The Development of Theileria Parva, the cause of East Coast Fever of Cattle in South Africa.

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PART II.

In the first part of my investigations concerning the development of *Theileria parva* (Arch. f. Protistenkunde 1910, and Report of the Government Veterinary Bacteriologist, Transvaal, 1909-10), I gave a short incomplete description of the life cycle of this important causal factor. Later I contributed a further report, accompanied by a sketch of the life cycle of the parasite (Zeitschr. f. Infekt. Parasit. Krankh. u. Hyg. der Haustiere, 1910, and Journal of Comp. Pathology and Therapeutics). My present communication deals with the forms of *Theileria parva* found in the blood corpuscles and their further development within the transmitting agent.

Details regarding the material and the methods used for investigations were given in the first half of this report. Something must, however, be added here concerning the treatment of the ticks. It is almost impossible to dissect an engorged rhipicephalus larva under the lens. Microscopical examinations are made in the following manner:—The larvae are either preserved *in toto* and examined in sections or they are pricked with a fine needle and squeezed out. The hind part of the abdomen has to be pricked, and the contents are pressed out with a clean strong cover-glass. The organs can thus be examined in either fresh, dry, or moist-fixed preparations. In order to study the whole organs of the tick it is advisable to cut off the head of the larva with a fine sharp lancet, and then to press the body and examine the contents. Nymphal and adult ticks are examined in the same way. With some practice the latter can easily be prepared for microscopical examination. This is done by fixing the nymphal or adult tick by means of a fine needle passed through the body of the tick, near the head, in a layer of wax; the wax is made adherent to a small dish and is immersed in physiological water. The back of the abdomen of the tick can thus be carefully severed with a sharp lancet. With fine needles and a pair of forceps single parts, such as intestines, glands, etc., can be dissected. The examination of such isolated organs facilitates the study of paraffin sections of ticks which have been preserved in their entirety.

In order to demonstrate the development of *Theileria parva* within the ticks more clearly, it is necessary to fall back upon the forms found in the blood.

There are two typical generations in cattle—the agamogonous and the gamogonous ones. The gamogonous stages, which lead up to the formation of gametocytes, result from the agamogonous ones by the process of reduction. The reduction aims at a separation of the vegetative nuclear substance from the generative one in the shape of chromidia. These chromidiae produce compact regular nuclei, which possess a strong affinity towards stains. It is mainly through the morphological structure of the nuclei that the gamogonous forms are easily distinguished from the agamogonous ones. The latter possess nuclei which are irregularly formed and loosely constructed. By means of gamogony (schizogony) the gamont is split up into its gametocytes, which infect the red blood corpuscles. This development, which takes place exclusively in the organs of cattle, reaches a certain conclusion with the formation of these gametocytes in the cattle.

The East Coast fever parasites having once reached the red blood corpuscles, develop in the blood into sexually differentiated parasites, which can develop further within the stomach of the tick. There is no parthenogenesis of macro-gametocytes, consequently there is no relapse in the case of cattle which have recovered from the disease. The East Coast fever parasite has no great injurious influence on the red blood corpuscles of the cattle, which, as is known, are very vulnerable. Only towards the end of the disease a slight anisocytosis is observed, which, however, might be caused by relapses of slight attacks of redwater or by the so-called "anaplasmosis".

The youngest gametocytes, reproduced by means of gamogony out of a gamont, measure fractions of a micron. They are round or pear-shaped, and when in movement change into "comma" forms. The nucleus has a regular round shape when alive strongly refractile, in stained preparations compact and deeply stained. If well differentiated a karyosome is visible. These youngest gametocytes attack the blood corpuscles, and are often seen in the shape of cross forms (*vide* Figs. 2 and 3). By further development two phases can morphologically be distinguished which are sexually differentiated, and represent micro and macro-gametocytes.

Some of the round or pear-shaped individuals (Figs. 1 to 6) grow into long narrow, so-called bacillary, or rod forms. Others absorb more liquid, become large ring and pear forms (Figs. 7 to 24), and later develop into coarser stages. The first represent the micro-gametocytes (Figs. 7-14), the latter the macro-gametocytes (Figs. 25-27).

The micro-gametocytes are mostly elongated and are often dented in different places (Figs. 8, 9, 10, and 14). The protoplasm appears to stain dark blue after *Giemsa*, and shows a delicate alveolar structure. With the growth of the parasite the nucleus gradually loses its round form, and increases considerably in size and chromatine so that it often protrudes beyond the protoplasmic body (Figs. 4, 7, and 9). It takes the red stain intensely when stained with *Giemsa*.

Frequently a second smaller nucleus occurs, especially with those parasites in which the nucleus has assumed rod forms and which is connected with the principal nucleus by means of a fine fibril (Figs. 8, 10, and 12). Chrithidia-like forms are also seen (Fig. 12), which are pointed towards the front. Thus we find here some relationship with the male forms of trypanosomes. The micro-gametocytes occur firstly as fine, thin, narrow individuals; then the nucleus becomes elongated, assuming the forms of a short narrow band or that of a rod (Figs. 8 and 10); and lastly there are forms which produce a second nucleus, i.e. a blepharoplast. Contrary to these male forms, the macro-gametocytes are larger, broader, and heavier looking. Their nuclei are not so rich in chromatin, and consequently if stained with *Giemsa* show a lighter red colour. The plasma absorbs a great deal of liquid, and thus leads to large alveolar forms which cause the formation of large characteristic ring and pear forms (Figs. 16-20). While the nucleus of the micro-gametocyte becomes richer in chromatin, the plasma of the macro-gametocyte (similar to other higher protozoa and metazoa) gains considerably. Thus within full-grown macro-gametocytes we find more compact plasma and more loosely constructed nuclei (Figs. 25 to 27). These are morphological conditions which may come into consideration if the gametocytes are to be distinguished from *Babesia mutans*. The latter parasite generally shows a more loose structure of nucleus and a plasma structure which is conspicuous by its large alveoles. Only the younger forms cannot be distinguished from *Theileria parva*.

As reported in another paper, it is very difficult to distinguish between the groups of four, or cross shape, of *Theileria parva* and *Babesia mutans*. The cross shapes of *Babesia mutans* result directly from the macro-gametes by means of parthenogenesis, whilst the quadruplet forms of *Theileria parva* are produced from gamonts by means of gamogony. In order to form a certain diagnosis, the examination of gland and spleen juice is preferable to an examination of blood.

Following the bite of a tick the above described gametocytes reach the stomach of the tick, where the further gamogonous development takes place. For the purposes of examination it is better to remove the ticks from cattle before they are ready to drop off, as it stands to reason that as soon as the gametocytes have arrived in the stomach of the tick they will very quickly leave the erythrocyte and commence their further development. When the engorged ticks have dropped off it is hardly possible to observe the formation of gametes and copulation. After having picked off the ticks preparations should be made at intervals of three to five hours, thus, if the living observation fails, the study of the stained preparations will lead to the right interpretation of the successive stages of development.

The parasites very soon leave the red blood corpuscles in the stomach of the tick. Almost within an hour after the erythrocytes have arrived free parasites are met with outside the blood corpuscles. Naturally the larger number perish, and it stands to reason that only the full-grown gametocytes are capable of developing into gametes, whilst the young ones die off and are digested.

The formation of an agglomeration indicates the death of undeveloped gametocytes. In the beginning the free parasites have rounded shapes, which soon become more or less elongated, and which gives the gametocyte the appearance of a small amoeba. Here we have mostly to deal with forms which are perishing, especially when several elongations are being formed (Figs. 3-4). I have never observed a further development. In the stained preparations I did not find any forms which indicated intermediate stages. I would leave it an open question if the stages of development in ticks (*vide* Literature Index, Part I), described by *Koch*, and later on by *Dschunskowsky* and *Luhs*, have anything to do with these amoebae.

As seen in the preceding note, according to analogy with other blood protozoa the micro-gametocytes have to be distinguished from the macro-gametocytes. The full-grown micro-gametocyte (Figs.

11-14) lengthen out (Figs. 28-30) after having left the erythrocytes, and the plasma is generally protruding on one side. The movements are very similar to those of limaxamoebae, namely, crawling and not so much jerking. Sometimes the prolongation may be taken for flagella. I would have been inclined to accept the latter if, in the stained preparations, I could have proved the existence of morphologically differentiated organella.

As shown by the figures, the nucleus undergoes a great change. The compact nucleus, which is rich in chromatin, becomes more loose (Fig. 28) and splits up into two portions of unequal size (Figs. 29-30), but whether amitotically or mitotically I could not prove. This unequal division probably represents the formation of the blepharoplast which has already partly occurred in the blood. The blepharoplast, which, as it is, plays the part of a centrosome in flagellates, has now to step in. If the division occurs across the longitudinal axis the plasma is pushed to one side, thus causing triangular figures, as shown in Fig. 30. After the formation of this second nucleus the micro-gametocyte has become a micro-gamete.

The macro-gametocytes (Figs. 26, 27) are rounded off when they have left the erythrocyte in the stomach of the tick (Figs. 31 and 33). Occasionally a prolongation may occur (Fig. 32). The nucleus of the macro-gametocyte is also subject to great changes which lead up into the formation of the gamete, and which mean a reduction of the nucleus.

The nucleus loosens considerably and divides unequally. Often the loosened nucleus, which is in a state of reduction, arranges itself in a circle around larger alveoles, the latter generally appearing in the centre (Figs. 32 and 33). With the reduction of the nucleus the macro-gamete matures; no movements of the macro-gamete could be proved.

The mature micro-gamete seeks the macro-gamete, and after copulation a blending of the plasma of the bodies takes place (Figs. 34-36). Before a karyomyxis is arrived at the two nuclei again have to undergo changes which relieve the nuclei of certain chromatin substances. The nucleus of the micro-gamete, as well as that of the macro-gamete, undergo an unequal, primitive mitosis (Figs. 35-36). The reduction bodies which are thus formed all make for the pointed end, in order to be thrown altogether out of the cell-body (Figs. 37-40). After this reduction the male and female nucleus come closer together, but before fusing with each other they have to undergo a further process of development, resulting in the appearance of very strange figures (Figs. 38-39), and which may be a second reduction.

After the complete fusion of the two nuclei (Figs. 41-48) the ookinete thus formed rounds itself off (Figs. 43-44). Also the globular ookinete is prone to changes which will lead to a temporary formation of a second nucleus (Fig. 45). These forms just described (Figs. 41-45) are found for some days after the ticks have dropped off. Only after several days (five to ten) the ookinetes lose their round shape, and, taking the shape of retorts (as seen in other blood parasites), they change into gregarine-like figures (Figs. 44-49). Our parasite remains a very long time in this stage. I could discern it until the process of moulting of the said infected tick larva or nympha started. The movements of these ookinetes are extremely active. They crawl and bend and stretch out rapidly.

So far I have not been able to trace intermediate forms between ookinetes and other forms which are met with only after the moulting of the infected tick larva or nymphae. In the salivary glands of infected adult ticks, and also in the caecum, I found large round cyst-like formations (Figs. 50 and 51) in connection with a large number of nuclei, which I could not prove in non-infected and cleansed ticks (*vide* Part I). Therefore I hold these shapes to be further forms of development of ookinetes. The cysts which correspond with the agamogonous stages in cattle, or with the agamogonous forms ot *Haemoproteus columbae* in the lungs of pigeons, are of different sizes. By successive nuclear division a considerable number of nuclei are produced which surround themselves in groups with plasma, as is the case with *Haemoproteus columbae,* and within the first cyst several smaller ones are called into existence. By the bursting of the cyst the smaller cysts are set free, and they result in producing minute parasites by means of schizogony (Fig. 52). With the formation of these individuals the sporozoites, or the youngest agametes of the first generation, the cycle of development of *Theileria parva* comes to a conclusion. These agametes only measure fractions of a micron, possess a clear nucleus, and have mostly the shape of the above-described youngest gametocytes. In closing, I should like to draw attention to the plates, which explain more minutely the morphology and the successive stages of development.

The following is a short résumé of the whole life cycle:—The gregarine-like ookinetes produce most probably cyst-like formations (the process has not been determined). These shapes appear after moulting of larva into nympha or of nympha into adults. Out of these cysts the agametes of the first generation result, and with the bite of the tick they enter the blood of cattle. The further development occurs almost entirely in the organs of cattle, especially in the lymphatic glands and in the haemo-lymphatic glands. The young agamete grows into an agamont, increases gradually by growth and formation of nuclei until it splits up into as many part-products as there are nuclei. The agamogonous generation results into a gamogonous one by means of reduction. The nuclei of agamonts form chromidiae out of which new nuclei come forth, which can be morphologically distinguished from the nuclei of the agamonts. The nuclei of agamonts are formed irregularly and show a loose structure, while the nuclei of the second gamogonous generation possess more regular and compact nuclei. By means of gamogony (schizogony) the gamonts produce small parasites, which attack the erythrocytes and grow into gametocytes in the blood. The gametocytes change into gametes as soon as they have reached the stomach of a transmitting tick. The micro-gamete approaches the macro-gamete and fusion takes place. The micro-gametocyte as well as the macro-gametocyte is subject to nuclear changes, which have to be considered as forming the blepharoplasts (centrosomes) or reductions respectively.

After the fusion of both gametes the nuclei are again reduced by a primitive mitosis. The reduction bodies thus formed are cast off at a later period. The ookinete "rounds off" after karyomyxis, and only after a further unequal nuclear division it passes from the retort form into the gregarine stage. Thus the generative cycle is concluded.

Description of Plate.

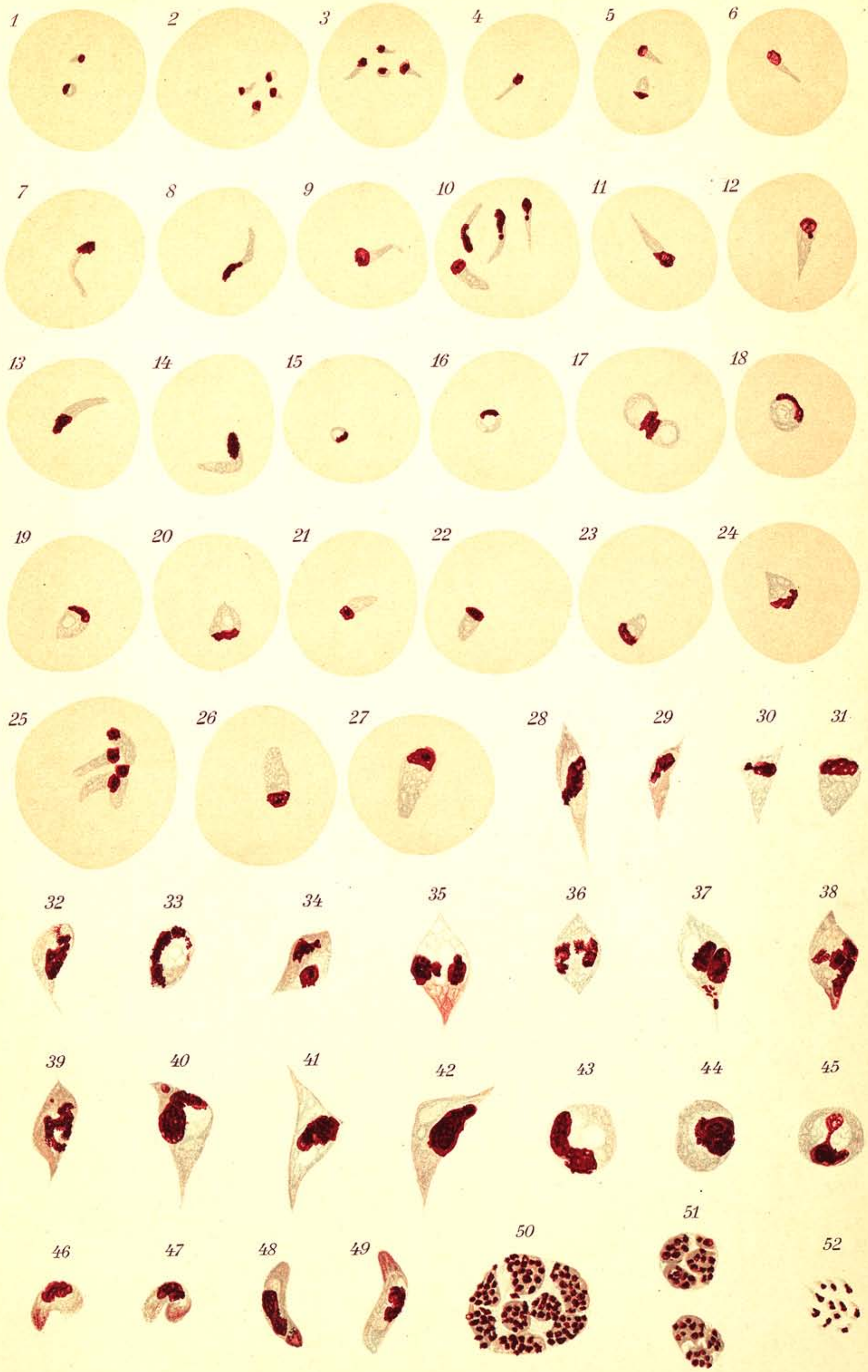
(All the figures were drawn with an Abbe apparatus ; Zeiss microscope, homog. oil immers ; apochro ; 2 mm. compens. ocular 18.)

Parasites in the Blood of a Sick Animal.

Fig. 1–5.—Young gametocytes which have entered the blood corpuscles.
Figs. 6–14.—Different successive stages of development of micro-gametocytes.
Fig. 7.—Bent form with large nucleus protruding from the cell body.
Figs. 8 and 10.—Rod-shaped micro-gametocytes, with a band-like main nucleus and a smaller one (blepharoplast).
Figs. 11–14.—Adult micro-gametocytes.
Figs. 15–27.—Different successive stages of development of macro-gametocytes.
Figs. 15–18.—Ring-shaped stages.
Figs. 19–25.—Pear-shaped stages.
Figs. 25–27.—Adult macro-gametocytes.

Development of Theileria Parva in the Rhipicephalus Tick.

Figs. 28–30.—Free micro-gametocytes forming a blepharoplast.
Fig. 31.—Free macro-gamete.
Figs. 32–33.—Macro-gametocytes changing into macro-gametes by means of reduction.
Figs. 34–37.—Copulation and formation of reduction-bodies.
Figs. 38–40.—Karyomyxis.
Figs. 41–42.—Full-grown ookinete.
Figs. 43–44.—" Rounded off " ookinete.
Fig. 45.—Formation of second nucleus (unequal).
Fig. 46.—Retort shape.
Figs. 47–49.—Gregarine-like ookinetes.
Figs. 50 and 51.—Formation of cysts (metagametic generation).
Fig. 52.—Agametes of the second generation (sporozoites).



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