Effect of Streptomycin on Susceptibility of Intestinal Tract to Experimental Salmonella Infection.* (21030)

MARJORIE BOHNHOFF, BARBARA L. DRAKE, AND C. PHILLIP MILLER.
From the Department of Medicine, University of Chicago, Chicago, Ill.

Among the complications of antibiotic therapy in man are the "secondary infections" of the mucous surfaces with microorganisms insensitive to the drug being administered (1-3). Such infections are really a replacement of the normal microflora by new microbic populations which may or may not be disease producing, depending upon their virulence, *e.g.*, monilia(4,5) and gram negative bacilli(6) in the oropharynx, or staphylococci (7,8) in the bowel. These regions are constantly exposed to contamination with such microorganisms which, nevertheless, are rarely able to establish themselves, except during antibiotic therapy. The antibiotic is presumed to render such a site vulnerable to the implantation of contaminating microorganisms by suppressing or eliminating some of its normal inhabitants; *i.e.*, by disturbing the ecology of the microflora(9). The question therefore arises, to what degree does the normal flora of the oropharynx or bowel hinder the establishment of contaminating microorganisms and thereby assist the host in its defense against infection?

As the initial step in an investigation of this problem, experiments were undertaken to determine the effect of streptomycin on the susceptibility of the mouse's intestinal tract to infection with one of its natural pathogens, *Salmonella enteritidis*. Susceptibility was measured by determining the number of Salmonella required to infect mice by oral inoculation, the natural route for this microorganism. It was found that infection could be initiated by much smaller numbers of Salmonella after treatment with a large dose of streptomycin; *i.e.*, susceptibility to infection was markedly enhanced by preliminary treatment with this antibiotic.

Materials and methods. Inoculations were made by introducing known numbers of Salmonella in .5 ml broth directly into the stomach by means of a small bent tube attached to a .5 ml tuberculin syringe. The tube was made from an 18 gauge needle, 2″ long, the point of which had been removed and replaced with a bead of silver. The tip of the tube was passed carefully down the esophagus into the stomach. After sufficient practice it was possible to carry out this procedure with a minimum of trauma. Whenever there was doubt about the position of the tube, the mouse was discarded. After inoculation the mice were kept in isolation in individual jars to prevent cross-infection. *Administration of streptomycin*† was always by mouth. Single doses (in .5 ml saline) were injected directly into the stomach by the method just described. Similar injections of normal saline the day before inoculation in a group of controls ruled out the possibility that trauma was responsible for the increased susceptibility described below. When streptomycin was administered for several days, a fresh solution, changed daily, was added to the animals' drinking water. The mice were found to drink the streptomycin solution as readily as tap water. *The strain of S. enteritidis*‡ used was highly resistant to streptomycin. It was chosen because this property a) precluded its being mistaken for any other strains of Salmonella, b) facilitated its recovery and identification, and c) prevented its being affected by any streptomycin which might still be present in the bowel. The strain was originally recovered from the heart's blood of a mouse. It grew readily on agar containing 10 mg streptomycin per ml, but was not streptomy-

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‡ For the type diagnosis of this and other strains of Salmonella the authors are indebted to the Division of Laboratories, Illinois Department of Public Health, Dr. H. J. Shaughnessy, Director.

cin-dependent. It was not highly virulent for this strain of mice, in which the LD_{50} (21 days) by intraperitoneal inoculation was 10^6. Virulence was maintained at this level by frequent passage through mice. *The mice* were Rockland "RAP" females,§ 9-10 weeks old, weighing 20-25 g. Their feed was Rockland mouse pellets. These mice were known to have been exposed to *S. enteritidis* because carrier surveys on representative numbers made at the time shipments were received in the laboratory showed a high incidence of positive fecal cultures, on occasion as high as 50%. It was found, however, that the feces of most of these carriers became Salmonella-free within a few days if the mice were segregated in individual jars. Illness and death attributable to Salmonella infection seldom occurred among stock mice, indicating a high degree of resistance—native or acquired. Although *S. typhimurium* was occasionally recovered from the feces of mice on arrival from the breeder, no epizootic caused by this microorganism occurred among the stock animals, although they were often held in reserve for several weeks, housed in groups of 32 to a cage. *Routine fecal cultures.* The mouse was put into a sterile 250 ml bottle, or a clean, unused (quart) cardboard container.‖ As soon as the mouse had defecated, the pellet of feces (average—20 mg dry weight) was transferred by means of a sterile platinum wire to a tube of 10 ml brain heart infusion broth containing 1 mg streptomycin per ml. The pellet of feces was mashed and evenly suspended by means of a sterile glass rod, flared at the end to fit the bottom of a test tube. Serial 10-fold dilutions were made in streptomycin broth. After 18 hours incubation, a loopful of each culture was streaked onto eosin-methylene-blue agar and/or brilliant green agar containing 1 mg streptomycin per ml. Autopsy cultures were made on 385 mice, including all of those tabulated in Table I. Before the mouse was killed, a pellet of feces was obtained and cultured by the methods just described. The mouse was chloroformed, autopsied under aseptic precautions and the heart's blood cultured in 10 ml brain heart infusion broth. The spleen was macerated in a tissue grinder, and cultured in broth. Positive cultures were streaked onto EMB and brilliant green-streptomycin agar. The entire gut was removed and ground in a Waring blendor with 50 ml streptomycin broth which was subcultured the following day onto EMB and brilliant green streptomycin agar.

As a check on the dependability of the method for detecting small numbers of Salmonella in the intestinal homogenate, the intestines of 20 normal mice were homogenized in streptomycin broth, pooled and divided into 50 ml aliquots. These were inoculated, in quintuplicate, with very small numbers of Salmonella, checked by plate counts and broth titration. The results showed that 1 or 2 microorganisms sufficed to initiate growth in the intestinal homogenate. Among the 264 experimental mice from which intestinal homogenate was cultured, the results in all but 8 confirmed those on a pellet of feces obtained shortly before the animal was killed. In these 8 instances (3%) the fecal cultures were negative and the intestinal homogenates positive.

Results. The results of a representative experiment are presented in Table I. Groups of 5 mice each were inoculated by mouth with the numbers of *S. enteritidis* indicated. The treated mice had been given 50 mg streptomycin by mouth 24 hours before inoculation. Not included in the table were uninoculated controls given 500 mg streptomycin without apparent ill effect. It will be seen that among the untreated mice no infection resulted from inoculations with fewer than 10^4 Salmonellae, and that among the 10 mice given the two largest inocula (10^4 and 10^5) only 4 showed Salmonella in their fecal cultures beyond the first day after inoculation. From only 3 of these 4 were Salmonellae recovered at the time of autopsy from intestinal homogenate and from the heart's blood and spleen.

Comparison of the foregoing with the results in the treated mice shows the effect of a single dose of streptomycin given 24 hours before inoculation. All treated mice inoculated with 42 or 420 Salmonellae became in-

§ Obtained directly from Rockland Farms, New City, N. Y.

‖ Made by Container Corporation of America.

TABLE I. Effect of a Single Dose of Streptomycin by Mouth on Establishment of Infection with *Salmonella enteritidis* 24 Hours Later.

	Fecal content of Salmonella* (logs)																	
	Streptomycin treated mice†									Control mice								
	Days after inoculation						Autopsy cultures			Days after inoculation						Autopsy cultures		
Inocula	1	2	4	6	8	11	Entire gut	Sp	HB	1	2	4	6	8	11	Entire gut	Sp	HB
.42	—	—	—	—	—	—	—	—	—									
	—	—	—	—	—	—	—	—	—									
	—	—	—	—	—	—	—	—	—									
	—	—	—	—	—	—	—	—	—									
	4	8	5	7	7	6		+	+									
4.2	—	—	—	—	—	—	—	—	—									
	4	—	—	2	—	—	—	—	—									
	4	2	4	5	6	—	—	—	—									
	5	4	7	6	3	6		+	+									
	2	7	5	4	5	7		+	+									
42.	5	8	5	4	4	5		+	—	—	—	—	—	—		—	—	—
	5	8	8	7	4	6		+	—	—	—	—	—	—		—	—	—
	3	4	6	6	6	7		+	—	—	—	—	—	—		—	—	—
	2	6	5	2	3	2		+	+	—	—	—	—	—		—	—	—
	6	5	7	6	5	7		+	+	—	2	—	—	—		—	—	—
420.	6	3	6	6	6	5		+	—	—	—	—	—	—		—	—	—
	3	6	8	5	4	4		+	+	—	—	—	—	—		—	—	—
	4	5	6	3	3	4		+	+	—	—	—	—	—		—	—	—
	2	5	4	5	3	3		+	+	—	—	—	—	—		—	—	—
	5	4	8	3	4	5		+	+	—	—	—	—	—		—	—	—
4.2×10^3										—	—	—	—	—		—	—	—
										—	—	—	—	—		—	—	—
										—	—	—	—	—		—	—	—
										—	—	—	—	—		—	—	—
										—	—	—	—	—		—	—	—
$\times 10^4$										—	—	—	—	—		—	—	—
										2	—	—	—	—		—	—	—
										2	—	—	—	—		—	—	—
										2	3	—	2	2	3	+	+	+
										2	4	4	4	3	3	+	+	+
$\times 10^5$										2	—	—	—	—	—	—	—	—
										4	—	—	—	—	—	—	—	—
										3	—	—	—	—	—	—	—	—
										3	3	2	2	3	—	—	—	—
										3	3	2	4	4	2	+	+	+

* Per pellet of feces. Numbers represent the log of the reciprocal of the highest dilution showing growth. —, culture negative for Salmonella. 2 indicates growth in the first tube only.
† 50 mg streptomycin introduced by mouth 24 hr before inoculation.

fected, as shown by the results of the routine fecal cultures and the positive cultures of heart's blood and/or spleen at autopsy. 6 of the 10 mice in these 2 groups had demonstrable Salmonella bacteremia. Definite infection also occurred in 2 of the 5 mice inoculated with approximately 4 Salmonellae, and in 1 of those with a 10-fold dilution of that suspension, indicated in the table as .42 Salmonella. The data also show that the numbers of Salmonella recovered from the serial fecal cultures (tabulated as the logarithm of the reciprocal of the highest dilution showing growth) were much greater (about 1000-fold) among the streptomycin treated mice than among the untreated controls.

Table II summarizes the results of all experiments in which mice had been treated with 50 mg streptomycin 24 hours before inoculation, and for comparison, the results on all untreated control mice. In those mice which were not autopsied for culture of blood, spleen and intestinal homogenate, the diagnosis of infection was based on the persistence of Salmonella in the serial fecal cultures beyond the 5th day after inoculation. It will be seen

TABLE II. Combined Results of Inoculations of Streptomycin-Treated and Control Mice.

No. Salmonella inoc.	Streptomycin-treated*			Controls		
	No. exps.	Total mice	Infected, %	No. exps.	Total mice	Infected, %
<1	4	36	14			
1 – 10	4	36	56	3	30	0
10 –100	4	36	83	7	66	1.5
10^2– 10^3	1	5	100	14	120	15
10^3– 10^4				8	97	27
10^4– 10^5				10	76	33
10^5– 10^6				5	47	50
10^6– 10^7				2	14	100

* 50 mg streptomycin by mouth 24 hr before inoculation.

that less than 10 Salmonellae sufficed to infect approximately half (56%) of the streptomycin treated mice and that 10^5 were required to infect half of the untreated controls. The ID_{50} (infective dose for 50%) was computed by Berkson's method (10)¶ to be 2.21 for the treated mice and 1.3 x 10^5 for the controls (see Table III). If it had been possible to autopsy all of the mice for evidence of visceral involvement and these results used as the criterion of infection, the ID_{50} would have been only slightly increased, since positive spleen cultures were obtained in 90% of the mice in which Salmonella persisted in the feces to the day of autopsy.

Effect of decreasing amounts of streptomycin. When the dose of streptomycin was reduced to 10, 7 or 5 mg, the effect produced was less pronounced, as measured by the ID_{50} of Salmonella. A dose of 1 mg was without effect.

Duration of the increased susceptibility to Salmonella infection following a single treatment of streptomycin. Groups of mice were treated by mouth with 50 mg streptomycin and at different times thereafter inoculated by mouth with graded doses of Salmonella. The numbers of Salmonella required to infect 50% (ID_{50}) were estimated by the method of Berkson. Despite the error inherent in the methods employed, the results presented in Table III show that susceptibility decreased with time after streptomycin treatment, but as long as 5 days after treatment mice still showed somewhat greater susceptibility than controls.

Effect of 50 mg streptomycin supplemented by continued treatment thereafter. Mice were treated with the standard dose of 50 mg streptomycin and thereafter given streptomycin in their drinking water in a concentration of 1 mg per ml for 3 or 4 days. The only effect of such additional treatment was an increase in the numbers of Salmonella recovered in the serial fecal cultures.

Discussion. These experiments were designed to make a rough quantitative estimate of the increase in susceptibility to an enteric infection which follows the administration of a large dose of streptomycin by mouth. This was done by determining the numbers of Salmonella required to initiate a definite infection in mice so treated and in untreated controls. The results show that the susceptibility of mice to infection with *S. enteritidis* inoculated by mouth, the natural portal of entry, was markedly enhanced by the oral administration of 50 mg streptomycin 24 hours before inoculation. Whereas approximately 10^5 Salmonellae were required by this method to establish infection in half of the untreated control mice, less than 3 microorganisms sufficed to infect 50% of the streptomycin treated mice. Moreover, the numbers of Salmonella recovered in the routine fecal cultures were much greater (about 1000-fold) in the mice which had been pre-treated with a single dose of streptomycin and still higher in the mice which had been given additional streptomycin for 3

TABLE III. Duration of Susceptibility following Oral Administration of 50 mg Streptomycin.

Days after treatment	No. mice	Infective dose 50*	95% confidence limit
1	113	2.21	1.18–4.13
2	48	3.97×10^2	1.03×10^2– 15.3×10^2
3	70	1.79×10^3	$.33 \times 10^3$– 9.61×10^3
4	60	2.3×10^4	$.037 \times 10^4$–147 $\times 10^4$
5	72	1.75×10^4	$.47 \times 10^4$– 6.5×10^4
Control	423	1.37×10^5	$.44 \times 10^5$– 4.32×10^5

* No. of Salmonella required to infect approximately 50% of the mice.

¶ For assistance in the statistical treatment of the data, the authors are indebted to K. A. Brownlee of the Committee on Statistics, The University of Chicago.

or 4 days after inoculation.

That the successful establishment of Salmonella in the intestinal tract represented a genuine infection was confirmed by the demonstration of visceral involvement in mice killed for culture. Of those with Salmonella still present in their feces, 90% had the inoculated strain in their spleens and 72% in heart's blood as well.

Smaller doses of streptomycin—10, 7 or 5 mg—were less effective than 50 mg, and 1 mg caused no increase in susceptibility.

The effect produced by 50 mg streptomycin did not persist. Susceptibility to infection diminished but was still detectable on the 5th day.

The mice used seemed to be particularly suitable for experiments designed to demonstrate increased susceptibility to Salmonella infection since they possessed a considerable degree of (natural or acquired) resistance to this microorganism. Despite a high carrier rate, they seldom showed diarrhea or other signs of Salmonellosis, even though they were often held in stock for several weeks before use. Moreover, the LD_{50} (21 days) by intraperitoneal inoculation was 10^6 for the strain of Salmonella used in these experiments. Their resistance to Salmonella infection was further demonstrated by the very low mortality during the period of observation (usually 2 weeks). Only 10 mice died in all experiments although bacteremia was a common occurrence in the infected mice.

No attempt was made to recover from feces any but the inoculated streptomycin-resistant strain of Salmonella. However, from heart's blood and spleen, which were always cultured on both streptomycin-containing and streptomycin-free media, streptomycin-sensitive Salmonella were recovered from about 6% of the animals autopsied. Most of these strains belonged to group D, which includes *S. enteritidis*, and a few to group B, which includes *S. typhimurium*. These naturally occurring (streptomycin-sensitive) Salmonellae have been disregarded in the tabulated data in which positive cultures refer only to the streptomycin-resistant strain used for inoculation.

The increased susceptibility of the mouse's intestinal tract to infection after treatment with streptomycin is believed to have been caused by changes in the enteric flora resulting from the antibacterial action of the drug rather than to any stimulating effect on the inoculated organism or toxic effect on the host.

To be sure, Welch, Price and Randall(11) observed that very small doses (.25-1.0 mg) of streptomycin increased the mortality of mice infected intraperitoneally with *S. typhosa*, and Jackson and Axelrood(12) have recently reported that the mortality of mice infected intraperitoneally with *Pseudomonas aeruginosa* was somewhat increased by subcutaneous treatment with 1 mg Chlortetracycline. They observed no such effect, however, in mice infected intraperitoneally with *Proteus vulgaris* or with a drug-resistant staphylococcus, although among the latter there was some increase in the severity of bacteremia 2 to 3 hours after inoculation.

In vitro stimulation of bacterial growth in the presence of subinhibitory concentrations of various antibiotics has been described by a number of workers(13). No evidence was obtained, however, that the growth of the strain used in these experiments was stimulated by streptomycin either *in vitro* or *in vivo*. Nor was any toxic effect apparent in mice treated by mouth with even larger doses of streptomycin. This was not surprising since little if any streptomycin is absorbed from the gastrointestinal tract(14). It is for these reasons that the effect of streptomycin herein described is attributed not to its action on the inoculated strain of Salmonella, nor on the tissues of the mouse, but rather to its antibacterial effect on the microflora within the intestinal tract. The nature of the changes in the microflora responsible for the increased susceptibility to infection are being investigated.

It seems possible that this method for increasing the susceptibility of a laboratory animal to infection by direct inoculation into the gastrointestinal tract may prove useful in the experimental study of other enteric infections.

Summary. 1. Preliminary treatment by mouth with a large dose (50 mg) of streptomycin increased the susceptibility of mice to infection following oral inoculation with a

streptomycin-resistant strain of *Salmonella enteritidis*. In mice treated with streptomycin 24 hours before inoculation, <3 Salmonella sufficed to initiate infection in 50% as compared with approximately 10^5 in untreated controls. 2. This effect of streptomycin decreased as the interval between treatment and inoculation was lengthened but was still detectable on the 5th day. Smaller doses of streptomycin (5-10 mg) resulted in smaller increases in susceptibility. 1 mg was ineffective. 3. Representative numbers of mice killed for culture showed the spleen to be infected in 90% and heart's blood in 72% of those with positive fecal cultures at the time of autopsy. 4. It is believed that this increase in susceptibility following streptomycin treatment resulted from a disturbance of the normal intestinal microflora caused by the antibacterial action of the drug. 5. It is suggested that this method may be applicable to the experimental study of other enteric infections.

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In vitro Conversion of Thyroxin to Triiodothyronine by Kidney Slices.* (21031)

EDWIN C. ALBRIGHT, FRANK C. LARSON, AND RALMOND H. TUST.
(Introduced by O. O. Meyer.)

From the Department of Medicine, University of Wisconsin Medical School and Veterans Administration Hospital, Madison, Wis.

Several reported observations suggest that 3-5-3′L-triiodothyronine is derived from thyroxin by deiodination in extrathyroidal tissues (1-3). This concept is supported by the following observations on kidney slices, demonstrating *in vitro* deiodination of thyroxine to triiodothyronine.

Methods. 150-200 g male rats (Sprague-Dawley strain) were used. The animals were sacrificed by decapitation, the kidneys aseptically removed and cut into thin slices. Slices representing approximately one-half of a kidney were placed in each of the Warburg flasks containing 3 ml of Krebs-Ringer phosphate solution supplemented with 0.03 mg glucose. 0.01 μg of chromatographically pure I^{131} labelled thyroxin† were added to each flask. The slices were incubated at 37°C for periods of 0, 3, 6, 9, and 12 hours. Controls were run as follows: To one flask incubated for 12 hours potassium cyanide was added to yield a final concentration of 0.01 M; to a second flask incubated 6 hours potassium iodide was added to yield a final concentration of 0.01 M; in a third flask incubated 12 hours kidney slices which had been boiled for 5 minutes were used; to a fourth flask incubated 12

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† Obtained from Abbott Laboratories.