tain some red cells, show a negative reaction for bile pigments, and a maximum absorption at 3700 Å, not shown by the normally colored urines. It is possible that hematuria and pigmented urine development are an expression of the same metabolic disturbance, which is responsible for the failure of these animals to develop ulcers. The role of the above pigments, or pigment derivatives is now under investigation.

Summary. The paper presents data on the degree of ulceration developed in a large number of rats treated according to the Shay technic, showing the incidence of esophageal changes, stomach acidity, and gastric liquid volume. The incidence of hematuria and pigmented urines is emphasized in their possible relationship to the etiology of Shay rat ulcers, as is the effect of preoperative diet in reducing hematuria and pigmented urines and increasing the incidence of ulceration.

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Received January 17, 1952. P.S.E.B.M., 1952, v79.

Effect of Oral Neomycin on Normal Intestinal Flora of Dogs and Man. (19369)

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Neomycin,* an antibiotic isolated from *Streptomyces fradiae* by Waksman and Lechevalier(1), has been reported to be effective *in vitro* and *in vivo* against a wide variety of gram positive and gram negative bacteria, including species resistant to other antibiotics (2-5). As the nephrotoxic and ototoxic effects of the drug(6) preclude for the present its parenteral administration, only the action after oral administration was investigated. The drug is so poorly absorbed from the gut that it is recovered nearly quantitatively and unchanged in the feces(4). This report deals with cultural data on the fecal flora after oral administration to man and to dogs.

In vitro studies. Seventy strains (*E. coli,* 10; *A. aerogenes,* 10; *Pseudomonas aeruginosa,* 10; *B. proteus vulgaris,* 10; hemolytic *Staphylococcus aureus,* 10; beta hemolytic streptococcus, 10; enterococcus, 5; and *Clostridium welchii,* 5), freshly isolated from human feces, were tested for sensitivity *in vitro* by a twofold tube serial dilution method, the concentrations of neomycin ranged from 200.0 to 0.006 μg per cc of nutrient broth. Each tube was inoculated with a bacterial suspension of 5,000-10,000 bacteria obtained by diluting an 18-24 hour broth culture of the particular strain. The lowest concentration of neomycin in μg/cc that showed no visible growth after 48 hours incubation at 37°C and did not yield living organisms in subculture was considered to be the bactericidal titer. *Comment.* In reading after 24 or 48 hours of incubation a series of tubes containing diminishing concentrations of neomycin, the last clear tube is followed by a tube just as cloudy as the control tube. In plating out this last clear tube there is no growth in the subculture. This indicates that *in vitro* neomycin has no bacteriostatic range and that the action of the drug is bactericidal—in sharp contrast to aureomycin which has a broad bacteriostatic range(7).

* The neomycin was provided by Dr. Earl L. Burbidge of Upjohn Co., Kalamazoo, Mich.

TABLE I. Bactericidal Titer of Neomycin in μg/ml in 48-Hr Nutrient Broth Cultures.

Organism	No. of strains	200	25	12.5	6.2	3.2	1.6	.8	.4	.2	.1	.05	.025
E. coli	10	—	—	1	—	—	—	1	—	—	—	1	7
A. aerogenes	10	—	—	—	—	2	1	2	—	4	—	1	—
Pseudomonas	10	—	—	—	1	1	1	2	—	3	2	—	—
B. proteus vulgaris	10	—	1	1	2	1	2	2	—	1	—	—	—
Staphylococcus aureus hemolyticus	10	—	—	—	1	—	1	2	—	1	—	2	3
β streptococcus hemolyticus	10	—	—	—	3	1	1	2	2	—	—	1	—
Enterococci	5	—	1	3	—	1	—	—	—	—	—	—	—
Clostridium welchii	5	5	—	—	—	—	—	—	—	—	—	—	—

Results. The results (Table I) confirm the reported data(2-4) that strains of *E. coli, A. aerogenes, Pseudomonas aeruginosa,* and hemolytic *Staphylococcus aureus* are very sensitive to neomycin, strains of *B. proteus* are moderately sensitive, enterococci are moderately resistant, and *Clostridium welchii* completely resistant to the drug. The strains of hemolytic streptococci herein reported were moderately sensitive to neomycin. This is in accord with some published data(4), while other reports indicate this species to be moderately resistant to the drug(5).

Effect of oral neomycin on the normal fecal flora of dogs. Method. Each of 37 healthy mongrel dogs received 0.4 g/kg of neomycin orally each day in 4 divided doses. In the first group of 17, the drug was given for 2 days; in the second group of 10 for 4 days; and in the third group of 10 for 10 days. The dogs were allowed to eat and drink as usual during the entire period of observation. No toxic reactions were observed. The fecal flora of all dogs was examined before the drug was started, daily during administration of the drug, and for a few days thereafter. The coliform bacilli were counted by the method of Spaulding *et al.*(8).

Results. 1. *Coliform bacilli.* In the 17 dogs treated for 2 days coliform bacilli disappeared from stool cultures in 1-3 days and then reappeared in steadily increasing numbers. In 8 of 10 dogs treated for 4 days the disappearance rate and reappearance following cessation of therapy were the same. In the remaining 2 there was only a marked reduction in the coliform count during and for 1-2 days after treatment. In 9 of 10 dogs which received the drug for 10 days, coliform bacilli disappeared within 1-4 days and were markedly reduced in the 10th. However, in 4 of these dogs *E. coli* reappeared on the 5th-6th day and rapidly reached pretreatment levels. These strains were tested *in vitro* and were found to be resistant to the drug (bactericidal titer 25 to 50 μg/cc). 2. *Pseudomonas* was present initially in the stool cultures of 18 of the 37 dogs disappearing in 9 of these after 2-6 days of neomycin. There was no appreciable change in the remaining 9 dogs. 3. *Proteus vulgaris.* Strains of this species, present only in 6 of the 37 dogs prior to neomycin, disappeared in 4 after 2-3 days of treatment. 4. *Enterococci* were found initially in the stools of only 7 dogs. They disappeared in all 7 after 2-4 days and remained absent during treatment and for 1-2 days thereafter. 5. *Yeasts* were found in many stools shortly after treatment was started. When all gram negative bacilli had been eliminated the aerobic plates frequently showed pure cultures of yeasts. However, they were never found in large numbers and there was no apparent increase during treatment. 6. *Clostridia* were present initially in the feces of 30 of 37 dogs. The number of colonies of these organisms on anaerobic blood plates from 24-hour thioglycollate subcultures was small to moderate, relative to the number of other fecal flora. The numbers of colonies was not appreciably reduced during treatment. Indeed, in several dogs all other bacteria were cleared from the feces and pure cultures of *Clostridia* remained. Hence *in vivo* resistance parallels the *in vitro* resistance of this species to neomycin.

Ten dogs were given a single dose of 6 g neomycin by gavage just prior to inducing hemorrhagic shock. Two died after 12 hours, 2 after 48 hours. The rest were sacrificed after 2 days. Cultures of small intestine and colon taken immediately after death showed the following: In the 2 dogs dead of shock after 12 hours, cultures from the jejunum yielded few to moderate number of colonies of *E. coli, Clostridia,* and enterococci. Cultures from the colon showed a large number of colonies of *E. coli,* a moderate number of colonies of *Clostridia* and a few colonies of enterococci. In all dogs dead or sacrificed after 48 hours cultures yielded no coliform bacilli in the jejunum or colon, but there were present moderate numbers of colonies of *Clostridia,* yeasts, and occasionally a few of *Pseudomonas.*

Comment. A single large dose of neomycin will eliminate coliform bacilli from the cultures of intestinal contents of the dog within 48 hours or less. This cannot be achieved so rapidly after oral administration of streptomycin, aureomycin, or sulfonamides(9).

Effect of oral neomycin on normal fecal flora of man. Three groups of patients without disease of the gastrointestinal tract received neomycin. In the first 8 patients, a single 6 g dose was given; in the second 8 patients a 6 g dose was given in 4 divided doses of 1.5 g every 6 hours for 24 hours; and in the third group of 9 patients, a 6 g dose was given daily for 3 days, 1.5 g every 6 hours. Two patients of the latter group had nausea or vomiting, and one had diarrhea. The pattern of the intestinal flora was determined in each case from stool cultures before treatment was started, and daily thereafter until the pretreatment status was restored. Quantitative changes in the coliform count were determined by the method of Spaulding(8).

Results. a) *Single 6 g dose—8 patients.* In 5 of the 8 patients the coliform bacilli disappeared from the cultures and remained absent for 48 hours in 4 of them. In the remaining 3 there was a marked reduction in the coliform count within 24 hours. In all 8 patients the count returned to pretreatment levels between the 4th and 6 day. Pseudomonas disappeared from the cultures in 2 patients and remained absent for 2 days thereafter. In 4 others there was no change, and in the remaining 2 none were cultured either before or after therapy. There was no appreciable change in the count of Clostridia or enterococci. b) *One and a half g every 6 hours for 1 day—8 patients.* Coliform bacilli disappeared from cultures in 24-48 hours after onset of therapy in 6 of the 8 patients, and remained absent for 24-72 hours thereafter. In the 7th patient there was only slight reduction and in the 8th no reduction in the coliform count. Clostridia, enterococci, and Pseudomonas were not significantly suppressed. c) *One and one-half g every 6 hours for 3 days—9 patients.* In all cases coliform bacilli disappeared from cultures within 24-48 hours. They remained absent for 2 days in 4 patients, for 3 days in 3, for 4 days in 1, and for 5 days in 1. Subsequently there was gradual return to pretreatment levels. Pseudomonas was suppressed after 2 days of neomycin therapy in 4 patients and remained absent from cultures for 1-2 days thereafter. The remaining 5 showed no change. Enterococci disappeared from the cultures in 3 after 2 days of neomycin administration. There was no change in 3 others. In the remaining 3 enterococci were absent before treatment was instituted. Clostridial growth was not altered in any instance.

Summary. 1. Ten random strains of each of the following species were sensitive *in vitro* (3 exceptions in 40 cultures) to 6.2 μg/cc or less of neomycin in a 48-hour nutrient broth culture: *E. coli, A. aerogenes, Pseudomonas aeruginosa, B. proteus vulgaris, Hemolytic staphylococcus aureus* and *Hemolytic streptococcus.* In 5 strains of enterococci the range of sensitivity was 25-3.2 μg neomycin/cc, while Clostridia were not sensitive at a concentration of 200 μg/cc or higher. When neomycin is effective, its antibacterial action is bactericidal and not bacteriostatic under the conditions of the test(8). 2. Oral administration of neomycin usually eliminates coliform bacilli from stool cultures in dogs and in man within 48 hours and frequently within 24 hours. Continuation of the drug for 5-6 days resulted in the appearance of resistant strains of *E. coli* in dogs. Such resistance was not

observed in 25 humans. 3. The numbers of *Enterococci, Ps. aeruginosa,* and *B. proteus vulgaris* in the stool are reduced in most cases by oral neomycin therapy. 4. Clostridia are not noticeably affected by neomycin.

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Received November 30, 1951. P.S.E.B.M., 1952, v79.