5·8	> 8 days
		9·2	SDD	2/4	3·9	2 days
		—	SDD/C	ND	—	—

* Mean population of bacteria excluding null values on the last day on which they were recovered. SDD = Selective decontamination of the digestive tract. SDD/C = SDD with parenteral cefotaxime for the first 3 days of treatment. ND, not done; N, not detected. Colonization defined as in methods.

origin, was kindly donated by Dr D. Platt (Dept of Bacteriology, Royal Infirmary, Glasgow, U.K.)

In both cases, single colonies from NRA plates were inoculated into NRB. After 24 h incubation, the cells were washed three times in nutrient broth, resuspended in one-third the original volume of nutrient broth plus 20% horse serum, dispensed in 1 ml aliquots into autoanalyser vials, and stored at −70 °C.

Estimation of the aerobic faecal flora

Freshly voided faecal pellets were collected from individual mice. The pellets were immediately weighted and homogenized in 3 ml of nutrient broth. Serial tenfold dilutions of the homogenates were made in the same broth and viable bacteria were estimated using a droplet count. (Miles, Misra & Irwin, 1938). Media used were MacConkey agar (Oxoid) without salt (MC) and Nutrient agar (Oxoid) plus 5% defibrinated horse blood (BA). Nalidixic acid-rifampicin agar was used to

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allow viable counts of Na- and Rp-resistant bacteria. Viable counts were reported as $\log_{10}$ of the colony forming units (c.f.u.) per gram of wet faeces.

Implantation of strains

Frozen 1 ml aliquots of the Na- and Rp-resistant mutants were thawed at 37 °C, and added to 2 ml of 100 mM $NaHCO_3$. Mice were given 0·2 ml of the cell suspension intragastrically using a feeding tube. Immediately after each experiment, the number of viable organisms in the buffered suspension was estimated using a droplet count (Miles Misra & Irwin, 1938). Colonization was defined as the recovery of viable implanted organisms from the faeces at levels $< 10^8$ c.f.u./g wet faeces. Recovery of greater than this number of implanted organisms was referred to as overgrowth.

Assay of antibiotic activity in faeces

Faecal pellets were collected from eight mice at various intervals after administration of antibiotics. Each time, the faeces collected were pooled, homogenized and compressed into 5×3 mm pellets. Four of the strains used in the implantation studies were cultured overnight in NRB. Molten nutrient agar was mixed with an equal volume of Mueller–Hinton Broth, cooled to 40 °C, and Na and Rp were added at a final concentration of 50 mg l^{-1}. Cultures were diluted $1:5 \times 10^5$ in the agar, which was added to 30 mm culture dishes (Flow Laboratories). One standardized faecal pellet was placed in the centre of each culture dish and the dish was then incubated overnight at 37 °C. An estimate of antimicrobial activity in the faeces was obtained by squaring the diameter of the zone of inhibition of bacterial growth. Each experiment was performed in duplicate.

Estimation of translocation

Spleens and mesenteric lymph nodes were removed aseptically from mice killed by cervical dislocation. The tissues were homogenized in 0·2 ml of sterile nutrient broth in sterile Griffith's tubes. Serial twofold dilutions of the homogenates were made in the same broth. The dilutions were incubated at 37 °C for 48 h, and droplets of each dilution were plated onto MC and BA (also onto NRA if required). Plates were scored for presence or absence of growth after 24 h at 37 °C. The viable count was reported as the reciprocal of the highest dilution to yield viable bacteria and is referred to as the titre.

RESULTS

Effect of SDD on the faecal flora of mice

As previously reported (Dubos *et al.* 1965), the aerobic faecal flora of the mice was found to be composed mainly of lactobacilli. The aerobic faecal flora of eight mice given SDD antibiotics but not Ctx may be seen in Fig. 1. The total aerobic viable count was not affected during SDD. However, this finding concealed significant changes in the relative concentration of certain aerobic species. Thus the lactose-fermenting gram-negative bacilli (LFC) were reduced to undetectable levels by the second day of SDD and remained low despite sporadic outgrowths of LFC in occasional mice. Lactose non-fermenting gram-negative bacilli (LNC)

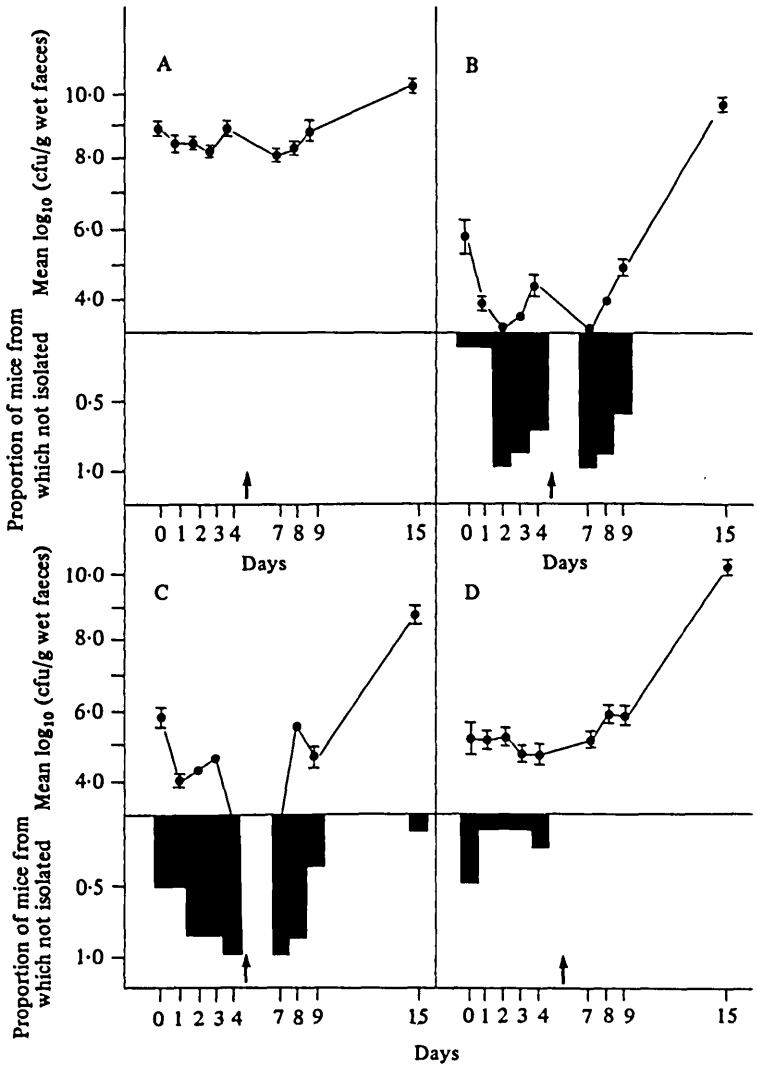

Fig. 1. Aerobic faecal flora of mice undergoing SDD. (A) Total aerobes, (B) lactose fermenting coliforms, (C) lactose non-fermenters, (D) Enterococci. Mean viable counts calculated only from positive cultures. Lower limit of detection *ca.* 10^3 c.f.u./g wet faeces. Solid arrow indicates termination of SDD. Mice not sampled on days not indicated on x-axis. $N = 8$ mice. Results shown ± standard error of the mean.

were similarly reduced. By contrast, enterococci were not significantly affected. These fluctuations within the aerobic intestinal flora were not seen in a group of eight control mice monitored over a period of 3 weeks. Isolates of LNC and LFC showing acquired resistance to the antibiotics used in SDD were not encountered in either control or treatment groups.

After discontinuation of SDD, the mice were reintroduced into a population of untreated mice. Initially, recovery of the aerobic flora was slow. However, after 1 week all bacterial populations studied were larger by two to four orders of magnitude than their size before SDD (Fig. 1). Surprisingly, the intestinal population of bacteria in the untreated cagemates was similarly affected. This rise was absent

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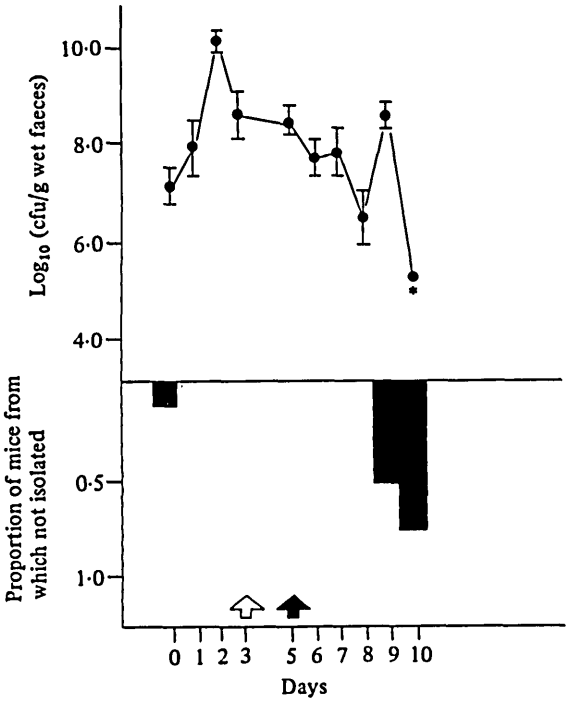

Fig. 2. Faecal enterococci of mice undergoing SDD and given cefotaxime. Mean viable counts calculated only from positive cultures. Lower limit of detection *ca.* 10^3c.f.u./g wet faeces. Open arrow indicates termination of Ctx. Solid arrow indicates termination of SDD. Mice sampled only on days indicated on x-axis. $N = 4$–14 mice. Results shown ± standard error of the mean. * = 1 of 4 mice only.

(despite monitoring for upto 15 days) when SDD mice were not reintroduced into a population of untreated mice but kept in separate cages.

Effect of the addition of Ctx to SDD on the faecal flora of mice

Additional effects were observed following the addition of Ctx to the SDD regime. The numbers of lactobacilli in the flora were reduced (Table 3) and this decrease was accompanied by a concomitant increase in the numbers of enterococci (Fig. 2). This change was reversed within 3 days of the removal of Ctx from the regime. Subsequent stopping of SDD was followed by a recovery of the faecal population similar to that seen in mice that underwent SDD without the addition of Ctx. The addition of Ctx also slightly accelerated the removal of LFC (data not shown).

Experimental recolonization of mice after SDD

Mice which had been treated with SDD alone or with SDD and Ctx until 24 h previously, and control mice were orally given suspensions of Na- and Rp-resistant mutants of five strains of aerobic gram-negative rods. The results may be seen in Table 2. Strains mS73-2 and mS76-2 were more successful in colonizing previously decontaminated mice than control mice. In the case of mS76-2, the number of mice colonized was significantly increased ($P < 0{\cdot}02$). Strain 9880-1 colonized

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Table 3. *Lactobacilli and enterococci as a proportion of the total aerobic flora in the faeces of eight mice treated with SDD or with SDD plus 3 days of cefotaxime*

Organism	Day	Ratio of organism viable count / total viable count: SDD	SDD/C
Lactobacilli	1	0·9±0·1	0·1±0·1
	2	0·9±0·1	0·1±0·1
	5	0·9±0·1	0·9±0·1
Enterococci	1	0·09±0·06	0·7±0·2
	2	0·03±0·02	0·6±0·2
	5	0·006±0·003	0·04±0·03

Results shown±standard error. SDD/C, SDD+parenteral cefotaxime. Cefotaxime was withdrawn after 3 days.

Table 4. In vitro *antimicrobial activity in murine faeces against nalidixic acid- and rifampicin-resistant bacteria after termination of SDD/C*

Time (h) after stopping SDD/C	Diameter²(mm²) of zone of inhibition of bacterial strains*: mS72-2	mS73-2	mS76-2	9880-1
2	0	0	0	0
5	307	256	145	256
7·5	421	273	157	289
19	0	t	t	0
24	0	0	0	0

* Strains used for implantation studies (Table 2). t, unmeasurable zone showing colonies of reduced size adjacent to the faecal pellet.

normal mice more efficiently than post-SDD mice. In no case was overgrowth with the implanted strain seen.

Measurement of antimicrobial activity in the faeces of mice treated with SDD and parenteral Ctx

The antimicrobial activity of murine faeces of mice treated with SDD and Ctx against all the implant strains except the *Flavobacterium* sp. (Table 2) was tested at selected times after the administration of antibiotics. Appreciable antimicrobial activity was detected in the faeces of mice after 5 h, after 19 h only a trace of antimicrobial activity against two of the strains remained, and no antimicrobial activity against any of the strains was observed after 24 h (Table 4).

Translocation associated with SDD

Translocation was not observed in control mice. However, enterococci were observed in the spleens and MLN of mice that had undergone SDD and had been given Ctx, and also in the MLN of two mice that had undergone SDD only (Table 5). Translocation in mice on SDD only was not related to any observed increase in the faecal population of enterococci before sampling, whereas in mice on SDD antibiotics and Ctx an appreciable increase in enterococcal numbers had been seen (Fig. 2). Translocation of implanted strains was not observed in any mice.

Table 5. *Translocation of viable bacteria to spleen and mesenteric lymph nodes of mice*

Treatment	No. of post-treatment	No. of mice	No. of spleen+ve	Titre	ID*	No. of MLN+ve	Titre	ID
NONE	—	11	0	—		0	—	—
SDD	48	4	0	—		1	2	EC
SDD	72	4	0	—		1	1	EC
SDD/C	48	4	1	1	EC	3	8	EC
SDD/C	120	4	4	2	EC	0	—	—

SDD Selective decontamination of the digestive tract. SDD/C = SDD+parenteral Ctx for the first 3 days of treatment.

* ID, bacterial identity by colonial and gram morphology. EC, enterococci. For definition of titre see Methods and Materials.

DISCUSSION

The results show that SDD regime described by Stoutenbeek *et al.* (1984) successfully removed potentially pathogenic aerobic gram-negative bacilli from the digestive tract of mice. Subsequent withdrawal of SDD was not associated with any obvious ill effects in the mice. Elevation of aerobic flora was seen within 10 days after stopping this regime, but only if the treated mice were housed with untreated mice. (The untreated mice also developed an increase in intestinal aerobes.) A similar elevation of enterobacteria in the faeces of mice after stopping decontamination was seen in a study by Rogers, Moore & Cohen (1985) and this was shown to be of a transient nature. The emergence of strains of aerobic gram-negative bacilli resistant to either the antibiotics used in SDD or to Ctx was not seen.

The variability of the implantation results indicate the difficulty of assessing colonization resistance except with a defined bacterial strain. Successful colonization was achieved by certain strains, but only to levels that were achieved in normal mice by van der Waaij *et al.* (1977), and overgrowth was never seen. Residual antibiotic activity was not detected in the murine faeces sampled at the time of challenge, a finding supported by the colonization achieved by drug-sensitive strains. Taken together, these data do not suggest a major impairment of CR 24 h after cessation of SDD. However, relatively minor effects cannot be excluded.

The consistent overgrowth of all aerobic bacteria observed within 10 days of ceasing SDD could be interpreted as indicating a reduction in CR. However, the untreated mice also showed this effect, an unexplained finding that again emphasizes the interpretative difficulties in this area. Also, the marked reduction in the concentration of certain aerobic genera during SDD must of itself have influenced events following drug withdrawal. More generally, CR must be considered in the context of the other factors influencing the gastrointestinal tract. Under the grossly abnormal conditions of SDD, it is claimed that CR prevents colonization with drug-resistant strains. This relatively simple hypothesis is of crucial importance for the clinical application of the technique, and is fully supported by the results reported here. However the role of CR in controlling aerobic faecal flora normally present in the absence of SDD or of other interference is much less clearly

defined and presents formidable interpretative problems. Irrespective of the mechanisms involved, the observed increase in aerobic flora is of potential clinical significance and further studies in both patients and experimental systems have been undertaken.

Transient outgrowth of enterococci with associated translocation to MLN and spleen was observed during treatment of mice given Ctx in conjunction with SDD. Translocation is not observed in normal mice (Berg & Garlington, 1979) and it is thought to be a function of the numbers of bacteria in the intestine rather than of their virulence (Steffen & Berg, 1983). The results therefore suggest that either the mice were immunocompromised, a condition known to increase translocation from the intestine (Owens & Berg, 1980; 1982), or the numbers of enterococci were raised sufficiently to overcome mucosal barriers transiently (Steffen & Berg, 1983). The limited data presented here suggest that outgrowth of enterococci may have been the major factor.

Cefotaxime has a relatively low biliary excretion rate (McKendrick, Geddes & Wise, 1980). Jones (1985) also reported that the incidence of enterococcal superinfection after Ctx therapy was lower than that in patients given other beta-lactam antibiotics. Cefotaxime is also thought to affect the human faecal flora less than other third generation cephalosporins (Knothe, Dette & Shah, 1985; Burdon *et al.* 1985). However, reported results in this area are difficult to evaluate: Lambert-Zechovsky *et al.* (1985) described a rise in the faecal enterococci in children treated with Ctx whereas Guggenbichler and co-workers (1985) demonstrated a similar rise in only 1 of 20 treated children.

The observed outgrowth by enterococci in mice may have implications for SDD in humans. Faecal enterococci have been used as an indicator of an intact anaerobic flora and hence of CR in mice (van der Waaij & Berghuis, 1974) and in humans (Sleijfer *et al.* 1980; de Vries-Hospers *et al.* 1981) as enterococci are resistant to the antibiotics used in SDD and are simpler to estimate than the intestinal anaerobic flora. If, as this study suggests, the addition of Ctx suppresses lactobacilli and thus allows overgrowth by the enterococcal population, monitoring of the latter as indicators of anaerobic flora and hence of CR may be inappropriate. In addition, outgrowth of enterococci and its associated translocation may have disease consequences. Although it is difficult to extrapolate directly from mice, similar outgrowth of enterococci after parenteral Ctx may occur in humans (Lambert-Zechovsky *et al.* 1985). Many patients to whom SDD is of potential benefit are severely immunocompromised (Guiot *et al.* 1983; Hargadon *et al.* 1981; Heimdahl *et al.* 1984; Sleijfer *et al.* 1980) and under these conditions a rise in any intestinal bacterial group with possibly enhanced translocation to spleen and lymph nodes may be hazardous.

In conclusion, this paper demonstrates the complexity of events associated with SDD supplemented with Ctx as used by Stoutenbeek *et al.* (1984). Such data are essential for a proper understanding of the striking clinical results described by these and other authors. Further studies are indicated, particularly concerning the events following cessation of SDD, and also the immunological effects of these regimes.

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