ON SOME HÆMOGREGARINES FROM AUSTRALIAN REPTILES.

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(From the Bureau of Microbiology, Sydney.)

(Plates xxxiv.-xxxv.)

Australian Hæmoprotozoa have been quite neglected until very recently. To this group there belong a number of families, to one of which, the *Hæmogregarinidæ*, the organisms described in the present paper refer. These are from reptiles, chiefly snakes.

The *Hæmogregarinidæ* constitute a family belonging to the Sporozoa, and the main characters, as given by L. W. Sambon,(1) are the possession of a definite form which is generally club-like, the wider end being regarded as anterior; the complete absence of black pigment; and the presence of a capsule, except in very young stages. The adult parasite commonly becomes somewhat bent on itself by the growing round of the posterior narrower end in such a way that it comes to lie against the main part of the organism.

The absence of pigment, and the presence of a definite form enclosed in a capsule, allow of the ready separation of hæmogregarines from species belonging to other hæmoprotozoan genera such as *Babesia* (*Piroplasma*), *Halteridium*, *Hæmocystidium*, and *Plasmodium*, including their very numerous synonyms such as *Proteosoma*, etc.

The genus *Leucocytozoon* is now regarded as a synonym of *Hæmogregarina*, since, in some hosts, the same kind of parasite may occur in the leucocytes as well as in the erythrocytes (*e.g.*, *Hæmogregarina colubri* Börner, and *H. rarefaciens* Samb. &

Seligm.). However, this is not commonly the case. Other genera have been proposed, whose characters are based mainly on the relative sizes of the host-cell and the parasite; but I intend to follow Sambon in regarding the family as consisting of a single genus, *Hæmogregarina* Danilewsky (Laveran emend.).

Sambon(1) states that these animals pass through a cycle in their life-history, which in many ways resembles that described for malaria parasites(*Plasmodium* spp.). Reproduction may be brought about by sporogony or by schizogony, *i.e.*, sexually or asexually, respectively. In the asexual stage, the cycle is passed through in the blood of a vertebrate (schizogony); whilst the sporogonic, or sexual phase, occurs in the body of some blood-sucking ectozoon such as a tick or louse, in the case of land-animals; or a leech, in the case of aquatic hosts. Thus the final or definitive host (*i.e.*, the host in which the parasites become sexually mature) is an ectoparasite, whilst the intermediate host is one of the higher animals. He also states, further, that in the intermediate host, two different adult organisms may be produced, viz., the schizont and the sporont. The schizont is known to break up into a number of merozoites which become liberated into the blood-plasma, and may infect blood-corpuscles of the same host. The sporont is destined for removal by the definitive host, on reaching whose gut it gives rise to micro- and macrogametocytes. These conjugate to become ookinites capable of again entering and reinfecting the vertebrate when occasion offers, *i.e.*, they may become extruded with the saliva of the ectoparasite, whilst it is sucking its host's blood. I have not seen schizonts in any of the films examined. Perhaps their appearance is periodic.

So far very few Hæmogregarines are known from Australia. They are

(a) *Hæmogregarina shattocki* Sambon and Seligmann,(1, 2) from an Australian diamond-snake, *Python spilotes* Lacép.

(b) *H. dasyuri* Welsh and Barling,(3) from the "Native Cat," *Dasyurus viverrinus* Shaw (New South Wales).

(c) *H. petauri* Welsh, Dalyell and Burfitt,(4) from a marsupial "squirrel," *Petaurus sciureus* Shaw (New South Wales).

(d) *H. amethystina* Johnston,(6) from the Northern carpet-snake *Python amethystinus* Schn.(North Queensland).

Of the above, the first and the fourth have been met with in our work at the Bureau of Microbiology, and in addition, *H.* (*Leucocytozoon*) *muris* Balfour (5),originally described from the Egyptian Sudan, has been met with on a few occasions(7) in the sewer-rat (*Mus decumanus* Pallas) in Sydney during our plague-investigation work. Dr. J. B. Cleland(8) recorded the occurrence of the same parasite from this rat in West Australia under the names *Leucocytozoon ratti*, and *L. balfouri* Laveran, the former name being a slip, whilst the latter name would be the correct one if further investigation should show that *H. balfouri* (*L. jaculi*) Lav., of the Jerboa, and *H. muris* of the rat, are specifically the same.

Although I have examined blood-films from a large number of birds, mammals, lizards, frogs, and fishes, the presence of hæmogregarines has not been detected in them, though species of other hæmatozoa, such as *Halteridium*, *Plasmodium*, *Babesia*, and *Hæmocystidium* were seen, many of them having been described recently by Dr. Cleland and myself. The Hæmogregarines described below were met with in our Bureau work.

Hæmogregarina shattocki Samb and Seligm.(2)

(Plate xxxiv, figs.13-20).

In 1907, Sambon and Seligmann(2) described the above species from the erythrocytes of an Australian diamond-snake, *Python spilotes* Lacép., which died in the Zoological Gardens, London. They mentioned(1) that this snake occurs in Australia, and New Guinea. The true diamond-snake has a very restricted range, being found only along the coastal districts from Broken Bay to Jervis Bay, and the adjacent mountain slopes; whilst the variety of it, *P. spilotes* var. *variegata* Gray, better known as the carpet-snake, is found all over the continent. I am inclined to take

the popular and scientific name mentioned by these authors. The range given will, in that case, need altering, and we may take it for granted that the specimen came from New South Wales.

Mr. H. Wasteneys, of Brisbane, recently forwarded several blood-films from carpet-snakes (*P. spilotes* var. *variegata* Gray) captured on the Enoggera Water-Reserve (near Brisbane). Two of the films showed the presence of comparatively few hæmogregarines, which I have identified as *H. shattocki*. Many of the adult stages described for this species were not present in the specimens examined, but the forms seen resembled those described sufficiently closely to justify the identification.

The youngest parasite seen was only about 6 μ long by about 2·5 μ wide. It did not possess the fairly characteristic shape of a young hæmogregarine. Its centrally situated nucleus was comparatively large, round, and deeply staining. The parasite was about as large as the host-nucleus. It is worth noting here that its host-cell harboured another much larger specimen, this being the only example of double infection seen in the several films examined. The other form was a typical crescent-shaped hæmogregarine. Many of the latter type were seen, their measurements being about 15 μ by 2·5 μ, measuring the length along the middle of the parasite. The ends gradually tapered for a short distance, and terminated in blunt, rounded extremities of equal size. Hence one could not distinguish definitely an anterior and a posterior end in each. The concavity of the crescent was usually facing the host-nucleus, which was not in any way displaced. The nucleus of the organism, when visible, appeared as a definite band across the body, usually slightly nearer one end than the other. A thin capsule could, in most cases, be detected.

These crescentic forms correspond fairly closely to Sambon and Seligmann's, which were from 11 to 15 μ long by 2 μ broad, and possessed rounded extremities differing only slightly in thickness.

In later stages, a capsule could be distinguished quite readily. Sometimes it lay some little distance from the parasite, especially on the concave side; sometimes the interval was very narrow.

These organisms were rather shorter and broader than the crescents, being from 12 to 14 μ long, by about 3 μ wide. Since the erythrocytes are only about 19 μ by 10 μ, the adult sporont (the stage represented in our specimens) necessarily becomes bent, as previously stated. Such forms are figured by Sambon.(1) That these later stages represented sporonts and not schizonts, was recognisable by their structural characters. They were more or less club-shaped, one end being, however, only slightly broader; their nuclei were situated, as a rule, near the middle, and were generally well defined, consisting of a rather open chromatin network extending across the organism in a band-like manner; and the cytoplasm did not possess the refractive granules characteristic of schizonts.

The host-cells were not distorted, though their nuclei were frequently pushed aside and lay close to the edge, often nearer one end of the erythrocyte.

Hæmogregarina moreliæ, n.sp.

(Plate xxxiv., figs. 1-12.)

My colleague, Dr. J. B. Cleland, handed over to me, for examination, a number of blood-films from Western Australian animals, including a tortoise, *Chelodina oblonga* Gray(?), and a carpet-snake, *Python spilotes* var. *variegata*.

The snake was captured on the Abrolhos Islands, a small group off the west coast of West Australia. A blood-film taken from it showed the presence of numerous hæmogregarines in the red cells. These appeared to me to differ from *H. shattocki* in several details, and, consequently, a new species is proposed for them. It is quite possible that further investigation of the hæmogregarines of the carpet-snake may lead to the fusion of this species with *H. shattocki*. The examination of the blood from parasitised carpet-snakes taken at localities between these two extreme parts (eastern and western) of the continent would settle the validity or otherwise of the proposed species. For the specific name, I have borrowed the old generic name (a synonym) of this reptile (*Morelia variegata* Gray).

The corpuscles were from 18-20 μ long, by 10 or 11 μ broad, with nuclei averaging 7 by 4·5 μ. The parasites varied from 10 to 19 μ in length, and from 1·5 to 5 or 6 μ in greatest breadth. The largest forms measured 20 μ by 4 μ, and 17·5 μ by 6 μ; whilst the smallest were 12 by 1·5 μ, and 10 by 4 μ.

Even the largest hæmogregarines, some of which occupied nearly the whole of the available space in the host-cell, *i.e.*, the nucleus excluded, did not cause any distortion of the erythrocyte, though the host-nucleus was usually displaced, even by moderately large parasites. Only exceptionally was its position so much altered that it rested against the edge of the red corpuscle.

Only one very young form, about 6 by 2 μ, was detected. It was placed transversely, towards one end of the host-cell. Crescentic parasites were rather uncommon, adult sporonts greatly predominating. The last varied considerably in shape. Some were club-shaped, with a very wide rounded anterior part which tapered to a much narrower, though still blunt, posterior end. Others were very long and somewhat narrow, the posterior end being bent round in an open curve. Others again were nearly uniform in breadth throughout, whilst still others possessed the typical adult-form in which the "tail" was bent round in such a way as to lie close to the "body." The parasites were usually of greatest breadth in the region of the nucleus, a distinct bulging being seen on the inner (*i.e.*, the concave) side.

The nucleus was generally broad and band-like, though occasionally it was small and irregular, or rounded. Its position was somewhat nearer one end.

The capsule was somewhat similar to that seen in *H. shattocki*, but appeared to be more delicate.

In brief, the main difference between *H. morelicæ* and *H. shattocki* is, that the sporonts of the former are rather shorter and much wider. *H. pococki* Sambon and Seligmann, from *Python molurus* Linn., is evidently a close ally to this species.

The type-slide has been presented to the Australian Museum, Sydney.

Hæmogregarina pseudechis, n.sp.

(Plate xxxv., figs. 13-20.)

A black snake, *Pseudechys porphyriacus* Shaw, obtained near Sydney, was found to be parasitised by hæmogregarines which were not by any means abundant.

The host-cells were about 15 μ by 10 μ, with nuclei 7·5 μ by 4 μ; whilst the parasites were generally about 14 μ by 3·5 μ. In addition to these typical hæmogregarines, there were present a number of very small parasites with a definite non-amœboid shape which was rounded, elongate or pyriform. These measured 3 or 4 μ long by 1·5 μ broad, and, no doubt, represented very early stages. If we except these very small forms, the youngest hæmogregarines appeared as large, slightly club-shaped bodies, with a thicker, rounded, anterior end tapering very gradually towards the blunt, and slightly curved, posterior extremity. These were from 12 to 14 μ long, by from 3 to 4 μ wide. A few specimens possessed a swelling on the concave side in the region of the nucleus. Their nuclei were broad and band-like, and were generally placed towards one or other end. The capsules were very delicate, and, as a rule, were not easily distinguishable. The only effect produced on the host-cell by the presence of the parasite was the displacement of the nucleus to the edge of the erythrocyte.

Adult forms were less bulky, and showed delicate but very distinct capsules which were not closely adherent to the parasites, especially on the concave side. Their sizes were 13 or 14 μ, by about 4 μ. In a few cases the posterior end was bent round in a manner similar to that mentioned in *H. moreliæ*. The central portion was bulged out on the inner side, the nucleus generally lying just anterior to it. As a rule, the concavity of the animal faced the host-nucleus, the latter being displaced laterally, though, in some instances, the displacement was towards one end of the red cell. No distortion of the host-cell was observed.

The generic name of the host has been borrowed as a specific name for the parasite. A type-slide has been presented to the Australian Museum, Sydney.

Hæmogregarina clelandi, n.sp.

(Plate xxxv., figs. 1-12.)

Dr. J. B. Cleland, while in Perth, took some blood-films from the common West Australian tortoise, *Chelodina oblonga*(?) Gray, the erythrocytes of which, on being stained with Giemsa, were seen to be rather heavily infected with a relatively broad hæmogregarine.

The sporonts were apparently of two types, which may represent some sexual differentiation. In the one type the parasites were lightly staining, and showed a number of structures resembling vacuoles. Sometimes there was only one, this being situated mostly at one end; sometimes there were one, two, or more at each end, their number, position, size, and shape being variable. Occasionally they were near the centre. The other forms were generally larger, more deeply staining, and non-vacuolated. Do the former represent male sporonts, and the latter female? These parasites ranged from 11 by 5 μ to 13 by 7 μ, the uninfected host-cells being from 18-20 μ long, by 10-12 μ broad; whilst infected host-cells were considerably larger, reaching from 20-24 μ in length, by from 10-13·5 μ in width.

There were many young stages represented, some of the parasites being only a little longer than the host-nucleus (7 μ by 4 μ). They were not encapsuled, and were usually vacuolated. The positions which they occupied in their hosts were very varied. Some lay transversely, with the concavity facing the host-nucleus in some cases, and remote from it in others. Sometimes they were placed longitudinally, either laterally, or along the median line of the host. The most usual position was somewhat oblique from the longitudinal axis of the erythrocyte. Even in the case of adult forms, it was quite exceptional to see any instances where the parasite was occupying the position usually taken up by hæmogregarines, *i.e.*, longitudinally between the nucleus (usually somewhat displaced laterally), and one side. Almost invariably was the host-nucleus displaced even by young forms. Another fact worth mentioning is that the displacement was in

such a direction that the nucleus came to lie at or near one end, instead of laterally. The infected cells were distorted along the axes, hence their shape was not much altered, though their size was considerably increased.

Each adult parasite possessed a very definite, wide capsule, generally elliptical in shape. There was a comparatively wide interval between it and the organism. The outline of the latter was much more regular than is usually the case in members of the genus, the parasites being rather plump. In only one instance, a short recurved "tail" was seen, lying close against the rest of the "body."

Many free forms were present in the plasma; but since they were encapsuled, and were generally adjacent to crushed nuclei, we may assume that the condition was produced in making the film. The leucocytes were not infected.

The name *Hæmogregarina clelandi* is proposed for this species, in recognition of Dr. Cleland's work on West Australian parasitology. The type-slide has been presented to the Australian Museum, Sydney.

Other hæmatozoa described from Australian tortoises are *Trypanosoma chelodina* Johnson,(10) from *Chelodina longicollis* Shaw, from Morgan, South Australia; and *Hæmocystidum chelodinæ* Johnston and Cleland,(9) from the same species, obtained near Sydney

As some authorities have stated that trypanosomes and hæmogregarines may be stages in the life-history of one organism, it will not be out of place to mention the main characters given by Dr. A. E. Johnson, of Adelaide, in his brief, unfigured account of *Trypanosoma chelodina*, especially as it appears in a medical journal which very probably may not be available to biologists in other parts of the world.

These parasites, which, in stained films, were bent in the form of a semicircle, were larger than the nucleated discs, measuring about 14 μ long by 1·5 μ wide; but, if the undulating membrane were included, the breadth was from 2·5 μ to 3 μ. The flagellum, which was 2 μ in length, was fringed, and ended at the centro-

some. The latter was situated at about one-third of the distance from the centre of the posterior end, the nucleus lying between it and the centre. A large vacuole appeared to be present in the deeply staining protoplasm (using Leishman's stain). Towards each end this protoplasm was replaced by some deeply staining granules.

There was no trace of any trypanosomes in any of the reptilian blood-films examined by me. *Trypanosoma lewisi* Kent, is fairly commonly met with in our rats (*Mus rattus* Linn., *M. alexandrinus* Geoffr., and *M. decumanus* Pall.). *Hæmogregarina muris* Balfour, also occurs in New South Wales, in *Mus decumanus* Pall. Though I have examined films containing each of these, no film has been seen with both of them present. There is most probably no connection whatever between them.

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EXPLANATION OF PLATES XXXIV.-XXXV.

Plate xxxiv.

Figs. 1-12.—*Hæmogregarina moreliæ*, in erythrocytes of *Python spilotes* var. *variegata*.

Fig. 13.—Erythrocyte of *Python spilotes* var. *variegata*.
Figs. 14-20.—*Hæmogregarina shattocki*, in erythrocytes of *Python spilotes* var. *variegata*.

Plate xxxv.

Fig. 1.—Erythrocyte of *Chelodina oblonga*.
Figs. 2-12.—*Hæmogregarina clelandi*.
Fig. 13.—Erythrocyte of *Pseudechys porphyriacus*.
Figs. 14-20.—*Hæmogregarina pseudechis*.
Figs. 14-15.— ,, ,, ; young forms.