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Colonization resistance of the digestive tract and the spread of bacteria to the lymphatic organs in mice

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SUMMARY

After oral contamination of conventional mice with high doses of *Escherichia coli*, *Klebsiella pneumoniae* or *Pseudomonas aeruginosa* the contaminant was recovered in abnormally high concentrations from the duodenum and caecum during the first few days. In this initial colonization phase, evidence of spread was obtained by culturing the cervical and mesenteric lymph nodes and spleen. Longer after contamination the intestinal concentration decreased to normal and spread stopped. In orally antibiotic-treated mice, the situation seen during the initial colonization phase in conventional mice occurred after a much lower oral contamination dose and persisted during the entire observation period of 2 weeks.

INTRODUCTION

In a previous paper (van der Waaij, Berghuis-de Vries & Lekkerkerk-van der Wees, 1971) experiments were described that indicated that the colonization resistance (CR) of the digestive tract is correlated with the presence of several anaerobic species of the intestinal flora in mice. The CR of the digestive tract for a foreign bacterial species was defined as the logarithm of that oral dose of bacteria that colonized the digestive tract for longer than 2 weeks in 50 % of the animals. For an *Escherichia coli* strain the CR was found to be 7, whereas it was above 9 for a *Klebsiella pneumoniae* and a *Pseudomona aeruginosa* strain. During and shortly after oral antibiotics were given, a rapid drop of the CR was found to extremely low values.

In the present study the colonization of the digestive tract is investigated as well as the spread of the contaminant into the lymphatic organs, i.e. the cervical and mesenteric lymph nodes and the spleen.

This study was performed because differences in survival time were found between contaminated conventional and antibiotic decontaminated mice after lethal irradiation. After oral contamination of irradiated mice with various Enterobacteriaceae species, bacteraemia due to the contaminant was found in 100 % of the decontaminated animals. In the conventional groups, bacteraemia due to the contaminant was dose-dependent and varied in frequency. Depending

on the interval between irradiation and contamination, this frequency varied between 40 and 75 %. These results suggested a difference in colonization pattern between conventional and decontaminated mice after experimental contamination.

MATERIALS AND METHODS

Mice

Conventional female ND2 mice and mice of the same stock treated with oral antibiotics were used. All animals were between 10 and 16 weeks of age. Housing, antibiotic decontamination and isolation procedure were identical with those described in a previous paper (van der Waaij *et al.* 1971).

Contamination

The suspensions for oral contamination were prepared in the same way as described before (van der Waaij *et al.* 1971). The streptomycin resistant (SR) strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the same as used in our previous publication (van der Waaij *et al.* 1971). In the conventional mice, contamination was performed with three different doses, namely 10^5, 10^7 and 10^9; the same doses as were used in the above-mentioned study. In the antibiotic-treated group of mice, however, only one dose of 10^5 cells was used.

Culturing

To determine the concentration of the contaminant in various parts of the digestive tract at various intervals after contamination, mice were killed in groups of 10 at days 1, 2, 3, 4, 7, 10 and 14. Immediately after death 0·1 g. samples of the contents of the duodenum and the caecum were taken and suspended in 0·9 ml. of brain–heart infusion broth (BHI) (DIFCO) to which 10 mg. of streptomycin per ml. was added to prevent growth of endogenous Enterobacteriaceae species in the conventional group. Subsequently the suspensions were tenfold serially diluted with 0·05 ml. diluting loops in the same medium. At autopsy also a throat swab was taken, and the cervical and mesenteric lymph nodes as well as the spleen were removed for culturing in streptomycin BHI-broth under strict aseptic conditions. Since previous experience had indicated that grinding of the organs gave the same results in culture as were obtained by cutting the organs in small pieces, the latter technique was applied in this investigation. All cultures were incubated at 37° C. and scored as negative when no growth was observed at the fourth day. Positive cultures were subinoculated on Endo-agar for subsequent identification.

RESULTS AND EXPERIMENTS

The colonization of the digestive tract after experimental oral contamination was investigated by culturing throat swabs and by determining the concentration of the contaminant in the duodenum and the caecum. Other parts of the digestive tract were not sampled in this study because in previous experiments it was found

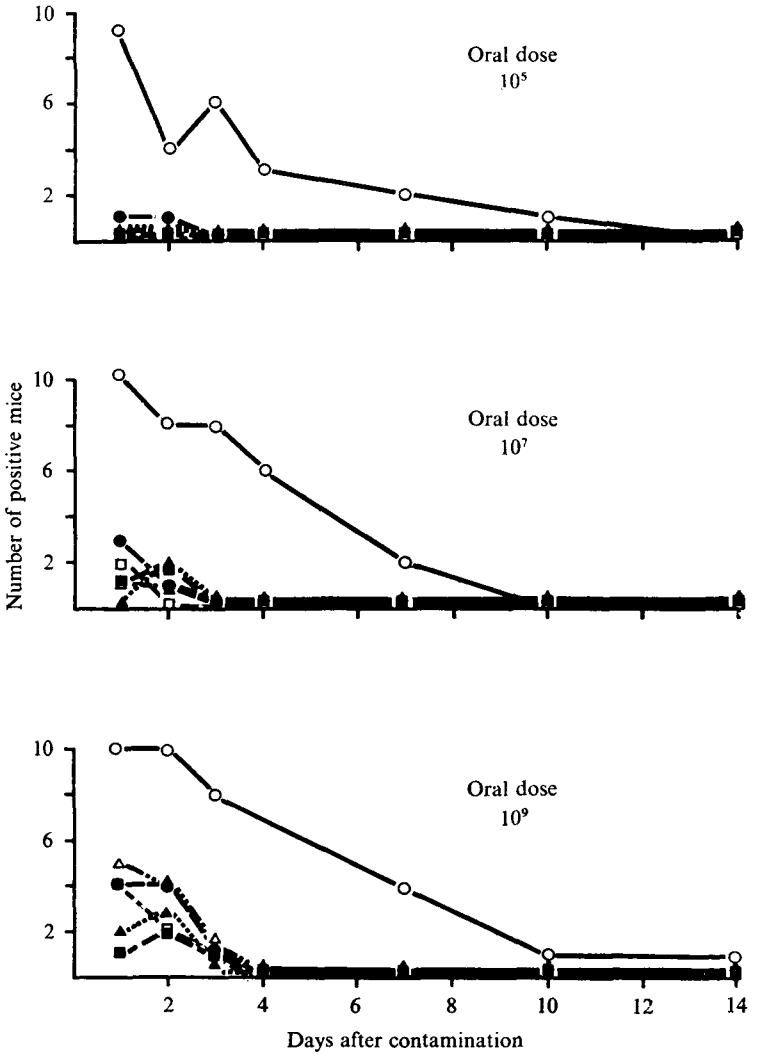

Fig. 1. Conventional mice contaminated with SR-*Escherichia coli*. ○—○, Throat swabs; ■– – –■, cervical lymph nodes; ●– – –●, duodenum concentration > 10^2/g. contents; △–··–·· △, mesenteric lymph nodes; □–·–·□, caecum concentrating > 10^6/g. contents; ▲···· ▲, spleen.

that these three parts of the digestive tract were representative of the colonization pattern in other parts (the stomach, the jejunum, the ileum and the colon). The spread of the strain used for contamination into the regional lymph nodes, i.e. the cervical and the mesenteric lymph nodes, and the spleen, was investigated by culturing these organs in broth.

The results of this study in conventional mice show that abnormally high concentrations of the contaminant in the digestive tract (> 10^2/g. duodenum contents and > 10^6/g. caecal contents) are seen the first 3–4 days following oral doses at the level of CR doses and at higher doses (Figs. 1–3). The CR for the *E. coli*, *Klebsiella* and *Pseudomonas* strains used for contamination were respec-

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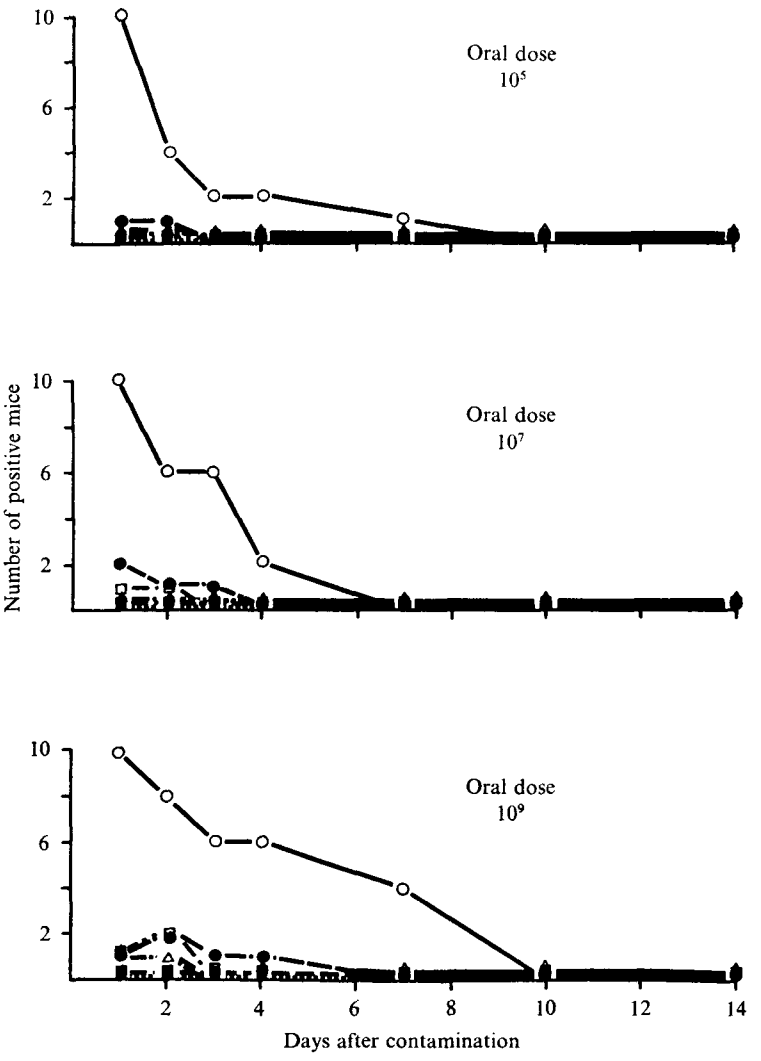

Fig. 2. Conventional mice contaminated with SR-*Klebsiella pneumoniae*. ○—○, Throat swabs; ■– – –■, cervical lymph nodes; ●– – –●, duodenum concentration $> 10^2$/g. contents; △–·–·–△, mesenteric lymph nodes; □–·–·–□, caecum concentration $> 10^6$/g. contents; ▲· · · ·▲, spleen.

tively 7, 10 and 11. More or less parallel with the 'abnormal' colonization pattern of increased concentration of the contaminant in the digestive tract, positive cultures were obtained from the lymph nodes and spleen of a number of animals. This was most obvious after contamination with SR-*E. coli*, the micro-organism with the lowest CR value (seven). Both other strains, SR-*Pseudomonas* and SR-*Klebsiella*, had a higher CR of above 9. Only after contamination with SR-*E. coli* were abnormally high concentrations and positive cultures of the lymphatic organs found during the first 3 days after contamination. Observations made in mice that were killed later after oral contamination revealed negative cultures of the

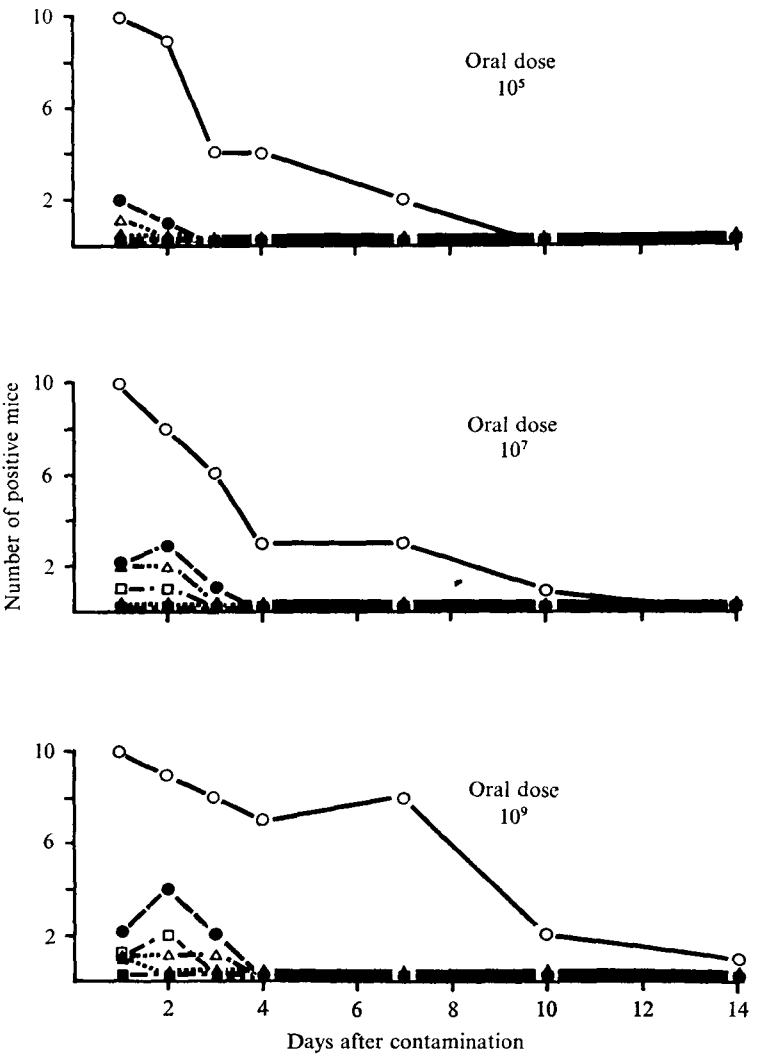

Fig. 3. Conventional mice contaminated with SR-*Pseudomonas aeruginosa*. ○—○, Throat swabs; ■– – –■, cervical lymph nodes; ●– – –●, duodenum concentration $> 10^2$/g. contents; △–··–·· △, mesenteric lymph nodes; □–·–· □, caecum concentration $> 10^6$/g. contents; ▲···· ▲, spleen.

lymphatic organs. In the first days after contamination a high percentage of animals had positive throat swabs. This, however, decreased rapidly.

In decontaminated mice abnormally high concentration of the contaminant and frequent positive lymphatic organ cultures were seen at all intervals after contamination with 10^5 cells (Fig. 4). This was most evident in the *E. coli* and in the *Kl. pneumoniae* contaminated mice. After contamination with *Ps. aeruginosa*, increased concentrations in the intestines and positive lymphatic organ cultures were found in a somewhat smaller percentage of the animals. Only the throat swabs remained positive in all decontaminated animals at all intervals after contamination.

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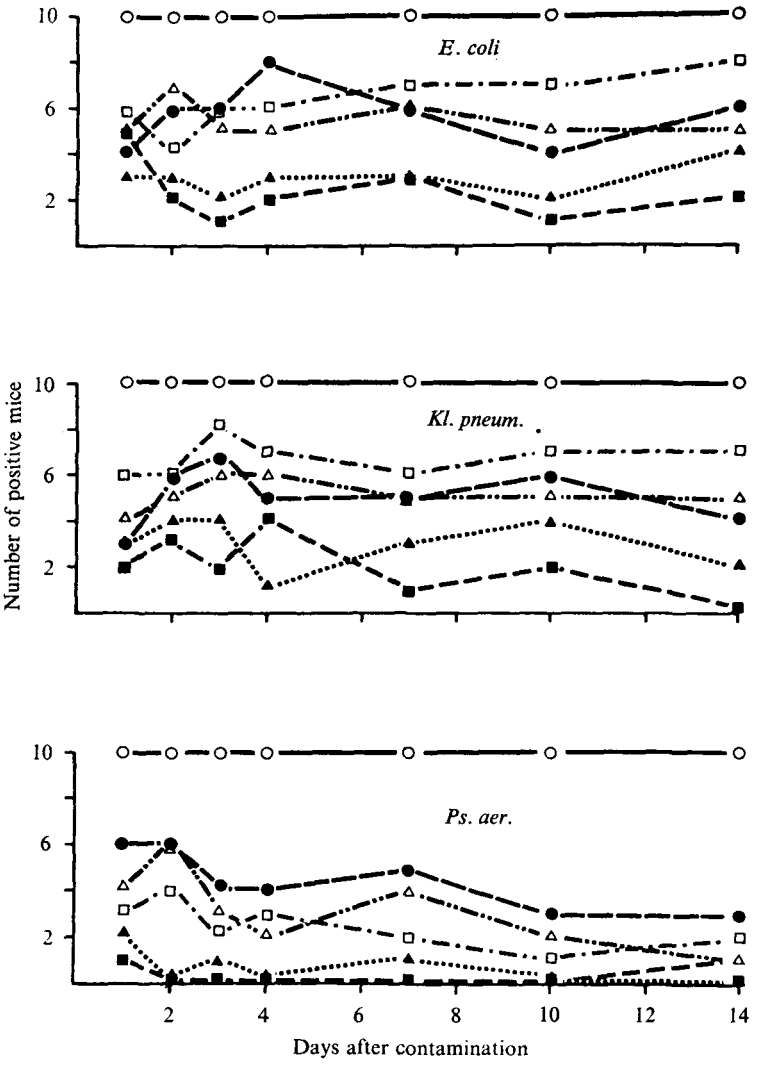

Fig. 4. Orally antibiotic-treated mice contaminated with 10^5 bacteria of three different species. ○—○, Throat swabs; ■– – –■, cervical lymph nodes; ●– – –●, duodenum concentration > 10^2/g. contents; △–·–·–△, mesenteric lymph nodes; □–·–·–□, caecum concentration > 10^6/g. contents; ▲···▲, spleen.

DISCUSSION

The present investigation has shown that in conventional mice abnormal colonization of the digestive tract (sometimes correlated with 'positive' lymph nodes and spleen) is only seen during the first 3–4 days following high oral doses with potentially pathogenic (p.p.) Gram-negative species. Abrams & Bishop (1966) have described a somewhat similar result after intragastric or intraduodenal challenge with *Salmonella typhimurium*.

After this initial colonization phase the situation becomes normal. Cultures of lymphatic organs were found negative in all animals and the concentrations in

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which the contaminant was found in the intestines had also decreased to normal values. After the lower contamination doses, in several mice the contaminant disappeared completely.

In mice whose intestinal flora had been greatly reduced by oral antibiotics the situation described above for the initial colonization phase persisted for much longer. Abnormal colonization and evidence of invasion was found during the entire period of suppression of the CR-responsible part of the intestinal flora (Dubos, Schaedler & Stephens, 1963; Savage & Dubos, 1968; van der Waaij *et al.* 1971) by oral antibiotic treatment. A comparable situation is also seen in monocontaminated germ-free mice, as has been described by Schaedler *et al.* (1965). Bonhoff & Miller (1962) described a similar difference in response between conventional and streptomycin-treated mice. The conventional animals required over 5×10^3 *Salmonella enteritidis* cells by stomach tube to make 50% of the animals positive for some time. With lower doses fewer animals, and with higher doses more animals became positive. Invasion of the spleen was found to occur more frequently after the higher doses and was particularly seen in animals in which the salmonella concentration in the faeces was above 10^2 per faecal pellet. In the streptomycin-treated groups, doses as low as 10 or less made 50% positive.

The mechanism responsible for the abnormal situation in 'initial colonization phase' seems to depend on an intact microflora. In conventional mice the CR mechanism is presumably temporarily overwhelmed by oral doses at or above the CR determining doses. When the CR is very low (as is seen in germ-free and orally antibiotic treated mice), it evidently cannot any longer control the colonization of the digestive tract, and is overwhelmed even after low oral contamination doses. Because invasion of the lymph nodes and spleen has only been seen in association with high intestinal concentration of the contaminant (both p.p. and pathogenic) we can assume that, in case an abnormal colonization pattern is seen, with positive throat swabs and high concentrations of the same species in the faeces, the mesenteric lymph nodes and the spleen are from time to time invaded by that particular species.

In conclusion we could state that the CR mechanism not only controls the microflora in the digestive tract but also directly or indirectly prevents spread of the endogenous p.p. species. The practical consequences of these observations are being investigated in irradiation experiments. Animals with a good CR due to our CRF flora (van der Waaij *et al.* 1971), which are contaminated and colonized with one p.p. bacterial species, may be less exposed to this p.p. species after irradiation than monocontaminated previously germ-free or antibiotic-decontaminated mice (van der Waaij & Sturm, 1968). These antibiotic-decontaminated mice have much greater numbers of the p.p. strain in their intestines than the CRF group. Also, the spread of infection in monocontaminated mice may be a disadvantage after irradiation or similar treatment has decreased the resistance to infection. Evidence has been obtained that this is the case since, particularly in experiments with the more 'pathogenic' p.p. species, the incidence of infection is much higher and the onset after irradiation earlier in monocontaminated mice than in CRF animals associated with that particular p.p. species (van der Waaij, unpublished data).

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