

usual way on April 23; D.P. received 11 bites and H.B. 17 bites. The first peripheral blood smears were examined on May 14 and thereafter on May 16, 20, 23, 27 and 30; all the smears were negative. On June 19 (6 weeks after the infective bites), 5.0 cc of blood were drawn from each of the subjects, pooled, and 0.1 cc at once injected into each of 5 canaries. The blood of these canaries remained negative through 16 days of smearing, but it subsequently became positive after the usual incubation period when the birds were challenged by the bites of infected mosquitoes.

Summary. The sporozoites of *P. cathemerium* 3H2, an organism causing severe infections in canaries, were injected intramuscularly in large numbers into full-grown barnyard hens. In the respective trials the injectum consisted of (a) the gauze filtrate of infected mosquitoes ground in fresh hen plasma; (b) the gauze filtrate of infected mos-

quitoes ground in hen plasma obtained after allowing the drawn blood to stand for one hour at room temperature; (c) the gauze filtrate of infected mosquitoes ground in Locke's solution; (d) whole canary blood to which was added the gauze filtrate of infected mosquitoes ground in Locke's solution, the mixture being incubated at the temperature of hen blood for 2½ hours before using for injection. None of the injected hens in any of the trials became infected and all of the canaries injected as controls did become infected.

Two human volunteers were bitten, respectively, by 11 and 17 mosquitoes infected with *P. cathemerium* 3H2; neither of the individuals became infected so far as could be determined by a study of peripheral blood smears and subinoculation of blood into canaries.

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Therapeutic Efficacy in Experimental Syphilis of Eight Daily Injections of Penicillin in Beeswax-Peanut Oil.*

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Many methods have been developed to prolong the therapeutic activity of penicillin preparations, but the most effective of these appears to be the use of calcium penicillin in beeswax and peanut oil.¹ Romansky² has demonstrated that therapeutic penicillin levels are maintained in man for approximately 24 hours following the single injection of 300,000 units of calcium penicillin in 4.8% beeswax

by weight in peanut oil, contained in 1 cc. From a practical standpoint the use of this preparation permits active therapy to be carried out on an ambulatory basis. The advantages of such a procedure in the treatment of syphilis are obvious.

The present report summarizes the results of an experiment designed to assay the effectiveness of calcium penicillin in beeswax and peanut oil in the treatment of experimental rabbit syphilis, when the calculated total dose is administered in 8 daily injections.

Material and Methods. Male rabbits weighing from 2.5 to 4.2 kilograms were utilized. They were housed in individual cages and maintained on commercial pellets

* This report represents work performed under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the New Britain General Hospital.

¹ Romansky, M. J., and Rittman, G. E., *Science*, 1944, **100**, 196.

² Romansky, M. J., and Rittman, G. E., *New England J. Med.*, 1945, **233**, 582.

augmented by a free supply of water. The environmental temperature was stabilized through air-conditioning at a level never exceeding 75°F. Bilateral intra-testicular inoculations were carried out with saline emulsions of active syphilitic orchitic material from routine passage animals infected with the Nichols strain of *Tr. pallidum*. The inoculated animals were then observed for the development of orchitis, and dark-field examinations were performed when clinically indicated. Only darkfield positive animals were included in the treatment groups.

Treatment was begun 6 weeks following inoculation. All animals were weighed on the day prior to the beginning of treatment and the total dosage for each animal was calculated on the basis of these weights. The total amount of drug was intramuscularly injected in 8 equally divided daily injections, through the shaved skin of the hind quarters. The preparation consisted of calcium penicillin in 4.8% beeswax (by weight) in peanut oil, prepared and kindly supplied to us by Dr. Harry Eagle. Three dilutions were utilized. The first 2 were labelled "Abbott-Eagle Lot No. 2" and contained 125 units and 1,000 units of calcium penicillin per cc respectively. The third contained 8,000 units of calcium penicillin per cc and was labelled "Squibb." The Squibb preparation was employed only in the highest dosage group, i.e., 16,000 units per kilo. The Abbott material was used in all other dosages.

The animals were observed for 4 months following the completion of treatment. During this period frequent clinical examinations were conducted and all suspicious lesions were examined by darkfield. If positive, the animal was considered to be a treatment failure and discarded. At the end of 4 months all surviving animals were sacrificed, and the aseptically removed popliteal lymph nodes from each animal were emulsified with equal parts of saline and rabbit serum. The resulting emulsion prepared from each rabbit was then inoculated into each testicle of 2 normal animals. These were held for 3 months and observed by frequent clinical examinations. Suspicious lesions were dark-

fielded, and when this examination was positive, the parent animal from whom the inoculated popliteal lymph nodes were secured, was deemed to be a treatment failure. At the end of 3 months the surviving transfer animals were sacrificed and the sites of inoculation examined by darkfield for the presence of treponemes. If these were found, the parent animal was considered to be a treatment failure. If this final examination was negative, the parent animal was listed as a treatment success.

There were 7 groups of treated animals. Treatment varied between groups from a total dosage of 250 units to 16,000 units per kilo of body weight. Two different experiments were conducted. The first series of animals was inoculated on February 14, 1946, and the second on September 6, 1946. The results in these 2 series were not significantly different. They were therefore combined and are reported here as a single experiment.

Results. The final results are summarized in the accompanying table. It will be noted that all of 4 rabbits receiving a total dose of 250 units per kilo were treatment failures, while all of 14 animals receiving 8,000 or more units per kilo total dose were treatment successes. There is a fairly uniform increase in the ratio of successes with increasing dosage. The CD_{50} or the amount of drug required to cure 50% of the animals, when calculated according to the Reed-Muench³ formula, was 1,070 units per kilo.

Discussion. Employing the identical method of assay, Eagle, Magnuson and Fleischman⁴ have reported a CD_{50} of 1,600 units per kilo when sodium penicillin in aqueous solution was employed, the total dosage having been given in 5 injections per day for 4 days. Penicillin G, injected every 4 hours for 24 treatments has been found⁵ to result in a CD_{50} of 1,450 and 1,600 units per kilo.

³ Reed, L. J., and Muench, W., *Am. J. Hyg.*, 1938, **27**, 493.

⁴ Eagle, H., Magnuson, H. J., and Fleischman, R., *Bull. Johns Hopkins Hosp.*, 1946, **79**, 168.

⁵ Arnold, R. C., Boak, R. A., Carpenter, C. M., Chesney, A. M., Fleming, W. L., Gueft, B., Mahoney, J. F., and Rosahn, P. D., *Am. J. Syph., Gon. and Ven. Dis.*, in press.

TABLE I.
Results of Treatment of Experimental Rabbit Syphilis with calcium penicillin in Beeswax and Peanut Oil. Total calculated dose given in 8 daily injections.

No. of animals	Total dosage units/kg	Failed	Cured	Total Failed*	Total Cured*	% cured*
4	250	4	0	16	0	0
7	500	5	2	12	2	14
9	1000	5	4	7	6	46
9	2000	1	8	2	14	87
9	4000	1	8	1	22	95
9	8000	0	9	0	31	100
5	16000	0	5	0	36	100

* After Reed and Muench.³

Our results with 8 daily injections of calcium penicillin in beeswax and peanut oil were essentially no different from these reported for sodium penicillin and for penicillin G.

Fleming⁶ assayed a commercial preparation of penicillin in beeswax and peanut oil in the same manner as that reported herein, but his schedule employed 16 daily injections instead of 8. The CD_{50} in his experiment was 1,400 units per kilo. This value is not significantly different from our own findings of 1,070 units per kilo, when the two results are compared by the method of Behrens⁷ (difference = 1.34 ± 1.07 , $t = 1.25$). It is apparent that

⁶ Fleming, W. L., presented at a symposium, *Recent Advances in the Investigation of Venereal Disease*, Washington, D.C., Apr. 17, 1947.

⁷ Behrens, B., quoted by Gaddum, J. H., Reports on Biological Standards III. Methods of Biological Assay Depending on a Quantal Response, *Medical Research Council, London*, Special Report Series, No. 183, 1933, p. 26.

the total curative dose (CD_{50}) is not significantly altered by increasing the number of daily injections from 8 to 16. This observation may have important clinical implications in the treatment of human syphilis.

Summary. Calcium penicillin in beeswax and peanut oil was employed to treat groups of rabbits infected with *Tr. pallidum*. The calculated total dose based on body weight was administered in 8 daily injections. A CD_{50} of 1,070 units per kilo of body weight was observed. This value does not differ significantly from a CD_{50} of 1,400 units per kilo reported by Fleming following administration of a similar preparation in 16 equal daily doses. It was concluded that when calcium penicillin in beeswax-peanut oil is employed in the treatment of rabbit syphilis, increasing the number of daily injections from 8 to 16 does not alter the total curative dose (CD_{50}).