

WESSELSBRON DISEASE. PATHOLOGICAL, HAEMATOLOGICAL AND CLINICAL STUDIES IN NATURAL CASES AND EXPERIMENTALLY INFECTED NEW-BORN LAMBS

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ABSTRACT

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This is a report on the clinical signs of Wesselsbron disease in 37 lambs and the pathology of 4 natural and 12 experimental cases. Generally the symptoms were ill-defined. At autopsy 13 of the 14 lambs revealed a mild to severe icterus and a slight to moderate hepatomegaly with discoloration of the liver. No foci of hepatic necrosis were observed macroscopically and, except for petechial and ecchymotic haemorrhages in the mucosa of the abomasum and generalized lymphadenopathy, no other obvious macroscopic lesions were noted. Perforation of the abomasum occurred in one lamb only.

Microscopy on the liver showed mild to extensive necrosis of the parenchyma. Degenerated and necrotic hepatocytes were diffusely scattered throughout the liver, but no definite well-circumscribed foci of necrosis were seen.

Mitotic figures and hepatocytes with large nuclei indicated that active regeneration of parenchymal cells had occurred in some of the livers. Other changes, for example, Kupffer cell proliferation, sinusoidal leucostasis, bile duct proliferation and infiltration of mononuclear cells in the portal triads, were frequently encountered. Moderate to severe cholestasis was a feature in 66% of the livers examined, while intranuclear inclusions and intracytoplasmic acidophilic or Councilman-like bodies were frequently observed.

A complete haematological study was carried out on 4 experimentally produced cases.

The gross and histopathological lesions in the liver are compared with those of Rift Valley fever in the new-born lamb.

Résumé

MALADIE DE WESSELSBRON. ÉTUDES PATHOLOGIQUES, HÉMATOLOGIQUES ET CLINIQUES SUR DES CAS SPONTANÉS ET EXPÉRIMENTAUX CHEZ DES AGNEAUX NOUVEAU-NÉS

Le présent article est un rapport sur les symptômes cliniques de la maladie de Wesselsbron chez 37 agneaux et sur la pathologie de 4 cas spontanés et de 12 cas expérimentaux. En général les symptômes sont mal définis. A l'autopsie, 13 agneaux sur 14 ont montré un ictère bénin à grave et une hépatomégalie légère à modérée, avec décoloration du foie. A l'examen microscopique, on n'a pas trouvé de foyers de nécrose hépatique; et, à l'exception d'hémorragies pétéchiiales et ecchymosiques dans la muqueuse de la caillette et d'une lymphoadénopathie généralisée, il n'y avait pas d'autres lésions macroscopiques évidentes. Un seul agneau présentait une perforation de la caillette.

L'examen microscopique du foie a révélé une légère nécrose parenchymateuse. Quoique des hépatocytes dégénérés et nécrotiques fussent répandus de manière diffuse à travers le foie, on n'a pas trouvé de foyers de nécrose bien délimités.

Des figures mitotiques et des hépatocytes à grand noyau dans certains foies ont témoigné d'une régénération active de cellules parenchymateuses. On a souvent rencontré d'autres alterations, par exemple la prolifération des cellules de Kupffer, la leucostase sinusoidale, la prolifération de canaux biliaires et l'infiltration de mononucléaires dans les triades portes. Les deux tiers des foies examinés présentaient de façon caractéristique une choléstase modérée à grave, et l'on a fréquemment observé des inclusions intranucléaires ainsi que des granules intracytoplasmiques, acidophiles ou "Councilman-like".

Une étude hématologique complète a été effectuée sur 4 des cas expérimentaux.

On compare les lésions macroscopiques et histopathologiques du foie avec celles que l'on observe dans les cas de fièvre de la Rift Valley chez l'agneau nouveau-né.

INTRODUCTION

Wesselsbron disease (WBD) virus was first isolated during the late summer of 1954/55 from an 8-day-old lamb which originated from the Wesselsbron district in the Orange Free State (Weiss, Haig & Alexander, 1956). New-born lambs in the flock from which this animal was submitted were reported to have died within a week after birth while some of the many ewes which aborted at about full term died. No deaths or clinical signs of disease were noted in wethers or yearlings.

Weiss *et al.* (1956) inoculated 32 pregnant ewes with the virus and experimentally produced one fatal case of WBD in a new-born lamb. Nine abortions (apparently full-term fetuses) and 4 weak lambs, which died or were killed *in extremis* within 24 hours

of birth, resulted from this experiment, and 6 of the pregnant ewes died. A post-mortem examination on the new-born lamb revealed a slight splenomegaly and degenerative changes in the liver and microscopy showed diffuse necrosis of hepatocytes with marked karyorrhexis. Besides infiltration of leucocytes in the liver, the organ showed karyorrhectic changes. Marked hepatomegaly with very pale-coloured and friable livers was seen macroscopically in the ewes while microscopy revealed changes in the liver similar to those in the new-born lamb but with marked fatty changes in addition. No mention was made by Weiss *et al.* (1956) of any lesions in the 4 weak lambs, the fetuses or in the original natural case.

Apart from the original outbreak of WBD described by Weiss *et al.* (1956), the only other case of the natural disease was recorded by Belonje (1958) who reported on a number of cases in sheep in the Middelburg district of the Cape Province in which peracute, acute, subacute, chronic and mild or abortive forms of the disease in older animals were present. According to Le Roux (1959), who studied the histopathology

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from specimens collected during this outbreak, the microscopic changes in the liver of lambs were fairly constant and typical. The main features he mentions are: fatty infiltration, bile pigmentation, necrobiosis of hepatocytes and infiltration of lymphocytes and neutrophils. However, he regarded the pathological changes in the livers of the adult sheep that died during the same outbreak as variable, rather confusing, and difficult to interpret. He pointed out that the disease in the adult animals was complicated with enzootic icterus (De Kock, 1928) and geeldikkop (Theiler, 1918).

Le Roux (1959) augmented this information when he described lesions in 2 experimentally produced cases of WBD in lambs. A 2-month-old lamb showed moderate diffuse fatty infiltration in the liver, odd hepatocytes with condensation and hyalinization of the cytoplasm and karyolysis. Some neutrophils in the process of disintegration were present in the sinusoids. A 5-month-old lamb showed no evidence of necrosis, but rather diffuse fatty changes of liver cells and prominent swollen reticuloendothelial cells, with phagocytosed pigment granules.

During the 1974/75 summer season numerous deaths of new-born lambs occurred in various parts of the Republic of South Africa (RSA), most of which, as reported by Coetzer (1977), were caused by Rift Valley fever (RVF). However, WBD virus was isolated from 2 lambs presented for autopsy from a farm in the Harrismith district of the Orange Free State on which mortalities in new-born lambs were occurring at the time.

The purpose of this communication is primarily to describe the lesions observed in natural and experimentally produced cases of WBD in new-born lambs and thus to augment the knowledge on the pathology of this disease.

MATERIALS AND METHODS

(A) Natural cases

Animals and histopathological specimens

A farmer from the Harrismith district submitted formalin-fixed tissues from 2 lambs for histopathological examination and 2 lambs for autopsy. The formalin-fixed tissues were routinely processed and embedded in paraffin wax and sections cut at 3-4 μm were stained with haematoxylin and eosin (HE). In addition, special staining techniques were applied to the liver sections (Table 1).

TABLE 1 Special stains and histochemical methods applied to the liver

Stained for	Method used
Ferric iron.....	Perl's reaction (BB) (Pearse, 1961)
Lipofuscin.....	Schmorl's technique (Pearse, 1961)
Bilirubin.....	Hall's method (Anon, 1968)
Mucopolysaccharides.....	Periodic acid Schiff (PAS) (Pearse, 1961)
Calcium.....	Dahl's Alizarin method (Anon, 1968)
Acid mucopolysaccharides	Alcian blue (Anon, 1968) Mowry's colloidal iron (Anon, 1968)
Deoxyribonucleic acid....	Feulgen's method (Pearse, 1961)

Autopsies were performed on the other 2 lambs and various tissues collected in 10% buffered formalin for histopathology.

Virology

(i) Virus isolation

The liver and spleen specimens collected from the 2 lambs at autopsy were homogenized and made up to a 10% suspension in buffer lactose peptone (BLP*). Day-old albino mice were injected with 0.03 ml of the suspension by the intracerebral (i.c.) route. The brains of the sick mice were harvested, ground, and suspended in BLP and saline.

(ii) Complement fixation test (CF test)

The CF test was performed according to the method of Cunningham (1960). A 20% mouse brain suspension in saline, prepared from both moribund mice infected with WBD virus and control mice, was centrifuged at 10 000 rpm for 30 minutes. The supernatant was used as a crude complement fixation (CF) antigen at a constant dilution of 1:2 against two-fold serial dilutions of a known positive WBD mouse ascitic fluid.

(iii) Serum neutralization test

The constant serum ten-fold virus dilution method was used (Cunningham, 1960). The virus was tested against known positive sera prepared in sheep to both WBD and RVF.

Equal volumes of serum and virus dilutions were mixed, incubated in a waterbath for 1 hour and used to inject day-old mice by the i.c. route, one family per dilution being used. The MLD₅₀ was calculated by the method of Reed & Muench (1938).

(B) Experimental cases

Animals

As they became available over a period of 2 months, 45 Dorper lambs, 1-3 days old, were placed in various experiments relating to WBD. Eight of these were used as controls and were kept with the infected lambs.

In each case the lambs were kept with their mothers in a reasonably insect-free environment. Blood for serological assay was collected in sterile 10 ml vacuum tubes from both ewes and lambs before experimental infection.

Virus

The WBD virus used as antigen to infect the experimental lambs was isolated from the 2 natural cases received from the Harrismith district. The virus was injected intracerebrally into day-old albino mice, the brains of which were harvested, ground, and made up as a 10% suspension in BLP. Ampoules with 0.5 ml of this suspension were lyophilized and sealed. The titre of this virus was 10^{6.6} MLD₅₀/ml.

This antigen was reconstituted to the original volume with distilled water and each experimental animal was inoculated intradermally over at least 5 different sites in the thigh with 0.5 ml of the suspension.

Temperature reactions and clinical symptoms

After the lambs had been inoculated with WBD virus, their temperature was taken 3 times daily and any clinical signs were recorded. The disease was allowed to follow its natural course.

* BLP: Final concentration of 1% peptone and 5% lactose in 1/10 M phosphate buffer

Pathology

Autopsies were performed as soon as possible after the death of each lamb. A wide range of tissues was collected in 10% buffered formalin and processed as described previously.

Haematological studies

Material was collected from lambs No. 4250, 4248, 4249 and 4251 for haematological studies, while 2 other non-infected lambs, No. 4255 and 4252, were used as controls in this experiment.

Every 24 h for 9 days, one ml of blood was collected in 5 ml vacuum tubes containing K⁺ EDTA from the jugular vein from both the infected and the control lambs.

A Coulter counter model FN, in conjunction with a Coulter diluter and haemoglobinometer, was used to assay all blood samples for white blood cell count (WCC), red blood cell count (RCC), packed cell volume (PCV), haemoglobin (Hb) and the mean corpuscular volume (MCV). Blood smears were prepared for differential counts within one hour of sampling and stained with the Diff-Quick stain*.

RESULTS

Virology

The mice that received the suspension of liver from the field cases became ill on the 9th day. Both CF and neutralization tests performed with brain material harvested from these mice showed that the field virus was identical with the known WBD virus.

The detailed virological results of the experimental lambs obtained during this study will be reported in a subsequent paper.

Temperature reactions, clinical symptoms and mortality

Ten of the 37 lambs inoculated with WBD virus died. In 7 cases a slight to moderate drop in temperature occurred 18–24 h post-inoculation. All the lambs developed a fever and in 73% of cases the febrile reaction was biphasic.

The first peak in temperature occurred 40–45 h after inoculation and temperatures of 40.5–41.7 °C were recorded, a reaction which lasted 2–30 h (average 14 h).

The second temperature reaction which was of longer duration was noted 74–128 h post-inoculation. On the average it persisted up to 153 h and in a few cases even as long as 160–173 h after infection. Body temperatures during this second peak were usually higher than those in the first peak.

While in 20 of the lambs a rise in body temperature was the only clinical sign recorded, the rest developed other clinical signs varying in severity, usually during the second temperature reaction. Seven lambs showed signs of listlessness with an increased respiratory rate. In the fatal cases the symptoms were more severe and included anorexia, staring coats, lethargy, general weakness, sunken flanks and an increased respiratory rate, the respiration being mostly abdominal. In 4 cases the perineum was soiled with soft, bright yellow to orange faeces which in one lamb showed traces of blood. Of the inoculated lambs, 10 died 92–133 h after infection or 18–41 h after the first signs of listlessness.

Of the 8 controls, 3 unexpectedly contracted WBD. Two of them showed severe symptoms and died 186 h and 208 h respectively after the start of the experiment, while the third recovered.

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Gross pathology of field and experimental cases

Thirteen of the 14 lambs autopsied revealed a moderate to severe icterus (Fig. 1).

Liver: In 2 field and in 2 experimental cases the liver was of normal size while in the other animals it was only slightly to moderately enlarged. The colour ranged from yellow to orange-brown and in some cases congested areas were distributed throughout the substance of the liver, giving it a mottled appearance (Fig. 2). The consistency of the liver was softer than normal and friable.

No lesions were seen in the gall-bladder though in some cases it contained almost no bile and in others it was filled with a greenish, watery bile.

Gastro-intestinal tract: The abomasal mucosa of one lamb appeared normal while in 10 lambs a few petechial and ecchymotic haemorrhages were seen. Three experimental animals revealed extensive petechial and ecchymotic haemorrhages (Fig. 3) and the abomasal content in these cases had a chocolate-brown colour (Fig. 4). In one of the latter the abomasum contained four raised necrotic haemorrhagic areas 1–2 cm in diameter which extended throughout the entire thickness of the wall. One of these areas was perforated, allowing the abomasal content to escape into the abdominal cavity (Fig. 5).

Petechial haemorrhages were seen on the serosal surface of the entire gut in a single animal and in 5 cases free blood or blood-tinged intestinal contents was found in the jejunum and ileum, the mucosa appearing hyperaemic.

Spleen: In 2 lambs the spleen was of normal size while in the others it was only slightly enlarged. Capsular petechial haemorrhages were noticed in one lamb only.

Lymph nodes: Both the peripheral and the visceral lymph nodes were enlarged, congested and oedematous. In 6 cases the caudal mediastinal lymph node appeared haemorrhagic while in 2 of these cases the lymph nodes and aorta were surrounded by an oedematous fluid. Petechiae were observed in the cortices of some mesenteric lymph nodes.

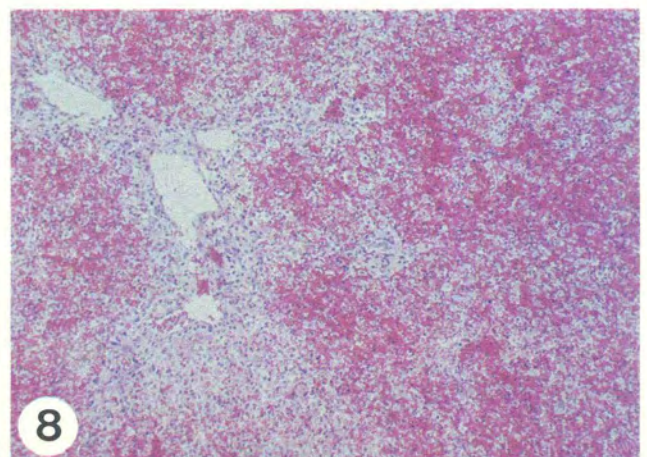
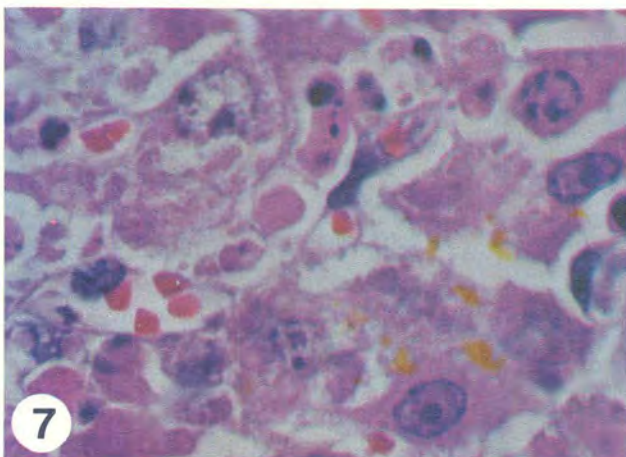
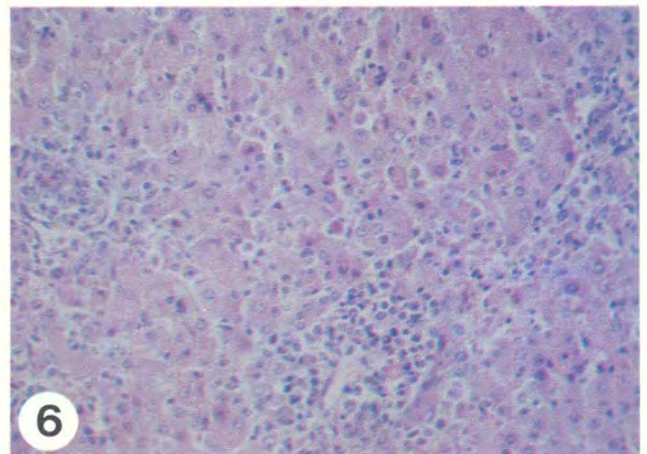
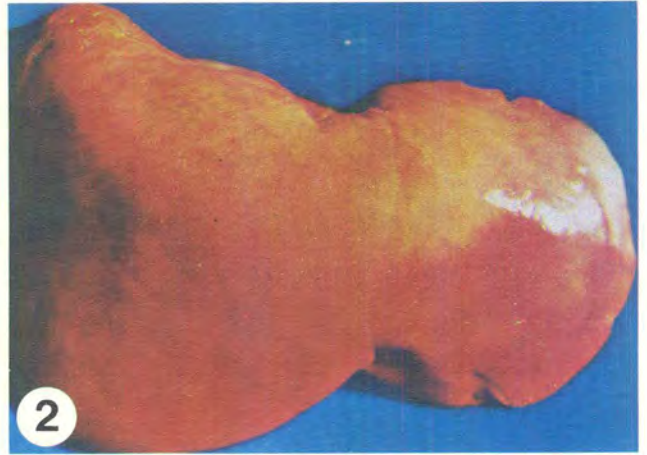
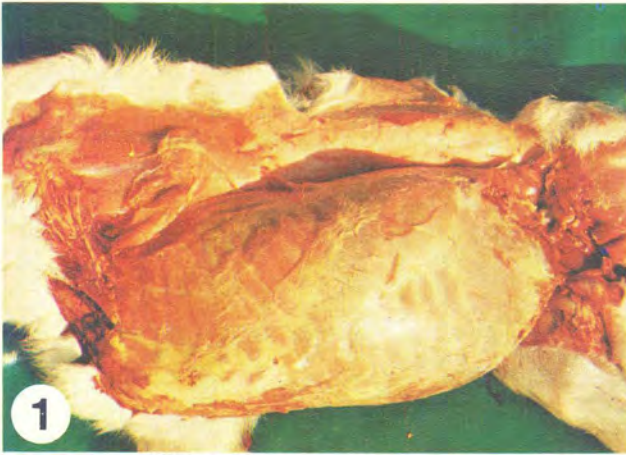
Kidneys: No consistent gross pathological changes were noted in the kidneys. Slight swelling with a yellow-brown discoloration, cortical petechial haemorrhages and severe congestion of the medulla were seen in only 3 of the animals.

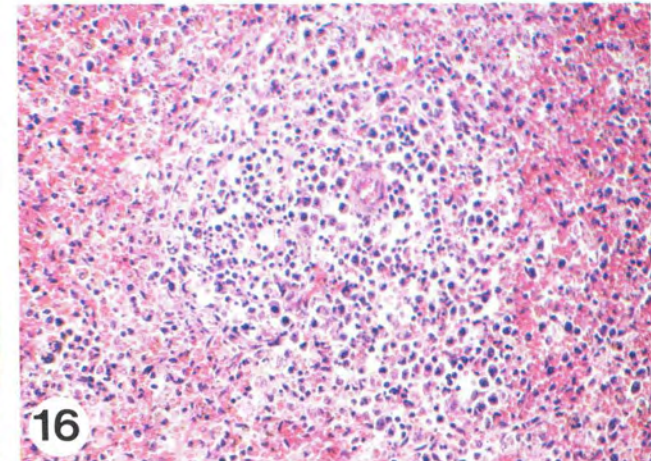
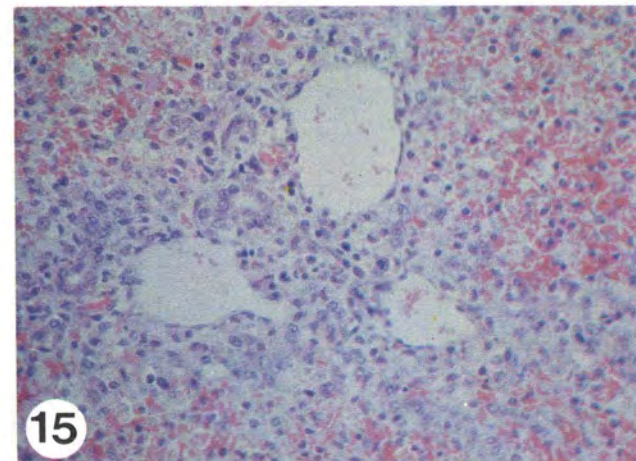
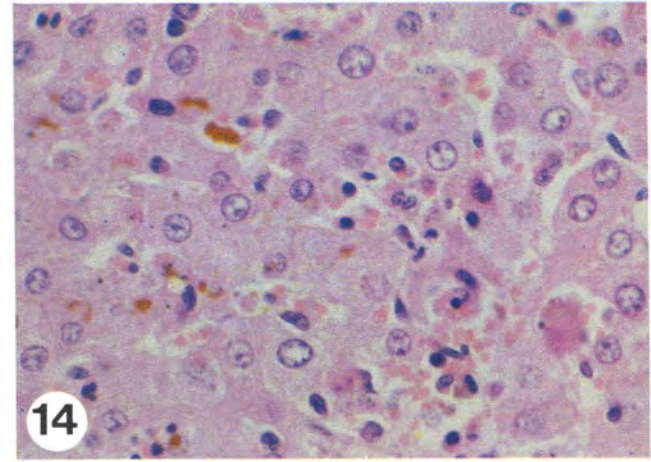
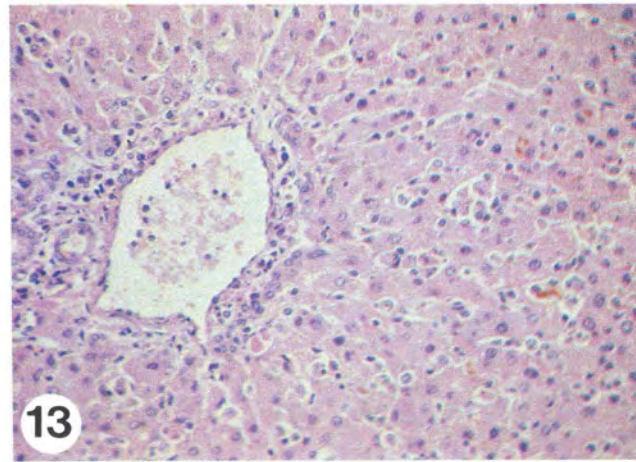
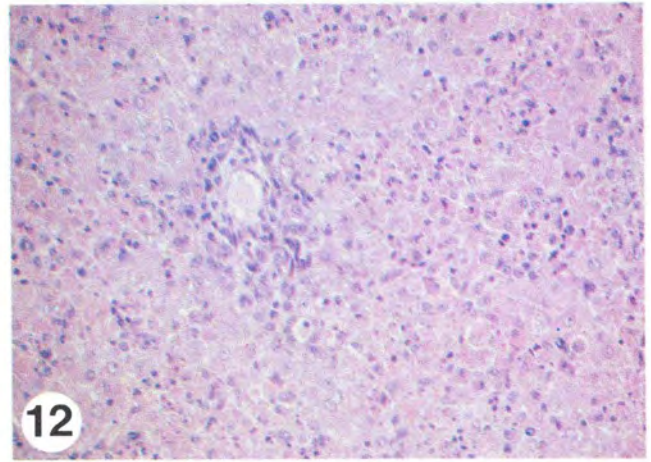
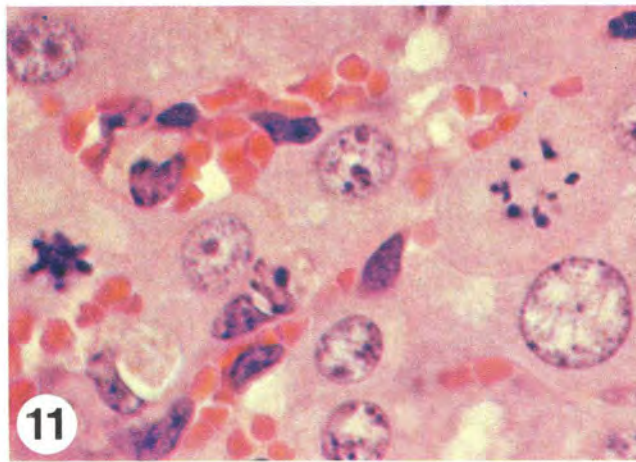
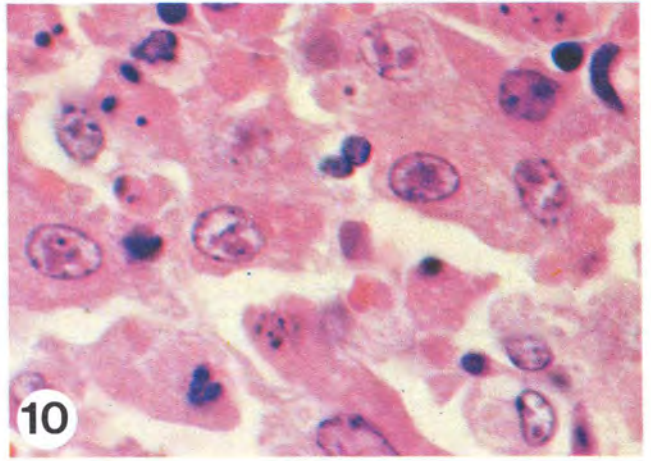
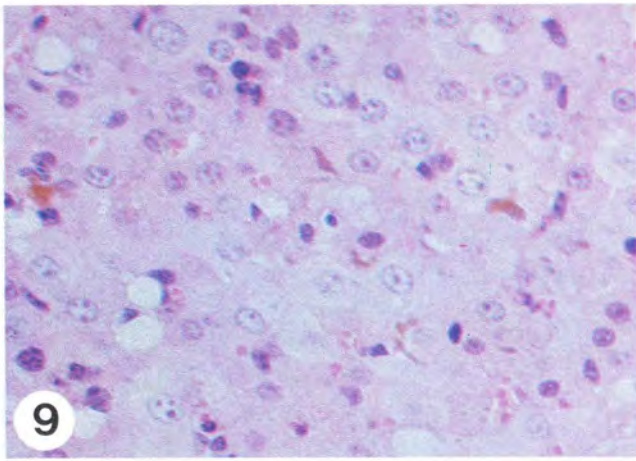
Other organs: Moderate congestion and oedema of the brain were noted in a few animals. Oedema of the lungs and subpleural petechiae were discernible in one lamb and the trachea contained a bright yellow, foamy material. The adrenals were enlarged in one case only. In 2 others, however, petechial haemorrhages were observed in the adrenal cortices. Small subcutaneous and subepicardial haemorrhages were seen in 2 experimental lambs.

Histopathology

Liver: Necrosis, which caused slight to extensive damage of the liver parenchyma, was a constant feature. In 2 natural and 2 experimental lambs the hepatic necrosis was mild and only scattered individual hepatocytes throughout the liver were affected (Fig. 6). More extensive necrosis involving numerous liver cells diffusely throughout the lobules was noted in the other 12 cases (2 natural and 10 experimental).

- FIG. 1 Skinned carcass showing icterus
- FIG. 2 Enlarged yellow-brown liver with congested areas
- FIG. 3 Petechial and ecchymotic haemorrhages in abomasal mucosa
- FIG. 4 Chocolate-brown abomasal content
- FIG. 5 Perforation of the abomasum
- FIG. 6 Scattered individual hepatocyte necrosis and portal reaction. HE \times 200
- FIG. 7 Necrotic hepatocytes resembling acidophilic or Councilman-like bodies. HE \times 1 200
- FIG. 8 Severe necrosis of hepatic parenchyma with marked portal reaction and blood pooling. HE \times 75
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- FIG. 9 Bile stasis and swollen hepatocytes resulting in partial occlusion of the sinusoids. HE \times 500
- FIG. 10 Irregular round intranuclear "inclusions". HE \times 1 200
- FIG. 11 Regenerating hepatocytes, showing enlarged nuclei and mitosis. HE \times 1 200
- FIG. 12 Scattered hepatocyte necrosis and Kupffer cell proliferation. HE \times 200
- FIG. 13 Kupffer cells with yellow-brown pigments and eosinophilic globules. Note portal reaction. HE \times 200
- FIG. 14 Individual hepatocyte necrosis and bile thrombi. HE \times 500
- FIG. 15 Proliferative portal reaction. HE \times 200
- FIG. 16 Malpighian body. Large basophilic cells scattered between pyknotic and karyorrhectic lymphocytes in spleen. HE \times 200





The cytoplasm of the necrotic hepatocytes stained intensely eosinophilic in the HE sections and appeared coagulated. Many of these cells which had a rounded-off appearance and were shrunken were detached from the adjacent hepatocytes and resembled acidophilic or Councilman bodies (Fig. 7). The nuclei were usually no longer recognizable as such and only fragmented and pyknotic chromatin material was seen in these cells. Lysis and disintegration of almost the complete liver parenchyma were seen in 4 of the experimental lambs (Fig. 8). Sinusoidal blood pooling resulting from pronounced hepatocyte destruction gave the organ a very congested appearance (Fig. 8).

In the 4 lambs that showed only slight liver necrosis the sinusoids were partially occluded as a result of swollen degenerated hepatocytes (Fig. 9). Small fat droplets were frequently noted in the cytoplasm of these cells.

The nuclei of degenerative and necrotic parenchymal cells appeared more vesicular, with vacuolation, margination and fragmentation of their chromatin. Frequently enlarged eosinophilic and eccentrically placed nucleoli were present in the vacuolated nuclei. In 10 lambs 1–15% of the affected hepatocytes displayed intranuclear "inclusions", while in 2 lambs about 30–40% of these hepatocytes revealed intranuclear structures (Fig. 10). No "inclusions" could be identified in the other 4 lambs. The "inclusions", stained eosinophilic in the HE sections, were very irregular in outline, inconsistent in shape and varied in size from small granular to more irregular round bodies filling most of the nuclear space.

An increased number of mitotic figures were seen in 3 of the livers (Fig. 11). The cytoplasm of these dividing hepatocytes was ballooned and foamy, and stained less intensely with eosin. Some hepatocytes with obviously enlarged nuclei had a cytoplasm with a similar appearance (Fig. 9 & 11). Occasionally liver cells with 2 nuclei were observed.

Besides the parenchymal changes already described, a fairly marked Kupffer cell proliferation was a constant feature (Fig. 6, 9 & 12). This was sometimes more pronounced around the central veins and was frequently accompanied by a slight mononuclear cell infiltration in this area. Sinusoidal leucostasis, comprising mostly neutrophils, some lymphocytes, histiocytes and plasma cells occurred, many of these infiltrating leucocytes also showing pyknosis and karyorrhexis. The cytoplasm of the proliferating Kupffer cells was in most instances packed with eosinophilic globules which probably originated from phagocytosed necrotic material and caused these cells to bulge into the sinusoids. Some of the Kupffer cell nuclei appeared to be pyknotic (Fig. 6, 10 & 13). The golden-yellow to yellow-brown pigments, present in most of the Kupffer cells, gave a positive reaction for both iron and lipofuscin. The same pigments which were particularly marked in 2 field and 3 experimentally infected lambs, occurred in the periportal hepatocytes and reticuloendothelial (RE) cells in the portal triads (Fig. 11 & 13).

Cholestasis was a conspicuous feature in all the field cases while bile thrombi were also seen in 6 of the 12 livers from the experimental cases (Fig. 9 & 14), even where the necrosis was less severe.

A striking feature in all the livers was the moderate to severe portal reaction. The portal triads were enlarged and appeared cellular as a result of a fairly marked bile duct and histiocytic proliferation (Fig. 13) extending into the periphery of the lobules (Fig. 15).

Occasionally neutrophils, a slight infiltration of lymphocytes and plasma cells were also present.

As a result of the bile duct proliferation, the Kupffer cell proliferation and the sinusoidal leucostasis, microscopy showed that the livers had a decidedly more cellular appearance.

Spleen: Pyknosis and karyorrhexis (Fig. 16) of the lymphocytes in the spleen were present in varying degrees. The field cases and 2 experimental lambs, however, revealed almost no necrosis of the lymphocytes, the destruction of which occurred in both the red and white pulp, though it was more pronounced in the latter.

An interesting observation in 9 cases (1 natural and 8 experimental) was the presence of many large cells with a basophilic cytoplasm, round to lobulated nuclei and large basophilic nucleoli scattered among the fragmented lymphocytes (Fig. 16). They were obvious in the Malpighian bodies and frequently revealed mitotic figures. These large cells are probably germinal blasts (Fliedner, Kesse, Cronkite & Robertson, 1964) taking part in the regenerative process following the necrosis. Reticuloendothelial cell proliferation was commonly observed in the spleen.

Lymph nodes: The karyorrhectic changes of the lymphocytes of the peripheral and visceral lymph nodes corresponded to those described for the spleen. Congestion, oedema and haemorrhages were most obvious in the medulla of the mediastinal lymph nodes. Scattered neutrophils and focal haemorrhages were frequently seen in the cortices and medullae of the other lymph nodes, most of which revealed a marked RE proliferation.

Kidneys: Cloudy swelling and hydropic and fatty degeneration of the convoluted tubules in the cortex were noted in 13 of the lambs, though many of the tubules were partially occluded by the swollen cells. Focal tubular necrosis was seen in 2 cases only. Pyknosis and karyorrhexis in individual glomerular cells, occasionally accompanied by slight hyalinization of the glomeruli, were sometimes observed. Congestion of the medulla was rarely observed.

Adrenals: Individual cell necrosis and haemorrhages with scattered neutrophils sometimes occurred in the adrenal cortex.

Abomasum: In cases where mucosal haemorrhages were noticed macroscopically, microscopy revealed haemorrhages and necrosis of individual epithelial cells between the gastric pits.

Brain and lungs: Apart from congestion and mild oedema of the brain in some of the lambs, no other lesions were found nor was encephalitis seen in any of the brain specimens. Lung oedema was infrequently noticed.

Other organs: No histopathological lesions were found in any of the other tissues examined, that is, in the thymus, pancreas, urinary bladder, salivary glands, skeletal muscle, eyes, costo-chondral junctions, gall-bladder and sternum.

Haematology

The most important changes in the leukocyte series and temperature reactions of the 4 infected and the 2 control lambs are illustrated in Fig. 17–21. Lambs 4250 and 4248 recovered, lambs 4249 and 4251 died while lambs 4255 and 4252 were used as controls.

The values of the different blood constituents are given in Tables 2 and 3.

TABLE 2 Haematological results of the control lambs

Hours post inoculation	Lamb 4252										Lamb 4255									
	0	24	48	72	96	120	144	168	192	0	24	48	72	96	120	144	168	192		
	WCC 10 ⁹	5,3	4,0	4,8	5,9	5,9	4,9	4,8	5,2	5,7	4,0	4,5	5,4	5,9	6,4	5,8	6,1	5,2	4,8	
N %.....	15	14	40	36	41	40	30	42	36	36	53	50	56	46	54	44	50	47		
L %.....	74	78	51	61	54	56	69	57	60	59	43	35	43	49	40	52	48	48		
M %.....	10	7	9	3	1	4	1	1	1	3	2	11	1	2	5	2		2		
E %.....	1	1			1					2	1	1		2	1	2	2	2		
B %.....					2						1			1				1		
RCC 10 ⁶	9,86	9,08	8,34	8,48	8,49	8,70	9,0	8,75	8,15	7,68	7,58	7,97	7,92	7,70	7,16	7,35	7,07	8,15		
PCV.....	31,5	28,7	28,5	29,8	29,4	28,2	28,9	28,2	29,0	26,2	25,2	24,9	25,7	24,7	24,0	25,0	24,2	26,5		
Hb mg%.....	14,9	13,3	13,1	13,1	13,5	12,7	12,9	12,5	12,4	12,1	11,8	11,4	11,6	11,2	11,1	11,1	10,6	11,0		
MCV.....	33,0	32,5	33,0	33,5	33,0	31,5	32,0	32,0	32,0	32,0	31,5	31,5	32,5	32,0	31,0	31,5	31,5	32,0		

TABLE 3 Haematological results of lambs infected with Wesselsbron disease virus

Hours post inoculation	Lamb 4250										Lamb 4248										Lamb 4251						Lamb 4249									
	0	24	48	72	96	120	144	168	192	0	24	48	72	96	120	144	168	192	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72	96	
	9,5	6,8	5,8	6,7	5,2	4,0	4,5	4,9	7,0	9,5	6,1	6,2	8,0	6,0	5,0	6,7	5,7	7,0	6,5	3,9	2,0	6,6	3,1	6,7	5,7	8,1	5,6	6,5	3,9	2,0	6,6	3,1	6,7	5,7	8,1	5,6
WCC 10 ⁶	45	49	95	70	56	46	21	22	25	54	50	64	44	76	59	42	38	31	52	47	65	81	48	52	61	87	88	52	47	65	81	48	52	61	87	88
N %.....	43	50	4	25	40	51	76	70	65	44	44	43	49	23	41	54	55	65	46	52	28	16	50	35	28	7	6	46	52	28	16	50	35	28	7	6
L %.....	9	1	1	3	4	1	2	3	7	3	4	3	5	1		2	7	3	1	1	7	3	18	12	10	5	6	1	1	7	3	18	12	10	5	6
M %.....	3					2	1	4	3	1	1					1		1	1	1	1		1	1	1	1		1	1	1		1	1	1	1	
E %.....				2				1					1			1																				
B %.....	9,08	8,24	8,51	8,04	8,58	7,46	7,76	7,49	8,12	9,6	10,66	8,52	9,58	9,53	8,85	9,11	8,93	8,92	9,57	9,02	7,8	8,79	9,06	7,48	6,92	7,14	7,6	9,57	9,02	7,8	8,79	9,06	7,48	6,92	7,14	7,6
RCC 10 ⁶	28,5	27,2	26,8	27,8	29,9	25,3	26,3	26,0	26,9	32,0	33,5	28,6	30,6	29,0	27,3	28,4	27,9	30,9	32,1	27,9	26,6	30,6	28,0	25,2	21,9	23,2	24,7	32,1	27,9	26,6	30,6	28,0	25,2	21,9	23,2	24,7
PCV.....	13,3	12,8	12,1	12,7	12,7	12,1	12,1	11,8	11,6	14,6	14,0	12,9	13,4	12,9	12,7	12,9	11,9	13,2	14,9	14,9	12,0	14,1	13,2	11,9	10,1	10,2	11,3	14,9	14,9	12,0	14,1	13,2	11,9	10,1	10,2	11,3
Hb mg%.....	32,5	31,0	32,0	33,0	33,5	31,0	32,0	32,0	33,0	32,5	31,5	32,0	32,0	32,0	30,5	31,5	31,0	32,5	32,5	31,5	32,0	33,0	31,5	32,0	32,0	32,5	32,5	32,5	31,5	32,0	33,0	31,5	32,0	32,0	32,5	32,5

WCC=White blood cell count
 N=Neutrophils
 L=Lymphocytes
 M=Monocytes
 E=Eosinophils
 B=Basophils
 RCC=Red blood cell count
 PCV=Packed cell volume
 Hb=Haemoglobin
 MCV=Mean corpuscular volume

Recovered cases

Lamb 4250: Lamb 4250 showed a sudden increase in the neutrophil percentage 24–48 h after infection (Fig. 17) and this was accompanied by a corresponding decrease in the percentage of lymphocytes. These haematological changes preceded the first temperature peak by 5–11 h. The neutrophilia and lymphopenia were accompanied by a gradual drop in the total WCC with a slight upward inclination 48–72 h after inoculation. The WCC dropped to its lowest level 120 h after infection. A lymphocytosis developed during convalescence while the neutrophil percentage dropped. The monocyte and eosinophil percentages remained within normal limits throughout the course of the disease.

This lamb showed no signs of illness other than a high temperature.

Lamb 4248: The haematological picture corresponded basically to that of lamb 4250 except that the neutrophilia and lymphopenia peaks were reached 96 h after infection (Fig. 18).

This lamb also showed no evidence of illness apart from a fever.

Fatal cases

Lambs 4251 and 4249: Lambs 4251 (Fig. 19) and 4249 (Fig. 20) died 109 h and 133 h after infection, respectively. As in the recovered cases, neutrophilia and lymphopenia developed during the acute phase of the disease. The sudden rise of the WCC in lamb 4251 shortly before death was an interesting phenomenon since it was associated with a dramatic increase in the percentage of neutrophils.

The WCC of lamb 4249 did not drop but rather increased, although there was a slight decrease just before death. As the WCC increased, the neutrophil reached the highest and the lymphocyte the lowest percentage recorded.

Controls

Lambs 4255 and 4252: The haematological values of both lambs 4255 and 4252 were almost the same but only that of lamb 4255 is illustrated (Fig. 21).

Other haematological changes

Compared with the 2 control lambs (Table 2), the infected lambs showed a slight to moderate decrease in the RCC, PCV and Hb (Table 3). A dramatic drop in the RCC and PCV 48 h after infection was seen in the 2 fatal cases (lambs 4251 and 4249). The MCV ranged within normal limits.

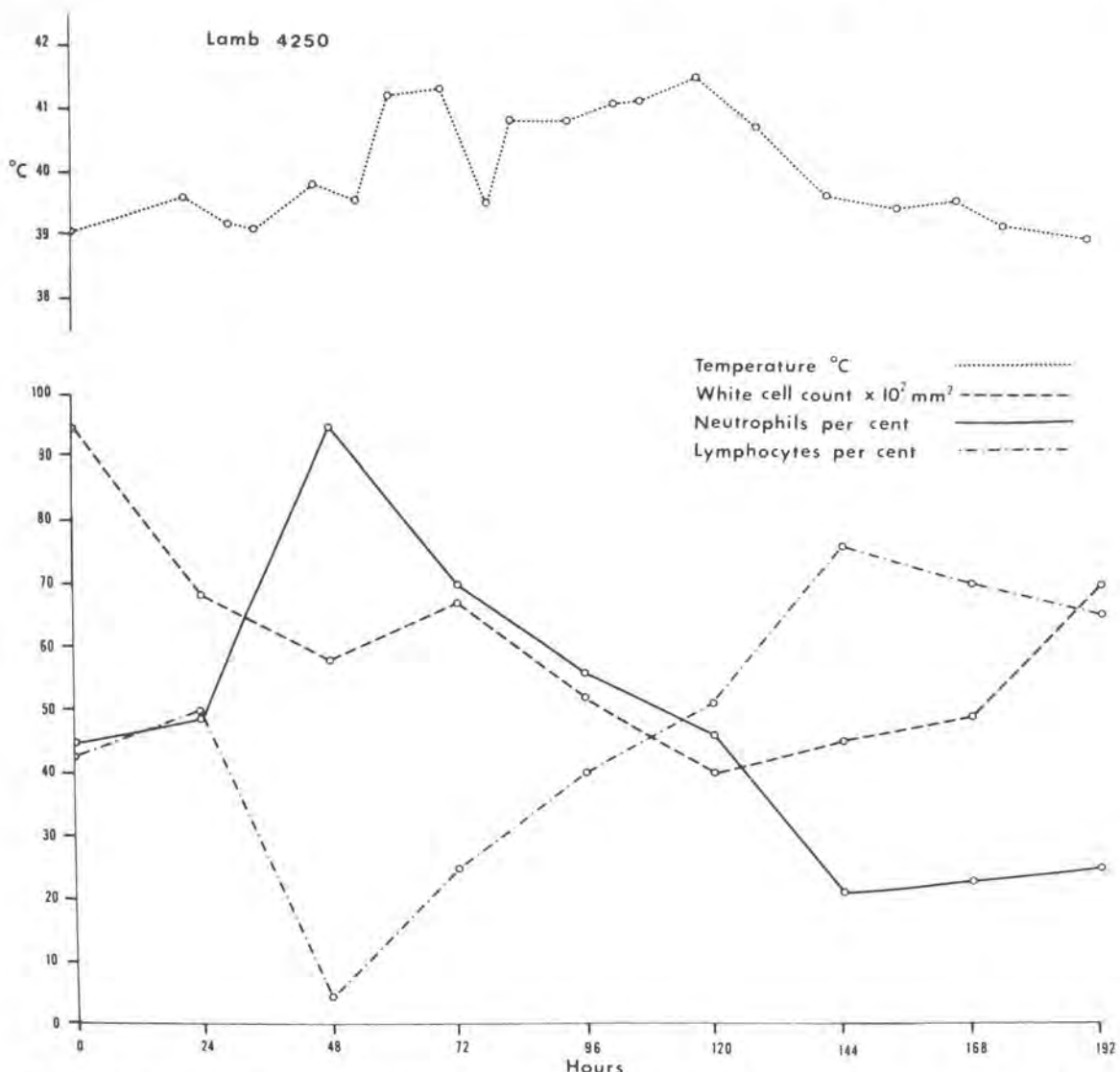


FIG. 17 Changes in the WCC, neutrophil and lymphocyte percentages in lamb 4250

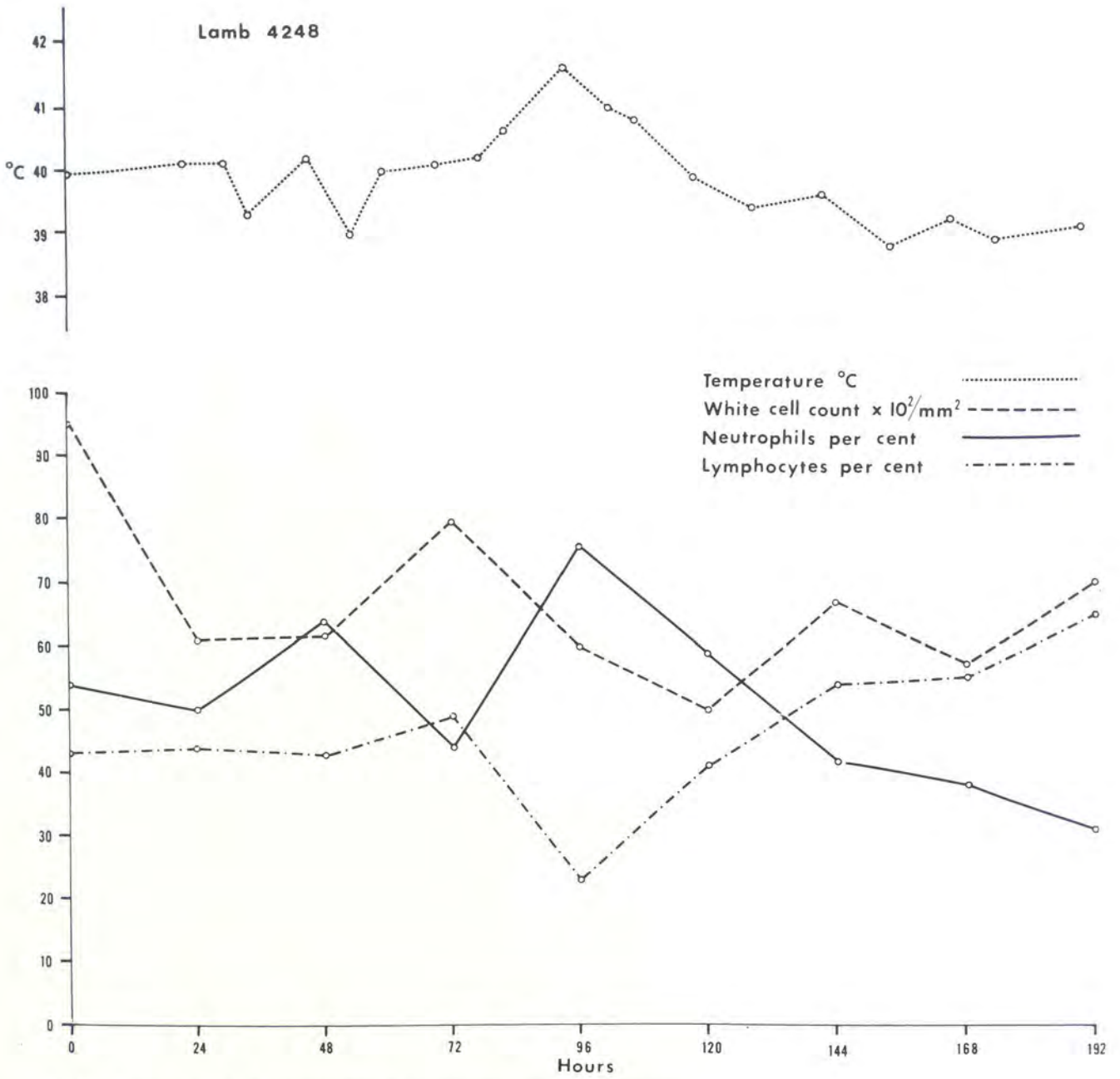


FIG. 18 Changes in the WCC, neutrophil and lymphocyte percentages in lamb 4248

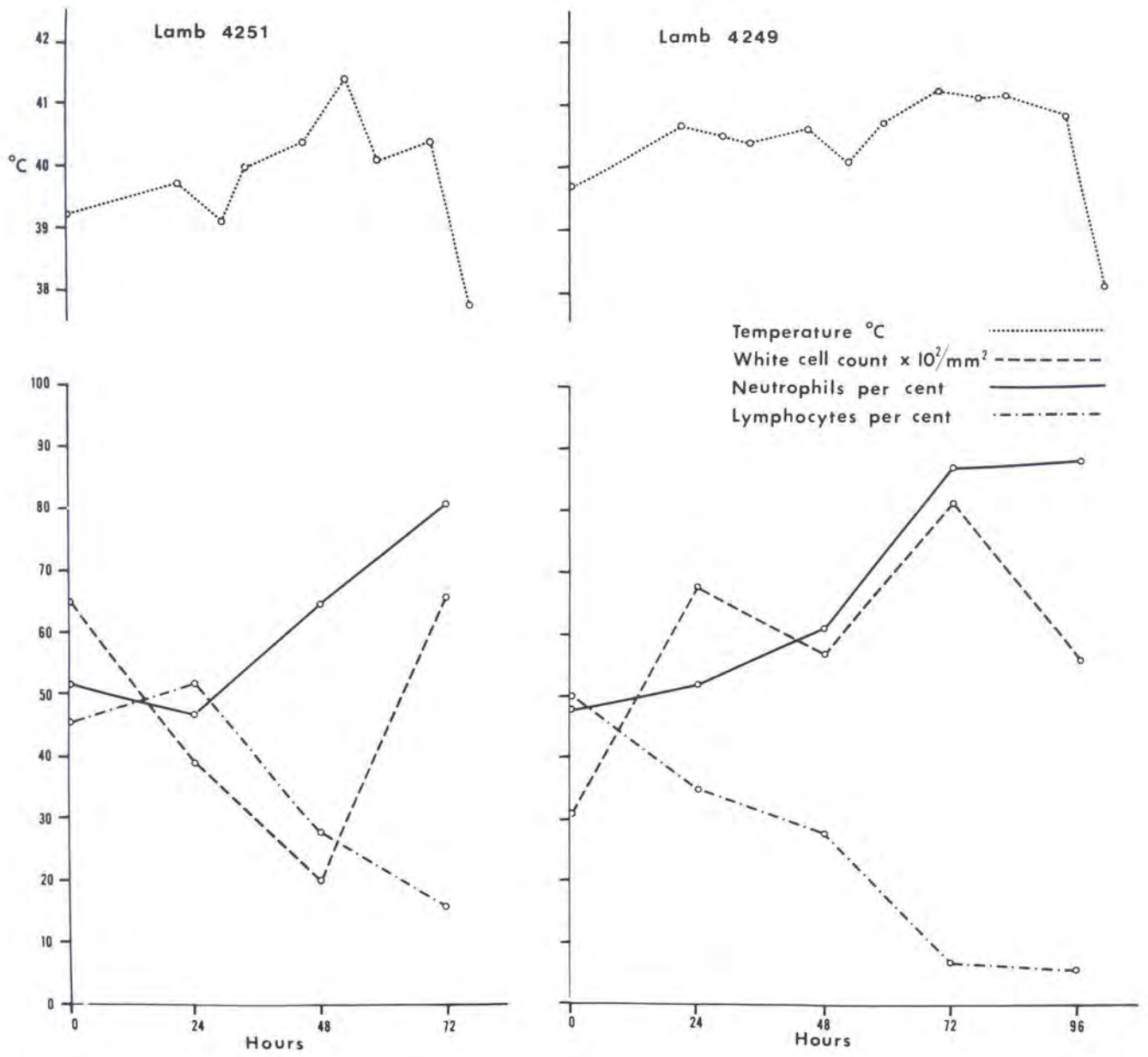


FIG. 19 & 20 The WCC, neutrophil and lymphocyte percentages in the fatal cases, lambs 4251 and 4249

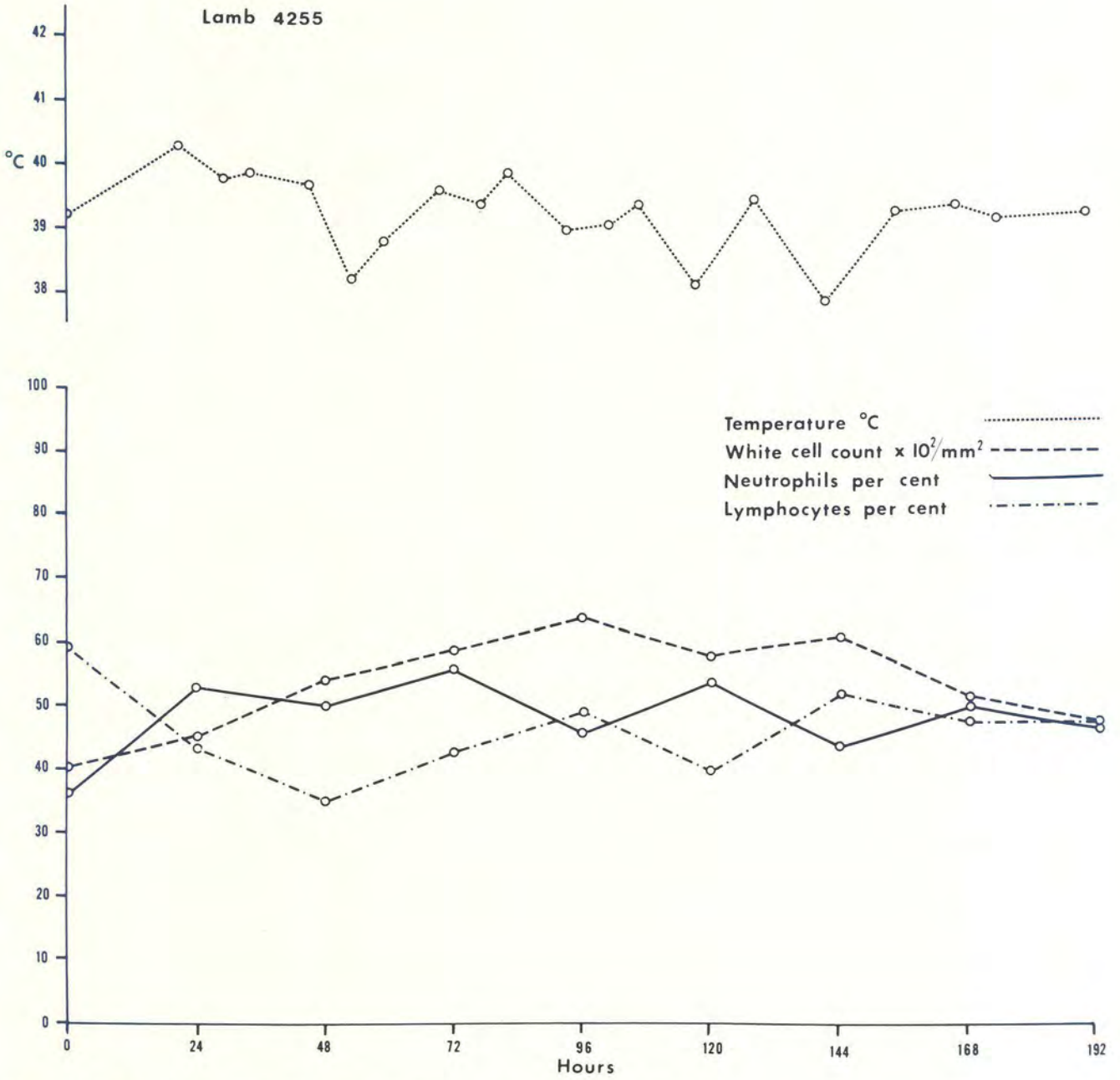


FIG. 21 The WCC, neutrophil and lymphocyte percentages in the control lamb 4255

DISCUSSION

The epidemiology of WBD is very similar to that of RVF and both diseases usually manifest themselves after abundant rains bring about an increase in the mosquito population. It has been shown that *Aedes caballus* (Kokernot, Paterson & De Meillon, 1958) and *Aedes circumluteolus* (Muspratt, Smithburn, Peterson & Kokernot, 1957; Smithburn, Kokernot, Weinbren & De Meillon, 1957) can transmit WBD virus. Optimum epidemiological conditions were prevalent during the summer months of 1974-1975 with the result that both diseases were diagnosed in the Republic.

It would appear that WBD virus is widespread in southern Africa and that many animal species are susceptible to this disease. Positive serum neutralization reactions against WBD have been described in sheep and cattle from countries such as Malawi, Rhodesia and Zambia (Weiss *et al.*, 1956). Serological evidence of the disease was also found in sheep and wild ruminants in Chad and Cameroon in northern Africa (Maurice, 1967). Smithburn *et al.* (1957) demonstrated positive titres against WBD virus in cattle, sheep, goats, dogs, donkeys and several species of wild birds in Tongaland, while Davis (1957) reported on 10 species of small wild mammals with positive titres from the same area. In the RSA Weiss *et al.* (1956) recorded serological evidence of the disease in sheep and cattle in the Wesselsbron, Wolmaransstad, Knysna and Johannesburg districts. Haemagglutination inhibition antibodies to WBD virus were also found in frugivorous bats (*Pteropus rufus*) in Madagascar (Coulanges, Robin, Le Gronidec, Mayoux & Bordahandy, 1974).

Apart from Smithburn *et al.* (1957), who were the first to isolate WBD virus from a naturally infected human being, various authors have also described the clinical symptoms of the disease in man (Weiss *et al.*, 1956; Smithburn *et al.*, 1957 and Heyman, Kokernot & De Meillon, 1958). Serological studies done by Kokernot, Smithburn, Gandara, McIntosh & Heyman (1960) amongst the inhabitants of Mozambique and Tongaland in 29 different localities from each of which blood was collected from 30 donors, provided only 4 sera without neutralizing antibodies against WBD virus. Rodhain, Ardoin, Metselaar, Salmon & Hannoun (1975) demonstrated a positive titre against WBD virus in the ethnic groups living in the basin of Lake Rudolf in Kenya.

Despite the serological evidence of the widespread occurrence of WBD virus in southern Africa, the disease has a very low incidence and appears to exist in an enzootic form over the greater part of the sub-continent. No further outbreaks of WBD were recorded after the original emergence of the disease described by Weiss *et al.* (1956) and the cases recorded by Belonje (1958). It is quite evident, however, that Belonje (1958) confused WBD with enzootic icterus (De Kock, 1928) and geeldikkop (Theiler, 1918) in adult sheep, as Le Roux (1959) pointed out. No material from new-born lambs was studied in the 1958 outbreak and Weiss *et al.* (1956) made mention only of one experimentally produced case in a new-born lamb. The present report is the first detailed description of the lesions in natural and experimental cases of WBD in new-born lambs.

Weiss (1957a) not only stated that the distribution and epizootiology of WBD and RVF appear to be similar but he also pointed out the remarkable resemblance between the clinical manifestations and

pathological anatomy of these two diseases. Both diseases give rise to outbreaks of abortions in ewes and mortality in new-born lambs, the most consistent lesion occurring in the liver. From the results of this and previous studies (Coetzer, 1977) it is apparent that haemorrhages in the mucosa of the abomasum, icterus and generalized lymphadenopathy may be seen in both diseases. Furthermore the possibility of intercurrent infections under field conditions cannot always be excluded.

However, in the new-born lamb, there seem to be definite differences in the pathology of the liver in these two diseases. The most important diagnostic gross lesion in RVF is the constant presence of scattered foci of necrosis 1-2 mm in diameter throughout the liver (Daubney, Hudson & Garnham, 1931; Findlay, 1932; Schulz, 1951; Easterday, McGavran, Rooney & Murphy, 1962; Coetzer, 1977) whereas macroscopically perceptible necrotic foci in the liver have not been described in WBD by previous workers (Weiss *et al.*, 1956; Weiss, 1957b; Le Roux, 1959), neither were any found in the material examined during the present study. The liver in cases of WBD showed a more intensely yellow to orange-brown discoloration but there is no record in WBD of the obvious haemorrhages frequently present in the liver in RVF.

Cases of WBD studied microscopically here failed to reveal lesions in the livers resembling the prominent, well-defined primary foci of coagulative necrosis which characterize RVF. Although severe hepatic necrosis was encountered in 4 of the WBD lambs, it was never focal in nature and consisted of individual necrotic hepatocytes scattered throughout the lobules. There was little or no evidence of Kupffer cell proliferation in the new-born lamb with RVF and the portal reactions were minimal, but the latter changes were seen in all the cases of WBD studied here. A particularly prominent distinguishing feature in WBD was the phagocytized cellular debris in the proliferated Kupffer cells, but no evidence of hepatocyte regeneration as indicated by mitotic figures and of enlarged nuclei as seen in some of these cases of WBD has been reported in RVF.

Icterus, a fairly consistent gross finding in WBD, occurred in 13 of the 14 lambs autopsied. Bile stasis, visible microscopically, was a frequent and prominent feature in this disease. Icterus seems to be less marked in RVF in the new-born lamb (Coetzer, 1977), while bile thrombi are also less common in the latter disease (Easterday *et al.*, 1962; Coetzer, 1977).

Whereas the RVF mortality rate in experimental and naturally infected new-born lambs could be 90% and over (Daubney *et al.*, 1931; Findlay, 1932; Coetzer, 1977), the WBD mortality rate in lambs in this study was much lower (29%). A rapid massive necrosis of the liver takes place in new-born lambs with RVF, and as a result these animals usually die peracutely from acute liver failure and shock. The necrotic process in WBD in the new-born lamb is neither as severe nor as destructive as in RVF and its apparently slower, more progressive character results in a lower mortality.

The diffuse nature of the necrotic process in WBD affecting isolated hepatocytes throughout the lobule, the karyorrhectic changes and the leucostasis as reported by Weiss *et al.* (1956) and Le Roux (1959) were confirmed in the present study. We also confirmed the description given by Le Roux (1959) of the phagocytosis of cellular debris by Kupffer cells, the

proliferation of these cells, the pigmentation of the liver, bile stasis and intranuclear inclusions, and his description of the haemorrhages in the intestine and diminution of the cellular content of the Malpighian bodies of the spleen. None of the earlier authors made any mention of the proliferative portal reaction which occurred in all the cases of the present study, although this microscopic lesion serves as one of the main features by which RVF can be distinguished from WBD. The regenerative changes as indicated by the increased mitotic figures and enlarged hepatocyte nuclei, seen in the present material, have likewise not been reported previously.

Basson, Morgenthal, Bilbrough, Marais, Kruger & Van der Merwe (1969) described pigments in the liver and various other organs of normal new-born lambs. According to their findings, some of the pigment was a lipoproteinaceous meconial bile pigment that changes both in colour and in biochemical nature with time, eventually staining brown in HE sections and giving a positive reaction with the Schmorl's technique for lipofuscin. In addition to these pigments, the livers of lambs dying from WBD also contained pigments which originated from bile stasis. These livers were more intensely pigmented as a result than those of unaffected lambs.

The only other fairly consistent finding was the scattered haemorrhages in the abomasal mucosa. This was very extensive in 3 of the lambs and in one of them the abomasal wall was perforated. Van der Linde (1953) also reported perforation of the abomasum and the caecum in fatal cases of RVF in lambs.

An interesting observation was that 3 of the control lambs contracted the disease spontaneously. In 2 of these cases contamination with infected blood could have played a role as these lambs were bled at the same time as the infected lambs. A separate needle and vacuum tube were used for each lamb, but not a new pair of protective rubber gloves. The other control lamb that became infected was not bled, neither were the infected lambs with which it was in contact.

These animals were handled only at the time when temperatures were taken. However, as this lamb shared its mother with its twin which had been inoculated with WBD virus, the possibility exists that the control lamb became infected by the aerosol route or through the oral mucous membrane when feeding at the same teat as its twin. Weiss *et al.* (1956) reported that not only laboratory workers contracted WBD through handling infected material, but that infant mice can also be infected by aspiration following intranasal instillation.

The leukopenia described in lambs and sheep with RVF (Easterday, Murphy & Bennett, 1962) and bluetongue virus (Luedke, Browne, Jochim & Doyle, 1964) was also a feature during the acute stage of WBD in the 2 lambs that survived the infection. Leukopenia has been reported with other viral diseases, for example, in pigs infected with hog cholera (Dunne, 1963), in cattle with virus diarrhoea (Baker, York, Gillespie & Mitchell, 1954), in cats with panleukopenia (Lawrence, Syverton, Shaw & Smith, 1940), in dogs with distemper (Lauder, Martin, Gordon, Lawson, Campbell & Watrach, 1954) and infectious canine hepatitis (Smith, 1951).

In contrast to the 2 lambs that recovered, the 2 that succumbed revealed a neutrophilic leukocytosis before they died, the latter changes being accompanied by

lymphopenia. However, this lymphopenia was masked by a marked neutrophilia which resulted in a gradual rise in the WCC. It is of interest to note that Mauser & Jones (1943) made a similar observation in respect of leukocytosis, mainly due to a neutrophilia, in uncomplicated cases of equine influenza.

The neutrophilia in WBD probably develops as a secondary effect of stress (Schalm, Jain & Carroll, 1975) and in response to the liver necrosis.

Lymphopenia, a consistent finding during the early stages of WBD, might be an expression of the widespread pyknosis and karyorrhexis of the lymphocytes in the spleen, lymph nodes and other lymphoid aggregates. However, the 2 lambs that recovered showed a lymphocytosis shortly after the temperature had returned to normal. This increase in lymphocytes is probably a reflection of the regenerative activity noted in the lymphoid tissue.

Apart from the changes observed in the leukocytic series, a drop in the RCC, PCV and Hb values also occurred. This was most marked in the fatal cases just before death and could probably be ascribed to shock and the severe haemorrhage seen in the abomasum and sometimes in the small intestine. It is thus evident that WBD virus affects mainly the leukocytic series but that certain changes also occur in the red blood cell values.

The macroscopic and microscopic features in the liver in new-born lambs dying from WBD can easily and definitely be distinguished from those of RVF. Furthermore, it would seem that a diagnosis based on the microscopic lesions in the liver in WBD in the new-born lamb can be made with a high degree of accuracy because of the constant and characteristic nature of these changes.

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