

SEQUENTIAL DEVELOPMENT OF THE LIVER LESIONS IN NEW-BORN LAMBS INFECTED WITH RIFT VALLEY FEVER VIRUS. I. MACROSCOPIC AND MICROSCOPIC PATHOLOGY

J. A. W. COETZER⁽¹⁾ and K. G. ISHAK⁽²⁾

ABSTRACT

COETZER, J. A. W. & ISHAK, K. G., 1982. Sequential development of the liver lesions in new-born lambs infected with Rift Valley fever virus. I. Macroscopic and microscopic pathology. *Onderstepoort Journal of Veterinary Research*, 49, 103-108 (1982).

Ten new-born lambs were necropsied at various intervals after artificial infection with Rift Valley fever virus for the study of the sequential development of the hepatic lesions.

During the late stage of the disease, the livers were slightly to moderately swollen and mottled yellow, orange-brown and red. Greyish-white foci, approximately 0,25-0,5 mm in diameter, were also scattered throughout the parenchyma.

Microscopically, the liver lesions progressed from sparsely distributed acidophilic bodies and hepatocytes, revealing acidophilic degeneration and necrosis 6-12 h post-inoculation, to small randomly scattered primary foci of necrosis 12-24 h after infection. At 30-36 h, these primary foci were larger and more circumscribed. In addition, numerous acidophilic bodies and necrotic hepatocytes were dispersed throughout the markedly degenerated parenchyma. The terminal stage of the disease (48-53 h after inoculation), was characterized by massive hepatic necrosis in which primary foci of necrosis could still be recognized as dense aggregates of cyto, asmic and nuclear debris.

INTRODUCTION

In the long recorded history of Rift Valley fever (RVF) in animals and man there are a number of very detailed studies of the liver lesions in new-born lambs (Daubney, Hudson & Garnham, 1931; Weiss, 1957; Easterday, 1965; Coetzer, 1977). There is general agreement that the hepatic lesions are highly characteristic of the disease in neonatal lambs. The liver is consistently affected and an accurate diagnosis of RVF can be made on the basis of the lesion in this organ.

Liver lesions have also been described in a wide range of other animal species, including sheep, cattle, goats, camels, dogs, cats and various laboratory animals (Daubney *et al.*, 1931; Weiss, 1957; Mitten, Remmele, Walker, Carter, Stephen & Klein, 1970). It appears that the principal microscopic features in the livers in these species are very similar and differ only in severity of involvement, depending on the susceptibility of the species and the age group affected. Recently, new insight was shed on the disease in man during the widespread outbreaks of RVF in South Africa and along the Nile Delta and Nile Valley in Egypt. Some of the patients presented with a fatal haemorrhagic disease accompanied by marked liver necrosis (Van Velden, Meyer, Oliver, Gear & McIntosh, 1977; Abdel-Wahab, El Baz, El Tayeb, Omar, Ossman & Yasin, 1978; Laughlin, Meegan, Strausbaugh, Morens & Watten, 1979).

The purpose of the present study was to follow the sequential development of the hepatic lesions in new-born lambs experimentally infected with RVF virus. This communication will deal with macroscopic and light microscopic findings, while ultrastructural changes will be reported in a subsequent paper (Coetzer, Ishak & Calvert, 1982).

MATERIALS AND METHODS

Ten new-born Merino lambs, 1-4 days old, and their mothers were placed in an insect-free stable. Blood for serological assay was collected in 10 ml vacuum tubes from both ewes and lambs before experimental inoculation to determine whether they were susceptible to RVF.

The RVF virus used to infect the lambs was isolated during the widespread epizootic of RVF in South Africa

during the summers of 1974 and 1975. 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 containing 0,5 ml of the virus in suspension were lyophilized and sealed, and the titre of the virus was determined to be $10^{7.5}$ MLD₅₀/0,25 ml.

The virus was reconstituted to the original volume with distilled water, and each lamb was inoculated subcutaneously over 3-5 different sites in the thigh with 0,5 ml of the suspension. The temperature of the lambs was taken 3 times daily and the clinical signs were recorded.

At 6 h post-inoculation, and then every 6 h thereafter until 48 h, one lamb was killed for necropsy at each interval. Three lambs were *in extremis* 51-53 h post-inoculation, and these were killed for the study of the terminal stage of the hepatic lesions. Five other new-born lambs were used as controls. Blocks from different parts of the liver as well as other organs and tissues from both the infected and control lambs were fixed in 10% buffered formalin. They were routinely processed and embedded in paraffin, and sections were cut at 4-6 μ m and stained with haematoxylin and eosin (HE). Special stains included Mallory's stain for iron, Fontana's stain for lipofuscin, Wilder's reticulin stain, periodic acid-Schiff stain for glycogen, and the Mallory's phosphotungstic acid haematoxylin method for fibrin (Luna, 1968).

RESULTS

Macroscopic pathology

No noteworthy lesions were seen in either the liver or any other organ of the lambs killed respectively at 6, 12, 18 and 24 h post-inoculation. At 30-36 h after infection the liver was slightly swollen and discoloured light-brown. In addition, a few small congested patches and ill-defined greyish-brown foci, "pinpoint" in size, were seen in the parenchyma. The livers of 4 lambs killed during the terminal stage of the disease (48-53 h post-inoculation) were slightly to moderately swollen and yellow to orange-brown in colour, with prominent areas of congestion. Greyish-white foci, approximately 0,25-0,5 mm in diameter, were scattered throughout the parenchyma. Other findings included splenomegaly, lymphadenopathy as well as haemorrhages and free, partially digested blood in the abomasum.

⁽¹⁾ Section of Pathology, Veterinary Research Institute, Onderstepoort, 0110

⁽²⁾ Hepatic Pathology Department, Armed Forces Institute of Pathology, Washington DC 20306 USA

Received 17 February 1982—Editor

* BLP: Final concentration of 1% peptone and 5% lactose in 0,1M phosphate buffer

Microscopic pathology

Minor hepatocellular changes were evident 6 h after inoculation with RVF virus. Parenchymal lesions consisted of acidophilic degeneration of isolated hepatocytes and scattered sinusoidal acidophilic bodies (Fig. 1). A few neutrophils were noted in the sinusoids.

At 12–18 h, the parenchyma revealed more advanced degenerative changes. Single necrotic hepatocytes as well as acidophilic bodies were seen in a number of the lobules. The acidophilic bodies varied in size, and some contained a pyknotic nucleus or nuclear fragments. They were seen in the liver plates, spaces of Disse or sinusoids. Mild Kupffer cell proliferation and sinusoidal neutrophilic infiltration accompanied the parenchymal changes.

In addition to the above-mentioned changes, there were small foci of necrosis (60–80 μm in diameter) randomly distributed throughout the liver 24 h after exposure to the virus. Such primary foci involved 5–8 hepatocytes and were composed of disintegrated necrotic hepatocytes and a few infiltrating neutrophils. Similar, but more advanced, parenchymal changes were noted 30–36 h after infection. However, the primary foci of necrosis were larger (120–160 μm in diameter) and more circumscribed (Fig. 2). They contained abundant cytoplasmic and nuclear debris, acidophilic bodies, fibrin and a few neutrophils and macrophages.

At 48 h most of the hepatocytes in the lobules were necrotic and were in the process of disintegration. Only narrow rims of hepatocytes around portal areas and small islets of hepatocytes within lobules were preserved, although these were markedly degenerated. Patchy centrilobular haemorrhages and/or blood pooling were present in some areas of the liver.

Three lambs, which were *in extremis* 51–53 h after infection, were necropsied for studying the terminal stage of the hepatic lesions. Lesions in the livers of these lambs were characterized by a massive eosinophilic necrosis (Fig. 3), accompanied by numerous randomly scattered primary foci of necrosis (*vide supra*) (Fig. 3 & 4). As a result of disintegration and cytolysis of necrotic hepatocytes the parenchyma had a porous appearance (Fig. 3). Degenerated hepatocytes were limited to a narrow periportal zone (Fig. 5). Most hepatocytic nuclei showed margination of chromatin, pyknosis, karyorrhexis or karyolysis. Bizarre nuclear profiles, including rhomboid chromatin spicules, empty and ballooned nuclear shells and abundant chromatin fragments, were scattered within and between disintegrated hepatocytes. Acidophilic bodies and a few neutrophils were dispersed throughout the affected parenchyma (Fig. 5 & 6). The sinusoidal lining cells were slightly swollen and activated.

The number and size of the randomly distributed primary foci of necrosis varied among the 4 livers studied 48–53 h after infection. Occasionally, these foci coalesced and occupied a $\frac{1}{4}$ to a $\frac{1}{3}$ of the lobule. When located centrilobularly or paracentrally, they often resulted in complete destruction or segmental vasculitis of central veins. The vasculitis was characterized by proliferation, swelling, degeneration or necrosis of endothelial cells as well as fibrinoid changes and neutrophilic infiltration of the wall. Similar vascular lesions were occasionally seen in collecting and intercalating veins when bounded by primary foci of necrosis. Cellular debris was often seen in these large vessels and central veins in livers, with marked destruction of the parenchyma. Small parenchymal haemorrhages, accompanied by patchy, centrilobular congestion, haemorrhage and/or blood pooling (Fig. 6), were discernible in lambs killed 48–53 h after infection.

The latter frequently involved only a few millimetres of the subcapsular parenchyma. Eosinophilic, filamentous to cigar-shaped intranuclear inclusions were seen in a low percentage of necrotic hepatocytes. Mineralization of isolated necrotic hepatocytes was also noted, but infrequently. Cholestasis was not a prominent feature and a few bile thrombi were seen in one liver, 36 h post-inoculation. In most livers, a yellowish-brown pigment, which stained positively for iron and lipofuscin, was present in hepatocytes, and to a lesser degree in Kupffer cells and macrophages within portal triads. The same pigment was also detected in the livers of some of the control lambs.

In general, the portal triads were only mildly affected, but showing minor localized changes (such as mild neutrophil and macrophage infiltration and degeneration of the connective tissue) when adjacent to primary foci of necrosis.

DISCUSSION

The microscopic hepatic lesions of RVF reported in different animal species are very similar (Daubney *et al.*, 1931; Findlay, 1932; Weiss, 1957; Mitten *et al.*, 1970). However, studies on the pathogenesis (Mims, 1957; Easterday, McGavran, Rooney & Murphy, 1962; McGavran & Easterday, 1963) of the lesions indicate that the hepatocellular changes could vary depending on the time interval after infection. Such variations could possibly be attributed to factors such as species and individual susceptibility, age, isolate of virus, route of inoculation and the size of the inoculum.

In the present study the lesions in the liver noticeably progressed from diffuse parenchymal degeneration with sparsely scattered, necrotic hepatocytes and acidophilic bodies 6–12 h after inoculation to small, randomly distributed, primary foci of necrosis 18–24 h after infection. At 30–36 h, the primary foci were larger and more circumscribed, while numerous acidophilic bodies and necrotic hepatocytes were dispersed throughout the markedly degenerated parenchyma. The terminal stage of the disease at 48–53 h after infection was characterized by massive hepatic necrosis in which residual primary foci of necrosis could still be recognized as dense aggregates of cytoplasmic and nuclear debris, infiltrated by a few neutrophils and macrophages.

Easterday *et al.* (1962) reported on the pathogenesis of the liver lesions in new-born lambs inoculated intraperitoneally with the Van Wyk strain of RVF virus. Although hepatocellular degeneration and necrosis were recorded much earlier, the sequential development of hepatic lesions in our study generally paralleled theirs. The differences can probably be attributed to some of the factors already mentioned. According to Easterday *et al.* (1962), apart from aggregations of polymorphonuclear leukocytes in the sinusoids, no altered hepatocytes were seen 12–24 h after infection. Small areas of necrosis were discernible for the first time 24–36 h post-inoculation. They reported that the remainder of the parenchyma was normal at 36–48 h, but that most of the hepatocytes were precipitously destroyed during the ensuing hours.

Both our results and those of Easterday *et al.* (1962) clearly demonstrated the rapidity with which the liver lesions progressed from the initial mild hepatocellular changes to the final massive necrosis. During the early stages of the disease (6–24 h) it became apparent that while some hepatocytes were mildly to moderately degenerated, others were already necrotic or in the process of disintegration. Thus, a whole range of changes was seen at any given time interval, suggesting that either all hepatocytes were not infected at the same time or that individual hepatocytes might react differently to infection.

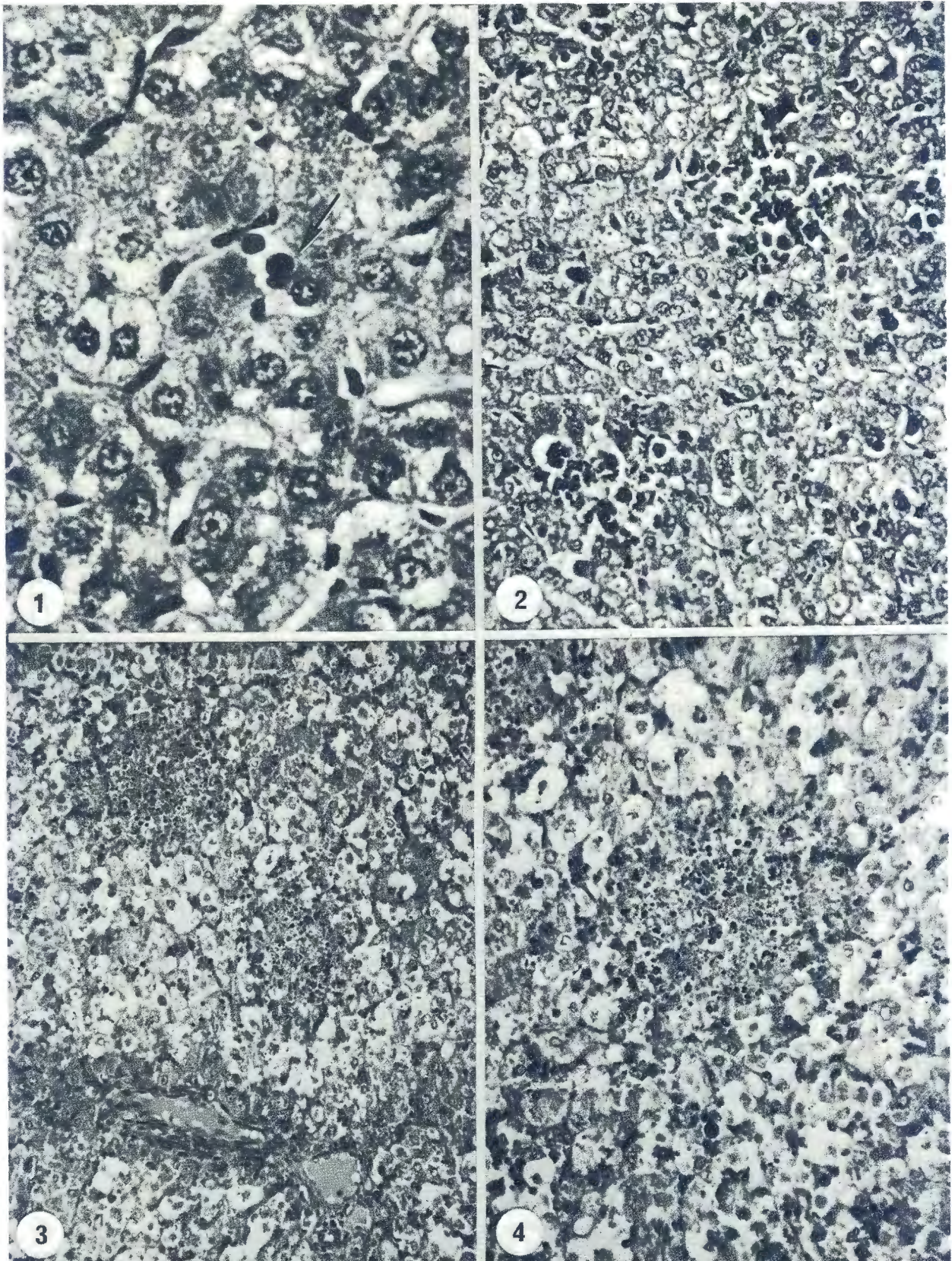


FIG. 1. Parenchymal degeneration and the presence of scattered acidophilic bodies (arrow) 6 h after inoculation: HE \times 400

FIG. 2. Scattered foci of hepatocytic necrosis and acidophilic bodies 30 h post-inoculation: HE \times 250

FIG. 3. Massive hepatic necrosis 52½ h after infection. Note primary foci of necrosis: HE \times 160

FIG. 4. Primary foci of necrosis containing abundant chromatin debris during the late stage of the disease. Remainder of the parenchyma showed marked disintegration: HE \times 250

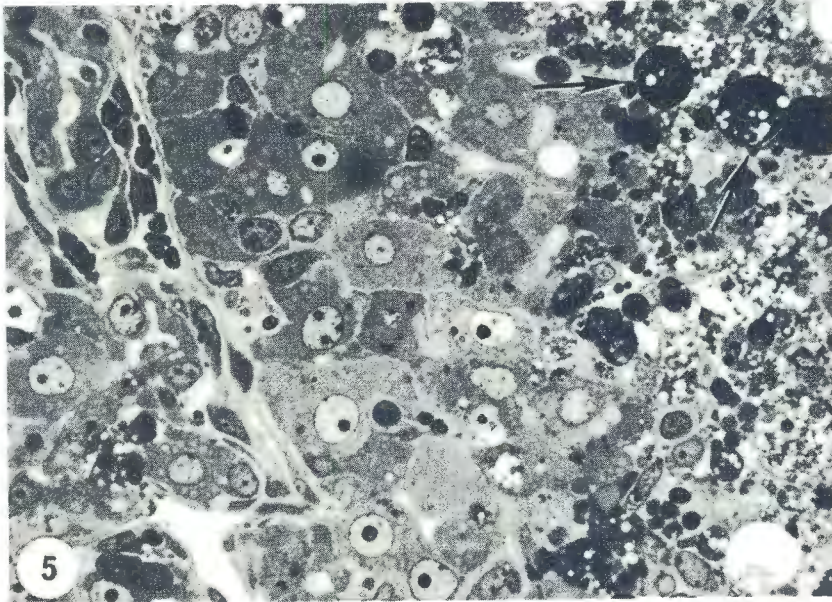


FIG. 5. Narrow rim of degenerated hepatocytes in the periportal area. Acidophilic bodies (arrow) are scattered among necrotic hepatocytes: Epon section, toluidine blue, $\times 630$

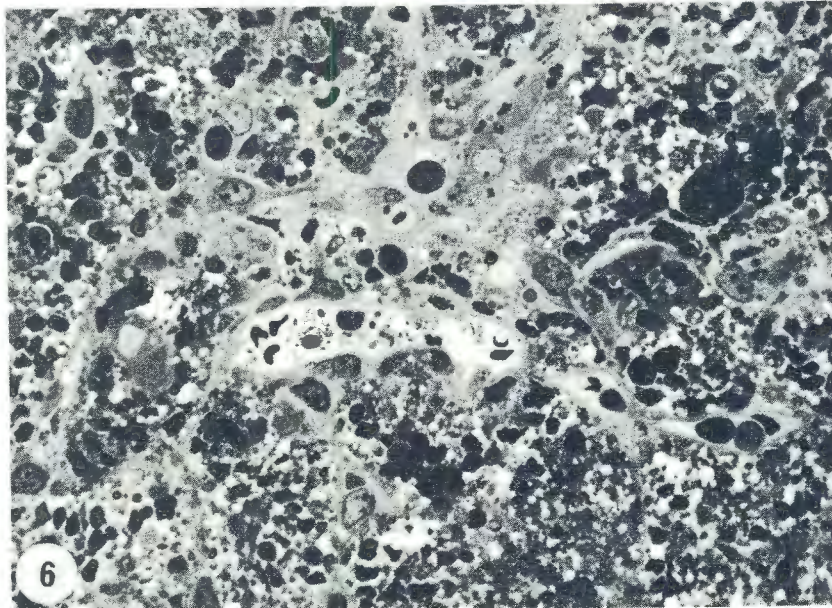


FIG. 6. Disintegration of most hepatocytes in the centrizonal area associated with haemorrhage: Epon section, toluidine blue, $\times 630$

Mims (1957) studied the histological changes in the livers of mice infected with RVF in relation to virus multiplication. He used a saturated inoculum ($10^{8.0}LD_{50}$ of RVF virus) in an attempt to infect all hepatocytes simultaneously. In that way it was possible to study the cellular changes during a single cycle of viral replication. He argued that, with smaller inocula, hepatocytes are infected at widely differing intervals after inoculation, and that these hepatocytes at any given time might be expected to show correspondingly different degrees of pathological changes. According to him many hepatic nuclei were swollen and showed margination of the chromatin 1 h after inoculation. These nuclear changes became more intense, and 2 h later inclusion bodies were seen in virtually every hepatocyte nucleus. At 4 h, nuclear and cytoplasmic disintegration was prominent in many hepatocytes and this disintegration process was maximal 6 h after infection. The mice were either sick or dead $6\frac{1}{2}$ -10 h post-inoculation. On the other hand, mice, receiving smaller virus inocula ($10^{1.0}$, $10^{5.0}$, $10^{7.0}LD_{50}$ of RVF virus), were moribund at 23-40 h, but the microscopic liver lesions were very similar to those seen 6-10 h after the saturated inoculum. Mims (1957) also showed that virus entered the hepatocytes from the blood almost immediately and became non-infective. About 75% of this adsorption took place within 30 min and the remainder within the next hour. With larger doses, he demonstrated that virus was released from affected hepatocytes as early as 3 h after inoculation in mice and that these newly released viruses could infect other hepatocytes. This would thus account for the different changes seen in them.

In the present study the first evidence of discrete, small foci of necrosis in the liver was seen 24 h after inoculation with the virus. They became more circumscribed and bigger and were clearly discernible 36-53 h after infection. Characteristic features of these primary foci of necrosis were that they contained abundant cellular debris, some fibrin, a few neutrophils and macrophages and the lobular architecture in them was completely disrupted. Coetzer (1977) studied the pathology of RVF in new-born lambs, with special reference to the hepatic lesions in 94 field cases. He found that the primary foci of necrosis accompanied the massive hepatic necrosis in all cases and these foci are regarded as a very significant diagnostic feature to differentiate the hepatic lesions of RVF from those of Wesselsbron disease in new-born lambs (Coetzer, Theodoridis & Van Heerden, 1978).

Rift Valley fever virus seems to have a very pronounced cytopathic effect on hepatocytes. Cytoplasmic disintegration as well as nuclear pyknosis, karyorrhexis and karyolysis of most hepatocytes were conspicuous changes during the later stage of the disease. The destruction of hepatocytes in the livers of new-born lambs was reported to be so prominent that the organ was hardly recognizable microscopically (Daubney *et al.*, 1931; Coetzer, 1977).

Intranuclear inclusion bodies have been described in degenerated and necrotic hepatocytes in a variety of animal species, as well as in humans (Daubney *et al.*, 1931; Findlay, 1932; Mims, 1957; Weiss, 1957; Coetzer, 1977; Mitten *et al.*, 1970; Van Velden *et al.*, 1977). Although they were not always discernible in every case of RVF, they were reported with some regularity. In some cases, 80-90% of the hepatocytes in livers of mice and neonatal lambs contained inclusions (Mims, 1957; Coetzer, 1977). In the present study they were only observed in a low percentage of affected hepatocytes. These inclusions were reported to be eosinophilic in liver sections stained with haematoxylin and eosin or phloxin

(Coetzer, 1977). They varied in shape from rod or cigar-shaped through oval to round or ring-shaped (Daubney *et al.*, 1931; Mims, 1957; Coetzer, 1977). A range of special stains and histochemical methods were applied to them in unsuccessful attempts at identifying their origin and composition (McGavran & Easterday, 1963; Coetzer, 1977). While some workers believe these inclusions may be viral in origin (Ellis, Simpson, Stamford & Abdel-Wahab, 1977; Swanepoel & Blackburn, 1977; Coetzer, Ishak & Calvert, 1982), others are of the opinion that they represent a degenerative phenomenon (McGavran & Easterday, 1963).

Although mineralization of necrotic hepatocytes was observed but infrequently during this study, it occurred in 60% of field cases of RVF in new-born lambs (Coetzer, 1977). It is apparently not a specific change and it has been associated with acute hepatocellular necrosis in murine viral hepatitis (Geldhill, Dick & Niven, 1955; Dick, Niven & Geldhill, 1956), in guinea-pigs experimentally infected with Marburg virus (Bechtelsheimer, Korb & Gedigk, 1972), and in some cases of human herpes simplex hepatitis (Ishak, 1981, personal observations).

ACKNOWLEDGEMENTS

We wish to express our appreciation for the assistance given by the Hepatic Pathology and Photography Departments of the Armed Forces Institute of Pathology, Washington DC 20306, with the preparation of the sections and photography respectively.

REFERENCES

- ABDEL-WAHAB, K. S. E., EL BAZ, L. M., EL TAYEB, E. M., OMAR, H., OSSMAN, M. A. & YASIN, W., 1978. Rift Valley fever virus infections in Egypt. Pathological and virological findings in man. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 72, 392-396.
- BECHTELSHEIMER, H., KORB, G. & GEDIGK, P., 1972. The morphology and pathogenesis of "Marburg virus" hepatitis. *Human Pathology*, 3, 255-264.
- COETZER, J. A. W., 1977. The pathology of Rift Valley fever. I. Lesions occurring in natural cases in new-born lambs. *Onderstepoort Journal of Veterinary Research*, 44, 205-212.
- COETZER, J. A. W., ISHAK, K. G. & CALVERT, R. C., 1982. Sequential development of the liver lesions in new-born lambs infected with Rift Valley fever virus. II. Ultrastructural findings. *Onderstepoort Journal of Veterinary Research*, 49, 109-122.
- COETZER, J. A. W., THEODORIDIS, A. & VAN HEERDEN, ANNELINE, 1978. Wesselsbron disease. Pathological, haematological and clinical studies in natural cases and experimentally infected new-born lambs. *Onderstepoort Journal of Veterinary Research*, 45, 93-106.
- DAUBNEY, R., HUDSON, J. R. & GARNHAM, P. C., 1931. Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep, cattle and man from East Africa. *Journal of Pathology and Bacteriology*, 34, 545-579.
- DICK, E. W. A., NIVEN, J. S. F. & GELDHILL, A. W., 1956. A virus related to that causing hepatitis in mice (MHV). *British Journal of Experimental Pathology*, 37, 90-144.
- EASTERDAY, B. C., 1965. Rift Valley fever. *Advances in Veterinary Science*, 10, 65-127.
- EASTERDAY, B. C., MCGAVRAN, M. H., ROONEY, J. R. & MURPHY, L. C., 1962. The pathogenesis of Rift Valley fever in lambs. *American Journal of Veterinary Research*, 23, 470-478.
- ELLIS, D. S., SIMPSON, D. I. H., STAMFORD, S. & ABDEL-WAHAB, K. S. E., 1977. Rift Valley fever: Some ultrastructural observations on material from the outbreaks in Egypt. *Journal of General Virology*, 42, 329-337.
- FINDLAY, G. M., 1932. Rift Valley fever or enzootic hepatitis. *Transactions of Royal Society of Tropical Medicine and Hygiene*, 25, 229-262.
- GELDHILL, A. W., DICK, G. W. A. & NIVEN, J. S. F., 1955. Mouse hepatitis virus and its pathogenic action. *Journal of Pathology and Bacteriology*, 69, 299-320.

- LAUGHLIN, L. W., MEEGAN, J. M., STRAUSBAUGH, L. J., MORENS, D. M. & WATTEN, H., 1979. Epidemic Rift Valley fever in Egypt: observations of the spectrum of human illness. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 73, 630-633.
- LUNA, L., 1968. Armed Forces Institute of Pathology, Washington, Manual on Histologic Staining Methods. 3rd ed. New York, Toronto, London, Sydney: McGraw-Hill.
- McGAVRAN, M. H. & EASTERDAY, B. C., 1963. Rift Valley fever. Light and electron microscopic studies in the mouse. *American Journal of Pathology*, 42, 587-607.
- MIMS, C. A., 1957. Rift Valley fever virus in mice. VI. Histological changes in the liver in relation to virus multiplication. *Australian Journal of Experimental Biology*, 35, 595-604.
- MITTEN, J. O., REMMELE, N. S., WALKER, J. S., CARTER, R. C., STEPHEN, E. L. & KLEIN, F., 1970. The clinical aspects of Rift Valley fever virus in household pets. III. Pathological changes in the dog and cat. *Journal of Infectious Diseases*, 121, 25-31.
- SWANEPOEL, R. & BLACKBURN, N. K., 1977. Demonstration of nuclear immunofluorescence in Rift Valley fever infected cells. *Journal of General Virology*, 34, 557-561.
- VAN VELDEN, D. J. J., MEYER, J. D., OLIVER, J., GEAR, J. H. S. & McINTOSH, B., 1977. Rift Valley fever affecting humans in South Africa. *South African Medical Journal*, 51, 867-871.
- WEISS, K. E., 1957. Rift Valley fever—a review. *Bulletin of Epizootic Diseases of Africa*, 5, 431-458.