Effect of Reduction of Bowel Flora on Experimental Staphylococcal Infection in Mice.* (25978)

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Reduction in total number of viable organisms in the gastrointestinal tract has been of interest experimentally and clinically for several years. In the laboratory it is possible to raise animals under exceptional conditions and thus disease-free (*i.e.* specific pathogen free) or even germ-free specimens may be obtained. Under clinical circumstances reduction in bowel flora is desirable when surgery is to be performed on the alimentary tract. This is usually achieved by use of an antimicrobial agent, most frequently neomycin.

Dubos and Schaedler(1,2) have demonstrated convincingly that mice reared with their alimentary tracts free of most common Gram negative enteric organisms are more susceptible to staphylococcal infections than their control counterparts. Since the strain used by Dubos and Schaedler is an inbred strain the authors suggest an alternate explanation for this phenomenon, namely, that this increased susceptibility to staphylococcal infection on a hereditary basis cannot be ruled out. It is the purpose of this investigation to determine whether or not reduction in bowel flora *per se* may be the important factor in permitting a more virulent infection to occur.

Materials and methods. Male Swiss Webster albino mice approximately 4 weeks old and weighing 16 to 20 g were housed in the usual steel mesh cages, 10 mice to a cage. As soon as the animals were obtained from the farm they were placed on ground food made by pulverizing commercial mouse pellets to accustom them to powdered food for one week before additives were included in the diet. In the groups of animals that received neomycin the drug was added to the diet by thorough mixing in a food tumbler. The experiments were divided into 4 groups.

Group I. *To evaluate effect of neomycin on intestinal flora of mice*: A control group and 3 dosage levels of neomycin were used, 0.05, 0.1, 0.2% of the diet. The mice were kept on this diet throughout the experiment. The animals were sacrificed periodically and the small intestine (from pylorus to cecum) was removed, homogenized, and an aliquot diluted and plated on agar pour plates. The method has been previously described(3).

Group II. These experiments measured survival of 160 mice. 0.2 cc of a 1:1 dilution of a strain of hemolytic *Staphylococcus aureus* (Giorgio) was given intravenously as the challenge. This strain is susceptible *in vitro* to penicillin concentrations of 3.1 μg/ml. The animals were divided into 4 groups: a control group; a group treated with neomycin (0.1% of diet) before challenge; a third group treated daily from day of challenge with 1.5 mg of aqueous penicillin G intramuscularly for 28 days; and a fourth group that received both penicillin and neomycin as above. Neomycin was added to the diet one hour prior to challenge. Survival was determined in each group.

Group III. These experiments were identical to those in Group II except that after 72 hours the groups of animals receiving neomycin were divided in half, and only one-half in each case continued on neomycin.

Group IV. This experiment was identical to Group II except that the microbial population of staphylococci in the kidneys of 200 mice was computed instead of survival. The technic is that of homogenizing the kidneys, and appropriately diluting and plating an aliquot. Total microbial census can be computed from this procedure(4).

Results. Group I. Oral neomycin significantly lowered the microbial census of the small intestine. In Fig. 1 it may be seen that 0.1% of the diet as neomycin caused the greatest fall in population. After a 72-hour period there was a gradual return of organ-

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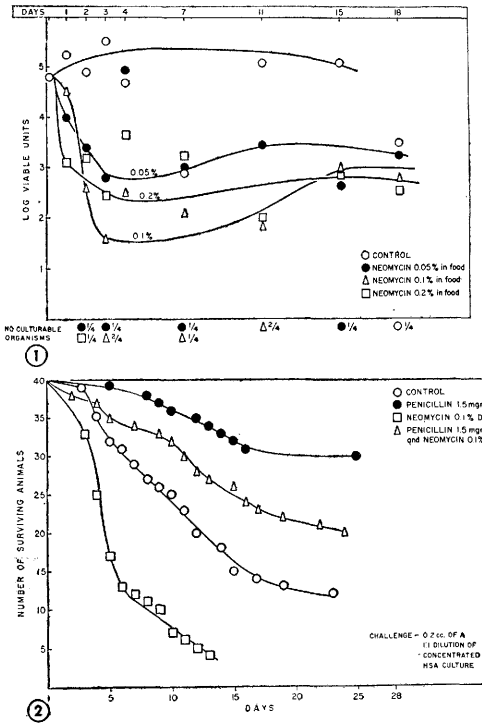

FIG. 1. Effect of neomycin on total microbial census of the small intestine of normal mice.

FIG. 2. Effect of neomycin bowel preparation on survival of mice challenged with hemolytic *Staphylococcus aureus* (Giorgio) (penicillin resistant) treated with penicillin.

isms. The rise and fall is qualitatively made up of Gram negative organisms, especially *Escherichia coli*. There were no deaths in this group and the animals appeared healthy.

Concentration of neomycin in blood was determined in all sacrificed animals. No neomycin activity could be shown by the tube dilution method using *Sarcina lutea* and hemolytic *Staphylococcus aureus* (Wolbeck) as test organisms. Both these strains are susceptible to neomycin concentration of 0.12 μg/ml. Several groups of mice were maintained on neomycin diet for 4 months. There were no deaths and all animals gained weight.

Neomycin as 0.1% of the diet was used exclusively throughout the remainder of the experiments described.

Group II. In the survival studies there was a definite increased susceptibility to staphylococcal infection of the neomycin-prepared animal as compared to control animals (Fig. 2). The SD-50 for the control was 14 days and for the neomycin group 5 days. Treatment with penicillin increased survival over the control animals when used alone or with neomycin.

Group III. Survival studies indicated that discontinuing neomycin administration after 72 hours has no effect on the outcome. The death rate remains constant and all animals are dead 15 days from time of challenge. The SD-50 of 5 days is the same as in animals continuing on neomycin.

Group IV. Microbial populations in the kidneys were highest in the group receiving neomycin alone. The census was significantly higher than the control animals. In the groups receiving penicillin the populations were lower than the control throughout the 28-day period.

Discussion. It is evident from these results that mice in which the intestinal flora are reduced by chemical means (neomycin) are more susceptible to intravenous infection with hemolytic staphylococci. This is shown not only by the higher mortality rate of the mice but also by the higher microbial populations in the kidneys. Dubos and Schaedler(2) have found the same phenomenon even though approached from an entirely different aspect. The only 2 things in common between their experiments and those described herein are the reduction in intestinal flora and the use of the Giorgio strain of hemolytic *Staphylococcus aureus*. Nevertheless, the results are strikingly similar.

The mechanism of this unexpected result must be either with the host defenses or with the parasite. Dubos and Schaedler(2) used *Klebsiella pneumoniae* as well as staphylococci as the challenge infection and found the same general pattern of increased susceptibility in the "clean" animals. It seems reasonable to conclude, therefore, that reduction (by use of drug) or absence (by careful raising of the mice) of Gram negative enteric organisms has a deleterious effect on the animals' capacity to withstand microbial challenge. Considered in its broadest sense, the unaltered microbial flora of the intestinal tract of an animal are a type of host defense mechanism. The manner in which this is mediated

is not at all clear. It certainly is alien to present concepts to associate a beneficial action with bacteria in relationship to the host. Except for the elaboration of Vit. K in the intestine there is no other evidence that the host is in any way helped by presence of these organisms.

It has been shown in this experimental model that penicillin has a beneficial effect on survival of mice infected with a penicillin resistant organism. The mechanism of this is discussed in detail by McCune(3,4) *et al.* In brief, studies of the Giorgio strain on agar plates with varying amounts of penicillin have revealed that this strain is composed of cells 90% of which are susceptible to 0.1 unit of penicillin per milliliter. However, even when organisms from one of the most resistant colonies are subcultured on plain agar or on penicillin containing agar the pattern of mixed resistance and susceptibility seen with the original culture is seen again. This would explain why penicillin is at least partially ef- Biology—One hundred seventy-nine Marks fective in treatment of this infection in mice.

Summary. Survival and microbial population studies in mice with their intestinal flora reduced by neomycin indicate that these animals are much more susceptible to intravenous staphylococcal infections than their littermate controls.

1. Dubos, R. S., Schaedler, R. W., *J. Exp. Med.*, 1959, v110, 935.

2. ———, *ibid.*, 1960, v111, 407.

3. McCune, R. M., Dineen, P., Batten, J. C., *J. Immunol.*, in press.

4. ———, *Ann. N. Y. Acad. Sc.*, 1956, v65, 91.

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Evaluation of Renal Function with Radio-Renogram.* (25979)

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Evaluation of renal function with I^{131} diodrast renograms has been reported(1,2). The value of the test is limited because the renogram could appear normal in presense of considerable kidney damage. This report deals with a modification of the I^{131} diodrast renograms designed to improve its effectiveness in both diagnosing and localizing renal disease.

Methods. Control Group. Standard I^{131} diodrast renograms were performed in 7 normal female mongrel dogs weighing between 10 and 17 kg. The animals were placed in a prone position and a catheter was inserted in the bladder. Scintillation detectors were placed over each kidney and over the heart. Output of the scintillation detector was connected to a count rate meter and a rectilinear strip chart recorder. Fifteen μc of I^{131} labeled diodrast (less than 3 mg) was injected intravenously. Levels of activity observed over each kidney and the heart were recorded. After completing the control renogram, an intravenous priming dose of para-amino-hippurate (PAH) calculated transiently to saturate the renal tubular mechanism was injected. The I^{131} diodrast renograms were repeated at frequent intervals as plasma level of PAH fell. Plasma levels of PAH were determined at each of these intervals. *Operative Group.* Routine radio-renograms were recorded in 8 dogs following which partial resection of their left kidneys were performed. Five dogs had about one-third, 2 had one-half and one had two-thirds of the left kidney excised. A bladder extrophy was also performed to facilitate study of indirect renal function in each kidney. One month or more post-operatively the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined simultaneously in both kidneys of each dog by measurement of creatinine and para-amino-hippurate (PAH)

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