

THE PATHOLOGY OF RIFT VALLEY FEVER. II. LESIONS OCCURRING IN FIELD CASES IN ADULT CATTLE, CALVES AND ABORTED FOETUSES

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ABSTRACT

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Since the original description of Rift Valley fever in sheep, cattle and man in the Rift Valley in Kenya in 1931, very little has been published on the disease in cattle. This report deals with the macroscopic and microscopic pathology of field cases of Rift Valley fever in 22 adult cattle, 8 calves and 8 aborted foetuses.

The microscopic liver lesions in 13 adult cattle were characterized by marked centri- and midzonal eosinophilic necrosis, involving almost $\frac{2}{3}$ of the lobules, and accompanied by sparsely distributed primary foci of necrosis. In 3 animals, however, the hepatic lesions were more focal in nature, while a massive hepatic necrosis was evident in 6 others.

In calves, the lesions in the liver ranged from cases showing numerous haphazardly scattered primary foci of necrosis to cases where the latter were accompanied by eosinophilic necrosis of the remaining hepatocytes in the lobules. Vascular lesions, thrombosis and sinusoidal fibrin deposits were sometimes seen in the livers of both calves and adult cattle.

Although the aborted foetuses were in a fairly advanced state of autolysis, it was still possible to make a diagnosis of Rift Valley fever from the characteristic lesions which were similar to those reported for new-born lambs.

Other noteworthy lesions in adult cattle and calves included pyknosis and karyorrhexis of lymphocytes in the spleen and lymph nodes, widespread serosal and visceral haemorrhages which were sometimes accompanied by copious free blood in the gastrointestinal tract, and a nephrosis.

INTRODUCTION

Since the original description of Rift Valley fever (RVF) in sheep, cattle and man in the Rift Valley in Kenya by Daubney, Hudson & Garnham (1931), very little has been published on the disease in cattle. According to these authors, the clinical signs in cattle during this first outbreak, apart from abortion, were very indefinite, while the necrosis in the liver of the few fatalities resembled that observed in adult sheep.

Subsequent reports on RVF in adult cattle, calves and aborted foetuses were mostly confined to clinical, virological and serological studies (Schulz, 1951; Scott, Weddell & Reid, 1956; Shone, 1958; Easterday, Murphy & Bennett, 1962; Petisca & Serra, 1971; Swanepoel, 1976). Easterday, Murphy & Bennett (1962) and Petisca & Serra (1971) reported briefly on the pathology of the disease in calves and adult cattle.

The purpose of this report is to describe the pathology of field cases of RVF in adult cattle, calves and aborted foetuses and to compare it with that in other animal species and in man.

MATERIALS AND METHODS

Animals

Although some of the cases were examined at autopsy, the majority were formalin-fixed tissues submitted with the case history reports of veterinarians from those areas where outbreaks of the disease occurred during the widespread epizootic of 1974/5 in South Africa. Most of the cases originated from farms where RVF had been confirmed virologically or histopathologically (in sheep and/or cattle), complete with a history of the disease.

Histopathological techniques

The formalin-fixed tissues were processed in a routine manner and embedded in paraffin wax. Sections were cut at 4-6 μm and stained with haematoxylin and eosin (HE).

In addition, special staining techniques such as the periodic acid-Schiff (PAS) reaction without digestion, Masson's trichrome, Wilder's reticulin, Fontana's and

Mallory's stains, and Mallory's phosphotungstic acid haematoxylin (PTAH) stain (Anon., 1968) were applied to the liver and various other tissues.

RESULTS

Macroscopic pathology

Adult cattle and calves

Liver: The livers in both adult cattle and calves were swollen, friable, discoloured orange-brown, and were sometimes accompanied by congested areas and haemorrhages in the parenchyma. While only a few haphazardly scattered, greyish-white foci of necrosis, approximately 0.5-1.0 mm in size, were seen in the livers of adult cattle, numerous foci were usually discernible in calves. Accentuation of the lobulation was often evident in the livers of these animals. In addition, the gall bladder wall was frequently oedematous and haemorrhagic.

Other organs: The spleen in some of the animals was normal in size, but in others it was slightly to moderately enlarged and congested. The lymph nodes were swollen and oedematous and showed petechial haemorrhages in the cortex and medulla. Other noteworthy lesions included: congestion and oedema of the lungs; haemorrhages on various serosal and visceral surfaces (especially the spleen, heart, rumen, small and large intestine); oedema of the abomasal folds accompanied by mucosal haemorrhages and copious amounts of fresh or partially digested blood in the lumen of the abomasum, the small intestine and sometimes also in the large intestine.

Aborted foetuses

Except that the livers were swollen and discoloured orange-brown, it was difficult to evaluate other macroscopic lesions because of advanced autolytic changes in most foetuses.

Microscopic pathology

Adult cattle

Liver: Although the liver was consistently affected, the degree of involvement varied among the 22 cases studied. Marked centrilobular eosinophilic necrosis and haemorrhage involving almost $\frac{2}{3}$ of the lobules were very conspicuous features in 13 out of the 22 livers

examined (Fig. 1 & 2). In 6 livers the periportal hepatocytes, being also affected, culminated in a massive hepatic necrosis (Fig. 3 & 4). Abundant chromatin debris derived from karyorrhectic and pyknotic hepatic nuclei was dispersed within and between necrotic parenchymal cells. Haemorrhage as well as neutrophils were frequently seen in these necrotic areas. However, the hepatic lesions remained focal in only 3 livers. They were made up of scattered foci of acidophilic necrosis and primary foci of necrosis (*vide infra*), sometimes associated with haemorrhage.

The hepatocytes in the rest of the lobules in these livers as well as the periportal hepatocytes in those livers with marked centrilobular necrosis were swollen and showed cloudy swelling, hydropic degeneration and mild fatty metamorphosis. Acidophilic bodies were scattered among the degenerative and necrotic hepatocytes (Fig. 2 & 4). In addition, intracytoplasmic hyalin droplets with a positive PAS reaction were also seen in some degenerated hepatocytes in the periportal zones in 4 livers (Fig. 5). The characteristic primary foci of necrosis (Easterday, McGavran, Rooney & Murphy, 1962; Coetzer, 1977) were discernible in 13 of the livers (Fig. 3 & 6). They often accompanied the extensive eosinophilic necrosis in the lobules (Fig. 3), were sparsely and haphazardly distributed but were not noticed in every section of the liver. These foci were sometimes ill-defined in HE sections (Fig. 6) and were more conspicuous in sections stained with PAS and PTAH.

Filamentous to cigar-shaped eosinophilic intranuclear inclusions were present in a few necrotic hepatocytes in 5 livers. They were usually more abundant and more easily discerned on the edges of the necrotic areas in the better preserved nuclei which revealed margination of the chromatin.

Apart from the parenchymal changes, abundant fibrin thrombi or deposits were especially prominent in the periportal and midzonal sinusoids (Fig. 7 & 8), but they were also evident in some central veins and blood vessels in portal triads, of 4 livers with submassive or massive hepatic necrosis. In these livers an oedematous fluid was present in the portal triads and around the larger blood vessels traversing the parenchyma.

Other organs: In addition to the constant hepatic lesions, a mild to marked pyknosis and karyorrhexis of lymphocytes in the spleen, lymph nodes and other lymphoid aggregates were almost always present. The red pulp in the spleen was congested, but was often severely depleted of lymphocytes. These changes were occasionally accompanied by small, dispersed fibrinoid foci which gave a positive reaction with the PTAH stain for fibrin. Scattered neutrophils were also seen in the red pulp.

A nephrosis, evident in most animals, consisted of tubular degeneration and necrosis, accompanied by proteinaceous fluid in the glomerular tufts, Bowman's spaces and the lumen of some tubules. Occasionally, isolated pyknotic and karyorrhectic nuclei and a slight increased glomerular cellularity were in evidence. Other noteworthy microscopic lesions included: scattered single cell necrosis or small foci of necrosis in the adrenal cortex; congestion, oedema, haemorrhage and emphysema of the lungs, rarely accompanied by a few fibrin thrombi in the alveolar walls (2 cases); and disseminated serosal and submucosal haemorrhages in the abomasum and small intestine.

Calves

Liver: The lesions in the livers of 8 calves ranged from cases where the necrotic process was generally limited to primary foci of necrosis, to others where the latter was accompanied by a massive hepatic necrosis (Table 1).

TABLE 1 Microscopic liver lesions in Rift Valley fever in adult cattle, calves and aborted foetuses

Hepatic lesions	Adult cattle (22 cases)	Calves (8 cases)	Foetuses (8 cases)
Focal hepatic necrosis	3	4	
Centrilobular eosinophilic necrosis	13	1	
Massive hepatic necrosis	6	4	8
Primary foci of necrosis	13	8	8
Cholestasis		1	2
Mineralization		1	6
Intranuclear inclusions	5	2	
Fibrin thrombi	4	2	

A notable feature in all the livers was the presence of many haphazardly scattered and well-circumscribed foci of primary necrosis (Fig. 9, 10 & 11). They were particularly conspicuous in 6 livers and occasionally coalescent, involving almost entire lobules. The architecture of the lobule within these foci was completely disrupted. On rare occasions foci extended partially into a portal triad or central vein, the latter becoming occluded or thrombosed (Fig. 11). Abundant cytoplasmic debris and fragmented nuclear material (Fig. 11) as well as some fibrin, neutrophils and macrophages were seen in them. Advanced disintegration and cytolysis of necrotic hepatocytes were evident in the primary foci in some livers, giving the foci a less cellular, "washed-out" and "meshy" appearance (Fig. 9 & 10). Although fibrin deposits were seen in the primary foci in all the livers, they were especially prominent in those revealing lytic changes (Fig. 12).

In 4 out of the 8 livers, the necrotic lesions were mostly confined to the primary foci of necrosis. The remaining parenchymal cells showed minor degenerative changes with individual or small groups of hepatocytic necrosis and acidophilic bodies scattered among them. However, in the other 4 cases the primary foci of necrosis were accompanied by eosinophilic necrosis of most of the remaining hepatocytes in the lobules. Fibrin thrombi were seen in sinusoids in one of these livers.

Other noteworthy lesions included: mild Kupffer cell activation accompanied by neutrophils in the sinusoids, mild portal inflammation including cholangiolar proliferation, portal oedema, and a mild lymphocytic and neutrophilic infiltration in 4 livers; eosinophilic and filamentous intranuclear inclusions in a low percentage of hepatocytes in 2 livers; vasculitis and thrombosis of central veins (Fig. 2 & 11) and blood vessels in the portal triads in 2 livers, and cholestasis as well as mineralization of isolated necrotic hepatocytes in one liver.

Other organs: The lesions in the lymphoid tissues, kidneys, adrenals and lungs were similar to those described for adult cattle. However, in 1 calf the vascular lesions in the lungs comprised of oedema and fibrinoid degeneration of the wall. These changes were occasionally accompanied by a mild neutrophilic infiltration and thrombosis. In addition to lung oedema, scattered neutrophils and a few pyknotic and karyorrhectic nuclei were also evident in the alveolar walls.

Aborted foetuses

Liver: Moderate autolytic changes were present in the 8 livers studied. The lesions in each of them were similar to those described for the new-born lamb (Daubney *et al.*, 1931; Coetzer, 1977) and were characterized by a massive hepatic necrosis accompanied by scattered primary foci of necrosis. Mineralization of isolated necrotic hepatocytes was noticed in 6 livers, while cholestasis occurred in only 2.

Other organs: Apart from pyknosis and karyorrhexis of lymphocytes in the spleen and lymph nodes, the other organs were too autolyzed for microscopic examination.

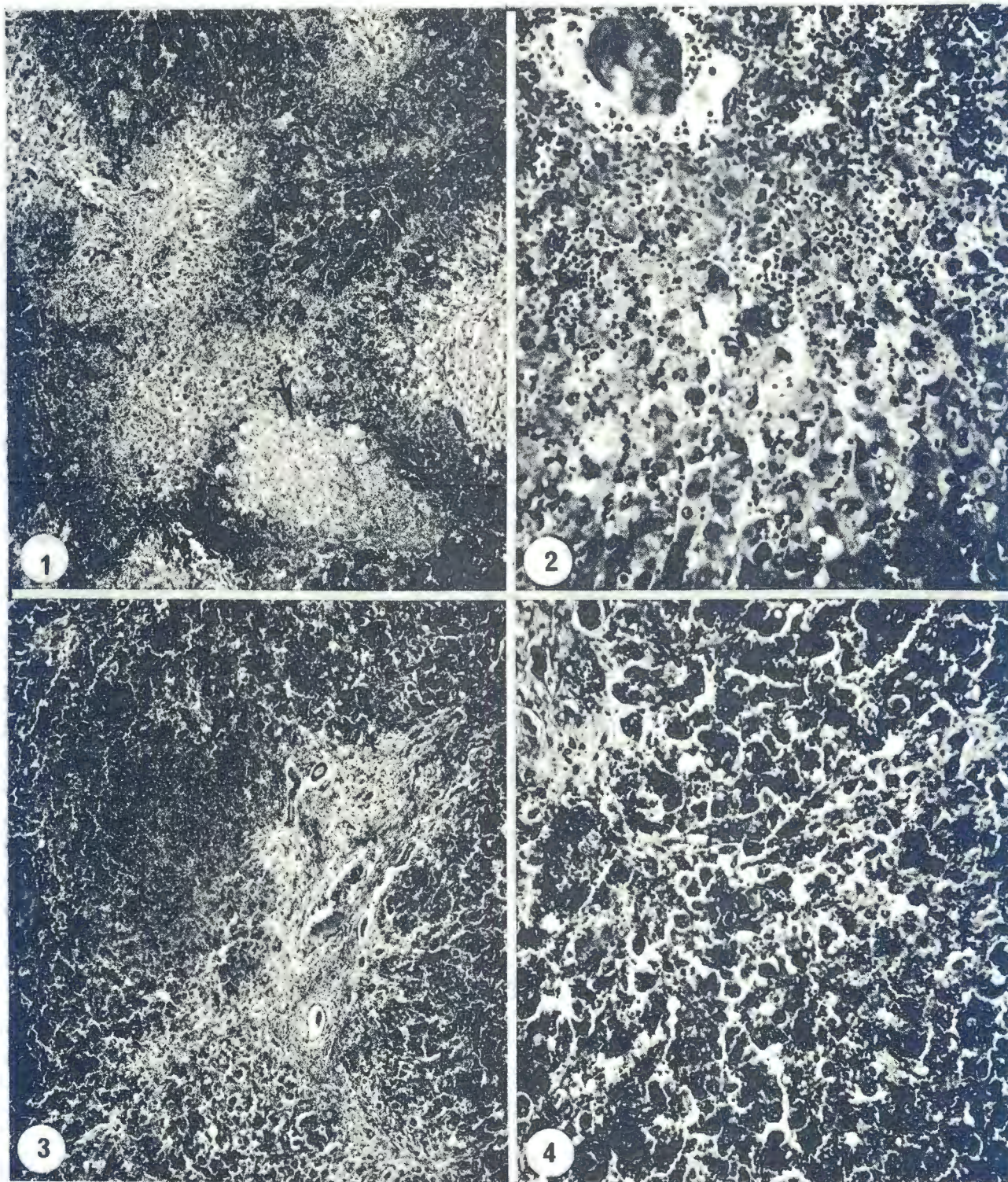


FIG. 1 Marked centrizonal eosinophilic necrosis and haemorrhage. Primary focus of necrosis (arrow): HE \times 40

FIG. 2 Centrizonal eosinophilic necrosis and haemorrhage involving almost $\frac{2}{3}$ of the lobules. Note scattered acidophilic bodies and fibrin thrombus in central vein: HE \times 250

FIG. 3 Massive hepatic necrosis. Primary focus of necrosis next to portal triad (arrow): HE \times 40

FIG. 4 Eosinophilic necrosis involving all the zones of the lobule: HE \times 160

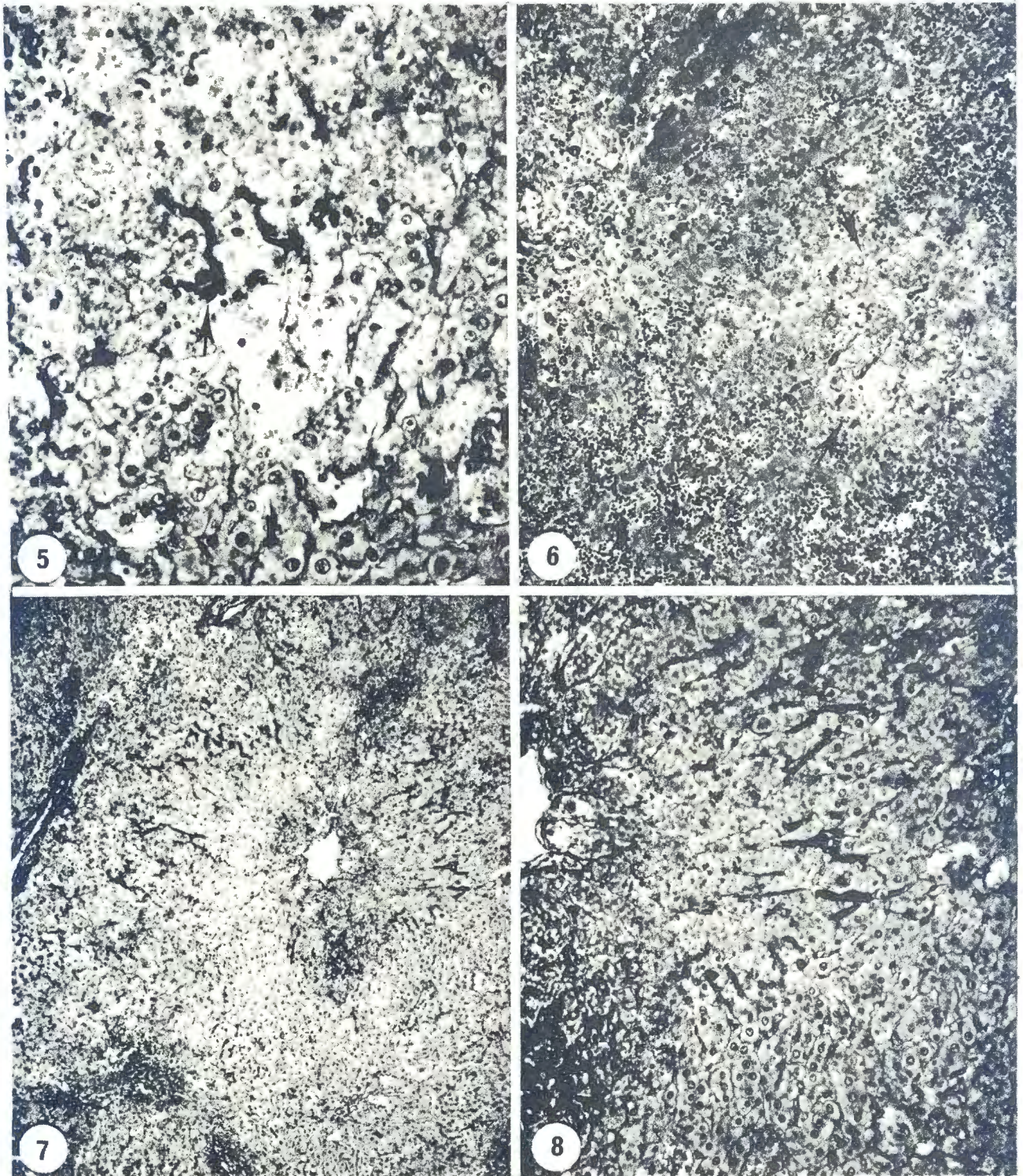


FIG. 5 Intracytoplasmic hyalin droplets in periportal hepatocytes (arrow): PAS \times 160

FIG. 6 Centrizonal necrosis accompanied by ill-defined primary focus of necrosis (arrow): HE \times 250

FIG. 7 & 8 Abundant periportal and midzonal sinusoidal fibrin thrombi: Note centrizonal necrosis: PAS \times 40 and \times 160

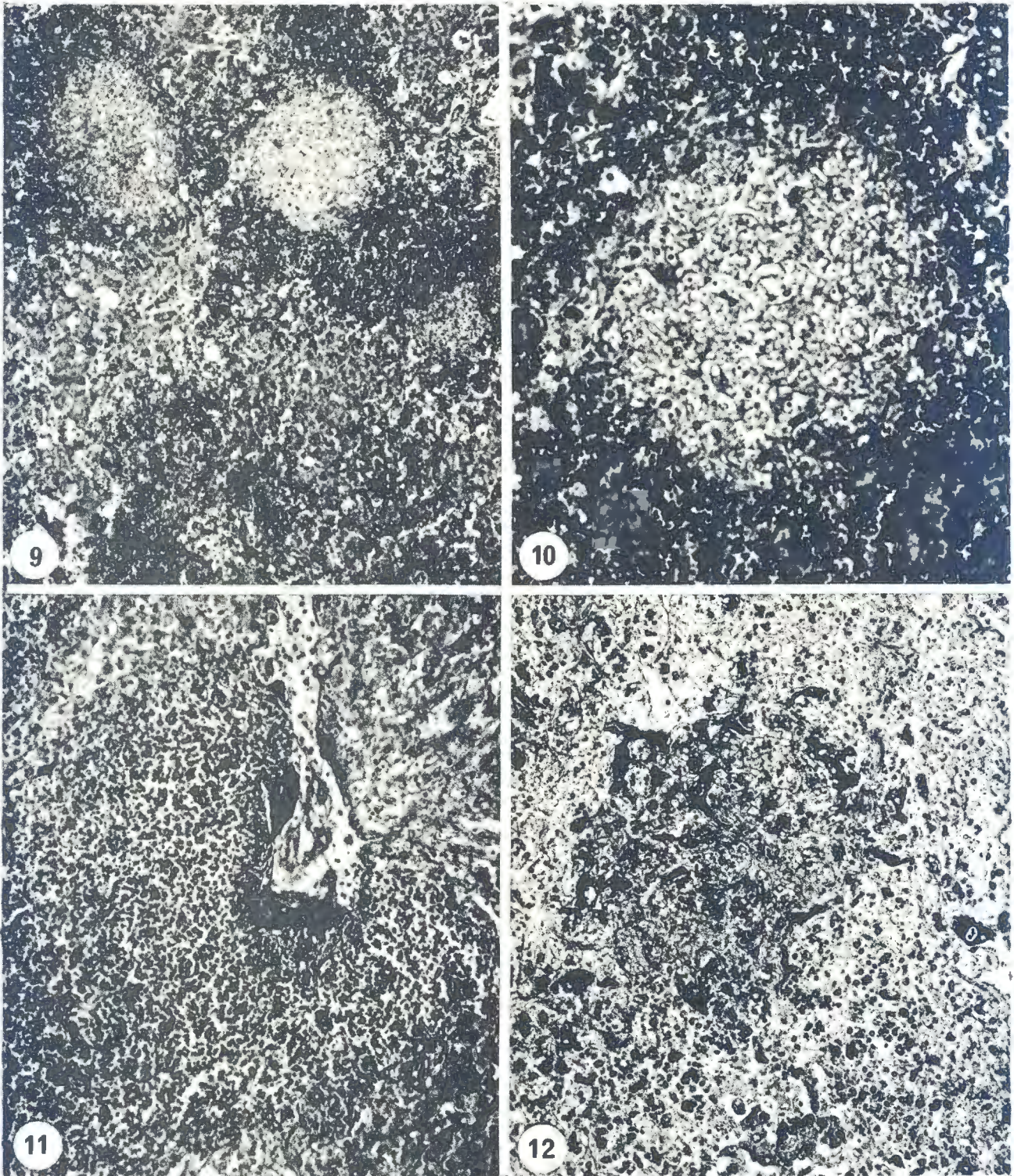


FIG. 9 & 10 Well-circumscribed primary foci of necrosis with "washed-out" and less cellular appearance. Note haemorrhages and congestion surrounding the foci: HE $\times 60$ and $\times 250$

FIG. 11 Paracentral primary focus of necrosis containing abundant karyorrhectic nuclear material. Vasculitis and mural thrombosis of central vein: HE $\times 60$

FIG. 12 Fibrin deposits in primary focus of necrosis: PTAH $\times 160$

DISCUSSION

Although there are a few detailed reports on the pathology of RVF in sheep, especially with regard to the hepatic lesions in the new-born lamb (Daubney *et al.*, 1931; Findlay, 1931; Easterday, McGavran, Rooney & Murphy, 1962, Coetzer, 1977), surprisingly little has been published on the pathology of the disease in cattle. Both Daubney *et al.* (1931) and Schulz (1951) reported that the liver lesions in adult cattle and sheep in the outbreaks studied by them were similar and were confined to focal areas of necrosis. The only report dealing with the pathology of the disease in calves is that by Easterday, Murphy & Bennett (1962). According to these authors, the experimentally infected new-born calves were just as susceptible to the disease as neonate lambs, nor did the lesions in the liver in these calves differ in any way from those described for the new-born lamb. The findings in this study reveal that the principal microscopic lesions in the livers of adult cattle and calves were similar to those described in all species, but there were certain noteworthy differences among the age groups.

In adult cattle the extent of hepatocellular involvement varied among different animals. A very conspicuous feature in 13 of the livers (59%) was the marked centrilobular eosinophilic necrosis and haemorrhage, frequently extending into the middle part of the lobules. Individual or small groups of necrotic hepatocytes as well as acidophilic bodies were dispersed among the intact but degenerated hepatocytes in the rest of the lobule. However, massive hepatic necrosis was evident in 6 animals (27%), while the necrosis remained focal in nature in only 3 livers.

Various workers (Daubney *et al.*, 1931; Schulz, 1951; Coetzer, 1977) emphasized the diagnostic significance of the hepatic lesions of the disease in sheep. Coetzer (1977) and Coetzer, Theodoridis & Van Heerden (1978) regarded the primary foci of necrosis seen in each of the 93 livers of field cases in new-born lambs as a very important diagnostic feature of RVF and a reliable aid in distinguishing RVF from Wesselsbron disease, a disease which also primarily affects the liver. The characteristic foci of necrosis were seen in 13 of the livers (59%) in adult cattle, but they were not nearly as numerous as in the new-born lamb and were only sparsely distributed throughout the parenchyma. While primary foci of necrosis were discernible in sections of some blocks of one liver, they were absent, however, in other liver blocks from the liver of the same animal.

In contrast to those of adult cattle, primary foci of necrosis were not only seen in the livers of every calf and aborted foetus, but they were particularly numerous and conspicuous in calves and were sometimes accompanied by a massive hepatic necrosis. These findings affirm the observations of Easterday, Murphy & Bennett (1962) that the liver lesions in new-born calves are very similar to those reported for the neonate lamb (Daubney *et al.*, 1931; Coetzer, 1977). However, in 4 of the older calves the hepatic lesions remained more focal in nature and did not progress to a massive hepatic necrosis.

In 4 adult cattle and 2 calves with submassive and massive hepatic necrosis, fibrin thrombi were noted in the sinusoids, central veins and the blood vessels in portal triads. Vascular lesions and thrombosis were also seen in the lungs in 1 of the calves. Although thrombosis is rarely associated with RVF, Easterday, McGavran, Rooney & Murphy (1962) and Coetzer (1977) described mural thrombosis in central veins in the liver of a few lambs infected with RVF.

Apart from the constant lesions in the liver, the lymphoid tissues revealed necrosis of lymphocytes. In addition, small fibrinoid foci, which were shown to contain abundant fibrin, were sometimes evident in the red pulp of the spleen, especially in animals with marked depletion of lymphocytes.

A fatal haemorrhagic disease, manifested by widespread serosal and visceral haemorrhages accompanied by copious amounts of fresh or partially digested free blood in parts of the gastrointestinal tract, was conspicuous in some of the adult cattle infected with RVF. A haemorrhagic diathesis has also been reported with the disease in sheep, mice and humans (Daubney *et al.*, 1931; Mims, 1956; Coetzer, 1977; Van Velden, Meyer, Oliver, Gear & McIntosh, 1977; Abdel-Wahab, El Baz, El Tayeb, Omar, Ossman & Yasin, 1978; Laughlin, Meegan, Strausbaugh, Morens & Watten, 1979). Mims (1956) demonstrated a reduction in prothrombin levels and a prolonged clotting time in mice infected with the pantropic RVF virus. However, none of these workers proposed disseminated intravascular coagulation (DIC) as an explanation for the bleeding tendencies. The fibrin thrombi seen in the liver and sometimes also in the lungs of some cases during this study suggest that DIC might have a role in the pathogenesis of this haemorrhagic state in animals and in man.

During the first outbreak of RVF in South Africa, Schulz (1951) described certain unusual symptoms associated with the disease in cattle. The symptoms he described included: a catarrhal stomatitis, accompanied by erosions; superficial necrosis of the skin of the scrotum of bulls and udders of cows; coronitis, laminitis, and haemorrhages and exungulation of the hooves; and marked ascites. Similar mouth lesions, as well as a *dermatitis crustosa* of the muzzle, face, perineum, udder, scrotum of pigmented and unpigmented skin were again seen in a herd of cattle in which RVF had been responsible for abortion and deaths amongst adult cattle and calves during the widespread epizootic in South Africa in 1974/75 (J. A. W. Coetzer & G. Imes, 1975, unpublished observations). It is still uncertain whether these lesions were indeed related to RVF or were a result of dual infection with other viral agents such as bluetongue and mucosal disease virus.

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