The Presence in Normal Serum of Inhibiting Substances Against *Bacillus subtilis*[1]

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In initiating an investigation of the absorption and excretion of streptomycin in humans, it was necessary to find an organism whose sensitivity to the antibiotic was such that it could be used as a test organism in determining the concentration of streptomycin in body fluids. *Bacillus subtilis,* No. 4R6259, received from the Merck Institute, appeared to have the required sensitivity and was selected for use in the study.

Before administering the streptomycin to patients chosen for the study a sample of blood was drawn from each as a control. In the first series of tests it was found that the serum inhibited the growth of *Bacillus subtilis* in dilutions varying up to 1:32.

Because of these findings, a titration of serum from 35 normal individuals was made in order to determine to what extent these inhibiting substances occurred. The sera were also tested to learn if inhibiting antibodies were present for a strain of *Staphylococcus aureus* (No. 209 P of the Food and Drug Administration). Unheated serum and serum inactivated at 56° C. for 30 minutes were used. Table 1 summarizes the results.

Thirty out of 35 of the sera inhibited the growth of *Bacillus subtilis,* while 5 of them failed to inhibit this organism. After inactivating the serum at 56° C. for 30 minutes, 10 of the sera lost their ability to inhibit, 15 partially lost their ability by a reduction in titer, and 4 were uninfluenced. (One sample, No. 32, was not tested in the inactivated state.)

TABLE 1

Titration of Inhibiting Substances in Normal Serum for *Staphylococcus aureus,* No. 209 P, and for *Bacillus subtilis,* No. 4R6259

Serum No.	Unheated serum		Serum heated 30 min. at 56° C.	
	Dilution at which organism is inhibited		Dilution at which organism is inhibited	
	Staph. aureus No. 209 P	*B. subtilis* No. 4R6259	*Staph. aureus* No. 209 P	*B. subtilis* No. 4R6259
1	No inhibition	No inhibition	No inhibition	No inhibition
2	No inhibition	1:2	No inhibition	Undiluted
3	No inhibition	1:8	No inhibition	1:2
4	No inhibition	No inhibition	No inhibition	No inhibition
5	No inhibition	1:32	No inhibition	1:32
6	No inhibition	1:8	No inhibition	1:4
7	No inhibition	1:8	No inhibition	No inhibition
8	No inhibition	1:4	No inhibition	No inhibition
9	No inhibition	No inhibition	No inhibition	No inhibition
10	No inhibition	1:8	No inhibition	1:4
11	No inhibition	1:4	No inhibition	1:2
12	No inhibition	1:4	No inhibition	1:2
13	No inhibition	Undiluted	No inhibition	No inhibition
14	No inhibition	1:4	No inhibition	1:2
15	No inhibition	1:4	No inhibition	1:2
16	No inhibition	1:8	No inhibition	No inhibition
17	No inhibition	1:8	No inhibition	1:2
18	No inhibition	1:8	No inhibition	No inhibition
19	No inhibition	No inhibition	No inhibition	No inhibition
20	No inhibition	1:2	No inhibition	No inhibition
21	Not tested	1:8	Not tested	No inhibition
22	No inhibition	1:2	No inhibition	No inhibition
23	No inhibition	1:4	No inhibition	No inhibition
24	No inhibition	1:8	No inhibition	1:4
25	No inhibition	1:8	No inhibition	1:2
26	No inhibition	1:8	No inhibition	1:2
27	No inhibition	1:4	No inhibition	1:2
28	No inhibition	No inhibition	No inhibition	No inhibition
29	No inhibition	1:2	No inhibition	1:2
30	No inhibition	1:2	No inhibition	No inhibition
31	No inhibition	1:4	No inhibition	1:2
32	Not tested	1:2	Not tested	Not tested
33	No inhibition	1:2	No inhibition	1:2
34	No inhibition	1:4	No inhibition	1:2
35	No inhibition	1:4	No inhibition	1:4

Two additional strains of *Bacillus subtilis* were tested in 5 of the sera listed in Table 1. Both of them were inhibited by each of these sera. The 5 sera which failed to inhibit strain No. 4R6259 were not tested for inhibiting substances against these 2 additional strains.

Thirty-three of the sera were tested against *Staphylococcus aureus,* No. 209 P. None of them possessed inhibiting substances against this organism. Some of the sera were tested against several other strains of

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staphylococci, but in no instance were inhibiting substances demonstrated.

Randall, Price, and Welch (*2*) have suggested the use of *Bacillus subtilis* as a test organism in assaying penicillin in various body fluids by a modification of the dilution method of Rammelkamp (*1*). This organism was proposed because of the ease with which it may be cultivated, the sharp reproducible end-points said to be obtained, and because its use obviates the employment of washed erythrocytes as required in the Rammelkamp technic. These investigators did not state, however, whether or not serum obtained from patients before the administration of penicillin inhibited their test organism.

The data presented in this paper would indicate that the results of any assay of serum for an antibiotic, using *Bacillus subtilis* as the test organism, are open to question, unless, prior to the administration of the antibiotic, the serum has been tested for the presence of natural inhibiting antibodies.

Summary: Sera from 35 normal persons who had received no previous medication were tested for inhibiting substances against *Bacillus subtilis* and *Staphylococcus aureus*. Thirty of the sera (85 per cent) inhibited *Bacillus subtilis* in dilutions varying up to 1:32, but in no instance was *Staphylococcus aureus* inhibited. The data presented would indicate that *Bacillus subtilis* is not a suitable organism for use in the assay of antibiotics in the presence of serum.

References

1. Rammelkamp, C. H. *Proc. Soc. exp. Biol. Med.*, 1942, **51**, 95–97.
2. Randall, W. A., Price, C. W., and Welch, H. *Science*, 1945, **101**, 365–366.

Preliminary Studies on the Absorption and Excretion of Streptomycin in Dogs

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Pharmacologic studies on three lots of streptomycin, ranging in potency from 105 to 500 units/mg., were done on 14 male and female dogs. The animals were starved or fed a liquid diet for 24 to 48 hours prior to administration of streptomycin. They were divided into four groups. Only the dogs in Group 1 were anesthetized, and these experiments were acute. Intravenous (saphenous vein) administration was employed in Groups 1 and 2. The intramuscular (gluteal) route was used in Group 3 and oral administration (stomach tube) in Group 4. Urine samples were collected by catheterization from all dogs of Group 1, from one dog of Group 3, and from two dogs of Group 4. All other urines were collected via the metabolism cage.

Plasma volumes and streptomycin levels were calculated from haematocrit determinations. By our method of assay (*3*), using a paper disc-agar plate, neither plasma nor urine in a twofold dilution with a 0.2-M phosphate buffer exerts an inhibitory effect on the test organism (*Bacillus subtilis*).

Results

In general, the rates of disappearance of streptomycin from the blood of anesthetized and unanesthetized dogs (Groups 1 and 2) following intravenous injections of 100,000 to 210,000 units were comparable. Only 18 to 29 per cent could be accounted for in the plasma during the period of 3 to 18 minutes postinjection. After 4 to 5 hours about 3 per cent could be detected in the plasma. The low total recovery of streptomycin (16 to 20 per cent of the amount injected) from the urines of the dogs in Group 1 may be due to the short experiment, or to anesthesia, since 45 to 65 per cent was recovered from the urines of Group 2. Approximately 35 to 55 per cent of the streptomycin injected was probably destroyed or inactivated. These results are in agreement with those found in humans (*1, 2, 4*). The purity of the three lots of streptomycin used apparently did not alter the results.

In Group 3, the highest plasma levels were 7.2 and 2.7 per cent of the injected amounts (100,000 units). These were reached after 1 and 1½ hours, respectively. After 5½ hours only a trace to 0.8 of one per cent could be detected. Therefore, the maintenance of plasma levels was no better with intramuscular than with intravenous injection. Only about 20 to 40 per cent of the stretopmycin administered could be recovered in the urines of this group.

In Group 4, streptomycin in doses from 210,000 to 420,000 units per dog could not be detected in the blood during the 24 hours following oral administration. The recovery in the urine of one dog of this group of 3.9 per cent of the drug administered, and lesser amounts in the others, indicates that streptomycin was absorbed to a small extent. The small percentage recovered in the urine is in agreement with the observations made on humans (*1, 2, 4*).

Summary

Streptomycin varying in potency from 100 to 500 units/mg. has been administered to 14 dogs intravenously, intramuscularly, and orally in amounts of 100,000 to 420,000 units.