

## A NEW FORM OF PARASITE FOUND IN THE RED BLOOD CORPUSCLES OF MOLES.

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AT the present time when the parasitic protozoa found in the blood are receiving great attention, it may be of interest to describe a form which appears not to have been noticed hitherto.

In examining the blood of various animals for the presence of *Piroplasma* the organisms about to be described were discovered in the blood corpuscles of a mole. In this specimen a considerable number of the red blood corpuscles were found to be infected, and it was therefore thought that by investigating a number of moles other infected specimens might be found.

In all the blood of 102 moles, caught in the neighbourhood of Cottenham, near Cambridge, was investigated. The moles were brought to the laboratory alive, and smear preparations were made from their hearts' blood and from the various organs. The organs were also preserved for histological purposes.

Parasites in greater or lesser numbers were found in the red blood corpuscles of ten (10%) of these moles.

### *Morphology of the parasites.*

The morphological characters of the parasites are best seen in specimens stained by Giemsa's method. They also stain well by Leishman's method.

The parasites appear as longer or shorter rods of irregular contour lying within the red blood corpuscles. The rods are never straight, and many have a marked curve in them, often near one of the extremities.

One or both ends of the longer forms are enlarged, giving the organisms a wedge- or club-shaped appearance. Some of the medium sized forms are definitely dumb-bell shaped, the very small ones almost round.

By Giemsa's stain the protoplasm of the organisms stains a light blue colour, with darker areas at the enlarged ends. In most of the longer forms some portions of the protoplasms stain more darkly than others, giving the organisms a banded appearance. Comparatively large masses of chromatin are frequently found usually situated near one of the dilated ends of the longer or dumb-bell shaped forms. The larger chromatin masses are round or oval in shape. Not infrequently small dots of chromatin can be seen near the ends of some of the organisms. Some of the longer forms, and the majority of the smaller, show no indications of the presence of chromatin.

The longest specimens vary from  $\frac{1}{4}$  to  $\frac{1}{3}$  of the length of the diameter of the red blood corpuscles, and the shortest are about  $\frac{1}{3}$  of the length of the long forms. The length varies between  $1\mu$  and  $1\mu$ .

Parasites are occasionally found free in the plasma. In such cases they are usually in groups as if recently liberated by the rupture of a corpuscle. Isolated free forms were rarely seen.

*Degree of infection in the 10 infected moles.*

The number of infected red blood corpuscles in smears from the heart's blood varied greatly in the 10 infected moles. In the following table they have been numbered according to the degree of infection.

*Table showing the degree of infection.*

Mole	I.	About	1%	of all red corpuscles infected.	
"	II.	"	3%	" " "	
"	III.	10 infected corpuscles found containing	8, 9, 22, 28, 30, 30, 32, 32, 33 and 42		
				parasites.	
"	IV.	4	"	"	6, 7, 20, 32 parasites.
"	V.	2	"	"	1 and 7 parasites.
"	VI.	2	"	"	18 and large numbers of parasites.
"	VII.	2	"	"	18 and 22 parasites.
"	VIII.	1	"	"	40 parasites.
"	IX.	1	"	"	Very numerous parasites.
"	X.	1	"	"	" " "

It will be seen from this table that in the blood of moles III to X, in which very few infected corpuscles were found, the number of parasites within such corpuscles was usually very high.

In smears taken from the heart of mole I the number of parasites contained in 1321 infected red corpuscles were counted, and the following table gives the total number of corpuscles containing various numbers of parasites, and their relative proportion to each other.

*Table showing condition of infected corpuscles in films from the heart blood of mole I.*

No. of parasites in infected corpuscle	No. of infected corpuscles counted	Percentage of each variety	No. of parasites in infected corpuscle	No. of infected corpuscles counted	Percentage of each variety
1	12	·98	19	8	·65
2	7	·52	20	15	1·12
3	9	·68	21	3	·22
4	14	1·06	22	5	·38
5	8	·65	23	1	·07
6	61	4·60	24	1	·07
7	64	4·83	25	2	·15
8	169	12·79	30	2	·15
9	132	9·99	38	1	·07
10	181	13·70	42	1	·07
11	122	9·23	45	2	·15
12	184	13·85	46	1	·07
13	94	7·11	50	2	·15
14	96	7·26	53	2	·15
15	36	2·72	56	1	·07
16	39	2·95	58	2	·15
17	19	1·43	79	1	·07
18	24	1·81		<u>1321</u>	

It will be seen that very few corpuscles were encountered containing between 1 and 5 parasites. Of all the infected corpuscles 94% contained between 6 and 20 parasites. More than 20 parasites were found in about 2% of the infected corpuscles. As far as it was possible to count them, 79 appeared to be the highest number of parasites found in one corpuscle.

Corpuscles containing very large numbers of parasites were slightly enlarged, but those containing moderate numbers, up to 20, were not enlarged.

#### *Smears from the organs.*

No infected corpuscles were found in smears made from the liver lung, spleen, kidney, or marrow of moles III, IV, V, VI, VIII, and IX. A few infected cells were found in the lung and spleen smears from moles I, II, VII, and X, in liver smears from moles II and III, and kidney

smears from moles I and II. Very numerous infected corpuscles were however encountered in smears from the liver of mole I.

In all cases the number of the parasites within the infected corpuscles was large, and corpuscles containing small numbers of parasites were never met with except in liver smears from mole I. Smears made from the marrow obtained from the shaft of the femur very seldom showed infected corpuscles.

For the sake of comparison with the previous table the number of parasites within 206 infected corpuscles were counted in liver smears from mole I.

*Table showing condition of infected corpuscles found in smears from the liver of mole I.*

No. of parasites in infected corpuscles	No. of infected corpuscles counted	Percentage of each variety	No. of parasites in infected corpuscles	No. of infected corpuscles counted	Percentage of each variety
2	4	1·8	12	27	13·1
3	1	·4	13	16	7·7
4	8	3·9	14	9	4·3
5	2	·9	15	7	3·4
6	10	4·8	16	7	3·4
7	11	5·3	17	2	·9
8	23	11·1	18	2	·9
9	28	13·6	19	1	·4
10	33	16·0	20	1	·4
11	13	6·3	48	1	·4

#### *Autopsies.*

No marked macroscopical lesions were found by which the organs of infected moles could be distinguished. Enlargement of the spleen was present to a marked degree in 12 moles, and of these one was found to be infected. A nodular cirrhotic appearance of the liver was noticed in two moles and cysts in the liver in two others. None of these were infected.

#### *Histology of the organs.*

The organs of all the infected moles were examined and compared with the organs of normal moles. No changes were discovered in the spleen, kidney, or heart muscle. In the lungs large collections of polymorphonuclear leucocytes were in some cases seen around the bronchi and larger vessels. In some of the infected moles there was a bronchopneumonic condition. Similar changes were seen, however, in the lungs of moles in whose blood no infected corpuscles were found.

The livers of all ten infected moles and of eight apparently healthy moles were examined. All but two of these were infected with *Coccidia* (90 %) in the bile-ducts. No other changes were found in the livers of moles III to X.

In the case of moles I and II, however, which showed the greatest degree of infection in the blood, the livers exhibited marked changes, probably therefore resulting from infection with this organism.

*Liver of mole I.*

Throughout considerable areas the substance of the liver appeared to be normal, and when stained by haematoxylin and van Gieson's stain the cells were well defined with yellowish protoplasm, and compact, round, blue, darkly staining nuclei. The appearance of the normal liver is shown in Plate XIV, Fig. 1.

Several degenerated, badly staining areas can, however, be seen in sections cut from various parts of the liver. In passing from the healthy liver substance towards the degenerated portion the following changes are seen. First the protoplasm of the cells begins to stain poorly, and the cell outlines become indistinct. A little further the cell outlines are lost, and many of the nuclei are badly stained. Here and there, however, in these areas are groups of cells or isolated cells, which still stain well and show apparently normal nuclei. Plate XIV, Fig. 2, represents the appearances seen in such areas as have just been described. Still further the remains of the nuclei can only be made out with difficulty, and small, rounded, clear spaces can frequently be seen in the granular remains of the cell substances. A badly staining granular mass, without a trace of cells or nuclei, is usually to be found in the centre of the affected area.

Into some of these degenerated areas haemorrhages had taken place.

In the liver of mole II the condition was much the same as in that of mole I, but was not so advanced. Completely degenerated areas in which the cells had totally disappeared were not found. In one lobe of the liver extensive haemorrhages, both old and recent, had taken place into some of the degenerated areas.

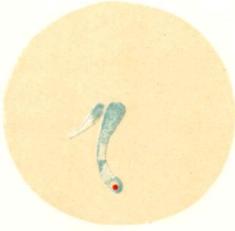
In suitably stained sections no bacteria were found, and sections treated by Leishman's and Giemsa's methods did not reveal the presence of parasites.

*External animal parasites on moles.*

The external animal parasites were recorded in all the moles which were investigated. Fleas and lice were found on all of them, mites on a considerable number, but ticks were never seen.

The parasites described do not appear to have any relation to *Piroplasma*. They differ markedly in morphology and in the numbers which are found within infected corpuscles. In the peripheral circulation in the course of the disease due to *Piroplasma canis* of all infected corpuscles those containing a single parasite are the most commonly found, but in this disease corpuscles containing less than six parasites are rare. In the former disease the parasites are usually found in even numbers in the greatly infected corpuscles, whereas in this case corpuscles containing odd numbers of parasites are nearly as common as those containing even numbers. In the former disease great multiplication takes place in the marrow, but very few parasites were encountered in smears made from the marrow of the infected moles.

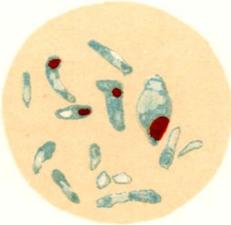
Owing to the difficulty of keeping the animals alive for any time no observations were made on the transmission of infection, or the effects or duration of the disease.



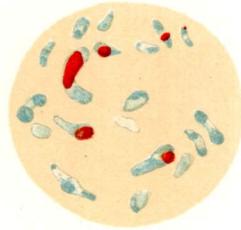
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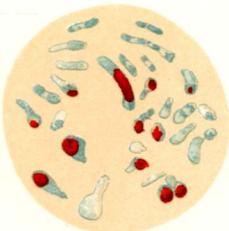
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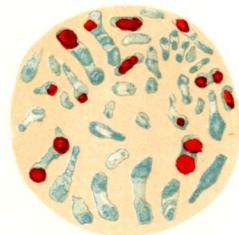
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5.



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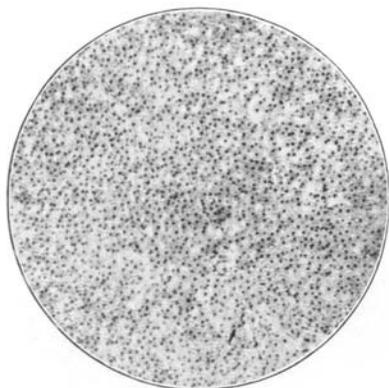


Fig. 1.

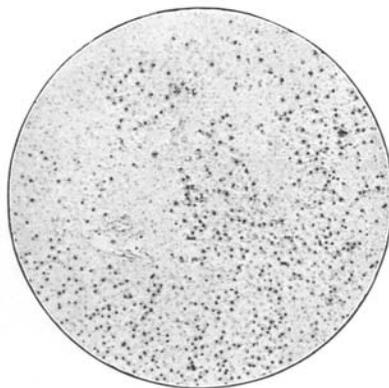


Fig. 2.

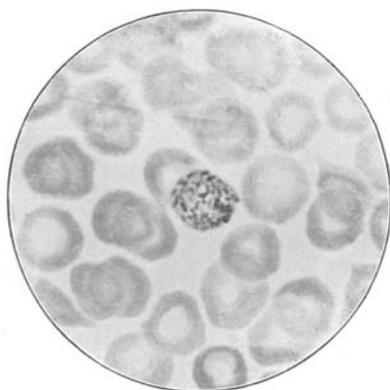


Fig. 3.

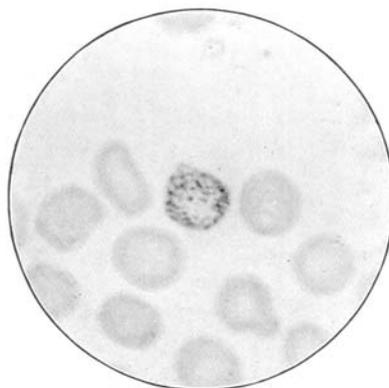


Fig. 4.

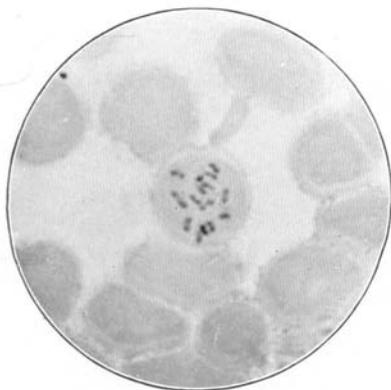


Fig. 5.

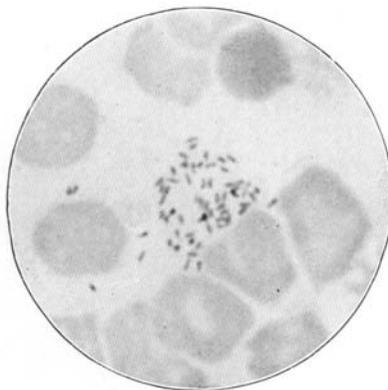


Fig. 6.



## EXPLANATION OF PLATES XIII AND XIV.

**PLATE XIII.** The figures represent the parasites as seen within the corpuscles of mole II in specimens stained by Giemsa's method. The differentiation between the chromatin masses and the rest of the protoplasm is well shown.

Fig. 1. Red blood corpuscle containing 2 parasites.

Fig. 2. " " " " 9 "

Fig. 3. " " " " 14 "

Fig. 4. " " " " 19 "

Fig. 5. " " " " 29 "

Fig. 6. " " " " 39 "

**PLATE XIV.** Fig. 1. Photograph ( $\times 100$ ) of normal liver substance of mole I.

Fig. 2. Photograph ( $\times 100$ ) of a portion of the liver of mole I near a degenerated patch. On one side of the specimen a considerable number of nuclei are still distinctly stained and the cells are distinguishable. Passing towards the degenerated area the cells become less distinct and only small numbers of the nuclei distinctly stained. The most advanced stage of degeneration is not here represented.

Fig. 3. Photograph ( $\times$  about 1500) of a greatly infected red blood corpuscle partly overlying a normal corpuscle. About 50 parasites are present in this corpuscle. (Stained by Giemsa's method.)

Fig. 4. Photograph ( $\times$  about 1500) of another greatly infected corpuscle. (Stained by Giemsa's method.)

Fig. 5. Photograph ( $\times 2000$ ) of a red blood corpuscle containing 15 parasites. (Stained by Leishman's method.)

Fig. 6. Photograph ( $\times 2000$ ) showing 59 free parasites in a group. (Stained by Leishman's method.)

I am indebted to Mr Walter Mitchell for these excellent microphotographs.