

HÆMOGREGARINA PETAURI: A HÆMOGREGARINE OF A MARSUPIAL FLYING SQUIRREL.¹

By D. A. WELSH, M.A., B.Sc., M.D., and J. E. V. BARLING, M.B., CH.M.

From the Pathological Laboratories, University of Sydney.

(PLATE XXXV. FIG. I.)

OUR object is to put on record our observations on what we believe to be an undescribed protozoon parasite which has a special interest, inasmuch as we believe it also to be the first of its kind to be discovered in a typical Australian mammal. For several years we made an examination of the blood of all the Australian mammals, birds, and reptiles to which we could gain access, and, although many individuals were investigated, the results were consistently negative so far as blood parasites were concerned. In October 1907, however, we had an opportunity of examining the blood of a family of a marsupial flying squirrel (of the genus *Petaurus*²) consisting of a male, a female, and one young. In the blood of the female and of the young we did not find any parasites, but in the blood of the male animal numerous unpigmented, non-amœboid protozoa were present. Unfortunately, within a few days after the blood was taken the animals died unexpectedly from exposure, and we were unable to secure the bodies for further examination. We have delayed publishing our results in the hope that we might obtain further material, but so far we have been unsuccessful. Our description and identification of the parasite are therefore based on an examination of the few blood films that we had secured in our preliminary investigation. The examination of the various tissues of the infected animal and the still more interesting examination of ecto-parasites to investigate the reproductive cycle of the protozoa have hitherto been impossible. Nevertheless, enough data are at our disposal to enable us to decide that the protozoon in question is, in

¹ Communicated to the Australasian Medical Congress, Melbourne, October 1908. [Received November 16, 1909.]

² We are indebted to Professor Haswell for his kindness in identifying the genus for us. As he did not see the animals he was unable to decide their species; but, from our description, he considers the species to be either *P. sciureus* or *P. breviceps*, and more probably the former.

all probability, a species of hæmogregarine, and to support the further conclusion that it is, to the best of our knowledge, an undescribed form. The latter conclusion is based on two facts—(1) That the protozoon presents striking morphological differences from the few other mammalian hæmogregarines described and figured by their original observers; and (2) that it infests the blood of an animal in which hæmogregarines have not previously been noted. On these grounds we have provisionally distinguished our parasite as a new species, *Hæmogregarina petauri*.

The hæmogregarines (cf. Minchin) form a genus closely allied to the genus *Plasmodium* (*Hæmamoeba*), of which the malarial parasite is the best known representative. They resemble malarial parasites in the partiality of the trophozoites for the red blood corpuscles of the vertebrate host, and in the manifestation of a distinct trophic phase (schizogony) passed within the tissues of the vertebrate host, alternating with a distinct reproductive phase (sporogony) probably passed within the body of an invertebrate host. It is true that in most instances the invertebrate host is not known, but the increasing number of cases in which it has been discovered render probable its existence in all cases. But in two important respects the hæmogregarines differ from the plasmodia: (1) in being non-amœboid, and (2) in being non-pigmented. Typically, the hæmogregarine presents itself as an endocellular parasite of a small worm-like form (vermicule), of fixed outline, invading the red blood cells. Some forms, being larger than the cells they inhabit, become curled up within them, the "tail" being sharply flexed upon the body. Other forms, being shorter than the diameter of the invaded red cells, present a short "sausage" form. Although non-amœboid, the vermicule may exhibit active movements, emerging from the red blood cells and gliding across the field of the microscope, when the blood is examined in the fresh condition. So far as is known, they are relatively innocuous parasites, and, at the most, produce only a minor degree of anæmia. Their interest and significance lie in their relationships to more important genera, and in the fact that recent discoveries have revealed a wider distribution of these parasites than was previously known.

Hæmogregarines have long been known as not uncommon parasites of cold-blooded vertebrates, and many forms have been described in the blood of fish, amphibians, and reptiles. Up to the year 1905, however, they had not been recognised as parasites of mammals. But in that year Christophers described *Hæmogregarina gerbilli*, a hæmogregarine infesting the red blood corpuscles of a species of Indian field rat, and in the same year Balfour discovered a similar but distinct parasite, *Hæmogregarina jaculi*, inhabiting the red blood cells of the jerboa, or desert rat, of the Soudan. More recently Laveran found the same parasite in jerboas from Tunis. So far no

other form of mammalian hæmogregarine parasitic on the red blood corpuscles has to our knowledge been described. The peculiar parasites discovered by Graham-Smith in the blood of the mole do not appear to belong to this genus. It is probable, however, that the recently described leucocytozoa of mammals may be included among the hæmogregarines, *e.g.* the leucocytozoa of dogs in Assam described in 1905 by James and by Bentley, the leucocytozoa described by Patton in the palm squirrel of India, the leucocytozoa found in rats infected by *Trypanosoma Lewisi*, and some others.

MORPHOLOGY OF *H. PETAURI*.

The parasites were elongated oval bodies, roughly cylindrical in outline, with a very uniform transverse diameter and rounded ends. The long axis of the parasite was slightly greater than the average diameter of the healthy red blood corpuscles, its transverse diameter was slightly less than half that length. Hence the general appearance of the vermicule was that of a short thick sausage. The sharply flexed "tail," characteristic of the longer forms of endo-corpuscular hæmogregarines, was not in any instance observed.

For about three-fifths of its length each parasite consisted of finely granular cytoplasm (coloured blue by the Leishman-Romanowsky stain), apparently of very uniform density throughout, though minute points of deeper staining affinity might, here and there, appear. Occasionally, small unstained areas, probably vacuoles, were noted in the cytoplasm, and occasionally also minute dots of chromatin were scattered in the neighbourhood of the nucleus. No evidence of pigment was at any time observed.

The remaining two-fifths of each parasite was occupied mainly by a relatively large terminal nucleus (coloured pink and red by the Leishman-Romanowsky stain). That extremity of the nucleus directed towards the centre of the parasite formed an almost straight line, whereas the polar extremity was more rounded. At the central end of the nucleus there was frequently to be seen a rarefaction of the cytoplasm indicated by a narrow pale zone adjacent to the nucleus; whereas the polar extremity of the nucleus was capped by a thin crescent of condensed cytoplasm, staining deep blue and forming one pole of the organism. With the exception of the polar cap of dense cytoplasm, the whole of this end of the parasite (equivalent to about two-fifths of the total length) was occupied by nuclear matter. The staining of the nucleus varied greatly in intensity, in different parts, from pale pink to scattered strands of deep red. The deepest red was often massed in the central portion of the nucleus and along a narrow strip at one side. Sometimes a slight constriction was apparent at the junction of the cytoplasmic three-fifths with the nuclear two-fifths of the parasite.

Each parasite had a sharp outline, as if limited by a definite membrane or cuticle. Moreover, the parasites showed practically no variation in general form, nor in the relative disposition of cytoplasm and nucleus. Slight variations in size, however, were not uncommon, the largest forms being about half as large again as the smallest. Although a very large number were, at one time or another, passed in review, no indication of sporulation was observed. Only forms corresponding to trophozoites were found.

In only one instance were two parasites found within the same red cell. In this case the two parasites were of equal size, but much smaller than the other hæmogregarines seen in the blood. They were alike in structure, but did not present all the morphological details in nucleus and in cytoplasm found in the isolated hæmogregarines of this species. They occupied the centre of

the red cell, lying in close contact, symmetrically disposed, and nearly parallel. The cytoplasmic portion of each was separately defined, but their nuclear extremities were partially overlapping or partially fused together. The appearances suggested not so much a multiple infection of the old cell as the longitudinal fission of a parasite within the cell and the formation of two daughter forms as yet imperfectly developed.

RELATION OF PARASITES TO RED CORPUSCLES.

Without exception the parasites were found to be either wholly or partly within (or upon) the red blood cells. None were found within leucocytes, and no free forms were observed. Most commonly the parasite was wholly within the corpuscle, and the invaded corpuscle was invariably larger than the average red cell of the blood. Hence, although the long axis of the parasite was longer than the average diameter of the healthy red cells, the parasite was usually shorter (and sometimes considerably shorter) than the diameter of the enlarged red cell containing it. The position of the parasite within the cell was usually eccentric, so that one end and one side of the parasite were nearer the circumference of the red cell than was the other end or side. Sometimes the invaded red cell formed a large oval, with the long axis of the parasite in the short axis of the oval, so that the ends or one end of the parasite might project beyond the margin of the red cell. Less commonly the parasite projected one-third or one-half of its length outside the red cell, and appeared to be in process of leaving (or entering) the corpuscle. Only two free vermicules were observed in the stained preparations, and, as already explained, there was no opportunity of examining the fresh blood. The free vermicules were of the same size and general structure as the endo-corpuscular forms.

The enlarged red cells containing the parasites were, as a rule, slightly paler than the average of the healthy red cells around, but this pallor did not appear to be greater than would be accounted for by the relative increase in size of the cell, and consequent distribution of the colouring matter over a larger area. In some of the invaded red cells no diminution of staining intensity could be noted, but in a few very considerable pallor was apparent. The invaded corpuscles appeared to be flabby and easily distorted; the corpuscles showing the greatest enlargement showed also the greatest distortion, and, in stained films at least, the outline of the enlarged corpuscle could not be said to follow the outline of the contained parasite.

In the great majority of instances no gap or break was distinguishable between the substance of the red cell and the substance of the parasite, the sharply defined outer membrane of the parasite being closely apposed to the colouring matter of the red cell. In particular there was no evidence of an unstaining "cyst," as described by Christophers in *H. gerbilli*. In a few instances a semicircular unstained area in the red cell, resembling a large vacuole, was found closely applied to the parasite.

The number of infected red cells was considerable. With a $\frac{1}{12}$ -inch Zeiss achromatic objective and No. 3 eye-piece, the majority of the fields were barren, but occasionally a field containing two, three, or rarely four parasites would present itself.

COMPARISON WITH OTHER MAMMALIAN HÆMOGREGARINES.

H. petauri, being a parasite of the red blood corpuscles alone, need not be compared in detail with the leucocytozoa, from which, owing to the difference in habitat, it may be assumed to be distinct. In regard to the only other forms of hæmogregarines known to infest the

red blood cells of mammals, the structural features of the endo-corpuseular trophozoites are so markedly different from those presented by *H. petauri* that a specific distinction must in our judgment be admitted to exist. *H. gerbilli*, for example, differs from *H. petauri* in being longer, in having a "tail" sharply flexed on the body in the endo-corpuseular form, in the nucleus not being polar or terminal in position, and in possessing an unstaining "cytocyte" separating the trophozoite from the colouring matter of the corpuscle. *H. jaculi* (vel *Balfouri*), on the other hand, more nearly resembles *H. petauri*, inasmuch as in both cases the endo-corpuseular stage assumes the short sausage form without flexion of the "tail." But the characteristic terminal position of the nucleus and the relatively stout, thick body of the trophozoite of *H. petauri* are in marked contrast to the centrally placed nucleus and relatively slender form of the corresponding stage of *H. jaculi*.

Apart, however, from these morphological distinctions, the fact that *H. petauri* is found in a marsupial is additional presumptive evidence of its specificity, since, as a general rule, it may be said that different mammalian genera are naturally infected by protozoa that are specifically distinct. At any rate, we believe that, having regard to the characters of the parasite and to the type of the host, we have made out a case for accepting the protozoon,—in the first place, as a hæmogregarine, and, in the second place, as a new species.

In conclusion, we may note the wide distribution of the hæmogregarines infesting the red blood corpuscles of mammals, since one has been found in Africa, one in India, and one in Australia, and it is interesting to note the further fact that all have so far been described only in rodents.

REFERENCES.

- BALFOUR "A Hæmogregarine of Mammals," Second Report, Wellcome Research Laboratories, Gordon Memorial College, Khartoum, 1906.
- BENTLEY "Preliminary Note upon a Leucocytozoon of the Dog," *Brit. Med. Journ.*, London, 1905, vol. i. p. 988.
- CHRISTOPHERS "Hæmogregarina Gerbilli," "Scientific Memoirs, Government of India," Calcutta, New Series, No. 18, 1905.
- GRAHAM-SMITH "A New Form of Parasite found in the Red Blood Corpuscles of Moles," *Journ. Hyg.*, Cambridge, 1905, vol. v. p. 453.
- JAMES "On a Parasite found in the White Corpuscles of the Blood of Dogs," "Scientific Memoirs, Government of India," Calcutta, New Series, No. 14, March 1905.
- LAVERAN Quoted by Balfour, *loc. cit.*

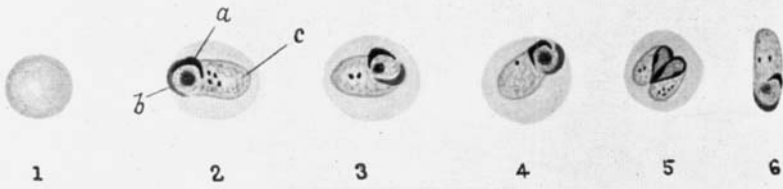


FIG. 1.

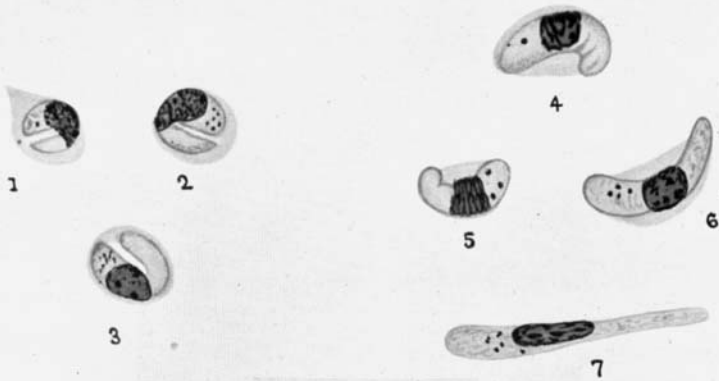


FIG. 2.



FIG. 3.



FIG. 4.

- MINCHIN Art. "Protozoa," Allbutt and Rolleston's "System of Medicine," London, 1907, Part 2, vol. ii. p. 82.
- PATTON Quoted by Balfour (*loc. cit.*), and by Stephens and Christophers, "Practical Study of Malaria," 3rd edit., London, 1909.

DESCRIPTION OF PLATE XXXV.

FIGURE I.

- No. 1.—Average healthy red blood cell.
- Nos. 2, 3, and 4.—Intracorpuseular trophozoites. Each parasite measured in the long axis from $7.5\ \mu$ to $8\ \mu$, in the transverse axis from $3.5\ \mu$ to $4\ \mu$. Note the enlargement and slight pallor of the invaded red cell, the characteristic form of the hæmogregarine with (*a*) its terminal (polar) nucleus, showing central and peripheral condensations; (*b*) its crescentic polar cap of condensed cytoplasm (which stains deep blue), scattered chromatin granules in the general cytoplasmic portion (*c*), etc.
- No. 5.—Two small parasites within one red cell. Each parasite measured $5\ \mu$ in the long axis, $2.5\ \mu$ in the transverse axis. Note their symmetrical arrangement, distinct cytoplasmic portions, and overlapping or fused nuclei. Note also the absence of some structural details visible in the isolated (mature) forms. The appearances are suggestive of longitudinal fission and immaturity.
- No. 6.—Free vermicule, measuring $7.5\ \mu$ longitudinally and $3.5\ \mu$ transversely, and exhibiting all the details of structure above described in 2, 3, and 4.
- (Leishman-Romanowsky stain.)