

Nine chicks have hatched and lived; one died emerging from the shell. The living chicks were killed with ether and examined at 28, 48, or 72 hours after hatching.

The yolk sac is normally taken into the abdominal cavity prior to hatching where it occupies a large part of the cavity in the newly hatched chick and lies in intimate contact with the organs. It persists for more than 2 weeks. That part of the sac which enters the cavity last is normally slightly adherent to the body wall at the umbilical area.

The tumor-bearing yolk sac was found in the normal position. It was more than normally adherent to the umbilical area. Most of the tumors were found near the point of

contact with the umbilical area, bulging into the yolk sac. In 2 cases they were scattered generally over the inner surface. In size they varied from less than 1 mm to about 10 mm. Larger masses were lobulated. Adjacent membranes were thickened. The tumors were usually firm, fleshy masses. In one case a cystic tumor was found.

In general appearance the tumors did not differ from those which we have examined in the late stages before hatching.

Of 9 living chicks from inoculated eggs, 7 showed gross tumors and 7 were confirmed on section.

Microscopically a majority of the tumors show alterations which suggest damage by the host.

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The Anti-Spirochetal Activity of Penicillin in Experimental Infections.

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1. *Activity of penicillin in experimental rabbit syphilis.* Mahoney, Arnold, and Harris¹ have reported on the anti-spirochetal activity of penicillin in rabbit syphilis. Definite spirocheticidal activity of penicillin *in vitro* tests has been reported more recently by Dunham and associates,² and by Eagle and Musselman.³

These findings form the experimental basis of the clinical results obtained with penicillin in human syphilis, as recently described by Mahoney and co-workers,¹ and by Bloomfield, Rantz, and Kirby.⁴

The purpose of our investigation was to establish the minimal healing and sterilizing

doses for penicillin in experimental spirochetal infections. The results are presented in this report.

Experimental. The strain of rabbit syphilis used in our investigation was received from the Laboratories of the U. S. Public Health Service in 1940 and has since been transferred at 2-3-month intervals. Rabbits infected intratesticularly 8 weeks before treatment with this strain of *Treponema pallidum* showed orchitis of both testicles besides chancriform lesions containing large numbers of spirochetes. Treatment consisted of intravenous injections of a penicillin sodium salt produced in the Roche chemical research laboratories. Some material not suitable for clinical use was at our disposal. The potency of the different samples as determined in the cup test varied between 100 and 300 units/mg. The drug was administered from 4 to 6 times over a period of 48-72 hours. The first injection was usually given in the evening, the lesions were examined for spirochetes the next morning, and thereafter the injections were repeated in

¹ Mahoney, J. F., Arnold, R. C., and Harris, A., *Am. J. Public Health*, 1943, **33**, 1387.

² Dunham, W. B., Hamre, D. M., McKee, C. M., and Rake, G., *Proc. Soc. Exp. Biol. and Med.*, 1944, **55**, 158; see also *J. Bacteriol.*, 1944, **47**, 22.

³ Eagle, H., and Musselman, A. D., *J. Bacteriol.*, 1944, **47**, 22.

⁴ Bloomfield, A. L., Rantz, L. A., and Kirby, W. M. M., *J. Am. Med. Assn.*, 1944, **124**, 627.

intervals of 3 to 18 hours.

Three rabbits received 4 to 6 intravenous injections of single doses ranging from 33,000 to 47,000 units/kg. Eighteen hours after a single injection with this dose a striking decrease of the number of spirochetes was noticed in the lesions of 2 rabbits, and complete disappearance of spirochetes in the third rabbit. After the third injection no spirochetes could be found in any of the lesions. Definite regression of the orchitis and of the lesions was observed 4-5 days after the first treatment. The primary lesions were completely healed 11-16 days after treatment was started. A fourth rabbit was treated with the same single dose 5 times at 2-hour intervals (total dose 150,000 units/kg). On the following day only a few spirochetes could be found in repeated examinations of the lesions. On the second day after treatment, none could be found while regression of the lesions became manifest. One week later the testicle was completely healed.

Three rabbits received 4 intravenous injections with single doses of 7,500-10,000 units/kg over a period of 48-72 hours. Eighteen hours after the first injection of this dose, a decrease in the number of spirochetes was found in the lesions of one of the 3 rabbits. After the third injection, spirochetes disappeared completely in the lesions of all 3. Dissolution of the lesions was apparent 5-6 days after the first treatment, while after 10-16 days healing of the primary lesions was complete.

Two rabbits were injected 4 times with single doses of 3,800-4,300 units/kg. No decrease in the number of spirochetes was noticed 18 hours after this treatment. Spirochetes did not disappear from the lesions until after the third injection. Marked regression of the orchitis was recorded 5 days after the initial treatment. Healing of the primary lesions occurred within 10 days in the first rabbit, and within 15 days in the second. At the same time 6 untreated control rabbits still showed orchitis and lesions containing spirochetes.

The activity of lower doses has not yet been established; neither can the question of permanent sterilization be answered satisfac-

torily, since the results of lymphnode transfers are not yet available.

The observations indicate that penicillin exerts a very definite activity on testicular infections of rabbits with *Treponema pallidum*. They are comparable to those observed in human cases.

2. *Activity of penicillin in relapsing fever (Borrelia novyi) in mice.* The spirochetes of relapsing fever (*Borrelia novyi*) seem to be approximately as sensitive to penicillin as is *Treponema pallidum*.

Heilman and Herrell⁵ found that a dose of 1,000 units was needed to prevent death as well as relapses in the majority of animals infected with *Borrelia novyi*. After administration of very high doses of penicillin, viz., 9,000 units per mouse, Augustin and his co-workers⁶ observed recovery of mice infected with this parasite. According to Eagle and Magnuson,⁷ the curative dose of penicillin in experimental infections of mice and rats with *Borrelia novyi* was 100,000 (50% cured animals) to 400,000 units (95% cured animals) per kg. The successfulness of treatment was ascertained by transfer of blood from treated to normal animals.

The purpose of our experimental work with *Borrelia novyi* in mice was to determine the minimal effective dose of penicillin in infections with the spirochetes of relapsing fever.

Experimental. The strain of *Borrelia novyi* used in these experiments was received from Dr. Malcolm H. Soule. Mice were infected intra-abdominally with a suspension of mouse blood containing 3 to 6 spirochetes per microscope field (dark field illumination). After 20 to 24 hours, spirochetes were present in the peripheral blood, generally 1 parasite per field. The mice were treated subcutaneously with penicillin using single doses ranging from 5,000 units/kg to 50,000 units/kg. In order to administer a high total dose (more than 50,000 units/kg), the treatment was given repeatedly in 3-hour intervals. Doses of

⁵ Heilman, D., and Herrell, W. E., *Proc. of the Staff Meetings of the Mayo Clinic*, 1943, **18**, 457.

⁶ Augustin, D. L., Weinman, D., and McAllister, J., *Science*, 1944, **99**, 19.

⁷ Eagle, H., and Magnuson, H. J., *J. Bacteriol.*, 1944, **47**, 21.

SPIROCHETICIDAL ACTIVITY OF PENICILLIN

TABLE I.
Activity of Penicillin Na Salt on *Borrelia Novyi* in Mice.

Penicillin, units/kg	No. of Mice			
	Treated	Blood cleared*	Relapses	Positive Reinfection
350,000†	2	2	0	0
200,000†	3	3	0	3
150,000†	3	3	1	0
100,000	3	3	0	—
50,000	17	17	9	2
25,000	6	6	1	0
12,500	5	1	1	0
5,000	3	0	—	—
Controls	10	0	6	0

* No spirochetes found by microscopic examination (dark field illumination) 20 hours after the treatment.

† Repeated treatment every 3 hours with single doses of 50,000 units/kg.

50,000 units/kg or less were given only once. Three to 4 weeks after treatment the mice were re-infected intra-abdominally with a heavy suspension of spirochetes. While we are aware of the fact that in spirochetal infections a positive re-infection is not absolute proof of previous successful sterilization, it might well provide some tentative information as to the curative effectiveness of antispiochetal agents. The results of the tests are given in Table I.

The minimal effective dose of penicillin in mice infected with *Borrelia novyi* was 25,000 units/kg, given subcutaneously one day after intra-abdominal infection; the spirochetes disappeared from peripheral blood within 4 hours after treatment. Relapses were observed only infrequently with doses up to 150,000 units/kg, and none occurred after treatment with doses ranging from 200,000 to 350,000 units/kg. Reinfection 3 to 4 weeks after the disappearance of parasites gave positive results in all mice treated with 200,000 units and in 2 out of 8 mice treated with 50,000 units. These results are in close agreement with the figures given by Eagle and Magnuson⁷ and indicate that penicillin possesses considerable spirocheticidal activity. No definite statement can be made as yet as to the permanently sterilizing effect of penicillin.

Summary. 1. The single intravenous dose of penicillin sodium salt required to produce disappearance of *Treponema pallidum* in experimental rabbit syphilis is 33,000-47,000 units/kg. The same effect can be obtained with about 16,000 units/kg if the drug is administered repeatedly over a period of 72 hours in doses of approximately 4,000 units/kg. The latter dose was found sufficient to produce healing of primary lesions in 10-15 days.

2. The minimal effective dose of penicillin sodium salt in *Borrelia novyi* infections of mice was found to be 25,000 units/kg mouse; doses of 200,000 units/kg and more might be considered as curative.

3. No definite statement as to the sterilizing potency of penicillin in spirochetal infections can be made at the present moment.

Addendum.

The results of the lymphnode transfer of the syphilitic rabbits are now available. The lymphnodes of 4 rabbits treated with the highest doses of penicillin used, viz., 132,000-282,000 units/kg, transferred into testicles of healthy animals, produced a syphilitic infection, indicating that even the high doses of penicillin were not sufficient to sterilize the animals.