THE INFLUENCE OF STREPTOMYCIN AND STREPTOTHRICIN
ON THE INTESTINAL FLORA OF MICE

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In the course of the past three years, two antibiotic agents named streptothricin (Waksman and Woodruff, 1942) and streptomycin (Schatz, Bugie, and Waksman, 1944) have been isolated and shown to be active against gram-negative and gram-positive bacteria in vitro. Additional studies (Robinson, Graessle, and Smith, 1944; Jones, Metzger, Schatz, and Waksman, 1944; Robinson, Smith, and Graessle, 1944) showed both drugs to be active in vivo as well as in vitro. The fact that both streptomycin and streptothricin are water-soluble, stable preparations which are not readily absorbed from the gastrointestinal tract following oral administration (Robinson, Stebbins, and Graessle, unpublished) suggests that these antibiotic agents might be of value in bacillary dysentery and other infections associated with the gastrointestinal tract.

In the present investigation, an attempt was made to determine the effect of streptomycin and streptothricin in enteric infections by measuring the effect of these agents on the intestinal flora of mice.

METHODS AND MATERIALS

White male mice (CFW strain) of 18 to 20 grams in weight were used. The streptothricin studies were made using 60 mice divided into groups of 5 mice each. The first 4 groups received 250 units of drug per gram of food, the second 4 groups received 500 units per gram, and the third 4 groups were kept on a normal control diet (table 1). In the studies on streptomycin 18 mice were used, these being distributed so that 2 groups of 3 mice each received 250 units per gram of food, another 2 groups received 2,500 units per gram and the remaining 2 groups of 3 mice were kept as controls. The sulfasuxidine (succinylsulfathiazole) and sulfaguanidine were studied simultaneously, using a total of 30 mice which were divided into 6 groups of 5 mice each. Two groups were given the normal control diet while each of the other 2 groups were fed diets containing 1 per cent of sulfasuxidine and sulfaguanidine respectively. The drug diet method of Bieter et al. (1939) was employed in order to maintain the drug in the intestinal tract throughout the period of therapy. The mice were allowed to eat ad libitum, and from a record of the daily food consumption it was computed that their average drug intake per day was 30,000 and 60,000 units per kg of streptothricin, 30,000 and 300,000 units per kg of streptomycin, and 2 g per kg of each of the sulfonamides.

At varying daily intervals, counts of the lactose-fermenting and non-lactose-fermenting organisms in the feces of the mice were made from a uniform stool
suspension prepared as follows: One pellet was collected from each mouse and the pellets from two similarly treated groups of mice were pooled. The freshly collected and pooled pellets, which were 6 to 10 in number depending on the number of mice in each group, were weighed and ground aseptically into 4 ml of sterile tap water using a glass homogenizer. This uniform suspension was diluted with sterile tap water so that 30 mg of pellet (wet weight) were contained in 10 ml of water. Serial tenfold dilutions of this stool suspension were then made in peptone water and 1 ml of each dilution was transferred to sterile fermentation tubes containing 9 ml of lactose broth (White, 1942). After 48 hours' incubation at 37 C, the coliform count was estimated on the basis of the greatest dilution at which fermentation of the lactose occurred, whereas the total count was based on

### TABLE 1

**Sensitivity of intestinal flora to streptothricin and streptomycin**

<table>
<thead>
<tr>
<th>ORGANISMS OCCURRING IN STOOLS OF NORMAL MICE*</th>
<th>UNITS† PER ML REQUIRED FOR INHIBITION</th>
<th>ORGANISMS OCCURRING IN STOOLS OF MICE AFTER STREPTOTHRICIN THERAPY (15 DAYS)</th>
<th>UNITS† PER ML REQUIRED FOR INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (communior)</td>
<td>10</td>
<td>B. mesentericus</td>
<td>5</td>
</tr>
<tr>
<td>E. coli (communior)</td>
<td>20</td>
<td>B. subtilis</td>
<td>20</td>
</tr>
<tr>
<td>Salmonella</td>
<td>10</td>
<td>B. subtilis</td>
<td>&gt;320</td>
</tr>
<tr>
<td>E. coli (acidilactici)</td>
<td>2.5</td>
<td>E. coli (neopolitanis)</td>
<td>160</td>
</tr>
<tr>
<td>E. coli (acidilactici)</td>
<td>10</td>
<td>P. aeruginosa</td>
<td>80</td>
</tr>
<tr>
<td>Sarcina lutea</td>
<td>10</td>
<td>P. aeruginosa</td>
<td>320</td>
</tr>
<tr>
<td>Shiga dysenteriae</td>
<td>2.5</td>
<td>S. marcescens</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. marcescens</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. albus</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
<td>160</td>
</tr>
</tbody>
</table>

* Formula of natural food ration (g per 100 g): yellow corn meal, 31; whole wheat (ground), 30; casein (technical), 10; soybean meal, 10; linseed oil meal, 7; brewers' yeast, 5; alfalfa meal, 2; NaCl, 0.5; CaCO3, 0.5; corn oil, 3.0; cod-liver oil, 1.0.
† Units of streptothricin or streptomycin.

the greatest dilution at which growth occurred. The nature of the intestinal flora was studied by staining procedures and by making agar plates from various dilutions of the stool. Colonies were then isolated and identified by the usual bacteriological techniques.

### RESULTS

**Low-dose therapy.** From the data obtained, streptomycin was found to be the most effective of the four drugs in both the rate and the extent of reduction of the numbers of intestinal organisms in the mice (figures 1 and 2). The effectiveness of streptomycin is particularly striking during the first 9 days. Within 24 hours after the beginning of treatment with 30,000 units of streptomycin per kg per
day, the coliform count (figure 1) had decreased from a normal of approximately 100,000 bacteria to 100 bacteria per 3 mg of feces. On the other hand, a similar
dose of streptothricin required from 3 to 4 days to produce an equivalent reduction in coliform bacteria. The sulfonamides showed still less activity in that
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neither drug produced as much as a 1,000-fold decrease. Sulfaguanidine was the more effective of the two sulfonamides since it brought about a 500-fold drop in the coliform count after 48 hours of administration in comparison with a 100-fold decrease induced by sulfasuxidine. The results with the sulfonamides are in agreement with those of other investigators (Gant, Ransome, McCoy, and Elvehjem, 1943; Miller, 1945).

Not only did streptomycin effect the most rapid reduction in the coliform count, but also it maintained this new low level throughout the period of treatment with very little daily fluctuation. However, rather wide ranges occurred in the coliform counts of the animals treated with streptothricin. The sulfonamides were even less effective inasmuch as the numbers of coliform bacteria returned to normal within 9 to 16 days after the initiation of treatment.

![Graph showing influence of streptomycin, streptothricin, sulfaguanidine, and sulfasuxidine on the coliform count of mice](http://jb.asm.org/)

**Fig. 3. Influence of Streptomycin, Streptothricin, Sulfaguanidine, and Sulfasuxidine on the Coliform Count of Mice**

A similarly significant reduction was produced by streptomycin in the numbers of non-lactose-fermenting organisms (figure 2). After only 24 hours of treatment there were about 5,000 organisms per 3 mg of feces, whereas before treatment there had been 100 million. Unlike the experience with the coliform bacteria in which streptothricin, if granted enough time, could reduce the count to as great a degree as did streptomycin, it was found that 30,000 units of streptothricin had no appreciable effect on the total numbers of organisms in the intestines. The sulfonamides also failed to reduce the numbers of organisms other than the lactose fermenters.

**High-dose therapy.** It was then decided to see if larger doses of streptothricin and streptomycin would produce still more pronounced reductions in organisms of all types. This was found to be true (figure 3); however, owing to the greater
toxicity of streptothricin it was not possible to administer this drug in so large a dose as could be used for streptomycin. Whereas streptomycin is tolerated orally in amounts at least as great as 6,000,000 units per kilogram, only a few mice were able to tolerate 60,000 units of streptothricin per kilogram. Although this dose of streptothricin caused a 10,000,000-fold decrease in coliform bacteria, it nevertheless failed to eliminate them completely. However, treatment with 300,000 units of streptomycin, which is 1/3 of the maximum tolerated dose, sterilized the feces of coliform bacteria within 24 hours. This condition persisted throughout the period of treatment which lasted three weeks.

In addition to the activity against intestinal organisms during treatment, streptomycin was found to have further value in that it delayed the increase in numbers of organisms following the removal of the animals from the drug (figure 4). When mice were returned to their normal diet after three weeks of treatment, the coliform counts were found to increase 1,000- to 1,500-fold within 48 hours. Those which received a small dose of drug thus reattained a normal count; however, those which received the larger dose had a subnormal count which persisted for at least an additional 6 days. In contrast, the counts done on sulfonamide-treated mice returned to normal during treatment. The return to normal following removal of streptothricin was not determined.

Nature of the intestinal flora. Investigation of the changes observed in the nature of the intestinal flora emphasized still further the superiority of streptomycin and streptothricin as chemotherapeutic agents. Streptomycin when consumed in an amount of 300,000 units per kg over a 24-hour period eliminated not only all coliform, but all gram-negative organisms, leaving only a small number of gram-positive sporeformers such as *Bacillus mesentericus*, *Bacillus megatherium*, and *Bacillus subtilis* (figure 5). A similar bacterial population developed in the
mice receiving 30,000 units, however, without such a complete elimination of the gram-negative organisms.

It is of interest to note that about four days after the beginning of treatment with 300,000 units of streptomycin, one gram-negative organism, Bacillus clos
teroides, appeared in considerable numbers. After about 1 week it disappeared. A test of its sensitivity to streptomycin proved that this organism was 30 times more resistant than Escherichia coli "W."

Streptothricin, likewise, caused a very definite change in the nature of the intestinal flora. However, the population developing after treatment with the drug was distinctly different from that seen with streptomycin. With the reduction in the coliform count in mice receiving 60,000 units per kg, there was an increase in the number not only of gram-positive sporeformers such as B. mesen
tericus, but also of certain gram-negative bacilli, in particular Pseudomonas aeruginosa. Within 6 days the P. aeruginosa appeared to be in relatively pure culture and to remain so for a period of 10 days. At this time, B. mesentericus
and similar sporeformers reappeared together with colonies of Staphylococcus albus and Staphylococcus aureus. The latter organism was in the majority when the experiment was ended. The flora in mice receiving 30,000 units per kg showed essentially the same general changes, except that it required about two weeks before P. aeruginosa formed the major part of the flora. In the transition from the normal population to one of P. aeruginosa, Serratia marcescens occurred in relatively large numbers, the plates made on the sixth day of treatment being heavily covered with colonies of this organism.

In order to obtain additional information concerning organisms such as the anaerobes which, if present, would not grow in the medium used, stained films were prepared from the original stool suspensions. These showed essentially the same conditions and relative numbers of organisms as were found in the plates and tube dilutions. In the case of streptothricin, yeasts were seen to be numerous, but these decreased greatly in about ten days.

*Sensitivity of various intestinal organisms.* Determination of the sensitivity to streptothricin and streptomycin of those cultures isolated from the feces before and during treatment showed that the most sensitive organisms were eliminated first. Those remaining belonged for the most part to the group of organisms known to be resistant to streptothricin and streptomycin in vitro (table 1). Since the resistance was of the order usually found for these organisms, it is assumed that there was essentially no development of fastness to the drug. One exception was *Serratia marcescens* which appeared to develop some resistance to streptothricin.

It is unfortunate that up to the present time there is no satisfactory way of inactivating the antibiotics in vitro as can be done with the sulfonamides by the use of para-aminobenzoic acid in the culture media. It has been reported (Denkewalter, Cook, and Tishler, 1945) that streptomycin can be inactivated by cysteine; the amount of cysteine required, however, would be sufficient to inhibit the growth of certain bacteria. Bearing in mind the possibility that sufficient drug might be present to inhibit the growth of some organisms in vitro, it was considered essential to determine the amount of streptothricin and streptomycin being excreted in the feces. The stool suspension containing 3 mg of feces per ml, as well as more concentrated preparations, were assayed for the drugs by means of the cup assay (Stebbins and Robinson, unpublished). Although considerable amounts of the drugs were being excreted in the feces, the stool suspension (30 mg wet pellet in 10 ml) was found to have been so diluted as to reduce the drug concentration to 0 to 0.5 units per ml, an amount below that required to inhibit the growth of sensitive organisms. Thus, the possibility of a bacteriostatic effect being exerted by the drug in vitro was eliminated.

In spite of the great reduction in coliform organisms in the intestines of the mice, no evidence of vitamin deficiencies, loss in weight, or any other signs of toxicity were observed with streptomycin. Some deaths did occur with the 60,000 unit dose of streptothricin without evidence of vitamin deficiencies. This lack of development of signs of nutritional deficiencies can probably best be explained by the fact that the diet used contained an adequate supply of all
growth-promoting factors including biotin and folic acid. Gant, Ransome, McCoy, and Elvehjem (1943) have stated that *E. coli* is mainly responsible for the synthesis of biotin and folic acid or related factors. As a consequence animals on a synthetic diet develop nutritional deficiencies when the numbers of coliform organisms are reduced and then later replaced by coliform bacteria which are unable to synthesize the important growth factors. With a diet containing the necessary growth factors, however, the mice are independent of the synthesis of vitamins by *E. coli*.

**DISCUSSION**

The results obtained in this study demonstrate the pronounced activity of streptothricin and especially streptomycin in the reduction of the number of organisms in the intestinal tract of mice. This reduction is particularly significant when compared with that caused by sulfasuxidine and sulfaguanidine. Not only were the numbers of the coliform organisms rapidly and effectively reduced, if not eliminated, but also in the case of streptomycin the total flora was strikingly decreased. Furthermore, this reduction was not a temporary condition but was maintained with little fluctuation for the period of treatment. Whether or not similar effects can be obtained in man and other animal species remains to be determined. In the case of streptothricin, it was found that even after 72 days of treatment the coliform count was still in the same range as it was during the first week. A similar result was obtained with streptomycin; however, treatment with this agent was stopped after one month. From this it would seem reasonable to assume that no resistance to either streptothricin or streptomycin had been developed by the intestinal organisms. This failure of the organisms to develop resistance in the intestines would be especially significant if streptothricin or streptomycin were being considered for treatment of chronic infections.

Owing to the toxicity of streptothricin, its use in man will, no doubt, be limited. It might, however, be of value in the treatment of enteric diseases in cattle and other animals.

Streptomycin, on the other hand, might be effective for both man and animals not only as a therapeutic agent for enteric diseases but also as a form of preventive medicine in the preoperative and postoperative treatment of surgical infections. Additional experiments with mice given oral doses of streptomycin have shown that administration of the drug for a 24-hour period is sufficient to eliminate the coliform bacteria and to reduce the total number of organisms greatly. Within limits, the oftener the drug is administered, the smaller is the dose that may be used. It can thus be seen that by the oral administration of streptomycin a few hours prior to surgery, the possibility of the development of infections due to intestinal organisms might be greatly reduced. Sulfasuxidine has been used (Behrend, 1945) for such a purpose and with some success. However, a considerable period of preoperative treatment is required. Thus it would seem that streptomycin by virtue of its low toxicity as well as its speed and effectiveness of action has many advantages to offer.
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SUMMARY

Streptothricin and especially streptomycin, when fed to mice in their diet, produced a very rapid and pronounced reduction in the numbers of both the coliform and the non-lactose-fermenting organisms in the feces.

The dose of streptothricin required for effective reduction was one-half the toxic dose. The dose of streptomycin required, however, was much below the toxic dose and maintained the reduced number of organisms as long as therapy was continued.

Both drugs produced a distinct change in the intestinal flora but did not appear to stimulate the development of resistance.

REFERENCES