A preliminary report on the early effects of plasmochin on *P. cathemerium.*

By

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(With Plate 1.)

During a study of the effects of plasmochin on the parasites of bird malaria, the following points were noted: 1. degenerative changes of the parasites became apparent somewhat earlier after treatment than has been heretofore shown; 2. asexual forms appeared to be, in the early stages of the plasmochin reaction, more susceptible than sexual forms; and 3. all asexual stages, young and old, appeared to be affected by the treatment, but the younger stages were more susceptible in the earlier part of the treatment. This study considers the blood from four hours after treatment until the parasites disappeared from the blood.

Plasmochin is a synthetic quinoline derative prepared by the German „Farbenfabriken". It is identified as n-diethylamino-isopentyl-8-amino-6-methoxy-quinoline. It is a tasteless yellow fine granular powder which dissolves in water (20° C) up to 0,03 %. It is converted to hydrochloride in the stomach. HÖRLEIN (1926) gives an account of its discovery and thinks plasmochin belongs "to the same class of body" as quinine, which (he holds) is a complicated alkalamine-6-methoxyquinoline body. Plasmochin was made

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public at the 89th meeting of the Society of German Naturalists and Doctors at Düsseldorf in 1926. It has been tested on bird malaria by ROEHL (1926) and HEGNER and MANWELL (1927) and has been used rather extensively in the treatment of human malaria. In human malaria MÜHLENS (1926) used it alone or in a compound tablet consisting of quinine 0,125 gms. and plasmochin 0,01 gm.

Six female canaries, *Serinus canarius*, were inoculated at the same time by injecting into the breast muscles of each 0,1 c. c. of citrated blood containing the bird malaria parasite *P. cathemerium* (HARTMAN, 1927). Parasites appeared in the blood of two birds in four days and in the other four birds in five days. Two birds were used as untreated controls, two birds were given quinine hydrochloride 2,0 mgs. per day, and the other two were given 0,1 mg. of plasmochin. Both drugs were given in solution so that 0,1 c. c. equaled the above amounts. The administration was by a bent steel esophageal tube attached to a tuberculin syringe. The hour set for the administration was 1:00 P.M. Treatment was begun on the third day (about 48 hours) after the appearance of the parasites in the blood. The drug was administered daily until parasites were no longer found.

Segmentation in *P. cathemerium* takes place regularly every twenty-four hours about six o'clock in the evening. The drugs were thus given five hours before the hour of maximum segmentation. The drug used was obtained by the School of Hygiene and Public Health from the Winthrop Chemical Company of New York. The contents of an ampule containing 0.02 gms. was diluted with tap water so that 0,1 c. c. equaled 0,1 mg. This same solution was used for the first series of birds for three days.

In both of the birds given plasmochin there was little change noticed in the parasites during the first four hours. There was slight vacuolation in some of the trophozoites and mature schizonts, but most of the organisms went on to maturity and segmented normally. The merozoites freed by the segmentation entered the red blood cells in about the normal proportion. From three to five hours after segmentation some of the parasites looked normal but most of them showed evidence of lack of proper growth or actual degeneration by parts of the parasite taking the stain feebly. The blue-staining cytoplasm was the first to show this degeneration. Later on, one or more vacuoles appeared in the parasite. Pigment as well as the chromatin was prominent in most of these abnormal forms. Less and less stain was taken up till there remained only

a thin red ring with a clear opening in the center. The ring was usually heavier at one or more points. This vacuole looked much as if a section of the cytoplasm of the cell had been punched out. The nucleus often remained pushed to one side of the cell as if the parasite were still there. The colored drawings (Fig. 1) accompanying the article will help make clear the appearance of these parasites untreated and in different stages of degeneration.

The action on the gametocytes was contrary to what was expected. The major proportion of both macro- and microgametocytes which could be found on slides where all the asexual forms were in some stage of dissolution were in an apparently healthy condition. Change was noticed in some of the gametocytes, however. They did not show vacuoles like the trophozoites but there was a general fading of both the chromatin and the cytoplasm which made the pigment granules more prominent than in normal gametocytes. Finally, degeneration reaches a stage where the pigment distribution is the only evidence that the parasite had been in the cell. The cytoplasm of the red cell seems to fill in the spaces vacated by the parasitic matter.

Later two more canaries were infected with the same parasite. When the infection was fairly heavy in one bird, it was given 0,2 mgs. plasmochin five hours before the time for maximum segmentation of the parasite, and the other was given 0,2 mgs. of plasmochin one and one-half hours after segmentation. Blood was taken every two hours from the time treatment was begun till the birds became rather anæmic, and after this every three or four hours until the parasites were very few.

The picture of the parasites in the blood of the bird treated with plasmochin five hours before segmentation was like that in the two birds treated with the same drug in the first experiment excepting that the great proportion of the second generation parasites did not develop as far as in the previous cases. This was perhaps due to the increased dose of the drug.

The second bird was given 0,2 mgs. of plasmochin at 7:30 P.M. This is shortly after the time for the maximum segmentation of this parasite. In this bird the merozoite stage went on into the trophozoite stage and developed but with a higher mortality rate than in the other birds after the first dose of the drug. All stages of trophozoites began to show loss of staining properties, and vacuoles appeared in most of them. By carefully looking over the slides taken twenty-two and twenty-seven hours after the first dose of the

drug, and two hours before and three hours after the second dose, no normal schizonts or segmenters were found. The disintegration of the parasites in the blood of this bird was similar to that of the other birds treated with plasmochin. The difference was that by giving the drug at about the time the trophozoites were beginning growth in the red blood cells, the parasites were acted upon in such a way or over so long a period of time that no segmenters could be found.

ROEHL gave large doses and noted in the first twenty-four hours slight changes, which he does not describe. He writes, "Within forty-eight hours the microscopic picture is entirely changed. The change consists in this that the adult forms of the parasite and also the gametocytes have completely disappeared and only small parasite forms are left." This study differs from ROEHL's in that small doses of plasmochin were used and in that the one bird given the drug just after the time for maximum segmentation all asexual forms of parasites had either disappeared or were undergoing a process of deterioration by the end of twenty-four hours. Only a few gametocytes were left and some of them were beginning to take the stain more lightly. The change he described in the smaller forms within forty-eight hours fits the picture for the first twenty-four hours in this study. It seems probable that the discrepancy is due to the fact that the strain of malarial parasite here employed was more susceptible to the action of plasmochin than the one used by ROEHL. Where the drug was given five hours before segmentation, only slight changes were noticed until after segmentation. At that time the appearance of the parasites was like that for the bird given the drug shortly after segmentation.

In the experiments of HEGNER and MANWELL, the large schizonts disappeared on the fifth day, which was the first day after treatment, but ring stages were still present. They were absent on the sixth day or two days after treatment was begun. Since *P. cathemerium* does not normally have a ring stage in the peripheral blood (HARTMAN, 1927), it was surmised that the ring stages mentioned by them were the disintegration phenomenon described in this paper. A personal communication from MANWELL states, "I have no doubt that the vacuolated forms which you mention in your letter represent precisely the same stages referred to in our paper as rings, although in the latter case we had reference only to what we regarded as more or less degenerate young forms."

This study corroborates the findings of RATCLIFFE (1927) that

the avian malarial parasite is intracellular. The place where the digested parasite had been remains for a short time in some cases as a vacuole in the cytoplasm of the red cell. If the parasites were on the outside of the cell, it would not be possible for them to displace the cytoplasm in this way.

Plasmochin is generally considered to have a definite action on the gametocytes. SCHULEMANN and MEMMI (1927) say, "It has a marked effect on sexual forms of *P. falciparum*". BROSIUS (1927) says it "promises well in that it apparently has a definite action on the gametocytes". HEGNER, ROOT and AUGUSTINE (1929) say, "Plasmochin appears to be particularly destructive to gametocytes and less injurious to the young trophozoites than quinine". In the light of the above statements it was surprising to find the gametocytes apparently normal on slides showing degenerative changes in all the asexual forms. These gametocytes, however, disappeared from the blood stream within another twenty-four hours and no new forms made their appearance.

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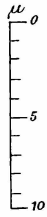
Explanation of Plate.

Drawings by Muriel McLatchie.
All camera lucida drawings magnification 1—2500. GIEMSA staining.

Plate 1.

Fig. 1. Normal young trophozoites.
Fig. 2. Two normal older trophozoites in red cell.
Fig. 3. Normal segmenter.
Figs. 4 and 5. Trophozoites starting to show the influence of the plasmochin.
Figs. 6, 7, 8. Schizonts and segmenters in process of disintegration.
Figs. 9, 10, 11. Further stages in the disintegration of the parasite.



Magnification 2500 diameters.

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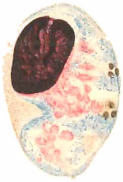

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