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Clearance of antibiotics from the intestines after termination of antibiotic decontamination

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SUMMARY

The clearance of neomycin and kanamycin from the intestines after stopping oral supply has been determined in mice. Both antibiotics, although given in different doses, were excreted in essentially the same way; the clearance being a little faster than logarithmically in both cases. The importance of this observation with regard to isolation and the moment of reconventionalization is discussed.

INTRODUCTION

The influence of the intestinal microflora on gastro-intestinal motility in mice has been previously investigated by Abrams & Bishop (1966, 1967). Using conventional and germ-free mice they demonstrated that the conventional microflora contributes to the control of the population of *Salmonella typhimurium* in the small intestine primarily by stimulating peristaltic emptying. The rate of propulsion of gastrointestinal contents compared in germ-free and conventional mice, using a non-absorbable radioactive substance yttrium 91, was found to be much higher in the conventional mice than in the germ-free animals. It took 24 hr. in the conventional mice before 100 % of the radioactive label was recovered in the faeces of germ-free animals.

In a previous communication (van der Waaij, Berghuis-de Vries & Lekkerkerk-van der Wees, 1971*b*), we have mentioned the possibility that also in antibiotic decontaminated animals a reduction of the enteric motility may explain the considerable decrease of the Colonization Resistance (C.R.) of the digestive tract which occurs during antibiotic decontamination.

In this paper we report attempts to measure the clearance of antibiotics from the intestines in decontaminated mice. This was accomplished by determining the concentration of non-absorbable antibiotics in the faeces daily after stopping oral treatment. The transit time in the upper part of the digestive tract was determined with a non-toxic non-absorbable dye, vermilion red.

MATERIALS AND METHODS

Animals

Conventional and germ-free ND2 female mice aged 12 weeks were used. Body weight varied between 32 and 40 g. During treatment, the animals were maintained in autoclaved cages with 4–5 animals to a cage inside a laminar cross-flow bench (van der Waaij & Andreas, 1971*a*). The chamber of the bench was peracetic acid sterilized and food, water and bedding were autoclaved. The animals were handled with sterile gloves. The germ-free mice used in one experiment were maintained similarly.

Antibiotics

On the basis of the outcome of an antibiotic sensitivity test of the faecal flora of the animals (van der Waaij, de Vries & Lekkerkerk, 1970), two combinations were used. In one experiment, performed with 10 animals, the combination of kanamycin and bacitracin was used while, in another experiment, a similar group was treated with neomycin and bacitracin. In the experiments in which the antibiotic concentration in the faeces was determined, these antibiotics were given for 2 weeks in the drinking water. Kanamycin was given at a concentration of 1 mg./ml., in combination with 1 mg./ml. bacitracin; neomycin was given at a concentration of 5 mg./ml. in combination with 5 mg./ml. bacitracin. In each instance 100 μg./ml. of pimaricin was added to suppress growth of yeasts and fungi.

The mice used in the vermilion red dye test were treated with neomycin and bacitracin both 2·5 mg./ml. and pimaricin 100 μg./ml. in the drinking water.

Antibiotic concentration assay

To determine the concentration of kanamycin and neomycin in the faeces of the mice, the microcup dilution method of Goss & Cimijotti (1968) was used after it was standardized and reference curves were made for a sensitive strain of *Escherichia coli*.

After stopping the oral supply of antibiotics, fresh faeces were sampled daily for 5 days after the last day of treatment. The faeces were suspended 1/10 in tryptose phosphate (T.P.) broth (Difco). After low-speed centrifugation, the supernatant of these suspensions was then assayed.

Vermilion red test

The propulsive activity of the small intestines was investigated by giving the animals 0·2 ml. of 2% vermilion red solution by stomach tube. At 1, 5, 10, 20, 40, 60 and 120 min intervals after administration the animals were killed in groups of 10 by cervical dislocation. The intestines were removed immediately after death and put in formaldehyde to stop further propulsion of the dye. Then the distance along which the dye had moved was measured. This experiment was performed with conventional, germ-free and decontaminated mice. The decontaminated animals were treated in two groups of 70. One group had been decontaminated for 4 days, the other for 2 weeks.

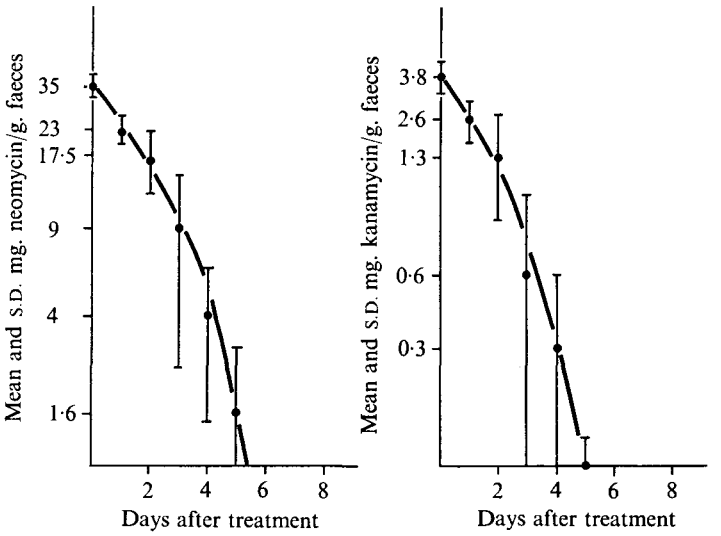

Fig. 1. Clearance of antibiotics from the intestines of mice expressed as the mean faecal concentration (and S.D.) at daily intervals after termination of treatment.

Caecal weight

The absolute and the relative weight of the caecum was determined at intervals of 2 days for 2 weeks after stopping oral antibiotic treatment, and thereafter weekly for 3 additional weeks. The animals were killed in groups of 8.

Bacteriological culturing

To investigate the effect of oral antibiotic treatment fresh faeces were sampled twice a week from each cage. The faeces from each cage were suspended in such a volume of Brain Heart Infusion broth (Difco) and Brewer's semi-solid thioglycollate that the antibiotic concentration existing in the faeces was diluted down to 1 μg./ml. or less. The suspensions were incubated at 37° C for at least 4 days before they were recorded as sterile. Also after antibiotic treatment was stopped mice were killed on alternate days in groups of 8 for aerobic culturing of intestinal contents.

RESULTS

The results of this study indicate that the clearance time of non-absorbable antibiotics from the gastro-intestinal tract is several days in decontaminated mice. In most of the animals treated with both the high dose (neomycin) and the low dose (kanamycin) it was more than 5 days before the antibiotics had disappeared from the faeces of all animals (Fig. 1).

The peristaltic activity of the small intestine was also much stronger in the conventional control mice than in those which were treated with antibiotics. The group of mice which were maintained decontaminated for 2 weeks (with neomycin and bacitracin) had a greatly decreased intestinal peristaltic activity. Peristaltic

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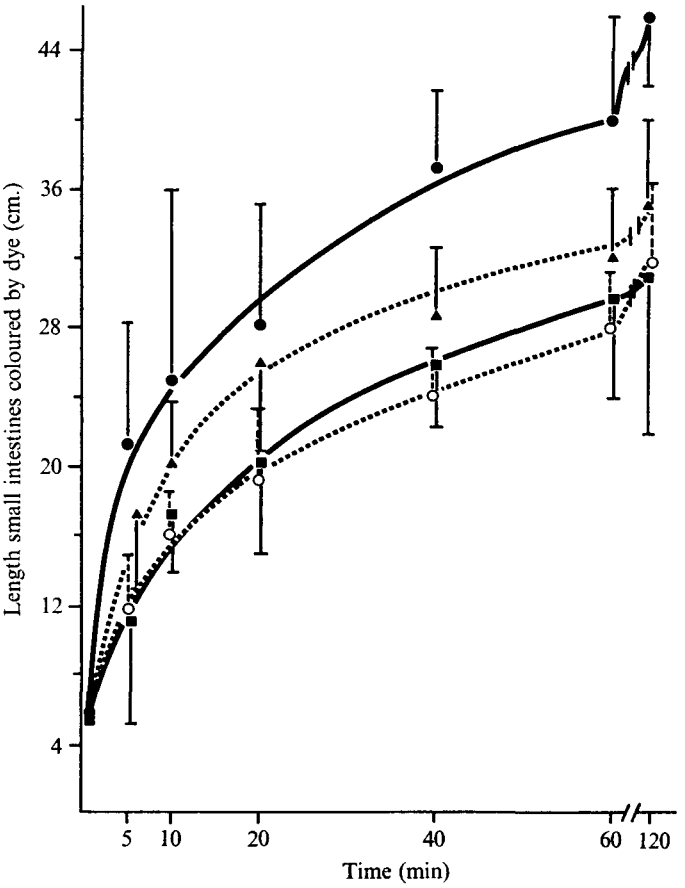

Fig. 2. Peristaltic activity of the small intestines expressed as the average distance (and S.D.) along which a dye was transported and the time. ●, Conventional mice; ■, germ-free mice; ▲, 4 days decontaminated mice; ○, 14 days decontaminated mice.

activity decreased already in the first week of treatment and was of the 'germ-free type' after 2 weeks (Fig. 2).

The results of cultures showed that decontamination had been successful in all animals in this study. Bacterial growth in the colon recurred only as late as 8 days after stopping treatment. However, the intestinal flora was still far from complete by that time, and the caecum was still enlarged. Reduction of the caecum started only 2 weeks after stopping treatment. The average caecal weight was back in the conventional range 3 weeks after antibiotic supply was stopped. The small intestines showed only occasionally positive cultures in animals killed in the first week after treatment. The percentage of positive cultures increased in the second week to become 100 % in the third.

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DISCUSSION

The present study indicates that the propulsion of contents in both the small intestines and the colon is much slower in mice that have been decontaminated for 2 weeks than in conventional animals. A similar conclusion about the role of the microflora in intestinal peristalsis was made by Abrams & Bishop (1967) on the basis of a study in germ-free and conventional mice. These authors applied a radioactive labelled non-absorbable substance httrium and determined the disappearance of the labelled material in the various parts of the gastro-intestinal tract. This means that the resultant of peristaltic and antiperistaltic activity was measured. Antiperistalsis may have mixed the remaining labelled material with later ingested food substances and in that way prolonged the excretion period. In our experiments in which the disappearance of aminoglycoside antibiotics from the colon contents was investigated, a prolonged excretion was seen, similar to that found by Abrams & Bishop in germ-free mice. Both curves derived from the results of our experiments have a similar shape. The excretion of antibiotic substance apparently does not occur logarithmically. The mean antibiotic concentrations in the faeces during the first days after the termination of treatment were a little higher, and those of days 4 and 5 were lower, than would be expected of the excretion had occurred logarithmically (Fig. 1). This has the important practical implication, that the clearance of big antibiotic residues from the intestines is relatively more rapid than that of small amounts of antibiotics.

The long-lasting increased volume of the caecum after termination of oral antibiotic treatment under isolation conditions could be explained by the fact that the anaerobic species which are responsible for the small conventional caecum in mice (van der Waaij *et al.* 1971*b*) only gradually found favourable circumstances for growth in the intestines in the second week after treatment. Since the caecum is the site where the contents from the small intestines are mixed with the caecal contents, the size of the caecum will significantly influence the speed of excretion of residual antibiotic substances in the caecum. Because the caecal size remained constant in the first week after termination of treatment, reduction of caecum size cannot explain why the antibiotic excretion was found to occur faster than exponentially after day 3. It seems unlikely that a repair of peristaltic activity in the upper part of the intestinal tract in the first week after treatment has enhanced the clearance later in the week, because the differences in transit time of the small intestines between conventional and decontaminated mice were small although significant. Also the concentration of the (aminoglycoside) antibiotics in the caecum and colon cannot have played a significant role in the clearance pattern, because both the high dose (neomycin) as well as the low dose (kanamycin) derived curves have essentially the same shape. In conclusion, the mechanism responsible for the stronger than logarithmic clearance of antibiotics from the intestines after termination of treatment is still unknown and requires further investigation.

The observations described in this paper underline the necessity of continued isolation after the cessation of oral antibiotic treatment for decontamination. When in previously decontaminated mice an adequate (anaerobic) microflora is

implanted from day 5 after treatment on 3 consecutive days, a normal Colonization Resistance will return in the second week as was described previously (van der Waaij *et al.* 1971*b*). Termination of isolation before that time will result in an abnormal colonization of the digestive tract which is associated with spread into the regional lymphatic organs (van der Waaij *et al.* 1972). When this occurs in animals with a not yet completely restored defence apparatus and a more pathogenic microorganism is involved, infection will occur.

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