CANINE PIROPLASMOSIS. V.

FURTHER STUDIES ON THE MORPHOLOGY AND LIFE-HISTORY OF THE PARASITE[1].

(Plates XI—XIII and 23 Diagrams.)

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INTRODUCTION.

IN the present paper we describe the results of further investigations on the morphology and staining characters of *Piroplasma canis*, as well as a large number of observations on the living parasites. Our previous papers (1905) contained the results of studies preliminary to the investigation of the life-history of the parasite, but further work was unavoidably interrupted owing to the difficulties attending this form of research—we unaccountably failed to transmit the disease after several passages through dogs, and met with great difficulties in rearing progeny from infected ticks. Consequently the observations had to be discontinued until, after several failures, infected ticks (*Haemaphysalis leachi*) capable of transmitting the disease, had been received from South Africa through the kindness of Mr C. P. Lounsbury, Government Entomologist, Cape Colony. With this fresh strain of the disease we have succeeded in demonstrating various points in the morphology of the parasite which have hitherto escaped observation, and have worked out its method of multiplication in the living blood. Further studies have also been made on the vitality of the parasite outside the body in culture fluids, but these and other studies on the cycle of development within the tick are reserved for future communications.

A. The structure of Piroplasma canis in stained preparations.

In previous publications we have described and figured most of the common forms of *P. canis* which are to be found in stained preparations both within the corpuscles and free in the plasma (Nuttall and Graham-Smith, VI. 05, p. 239) and have further given tables showing the relative frequency of these forms in blood and organ smears (Graham-Smith, VI. 05, pp. 251—266). In the present paper we therefore only propose to deal with certain special points in the morphology of the parasites and the structure of their protoplasm and chromatin masses.

Method of Staining. The preparations, which we have recently studied, were made in the following way. Very thin blood smears were made on perfectly clean glass slides by placing a drop of blood on one end of the slide and drawing the edge of another slide along it. The first slide was then rapidly waved about in the air for several seconds until the smear was quite dry and then placed in absolute alcohol for 12 hours. The film was then stained with Giemsa's stain (1 : 12 in distilled water) for $1\frac{1}{2}$ to 3 hours, and rapidly washed in distilled water and dried with filter paper. The slide was then immersed for a short time, according to the condition of staining, in strong methylated spirit, and again dried with filter paper. By this means some of the blue was removed from the parasites leaving the protoplasm stained a light but distinct blue, and the chromatin masses deep or light red according to their structure (Plate XI). The preparations were examined by placing immersion oil on the film and were not mounted with Canada balsam. After the examination the oil is removed with xylol.

Smears from the organs were treated in the same way.

I. The structure of the protoplasm.

In our original description (1905, p. 239, and Plate IX, Figs. 5, 13, 34, etc), we noted that the blue staining protoplasm has a vacuolated appearance, and Lühe (1906, p. 47) remarks that this is especially the case in the pyriform bodies. In the present studies these vacuoles were frequently very well-marked and typical examples are illustrated in Plate XI, Figs. 2, 3, 18, and 19, while in Fig. 29 and Diagram 1, Figs. 4 and 11 a vacuole, which perhaps has some special significance, is figured. This vacuole, which is usually oval in shape, lies between the nucleus and a secondary mass of chromatin, and generally possesses extremely well-defined edges. The ring-like forms of the parasite (1905,

Plate IX, Fig. 2; and in this paper Plate XI, Fig. 26, and Diagram 2, Fig. 3) often show a clear central space, which Lühe attributes to the presence of a rounded central vacuole. In some forms of the parasite several vacuole-like clear spaces, usually rounded or oval in shape, may be found. In many parasites the protoplasm presents a delicate trabecular structure, which is so fine that it is scarcely suggested in the reproductions of our original drawings (1905), but which gives the amoeboid forms of the parasite the "foamy protoplasm" which as Lühe states may coexist with a vacuole.

The vacuoles and the reticular structure of the protoplasm are often extremely well-marked in free parasites, which appear to be undergoing degenerative changes, found in liver and spleen smears and preparations made from defibrinated blood kept at room temperature for 48 hours.

II. THE DISTRIBUTION AND STRUCTURE OF THE CHROMATIN MASSES.

(1) *Previous observations.*

Ziemann (1898, p. 125) and Laveran and Nicolle (1899) appear to have been the first observers to demonstrate the presence of the chromatin masses in *P. bovis*, which have subsequently been described by all writers.

Schaudinn (1904, p. 438) was the first to shortly call attention to the occurrence of nuclear dimorphism in *P. canis* and *bovis*, and Lühe (1906, p. 47) confirmed this observation and went more fully into the question. The latter distinguishes the large nucleus usually figured by authors, and a smaller compact almost punctiform body, the blepharoplast of Schaudinn. He was unable to determine any structure in the large nucleus, which usually appears round and compact. Rarely two or three closely aggregated grains may represent the nucleus, in which case they are surrounded by an achromatic zone. In our previous studies we have figured various forms of this nucleus, and parasites containing two or more nuclei (1905, Plate IX, Figs. 4, 11, 16, 43, 44), as well as parasites with certain punctiform dense masses of chromatin lying in the neighbourhood of the nucleus (Plate IX, Figs. 18, 22, 58). The latter bodies are probably identical with those described by Schaudinn and Lühe under the name of the blepharoplast, but owing to their relative rarity we did not attach any special importance to them. Lühe (p. 52) too states that he was unable to constantly demonstrate the presence of the blepharoplast, especially in amoeboid, oval and round forms. Though usually situated near the nucleus he sometimes found it at the opposite pole of the parasite.

Lühe further met with parasites with rod-like nuclei, like one previously figured by us (1905, Plate IX, Fig. 57), which he thought were about to divide. He found that the rods were not smooth but apparently made up of closely packed grains. In other oval forms which he also considered to be undergoing division he found two nuclei and two blepharoplasts, and on one occasion saw a form with one nucleus and two blepharoplasts. On one occasion he also observed a form which had apparently completed the nuclear division, and contained two closely approximated nuclei of dense chromatin and two blepharoplasts still connected by a delicate thread of chromatin, and on another occasion a spherical body with a marginal mass of dense chromatin from which a faint arcuate chromatin band traversed the width of the parasite. In consequence of these observations he came to the conclusion that the nucleus gave rise to the blepharoplast. No other observers appear to have noticed this body.

Dschunkowsky and Luhs (1904, p. 488) mention that in *P. parvum* the chromatin mass may be basal and at times a small particle may occur at the apex.

(2) *Our own observations.*

In the following pages the distribution and structure of the chromatin in various forms of parasites, which have been seen in blood and organ smears, are described, using as far as possible the nomenclature of Lühe. In many of the parasites three distinct masses of chromatin were found, a large dense compact mass, the nucleus; a minute dense punctiform mass usually lying in the immediate neighbourhood of the nucleus, the blepharoplast; and a third lightly staining, irregular, loosely packed, or reticulated mass, which appears to have hitherto escaped observation. Other parasites contained long rods of dense chromatin, several small masses of chromatin, or aggregations of minute particles.

Our studies on the living parasites have convinced us that the appearances seen in stained preparations are often very misleading, and we feel that it would be premature to hazard any definite opinion as to the significance of these structures until our studies on the life cycle of the parasite within the tick and its possible development in cultures outside the body of the mammalian host have been concluded. We have therefore only described the various types which have been encountered, without comment.

(*a*) *The distribution of chromatin in single free pyriform parasites*[1].

The following figures (Diagram 1, Figs. 1—25) show that the distribution and character of the chromatin varies in the single pyriform bodies lying in the plasma. The figures selected are from a series representing over 200 parasites consecutively observed in smear preparations made from the liver immediately after death and in blood films prepared

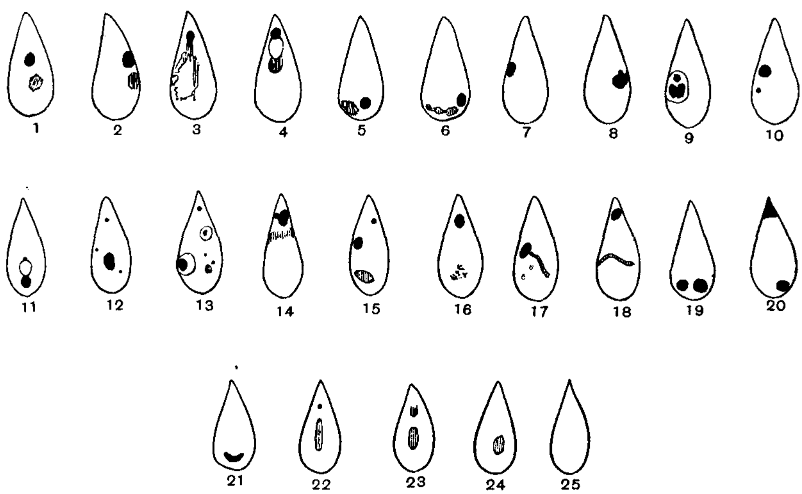

Diagram 1.

the day before death. The commonest type, which included about one-quarter of the parasites, is represented in Figs. 1, 2 and 5. In this type a mass of loose chromatin lay posterior to the nucleus, either closely apposed to it or slightly separated from it. In a few instances a vacuole clearly separated the two masses (Fig. 4), whilst in others the loose chromatin appeared to be directly connected to the nucleus, in some instances by two arms surrounding a clear central space (Fig. 3). Occasionally the loose chromatin was aggregated into smaller and denser masses (Fig. 6). In another type the loose chromatin is widely separated from the nucleus (Fig. 16). A common type is represented by Fig. 7, in which a dense mass of chromatin is placed at one side or in the middle of the parasite. In a few instances it is situated relatively close to the pointed extremity, in others nearer the blunt end. The types

[1] In the following diagrams the contours only of the parasites are figured together with dense (black) and loose (shaded) chromatin and an occasional vacuole. Intracorpuscular forms are figured inside circles representing the contours of the infected corpuscles.

represented in Figs. 8, 9, 10, 17 and 18 are less frequent, and the other types are rare. In the types shown in Figs. 17 and 18 the loose chromatin has a roughly rod-like form. The so-called blepharoplast is seen in Figs. 8, 9, 10, 11 (separated by a vacuole), 14 and 15, and is in most cases distinct from the nucleus. In the latter two types nucleus, blepharoplast and loose chromatin are all present, in one case lying close together (Fig. 14), and in others widely separated. In some cases the nucleus and blepharoplast may lie in a halo of achromatic substance (Figs. 9, 13). In some forms two nuclei occur which may lie side by side (Fig. 19) or at opposite poles (Fig. 20).

Forms like those represented in Figs. 12 and 13 were occasionally encountered in which, besides the nucleus, several small dense masses of chromatin occurred which may represent two dividing blepharoplasts and reduction bodies. The rarest forms are shown in the last five Figures (21—25). Fig. 21 may possibly represent a form with the nucleus about to divide, while the others probably represent degenerating parasites, whose chromatin has been lost. Only two forms completely devoid of chromatin were encountered.

It was noticed that a larger proportion of the free pyriform parasites found in films prepared from peripheral blood contained loose masses of chromatin than of those found in liver and spleen smears. Such parasites are figured in Plate XI (Figs. 28—31. In Fig. 30 the loose chromatin should not protrude beyond the blue-stained protoplasm).

(*b*) *The distribution of chromatin in single pyriform intracorpuscular parasites.*

Specimens of single pyriform intracorpuscular parasites showed the various masses of chromatin arranged in the same manner as in the free pyriform bodies just described. All the common types of the latter series were observed.

(*c*) *The distribution of chromatin in single rounded or irregular intracorpuscular parasites.*

The distribution and character of the chromatin in single rounded or irregular intracorpuscular parasites observed in smears made from blood before death and in liver and spleen smears are shown in the following figures (Diagram 2, Figs. 1—18). Some of these forms show a single dense mass of chromatin (nucleus) (Fig. 1), others show besides a well-marked blepharoplast situated either close to the latter (Fig. 12) or applied to it (Fig. 11). Occasionally two masses of dense chromatin are seen joined by a thread (Figs. 2, 16), or a single mass may be continued into a long thread (Fig. 7) which sometimes leads to a mass of loose chromatin (Fig. 8). Two chromatin masses, one dense, the other loosely packed, arranged in the same manner as in the single free parasites, are common (Figs. 4, 5, 6). More rarely forms are met with containing several minute dots of chromatin (Fig. 9) or masses of loosely packed chromatin only (Fig. 10). In some forms one or more of the chromatin masses are situated in achromatic areas (Figs. 11, 14, 17). More rarely very small parasites are seen (Fig. 17), or specimens which appear to be undergoing division (Figs. 13, 18).

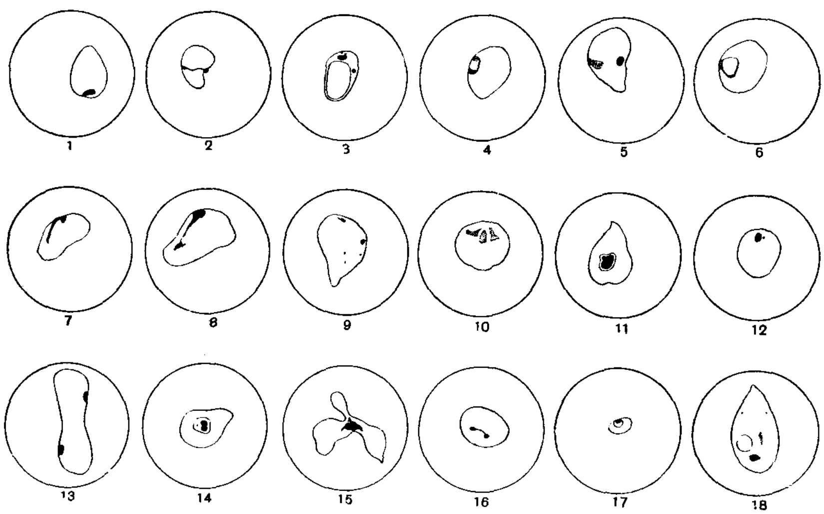

Diagram 2.

Types occasionally seen are illustrated in Figs. 3 and 15. In the latter the irregularly shaped parasite contains a single mass of deeply staining chromatin, which is prolonged into each of the extensions of the parasite, and in the former the organism is ring-shaped.

Types 1, 5, 4 and 6 are common in blood and organ smears, 7, 8, 11, 12 are seldom seen in blood smears, but are not uncommon in smears from the liver and spleen, and the other types are rare.

(*d*) *The distribution of chromatin in double pyriform intracorpuscular parasites.*

The individuals composing many pairs of pyriform intracorpuscular parasites appear to have reached the same stage of development, probably indicating that they have developed by fission from a single

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parasite. In some of these forms the number and arrangement of the chromatin masses in the two individuals is identical, while in others similar masses occur but they are arranged differently, while in others again both the number and arrangement is decidedly different.

In Diagram 3, Figs. 1—24 represent various types encountered during the examination of a single blood film. In the commonest forms (Figs. 2, 3, 4, 6) a loose or granular mass of chromatin is present either apparently connected to the nucleus (2), or situated close to it (3, 4) or at a distance from it (6). Occasionally the nucleus appears to be surrounded by loose chromatin (1). More rarely forms are encountered in which the loose chromatin is arranged in a rod-like manner (5) or is represented by an aggregation of coarse particles (8).

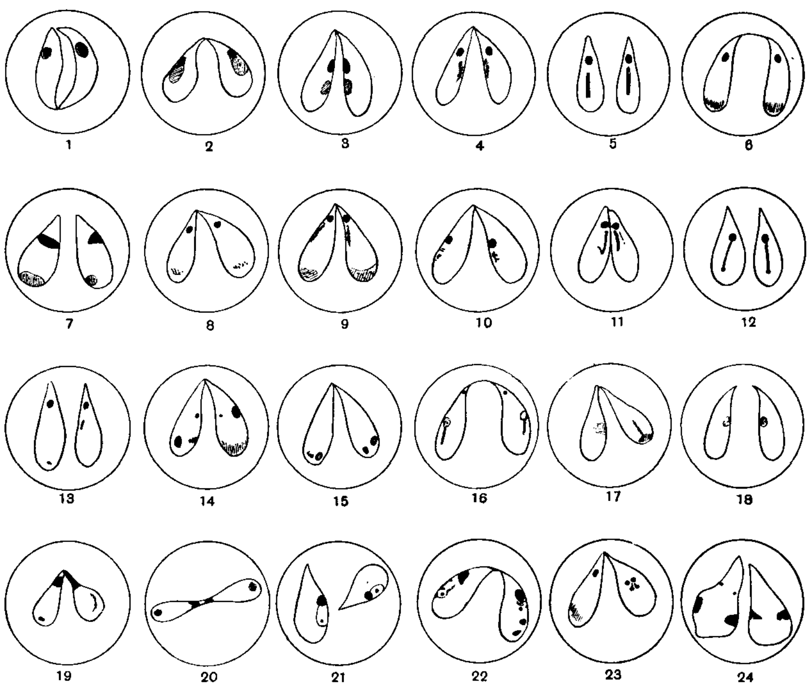

Diagram 3.

The remaining forms (9—13, 15—24) are rare. Fig. 12 shows an interesting type in which a loose chromatin strand joins the nucleus and blepharoplast. In 9 the loose chromatin contains small granules, and in 11 the mass is represented by a number of compact grains arranged in the form of a rod. In Fig. 24 one form shows two nuclei and two blepharoplasts, suggesting the process of division.

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Many of the types here shown correspond closely to the free pyriform parasites illustrated in Diagram 1. And it may be pointed out that the commonest types in each case are similar, namely 2, 3 and 4, Diagram 3 and 1, 2, 5, Diagram 1. One common double type (6) is however not found amongst the free forms.

Rarely very small double pyriform bodies may be found either within corpuscles or free in the plasma. These are shown in Diagram 4.

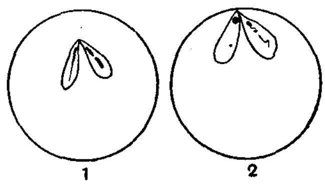

Diagram 4.

(*e*) *The distribution of chromatin in rounded and irregular double intracorpuscular parasites.*

Various forms of rounded and irregular double intracorpuscular parasites from blood, liver and spleen smears are shown in Diagram 5. Some

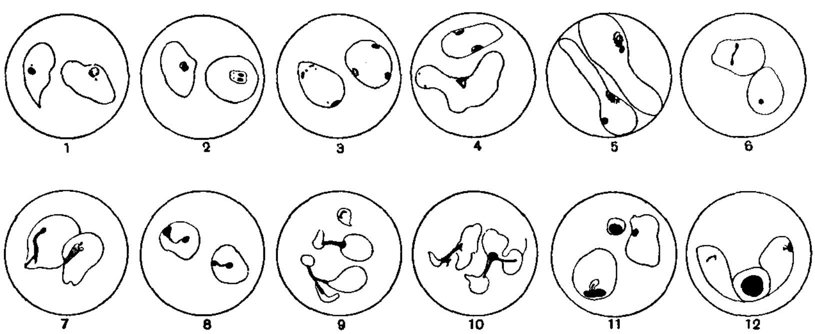

Diagram 5.

show single masses of dense chromatin (Figs. 6, 11), others long dense rod-like masses (Figs. 6, 7, 9, 10), while others contain in addition a blepharoplast (Fig. 1). Specimens showing both dense and loosely packed chromatin are common (Figs. 2, 5, 7), but those showing a blepharoplast also are rare (Figs. 1, 11), and examples with two masses of dense chromatin joined by a bar are occasionally seen (Fig. 8).

Forms apparently undergoing division are very rare (Fig. 2). Two corpuscles were seen, one in a spleen smear (Fig. 11), and the other in a liver smear (Fig. 12), which contained besides double irregular parasites, round organisms consisting of a small amount of protoplasm enclosing a very large round densely staining mass of chromatin.

(*f*) *The distribution of chromatin in the parasites present in multiple infection of the corpuscles.*

In our original plate (1905, Plate IX, Figs. 15—32) we showed that most of the individual parasites in corpuscles containing three or more organisms possessed a single well-marked nucleus. Rare types only are therefore shown in Diagram 6 Figs. 1—6 apparently represent

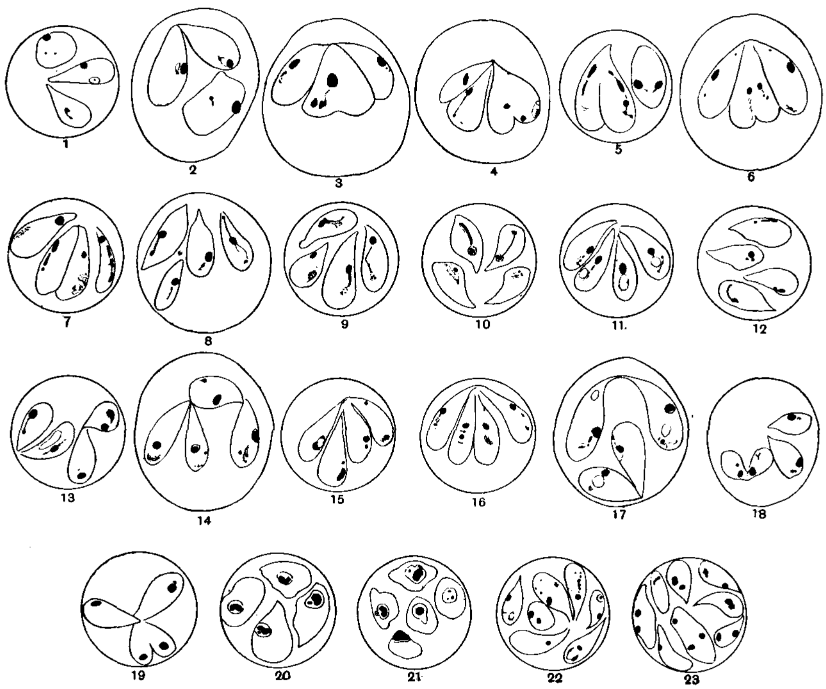

Diagram 6.

parasites in the process of division, and types already shown in Diagrams 1 and 3 may be seen. Fig. 10 shows a very striking example of the rod-like arrangement of chromatin.

(*g*) *The extrusion of chromatin from the parasite.*

Certain specimens were encountered which suggested that the parasites occasionally extrude masses of chromatin into the substance of the red blood corpuscles. These are illustrated in Diagram 7. Fig. 1

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shows a parasite containing two dense masses of chromatin, one (rod-like) situated within its body, the other at the extremity of a long process. In Figs. 2 and 3 the main mass of chromatin occupies the whole of the extremity of a process, while in Fig. 4 a large mass of chromatin is

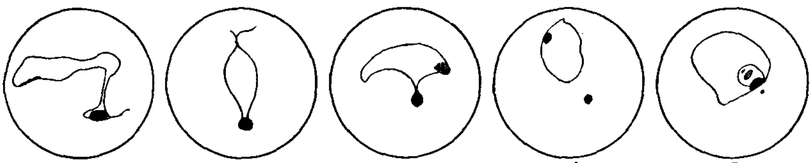

Diagram 7.

seen definitely detached from the parasite. In Fig. 5 the blepharoplast has apparently been extruded. All the conditions here figured are rare.

III. SOME OTHER POINTS IN THE MORPHOLOGY OF THE PARASITE.

(*a*) *Free parasites with flagella-like processes.*

Free parasites with short processes protruding from the pointed extremity have occasionally been described; but forms with long terminal flagella have seldom been recorded.

Pound (1. IX. 97), Bowhill and Le Doux (1904, Fig. 6) and Bowhill (1905, Fig. 14) illustrate forms resembling Fig. 9 (Diagram 8) which were seen in preparations from cattle infected with *P. bovis*, and Bowhill and Le Doux (1904, Fig. 4), illustrate similar parasites from the blood of dogs infected with *P. canis*. Lignières (1901, p. 123) mentions the occurrence of free flagellated pyriform *P. bovis*.

We have only rarely seen free parasites in stained preparations which show long flagella-like processes, but numerous examples have been observed with short thin processes and finely pointed extremities.

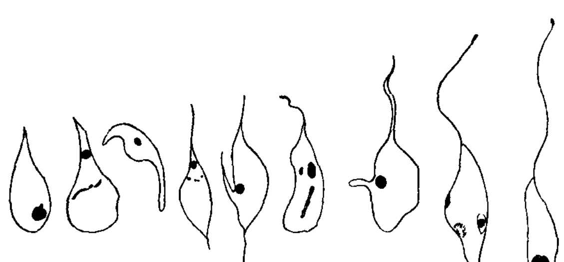

Diagram 8.

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Diagram 8, Fig. 1 illustrates a common type of free parasite with one extremity ending in a fine point. Forms with still more elongated extremities are illustrated in Figs. 2 and 3. Figs. 4, 5 and Plate XI, Figs. 10, 11, 12, 13 show parasites with definite thin but rather short flagella-like processes which are frequently met with in blood films and organ smears. Diagram 8, Fig. 7 illustrates a type with a thicker flagellum and Figs. 8 (blood) and 9 (liver smear) parasites with very long thin flagella ending in rounded blunt extremities.

In spite of the fact that flagellated free forms are uncommon in stained preparations their frequent occurrence has been definitely proved by observations on living forms (p. 625). Their rarity in stained specimens is probably due to two causes, the difficulty with which the fine process takes the stain and the retraction of the flagellum by the parasite when it is dying.

(*b*) *Intracorpuscular parasites showing processes.*

Intracorpuscular parasites showing one or more definite processes are commonly met with, especially in smears made from the organs. These processes are of various types. Some are short and straight, and others long and curved; the majority terminate in rounded slightly enlarged extremities, but a few have sharply pointed ends. These processes represent the pseudopodia seen in the living amoeboid forms (Diagrams 14, 15, 18). Diagram 9, Figs. 1—9 illustrates single intracorpuscular parasites with processes of this type. Certain parasites show extremely thin, often very long wavy processes resembling flagella[1]. Such flagella-like processes usually end in a minute knob (Figs. 12, 13, 14, 16 and Plate XI, Figs. 8 and 9), but some end in a fine point (Figs. 10, 15—18 and 23 and Plate XI, Figs. 2—7).

Rarely minute knobs or irregular masses are found along the length of the flagella (Figs. 12, 14, 17). Parasites showing blunt pseudopodia and flagella are occasionally seen (Figs. 10, 11, 12, 23). Chromatin is sometimes found in the larger processes (Figs. 19, 20 and Diagram 7, Figs. 1, 2, 3), but has only been seen three times in the delicate flagella-like processes (Figs. 21, 22, 23). Double intracorpuscular pyriform bodies are frequently joined together at their pointed extremities, and the connecting matter may occasionally be represented by a long curved thin strand of protoplasm (Plate XI, Fig. 1). Both pseudopodia and flagella may be possessed by one or more of several

[1] These long thin processes we call "flagella" to distinguish them from the blunt pseudopodia.

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parasites within a single red blood corpuscle (Plate XI, Figs. 7, 8, 9). Rarely the flagella-like processes show bifurcated extremities (Plate XI, Fig. 8). In no case have we observed a flagellum extending beyond the red corpuscle as figured by Baruchello and Mori (1905, Figs. 64, 67) and Bowhill (1905) in *P. equi.* The former illustrate a flagellum passing out of the infected corpuscle and indenting an adjacent normal corpuscle.

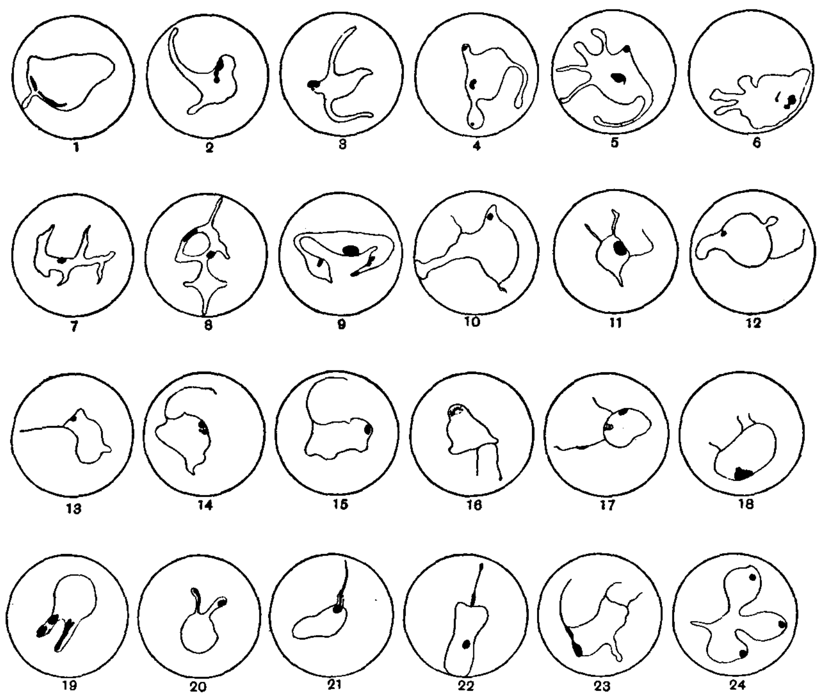

Diagram 9.

Many observers have described intracorpuscular parasites with pseudopodia, and we figured several of these forms in our previous paper (1905, Plate IX, Figs. 26, 33—39), but forms showing flagella-like processes have very seldom been recorded.

Baruchello and Mori (1905, Figs. 45, 47, 48, 51 and 66), Bowhill (1905), and Edington (1905, Fig. 5), describe or figure flagella-like processes of the intracorpuscular *P. equi,* some of which closely correspond to Plate XI, Figs 1—9. Lignières (1900) describes similar forms in *P. bovis* and Webb (1906) and Bowhill and Le Doux (1904) in *P. canis.*

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Webb illustrates parasites resembling those shown in Plate XI, Figs. 1, 2, 4 and Diagram 9, Fig. 11, and Bowhill and Le Doux forms like those shown in Plate XI, Figs. 1, 6, 8, 9. The latter observers mention (p. 218) that "several endoglobular parasites were observed which showed long flagella-like processes, some with two bulbs on the flagellum and some with only one at the end."

Lignières also states that he has met with infected red corpuscles showing intracorpuscular pyriform parasites and long flagella-like processes extending from the surface of the corpuscle without any apparent connection with the parasites (see under Section VII, p. 641). We have never observed anything resembling this condition.

(c) *The structure of rounded and irregular free parasites found in organ smears.*

Various forms of free parasites were encountered in smears made from the liver and spleen and are illustrated in Diagram 10 (Figs. 1—12, 23, 24, 28, 31 from liver, 13—22, 26, 27, 29, 30 from spleen).

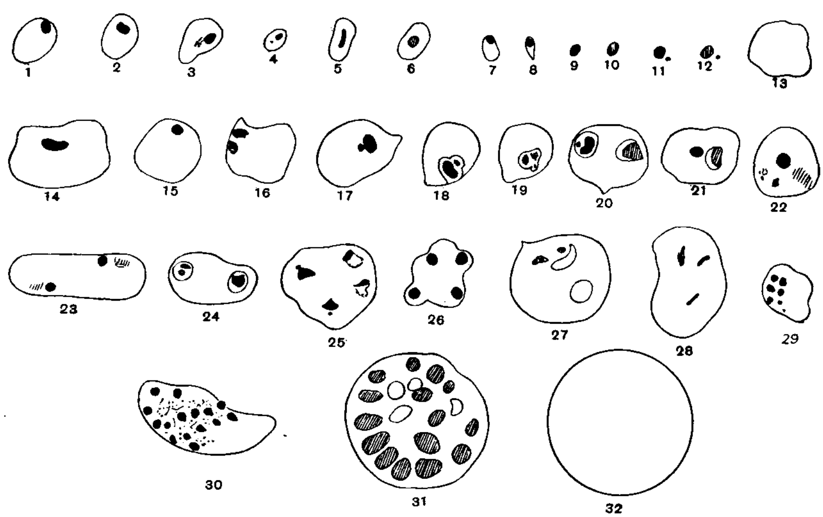

Diagram 10.

The commonest type (1, 2) showing a rounded or undulating margin, and a single mass of dense chromatin lying centrally or peripherally within the blue staining protoplasm, is also found within the corpuscles (1905, Plate XI, Figs. 7, 47, 48, 49). Forms resembling these in general appearance but containing also a mass of loose

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chromatin (3) or smaller forms showing a blepharoplast (4) are occasionally seen. Figs. 5, 6 and 13 (without chromatin) represent forms apparently undergoing degeneration and correspond to Figs. 24, 25 in Diagram 1, p. 591.

Forms with little blue staining protoplasm (7, 8) are frequently seen in both spleen and liver smears and may either represent young parasites or degenerating forms. Free masses of dense or loose chromatin, corresponding in size to the masses observed in several of the free forms of the parasites are frequently seen (9, 10), and more rarely a free nucleus and blepharoplast may be found (11, 12).

In these preparations larger types of free parasites are very common with rounded or undulating margins, and containing one or more masses of chromatin distributed in the same manner as in the free pyriform bodies (Diagram 1). The figures show specimens with a single nucleus, two nuclei, a nucleus and a blepharoplast, a nucleus and a loose mass of chromatin, and others containing all three. Not infrequently the chromatin masses are surrounded by a colourless halo (18—21).

Figs. 23—26 represent exceptionally large free parasites with two to four large masses of dense chromatin, in one of which (25) there are two blepharoplasts. Figs. 27, 28 represent forms which seem to be undergoing degeneration.

Besides these forms we have also observed three very large organisms which differ from any which have hitherto been described. The bodies illustrated in Figs. 29, 30 contained a large number of dense chromatin masses of fairly uniform size, and one of them (30) showed between these masses a large number of minute chromatin granules apparently forming part of a chromatin network. Fig. 31 represents a roughly spherical body composed of pale staining protoplasm containing several vacuoles and fifteen large rounded or ovoid masses of loose chromatin.

Fig. 32 represents a red blood corpuscle to give some idea of the relative sizes of these bodies.

These various types are illustrated for comparison with the types found in the peripheral blood, but we intend to make further studies on these forms both in the living condition and in dried preparations. For the present we may say that some of these bodies, especially those in which the protoplasm is much reduced and stains poorly and the free masses of chromatin, probably represent degenerating parasites, while others seem to represent dividing forms. We can offer no explanation of the forms shown in Figs. 29—31.

(*d*) *Forms of parasites found in defibrinated blood.*

Blood taken from the heart immediately after death and defibrinated in sterile bottles and kept at room temperature in the dark shows interesting changes in the parasites.

After 24 hours the majority of the parasites are free in the serum, some being motionless but others actively motile, and many have become enclosed within the leucocytes. In stained preparations various changes in the condition of the protoplasm and chromatin were observed.

After 48 hours very few of the parasites are still found within the corpuscles and the changes are more marked. Some of the parasites are still motile. The following description is taken from stained films prepared from specimens 48 hours old.

Most of the corpuscles have retained their shape remarkably well but are of a pale colour and only a small number contain parasites. The intracorpuscular parasites show very little change in their appearance or staining properties, many of them having well-marked processes and flagella, and chromatin masses indistinguishable from those found in parasites seen in fresh films. Occasionally the parasites are situated in a light-coloured zone, and less frequently in very pale corpuscles, of which only the rim can be clearly defined.

Free forms are very numerous and frequently occur in groups (Plate XIII, Fig. 4), some of which are very large, containing as many as 30 parasites. The specimens composing these groups show considerable changes. Most of them are irregular or rounded in shape and vary in size from minute organisms about one-sixth of the diameter of a corpuscle to specimens almost as large as corpuscles. In some the protoplasm stains well and to the same degree as that of specimens obtained from fresh blood, showing the usual lighter and darker areas. In others the protoplasm stains less deeply and shows less differentiation, while in some it stains very faintly. In some of the larger very pale forms the protoplasm has a curious reticulate appearance. Generally, corresponding with the condition of the protoplasm, the chromatin shows various changes. In some of those showing the least change a single, deeply staining mass of chromatin is seen (Diagram 11, Fig. 1), in others a similar mass situated within an achromatic zone (Fig. 2). Others again show two deeply stained masses (Fig. 3), while in some the blepharoplast is well defined (Figs. 4, 5), and in others, besides the deeply stained mass a less deeply stained, larger, often reticulate mass can also be found (Fig. 6). In other forms, usually those in which the protoplasm stains faintly, only reticulate, feebly staining, loosely packed chromatin can be seen, occasionally situated in an achromatic zone (Figs. 7, 8). Rounded forms without a trace of chromatic substance are not rare, (Fig 11) and occasionally very large forms which contain two or more masses of faintly staining chromatin are found (Figs. 9, 10). Amongst these forms masses of chromatic substance without any protoplasm surrounding them are frequently seen, some of which are small and deeply staining, others larger and less deeply staining, and others still larger, staining a pink colour and showing a slightly reticulated appearance (Fig. 12). The latter are the most common. All these forms may occur in the same group.

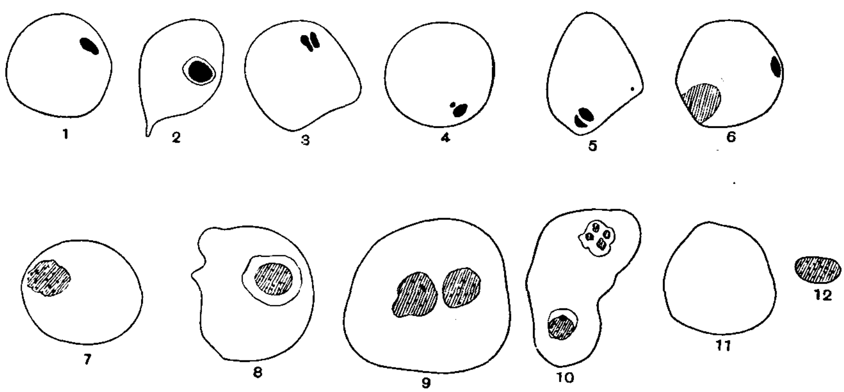

Diagram 11.

Of these forms those numbered 1, 6, 7, and 12 in Diagram 11 are the most common, 2, 3, 4, 5 and 11 are less common, while 8, 9 and 10 are rare. Free forms showing flagella or pseudopodia are only rarely seen.

Most of these forms are probably due to degenerative changes, and have been mentioned for the sake of comparison with the free forms found in liver and spleen smears. Many of the leucocytes contain enormous numbers of parasites, representing nearly all the forms which have been described (Plate XIII, Fig. 3).

(*e*) *Forms apparently applied to the surfaces of the corpuscles.*

In stained preparations parasites which appear to be adhering to or indenting the surface of corpuscles are occasionally encountered, and several instances are illustrated in Plate XI, Figs. 15—23. The explanation of these appearances can only be derived from the study of living forms, which are given at length later (see Section VI, p. 610). These studies show that the intracorpuscular forms are flattened, and Fig. 19, in which the parasite is probably presenting its thin surface, seems to prove that they have a similar shape in their extracorpuscular stage.

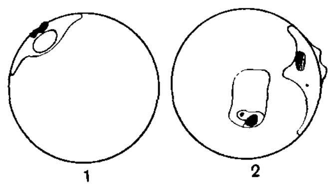

Diagram 12.

Plate XI, Fig. 15 and Diagram 12, Figs. 1 and 2 illustrate amoeboid parasites apparently applied to the corpuscles. We have no doubt, however, that these appearances are deceptive and that the parasites are internal and enclosed within the corpuscular membrane, since in living specimens intracorpuscular amoeboid parasites often assume this position, and free amoeboid parasites have never been observed.

B. The examination of the living blood.

IV. Technique: the preparation and examination of fresh blood films.

A perfectly fresh drop of blood is obtained from a puncture near the posterior margin of the ear and transferred by means of a small sterile platinum loop to a clean glass slide. A cover-glass is then placed over the drop and slight pressure is applied. The drop of blood required is very small and the glass slide and cover-glass ought to be perfectly clean. Under these circumstances the blood immediately spreads out, except at the edges of the film, into a very thin layer showing the blood corpuscles separate and distinct in outline. Immediately after the film has been prepared a small quantity of vaseline is applied round the edges of the cover-glass by means of a brush.

In some cases a small quantity of tap water was added to the blood. Immediately before the drop of blood is placed on the slide a damp cloth is rubbed across it, leaving a small quantity of moisture on which the drop is placed, or the blood is placed in a very minute drop of water. The preparation is finished in the same way as before. If properly prepared in this way the films show the corpuscles and their contents extremely well.

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The films were examined by either natural or artificial light under a $\frac{1}{12}$ inch oil immersion lens with the condenser lowered. Some of the preparations were examined at room temperature and others at 35°—40° C.

Unless otherwise stated the descriptions refer to examinations made at room temperature of films prepared without the addition of water.

V. OBSERVATIONS ON FRESH NORMAL DOG'S BLOOD.

(*a*) *The corpuscles.*

A number of examinations were made of the blood of normal dogs. These examinations proved of considerable interest in helping to eliminate certain possible sources of error.

Many of the red blood corpuscles were found to retain their shape, colour, and normal appearance for many hours or even days in properly prepared and sealed specimens. Some crenated examples can, however, be found in all preparations. Badly crenated corpuscles present a very characteristic and unmistakable appearance and therefore need no description, but examples undergoing the earlier stages of crenation might be mistaken for infected corpuscles. Some of the latter show a perfectly sharp round or ovoid outline, but the appearance of the central portion is altered. In some cases a light round area may be seen, up to one-third of the diameter of the corpuscle, situated in the centre or near the periphery. This at first sight might be mistaken for a round parasite within the corpuscle. On careful focussing, however, the margins of this area become darker and the central light parts smaller, until the latter becomes reduced to a mere speck. This appearance is due to an elevation on the surface of the corpuscle. Several such elevations may be found near or on the margins of otherwise normal looking corpuscles. Other corpuscles again which show a normal contour present irregular wavy bright lines on the surface. Such lines may be short and single or numerous and long. These lines may change their position slowly and become more numerous. The former variety may be mistaken for single elongated amoeboid parasites, but as in the case of the rounded elevations, careful focussing shows that they are due to elevations on the surface of the corpuscles.

The production and multiplication of elevations on the surfaces of these corpuscles occasionally lead to slight movements and changes in position.

Occasionally distorted corpuscles without crenations are encountered which possess one or more long thin processes. These processes almost invariably show slight but definite vibratory movements, which may cause them to be mistaken for parasites attached to the corpuscles. Careful definition, however, immediately shows that the substance of the corpuscle is continued into the process.

Cropper's bodies.

All the structures hitherto described, which might be mistaken for intracorpuscular parasites can be readily distinguished by careful definition. There occur, however, within the red cells certain bodies described by Cropper (1. v. 1905) and later by Smith (7. x. 1905) which closely resemble parasites. In fact the latter observer describes them as parasites and the former was at first inclined to regard them as such. Cropper observed these bodies most commonly in the corpuscles of children suffering from fever in Palestine, but also observed them in the corpuscles of adults suffering from malaria, and in small numbers in the blood of healthy persons. He states that their "number frequently coincides with the gravity of the case." Cropper[1] describes two forms, pale rings (Diagram 13, upper row, Figs. 1, 2) which may be motionless or

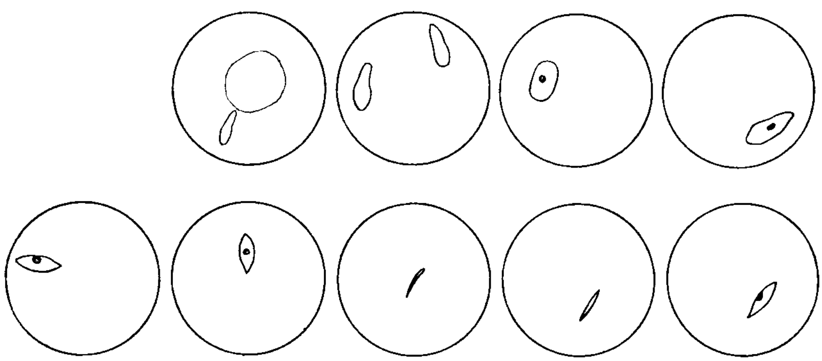

Diagram 13. Row 1 after Cropper (1. v. 1905). Row 2 after Smith (7. x. 1905).

possess slight flowing motion, and larger forms (Figs. 3, 4) which are melon seed shaped, and one-fourth to one-third the diameter of the red corpuscles in length. These bodies frequently possess a central dark dot, which gives them an "eye"-like appearance. The larger form may be motionless or actively motile. Cropper describes their movements in the following sentence.

[1] Cropper, T. (1. v. 05). "Note on a form of malarial parasite found in and around Jerusalem." *Journ. of Tropical Med.* VIII. p. 132.

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"*Movement.* This is characteristic, and totally different from the amoeboid, slow flowing motion seen in the case of the pale rings. The amoeba does not alter its shape to any appreciable extent, but at most has a slight oscillatory character, the chief point being the alteration of the direction of its axis and its position in the cell, which takes place fairly quickly and to a considerable extent. After some hours the movement gradually ceases."

Occasionally two or more of these bodies are found in an affected corpuscle.

Smith[1] saw similar bodies in the corpuscles of patients thought to be suffering from pernicious malaria, and gives the following excellent description of his observations.

"The blood of these 119 patients showed only hyaline, non-pigmented, intracellular bodies in the protoplasm of the red blood corpuscles. At no time, either before or after death, were crescents, extracellular bodies, segmenting forms, or other forms usually to be found in malarial blood, discovered in the blood of these patients.

"The parasites observed are small hyaline discs of an oval spindle form. They have a very sharply defined outline, are highly refractive, and in the centre of each form is a small round dot of haemoglobin. Their short diameters vary from a fifth to a tenth the diameter of the infected red blood corpuscles, their long diameters being about twice as great. These bodies taper gradually toward each end, and on account of the spindle shape which they invariably show they were designated on the hospital records as 'spindle forms.'

"They have no amoeboid motion whatever, but possess a motility of their own, moving around the periphery of the infected corpuscles or across them in any direction. They move by revolving on their long axis and also by swinging around on their short axis, using one end as a fixed point or pivot, as it were. When revolving on their long axis they gradually change from their oval spindle form to a linear one as the side or edge comes uppermost, and back again to their original form as the revolution is completed, indicating that this parasite is more or less flattened like a melon seed as it lies in the infected corpuscle. The motion is generally slow but easily perceptible and is the most peculiar characteristic of the parasite.

"There are also larger non-motile 'spindle forms,' sometimes associated with the motile parasites and sometimes not. Whether these non-motile forms differ sexually from the others or are a different stage or age or condition of the same organism, I am unable to say. At any rate, only these non-motile forms were found a number of times in patients suffering with the same symptoms, but in a milder degree, as the patients in whose blood the motile forms appeared. There seems to be some connection between the motility of the parasites and the severity of the infection, the more motile the forms the more severe the symptoms. As many as three parasites have been observed in one blood corpuscle, but they usually appear singly."

[1] Smith, H. M. (7. x. 05). "An apparently distinct and hitherto undescribed type of parasite in pernicious malaria." *American Medicine*, x. p. 607.

The lower row of figures in our Diagram 13 "show the consecutive changes in position of the parasite moving across the corpuscle, changes occurring during a 20 minutes' observation." The third figure represents the linear form referred to.

"They stain with great difficulty, and even then it is only the periphery of the parasite, which takes on a methylene-blue or methyl-violet stain to a slight extent. No nucleus or chromatin dot stains in the periphery or elsewhere, the haemoglobin central dot taking the usual eosin stain with eosin mixtures. Numerous stains and modifications of the same were used, but all gave practically negative results.

"When kept and observed under the microscope for as long as four days, no development or change of any nature has been observed in these parasites, except a gradual loss of motility in the case of the motile forms, some retaining their motility for nearly 48 hours. Repeated examinations of the blood of these patients were made, but at no time were any parasitic forms beside these 'spindle forms' ever discovered, and in no case did these parasites give any evidence of the formation of pigment.

"The infected corpuscles frequently showed no change in colour or form, while again they were often of a greenish hue and showed considerable shrinking and crenation. In some cases many corpuscles were infected, while in others prolonged examination would discover only a few parasites.

"These forms have been found frequently in patients with a normal temperature, existing thus in a masked or latent form. After surgical operations such patients—and they all had uninfected wounds—almost invariably developed a fever, which was always controlled by quinine.

"The clinical history of these patients corresponds closely to that of cases of pernicious malaria. A remittent fever, chilliness, occasional chills, headache, vomiting, frequently severe pains in the back, arms, legs, and joints, enlarged spleen in many cases, absence of skin eruption, with delirium, convulsions and coma in severe cases, make up a clinical picture common to most of these cases.

"These patients were at once given quinine by mouth. While some were amenable to such treatment, the great majority entirely resisted quinine administered in this manner and required large doses of quinine hypodermically, from 1 gm. to 5 gm. of quinine sulphate daily. Many of the cases responded promptly to quinine hypodermically, and the 'spindle forms' disappeared from the blood. In other cases there was no response until this treatment had been continued from 5 to 15 days, these forms persisting in the blood throughout this entire time, but finally disappearing, except in four fatal cases. A few mild relapses were observed.

"In the four cases that proved fatal no parasitic forms but these 'spindle forms' could be found in the blood before death, or in the blood and organs after death. The necropsies showed that in one case terminal bronchopneumonia and purulent pericarditis, and in another case terminal bronchopneumonia, could have been the immediate cause of death. The other organs showed no important pathological lesions except the spleens, which were much enlarged and congested. In the other two cases the pathologic findings were practically negative except for the very large congested spleens in both cases, and, in addition, in one of these, in which coma, delirium, and convulsions had been severe, oedema of the base of the brain with punctate haemorrhages throughout the white matter of the brain. Smears from the blood and spleens in the four fatal cases showed only these hyaline non-pigmented 'spindle forms' described herein."

We have observed the bodies described by Cropper and Smith both in the blood of normal dogs and of those suffering from the disease. In the former case both oval and melon seed bodies, with or without the central dot, have been noted, but motile forms were seldom seen. In the latter all forms, including motile examples, were occasionally observed, especially at the commencement of the fever, which showed the movements and changes in shape illustrated in Diagram 13 exceedingly well. In normal dog's blood these bodies are rarely found, and were never common even at the height of the fever in the diseased dogs. Cropper mentioned in his paper that he had seen them in the blood of normal dogs and demonstrated them to us during a visit to Cambridge.

We have been unable to stain these bodies in dried preparations, and at present are unable to offer any opinion as to their nature. In fresh blood they may be easily mistaken for solitary intracorpuscular forms of *Piroplasma.*

(*b*) *The plasma.*

Various bodies liable to be mistaken for free forms of *Piroplasma* may be encountered in the fluid between the cells. Minute highly refractile particles with active dancing movements are the most common. The particles may either oscillate about one point or may actively move about the field. One frequently attaches itself to a corpuscle and either oscillates about one point on its circumference or gradually moves round it. After a longer or shorter period it not infrequently leaves the corpuscle and attaches itself to another. Minute dumbbell shaped bodies are also met with which behave in a similar manner. These bodies might be mistaken for free parasites apparently attacking corpuscles.

Besides these minute bodies round, highly refractile, larger bodies, having a diameter of one-fourth to one-third of a red blood corpuscle are not infrequently seen. Occasionally they remain motionless, but more frequently they move slowly about the field. Some of them have a greenish tint. These bodies may be easily mistaken for round free forms of *Piroplasma.*

Other bodies of various shapes, probably fragments of leucocytes, are occasionally seen, which may be motionless, or move slowly with a flowing motion or show slight amoeboid movement.

VI. Observations on the living blood of infected dogs.

(1) *Before the appearance of the parasites.*

Daily observations were made on the living blood of three dogs from the day of inoculation to the day of death, and in each case parasites were first observed a few days before death. For several days no changes were noticed. Later Cropper's bodies and the various extra-corpuscular motile bodies which have been described appeared to become more numerous, and distorted corpuscles were more frequently seen. The most striking feature in these examinations was the rapidity with which the parasites appeared. In one case very numerous films were carefully examined for parasites on the seventh day after inoculation, but none were found. On the eighth day two doubtful intracorpuscular forms only were seen, but on the ninth day, several infected corpuscles could be seen in almost every field.

(2) *Previous observations on living Piroplasmata.*

Very few observers have hitherto attempted to study the parasites in the living blood, and those who have done so seldom appear to have given much attention to the subject. Nevertheless many hypotheses as to their movements, mode of division and cycle of development have been framed solely on the appearances observed in stained preparations.

P. bovis.

Smith and Kilborne (1893), to whom we owe the discovery of *P. bovis* noted that single forms may be highly motile, changing their shape with lightning-like rapidity, and retaining their power of movement for hours at room temperature in summer. Later Pound (1. IX. 97), encountered free forms swimming by means of a flagellum, which he thought they used to penetrate red blood corpuscles. Lignières (1900), considered that Smith and Kilborne had mistaken rotation of the pyriform bodies for alteration in form. From his studies on living blood he thought that free pyriform parasites were incapable of entering fresh corpuscles, but had first to be reduced to circular forms. He states that the single pyriform bodies are actively motile by virtue of a long flagellum situated at the anterior extremity, and that the coupled pyriform bodies cohere by means of their flagella. He adds that all pyriform bodies observed in extravascular blood become spherical after 3—10 hours, the connecting filament growing proportionately longer. Such spherical

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bodies never regain their pyriform shape. His theory of germ production is given at length later (see Section VII).

Ziemann (1902) considered that the minute hyaline intracorpuscular actively motile bodies which he saw grew into amoeboid bodies, became larger and developed into inactive large pale bodies measuring 2·5—3μ. He thought that the coupled pyriform intracorpuscular bodies were non-motile.

P. ovis.

Bonome (4. I. 1895, p. 6) examined the fresh blood of sheep infected with *P. ovis.* He observed rounded and pyriform parasites within the corpuscles, which at times were actively motile, even causing the corpuscles to move. He also observed free parasites, the larger forms often showing one or two refractive points centrally. He states that under favourable conditions of heat and moisture the parasite appears "to leave the surface of the erythrocyte and to return to it later without changing its form." Motas (1904, p. 31) observed a rolling motion of the infected corpuscles due to the motion of the parasites within.

P. canis.

Piana (1896) described and figured the motions of a highly amoeboid intracorpusclar parasite during half an hour. Nocard and Motas (1902, p. 265), observed that some intracorpuscular parasites changed their shape and protruded processes, often two or three in number, towards the periphery of the corpuscle, at times causing the infected corpuscles to rotate. They state that the infected corpuscles are enlarged and pale, and that the contained parasites have a dark contour and central refractive portion. They also observed free parasites in the plasma. Finding some difficulty in distinguishing the parasites they made examinations of blood suspended in salt solution faintly tinted with methylene blue. By this means the parasites are faintly stained, but are not killed.

All these appearances they say are best observed after the fall of the fever, the only time when the parasites are numerous and motile.

P. parvum[1].

Dschunkowsky and Luhs (1904) found bacillary, ring-like and puncti-

[1] The differences which separate *P. parvum* and *P. equi* from the typical piroplasmata, *P. bovis*, *ovis* and *canis* are given later (see Notes I and II at the end of this paper). The observations on living examples of those species are given here for the sake of completeness, but no inferences as to the life-history of *P. canis* can be drawn from them.

form types of *Piroplasma* in Transcaucasian cattle, all of which were motile, exhibiting both rotatory and migratory movements.

P. equi.

Theiler (31. III. 02), has carefully studied the movements of *Piroplasma equi* in the living blood, and describes his observations in the following words.

"The smallest parasites take the form of little points, the largest of which are about from one-third to one-fifth of the size of a red blood corpuscle; only exceptionally do they appear larger. They present themselves as clear, strongly refractile round discs, standing out prominently against the dark background of the blood corpuscles.

"The normal form is that of a sphere. The edge appears to be thickened as the centre is clearer. In addition to the round forms, other shapes occur, such as oval, pear-shaped, spindle-shaped and rod-like.

"The very small forms are motile. Within the blood corpuscle they show a change of position resembling Brownian movement. The somewhat larger forms are less actively motile, and the fully developed forms are quite motionless. In the case of the medium-sized parasites one can often observe that they very slowly change their position within the blood corpuscles, approaching sometimes to the one or other edge, and sometimes to the centre. I have not been able to observe the protrusion of protoplasmic processes, although appearances observable in stained preparations suggest the possibility of such a thing.

"The method of development of a parasite would therefore appear to be as follows. A young individual which has escaped from a blood corpuscle seeks out another corpuscle. In virtue of its amoeboid movement it sinks into the latter. Its movements become slower and soon cease altogether, there being no further occasion for them. The motionless round discs of largest dimensions represent the parasite before division. Then follows division, and the cycle begins again."

(3) *Our observations on living parasites*[1].

A very large number of observations were made on living parasites both free and intracorpuscular, at room temperature and at 35°—41°C.

[1] We see from these references that very little work has hitherto been done on typical living piroplasmata and that no part of the cycle of their development within the mammalian host has yet been worked out.

Fresh films were made in the manner previously described and immediately placed under the microscopes, which in the case of the higher temperature observations were placed with Nuttall thermostats. The time at which the blood film was made was accurately noted and all observations were dated from this time. The organisms selected for observation were drawn as accurately as possible and the results of all movements or changes were also drawn, and the time of occurrence noted. In this way a very large series of drawings representing the living parasites were obtained, and the following diagrams have been selected from amongst them to illustrate the more typical events. Some of the continuous observations lasted as long as $2\frac{1}{2}$ hours.

To avoid confusion the movements of single parasites within corpuscles are first described, next the movements of two or more parasites within the same corpuscle, and later the escape of the parasites from the infected corpuscle. In another section the movements of free parasites immediately after their escape from the corpuscles, the movements of forms which were not detected in the act of escaping, and the entry of parasites into corpuscles are described. Certain observations on the appearance of the infected corpuscles follow, and lastly the whole subject of the relationship of the parasites to the corpuscles on evidence derived both from living and stained specimens is discussed.

An infected corpuscle (especially when it contains more than one parasite) usually appears darker than uninfected corpuscles. This appearance may be due to the contained parasite causing the corpuscle to assume a more spherical shape. The contained parasite is seen as a lighter body with definite well-defined margins, and may at times show within it a more refractive area, which we believe to be a vacuole. Extracorpuscular parasites appear as darker bodies on a light background.

Free parasites swimming over the surfaces of normal corpuscles have a characteristic appearance and can be differentiated from internal parasites. They appear as dark bodies surrounded by a light halo which gradually fades into the dark surface of the corpuscle. The halo is no doubt caused by the depression of the surface of the underlying corpuscle.

(*a*) *Single intracorpuscular parasites which did not at any time show very marked pseudopodia.*

Diagram 14 represents a series of sketches of two single intracorpuscular parasites (Series *A* and *B*) which were kept under continuous observation at room temperature (22° C.). The numbers below the successive figures denote the minutes which elapsed in each case from the time the film was prepared. The dog died 24 hours later.

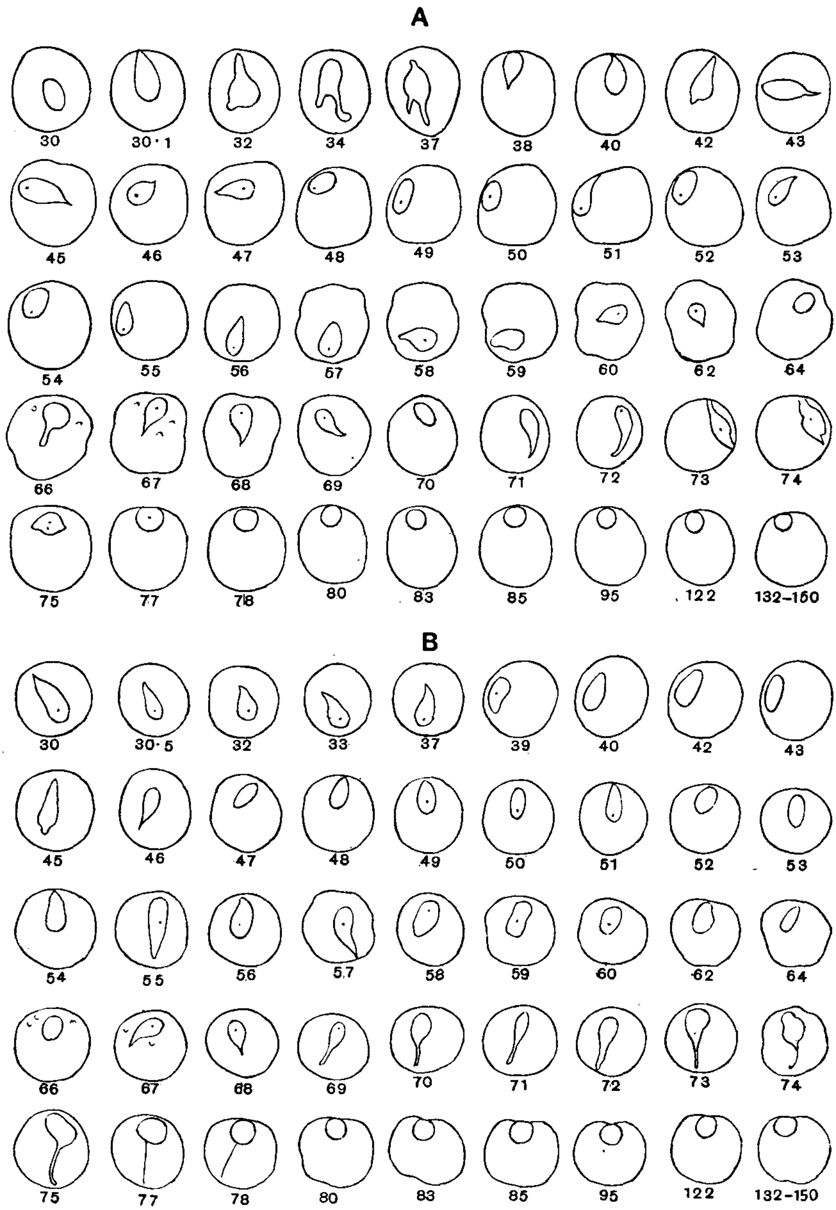

Diagram 14.

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Series A. In this series the infected corpuscle was first noticed 30 minutes after the preparation was made, the parasite then being ovoid. Almost immediately it became pyriform, and then protruded pseudopodia (32—37′). Later the pyriform shape was resumed (38′), and for the next fourteen minutes the parasite moved about the corpuscle, at times appearing pyriform and at other times oval. After 64 minutes it became rounded and then protruded a fine process (66′) which was almost immediately withdrawn and the parasite again became pyriform, causing irregularities on the surface of the corpuscle at the points indicated (67′). The parasite next became elongated (71′, 72′), moved to the side of the corpuscle, and became irregular in contour (73′, 74′) and later rounded (75′). After 77 minutes it became spherical and lay near the edge of the corpuscle. Although the corpuscle was kept under observation up to 150 minutes no further motion or alteration in shape was noticed.

Series B. This parasite also first came under observation 30 minutes after the preparation of the film. At first it was a pyriform body which moved about the corpuscle, and at times seemed to be oval. At certain times (66′, 67′) its movements caused irregularities on the surface of the corpuscle. After 69 minutes it became elongated, and during the next 9 minutes one extremity gradually became thinner and the other thicker and more rounded, till after 77 minutes the parasite appeared as a spherical body with a long thin process. The filament was then retracted and the organism became motionless 80 minutes after the blood was drawn. During the next 70 minutes no changes in shape or position were noticed.

In another similar observation an active elongated parasite was observed within a corpuscle 18 minutes after the blood was drawn. It first became irregularly rounded (19′), then rod-like (20′), roughly triangular (24′, 25′) irregularly fusiform (27—40′) and eventually shortened, leaving a delicate filament protruding from one end (42′), which soon disappeared (43′). Later (45—49′) the parasite assumed a typical pyriform shape and eventually became spherical (50′) when all motion ceased.

In still another observation a large spindle-shaped parasite which stretched across the corpuscle was watched for nearly an hour. For nearly 10 minutes it remained almost motionless though slight changes in contour occurred. Then it suddenly altered its position in the corpuscle, still retaining its original shape. Later the central parts became thicker and the ends were withdrawn, so that the parasite became oval and lay in the centre of the corpuscle. Later the parasite moved towards the

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edge of the corpuscle, becoming at the same time pear-shaped. Finally it became round and moved to the edge of the corpuscle and remained motionless in this position until the observation ceased. Several such cases were noted.

Many observations of this nature were made in which single parasites which never showed very actively moving pseudopodia came to rest as spherical bodies near the edge of the infected corpuscles.

(*b*) *Single parasites showing well-marked pseudopodia.*

In Diagram 15, Series *A* is figured an exceedingly active single parasite, the first nine figures being all drawn within 2 minutes. In fact it was impossible to draw fast enough to indicate the exceedingly active movements of the parasite. In Figs. 3 and 4 it is seen that the corpuscle was bulged by the parasite and much distorted, reminding the observer of a cat in a bag. This specimen was first observed 100 minutes after the preparation was made, and the movements, though very violent for a short time, suddenly ceased and the parasite became spherical.

In Series *B* another very active parasite is shown which was first observed 7 minutes after the blood was drawn. At first this parasite possessed two very long pseudopodia with blunt slightly enlarged extremities which were gradually withdrawn. During the first ten minutes of the observation these pseudopodia exhibited rapid side to side vibratory motion. After 4·5 minutes the parasite was round with a short process and first showed a more refractile central spot, which later became very clearly defined and gradually enlarged (20′), and later became smaller again (41′). This object is apparently a vacuole. For 34 minutes the parasite kept changing its shape, but at length became pyriform. Eventually it became oval and closely applied to the edge of the corpuscle, and remained in this condition until the observation ceased, 107 minutes after the blood was drawn.

In Series *C* an elongated parasite is shown which very rapidly changed its shape for 20 minutes, occasionally showing well-marked pseudopodia (13′, 21′), and twice becoming almost spherical. Finally it became spherical and remained motionless, showing a central refractile area.

All the above observations were made at room temperature, but several others were made at 35—41° C. which showed the same changes. In several cases the vacuole-like structure was noticed to gradually

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enlarge, then elongate, and finally divide into two minute refractile areas.

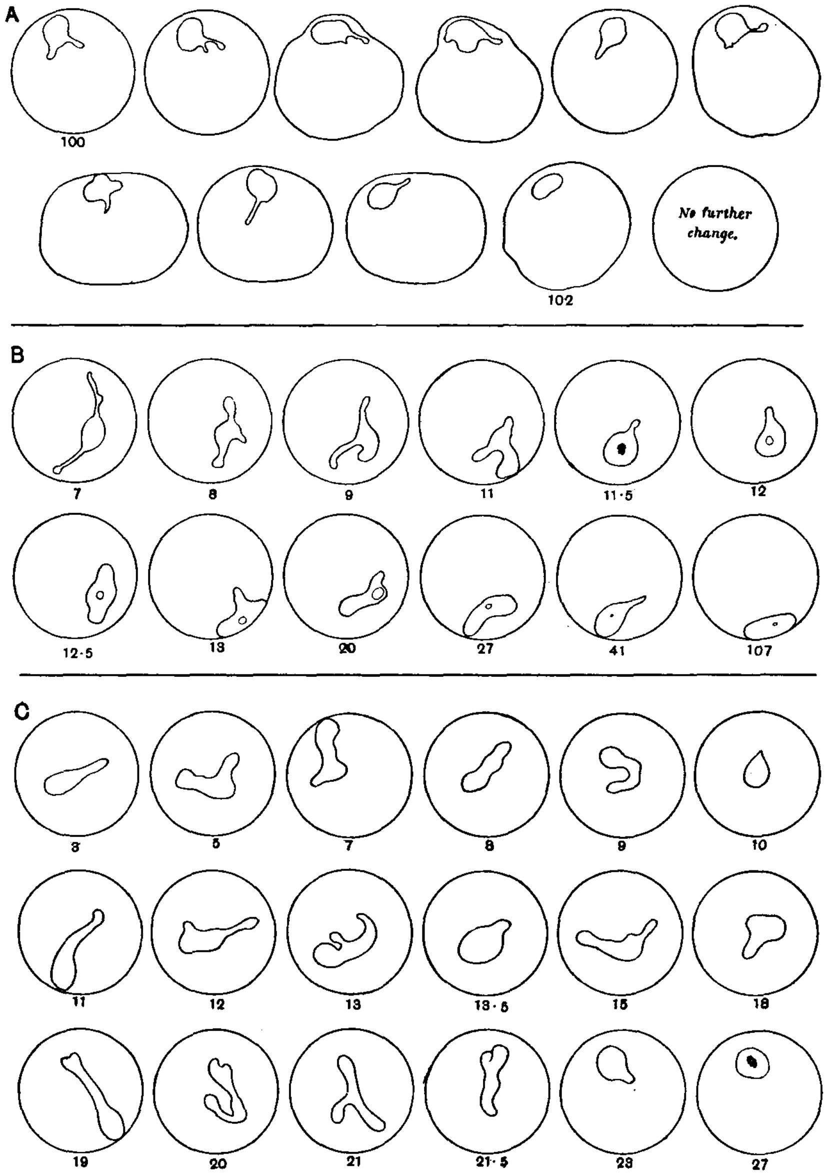

Diagram 15.

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(*c*) *Observations on double pyriform intracorpuscular bodies.*

Diagram 16, Series *A*, reproduces a series of sketches made during the observation of a pair of conjoined intracorpuscular bodies. The corpuscle was first noticed 14 minutes after the preparation was made and the parasites appeared as two pyriform bodies attached by their apices to a small rounded mass. This condition was very frequently

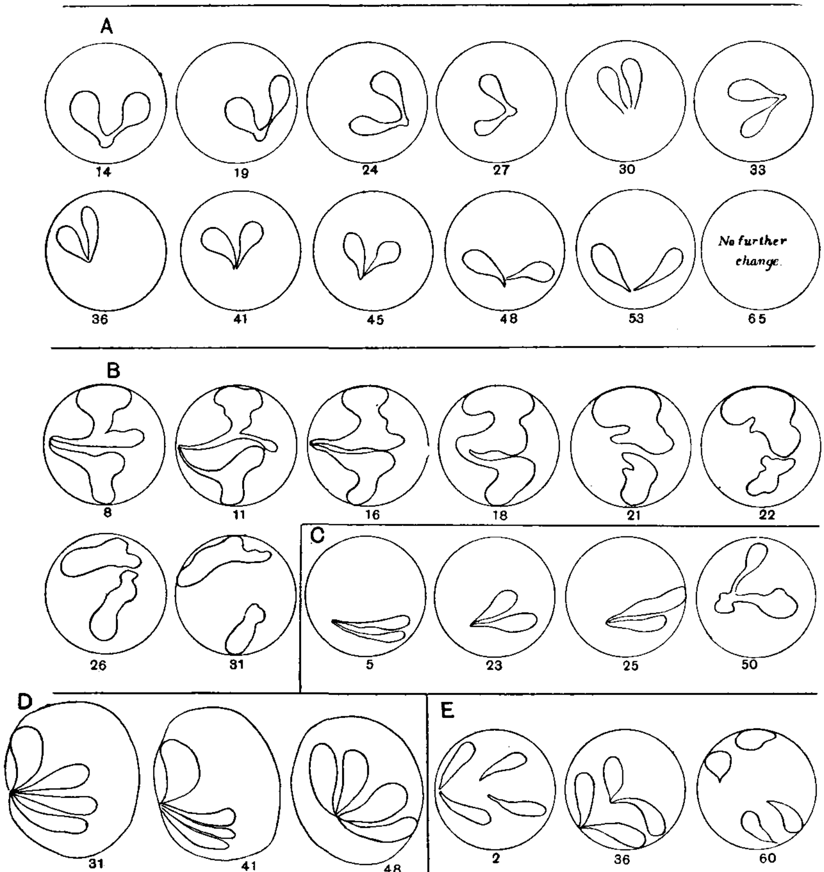

Diagram 16.

noticed and is of some importance in view of another observation on the development of these bodies (p. 621). The parasites gradually altered their position in the corpuscle and in relation to one another, and the thickened mass at their junction gradually disappeared (30′). The

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parasites appear broader in one position than they do in another, which may be explained by their having a somewhat flattened form. This fact was noticed on several occasions and is again represented in Series *C* and *D*, in the former of which the parasites not only alter their relative positions, but increase in size and appear to acquire a rounded body at their apices. The changes in the relative positions of the parasites consist in their bases approaching one another or moving apart, and their opposing surface contours appearing alternately convex and concave. The changes illustrated in this diagram were noticed on several occasions (Series *C*).

Series *B* illustrates the changes which occurred in an extremely amoeboid parasite within a corpuscle. The parasite which was composed of the large portions joined together at the end of two long processes was first observed eight minutes after the preparation of the film. At the end of ten minutes observation (18′) the large portions were more definitely united, but within three minutes (21′) the two portions had separated but still showed the remains of the uniting processes. Eventually all pseudopodia were retracted and the two parasites which had developed were separated by a considerable interval (31′).

(*d*) *Observations on double amoeboid intracorpuscular parasites.*

Several corpuscles containing double amoeboid parasites have been noticed during the course of these observations, the parasites undergoing similar changes to those which have been described and eventually coming to rest as spherical bodies. One specimen, however, deserves special mention, as one of the two parasites threw out on several occasions long pseudopodia which passed between the other parasite and the surface of the corpuscle.

(*e*) *Observations on corpuscles containing more than two parasites.*

Diagram 16, Series *D*, illustrates the change which took place in the relative positions of four pyriform parasites contained within one corpuscle. When first observed 31 minutes after the preparation of the film one of the parasites was much broader than the others. During the next 10 minutes this parasite seemed to become still broader, while the others appeared smaller and became closely crowded together. Later however (48′) their positions again changed and the four became symmetrically arranged, and all of the same size and shape. These changes in form appear to indicate that such pyriform parasites are flattened leaf-like bodies which may at one time present the broad surface and at another the narrow surface.

Diagram 16, Series *E*, illustrates another form of movement within a similar infected corpuscle. In this too the parasites, which were first observed two minutes after the preparation of the specimen, showed definite changes in contour at the end of 34 minutes observation. During the next half-hour the individuals of the pairs seemed to become detached from each other and all moved to the edge of the corpuscle. One parasite turned completely round, its blunt end being placed where the apex originally was. Some of the sketches made during the intervening periods have not been reproduced.

Such slowly occurring motions have frequently been noticed in similar specimens.

Still more rapid and remarkable changes in four parasites occupying a corpuscle are illustrated in Diagram 17, Series *A*. The infected corpuscle was first noticed within two minutes of the time the preparation was made, but the parasites remained motionless for some time. Later

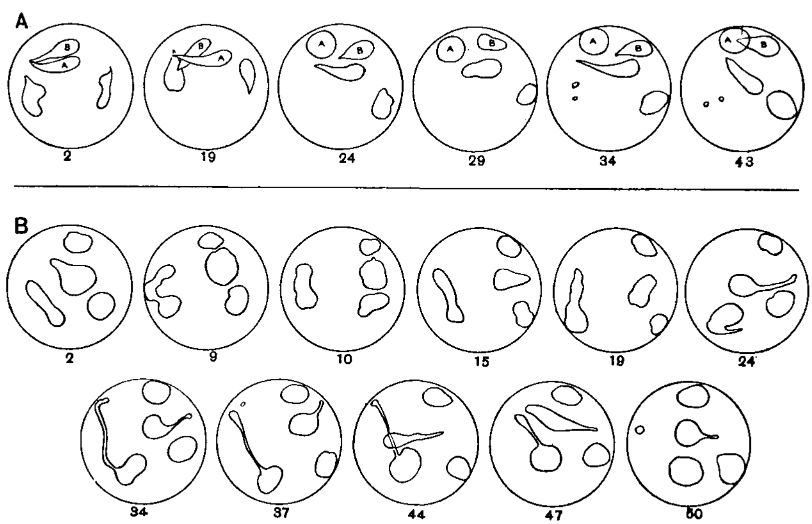

Diagram 17.

all became pyriform (19′) and the apices of three became superimposed. Within the next five minutes (24′) one of the pyriform bodies (*A*) became spherical and remained in this condition until the end of the observation. After 27 minutes observation all the organisms were roughly spherical or oval, but within five minutes one had become a typical

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pyriform body and another long and thin. At the same time two small objects appeared in the substance of the corpuscle, which were possibly fragments detached from the parasites or bodies extruded by them. No further changes of importance occurred, although the corpuscle was watched for 65 minutes.

Diagram 17, Series *B*, represents still more marked changes. This corpuscle was first observed two minutes after the preparation of the film and contained the four parasites shown (2′). Within seven minutes (9′) three parasites had become spherical but the other had assumed an irregular shape, but soon became long and thick (10′). Soon after (19′) this parasite lengthened, while the others remained in almost the same condition. At the end of 22 minutes observation (24′), however, the former and one of the latter developed pseudopodia, which increased in length (37′), both terminating in rounded bulbs. One of these parasites then became long and thin while the other became larger and retracted its pseudopodium, leaving however the end apparently detached as a round mass (50′).

(*f*) *The multiplication of parasites within corpuscles.*

It has generally been assumed from the study of stained preparations that the parasites multiply within the corpuscles. We have, however, been able to follow the process in the living parasite[1]. One example has been illustrated in Diagram 16, Series *B*, and another is shown in

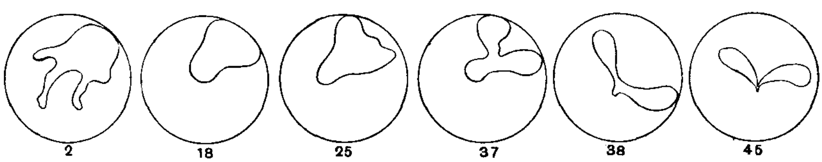

Diagram 18.

Diagram 18. In this case an extremely active amoeboid parasite was noticed within a corpuscle two minutes after the preparation of the film. The movements gradually ceased and the parasite became irregularly pear-shaped (18′). After a period of 7 minutes an indentation appeared on the blunt end of the pear (25′) which gradually increased in depth until the parasite had a trilobed appearance (37′). This developed into

[1] *Note whilst going through the press:*—We have since had several opportunities of observing the complete development of intracorpuscular paired pyriform parasites from bodies resembling Figs. 2 and 18 in Diagram 18. We shall describe the process in detail in a future paper.

the double pyriform type joined by their apices to a small mass (38′), and finally into typical double pyriform bodies (45′).

This observation was made at 35° C.

Large numbers of corpuscles containing parasites resembling those shown in Figs. 37, 38 have been noticed, and their further development has already been illustrated in Diagram 16, Series *A*.

On many occasions also the earlier stages of this process have been observed, but apparently owing to the experimental conditions, its completion has not been always followed.

(*g*) *The escape of the parasites from the infected corpuscles.*

On many occasions the parasites have been seen to escape from the infected corpuscles, and it has been noticed that three different methods may apparently be adopted. (1) Most commonly the parasite or parasites leave the corpuscle, and the latter immediately becomes pale and finally disappears. (2) Less commonly the corpuscle first becomes pale and then the parasite escapes. (3) Still more rarely the parasite appears to leave the corpuscle without apparently injuring it.

(1) The mode of escape about to be described in detail is undoubtedly the most common one under experimental conditions, and satisfactorily accounts for the watery condition of the blood during the last hours of life as well as the haemoglobinuria.

We have already pointed out that an intracorpuscular parasite appears as a well-defined, light body surrounded by the dark corpuscle. No intervening light halo is seen. At times, prior to the escape of the parasite, without any apparent disturbance of the surface of the corpuscle, the parasite seems to disappear as into a fog. Though its general form can still be defined, its outline is no longer sharp, and the colour of the organism approaches more nearly to that of the corpuscle. This appearance may be due to the corpuscle assuming a more spherical form owing to the absorption of fluid. Gradually, or at other times rapidly, the parasites become more distinct, show active movements and simultaneously pass out of the corpuscles, often without apparently encountering any very great resistance, and swim away. The corpuscle then rapidly loses its colour and almost disappears, although its margin can generally still be defined by careful focussing. Occasionally, however, no trace of it remains.

Slight differences are noticed in various cases, for example the foggy stage may not be observed, or the organisms may distort the corpuscle to some extent before their escape as if the envelope offered considerable resistance. At other times the whole process is so rapid that the various changes can scarcely be followed.

(2) On several occasions the corpuscles even seem to become pale or almost invisible before the escape of the parasites. Under these conditions the parasites frequently perform remarkable gyratory movements before leaving the remains of the corpuscle, two instances of which are described in detail.

In one instance the parasite swam rapidly round and round, blunt end foremost, following the round contour of the cell. After two minutes the movements became irregular, and the pointed end appeared to feel about the margin of the corpuscle, and after a further interval of five minutes the parasite suddenly issued. In another case a pair of pyriform parasites were observed within a corpuscle, which suddenly became pale 78 minutes after the preparation of the film. One parasite immediately left the corpuscle, while the other rapidly swam round the inner margin. After six revolutions it suddenly left the corpuscle, blunt end first, the contour of the corpuscle remaining unaltered and still faintly visible.

(3) Two instances of the escape of parasites have been observed in which the affected corpuscles retained their normal appearance.

Diagram 19, Series *A*, illustrates the escape of a single pyriform parasite from a corpuscle. When first noticed the parasite was of the typical pyriform type and was exceedingly active, constantly changing its position within the corpuscle, and sometimes rapidly moving across the corpuscle. These movements often violently agitated the corpuscle and caused it to assume all sorts of shapes, and during these periods it was hard to define the parasite. At intervals however it became fairly quiet. After 30 minutes it gradually altered its shape and became very irregular, and its movements during a period of two minutes were extremely violent, producing great alterations in the shape of the corpuscle. At the end of this period the parasite became spherical and motionless, and the corpuscle regained its original shape and general appearance. After a short time the parasite gradually approached the edge of the corpuscle and seemed to protrude beyond it, and for some time nearly half the parasite appeared outside. Suddenly very violent movements occurred and the parasite became free (45 minutes) and pear-shaped, and the corpuscle immediately resumed its normal shape. The free parasite remained near the corpuscle, but showed a constant vibratory movement of its pointed extremity.

It then swam away, and in turn attached itself to other corpuscles, behaving in the manner shown in Diagram 22.

Although watched for more than an hour this parasite did not enter another corpuscle.

Diagram 19, Series *B*, represents the escape of one of two parasites contained in an infected corpuscle. The corpuscle was first observed 70 minutes after the preparation of the film and the process was complete within five minutes. The irregular parasite was at first in close

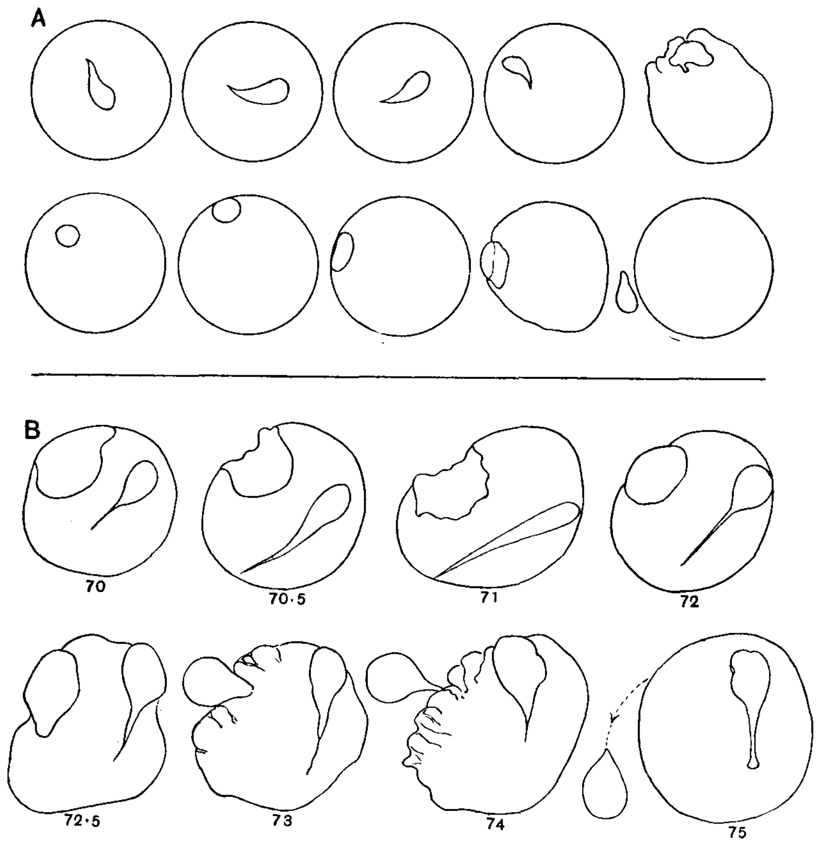

Diagram 19.

contact with the edge of the corpuscle and produced a slight prominence on the surface. Later (70·5—71′) the surface of the corpuscle and parasite became irregular and depressed, but within another minute the parasite again caused a prominence on the surface, and gradually assumed a pyriform shape. Then the parasite shook

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violently and caused great distortion of the corpuscle, at the same time causing its blunt end to protrude beyond the corpuscle (73'). Later the parasite became almost free, being only attached to the corpuscle by its apex (74'). During this period the violent movements were still shown, and half the corpuscle was much distorted. Eventually the parasite broke loose as a pyriform body (75') and the corpuscle resumed its normal appearance. During the whole period the other parasite seemed to undergo slight changes in shape. Finally the free parasite, after a short quiescent stage, swam away and attached itself to another corpuscle but did not enter.

In spite of the latter two observations we still consider it doubtful whether parasites ever escape without causing the destruction of the corpuscle. In the first case the observation was made before the characteristic difference between a parasite on the surface of a red corpuscle and an intracorpuscular parasite had been fully realised. Consequently we are doubtful whether a parasite swimming on the surface of a corpuscle and confined within its concave surface was not mistaken in this instance for an intracorpuscular form. The second observation is also open to doubt in that the parasite which eventually escaped was never seen completely within the corpuscle. In this case the parasite may have only made a depression for itself in the surface of the corpuscle, as other forms have been seen to do (Diagram 22). Until these observations have been confirmed we therefore hesitate to definitely state that intracorpuscular forms can leave the corpuscles without causing their destruction.

(*h*) *The behaviour of free parasites.*

On several occasions parasites which had been seen to escape from infected corpuscles were watched, and their behaviour varied considerably. All immediately after their escape were roughly pyriform, some being longer and thinner and others shorter and with a more rounded blunt extremity. It was also noticed that the blunt extremity almost invariably showed a slight but definite pointed process as illustrated in Diagram 20, Fig. A 5; and that they often possessed two shoulder-like, blunt prominences at the junction of the upper and middle third of the parasite. Some parasites after their escape remained almost motionless in the same position for a long period, others remained there for some time, but frequently altered their position in relation to the neighbouring corpuscles by sudden jerky movements; others again after a short quiescent period swam away, while others immediately swam

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off with great rapidity. All these conditions were noticed in comparatively fresh preparations observed at 35° C., consequently the inactive condition cannot be entirely due to lack of vitality on the part of the parasite in old preparations.

Even when free pyriform parasites do not leave their original position they exhibit slight movements. The pointed extremity of the parasite is almost invariably in more or less rapid vibratory motion, closely resembling a fish moving its tail fin but otherwise motionless. Sometimes the neighbouring fluid appears to be agitated by a flagellum, but such a structure can very seldom be defined. The sudden jerking movements of the parasite which often cause them to rotate through a part of a circle are usually accompanied by a violent movement of the whole pointed extremity. Sometimes such movements cause the parasites to suddenly dart forward up to a distance equal to two or three times their own length. Both these forms of movement again forcibly remind the observer of the sudden movements of a fish brought about by rapid contractions of the tail. Free swimming parasites move along with fair rapidity, usually with the blunt end first, though they are undoubtedly capable of moving with the apex first. This swimming motion is accompanied by slight side to side motions of the tip of the pointed extremity. They may traverse a field in about one minute. The parasites may move in a straight line or in various directions, but do not seem to purposely avoid corpuscles and other obstacles.

Occasionally parasites which have just escaped from a corpuscle swim with great rapidity for a short distance, and usually immediately enter other corpuscles. Specimens which behave in this manner generally belong to a large type with a long tapering extremity, which shows rapid side to side movements, and resemble fish suddenly darting forward.

In most preparations free parasites can be found, which have not been seen to leave corpuscles, and their behaviour has been watched on several occasions. They may either remain in the same position, or swim slowly about the field, or attach themselves to red corpuscles (see p. 629) or passing leucocytes (p. 632), or several may collect into a mass.

Those which remain in the same position, as well as those which swim slowly about the field, gradually lose their pyriform shape and become more rounded, finally becoming spherical bodies. Diagram 20, Series *A*, illustrates the changes which occurred in a very large free form which was kept under observation for two hours. The organism, which was at first long and possessed a flagellum, after 35 minutes (40′)

became round and showed a thicker flagellum. After two hours the organism was spherical, and had lost its flagellum. In specimens obtained during the last hours of life very large numbers of free pyriform parasites are present, and sometimes several individuals may

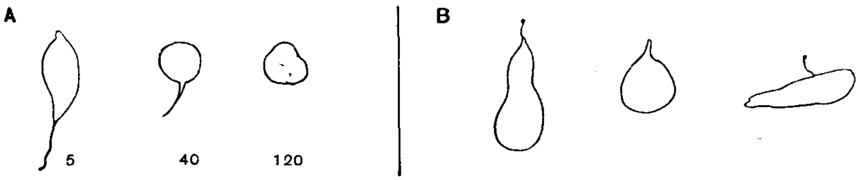

Diagram 20.

be seen to agglutinate, apparently joined by their pointed extremities. Sometimes irregular bodies have been noticed in blood taken at this period, which look as if they might have been derived by a partial process of fusion from an agglutinated mass of four or five parasites.

At this period also certain very remarkable forms were noticed which are illustrated in Diagram 21.

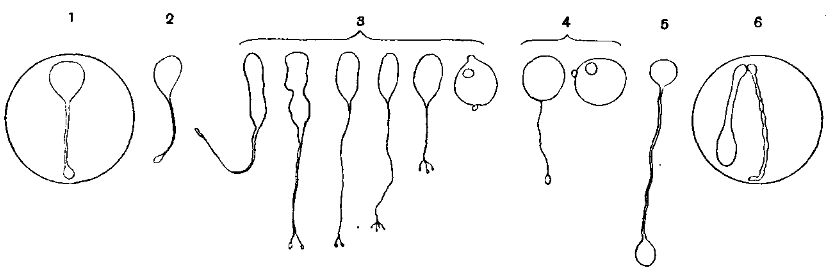

Diagram 21.

Fig. 1 shows within a corpuscle a parasite with a large rounded blunt end and a long thin process ending in a distinct rounded mass, and Fig. 2 a similar organism free in the plasma. Both these forms were common, and though the escape of the latter form from a corpuscle was not observed there can be little doubt that they were derived from intracorpuscular forms. Some of these flagellated forms move slowly, but others remain in the same position. In both cases they show slight or even fairly well-marked lashing movements of the flagellum. Fig. 3 reproduces a series of sketches made of a flagellated form during a prolonged observation.

At first the organism showed one greatly enlarged extremity, divided by a slight constriction, and a long flagellum, apparently ending in two slight bulbs. Later the flagellum became thinner, and the end was distinctly branched, each branch ending in a small enlargement. Gradually the flagellum shortened and the extremity became more branched. Finally after 10 hours the flagellum was almost completely withdrawn, and was only represented by an ill-defined mass, and the organism had become almost round and showed a large vacuole. In Fig. 4 a somewhat similar organism is illustrated as at first seen and 10 hours later. In all cases slow lashing movements of the flagellum took place.

Fig. 5 represents a type of which several specimens were observed, an organism consisting of two almost equal sized round masses joined by a thin, long, irregular band. This type moves slowly with an undulating motion.

In Fig. 6 one out of a long series of sketches is reproduced. An amoeboid intracorpuscular parasite with a long slender process was first noticed, which gradually altered in form until the larger portion remained as an elongated body, and the process became very long and distinct and possessed a series of irregular nodes. Eventually the process was represented by a series of seven minute enlargements joined together by a fine thread. A considerable number of corpuscles infected by similar parasites were seen, and it was thought possible that the processes might be extruded and develop into the organisms represented in Fig. 5. No instance of the escape of such a process was however noticed.

Free amoeboid parasites have not been noticed under any conditions.

Diagram 20, *B*, represents other forms of free parasites seen in the living blood. Fig. 1 shows a long irregularly pyriform parasite with a short flagellum ending in a minute knob, and Fig. 2 a rounded parasite with a spike-shaped process. Both these forms are capable of locomotion, and even when stationary usually exhibit slight movements in the flagellum or process. A third and uncommon form is represented in Fig. 3. This form is actively motile, and also shows a movement of rotation on its long axis. Occasionally, when the movements are less active and the parasite momentarily pauses in a suitable position, an object has been seen on one side, which was apparently a short flagellum ending in a minute knob.

(*i*) *Observations of free parasites apparently attacking corpuscles.*

In a considerable number of freshly-made preparations we have seen pyriform parasites attach themselves to or attack corpuscles.

Typical instances of the changes which are seen in the corpuscle and attacking parasites are illustrated in Diagram 22, Series *A* and *B*.

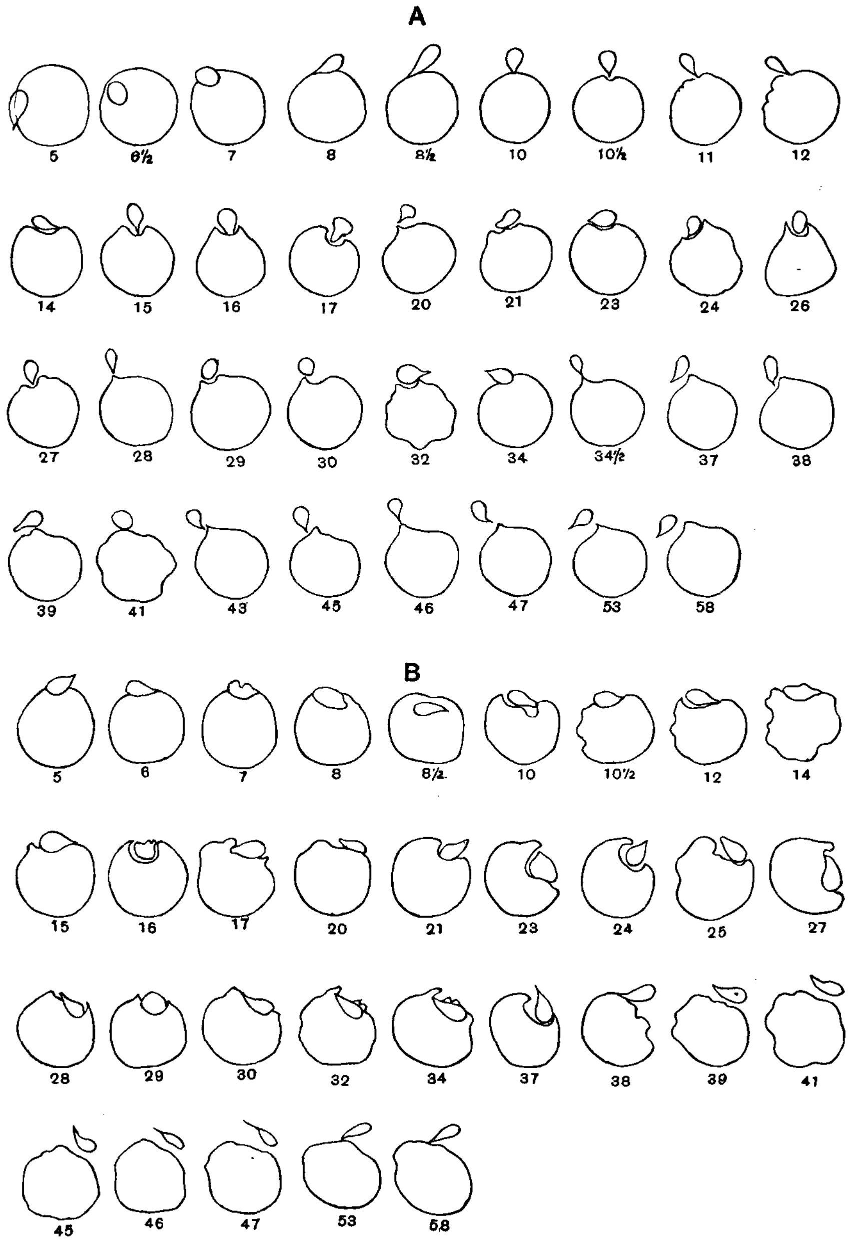

Diagram 22.

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Series A. Within five minutes of the preparation of the film a pyriform parasite was seen applied along its length to a corpuscle. Shortly afterwards the parasite seemed to become rounded ($6\frac{1}{2}'$) and indented the corpuscle, but very soon again drew away ($8'$), resuming its pyriform shape. During the next four minutes (8—$12'$) it remained attached to the corpuscle, by its pointed extremity, at a place indicated by a slight projection, while slight side to side movements occurred. Next the parasite applied one side to the corpuscle, causing a considerable indentation ($14'$—$17'$), but after a few minutes again only remained attached by its apex, this time drawing out the surface of the corpuscle to a sharp point ($20'$). Subsequently the same series of movements were repeated, and at the end of an hour the parasite was still attached to the corpuscle. It was noticed throughout that even when the parasite was almost stationary an active movement was visible at the point which was adhering to the corpuscle. The apparent rounded form of the parasite at times was probably due to an alteration in its position.

Series B illustrates a similar series of events, with slight differences. The parasite was first noticed with its rounded extremity applied to the corpuscle, and later occasionally seemed to show amoeboid movement ($7'$, $14'$, $16'$). The latter appearances may however have been caused by crenations of the corpuscle while it was being indented by the parasite, since distinct temporary crenations of the corpuscle were seen on other occasions ($32'$, $34'$). At one time the parasite became apparently detached ($39'$), but later again attached itself by its apex ($53'$).

A very large series of observations and drawings have been made of those forms which are apparently attacking corpuscles, but in the great majority of cases the parasite has been unable to effect an entrance. Sometimes the parasite remains almost motionless with its blunt or pointed extremity, or its side closely applied to the corpuscle, without altering the shape of the latter. Plate XI, Figs. 15, 16, 18, represent this condition in stained preparations made from blood taken several days before death. On the other hand the parasite may come to rest for a time, either indenting the corpuscle or distorting it by drawing out one side (Plate XI, Figs. 17, 18, 19, 21, 22, 23). In the latter case the pointed extremity is nearest the corpuscle and is probably attached to it by a fine process, which usually cannot be seen either in fresh or stained preparations.

Other observations appear to show that the parasite is capable of affecting the corpuscle in some manner by the application of its pointed extremity. In some instances almost as soon as the pointed extremity of the parasite had come into contact with the corpuscle the latter suddenly became very pale, and "vanished," and its presence could only be determined by carefully looking for the rim, which could still be just made out. We must point out, however, that in smears made shortly before death, when the plasma is tinged with haemoglobin, corpuscles have been seen to behave in a similar manner without the approach of a parasite. This phenomenon has not however been often observed in smears made at an earlier period. The specimen figured in Plate XI, Fig. 22, shows that the parasite had caused some disturbance within the corpuscle. The appearances figured in Plate XI (Figs. 16—23) might be considered accidental but for the facts we have established with regard to the behaviour of the parasites in fresh blood. The chain of events depicted in Diagram 22 coincides very closely with what is shown in the Plate.

(*j*) *The entry of parasites into red blood corpuscles.*

Parasites have on many occasions been seen to enter corpuscles, and in all cases the entrance has been effected by pyriform or long thin parasites, never by rounded forms. In most instances the parasites which have entered corpuscles have been actively motile, and on several occasions they have been seen to leave other corpuscles a very short time before. Sometimes four parasites leave an infected corpuscle and moving with great rapidity some of them enter other corpuscles. In one case three out of the four recently liberated parasites immediately entered three fresh corpuscles, while the other remained free and gradually became quiescent. In another case a pair of conjoined pyriform parasites escaped from a corpuscle which promptly faded. The parasites very soon separated and at once entered two fresh corpuscles, after which the parasites became spherical. Usually the parasite approaches the corpuscle with its blunt extremity foremost and rapidly indents the surface. Then violent movement of the thin end of the parasite occurs, and the side of the corpuscle becomes greatly distorted and it may be caused to oscillate or may even be moved from its original position. Gradually the parasite sinks more deeply into the corpuscle and finally disappears within it, when the movements of the corpuscle cease and it resumes its rounded shape. At this time it is generally hard to define the parasite within the corpuscle, which seems to become darker in colour. Gradually however the parasite becomes more visible, and its shape changes from pyriform to oval.

(See p. 613 regarding the differences in the appearance between parasites outside and inside corpuscles.)

Diagram 23 illustrates a peculiarly interesting observation.

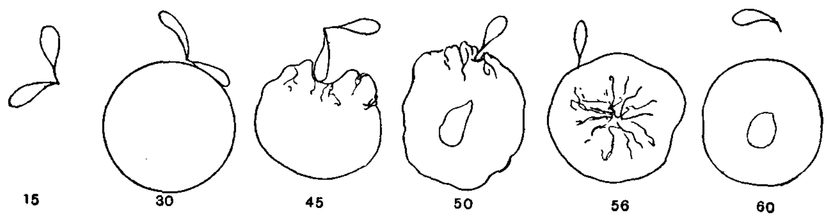

Diagram 23.

Serum containing numerous free parasites was obtained from an infected dog shortly before death and added to a small quantity of normal dog's blood. Shortly after the preparation was made two pyriform parasites joined by their pointed ends were noticed swimming free in the fluid between the corpuscles, and frequently altering their relative positions to one another. Fifteen minutes later one of the pair applied itself by its side to the edge of a corpuscle, while the other swung freely (30′). In this condition they remained for fifteen minutes, when the applied parasite rapidly changed its position and indented the corpuscle with its blunt extremity. Very violent movements then occurred, during which the side of the corpuscle was greatly distorted, but after five minutes the parasite passed into the corpuscle (50′) leaving the other with its apex attached to the distorted wall of the corpuscle. Soon afterwards the edge of the corpuscle became well defined, but the surface of the central portion was still disturbed by the movements of the internal parasite (56′). Shortly afterwards all motion ceased and the corpuscle resumed its normal appearance, but showed a rounded parasite within it. Shortly before this the other parasite became free and swam away. It attacked another corpuscle but did not enter it.

(*k*) *Observations on the actions of the leucocytes in the blood of infected dogs.*

During observations on fresh blood preparations parasites have been seen on several occasions to swim towards and adhere to passing leucocytes. The parasite becomes attached by its pointed extremity and may be dragged about after the leucocyte for a considerable time, or may tear itself free. On one occasion a leucocyte was seen dragging after it a parasite attached to a long pseudopodium. Soon the leucocyte

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passed between two red corpuscles which afterwards came together, tearing loose the parasite.

Under some conditions, however, the phagocytes rapidly take up parasites. Phagocytosis has been especially noticed in preparations made within 24 hours of the animal's death, in those which have been kept under observation for a long time, and in preparations made from sterile defibrinated blood kept in the dark at room temperature for 24 to 48 hours (Plate XIII, Fig. 3.)

Even if the process of phagocytosis is carefully watched it is often very difficult afterwards to locate the parasite within the leucocyte. Sometimes however, the contour of the parasite seems to be visible for some time, and leucocytes have been observed which apparently contained several parasites. Occasionally phagocytes enclose whole infected corpuscles, a condition which we illustrated in a previous paper (1905, Plate IX, Figs. 63—67).

Stained preparations show that the parasites rapidly undergo degenerative changes within the leucocytes.

(*l*) *Observations made at night on the living blood.*

Bearing in mind Weber's and Schaudinn's observations (p. 642) we made a number of examinations of the living blood at night, to determine whether other forms of the parasite appeared in the peripheral circulation at that time. No trypanosome or spirochaete-like types were however encountered, and the parasites behaved in exactly the same manner as those observed in the daytime.

(*m*) *Observations on living blood some hours after the preparation of the specimens.*

After the lapse of two or three hours or more specimens of living blood present certain peculiar features. Many of the parasites both free and intracorpuscular become rounded, the number of red corpuscles is diminished owing to the rupture of many of them by the escaping parasites, and a certain proportion of the corpuscles become crenated. One phenomenon, however, deserves special attention, namely the positions assumed by parasites within crenating corpuscles. It has already been shown that the parasites in old preparations tend to become spherical and come to rest at the edge of the corpuscles. When the latter begin to crenate, these parasites often appear to project beyond the margins of the corpuscles, while still remaining enclosed within the corpuscular envelope. Under similar conditions the rounded extremities

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of single or double pyriform bodies may also appear to project. These appearances are of some importance, for similar forms, whose history is unknown, are encountered in stained preparations, both in this disease and in malaria. In the latter case Argutinsky (1902, p. 345) after the study of stained preparations came to the conclusion that the parasites were only applied owing to the fact that a limited number protruded beyond the edge of the corpuscles. We refer to this question on pp. 635—639.

(*n*) *Observations on the living blood within the last few hours of life.*

Blood obtained from dogs within the last 12 hours of life has a peculiar thin watery appearance, and flows very readily. Preparations made from such blood show enormous numbers of parasites both free and in the corpuscles. Frequently almost every corpuscle in the field is infected, often with more than one parasite, as shown in Plate XII, Fig. 5. Many of the intracorpuscular parasites exhibit very active amoeboid movements, while others constantly change their positions in the corpuscles. Immediately after the preparation of the film many of the pyriform parasites are seen to leave the corpuscles, usually in the following way. Either active movements may be seen in the corpuscles infected by one, two or four pyriform parasites, or the parasites remain almost motionless, and suddenly emerge from one side of the corpuscle and rapidly swim away. Immediately after the escape of the parasite the corpuscles begin to lose their colour, and within a very short time become very pale and finally almost disappear, so that the outline can only be defined with great difficulty. At other times they disappear so completely that their remains cannot be detected with certainty. Not infrequently the exit of the parasites is followed so rapidly by the disappearance of the corpuscles, that the observer if he happens at the time to be concentrating his attention on neighbouring corpuscles only becomes aware of free parasites near an area originally occupied by an infected corpuscle. More rarely a corpuscle gradually or suddenly fades and the contained parasites execute a remarkable gyratory movement in the shadowy corpuscles (see p. 623). As time goes on the number of free parasites in the preparation increases greatly and in some places very few corpuscles remain. In other similar preparations the destruction of the corpuscles is not so great and free forms do not become so numerous. We are unable to give any reason for these differences.

In these films it is especially noticeable that many of the intra-

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corpuscular forms possess long thin flagella such as those illustrated in Diagram 20. After some hours the flagella are retracted or lost and the parasites become rounded, and frequently show a central vacuole.

Several films prepared 12 hours before death were kept under the microscopes for 10 hours. At the end of this period the number of corpuscles had diminished, but those which remained were still well-preserved. The active amoeboid movements of the intracorpuscular forms had ceased, and nearly all such intracorpuscular parasites seen were spherical in shape and many showed a large vacuole. The free flagellated and pyriform parasites which had been present in great numbers at the end of one hour were also represented by spherical bodies, frequently showing vacuoles. Only a few motile pyriform bodies or rounded forms with short flagella were noticed. Many of the leucocytes contained numbers of rounded parasites which could be easily defined.

(o) *The relation of the parasites to the corpuscles.*

We have throughout followed the usual custom and described those parasites which appeared to be within the corpuscles as intracorpuscular forms. We think it however desirable, in view of the various opinions which have been maintained on the relationship of human malaria parasites to the corpuscles and Schaudinn's recent observations on *Haemoproteus*, to state at length the evidence in favour of the intracorpuscular situation of some forms.

In the case of human malaria the majority of observers agree that in certain stages the parasites are intracorpuscular, and attribute the beneficial effects of quinine in cutting short the attacks of fever to the action of the drug upon the free spore forms of the parasite, which are no longer protected by the envelope of the corpuscle. On the other hand some investigators believe that the parasites are epicorpuscular. Argutinsky[1] for example claims that the parasites of tertian malaria after applying themselves to the corpuscles gradually sink into and absorb the surface.

Schaudinn[2] (1904, p. 402) in his description of the indifferent form of *Trypanosoma noctuae*, which he states develops into the *Haemoproteus* (= *Halteridium*) of the stone owl (*Athene noctua*) says, that the parasite attaches itself to the corpuscle in the following manner. The trypanosome applies its flagellum flatly upon the corpuscle. The

[1] Argutinsky, P. (1902). "Malariastudien." *Arch. f. mikr. Anat.* vol. 59, p. 315. Pl. 18—21.

[2] See Note IV, p. 646.

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flagellum then withers and the parasite sinks into a pit on the surface of the corpuscle, which it again leaves after it has grown to some extent. In support of the view that the gregariniform stage of *Haemoproteus* is epicorpuscular he cites the fact that the affected corpuscles do not seem to be injured after they have been abandoned by the parasite.

In the case of *Piroplasma* some forms are obviously free swimming, and all observers, with the exception of Lühe (1906) speak of the other forms as intracorpuscular. This observer thinks that of the apparently intracorpuscular forms only the pyriform bodies are truly within the corpuscles.

As we have seen (p. 610) very few observers have made any attempt to study the parasites in the living condition, and the majority base their opinions solely on examinations of stained preparations, which owing to the manipulations they have undergone, often present very deceptive appearances. Moreover none of them clearly state the reasons which lead them to believe in an intracorpuscular stage. In our opinion the view that the parasite has an iutracorpuscular stage is correct, and we are able to adduce in favour of it evidence derived from a prolonged study of the parasites in a living condition.

All physiologists agree that the mammalian red blood corpuscle has a distinct envelope. While Schäfer and others think that the corpuscle consists of an envelope containing a fluid mass, most physiologists regard them as consisting of an envelope and a network or stroma whose meshes surround a fluid or semi-fluid mass. The capacity possessed by corpuscles of returning to their original shape after great distortion is well known. Peskind[1] (1904) has demonstrated by means of experiments with hydroxylamine hydrochlorate that the envelope can be separated from the stroma by bubbles of nitrogen gas generated within the corpuscle. According to this observer the envelope is exceedingly thin, elastic, colourless and semi-fluid, and some of his figures of intracorpuscular gas bubbles closely resemble certain intracorpuscular forms of *Piroplasma*, which appear to project beyond the corpuscles.

Our observations lead us to think that the corpuscle consists of an exceedingly delicate and elastic envelope enclosing material which offers very little resistance to the movements of such parasites as *Piroplasma*.

Very strong evidence in favour of the intracorpuscular condition is afforded by the movements of the amoeboid forms. These forms send

[1] Peskind, S. (vi. 04). "The envelope of the red blood corpuscle and its rôle in haemolysis and agglutination." *Amer. Journ. Med. Sci.* (Reprint, 16 pp.).

out pseudopodia which, however, never project beyond the margins of the corpuscles, and moreover they are capable of producing marked distortion of the corpuscle by the impact of their bodies against the wall (Diagram 15, Series *A*). Motile but non-amoeboid forms can also produce distortion in a similar manner. Again the extraordinary gyratory movements of certain pyriform parasites before they leave the remains of a ruptured corpuscle (p. 623) show that their movements are restrained by the remains of the envelope. Further a great difference exists between the appearances presented by free corpuscles swimming over the surfaces of intact corpuscles and those presented by parasites which are apparently within corpuscles. These appearances have already been described (p. 613), but may be briefly recapitulated. In the former case the superimposed parasite appears as a dark body, surrounded by a light halo on the dark background of the corpuscle, while in the latter case the parasite is seen as a light body with a distinct contour immediately surrounded by the darker corpuscular substance. Moreover infected corpuscles usually appear darker than others owing to their being more inflated through the displacement of their contents by the body of the parasite.

It is difficult to explain any of these appearances on the supposition that the parasites are merely epicorpuscular. If the parasites were epicorpuscular it is scarcely conceivable that the amoeboid forms would not protrude processes beyond the periphery of the corpuscle, or that they should cause the envelope of the corpuscle to protrude in the manner figured in Diagram 15, Series *A*. The gyrating movements of parasites in dehaemoglobinised corpuscles prior to their escape is most difficult to explain except on the hypothesis that they lie enclosed within the corpuscular membrane. It is true that sometimes the delicate membrane of the vanished corpuscle appears to offer no resistance to the exit of the parasite when the gyrating movements cease, nor is any visible break observed in the circular contour. The almost invisible character of the vanished corpuscle makes it impossible however to state that no rupture of the membrane takes place, and it may have reached such a degree of tenuity as to offer no appreciable impediment to the exit of the parasite.

Again, if the parasites were epicorpuscular their movements ought to produce undulations on the surface. These, however, are not seen unless the movements are very violent. On the other hand truly epicorpuscular parasites do cause undulations of the surface of the underlying corpuscle, and are invariably surrounded by a light halo.

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The fact that pyriform or oval intracorpuscular parasites may in old preparations apparently project beyond the edges of the corpuscles constitutes no evidence that the parasites are not enclosed within the delicate and elastic corpuscular envelope. In support of this view Peskind's observations are of great importance, since he was able to demonstrate that intracorpuscular gas bubbles enclosed by the corpuscular envelope may apparently project beyond the contour of the corpuscle.

Parasites which have been seen to apparently enter corpuscles and others which have been seen to attack corpuscles but have failed to enter produce great distortions in their walls. It seems improbable that the parasites would display such violent movements unless it was necessary for them to effect an entrance, or produce a deep depression for themselves on the side of the corpuscle. If, however, they merely indented the surface and came to rest in a pit the neighbouring walls of the corpuscle would show distortion, but this is not the case when the parasites truly enter the corpuscle, and the latter resumes its original contour.

It may be objected that a relatively large pyriform parasite could scarcely enter a corpuscle without producing a rupture and causing the latter to void its contents. This argument can we believe be met by the following observations. The corpuscular envelope is very elastic, allowing the corpuscles to be greatly distorted when passing through capillaries, or when attacked by parasites, which may even bury themselves in deep depressions, and causing the corpuscles to regain their original shape with great rapidity after the disturbing influence is removed. We think therefore that the envelope behaves during the passage of the fish-shaped parasite like a thin sheet of rubber, which may allow a relatively large object to pass through yet after regaining its original form shows hardly any indications of the rupture. If this is the case once the parasite has affected an entrance, little if any leakage may occur through the puncture, and even if a certain amount of leakage does take place the cycle of development of the parasite within the cell is probably so rapid, and the facility with which it can leave one corpuscle and enter another so great, that it would not be a matter of great importance.

We conclude then that the parasite has a truly intracorpuscular stage in its life history, and may further add that these observations seem to be of considerable interest in relation to the structure of the red blood corpuscles. They appear to indicate that the latter have an exceedingly delicate, elastic, colourless envelope, and contents which offer very little resistance to the free movements of parasites within them.

(*p*) *Summary of observations on living blood.*

An extended experience of the examination of living blood containing *Piroplasma canis* has convinced us that many of the appearances seen in stained preparations are misleading, and that trustworthy deductions cannot be made from them, without the assistance afforded by the study of the living parasite. For example, though extra-corpuscular flagellated forms are fairly common in the living blood they are seldom seen in stained preparations, either because the flagellum seldom takes the stain or because it is withdrawn into the dying parasite during the process of preparing the specimen.

The principal facts derived from our study may be briefly summarised as follows:

Piroplasma canis has a free and an intracorpuscular stage in its life-history. During the free stage the parasites may be fusiform, pyriform, or roughly ovoid with a short blunt process, and frequently show short or more rarely long flagella. These forms are all motile, and some specimens are capable of moving at a considerable rate. Rounded free forms are usually, if not always, derived from these forms during the process of degeneration. Amoeboid free forms have never been encountered. The free pyriform parasites attack and enter red blood corpuscles.

Numerous intracorpuscular types, round, irregular, and pyriform, have been encountered, all of which possess the power of movement to some degree. The round forms are capable of changing their position within the corpuscle and have frequently been observed to develop into pyriform or into irregular types. The irregular types are usually amoeboid and finally give rise to pyriform, or round types, the former apparently being the process of development under normal conditions, and the latter the process of degeneration under artificial conditions. After an uncertain period within the corpuscles the fully developed pyriform bodies escape from the corpuscles, become free and enter other corpuscles. In the few instances in which round forms have been seen to escape they have immediately assumed the pyriform type. In old preparations certain round forms represent dying parasites.

Intracorpuscular pyriform parasites are undoubtedly flattened and

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leaf-like structures, but in the free condition they seem to become more ovoid in section.

From our observations we believe we are justified in concluding that the asexual cycle of development within the peripheral blood takes place in the following manner.

A free pyriform body which has just left a blood corpuscle enters another corpuscle and soon assumes a round form, usually remaining quiescent for a time. The round body then becomes actively amoeboid, and grows. After a longer or shorter time it either assumes a pyriform shape and escapes from the cell to repeat the process, or it divides and gives rise to two or more pyriform bodies, which are for some time joined together by a thin process or processes, but ultimately become separate, escape from the corpuscle, and invade other corpuscles. The invaded corpuscle ruptures and disintegrates after the escape of the parasite.

Although we have not as yet been able to follow the complete cycle of any particular parasite, we have repeatedly observed each of the various stages and often followed a single parasite through two stages. Further we have never observed any indications of a sexual process, and have as yet no evidence to indicate that any other form of multiplication occurs within the internal organs.

The process of multiplication takes place with great rapidity in the blood of the infected animal in acute cases, for the parasites often become very numerous within 24 hours of their first appearance in the peripheral circulation, and increase with such rapidity that within 48 hours the great majority of the corpuscles are infected. The condition of the blood and urine shortly before death is further evidence in support of the rapid multiplication and consequent destruction of corpuscles. The former is very thin and watery and the serum which separates from it is deeply tinged with haemoglobin, while the latter often resembles blood in colour.

In spite of the lack of evidence we cannot, however, definitely assert that no other form of multiplication occurs in the organs at an early stage of the disease, and believe that a sexual[1] cycle of development within the tick probably exists. These points and others relating to

[1] In a previous paper (1905, p. 244, Plate IX, Figs. 59—62) we described and figured certain sausage shaped organisms which we had encountered in the peripheral blood and organs of one dog. We thought at the time that they might possibly be sexual forms, but we have never encountered them since that time, and we are now inclined to regard them as a separate species of parasite. Ziemann (1898, p. 125) states that he once encountered a crescent-like body, but makes no comments on it. Jackschath's (1903) views are quoted in the following section (p. 642 [3]).

significance of the various chromatin structures can only be ascertained by further studies, including the artificial cultivation of the parasite, and the examination of infected ticks, on which we are at present engaged.

VII. HYPOTHESES REGARDING THE DEVELOPMENT OF PIROPLASMA.

Finally we think it may be desirable to briefly summarise the hypotheses which have been advanced regarding the significance of various forms of *Piroplasma,* although most of them have been entirely based on the study of stained preparations.

(1) Lignières (1900, and 1903, p. 400) advanced the view that those pyriform, amoeboid, and rounded parasites, which contain two or more masses of chromatin give rise to minute "corpuscules germes." These minute bodies are most numerous in the internal organs, and in the blood a few hours before the death of the animal. Outside the body of the mammalian host they occur in blood kept for several days on ice, or in serum containing much haemoglobin (2 weeks) and in the gut of ticks fed on infected blood. He brings forward no evidence that his "corpuscules germes" are not degeneration forms, nor that the free masses of chromatin are not produced by the maceration of the parasites.

In another paper Lignières (1901) illustrated in a coloured plate a corpuscle stained a pink colour with flagella-like processes of the same colour projecting from it. This corpuscle contained two blue stained pyriform parasites, which showed no chromatin, and had no connection with the external processes. Lignières himself thought that the flagella-like processes were due to the degeneration of the corpuscle, and were not connected in any way with the presence of the parasites. Doflein, however, reproduced this figure in his text-book, and based an hypothesis on it.

(2) Doflein (1901, p. 153) after studying Lignières' description and figures came to the conclusion that the large pyriform bodies represent gametocytes, comparable to the malaria crescents, because (1) with time they become spherical in extravascular blood, and (2) because the chromatin mass is often placed peripherally in such spheres, from which it may be extruded (= germ of Lignières, expulsion of karyosome according to Doflein). He thinks that evidence to support this view may be found in stained preparations made from fresh, and recently defibrinated blood. Contrary to Lignières he thinks that the forms

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found in old defibrinated blood are due to degeneration. He also believes that the flagellate bodies which Lignières described (see above) were not due to degeneration, but represented male sexual forms exactly comparable to those of the *Haemosporidia.* He further assumes that the cycle of development in the tick is similar to that of the malaria parasite in the mosquito.

As Doflein's hypothesis was not based on personal observation it scarcely needs further consideration.

(3) Jachschath (1903) thinks that the paired pyriform intracorpuscular individuals differ, and are due to double infection of the corpuscle in which they occur, and that they represent male and female gametes which copulate within the corpuscle. In view of our observations it seems needless to consider Jackschath's hypothesis, founded on stained preparations, at greater length.

(4) Schaudinn (1904, p. 438) as the result of his study on *Haemoproteus noctuae*[1] and an examination of Weber's specimens brought forward a "working hypothesis." Weber in 1900 had prepared films two hours before death from a cow dying of piroplasmosis. The animal had been kept in a dark stall into which light only penetrated when the door was opened. In these films Weber found besides typical examples of *P. bovis,* trypanosome-like forms of smaller size than *T. evansi.* Schaudinn himself later examined the old films and thought that the trypanosome-like bodies in their size, appearance and nuclear structure looked as if they might be related to the *Piroplasma.* This view received some support by the results of the examination of old films made by Kossel and Weber from the gut contents of ticks taken from cattle suffering from piroplasmosis, for in these also trypanosome-like bodies were found. Schaudinn therefore thought that *P. canis* and *bovis* multiply in the same way as *Haemoproteus,* possessing trypanosome and spirochaete-like stages. This hypothesis he considered received support from certain observations of Theiler's (1904, c.) on African cattle[2].

It seems hardly necessary to point out that the mere presence of two or more forms of parasites, such as piroplasmata, trypanosomes, and spirochaetes, in the same animal affords no evidence of their develop-

[1] See Note IV, p. 646.

[2] Theiler discovered *Spirochaeta theileri* in six head of cattle. Three of these animals also harboured *P. parvum,* and two others *P. bovis.* In one animal the *Spirochaetae* alone were present. He has since found that *Rhipicephalus decoloratus* larvae, the progeny of female ticks fed on animals suffering from a double infection are capable of communicating infection with both parasites. This fact has been confirmed by Laveran and Vallée (5. VI. 05) at Alfort in France, where the diseases are not endemic.

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mental relationship. In fact mixed infections with protozoal diseases are extremely common. Cattle for example may be infected with *P. bovis* and *P. parvum*, organisms which have been proved to belong to distinct species, and *Spirochaeta theileri* may be present alone, or coexist with either or both these parasites. Again horses may suffer from piroplasmosis alone or combined with trypanosomiasis, or horse-sickness or both.

In this connection an experiment of the greatest significance is published by Wenyon (x. 06) in the present number of this *Journal*. He has observed the coexistence of *Trypanosoma dimorphon* and *Spirochaeta muris* in mice, and has been able to separate and clearly distinguish the two parasites. Finally the correctness of Schaudinn's observations on the development of *Haemoproteus* on which his hypothesis is founded has not been accepted by many protozoologists.

According to Schaudinn's hypothesis a trypanosome-like stage should follow the separation of the individuals forming the intra-corpuscular pyriform bodies. We have observed that the pyriform bodies become motile and possess flagella, but they cannot be described as trypanosome-like.

(5) Lühe (1906, p. 49) studied stained preparations obtained from Theiler (Pretoria) and Ziemann (Dualla, Cameroon) and expressed his belief in the existence of Schaudinn's hypothetical flagellate body, but thought that it could not bear the same relationship to the intra-corpuscular form as does the flagellate form of *Haemoproteus*. He further thinks that Schaudinn's view cannot account for the presence of groups (4, 8 etc.) of parasites in corpuscles.

None of the hypotheses which we have briefly summarised receive any support from our studies on living parasites.

VIII. GENERAL CONCLUSIONS.

(1) Nearly all forms of *Piroplasma* show one densely staining mass of chromatin, the nucleus, many also show a second punctiform dense mass generally situated near the nucleus, the blepharoplast, and a considerable number show a third loose mass which has not been previously observed. These masses may occupy various positions, or assume various shapes, the significance of which we have not yet been able to determine.

(2) Many intracorpuscular forms in stained preparations show both pseudopodia and flagella-like processes, and many of the free forms possess distinct flagella.

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(3) Round forms, apparently in a degenerating condition, are common in liver and spleen smears. In these situations free masses of chromatin also occur probably derived from degenerated parasites.

(4) Many of the appearances seen in stained preparations are extremely deceptive, and deductions made from them are frequently not confirmed by the study of living forms.

(5) Various bodies occur in normal dog's blood, which may readily be mistaken for piroplasmata.

(6) *Piroplasma canis* has a truly intracorpuscular and an extracorpuscular stage. In the former condition round, amoeboid and pyriform bodies occur, all of which are to some extent motile, and in the latter long and pyriform, frequently flagellated free swimming bodies. Amoeboid extracorpuscular forms are never seen.

(7) Within the peripheral blood a definite cycle of development occurs. Free pyriform bodies invade the corpuscles, become round and later amoeboid. The amoeboid bodies according to their size either again form intracorpuscular pyriform bodies or divide and form two or more pyriform bodies. The pyriform bodies leave the corpuscles, and in doing so, rupture them, and enter other corpuscles.

(8) Our observations lend no support to any of the theories of development which have hitherto been put forward[1].

Notes.

Note I. Piroplasma equi[2].

This parasite differs in several respects from the typical piroplasmata, *P. bovis*, *ovis* and *canis*, and in its morphology and pathogenic action offers certain points of resemblance to *P. parvum*. It has been found in the horse, mule, and donkey and according to Koch by Kudicke in the zebra. Several observers including Laveran (1901, p. 385) and Koch (1905, p. 1867) lay stress on the frequent occurrence of groups of four parasites in the infected corpuscles, which is not the case in the other piroplasmata. Koch believes that equines suffer from two diseases, one due to parasites frequently arranged in groups of four, and the other to parasites corresponding in type to *P. bovis* and *canis*. Theiler's discovery that Rhodesian cattle suffer from two diseases, East Coast Fever due to *P. parvum*, and Redwater, due to *P. bovis*, lends some support to this view.

Laveran (1901) studied material obtained from Theiler and states that *P. equi*

[1] As most of the sections are summarised at the end we feel that it is unnecessary to give our conclusions at greater length.

[2] The earlier literature is given by Nuttall (1904, p. 250) and Bowhill (1905, p. 16).

is almost always intracorpuscular, and occurs as rounded or ovoid, rarely pyriform, bodies, most numerous in the spleen. Russi (1902, p. 32) who observed *P. equi* in Italy, only observed pyriform types on three occasions, and rarely encountered free parasites.

Theiler (1904, b, p. 383) observed that in the donkey the parasites could only be discovered at the beginning of the disease and while the animal was suffering from acute symptoms, and were never so numerous as in the horse. As in the horse groups of four are common, and the parasites are most numerous in the spleen. Edington (1904, p. 21 and Plate I) figures groups of four and also pyriform bodies (fig. 5), and Bowhill (1905, p. 16, Plates I, II) also figures them in a series of 10 microphotographs of which two (9, 10) represent flagellate forms. He observed spherical, pyriform, and rod-like bodies belonging to large and small types, as well as groups of four and flagellate bodies. Baruchello and Mori (1905, p. 6) observed *P. equi* in and about Rome, and like Bowhill, described the symptoms and pathology of the disease. They state that the parasites are best seen at the beginning of the disease and are hard to find when the temperature falls. At times up to 50 or 60 $^0/_0$ of the corpuscles may be infected. They distinguish four chief types of parasites. The commonest type is represented by round, large or small intracorpuscular parasites occurring singly or in groups of two, three or four individuals, but never more. Elongated more or less regular types are frequent which may assume pyriform, clubbed, crescentic, or bacillary forms. Sometimes they are of large size and extend across the corpuscle. Double pyriform bodies occasionally occur. The free pyriforms and flagellate forms are scarce. They have noticed the occurrence of mixed forms, round, amoeboid or pyriform, in the same corpuscle.

Theiler (1902) failed to transmit the disease by blood inoculations, and Koch (1904) only seems to have succeeded once.

Note II. Piroplasma parvum.

This parasite differs from *P. bovis* in its morphology and pathogenic action. The parasite is transmitted by *Rhipicephalus appendiculatus* and *R. simus*. Contrary to what has been observed in dealing with the typical piroplasmata the disease cannot be communicated by blood inoculation. Morphologically the frequent occurrence of bacillary forms and its minute size separate this parasite from *P. bovis* (Theiler, XII. 04). Prior to the appearance of the parasites in the peripheral circulation Koch (1905, p. 1867) discovered in the spleen and lymph glands a special form of the parasite, which when stained by a modification of the Romanowsky method shows a blue protoplasm containing a number of chromatin masses.

Note III.

Lingard and Jennings (1904) in a preliminary note state that they discovered piroplasmata in almost all the animals they have examined, including men, elephants and lizards. They also found them in dirty water, and in the eggs, larvae and pupae of mosquitoes which derived them from the water. Moreover they describe micro- and macro-gametes, which they discovered in the body of a tick which had been dead some weeks and had become dry and shrivelled. A cycle of development in ticks and mosquitoes is described and illustrated. Until further evidence is produced we cannot seriously consider these statements.

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Note IV.

According to Schaudinn (1904) the *Haemoproteus* (= *Halteridium*) of *Athene noctua* represents only a stage in the life-history of *Trypanosoma noctuae* (Celli and San Felice). Schaudinn states that the "travelling vermicule" (ookinet) discovered by MacCallum may develop in three ways, giving rise to (1) indifferent, (2) bisexual, and (3) sexual forms. The indifferent forms multiply by division and the female forms may through parthenogenesis reproduce all the three forms enumerated. For our purpose it will suffice to consider the indifferent form. Here the *Karyosome* (achromatic) makes amoeboid movements and mixes itself with the chromatic elements of the dividing nucleus. The nucleus divides by heteropolar mitosis into two bodies of unequal size, the larger constituting the *nucleus* (1) of *Trypanosoma*, the smaller a body, which he has termed the *blepharoplast* (2). Both of these bodies contain eight chromosomes and they remain organically connected by means of an achromatic thread running into the central mass of each body. The blepharoplast is therefore a nucleus and not simply a centrosome, karyosome, or nucleolus as stated by various authors. The staining reactions of the two bodies differ however for the nucleus stains red, the blepharoplast violet-red by Giemsa's method. As the typical trypanosome form develops the blepharoplast in turn divides in the same way as the primary nucleus giving rise to a third and smaller nuclear body (3) which again divides giving rise to a fourth body (4). The nuclear structures 1, 2, 3, remain connected by an achromatic filament, whilst 3, 4 move widely apart and are connected by a chromatin filament which forms the edge of the undulating membrane. The eight chromosomes developed during the division of bodies 3 and 4 form the myonemes of the ectoplasm.

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DESCRIPTION OF PLATES.

PLATE XI.

Fig. 1. Fusiform and pyriform bodies connected by a long filament.

Fig. 2. Oval and rounded bodies joined by a delicate filament and possessing short processes. One body vacuolated.

Fig. 3. Body containing two processes, two nuclei and an oval vacuole.

Figs. 4–5. Bodies with flagella-like processes.

Fig. 6. Body with a relatively large mass of chromatin and distinctly wavy flagellum.

Fig. 7. Corpuscle containing a flagellate and round body.

Fig. 8. Two flagellated bodies, the one showing a bifurcated process.

Fig. 9. Four flagellated bodies.

Figs. 10–13. Free bodies showing long and short processes.

Fig. 14. Three parasites and five separate masses of blue staining detached protoplasm (see Diagram 17).

Fig. 15. Parasite which would appear to be applied to the corpuscle (see p. 612, and Diagram 12).

Figs. 16–21. Probably represent attacking forms indenting the corpuscles. In Fig. 16 the parasite may be seen "end on," in Fig. 19 from the side, in Fig. 20 when presenting its broad surface.

Fig. 22. Parasite indenting a corpuscle and disturbing its contents.

Fig. 23. Parasite distorting the corpuscle by drawing back after indenting it (see p. 629, and Diagram 22).

Figs. 24–27. Paired pyriform and single spherical intracorpuscular parasites.

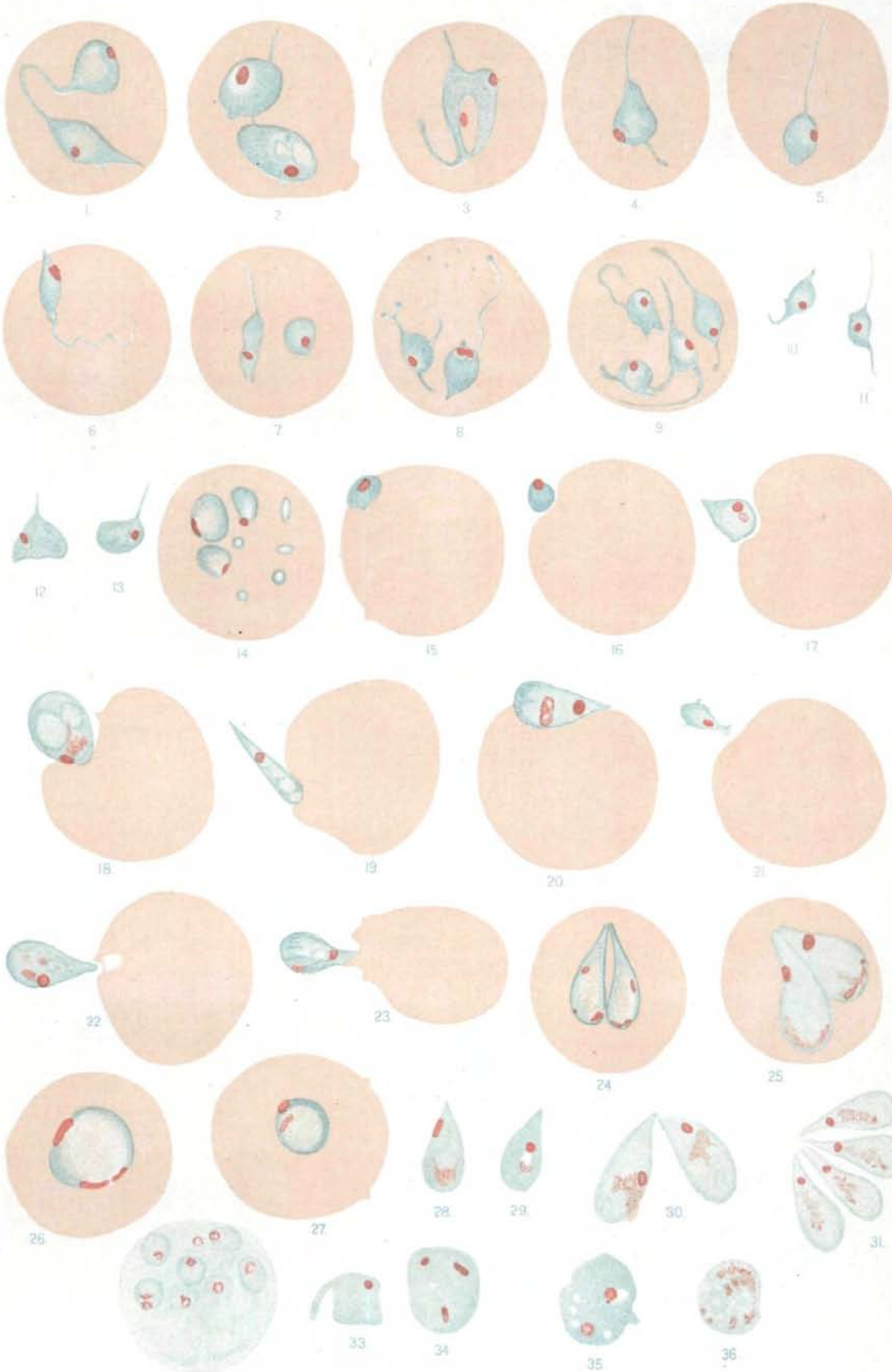
Figs. 28–31. Free pyriform parasites, singly, paired, a group of four. (In Fig. 30 the loose chromatin should not protrude outside the blue staining parasite.)

In the foregoing figures the ordinary single masses of dense chromatin (nuclei) are seen in Figs. 1—16, 19, 21. In Figs. 3, 24, 26 two or more dense masses of chromatin occur. In Figs. 17, 18, 20, 22, 23, 25, 27—31 besides the nucleus, a second mass of loose chromatin is figured. In Figs. 17, 20, 27, this structure appears more regular in form and compact, whilst it appears more scattered and irregular in amount in some of the other parasites. In Fig. 29 it is separated from the nucleus by a vacuole, vacuoles are shown in Figs. 2, 3, 18, 29 and 35.

Fig. 32. A degenerated corpuscle which stains a faint blue colour and contains 8 parasites with loose chromatin.

Figs. 33–36. Free parasites from the spleen, 33 with one mass of dense chromatin, 34 with rod-like masses of chromatin, 35 with loose chromatin, and 36 with diffuse chromatin.

The so-called blepharoplast is not figured in this Plate. Figs. 1, 3, 4, 9, 11, 12, are from kidney smears; 2, 5, 7, 10, 13, 15, 16, 21 from heart smears; 6, 8 from spleen smears; 14 from marrow; 16—20, 22—31 from a film prepared during life from the peripheral blood; 32—36 from defibrinated blood 48 hours at room temperature.



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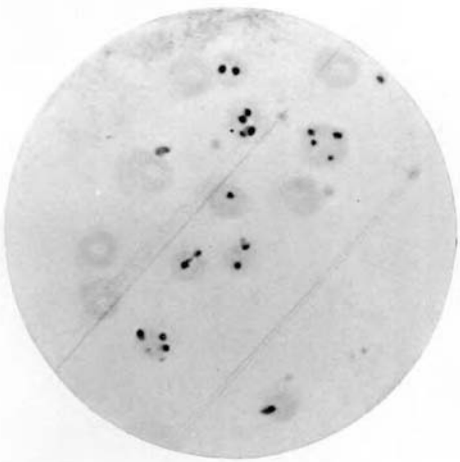


Fig. 1.

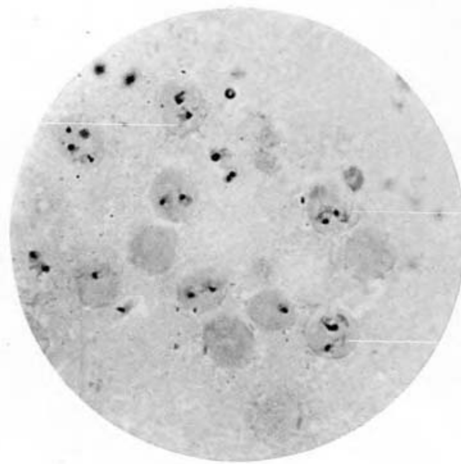
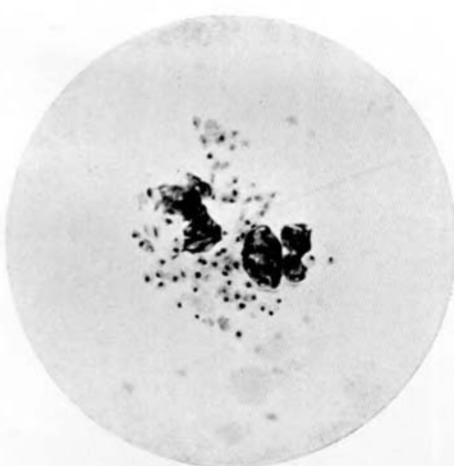


Fig. 2.



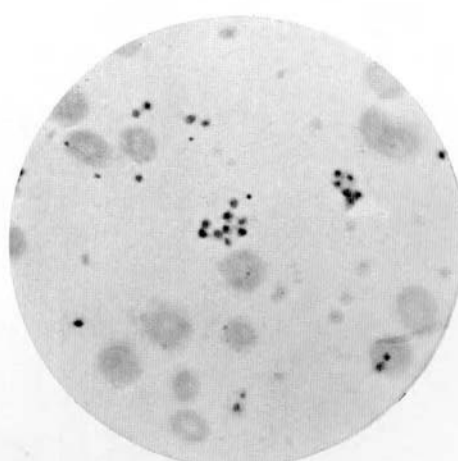


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PLATE XII.

Figs. 4 and 3 illustrate the appearance of the external surface and a median section of the kidney of a dog, which had succumbed to acute piroplasmosis. The blood vessels of the organ, especially the minute capillaries were greatly congested, the renal epithelium showed cloudy swelling and fatty changes, and the collecting tubes contained much granular matter and blood casts. The urine of this animal looked like dark blood previous to death.

Figs. 2 and 1 in contrast to the above illustrate the appearance of the kidney of a dog suffering from a more chronic form of the disease. The surface shows greenish yellow irregular apparently bile stained patches, and streaks of a similar colour are seen on section. The fatty changes were more advanced and the vessels were not markedly congested.

Fig. 5 reproduces an accurate drawing of part of a field of a preparation of living blood made a few hours before death. Almost every corpuscle is infected, many with more than one parasite and free parasites are common.

A. a leucocyte.
B. a corpuscle containing a small spherical parasite.
C. a corpuscle containing a single pyriform body.
D. a corpuscle containing two pyriform bodies.
E. Crenated corpuscle.

PLATE XIII.

Fig. 1 is a photograph of a stained (Giemsa) preparation of the peripheral blood made shortly before the death of the animal. It shows the high proportion of infected corpuscles.

Fig. 2 is a photograph of a stained (Giemsa) smear from the kidney. The original specimen contained many intracorpuscular forms with long pseudopodia, but these are very indistinctly shown in the reproduction. The fine white lines indicate the positions of some of the processes.

Fig. 3 is a photograph of a preparation made from defibrinated blood kept for 48 hours at room temperature in the dark. It illustrates a leucocyte containing a large number of parasites in various stages of degeneration (Giemsa).

Fig. 4 is a photograph of another portion of the same film from which Fig. 3 was taken. It illustrates large groups of free parasites in various stages of degeneration.

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