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Treatment of Experimental Syphilis in Rabbits with Streptomycin.

STURE A. M. JOHNSON AND JOHN D. ADCOCK. (Introduced by C. C. Sturgis).

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Streptomycin, discovered by Schatz, Bugie, and Waksman,[1] was shown by Heilman[2] to have an effect on experimental relapsing fever and leptospirosis. For this reason the present preliminary study was undertaken to determine the effect of streptomycin on the course of experimental syphilis in the rabbit.

Procedure and results. Three white, adult, male rabbits were inoculated intratesticularly with darkfield positive testicular material obtained from a rabbit previously infected with the Nichols' strain of *Spirocheta pallida*. All 3 rabbits so inoculated developed darkfield positive lesions within 23 days. Repeat darkfield examinations of the same 3 rabbits were still positive 24 days later. The quantitative Kahn reactions varied from 20 to 160 units. At this time 2 of the rabbits (No. 244 and No. 247) were weighed* and streptomycin therapy was started. Each of these 2 rabbits received 30 mg (30,000 units) of streptomycin† at 8:00 A.M., 12:00 M., 4:00 P.M., 8:00 P.M. and 80 mg at 12:00 midnight, making a total of 200 mg of streptomycin daily. The material was injected into the deep muscles of the back. After 24 hours of therapy one testicle of rabbit No. 244 was removed and found to contain motile spirochetes by darkfield examination. The quantitative Kahn reaction was 20 units. A normal saline emulsion was made of the testicle and injected intratesticularly into a normal rabbit which developed darkfield positive lesions in 40 days, with a Kahn reaction of 40 units.

The dosage schedule was continued unchanged for both rabbits and at the end of 48 hours of therapy one testicle of the second rabbit (No. 247) was removed. Darkfield examination revealed motile spirochetes. The quantitative Kahn reaction was 160 units. Again a testicular emulsion was prepared and inoculated into another rabbit which developed darkfield positive lesions in 40 days with a Kahn reaction of 320 units. No change in the dosage schedule was made and at the end of 72 hours of treatment, the remaining testicle of rabbit No. 244 was removed and therapy was discontinued in this animal. No spirochetes were seen on darkfield examination of this testicle. The quantitative Kahn reaction was 40 units. A testicular emulsion was prepared and inoculated into 2 rabbits. One rabbit failed to show clinical or serological evidence of syphilis in 94 days when it died. The second rabbit failed to show evidence of syphilis in 137 days. It was later successfully inoculated intratesticularly with darkfield positive rabbit material. Treatment was continued to rabbit No. 247 for a total of 96 hours. At that time, the remaining testicle was removed. There were 6 non-motile spirochetes per 100 darkfields. The quantitative Kahn reaction was 400 units. An emulsion of the testicle was inoculated into 2 rabbits. Both of these rabbits developed darkfield positive lesions, which did not appear, however, until 120 days had elapsed. The quantitative Kahn

1 Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

2 Heilman, F. R., *Proc. Staff Meet., The Mayo Clinic*, 1945, **20**, 169.

* Rabbit No. 244 weighed 4.7 kg and No. 247 weighed 4.4 kg.

† Amounts of streptomycin are reported as equivalents of pure streptomycin base according to the recently adopted plan whereby 1 unit equals 1.0 μg of pure base. The streptomycin used in these experiments was furnished by Merck and Company, Inc., Rahway, N.J. The material was supplied as a partially purified dry powder containing the equivalent of 150 μg of pure streptomycin base per mg of solid. It was dissolved in sterile distilled water so that each cubic centimeter contained the equivalent of 50 mg of streptomycin base.

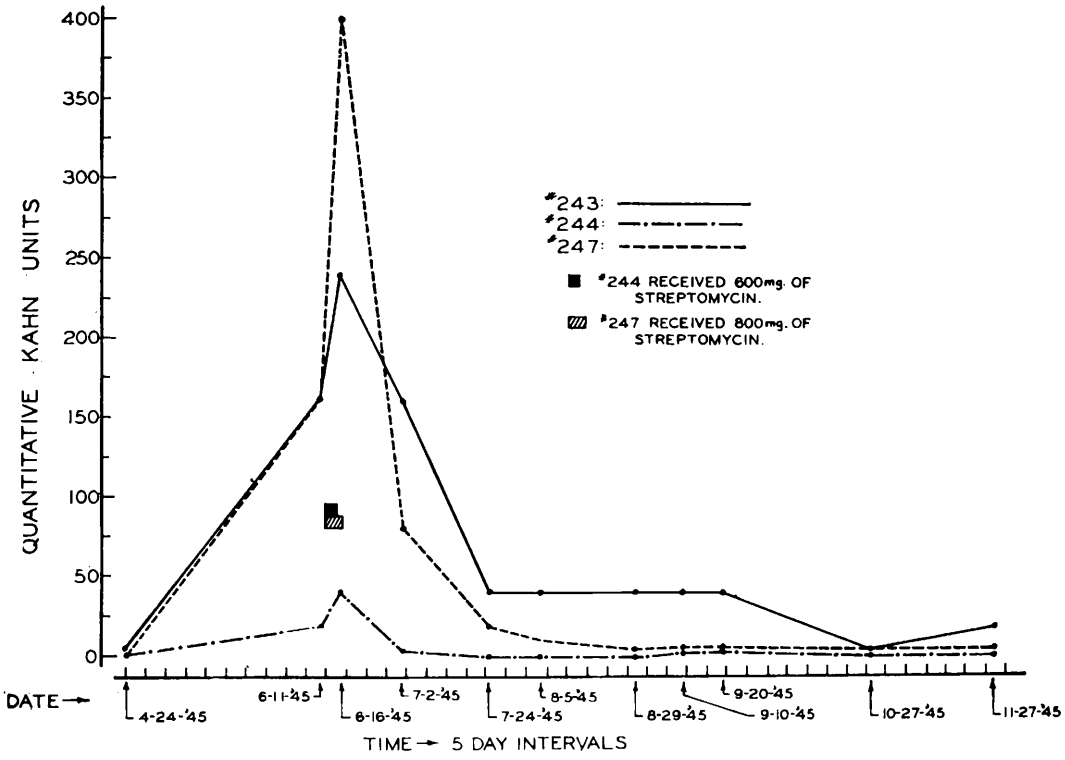

Fig. 1.
The results of the quantitative Kahn reactions on the blood of the treated and control rabbits.

reactions were 20 and 60 units respectively. At the time of removal of the second testicle of rabbit No. 247, both testicles of the third rabbit No. 243, which served as a control, were removed. An emulsion of one of these 2 testicles caused darkfield positive testicular lesions in a recipient rabbit 40 days later. Repeated quantitative Kahn reactions were done on the 2 streptomycin treated rabbits and on the control. The results are shown in Fig. 1.

Six months following treatment the popliteal nodes of the original 3 rabbits (No. 243, 244, and 247) were removed and 3 separate normal saline emulsions prepared. These were inoculated intratesticularly into each of 3 normal rabbits. The popliteal nodes of rabbit No. 244, which had received 72 hours of streptomycin failed to cause syphilitic lesions or a positive Kahn reaction in a recipient rabbit in 141 days. At this time the rabbit died so that further study could not be carried out. The popliteal nodes of rabbit No. 247, which had received 96 hours of therapy likewise failed to produce clinical or serological evidence of syphilis in the recipient rabbit in 141 days. This rabbit subsequently developed testicular chancres, following inoculation with darkfield positive material. The rabbit inoculated with the popliteal glands of the control rabbit (No. 243) developed darkfield positive lesions in 41 days and a quantitative Kahn reaction of 800 units.

Comment. One report has already appeared regarding the use of streptomycin in 4 cases of human syphilis by Herrell and Nichols in collaboration with O'Leary.[3] Each patient received treatment for 10 days with a total dosage varying from 3.2 g to 10.0 g. Assuming that these patients were of average weight, approximately 60 kg, the dosage varied from 5.3 mg to 16.6 mg per kg per day. In the 3 cases with darkfield positive

[3] Herrell, W. E., and Nichols, D. R., *Proc. Staff Meet., The Mayo Clinic*, 1945, **20**, 449.

lesions, early improvement was noted in that the lesions became free of spirochetes. However, all 3 of these cases of syphilis later developed darkfield positive relapses.

The 2 rabbits here reported received approximately 3 to 5 times as much streptomycin on a daily kilogram basis as the above patients. In one rabbit there was apparent cure of the disease after 72 hours of treatment while in the other rabbit 96 hours of treatment failed to cure as demonstrated by testicular transfers. On the other hand, popliteal transfers of both rabbits performed 6 months after streptomycin therapy were negative. Employing the dosage schedule used in these 2 rabbits, the daily dose in a man weighing approximately 60 kg would be 2.5 g which does not represent excessive amounts of streptomycin. The drop in the quantitative Kahn reaction in the 2 rabbits which had received streptomycin is of significance when compared with the control. This same phenomenon was reported as having occurred in one of the patients of the Mayo group. It is important to note that the testicular emulsion of the rabbit which received 96 hours of treatment contained nonmotile spirochetes which took 120 days to cause syphilitic lesions in 2 recipient rabbits, whereas the 24- and 48-hour post treatment testicular transfers, as well as the control, required only 40 days.

In a recent report, Dunham and Rake[4] have likewise shown that streptomycin exerts an antisyphilitic effect in experimental syphilis in the rabbit. While their method of study differed entirely from that employed in these studies, the results are roughly comparable.

Summary. 1. Two syphilitic rabbits were treated with streptomycin for 72 hours and 96 hours respectively, with 42.5 mg per kg and 45.5 mg per kg per day respectively. 2. Darkfield examinations were positive at the end of 24 and 48 hours of treatment and transfers of testicular material from both rabbits caused syphilitic lesions in other rabbits in 40 days. 3. Darkfield examination was negative after 72 hours of treatment in the rabbit which had received 42.5 mg of streptomycin per kg per day. Transfer of one of this rabbit's testicles to 2 rabbits failed to cause syphilitic changes. 4. The rabbit which was treated for 96 hours with 45.5 mg of streptomycin per kg per day showed nonmotile spirochetes which were capable of causing syphilis in 2 rabbits. However, the lesions did not develop until 120 days after inoculation. 5. Popliteal transfers of the streptomycin-treated rabbits, performed 6 months after therapy, failed to cause syphilitic lesions in 141 days. The control's popliteal nodes caused changes in 41 days. 6. The quantitative Kahn reaction for syphilis showed a rapid drop in the rabbits which had received streptomycin. This eventuated in negativity whereas the control remained positive. 7. The results obtained in this preliminary experiment justify further investigation of the effects of streptomycin in experimental syphilis.

[4] Dunham, W. B., and Rake, G., *Science*, 1946, **103**, 365.

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Inositol, a Constituent of Thyroid Gland Effect on Perfused Rabbit's Heart.

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In connection with an investigation of physiologically active factors contained in thyroid substance, a commercial powder of desiccated thyroid gland (Wilson) of an iodine content of 0.55% was extracted with 95% ethyl alcohol in a Soxhlet apparatus. The extraction was so conducted that the alcohol in contact with the gland powder had a temperature of about 50° and was continued for at least 3 days. The brown alcoholic extract was left standing for 24 hours, after which time a solid substance was found at the bot-