EXPERIMENTAL TRANSMISSION OF THE "3m" STRAIN OF PLASMODIUM CATHERMERIUM TO THE DUCK AND ITS CHEMOTHERAPEUTIC SUITABILITY FOR ROUTINE ANTIMALARIAL SCREENING

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EXPERIMENTAL TRANSMISSION OF THE "3M" STRAIN OF PLASMODIUM CATHERMERIUM TO THE DUCK AND ITS CHEMOTHERAPEUTIC SUITABILITY FOR ROUTINE ANTIMALARIAL SCREENING

BY

DONALD LEE BUSH

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Coatney and Roudabush (1937) in the fall of 1936 made a survey of various wild birds in Nebraska by means of blood films and subinoculations, and noted the incidence of red blood cell parasites of birds. A magpie (Pica pica hudsonia) was found by them to be heavily parasitized with plasmodiae. Two types of gametocytes were noted in the blood smears, one was round and the other was of the elongate type. The blood from this magpie was subinoculated into a canary without previous infection, and the only type of parasite to appear was that of the round gametocyte type. This strain of plasmodium was tentatively identified as Plasmodium cathemerium, which was later confirmed by Huff of the University of Chicago. This strain was reported to be decidedly pathogenic for the canary, and has hence been designated as the "3M" strain by the Committee on Terminology of Strains of Avian Malaria.

Purpose

The purpose of this thesis is: (1) to show the method of passing the "3M" strain of Plasmodium cathermerium from the canary to the duck, (2) to determine if it is too virulent for the duck, (3) to look for exo-erythrocytic stages, (4) to standardize the parasitemia so a routine high percentage of the red blood cells will be parasitized, (5) to determine if the "3M" strain of Plasmodium cathermerium in ducks is a suitable malaria parasite for the chemotherapeutic screening of antimalarials, (6) to determine the presence or absence of a filterable lethal agent for ducks associated with *Plasmodium cathermerium* (Dearborn 1946), (7) to make observations on the periodicity, synchronicity, course of infection and anemia produced, and (8) to determine if the "3M" strain of *Plasmodium cathermerium* could be established in the chick.

Materials

Laboratory hosts consisted of adult female canaries (*Serinus canarius*) weighing about 15 grams, and white Pekin ducks obtained when one day old, and raised until they were about 100 grams. The strain birds used were of a much greater weight, usually 300-500 grams. This insured an ample supply of infective blood when it was needed. Chicks used were 3-5 day old white Leghorns weighing 40-50 grams.

All feed for both chicks and ducks was fortified broiler mash. Canaries received the usual mixture of seeds.

A rotary feed mixing machine was used to mix the drug diets (Litchfield 1939) employed in the chemotherapeutic study. Thorough mixing was achieved in 30 minutes. Previous tests were made with water soluble dyes added to the feed and aliquot portions of feed taken out and compared on a Lumetron colorimeter with a known standard of the same dye. In this way it was determined that proper mixing was accomplished in 30 minutes.

For the periodicity experiment, the birds were kept on a 12 hour light and a 12 hour dark cycle in a regular type brooder pen; 4 birds were used in this experiment.

Separate containers for running water and food were designed to accomodate one group of 3 ducks for the chemotherapeutic tests. Thus it was impossible for the running water and the feed to become mixed with that of another group of ducks on test. Ten groups of ducks were put on test at one time. The duck pens were placed on metal racks with one-half inch hardware cloth floors, thus allowing the fecal material to fall on a metal apron. The fecal material automatically passed down an outlet to the sewer by the action of the continuously running water, which was the overflow from the drinking troughs.

Birds that were kept for relapse studies were put in finishing batteries also equipted with running water.

The Lumetron colorimeter, calibrated against an Evelyn hemoglobinometer, was used to determine the hemoglobin as oxyhemoglobin.

Methods

Experimental Hosts

Adult female canaries have been used routinely as experimental hosts for the "3M" strain of *Plasmodium cathermerium*. However, this strain is virulent for the canary and initial infections or relapses result in deaths in many cases.

With this in mind, and, also, the report by Dearborn (1946) that an agent lethal for ducks is associated with the "3T" strain of Plasmodium catnermerium in ducks, it was decided to determine the susceptibility of white Pekin ducks to the "3M" strain; and to possibly make available another strain in the duck which would have possibilities for routine antimalarial screening.

Canary no. 80 was sacrificed at the peak of infection and 1 ml. of blood obtained from the heart. 0.5 ml. of the blood containing 40×10^6 parasites was quickly frozen in small brown glass tubes with very thin walls by immersing them in a mixture containing dry ice and acetone. This was later placed in a deep freeze unit at -70degrees F. The remainder of the blood containing 40×10^6 parasites was given to two day old ducks via the tarso-metatarsal vein, each duck receiving 20×10^6 parasites. These ducks were designated 1C and 2C. Blood smears were made daily but the parasites were not sufficiently numerous to count. The third day after inoculation 1 ml. and 2 ml. of blood, respectively, were inoculated into two uninfected ducks from duck 2C and the course of the parasitemia was observed in duck 1C to determine the best time to transfer the malaria blood. Definite dosages were not attempted until the 5th transfer when 145×10^6 parasites were given to a 100 gram duck. The peak parasitemia was reached on the 4th day with 38% of the erythrocytes parasitized.

These preliminary observations suggested that the duck would be a suitable host, and therefore, the strain was maintained by subinoculations every 4th day into young ducks.

After the transmission of the "3M" strain of Plasmodium cathermerium to ducks and its adaptation to that host, larger birds were used for maintenance of the strain.

Method of Counting Parasites

The method of counting the number of parasites in relation to red blood cells is that calculated by Hartman (1927a) and Gingrich (1932) according to the following formula: $N = 45.954 \frac{I-P}{P}$ where N is the number of red cells to be counted, P is the number of parasites per sample and I is the sample unit (10,000 RBC). This gives a parasite - red cell ratio which has a probable error of 10% of the observed values.

A second method was used which gave a probable error of 20% of the observed values with 10,000 red blood cells used as a sample unit. The formula is as follows: $N = 11.373 \frac{I-P}{P}$; letters indicate the same as the above.

A third method used in counting was that of determining the number of parasites per field when the average field contained 100 red blood cells. 100 fields were counted. The probable error was estimated at less than 20%.

A Howard disc was placed in the left ocular of a Bausch and Lomb binocular microscope to facilitate counting.

When blood was pooled in sterile rubber stoppered bottles, the parasite count was done directly from the bottle by making a blood smear in the usual manner and then, at the same time, a sample was taken for the red blood cell count. Smears were stained individually on a staining rack with Giemsa's solution.

<u>Method of Obtaining Infective Blood</u>

The method of obtaining malaria blood from canary 80 was intracardially. The boundaries for the insertion of the needle was at a 45 degree angle between the clavicle and centrally into the fossa formed by the clavicle. This method was also used in obtaining blood from chickens.

The method of obtaining malaria blood from ducks consisted of palpation over the sternum until the strongest heartbeat was felt, and then, merely inserting the needle through the sternum, using gentle traction on the plunger of the syringe until the blood appeared in the syringe.

<u>Method of Transfusing Malaria Blood</u>

Venous transfision was used in every instance. Chicks used were 5 days old, and easily handled. The site of injection was midway between the proximal and distal end of the metatarsus just above the bony core of the spur. Approximately one chick can be inoculated every 30 seconds.

Venous transfusion was less difficult in ducks. The same vein was used and would appear quite prominent even in day old ducks.

<u>Standardization of the Parasitemia</u>

Once the strain was well established in the duck initial experiments were carried out using 1×10^6 parasites per gram of body weight. This produced a sufficiently high parasitemia for a periodicity study, but was considered low for chemotherapeutic studies. After several experiments it was decided to use 1.5×10^6 parasites per gram of body weight for the chemotherapeutic studies. This size dosage produced a parasitemia with a range of 3800-6500 parasites per 10,000 red blood cells. After 300 transfers the virulence does not seem to have been increased when standardized dosages of parasites were given.

Experimental Data

<u>Parasitological Periods</u>

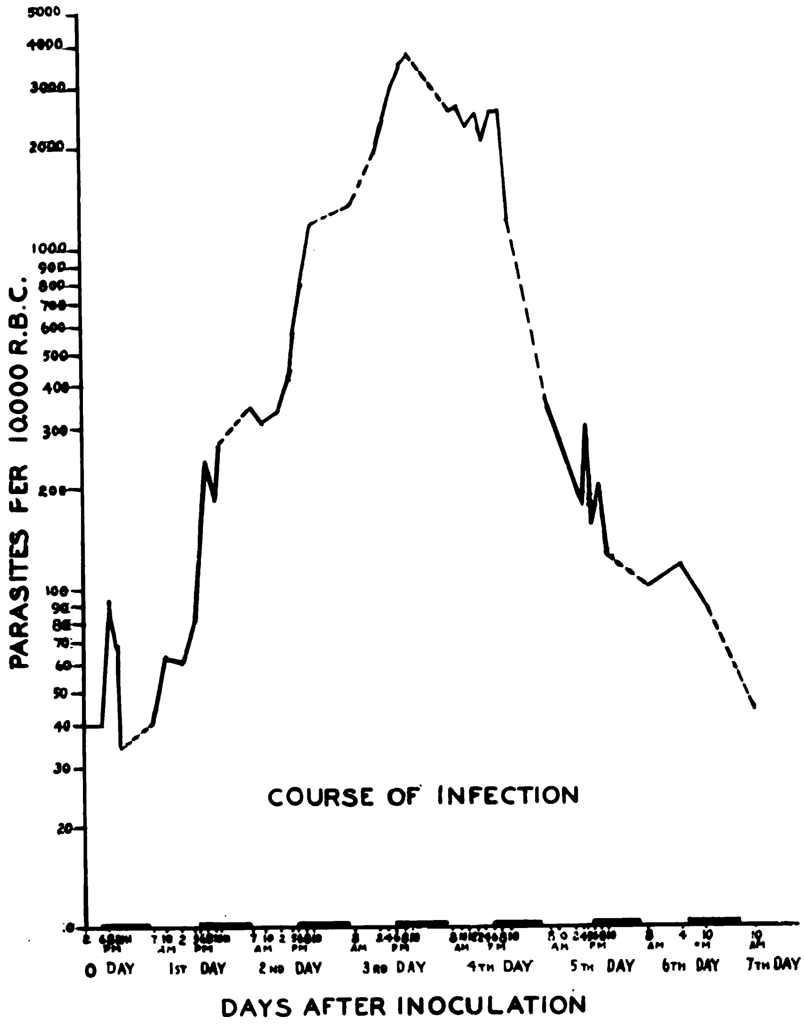
Parasitological periods (Hewitt 1940) pertains to the relationship between the parasite and its host, and may be divided as follows: (1) prepatent period, which is the period of entrance of the malarial parasite into the body until it can be demonstrated by ordinary diagnostic means, (2) patent periods, is the time interval is the time interval when the parasite can be demonstrated in the blood, and (3) the subpatent period when parasites are not demonstrable by the usual means, but may be present in minute numbers and could be demonstrated by subinoculations.

In standardized doses of 1.5×10^6 parasites per gram of body weight, the prepatent period is completely eliminated, thus, making the infection available for immediate study (Boyd 1925).

<u>Peak of Infection</u>

The peak of infection was determined in the following manner. Slides were made at two hour intervals during the day in order that the periodicity could be determined at the same time. Counts were made by random sampling, and the size of the sample unit for the probable error to equal 10% of the observed values was maintained throughout. Four ducks were used in the combined peak of infection and periodicity study. The ducks were injected with 1×10^6 parasites per gram of body weight at 11:00 A.M. and smears were taken at 2:00 P.M. the same day and every 2 hours until 10:00 P.M. and in some instances 11:00 P.M. Initial counts showed that all the birds had received desirable amounts of parasites and that the parasitemia of the various birds were coming up with a close correlation. However, due to individual response to malarial infection and biological variations in phagocytosis and immunity, it was decided that the geometric mean gave a better overall picture because it is a calculated value and is dependent upon the size of all the values. The advantages of the geometric mean are several (Arkin and Colton 1946); it is less affected by extreme items, it is a more typical average than the arithmatic mean, since it is less affected by the extremes and it can be manipulated algebraically. The formula when reduced to its logrithmic form is: $\text{Log. Gm} = \text{Log } X_1 + \log X_2 + \log X_3 + \ldots \ldots \log X_n$ divided by N.

Graph 1 represents the mean course of the infection in 4 ducks. The peak parasitemia was reached the third day at 10:00 P.M. following the injection of 1×10^6 parasites per gram of body weight in all the ducks studied. The blood used in the experiment was from ducks which were on a 12 hour light and a 12 hour dark schedule (Boyd 1929). These data correspond with the results Wolfson (1943) found with the "3T" strain of Plasmodium cathermerium in the duck. However, Wolfson (1943) states . . . "that further rapid passage of this strain through the duck is likely to increase the degree of parasitemia, to shift the peak to a later time in the infection, and to cause more frequent deaths of the host." It has been found by the author that if the number of parasites injected remains constant, there was no increase in the degree of virulence of the "3M" strain of Plasmodium cathermerium in over 300 transfers. The degree of virulence appears to be, to a certain


PARASITES PER 10,000 R.B.C.
5000
4000
3000
2000
1000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
COURSE OF INFECTION
0 DAY
1ST DAY
2ND DAY
3RD DAY
4TH DAY
5TH DAY
6TH DAY
7TH DAY
DAYS AFTER INOCULATION

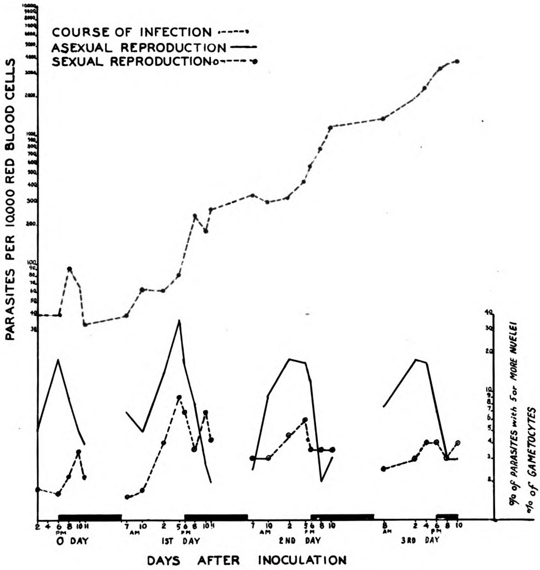
extent, proportional to the number of parasites injected intravenously when the hosts are in good condition and the parasites are obtained from a donor before the peak of the parasitemia is reached.

<u>Periodicity and Synchronicity</u>

Periodicity of the "3H" strain of <u>Plasmodium cathemerium</u> was first studied by L. G. Taliferro (1925) in the canary. She discovered that <u>P. cathemerium</u>, "3H" strain, had a 24 hour periodicity with schizogony occurring largely from 6:00 to 9:00 P.M. This was later confirmed by Drensky and Hegner (1926), Boyd (1929), Hartman (1927) and others. Wolfson (1938) first maintained a strain, designated as the "3T" strain of <u>P. cathemerium</u> in the duck and Wolfson (1943) reported further studies of that strain in the duck. She found that the period of greatest segmentation occurred between 8:00 P.M. and midnight daily, and the length of the asexual cycle to be about 24 hours in length. The peak of old schizonts occurred at about 8:00 or 10:00 P.M. daily.

The method of making the present periodicity study was as follows; smears were made at 2 hour intervals, and in some instances at one hour intervals, and stained with Giemsa's solution. During the period between 4:00 P.M. and 8:00 P.M., smears were usually made every hour. From a random sample of 100 parasites from each blood smear the percentage of each of the following groups was determined: (1) stages containing 5 or more nuclei were designated as old schizonts, (2) microgametocytes, and (3) macrogametocytes. There was a great deal of similarity in the 4 birds studied and graph 2 shows the results of the periodicity study. The periodicity was essentially 24 hours in length. On the day of transfusion, the maximum number of schizonts with 5 or more nuclei occurred at 6:00 P.M.; the day after inoculation, or the first day of the infection the maximum number of these schizonts occurred at 5:00 P.M. The second day of the infection the peak becomes flattened somewhat and the maximum number of old schizonts occurred at 2:00 P.M., and the third day was like that of the second day. Segmentation occurring earlier in the afternoon in the peripheral blood was noted by Wolfson (1938), by Redmond for the "3A" strain, and by Coatney for the "3M" strain (unpublished) and by Porter (unpublished) for the "3M" strain. This observation of earlier segmentation for the various strains mentioned were infections in the canary.

Periodicity of the gametocytes was also studied and recorded on graph 2. The male and female gametocytes appeared in the peripheral blood circulation simultaneously with the asexual forms which might be expected with the use of a large inoculum. The gametocytes were observed to show a periodicity quite similiar to that of the asexual forms. However, the maximum number of



COURSE OF INFECTION
ASEXUAL REPRODUCTION
SEXUAL REPRODUCTION
PARASITES PER 10,000 RED BLOOD CELLS
% of PARASITES with 5 or MORE NUELEI
% of GAMETOCYTES
0 DAY
1ST DAY
2ND DAY
3RD DAY
DAYS AFTER INOCULATION

gametocytes appeared at a later hour than the old schizonts. This would tend to indicate that the schizonts and gametocytes reached maturity at nearly the same time. These observations were in agreement with those of Shah (1934). Criteria for distinguishing pregametocytes, microgametocytes, and macrogametocytes were based on the cytological characteristics offered by Gambrell (1937).

Synchronicity appeared to be quite high dispite the fact that segmentation occurred at an earlier hour on the third and fourth days of the infection. The synchronicity was much sharper on the first and second day of the infection with the synchronicity appearing to be low near the peak of the infection. Following the peak of the infection, synchronicity was nearly broken up completely, and multiple parasites were found in the young red blood cells, the polychromatophilic erythroblasts, (Hegner and Hewitt 1938),(Hegner 1938a), (Hewitt 1939a) more than at any other time.

Number of Merozoites Produced per Schizont

Thirty segmenters were counted in each of the 4 slides representing ducks 16C, 17C, 18C, and 19C. Blood smears chosen were 77 hours after the ducks were injected intravenously with the malaria blood.

The method of choosing the segmenters was by random sampling. Only those segmenters were counted which showed complete division of the cytoplasm and no evidence of further division. The merozoite counts were not made during the first and second days of the infection for several reasons which were; the first day counts may be unduly influenced by such factors as nutrition, etc., imposed upon it by the donor bird, and immunity near the peak of the infection in the donor bird, all of which might be reflected in the recipient at such an early date. The following table shows the results obtained:

Bird No.	Am	Range	Std. Dev.	Std. Er.	Co. of Var.	Std. Er. Co. of Var.
16C	11.00	8-14	1.79	±0.32	16.2%	±2.1
17C	10.83	6-16	2.73	±0.49	25.0%	± 3.4
18C	13.17	8-24	3.95	±0.72	29.0%	± 4.0
19C	12.37	6-18	3.12	±0.56	25.0%	± 3.4

The merozoite mean in the 4 ducks ranged from 10.83 to 13.17, and the actual numbers ranged from 6 to 24. The average number of merozoites for the 4 birds was 11.84 ± 0.29 with a standard deviation of 3.13.

Statistical formulae (Arkin and Colton 1946) used to determine the arithmatic mean, standard deviation, standard error of the mean, coefficient of variation, and standard error of the coefficient of variation were as follows: arithmatic mean (ungrouped data) $\bar{X} = \frac{\Sigma(X)}{N}$ where $\bar{X}$ equals the arithmatic mean, Σ equals the "sum of," X equals the data expressed as individual items, and N is the number of items; standard deviation formula grouped data is $\sigma = \frac{\sqrt{\Sigma f(x^2)}}{N}$ where σ equals the standard deviation, f the frequency, and x the deviation of individual values from its arithmatic mean; standard error of the arithmatic mean, $\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}}$ where $\sigma_{\bar{x}}$ is the standard error of the mean and σ is the standard deviation of a sample; the coefficient of variation formula, where V is the coefficient of variation, σ is the standard deviation, and $\bar{X}$ is the arithmatic mean, is $V = \frac{\sigma}{\bar{X}} 100$ This formula was used to relate the measure of dispersion to the average, and to convert it to the percentage form, thus, solving the problem presented by the differing units; the standard error of the coefficient of variation formula is, $\sigma_V = \frac{V}{\sqrt{2N}} \sqrt{1 + \frac{2(V)^2}{10^4}}$

The destruction rate of the parasite has been determined by Boyd (1939) for the "H" strain of P. cathermerium in the canary. He found that at the peak of infection the destruction rate was around 90%. The destruction rate was determined for the "3M" strain of P. cathermerium in the duck in the following manner. The number of parasites per 10,000 red blood cells was determ'ned from smears made at 2:00 P.M. on the third day of the infection. Merozoite counts were made at 4:00 P.M. on the same day, and the two multiplied together. This should give the population per 10,000 red blood cells which should theoretically exist in the next 24 hour period. The number of parasites determined ror ducks 16C, 17C, 18C, and 19C was 1400, 2305, 3082, and 1449 respectively. The number of merozoites has been reported for these ducks. Hence, the per cent of destruction was as follows: duck 16C, 90%; 17C, 90.1%; 18C, 96.7%; and 19C, 59%. This high ratio of parasite destruction indicated the extent of acquired immunity at the crisis of the infection.

Exoerythrocytic Stages

Corrodetti (1940) drew the conclusion that the exoerythrocytic cycle was a test of the degree of adaptation reached by each species in its relation to its respective host. Hewitt (1940) stated that, "if an exoerythrocytic schizogonic cycle does form a part of the life cycle of some strains of avian plasmodia, the factors which govern the appearance of such a cycle are biologically unstable." Wolfson (1940a) observed that exoerythrocytic stages were prevented in the canary by passage through ducks, and no exoerythrocytic stages were found in the duck associated with the "3T" strain. Porter (1942) found a similarity between strains "3M", "3A", and "3T" of Plasmodium cathermerium, as all showed exoerythrocytic schizogony during blood passage after recent passage through mosquitoes, and all were markedly virulent for canaries.

In the present search for exoerythrocytic stages, 24 birds have been examined. The organs routinely examined were the brain, heart, lung, liver, spleen, bone marrow, and occasionally the muscle. No exoerythrocytic stages were found. However, many parasites were seen outside the red blood cells, and in the brain especially. The parasites seen were usually immature schizonts and gametocytes. These were undoubtedly the result of rupture of the red blood cells, because they contained malarial pigment. These were stained as brightly by Giemsa's solution as were parasites in the red blood cells, and did not appear to be undergoing any degenerative changes when observed.

<u>Induced "3M" strain of Plasmodium cathermerium in Chicks</u>

Six chicks were used in the experiment, ranging from 40 to 50 grams. The dosage of parasites ranged from 1×10^6 parasites per gram of body weight to 450×10^6 per gram of body weight. In all cases the results were the same. Parasite counts remained nearly constant in all of the chicks for 3 days; thereafter, there was a marked decline until their disappearance. This evidence of the resistance of chicks to the "3M" strain coincided with the results of Manwell (1933a), and Hegner and West (1941b) using various other strains of *P. cathermerium*.

<u>Chemotherapeutic Experiments</u>

Possible methods available for testing antimalarial drugs are three in number; (1) in vitro, (2) in humans, and (3) in animals. The first named method has not been adapted for routine screening of antimalarial compounds at the present time. Testing in human beings may be done in induced malarial infections on paretics or on natives where the natural infections are found. It would be an impossibility to screen all new drugs against human malarials; therefore, some experimental animal is necessary as a test for the assay of possible antimalarial drugs. Routine screening for antimalarials using canaries, chicks, and ducks, has a disadvantage in that both the host and parasite are different from human malarias. A better strain of malaria with which to test drugs would be one that would infect the rat or some other economical mammal. Moreover, the problem of proper evaluation of host and species specificities is one of the most serious connected with reaching a solution to malaria problems (Elderfield 1946).

From the laboratory viewpoint, the parasitemia produced in the avian host is much greater and much more easily followed that in the human.

Drugs on test were made up in the feed (Litchfield 1939). As the ducks ate only during periods of light, a 4 hour light and dark schedule was followed. The effect of this method of feeding was similar to taking doses of drugs every 4 hours in the human, and uniform blood levels may be maintained in this manner.

The time of initiation of the treatment was 24 hours before infection. The duration of treatment with the "3M" strain of P. cathermerium was 5 days. The dosage of drug was a maximum of 0.4% in the diet for new drugs and less for small drug samples in the routine screening of new drugs.

The size of the inoculum was 1.5×10^6 parasites per gram of duck, which was given intravenously by the method described. The blood smears were made on the third and fourth days after injection of the parasites. From 30% to 60% of the cells were parasitized in the untreated controls. The arbitary criteria of drug activity was 50% parasite suppression at the peak of the parasitemia on the third or fourth day after the inoculum was given. The minimum effective diet per cent of quinine was 0.0125% of the diet figuring on the base of the drug in every instance. Then, the ratio of the test drug to that of quinine producing a comparable suppression of the parasitemia was designated as the quinine equivalent.

The present chemotherapeutic study was carried out by using 30 ducks weighing 84 to 142 grams with a mean weight of 119 grams. The hemoglobins were determined on a Lumetron colorimeter by the oxyhemoglobin method (Coffin 1946). This method of determining the hemoglobin was used for several reasons; (1) hemoglobins are determined quickly and by simple methods, and, thus, large numbers of samples can be run in a few minutes time, (2) color changes form almost immediately, avoiding delay and inherent error in the slow color development of acid hematin methods, (3) elimination of the personal factor in color matching and, (4) a correction factor does not have to be worked out with the oxyhemoglobin method, as it does in the acid hematin method due to the nucleated red blood cells; therefore, acid hematin values give higher results which are erroneous and of a relative value only. The mean hemoglobin values for the 30 ducks was 9.7 gms.%, with a range of 8.9 to 11.87 gms.%. Six ducks (groups 1 and 2) were used as controls, and the 24 remaining ducks were placed 3 to a pen and put on the following drug diets:

Bird Group No.	Drug	Drug-Diet %
1.	control	0
2.	control	0
3.	quinine	0.0125
4.	quinine	0.025
5.	quinine	0.05
6.	PAM 23*	0.0125
7.	Sulfadiazine	0.8
8.	PAM 2-4-2*	0.0008
9.	PAM 3-4-b*	0.00078
10.	Atebrin	0.0014

Hemoglobin readings were taken before inoculation and on the third, fourth, fifth, sixth, seventh, and twelth day of the infection at 10.00 A.M.

Thin blood smears were made at 8.00 A.M. on the first, third, fourth, and fifth days after injection of 1.5 x 10^6 parasites per gram of body weight.

Groups 1 and 2 (controls) reached a parasitemia peak on the fourth day of the infection with a count

* Confidential Parke, Davis Antimalarials

of 3352 and 3705 parasites per 10,000 erythrocytes. The geometric mean of the two groups was 3524.

Groups 3,4,and 5 (quinines) reached a peak on the third day. Group 3 ducks on 0.0125% drug diet (minimum effective dose) had a parasitemia of 1695 parasites per 10,000 red blood cells. Group 4 receiving 0.025% quinine had a peak of 1409 parasites, while group 5 on 0.05% quinine had a peak of 439 parasites per 10,000 red blood cells.

Ducks on group 6, PAM 23, 0.0125%, reached a peak on the fourth day, as did the controls with a peak parasite count of 4031. This dosage of drug was apparently a subeffective level.

Group 7 on sulfadiazine reached a peak on the fourth day with a count of 2039 parasites, although the peak tended to be flattened out. Sulfadiazine was put on test as a check to determine if this strain of parasite was sulfa susceptible.

Group 8 on PAM 2.4.2 received 0.0008% drug in the diet. This group of ducks reached a peak parasitemia on the fourth day of the infection with 2134 parasites. Marked morphological changes were noted in the parasites, many appearing "punched out" and dark in appearence. The number of merozoites were noted to be fewer in number and there appeared to be good gametocidal action. The dosage of this drug was the minimum effective level. The counts were suppressed 50% that of the controls and the quinine equivalent was 15, when the ratio of PAM 2.4.2 was compared to that of quinine (MED).

Group 9 on PAM 3.4.b, 0.00078% drug never reached a peak. The amount of drug employed completely suppressed the infection, preventing a peak of any kind. From this preliminary test, however, the drug had a Q64+. Gametocidal and schizonticidal action was most apparent. Gametocytes and schizonts appeared as dark shrunken spots in the red blood cells. Morphological characteristics were obliterated. The maximun number of merozoites discernible was 4 or 5, and this number could be considered as only approximately accurate as the schizonts were so dark.

Ducks in group 10 receiving 0.0014% atebrin reached a peak on the fourth day with 1240 parasites per 10,000 red blood cells. Thus a Q17 is in the range reported by other workers. This drug was put on test as merely a drug control, the same as sulfadiazine, to determine if there were any manifestations of hypersensitiveness or idiosynchrosis to drugs already reported on.

Worthy of mention was the ducks on PAM 23, sulfadiazine, PAM 2.4.2, atebrin, and the controls all reached a peak on the fourth day of the infection and all showed the same type of parasitemia curve. That is, the parasitemia progressed to the third day with a straight line approach and then flattened out before the peak and dropped rapidly. In contrast, the quinine curves showed a rapid rise to a peak and then flattened out on the other side of the peak. The characteristics of the two types of parasitemia curves may or may not be significant. It must be kept in mind that in the untreated controls the "3M" strain of P. cathermerium reached a peak on the fourth day irrespective of the size of the dosage of parasites inoculated. Therefore, when ducks reached a peak on the third day of the infection on the quinine diet it became apparent that third day counts must be shown consideration in the evaluation of a drug when they are compared by the ratio method to that of the quinines. Then too, the peak of anemia of the quinine birds occurred on the day after the peak of the parasitemia, even though the peak occurred one day earlier.

Also worthy of mention is the variation in the morphology of the avian malaria parasites under drug therapy. The avian malarial parasites in ducks receiving quinine exhibited a definite and consistant chemotherapeutic damage. That is, they appeared "punched out" and somewhat faded. In contrast, the parasites in ducks receiving PAM 3.4.b showed a different type of damage. Their morphology was altered from the normal in that they were extremely dark and some were black, and appeared to be, what I shall term, "chemotherapeutically burned." It is therefore suggested that the morphological alterations of the avian malarial parasite under drug therapy may be a significant factor both from the chemical and biological viewpoint as; desirable type of drug action, i.e. detrimental to a particular system of the parasite which would be irreversible, etc., drug resistance could be noted, and reproductive ability, both sexual and asexual could be noted.

Ben Harel (1923), Young (1937), Terzian (1941), and Hewitt (1942) demonstrated a fall in red cell number accompanied by a diminutuion in hemoglobin. Hewitt, Richardson, and Seager (1942) conducted experiments involving a considerable number of ducks and concluded that "the fall in hemoglobin following different doses of parasites follows the parasite curve very closely ..... and serves as a supplementary method for estimating the degree of parasitemia." Then too, they found that the sharp drop in hemoglobin on the day after the peak (*P. lophurae*) was evidence *per se* to denote the day on which the highest number of parasites was reached, even though parasite counts were not made.

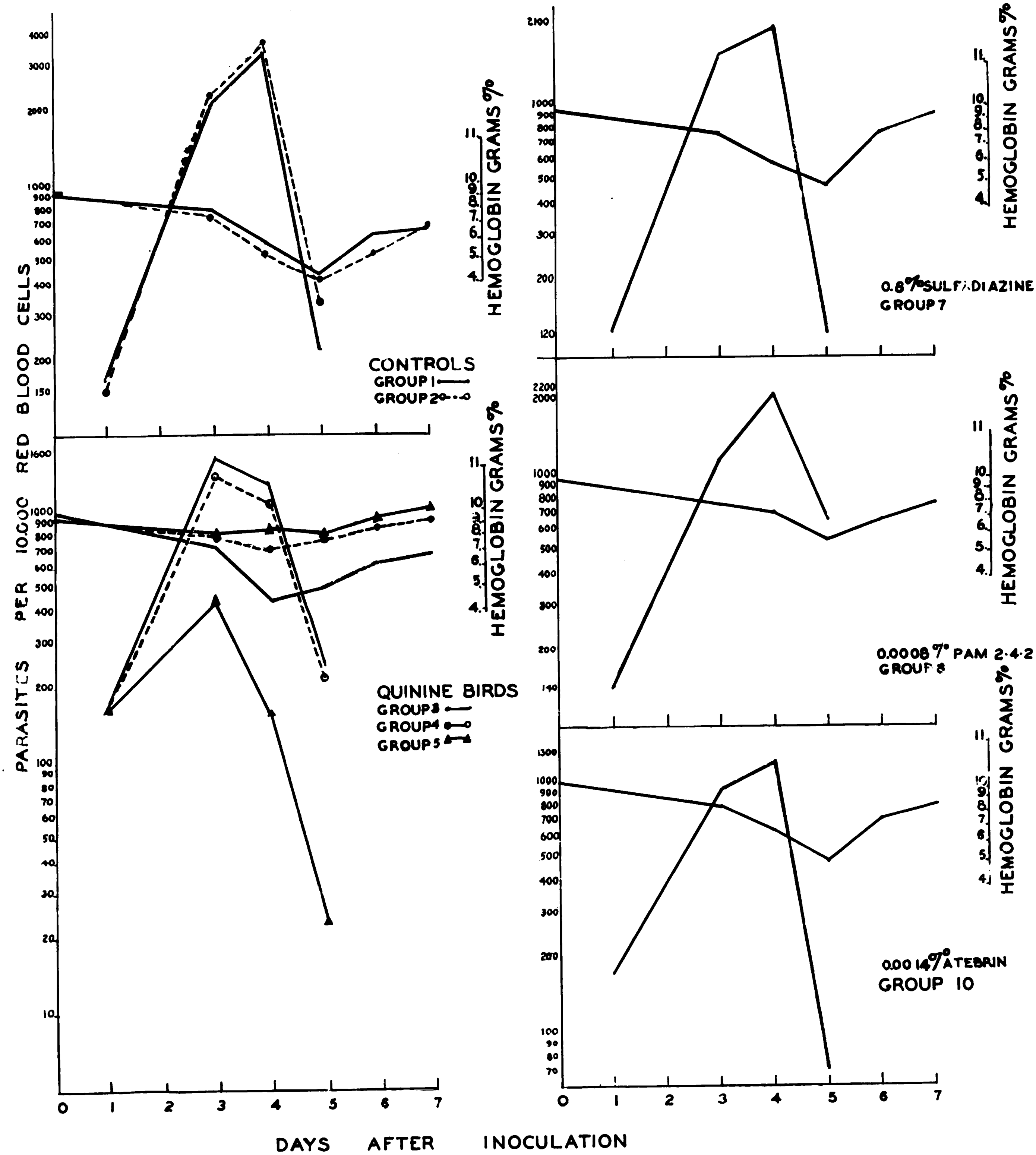
Anemia and chemotherapeutic experiments run concurrently confirmed the results of Hewitt, Richardson, and Seager using the "3M" strain of *P. cathermerium*. The anemia and parasitemia curves of the controls and the ducks on test agreed very closely. The lowest hemoglobin values were on the fifth day of the infection, which was the day following the peak of the infection in all cases except the birds on quinine diets. These quinine

birds (groups 3,4,5) reached a peak parasitemia on the third day of the infection, and the greatest anemia occurred on the following day(4th day). In contrast _P_. _lophurae_ infections in the duck are quite asynchronous, and the peak of the infection using 1×10^6 parasites per gram of body weight may be on the fifth or sixth day, while _P_. _cathermerium_ was constant in that the peak was always reached on the fourth day.

Results of the chemotherapeutic and anemia study are shown on graph no. 3. On the fifth day the hemoglobins of group 1 had dropped from 9.34 gms.% to 4.4 gms. %. This represents a hemoglobin loss of 52.9%. Group 2 ducks had a hemoglobin level of 9.45 gms.% before inoculation and on the fifth day it was 4.2 gms.%, a loss of 55.6 gms.%. of the hemoglobin originally present.

Group 3 of the quinine ducks (0.0125%) averaged 9.87 gms.% before inoculation, and at the peak of their anemia on the fourth day the hemoglobin was 4.51 gms.%. A loss of 54.4 gms.% of hemoglobin. Group 4 ducks on 0.025% quinine had hemoglobin values of 9.56 gms.%, and on the fourth day it was 7.15 gms.%, a loss of 25.3%. Group 5 ducks receiving 0.05% quinine lost 15.2% of their hemoglobin. The initial reading before inoculation being 9.45 gms.%, and it was 8.02 gms.% on the fourth day.

Group 6 ducks on PAM 23 lost 54.8% of their hemo-


CONTROLS
GROUP 1
GROUP 2
QUININE BIRDS
GROUP 3
GROUP 4
GROUP 5
0.8% SULFADIAZINE
GROUP 7
0.0008% PAM 2-4-2
GROUP 8
0.0014% ATEBRIN
GROUP 10
PARASITES PER 10,000 RED BLOOD CELLS
HEMOGLOBIN GRAMS %
DAYS AFTER INOCULATION

globin. This drug was at a subeffective level and therefore was not included on the graph. PAM 3.4.b was not included on the graph because the level of the drug was above the minimum effective dose.

Group 7 ducks on 0.8% sulfadiazine had 9.45 gms.% hemoglobin prior to inoculation, but dropped to 4.90 gms.% on the fifth day of the infection, representing a 48.2% loss in hemoglobin.

Group 8 on PAM 2.4.2 receiving 0.0008% drug in the diet had hemoglobin values of 9.56 gms.% before inoculation, but on the fifth day it was 5.60 gms.%, a loss of 41.5%.

Group 9 on a highly effective antimalarial drug for the "3M" strain of P. cathermerium, only 0.00078% in the diet had a hemoglobin level of 9.56 gms.% before injection and on the fifth day it was found to have lost only 9.0% hemoglobin.

Group 10 ducks on 0.0014% atebrin had a hemoglobin reading of 10.11 gms.% before inoculation and were 5.04 gms.% on the fifth day of the infection indicating a loss of 50.2% hemoglobin.

By the seventh day many of the ducks had hemoglobin values which were near their preinoculation levels, and on the twelth day all of the ducks were at their preinoculation levels, and many were above those levels.

It has been established that hemoglobin values reveal much in the course of untreated and treated infections with malaria parasites in birds. The plotting of regression curves during the course of infections when animals are on test should be of added value by determining alterations in the exponential growth curves. Furthermore, the altered morphological properties of the parasites may be significant.

Discussion

The "3M" strain of P. cathermerium has been adapted to the duck and some of its outstanding characteristics are presented. The "3M" strain of Plasmodium cathermerium is very virulent for the canary and often causes death during the acute attack, and also, during relapses. This strain is also quite virulent for the duck as the infection produced a marked degree of anemia in untreated birds. However, once the peak of infection is reached, the parasites disappeared from the blood rapidly.

Exoerythrocytic stages have been observed by other workers in the canary during mosquito and also blood passage, but none have been found in the duck in these experiments.

The parasitemia has been standardized so that about 40% of the red blood cells are parasitized. For studies such as periodicity 1×10^6 parasites per gram of body weight produced a satisfactory infection, which was easily followed and studied. However, for routine antimalarial screening a dosage of 1.5×10^6 parasites per gram of body weight was more desirable.

Known antimalarial drugs were used in the chemotherapeutic studies to determine if the "3M" strain would reveal drug hypersentiveness or idiosyncrasies. Moreover, it was found that this strain was not sulfa susceptible and that atebrin and sulfadiazine had a Q equivalent the same as has been reported on for the "3T" strain. Therefore, it is believed that the "3M" strain is a suitable malarial parasite in the duck for routine antimalarial screening using the method and dosage outlined.

Present observations indicated that there was no lethal agent associated with the "3M" strain of P. cathermerium. Untreated controls and ducks surviving initial attacks have been put in finishing batteries and none have died except by euthanasia, when crowded conditions deemed it necessary.

Asexual periodicity of the "3M" strain of P. cathermerium has been studied and was found to be essentially 24 hours in length. Gametocyte periodicity followed that of the asexual cycle very closely, but the maximum number of gametocytes produced appeared to be about an hour later. Synchronicity was very high during the period before the peak of infection was reached. Near the peak and thereafter, the synchronicity was broken up to a great extent. Also, multiple infected immature red cells were more numerous than at any other time.

The number of merozoites has been found to be 11.84 ± 0.29, 77 hours after inoculation.

The course of the infection has been studied and it was observed that the peak was reached at about 10:00 P.M. on the third day of the infection. Therefore, when third and fourth day counts were made it was found that the fourth day counts were the peak counts. So, it should be kept in mind that the fourth day counts on the controls are already past the peak of the infection. Moreover, the peak of the infection will occur on the fourth day irrespective of the dosage of parasites inoculated intravenously.

Of considerable interest was the fact that if a line were drawn through the points at 2:00 P.M. on the first, second, and third days of the course of infection graphs, a straight line would result. Thus, the course of the infection followed the exponential growth law, since the parasite counts were plotted on semi-log graph paper. The use of semi-log paper obviates the necessity of looking up the log of Y. The horizontal rulings on the paper are drawn to such a scale that the plotting of the original data results in a straight line, if the data follows the exponential growth law(Snedecor 1946).

Hemoglobins had been determined on 30 uninfected ducks before inoculation and found to average 9.7 gms.% for ducks with a mean weight of 119 grams. The oxyhemoglobin method of determining hemoglobins was used in all instances. The shortcomings of the acid hematin methods were pointed out when used as a test for bird blood hemoglobins. Graphs presented illustrate the close inverse relationship between the parasitemia and anemia during untreated and treated infections. It had been suggested that much can be learned by plotting hemoglobin levels and regression curves of parasite counts.

Experiments with chicks indicated that they were unsuitable hosts for the "3M" strain of _P. cathermerium_ as the infection was transient in nature.

Summary and Conclusions

The "3M" strain of _P. cathermerium_ has been successfully passed to the duck and has been transfered over 300 times.

The outstanding biological characteristics studied included: periodicity, synchronicity, number of merozoites produced per schizont, destruction rate of the parasite 77 hours after inoculation, observations for exoerythrocytic stages in the duck, and observations on the induced infections in chicks.

Chemotherapeutic studies indicated that the "3M" strain did not have any idiosyncrasis against atebrin or sulfadiazine and that it was a suitable malarial parasite for the routine screening of antimalarials.

Then too, it was pointed out that during antimalarial screening work the plotting of regression curves would indicate alterations in the exponential growth law. It was also suggested that hemoglobin determinations may be a rapid method of screening due to the close inverse relationship between the parasitemia and the anemia. Furthermore, the morphological characteristics of the parasite under drug therapy may be highly significant.

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