tively heliotropic *Gammarus* into a positively heliotropic animal is produced differs for different substances and inasmuch as the transformation is brought about most promptly by such substances as diffuse most rapidly into the tissues, *we must conclude that we are not dealing here with an osmotic, but with a chemical effect.*

4 (50). **"Trypanosomes and bird malaria": F. G. NOVY** and **W. J. MACNEAL.** (Presented by **GARY N. CALKINS.**)

The studies made heretofore upon the malarial parasites of birds have shown the existence of four species or types. These are:

Proteosoma.

Halteridium.

Hæmamœba majoris, Lav.

Hæmamœba Ziemanni, Lav.

In the course of an extended study of the parasites of birds, the authors encountered several new species, and, since the number is likely to be still further increased, it seemed desirable to attempt a classification. The authors based their classification largely upon the type of multiplication and the habitat of the parasite. Two genera were given; one, *Plasmodium*, characterized by formation of segmenting forms in the peripheral blood and invasion of fully developed red blood cells. The injection of blood having these parasites results in an infection. For the other genus the authors used the priority name of Kruse's, *Hæmoproteus*. This genus is characterized by an entire absence of segmentation-forms in the peripheral blood, and, with the exception of two species which form a transition as it were between the two genera, invasion of young erythroblasts is the rule. Injection of blood having these parasites does not lead to infection.

With this division, the species are arranged as follows:

A. Plasmodium, including parasites of man, some of birds, and very probably some of cold-blooded animals.

1. — *Plasmodium relictum*, syn. *Hæmamœba relicta*, Proteosoma.

2. — *Plasmodium vaughani*, n. sp.

B. Hæmoproteus, including chiefly parasites of birds, and probably offering transitional forms to the hemogregarines of cold-blooded animals.

1. — *Hæmoproteus danilewskyi*, syn. Halteridium.
2. — " *maccallumi*, n. sp.
3. — " *sacharovi*, n. sp.
4. — " *majoris*, Lav.
5. — " *ziemanni*, Lav.
6. — " *rouxii*, n. sp.

Plasmodium vaughani, n. sp., is common in robins; it resembles proteosoma, and may be easily mistaken for the latter. The hyaline body is smaller than that of proteosoma, does not displace the nucleus, contains one large pigment granule, and is readily recognizable by the presence of a large, bright, refractile, colorless globule. It segments and usually forms four cells. Canaries may be infected; apparently non-fatal.

Hæmoproteus maccallumi, n. sp., found in mourning doves. Like *halteridium*, which it resembles, it infects erythrocytes. Grows on one side, or may completely surround the nucleus. The fully developed sexual forms fill and somewhat distend the blood cells. Microgamete formation observed. The infection cannot be transferred by blood injection.

Hæmoproteus sacharovi, n. sp. This species, probably first observed by Sacharoff, who regarded it as a "leucocytozoön," is related to that of Danilewsky. Found in young mourning doves and elsewhere. Invasion begins with an infection of very young erythroblasts. As the parasite grows it pushes the nucleus to the periphery, where it is seen in the adult form on the outer edge as a cap, which is but a trifle larger than the nucleus of a red blood cell. The parasite is spherical, male and female forms common, latter predominate; blepharoplast distinct, adjoining or over the nucleus. Microgamete formation common. Infection not transferable by the blood.

Hæmoproteus majoris, Lav. This was found once by Laveran in a titmouse. This species is extremely common in robins and other birds. As with preceding species, invasion at early stage shows infection of very young erythroblasts, the small parasite lying next to the large round nucleus. As the parasite grows it pushes into the nucleus, which becomes crescentic and may almost wholly surround the parasite. The adult sexual forms are large, about 10 to 12 microns in diameter, and are readily recognizable

by the peripheral ring of the nucleus of the host cell. This cap may extend, and usually does, around two-thirds of the cell, and even more. The blepharoplast is easily seen in female cells; microgamete formation common. No infection of other birds by injection of blood swarming with these forms.

Hæmoproteus ziemanni, Lav., has been studied by Danilewsky, Ziemann, Laveran, Schaudinn, and others. Forms long spindle-shaped bodies, which are 30 to 50 microns in length. The authors have found this species, or one closely related to it, in the blood of a hawk. Sexual forms easily recognized.

Hæmoproteus rouxii, n. sp., is very common in sparrows, and represents the very earliest possible infection of young erythroblasts, so much so that it is not feasible to exclude the possibility of their being leucocytes. As the parasite grows it pushes into the nucleus, which assumes the form of a thick crescent. The parasite measures from 4 to 6 microns. Its plasma does not stain readily, and sexual forms have not as yet been recognized. Apparently always associated with this cytozoön are minute crescentic free hemogregarines. These are but 4 microns long, and are motile, crawling over the red blood cells in characteristic manner. Larger motile crescents, about 10 microns long, at times are present. Both large and small crescents are free and motile, and, it is important to note, are present in the fresh blood at the moment when drawn. They cannot, therefore, be considered as oökinetes. They are hemogregarines, and presumably constitute the extracellular stage of *H. rouxii*.

It is to be noted that the last four mentioned forms all exert pressure on the nucleus of the erythroblasts, and, as a result, give rise to very peculiar types. They are all "*leucocytozoa*" of which, thus far, there has been but one recognized type, that of *H. ziemanni*. The view of Schaudinn that the latter is a trypanosome which ingests an entire erythroblast by attaching itself to such by one end becomes untenable, inasmuch as all stages of infection from the earliest to the latest can be readily observed in the case of *H. sacharovi* and *H. majoris*. The authors regard the large spindle-shaped *H. ziemanni* as an infection of an erythroblast, and the elongated form as consequent upon an alteration of the wall of the host cell leading to increased osmotic pressure, which, acting on the poles, gives rise to the spindle-shaped forms.

In addition to the parasites mentioned, birds harbor very frequently *filaria* and *trypanosomes*. In the case of the filaria, while the peripheral blood may contain but a few, the heart blood may contain large numbers.

Trypanosomatic infection of birds is far more common than has been supposed. The largest number of infected birds seen by any one observer was eight, which were found by Dutton and Todd in Senegambia. The reason why they have not been found more commonly is because they are present in very small numbers, usually not more than one or two flagellates in a drop of blood. By microscopic and cultural methods the authors have been able to detect trypanosomes in the blood of thirty-three birds. Of this number, eighteen were detected by direct blood examination (of the eighteen birds, ten were tested culturally and gave growths); and fifteen by means of their cultivation method — that is to say, in fifteen cases where the microscope failed to show trypanosomes, the culture method showed them to be present. This shows that, as in the case of the bacteria, the cultural method is a more delicate means of detection of small numbers of parasites than is the microscope.

The occurrence of these trypanosomes with reference to the cytozoa mentioned is of special interest. Thus in thirteen of the thirty-three cases, trypanosomes were unaccompanied by any intracellular parasite, while in twenty they were associated with one or more kinds of cytozoa. It was not an uncommon thing to find multiple infections, that is, the same blood harboring, at one time, in addition to trypanosomes, two or three different species of intracellular parasites. Again, in addition to the fact that trypanosomes may be present without another parasite, is the interesting fact that when thus associated there is no constant relation between the two. In other words, the same trypanosome may be found at one time with a *proteosoma*, at another time with a *halteridium*, or with *H. sacharovi*, or with *H. majoris*, etc.

In twenty-five of the thirty-three cases mentioned, cultures were obtained. Nearly all of these have been carried through a series of subcultures or new generations. Here another important fact was brought out. The cultural method is not only the best means of detecting trypanosomes in the blood, but it is the best

means of differentiating them into species. A study of the twenty-five cultures obtained showed that they represented three, if not four, and possibly more distinct species. The cultural characteristics were extremely well marked, and offered an admirable means of differentiation. One species, more common than any of the others, was specially mentioned at this point. Its cultures present an extremely interesting appearance and are unusually luxuriant. They show two types of cells. One of these is round or in short spindles, which always occur in rosettes, with the flagella directed centrally, as in the case of *Trypanosoma lewisi.* The other type is long and very slender, almost a mere line, and is extremely motile, traveling forward and backward, with great rapidity. Very often two cells unite by their posterior ends; at times agglutinations are found and in these the whips are found situated on the outside of the mass. This long, slender type corresponds exactly to the *Spirochæte* described by Schaudinn, while the other type agrees with his *Trypanosoma.*

It is noteworthy that inoculation of the trypanosome cultures, even in large amounts, into birds failed to produce any cytozoa. In one case a canary infected with such a culture showed trypanosomes in its blood for three months without any sign of an intracellular parasite.

These facts are of importance, because of their bearing on the recent views of Schaudinn regarding the relation of trypanosomes to the intracellular parasites. As is well known, this distinguished protozoölogist believes that in the case of *halteridium* the sexual forms unite in the stomach of the mosquito to form oökinetes, which then develop into indifferent, male and female trypanosomes. This type agglutinates with the flagella directed toward the center. In the case of *H. ziemanni,* he holds that a similar change occurs in the mosquito, giving rise to long, slender spirochætes, which agglutinate with the flagella directed outward. Injections of suspensions of such infected mosquitoes produced the characteristic infection with the hemocytozoa.

It will be seen that the results obtained by the authors do not bear out Schaudinn's conclusion. The authors have shown that birds may harbor trypanosomes, even for months, without showing any intracellular parasites. On the other hand, birds rich in

such cytozoa may contain no trypanosomes. Thus, cultures attempted from twenty-six of such heavily infected birds failed to show any growth. Again, the presence of trypanosomes is not associated with any one form of intracellular parasite. Furthermore, the cultural method shows the existence of several distinct species of trypanosomes, and among these is one which presents at the same time both types described by Schaudinn as stages on the one hand for *halteridium* and on the other hand for the "leucocytozoön" of Danilewsky.

The authors therefore conclude that trypanosomes in birds may be met with as several distinct species wholly unrelated to the intracellular parasites. The greatly diverging conclusions reached by Schaudinn and the authors must be ascribed to the fact that Schaudinn worked with *mixed cultures* as developed in the body of the mosquito, whereas the authors have employed strictly *pure cultures* of these flagellates.

[The authors published the full details of a part of this investigation in the March issue (1905) of the *Journal of Infectious Diseases*. Additional papers will appear in later issues.]

5 (51). **"The gradual decrease in bacteria of the production of agglutinable substance": WILLIAM H. PARK.**

At the last meeting of the Society of American Pathologists and Bacteriologists an informal statement of this fact was made by Dr. Welch for Drs. Marshall and Knox. The experiments of Dr. Collins and the author are reported here because they were undertaken in a slightly different way and also because a certain number of confirmatory observations are of value.

The maltose-fermenting paradysentery bacillus of Flexner was grown for twenty-four hours on each of eleven consecutive days in fresh bouillon solutions of the serum from a horse immunized through oft-repeated injections of the bacillus. The serum strength in the solutions used was 1.5%, 4% and 15%. The serum agglutinated the culture before its growth in the solutions in dilutions up to 1 in 800, and was strongly bactericidal in animals. After eleven transfers the culture grown in the 15% solution ceased to be distinctly agglutinated by the serum in any dilution and ceased to absorb from the serum any appreciable amount of the agglutinins acting upon the original culture. The