

SOME REMARKS ON THE GENUS LEUCOCYTOZOON.

By C. M. WENYON, B.Sc., M.B., B.S.

Protozoologist to the London School of Tropical Medicine.

NOTE. A reply to the criticisms contained in Dr Wenyon's paper will be published by Miss Porter in the next number of "Parasitology".

A GOOD deal of doubt still exists in many quarters as to the exact meaning of the term *Leucocytozoon* applied to certain Haematozoa. The term *Leucocytozoaire* was first used by Danilewsky in writing of certain parasites he had found in the blood of birds. In a later publication he uses the term *Leucocytozoon* for the same parasites though he does not employ it as a true generic title. In this latter sense it was first employed by Ziemann who named the parasite of an owl *Leucocytozoon danilewskyi*, thus establishing this parasite the type species of the new genus *Leucocytozoon*. It is perhaps hardly necessary to mention that Danilewsky and Ziemann both used this name because they considered the parasite in question to inhabit a leucocyte of the bird's blood. There has arisen some doubt as to the exact nature of this host-cell. Some authorities consider it to be a very much altered red blood corpuscle, some perhaps more correctly an immature red blood corpuscle, while others adhere to the original view of Danilewsky as to its leucocytic nature. It must be clearly borne in mind that the nature of the host-cell does not in any way affect the generic name *Leucocytozoon*. If it could be conclusively proved that the host-cell is in every case a red blood corpuscle the name *Leucocytozoon* would still remain as the generic title though it would have ceased to be descriptive.

Apparently it was a want of knowledge on this point that led James to apply the name *Leucocytozoon* to a totally distinct parasite which had been found by Bentley in India to attack certain leucocytes in the blood of dogs. In his paper describing this leucocytic parasite of dogs James discusses the various views as to the nature of the host-cell of the *Leucocytozoon* of birds and as he can find no agreement on this point he erroneously considers this sufficient ground for removing the name *Leucocytozoon* from the bird-parasite and applying it to the parasite of the dog which undoubtedly is within a leucocyte. Since the

discovery by Bentley of the leucocytic parasite of dogs similar parasites have been described from the rat, mouse, cat, palm-squirrel and hare and for these the name *Leucocytozoon* has generally been employed, in spite of the fact that it has been constantly pointed out by Mesnil, Sambon and others that this name strictly belongs to the totally distinct parasite of birds.

Patton who has described several of these leucocytic parasites of mammals employs for them the generic name *Leucocytozoon*. Neither he nor James regards the leucocytic parasite of mammals as allied to the *Leucocytozoon* of birds. As James incorrectly justifies his use of the generic name *Leucocytozoon* for the dog-parasite by pointing out the doubt existing as to the true nature of the host-cell of the bird-parasite so Patton considers he is also justified in so doing, and he agrees with Laveran in considering the bird's parasites as belonging to the genus *Haemamoeba*, a genus in which Laveran includes the pigmented malarial parasites and their allies. It is evident that Patton thinks this would liberate the name *Leucocytozoon* from the bird-parasite. This is quite out of accord with the rules of nomenclature. Even if Laveran and Patton who follows him are correct in their view the name *Leucocytozoon* would remain as a synonym of the bird-parasite and could not be employed for any other form. As we shall see below the view that the *Leucocytozoon* of birds is really of the same genus as the pigmented parasites of malaria cannot be accepted.

Quite recently in *Science Progress* Miss Porter has reviewed our knowledge of the Leucocytozoa. She has attempted, as no one has done hitherto, to describe under this one name both the parasites of birds and the totally distinct parasite of mammals, with what success those who read the review will see. Such a grouping, it must be evident to anyone who has studied and compared both these parasites, can lead to nothing but confusion. Towards the close of her paper Miss Porter makes this statement "the name *Leucocytozoa* should be retained for *all* protozoal parasites of the leucocytes of vertebrates" in spite of the fact that the leucocytes of mammals are known to harbour several totally distinct parasites¹. It is evident that Miss Porter has

¹ The following parasites have been described from the leucocytes of mammals:—

1. Leishman-Donovan bodies.
2. The haemogregarines of dog, cat, rat, etc.
3. The *Toxoplasma gondii* of Nicolle and Marceaux.
4. A similar parasite described by A. Splendvie from the rabbit.
5. The curious trypanosome described by Cruz in Brazil.
6. The parasite of equine lymphangitis described by Dougloux.

no knowledge of the *Leucocytozoon* of birds for her survey of the group is inaccurate and contradictory. With reference to the bird-parasites it is stated "Avian Leucocytozoa are said to exhibit sexual characters but this is somewhat doubtful" though earlier in the paper male and female forms are described. As a matter of fact the presence of both male and female forms in the peripheral circulation is one of the chief characteristics of the *Leucocytozoon* of birds. In this respect it agrees with the pigmented *Halteridium*. Again at the beginning of the paper Danilewsky's records are completely misinterpreted. In writing of the bird-parasites Danilewsky mentions having observed two forms of Polymitus in birds' blood, one of ordinary size and with grains of melanin, the other without grains of melanin but of very large dimensions. In a later publication he says he has found that the "leucocytozoaire" is only an intracellular stage in the development of the large Polymitus but he goes on to say that not all the "leucocytozoaires" develop into Polymitus. This is exactly what is known to occur and speaks for the accuracy of observation of this investigator. The *Leucocytozoon* of birds exhibits marked sexual characters. The male gametocyte gives rise to male gametes in much the same manner as the male forms of the malarial parasites while the female forms become rounded preparatory to fertilization by the male gamete. We thus find that Danilewsky was quite correct in his observation that only some of the "leucocytozoaire" develop into Polymitus. In view of these facts it is unfortunate that Miss Porter should state that "It is now known that the Leucocytozoa and the Polymitus of malaria have no connection." It is evident that Miss Porter has not read Danilewsky's papers with sufficient care.

For the sake of clearness I will give the characters of *Leucocytozoon* :—

1. They are exclusively parasites of birds for though Carini has described a *Leucocytozoon* from an amphibian it is exceedingly doubtful if this is a *Leucocytozoon* as it does not exhibit the sexual dimorphism which is such a distinctive characteristic of the avian parasites.

2. They are parasitic in cells which have been variously interpreted as leucocytes, red blood corpuscles, immature red blood corpuscles, etc.

3. They have the peculiar property of causing the host-cell to assume a characteristic spindle form¹. The parasites themselves sur-

¹ Miss Porter quotes me as admitting that sometimes the host-cell may have a spindle shape. If she will refer to my article she will see that I carefully state that there is practically always a spindle shape.

round the nucleus of host-cell which is found at the middle of the spindle.

4. Three forms of parasite can generally be recognised in the peripheral blood,—large deeply staining parasites which are the female gametocytes, pale staining parasites which are male gametocytes and lastly young forms which are possibly immature gametocytes.

5. The male gametocyte develops into a Polymitus or in other words it forms male gametes which are narrow whip-like organisms resembling the male gametes of the malarial parasites. These male gametes fertilize the female gamete and a motile zygote results.

6. The nucleus resembles that of *Halteridium*. The male nucleus consists of chromatin granules scattered about the centre of the parasite. The female nucleus is a more compact group of chromatin granules in the midst of which or to one side is a granule¹ definitely marked off from the rest by its larger size. It is possible that this granule is a karyosome.

¹ There is a tendency for those who wish to establish the trypanosome nature of the intracorpuseular blood parasites, to describe any granule of chromatin which is larger than those of the main nuclear mass as homologous with the smaller chromatin body of trypanosomes. It must be remembered that extranuclear chromatin is of common occurrence in Protozoa so that it is exceedingly misleading to homologise any such granule of an intracorpuseular parasite with the definite micronucleus of trypanosomes. Woodcock has considered that his discovery of such a granule in *Halteridium* is the first definite evidence which has been brought forward in support of Schaudinn's contention that *Halteridium* has a trypanosome stage in its life history. It appears to me that Woodcock has failed to prove the one point necessary to support this hypothesis. It is not so much the discovery of such a granule that is required but the conclusive evidence that this granule is homologous with the micronucleus of a trypanosome. This evidence Woodcock has failed to produce. As a matter of fact similar granules had been described by several observers before Woodcock's discovery. In the Third Report of the Wellcome Research Laboratories, Khartoum, I have described this granule in the *Leucocytozoon* of the guinea-fowl and have figured it in the *Halteridium* on Plate XIII and again in the *Haemocystidium* on Plate XIV. It is equally undesirable to consider the similar granule in the piroplasmata as a micronucleus till it has definitely been proved to give rise to a flagellum or to have originally done so. In the case of the piroplasmata such a view has already produced considerable confusion with the Leishman-Donovan bodies. I quite agree with Calkins (Chap. IX, p. 269) who says in reference to the present tendency to group the Haemosporidia with the Mastigophora, "It is possible that future research will justify this step and that the large, relatively immobile blood parasites like Lankesterella of the frog, haemogregarines of turtles and tortoises, Karyolysus of lizards, haemoproteus of birds and plasmodium of man are like the Leishman-Donovan bodies only passing phases of some flagellated protozoon, but at the present time the evidence is not weighty enough to warrant such a step even as a working hypothesis. The weakness of the evidence, apparent as soon as reviewed may be briefly summarised as follows, etc.," and again on p. 270. "For these reasons, therefore, I believe it premature to separate the haemosporidia from the sporozoa."

7. The parasites are devoid of pigment, they are not enclosed in any cyst, they never leave the host-cell to move about in the blood plasma as do the haemogregarines, the asexual mode of reproduction is unknown nor is there any knowledge of the further development of the zygote and of the mode of transmission.

Such are the characters of the *Leucocytozoon* of birds. It will be evident to anyone who knows the group that its nearest relative is *Haemoproteus* (*Halteridium*) of the same host. It differs from *Halteridium* however in its large size, the absence of melanin pigment and the peculiar action it has upon its host-cell. It will be equally evident that it has no connection with the haemogregarines nor with the closely allied leucocytic parasites of mammals though Miss Porter would group the latter with the avian parasites.

The characters of these leucocytic parasites of mammals are as follows:—

1. They are parasitic in leucocytes of mammals.
2. They do not alter the shape of the host-cell to any extent though they may cause considerable change in its nucleus.
3. There is no sexual differentiation to be compared with that existing in the bird-parasites.
4. The nucleus is characteristic and resembles that of haemogregarines. It is quite unlike the nucleus of the *Leucocytozoon* and in staining reaction approaches that of the nucleus of the host-cell.
5. A cyst (cytocyte) is frequently though not invariably present. Within the cyst the parasite lies as a cylindrical body completely filling the cyst or as an elongate vermicule doubled upon itself in the shape of a **U**.
6. The parasites may leave their host-cell and their cyst and move about in the plasma as minute gregarines.
7. Asexual multiplication is by schizogony in a cell of some internal organ.
8. Sexual development has only been completely described in one case namely in the case of the leucocytic parasite of the rat. It has also been described but less completely for the similar parasite of dogs.

It is quite clear that these parasites are very closely allied to the haemogregarines and far removed from the *Leucocytozoon* of birds. Indeed if we look into these characters just enumerated we find there is not a single one of them which may not be applied to some typical haemogregarine. The fact that the host-cell is a leucocyte can hardly be regarded as a generic character and unless some marked difference

in the life cycle of the leucocytic parasite of mammals and the haemogregarines of cold-blooded animals is discovered there is no reasonable course open but to include them in the same genus.

Miss Porter states that "The Leucocytozoa of mammals are very similar in general morphology to the Haemogregarina found in the red blood corpuscles of certain mammals" but she continues "surely the difference in habitat is sufficiently striking to separate the genera of strict mammalian Haemogregarines and Leucocytozoa." It is difficult to understand how mere habitat alone can in any way be converted into a generic character. In this, Miss Porter follows James and Patton who are stated to have the advantage, over Laveran and Mesnil, of first-hand knowledge of the group. Apparently Miss Porter imagines that Laveran and Mesnil and possibly others have not this first-hand knowledge, but I can assure her that in this she is mistaken.

In his paper on the leucocytic parasite of dogs James remarks "while it undoubtedly has affinities with the haemogregarines the fact that it is parasitic upon the leucocytes and that it represents a hitherto entirely unknown form of mammalian blood-infection would seem to justify its recognition as the type of a new genus," and Patton at the close of his description of the leucocytic parasite of the palm-squirrel makes this assertion, "It will be seen that the description of this parasite agrees in every detail with that of a haemogregarine." In spite of this complete agreement Patton regards the parasite as belonging to a distinct genus presumably because it is within a leucocyte. The question resolves itself into this—Can mere habitat be regarded as a generic character to separate one parasite from another when in other respects there is "agreement in every detail"?

It is very difficult to understand Patton's attitude towards his two groups *Haemamoebina* and *Haemogregarina*. In the genus *Haemamoeba* are included the malarial parasites of man the life cycles of which are so well known, the similar parasites of birds (*Proteosoma*), the *Halteridium* of birds which differs very much from the malarial parasites both in their asexual multiplication and mode of transmission and finally in this same genus he prefers to include the *Leucocytozoon* of birds though it displays such marked differences from the pigmented malarial parasites. A grouping together in a single genus of such diverse forms is a view accepted by very few authorities. If one compares such a parasite as the pigmented benign tertian parasite of malaria with a *Leucocytozoon* of a bird one can see at a glance that they are quite distinct.

The group *Leucocytozoon* of birds is a homogeneous one, the individual members of which show only slight variation so much so that it is exceedingly difficult to separate the species. There is no possibility of confusing any *Leucocytozoon* with the pigmented parasites with which Patton would group them so that the inclusion of such distinct forms in a single genus seems quite unnecessary. On the same line of argument one would be compelled to do away with all generic distinction in the Haemosporidia and to establish a single genus for the whole heterogeneous assemblage. It is certain that some of the Piroplasmata could be more easily confused with the malarial parasites than an avian *Leucocytozoon* should escape recognition. However it is to no purpose to pursue the argument further as Patton states that he prefers to consider the avian parasites as *Haemamoebae*. We find however that when Patton comes to consider the leucocytic parasite of the palm-squirrel though it "agrees in every detail with the haemogregarines" it is placed in a separate genus. I do not think even Patton would maintain that the *Leucocytozoon* of birds agrees in every detail with the parasites of malaria¹.

The most complete account of the development of a haemogregarine is that of the late Dr Miller of the parasite which he found in the leucocytes of rats. He seems to have been singularly fortunate in having at his disposal an unlimited amount of material. As a result of his researches he was able to follow the complete cycle of this parasite both in the rat and in the intermediate host—*Lelaps echidninus*. Now it occurred to him that the life cycle of this parasite of the rat would most probably be found to be very different from that of the type species of haemogregarine namely *Haemogregarina minima* of the frog,

¹ In the case of the Leishman-Donovan bodies and the allied parasites, Patton has suggested that the generic name should be *Herpetomonas* and that the name *Leishmania* is to be abandoned. The evidence in support of this action is that certain *Herpetomonas* of the intestinal tract of non-biting arthropods exhibit stages in their development which are indistinguishable from the Leishman-Donovan bodies. There is no question of this *Herpetomonas* passing any part of its life-history in different hosts. The infection of an arthropod is by the accidental ingestion of cysts which have escaped from the intestine of an infected individual of the same species. In the case of the Leishman-Donovan body the condition is quite different. Part of its life-history is passed like a *Herpetomonas* in the intestine of some biting arthropod (bed-bug) but the other as a parasite within certain cells of a mammalian host. In the life-history of the Leishman-Donovan body there is then a definite alternation of hosts in one of which it exists as an intracellular parasite. This is a profound distinction which undoubtedly justifies its inclusion in a distinct genus. As the generic title of the Leishman-Donovan body and its allies the name *Leishmania* may well remain.

though the life cycle of the latter had not yet been discovered. The great difference in the two hosts seemed to warrant the conclusion that the life-cycles would be so distinct as to justify their inclusion in different genera. Accordingly Miller suggests for the rat-parasite the name *Hepatozoon perniciosum*. It must be very doubtful whether one is at liberty to create a new generic name on a mere probability for it must be remembered that the life cycle of the frog haemogregarine is not known. But the point I should like to emphasise is this that if the leucocytic parasites of mammals are to be separated from the haemogregarines the generic name will be *Hepatozoon*. We have seen that the generic name *Leucocytozoon* cannot be employed for the mammalian parasites as it is the true generic name of a distinct avian parasite. Towards the end of her paper Miss Porter says "However as the structure and life-history of avian leucocytozoa are still subjects of controversy and as the name *Leucocytozoon* was first applied to parasites of birds, and Lühe seems to restrict the name thereto, the generic name *Leucocytogregarina* might be used for the highly specialised parasites of mammalian leucocytes which have a different habitat from the strict Haemogregarines of red corpuscles." Miss Porter has still further confused the matter by introducing another name though it must be evident to her that if the leucocytic parasites of mammals require a distinct generic name the recently suggested name *Hepatozoon* of Miller will have priority.

In an earlier paper in which Miss Porter describes a leucocytic parasite of the mouse she refers to recently described leucocytozoa and mentions one discovered by Sambon in the grouse. It was evident that here there was a misapprehension that the *Leucocytozoon* of birds was a parasite closely allied if not identical with the leucocytic parasites of mammals. As far as I can recall the literature this is the first instance in which such a mistake has been made. In her recent review Miss Porter has still further extended this mistake though the suggestion at the end of the review of a new generic name seems to indicate some doubt even in Miss Porter's mind as to the validity of this grouping.

All recent writers on the bird-parasites employ the true name *Leucocytozoon* and it is for these bird-parasites that this name must be used and not for the leucocytic parasite of mammals. As the complete life cycle of the haemogregarines has been followed in only one instance there are insufficient data for splitting up the group, so that it is at present safer to include in the group *Haemogregarina* the haemogregarines of the cold-blooded animals and the very similar parasites of the

red blood corpuscles and leucocytes of mammals. If it be found that the life-history of the leucocytic parasites of mammals differs markedly from that of the type species of haemogregarine (*H. minima*) then the name *Hepatozoon* of Miller will have priority.

REFERENCES.

- ADIE, J. R. (1906). Note on a Leucocytozoon found in *Mus rattus* in the Punjab. *Journ. Trop. Med.* ix. p. 325.
- BALFOUR, A. (1905-6). A haemogregarine of mammals. *Ibid.* viii. 241-244 and ix. p. 82.
- (1906). Leucocytozoon muris. *Report of the Wellcome Research Lab.* Khartoum, ii. p. 110.
- BENTLEY, C. (1905). Preliminary note on a Leucocytozoon of the Dog. *B.M.J.* p. 988.
- BERESTNEFF, N. (1904). Ueber das *Leucocytozoon Danilewskyi*. *Archiv für Protistenkunde*, iii. p. 376.
- CARINI, A. (1907). Un leucocitozoon de *Leptodactylus ocellatus*. *Rev. da Soc. Scient. de San Paolo*, viii.
- CHRISTOPHERS, S. R. (1906). *Leucocytozoon canis*. *Scient. Mem. Gov. India*, No. xxvi.
- (1907). The sexual cycle of *Leucocytozoon canis* in the tick. *Ibid.* No. xxviii.
- CLELAND, J. B. (1906). The Haemogregarine of Mammals (*H. balfouri*) and some notes on Rats. *Journ. Trop. Med.* ix. p. 296.
- DANILEWSKY, B. (1889). La parasitologie comparée du sang. Kharkoff.
- (1890). Développement des parasites malariques dans les leucocytes des oiseaux (Leucocytozoaires). *Ann. de l'Inst. Pasteur*, Paris, iv. p. 247.
- (1891). Ueber den Polymitus malariae. *Centralb. f. Bakteriol. u. Parasitol.* ix. p. 397.
- DOUCLOUX, E. (1908). Sur un Protozoaire dans la lymphangite épizootique du mulet en Tunisie. *C. R. Soc. Biol. Paris*, lxiv. p. 593.
- DUTTON, J. E., TODD, J. L. and TOBEY, E. N. (1907). Concerning certain parasitic Protozoa observed in Africa. *An. Trop. Med. and Parasitol.* i. No. 3.
- GERRARD, P. N. (1906). On a Protozoon parasite found in the Polymorphonuclear Leucocytes of a Dog. *Journ. Hygiene*, vi. p. 229.
- JAMES, S. P. (1905). On a Parasite found in the White Corpuscles of the Blood of Dogs. *Scient. Mem. Gov. India*, No. xiv.
- KÉRANDLE, J. (1909). Sur quelques hématozoaires observés au Congo (Haute-Sangha-Logone). *Bul. Soc. Path. Exot.* ii. p. 206.
- KEYSSELITZ, G. and MAYER, M. (1909). Ueber ein *Leucocytozoon* bei einem ostafrikanischen Perlhuhn (*Guttera pucherani*). *Archiv für Protistenkunde*, xvi.
- LAVERAN, A. (1902). Sur un Haemamoeba d'une mésange (*Parva major*). *C. R. Soc. Biol. Paris*, liv. p. 1123.
- (1903). Contribution à l'étude de *Haemamoeba ziemanni*. *Ibid.* lv. p. 620.

- LUKE, M. (1906). Leucocytozoon. *Handbuch der Tropenkrankheiten, C. Meuse, Leipzig.*
- MATHIS, C. et LEGER, M. (1909). Présence d'un Leucocytozoaire chez les chiens du Tonkin. *C. R. Soc. Biol.* LXVII. p. 98.
- (1909). *Leucocytozoon* de la Perdrix du Tonkin. *An. de l'Inst. Pasteur*, XXIII. p. 740.
- MEZINCESCU, D. (1909). Leucocytozoon *ziemanni* et trypanosomes chez l'épervier (*Falco nisus*). *C. R. Soc. Biol. Paris*, LXVI. p. 328.
- MILLER, W. W. (1908). *Hepatozoon perniciosum* (N.G., N.Sp.); A haemogregarine pathogenic for White Rats; with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*). *Treas. Depart. Pub. Health and Marine-Hospital Service of U.S. Hyg. Lab. Bulletin*, XLVI.
- NEAVE, S. (1906). *Haemamoeba neavei*. *Report of the Wellcome Research Lab. Khartoum*, II.
- (1909). An avian haemoprotozoon. *Journ. Trop. Med.* XII. p. 79.
- PATTON, W. S. (1906). On a parasite found in the white blood corpuscles of Palm Squirrels. *Scient. Mem. Gov. India*, No. XXIV.
- (1907). Mammalian Leucocytozoa. *An. Report King Inst. Preventive Medicine, Madras.*
- (1909). The Parasite of Kala Azar and allied organisms. *Lancet*.
- PORTER, A. (1908). *Leucocytozoon musculi* sp. n., a parasitic protozoon from the blood of white mice. *Proceedings Zoo. Soc. London*, p. 703.
- (1909). Leucocytozoa. *Science Progress*, III.
- SACHAROFF, F. (1893). Recherches sur les hématozoaires des oiseaux. *Ann. de l'Inst. Pasteur, Paris*, XII. p. 801.
- (1895). Ueber die selbständige Bewegung der Chromosomen bei Malaria-parasiten. *Centralb. f. Bakt. u. Parasit.* XVIII. p. 374.
- SAMBON, L. W. (1908). Remarks on the Avian Haemoprotozoa of the genus *Leucocytozoon*, Danilewsky. *Journ. Trop. Med.* XI. pp. 245—8 and 325—8: XII. pp. 37—8.
- SCHAUDINN, F. (1904). Generations- und Wirtswechsel bei Trypanosoma und Spirochaete. *Arb. a. d. Kaiserl. Gesundheitsamte*, XX.
- SELIGMANN, C. and SAMBON, L. (1907). Preliminary note on a *Leucocytozoon* found in the blood of red grouse (*Lagopus scoticus*). *Lancet*, p. 829.
- SERGENT, Ed. et Et. (1904). Evolution des hématozoaires, de *Athene noctua*, d'après Schaudinn. *C. R. Soc. Biol. Paris*, LVII. p. 164.
- WENYON, C. M. (1906). Report on Dr P. N. Gerrard's preparations of a protozoon parasite found in the polymorphonuclear leucocytes of a dog. *Journ. Hygiene*, VI. p. 231.
- (1908). *Leucocytozoon neavei*. *Report of the Wellcome Research Lab. Khartoum*, III. p. 157.
- WOODCOCK, H. M. (1909). On the occurrence of nuclear dimorphism in a Halteridium parasitic in the Chaffinch. *Quart. Journ. Microsc. Sci.* LIII. p. 339.
- ZIEMANN, H. (1898). Ueber Malaria und andere Blutparasiten. Jena.