

NOTES ON THE NATURE OF KOCH'S GRANULES AND THEIR RÔLE IN THE PATHOGENESIS OF EAST COAST FEVER.

BY DR. K. F. MEYER.

IN his first report on East Coast fever, *Robert Koch* mentioned bodies which he found in the different organs of an animal which died of this disease. He wrote on this subject as follows:—"That this is caused by the action of the parasite is proved on microscopic examination, as we find in all these different parts the organisms in unusual abundance, and there we have observed a peculiar form which up to now has not been described, a form which leads to the conclusion that an increase of parasites takes place here."

These bodies, which are generally called "Koch's granules", "Blue bodies", or "Koch's plasmakugeln", were illustrated in his reports, and nobody had any doubt that they were developmental stages of piroplasma. The discovery of Koch was confirmed by several investigators, and these bodies became very important in the diagnosis of East Coast fever.

Theiler reported on these bodies in connection with *P. mutans*, and he recognized their high value in the differentiation of the organisms belonging to the group of small piroplasms. It is a well-known fact that *P. mutans* and *P. parvum* morphologically are difficult to differentiate, and only the demonstration of Koch's granules justifies at present the microscopical diagnosis of East Coast fever.

J. Walker then collected the observations of *Theiler*, *Lichtenheld*, and others, but shortly before this *Martin Mayer* published, in a paper on *Spirochaeta duttoni*, a few notes on "Koch's plasmakugeln". There he stated that he found similar bodies in other diseases also, and very rarely also in healthy animals. In his opinion the presence of "Koch's granules" in pathological lesions typical of East Coast fever was probably due to nothing other (as he stated in a lecture) than reactions, and were products of reactions caused by ultra visible virus.

Several investigators (*Fülleborn*, *Ollwig*, and others) stated that East Coast fever was caused by an ultra visible virus, but *Kleine*, in an article, defended the parasitic nature of Koch's granules, stating that this was more or less proved, and that they were specific for the disease and could easily be compared with the schizogony of other blood parasites. At the same time he noted the presence of similar plasma bodies in the Egyptian and Russian piroplasmoses of cattle, diseases similar to East Coast fever. Of the Russian disease, *Dschunskowsky* demonstrated specimens at the Congress at the Hague in 1909. Reports on this subject are to be found in "Notes on Stock Diseases of German and British East Africa and Uganda, etc., by

Theiler". A short time ago *Colonel Sir David Bruce* and others demonstrated the presence of "Koch's plasmakugeln" in a disease of calves in Uganda, called "Amakebe". As this disease is doubtless East Coast fever of the young animals, the presence of Koch's granules is not very remarkable, but in any case these granules led to the identification of that disease. I personally had the good fortune to see specimens of organ smears from this disease which were sent to this institute. From a morphological point of view they are identical with the Koch's granules found in cases of East Coast fever in South Africa. At the end of December, 1908, I came into contact with the question of East Coast fever, as *J. Walker*, who was engaged in the study of Koch's granules, allowed me to examine nearly all of his preparations. Later I obtained permission to examine the animals which were used in East Coast fever experiments at the institute here, and by this means I obtained a very rich and variable material. From the first examination we found that organ smears of animals dead longer than half an hour are nearly useless for exact investigations. The animals, about fifteen in number, were therefore killed *in extremis* and used for experiments. At a later period, when the question arose as to the date on which the Koch's granules would appear in the organs, we used the extirpation of small pieces of the lymphatic glands, the puncture of the glands, and afterwards the puncture of the spleen, of about another 30 to 40 animals. All the blood and organ smears sent in from the districts were used for my research (about 4000 preparations). Some methods of puncturing glands and spleen gave such satisfactory results at this institute that a few notes on the technique will be given.

Puncture of the Lymphatic Glands.—All the glands which can easily be detected under the skin can be used for puncture, but the prescapular and precrural lymphatic glands are generally the best. The fat tissue in cattle is often increased, and then the glands are difficult to locate. In such cases where the swelling of the retro-pharyngeal glands is very pronounced, these glands can be used for puncture, but where one or two punctures are carried out daily, especially in experimental investigations, the use of these glands has to be avoided, because small haemorrhages are to be found in the medulla and the sinus, as a sequel of the puncture, and they then will not give satisfactory preparations. After disinfection of the skin with acetone-alcohol, the glands are fixed with one hand, and the needle is then pushed into the gland. Often with a slight compression of the gland, a small quantity of lymph runs through the needle channel and can be used for preparations. In other cases the material must be aspirated by means of a syringe. For all punctures I used a sterilized, dry, strong needle of the same length as that generally used for hypodermic injections. All the syringes used should be absolutely dry, otherwise one risks a deformation of the aspirated lymphocytes and blood corpuscles. The channel of the puncture is closed by means of iodoform collodion.

The Puncture of the Spleen.—*Stordy* reports on this method, which was introduced in British East Africa. Since September, 1909, this method is now also used to a great extent in the laboratory. The technique is as follows:—On the standing animal the hair between the eleventh and twelfth ribs on the left side of the body, at the height of the tuber coxae, is shaved off, the skin disinfected with

alcohol, and a small incision made in the middle of the intercostal space. A strong needle (for small cattle about 5 centimeters long and for big oxen from about 8 to 10 centimetres long) is pushed through the wound along the *musculus longissimus dorsi* in a ventral and slightly cranial direction directly into the spleen, which lies rather superficially at this place. As soon as the spleen is touched, a small quantity of blood escapes; if the pulp is dry only aspiration by means of a syringe will show whether the needle is really in the spleen. After a short training it is easy to puncture the spleen, but it is advisable to take care that the animal which is being operated upon is unable to move. The contents of the syringe are ejected on to a slide, and the pulp particles, which can easily be detected, are used for smears. The wound is closed with collodion. The operation can be carried out daily without any risk. I controlled several cases by *post-mortem* examination, and found only small haemotomata at the seat of the puncture. Both methods are also used for the inoculation and artificial transmission of East Coast fever.

From the organs of an animal killed and opened shortly afterwards, smears were made for the purpose of studying the distribution and numbers of Koch's granules. Generally the haemotopoietic organs were considered of importance, as intraperitoneal operations such as puncture of the liver and kidney in two places gave proof that these organs are infected with Koch's granules at a later date than the lymphatic glands and the spleen. Several *post-mortem* examinations show that the character, and especially the course of the disease, are responsible for the formation of "infarcts" in the kidney, liver, myocardium, and lungs. I shall report in a later part of this paper on the presence of Koch's granules and their importance in these pathological lesions.

All smears made by the different methods were fixed by either the moist or dry method, the first in sublimate alcohol and the latter in methyl-alcohol. They were stained after Giemsa, Leishman, or May-Grünwald-Giemsa (Pappenheim) for panoptic stains, with haemotoxylin (Delafield or Heidenhein). In several cases also sections were used. Generally, the well-known facts of a morphological nature were confirmed in all smears taken from animals which died of East Coast fever. Koch's granules in an unstained preparation are round or oval bodies which contain in their plasma a few or several strongly refractile granules, which become enlarged under the influence of alkali, and which are never dissolved by sodium taurocholate, but often become slightly enlarged. In a stained smear they are light blue or dark violet discs or spheres, which contain a variable number of round or irregular light reddish or more bluish violet granules, which microchemically are pure chromatin. Generally these spheres are not only found free in the lymph, of different sizes (from 1 to 10 μ in diameter), but also are very frequent in the plasma of cells which have the character of lymphoid elements, generally mononuclear leucocytes. Shortly before death, cells of the same character are often found in the circulating blood, as in East Coast fever a leucopenia is followed by a lymphocytosis. These leucocytes with the granules, when found in blood smears, can be used in doubtful cases for diagnosis. If one of these elements is found in a blood smear where small piroplasms are fairly frequent,

P. mutans can be excluded. In one case I observed free Koch's granules on the fifth day after the rise of temperature. The Koch's granules were always present when the *post mortem* examination revealed East Coast fever. In all cases where Koch's granules can be detected only in rare numbers in the haemotopoetic organs, spleen, and lymphatic glands, every organ should be examined for these bodies. I know of one case where the temperature reaction, as a sequel to infection with ticks, was so typical of East Coast fever that I was astonished to find only a few Koch's granules in the lymphatic glands and the spleen. I therefore trepanned one of the ribs, and found Koch's granules in considerable numbers. In all atypical cases of East Coast fever, the Koch's granules are always found in the first stages of development, and comparatively rare in number. The piroplasms found in these cases may be *P. mutans* and *P. parvum*. The question arises as to when the first plasma bodies may be found in the organs of an animal suffering from East Coast fever. From a technical point of view this question could not be solved entirely, because only lymphatic glands and spleen could be examined daily, and the other organs which may also be of importance had to be excluded. A daily puncture of lymphatic glands will be demonstrated in the following report.

Cattle 686.—Infected with ticks on 13th July, 1909.

28th July, 1909. Left prescapular glands normal; blood normal. Temperature 101.8° F. to 103° F.

29th July, 1909. Right prescapular glands normal; blood normal. Temperature 102° F. to 100.4° F.

30th July, 1909. Right prescapular glands normal; blood normal. Temperature 102° F. to 101.8° F.

31st July, 1909. Left prescapular lymphatic glands, a few lymphocytes with granules; blood normal. Temperature 102.6° F. to 104.2° F.

1st August, 1909. Left prescapular lymphatic glands, the same as noted on 31st July. Temperature 104° F. to 106.4° F.

2nd August, 1909. Right prescapular lymphatic glands, in several smears Koch's granules. Temperature 106° F. to 107° F.; blood normal.

3rd August, 1909. Right prescapular glands, Koch's granules intra- and extra-cellular very frequent; blood a few piroplasms. Temperature 106° F. to 106° F.

4th August, 1909. Left prescapular lymphatic glands, Koch's granules frequent. Temperature 106.6° F. to 106.6° F.; blood a few piroplasms.

5th August, 1909. Blood examination normal. Temperature 105° F. to 107° F.

6th August, 1909. Blood normal. Temperature 107° F. to 107.6° F.

7th August, 1909. Blood *P. parvum*, 1 to 300. Temperature 106.2° F.

The animal was killed on the 8th August, and showed in all haemotopoetic organs Koch's granules very frequent.

Epicrisis.—The presence of Koch's granules was noted the second day after the rise of temperature, i.e. twenty-one days after infestation; *P. parvum* on the third day.

Several animals examined in the same way showed that the appearance of Koch's granules is not bound to a certain time.

In no cases so far as we could study this question in smears from the lymphatic glands and the spleen were they present at the date when the temperature increased. Also on the second day they were generally not present, but on the third day a few intracellular and free forms were found. On the fourth day the swarming forms were often present. The following days Koch's granules increased in number, and we obtained smears from the organs resembling those from dead animals. In very rare cases a few small piroplasms were present in the blood when only a few Koch's granules were found in the lymphatic glands. Often a few days passed after the first appearance of Koch's granules until parasites in the blood were noted. We have, therefore, only the fact that the Koch's granules were not present at the beginning of the fever, but they were generally found on the third day, and were always followed by *P. parvum* in increasing numbers; *P. mutans* may have been in the blood, but did not increase in number. No *Piroplasma parvum* was present in the blood if Koch's granules could not be demonstrated in the lymphatic glands. The appearance of Koch's granules in the lymphatic glands runs parallel to that in the spleen, as was shown in several cases. In one exception where another component of the haematopoetic system was more affected, I reported as above. The question whether Koch's granules are found in healthy animals or in cattle affected with other piroplasmosis will be answered in the following lines.

The notes from *Martin Mayer*, stating that he found similar bodies in trypanosomiasis in piroplasmosis of the dog and other diseases may be true, but in cattle I never saw Koch's granules in any disease other than in East Coast fever, and I think that it is impossible to apply to cattle conclusions drawn from experiments on small animals such as guinea-pigs, rabbits, etc. *J. Walker* examined about fifty spleens, lymphatic glands, and kidneys of healthy animals for the presence of Koch's granules with negative results. I was able to see these smears, and am therefore in the position to confirm his statement entirely. In other piroplasmosis or other cattle diseases several organs were examined on *post-mortem* with the same result. In several cases of redwater (631 and 643, etc.) the glands were punctured and examined during an acute infection with *P. bigeminum*, also with negative results. In several cases where *P. mutans* (642, 655, 656, and 777, etc.) was very frequent in the blood, small pieces of the lymphatic glands—and in one case of the spleen—were extirpated, but no Koch's granules could be found in smears made from this organ. This fact was confirmed by all the blood smears sent in from the districts where pronounced infection of *P. mutans* never showed Koch's granules in the organ smears sent from the same animals. The presence of Koch's granules was observed by me for a long period, but I was always in doubt as to their nature and their connection with the piroplasma until the artificial transmission of this disease threw some light on the subject. Two forms of Koch's granules were of importance—(1) The commencing forms in the shape of small granules in the protoplasm of lymphocytes; and (2) the end of the development. The investigation of the first forms was very difficult as these granules resemble at the beginning the azurophile granulations of the lymphoid elements.

The latter forms I called "swarming" forms, which were probably previously observed by other investigators, but they could not be identified as belonging to the piroplasma until definite proof was given in another way, as explained later. With the first appearance of Koch's granules large granular infiltrations in the plasma of cells of the endothelial type, or of lymphoid elements—in rare cases also in polynuclear leucocytes—are observed. It may be mentioned here that the affection of the polynuclear leucocytes is very small, and was only pronounced in one case, No. 337, where phagocytosis was very frequent. Two or more of these granules, which take up the haemotoxylin stain, are at the commencement surrounded by protoplasma, which is differentiated in a more or less distinct manner. The presence of these granules in the protoplasma of the cell is important, as it decides the question whether all Koch's granules have an intracellular stage or whether there are some which develop free. I observed cases where the intracellular stage was predominant during the first days. As these stages were always followed by a breakdown of the lymphoid elements it was thought that by this means the well-formed Koch's granules in the plasma get free and develop independently. We often observe small spheres which contain only one or two nuclei similar to those illustrated by *Sir David Bruce* in his paper on "Amakebe", and therefore probably a certain number of Koch's granules develop without any parasitism in another cell. Logically all parasites develop free; they are brought to the organ like the "sporozoites" in malaria, and are in the blood or lymph. The development in the cells is of a secondary nature, and probably only a smaller number of parasites develop there. The free and round spheres or discs are light blue (Giemsa stain) or dark violet, and contain a variable number of round compact or irregular slightly stainable chromatin particles, which lie irregularly in the plasma of the body. At the same time a great number of pyenotic and cariorrhctic nuclei of the endothelial and lymphoid elements, especially of the cells which contain one or two small Koch's granules, were detected. The pictures formed by the breakdown of the nuclei can never be mistaken for real "blue bodies". Often a Koch's granule can be observed at the moment when it is pressed out from the plasma of the cell. The formation of new cells in the lymphatic glands and in the spleen is always noted, and cells with caryokinetic pictures are very frequent. Often giant cells (*Riesenzelle*) with mitosis have a similarity to Koch's granules.

Lately, Captain I. D. Holmes published, under "Notes on a Giant Polynuclear Cell", bodies he found in blood smears from horses which died of surra and in pus smears from animals suffering from "bursati"; these "mulberry"-like blood elements—as he states—resemble the "plasma kugeln", and he identifies them with these. His description and the sketches made of these cells are, in my opinion, nothing else than eosinophiles cells generally found in horses in an increased number when the animal is suffering from anaemia and intestinal parasites such as worms, etc. Such cells cannot be mistaken for Koch's granules by an investigator who knows these elements.

In the following days forms are observed which are of importance to decide that the Koch's granules are related to the piroplasms. The presence of these bodies is probably dependent on certain conditions, since they are often rarely met with in smears. Under such conditions it is easily understood that by superficial examination they may

be overlooked. When these forms are found in great numbers the parasites are always present in the blood. Two different forms can be observed. First, cells of the type of mononuclear leucocytes [*vide* Figs. 1 and 4 (12-16 μ and nuclei of 12½ to 14 μ)]. In the protoplasmatic part of such a cell we find dark red or violet chromatin particles, which in an advanced stage of development show characteristic circular or star-like formation, surrounded by a light bluish plasma body. Often up to 100 of such chromatin particles may be observed in the entirely preserved cell (Fig. 1). In an advanced stage of development we find similar cells in which the protoplasm has disappeared and piroplasma-like protozoa are swarming. These piroplasma-like bodies consist of small nuclei, to which is attached a small protoplasm body well differentiated. Secondly, we find groups of similar piroplasma-like bodies, morphologically like those described, but free in the lymph, and in no way connected with parts of any cell (Fig. 3). That these are advanced stages of free Koch's granules can be shown in cells which contain a small round or irregular protoplasm rest in the centre, which is nothing else than the residual plasma surrounded by piroplasma-like bodies (Fig. 2). The number of these is usually small. Later, as can be shown in smears, piroplasma-like bodies are swarming out, and we find them often entering into the red blood corpuscles.

Lichtenheld saw similar bodies which he described under the name of "Steckapfel" form. Endothelial cells with two or three Koch's granules in the different stages of development are often observed. One Koch's sphere may be so far advanced that the piroplasmas are well differentiated, whereas another may contain the undifferentiated chromatin particles. Whether the two forms described above are separate types of Koch's granules (the intracellular and the free ones show variations in the number) is not my duty to decide. If we compare all these facts with those known in other protozoa diseases we find an explanation for them. At the beginning of the fever no piroplasmas are present in the blood. Three or four days afterwards *P. parvum* appears in the circulating blood, and increases to such a degree that 80 per cent. of the red blood corpuscles (with at least two or three parasites in each) are infected. This appearance succeeds the development in the organs and finds thereby a natural explanation. The Koch's granules are, therefore, specific, and are developmental stages of the parasite *P. parvum*. They can never be detected in other piroplasmosis. The inoculation of organs or pieces of an organ containing Koch's granules produces East Coast fever. To explain the transition of the different Koch's granules the development of *P. parvum* in the tick has to be studied, and this knowledge would help us for the classification of our piroplasmas. Comparing the Koch's granules with other protozoa where the development of the parasite takes place in the internal organs, we may state that they represent an asexual stage in the development, the "schizogony" and the free swarming piroplasma-like bodies could be compared with the "schizonts". As was noted above, the Koch's granules are only noted in East Coast fever, and not in animals infected with *P. mutans*, and therefore the different theories as to the nature of the small piroplasma cannot find a solution. To everybody who knows the experiments of *Theiler* on this subject it is not astonishing that *P. parvum* and *P. mutans* are two separate piroplasmidae.

The observed enlargement of Koch's spheres, the subsequent fragmentation of the nuclei, indicating a typical growth, can not be compared with the products caused by toxic reaction. If *Martin Mayer* tries to prove his theory by means of demonstration of intranuclear bodies, I can state here that they do not resemble Koch's granules. The organs in which Koch's granules are found are always in a state of hyperplasia caused by the growth and increase of the parasite. Probably a considerable number of cells attacked by the parasite have to be replaced, and we found, therefore, in simple smears, forms and artefactes of chromatin particles which may be mistaken for Koch's granules. In the pathological histology of tumours such forms are also well known; they are nothing else than abnormal development stages of rapidly growing cells. But the objection of *Martin Mayer* is disproved by demonstrating that Koch's granules represent development stages of the *Piroplasma parvum*.

The question of the rôle of Koch's granules in the pathogenesis of East Coast fever is of the greatest interest.

Unfortunately I could only enter into this question superficially. Studies were made on sections from organs preserved in sublimate alcohol in the solution of *Helly*, *Borrel*, *Flemming*, *Herman*, and also in formaline, for staining haemotoxylin in different solutions with eosin or van Gieson, as contra stains were used. These staining solutions gave for the pathological investigations better and more distinct results than the combination of methylene blue, azur, and eosin.

Martin Mayer stated that the macroscopic lesions found in kidney, liver, etc., are due to an ultra visible virus. As far as the single cell is concerned I think this theory is disproved, and for complexes of cells the following notes prove the inaccuracy of his views. I presume that the macroscopical lesions in an animal which has died of East Coast fever (typical and atypical case) are known. They vary, corresponding with the course and length of the disease. We have cases where pulmonary oedema causes death at an early date and "infarcts" are never present. These are atypical cases, which are sometimes met with in practice, but very rarely in the laboratories, where the animals are kept free from climatic influence (*vide Gray*). It was rather difficult to receive full details on the presence of the piroplasms and Koch's granules in such cases. I had the opportunity of examining some cases which ended fatally in a short time. *P. parvum* was never, or only in a small number, present in the blood. Koch's granules were never missed, and were fairly frequent in the haemotopoetic organs and also in the haemorrhages of the serous membranes. The plasma of these is light bluish, the nuclei rather irregular and incompact. They were never present in the exudates of the pericardium, the pleura, and the peritoneum.

In typical cases of East Coast fever the alterations in the parenchymatous organs are very pronounced. They are more distinct in prolonged cases of the disease where more parasites and Koch's granules are present in the blood or in the organs. The distribution of Koch's granules in a dead animal is best illustrated by the following case of an animal which died twenty-eight days after having been infected with ticks (674):—

Spleen.—The granules were very frequent; three and four cells were observed in one field. (Zeiss apochromat 2 mm. ocular 4.)

Lymphatic Glands.—Here the prescapular, the precrural, submaxillary, retropharyngeal, iliaca, and portal glands were the most affected.

We observed often ten granules in one field. In the mesenteric and mediastinal lymphatic glands we could also find them. In the tonsilli their number was about one in one field. In the haemolymphatic glands they were very frequent, about twenty to one field.

In the *bone marrow* of the femur and humerus they number one to two fields. In the ribs similar conditions to those in the spleen were met with.

Liver.—In the typical star-like whitish foci of the interstitium they were very frequent, about five to one field.

Kidney.—In the parenchyma about one to two fields. In the white round or irregular shaped foci they were fifteen to twenty in one field. In the *supraenal glands* about one to twenty in a field. In the *pancreas* one to twenty fields.

Stomach and Intestinal Tract.—They were frequent in the small ulcers of the fourth stomach and in the colon. In the peyers patches they were very frequent.

Sexual Organs (details from cow 830).—In the ovaries, cotyledones, they were not very frequent, about one to three fields.

Lungs.—They were fairly rare, about one to twenty fields.

Heart.—In the myocardium they were not frequent, about one to twenty-five fields.

Blood-Vessels.—On the intima of several large vessels they could be detected.

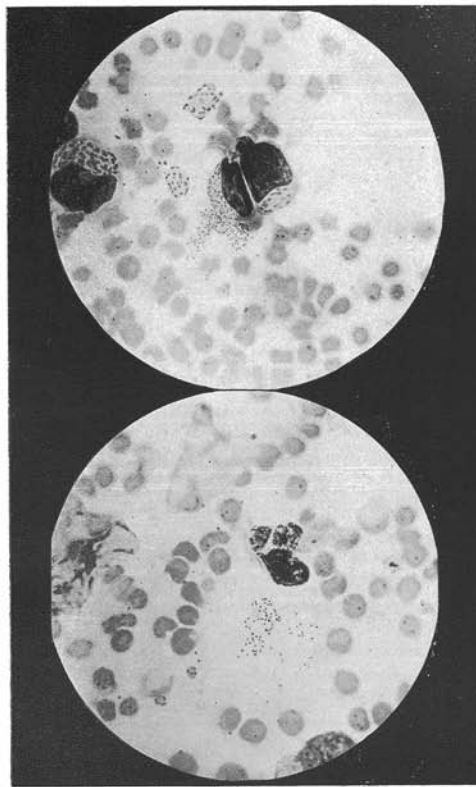
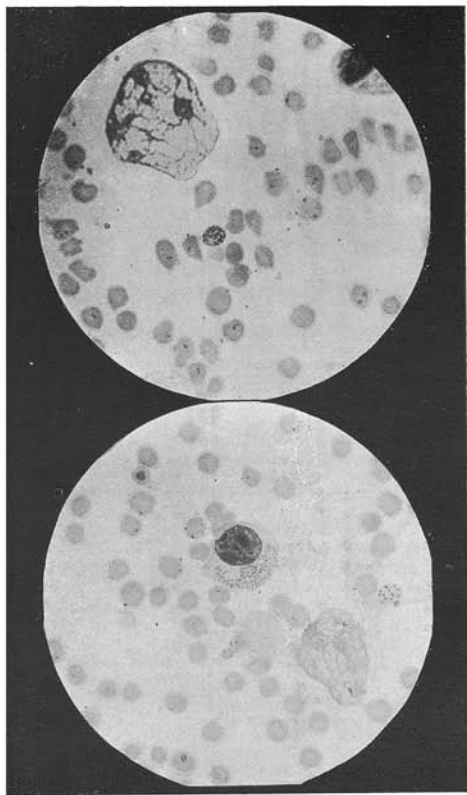
Arachnoidea.—In the small capillaries, at the base, they were about one to five fields.

Skull and Spinal Cord.—They were very rare, one to sixty fields.

In the *skin* they were comparatively frequent. Smears made by pressing the cut surface showed them in about one to two fields. Also in the blood of the heart the Koch's granules were present. In atypical cases Koch's granules were only found in the lymphatic glands, the spleen, and bone marrow. They were rare in the liver, kidneys, intestines, lungs, heart, and blood-vessels.

Two observations on the presence of Koch's granules in the foetus (intra-uterine and postpartum) were noted. In one case the *post-mortem* examination of a cow which died of acute East Coast fever showed in her organs and also in the cotyledones Koch's granules very frequent. Several smears from the organs of the foetus gave negative results. The second case, cow 830, infected with ticks, gave a good East Coast fever reaction, and at the height of the reaction a fully developed calf was born (894). This was punctured shortly after birth, but no Koch's granules could be detected. The animal is still living at the date of writing, and has never showed any disease.

If we compared the distribution of the number of Koch's granules in the different organs we observed that the haemotopoetic organs were the most affected. The secondary characteristic lesions in the liver, kidneys, lungs, heart, and stomach often showed the same bodies in such numbers, suggesting their being metastases. The examination of the bone marrow showed that in several capillaries the intimacells were projected into the lumen, and showed Koch's granules of different sizes. Similar conditions were found in the liver, kidneys, etc.,



East Coast Fever (by Dr. K. F. Meyer). *Piroplasma parvum* and Koch's plasma bodies. *Plate No. 1.*

especially in small haemorrhages. By scraping off the intima from several large blood-vessels I could demonstrate endothelial cells containing Koch's granules.

Collaud states in his paper that pathological processes in the intima of the arteries in the kidney are always present in East Coast fever. His statement concerning the presence of an endarteritis obliterans could not be proved on my material, but nevertheless I quite believe that it may be present. All the capillary haemorrhages which are observed at the beginning and the secondary regenerative processes in the different stages were described by *Collaud*. He did not know Koch's granules, and for the explanation of the lesions he considered a toxin to be responsible, probably secreted by *P. parvum*. My examinations of the kidney, liver, lungs, etc., always showed a swelling of the intima cells in the capillaries of the interstitial of the kidney, etc. As a sequel, a small haemorrhage per diapedesin is observed. These lesions we found in atypical cases not only on the serous membranes but also in the parenchymatous organ, where Koch's granules act on the epithelae of the capillaries. The haemorrhages undergo changes under the influence of proliferating lymphocytes, connective tissues, etc. Koch's granules are washed into these lesions and an accelerating growth of the parasites takes place. Such a focus grows larger and resembles more or less a lymphoma or a metastasis of a growth. But these foci are nothing else than metastatic conglomerations of Koch's granules as a sequel of the lesions in the blood-vessels. A reparation often takes place, and a defect will heal up by growth of connective tissue. The predisposition of the small capillaries in kidney, liver, lungs, etc., for metastasis is well known in pathology, and is known to occur in protozoal diseases such as malaria and kala-a-azar. It may be stated here that the morbid anatomy of East Coast fever resembles to a great extent the lesions found in leucaemic diseases. Similar to this process in the kidney where the small haemorrhages are followed by metastasis we observe lesions in the interstitiae of the liver and the lung. In the liver the star-like metastasis are often so pronounced that the organ has macroscopically a marble appearance. The interstitiae are in the state of an acute lymphocyte infiltration. These "lymphoma" are separated from the acini, and enter irregularly between the trabeculae of the liver. Similar foci are found in the lungs, where they are under the pleura and near the small bronchi. In several cases I also saw whitish foci in the myocardium. The histological examination of the ulcers in the fourth stomach showed a great number of Koch's granules as was described before by *Lichtenheld*, and similar lesions in the capillaries indicate that the same noxis is present. The alterations in the circulation are macroscopically demonstrated by the small punctiform haemorrhages, which are always observed in acute East Coast fever cases. In the stomach these small foci, reduced in their vitality, are dissolved under the influence of the ferments, and the defects which show at the beginning no reactions enlarge under the influence of bacteria. Later on regeneration may take place, and the irregular margins of the ulcer show the beginning of cicatrization. Generally speaking, in East Coast fever the lesions in the stomach are fresh, indicating that they are caused by the disease. Often I found similar ulcers in the colon. The haemorrhages in the yellow bone marrow have also their origin from the endothelial affections. I

explained that all the metastatic foci are composed of lymphoblasts, lymphocytes, and lymphocytoid elements. I think the theory that the several foci in the kidney, the liver, etc., originate in the lymphatic tissue of these organs can be disproved. First, the primary lesions of the capillaries, the haemorrhages, are always present in places where no lymphatic tissue has ever normally existed. Second, for all diseases of the blood where perivascular growths of a lymphoid character (leucaemia and pseudoleucaemia) are produced, the autochthone formation is well known. Have these alterations of a macroscopic or microscopic nature a toxin as cause, or are the protozoon in the form of Koch's granules to be accused? Until further proofs for the first hypothesis are given I am inclined to adopt the latter view. The fact that large quantities of blood, up to as much as 10 litres, of an East Coast fever animal, transfused to a healthy animal, give no reaction is remarkable. If toxins were present, I think in any case some symptoms would have been observed, because if the blood-vessels of the sick animal become affected under the influence of the toxin why should not the transfused animal show similar symptoms? All the characteristic lesions of the action of a toxin are missing. The parasite in the blood, which probably never increases in the blood corpuscle, is harmless and never causes a breakdown of the red corpuscles as is generally observed in other piroplasmosis. The symptoms of degeneration, so far as I could control my observations on one animal (which previously had never passed through any other piroplasmosis), are insignificant. I refer here especially to the fatty amyloid and hyalin degeneration. The first is generally found in the liver and the kidney of animals which have died of redwater, piroplasmosis in horses, and of ultra visible vira. Alterations in the nutrition of the tissue, fever, etc., may cause hyalin degeneration in the epitheliae, but it is not necessary to take for the explanation of their presence a toxin secreted by the parasites, because in advanced cases of East Coast fever, when Koch's granules and piroplasms are very frequent, the lesions of degeneration are almost the same as those found in atypical cases. Necrosis especially could never be observed. If East Coast fever caused progressive degeneration it would be very astonishing for animals suffering from this disease to pass through an attack and become immune without showing any remarkable internal alterations. It may be that autolysines (metabolic or histogene toxines), due to the breakdown of nuclei, may be engaged in the degenerative alterations of the epitheliae (especially kidneys). Proofs that a toxin really causes all the alterations in the capillaries cannot be given, but all the typical and atypical cases of East Coast fever and lesions of a pathological anatomical character can be explained by the presence of Koch's granules in the blood-vessels of the interstitiae. In the atypical cases we have the acute affection of the blood-vessels, the diffuse haemorrhagic diapedesis with affection of the heart, and no metastasis. In the typical cases one meets instead with reparation processes and secondary metastasis in all the organs. Probably in this generalized affection of the lymphatic apparatus also the lymphocyte groups of the adventitia of the blood-vessels may become affected by the parasite, and some of the lymphoma may originate from these. From the main observations we conclude that the haematopoetic organs, the blood-vessels, and the blood itself are the organs most affected in East Coast fever. It is therefore astonishing that up till now a transmission by means of blood has

never successfully been carried out. We saw that the development of the parasite takes place in the organs and that the parasite in the blood is harmless, but often we find Koch's granules in the blood, and they could transmit the disease if their number was not too small. In piroplasmosis the fact is known that the red blood corpuscles infected with the parasites always lie along the blood-vessels. By making smears from a capillary of the skin, and by pressing on the blood-vessels, we succeed in demonstrating more parasites in such a slide than in one made from blood obtained from the jugular vein. Similar conditions were found in East Coast fever, where often Koch's granules were found in blood smears from the skin, when none of them were present in the blood from the jugular vein. In practice the blood is generally collected from a wound in the skin, and this fact alone shows that in such blood smears mononuclear leucocytes or lymphocytes with Koch's granules are more frequent than in blood smears from animals of the institute, where the blood for the smears was usually collected from the jugular vein. I proved this in two cases on the station (875, 700), and I succeeded in finding Koch's granules in the blood when not one parasite was found in the red blood corpuscles. I think, therefore, that for a transmission experiment it would be better to collect the blood from a wound in the skin and inoculate it directly into the organ disposed to the growth of Koch's granules. The presence of Koch's granules in the blood shortly before death is partly due to the increase and partly to the decrease in the tonus of the blood-vessels.

CONCLUSIONS.

Koch's granules are a developmental stage of the blood parasite *P. parvum*. They are, therefore, specific for East Coast fever. These granules are engaged in the formation of the secondary metastasis in the liver, kidney, lungs, heart (ulceration in stomach, etc.). The endothelial cells of the small capillaries become affected under the influence of Koch's granules. The secondary endarteriitis gives rise to haemorrhages, invasion of cells, and proliferation of lymphoid elements with increase of Koch's granules. The theory of *Martin Mayer* as to their non-specific character is herewith disproved.

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