ANAPLASMA MARGINALE (Theiler).

By Hans Sieber, Ph.D.

In the last annual report of the Government Veterinary Bacteriologist, Transvaal, 1908-09, *Theiler* gives the results of his investigations, continued for many years, concerning gall-sickness. He has now come to the conclusion that *Trypanosoma theileri* and *Babesia mutans* which, at one time, were considered to be closely connected with gall-sickness, do not play the primary rôle as was thought first, but are parasites accidentally found in gall-sickness. They are not independent, typical causal factors of that disease, but are able to influence its course, generally unfavourably.

As shown by *Theiler's* report, gall-sickness is a type of disease which spontaneously is rarely observed in a pure state, i.e. showing but one species of parasite, the *Anaplasma marginale*, which has now been determined to be the cause of it. This parasite, a new genus which so far has not yet been described in protozoology, is frequently associated with the above-mentioned parasites, *Trypanosoma theileri*, *Babesia mutans*, as well as *Spirochaete theileri* and *Babesia bigemina*, which accounts for the uncertainty hitherto prevailing concerning the etiology of gall-sickness.

Former attempts to cause a disease identical with gall-sickness by means of *Trypanosoma theileri*, or *Babesia mutans*, were not successful in every instance. Now *Theiler* has succeeded in producing a disease, absolutely free from the parasites mentioned above, in cattle freshly imported from England and which is undoubtedly gall-sickness proper. In the course of it the actual agency, anaplasm, was found in a pure state in the blood.

Undoubtedly anaplasms have been seen by different observers, not only in Africa (Lichtenheld),* but also in America (Knuth)† (Smith and *Kilborne*)‡ and Asia (*Dschunkowsky*).§ As to their nature, these investigators consider these parasites to be forms belonging to the cycle of development of another micro-organism, as, for instance, *Babesia bigemina*, etc. As to the form of these parasites and the effect they have on the blood, these authors only state that they represent peripheral strong basophile if not chromatophile points. Further characteristics have not been described.

Anaplasms have been found at different times in South Africa. However, so far, they have not been determined precisely.

One instance is given by *Kolle* in the "Zeitschrift fur Hygiene u. Infektionskrankheiten", Vol. 27, No. 1, regarding a new parasite in the blood of cattle in South Africa. *Kolle* describes a disease which he observed in his experiments with rinderpest in cattle and

* Lichtenheld, Zeitschr. f. Hyg. u. Infektionskrankh. Vol 65, No. 3.

† Knuth, Experiment. Studien uber das Texasfieber (La Tristezza) der Rinder in den La Plate Staaten. Berlin, 1905.

‡ Smith and Kilborne, 8th and 9th Annual Report of the Bureau of Animal Industry. Washington, 1893.

§ Dschunkowsky and Luhs, Die Piraplasmosis der Rinder, Centralbl. f. Bacteriologie. Vol. 35, 1904.

which he calls *Febris malariaformis*, the symptoms of which are identical with anaplosmosis. In a coloured illustration by *Dr. Turner* (page 46), the exact reproduction of which cannot be questioned (especially as *Kolle* draws attention to it in a footnote) an erythrocyte is found showing two anaplasms. *Kolle* considers them to be young parasites. The nucleus of a normoblast is likewise described as a young parasite. Three further normoblasts represent, according to *Kolle*, erythrocytes, the nuclei of which are supposed to be growing parasites. Through the loss of the nucleole the normoblasts possess clear spaces, *Kolle* holds them to be "vacuolated adult forms". A basophile erythrocyte is called a "pigment cell", a dying-off erythrocyte, a so-called blood corpuscle shade is designated a "free parasite".

Considering the knowledge prevailing at that time of blood pathology and the technique of staining, we can, to-day, understand this almost incomprehensible lapse. The dates mentioned by *Kolle* in another publication* justify the conclusion that anaplasmosis has been observed by him. He inoculated (*vide* page 333) on Robben Island (8th December, 1897) a number of cattle for the purpose of rendering them immune against rinderpest. By the 17th January, 1898, a larger mortality is reported by him to have occurred amongst the inoculated animals. The autopsy indicated the presence of lesions as we have observed them in anaplasmosis. The time between inoculation and outbreak of the disease coincides with our knowledge of the incubation time in regard to anaplasmosis, and we may be sure that he himself had transmitted the anaplasm in spite of his attempts to prove the contrary.

On page 339 we read that he examined the blood of diseased animals. He found the intracellular parasite discovered by him. The microscopical examination of smears did not leave any doubt as to the nature of the disease. Some cattle escaped the disease, a circumstance which *Kolle* mentions particularly in order to avoid incurring the reproach of having infected the cattle by inoculation, but this is not conclusive, as we are aware in South Africa that immunity exists against anaplasmosis.

During the experiments of *Theiler* to isolate the anaplasm by means of ticks and to infect cattle with them, I have been able to make the following observations and investigations on the changes of the blood, and more especially on the morphological and biological peculiarities of the parasite.

Pathological Changes in the Blood.

The occurrence of the anaplasm is preceded by an incubation time of at least sixteen days. According to *Theiler* the duration of incubation is shorter after an experimental injection (blood injection) averaging about sixteen to forty days and longer after a spontaneous tick infection (*Boophilus decoloratus*) which, according to *Theiler* is one of the transmittors, varying from sixty to eighty days.

In a case of intravenous infection (calf 894—compare Temperature Chart 1) the blood appeared to be normal up to the eighteenth day. It was examined daily once or twice in dry preparations and stained according to *Giemsa*. On this date the anaplasms were first seen in

* Kolle and Turner, Ueber Schutzimpfungen u. Heilserum bei Rinderpest. Zeitschrift f. Hygiene und Infektionskrankheiten, Vol. 29, No. 2, 1898.

the erythrocytes of the spleen pulp. As a rule I could observe them endoglobularly as well as free—there was but one anaplasm on the periphery of a red corpuscle. Free forms were seen less frequently in the spleen. Only on the seventeenth day were anaplasms detected in the peripheral blood. Gradually small parasites developed into larger bodies. Generally they were to be seen on the margin of the erythrocytes, near the central depression; some, however, seemed to be reaching over the margin or adhering to it. The number of infected blood corpuscles was relatively small—about 2 per cent. to 3 per cent. On the eighteenth day the percentage increased (about 4 per cent. to 5 per cent.). Smaller anaplasms were found together with larger ones. Occasionally double parasites were met with, similar to diplo-cocci; however one of the two round corpuscles constantly appeared to be smaller and more diffusely stained. The blood picture was still unaltered up to the twentieth day. Then the number of anaplasms increased; up to 15 per cent. of erythrocytes were infected. Large and small parasites occurred; a rapid multiplication of blood platelets was noticeable. The next day (twenty-first) large parasites were found after staining with *Giemsa*, showing distinctly a central body; there were also smaller forms met with. The blood corpuscles showed sometimes double or triple infections. On the twenty-second day a strong anisocytosis appeared; the frequency of the infection continued to increase. Sometimes three, four and more parasites were observed in one blood corpuscle. A remarkable fact was noted in the appearance of so-called flagellated forms of blood platelets. On the twenty-third day the anisocytosis was more pronounced, anaplasms having again increased relatively. As the number of erythrocytes appeared to decrease I punctured the spleen. In the smear of the spleen pulp (I examined preparations fixed with absolute alcohol or methyl alcohol and acetone, followed by *Giemsa* staining, and preparations fixed moist with hot sublimate alcohol) very few splenocytes were found. The spleen was enormously gorged with blood. There were a great number of slightly deformed blood corpuscles, the latter being enormously infected. Many remains of perished blood platelets were noticeable. There was also an anisocytosis of the erythrocytes of the spleen. Polychromatic or basophile erythrocytes were not yet present.

In order to find out whether any multiplication or reproduction forms are found in the lymphatic system, I repeatedly made punctures of the larger lymphatic glands, but did not discern any parasites in the smears.

On the twenty-fourth day the parasites had still more increased. The lesions of anisocytosis had progressed in such a way that in one field there were hardly two blood corpuscles of the same size. A further increase of parasites was again observed on the twenty-fifth day. There were 20-25 per cent. of infected erythrocytes (4-6 anaplasms of different size and differently stained in one corpuscle).

On the twenty-sixth day a slight polychromasia set in, the number of infected corpuscles reached 25-30 per cent., the anisocytosis continued. The general aspect of the blood was bad, the blood becoming more and more watery (oligocythaemia). *Giemsa* preparations showed the presence of crenated forms. On the twenty-seventh day polychromasia had advanced considerably, crenated forms were still present. The blood became more watery and the blood

corpuscles were heavily infected. Numerous parasites were observed outside the blood corpuscles, appearing either as single or double parasites. The next morning fully 30 per cent. of the blood corpuscles were infected. In the evening of the same day the infection of the blood corpuscles seemed to have lessened, polychromasia increased. In the dark polychromatic erythrocytes small dots were observed when stained with *Giemsa* (transition to basophilia). In *Giemsa* moist preparations were many extracellular anaplasms. Basophilia was well advanced on the twenty-eighth day; polychromasia had developed into a picture bearing a strong resemblance to that of a kaleidoscope. Anisocytosis and poikilocytosis persisted and a strong increase of blood platelets took place. Further now and then normoblasts put in an appearance, and an increased leucocytosis seemed to bring a change in the picture of the blood.

On the twenty-ninth day the same observations were made. The leucocytes with oxyphile granulations and the normoblasts increased; the number of parasites greatly decreased. The decrease of parasites persisted up to the thirtieth day, basophilia continued, normoblasts increased, and single megaloblasts appeared. Now an important improvement of the blood picture seemed to set in. The normoblasts and megaloblasts disappeared out of the peripheral blood, basophilia and polychromasia receded. The infection of the blood diminished slowly, until on the fortieth day the blood seemed free from *anaplasma*. Slight remissions occurred on the fifty-second day, on which date, however, very few parasites were met with.

On the eighty-fifth, eighty-sixth, and eighty-seventh days some anaplasms were again seen. The blood picture itself did not appear to be abnormal, with the exception of a slight anisocytosis. In this case the infection resulted in recovery.

In another case, resulting in death (calf 936), after subcutaneous injection of 10 ccm. defibrinated blood (from calf 905—anaplasmosis—*vide* Temperature Chart 2) anaplasms were first detected on the nineteenth day (Fig. 1). On the twenty-second day (Fig. 2) 20 per cent. of the blood corpuscles were infected. At the same time a strong anisocytosis and poikilocytosis occurred. While these alterations were dominant during the next and following days, on the evening of the last date (twenty-fourth day) a strong polychromasia became marked (Fig. 3). The same day some basophile blood cells could be observed, the parasites having infected about 25 per cent. of corpuscles. On the twenty-fifth day basophilia progressed simultaneously with the infection of the blood corpuscles. The anaplasms invaded up to 30 per cent. of the blood corpuscles. The twenty-sixth day showed a marked basophilia, the infection increased (30-35 per cent.); the blood was very poor in erythrocytes, showing itself in the increasing distance between the red blood corpuscles in the smear (Fig. 4). Often I could observe pale bluish stained erythrocytes in a state of disintegration, having indistinct fringed margins as described by *E. and E. Sergent* (Comptes Rendus Hebd. Société de Biologie, vol. 58, No. 2, 1905). It may be mentioned, however, that the authors consider these forms to have a certain specificity for malaria. I have mentioned before that *Kolle* held these forms to be free parasites of *Febris malariaformis*.

Also *Maurer* (Archiv f. Schiffs-u. Tropenhygiene, 1910, No. 11) in his review on corpuscular elements of blood gives pictures which

are designated as corpuscles containing nuclei. I am not of the same opinion, and consider them to be erythrocytes in a state of dissolution, so-called blood shades. I likewise do not agree with his explanations concerning the conception of polychromasia and basophilia.

It is interesting to note the appearance of different forms of blood platelets with and without flagellates (tailed forms) as described by several authors in connection with many blood diseases. (Among others, Holmes: Report of the Imperial Bacteriologist, Muktesar, 1908-09.)

The twenty-seventh day showed the above-mentioned blood changes of anisocytosis, poikilocytosis, polychromasia, and basophilia as on the previous day, only the number of infected blood corpuscles had increased to fully 35 per cent.; some normoblasts were likewise noticeable in the blood.

On the following day, the twenty-eighth, the infection decreased, while normoblasts and megaloblasts increased (Fig. 5). This proportion augmented from day to day, the normoblasts and megaloblasts increased, and the number of infected erythrocytes and the red blood corpuscles decreased. The blood itself became more watery and poorer in form elements (oligocythaemia), until on the thirty-third day not one normal-looking erythrocyte was visible. There were very few parasites to be found, and these did not take the stain well. There were some oxyphile polynuclear leucocytes.

Thirty-fourth day.—General anaemia pronounced, and death occurred.

The *post-mortem* examination was as follows:—

Calf 936.—Sussex heifer, age fourteen months; colour: red; condition: fair; rigor mortis: not present; appearance of flesh: pale and bloodless, at back and hind quarters yellow, in some parts a gelatinous infiltration was noticed; blood: watery and thin; external lymphatic glands: swollen; pericardium contained only a small quantity of serous liquid. The lungs had not collapsed. Pleura: infiltrated with oedematous liquid; parenchyma: strongly oedematous; on section a large quantity of serous liquid escaped; bronchial and mediastinal glands: enlarged and juicy; retropharyngeal glands: considerably swollen; mucous membrane of the trachea: showed ramifications and was filled with foam; heart: soft; left endocardium: bluish colour; right endocardium: bloodless and pale; coagulated blood in both ventricles; liver weighed 12 lb., enlarged; colour: light brown, the sections showed a yellow saffron tinge; lobules: strongly marked; parenchyma: bloodless, soft and brittle; gall bladder contents: thick, gelatinous, greenish-brown liquid; spleen weighed 3 lb.; size 50 × 17 cm., swelling considerable; pulp: soft and friable, protruding on section; trabeculae: indistinct; a small quantity of contents in the fourth stomach; contents of omasum: moist and soft; abomasum: almost empty; folds of mucous membrane swollen; mucous membrane: slate coloured; small intestine: serosa: strongly injected; mucous membrane: slightly swollen and greyish; large intestine: slate coloured; mucous membrane: swollen. Kidneys: capsule easily detachable, cortex striped, tubuli recti distinctly visible. Medulla: opaque and pale, gelatinous contents in the pelvis; urine: clear, brown yellow; bone marrow: yellow gelatinous infiltrations. Pathological anatomical diagnosis: *Anaplasmosis.*

The erythrocytes, as can be noted in both cases, are in no way influenced during the incubation time. Only when the parasites appear in the peripheral circulation the first reaction of the blood is noted by anisocytosis and poikilocytosis. The difference in size fluctuating between 3–4 μ. and 12–15 μ. is far greater than the variations observed in the normal blood. Apparently an insufficient blood renovation seems to take place in the blood forming centres culminating in the appearance of immature blood elements. As an accumulation of infected erythrocytes (from the portal circulation) and in connection therewith perhaps followed by a destruction of them,

takes place in the spleen (as is shown in preparations obtained by spleen punctures) the peripheral blood contrives to find recompensation from the bone-marrow. The possibility of restoring blood from the bone-marrow has a definite limit, which is soon exhausted, and we perceive blood elements which have not yet attained the differentiation of normal erythrocytes. At first we discern polychromatic or polychromatophile blood cells. These erythrocytes contain less haemoglobin, and protoplasmatically they are less differentiated than the rest, i.e. the healthy blood corpuscles. Their margin is also less defined, but they take basic strains easily, and in different degrees, so that with *Giemsa* and methylene blue stains, the colouring of these blood corpuscles fluctuates from the palest to the deepest blue or bluish green.

As to the nature of polychromasia the opinions of authors vary a great deal. *Ehrlich*, *Maragliano*, and *Castellino* consider the blood corpuscles, and especially the older forms, to be in a dying off state, i.e. an anaemia preceding a degeneration which ultimately leads to a coagulation-necrosis of the discoplasma (as indicated by the appearance of basophile portions in the polychromatic erythrocyte). The discoplasma is, according to them, laden with albuminoid substances, and by this becomes enabled to take the chromatine stains. At the same time the discoplasma loses its power to withhold the haemoglobin and passes it on to the blood plasma according to the progressive pathological changes. *Gabrischewsky* and others, on the other hand, hold the polychromatic elements to be young formations, as polychromatophilia is to be found in non-degenerated normoblasts and in karyokinetic forms of division.

Indeed, according to recent research, young corpuscles containing nuclei can show different degrees of polychromasia without any sign of degeneration; in dying elements (*Pappenheim* also supports *Ehrlich's* views) a similar but degenerative process cannot be denied. *Türk* wants to combine the two views in accepting a typical polychromasia to be the product of the joint-operation of two components, on the one hand resulting from an extraordinary basophilia of cytoplasma, on the other hand due to an abnormal lack of haemoglobin, a normal amount of haemoglobin being able to efface the basophilia of the cytoplasma. These two conditions can coincide under very different circumstances. The opinion expressed by *Ehrlich* in regard to a degenerative polychromasia appears correct concerning the changes one observes after anaemias due to loss of blood.

In this case the phenomenon of a rapid degeneration of cells seems to be going hand in hand with a very pronounced polychromasia. Simultaneously the blood cells indicate a loss of haemoglobin. On the other hand the cytoplasma of young undeveloped, i.e. not yet differentiated erythroblasts shows a well developed capability for taking basic stains. And in this case a degeneration does not come into consideration, but the polychromasia or the basophilia seem to be the outcome of an incomplete differentiation process of the actively proliferating blood elements which as yet do not possess any haemoglobin. *Türk* has observed in such blood cells mytosis which showed polychromatic protoplasm to a very great extent. *Pappenheim* also endeavours to combine the two opinions inasmuch as he considers that polychromasia is neither the expression of youth nor degeneration, but may accompany either.

It is different with the phenomenon of basophilia which is observed in conjunction with the polychromatophile alterations. In the beginning they are seen as light, discreet punctuations of the polychromatic erythrocytes, reaching the size of coarse granulations, and growing into lumps. By means of chronic lead poisoning basophile cells can be produced experimentally in the blood of guinea-pigs.

Some authors (*Askanazy*, *Schaumann*, *Lazarus*, *Engel*, and others) considered these granulations to be karyokinetic products. *Plehn* described them as karyochromatophile bodies in the blood of a malaria patient, and holds them to be juvenile forms of agamogonous malaria parasites.

Grawitz understands under basophilia a degeneration of protoplasm which is caused by any toxins in the blood. He considers the appearance of basophile granules to be an early symptom of a degenerative noxis in the blood.

Some authors tried to draw from the difference between the coarse and fine grained granules the conclusion that they are the expression of a juvenile cytoplasm in the latter or that they represent the rest of a nucleus undergoing disintegration or resorption in the former case. So far, however, a basophile granulation has not been observed in the case of blood cells which are undoubtedly perishing.

Pappenheim has succeeded, by means of a special method, in proving the existence of erythroblasts in the bone-marrow. A new pathological blood element appears when basophilia has reached its height, viz., the nucleated *blood corpuscle* or *erythroblast*. This is considered to be the preparatory stage of the red blood corpuscles still having the characteristics of a cell—protoplasm and nucleus. Whereas with lower vertebrates the nucleus even in the adult blood corpuscles persists with higher vertebrates the nucleus is dispensed with in blood corpuscle forming organs, and only such blood corpuscles are supplied which are destined for their special function in the blood stream. Naturally multiplication ceases here. The forerunner of the erythrocyte, that is the nucleus bearing erythroblast, now undertakes this function, and is invisible in normal peripheral blood, but develops and matures in the bone-marrow.

The opinions on the evolution of the so-called white corpuscles vary considerably, and I do not wish to enter into any discussion on that point. The views on the development of erythrocytes are so far clear that normoblasts and megaloblasts (*Ehrlich*) are considered to be the younger forms. *Pappenheim* does not lay much importance on size, but on the richness in chromatine, and accordingly distinguishes two classes, amblychromatic and trachychromatic forms. Where and how an enucleation takes place is unknown. It is not yet decided whether the nucleus leaves the corpuscles—the depression indicating the place of exit (according to *Rindfleisch* the nucleus is capable of surrounding itself with another protoplasma body)—or whether it dissolves and is resorbed. Undoubtedly both processes have been mentioned to occur.

The escaped nuclei are now and again observed in the free blood stream, and owing to their karyorhectic disintegration appear as coarsely or finely granular forms. They are often found in blood smears of cattle suffering from East Coast fever, also in animals affected with other diseases, and it is not to be wondered at if they have been taken for *Koch's* granules, the latter belonging to the life cycle of *Theileria parva*.

The majority of authors are of opinion that the dissolution and resorption of nuclei represent the normal enucleation process of erythroblasts. *Arnold* and *Pappenheim* have succeeded in proving the existence of a body within the erythrocyte which has to be regarded as transformed remains of the nucleus in the cell. They call it " nucleoid "; according to *Arnold* it is surrounded by a delicate paraplasma. These remains of the nucleus can no longer be stained in the unaltered erythrocyte and can later escape into the blood plasma (*Arnold*).

I have observed these so-called " nucleoides ". In the course of experiments into the cause of heartwater, I saw these small corpuscles in erythrocytes of goats, sheep, and calves, which can hardly be anything else than such " nucleoides ". When the haemoglobine is removed from a blood smear by means of diluted acetic acid and subsequent alkalinisation by means of ammonia and then stained with iron haemotoxylin (after *Heidenhain* or *Rosenbusch*) round or polygonal bodies may result which are lodged either on the margin or more towards the centre of the red corpuscles. When this preparation is stained with *Giemsa* delicate protoplasma structures are obtained, directed towards such bodies and which in their totality represent the erythrocyte-stroma. At first I was inclined to connect these corpuscles with heartwater. But when I discovered them in the blood of normal animals I managed to define them as the above described nucleoides.

The normoblasts and megaloblasts do not occur in the normal blood. They represent globular cells of the approximate diameter of a normocyte and possess a sharply defined nucleus very rich in chromatin. This nucleus is generally concentric. The proportion as to the size and length of protoplasma and nucleus fluctuates considerably. The protoplasma margin is not always clearly defined and round, sometimes it is angular and fringed. The normoblasts, as well as megaloblasts and gigantoblasts take *Giemsa* stain very well, the protoplasma appearing to be stained polychromatically and the nucleus to be of a bright purple colour .

The process of anaemia appearing with *anaplasmosis* seems to be the following :—

The erythrocytes, invaded by anaplasms, are kept back by the spleen, where they will eventually be dissolved. By degrees more and more affected blood corpuscles are removed from the blood, and the body has to be replenished from the haematopoetic organs. Since the renovation of the corpuscles does not keep pace with their destruction, immature blood elements are sent into the blood stream and gradually polychromatic cells put in an appearance, that is to say, cells in which the transformation of protoplasma into haemoglobin is still incomplete. Subsequently the basophile cells have to be made use of, and finally even the most elementary erythroblasts replace the destroyed blood cells. The appearance of normoblasts and megaloblasts forms the height of the anaemia. In cases resulting in recovery the olygocytic blood improves visibly, and slowly returns to its normal appearance. Some remissions are noted at intervals of from eight to ten days.

In cases ending with death an increase of normoblasts takes place. An increase of polynuclear leucocytes with eosine or oxyphile granulation is very pronounced, the latter are rarely seen in normal blood.

The parasites diminish in number even considering the relative decrease of the erythrocytes, both in cases ending in recovery and fatally.

Morphological and Biological Investigations into Anaplasma Marginale.

The best universal method of staining for daily investigations, with consideration for blood changes, is obtained with *Giemsa* solutions. The parasites appear as purple-bluish granules of different sizes, which, with a good illumination, show (after lengthy staining) a small black body in the centre or on the margin of the granule. Sometimes marginal anaplasms are observed which seem to cast off a plasmatic portion (plastin). In order to obtain a good picture of the blood and its relation to the appearance and disappearance of anaplasms, *Giemsa* staining cannot be dispensed with. The usual aniline dyes may also be serviceable, as experiments with nearly all stains used in bacteriological technique have proved to be. Generally the blood corpuscles themselves become stained so intensely that a characteristic demonstration of parasites cannot be obtained. This is also the case in preparations where before staining the haemoglobin had been washed out.

Fair results are obtainable with methylene blue or water blue or methyl blue (plasma stains) in a watery solution. Water blue with safranin gives good contrasts. Bad results are obtained with gentian violet and fluorescin, also gentian violet with eosin. Staining with *Delafield's* haematoxilin and following eosine treatment resulted negatively. *Betegh's* trichromin was used also. (Centralblatt fuer Bacteriologie, Vol. 52, page 568.) This method, however, has not any advantage over *Giemsa* staining. The fixation with absolute alcohol or methyl alcohol acetone, although an improvement on the old venerable fixation by means of a flame, does not suffice for a more detailed examination of the structure. The blood corpuscles no longer represent on the slide what they are in the blood, that is to say, well characterized structures, but they appear to be flat discs due to the retraction caused by dessication.

Naturally, the parasites whose structure is chemically and morphologically different from that of the blood corpuscles cannot be represented as they are in reality. Dry fixing on the slides is only a makeshift, although indispensable. For closer examination the living object gives the best idea as to the nature of the parasite; if staining is resorted to, vital staining and "wet fixing" are preferable methods. I had the best results with this last one. *Giemsa* has modified the ordinary fixation method in the following manner:— Cover glasses have to be cleaned well in alcohol, then a thin film of blood is spread on, and, with the smeared side downwards, they are thrown into hot sublimate alcohol (concentrated watery sublimate lotion two parts, alcohol one part). The preparations remain in this solution from two to twenty-four hours. Then they are taken out with small horn forceps, well rinsed, and, in order to remove the sublimate, thrown into a solution of 2 per cent. iodide of potash (100 parts) and *Lugol's* solution (3 parts); rinsed again after ten to fifteen minutes, and in order to remove the iodine put into a watery solution of sodium hyposulphite (0.5 per cent.). The preparations having

become colourless by the solution of iodine (after five to ten minutes) are now carefully rinsed in water and fit for other staining manipulations.

This wet fixing is suitable for *Giemsa* staining, also for treatment with haematoxylin according to *Heidenhain* or *Rosenbusch-Hartmann*, *Borell's* mixture or *Mallory*, etc.

Similar results are obtained by *Hermann's* solution (platinic-chloride-osmic-acid) or *Flemming's* solution. In order to avoid drying or shrinking of blood smears the preparations must be put directly from one dilution into another.

For *Giemsa* staining of the above-mentioned smears, diluted *Giemsa* lotion is used ($\frac{1}{2}$ to 1 drop in 1 ccm. aq. dist.). This liquid has to be changed several times during the first two hours, then the preparations are left in the solution for four to twenty-four hours. Then they are well rinsed and brought through the following:—

Xylol 5 acetone 95
,, 30 ,, 70
,, 70 ,, 30
Xylol pure.

According to the degree of differentiation, the preparations are left for a longer or shorter time in the acetone liquids. The preparations are taken out of the pure xylol and placed at once in oil of cedarwood (not Canada balsam).

This staining gives good results in regard to differentiating the parasites and their structure. A small central body or two bodies in the case of oval forms are distinctly visible, and these bodies are surrounded by a homogonous lighter tinted, coat-like cover (Fig. 6).

Similar results are obtained with *Heidenhain's* haematoxylin stain. In this case the preparations have to be differentiated for a considerable time in iron mordant. The central bodies are seen to advantage if the preparations are put into diluted bordeaux red solution for some hours directly after fixing. The modification, according to *Rosenbusch-Hartmann*, did not answer (iron mordant, then 1 per cent. haematoxylin in 95 per cent. alcohol with conc. lithium carbonate). I have also had negative results with *Borell's* mixture and *Mallory* staining. When *Borell's* stain is used the fixed and washed preparations are put into a concentrated watery solution of magenta red, then rinsed and brought into a mixture of equal parts of concentrated, watery picric acid solution and concentrated watery solution of indigo-carmine (a green mixture). The latter mixture is used for the purpose of differentiation, and as for the time it takes this has to be found out by actual experimenting. With *Mallory* stains the preparations are put for ten to fifteen minutes into a 1 per cent. watery acid fuchsin solution, then for about five to ten minutes in 1 per cent. phospho-molybdic acid, and finally into the *Mallory* mixture:—Equal parts of aniline blue solution (0.5 per cent.), orange "g" (2 per cent.), oxalic acid (2 per cent.) for about ten to twenty minutes, to be rinsed in water.

The small central bodies are on the margin or in the centre of the parasites. In double forms a line of connection between the two central bodies is distinctly visible, showing dumb-bell formations. The central bodies are particularly well discerned if *Loeffler's* cold flagellum mordant is applied (about three to four hours) and followed by aniline fuchsin. The pointed or dumb-bell shaped central bodies

which later appear like diplococci are of a deep black in a lighter zone, while the blood corpuscles take a red tinge (Fig. 7). If fuchsin is taken out of these preparations by means of alcohol and some *Giemsa* solution applied (one drop by means of a glass capillary tube on the margin of the cover glass), the formation of a blue coat round the central or dumb-bell shaped bodies is distinctly noticeable. The latter attains the size and intensity as observed in the case of anaplasma after the usual *Giemsa* dry fixing. The experiments to isolate anaplasms support the above results. Blood corpuscles dissolved in distilled water and centrifugalised contain slightly swollen anaplasms in the stroma, and take the staining in the same way as the well differentiated anaplasms obtained by the *Giemsa* method.

On the living unstained preparations only slight elevations on the erythrocytes are discernable. They are better visible in the dark-ground illumination. Treated with weak saponine or taurocholic sodium solution (1 per cent.), dissolution of erythrocytes as well as the coat of the anaplasm is observed in the dark-ground illumination, and the central bodies appear as small, refractile points. If some Chinese ink is added in transmitted light the outlines of blood corpuscles are seen as faint light discs in the greyish medium; the central bodies are observed in the blood corpuscle shades as strongly refractile points.

Experiments to dissolve the blood corpuscles by means of a haemolytic serum (dog serum and haemolytic serum for cattle blood obtained from rabbits) and to isolate the anaplasms were not successful. Vital staining with neutral red did not succeed. However, if a weak methylene blue solution was added, anaplasms put in an appearance. If a small quantity of saponine or quinine solution is brought in contact under the cover glass with some blood containing anaplasma, a light zone is formed on the margin of anaplasms, while the parasites appear swollen. Concentrated salt solution causes a considerable swelling of the plastine substance. If a quinine solution is added (under the cover glass) to some blood containing anaplasms, the plastine substance will expand. If later a thin solution of neutral red is added, a slight red stain is produced, which lodges on the outer margin of the anaplasm.

To classify the causal factor, called *Anaplasma* by *Theiler*, in the zoological system is impossible at present. However, there is no doubt that these forms are of a parasitic nature. We do not know of any pathological change of blood in the course of which bodies appear which are similar to anaplasms. *Theiler* himself considers the anaplasm to be a phylogenetically retrogressive organism which, under the reduction of special organella, has gradually adapted itself as an endogenous parasite to its host cell, and throws off the function of its original protoplasm on to the host cell in the same degree as it loses its own protoplasm. Undoubtedly there are numerous analogies in parasitology.

Based on the results of my morphological experiments, I consider I am justified in comparing them with formations which *Prowazek*, under the name of "chlamydozoa", places in one group. These are micro-organisms, which in regard to their parasitism adapt themselves very easily, and whose attacks on the host cell result in formations of peculiar reaction products. By means of nuclear staining their existence is generally proved.

We have met these causal factors in connection with variola, vaccinia, hydrophobia, fowl plague, scarlatina, and a host of other diseases of a so-called invisible origin. These bodies are known as *Guarniery's* bodies, after their discoverer, in the case of variola and vaccinia, *Negri's* bodies with hydrophobia, *Mallory's* cyclasterium with scarlatina, etc. *Lenz* has proved the existence of intracellular bodies of the nervous distemper in dogs, and *Siegel* the existence of intracellular bodies (cytorrhyctes) in foot and mouth disease. Recently *Prowazek* has described the causal factor of trachoma, and considers it to be a chlamydozoon. (*).

Borell and *Liebschuetz* discovered a chlamydozoon in *Molluscum contagiosum*, *Loewenthal* found the same causal factors with carp pox (Karpfenpocke), *Keysselitz* with barbel pox (Barbenpocke), *Keysselitz* and *Meyer* with chicken pox, *Burnett* and *Loewenthal* with epithelioma of birds.

In most of the above-mentioned diseases the virus is in the first instance localized in the host cell, the reaction results in the formation of special morphologically differentiated cell substances. These reaction products (*Prowazek* calls them plastine bodies) are defensive reactions of the invaded cells, and do not belong to the parasite proper, however they seem to take the place of plasma which surrounds the nucleus of protozoa. The small central bodies (according to *Prowazek* they are considered to be the cause—initial bodies) have morphologically few typical characteristics.

Cocci, diplococci, or dumb-bell formations are alone recognizable. The latter are not unlike karyokinetic figures of flagellates, and it does not seem improbable that we have to deal with parasitic protists which manifest themselves in the nuclear forms devoid of plasma.

If the above-mentioned chlamydozoa are localized generally in cells of the primary ectoderm, as is the case in trachoma, scarlatina, vaccinia, molluscum contagiosum, epithelioma of birds attacking the epithelium, hydrophobia, fowl plague and distemper, influencing the brain, anaplasma would be a parasite which damages and invades the cells of mesenchymatic origin. The confirmation of this supposition would open a far-reaching perspective on numerous ultravisible diseases of the blood (horse-sickness, heartwater, equine influenza, and typhoid fever, etc.). Only the results of experiments will prove eventually if, on the one hand, certain filtrable causal factors of infection (so far unknown) are able to produce such cell reaction (for instance in horse-sickness), and, on the other hand, if there are free, perhaps filtrable, forms in serum (for instance with anaplasma) which might cause the appearance of bodies of the anaplasma type. However, these experiments are being carried on at present, and reports on them will follow later.

Explanation of Plates.

Appearance of the blood of calf 936.

Fig. 1.—19th day. Erythrocytes mostly of normal size, one mononuclear leucocyte. Blood platelets. Anaplasms, one or two in a blood corpuscle cell. On the left a projecting plasma body.

(Zeiss Comp. ocular 4, Homog. Imm. 2 mm., n. apert. 1, 30.)

(*) In a recent report by Herzog (*Deutsche Med. Wochenschr*, 1910, *No.* 23) an involution state of cocci is conjectured which results in dumb-bell shapes. It has to be seen if he is able to furnish proofs concerning the bacterial nature of these bodies in his forthcoming monography.

Fig. 2.—22nd day. Anisocytosis. Strong infection; blood platelets increased; so-called "tailed" forms in centre. One polynuclear leucocyte; free parasites.
(Zeiss Comp. ocular 4, Homog. Imm. 2 mm., n. apert. 1, 30.)
Fig. 3.—24th day. Anisocytosis, polychromasia, basophile erythrocyte, strong infection.
(Zeiss Comp. ocular 4, Homog. Imm. 2 mm., n. apert. 1, 30.)
Fig. 4.—26th day. Anisocytosis increased, crenated forms, oligocythaemia, blood platelets showing "tailed" forms, strong basophilia.
(Zeiss Comp. ocular 4, Homog. Imm. 2 mm., n. apert. 1, 30.)
Fig. 5.—29th day. Anisocytosis, poikilocytosis; few normal blood corpuscles; megaloblasts; normoblasts; polychromasia; basophilia; a polynuclear leucocyte with oxyphile granulations.
(Zeiss Comp. ocular 4, Homog. Imm. 2 mm., n. apert. 1, 30.)
Fig. 6.—22nd day. *Giemsa* moist preparation; anaplasms with distinct central bodies (initial bodies).
(Zeiss Comp. ocular 4, Homog. Imm. 1, 5, n. apert. 1, 30.)
Fig. 7.—Dry fixation after *Loeffler's* flagellum mordant by means of aniline fuchsin; central bodies visible without envelope.
(Zeiss Comp. ocular 4, Homog. Imm. 2 mm., n. apert. 1, 30.)
Fig. 8.—Section of heart muscle showing anaplasms (*Giemsa* stain).
(Zeiss Comp. ocular 4, Homog. Imm. 2 mm. n. apert. 1, 30.)