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The dose at which neomycin and polymyxin B can be applied for selective decontamination of the digestive tract in mice

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SUMMARY

Oral treatment of mice with various doses of neomycin or polymyxin B was performed in order to determine which dose caused substantial suppression of aerobic gram-negative rods. In addition the effect of the various doses on *Streptococcus faecalis* and on other factors of the colonization resistance (CR) of the digestive tract were studied. It was found that polymyxin B was effective in suppressing sensitive gram-negative bacteria following daily doses of 3·2 mg/mouse, and that even extremely high daily doses of 9·7 mg/mouse did not affect the CR. Neomycin was effective in suppressing Enterobacteriaceae species following oral daily doses of 5·4 mg/mouse. With this dose, however, the CR was somewhat decreased which was also evidenced by the increased concentration of beta-aspartylglycine in the faeces and the increased size (weight) of the caecum in these animals. Suppression of *Str. faecalis* was seen from doses of 24 mg/mouse on.

INTRODUCTION

Because so-called 'gut sterilization' in leucopenic patients has proved to be not as successful as it is in general in animals (Van der Waaij & Sturm, 1971; Dankert *et al.* 1978) we decided to investigate whether the arsenal of antimicrobial agents available for so-called 'selective decontamination' could be extended. The latter aims at selectively eliminating the potentially pathogenic bacteria and yeasts from the digestive tract, leaving the anaerobic bacteria, which contribute greatly to the colonization resistance of the digestive tract (CR), unaffected.

The CR of the digestive tract is a rather complex mechanism in which host and microflora co-operate in preventing colonization of the g.i. tract by exogenous potentially pathogenic microorganisms (Van der Waaij, Berghuis-de Vries & Lekkerkerk-van der Wees, 1972; Van der Waaij & Berghuis, 1974). This approach to infection-prevention by selective decontamination has meanwhile been quite successful in man (Mulder *et al.* 1979; De Vries-Hospers *et al.* 1979). The advantage of selective decontamination over gut sterilization is that the isolation precautions can be much less stringent because the colonization resistance remains unaffected.

Although polymyxin B and neomycin have already been used elsewhere in a

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Table 1. *Oral doses of neomycin and polymyxin B given to the respective subgroups of mice*

	Treatment group	
Subgroup	(A) Neomycin (mg/mouse*/day)	(B) Polymyxin B (mg/mouse*/day)
C	0	0
1	0·20	0·04
2	0·60	0·12
3	1·80	0·36
4	5·40	1·08
5	16·20	3·20
6	24·00	9·70
7	32·00	—

* Mean daily oral intake of drinking water was 4 ml.

small number of patients (Guiot & Van Furth, 1977) we decided at first to investigate which oral dose of these antibiotics would begin to cause a decrease of CR of the digestive tract in mice. We aimed at obtaining an impression as to which dose range of these drugs can safely be used in mice without decreasing the CR. The mouse was selected as experimental animal because the mouse intestinal flora has been found to be interchangeable with the human intestinal flora as far as its contribution to the CR is concerned (Van der Waaij *et al.* 1977*b*).

MATERIALS AND METHODS

Mice

Conventional female Swiss mice of 8–12 weeks and a mean body weight of 28 g were used. The animals were housed four per cage. Food pellets were supplied *ad libitum* and during the antibiotic treatment period antibiotics were added to the drinking water.

Antibiotic treatment

Two groups (A and B) of 32 and 28 mice respectively were subdivided into eight and seven subgroups of four animals. During the first 2 weeks the animals were not treated, but only observed bacteriologically. During this period the faecal flora of the animals was inventoried as far as the Enterobacteriaceae species and *Str. faecalis* were concerned. In the third, fourth, fifth and sixth week, neomycin sulphate (treatment group A) and polymyxin B (treatment group B) were given in increasing doses to six and seven subgroups respectively. In each treatment group one subgroup remained untreated to serve as a control group. The water intake was determined daily per cage. The various doses applied are shown in Table 1.

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Bacteriological procedures

Fresh faeces were collected three times a week from each individual mouse. Within two hours after collection the faeces were added to Brain Heart Infusion broth (DIFCO) to make a 10% (w/v) suspension. From those suspensions eight serial tenfold dilutions were made, also in BHI broth. After overnight incubation each dilution was subcultured on MacConkey agar for isolation and counting of the Enterobacteriaceae species, while subculture on aesculin-azide agar (Sneath, 1956) provided information concerning the concentration of *Str. faecalis*. This provided mean values of the concentration of the Enterobacteriaceae species and *Str. faecalis* in the faeces per group of four animals in each week. Consequently each mean was calculated from 12 values. In the initial observation period of 2 weeks, the biotype of the Enterobacteriaceae species which were isolated was determined.

The procedure for isolation and subsequent biotyping to allow a complete inventory, has been described previously (Van der Waaij, Speltie & Vossen, 1972; Van der Waaij, Tieleman-Speltie & De Roeck-Houben, 1977*a*). On the first day of the fifth week of the experiment (third week of antibiotic treatment) all animals (including the control group) were contaminated with an *Escherichia coli* strain which was resistant to the antibiotics used (neomycin, polymyxin B) and was also resistant to cephaloridin. Selective isolation of the contaminant strain from the faeces was obtained by adding cephaloridin in a concentration of 100 μg/ml to the MacConkey agar. The endogenous gram-negative flora was completely sensitive to cephaloridin, while the resistant strains were not inhibited by 1 mg/ml of all three antibiotics mentioned.

Oral contamination

The mice were contaminated with 0·2 ml of an overnight broth culture of the appropriate strains containing approximately 10^9 bacteria/ml.

Caecal weight

At the end of the experiment the body weight as well as the wet weight of the filled caecum was determined. This made calculation of the 'relative caecal weight' (weight of caecum/body weight) possible. This is another measure for CR (Koopman & Janssen, 1975).

Beta-aspartylglycine

This substance present in the caecum contents of germfree and 'gut sterilized' mice is absent from the caecum and faeces of conventional animals. Its concentration has been found to correlate well with the degree of CR of the digestive tract (Welling, 1979). The caecal contents of all neomycin-treated mice were suspended and processed as described by Welling & Groen (1978) to determine its beta-aspartylglycine concentration.

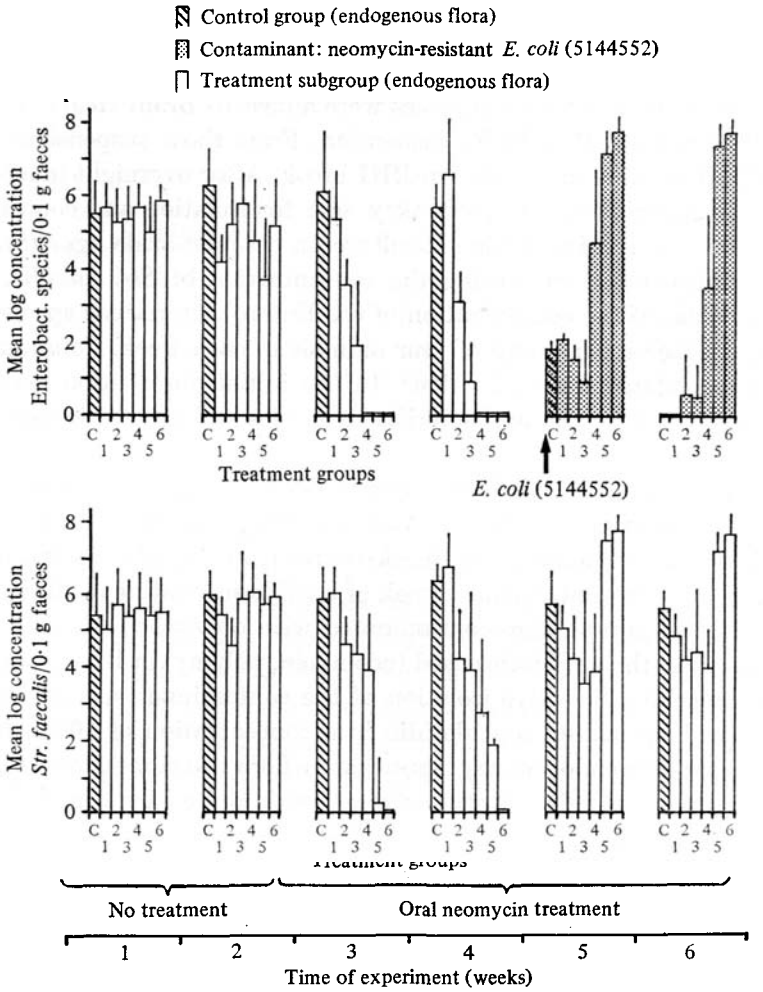

Fig. 1. Concentration of Enterobacteriaceae species and *Streptococcus faecalis* in the faeces of mice before and during oral treatment with neomycin in doses ranging from 0·05 to 6 mg/ml of water.

Neomycin concentration in the caecal contents

This was determined by serial dilution of 1/5 suspension of caecal contents in micro-trays filled with tryptose phosphate broth, with tetrazolium tetrachloride as indicator. After serial dilution a sensitive *E. coli* strain with a known M.I.C. was added to each cup as described earlier (Van der Waaij, Berghuis-de Vries & Korthals-Altes, 1974).

RESULTS

The biotypes of Enterobacteriaceae species isolated during the inventory phase were mainly biotypes of *E. coli*. They were all sensitive to neomycin and polymyxin B. The mean endogenous Enterobacteriaceae concentrations as well as the mean *Str. faecalis* concentrations in the faeces of the control group are shown for

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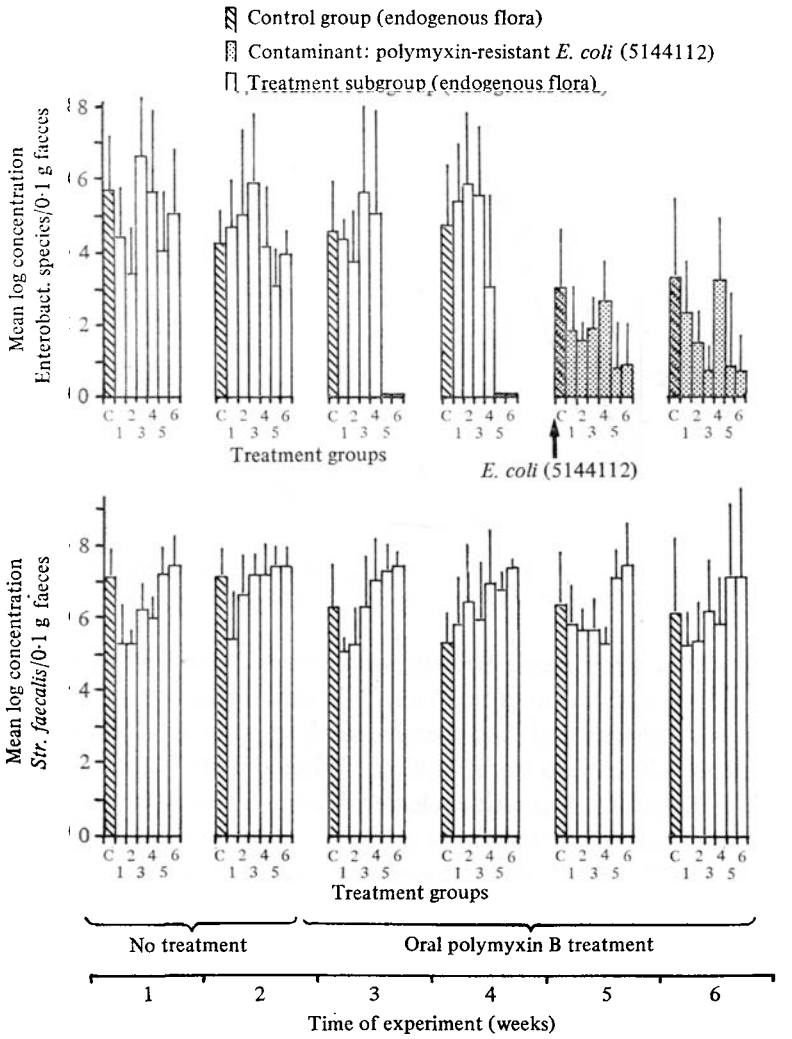

Fig. 2. Concentration of Enterobacteriaceae species and *Streptococcus faecalis* in the faeces of mice before and during oral treatment with polymyxin B in doses ranging from 0·01 to 2·5 mg/ml of water.

the week before and during treatment with neomycin (Fig. 1). *Str. faecalis* had become resistant to neomycin during treatment in subgroup 5 in the second week of treatment and was thereafter found in increased concentration. The effect of polymixin B treatment is presented in the same way in Fig. 2. The results obtained in the neomycin treated subgroup 7 did not differ from those seen in the subgroups 5 and 6 and is not presented in Fig. 1 for the sake of simplicity.

Neomycin-treated mice

These results show in the neomycin treated group in the first week an obvious and in the second week a significant decrease of the Enterobacteriaceae concentration in subgroup 3. The subsequent subgroups 4, 5 and 6 had negative gram-

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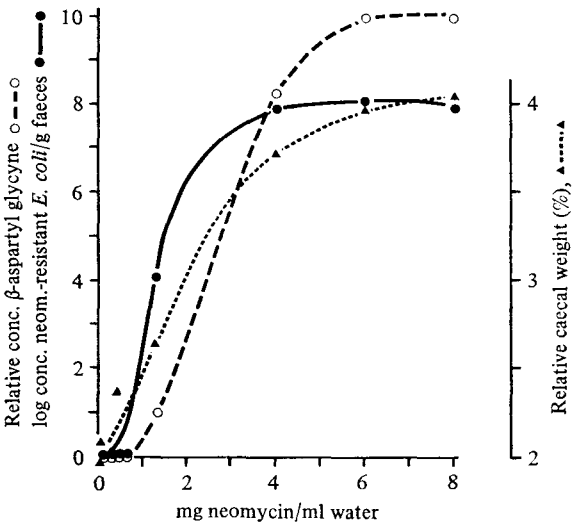

Fig. 3. Three different parameters of the colonization resistance of the digestive tract in relation to the oral dose of neomycin.

negative cultures within a few days. *Str. faecalis* decreased only during treatment with the higher oral doses, from subgroup 5 on. This means that for the elimination of Enterobacteriaceae species and *Str. faecalis* in these mice oral daily doses as high as 16·2 mg neomycin/mouse were required. When only elimination of gram-negative bacteria is aimed at, three times lower doses may be sufficient.

The results of experimental contamination of the neomycin-treated mice with a resistant *E. coli* strain (Fig. 1) show increased concentrations in comparison with the control group from subgroup 4 on. This means that the CR was somewhat decreased in subgroup 4 but appeared to be very strongly decreased following higher oral doses in subgroups 5 and 6. It is interesting to notice that the decrease of the CR in the latter subgroup was also evidenced by the higher concentration of *Str. faecalis* which had become resistant to neomycin in subgroup 5 in the second week of treatment.

Polymyxin-treated mice

In the polymyxin B treated subgroups the Enterobacteriaceae species (no *Proteus* strains) disappeared promptly and completely in the subgroups 5 and 6, while *Str. faecalis* neither increased nor decreased in concentration during treatment with these high doses (Fig. 2). This means that polymyxin B can only be used for the elimination of sensitive Enterobacteriaceae from the intestines and that for this purpose in the present experiment daily oral doses of at least 3·2 mg/mouse were required. The results of experimental contamination with an *E. coli* strain resistant to polymyxin B revealed no change in response between the control group and the various subgroups. This means that the CR in these mice was apparently not affected by oral polymyxin B treatment, even with daily doses as high as 8 mg/mouse.

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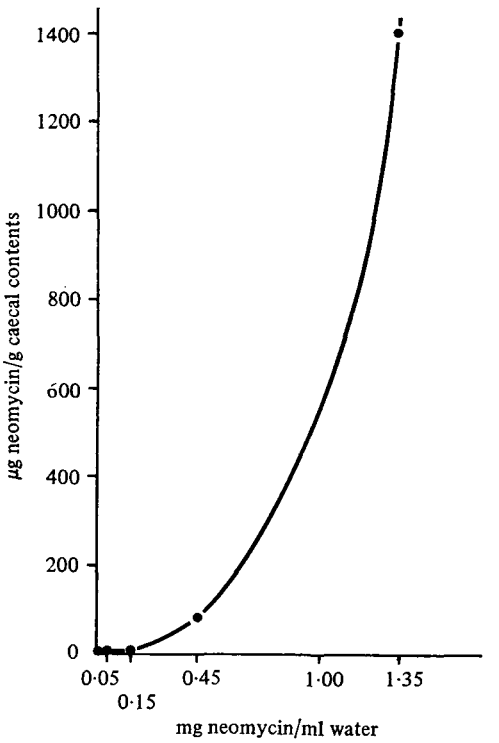

Fig. 4. Relation between the oral dose of neomycin and the mean neomycin concentration found in caecal contents.

Beta-aspartylglycine concentration in the caecum

The results of the mean concentrations found in the caecum at necropsy of the neomycin-treated mice are presented in Fig. 3. As found by experimental oral contamination, evidence was seen of a decrease of the CR starting from subgroup 4 on. In the mice treated with polymyxin B no beta-aspartylglycine was found which supports other evidence that the CR is not affected by polymyxin B even when the drug is given in high oral doses.

Neomycin concentration in the caecum

The results of the determination of the neomycin concentration are presented in Fig. 4.

Relative caecal weight

The mean relative caecal weight after 4 weeks of neomycin treatment are presented in Table 2 and in Fig. 3. The relative caecal weight was found to start to increase from subgroup 3 on. The increase was only significant ($P < 0{\cdot}01$) from subgroup 5 on.

At the end of the fourth week of treatment all the polymyxin-treated mice showed a mean relative caecal weight similar to that of the control group.

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Table 2. *Effect of 4 weeks oral treatment with neomycin on the mean and the relative caecal weight**

Subgroup	mg neomycine per ml of drinking water	Mean values of: Body weight (g)	Caecal weight (g)	Relative caecal weight (%)
C	0	29·6	0·6	2·0
1	0·05	25·5	0·6	2·4
2	0·15	27·6	0·5	1·8
3	0·45	25·2	0·6	2·4
4	1·35	25·7	0·7	2·7
5	4·05	25·4	1·0	3·8
6	6·0	27·6	1·1	4·0
7	8·0	25·3	1·1	4·3

* Relative caecal weight $= \frac{\text{caecal weight}}{\text{body weight}} \times 100\%$.

DISCUSSION

The results of this study indicate that both neomycin and polymyxin B can be applied for selective decontamination of the digestive tract. However, both antimicrobial drugs have their limitations for this purpose. Neomycin could only safely be applied in daily doses lower than 5·4 mg/mouse, a dose too low to suppress *Str. faecalis* to a noticeable extent. The Enterobacteriaceae species on the other hand did show a noticeable suppression following the next lower dose given, i.e. 1·8 mg/day.

Polymyxin B appeared not to influence the CR even after relatively high oral doses of 9·7 mg/day. It did on the other hand not noticeably suppress the *Str. faecalis* population. However, this was, on the basis of the natural sensitivity range of this bacterial species, also not to be expected. For the same reason polymyxin B may not be effective in suppression of *Proteus* species, which were not found in the experiments described here.

These observations suggest that for selective decontamination in mice neomycin and polymyxin B could be most effectively used in combination. Since they do not completely overlap (polymyxin B is in general effective against *Pseudomonas* species, whereas neomycin is in general active against *Proteus* species) these antibiotics could supplement each other for a certain part of their activity range. Whether addition or even synergism occurs *in vivo* inside the digestive tract remains to be investigated.

The beta-aspartylglycine test is confirmed in this study by the earlier observations of Welling (1979). This and the fact that Welling & Groen (1978) have also reported evidence that beta-L-aspartylglycine could be used as an indicator for the intactness of the CR, makes it probable that when neomycin (and polymyxin B) is applied clinically for selective decontamination, it may become a very valuable rapid diagnostic aid in the determination of the (average) CR-indifferent dose level

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in individual patients. This seems particularly important for neomycin as it, at least in mice, affects the CR at quite a low dose.

The mean neomycin concentrations in the caecum of mice found at necropsy following the various oral doses correlate with our bacteriological findings. Only following daily oral doses of 1·8 mg was the mean caecal concentration sufficiently high to excert an effect on the relatively sensitive Enterobacteriaceae species.

The culturing results of endogenous Enterobacteriaceae species show that in the first week of neomycin treatment the concentration of these bacteria in the faeces was less suppressed than in the second week. This could be explained by observations made in an earlier study in which it was found that it takes about a week after the onset of treatment before neomycin concentration in the faeces reaches a plateau; this was particularly the case following such relatively low oral doses (Van der Waaij *et al.* 1974*b*). The less-sensitive *Str. faecalis* apparently requires fairly high intestinal neomycin concentrations for a substantial suppression. In the study reported here persistent suppression was not achieved even following the highest oral dose. Resistance occurred and already in the third week this led to peak concentrations of 10^8 bacteria/0·1 g of faeces.

In conclusion we could state that both neomycin and polymyxin B can be used for selective decontamination in mice. They will, however, only affect the gram-negative aerobic flora and the daily dose of neomycin per mouse should be kept lower than 5·4 mg. Higher oral doses of neomycin will affect the CR and, with that, the aim of selective decontamination.

Whether these observations are also valid in man is still uncertain. Information on oral administration of neomycin and polymyxin B in low doses aiming at selective decontamination is limited to one publication by Guiot & Van Furth (1977). Further studies in patients will be necessary in which, in addition to bacteriological observations, determination of beta-aspartylglycine in the faeces may be of great value.

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