CANINE PIROPLASMOSIS. II.

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IN a previous paper¹ one of us has summarized what is known with regard to canine piroplasmosis, and presented the results of infection experiments carried on in Cambridge with infected ticks (adult *Haemaphysalis leachi* Andouin) imported from South Africa. In the present paper we propose to describe and figure the parasite as observed in stained preparations, and to state what is known regarding its biology. The supply of infected ticks having unfortunately been exhausted and our last attempt at the transmission of the disease by infected blood inoculation having failed, we are obliged, for the present, to postpone a detailed description of the living parasite.

Piroplasma canis.

The history of the discovery of the parasite has been given in the paper above referred to (p. 223), nevertheless there are still a number of interesting facts to be considered which are stated in papers by other authors². It appears advisable for this reason to briefly summarize what is known with regard to the parasite.

¹ Nuttall, G. H. F. (rv. 1904), Canine Piroplasmosis, I., *Journ. of Hygiene*, iv. pp. 219—257, Plates XII—XIII, 8 Temperature Charts.

² The papers here cited are given in the bibliography accompanying the previous paper (pp. 250—252).

The position of the Piroplasmata among the Protozoa has not as yet been satisfactorily determined. The generic name Piroplasma was given to them for the reason that they frequently are pear-shaped. They are endoglobular parasites, growing and multiplying within the red blood corpuscles. They do not produce melanin granules. The parasites are also encountered in a free state in the plasma. The existence of amoeboid forms was first noted and figured by Piana and Galli-Valerio (1895 and 1896), and has been repeatedly observed since by various workers. Irregular, spherical, and typical pear-shaped parasites have been also observed in the disease as studied in different countries. It is premature to assume that the *Piroplasma canis* of South Africa is identical with that of Europe, or India, nevertheless there is at present no proof that they are different species.

Piroplasma canis in Italy is supposed to be conveyed by *Ixodes reduvius*. It is found in 3—4 % of red blood corpuscles; free forms also occur. The parasites measure 3·5—2·5 μ . Piana and Galli-Valerio stated that the corpuscles contained 2—5 pyriform parasites.

Piroplasma canis in France is supposed to be conveyed by *Dermacentor reticulatus*. The parasites (Almy, 1901, p. 375) are found in a variable percentage of red blood corpuscles, and also as free forms. They measure 2—4 μ . At the beginning of the disease only single large, round parasites are found in the infected corpuscles, later from 2—16 occur in the corpuscles in the acute cases. Under these conditions the parasites are small, irregular, and seldom pyriform (Nocard and Motas, 1902, p. 265). Many parasites, mostly small and spherical, occur in the heart and internal organs (*Ibid.* p. 269). Groups of 10—12, apparently free, parasites seem to lie in spaces which are probably the remains of corpuscles (Marchoux, 1900).

Piroplasma canis in South Africa is certainly conveyed by *Haemaphysalis leachi* Andouin in the adult stage (see the previous paper). Its essential characters are similar to those of the parasite observed in Europe. This is the parasite which we describe in the following pages.

Time when the Parasites appear in the Blood after experimental Infection.

According to Nocard and Motas (1902, p. 273) infected corpuscles are rarely encountered within 36 hours after inoculation. Even after intravenous inoculation they usually do not appear until the end of 48 hours.

Robertson (1902 a, p. 331) in South Africa did not find the parasites

microscopically in the blood before the fourth day after subcutaneous inoculation. That they were present earlier was proved by the fact that blood taken from a dog three days after inoculation proved infective. The parasites were present singly or in pairs. In our experiments the parasites, whether infection was produced by means of ticks or by blood inoculations, made their appearance in appreciable numbers immediately before the onset of fever.

Persistence of the Parasites in the Blood.

We found that the number of parasites present in the peripheral blood varied considerably, irrespective of the attacks of fever. From this it appears reasonable to conclude that the multiplication may be taking place in the internal organs when few parasites are present in the peripheral blood. The persistence of the parasites in the blood of "salted" dogs (animals which have recovered), has already been referred to in the previous paper under the heading of Immunity (p. 246). We have had the opportunity of studying acute cases only, in which the parasites are moderately or very plentiful. Nocard and Motas (1902, p. 265) state that the parasites are scarce in chronic, as compared with acute cases.

Structure of the Parasite.

Stained by Leishman's method, the parasite appears as a blue-staining body containing usually a single homogeneous nucleus (karyosome) which stains a vivid red. The blue-staining protoplasm frequently presents a delicate vacuolated or trabecular structure, and is chiefly condensed about the periphery. These appearances are especially marked in the larger forms. Young spherical parasites often appear as "rings" resembling young malarial parasites in man, in which the pink colour of the blood corpuscle may be seen in the central clear space within the blue-staining ring. In dividing forms a delicate protoplasmic thread may persist for some time as a connecting link between daughter cells as shown in Figs. 12, 15, 28, 55, 63, and 64. Where paired pyriform parasites occur this connecting thread is as a rule situated at their pointed extremities. In other cases the connecting threads are somewhat irregularly distributed, as in Figs. 25, 26, and 41. In the latter figure and in Fig. 42 the parasites appear to be amoeboid. Occasionally (as shown in Figs. 24, 43, and more especially Fig. 40)

apparently detached masses of blue-staining protoplasm are encountered in the corpuscle. In some cases these appeared to be connected by very delicate faint threads to each other and the parasite. In forms which appear to be the amoeboid (Plate IX, Figs. 35—36) portions of the protoplasm stain a more intense blue than do others. At times the processes may appear fragmented as in Fig. 35. The chromatin mass is usually spherical and is generally situated in the centre of the main mass of protoplasm, although frequently placed excentrically and at times peripherally. In some cases it appears to protrude from the protoplasm (Figs. 1—2, 35—39). The chromatin mass is frequently surrounded by an unstained halo, representing what appears to be a vesicular nucleus (Figs. 1, 13, 19, etc.). In dividing forms the chromatin mass assumes an elongated form before breaking up into two portions (Figs. 2, 3, 11, 57), and this separation of the nuclear masses immediately precedes the division of the protoplasm (Figs. 2, 4, 5, 6, 10, 12, 16, 18, 50—54). When about to divide the chromatin mass frequently takes up a peripheral position (Figs. 2, 3, 9, 11). The smallest chromatin masses measured about $\cdot 2 \mu$, the largest $\cdot 6$ — $\cdot 8 \mu$ in length, in the same specimen. Undoubtedly the size of these masses varies according to the method of staining employed.

Resemblance of the Parasite to other Piroplasmata.

It is obvious from the appearance of the canine parasite that it offers a great resemblance to other Piroplasmata, notably that of Texas fever (*P. bigeminum*) in cattle and that of Redwater in sheep (*P. ovis*). According to Marchoux (27. i. 1900) *P. canis* in France is larger than *P. bigeminum*, and differs in being less constantly bigeminate and in the fact that single parasites occur more frequently. In the former disease corpuscles containing 10—12 parasites may occur, as well as extra-cellular forms in pairs or in groups up to 8—10 individuals. On the other hand Piana and Galli-Valerio (1895), who first discovered the parasite in Italy, found it to resemble the Texas fever parasite so closely that they named it *Pyrosoma bigeminum*, var. *canis*, and Nocard and Motas (1902, p. 275) say that the French parasite is morphologically identical with that of Texas fever. The resemblance of the South African parasite to *P. bigeminum* is also well-marked, but Robertson (1902, a, p. 331) states that it is larger, and is more oat-shaped than pyriform. From specimens, which one of us has studied, the dog parasite does appear to differ from the Texas fever organism as stated by Robertson.

Size of the Parasites.

The canine parasites observed in Italy by Piana and Galli-Valerio (1895) measured 2·5—3·5 μ in their largest diameter, those observed by Marchoux (1900) in France 2—4 μ . Nocard and Motas (1902, p. 269) state that the parasites are larger in young dogs, which are more susceptible and may fill as much as half of the corpuscle. In adult dogs near the end of the disease they may be very small, being almost reduced to a nucleus.

Our measurements show the African parasite to be somewhat smaller. The smallest specimens measure from ·7 to 1·2 μ , the largest about 3·6 μ . Rarely pyriform bodies measuring up to 4·5—5 μ were encountered. The variations in size are however considerable, and are best seen by reference to Plate IX, where the red blood corpuscles of normal size (Figs. 1—12, 58 etc.), measuring on an average 7·2 μ , may be used as a standard for comparison. The large gamete-like bodies (Figs. 58—62) not hitherto described by other authors, measured 10·3—10·7 μ in length by 1·4—1·7 in width in their greatest diameter. All our measurements were made on stained preparations.

The above measurements (excepting the last) may be compared with those given for *P. bigeminum*. Smith and Kilborne (1893) give the size of the smallest parasites as 0·5 μ . Laveran and Nicolle (1899) working about Constantinople found the smaller parasites measured 1—2·5 μ , the largest up to 3·5 by ·8—1·2 across the base. Ziemann (1902) in Venezuela found small parasites measuring ·75—1 μ , and larger ones up to 2·5—3 μ . The measurements for *P. ovis* show that the parasites are about the same size. Bonome (1895) in Italy for example found parasites measuring from 1—3 μ up to 2—2·5 μ , and Laveran and Nicolle (1899) give the measurements for the parasites observed in sheep near Constantinople at 1—1·5, and occasionally 2 μ (in the spleen).

Parasites viewed in Fresh Blood.

Where the parasites are not numerous, they are difficult to find in fresh films. Nocard and Motas (1902, p. 265) state that they are best observed immediately after the fall of fever, the only time when they are numerous and motile. Examination is facilitated by diluting the blood with aqueous humour or saline solution in a hanging drop and using the warm stage. The infected corpuscles appear somewhat enlarged and pale, the parasites are irregular and have a dark contour, with a

central refractive portion. The French observers saw amoeboid bodies with processes, sometimes 2 or 3 in number, protruded toward the periphery of the corpuscle. Rapid movement caused the corpuscle to revolve on its axis. At times the parasite appears globular, and non-motile, and lies centrally in the corpuscle, and small, very actively moving bodies are seen dancing about it. They state that amoeboid motion quickly ceases after the febrile stage, the parasites then remaining spherical and quiescent. Free parasites are difficult to distinguish from cellular detritus, and are best seen in saline tinted faintly with methylene blue. This method is also an aid in the examination of intracellular parasites, as the Piroplasmata become faintly stained without being killed. These observations naturally explain the variations in form observed in stained blood films.

In our dogs, infected with the South African form of the disease, parasites were observed a few times in fresh blood films, but unfortunately the parasites were very scarce on these occasions, and we did not pursue the matter further at the time. We have since lost the strain, but we hope to make further investigation on this point.

Multiplication of P. canis.

It is obvious that one mode of multiplication is by direct fission, in the manner represented in the successive Figures 1—8, and 9—32 in Plate IX. The parasite, having penetrated the corpuscle, grows in size and then successively divides (usually) into 2, 4, 8, and 16 individuals. Owing to irregularities in division, groups of 3, 5, 6, 7, 9, 10, 12, 14, 15, 18, and even 21 may be encountered. Division forms giving multiples of 2 (2—16) are by far the most numerous, and the usual number reached before the corpuscle ruptures and liberates its contents, appears to be 16. The corpuscles containing numerous parasites, 8—16 or more, are more frequently encountered in the blood from the internal organs, notably the brain, lymphatic glands, bone-marrow, etc., and it is doubtless due to their accumulation in the brain capillaries that the animals become comatose in a manner similar to that which has been observed in severe cases of human malaria¹. Robertson (1902, a, p. 332) has already noted this blocking of the cerebral capillaries by infected

¹ Bowhill gives two photomicrographs of *P. canis* on Plate III, Figs. 11—12 of this volume. Fig. 11 shows a group of parasite-containing corpuscles in a kidney capillary, Fig. 12 eight parasites with large chromatic masses lying in a corpuscle. See also Vol. IV, Plate XI.

corpuscles. In smears from the internal organs groups of 8—16 or more parasites are frequently encountered, sometimes embedded in the faintly-staining detritus of a ruptured corpuscle, while in other cases all traces of corpuscular substance have disappeared. A group of free parasites is shown in Figure 46. The parasites decrease in size as their number increases within the infected corpuscle. The small free parasites correspond in size with the youngest forms encountered in corpuscles. From this we may conclude that the parasites which escape from ruptured corpuscles again attack and penetrate new corpuscles. In one case we observed two parasites lying in a nucleated red blood cell (Fig. 45).

Most authors are agreed that this is a mode of multiplication in other *Piroplasmata*. Other modes of multiplication are unknown. Marchoux (27, I. 1900) working with the French parasite represents multiplication by fission of single spherical forms into 2—4—6—12 pyriform parasites, when the corpuscle ruptures and sets them free. He states that single parasites in corpuscles are usually round or oval, pairs of parasites usually pyriform. Multiplication along the lines indicated has also been described by Nocard and Motas (1902, p. 269), who figure the French parasites. They state that multiplication is most active during fever periods and that it is not well observed in the peripheral blood, where, they assume, it takes place too rapidly and irregularly. They figure two series of multiplication forms from the blood and internal organs respectively, which are in general agreement with our figures above described. According to these authors our Figure 47, with its central spherical nucleus, represents a parasite at rest. They consider that the nucleus next elongates and moves to the periphery, where it divides as we have shown in Fig. 2; that the nuclei next move to opposite poles as in Fig. 5 and fission takes place as in Fig. 6. They give some figures in a series of drawings they made from peripheral blood showing curved rod-like masses of chromatin lying close to the periphery of the parasites. We have failed to observe such forms. On the other hand they do not figure dividing pyriform parasites such as we represent in Figs. 9, 10, 11, 53, 54. Their reason for stating that the globular forms represent "*l'état normal du parasite*" does not appear quite clear to us. They observed 1, 2, 4, 8, 16, and 18 parasites in infected corpuscles, and 3, 6, 12, and 14 if one failed to develop. In the vast majority of cases they noted parasites in even numbers. They found kidney smears to show the greatest number of corpuscles containing numerous (12—14—16—18) parasites. During

the febrile stage of the disease they encountered round parasites with elongated excentric nuclei, measuring $\frac{1}{2}$ the length of the margin of the parasites—these represent the rapidly multiplying forms above noted. According to these authors the most active multiplication, judged by the number of infected corpuscles observed, takes place in the following order; kidney, spleen, liver, marrow, lung, heart, lymphatic glands, intestinal mucosa, central nervous system. Robertson (1902, a, p. 331) in South Africa also noted the great number of parasites in smear preparations made from the liver and spleen.

Free Parasites.

We have noted that the parasites escape into the plasma from the disintegrated corpuscles and that they may at times be found in groups similar to those seen in corpuscles. These groups soon break up, and then the parasites are found singly or in coherent pairs as typical bigeminate pyriform or ovoid bodies. Very small ovoid or spherical bodies are also encountered in a free state (Figs. 46—58). Robertson (1902, a, p. 331) especially noted free parasites in the blood in advanced stages of the disease. He gives rough sketches of very small size in which the free parasites appear to be of variable shape and size, all single, spherical, irregular or pyriform. Nocard and Motas (1902, p. 269) note the fact that free parasites may appear somewhat larger than the endoglobular forms. We have made similar observations.

Sexual Forms?

In Volume iv of this *Journal* (Plate XI) Bowhill and Le Doux stated that they observed what appeared to be flagellate bodies in the blood of dogs suffering from Piroplasmosis in South Africa. We have not as yet observed such forms which are similar to bodies noted by Lignières (1900) in Piroplasmosis in cattle in Buenos Aires. Bowhill has since described in the current volume of this *Journal* (p. 16, and Plates I—III), similar bodies in connection with *Piroplasma equi*, and *Piroplasma bigeminum* of South African Redwater. Possibly these bodies represent microgametes.

On the other hand we have observed a body which is so strikingly suggestive of a gamete that we especially draw attention to it. These bodies were encountered in the blood of the first dog experimentally infected in Cambridge, being found on the 4th, 5th, and 10th days after

the onset of fever. These bodies which somewhat resemble the crescents of the human aestivo-autumnal malarial parasites are shown on Plate IX, Figs. 58—62. Only four have been encountered in the peripheral blood after prolonged search in stained films. Three others have been seen in smear preparations from the organs, one in a lung smear, and two in kidney smears. These bodies are sausage shaped, of fairly uniform width, and rounded, or tapering at their extremities. Some are markedly vacuolated. In these cases, when the vacuoles happen to lie upon red blood corpuscles (Fig. 58) the contour and colour of the corpuscle can be seen through the vacuole. In three out of the four specimens figured the chromatin is almost entirely concentrated in the middle of the body, and in two appears to be of loose texture. In Fig. 58 particles of chromatin of fairly uniform size are distributed about irregularly, mostly near the periphery. These bodies measure 10.4 to 10.7μ in length by 1.4 to 1.7μ in width. They are faithfully reproduced in the figures as they appeared in films stained by Leishman's method. These bodies certainly merit further investigation. It is at present impossible to say if they stand in any relation to the bodies shown in Figs. 43—44, the latter of which is very peculiar. Nocard and Motas figure bodies somewhat like Fig. 43 showing 1—4 fine blue-staining processes, evidently amoeboid bodies.

Behaviour of the Parasites outside the Body.

Working with the French parasite Nocard and Motas (iv. 1902, p. 274) found that it remained alive and virulent in blood preserved in the dark and cold (during winter) for 25 days. Blood kept in the dark at summer temperature lost its virulence after 14 days. Blood heated to 43°C. for 90 minutes was still virulent, but became non-virulent after being heated to 44°C. for 75 minutes, or 45°C. for 60 minutes, or 50°C. for 30 minutes. They state that heating to 45°C. or above for an hour (p. 287), renders the blood non-virulent, whilst heating to 44°C. for the same time lowers its virulence slightly. Animals inoculated with the latter die more slowly than usual. Heating the blood to 44°C. for 75 to 90 minutes rendered it non-virulent.

Attempts at cultivation (p. 275) have failed on media composed of defibrinated dog's blood, serum rich in haemoglobin, and blood rendered incoagulable by the addition of leech-extract. Leeches which had fed on highly infected dog's blood have been kept at 22°C. , but after a week only the nuclei of the parasites could be detected.

Observations on defibrinated blood kept at 37° C. showed that the infected corpuscles were taken up by leucocytes. The parasites became spherical, their nuclei took up a central position, and their protoplasm appeared to gradually shrivel away and set free the nuclei. Similar changes occurred in blood kept at 22° C. but more slowly, so that a little protoplasm still remained surrounding the nuclei after 5—6 days. Nuclei resembling micrococci were alone found within degenerated corpuscles after some weeks.

Robertson (5. iv. 1902, p. 685), working with the South African parasites, states that he unsuccessfully tried in many ways to preserve blood in a virulent condition outside the body. He found that such blood either killed or did not kill, but in the latter case did not confer immunity. One of us successfully infected a dog with blood which had been kept in ice for 24 hours in the dark. Inoculations with older blood were not tried. Similar changes to those described by Nocard and Motas were observed in stored blood.

Changes in the Red Blood Corpuscles.

The infected corpuscles do not alter much in size. Those containing 1—6 parasites are about the same size as normal corpuscles, namely 7.1 μ . Corpuscles containing 7—10 parasites measure on an average 8 μ , while those containing 11—16 parasites measure 9 μ . These figures are based on a large number of measurements, the mean being taken in cases where the corpuscles were altered in form. Corpuscles containing 12 or more parasites, as is indicated in Plate IX, Figs. 26—31, show a tendency to stain more feebly and to lose their round contour. As the disease progresses, nucleated red cells appear occasionally in the peripheral blood, and pale-staining, often enlarged corpuscles are encountered. In fresh blood these corpuscles may at times be almost invisible owing to their containing little haemoglobin. That the infected corpuscles soon begin to undergo some degenerative change is indicated by the fact that they are taken up by leucocytes upon which they must exert a positive chemiotactic action, possibly by the diffusion of their substance into the plasma. The changes in the blood count observed by Nocard and Motas in the French disease have already been referred to in the previous paper (Vol. iv, p. 238) and have been studied by Dr Wright in connection with our work. The relative number of infected corpuscles found under different circumstances will be treated of in Part IV.

Leucocytes.

In Figs. 63—67, Plate IX, infected corpuscles are depicted within leucocytes, together with the progressive changes the former undergo. It will be seen that the corpuscles stain a pale yellowish colour and shrink in size, the parasites they contain also degenerate and no longer show the characteristic blue-staining protoplasm (Fig. 65). Later they cease to take up the blue (Fig. 66), and finally all traces of them except the resistant chromatin masses, which show out clearly in the blue-staining protoplasm of the leucocyte, are lost (Fig. 67).

Staining Method employed.

Piana and Galli-Valerio (1895 and 1896) in Italy stained blood films by means of thymolmethylene blue, the corpuscles appearing pale blue, and the parasites dark blue, with an unstained spot representing the vesicular nucleus. Their appearance is figured in a coloured plate. Marchoux (1900), in France, used Laveran's stain (*Compt. rend. Soc. de Biol.* 15. iv. 1899) which stains the chromatin violet-red. He described the nucleus as round or elongated, and situated peripherally (its position not being as constant as in the parasites of Texas fever), lying between the margin of the parasite and a line of blue-staining protoplasm which borders the central unstained part. Nocard and Motas (1902, p. 265) stained by means of Nicolle's carbol-thionin. They dried the film in the air, fixed it in alcohol-ether or absolute alcohol, stained for 30 seconds, washed, dried, and mounted in balsam. With this stain the corpuscles appear pale green, the parasites dark blue and sharply defined, their central portion remaining unstained or staining a pale blue. They also used a modification (pp. 266—267) of Laveran's stain.

Robertson (1902 a, p. 331), in South Africa, recommends carbol-thionin blue, methylene blue, and the double stain of Plehn and Czinziuski. He noted that the parasites stained more deeply at places about the margin, and that there was in the centre an unstained area.

We obtained such excellent results with Leishman's modification of the Romanowski stain that we have employed this method throughout. This well-known stain colours the red cells yellowish-pink or pink. The corpuscles usually appear paler (see Plate IX) when occupied by numerous parasites, and more yellow when included in leucocytes within

which they progressively shrink and degenerate (Plate IX, Figs. 63—67). The protoplasm of the leucocytes appears blue, and the nuclei of the leucocyte and nucleated red corpuscles appear violet.

Further Note on the Pathogenicity of P. canis.

On page 245, Vol. IV, of this *Journal*, the results were recorded of inoculation experiments of different animals made by Robertson in South Africa, and by Nocard and Motas in France. These experimenters only succeeded in infecting dogs with *Piroplasma canis*. With a view to further testing the question we inoculated cats, ferrets, hedgehogs, guinea-pigs and white rats, two examples of each species being employed. The results were all negative. We had hoped to experiment with other Carnivora, especially foxes and other Canidae, but were unable to obtain them. It would be of interest to determine what other animals besides dogs are susceptible to infection with this parasite.

Piroplasma-like Parasite of the Mole.

Whilst investigating blood films prepared from a variety of animals from the vicinity of Cambridge, one of us discovered a piroplasma-like parasite in the mole. We wish but to record the fact, and to state that the new parasite will be described in the next number of this *Journal*.

EXPLANATION OF PLATE IX.

Piroplasma canis in the blood of dogs experimentally infected in Cambridge with the South African disease. The dogs were infected by ticks and by blood inoculations. Stained by Leishman's method. Drawn with Zeiss Apochr. $\frac{1}{2}$ oil immersion objective and eyepiece 12, larger than they were seen because of the impossibility of reproducing the details in smaller figures. The figures in the Plate consequently all appear magnified about 3400. Drawn by G. H. F. N.

Figs. 1—8. Young intracorpuseular forms, rings, and dividing ovoid bodies ending in a group of four. Blood obtained chiefly from the peripheral circulation, also from spleen and lung smears.

Figs. 9—32. Series of intracorpuseular parasites beginning with a large single oval parasite. Longitudinal fission leading to groups of typical pyriform bodies etc., in groups of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 21. The nuclear division is seen to precede the separation of the daughter cells. Figs. 9 and 10 from liver and spleen smears; 12—15, from peripheral blood, the rest chiefly from spleen, bone-marrow, lung, and liver smears.

Figs. 33—42. Amoeboid forms in spleen and marrow smears.

Fig. 43. Large amoeboid form in peripheral blood.

Fig. 44. Large spherical form with exceptionally large chromatin masses. Developing sexual form? (brain).

Fig. 45. Nucleated red corpuscle containing two parasites.

Fig. 46. Group of 16 free parasites liberated from a corpuscle of which all trace has disappeared (lung).

Figs. 47—58. Parasites in various stages of development and lying free in the plasma (lung and spleen).

Figs. 59—62. Sausage-shaped gametes (?) lying free in the plasma in peripheral blood.

Figs. 63—67. Infected corpuscles taken up by leucocytes and undergoing progressive degeneration in order of the figures from left to right. In Fig. 67 only the chromatin masses of the parasites are left.