Cytological Studies on Heartwater.

I.—The Observation of *Rickettsia ruminantium* in the Tissues of Infected Animals (1).

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Heartwater is defined by Spreull (2) as "a specific febrile disease affecting sheep, goats, and cattle in South Africa and due to an ultra-visible virus transmitted by the bont-tick, *Amblyomma hebraeum* (Koch)."

The name "Heartwater" suggests the most characteristic lesion which is usually met with at autopsy, namely, a variable degree of hydropericardium.

According to Hutcheon (3) the disease was first noticed in South Africa in 1860 coincident with the appearance of the bont-tick, *Amblyomma hebraeum* (4). Owing to the severe economic losses which it has entailed, it has since been the subject of much experimentation by Hutcheon (5) and others after him. Edington (6) was the first to transmit heartwater to susceptible cattle by the inoculation of blood containing the virus. In the same year of 1899, Lounsbury transmitted it to sheep and goats, and also to cattle in 1902. He demonstrated, in a series of contributions (7), that normally the tick does not carry the virus, but that when it is allowed to feed upon a diseased animal it will pick up the virus, carry it through a moulting period (from larva to nymph or from nymph to adult) and transmit it to its next susceptible host. Lounsbury also discovered a most interesting fact, namely, that a larva having obtained the virus and having fed as a nymph upon an insusceptible host, is still capable as an adult of infecting its next host if the host is susceptible. In this respect the virus differs from that of East Coast Fever and also in so far that it is not inherited from one generation to another through the eggs.

The period of incubation is about 14 days, when the disease is induced by ticks, somewhat shorter after blood inoculation. The febrile reaction is characteristic. The temperature usually rises abruptly to between 105° and 108° F., where it remains for several days or as long as a week and then often drops to sub-normal before death. Occasionally there are some nervous symptoms, such as muscular twitching, tetanic seizures, squinting of the eyes, excessive salivation, and galloping movements after the animal has fallen to

the ground. As Theiler (8) showed in 1903, the symptoms can only be explained on the supposition that we have to do with a micro-organism present in the circulating blood. In his opinion the virus does not pass through a Berkefeld or Chamberland filter (9). The mortality is high (over 50 per cent.), and there is no satisfactory method of establishing protective immunity.

At the suggestion of Sir Arnold Theiler that the disease might be due to a Rickettsia, and with the approval of Dr. Simon Flexner, a cytological study of the tissues of affected animals was made by methods which have proved useful in the investigation of Rickettsia. It was Dr. Flexner's belief that information might be secured which would have an interesting though indirect bearing upon the nature of Rocky Mountain spotted fever, typhus fever, and other diseases of man associated with Rickettsia, the viruses of which, like that of heartwater, have thus far resisted all attempts at artificial cultivation and of which arthropods are the vectors.

The Experiments.

The heartwater virus employed had reacted with great constancy in passage through many animals, as indicated in the following history chart.

Infective blood was injected intrajugularily into goats, sheep, and cattle. After a period of inoculation of about 10 days the temperature rose suddenly from 106° to 108° F. and then subsided when the animals usually either died or were killed. In order to have full information regarding them, careful autopsies were made by the veterinary officers on duty (see Table 2). For routine purposes tissues were fixed in Zenker's fluid and were coloured by Giemsa's stain, but other special fixatives and stains were also employed, as will be mentioned subsequently.

Table 1.

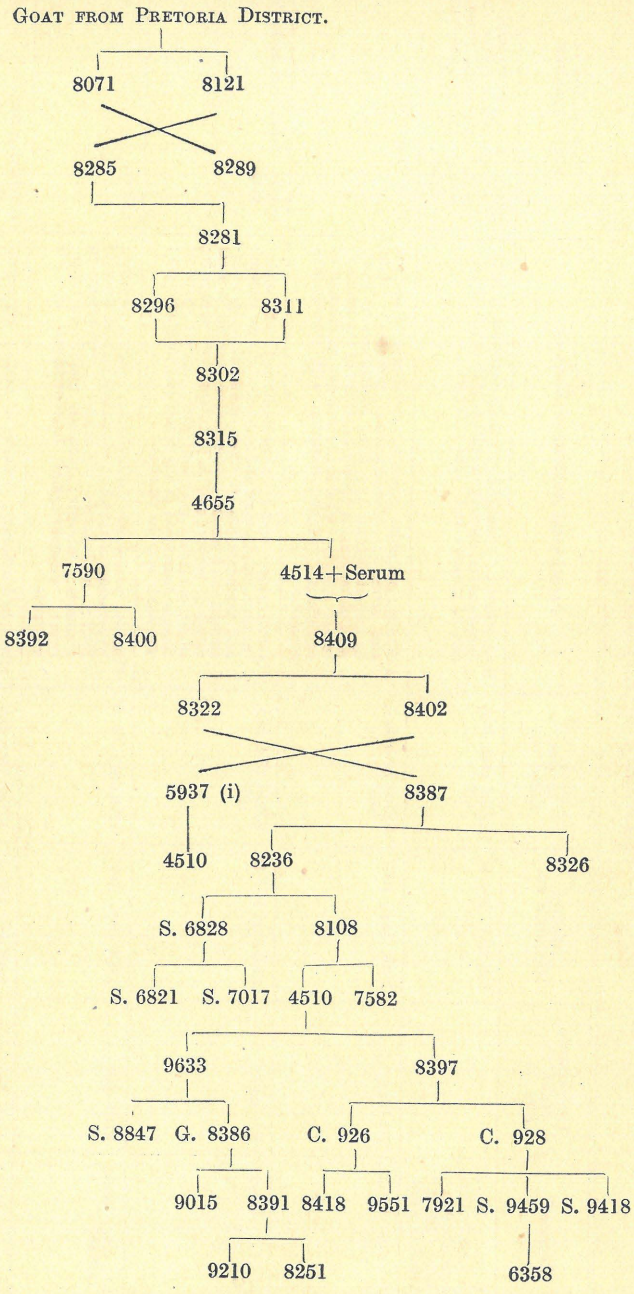
Goat from Pretoria District.
8071
8121
8285
8289
8281
8296
8311
8302
8315
4655
7590
4514+Serum
8392
8400
8409
8322
8402
5937 (i)
8387
4510
8236
8326
S. 6828
8108
S. 6821
S. 7017
4510
7582
9633
8397
S. 8847
G. 8386
C. 926
C. 928
9015
8391
8418
9551
7921
S. 9459
S. 9418
9210
8251
6358

Table 2.

Summary of Experiments.

No.	Received.	Temperature began to Rise on.	Attained Maximum on.	Animal Died or was Killed on.	Pathological Anatomical Diagnosis.	Distribution of Micro-organisms.
G. 4510	10 c.c. blood G. 5937, no reaction: 10 days later 10 c.c. blood G. 8108	—	8th day, 106·8°	Killed 14th day, 103°	Hydrothorax; marked hydropericardium, fatty degeneration, and pigmentation of liver; hyperaemia of kidneys and slight enteritis	Spleen, kidneys, cerebral cortex, cerebellar cortex, midbrain, suprarenal, pancreas, lymph gland, and heart muscle.
G. 7582	10 c.c. blood G. 8108	7th day.....	10th day, 108°	Killed 16th day, 105°	Anaemia; petechiae in mucous membrane of trachea and bronchi; subendocardial petechia; slight hydropericardium and fatty infiltration of liver, with pigmentation chiefly in centre of the lobules; tumor splenis; erosions in mucous membrane of oesophagus; slight infestation with *Haemonchus contortus*	None observed.
G. 8108	50 c.c. blood G. 8236	8th day.....	12th day, 107·2°	Killed 13th day, 107°	Haemorrhages under epicardium and left endocardium, emphysema and oedema of lungs; oedema and pigmentation of liver (infiltration of interstitium and green pigmentation around central veins); tumor splenis; interstitial nephritis; slight catarrhal gastroenteritis; larval cysts of *Taenia hydatigena* in omentum and mediastinum; *Trichuris ovis* in caecum	Spleen, suprarenal and lymph gland.
G. 8236	10 c.c. blood G. 8387	11th day.....	12th day, 108°	12th day, 105·6°	Fibrous pericardial adhesions; tumor splenis; hyperaemia of kidneys; stasis and pigmentation of liver; hyperaemia of lungs; wireworms in stomach; enteritis; parasitic nodules in omentum; subendocardial haemorrhages in left ventricle	Spleen.
G. 8326	10 c.c. blood G. 8387	11th day.....	12th day, 106·4°	Killed 13th day, 105°	Slight hydrothorax; oedema of lungs; pigmentation and enlargement of liver; degeneration of myocardium and kidneys	Cerebral cortex.
G. 8386	10 c.c. blood, G. 9633	10th day.....	13th day, 106·6°	Killed 13th day, 106·6°	Oedema of lungs; areas of fibrosis in liver capsule as a result of perihepatitis chronica tumor splenis; nephritis (?); slight hydropericardium; caseous lymphadenitis of mediastinal lymph glands; *Helminthiasis nodularis* chiefly in large intestine	Kidney, cerebral cortex, midbrain, suprarenal and lymph gland.

TABLE 2—(*continued*).

No.	Received.	Temperature began to Rise on.	Attained Maximum on.	Animal Died or was Killed on.	Pathological Anatomical Diagnosis.	Distribution of Micro-organisms.
G. 8387	10 c.c. blood, G. 8322, G. 8402	7th day.....	10th day, 107°	Killed 12th day, 105°	Hydropericardium; epicarditis fibrosa; oedema of lungs with marked interstitial oedema; chronic cholangitis of intrahepatic bile-ducts; *Stilesia hepatica* in the lumina; tumor splenis; fatty infiltration of kidneys; few *Haemonchus contortus* in abomasum; few foci of coccidiosis and *Oesophagostomum columbianum* nodules in intestine; 2 larval *hydatigena* attached to serosa of bladder	Cerebral cortex.
G. 8391	10 c.c. blood, G. 8386	10th day.....	11th day, 107·6° excised lymph gland under chloral hydrate	Died 17th day, 100°	Interval after death, 7 to 10 hours; hyperaemia of lungs; oedema and emphysema of right auricle and left endocardium; marked tumor splenis	Kidney.
G. 8397	10 c.c. blood, G. 4510	10th day.....	12th day, 106°	Killed 12th day, 106°	Acute anaemia (due to bleeding); slight hydropericardium; degenerative changes in liver and kidneys; tumor splenis; slight nodular worm infestation	Kidney, medulla oblongata, corpus luteum and lymph gland.
G. 8418	10 c.c. blood, C. 926	11th day.....	12th day, 106·4°	Died 14th day, 104·2°	Interval 1½ hour, slight hydrothorax and hydropericardium; petechiae in left ventricle; fibrous adhesions of liver and diaphragm; tumor splenis; parasitic nodules in large intestine	Kidney, corpus luteum.
S. 8847	10 c.c. blood, G. 9633	11th day.....	12th day.....	Killed 13th day, 106°	Slight hydropericardium; slight oedema of lungs; few small calcareous nodules under the capsule; tumor splenis; oedema of kidneys; slight infestation with *Haemonchus contortus*	Kidney.
G. 9633	10 c.c. blood, G. 4510	5th day.....	7th day, 107°	8th day 105·8°	Hydropericardium; subendocardial haemorrhages; tumor splenis; wireworm infestation; old *Oesophagostomum* nodules	None observed.
S. 6821	50 c.c. blood, G. 5937	13th day.....	14th day, 106·8°	Killed 18th day, 105·6°	Slight hydrothorax; slight hyperaemia and oedema of lungs; infiltration and slight pigmentation of the liver; parasitic nodules in liver; subserosal haemorrhages in caecum, *Oesophagostomum columbianum* nodules	Spleen, kidney, cerebral cortex and salivary gland.
S. 7017	10 c.c. blood, S. 6828	6th day.....	10th day, 107·6°	14th day normal, animal recovered; killed on 30th day	Hydropericardium; general anaemia; *Oesophagostomum columbianum* nodules	None observed.

TABLE 2—(*continued*).

No.	Received.	Temperature began to Rise on.	Attained Maximum on.	Animal Died or was Killed on.	Pathological Anatomical Diagnosis.	Distribution of Micro-organisms.
S. 9015	10 c.c. blood, G. 8386	10th day.....	13th day, 107°	Died 14th day	Interval after death, 6 hours. Anaemia; sutured wound in left flank; slight hydrothorax and hydropericardium; hypostasis of right lung; fair number of *Oesophagostomum columbianum* nodules in intestines; larval cysts of *Taenia hy atigena* in omentum	Kidney.
C. 926	20 c.c. blood, G. 8397	11th day.....	13th day, 106°	Killed 14th day, 104°	Acute anaemia due to bleeding; hydropericardium; oedema of lungs; hyperaemia of liver; tumor splenis	Kidney and cerebral cortex.
G. 8251	10 c.c. blood, G. 8391	7th day.....	9th day, 103°	Killed 9th day, 103°	Slight stasis in lungs; ecchymosis in left endocardium	Kidney (only a single clump).
G. 7921	10 c.c. blood, C. 928	8th day.....	12th day 107°	Died 14th day, 103·2°	Hydropericardium; hydrothorax; ecchymosis under left endocardium; petechia and oedema of lungs; stasis and fatty infiltration of liver; marked tumor splenis; slight gastritis	Kidney, ovary, corpus luteum cerebellar cortex, corpus striatum, cerebral cortex. (Organisms not well stained, particularly in kidney and corpus luteum.)
S. 9210	10 c.c. blood, G. 8391	—	—	Killed 11th day, 104·8°	Slight lymphatic hyperplasia of spleen; *Oesophagostomum columbianum* nodules; *Haemonchus contortus*	None observed.
S. 9557	10 c.c. blood, C. 926	10th day.....	13th day, 107°	18th day temperature normal; killed 24th day	Parasitic nodules and fatty degeneration of liver; slight fatty degeneration of kidneys; *Oesophagostomum columbianum* nodules in intestines	None observed.
S. 9459	10 c.c. blood, C. 928	8th day.....	11th day, 107·6°	17th day temperature normal; killed 20th day	Hydropericardium; general anaemia; infarcts in kidneys; parasitic nodules in intestines	None observed.
S. 6358	20 c.c. blood, S.9459, 105°F; temperature fell to 102° 2nd day	12th day.....	—	Killed 13th day, 104°	Tumor splenis; hyperplasia of follicles in spleen; *Oesophagostomum columbianum* nodules; *Ecchinococcus granulosus* in lungs; *Stilesia hepatica* in bile-ducts	None observed.
S. 9418	20 c.c. blood, C. 928	11th day.....	15th day, 107·6°	Died or killed 17th day, 107°	Hydropericardium: slight ascitis; degeneration of myocardium; tumor splenis; swelling and fatty infiltration of kidneys, oedema of caecum; two *Haemonchus contortus*; *Oesophagostomum columbianum* nodules; calcified cyst of *Cysticercus tenuicollis*; few foci of coccidiosis in small intestines	Medulla oblongata, cerebellum.

Controls.

The following controls were examined and proved negative for the micro-organisms under discussion:—

3 Sheep and 3 goats, which were held under careful observation for several days before they were killed in order to make certain that they exhibited no signs whatever of disease. (For this material I am indebted to Dr. Steck.) 12 Sheep which died from bleeding in the preparation of blue-tongue vaccine; 4 sheep killed in an advanced stage of jagsiekte (10); 2 cattle which died of snotsiekte (11); and 1 which died of lamsiekte (12).

The Observation of Micro-organisms in Animals Experimentally Infected with Heartwater.

Fresh blood was examined by direct and oblique illumination and in smears stained in a variety of ways at intervals during the febrile reaction without revealing any trace of micro-organisms, although it is known that the blood is infective at this time. Smears of the several organs taken at autopsy were likewise examined without results. It was only in the fixed and stained tissues that peculiar Gram negative cocci were found first in the spleen and later in the other organs. In making the histological examinations it was often necessary to spend hours going over entire sections with the help of a mechanical stage, as is also the case in the study of Rickettsia in the tissues of animals suffering from Rocky Mountain spotted fever (13). For this purpose a 1.5 mm. apochromatic objective and a No. 8 compensating ocular were employed.

The micro-organisms were most easily detected in the endothelial cells of the capillaries of the renal glomeruli (Figs. 3 and 11) and in the superficial grey matter of the cerebral cortex (Figs. 4, 6 to 8, 10, and 15), which latter, it will be remembered, is the location where Wolbach, Todd, and Palfrey (14) suggest that search be made for the Rickettsia of typhus fever in experimentally infected guinea-pigs. The micro-organisms were also found in the following tissues in order of frequency: spleen (Figs. 1 and 2), lymph glands, corpus luteum, cerebellar cortex, suprarenals, mid-brain, medulla oblongata, ovaris, corpus striatum, salivary glands, pancreas, and heart-muscle. Since they were never found in either the liver or the lungs, it is safe to conclude that in these organs they are either of very rare occurrence or else wholly absent. Testicles were seldom available for examination, as most of the males had been castrated.

The observation of micro-organisms in the tissues was used successfully as a rapid and inexpensive method of diagnosis in the case of sheep suffering from anaplasmosis and dying from a superimposed attack of heartwater.

In this connexion the further observations were made that after death the micro-organisms remained in the tissues for at least 6 hours, as in the case of sheep 9015, which died in the early morning, and in which they were demonstrated in the kidneys, but with considerable difficulty, since they had lost their affinity for basic dyes and were recognizable only by their position and morphology. Micro-organisms were also detected in fragments of spleen taken from this sheep and kept for a further interval of 11½ hours at room temperature, but here also they were seldom of entirely normal appearance. This failure of the micro-organisms to survive for more than a few hours also runs parallel with the rapid loss of infectivity of the blood and tissues after death.

The Association of the Micro-organisms with the Febrile Reaction and with the Lesions.

A very close correspondence was noted between the presence of micro-organisms and the febrile reaction. They were never seen in the incubation period, but invaded the endothelial cells before the maximum temperature was reached and as early as two days after the initial rise in temperature. It was found that the most favourable time to search for them was when the temperature was subsiding, from two to four days after the maximum temperature had been reached. They usually remained, however, for a period of six days, when the temperature had generally fallen to normal. Failure to observe them later than six days after the maximum temperature was in general accordance with the loss in infectivity of the blood when inoculated into susceptible animals.

Goat 9633 seems to have been an exception in so far that no micro-organisms were seen, although it was killed the first day of the decline in temperature. The incubation period in this case was atypical, being reduced to five days, but there was every reason to believe that the animal was actually suffering from heartwater.

Detailed information regarding the relation of the micro-organisms to the febrile reaction has been summarized in Table 3. In the first column, the number of the animal is recorded; in the second, the temperature when it died or was killed; in the third, the time relative to the maximum temperature reached; and in the fourth, the number of organs in which the micro-organisms were found is indicated by plus signs. It will be noted that the number, roughly estimated in this way, was subject to wide variation.

Table 3.

Relation between Febrile Reaction and Number of Micro-organisms.

No.	Temperature.	Days After Maximum Temperature.	Micro-organisms.
	°		
S. 6358	Rising 104	Maximum not reached	−
S. 9210	„ 104·8	Maximum not reached	−
G. 8251	„ 103	„ „ „	+
G. 8397	„ 106	Perhaps maximum	++++
G. 8386	„ 106·6	„ „ „	+++++
G. 8236	Falling 105·6	Same day as maximum of 108	+
G. 8108	„ 107	1 day after maximum of 107·2	+++
S. 8847	„ 106	1 „ „ „ „ 107·1	+
G. 9633	„ 105·8	1 „ „ „ „ 107	−
G. 8326	„ 105	1 „ „ „ „ 106·4	+
C. 926	„ 104	1 „ „ „ „ 106	++
S. 9015	„ —	1 „ „ „ „ 107	+
S. 9418	„ 107	2 „ „ „ „ 107·6	++
G. 8387	„ 105	2 „ „ „ „ 107	+
G. 8418	„ 104·2	2 „ „ „ „ 106·6	++
G. 9721	„ 103·2	2 „ „ „ „ 107	++++++
S. 6821	„ 105·6	4 „ „ „ „ 106·8	++++
G. 4510	„ 103	6 „ „ „ „ 106·8	+++++++++
G. 8391	„ 100	6 „ „ „ „ 107	+
G. 7582	„ 105	6 „ „ „ „ 108	−
S. 9459	Normal	9 „ „ „ „ 107·6	−
S. 9551	„	11 „ „ „ „ 107	−
S. 7017	„	20 „ „ „ „ 107·6	−

The most characteristic lesion invariably associated with the presence of micro-organisms was a marked swelling of the endothelial cells. They were seen within the endothelial cells in dense masses varying from a few individuals up to several hundreds, as is represented in Figs. 1 to 9 and in the photomicrographs, Figs.

10 to 15. The clumps of micro-organisms were always found to be surrounded by a halo of cytoplasm staining very lightly or not at all. Single, isolated micro-organisms were never observed. The endothelial enlargements were found in some cases to be so extensive as to entirely block up the lumina of the capillaries—a condition illustrated in Fig. 1. In other cases portions of the cells containing micro-organisms became detached and passed into the circulation, or the cells ruptured with the discharge of micro-organisms into the blood-stream (Fig. 3). The fact, already mentioned, that attempts by many methods to detect the micro-organisms in the circulating blood during the febrile reaction were unsuccessful, may have been due to the splitting up of these masses of micro-organisms into single individuals which, owing to their spherical form, would be difficult to identify in the living state. Moreover, in fixed preparations the stain would be very easily extracted during differentiation on account of their small size.

Except for the presence of the micro-organisms, the cytoplasm of the endothelial cells showed no deviation from the normal and never contained, in addition, products of the phagocytosis of haemoglobin or fatty inclusions, or any of the basophilic granulations reported in typhus fever.

Neighbouring endothelial cells without micro-organisms appeared to be perfectly normal and were not enlarged. No evidence was found of undue multiplication of the endothelial cells. No thrombosis was noted, and the micro-organisms were never seen in association with any kind of leucocytic infiltration or in any extravascular location. In other words, they seemed to excite no detectable local tissue reaction other than the physical distention of the cells to accommodate them in large number (15).

The Morphology of the Micro-organisms.

The micro-organisms were found to be very uniform coccus-shaped bodies, 0.2 to 0.5 μ in diameter as measured after fixation in Zenker's fluid or Regaud's fluid and coloration by Giemsa's stain, or by any basic aniline dye. Their spherical shape is well shown in Figs. 1 to 6. No agglutinations or marked irregularities in morphology suggestive of intracellular digestion were observed. In a single clump the micro-organisms were, as far as could be ascertained by microscopic examination, of the same size; but adjacent clumps in the same section occasionally differed slightly in the size of the individuals composing them, within the limits specified above, as represented in Fig. 1. The micro-organisms had the appearance of being slightly larger when present in small masses and when not closely crowded together, as in the case of accumulations of many hundreds; but the difference was not sufficiently marked to exclude the possibility that it may have been merely an optical illusion. Occasionally the micro-organisms were observed in diplo-formation (Fig. 11), but this was the exception rather than the rule. No morphological evidence was detected of any multiplicative phase other than this diplo-formation indicating the likelihood of simple division. The shape and size of the micro-organisms remained constant throughout the febrile reaction.

Microchemical Reactions of the Micro-organisms.

The micro-organisms were well preserved after fixation in Regaud's fluid (16) and Zenker's fluid, both with and without acetic

acid, but all the fixatives commonly used for bacteria were suitable. They were coloured deep clear blue by Giemsa's method. Those in diplo-formation showed no trace of red staining material between the two halves, as has often been described in Rickettsia in lice, but sometimes exhibited not too plain halos. They were likewise easily stained by Löffler's methylene blue and other basic aniline dyes. When treated with Unna-Pappenheim's methyl-green pyronin mixture, they usually stained light red, but sometimes acquired a slightly greenish tint, depending upon the method of staining. They were Gram-negative and stained readily with Fuchsin, but did not retain it on differentiation. They were nicely stained with iron haematoxylin, but on differentiation became bleached before the nuclear chromatin and erythrocytes. Some resisted decolorization more than their neighbours and remained jet-black, while the others became light-grey, but this may have been occasioned by irregular washing out of the mordant (iron alum) and may not indicate the existence of a true qualitative difference *inter se*. When autolysis of the tissue was allowed to proceed, their affinity for basic dyes was lost before that of the nuclear chromatin.

Distinction from Normal Cellular Components and the Products of Degeneration and Phagocytosis.

Although the association of the micro-organisms with Heartwater, their morphology and their staining reactions were found to be so definite, it was decided to compare them carefully with normal cellular components and with the products of degeneration and phagocytosis, since, up to the present time, like most Rickettsia, their status as living organisms has not been proved by methods of artificial cultivation.

When they were first detected in the spleen (Figs. 1 and 2), the possibility of confusion with some unfamiliar granular type of blood or bone-marrow cell was carefully considered on account of their characteristic tendency to occur in dense clumps, but this was soon definitely excluded by the observations already alluded to: that they seemed to be identical in three species (i.e. goats, sheep, and cattle); that they occurred only in a certain phase of the febrile reaction, being absent in controls; and that in distribution they were restricted to the cytoplasm of endothelial cells being surrounded by a distinct zone of rarifaction. Another point of distinction was that the micro-organisms were always disposed in clumps to one side of the nucleus. The nucleus was never surrounded on all sides by them, as is usually the case by the specific granules in granular leucocytes. Although a search was made of the bone-marrow, no cells containing granules at all resembling them were found.

Unlike mitochondria, they were readily preserved by Zenker's fluid, containing the usual 5 per cent. of acetic acid. They were basophilic, whereas the mitochondria are acidophilic. In shape also they were different from mitochondria, because no rod-like or filamentous forms were seen. They were present in the cytoplasm in large and compact masses in contrast to the mitochondria which are never so abundant and are not grouped in the same way. Finally, by their restriction to the endothelium they were clearly differentiated from mitochondria and all mitochondrial products (17).

The micro-organisms were also distinguished from the granules of most cells by their uniformity in size and shape, by the fact that

they generally stained clear blue instead of purple by Giemsa's method and similarly by their restriction to the cytoplasm of endothelial cells. They were never found outside the blood-vessels.

Neither were the micro-organisms to be confused with products of the phagocytosis of haemaglobin—an explanation of the nature of Rickettsia advanced by Woodcock (18). In the first place, phagocytosis of fragments of red blood-cells by the endothelium was not a process commonly met with in heartwater. In the rare instances in which it did occur the resultant inclusions took the form of droplets grading from about the size of a red blood-corpuscle down to a few microns in diameter and stained much more strongly than did the micro-organisms with iron haematoxylin. When coloured by Giemsa's method, these inclusions frequently exhibited a yellowish or greenish tinge, due to the superposition of pigment and stain. They were never clear blue, like the micro-organisms, nor were they clumped in so characteristic a fashion.

The micro-organisms stained much the same colour as nuclear chromatin, but were easily differentiated from it or any of its degenerative products. As is indicated by the figures, the nuclei of the endothelial cells containing them seldom revealed any traces of division or degeneration, so that chromatin was not being emitted in a form detectable morphologically. It was to be noted that except for the presence of the micro-organisms the cytoplasm of the endothelial cells showed no microscopically visible modifications. In this respect the condition of the vascular endothelium in heartwater differed sharply from that found in typhus fever as described by Wolbach and his associates (14, 19).

Discussion.

It is interesting to compare the above-mentioned micro-organisms with Rickettsia, but to do so consistently is by no means a simple matter, because there is so little unanimity of opinion as to what distinguishing features Rickettsia actually possess. If the suggestion made in an earlier paper (20), that in the identification of Rickettsia stress may properly be placed upon "the ability of the organisms to lead an intercellular existence, their location in the tissues, their host specificity, their Gram-negative properties, and their bacterium-like morphology" be accepted by others, as it has been by Hertig and Wolbach (21), we are clearly justified in including these micro-organisms in the general category of Rickettsia. These authors, in practice have, however, slightly modified the definition to read as follows:—"Gram-negative, intracellular, bacterium-like organisms found in arthropods." This inclusion of the word "arthropod" necessitates mention of the fact that similar micro-organisms have been found in the insect vector of heartwater (22), so that the Rickettsia under consideration fulfil this proviso also. Discussion in this paper will be limited to the micro-organisms as seen in mammalian tissues.

In a recent critical review of "the position of Rickettsia as an aetiological factor in disease," Arkwright (23) expressed the opinion that Rickettsia are only known to be concerned with three diseases, namely, Rocky Mountain spotted fever, typhus fever, and trench fever. They have been frequently seen in the invertebrate ectoparasitic hosts in association with all three. In mammalian tissues they have also

been reported in Rocky Mountain spotted fever, chiefly by Wolbach (24, 25), and in typhus fever by Kuczinski (26), by Wolbach and Todd (18), by Stevenson and Balfour (27), with certain reservations, and more emphatically by Wolbach, Todd, and Palfrey (14); but it is questionable whether they have been observed in trench fever.

The kindness of Professor Wolbach and of Dr. Nicholson in giving me slides containing the Rickettsia of spotted fever and of Professor Wolbach in giving me tissues from cases of typhus fever, which I have sectioned and stained myself, makes it possible for me to compare these Rickettsia very closely with the micro-organisms in heartwater. For convience, this comparison is given in tabular form. Rocky Mountain spotted fever is listed first as the only disease in which Rickettsia have been proved to be the etiological agents and in which they may invariably be detected in the tissues; then typhus fever, in which Wolbach and his colleagues have presented valuable evidence in favour of Rickettsia as the causative agents; and lastly, heartwater.

TABLE 4.

Comparison of the Rickettsia of Rocky Mountain Spotted Fever and of Tyhus Fever with the Micro-organisms in Heartwater as seen in Mammalian Tissues.

	Spotted Fever.	Typhus Fever.	Heartwater.
Morphology.....	(*a*) Paired 0·2 to 0·3 by 1 μ often surrounded by halo (*b*) Rod-like forms 1 μ in length and often possessed of polar granules (*c*) Rounded forms (2)	(*a*) Ovoid, somewhat lanceolate bodies in pairs. The pairs measure slightly over 1 μ by 0·2 to 0·3 μ (*b*) Smaller coccoid bodies (14)	Very uniform coccoid bodies, 0·2 to 0·4 μ in diameter, rarely in pairs.
Phases of multiplication	None detectable in mammalian tissues other than possibly simple division	The same.	The same.
Microchemical reactions	Best seen after fixation in Zenker's fluid and coloration by Giemsa's stain. Easily stained by basic aniline dyes. Gram-negative and not acid-resistant	The largest paired bodies stain more readily than the cocci and are surrounded by a slight halo (14). Gram-negative and not acid-resistant	Stain more easily, otherwise the same.
Detection.....	Not visible, or visible with difficulty, in living cells unstained	Not reported in living cells	Not seen in living cells.
	Demonstrated in teased cells (24)	Not reported in teased cells	Not as yet seen in teased cells.
	Occurs in blood in exceedingly small numbers [Ricketts confirmed by Wolbach (24)]	Not as yet clearly seen in blood (28)	Observed occasionally within vascular lumen in sections, but not in blood smears.
	Detectable in sections after careful search	More difficult to detect in sections than spotted fever organism	Easier to observe in sections than either of the other.
Position.....	Chiefly within the cytoplasm of endothelial cells, but also in the smooth muscle of the media (14); in endothelial cells which collect in and around the adventitia (24); in vascular lumina, giant cells, liver cells, and mononuclear leucocytes (13)	Restricted to the cytoplasm of endothelial cells (29)	Definitely restricted to the cytoplasm of endothelial cells and to portions of them broken off into the vascular lumen. Occasionally the cells rupture and discharge single micro-organisms into the blood stream.

TABLE 4—(*continued*).

	Spotted Fever.	Typhus Fever.	Heartwater.
Arrangement.....	Single and in clumps of varying size	"Globular massing of organisms is the most characteristic appearance of Rickettsia in human lesions" (14)	Spherical clumps are more marked than in either spotted fever or typhus, and attain a much larger size. They are often multiple, several discrete clumps being present within a single endothelial cell.
Distribution.....	Skin, scrotum, epididymis, testis, thyroid, spleen, lungs, and skeletal muscle (25), and in addition in heart, adrenals, lymph glands, and liver (13)	Skin in 27 cases, kidneys in 2 cases, femoral vein in 1, testes and adnexa in 5 cases, and brain in 7 (14)	Kidney in 11 cases, cerebral cortex in 7, spleen in 4, lymph glands in 4, cerebellar cortex in 3, suprarenals in 3, corpus luteum in 3, mid-brain in 2, medulla oblongata in 2, pancreas in 1, heart-muscle in 1, corpus striatum in 1, ovary in 1, salivary glands in 1.
Association with febrile reaction	Increase in number progressively with development of the lesions from 1st to 5th or last day of fever, when both attained a maximum. Early in reaction, diplobacillary becoming bacillary in later stages (13)	Found in every case (i.e. 25) where post-mortem examination was made before the 13th day of the disease and while the body was in a fresh condition (14)	Found in 16 cases, most frequently in the six-day period after the temperature has commenced to decline (details given in Table 3).
Association with lesions	Direct injury caused by parasite shown by degenerative changes in endothelial cells and smooth muscle cells of media (24)	Similar swelling and degeneration of endothelial cells	Endothelial cells swollen, but in contrast show no sign of degeneration.
	Thrombosis, necrosis, and perivascular infiltration chiefly in subcutaneous tissue associated with the development of a rash	Similar.	No thrombosis, necrosis, or perivascular infiltration, and consequently no rash. The most characteristic lesion is a variable degree of hydropericardium.

In addition to this close resemblance between the three micro-organisms, brief reference may be made to certain points of similarity between the viruses of the three diseases. In each case the virus may be transmitted by the bites of infective insects (or arachnids) or by the inoculation of infective blood in which Rickettsia are not demonstrable or may be seen with difficulty (Rocky Mountain spotted fever). All three occasion a high temperature reaction and a heavy mortality. The virus of heartwater seems to differ from that of Rocky Mountain spotted fever and to resemble that of typhus fever in respect of the fact that it is not inherited through successive generations in the eggs of the invertebrate vector. All three viruses are unfilterable and as yet uncultivatable (30). They do not retain their vitality even under the most favourable conditions *in vitro* for more than a few days, and one attack of the disease confers a permanent immunity or one lasting for several years.

A divergence in clinical symptoms between heartwater on the one hand and spotted fever and typhus fever on the other, is to be expected, because heartwater is by contrast a disease of ruminants

only. In all three, however, functional disturbances of the nervous system are of common occurrence. The absence of a rash in heartwater is probably due to the non-involvement of the cutaneous blood-vessels. It is this property, that the vascular endothelium in all parts of the body retains its normal vitality and is not injured by the action of the virus, which makes heartwater so very favourable for the study of the general problem of the relation of Rickettsia to disease. In heartwater the Rickettsia may be easily studied in endothelial cells which are to all appearances normal except for the mechanical distension which they have undergone to accommodate the Rickettsia in large numbers. Whereas in both spotted fever and typhus fever the blood-vessels are the seat of severe lesions, and the endothelium is much involved. This injury results in the appearance of numerous granules within the endothelial cells which mask the organisms. In consequence of this fact, investigators have been slow to accept conclusions based wholly upon the histological study of Rickettsia, particularly so since the Rickettsia of both spotted fever and typhus have resisted many and repeated attempts at artificial cultivation. Not only is the study of heartwater relieved from this troublesome handicap, but in addition the Rickettsia may be rapidly demonstrated by much more simple methods of technique.

Results.

A Gram-negative, intracellular, coccus-like micro-organism was found in cases of heartwater in the three species which are susceptible to the disease, namely, goats, sheep, and cattle. The presence of this micro-organism was definitely related to the febrile reaction, and it was absent in controls. It probably occurred throughout the body, but was most easily detected in the renal glomeruli and in the small capillaries of the cerebral cortex. The micro-organism was a typical endothelial parasite, being restricted in distribution to the endothelial cells of the smaller blood-vessels and to portions of them which broke off into the blood stream. It was never observed to cause any injury to the endothelial cells other than mechanical distension through accumulation in large densely packed masses which were characteristically spherical. A typical attribute was the presence of several of these masses with the cytoplasm of a single endothelial cell. In view of the association of this micro-organism with heartwater, which is a disease of ruminants, and thus far the only one in which micro-organisms resembling Rickettsia have been reported, the designation *Rickettsia ruminantium* is proposed.

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(10) Jagsiekte is a contagious chronic catarrhal pneumonia of sheep, the causative agent of which is unknown [Mitchell, D. T., Report of the Director of Veterinary Education and Research (Sir Arnold Theiler), 1915, 585].

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(12) "Lamsiekte is a disease of cattle caused by poisoning through the ingestion of a toxin produced by 'an anaerobic bacterium reminiscent of but not identical with *Bacillus botulinus*'" (Sir Arnold Theiler, H. H. Green, and P. J. du Toit, *J. Agric.*, Union of South Africa, 1924, 4).

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(15) The histopathology of heartwater will soon be made the subject of a special paper by Dr. Steck, who has also enjoyed the privilege of working in Sir Arnold Theiler's laboratory.

(16) Four parts of 3 per cent. solution of potassium bichromate and one part of commercial formalin—a fixative intended primarily for the study of Mitochondria.

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(26) Kuczinski, *Centralblatt für Allgem. Path. und Path. Anatomie*, 1919, XXX (quoted from Arkwright, J. A., "Typhus Fever," in Bryam and Archibald: "The Practice of Medicine in the Tropics," Oxford Medical Publications, 1923, III, 2078).

(27) Stevenson, A. C., and Balfour, Andrew, *J. Path. and Bact.*, 1921, XXIV, 289.

(28) According to Wolbach, Todd, and Palfrey (14), Rickett's observation of bipolar micro-organisms in the blood of typhus-fever patients has not been confirmed.

(29) On page 189, Wolbach, Todd, and Palfrey (14) state that "they (the Rickettsia) are found only in the endothelium and never, as in Rocky Mountain spotted fever, in the smooth muscle of the media." On the next page, however, they refer to the finding of Rickettsia in the mononuclear cells of the perivascular nodules. On page 192, the localization of Rickettsia in endothelial cells is again emphasized.

(30) In this respect they apparently differ from—

(a) *Rickettsia melophagi* (Nöller, W., Arch. Schiffs- und Tropen-Hygiene, 1917, XXI, 53);

(b) *Rickettsia Rocha-Limae* (Weigl., R., *Przeglad. Epidemjol.*, 1921, 1, 373); and

(c) *Rickettsia nipponica* (Sellards, A. W., *Am. J. Trop. Med.*, 1923, III, 529).

EXPLANATION OF PLATES.

Plate I.

Micro-organisms in the endothelial lining of small blood-vessels are illustrated in all the figures as seen after coloration by Giemsa's stain. The drawings were made with Zeiss apochromatic objective 1.5 mm., compensating ocular 8, and camera lucida, giving a magnification of 1,940 diameters.

Fig. 1.—Spleen of Goat 4510; fixed in Regaud's fluid. A small branching capillary is shown, the lumen of which is occluded by the enlargement of endothelial cells containing micro-organisms.

Fig. 2.—Spleen of Sheep 6821; fixed in Zenker's fluid. A swollen endothelial cell is illustrated containing a roughly spherical mass of micro-organisms embedded in chromophobic cytoplasm.

Fig. 3.—Kidney of Goat 4510; fixed in Regaud's fluid. A clump of micro-organisms is seen discharging into the blood stream.

Fig. 4.—Cerebral cortex of Goat 8386; fixed in Zenker's fluid. A clump of micro-organisms in the endothelial lining of a small blood-vessel.

Fig. 5.—Kidney of Goat 4510; fixed in Regaud's fluid. An endothelial cell containing two masses of micro-organisms with the lumen (at the base of the drawing) almost blocked. In contact with it is an histogenous mast-cell possessing granules which are of irregular size and shape and have been coloured deep red.

Figs. 6 *and* 7.—Cerebral cortex of Goat 4510; fixed in Zenker's fluid. Two capillaries with endothelial cells containing clumps of micro-organisms surrounded by zones of unstained cytoplasm.

Fig. 8.—The same. A rather large blood-vessel with two endothelial cells in different stages of engorgement.

Fig. 9.—Kidney of Goat 4510. A still larger blood-vessel with engorged endothelial cells, in which the micro-organisms are arranged in multiple sharply outlined clumps, which were found to be very characteristic of heartwater.

Plate II.

The photomicrographs were taken with Zeiss apochromatic objective 3 mm., 1.40 aperture, and compensating ocular 8, giving a magnification of 1,400 diameters. The preparations were fixed in Zenker's fluid and coloured by Giemsa's stain.

Fig. 10.—A small blood-vessel in the cerebral cortex of Goat 4510, in which two clumps of micro-organisms are seen within the cytoplasm of the endothelial cells. The largest is uppermost and extends downward completely occluding the lumen of the vessel. The smaller of the two occupies the next endothelial cell and is slightly lower to the left. It is coloured rather more intensely since the micro-organisms are more closely packed together. Both clumps are encircled by a halo of lightly stained cytoplasm.

Fig. 11.—A clump of micro-organisms within an endothelial cell of a renal glomerulus of Goat 8386. In some places it may be seen that they are grouped in pairs and that each is surrounded by a halo.

Fig. 12.—Four clumps of micro-organisms within an endothelial cell of a capillary between the renal tubules of Goat 4510. The fourth clump on the left is slightly out of focus. The nucleus is flattened and lies just below the micro-organisms.

Fig. 13.—A clump of micro-organisms in an endothelial cell of a somewhat larger blood-vessel of the same kidney.

Fig. 14.—Another group of micro-organisms within an endothelial cell of a capillary of the same kidney.

Fig. 15.—A clump of micro-organisms in an endothelial cell of a small blood-vessel of the cerebral cortex of Goat 8386.

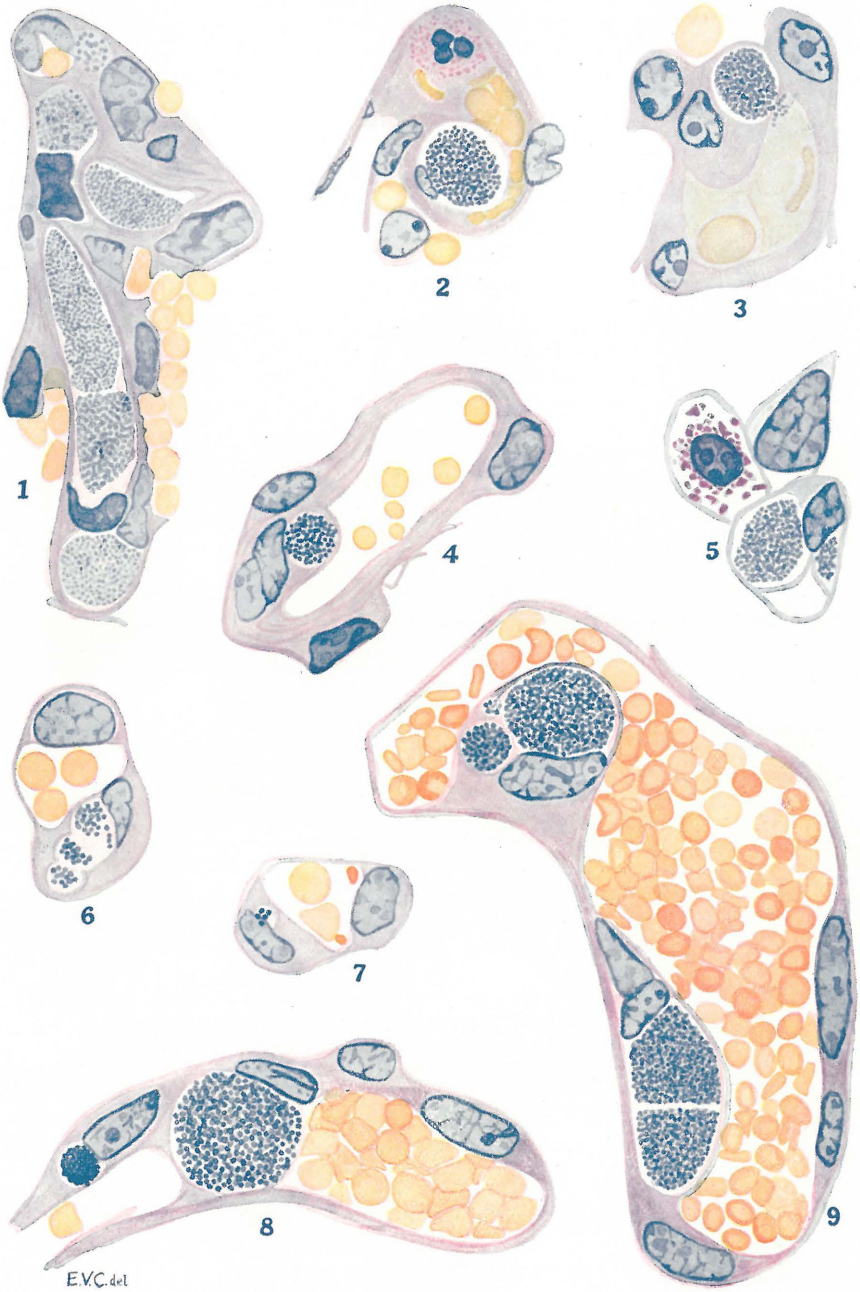

PLATE I.

Heartwater I.] [*E. V. Cowdry.*

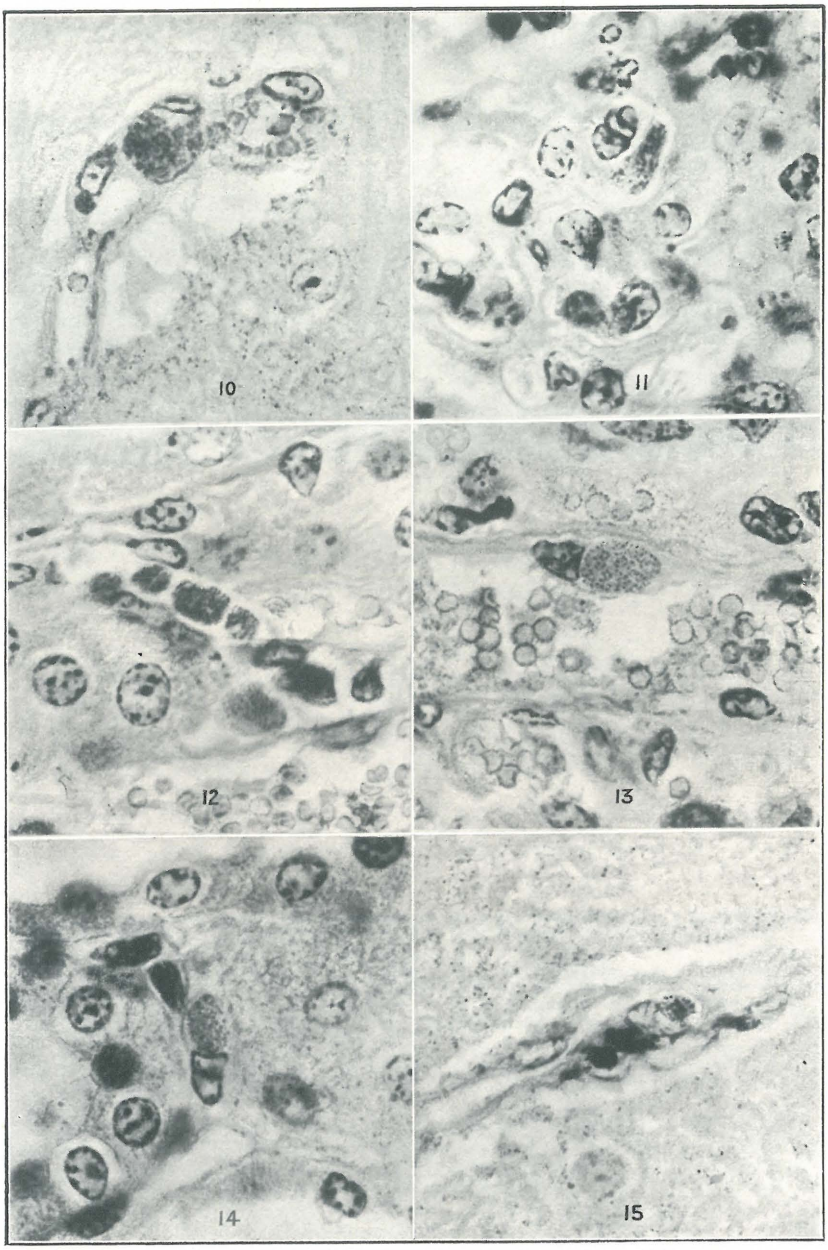

Plate II.] [*E. V. Cowdry.*

HEARTWATER I.