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SEQUENTIAL DEVELOPMENT OF THE LIVER LESIONS IN NEW-BORN LAMBS INFECTED WITH RIFT VALLEY FEVER VIRUS. II. ULTRASTRUCTURAL FINDINGS

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ABSTRACT

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The macroscopic and microscopic lesions in livers of new-born lambs experimentally infected with Rift Valley fever virus and killed at various intervals between 6–53 h after inoculation, were described in a previous paper. This communication gives an overview of the ultrastructural changes affecting hepatocytes, sinusoids and spaces of Disse, biliary tree and portal triads as well as observations on the morphology and morphogenesis of the virus.

Hepatocytes were those primarily affected, while inflammatory and architectural changes were secondary. The changes included prominent nuclear alterations, fragmentation or disintegration of necrotic hepatocytes, focal cytoplasmic degradation and sequestration, and the presence of acidophilic bodies. The ultrastructure and origin of the intranuclear inclusions are discussed.

INTRODUCTION

The wild-type Rift Valley fever (RVF) virus has specific tropism for the liver as well as latent neurotropic properties (Findlay, MacKenzie & Stern, 1936; Weiss, 1957; Coetzer & Barnard, 1977). For that reason characteristic hepatic lesions are consistently present in animals infected with this virus (Daubney, Hudson & Garnham, 1931; Easterday, 1965; Coetzer, 1977). Recently, extensive liver damage has also been described in humans who died of the disease (Van Velden, Meyer, Oliver, Gear & McIntosh, 1977; Abdel-Wahab, El Baz, El Tayeb, Omar, Ossman & Yasin, 1978; Laughlin, Meegan, Strausbaugh, Morens & Watten, 1979). The hepatic lesions in animals and man are frequently associated with a haemorrhagic diathesis and involvement of other organ systems (Daubney *et al.*, 1931; Weiss, 1957; Mitten, Remmele, Walker, Carter, Stephen & Klein, 1970; Coetzer, 1977).

Although the light microscopic pathology of RVF is well documented, especially with regard to the liver lesions in animals, there are only a few ultrastructural reports on the changes seen in mice and cell cultures (McGavran & Easterday, 1963; Lecatsas & Weiss, 1968; Ellis, Simpson, Stamford & Abdel-Wahab, 1979). The studies of these workers concentrated mostly on viral morphology and morphogenesis. The present study is therefore the first detailed description of the ultrastructural changes in the livers of new-born lambs killed at different intervals of the disease. The light microscopic changes have been previously reported (Coetzer & Ishak, 1982) and will be correlated with the ultrastructural findings.

MATERIALS AND METHODS

The experimental animals, the procedures and the light microscopic changes have been described in a previous report (Coetzer & Ishak, 1982). Liver blocks approximately 1 mm^3 were collected from each lamb in 4% phosphate buffered glutaraldehyde (pH 7,35) at 4 °C and were stored at the same temperature for electron microscopy. The tissues were then buffer-washed and post-fixed in 1% OsO_4 for 1 h in Millonig's phosphate buffer (Millonigs, 1961). All the samples were dehydrated in an ethanol gradient, cleared in propylene oxide and embedded in Epon 812. For tissue orientation, sections were cut 1–2 μm thick and stained with 1% toluidine blue (Hayat, 1970). Thin sections (60 nm) were cut with a diamond knife on a Sorvall MT-2 or MT-5000 Ultramicrotome, mounted on copper grids and post-stained with 4% uranyl acetate in 50% methanol for 1 min. This procedure was followed by lead citrate staining for another minute. Samples were then examined in a Siemens 101 electron microscope.

RESULTS

Although the extent of hepatocytic involvement varied as the lesions progressed from isolated necrotic hepatocytes 6–12 h post-inoculation to massive necrosis 48–53 h after infection, the ultrastructural findings during the various time intervals were very similar. For that reason we chose not to describe the findings in each liver separately, but rather to give an overview of the changes in hepatocytes, sinusoids and spaces of Disse, biliary tree and portal triads as well as a description of virus morphology and morphogenesis.

Hepatocytes

A range of degenerative and necrotic changes was seen in hepatocytes in lambs killed 6–36 h after infection. During the earlier stages of the disease (6–36 h), necrosis was confined to scattered single cells or small foci of hepatocytes (Fig. 1) while the remainder of the parenchyma was degenerated and depleted of glycogen. Focal cytoplasmic degradation *(vide infra)* was also in evidence in some of the degenerated hepatocytes. The intercellular spaces were widened and often contained sequestered portions of cytoplasm and degradation products of hepatocytes as well as acidophilic bodies *(vide infra)* (Fig. 2). The terminal stage of the disease 48–53 h post-inoculation was characterized by massive necrosis, culminating in distortion of liver plates. Only narrow rims of periportal and isolated islets of degenerated hepatocytes were spared.

Nuclear changes included clumping and margination of chromatin, pyknosis, karyorrhexis and karyolysis (Fig. 3–6). Some nuclei revealed a combination of these changes. Karyorrhexis, a very conspicuous change, was preceded either by condensation or lysis of the karyoplasm and chromatin. Nuclear debris was dispersed within and between necrotic hepatocytes. Two distinct morphological expressions of karyolysis were noted. In the first form, which was rarely seen, the nuclei were preserved, but the nuclear content had a faded and diluted appearance. The second form was characterized by grossly disfigured nuclear profiles with marked focal or diffuse lysis (Fig. 5 & 6). In both forms the cytoplasm usually had a ballooned and rarified appearance.

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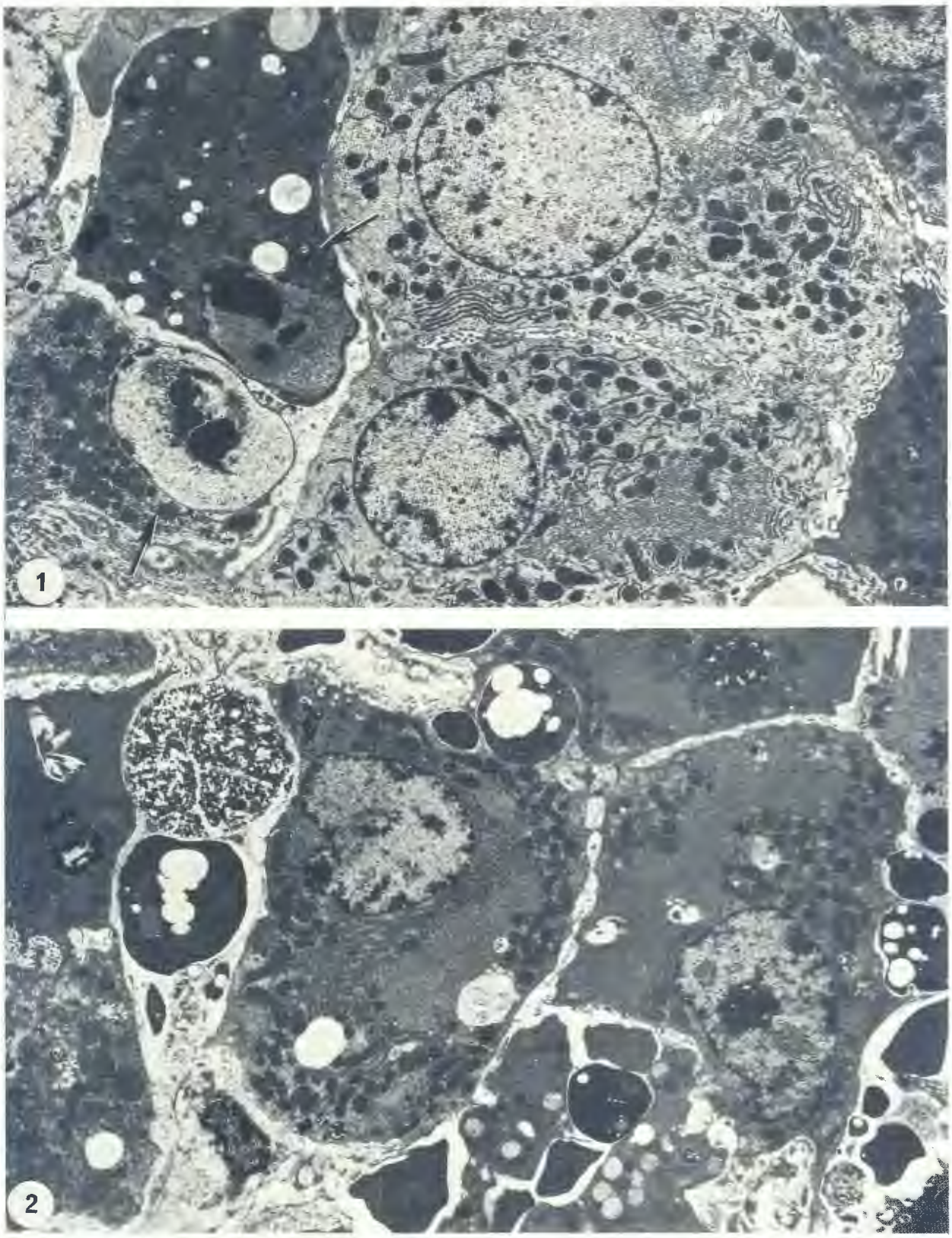


FIG. 1 Necrosis and detachment of two hepatocytes (arrow). Note difference in electron density of hepatocytes: 5 000 ×

FIG. 2 Widened intercellular spaces which contain products of degradation and debris derived from hepatocytes. The cytoplasm of the hepatocytes are condensed ("dark hepatocytes"): 4 800 ×

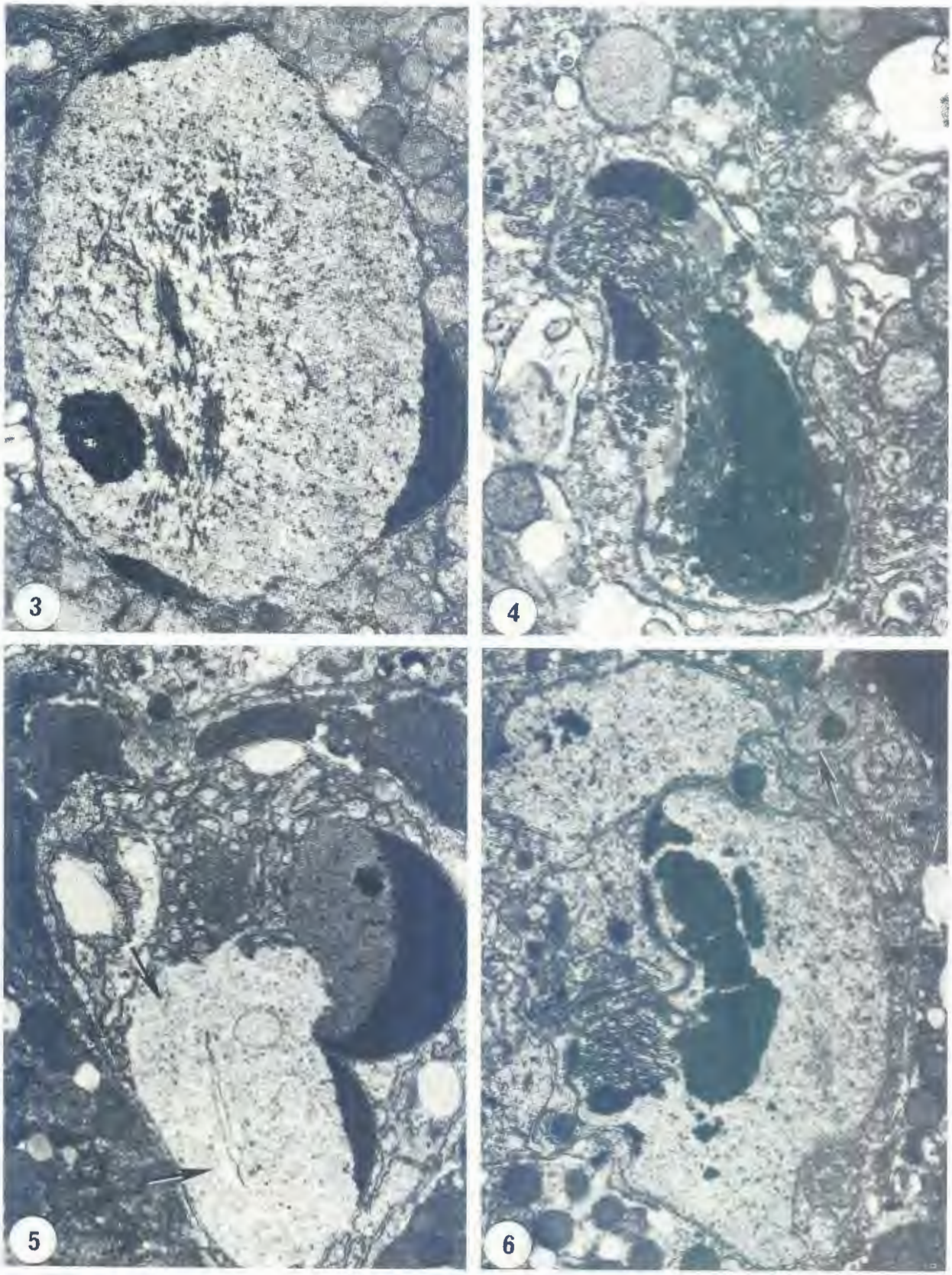


FIG. 3 Margination of the chromatin with partial collapse of the nuclear envelope adjacent to chromatin fragments. Aggregate of microfilamentous (inclusion) is seen in nucleus: 12 000 ×

FIG. 4 Pyknosis of hepatocyte nucleus. Segmental fusion of the inner and outer nuclear membranes as well as papilliform projections of the latter in the perinuclear cisterna. Note microfilamentous inclusion and relatively large condensed nucleolus: 15 000 ×

FIG. 5 Grossly disfigured hepatocytic nucleus showing lysis of part of the nucleus (arrow): 9 000 ×

FIG. 6 Lobulation of a markedly affected parenchymal cell nucleus. Bleb-like protuberances of the outer nuclear membrane (arrow). Note microfilaments in association with clumped chromatin: 9 000 ×

A range of nuclear envelope and karyoplasmic changes was seen (Fig. 4–6). The perinuclear cisternae were frequently irregularly widened and contained an amorphous and slightly granular material. Alterations of the nuclear envelope were often accompanied by focal or diffuse lysis and degeneration of the nuclear content. The latter was composed of fine, granular matrix, interspersed with discrete electron-dense granules (50–75 nm in diameter) and inclusion bodies made up of a parallel arrangement of microfilaments (Fig. 3, 4 & 7). These microfilament aggregates were generally located near the centre of the nucleus and in only a few instances were they seen in association with marginated and clumped chromatin.

No morphological alterations were observed in the majority of nucleoli other than eccentric positioning and condensation or disintegration.

These conspicuous nuclear alterations were accompanied by a range of cytoplasmic changes. While some hepatocytes had a less dense or lighter appearance, others were more condensed and appeared as "dark hepatocytes" (Fig. 1 & 8). Marked ballooning of the cytoplasm was seen only in odd hepatocytes. The "dark hepatocytes" seemed to have shrunk and to have increased in density. They were often fragmented into many smaller pieces and were thus seen lying free among degenerated parenchymal cells (Fig. 8 & 9). On the other hand, the cytoplasm of the less dense hepatocytes had become more rarified and disintegrated to release their contents.

A variety of changes was discerned in the cytoplasmic organelles. The mitochondria were markedly swollen and often interdigited. Floccular deposits, occasionally associated with highly electron-dense needle-like spicules, were in the mitochondrial matrix of some of the rarified hepatocytes. The rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and Golgi complexes were usually dilated. In some hepatocytes the cisternae of the RER were markedly widened, giving the cell a cribiform appearance.

An assortment of tubular arrays, derived from proliferated RER and/or SER, were observed only rarely in necrotic hepatocytes (or fragments of them) in markedly involved livers (Fig. 10). While most of these fragments containing the tubular profiles were lined by a membrane, a few were not. The tubules at the periphery of the whorl-like configuration were loosely spaced, and ran in a parallel fashion. They appeared to be pinched-off at irregular intervals but continued to spiral to the centre of the whorl, which consisted of densely packed and intermeshing short and branching tubules. Some tubules were also arranged in single or a few laminated circular patterns. Isolated mitochondria and fat droplets were trapped between these tubules. A notable feature of the endoplasmic reticulum was that it did not show any dilatation. Evidence of degranulation of RER was seen in some hepatocytes.

Focal cytoplasmic degradation and sequestration was a distinctive change in many degenerated and necrotic hepatocytes, especially during the late stage of the disease (Fig. 8 & 11). Although different morphological expressions of this phenomenon were seen, there appeared to be two main types, namely degradation as a result of cytoplasmic condensation or mummification and degradation through dissolution or lysis. The latter would seem to have encompassed 3 different forms. The first form was depicted as round to ovoid cytoplasmic vacuoles containing scanty, amorphous material intermingled with sparse myelin figures, electron-dense granular deposits, pigment granules as well as degenerated organelles (Fig. 12). The vacuoles were frequently located in areas where haemosiderin or lipofuscin had accumulated. The second form consisted of bodies containing numerous sequestered parts of degenerated cytoplasm and granular material (Fig. 13). The organelles in these bodies were degenerated, although their outlines were still reasonably preserved. Pigment granules and floccular deposits were sometimes noted in them. The third form was regularly seen and was especially prevalent in markedly necrotic hepatocytes (Fig. 11 & 14). They were made up of rounded bodies which varied in size from approximately 0,2–0,8 μm in diameter and contained abundant electron-dense granular to floccular material, pigment granules and debris of organelles.

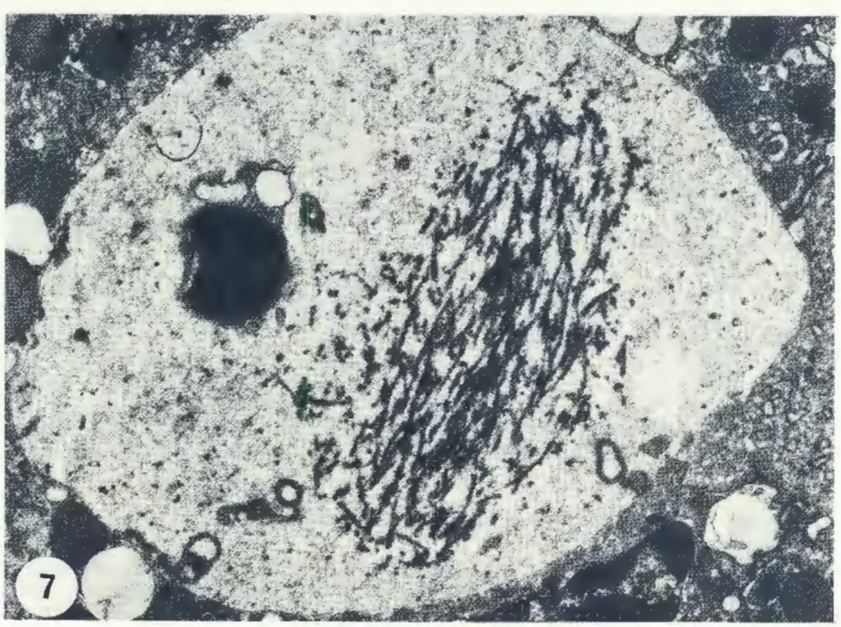


FIG. 7 Microfilamentous inclusion in a karyolytic nucleus: 9 000 ×

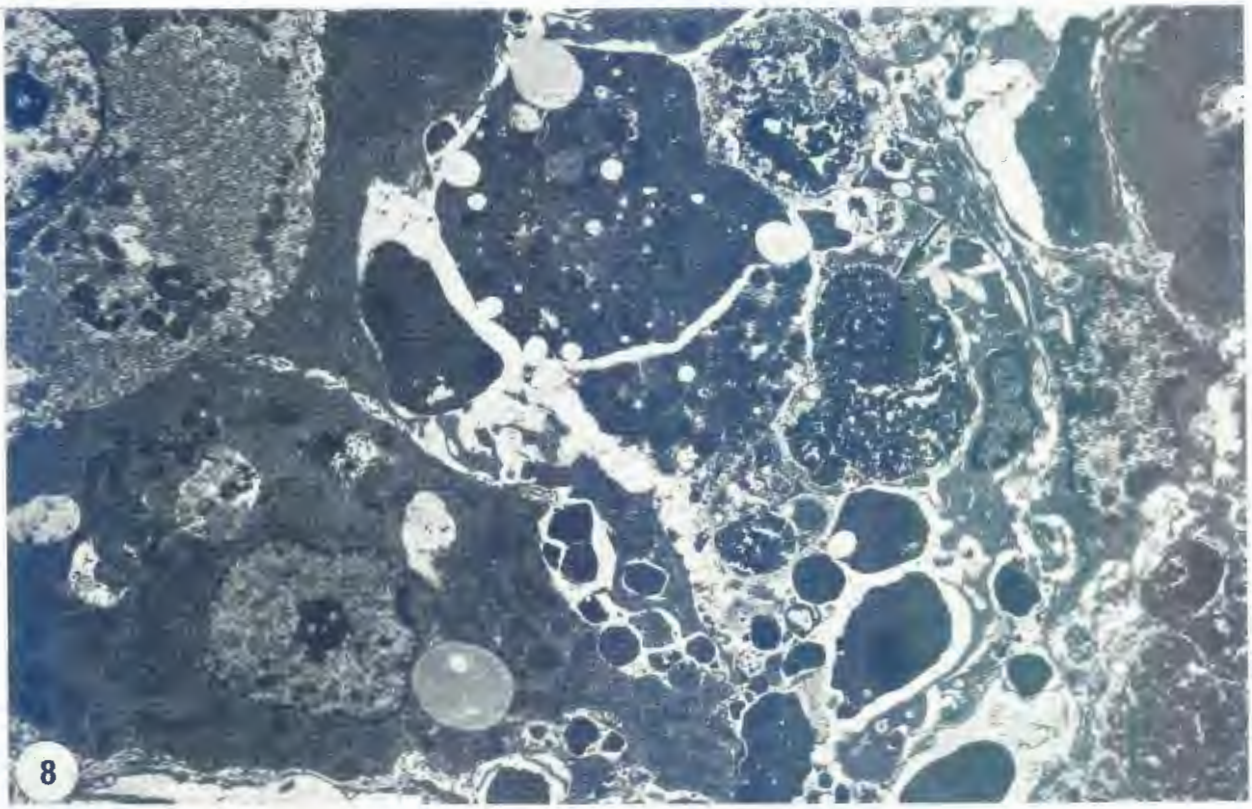


FIG. 8 Fragmentation of a necrotic hepatocyte. Cellular debris such as pieces of disintegrated hepatocytes and amorphous bodies (arrow) located between "light" and "dark" hepatocytes: 4 600 ×

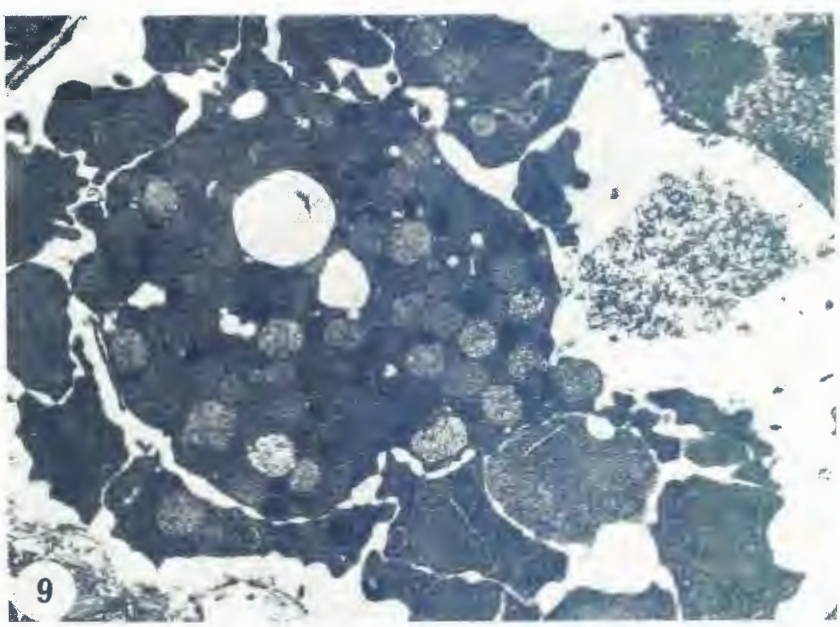


FIG. 9 Necrotic hepatocyte in the process of breaking up. Note some of the pinched-off pieces of cytoplasm which are still attached by a short stalk: 6 000 ×

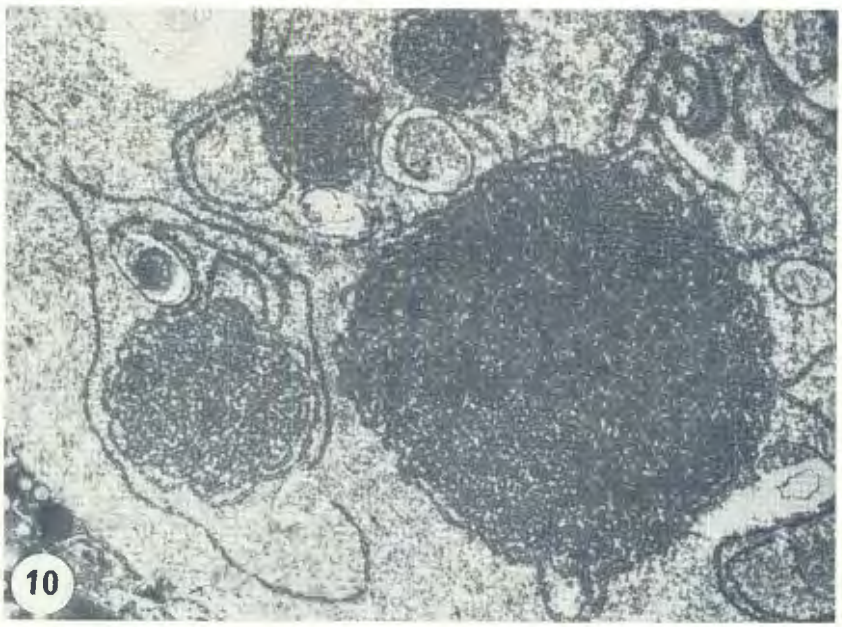


FIG. 10 Whorl-like proliferation of endoplasmic reticulum: 7 500 ×

One to 4 areas of degradation of one or both major morphological types were sometimes present in a single hepatocyte. Most of these cytoplasmic bodies were not clearly membrane-bound. They were often located near the periphery of the cell, lay free in intercellular spaces or spaces of Disse or were dispersed among the cellular debris (Fig. 2, 8, 12, 13 & 15). Completely extruded bodies or portions of them were sometimes phagocytosed by macrophages, neutrophils and Kupffer cells (Fig. 11).

Occasional acidophilic bodies (Councilman or "dark bodies") were seen as early as 6 h after infection with RVF virus but many were noted 18–52 h post-inoculation (Fig. 2, 15 & 16). During the earlier stages of development of the hepatic lesions, they were seen lying free between degenerated hepatocytes in the liver plates or were dispersed among the cellular debris in primary foci of necrosis (Coetzer, 1977; Coetzer & Ishak, 1982). These bodies varied in size from approximately 0,028–1,0 μm in diameter. Although they were pleomorphic, some had round to ovoid shapes with smooth or scalloped borders. Only a few were contained within a membrane. The cytoplasm which comprised these bodies had a condensed or "mummified" appearance and included degenerated organelles which were usually tightly packed, pyknotic and karyorrhectic nuclear debris, a few fat droplets, pigments granules (haemosiderin and/or lipofuscin) and autophagolysosomes. They did not contain any glycogen. A number of acidophilic bodies fragmented, a process which involved either the entire body or the periphery. Fragments often lay free in the liver plates, intercellular spaces, spaces of Disse, sinusoids or central veins. Some were partly or completely engulfed by macrophages and neutrophils in the parenchyma or by Kupffer cells.

Hepatocellular disintegration and cytolysis with loss of lobular architecture were very prominent features of primary foci of necrosis (Fig. 17). The foci generally had a less dense appearance and contained abundant cellular and nuclear debris. Abundant fibrin as well as neutrophils and macrophages engulfing necrotic tissue were also seen (Fig. 17).

Biliary tree and portal triads

In areas where the boundaries of affected hepatocytes were still preserved, bile canaliculi were usually mildly to moderately dilated, and showed loss of microvilli and "lipping" at the junctional areas. Oedematous microvilli, bleb-like cytoplasmic protrusions and small amounts of bile were occasionally observed in the lumen. In livers with marked hepatocellular disintegration, the canaliculi were either not recognizable or were grossly disfigured, and revealed similar but more advanced changes to those described above (Fig. 18). In addition, desquamated microvilli and small fragments of sequestrated cytoplasm were seen in the lumen. Changes similar to those described for the bile canaliculi were also noted in canals of Hering.

Apart from cellular debris in lymphatics along with a few neutrophils and macrophages containing phagocytosed material, no other noteworthy changes occurred in the portal triads.

Sinusoids and spaces of Disse

The sinusoids generally were preserved even during the late stage of the disease, when most of the bordering hepatocytes were necrotic. Sinusoidal collapse was seen in only a few places. Apart from isolated necrotic endothelial cells the majority revealed minor degeneration (Fig. 19). The Kupffer cells appeared not to be affected but a mixture of phagocytosed fibrin and cellular debris was seen in some of them. Occasionally amoeboid processes of Kupffer cells extended through the fenestrations in the sinusoidal wall to phagocytose necrotic tissue in the spaces of Disse or liver plates. Segments of the basement membrane of the sinusoids were only overtly altered in livers with marked hepatocellular necrosis, and in these it was either in the process of dissolution or was slightly thickened and "fuzzy" in appearance.

Neutrophils, macrophages, cellular debris and sparse fibrin deposits were especially prominent in some of the sinusoids in livers with extensive parenchymal involvement. In a few instances the lumen of the sinusoids was almost completely obliterated by fibrin (Fig. 20).

Though no abrupt break in the wall could be demonstrated in most sinusoids, it appeared that plasma had percolated profusely through the affected wall in some areas excavating necrotic hepatocytes (Fig. 19). The perisinusoidal cavitations thus created contained plasma (fine granular material) as well as fibrin and pieces of parenchymal cells. In a few areas the sinusoidal wall was disrupted and associated with haemorrhages in the parenchyma.

Depending on the extent of damage to the hepatocytes, the spaces of Disse were either markedly narrowed or widened, or alternatively, were not clearly delineated. The vascular pole of affected hepatocytes was often almost entirely denuded of microvilli. Occasionally, cytoplasmic protuberances extended from their surfaces into the spaces of Disse. In addition to sparse reticulin fibres, desquamated microvilli, cellular debris, fibrin and viral particles were seen in these spaces.

Viral morphology and morphogenesis

Most hepatocytes were either degenerated or necrotic during the late stage of the disease. However, viral particles were only demonstrable in the cytoplasm of a low percentage of them. Virions were also discernible within fragments of disintegrated hepatocytes, and were free among cellular debris in the lobules, sinusoids, intercellular and spaces of Disse. Only rarely were viral particles noted in acidophilic bodies, products of cytoplasmic degradation or in association with phagocytosed necrotic material within Kupffer cells, neutrophils and macrophages.

Viral particles measured approximately 90 ± 5 nm in diameter, were spherical to hexagonal in shape and surrounded by an envelope. Single or small groups of viruses were noted free among the cytoplasmic organelles or were contained in smooth membrane-bound vesicular or tubular structures in the cytoplasm (Fig. 21). In the latter, they were usually closely arranged in single file in close proximity to the membrane or were composed of aggregates of double filed viral particles. Virus budding from these membranes was only occasionally observed.

Discussion

This study has shown that RVF virus primarily affects hepatocytes, while inflammatory and architectural changes are secondary. The most conspicuous hepatocytic changes included prominent nuclear changes, cytoplasmic fragmentation or disintegration, focal cytoplasmic degradation and sequestration, and the formation of acidophilic or "dark bodies".

The various nuclear alterations were not always clearly separable, making it difficult to propose a sequence for their development. It appeared that clumping and margination of the chromatin were among the earliest changes, and these were followed by pyknosis, karyorrhexis or karyolysis. Some of the affected nuclei did not fit the description of any of these specific alterations but rather exhibited a combination of these pathological changes which resulted in the formation of bizarre nuclear profiles. A variety of nuclear envelope, karyoplasmic and nucleolar changes were seen, but the most significant changes were the microfilamentous inclusion bodies in the centre of affected nuclei. Although the impression was gained in a few nuclei that the microfilaments were originating from degenerated chromatin, we concluded that they were most probably viral-associated. Basing our opinion on the uniformity and parallel arrangement of the microfilaments and on the negative reaction of these inclusions with the Feulgen stain (McGavran & Easterday, 1963; Coetzer, 1977), we support the hypothesis of Lecatsas & Weiss (1968) that it could be nuclear RNA and probably precursor material for cytoplasmic viral assembly. Recently, positive immunofluorescence was associated with the intranuclear inclusions (Swanepoel & Blackburn, 1977). It should be emphasized that at no time during the progression of the liver lesions could virus be demonstrated in hepatocytic nuclei in our study. On the other hand, McGavran & Easterday (1963) failed to demonstrate positive immunofluorescence in association with the inclusions. They were of the opinion that the inclusions represented a degenerative phenomenon.

Ultrastructurally, the cytoplasm of many of the necrotic hepatocytes had a shrunken and more electron-dense appearance ("dark hepatocytes"), a change that corresponded to the eosinophilic degeneration and necrosis seen with light microscopy (Coetzer & Ishak, 1982). Most of the affected hepatocytes fragmented into numerous smaller parts, or pieces of necrotic cytoplasm were seen breaking off from the cell periphery. The changes became more prominent and widespread as the disease progressed. Thus at 6–30 h post-inoculation with RVF virus individual or small groups of hepatocytes were affected while at 48–53 h almost all the cells were necrotic and fragmented. This fragmentation process could possibly best be explained by the phenomenon of "apoptosis", which is a form of cell deletion (Kerr, Wyllie & Currie, 1972). This process can be initiated by a variety of physiological and pathological stimuli and takes place in 2 stages, the first being characterized by condensation of the cytoplasm and fragmentation of the cell into a number of membrane-bound, well-preserved fragments. During the 2nd stage the "apoptotic bodies" are shed from the surface of the cell and are phagocytosed and degraded by other cells. The larger "apoptotic bodies" are visible by light microscopy as acidophilic bodies (Kerr *et al.*, 1972).

Focal cytoplasmic degradation and sequestration were conspicuous changes in many degenerated and necrotic hepatocytes and were seen with a greater frequency during the late stages of the disease. There appeared to be at least 2 main morphological expressions of degradation. The first type of degradation was characterized by a range of changes, all of which were interpreted as different stages of cytoplasmic degradation through dissolution or lysis. These changes were characterized either by vacuoles containing sparse myelin figures, degenerated but intact organelles and particulated matter, or amorphous bodies densely packed with abundant electron-dense granular to floccular material as well as debris of organelles and pigments. In contrast, the 2nd type was distinctly characterized by focal cytoplasmic condensation or mummification; this form occurred far less frequently. The electron density of both types of degradation made it difficult to differentiate or detect viral particles from particulated matter. Isolated virions were thus only occasionally identified in some of the amorphous bodies but never in intracytoplasmic mummified fragments (acidophilic bodies). These bodies of degradation were extruded or released when the cell disintegrated into intercellular and spaces of Disse or sinusoids while some were phagocytosed by macrophages, neutrophils or Kupffer cells.

Alternatively, it is possible that some intracellular products of degradation did not originate in a given hepatocyte, but were phagocytosed. Injured liver cells can apparently phagocytose cellular debris derived from adjacent necrotic cells (heterophagy) in certain hepatic injuries associated with focal or zonal necrosis (Biava & Muklova-Montiel, 1965; Kerr, 1969; Kerr, 1970a; Kerr, 1970b). Evidence that phagocytosis had possibly

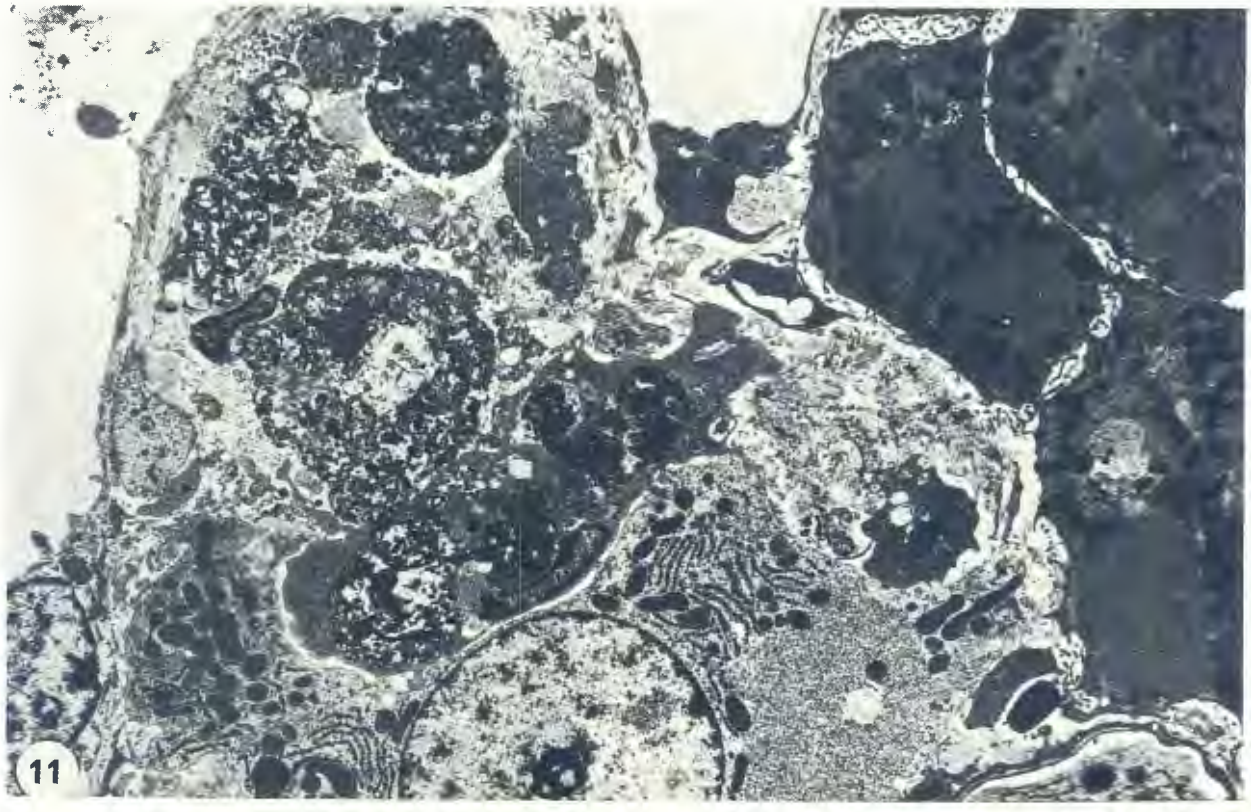


FIG. 11 Numerous free and phagocytosed amorphous bodies between "light" and "dark" hepatocytes: 4 888 ×

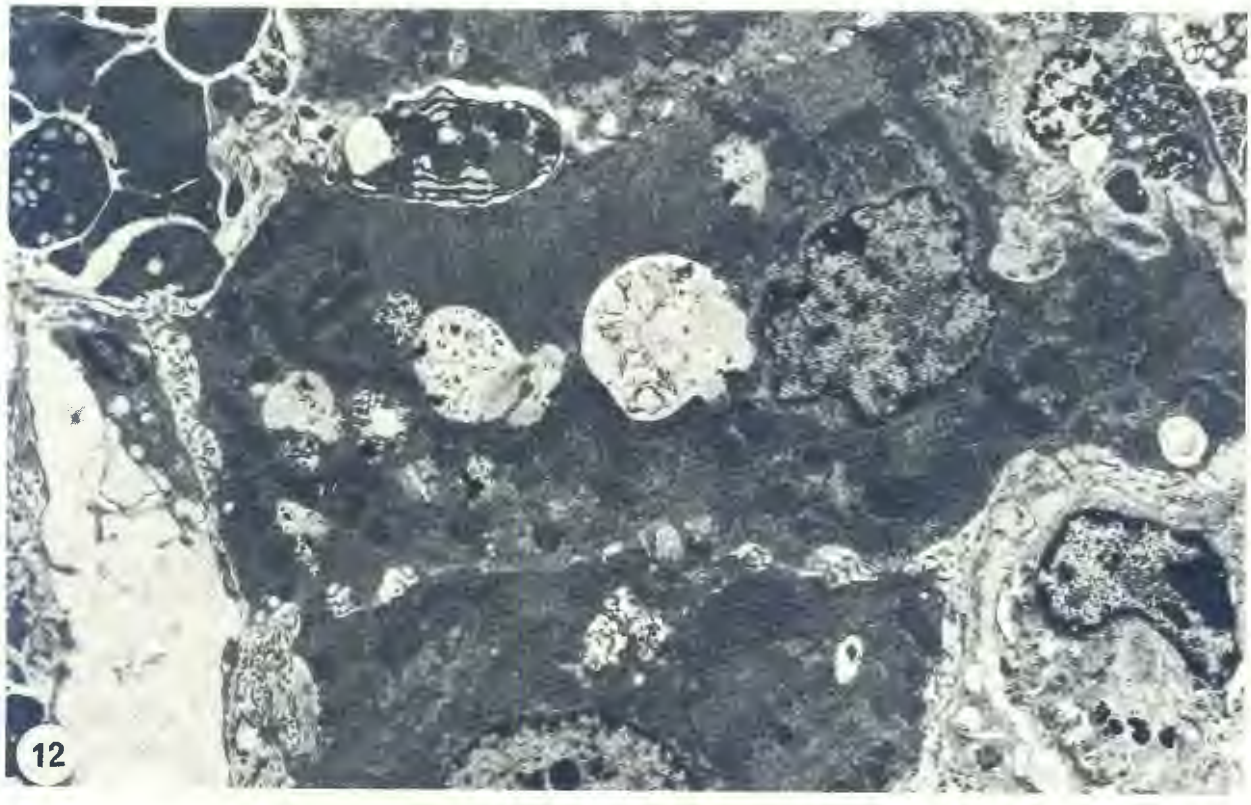
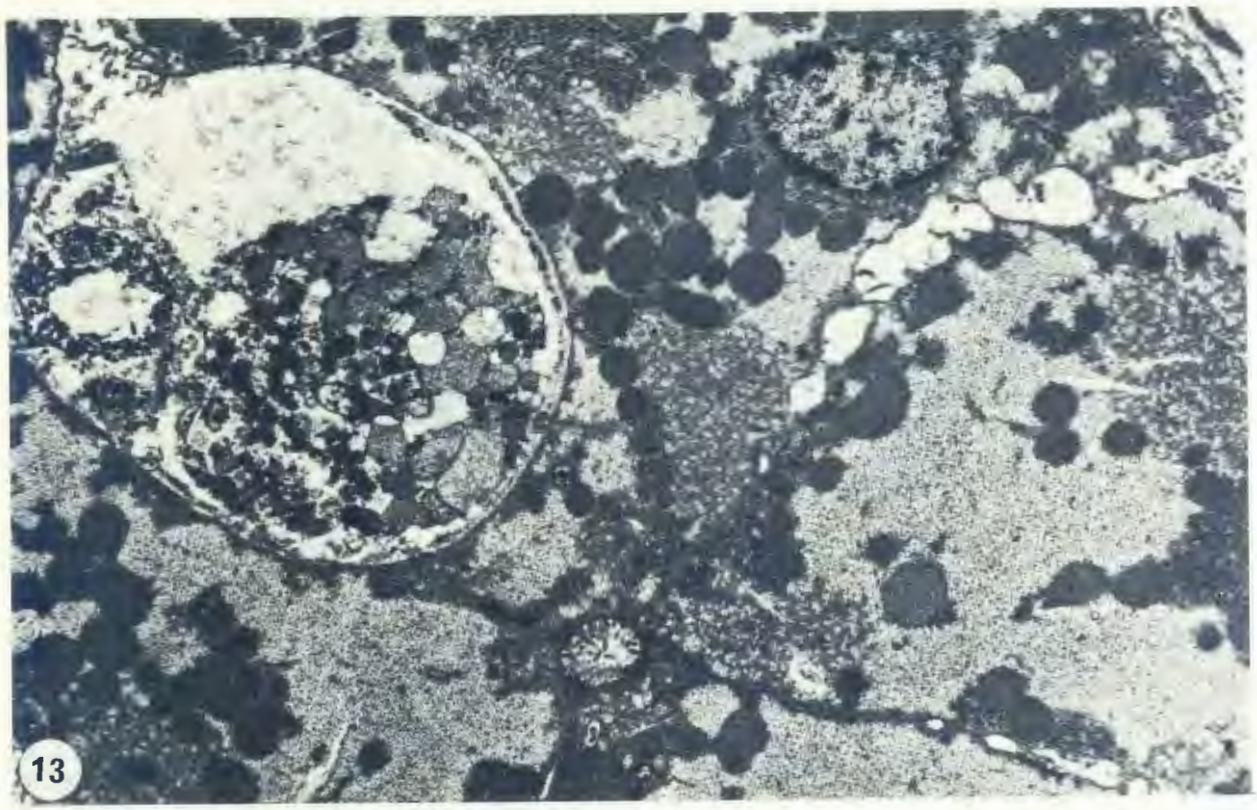
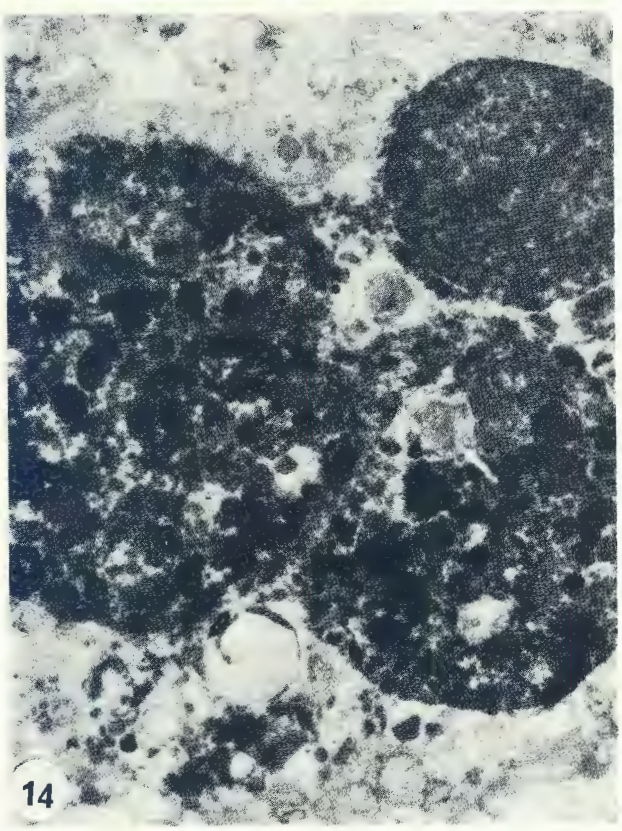


FIG. 12 Autophagolysosomes, containing myelin figures and particulated matter: 5 200 ×





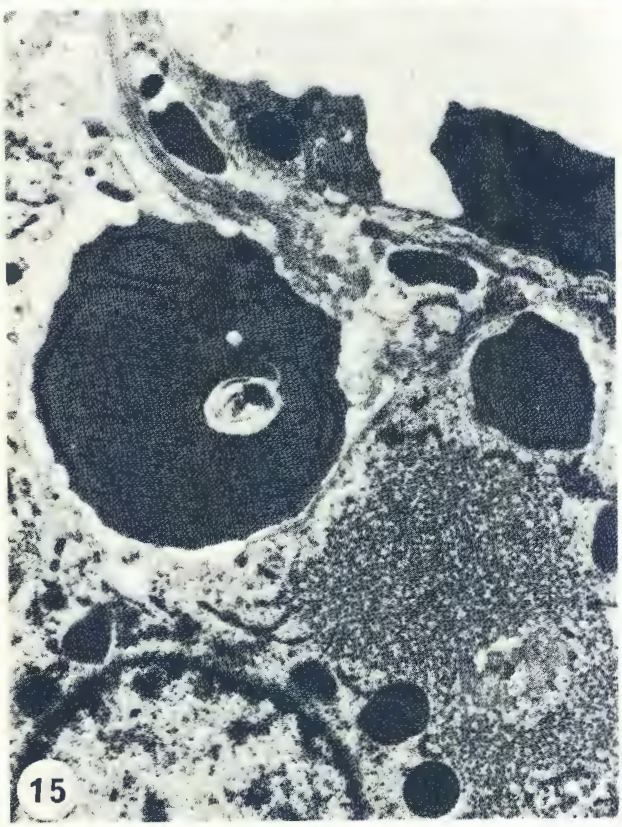


FIG. 13 Body of degradation comprising degenerated organelles and electron-dense granular and floccular material in widened intercellular space: 6 000 ×

FIG. 14 Spherical amorphous bodies of degradation: 11 700 ×

FIG. 15 Mummified pieces of cytoplasm (acidophilic bodies) in space of Disse: 6 000 ×

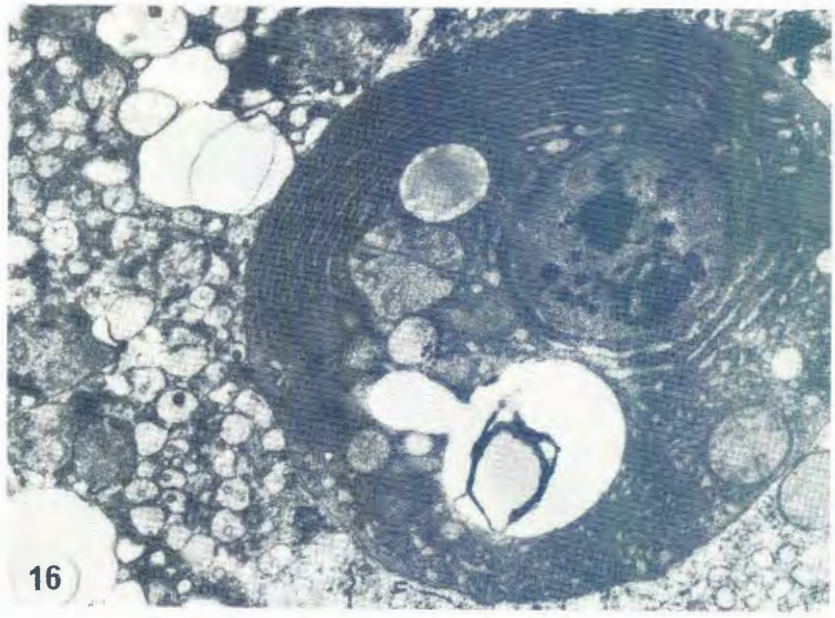


FIG. 16 Membrane-bound acidophilic body. Note tightly arranged degenerated organelles as well as fat droplets and nuclear debris: 12 000 ×

occurred was seen in our study. Electron-dense amorphous bodies and fragments of mummified cytoplasm located in the intercellular spaces were frequently seen to indent and protrude into neighbouring, mildly degenerated hepatocytes.

The light microscopic appearance and ultrastructure of acidophilic bodies have been described in many human hepatic disorders with a variety of aetiologies, including most of the viral haemorrhagic fevers (Biava & Muklova-Montiel, 1965; Klion & Schaffner, 1966; Proctor & Ruiz, 1968; Zuckerman & Simpson, 1979). These bodies are reported to be a distinctive feature of the light microscopic hepatic lesions of RVF in animals and man (Weiss, 1957; Coetzer, 1977; Van Velden *et al.*, 1977; Abdel-Wahab *et al.*, 1978; Coetzer & Ishak, 1982), although their ultrastructure has not been studied. With regard to the fine structure of acidophilic bodies, our findings generally corroborate those reported in humans with different liver disorders, namely, that they represent entire or portions of condensed and dehydrated cytoplasm containing degenerated organelles, altered nuclear material, pigments and fat droplets, but usually no glycogen. It was not always possible ultrastructurally to distinguish between fragments derived from disintegration of the so-called "dark hepatocytes" or from acidophilic bodies. The changes of the hyaloplasm and organelles in both were very similar and sometimes differed only in degree of condensation or dehydration. Furthermore, some of the bodies which were interpreted as products of cytoplasmic degradation might also be considered as acidophilic bodies when seen in HE sections.

Compared with the parenchymal changes, the sinusoids were generally not as markedly affected. The spaces of Disse were often not discernible as a result of the pronounced destruction of hepatocytes during the terminal stage of the disease. In addition, the supportive function of the hepatocytes on the sinusoids was lost and this culminated in localized dysfunction or disruption of the microvasculature. The haemodynamics of the sinusoids and spaces of Disse in these areas were therefore altered, allowing plasma and/or red blood cells to percolate out and excavate the necrotic parenchyma.

Hepatocellular fragmentation and disintegration were even more marked in primary foci of necrosis compared with those in other parts of the lobule. Sinusoids were destroyed and abundant fibrin was constantly present among the cellular debris in the foci. On the other hand, fibrin deposits were seen in only a few areas between disintegrating hepatocytes and in sinusoids in the remainder of the liver. The fibrin deposits could have formed in response to hepatocellular necrosis or sinusoidal damage or both, or they might have resulted from disseminated intravascular coagulation (DIC). In our opinion, all 3 pathogenetic factors could have been contributory.

Our studies did not include clinical pathological parameters such as thrombocyte counts, prothrombin time and fibrinogen levels to confirm DIC. However, the haemorrhagic diathesis associated with RVF infection in sheep, cattle, mice and man (Daubney *et al.*, 1931; Mims, 1956; Coetzer, 1977; Van Velden *et al.*, 1977; Abdel-Wahab *et al.*, 1978; Laughlin *et al.*, 1979), a reduction in prothrombin levels and a prolonged clotting time in mice (Mims, 1956) suggest that DIC may be a feature of RVF, as it is in some other viral haemorrhagic fevers (McKay & Margaretten, 1967). Furthermore, hepatic synthesis of coagulation or fibrinolytic enzyme precursors may be impaired as a result of extensive hepatocellular necrosis caused by this virus in animals and man.

Single or small groups of virions, measuring approximately 90 ± 5 nm in diameter, were observed free among the cytoplasmic organelles or were contained in membrane-bound vesicular or tubular structures in the cytoplasm. Rift Valley fever virus, 90–110 nm in size, has also been described in membrane-limited systems resembling Golgi and reticulum organelles (McGavran & Easterday, 1963; Lecatsas & Weiss, 1968; Murphy, Harrison & Whitfield, 1973; Ellis *et al.*, 1979). In general, virus was demonstrated intracellularly or between necrotic hepatocytes in a low percentage of affected parenchymal cells at any given time interval after infection. They were also occasionally seen in cellular debris or lying free in the intercellular and spaces of Disse, sinusoids or in association with phagocytosed debris in Kupffer cells. While numerous viral particles were discerned in some affected hepatocytes, few or no virions were seen in others. It might be that many of the

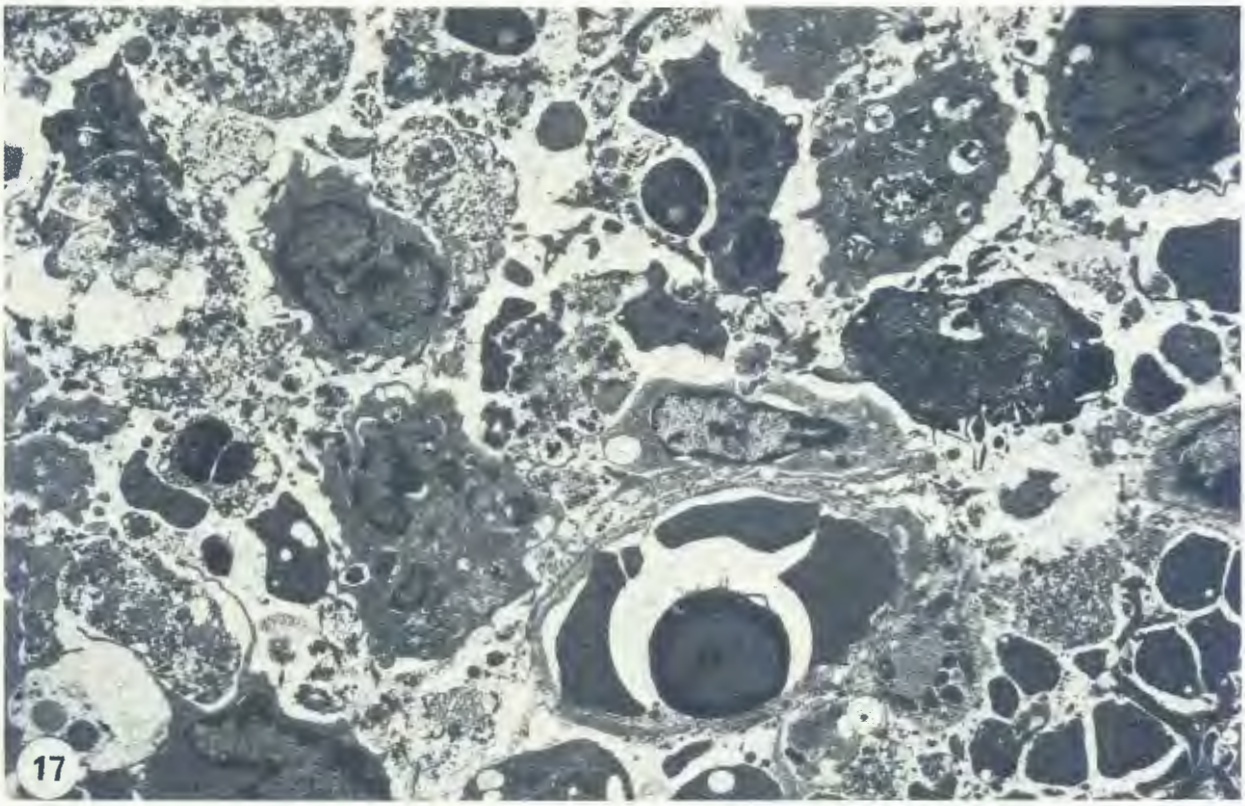


FIG. 17 Primary focus of necrosis comprising abundant cellular debris derived from necrotic hepatocytes, fibrin and a few macrophages and neutrophils. Extruded hepatocytic nucleus in sinusoid: 4 800 ×

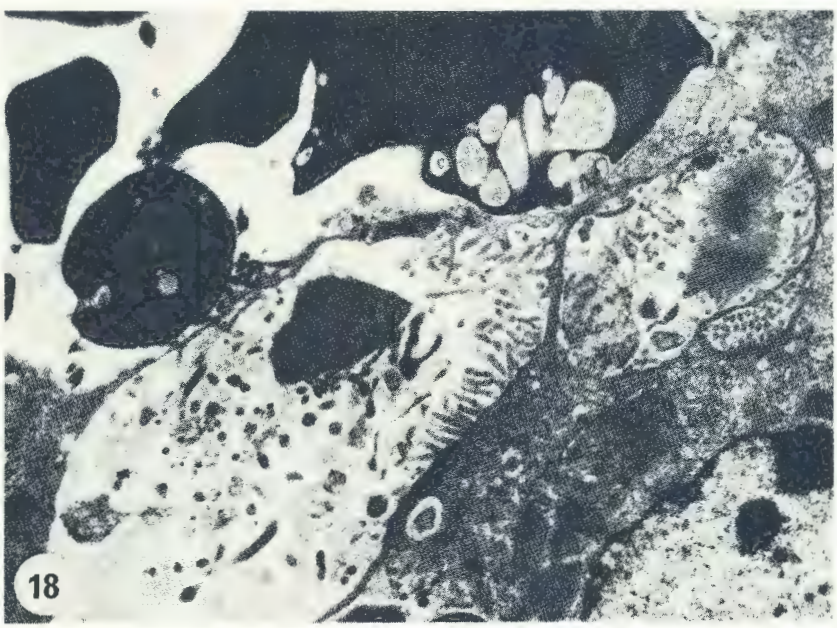


FIG. 18 Grossly distorted bile canaliculi with desquamated microvilli, sequestrated pieces of cytoplasm and small amounts of bile in its lumen: 7 500 ×

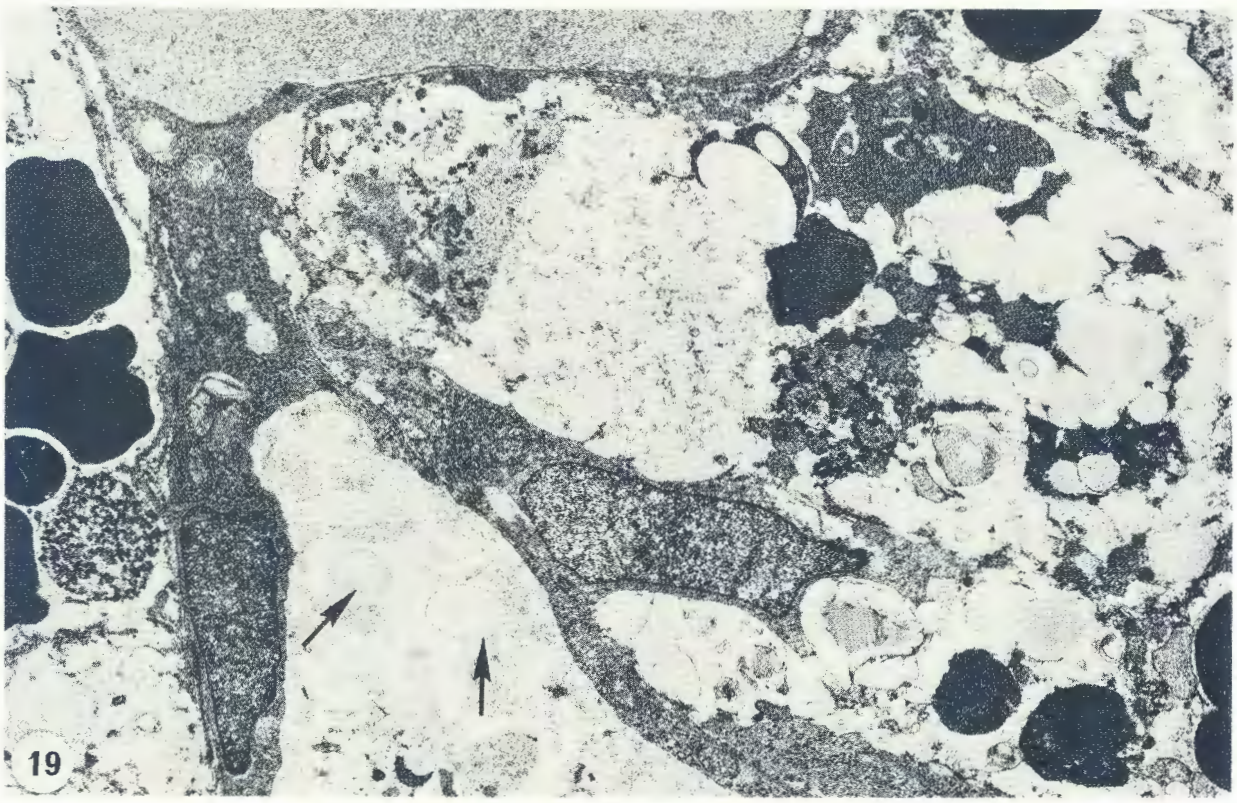


FIG. 19 Perisinusoidal cavities filled with plasma, red blood cells and remnants of disintegrated hepatocytes. Note cellular debris in sinusoids (arrow): 5 600 ×

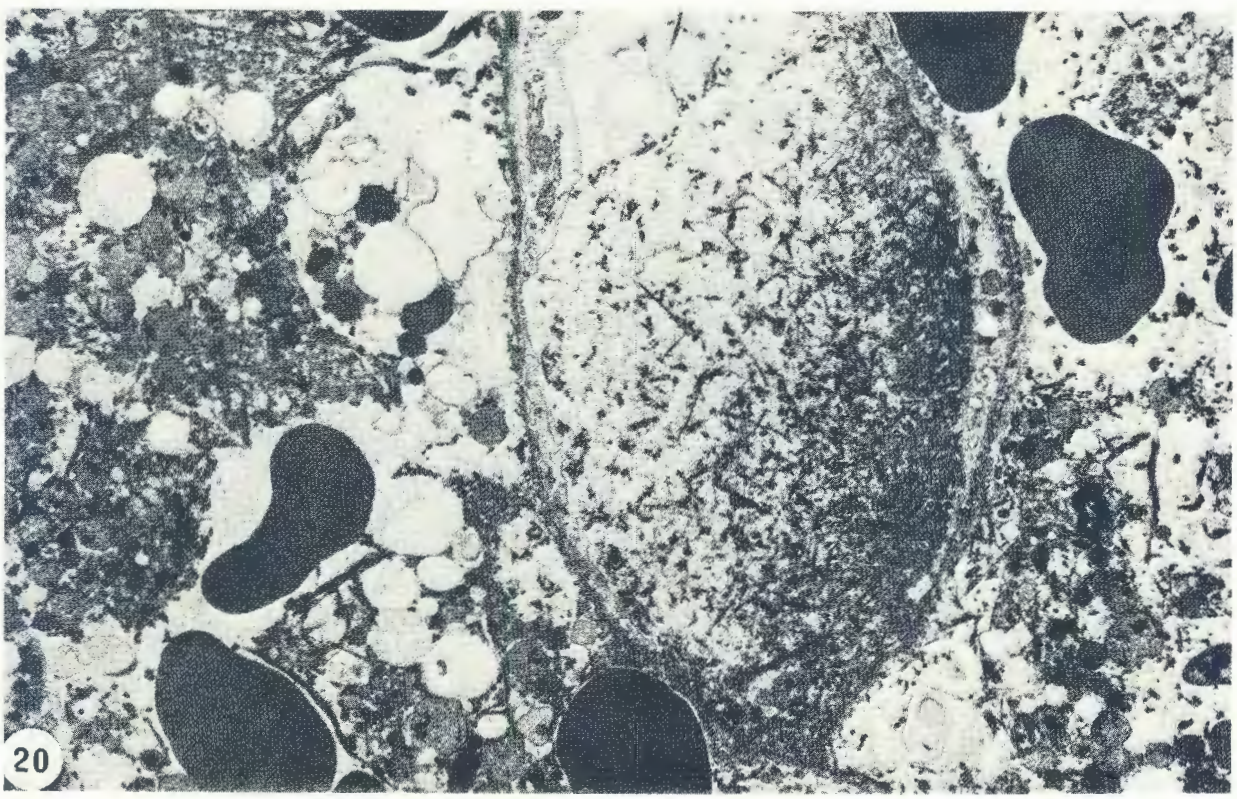


FIG. 20 Abundant intrasinusoidal and perisinusoidal fibrin deposition: 6 000 ×

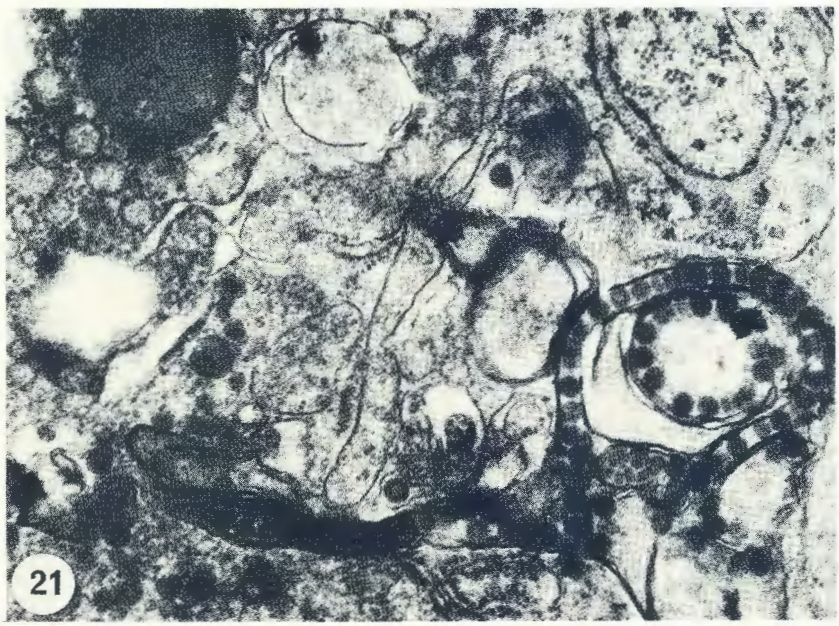


FIG. 21 Viral particles contained in vesicular and tubular membrane structures: 30 000 ×

affected hepatocytes contained such a low concentration of virions that they were not demonstrable in thin sections in that specific field in every necrotic parenchymal cell. Alternatively, the virus might already have been released or products of it could have triggered cellular degeneration and necrosis without infecting the cells *perse*.

In conclusion, there appear to be direct and indirect mechanisms which play a role in the development of the hepatic lesions in RVF. The direct mechanism involves the penetration and multiplication of virus in hepatocytes, and this interferes with normal functions of the cell. Indirect mechanisms include the effects of inflammatory cells (predominantly neutrophils and macrophages) on infected and non-infected parenchymal cells as well as changes resulting from microvasculature disturbances.

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