Pathological Studies on Heartwater.

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CONTENTS.

Introduction.

A good description of the disease heartwater will be found in Theiler's report of 1904 * and in the handbook by Knuth and Du Toit.† Cowdry‡ has studied the disease from an etiological point of view. His findings, which will be referred to repeatedly, deserve special mention. In all cases of heartwater he found intracellular parasites—gram-negative cocci. They live in the endothelial cells of small blood-vessels in various organs of the body, particularly frequently in the cortex of the brain and in the kidney. They are easily demonstrable with basic aniline dyes. Cowdry describes them as *Rickettsia ruminantium.*

Grosspathology.

The following is a summary of the observations carried out on over 228 cases of heartwater by my colleagues of this Division and myself. The material consists of 29 bovines, 82 goats, and 117 sheep.

The lesions are fairly uniform in bovines, goats, and sheep, although some local changes are more frequently met in certain species than in others. Certain observations seem to indicate that the nature of the strain of heartwater virus has some influence on the pathological picture.

Complications are rare, most of the lesions observed in an animal stand in a direct relation to the disease.

The *condition* is fair to good, i.e. apparently little altered during the short course of the disease.

The *general external aspect* of the carcass has nothing characteristic, rigor mortis sets in at a normal time and the abdomen is only slightly distended or sunken.

The *integument* shows nothing particular. Ticks are present in animals coming from farms, *Amblyomma hebraeum*, the transmitter of the disease, among them.

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* *Report of the Veterinary Bacteriologist, Transvaal.* 1904. Pretoria: Government Printer.

† Knuth and Du Toit. *Handbuch der Tropenkrankheiten der Haustiere.* Leipzig: Barth. 1921.

‡ Cowdry, E. V. *Journal of Experimental Medicine.* Vol. 42. 1925. P. 231.

The *visible mucous membranes* have the usual bluish-pink colour, rarely are haemorrhages present, mainly in the conjunctiva. In a few experimental cases all the mucous membranes and, more or less, the organs are discoloured on account of vital impregnation with lithioncarmine, trypan blue, and other stains. These alterations will be disregarded in the following descriptions.

In goats and sheep the lymph follicles of the conjunctiva are often prominent. This condition is frequently met in apparently healthy small ruminants and associated with large Malpighian bodies of the spleen, etc. It has nothing to do with heartwater.

A slight mucous or serous discharge from the nostrils is not rare.

The *blood* stains well, has a normal colour, and coagulates readily.

Flesh and subcutaneous tissue show no marked alterations. They may even be dry, while there are extensive serous infiltrations in the lungs and serous cavities.

Peritoneal Cavity.—In bovines there is usually an abnormally large amount (up to litres) of a yellowish, transparent, sometimes slightly turbid or blood-stained fluid present. This same alteration is less frequently met in goats and sheep, occurring in about a quarter of the cases only, but in an individual of these species it may be very marked. In a sheep one can find occasionally as much as 500 c.c. of fluid in the peritoneal cavity.

The serosa itself is smooth, glistening, and transparent. In some cases small haemorrhages are present. When the transudation is extensive there can be a marked serous infiltration of the connective tissue in which the perirenal fat tissue is embedded.

Pleural Cavities.—In about half the goats and two-thirds of the bovines and sheep, the liquid-content of the pleural cavities is increased, sometimes considerably, so that the diaphragm is markedly displaced. There may be several litres of fluid in one pleural cavity of a bovine and up to half a litre in the case of a sheep. In goats the quantity rarely exceeds 20 c.c. The parietal pleura itself is generally smooth, glistening, and transparent.

The connective tissue of the mediastinum often shows a marked serous infiltration which may be particularly pronounced in the connective tissue surrounding the lobules of the thymus.

The *pericardial sac* is the seat of the alteration which has given the disease its name, but in all three species it is absent in a few per cent. In general, the liquid-content is relatively more abundant in sheep and goats than it is in bovines. The content of the pericardial sac is a transparent or only slightly turbid, light yellowish fluid. Very frequently it coagulates spontaneously, and the cavity may be filled with a soft pale clot. In most post-mortem examinations it is found liquid in the main and containing some soft elastic fibrinous strings. The quantity of fluid can be considerable, especially in sheep and goats. The pericardial sac is then distended by its content. The pleura-pericardium itself is sometimes infiltrated.

The *salivary glands* do not show any alterations.

In the *thyroids* small haemorrhages have been found in rare cases.

The *lymph nodes* in various parts of the body are swollen, unusually moist on section, and often reddened through a fine ramiform injection or infiltrated with extravasated blood in small foci.

These changes are more or less pronounced in most cases of all three species. The retropharyngeal, submaxillary, cervical, bronchial, and mediastinal lymph glands are particularly frequently involved. The enlargement is not excessive. In the cases I observed myself, it never seemed to exceed 100 per cent. and very rarely 50 per cent.

The portal and mesenteric lymph glands appear less affected than the rest. In the majority of cases the mesenterial lymph glands—which are fairly large in normal ruminants—show no striking alterations and the portal lymph glands are, if somewhat enlarged, not hyperaemic.

Tongue and *oesophagus* do not show any marked or characteristic changes.

The mucous membrane of the *pharynx* is hyperaemic in some cases.

Larynx.—In all three species and almost half the cases the larynx shows ramiform and diffuse reddening, sometimes associated with enlargement of the lymph follicles. Small petechiae in the mucous membrane are frequently present. Occasionally the mucous membrane is covered with an unusually large amount of mucus.

Trachea.—Distinct alterations are found in about half the cases. They consist of vascular injection, haemorrhages, increased amount of mucus, and swelling of the mucous membrane. The changes are generally least pronounced in the oral, most in the aboral part of the trachea, or even entirely restricted to that part. In many cases the reddening of the mucous membrane is very pronounced, and the lesion one of the most marked in the whole animal.

In the great majority of the cases (over 90 per cent.) the *lungs* are the seat of more or less pronounced oedema. Size and shape are those of inspiration or, at least, incomplete expiration. The pleura is generally smooth, glistening, and transparent. If occasionally there are fibrous adhesions (which we believe are not the result of the disease in question), these often are infiltrated with a clear yellowish fluid, and so appear as semi-transparent gelatinous coats. This condition is more frequently met in sheep. Small punctiform haemorrhages are occasionally present. By a marked serous infiltration of the interstitium, which will be described later, the pleura may be separated from the underlying parenchyma, especially in the lower parts.

When the oedema is well developed the edges appear everted on section into the organ, and from the cut surface a frothy serous fluid may be pouring down in a large amount. The cut surface generally shows a purplish-red colour which soon turns brick red under the influence of the air. Haemorrhages are present in some cases.

In bovines, as a rule, less frequently in sheep and still less commonly in goats, the subpleural, sublobar, and peribronchial interstitium is infiltrated with a clear yellow fluid. It is much widened in some cases and on account of a slight opalescence shows a green colour.

The main bronchi contain a frothy liquid which is frequently slightly blood-stained and often contains strings of fibrin. The mucous membrane

may not show any striking alterations, but in about half the cases there is a ramiform reddening and sometimes numerous punctiform haemorrhages. It is frequently covered with an increased amount of grey transparent mucus.

Occasionally parts of the lung, most frequently the cardiac lobe, are the seat of a broncho-pneumonia or the ventral parts of all lobes are atelectatic. The first of these changes appears to have only an accidental relation to heartwater.

Fibrous adhesions and filaments, parasitic lesions (*Cysticercus tenuicollis*, *Ecchinococcus granulosus*) occur in the same number of cases as in all the animals of this station.

Heart.—Size and shape show nothing unusual; the right ventricle is in the state of incomplete diastole, the left ventricle in incomplete systole. In agreement with the general condition of the animal the epicardial fat tissue is well developed and appears normal. Haemorrhages from pin-point size to a few centimetres diameter are frequently found on the epicard. Not infrequently a fibrinous coat is present, a precipitate it seems from the serofibrinous content of the pericardial sac. Occasionally the epicard is found thickened in an area of 1–3 cm. diameter and there infiltrated with a serous fluid, an alteration similar to that found in the pleura. The ostia show nothing abnormal. The endocards are almost invariably the seat of haemorrhages up to centimetres in diameter. They are always much more extensive in the left heart and frequently restricted to that. Some of these haemorrhagic infiltrations extend into the valves (left atrioventricular and aortic valves). On section they can be followed extending into the myocard for a few millimetres.

The *myocard* has the normal thickness on both sides. Usually it shows a normal coloration; only exceptionally does it appear mottled. In some cases it is opaque and possesses a lowered consistence, but in more than half of the cases transparent and firm.

The pathological changes in the *liver* are subject to considerable variation. If they are well developed a characteristic picture results. This is most frequent, i.e. in more than half of the cases, found in bovines, less commonly in sheep and goats. The liver in such a case appears enlarged, the edges rounded. A higher blood-content shows itself in the deep red colour of the surface. On close examination a red network might be detected, but that is more distinct on section. The edges of the incision are everted, a large amount of blood flows off. The cut surface has a glossy appearance and more red tint than normally. Lobulation is distinct, marked out by a red network on a more greyish brown background. But the lobules are those of Sabourin. The centre is formed by the periportal interstitia. These sometimes are distinctly enlarged, greyish, semi-transparent. The consistency in these typical cases is firm, sometimes slightly friable.

The variations in the aspect of the organ are manifold. Firstly, all the changes mentioned may be much less developed, the network not distinct, the centres not enlarged, grey, etc. In addition there may be yellowish discoloration associated with a more friable consistence, i.e. due to fatty infiltration.

Pigmentation is not more frequent or more marked than in normal cases. In certain animals *Stilesia hepatica* is found in the intrahepatic bile ducts, and their presence is associated with an enlarged, light periportal interstitium and a distinctly increased consistence of the whole organ.

Extra hepatic bile ducts, gall bladder and pancreas show no characteristic lesions.

Swelling of the *spleen* is one of the most constant changes. It is almost invariably (more than 90 per cent.) present in sheep and goats and in about three-quarters of the bovines.

In the small ruminants the size may be well six times the normal; in bovines the enlargement rarely exceeds 100 per cent.

The edges appear rounded, the capsule is smooth, otherwise without abnormalities. On section the cut surface bulges out. It has a coarse granular appearance, reminding one of raspberries, and a deep red or brownish-red colour. It cannot easily be scraped off. The trabeculae are generally not distinct, the Malpighian bodies in the majority of cases are invisible to the naked eye. The consistence is in most cases normally firm.

In the majority of cases the changes in the *kidneys* are not striking. Glomeruli and interlobular vessels of the cortex are often prominent, but otherwise structure, colour, and consistence are normal. In some cases, however (a quarter or less), the organ appears swollen, the capsule glides off with unusual ease, the surface appears somewhat more greyish (in *small ruminants*) or more reddish in colour (in bovines). It often appears mottled, due to the presence of greyish and wine-red areas of few millimetres diameter, besides numerous ill-defined spots of these same colours which become evident on closer examination. The consistence is then decreased.

The pericapsular fat tissue is generally well developed.

The *adrenals* show small haemorrhages in exceptional cases, more frequently hyperaemia, particularly of the central portion of the cortex.

The first three *stomachs* appear normal, with this exception that the contents of the omasum often are more dry than normal.

The abomasum may show swelling of the mucous membrane, hyperaemia, and haemorrhages. These changes are met with in most of the bovine cases (ca. 9/10), but only in about one-third of the small ruminants. They are also more extensive in bovines. The mucous membrane may be much thickened, on section infiltrated with a transparent fluid. Aside from a diffuse reddening, which is usually more marked towards the pylorus, there are deep red spots from pin-point size to few millimetres in diameter, irregular in outlines, particularly numerous on the folds. The contents are semi-liquid, of normal colour.

The changes in the *small intestine* resemble those in the abomasum; Swelling, patchy hyperaemia with ramiform injection of venules and haemorrhages. They are again more frequent and more extensive in bovines than in the small ruminants. Patchy reddening was distinct in two-thirds of the bovines, one-quarter of the sheep, and one-third of the goats; slight in one-ninth of the bovines, a quarter of the sheep and goats, and entirely absent in a quarter of the bovines, half of the sheep, and a little less than half of the goats.

Generally the first portion of the duodenum resembles the abomasum; then the changes become less pronounced to increase in extent again towards the ileum. The ramiform injection is not rarely confined to successive transverse bands which produce a zebra marking. Necrosis has not been observed. The contents are semi-liquid, sometimes mixed with an increased amount of mucus. In bovines they are often more brownish than normal.

The *large intestine* shows changes similar to those met with in the small intestines, but they are much less pronounced.

In the whole of the intestinal tract the usual parasitic invasion has been observed. It seemed, as far as could be ascertained, not influenced by the disease one way or the other.

Bladder, *urethra*, and *sexual organs* show nothing unusual, with the exception of occasional small haemorrhages in the mucous membranes in few cases.

No striking characteristic lesions were found in the *central nervous system* and the *skeleton* (*bone marrow*).

Microscopical Pathology.

*Material.**—It will be pointed out that the lesions in many organs were not very striking. It seemed, therefore, necessary to establish definitely certain histological details in healthy animals of this country, or at least in animals not suffering from infectious diseases. In addition to the histological material available in the collections of the Institute, I collected organs from the following animals to serve as control material.

Table I.

Control material collected from animals free from infectious or toxic lesions :—

Species.	*Spec. No.*	
Goat.....	4070	Normal, died immediately after the first injection of lithioncarmine.
Goat.....	4070	Normal, killed.
Goat.....	4087	Normal, treated with lithioncarmine, killed.
Goat.....	4309	Normal, treated with lithioncarmine, killed.
Goat.....	4388	Normal, treated with lithioncarmine, killed.
Bovine.....	3819	Arthritis chronica deformans, killed.
Bovine.....	3800	Died of " shock " immediately after blood transfusion.
Bovine.....	3694	Died of acute tympanitis.
Bovine.....	4121	Styfsiekte, killed.
Bovine.....	4111	Styfsiekte, killed.
Bovine.....	4110	Healthy, killed.
Bovine.....	2945	Healthy, treated with lithioncarmine, killed.

The pathological material is given in Table II.

* The investigations were originally planned on a larger scale, but owing to the writer's transfer to the University of Berne a reduction had to be made.

† The sections Dr. Cowdry kindly lent me from his material are not contained in the table.

Table II.

Species.	No.	Sex.	Age.	Disease Acquired.	Death.	Dead, Days after Onset of Fever.	Organs Examined.
Bovine.	4374	Ox.....	6 yrs.	Spont.	Spont.	—	Liver, lung, kidney, saliv. glands, adrenals.
Bovine.	399	Ox.....	1 yr.	Spont.	Spont.	—	Liver, lung, myocard, spleen.
Bovine.	4769	Cow....	6 yrs.	Spont.	Spont.	—	Spleen, myocard, liver, lung, kidney.
Bovine.	400	Ox.....	1 yr.	Spont.	Spont.	—	Spleen, myocard, liver, lung, kidney.
Bovine.	397	Bull....	1 yr.	Spont.	Spont.	—	Liver, lung, kidney, skeleton muscle.
Bovine.	593	Cow....	5 yrs.	Spont.	Spont.	—	Liver, lung, kidney, myocard, spleen, skeleton muscle, var. lymph glands.
Bovine.	425	Heifer..	1 yr.	Spont.	Spont.	—	Liver, kidney, myocard.
Bovine.	391	Cow....	7 yrs.	—	—	—	Liver, lung, kidney, myocard, spleen, lymph glands, saliv. glands.
Bovine.	614	Heifer..	1 yr.	Blood inoc.	Spont.	9	Liver, kidney, myocard, lymph glands, adrenals, small intestine.
Bovine.	542	Ox.....	2 yrs.	Blood inoc.	Killed	9	Liver, kidney, spleen, myocard.
Bovine.	2907	Cow....	11 yrs.	Spont.	Spont.	—	Liver, kidney, myocard, adrenals, lymph glands, small intestine.
Bovine.	620	Heifer..	15 mths.	Blood inoc.	Killed	11	Liver, lung, spleen, myocard, oesophagus.
Bovine.	587	Heifer..	2 wks.	Spont.	Spont.	—	Liver, kidney.
Bovine.	926	Heifer..	1½ yrs.	Blood inoc.	Killed	2	Liver, lung, kidney, pallium, lymph glands, small intestine, colon, adrenals, myocard, tongue.
Bovine.	Private	Bull....	8 wks.	Spont.	Spont.	—	Myocard, liver, kidney, lung, abomasum.
Bovine.	1071	Bull....	4 wks.	Blood inoc.	Spont.	11	Liver, kidney, myocard, adrenals.
Bovine.	—	—	—	Spont.	Spont.	—	Liver, kidney, myocard, spleen.
Bovine.	Dist.	—	—	Spont.	Spont.	—	Lung, liver, kidney.
Bovine.	Dist.	—	—	Spont.	Spont.	—	Liver.
Goat...	4561	Kapater	3 yrs.	Blood inoc.	Spont.	3	Liver, lung, kidney, myocard, spleen.
Goat...	5742	Kapater	1 yr.	Blood inoc.	Spont.	2	Liver, kidney, adrenals.
Goat...	5714	Ewe...	2 yrs.	Blood inoc.	Spont.	2	Liver, lung, kidney, myocard, lymph glands, thyroid, large intestine, small intestine.
Goat...	7928	Kapater	1 yr.	Blood inoc.	Spont.	2	Liver, lung, kidney, spleen, myocard.
Goat...	5673	Ewe...	2 yrs.	Blood inoc.	Spont.	3	Liver, lung, kidney, spleen.
Goat...	6673	Kapater	3 yrs.	Blood inoc.	Spont.	3	Liver, kidney, myocard.
Goat...	8122	Ewe...	2 yrs.	Blood inoc.	Spont.	2	Kidney.

Table II—continued.

Species.	No.	Sex.	Age.	Disease Acquired.	Death.	Dead, Days after Onset of Fever.	Organs Examined.
Goat...	6402	Kapater	3 yrs.	Blood inoc.	Spont.	7	Liver, lung, kidney, spleen, lymph glands, adrenals, small intestine, large intestine.
Goat...	5656	Ewe...	2 yrs.	Spont.	Spont.	—	Kidney.
Goat...	7576	Kapater	3 yrs.	Blood	Spont.	5	Liver, lung, kidneys.
Goat...	8290	Ewe...	1 yr.	Blood	Spont.	5	Liver, lung, kidney.
Goat...	8259	Ewe...	3 yrs.	Blood	Spont.	6	Liver, lung.
Goat...	8233	Ewe...	4 yrs.	Blood	Spont.	3	Liver, lung, kidney, colon, adrenal.
Goat...	4514	Ewe...	5 yrs.	Blood	Spont.	3	Liver, lung, kidney, myocard, spleen.
Goat...	8384	Ewe...	3 yrs.	Blood	Spont.	7	Liver.
Goat...	7590	Kapater	4 yrs.	Blood	Spont.	8	Liver, lung, kidney, spleen, colon, adrenals.
Goat...	8400	Ewe...	3 yrs.	Blood	Spont.	3	Liver, lung, kidney, spleen, myocard, caecum, adrenal, trachea.
Goat...	7919	Kapater	4 yrs.	Spont.	Spont.	—	Liver, lung, kidney, small intestine.
Goat...	8386	Ewe...	3 yrs.	Blood	Spont.	2	Liver, lung, kidney, myocard, abomasum, small intestine, caecum, colon, rumen, adrenal, thyroid, lymph gland, tonsils, trachea, lip, thymus.
Goat...	9657	Kapater	1 yr.	Ticks	Spont.	5	Liver, lung, kidney, myocard, spleen, thyroid, parathyroid, adrenals, lips, tonsils, pharynx, oesophagus, rumen, reticulum, omasum, abomasum, small intestine, caecum, colon, pancreas, trachea, skeleton muscle, bladder, pallium, corpus striatum, cornu ammonis, cerebellum med. oblongata, spinal cord, presc. lymph glands, portal lymph glands, renal lymph gland, suprapharyng. lymph gland, mesent. lymph gland, ing. lymph gland, haemo lymph gland.
Goat. .	9665	Kapater	1 yr.	Ticks	Spont.	4	Skin, lung, kidney, adrenal, reticulum, colon, pallium, corpus striatum.
Goat...	9675	Kapater	3 yrs.	Blood	Spont.	6	Kidney, liver, adrenal, pallium.
Goat...	11567	Kapater	1 yr.	Blood	Spont.	12	Liver.
Goat...	14029	Ewe...	3 yrs.	Blood	Spont.	4	Liver.
Goat...	8235	Ewe...	4 yrs.	Ticks	Killed	4	Liver, lung, kidney, myocard, spleen, pancreas, thyroid, adrenal, thymus, tonsils, rumen, reticulum, omasum, abomasum, small intestine, colon, vagina, uterus, skin, pallium, corpus striatum, cerebellum, cornu ammonis, trachea, oesophagus, skin, supraph. gland, prescap. gland, renal gland, precrur. gland.

Table II—continued.

Species.	No.	Sex.	Age.	Disease acquired.	Death.	Dead, Days after Onset of Fever.	Organs Examined.
Goat...	Private	Ewe...	3 yrs.	Spont.	Spont.	—	Liver, adrenals.
Goat...	10275	Kapater	2 yrs.	Blood	Spont.	6	Liver, kidney, myocard, adrenals, pallium, cerebellum.
Goat...	8338	Kapater	2 yrs.	Spont.	Sport.	—	Kidney, liver, adrenal, skin.
Goat...	9662	Ewe...	3 yrs.	Ticks	Spont.	—	Lung, liver, kidney, myocard, pharynx, tonsils, oesophagus, rumen, colon, thyroid, pallium, corpus striatum, cerebellum, spinal cord, cornu ammonis, skin, ovary, uterus.
Sheep..	5282	Hamel.	2 yrs.	Blood	Spont.	8	Liver, lung.
Sheep..	9224	Hamel.	3 yrs.	Spont.	Spont.	—	Caecum.
Sheep..	8847	Hamel.	2 yrs.	Blood	Killed	6	Liver, lung, kidney, spleen, myocard, rumen, small intestine, colon, tonsils, adrenals, thymus, Peyer's plaques, lymph gland, skeleton muscle.
Sheep..	7017	Hamel.	4 yrs.	Blood	Killed	21	Liver, lung, kidney, spleen, myocard, thymus, thyroid, skeleton muscle, pallium, skin, lymph glands.
Sheep..	9418	Hamel.	4 yrs.	Blood	Killed	6	Lung, adrenal, small intestine.
Sheep..	6938	Hamel.	2 yrs.	Blood	Spont.	—	Kidney, lung.
Sheep..	8735	Hamel.	3 yrs.	Blood	Killed	8	Liver, lung, kidney, spleen, myocard.
Sheep..	8635	Hamel.	3 yrs.	Blood	Spont.	8	Liver, lung, kidney, adrenals, myocard, spleen, lymph gland.
Sheep..	10397	Hamel.	4 yrs.	Spont.	Spont.	—	Kidney, liver, myocard.
Sheep..	11642	Ewe...	4 yrs.	Spont.	Spont.	—	Liver, lung, kidney, myocard, adrenal.
Sheep..	10996	Hamel.	4 yrs.	Blood	Spont.	7	Liver, adrenal.
Sheep..	10777	Hamel.	4 yrs.	Blood	Spont.	8	Liver, lung, kidney.
Sheep..	13949	Hamel.	3 yrs.	Blood	Spont.	8	Liver.

Methods.—The fixation methods employed were formalin 1/9th, Zenker's fluid, and sublimate alcohol.

Both freezing and paraffin sections were used and the following staining methods applied: haemalum, haemalum-eosin, haematoxyline-Van Gieson, ferrocyanide of potassium for iron (prussian blue reaction), sudan and occasionally nilbluesulphate for fat, Bielschowsky-Maresch gold impregnation of fine collagen fibrils, giemsa in Helly's modification, oxydase reaction (Graef-v. Gierke-Schultz).

A good giemsa stain was obtained from freezing sections with the following procedure, which will be described in more detail elsewhere; the material may be fixed with any of the current methods. Freezing sections come from distilled water into the following solution for 24 or more hours: Giemsa stock solution, 10 drops; distilled water 20 c.c. The water should be neutral; if necessary this may be by addition of a few drops of a phosphate-buffer solution. From tap-water the sections are caught on a slide one by one, the water removed and blotted away, 2–3 drops of acetone are allowed to run over the section and before it is dry 2–3 drops of cedar wood oil. This is removed by blotting and the section mounted in neutral canada balsam.

The stain gives a good Romanowsky effect, good metachromasia of mucine, granules in the adrenal medulla, fat, myeline, etc. If it is prolonged the fine collagen fibrils, e.g. the so-called "Gitterfasern" of the liver, can be brought out sharply.

Results.—The histological alterations found in fatal cases of heartwater are fairly characteristic when they are well developed, but in many cases they are extremely slight.

They are most pronounced in kidneys, liver, and lungs, less in the adrenals, and rare in other organs. As Cowdry has pointed out already, there is no local reaction to the presence of Rickettsias in the vessel walls. The histological changes are diffuse or stand in relation to certain normal structures, quite irrespective of whether these are visibly infected or not.

The parenchymatous elements may show moderate degenerative changes. But I have never seen necrotic foci. The small blood-vessels, arterioles, capillary vessels, and venules contain an abnormally large number of nucleated cells and in general among them a strikingly large proportion of macrophages. In the interstitium one meets perivascular cell accumulations which mainly consist of plasma cells.

The upper portion of the *intestinal tract*, i.e. mouth cavity, pharynx and tonsils, oesophagus, rumen, reticulum, and omasum, shows nothing particular. In the abomasum, the small and the large intestine, the small blood-vessels of the propria may be congested. Sometimes there is even a haemorrhagic infiltration of the propria. The superficial epithelial layer may be removed. Comparison with normal material did not reveal any definite qualitative or quantitative alteration in the cellular infiltration of the propria.

The *liver* (Figs. 1, 3, 4), however, shows definite changes in more than three-quarters of the cases. The stasis referred to in the macroscopical description may be very marked, so that the lobule of Sabourin which is visible to the naked eye is also marked off in the microscopical picture.

In the intralobular capillary vessels the nucleated cells are increased in number. We find lymphocytes, neutrophile leucocytes, macrophages, less frequently plasma cells and eosinophiles. Any one of the three first mentioned types may be prevalent, most frequently the macrophage, least the lymphocytes. Most of these cells appear intact, but it is not uncommon to find karyorrhexis in neutrophiles and lymphocytes.

A small proportion of these macrophages contain pigment granules, iron free or with detectable iron, also acidophile débris and even whole red cells. There is doubtlessly an increased erythrophagocytosis, but never to the same extent as in piroplasmosis or equine infectious anaemia.

The leucostasis is also quite different from that observed in East Coast fever. The lymphocytes are much less prominent and free from visible micro-organisms.

Some of the stern cells of Kupffer contain a brownish pigment in coarse granules generally with detectable iron. I have never seen Rickettsias in Kupffer cells but once in the endothelial cell of a small arteriole in the periphery of a lobule, apparently in a small branch of the hepatic artery.

The parenchyma cells generally show no marked changes. But in some cases there is a diffuse fatty infiltration with small fat droplets. Occasionally I met with large hyaline drops, staining diffusely, but not with Sudan III or nilbluesulphate. Pycnosis in the nuclei is not rare. Sometimes, on the other hand, the nuclei are exceptionally large and deeply stained. With regard to the presence of pigment granules, there is no difference between these and normal animals.

In more than half the cases there is an increased cellular infiltration of the interstitium. As I have pointed out at another occasion, normal bovines and goats in this part of the country do not show any perivascular cellulation around the branches of the portal vein or the hepatic artery. Any definite cellulation in these places must be the result of a noxe to which the " normal " animal evidently is not exposed.

In about a quarter of the cases, the periportal cellular infiltration is very marked and the interstitia stand out prominently. In its general aspect the infiltration differs from that found in East Coast fever or other diseases of a lymph-adenotic nature. It is less sharply marked off from the peripheries of the adjoining lobules. At first sight it looks like an inflammatory infiltration.

The qualitative study confirms this. There is a cellular reticulum with cells similar to those found in normal lymph follicles, but they are less numerous than in East Coast fever. The free cells dominate, mainly cells with a compact spherical trachychromatic nucleus which may show a wheel-spoke pattern and a basophile cytoplasm: lymphocytes and especially numerous plasma cells. Less frequent are: histiocytes with a small trachychromatic nucleus and a large often elongated polymorph, carminophile (vital stain) cell body, mast cells, still less numerous: neutrophiles and macrophages.

In all specimens, but in small number only, there are found alongside lymphocytes and plasma cells, apparently free cells with a large spherical or slightly irregular nucleus of about liver cell nucleus dimensions and a large polygonal cell body up to 40–50 μ in diameter. The cytoplasm in most of the cells of this habitus is markedly basophile as much as that of the lymphocytes and plasma cells. The nucleus is very deeply stained in coarse granules and not infrequently exhibits the individual chromosomes as in mitosis. Around the nucleus there may be a lighter halo. As was said already, the cell appears to be lying free and there is no evidence of new formation of collagen in the neighbourhood. With the exception of cases associated with *Stilesia hepatica* in the intrahepatic bile ducts, new formation of connective tissue seems to be very slight or absent.

The *respiratory tract* like the digestive tract is exposed to so frequent injuries that normally already we find the tissues in a state of visible defence, which is manifested in the microscopical picture.

The trachea, e.g., shows fairly marked cellulation underneath the epithelial layer and around the small blood-vessels of the propria mucosae. Although I gained the impression that this cellulation (mainly lymphocytes, plasma cells, less frequently neutrophiles) is more marked in some cases of heartwater where it is associated with increased injection of small blood-vessels, this fact is of little diagnostic value. Normally already the cellular infiltration seems to vary within fairly wide limits.

The same is true for the peribronchial development of lymphoid tissue in the lungs.

The most marked changes in this organ are leucostasis and alveolar and interstitial oedema. Among the nucleated cells in the capillary vessels, the macrophages are again the most numerous, besides there are neutrophiles and lymphocytes, less frequently eosinophiles.

The alveoles are filled with serum and a varying, sometimes in places quite considerable, amount of fibrin. There are only few cells in the lumina, erythrocytes, cells of macrophage habitus and desquamated epithelial cells.

In the interlobular and peribronchial interstitia the connective tissue fibrils are separated by a serum-like fluid. The fibres themselves may appear somewhat swollen. The lymph-vessels are not particularly prominent. There is a slight cellulation. In the neighbourhood of small blood-vessels mainly, one finds cells of the macrophage type and the lymphocyte series, sometimes with necrobiotic nuclei.

The interstitia are much enlarged by this infiltration, so that they become very prominent in the macroscopical picture.

In more than half the cases of heartwater, *the kidney* (Figs. 2, 5) is the seat of distinct and fairly characteristic lesion.

Among the parenchymatous elements the tubuli contorti are mainly affected. Some of the nuclei show pycnosis, less frequently karyorrhexis. The changes, however, are not very extensive. In an altered tubule there are besides degenerated nuclei always some of normal appearance. The cytoplasm may contain fat droplets, some of the tubuli have all the cells filled with hyaline droplets of up to half nuclear diameter. Albuminous material may be present in the lumina in the form of hyaline masses. Less frequently cells of the lining are shed off into the lumina. Some of the tubules are distinctly more altered than others, but not groups of them. It never comes to the formation of a necrotic focus.

The glomeruli are less frequently affected and only in a small number of cases do the capsules of Bowman contain albumen. This is particularly interesting since the intracellular cocci are most frequent in the glomeruli.

The blood-vessels are frequently distended, especially the capillary vessels and the small veins; occasionally the stasis may be accompanied by diapedesis of red-blood corpuscles. In the great majority of cases the number of nucleated cells in the small vessels is considerably increased. Macrophages stand in the foreground and may constitute more than three-fourths of the nucleated cells, but lymphocytes and neutrophiles are also more abundant than in the normal controls. Few of the macrophages contain fragments of red corpuscles. Parasites could not be detected in their cytoplasm. As Cowdry had shown, the endothelial cells of small blood-vessels are frequently infested with a Rickettsia, especially those of the glomerular vessels. I have found them there in great numbers in some cases, but also in other arterioles, and once in the endothelial cell of an arteria arcuata. The simple technique described above for the application of the Romanowsky stain to freezing sections brings out these cocci very clearly.

As Cowdry has pointed out, the infected cells, as well as those in their neighbourhood, are not visibly affected except for the displacement of the nucleus by the mass of the conglomeration of cocci. Cowdry described a halo of rarefaction around the granules. He does not consider it a pathological change; indeed, it is probably a staining-artefact and due to the deprivation of precipitable basic dye by the strongly basophile granules (similar to the perinuclear halo in lymphocytes and plasma cells).

The changes in the interstitium have the character of an interstitial inflammatory reaction. Mainly concentrated around small arteries (interlobulares and their branches) we find typical lymphocytes and plasma cells. They lie in a network consisting of fine preformed collagen fibrils and sessile adventitious cells of mainly three types : carmine storing polymorph-cellular histiocytes, mast cells, and cells similar to those forming the reticulum of the lymph follicles. Neutrophiles and macrophages are much less frequent in an extravascular position and eosinophiles still rarer. Besides these, we find regularly and more frequently than in the liver the large basophile cells described in connexion with the periportal interstitia (Figure 5). New formation of connective tissue does not seem to take place.

A correlation between these interstitial changes and the presence of Rickettsias goes only so far that in general Rickettsias are present in a number to be easily detected when the interstitial changes are well pronounced. But there may be many Rickettsias accompanied by slight interstitial changes and also the opposite, few Rickettsias with marked interstitial changes. As far as the localization is concerned, there is no correlation at all.

The interstitial cellulation is not a local reaction to the presence of microscopically detectable Rickettsias.

Urethra and bladder show no definite alterations.

Among the *glands of internal secretion* thyroids, parathyroids, and adrenals have been examined. In the thyroids and parathyroids, I have never been able to detect any striking alterations except leucostasis as in so many other organs.

The adrenals show alterations only in a small percentage of cases. They affect the medullary portion more frequently than the cortex. In the medulla smaller arteries may be surrounded by a marked cellular infiltration which resembles very closely that found in liver and kidneys. The parenchyma cells may show pycnosis of nuclei, even karyorrhexis, but always less than, e.g., in the kidneys. A marked leucostasis with macrophages as main type completes the picture, but leucostasis is very frequently present without any distinct interstitial changes.

In two cases I found in the cortex small foci of extravascular lymphocyte and plasma cell accumulation associated with marked degeneration of the parenchyma cells in their immediate neighbourhood. This alteration falls somewhat out of the typical histological picture of heart-water. It may be due to an accidental injury of a different nature.

Heart Muscle.—Haemorrhages from the endo- or epicard may extend into the depth of the myocard separating the muscle fibres. In such foci of haemorrhagic infiltration there are, besides the extravascular red cells, considerable numbers of nucleated cells scattered between the fibres, particularly in the neighbourhood of small blood-vessels. Macrophages are the dominant type, lymphocytes, plasma cells, and leucocytes less numerous. Otherwise the changes in the myocard are slight. Occasionally one finds fatty infiltration of the sarcolemma and pycnotic nuclei. The interstitial and, especially, the perivascular cellulation does rarely exceed that found in many normal myocards. In few cases, however, it is distinctly increased. The cells are mainly macrophages and members of the

lymphocytic series. Leucostasis is marked in many cases, the main type being the macrophage.

The most striking and regular alteration in the *lymph glands* is injection of the small blood-vessels of the cortex. They stand out most prominently in many sections, especially since they are filled mainly with red cells. Leucostasis is often far less pronounced than in other organs.

The other alterations commonly found, a large number of plasma cells in the medullary sinuses, sharply defined secondary follicles, mitosis, karyorrhexis in the large reticulum cells of the secondary follicles, have been so regular in the apparently normal controls that they cannot be brought into relation with heartwater.

The *spleen* shows a pulp crowded with cells; red cells and macrophages are the prominent types. Pigment is present in the usual amount.

The follicles are often reduced in size, rarely enlarged. In larger follicles the secondary follicles may be prominent and exhibit the same changes as have already been discussed in connexion with the lymph glands.

The *bone marrow* has not been studied on satisfactory material.

The *sexual organs* show nothing particular. Rickettsias have been found in uterus and ovaries.

In the *central nervous system* cortex cerebri, cerebellum, corpus striatum, cornu ammonis, medulla oblongata, and spinal cord all show more or less pronounced leucostasis. As in other organs small blood-vessels may be packed with macrophages. In the cortex of the cerebrum and in the cerebellum intraendothelial Rickettsias may be exceedingly frequent. Here I have seen them exceptionally stained metachromatically purple with giemsa when the localization and morphology excluded any confusion with leucocytic or mast cell granula. Since they were conglomerates of medium size, I am inclined to believe that the metachromasia was determined rather by the conditions prevailing during the process of staining than by a peculiar property of these particular cocci. In general the conditions for a good azure stain seem to be better in freezing sections than in paraffin material.

In spite of the presence of so numerous Rickettsias, in some cases there is again no local reaction.

The *skin* shows no characteristic lesions, but I have seen an intracellular Rickettsia in the wall of a capillary vessel passing within some 10 μ from the epidermis.

Haematological Observations.*

The results of observations made in normal goats treated with lithioncarmine, heartwater goats, and heartwater goats treated with lithioncarmine are recorded in Tables III–IX and Graphs 1–3.†

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* These studies originally formed part of a much more extended programme.

† Abbreviations :—

R.C.	Red count.
R.P.	Red percentage volume.
L.	Lymphocytes.
N.	Neutrophiles.
M.	Monocytes.
E.	Eosinophiles.
B.	Basophiles

It will be seen that in some cases a monocytosis develops, in others there is no appreciable change in the blood picture apparently because the monocytes, although increased in number, are kept back in the organs.

Summary.

Besides the presence of Rickettsia ruminantium (Cowdry), the characteristic changes in heartwater are leucostasis and perivascular cellulation. The former occurs in all organs and the macrophage is the prominent cell, but lymphocytes and neutrophiles are also numerous. The latter is pronounced in liver, kidney, and sometimes the adrenal glands. All the cells of the mesenchyma may take part in this reaction, but mainly those of the lymphocytic series: lymphocytes and plasma cells.

It seems most likely that these alterations are due to a noxe which is spread diffusely by the blood-stream. It must remain for further investigation to determine the nature of this noxe.