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Attempted Infection of the Hen and Man with the Sporozoites of *Plasmodium cathemerium* 3H2.*

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Huff and Coulston¹ reported their inability to find cryptozoites in canaries inoculated with the sporozoites of *Plasmodium gallinaceum*, a parasite to which the chick is quite susceptible, and also their failure to establish infections in canaries by the inoculation of blood from chickens infected with this organism. The present communication deals with a reversed experiment, *i.e.*, the attempt to infect the chicken with both the sporozoites and the trophozoites of a parasite that is highly virulent in canaries. The organism used in these trials was the *P. cathemerium* 3H2 that has been continuously maintained through mosquito-canary-mosquito passages in this laboratory since February, 1937. In addition there will be placed on record here an attempt to infect man with this organism, which I believe has not previously been reported.

First Trial. Using heparin as anticoagulant, 5 cc of blood was drawn by cardiac puncture from each of 3 full-grown white Leghorn hens and the plasma obtained after pooling the samples. Then 100 *Culex pipiens* mosquitoes that had fed two weeks previously on canaries whose blood contained numerous gametocytes of *P. cathemerium* 3H2 were stunned in a flask, ground in 2.5 cc of the plasma, and washed through 3 layers of gauze with another 2.0 cc of plasma; the volume of the filtrate was then made up to 5 cc with additional plasma. Of this filtrate, 0.1 cc (representing the sporozoites from 2 mosquitoes) was immediately injected intramuscularly into each of 4 clean canaries and 0.3

cc (6 mosquitoes) intramuscularly into each of 4 white Leghorn hens. Plasmodia were found in the peripheral blood of the canaries on the 7th, 7th, 8th and 9th days, respectively, but the hens remained negative through 17 days. On the 18th day the blood drawn by cardiac puncture from each of the 4 hens was pooled and 0.1 cc injected intramuscularly into each of 4 clean canaries; the blood of these birds remained negative through 16 days but all 4 birds subsequently became infected when challenged by the bites of infected mosquitoes.

Second Trial. Hen blood was obtained as in the preceding trial but it was allowed to stand after cooling at room temperature for one hour before centrifuging to obtain the plasma. Thereafter all steps were as in the preceding trial and the results were precisely the same.

Third Trial. One hundred infected mosquitoes were ground in 1.0 cc of Locke's solution for 30 seconds and the filtrate made up to 1.0 cc with Locke's solution; 0.2 cc of this filtrate was then diluted to 2.0 cc with Locke's solution and 2 hens were each injected intramuscularly with 0.5 cc (5 mosquitoes) of this mixture and 2 canaries with 0.1 cc (1 mosquito). Again the same results were obtained.

Fourth and Fifth Trials. These were repetitions of the immediately preceding trial, with the same results.

Sixth Trial. In this trial the mosquito-Locke filtrate was added to whole canary blood and rotated in the incubator at 41.5°C for 2½ hours before injecting into the hens and canaries. The results were the same as in all the preceding trials.

Seventh Trial. On May 8, 1947, D.P. and H.B. were bitten on the forearm by mosquitoes infected with *P. cathemerium* 3H2 in the

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¹ Huff, C. G., and Coulston, F., *J. Infect. Dis.*, 1946, **78**, 99.

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

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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.