

CELL-INCLUSIONS IN THE BLOOD IN BLACKWATER FEVER.

SECOND NOTE.

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SINCE the publication of my first note on this subject [1] I have had, thanks to the kindness of Sir Almroth Wright and Sir Ronald Ross, the opportunity of studying closely blood-films from two other cases of blackwater fever. It appears desirable to place the result of the examination of this further material on record, on the one hand because it adds somewhat to the significance of the inclusions described in the first case, and, on the other, because it is obvious that, should further experience prove them to have no connection with the disease, the sooner this is established the better, in order that the ground may be cleared for research in other directions.

As the material with which the first note was concerned was derived from a single case, it was naturally encouraging to find in these new cases the same cells and the same inclusions which were described in the first. At the same time, failure to find them would not necessarily have lessened the possible correctness of my suggestions as to their nature, since it is quite conceivable that the bodies found in the cells, whether of Chlamydozoal nature or not, may only rarely be encountered in the peripheral blood, while constantly present in some other situation.

The cases will be briefly described as "Case 2" and "Case 3," that dealt with in the first note being alluded to as "Case 1."

Case 2.—This consisted of a single blood-film, stained by Giemsa's method, which was taken by Dr. Dodgson from a native "boy" suffering from blackwater fever in one of the outlying mines of the Rand, and was most kindly sent to me by my old chief, Sir Almroth Wright. The film showed a very intense degree of leucocytosis; in fact, at first sight, and with a low power, it was suggestive of spleno-medullary leucocythæmia. On closer study, however, the blood picture differed in many respects from that disease. I have no record of the blood count, but a census gave the following relative proportions of the white cells. In making this census there was considerable difficulty at times in assigning a particular mononuclear cell to one of the four heads of "hyalines," "transitionals," "neutrophile myelocytes," and what I classed in connexion with Case 1 as "endothelial cells." Too much stress, then, is not to be

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laid upon the strict accuracy of the percentages in connexion with these four groups of cells. There will be noted also a separate heading for what I have called "chrome cells," for the reasons given below.

Blood Census, Case 2.

Polynuclears	63.5 per cent
Lymphocytes	12.5 "
Hyalines	2.5 "
Eosinophiles	1.5 "
Transitionals	5.5 "
Myelocytes (neutrophile)	5.0 "
Turck's cells	2.0 "
Endothelial cells	5.5 "
"Chrome" cells	2.0 "

In addition to the above, very large numbers of nucleated red cells were present, in the proportion of one megaloblast and four normoblasts to every 100 white cells.

This blood picture makes it evident that the bone marrow in this case was gravely affected, a sign-post which might possibly indicate a useful path of exploration in the future.

No useful purpose would be served by an elaborate analysis of the census, and attention, both in this case and in Case 3, will be confined to certain special points and to the fresh features, or at least features fresh to myself, disclosed in each instance!

The cell-inclusions, described and sketched in connexion with Case 1 were found to be plentiful in this film. The cells in which they were encountered appeared to be of the same nature as those which were classed as endothelial cells in Case 1, and none were encountered in any of the ordinary leucocytes found in normal blood. The type of inclusion most commonly met with was that figured in Nos. 1 to 4 of the coloured plate, to which reference may be made. They stained a varying depth of pink or red, and were almost invariably clear cut and circular in contour. The forms were mostly homogeneous, the ring forms which were fairly common in Case 1 being rare in Case 2. None of the large forms, as shown in fig. 13 of the coloured plate, were found in this film.

In connexion with the various possibilities as to their origin, analysed in the former note, it was stated that the greatest difficulty was found in deciding whether they might not be altered or fragmented red cells which had undergone phagocytosis: this difficulty was even more apparent here, since undoubted phagocytosed reds were found in some of these large mononucleated cells and a few even in cells which contained inclusions. In spite of this fact I

still consider that the inclusions are not explicable on this ground, and the more I have seen and studied them the more do I feel convinced of this.

The granules mentioned in the former note, as occurring in many of the cells which showed inclusions, were also found in this instance, though by no means invariably. Comparison between the granules of the two cases was rendered difficult by reason of the different staining methods employed. In Case 2, however, a remarkable feature of many of the mononucleated cells was the presence, often in great numbers, of granules in the cytoplasm which I was unable to refer to any of the types of Ehrlich or to the azure granules of normal lymphocytes or hyalines. These granules were deep red, and displayed an intense affinity for the chromatin element of the dye; they were a little larger than neutrophile granules, though not nearly so large as coarse eosinophile or basophile granules. Their distribution in the cell cytoplasm was patchy; while some cells were almost filled with them, others would show only a small clump localized in one portion of the cytoplasm. These granules were encountered in cells of several different types, but were never seen in polynuclears or in eosinophiles. It is possible that they represent a stage in the history of a Chlamydozoon, but this remains at present purely conjectural.

In both this case and Case 3 certain curious cells were found which I have never previously encountered, either in blood-films or in plates illustrating cytological work. These cells were not uncommon and could be readily distinguished from all others, even with a low power, by the deep chromatin tint of the whole cell; they were of the size of ordinary polynuclears and their nuclei were sometimes of that type, sometimes mononuclear. The cytoplasm appeared more or less completely filled with material which showed the chromatin reaction and, in almost every instance, the red colour was most intense at the periphery, giving the cell an appearance of being capsulated. In other instances the partial disruption of the cell permitted it to be seen that the red-staining material consisted of a mass of chromatin bodies, sometimes of quite irregular shape and size, but occasionally showing a tendency to ring form. It is difficult to convey the appearance of these cells apart from a coloured sketch, but the accompanying photographs (see figs. 1 and 2) give a fairly good idea of their general appearance. In each instance a cell was selected which was close to an ordinary polynuclear that the latter might serve as an index of comparison. For the sake of avoiding frequent periphrases I may perhaps be pardoned labelling

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them provisionally "chrome cells." They will be mentioned again in connexion with Case 3.

Case 3.—The two stained films from this case were lent to me by Sir Ronald Ross, to whom I am further indebted for his interest in and criticism of my former note. These films have the further interest of being derived from the interesting case of which he published particulars, with Drs. D. Thomson and G. C. E. Simpson, at the end of 1910 [2]. In view of the full details there given, I need only mention that this case occurred under their own observation at Liverpool, and led them to the conclusion that neither the hæmoglobinuria nor the subsequent attacks of fever from which the patient suffered could be attributed to the toxins of malarial parasites.

Each of the films had been stained by Giemsa's method. As recorded in the chart of the case, 3,000 white cells per cubic millimetre were found on the day on which the films were taken, which was at the commencement of the third attack of fever, during which there was no hæmoglobinuria and no malarial parasites were found. For the sake of uniformity I may give the result of my own census of the white cells:—

<i>Blood Census (Case 3).</i>					
Polynuclears	63·0 per cent
Lymphocytes	13·5 "
Hyalines	7·0 "
Eosinophiles	1·5 "
Transitionals	7·0 "
Myelocytes (neutrophile)	3·0 "
Endothelial cells	4·5 "
"Chrome" cells	0·5 "

In this case, possibly owing to the leucopenia which existed, and the smaller number of endothelial cells, inclusions of the type found in Cases 1 and 2 were rare, only two or three cells showing them; those found were of the small homogeneous type figured in Nos. 1—4 of the coloured plate.

The special interest, however, of these two films was: 1st, that each showed a fair number of the "chrome cells" described in connexion with Case 2 and precisely identical with those in appearance, size and staining reaction; and, 2nd, that, in the case of one of the films, somewhat more deeply stained than the other, two cells were encountered which appear to suggest a possible connexion between the inclusions, so frequently alluded to, and the "chrome cells." Each of these cells I have photographed (see figs. 3 and 4), and the reproduction will show that, in each instance,



FIG. 1.

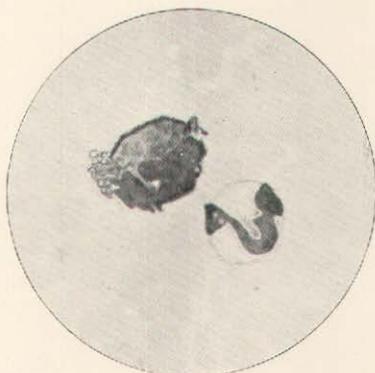


FIG. 2.

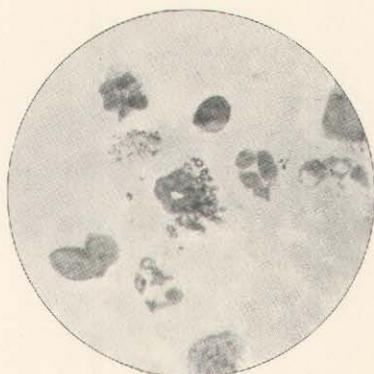


FIG. 3.

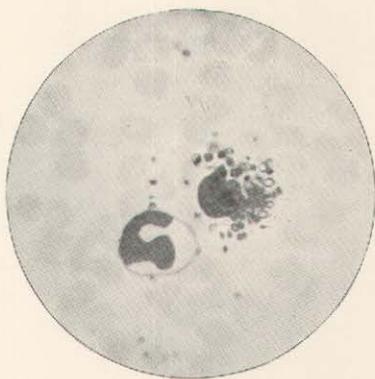


FIG. 4.

To illustrate "Cell-inclusions in the Blood in Blackwater Fever."

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the cell wall has been ruptured and has liberated a number of deep-staining chromatin bodies, of varying size and showing a pronounced tendency to ring form. Contrasting these with the photograph of the "chrome cell" in fig. 3, where some of the chromatin contents of the cell are sufficiently isolated to show a tendency to similar ring form, it is hard to resist the impression that the well-marked bodies seen in the disrupted cells in figs. 3 and 4 represent a further stage in development of the elements which compose the red staining mass which fills the protoplasm of the "chrome cells" found in Cases 2 and 3. Again, as far as one can judge by staining reaction, size and shape, criteria of admitted insufficiency, these chromatin rings are the same as the ring forms described in the endothelial cells in Case 1. I may add, since I have used the term "ring form," that the bodies in question show no resemblance to the ring forms of malarial parasites and could not possibly be confused with the latter.

As to the possible occurrence of similar inclusions in other conditions, Dr. G. C. Low [3] has recently recorded that he has seen bodies, apparently similar to those which I described, first in some cases of fever from Borneo, and second, in the blood of pellagra cases recently brought home by Dr. Sambon from Italy. I have not seen the specimens alluded to in Dr. Low's article, but I had recently an opportunity of discussing the subject with him, and of showing him a few of the inclusions from Case 1, and I think it possible that the smaller granules which he noted and has figured in mononuclear cells may have been azure granules. In these three cases of blackwater, azure granules were common in both lymphocytes and hyalines, but were quite distinct from the inclusions. It is quite possible that the larger inclusions of which Dr. Low speaks may resemble those in question here, but without seeing them I can express no opinion.

Major W. S. Harrison has, however, shown me a blood-film from a case of chronic malaria, in which there were present in large cells, of mononuclear type, homogeneous, pink-staining inclusions, which I agree with him in regarding as identical with the blackwater ones. In this case he said there was no history of blackwater and no probability of its development, but it appears to me of some significance that the officer in question had recently returned from Nigeria, where he contracted his malaria, and that blackwater fever is common in that country. Making the large assumption that the inclusions may eventually prove to be, or to be due to, the specific cause of this disease, it is quite probable that

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it is only under certain, as yet unknown, conditions that they give rise to the symptom of hæmoglobinuria.

Should the further material which I hope to receive before long disclose any facts of fresh interest, either favourable or inimical to my views, they will form the subject of another note; the present one, as may be seen, is no more than a progress report, and any attempt to expand the hypothesis put forward in my former article appears unjustifiable in view of the paucity of the material.

REFERENCES.

- [1] Lieutenant-Colonel Sir WILLIAM LEISHMAN, R.A.M.C. "Cell-inclusions in the Blood of a Case of Blackwater Fever," *JOURNAL OF THE ROYAL ARMY MEDICAL CORPS*, vol. xviii, p. 493, 1912.
- [2] Sir RONALD ROSS, D. THOMSON AND G. C. E. SIMPSON. "A Case of Blackwater Fever followed by a peculiar Relapse without Hæmoglobinuria or detectable Plasmodia," *Annals of Tropical Medicine and Parasitology*, vol. iv, p. 308, 1910.
- [3] G. C. Low. "Cell-inclusions in the Blood of Blackwater Fever and other Tropical Diseases," *Journal of Tropical Medicine and Hygiene*, June 1, 1912.