A Study of the Reticulo-endothelial System of the Sheep.

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The author was afforded an opportunity, while studying the pathology of Anaplasmosis and Splenectomy in sheep, to consider two other conditions associated with abnormal pigmentation, viz., Enzootic Icterus and Bacterial Icterus of sheep. In all these conditions the reticulo-endothelial system was more or less affected, and this yielded material for a more comprehensive study of this system.

It is proposed to treat the subject matter as follows.—

(*a*) A brief general survey of the literature, dealing only with the important points raised in connection with the reticulo-endothelial system.
(*b*) Technique used in the study.
(*c*) A consideration of the three conditions named, viz.:—
  (1) Anaplasmosis and Splenectomy.
  (2) Enzootic Icterus.
  (3) Bacterial Icterus.
(*d*) General discussion and conclusions.
(*e*) References.
(*f*) Appendices I, II and III.
(*g*) Plates, Nos. 1 to 6.

(a) *The Literature.*

Seeing that the literature in connection with the study of the Reticulo-endothelial apparatus has from time to time been fully reviewed by many authors, it is proposed in this paper only to mention the more important points which have a bearing on the present study.

Aschoff (2) gives a very excellent review of the progress made in connection with the study of the Reticulo-endothelial apparatus. He commences with the work of Metschnikoff, "System des Makrophagen," deals with the findings of Ribbert in connection with vital staining, and mentions that Ribbert showed that these cells not only hoarded carmine granules, but also iron and fat. With reference to this "hoarding property" of pigment granules, Aschoff recognises the following important groups: (*a*) *endothelials* of blood and lymph vessels which hoard in case of far-reaching vital staining; (*b*) *fibrocytes*: also hoard fine granules; (*c*) *reticulum cells* of the spleen pulpa and of the cortical nodules and medullary strands of lymph glands hoard relatively easily, more so than fibrocytes, but remains behind the next group; (*d*) *reticulo-endothelials* of the lymph sinuses, of the blood sinuses of the spleen, of the capillaries of the liver lobules and of the capillaries of bone-marrow, adrenals and hypophyses; (*e*) *histiocytes* i.e. wandering cells of connective tissue in distinction to the fibrocytes; (*f*) *splenocytes* and *monocytes* derived from histiocytes and reticulo-endothelials, and found in the blood stream.

Kiyono (3) is of opinion that the distribution and arrangement of this system varies in the different animals. In the case of many (rat, mouse), the R.E. system of the liver reacts prominently on splenectomy, whereas in others (rabbit, guinea-pig) the reaction is only slight, [Schmidt (4), Lepehne (5)], and in the case of the dog and the mouse there is, after splenectomy, active phagocytosis of erythrocytes in the R.E. system of the mesenteric and retropharyngeal lymph glands.

Benjamin Sacks (6) in his review states that there is still much dispute concerning the relationship of the endothelial and reticular cells of the spleen and lymph nodes, and the exact status of the Kupffer cells of the liver. In a more recent publication Aschoff stated that the reticulum and endothelials of the lymph-nodes are not quite identical, since they react differently towards particulate material.

Kiyono, with reference to the question of the nature of monocytes, maintains that he can distinguish three types of Mononuclears of the blood:—

(*a*) blood histiocytes ("histiogene monozyten"), e.g. from stern cells; (*b*) those derived from myelogenous tissue, i.e. transitionals of Ehrlich; and (*c*) mononuclears of lymphatic origin.

Aschoff comes to the conclusion that further experiments are needed to ascertain whether there are two mononuclear cell forms (lymphocytes, histiocytes) or three (lymphocytes, histiocytes, myelocytes). According to Sabin and Doan (28) there is a practically constant desquamation of endothelials into the circulating blood in rabbits and man. These desquamated cells can be discriminated from monocytes.

Warthin (9) in his observations on splenectomy in sheep and goats confines himself to the lymphoid tissues, and makes very little reference to other organs. After total splenectomy in the sheep there is no evidence of regeneration of the primitive spleen, or of the new formation of splenic tissue. The structural alterations following splenectomy are: hyperplasia of existing lymphoid tissues, transformation of haemolymph nodes into ordinary lymphatic glands, and a new formation of haemolymph nodes out of the lobules of the fat tissue, and a later proliferation of bone marrow. The presence of great numbers of eosinophiles in the glands, showing great destruction of red cells, seems to point to some relationship between these cells and haemolysis.

Lauda (8) maintains that splenectomy in case of man and animal is tolerated without damage. Rats stand the operation well, but it is followed by quick emaciation, anaemia, not seldom haematuria, haemoglobinaemia, with early death. A study of the blood reveals acute anaemia and erythrophagocytosis. Most acute lesions are found in the liver, especially in connection with the R.E. system, i.e. the stern cells. There is swelling of the stern cells, erythrophagocytosis and desquamation. In the stellate cells one can either recognise erythrocytes, or haemoglobin drops, or haemosiderin. Vacuoles are also present. The changes in the liver cells are of the nature of a small fat droplet degeneration. In the tubuli of the kidney, haemoglobin cylinders are present, less frequently cylinders of erythrocytes. With reference to the lungs it would appear that erythrophagocytosis takes place in the endothelials of capillaries, and in the fixed cells of the alveolar septa. It is difficult to say whether they have dropped in from the circulation, or whether they have emanated in the lungs.

The sinus-endothelials of lymph nodes show erythrophagocytosis or haemoglobin drops, or haemosiderin. Lauda comes to the conclusion in his paper that the lesions following splenectomy in rats are due to a virus, which only becomes active after removal of the spleen. This is analogous with the observations of De Kock and Quinlan (1) in connection with splenectomy of sheep as related to Anaplasmosis. Rich (11) maintains that direct observations, as well as microchemical reactions for iron, leave no doubt that haemoglobin of the engulfed erythrocytes is broken down within these cells into simpler substances.

Cells of the reticulo-endothelial system can be traced to the lung arteries, and even to the lung veins, rapidly disorganising in the left heart, because the peripheral arterial blood contains a few of these elements. According to Aschoff (7) some of the material hoarded in the R.E. cells becomes free in the lungs. Georg Seemann (22) holds that the hoarding of colloidal substances in the lung tissues depends on the port of entry, and the colloidal state of the substance. Finely dispersed colloidal substance (e.g. Trypan Blue) is hoarded by histiocytes, and alveolar epithelium, whereas coarsely dispersed colloidal substances are not hoarded but mechanically retained. Foot (24) in his recent article on the origin of the pulmonary " dust cell " summarises the question as follows: " The wealth of evidence adduced from the literature and the experiments described by him go far towards proving that the most likely origin of the pulmonary dust cell is from the blood stream and more especially, the monocytes thereof. That various reticulo-endothelial elements share to a degree in the production of dust cells cannot be denied; that they play the chief part in this production, however, seems improbable. That the vascular endothelium gives rise to dust cells under ordinary conditions seems entirely unlikely . . . ."

With reference to the breaking up of erythrocytes under pathological conditions, Lepehne (10) recognises two processes:—(*a*) *erythrolysis*: with haemoglobinaemia; (*b*) *erythrorhexis*: breaking up of erythrocytes into smaller particles. In Weils disease it is of the nature of erythrorhexis. If there was an haemoglobinaemia, changes in the kidney would have been noted. In erythrorhexis it would appear that the breaking up of phagocyted erythrocytes takes place inside the cells of the reticulo-endothelial apparatus, and according to Lepehne in Weils disease there exists a direct icterogenous function in the reticulo-endothelial apparatus. The bile pigment comes into the blood, and when it reaches a certain level, gives rise to skin icterus and bilirubinaemia. Heitzmann maintains that in experimental yellow-fever, Weils disease, Sumatra-infection, there is an extensive intravascular destruction of erythrocytes. Moreover, in the majority of cases small miliary and submiliary necrotic foci in the liver could be identified.

Rich (11) maintains that for the first time it has been proven beyond a doubt and without a source of error that bile pigment may continue to be formed even to the point of jaundice in an animal from which the liver has been removed. The view that the reticulo-endothelial cells are the site of origin of bile pigment is at present represented, especially by Aschoff, Lepehne and Eppinger. According to Mann and Magath (12) the functional activity of the liver with reference to bile pigment is mainly excretory.

Rich holds that haemoglobin spilled into the tissues almost anywhere in the body may be transformed into bile pigment, and the presence of excess of haemoglobin in the circulation, whether introduced in pure form experimentally, or liberated during haemolysis in pathologic conditions, is regularly followed by an increased formation of bile pigment. Shimura (25) holds that when a large quantity of haemoglobin is injected intravenously into dogs or rabbits then this haemoglobin is excreted by the liver and kidneys unchanged. This occurs through the convoluted tubules. The glomerulus plays no part in this excretion. The rest of the haemoglobin is taken up by the spleen, bone marrow, lymph glands and certain other organs, and transformed into haemosiderin, some of which lands in the circulation, and becomes excreted by the epithelium of the urinary canals. In the lungs there is little iron pigment.

In connection with the presence of lipoids in various cellular elements Warren and Root, (13), in the average spleen removed at necropsy, found little evidence of lipoids. Poschanisky demonstrated fat to be present in spleens associated with many different diseases. Kusunoki records the frequent occurrence of lipoid containing cells in the spleen, and showed that most of the lipoid was contained in cells of the reticulum. As regards the lipoid content of eosinophiles Neumann (26) is of the opinion that the lipoid body is attached to the granules of eosinophiles in a special way. Many authors according to Sacks, have confused the general lipoid infiltrations of the reticulo-endothelial system with *Gaucher's disease,* in which case the reticulo-endothelial cells of the liver, spleen, bone marrow and certain lymph nodes may be involved. Mandlebaum and Downey (27) have shown that Gaucher cells do not contain any fats or lipoids which are demonstrable by the usual microchemical methods. The careful histological studies of these authors led them to the conclusion that the Gaucher cells in the spleen are derived from the reticulum cells. In the liver Gaucher cells arise in the histiocytes of Glisson's capsule, and in the adventitial and periadventitial connective tissue cells of the central veins of the lobules, whereas in the bone marrow and lymph nodes they arise in the reticular cells. The interlobular tissue of the liver in a case examined by Mandlebaum and Downey contain the same long strands and syncytial masses of Gaucher's cells described in the lymph nodes. These elongated cells and strands are particularly numerous just beyond the wall of larger veins. . . . The stellate cells always appear normal even in those cases where there are numerous Gaucher cells. There is *no* evidence for the assumption that Gaucher cells are derived from stellate cells. Epstein and Lich's investigations led them to the conclusion that the chief substance in these cells is a complex substance of the nature of a cerebrocide, and that phosphatids are also present. According to Schittenhelm (14) the more important features of Gaucher's disease are: marked tumour splenis; marked enlargement of the liver and anaemia; the disease seems to run in families; peculiar pigmentation of the skin, viz.: yellow brown to ochre brown; the presence of the characteristic "Gaucher" cells in definite organs, namely, spleen, liver, lymph glands, and bone marrow. Gaucher's disease is of the nature of a system disease of the reticular apparatus, without the apparent implication of the endothelials. It is characterized by large cells which become filled with a peculiar substance, about the nature of which there is no uniform opinion at the present. Good descriptions of these Gaucher cells appear in the paper of Epstein (15). With haemalum-eosin the cytoplasm stains homogeneous bright greyish blue of different intensity, in other cases it is of a reddish soft tone. They are large clear cells, with a " foam-like " cytoplasm, and a small round nucleus situated in the centre or along the periphery etc. The lesions in the liver are characterized by enlargement of the interlobular connective tissue and in the spaces groups of Gaucher cells can be seen, probably derived from the pre-existing clasmatocytes, etc., etc. The disease has a chronic course, and death is usually associated with some intercurrent disease.

(*b*) *Technique*.—The material collected at post-mortem was either taken from animals that had died in the various experiments, or were killed for the purpose of obtaining specimens in various stages of the disease. The haematology of the majority of these sheep was studied prior to death, and in many instances, especially in the anaplasmosis and splenectomy experiments, the blood picture was also obtained prior to infection or splenectomy. In some instances animals were chloroformed, and a study made of the cellular elements collected at various situations, e.g. from the jugular, vena portae, vena cava caudalis, aorta. Such animals usually succumbed from the effects of anaesthesia, and internal haemorrhage.

A number of the usual fixatives were tried, and it was found that the best results were obtained with material fixed in 10 per cent. formalin, and cut by the freezing method. Numerous stains were used to study the nature of the cells, pigment, etc., etc., e.g., haemalum-eosin (Böhmers), haemalum-Sudan III, Van Gieson, Berlin Blue, Turnbull's Blue, Giemsa, etc. In connection with some of these the following remarks may be made:—Haemalum-Sudan III method at first gave very unsatisfactory results as regards the capability of the Sudan III to stain the neutral fats and lipoids. The colour and intensity of the stain varied from one group of sections to another. Various brands of Sudan III were tried, and it was found that the stain from Grüblers, Leipzig, gave the most uniform results. With Grüblers, fats stained an intense brick red colour, haemosiderin reddish yellow-brown and bile pigment greenish yellow. As far as possible controls were included in each group of sections prepared.

The greatest difficulty was experienced in connection with the staining of haemosiderin. It was found that material should be as fresh as possible in order to obtain the best results. Turnbull's method was not so satisfactory as the Berlin blue modification of Abbott (17). The staining reaction gradually became less intense as the material became older. The majority of specimens collected a year ago failed to give a reaction. A few months in some instances already made a difference. The best results were obtained with sections cut by the freezing method.

In connection with Giemsa numerous methods were tried, and the following modification with sections by the freezing method from material fixed in 10 per cent. formalin, gave very satisfactory results. These sections were stained according to the Pappenheim modification of the Giemsa method, viz., stain with Giemsa (2 drops to 1 c.c. distilled water) for one hour, wash in tap water, differentiate in absolute alcohol containing a trace of acetic acid (30 c.c. absolute alcohol plus 5-8 drops of 1 per cent. acetic acid), until the sections assume a pinkish blue colour. The sections were cleared in bergamot oil. This agent was found to give more satisfactory results than xylol.

In the majority of cases organ smears were prepared at the time when the material was collected for sections. Such smears proved to be of great assistance in determining the nature of some of the cells, which were not clear from the section examined. Sufficient cells were found on the glass slide to identify them, and study them as regards their occurrence in the different organs. Two methods of preparing such organ smears were tried. The first method was found to be not so successful as the second. In the former the organ on section was freed from blood, as far as possible by scraping with the knife. The surface was then scraped with a glass slide and the contents on this slide were smeared on a second slide, which was then used for the examination. By this method cells were frequently destroyed in the preparation. In the second method a glass slide was quickly drawn across the cut surface of the organ which was freed from blood.

These smears were stained according to Pappenheim's modification of Giemsa's method.

(1) *The Pathology of Anaplasmosis and Splenectomy in Sheep.*

In a paper on splenectomy in domesticated animals and its sequelae, De Kock and Quinlan (1) described the occurrence of a hitherto unknown disease in sheep in South Africa, i.e. Anaplasmosis, folowing splenectomy. It was subsequently shown that a certain percentage of South African sheep kept locally became "carriers" of Anaplasma. The course of the disease in such sheep, was, however, of such a mild nature that it was never identified. When sheep introduced from some other centres in the Union were infected with the blood of such carriers, mild reactions of Anaplasmosis were set up in the susceptible sheep. On the other hand grave symptoms of Anaplasmosis, which proved fatal in some instances, followed the splenectomy of carriers of Anaplasma. The reactions of Anaplasmosis in the majority of the splenectomized sheep, which did not succumb, were protracted over long periods (in cases of sheep 8427, 8428, 8429 for more than 2½ years, the latter two are still reacting), with remissions from time to time. The blood changes in the splenectomized infected sheep were associated with a marked oligocythaemia, and the appearance in the blood of erythroblasts, normoblasts and Jolly bodies. With reference to leucocytes first a neutrophilia was observed (probably associated with the operation of splenectomy), this was followed by a monocytosis accompanied by an erythrophagocytosis).

It is proposed to consider the pathology of this section under the following heads:—

(x) *Anaplasmosis in non-splenectomized sheep.*
(y) *Healthy sheep—splenectomized.*
(z) *Anaplasmosis in splenectomized sheep.*

(x) *Anaplasmosis in Non-splenectomized Sheep.*

As was shown in the paper of De Kock and Quinlan, susceptible sheep infected with Anaplasma, showed very little impairment.

Such a disease as regards its course and symptoms resembles an extremely mild form of bovine Anaplasmosis. At post-mortem of sheep killed, since no cases proved fatal, the only fairly characteristic lesion was a general anaemia, evidenced by the pallor of the mucous membranes. There was slight enlargement of some of the lymph glands.

Microscopically.—No lesions were detected. In one case (Sheep No. 8463) there seems to have been a complication of indefinite necrotic foci in the liver. This case also showed slight fatty changes in the liver cells. No abnormal pigmentation was observed.

In some of the lymph glands there was a number of eosinophiles, but such cells in fair numbers were also observed in the control sheep. It may be stated here that the granules of these eosinophiles stain an intense brick red colour with Sudan III, yet these granules are not soluble in alcohol, seeing that they stain intensely with eosin in the haemalum-eosin method. A comparative study was made of a large number of lymphatic glands. The glands examined were: retropharyngeal, mediastinal, periportal, renal, iliac, mesenteric, prescapular, and some haemolymph glands as well. It was found that the picture, especially as regards the presence of pigment, varied markedly, and that in the study of the various cases no uniformity occurred as regards any particular gland. In these cases it was noted that in some of the glands, rounded-off sinus cells were detected which contained a yellow-brown granular pigment. When examining the glands of control sheep it was found that some of the glands showed the presence of pigment in the sinus cells, and mainly those associated with the lymphoid strands of the medulla. Different stains were employed to study the nature of the pigment. With haemalum-eosin it had a yellow brown appearance, with Sudan III a yellow brown (golden), and with Berlin blue greenish yellow brown. In some cells the pigment granules were few and varied in size, whereas in others they were more frequent. Their presence became more prominent, sometimes with the one stain, at other times when one of the other stains was used. It became evident that some of the sinus cells, besides the above pigment, also contained fatty substances and this was especially manifested in case of the Sudan III stain. These droplets were found lying in between the pigment granules described above. With this stain they stand out as brick red droplets, and in some cases more of these droplets were detected than pigment granules. The character and arrangement of these sinus cells with regard to the pigment is very well depicted in plates III and IV. In the control cases, as well as in the Anaplasmosis sheep, there was *no* evidence of haemosiderin in the lymph glands examined. In none of the organs were any specific lesions detected.

(y) *Healthy Sheep—Splenectomized.*

De Kock and Quinlan (1) maintain that the operation of splenectomy in susceptible equines, bovines, goats and ovines can be carried out practically without any mortality. A temporary polyglobuly and neutrophilia were seen in some of the 22 cases of sheep splenectomized, some of which were subsequently used for Anaplasmosis experiments. Four sheep (viz., Nos. 8431, 8455, 8458, 10946) died as a result of the operation. In one case death occurred 18 hours afterwards, whereas one survived as long as seven days. It may be stated that in two of these cases (sheep Nos. 8455, 8458) splenectomy was carried out immediately after the animals had recovered from a severe form of "drug" anaemia, and the blood picture had more or less returned to normal. As a result of this these sheep may have acquired a lowered resistance to chloroform anaesthesia. Besides these four sheep referred to, sheep No. 9414, which showed no reaction after splenectomy was killed seven days after the operation for the collection of material for microscopic examination.

At post-mortem these cases revealed no specific changes. The following alterations were noted:—Healing wounds in the left iliac region, absence of the spleen, evidence of fibroblastic peritonitis associated with the seat of operation, in some cases degenerative changes in the liver and myocardium, slight hyperaemia in some of the organs, e.g., liver, kidney. In one case (sheep No. 8431) there was a broncho-pneumonia.

No marked abnormality was noted in the haemal lymph glands as described by Warthin. The haemal lymph glands in some cases were undoubtedly conspicuous and appeared more numerous, but a similar state of affairs has frequently been observed in the post-mortem of sheep, which had died of diseases (heart-water, etc.), in which the blood-forming organs were in no way primarily affected.

Furthermore the seat of operation was not in a single case associated with any purulent inflammatory process. Death in these cases was evidently due to shock as a result of the operation, or was connected with the anaesthesia.

Microscopical Changes.—These cases afforded sufficient material for observing the effect of splenectomy on the blood-forming organs. Splenectomy in sheep caused no characteristic lesions in any of the organs. Practically all the organs were at one time or other examined. Some of the cases showed fatty changes in the liver, myocardium, and kidneys, but in none of these organs were they extensive. As regards the circulation, venous hyperaemia was observed in a few cases in the liver and kidneys. In a few instances necrobiosis was observed in the liver and kidneys. In the epithelium of the tubules (mainly convoluted) haemosiderin granules were observed in two cases (sheep Nos. 8455, 8458). This was only present to a slight degree, and it is possible that this was associated with the oligocythaemia set up by drugs from which these sheep had almost recovered. In the lymphatic glands a yellow brown pigment was noted in the sinus cells. In one case (sheep No. 8458) some of this pigment in the sinus cells was shown to be haemosiderin.

(z) *Anaplasmosis in Splenectomized Sheep.*

This was studied in two groups of sheep, viz.:—

(i) *Carriers of Anaplasma splenectomized* (sheep Nos. 7369, 7443, 10656 and 13853 were natural cases, whereas sheep Nos. 8427 and 8434 were previously infected with Anaplasma).

(ii) *Healthy sheep splenectomized*, and then infected with Anaplasma, viz., sheep Nos. 8457, 8430 and 8456.

In both groups acute symptoms of Anaplasmosis were observed, which in one instance proved fatal. In some cases the disease was protracted, especially as regards blood changes, over long periods (1924-1927), with remissions from time to time (sheep No. 8429). These remissions were sometimes only detected by an examination of the blood, which revealed an oligocythaemia and the presence of Anaplasma. Some sheep recovered, but it took a considerable period before they reached their original erythrocyte count.

The blood changes in the splenectomized infected sheep were associated with a marked oligocythaemia, the appearance of erythroblasts, normoblasts and Jolly bodies in the blood, and the presence of Anaplasma. With reference to the leucocytes, first a neutrophilia was noted, then a monocytosis with erythrophagocytosis, and subsequently a lymphocytosis developed. In some instances an eosinophilia occurred in convalescent cases. It would appear that the neutrophilia was associated with the operation of splenectomy, while the monocytosis was related to the removal of degenerated and damaged erythrocytes, etc.

Anaplasmosis in splenectomized sheep was associated with fairly characteristic lesions. As was shown above, a healthy sheep, not a carrier of disease, can easily adapt itself after splenectomy, without showing alterations in any of the organs. The reticulo-endothelial apparatus of the sheep is, therefore, so constructed that it is capable of dealing with the degenerated and damaged erythrocytes so completely that it leaves no trace of the extra function thrown on to this system as the result of the splenectomy. Although the spleen in sheep does not normally show haemosiderosis to the extent observed in other species of animals, yet there is sufficient activity of erythrophagocytosis to show that the spleen plays an important role in the breaking up of erythrocytes [c.f. Ziegler and Wolf (16)]. It may be stated that in some of the sheep referred to in this study the presence of haemosiderin in the spleen was decidedly limited. It is at present not definitely known what exactly controls the formation of haemosiderin in the spleen.

The most characteristic lesions were, however, observed in the liver. This was characterized by extensive desquamation of the stern cells with erythrophagocytosis. Phagocyted erythrocytes were present in all stages, from recently ingested red cells to mere pigment granules. The character and nature of these stern cells can be well studied from the organ smears of the liver (see later). As soon as the erythrocytes are phagocyted their haemoglobin becomes transformed into haemosiderin, and with the Berlin blue reaction appear out as blue blotches or masses corresponding to the state of degeneration of the phagocyted erythrocyte.

With haemalum-eosin the phagocyted erythrocytes could be identified as discs in the cytoplasm of the cell, but varying in staining intensity from the normal red cells of the blood. The colour was usually a reddish brown. With Sudan III the cytoplasm of a number of stern cells revealed besides the remains of erythrocytes, the presence of fat droplets of various sizes.

These desquamated cell accumulations vary in the different parts of the lobule, and in the different cases examined. They are more frequent towards the central vein, and in respect of this they cause atrophy of the liver cells. A number of these cells usually lie free in the central veins.

These cells may to a certain extent be accompanied by other leucocytes and round cells, but such cells are, as a rule, not numerous.

Besides atrophy of liver cells, fatty changes were also present. In some of the cases the latter was rather prominent affecting the greater portion of the liver lobule. The droplets present varied from small ones to those practically filling the whole liver cell. They were, however, usually medium sized.

In connection with the periphery of the lobule changes might be referred to which were not only detected in sheep in these experiments, but were also observed in clinically healthy ones, viz., Glisson's capsule in places was widened and stood out more prominently. This was due to the presence of cellular elements which had accumulated there. These cells were of the nature of round cells, amongst which lymphocytes could be detected. There was also an increase in the fibrillae of the connective tissue. These round cells were lying free in the reticulum. It might be pointed out that proliferation of bile ducts was not observed in these instances. The nature of these changes in Glisson's capsule of sheep has not yet been explained. It is not certain whether it is a normal occurrence, or whether it is associated with some pathological process. It will be necessary to make a careful study of Glisson's capsule in various types of clinically healthy sheep, including sheep artificially reared.

The lungs showed well marked evidences of pigmentation associated with the blood vessels in the alveolar walls. This pigment happened to be haemosiderin and showed the same peculiarities with the various stains, referred to in case of the liver. With the Berlin-blue-method these cells with pigment stood out as blue blotches in the capillaries, and on account of their numbers formed a pretty bluish network (see plate VIII). In some instances this pigment was present in such large amounts that the periphery of practically every alveolus was marked off as a blue line of cells. In the organ smears these cells resemble stern cells very closely, but it was not clear whether they originate from:—

(*a*) the vascular endothelium or the histiocytes of the lung; or

(*b*) *the reticulo-endothelial system outside the lung*, i.e., that the cells observed in the lungs are the desquamated endothelials which have drifted to the lung with the blood from other centres. If this is so then the majority of these cells have reached the lung via the vena cava caudalis and the heart. Others may have reached the lung from the cranial aspect of the body via the jugularis. That the latter actually occurred was shown by the presence of these cells in jugular blood. It is possible that the cells observed in the jugular blood previously escaped from the lung, and so landed in the systemic circulation.

In comparing the internal blood picture in a few cases, it was found that the monocytic count as such varied to a very slight extent in the various positions examined, viz., jugular, vena cava caudalis, vena portae and aorta.

From the evidence in the above cases it would appear that in the case of extensive erythrophagocytosis numbers of monocytes are encountered in various parts of the body. The actual position where R.E. cells are broken down is not clear, at any rate the lung cannot be regarded as such a centre, seeing that the monocytic count before (vena cava caudalis), and after (aorta), varied only to a small degree. In these desquamated endothelials a certain amount of katabolism of erythrocytes takes place. It is believed that these cells after desquamation carry on the breaking down of erythrocytes entangled in their cytoplasm. It is not clear whether these cells extrude the broken down remains of erythrocytes, i.e. the pigment into the circulation and pass on to form the monocytes of the blood, or whether this pigment only becomes freed when the cell disintegrates somewhere in the body.

It could not be definitely decided whether the lung is associated with an erythrophagocytosis, i.e. whether the vascular endothelium or the histiocytes of the lung function in the same way as the stern cells of the liver. In the various sections and the organ smears examined the impression given is that these desquamated cells encountered are not derived from the lung tissues, but that these cells have drifted into the lung from elsewhere. In studying the smears from the liver, lungs, glands, etc., it will be seen that those cells showing erythrophagocytosis are all more or less identical (see plate II). The distribution of these cells in the lungs is uniform and always seems to stand in direct proportion to the amount of erythrophagocytosis, observed in the liver.

It was, however, felt that a biochemical study of the blood and the tissues should be undertaken at the same time as a morphological survey, and that the pigment values should be determined in different parts of the body. In this way it may be possible to solve some of the problems referred to above.

In the lymphatic glands no uniform picture was obtained in the different cases studied. A large number of glands examined showed the presence of erythrophagocytosis associated with the sinus cells. With the Berlin blue stain these phagocyted red cells and their remains manifested themselves as bluish blotches, i.e. similar to those cells observed in the liver and lungs. In several instances sinus cells showed the presence of two types of pigment besides a few small fat droplets. The pigments were (i) haemosiderin, and (ii) a yellow brown granular pigment similar to that described in the cases above. In a few of the lymphatic glands eosinophiles were observed in large numbers. (c.f. Warthin.) Their granules with Sudan III stained an intense brick red colour.

In addition to the endothelials and monocytes, great difficulty was experienced in definitely classifying a type of mononuclear, which revealed certain lymphocytic characters especially as regards the structure of their nuclei. These cells were seen in the various smears prepared from the blood and organs. The type of cell implicated resembled a large lymphocyte, except that the nucleus was more leptochromatic in character, and the cytoplasm was more of a light iron grey than a blue. These cells frequently showed the presence of large chromatin stained masses, often triangular in shape. (See plate I, and also plates in the paper of De Kock and Quinlan). There were also cells resembling lymphocytes which showed erythrophagocytosis.

Besides the peculiar cells referred to above, others were encountered in the organ smears which gave rise to difficulty in classifying, e.g.,

(*a*) a spherical cell, larger than a large lymphocyte, with an intensely blue stained cytoplasm, and a large intensely stained spherical nucleus; its nucleus in some cells resembled that of an endothelial (see plates I and III), and in others it was like the nucleus of a lymphocyte.

(*b*) a small spherical cell about the size of a medium-sized lymphocyte, with the cytoplasm and nucleus of a lymphocyte, except that these were more intensely stained, and the nucleus eccentrically placed. The cytoplasm near the nucleus usually revealed a well circumscribed lighter rose-pink area. These (*b*) cells resemble the plasma cells depicted by Naegeli (30) and others. (See plate I.)

SUMMARY.

X. (i) The only alteration of any importance in anaplasmosis of sheep was a slight oligocythaemia, evidenced by the pallor of the visible mucous membranes. Signs of anaemia besides the presence of anaplasma were detected in the study of the blood.

(ii) Variations occurred as regards the presence of pigments in the sinus cells of the medullary strands of the lymphatic glands. This, however, was observed in anaplasmosis as well as in clinically healthy sheep.

(iii) The pigment in these sinus cells showed the following peculiarities: with haemalum-eosin it stained a light reddish brown; with Sudan III-haemalum yellow brown; with the Berlin blue method light brown with a slight tinge of green.

(iv) Some of the sinus cells, besides this pigment referred to, showed the presence of fatty substances which with the Sudan III-haemalum stain showed up as brick red droplets of different sizes.

(v) In anaplasmosis there was no evidence of haemosiderin in the lymphatic glands.

Y. (i) No specific pathological-anatomical changes were observed in clinically healthy splenectomised sheep, which died or were killed for the collection of material.

(ii) Microscopically no specific lesions were detected. Pigment changes noted in clinically healthy sheep were also seen in lymphatic glands of splenectomised sheep, and in one instance there was a distinct haemosiderosis in connection with the sinus cells.

(iii) It would appear that the reticulo-endothelial apparatus of the sheep is so developed that it can adapt itself, after the removal of the spleen, to deal with normal blood destruction without revealing specific lesions in any of the haemopoietic organs.

Z. (i) The blood changes observed in case of Anaplasmosis in splenectomised sheep were fully described in the paper of De Kock and Quinlan.

(ii) The most characteristic lesions were encountered in the liver, and were characterised by extensive erythrophagocytosis and desquamation of the stern cells of Kupffer. In the stern cells phagocyted erythrocytes were found in all stages of "digestion," i.e. from recently phagocyted erythrocytes to haemosiderin pigment granules and vacuoles.

(iii) In some instances the accumulated desquamated stern cells were responsible for a good deal of atrophy of the liver parenchyma. Fatty changes of a variable character were also observed.

(iv) In groups x, y and z, a localised widening of Glisson's capsule with the presence of round cells was observed in a number of cases. It is not clear whether this can be regarded as a lesion in the liver of sheep, seeing that it also occurred in clinically healthy sheep.

(v) Desquamated "endothelials (?)" with well-marked erythrophagocytosis and haemosiderosis were very prominent in the capillaries of the alveolar walls of the lungs. Although these cells in the organ smears from the lung resemble stern cells, yet it was not clear whether they were derived from the vascular endothelium of the lung, or whether they were cells of the R.E. system, which had drifted into the lung with the blood from elsewhere.

(vi) It would appear that the lung does not play a prominent part in the destruction of desquamated phagocytic "endothelial" cells, seeing that the blood counts before and after the lung showed very little difference as regards the presence of these cells, and no remains of such cells could be detected in the lung.

(vii) Katabolism of the phagocyted erythrocyte probably takes place in the cytoplasm of the R.E. cell. It is not clear whether the pigment formed is extruded into the circulation, while the cell continues its journey in the circulation as a monocyte of the blood or whether the pigment formed in the cytoplasm of the cell only becomes freed when the cell itself disintegrates. Pigment granules were frequently observed in the monocytes of the blood.

(viii) Haemosiderosis occurred in connection with a number of the lymphatic glands.

(ix) There were certain "mononuclears" which could not be definitely classified as "monocytes" or "lymphocytes." Some of these cells with distinct lymphocytic characters showed erythrophagocytosis. Cells resembling plasma cells were also encountered.

2. *Enzootic Icterus.*

Aetiology.—Enzootic Icterus of sheep was first identified as such at the Laboratory towards the end of July, 1924. It was characterised by an extensive general icterus and marked pigmentation of the kidneys. The disease seems to have a most remarkable distribution. Reports of the occurrence of this disease have also come from a few other centres, viz., Grahamstown, October, 1924, Abattoirs, Maritzburg, October, 1925, and Nooitgedacht, Ermelo, 1926.

In all these instances, with the exception of the last, the cases presented a similar history, namely that the sheep had recently arrived from the Karroo. The districts particularly mentioned were Carnarvon, Philipstown, Colesberg, and Middelburg. It may be mentioned that isolated cases of this disease have been reported (?) to have occurred in some of these centres named, but always limited to a few cases. Some farmers in that vicinity maintain that they may observe one or two cases one year followed by an interval of a few years when no cases occurred. From 1924-1926, several attempts have been made by the Department to study this disease in the locality in which it is said to occur. Up to the present, no cases were reported from these areas, although in the batches of sheep brought to the Laboratory from these areas, numerous cases were observed.

Sheep are bought in the Karroo areas and brought to Onderstepoort for the preparation of Blue Tongue Vaccine. These sheep are mainly supplied by two dealers, viz., Edwards, Schoombie, Middelburg, C.P., and Conroy, Philipstown, C.P. They buy respective batches of sheep for Laboratory requirements in the districts immediately surrounding their own. In Table I the number of sheep purchased from them during the years 1924-1926 is given.

TABLE I.

The number of sheep purchased for the preparation of Blue Tongue Vaccine during the years when Enzootic Icterus was observed at Onderstepoort.

Year.	Month.	Conroy, Philipstown, C.P.	Edwards, Schoombie, Middelburg, C.P.
1924	May	497	—
	July	—	500
	December	561	—
	TOTAL	1,058	500
1925	January	500	—
	March	49	—
	April	50	—
	August	250	580
	September	500	420
	December	42	—
	TOTAL	1,391	1,000
1926	January	50	—
	July	—	50
	August	250	—
	September	600	—
	December	100	—
	TOTAL	1,000	50

Table II.

In Table II a list has been compiled of the number of cases of Enzootic Icterus observed in the various batches of sheep brought to Onderstepoort for vaccine purposes.

The following information is given in the various columns of Table II:—

(i) Year.

(ii) and (iii) D.O.B. (i.e. tag numbers of the Department) numbers of the sheep bought from Conroy or Edwards, and affected with Enzootic Icterus.

(iv) Date of arrival of the sheep at Onderstepoort.

(v) Whether the sheep was affected with Enzootic Icterus before (available) it was utilised for the preparation of Blue Tongue Vaccine, or when it was or had been in Blue Tongue experiment (B.T.)

(vi) Date of death of the sheep.

(vii) The interval between date of arrival at Onderstepoort, and date of death.

Year.	Conroy D.O.B. No.	Edwards D.O.B. No.	Date of Arrival.	Expt. or Available.	Date of Death.	Interval between Date of Arrival and Death.
1924.....		9153	9. 7.24	B.T.	8. 8 24	30 days.
		9180	,,	Available.	6. 8.24	28 ,,
		9182	,,	,,	4. 8.24	26 ,,
		9192	,,	,,	30. 7.24	21 ,,
		9225	,,	B.T.	4. 8.24	26 ,,
		9228	,,	Available	18. 8.24	40 ,,
		9258	,,	,,	5. 8.24	27 ,,
		9283	,,	B.T.	12. 9.24	65 ,,
		9286	,,	Available	28. 7.24	19 ,,
		9288	,,	,,	13. 8.24	35 ,,
		9295	,,	,,	9. 8.24	31 ,,
		9300	,,	,,	6. 8.24	28 ,,
		9336	14. 7.24	,,	7. 8.24	24 ,,
		9364	,,	,,	31. 7.24	17 ,,
		9388	,,	,,	19. 8.24	36 ,,
		9408	,,	B.T.	11. 8.24	28 ,,
		9411	,,	Available	19. 8.24	36 ,,
		9438	,,	,,	28. 7.24	14 ,,
		9492	,,	,,	17. 8.24	34 ,,
		9563	,,	,,	2. 8.24	19 ,,
		9571	,,	,,	13. 8.24	30 ,,
		9598	,,	B.T. (complicated with pneumonia)	31.12.24	— 170 ,,
	10354		15.12.24	B.T.	7. 1.26	— 388 ,,
	10477		19.12.24	,,	12. 1.25	24 ,
	10580		19.12.24	,,	21. 1.25	33 ,,
	10655		,,	,,	14. 1.25	26 ,,
	10672		,,	Available	12. 1.25	24 ,,
1925.....	10932	(J. Sykes, O.F.S.)	7. 1.25	Icterus ?	20. 4.25	— 103 ,,
	10980		14. 1.25	B.T.	1. 3.25	46 ,,
	11250		19. 1.25	,,	5. 2.25	17 ,,
	11307		,,	Available	11. 3.25	51 ,,
	11377		,,	B.T.	3. 2.25	15 ,,

TABLE II (*continued*).

Year.	Conroy D.O.B. No.	Ecwards D.O.B. No.	Date of Arrival.	Expt. or Available.	Date of Death.	Interval between Date of Arrival and Death.
1925......	11418		19. 1.25	B.T.	27. 1.25	8 days.
	11444		,,	,,	2. 2.25	14 ,,
	11700		20. 3.25	,,	25. 4.25	36 ,,
		12419	22. 8.25	,,	2. 9.25	11 ,,
		13200	5. 9.25	,,	9. 1.26	— 126 N
		13330	7. 9.25	,,	7. 1.26	— 122 N
		13369	,,	,,	9. 1.26	— 124 N
		13489	17. 9.25	,,	13. 1.26	— 118 N
1926.....	14342		14. 1.26	H.W.	11. 2.26	28 days.
	14708		5. 8.26	B.T.	30. 8.26	25 ,,
	14709		,,	,,	24. 8.26	19 ,,
	14733		,,	,,	22. 8.26	17 ,,
	14734		,,	,,	25. 8.26	20 ,,
	14739		,,	,,	2. 9.26	28 ,,
	14795		19. 8.26	,,	3. 9.26	15 ,,
	14807		,,	,,	1. 9.26	13 ,,
	14814		,,	Available	7. 9.26	19 ,,
	14887		,,	B.T.	13. 9.26	25 ,,
	14922		,,	,,	9. 9.26	21 ,,
	14966		,,	,,	12. 9.26	24 ,,
	15156		9. 9.26	Malig. Oedema	29.11.26	— 82 ,,
	15160		,,	B.T.	27. 9.26	18 ,,
	15193		,,	,,	30. 9.26	21 ,,
	15196		,,	Available	27. 9.26	18 ,,
	15533		30. 9.26	B.T.	20.10.26	20 ,,
	15788		17.12.26	,,	5. 1.27	19 ,,
	15791		,,	,,	6. 1.27	20 ,,

The purchase of sheep from these areas for the preparation of Blue Tongue Vaccine has now been carried out for a number of years. These sheep at the Laboratory have been kept under close observation, and no cases of Enzootic Icterus were observed until July, 1924, when a number of cases occurred in the respective batches introduced. The occurrence of this disease was most remarkable as regards the suddenness of its appearance at the Laboratory. In May, 1924, 497 sheep were introduced without any mortality, whereas in the July batch of 500 sheep there were no less than 21 cases of Enzootic Icterus. Since then cases occurred in every batch brought from the Karroo to Onderstepoort for the preparation of Blue Tongue Vaccine.

From July, 1924, to December, 1926, 4,000 sheep arrived at Onderstepoort from these Karroo areas, and amongst these sheep there were about 50 cases, i.e. slightly more than 1 per cent. It will be seen that most of these cases were in the batch of 500 which arrived here during July, 1924, i.e. 21 cases. In August, 1925, amongst 830 sheep one case occurred, whereas in August, 1926, amongst 250 sheep there were 11 cases. It is difficult to say at what time of the year the disease is most prevalent, seeing that there was no regular supply of sheep throughout the year. The sheep were bought to suit the requirements of vaccine production. The shortest time after arrival here which elapsed before the disease made its appearance was eight days.

From the tables it will be seen that 20 cases occurred after an interval of about 10-20 days, 19 cases after 21-30 days, 9 cases after 82-388days. Were these in any way associated with an earlier grazing case of eight sheep, the intervals were 82, 103, 118, 122, 124, 126, 120 and 388 days respectively. It is difficult to say exactly what is the origin of these latter cases, seeing that the interval varied from 82-388 days. Were these in any way associated with an earlier grazing over Karroo veld, or were some of these actually connected with Laboratory conditions? The interesting fact is that the majority of the cases occurred shortly after arrival here, viz., 20 cases after an interval of 10-20 days, 19 cases after 21-30 days, and 9 cases after 31-40 days.

From Table II it will be seen that some cases appeared in sheep which were not yet treated, i.e. available sheep, whereas other cases occurred in sheep which were injected with Blue Tongue Virus before and after bleeding for Blue Tongue Vaccine. It would, therefore, appear that this treatment had no influence on the occurrence of the disease.

Various experiments were carried out to ascertain whether the disease could be transmitted by blood, or by extracts from various organs. In not one instance could a case be produced artificially (vide Experiments S. 2096, and S. 1776). Cultures were made from the blood and organs, for the identification of spirochaetes, or other organisms, but always with negative results. The numerous smears examined, stained and by dark field illumination, were negative.

At present we are, therefore. not in a position to throw any light on the aetiology of the disease. It may, however, be pointed out that no opportunity offered itself for studying this disease under natural conditions.

Is it in some way associated with dietetics? Sheep brought from a dry Karroo veld are stabled and given a diet of grass and mealies. This has probably to be ruled out, for if that is so, why did the disease not make its apearance before 1924, when such sheep were subjected to similar treatment. The aetiology of this disease was briefly referred to here mainly on account of its slight bearing on the much discussed Gaucher's disease in man.

Symptoms.—The disease is usually rapidly fatal, i.e. sheep die quickly after the appearance of jaundice noticed in connection with the visible mucous membranes. Besides jaundice there is general prostration, loss of appetite and hurried respiration. Those cases which we were lucky to obtain early, i.e. about 12-24 hours prior to death, only revealed slight fever. Sheep may be perfectly healthy in the morning, at noon show marked weakness and hurried respiration, jaundice manifests itself in the afternoon, and the animal dies that evening or during the night. Sometimes the symptoms of icterus may be present for 24 hours before death occurs. No cases of recovery are recorded. Another change which was observed in the majority of cases was the appearance of haemoglobinaemia. On account of the suddenness of the disease and the difficulty experienced in making an early diagnosis the blood picture could only be followed in a few cases (given in Table III).