REPORT ON DR P. N. GERRARD'S PREPARATIONS ACCOMPANYING THE FOREGOING COMMUNICATION.

(Plate II.)

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THE parasite referred to in the preceding paper was found by Dr Gerrard in the polymorphonuclear leucocytes of a dog. As is stated by Dr Gerrard, the parasite is evidently similar to that discovered by Bentley and described by him, and afterwards by James, as the *Leucocytozoon canis*. Dr Gerrard sent some films, stained by him by Leishman's method, to Sir Patrick Manson, and they were handed on to me for description and identification. These films had faded in transit, they were therefore decolorised and restained by various methods. All the staining reactions mentioned in this description refer to these old films.

The parasite is found as an elongated cyst with rounded ends, enclosing granular protoplasm and a nucleus. These cysts are contained in the polymorphonuclear leucocytes, of which about 3% are infected.

Position of cysts. The cysts occur in the polymorphonuclear leucocytes only. They lie in the protoplasm which is stretched tightly round the ends of the cyst and the nucleus of the leucocyte. In many instances free cysts were observed lying in the plasma but, with one or two exceptions, in every instance, the remains of the broken down leucocytes were near them. It would appear therefore that the natural position of the parasite is intracorpuscular.

Cyst. This is a definite structure and is always present. The cyst is an elongated structure with rounded ends and its length (10 μ) is almost exactly twice the breadth. There is very little variation in the dimen-

sions of different cysts. The material of the cyst resembles that of coccidian spores, though it is not so thick and impenetrable to stains. It is a resistant structure. In the process of drying the film, several of the cysts have been caused to crinkle whereby their nature can very well be made out. The cyst is quite smooth, transparent and homogeneous and shows no sign of any operculum, valve, suture or other marking.

Contents of the cyst. These have been most satisfactorily brought out by staining with haematoxylin, then with carbol fuchsin, and differentiating with absolute alcohol. This method has the advantage of staining the nucleus of the parasite a different colour from that of the infected leucocyte. Leishman's stain does not give a satisfactory picture of the parasite Giemsa's stain is better, but colours the nucleus of the parasite the same as that of the leucocyte; it however has the advantage of showing more clearly the granules in the protoplasm.

Protoplasm. The protoplasm completely fills the cyst in almost every case. It stains more deeply round the margins of the cyst than towards its centre. The protoplasm shows no structure save for some very fine granules which stain deeply. These granules are more clearly defined by Giemsa's stain.

Nucleus. This is stained a deep crimson with carbol fuchsin. It is usually situated at one pole of the parasite, and in the vast majority is roughly U-shaped. In other cases it is irregularly spherical or lying along one side of the parasite as an elongated body. It may be almost divided into two parts, though in these cases the two parts are invariably connected by strands of nuclear material. The nucleus is of a reticulate structure containing some deeply staining granules.

I have failed to find any trace of a micro-nucleus.

Two parasites were seen which did not completely fill the cyst. The protoplasm was in the form of a loop with one extremity rounded and blunt, and the other narrow and tapering (Plate II. Fig. 6). The nucleus is situated at the bend of the loop and is U-shaped. These forms are important as they throw some light on the nature of the organism.

Effect on the host cell. The only change produced by the presence of the parasite in the polymorphonuclear leucocyte is a purely mechanical one. The various parts of the polymorphic nucleus are displaced by the large cyst. In some leucocytes the nucleus is crowded into one mass, in

others the parts are widely separated, though connected by strands stretching across the parasite, while in others they are lying over the parasite and between it and the slide. If the last is the case the nucleus is protected from staining by the presence of the cyst, though it shows through the cyst as a lightly stained area (Figs. 1, 2, 3, 6, 7).

There is no sign of degenerative change either in the protoplasm or nucleus of the leucocyte both staining as do the uninfected cells. Some of the infected cells are slightly larger than the uninfected ones, but this is probably a purely mechanical effect.

A certain number of the infected leucocytes have broken down in the process of film making and those that are entire appear stretched to their utmost capacity. In the circulation the infected cells would approximate as nearly as possible to a sphere, as that would then be the greatest volume to the minimum surface strain. When flattened out on a film the spherical condition being no longer possible the leucocyte ruptures.

Blood count. There is a marked eosinophilia. The following are two counts made from two different slides:

		Percentages	
Polymorphonuclears	infected	2	4
	uninfected	61	58
Small lymphocytes		12	15
Large lymphocytes		1	2
Eosinophiles		23	19
Transitional forms		1	2

Affinities. This parasite is probably identical with that discovered by Bentley in dogs in Assam.

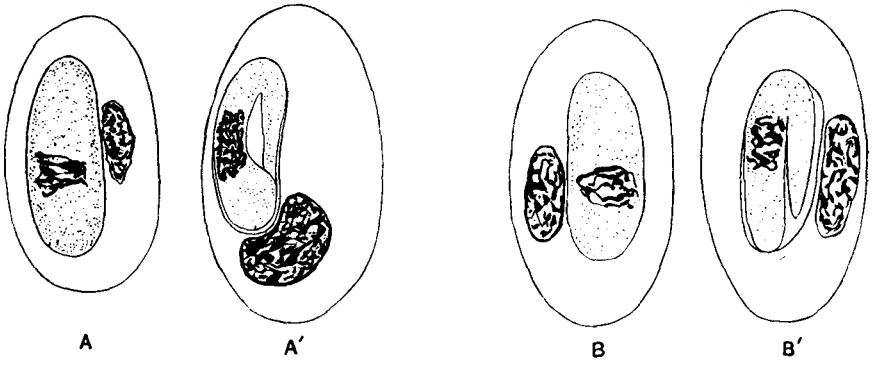
Figs. 8 and 9 are two of Bentley's parasites drawn from a film, kindly presented to the school by Capt. James, I.M.S. The film had been stained by Giemsa's stain.

The protoplasm of the parasite is stained a pale blue with red granules scattered through its substance but becoming more concentrated at one pole. At the opposite pole is the nucleus, which in the majority is irregularly spherical or compact (Fig. 8). In some however the nucleus is U-shaped (Fig. 9).

In these parasites I have also failed to find the large granule which James describes as resembling a micro-nucleus. The resemblance between these forms and those of Gerrard's is very striking, the main difference being in the granules in the protoplasm and in the shape of

the nucleus. The fact that in Gerrard's parasite the granules are not so clear is most probably due to the films being old ones. They are most clearly brought out by Giemsa's stain. The nucleus of Gerrard's parasite is usually U-shaped, though some have a more compact nucleus (Fig. 4). In Bentley's parasites the reverse is the case. This is probably due to the two parasites being in somewhat different stages of development.

The parasites appear to be nearly related to the Haemogregarines. The cysts with their contents resemble closely the encysted forms of Haemogregarines found in the blood and more especially the internal organs of Reptiles.



Figs. *A* and *A′*. Haemogregarines in blood of *Crocodilus cataphractus*.
Figs. *B* and *B′*. Haemogregarines in blood of *Lacerta ocellata*.

The above figures are drawn from blood films of the long-nosed Crocodile (*Crocodilus cataphractus*, W. Africa) and the Green Lizard (*Lacerta ocellata*, Europe). At first the Haemogregarines are looped, but gradually the loops become more and more unequal in size till finally they are indistinguishable and the encysted forms as in Figs. *B* and *B′* above are produced. This process has been described by Billet in the Haemogregarine of a North African Toad and in *Emys leprosa*. On examining some films from the blood of a Jerboa infected with Balfour's Haemogregarine there are indications that a similar process maintains here also. Plate II. Figs. 10 and 12 are drawings from this film which had been stained by Leishman's stain, and show very well the disappearance of the smaller loop. The looped forms of Gerrard's parasite (Fig. 6) resemble the looped forms of these Haemogregarines, and its U-shaped nucleus may be explained by its situation at the bend of the loop.

With the disappearance of the smaller loop the nucleus would still remain U-shaped and the forms of parasite figured at Figs. 1, 2, and 7 would be produced. At a later stage the nucleus loses its U-shape and becomes more compact (Fig. 4). Should however the nucleus not lie at the bend of the loop it would not be U-shaped, and when the two limbs ceased to be distinct it would still remain as an elongated body on one side of the parasite (Fig. 5). It would appear then that the parasite is really a Haemogregarine in the encysted condition. No young forms could be found which would correspond with the young forms of Balfour's Haemogregarine (Figs. 13 and 14). The position of a Haemogregarine in a leucocyte is unusual, but it is known that in Reptiles, where the red cells are chiefly infected, occasionally they occur in the white corpuscles also.

James on one occasion observed the escape of a parasite from a leucocyte. It remained near the leucocyte, and performed slow gregariniform movements for some time. This is all that is known of the parasite outside its encysted condition.

As far as is known no pathological symptoms are produced in the dog by its presence.

James suggests in his paper the name *Leucocytozoon canis*, though he states at the commencement that this parasite has nothing to do with the parasites described by Danilewsky as Leucocytozoa. Till more is known of the life cycle the exact position of this parasite cannot be determined.

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DESCRIPTION OF PLATE II.

Gerrard's Dog Parasite.

Figs. 1 and 2. Two parasites with the U-shaped nuclei. The nucleus of the leucocyte is faintly stained where it is lying between the cyst and the slide.

Fig. 3. Form of parasite with nucleus almost separated into two parts.

Fig. 4. Parasite with compact nucleus.

Fig. 5. Parasite with elongate nucleus.

Fig. 6. Parasite in form of a loop with U-shaped nucleus at bend of loop.

Fig. 7. Parasite partly covered by nucleus of leucocyte.

Bentley's Dog Parasite.

Figs. 8 and 9. Two of Bentley's parasites, one with a compact, and the other with a U-shaped nucleus. Protoplasm showing granules concentrated at one pole.

Balfour's Jerboa Parasites.

Figs. 10, 11, 12. Three of Balfour's parasites in the red corpuscles of the Jerboa, showing the change from the looped to the solid encysted form.

Figs. 13, 14. Young forms of Balfour's parasite.

Note.

Figs. 1—7. Stained by haematoxylin, overstained by carbol fuchsin and differentiated in absolute alcohol.

Figs. 8, 9. Stained by Giemsa's stain.

Figs. 10—14. Stained by Leishman's stain.

All specimens drawn under Zeiss objective $\frac{1}{12}$ and ocular 4.

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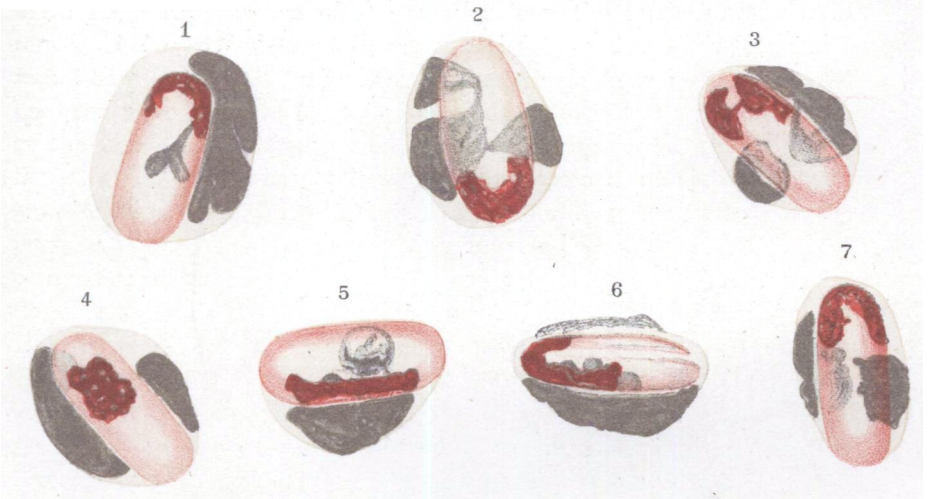
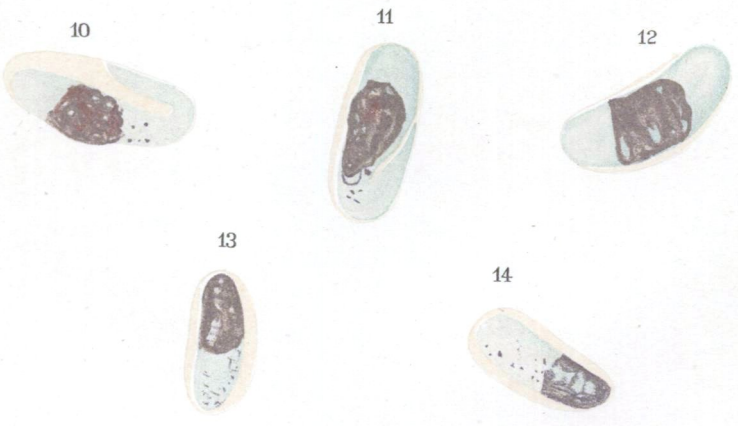
Gerrard's Dog Parasite



Bentley's Dog Parasite

Balfour's Jerboa Parasite



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