THE DEVELOPMENT OF A LEUCOCYTOZOON OF GUINEA-PIGS*

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PLATE VI

The presence of 'bodies' within the large mononuclear leucocytes of guinea-pigs was first noticed by Kurloff (1898). He described them as inclusions; for in a drop of guinea-pig's blood he noted that many of the large lymphocytes contained, within their cytoplasm, clear, spherical vacuoles which were distinct from the nucleus, and which had not been described before; and he suggested the possibility of these bodies being accessory nuclei. Since their discovery by Kurloff they have been subjected to much research; and papers describing various observations concerning them have been published by Burnett (1904), Staubli (1905), Goldhorn (1905), Ledingham (1906), Howard (1907), Pappenheim (1908), Patella (1908), Hunter (1909), and Schilling (1911).

Kurloff noticed that when the blood containing these bodies was fixed and stained, they contained a nucleus-like structure staining with nuclear dyes, but he believed them to be vacuoles formed by a secretion product of the cells which held them. Ehrlich (1906) also thought that Kurloff's bodies represented some 'Secretstoff.' Dr. Ledingham, to whom I am indebted for much information, seems to have been the first to suggest the possibility of their parasitic nature, and he mooted an analogy to the *Cytorryctes variolae* or *vaccinae*. Goldhorn (1905) boldly called them leucocytozoa. The most recent work published on the subject is that of Schilling (1911). He has examined these bodies by 'vital' staining with Azur, and he has described some of the earlier stages of their development while in the mononuclear leucocytes

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(lymphocytes). He believes that the rod stage precedes the granule stages, and this has caused him to adhere to the opinion that Kurloff's bodies must be classed with the Chlamydozoa, symbiotic structures, or vaccine inclusions.

Early in 1911, while examining a guinea-pig's blood by a new jelly method of examination of blood cells, H. C. Ross saw Kurloff's bodies, and pointed out to me that the method demonstrated the probability of their parasitic nature. The new method, which was devised partly at the suggestion of Sir Ronald Ross, K.C.B., has already been fully described (H. C. Ross, 1909); the bodies then seen were in the earlier stages of their development. But the inclusions stood out so clearly by this method that I determined to continue the observations, for this technique seemed to show details of structure which had not been described before; and since by the new process the bodies can be subjected to the action of various stains and chemical agents there was a possibility of the phases of their development being observed. I may state that I have now been able to convince myself that these bodies are living parasites of the mononuclear white corpuscles (lymphocytes), and henceforth in this paper I propose to call them such.

I use a modification of the original jelly method—it is as follows:—A 2 % solution of agar in water is boiled, sterilised and filtered. To 5 c.c. of the filtrate is added 0·5 c.c. of a 10 % solution of sodium chloride in water, and 0·5 c.c. of a 1 % solution of Azur II in water. The total bulk of the mixture is made up to 10 c.c. in a test tube. When molten, a small quantity of the jelly is allowed to spread itself in a thin film on a microscope slide and to cool and set. Then a drop of guinea-pig's blood (or citrated blood) containing Kurloff's bodies (about 90 per cent. of the guinea-pigs examined by me, and which were obtained from dealers in England, are infected) is placed upon a cover-glass, and this is inverted on to the set jelly. The blood spreads out between the cover-glass and the surface of the jelly, and, after an interval of five minutes, during which the blood corpuscles come to rest, the specimen may be examined under the higher powers of the microscope. After a further interval of a few minutes—the exact period varying slightly with the temperature of the room—the granules of the leucocytes begin to stain, after which their nuclei gradually stain a deep blue;

the contours of the erythrocytes, as well as those of the leucocytes, show up clearly, and the method is a pretty example of *in vitro* staining. In some of the larger mononuclear cells the colourless parasites will be noticed at one side of the protoplasm. These parasites are inside the cell, because the shape of the nucleus of the lymphocytes is moulded according to the size of the parasite, which grows larger as it develops—in its youngest stages it is small, while in its last intracellular stages it bulges the lymphocyte cell well and squeezes the nucleus into a small space; this point is of interest because, as Hunter has shown, Patella claimed that Kurloff's bodies lie upon and not in the lymphocytes. In cells containing the larger parasites smaller vacuoles can also be seen; these latter always remain clear and transparent even when examined on stain-containing jellies, and they vary in numbers, and slightly in size, in different examples. It has been suggested that these smaller, subsidiary vacuoles are polar bodies, but more probably they contain excretory products of the lymphocytozoa into the cytoplasm of their hosts, for they become larger and more numerous as the parasite grows.

When examined on the jelly, and immediately before the staining of the nucleus of the leucocytes, the contents of the parasites begin to stain*—the internal chromatin structure of the spherical sac embedded in the lymphocytes' cytoplasm becomes purple and remains stained for several hours, so that its examination is readily made. If the bloods of a number of infected guinea-pigs are watched in this manner from day to day what appear to be the successive stages of the growth of the parasite in the lymphocyte can be seen and drawn; but the leucocytes of a single animal at any particular moment contain, usually but not always, parasites in the same stage of development. The cycle, however, can be followed by observing the blood of one guinea-pig hourly.

*It must be emphasised that if the jelly contains excess of salts or impure stains, the wall of the parasite will stain in an irregular manner, and then patches of stain will hide its contents. Furthermore, if the blood on the jelly dries, or if the blood is fixed in any way, the same thing occurs. Similarly, patchy staining is obtained by the various fixed film methods in vogue, as, for example, Romanowsky's or Jenner's stains. Even Azur stain, when applied to the dried or fixed films of blood, will not demonstrate the details of the development of the parasite. No alkali should be added to the jelly.

The interpretation which I place upon the appearances I have seen are as follows:—The parasite presents itself, in the smallest phase of its intracorpuscular cycle, as a tiny translucent body embedded within the cytoplasm of the larger mononuclear blood corpuscles and near the periphery of those cells. Usually one of such bodies is present in any one cell, but occasionally two or even three parasites may occur in the same cell. The parasite, in this early stage, contains a double purple dot (Pl. VI, figs. 1, 2); in this phase it resembles the Leishman-Donovan bodies found in human leucocytes in cases of Kala Azar. When first seen the dot is motionless, but after a time on the jelly, as the lymphocyte host becomes disorganised, it may show some Brownian movement. In the next stage the parasite is larger, and the chromatin dot has divided into two or more dots until the sphere-like sac may be packed with them (fig. 3). Then each dot becomes dumb-bell shaped (fig. 4), and again, by a simple process of elongation, rod shaped (figs. 5, 6, 7). The parasite may contain one of these rods (fig. 9), or it may be full of them—the actual numbers varying in different examples. Sometimes a parasite may contain one or more rods, some dumb-bells, and some dots. But the size of the parasites increases steadily with these successive stages of the development of their contained chromatin (compare figs. 1 and 15). During the rod formation, the smaller subsidiary vacuoles already mentioned appear in the cytoplasm of the host cell (figs. 3, 5, 12); they never contain any chromatin and remain unstained. With its growth the parasite begins to compress the nucleus of the lymphocyte (figs. 13, 14), and the wall of the latter can be seen as a shell enclosing the parasite (figs. 14, 15, 16). The rods grow longer and thicker (figs. 8, 9, 10) until they stretch across the parasite, and their ends may be doubled against its wall, and they may then present in optical section an erroneous impression of flattening or a terminal bulging (figs. 8, 13, 14). In the next stage a stout flagellum grows out from both ends of the rod (figs. 8,11, 12, 13), which becomes rolled up in a coil within the sphere (figs. 13, 14, 15). The rod with its two flagella splits longitudinally in its whole length (figs. 8, 12), and this process of splitting takes place again and again. The fission throughout is always lengthwise, never transverse. A specimen in this stage will show the parasite, now equal in size to

the original dimensions of its host-cell, bulging the wall of the latter, compressing the nucleus into a small space, and containing within its interior a mass of woven, twisted, and intertwined purple threads, a conglomerate maze of worm-like spirilla stained red by the Azur dye (figs. 15, 16).

Arrived at its maturity, the parasite breaks away from the shell of its host-cell and then bursts, setting free the threads into the plasma (fig. 17). But the flagellate forms, owing to the fact that they are stained, are dead and motionless, and they may remain attached to the shrunken sphere sac, their ends waving in the currents set up.

It was found very difficult to demonstrate the motile, flagellate forms of the parasite when free in the blood. They cannot be seen then by the jelly method, because, probably, they stain momentarily as the trypanosomes do, and immediately die and become achromatic, and unless stained they are not visible. By the examination of ordinary wet films of the blood I was unable to demonstrate the presence of these free flagella, although a disturbance of the corpuscles was frequently seen. But the blood of some infected guinea-pigs, drawn under all aseptic precautions and examined by the dark ground illumination, showed free-swimming spirochaete-like bodies. It was not until the blood of highly infected guinea-pigs containing full matured lymphocytozoa was treated with an equal part of a 1 % solution of 'globin'* and incubated at 37° C. for eight hours that the free flagellate forms in the blood plasma could be fixed and stained by ordinary methods (fig. 18). Even by this process it is not always possible to demonstrate them, and the maceration involved gives them the appearance of spirilla with blunt ends. However, some of the spirilla obtained after the treatment with the 'globin' show the wavy outline of spirochaetes. Sir Ronald Ross was the first to suggest that these flagellate forms constitute the gametes of the parasite; this seems quite probable, though no separate female form has yet been noticed. It will be remembered that Lewis suggested that trypanosomes are sperms, and, perhaps, these spirochaete-like bodies are similar stages of a larger parasite.

*The filtrate of a solution of haemoglobin which has been precipitated by heat. H. C. Ross claims that this substance induces the division of certain cells.

What may possibly be the last phase of this parasite has occasionally been seen in preparations which had been submitted to the action of 'globin' for a further period of four hours. It is an object which resembles somewhat the trypanosome 'latent bodies' described by Moore and Breinl (fig. 19). Hunter has also mentioned the presence of amoeboid forms of this parasite being free in the plasma, but he does not picture them. These may be the form now drawn (fig. 19).

Dr. J. W. Cropper and I have repeated and can confirm the experiments of Ledingham (1906) and Hunter (1909), namely, that newly-born guinea-pigs do not show these lymphocytozoa in their blood. Although a pregnant animal may be markedly infected, the young, when born, possess no parasites. As has been already observed by these writers and by Schilling (1911), the number of parasites found in both the peripheral blood and in that of the internal organs of any one infected guinea-pig varies greatly from day to day. The parasites seem to appear in large numbers, to diminish, to disappear, and then, after a varying period of time, to reappear. Except for a slight anaemia, shown by the presence of an increased number of erythroblasts in the peripheral blood, the guinea-pigs do not suffer apparently. The livers of many of these infected animals show, however, single or multiple white patches of necrosis varying in size between that of a pin's head to that of a large pea, and extending into the substance of the organ. But we have no proof, as yet, of their direct relation to the parasite.

Fixed specimens of the various stages of the development of this parasite may be made by substituting an equal amount of a 1 % solution of caustic soda in the jelly for the sodium chloride solution. By this means the red blood corpuscles are laked, but the nuclei of the leucocytes and the chromatin of the lymphocytozoa stain well. The cover-glass can then be lifted from the jelly and mounted while still wet in Canada balsam. Many of the leucocytes with the contained parasites will adhere to the cover-glass and will retain their stain.

Since writing this paper, Hindle has published a preliminary note (Hindle, 1911), 'On the Life-cycle of *Spirochaeta gallinarum*.' He asserts that these spirochaetes possess an intracellular stage within the cells of the Malpighian tubes of the tick, *Argas persicus*.

In view of the life-history of this lymphocytozoon of guinea-pigs his work is of great interest.

I have to express my indebtedness to Dr. J. W. Cropper and to Dr. H. Bayon for their help in these researches; the latter was the first to recognise the free-swimming, spirochaete-like bodies. I also wish to thank Professor Minchin and Dr. Martin for much help and advice and the interest they have taken in this work.

SUMMARY

Kurloff's bodies are parasites, lymphocytozoa inhabiting only the mononuclear cells of the guinea-pig's blood.

These lymphocytozoa have an intracorpuscular stage, and ultimately give rise to free-swimming, spirochaete-like bodies, which may be gametes.

The development of the spirochaete-like body is demonstrated.

The name *Lymphocytozoon cobayae* is suggested for this parasite.

REFERENCES

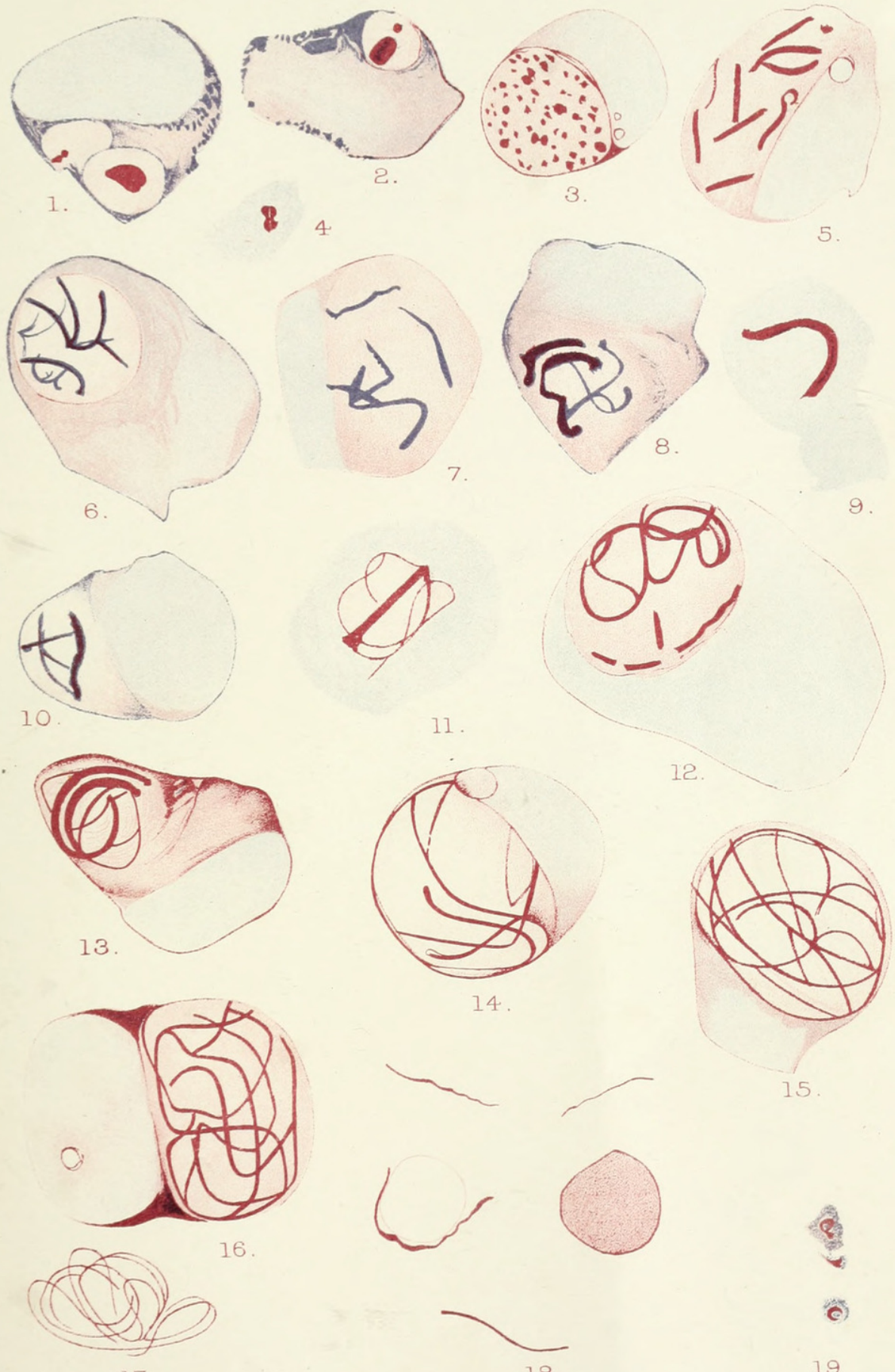
Burnett (1904). Journ. Med. Research.
Ehrlich (1906). Quoted by Ledingham, Lancet, London, June 16.
Goldborn (1905). New York Path. Soc. Proc.
Hindle (1911). Parasitology, vol. 4, No. 4.
Howard (1907). Journ. Med. Research.
Hunter (1909). Univ. Penn. Med. Bull., Phila., vol. 22.
Kurloff (1891). Ehrlich's ' Die Anaemie.'
Ledingham (1906). Lancet, London, June 16.
Pappenheim (1908). ' Folia Haematologica.'
Patella (1908). Berliner klin. Woch.
Ross, H. C. (1909). Roy. Soc. Proc., B, vol. 81, p. 97.
Schilling (1911). Centralb. f. Bakt., orig., vol. 58, Part 4.
Staubli (1905). Deut. Archiv f. klin. Med.

PLATE VI

Lymphocytozoon cobayae.

For Explanation of Plate see text.

76 a



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