PART II.

THE MARGINAL POINTS IN THE LITERATURE ON PIROPLASMOSIS.

(*a*) Observations in North America by Smith and Kilborne.

The new conception of the marginal points being a protozoon, distinct from *Piroplasma bigeminum*, will give quite a different aspect to the question of Texas fever, and a perusal of the literature on the subject will prove interesting.

In an article on *Piroplasma nutans* in my Annual Report for 1905-06, I alluded to the marginal points noticed by other observers who found them in connection with their investigations into Texas fever, tristezza, etc. At that time I shared the general opinion that the marginal points or the so-called "peripheral points, coccus-like bodies", etc., were forms belonging to the life-cycle of *Piroplasma bigeminum*, and in that article I related some experiments seemingly in support of this view which was formed by the first investigators into Texas fever, namely *Kilborne* and *Smith* of the United States Department of Agriculture, Washington.

These scientists, in their publication in the Eighth Annual Report of the Bureau of Animal Industry, in describing the micro-organism of Texas fever, distinguish two types of the disease, an acute and a mild one. Concerning these two types, I wish to extract some statements in their own words.

On page 220, referring to the acute form, they say: ". . . The forms of the micro-parasite are pyriform and fusiform bodies, chiefly intraglobular, occasionally free. The *post-mortem* forms are roundish. In size the pyriform bodies are quite large, and the question arises, are there any smaller forms to be found? For these we must turn to the mild (usually autumnal) cases of the disease. It is an interesting fact that these cases are characterized by the presence of the smaller stages of the parasite. While the pyriform bodies are not entirely absent, they are very rare. In the acute type only the latter and not the former are seen.

"In the mild type we have from 5 to 50 per cent. of the red corpuscles in the circulating blood infected for a period of from one to five weeks. In the acute type, on the other hand, the circulating blood contains usually from $\frac{1}{2}$ to 2 per cent. of infected corpuscles: 10 to 15 per cent. is a rare occurrence, usually just before death. In the fresh preparations of blood this small stage of the parasite is, as a rule, invisible. Rarely we may observe it on the very border of the corpuscle as a round pale spot about 0·5 μ in diameter, which does not change its place. When dried films of blood are stained in alkaline methylene blue, the parasites appear as round coccus-like bodies from 0·2 to 0·5 μ in diameter, and situated within the corpuscle on its border. They sometimes appear as if situated on the border, but outside the corpuscle. As a rule only one is found in a corpuscle. In many cases a division of the coccus-like body into two could be clearly made out. The separation was noticeable as a paler line, and a constriction at either end similar to the division of certain micrococci.

This division usually appeared in all bodies of a preparation from one case, but could not be noticed in any preparation of perhaps the next case".

Again on page 221 :—

". . . It has already been stated that these bodies are characteristic of the mild autumnal type of the disease. A glance at the appendix will show how numerous these cases may be. This stage of the parasite is there indicated provisionally as 'peripheral bodies' or 'peripheral coccus-like bodies'. A more careful examination of these cases will reveal three groups :

" (1) Animals exposed to Texas fever late in the season (October and November).

" (2) Animals which have passed through an acute attack earlier in the summer (second attack or relapse in October and November).

" (3) Animals which contract a mild disease during or previous to the season of the acute disease.

" In the first group the disease is mild and may pass unnoticed. The corpuscles with peripheral bodies appear in the blood as the number of the corpuscles begins to fall and disappear when it again begins to rise.

" *Rarely a corpuscle with a pair of large pyriform bodies is detected.*

" In the second group the phenomena are the same.

" To the third group belong a few cases which showed a blood infection several weeks before the fever appeared among all the susceptible animals in the infected field. In two cases the infection was at first by peripheral coccus-like bodies. This, after a week's time, developed into an acute fatal infection, in which only the large forms were found after death. In another the infection by peripheral cocci was noticed as early as 7th August. From 10 to 20 per cent. of infected corpuscles circulated in the blood until 19th August, when some large pyriform bodies made their appearance. The blood contained both small and large parasites until 25th August, when the animal was killed in a dying condition".

I do not think it necessary to prove here that the peripheral coccus-like bodies of *Kilborne* and *Smith* are identical with our marginal points. Their description and mine given in the mentioned report only allows of the one conclusion that they are identical. From our new point of view, the observations of the Americans will have to be interpreted differently.

(1) *Animals exposed to Texas fever late in the season.*

It is possible that the ticks which are responsible for the transmission of the mild form of Texas fever were only infected with *Anaplasma marginale* and not with *Piroplasma bigeminum* at all, or that the attack of Texas fever due to *Piroplasma bigeminum* was so mild that it passed unnoticed or, finally, that animals immune to *Piroplasma bigeminum* were exposed. To judge from the subsequent note referring to the statement that " Rarely a corpuscle with a pair of large pyriform bodies is detected ", the two latter views are therefore probably the correct ones. We have noticed in some of our experiments that during the reaction to anaplasma, *Piroplasma bigeminum* may be present, a fact which is easily explained by the breakdown of immunity against the former parasite by the presence of the latter one. We must remember that the incubation time of anaplasma is much longer than that of *Piroplasma bigeminum* ; indeed, it will be shown later, after tick infection it can be exceptionally long, so that anaplasma would appear later in the season, even when the animals were exposed in the summer.

(2) *Animals which have passed through an earlier attack in the summer, etc.*

The explanation of this is similar to the former. The first attack was the real attack of Texas fever due to *Piroplasma bigeminum* and which, unlike the

above, was noted. It did not give any immunity against the so-called relapses in October and November, these latter being due to the anaplasma, either freshly acquired or appearing after the long incubation time.

(3) *Animals which contract a mild disease during or previous to the season of the acute disease.*

In these cases the reverse has taken place. The animal became infected first by the disease caused by *Anaplasma marginale*, which did not give them any immunity against Texas fever contracted later. There were also mixed infections breaking out at the same time due to breaking down of immunity or to two separate infections, *Piroplasma bigeminum* succeeding the anaplasma.

Already *Smith* and *Kilborne* had some doubts as to the true nature of the peripheral points or coccus-like bodies. On page 222 they say :—

". . . If we admit their parasitic nature as highly probable, we have still the question before us whether they are stages of the Texas fever parasite or *another parasite transmitted with it.* This question cannot be positively answered until by methods akin to those of bacteriology we shall be enabled to isolate the Texas fever organism and observe the transformation of one stage into the other, either in cultures or in the blood of inoculated animals. In the absence of such rigorous proof, the presumption is nevertheless strongly in favour of the unity of this and the larger forms already described. We observe in the first place the appearance of both types of the disease in all outbreaks studied at the experimental station since 1889, though at different periods of the same season, the coccus-like bodies being chiefly associated with cool weather. An outbreak produced after the middle of September in 1889 developed cases containing the coccus-like bodies only. In one of these cases, killed in a dying condition, the spleen and the liver were affected as in acute cases, *but haemoglobinuria was absent.* Several cases were observed in which there is a transformation of the mild into the acute type with a corresponding change in the form of the parasite.

" Perhaps the strongest proof that the coccus-like bodies and the pyriform amæboid bodies are stages of the same parasite was furnished recently in an unexpected manner. Two cows inoculated with blood from healthy North Carolina cattle early in July, 1892, developed the acute type of Texas fever with the appearance of pyriform parasites within the red corpuscles. Both recovered, and the number of corpuscles was rising towards the normal when at the end of August a relapse was detected in both animals. The number of corpuscles was rapidly falling again, and many were infected with the coccus-like bodies. Re-infection from without can hardly be considered in these cases, as there were no ticks in the field and two control animals had normal blood throughout the season. . . ."

From my point of view the facts recorded mean that in some experiments the Americans had the pure picture of redwater, and in others that due to anaplasmosis. The absence of haemoglobinuria in the latter one agrees with my observations. What the investigators consider as a proof that the coccus-like bodies and the pyriform amoeboid bodies are stages of the same parasite is, of course, just the reverse of a proof. The two cows were inoculated with blood of an animal which was infected at the same time with both diseases ; *Piroplasma bigeminum* having a short incubation time appeared first, anaplasma, having a long one, appeared later, and the intervals between the two attacks correspond with my observations.

In their notes on page 223 on the probable life history of the micro-organism the authors distinguish three stages, namely (1) the (hypothetical) swarming or motile stage (intraglobular) ; (2) the stage of the peripheral coccus-like

bodies; and (3) the stage of the larger forms (pyriform and spindle-shaped bodies).

Referring to the second stage, they say as follows:—

". . . After the (hypothetical) swarm spore has penetrated into the corpuscle it comes to rest, loses its bright refrangent appearance, and attaches itself near the periphery of the corpuscle as a pale body which is only detected with difficulty in the unstained corpuscle. This body next undergoes division which is probably incomplete, for in the more advanced stages the two resulting bodies are as a rule still attached to each other. These remain close together while the infected corpuscle is circulating in the blood. This stage of the coccus-like body, like the preceding hypothetical stage, must be regarded as recognizable, because of a retarded development of the micro-parasite. It is probable that this retardation of development in susceptible animals is due to *meteorological conditions, such as low temperature of the air, and to partial immunity.* In acute attacks the enormous multiplication of the parasite in the blood shows how rapid in such cases its development, and how ephemeral these intermediate stages must be. The period of retardation may vary in length, but it seems probable that this stage may remain in the circulation at least several days. . . ."

This interpretation of the life cycle of the blood parasite causing Texas fever does not correspond to what actually takes place. In all cases we notice that the typical piroplasma form appears first as such, and not in a coccus-like form. Indeed, these coccus-like bodies only appearing after a longer incubation time would represent a later stage of the parasite if they really belonged to its cycle.

My experiments were carried out almost at the hottest time of the year, and therefore no meteorological changes, such as low temperature of the air, could have had any influence on *Piroplasma bigeminum.*

In the interpretation given by *Nuttall* and *Smith* of the life cycle of *Piroplasma bigeminum*, they do not mention any coccus-like bodies, and their observations have been supported by other observers in England and on the Continent.

With regard to the immunity tests given on pages 275–277 the deductions which the American investigators made was that an animal which had gone through a previous attack of Texas fever may contract the disease again by subsequent exposure. We know that this is sometimes the case in redwater, when one attack does not necessarily give immunity to a subsequent one. The fact quoted that an animal which goes through the severe attack of the disease is not protected against the mild attack later in the season, or vice versa, finds a better explanation, from my point of view, than that given. We deal with two different species of parasites, recovery from one disease does not protect against an attack of the other.

On scrutinizing the appendix, we find further facts which support our view of the duality of the two parasites.

(1) Native animal 49 (page 292), which was exposed on the 4th July, 1890. On the 21st August—forty-eight days later—it first showed large parasites (*Piroplasma bigeminum*); they were last seen on the 2nd September. On the 14th October, 5 to 10 per cent. peripheral cocci were seen. Thus, in this case, the peripheral bodies were also seen a long time after the appearance of *Piroplasma bigeminum*, namely fifty-three days later.

(2) Native animal 56. Exposed on the 8th September, 1889, then again from September, 1890; all examinations negative up to the 22nd October. On this date 10 to 20 per cent. of peripheral cocci were seen; on the 25th and

30th they were present in the same numbers; on the 6th November, 20 to 30 per cent. of the corpuscles were infected; on the 8th, 30 per cent., and on the 10th November; on the 13th and 15th November, 10 per cent.; on the 17th 1 to 2 per cent. of corpuscles were infected; a few were noted on the 21st; on the 26th the examination was negative; *on the 28th one pair of large parasites were noted.*

I also consider these cases as a *Piroplasma bigeminum* infection in the first instance, probably contracted in 1889, which was so mild that it escaped notice. Forty-four days after the second exposure the peripheral bodies appeared, corresponding to the incubation time. The fact that only one pair of large parasites were seen as late as the 28th November proves that the animal had gone previously through a mild attack of Texas fever; if in this case the peripheral cocci had been the precedents, out of which the *Piroplasma bigeminum* developed, we would have a right to ask "How is it that only one *Piroplasma bigeminum* was noted when there were such numbers of peripheral bodies?" If peripheral bodies would develop into *Piroplasma bigeminum* we would meet more *Piroplasma bigeminum* than we actually do, and it could be expected that they would both stand in direct numerical relation to each other, which is not the case.

(1) Animal 198. This was an animal injected on 6th July with blood of immune Carolina cow 217. In this case the parasites were first seen on the 16th July, paired and un-paired, that is the typical *Piroplasma bigeminum*, but no marginal points were noted. The animal died on 19th July.

This is another case which proves that also in America, *Piroplasma bigeminum* appears first, and is not preceded by marginal points in typical cases of Texas fever.

CONCLUSION.

The conclusion from these notes is:—

Texas fever is the name for two diseases, the one due to *Piroplasma bigeminum*, representing Texas fever, *sensu stricto* and identical with our red-water, the other one due to *Anaplasma marginale* and corresponding to Anaplasmosis.

(*b*) Observations in South America.

With regard to the observations made by *Dr. Knuth* * in the La Plata States in 1904 on "Tristeza", I wish to refer to his investigation on the peripheral bodies in his own words:—

"Besondere Aufmerksamkeit habe ich den punktfoermigem Parasiten, welche teils auf den roten Blutkoerperchen, teils an ihrem Rande lagen, gewidmet

"*Smith* und *Kilborne* erwaehnten die punktfoermigen Parasiten (coccus-like bodies) in ihrer klassischen Arbeit und hielten sie fuer Entwicklungs stadien des Texasfiebers.

"Ich habe dieselben sehr hauefig festellen koennen, hauptsaechlich nach ueberstandenem Texasfieber. Die Puenktchen faerben sich nach Romanovsky rot, bestehen also aus Chromatin. Sie sind daher nicht als Zerfallsprodukte und auch nicht als Coccen anzusehen. Am wahrscheinlichsten scheint es mir, dass die punktfoermigen Parasiten Schizonten darstellen.

"Bei Tieren aus der zeckenfreien Zone habe ich die punktfoermigen Parasiten so lange vermisst, bis sie entweder auf natuerlichem oder kuenstlichem Wege mit Piroplasma infiziert wurden.

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* *Experimentelle Studien ueber das Texasfieber der Rinder.*

" Im uebrigen habe ich sie bei Tieren aus der zeckenfreien Zone nach natuerlicher, wie nach kuenstlicher Infektion gleich hauefig gefunden. Bei einigen dieser Tiere waren die punktfoermigen Parasiten zeitweise sehr hauefig, um dann wieder fast zu verschwinden.

" Am zahlreichsten fand ich sie aber bei Staerke No. 38. Die hierueber gemachten Aufzeichnungen moegen deshalb hier Plaz finden.

" Die Staerke No. 38 war als Kontrolltier zusammen mit No. 42 mit virulentem Blute von No. 44 geimpft worden, in der Absicht, den Grad der Infektiositaet des Blutes von No. 44 zu pruefen. Staerke No. 44 war aber am 22 August mit Blute von Kalb No. 66 geimpft worden. Kalb No. 66 war im Zeckengebiet geboren. In seinem Blute hatte ich einige Piroplasmen am 19 August 1902, gefunden. Somit sind die Piroplasmen von No. 38 die zweite Impfgeneration aus Kalb No. 66.

Die Impfung von No. 38 war ganz normal verlaufen. 24 Tage nach der Infektion schienen auch die letzten getuepfelten Zellen verschwunden zu sein.

" Da traten ploetzlich am 16 Oktober (41 Tage nach der Infektion) unter hohem Fieber im Blute je einzeln am Rande der roten Blutkoerperchen liegende punktfoermige Texasfieber-Parasiten auf. Am 18 Oktober waren dieselben, ebenfalls unter Fieber, in so ausserordentlich grosser Menge vorhanden, wie ich sie nie zuvor bei Rindern beobachtet habe. Oft sah man 2–4 Puenktchen an oder auf einem roten Blutkoerperchen. Zuweilen lagen auch 2 Puenktchen ziemlich nahe beieinander.

" Am 22 Oktober erkrankte No. 38 schwer, trotzdem die Mastdarmtemperatur nur 38·4° C. betrug. In den Blutausstrichen fand ich den hoechsten Grad der Blutaufloesung den ich bisher gesehen habe. Man sah im Praeparate eine fast homogene roetliche Masse, aus der sich nur wenige unverletzte rote Blutkoerperchen abhoben. Ferner fanden sich darin sehr viele feingetuepfelte Zellen und eine grosse Anzahl von Leukocyten. Ich entnahm an diesem Tage ca. 30 ccm. Blut, um damit sechs andere Tiere zu impfen.

" Am 23 Oktober starb No. 38. Aus dem Sektionsbefunde ist folgendes von Interesse :—

" Das spaerlich vorhandene Unterhautfettgewebe postgelb. Blut sehr waessrig. Im Herzbeutel eine seroese, gelbliche Fluessigkeit mit groesseren Gerinnseln. Am Epi- und Endocard fehlen die sonst bei Texasfieberfaellen fast regelmaesig beobachteten Blutflecken. Milz, Leber, Galle und Niere zeigen aber die fuer Texasfieber charakteristischen Veraenderungen in hohem Grade. Im 4 Magen reichlich mit Futterbrei gemischte Erde. *In der Harnblase ein klarer, hellgelblicher Harn.* Der Darmtraktus frei von auffaelligen Veraenderungen. Im Mastdarm einige kleine feste Kotballen von grauroetlicher Farbe und von schaumigen Schleime umhuellt.

" Mit dem am 22 Oktober von No. 38 entnommenen defibrinierten Blute impfte ich sofort subkutan zwei aeltere Kuehe, No. 99 und No. 100, 2 Kaelber *No.* 101 (6 *Monate alt*) und No. 102 (9 Monate alt) und 2 jungrinder, welche einige Wochen vorher aus der Zeckenfreien Gegend von Buenos Aires nach Fray Bentos grbracht worden waren, No. 72 und No. 83. Die sechs geimpften Tiere stellten drei verschiedene Grade von Empfaenglichkeit fuer Piroplasmen dar.

" Bei Staerke No. 42, welche mit demselben Materiale wie No. 38 geimpft worden warem, traten seit dem 22 Oktober (46 Tage nach der Infektion ebenfalls unter leichter Erhoehung der Koerpertemperatur sehr viele punktfoermige Parasiten an den roten Blutkoerperchen auf, und es kam bei No. 42 nicht zu dem stuermischen Krankheitsverlaufe wie bei No. 38.

" Ueber den Verlauf der Infektion bei den 6 Tieren, welche mit Blut von No. 38 geimpft worden waren, ist folgendes zu sagen :—

" Kuh No. 99 und No. 100, ferner Kalb No. 101 und No. 102 erwiesen sich als voellig resistent gegen dieses Impfmaterial. Dagegen waren bei den fuer Texasfieber empfaenglichen Jungrinder No. 72 und No. 83 folgende sehr bemerkenswerte Beobachtungen zu machen:

" Waehrend bei meinen bisherigen Piroplasmen-Infektionen ca. am 5–7 Tage Piroplasmen im Blute auftraten, war dies bei No. 72 und No. 83 nicht der Fall. Dagegen konstatierte ich am 10 Tage bei No. 72 und am 18 Tage bei No. 83 die bekannten punktfoermigen Parasiten an den roten Blutkoerperchen. Am 20 Tage nach der Infektion waren bei beiden Tieren diese punktfoermigen Parasiten in sehr grosser Zahl verhanden, und am 28 Tage der Infektion hatten beide Tiere sehr viele getuepfelte Zellen. Leider habe ich, durch eine Reise verhindert, zwischen dem 20 und 28 Tage nach der Infektion von den Tieren kein Blut entnehmen koennen, so dass ich nicht sicher zu sagen vermag ob nicht vielleicht in dieser Zeit Piroplasmen aufgetreten sind. Doch kann ich durch Kombination mit anderen Beobachtungen bei meinen Versuchstieren folgern, dass auch in dieser Zwischenheit wahrscheinlich keine Piroplasmen vorhanden waren, so dass also die getuepfelten Zellen sich unmittelbar an das Auftreten der punktfoermigen Parasiten aehnlich wie bei dem Ausgangstier No. 38 angeschlossen haben.

Die bei Staerke No. 38 gemachten Beobachtungen haben in mancher " Beziehung eine gewisse Aehnlichkeit mit dem Rhodesischen oder Ostafrikanischen Kuestenfieber nach *Koch* und der tropischen Piroplasmose nach *Dschunkowski* und *Luhs* ergeben. Vermisst wurde in Fray Bentos bei solchen Tieren die von *R. Koch* beschriebenen Lungenveraenderungen und Schwellungen der Lymphdruesen. Auch die Hoehe der Mortalitaet ist in Fray Bentos bei allen Versuchstieren, welche waehrend meines Aufenthaltes einer dauernden Blutkontrolle unterworfen wurden, nur gering gewesen im Gegensatze zu den Suedafrikanischen Erfahrungen. Uebereinstimmend mit jenen aber ist das gleichzeitige Vorkommen von punktfoermigen Parasiten mit typischen birnfoermigen Piroplasmen, das zeitweise ausserordentlich haeufige Vorkommen der punktfoermigen Parasiten auf den roten Blutpoerperchen und das Fehlen der Haemoglobinurie.

" Nach meinen eigenen Beobachtungen, welche ich bei der Staerke No. 38 schon im Oktober 1902 gemacht und *Herrn Professor Dr. Kolle* in Berlin mitgeteilt habe rechne ich somit die punktfoermigen Parasiten zu den Entwicklungsformen des *Piroplasma bigeminum* und sehe sie nicht als besondere Erreger an."

The observations of *Knuth* tally completely with mine. He observed as a general rule the *Piroplasma bigeminum* in the first instance and soon after the inoculation and anaplasma after a longer incubation time. The last two cases, 72 and 83, by *Knuth* were undoubtedly pure infection of *Anaplasma marginale*. He emphasises the fact of the absence of red urine in the case on which he made a *post-mortem* examination.

The experiments which *Knuth* made to transmit the disease into animals immune to Texas fever were negative, apparently because these animals were not only proof against an infection of *Piroplasma bigeminum* but also *Anaplasma marginale*, which finds in this way its proper explanation.

Knuth comes finally to the conclusion that these punctiform parasites represent a form in the life cycle of *Piroplasma bigeminum* and is inclined to consider them as schizonts.

Conclusions.

The conclusion concerning Texas fever also holds good for tristeza. Here again this term may include the two infections, piroplasma and anaplasma.

(c) The Observations of Lignieres in the Argentine.

In 1900 *Lignieres* published his investigations on " La Tristeza or Malaria Bovine dans la Republique argentine."

On page 66 under the title " L'Evolution benigne de la malaria bovine est-elle due a une forme speciale du parasite ? ", he refers to *Smith* and *Kilborne's* observation concerning the periphal bodies as follows :—

" *Smith* et *Kilborne* ont, en effet, rattaché la forme benigne de la maladie á l'existence dans les globules, surtout a la périphére, de tres petits hematozoaires punktiformes au nombre de un, deux ou trois, qui infecteraient 50 per cent. des hematies. Pour moi, les savants auteurs americains ont été trompés par leur procédé de coloration qui donne parfois un grand nombre de granulations intra-globulaires ressemblant, en effet, à des parasites. Cependant on peut remarquer que ces granulations sont tres irrégulièrement distribuées sur la préparation ; certaines parties en sont couvertes, tandis que d'autres en sont totalement dépourvues. Si l'on colore le même sang a l'aide de différentes methodes, notamment par le bleu de méthylène seul, comme je l'ai indiqué, on ne trouve plus ces granulations. Elles réprésentent donc un accident de préparation.

" En réalité, la forme bénigne, aussi bien que la forme grave de maladie, sont causée par le même parasite. Seulement, dans le premier cas, l'infection globulaire s'arrète tres vite et n'atteint qu'un nombre relativement restreint de globules ; dans le second, au contraire, elle est rapide et générale."

Undoubtedly *Lignieres* has not seen the bodies described by the American savants and his interpretation of their observation is wrong. We can therefore safely conclude that our learned friend in the Argentine Republic has not yet seen our *Anaplasma marginale*. In other words that his experiments were carried out with a pure infection of *Piroplasma bigeminum*, thus proving that also in South America both parasites may be found disassociated.

(d) Observations in the Trans-Caucasus by Dschunkowsky and Luhs.

(Centralblatt fuer Bacteriologie Originale xxxv, Bd. No. 4.)

In their article " Die Piroplasmosen der Rinder " *Dschunkowsky* and *Luhs* describe a " Tropical Piroplasmosis " of which they distinguish an acute and a cachectical form.

The tropical piroplasmosis is due to the presence of a small piroplasm, *Piroplasma annulatum*, almost identical with *Piroplasma parvum*.

About the cachectical form, on pages 487–488, they say as follows :—

". . . In kachektischen Faellen beobachtet man in Labmagen, besonders an der Pfoertnerhoehle, an den Raendern der Falten, flache Schleimhautdefekte in Gestalt von Streifen, Leisten und laenglichen Flaechen mit ocker-gelbem oder dunklem, bisweilen auch blaugelbem Grunde. Sie haben viel Aehnlichkeit mit braunlichen Krusten wie sie durch energisches Brennen mit gluehendem Eisen auf pigmentloser Haut entstehen.

" Die parenchymatoesen Organe sind stark vergroessert. Die Leber meistenteils lehmfarbig, die Galle orangegelb mit roetlichen Flocken, von der Konsistenz eines dicken Breies, sehr aehnlich einer dicken Tomatensauce. Die Milz ist stark (2–3 mal) vergroessert, die Pulpa derselben zerfliessend. Die Nieren jedoch zeigen ausser den Haemorrhagieen in der Kapsel und im Parenchym keine Veraenderungen. In der Harnblase findet *man klaren oder leicht getruebten Harn*. Das subkutane und Zwischenmuskelbindegewebe ist

besonders in der Gegend der Schulterblaetter durchtraenkt von einen gelbichen, gallertartigen Infiltrat. Dasselbe kann man um das Duodenum herum und am Grunde der Gallenblase beobachten. In der Trachea und in den Bronchien findet man zuweilen eine reichliche Quantitaet einer blutig-schaumigen Fluessigkeit. Die Lungen sind oft emphysematoes.

"Neben dem eben beschriebenen pathologischen Bilde haben wir auch, besonders bei Rindern, welche aus verschiedenen Gegenden des noerdlichen Kaukasus nach Transkaukasien gebracht wurden, Mischformen beobachtet. In solchen Faellen fanden wir im Blute sowohl grosse typische *Piroplasma bigeminum* als auch die kleinen Parasiten unserer tropischen Form. . . ."

These symptoms undoubtedly correspond with those which I have given before in anaplasmosis. It has to be emphasised that the spleen was found to be enlarged, being twice or thrice normal size, and that the pulpa was very soft and the urine clear.

In this cachectical piroplasmosis the authors found the peripheral points of which they say as follows (p. 489) :—

"Bei der Kachexie der tropischen Piroplasmose haben wir die Parasiten fast immer im Form von runden oder leicht ovalen Punkten, welche aus kompakten Chromatin bestehen beobachtet. Das Protoplasma konnte bei ihnen vorlaeufig nicht nachgewiesen werden. Sehr oft scheint ein solcher punktfoermiger Parasit aus zwei gleichen, sehr nahe beieinander liegenden Haelften zu bestehen in Gestalt eines Diplococcus. Die feine Spalte zwischen beiden Haelften war nur bemerkbar bei schwacher Faerbung des Praeparates, wogegen sie bei intensiver Faerbung immer verschwand. Ferner wurde mitunter in manchen Punkten eine kleine nicht gefaerbte Stelle wahrgenommen. Im hangenden Blutstropfen betrachtet, erschienen die Parasiten als stark lichtbrechende, runde Koernchen, jedoch ohne Eigenbewegung. Sie scheinen eine resistentere Form des Parasiten darzustellen."

Thus the Russian savants consider the punctiform parasites to be a more resistant form of the parasite of tropical piroplasmosis, viz., of *Piroplasma annulatum*. They also state that in the cachectical piroplasmosis, 10 to 40 per cent. of large erythrocytes contained these parasites.

To understand the explanation of *Dschunkowsky* and *Luhs* it must be further stated that they distinguish in Russia three forms of piroplasmosis, namely, the piroplasmosis of Northern Russia, the piroplasmosis of the Caucasus, and the piroplasmosis of the Transcaucasus.

The former two piroplasmoses are probably identical with *Piroplasma bigeminum*, and the latter one is the tropical form. They do not state that they have seen marginal points in either of the former two. They have also found in the Trans-Caucasus the presence of *Piroplasma bigeminum*. Notwithstanding this fact they do not identify the peripheral points with the *Piroplasma bigeminum*, but with the *Piroplasma annulatum*. Apparently in their experiments they have been working with cattle which were immune against *Piroplasma bigeminum* and not immune against the anaplasma infection; hence they did not see the former but the latter infection occurring in the animals which were also susceptible to tropical piroplasmosis. Also here our contention is the right explanation of the facts observed by the Russians. They have three diseases to deal with, namely, tropical piroplasmosis, redwater, and the disease under discussion, due to *Anaplasma marginale*.

Conclusion.

The cachetical form of tropical piroplasmosis is identical with our anaplasmosis.

(e) Observations in Europe.

We have to take into consideration the observation concerning *Piroplasma bovis* of Europe, a parasite closely related to *Piroplasma bigeminum*, if not identical. We have a right to expect that, if the peripheral bodies belong to the cycle of development of *Piroplasma bigeminum*, they would also appear in the cases of *Piroplasma bovis* of Europe. The fact is that in Europe no observers have ever described the presence of chromatic bodies resembling our marginal points or the peripheral bodies of the Americans.

Referring more especially to the work "Ueber die Hamoglobinurie der Rinder in Deutschland", by *Kossel, Schutz, Weber*, and *Miessner* (published in the "Arbeiten aus dem Kaiserlichen Gesundheitsamte", Vol. 20, 1904), a number of cases are enumerated where a careful examination of blood was undertaken and which was continued for some length of time, or at intervals during the course of two years, yet in no instance do we see any mention of peripheral points.

On page 67, an instance is given (Case No. 4) of a cow which was injected on the 20th of October, 1900, with 20 c.c. fresh blood, and we notice the appearance of piroplasms four days later. They remained present until the 12th day, during which time there was a high fever reaction. The temperature of the animal is recorded during November and December. During December on thirteen days subsequent to the fifth (after a second inoculation of blood on the 2nd December), no peripheral points were registered. If these peripheral points belonged to the cycle of the piroplasm they would have shown here, as sufficient length of time had elapsed for them to make their appearance, or if the animal had suffered subsequently, it could not have escaped notice as it was under constant observation for a long period. From the interpretation of these facts it is evident that in Europe the piroplasm due to *Piroplasma bovis* is not complicated by the anaplasma. The disease corresponding to the anaplasmosis has not yet been described there.

Résumé of Conclusions.

The conclusion of this review is that the observations both in North and South America concerning the peripheral bodies found in Texas fever infected areas are identical with those found in the Transvaal. The facts quoted find their natural interpretation by accepting my contention of the dual nature of both parasites. In the Trans-Caucasus the coccus-like bodies have never been identified with Texas fever but with tropical piroplasmosis.

In Europe, finally, the genus piroplasma is found, but not associated with anaplasma, which should rightly be expected to be there if it represented a stage in the cycle of the piroplasma. The presence of piroplasma and the absence of *Anaplasma marginale* in Europe therefore is a further and strong support of my views.