Onderstepoort J. vet. Res., 50, 59–71 (1983)

PHOTOSENSITIVITY IN SOUTH AFRICA. V. A COMPARATIVE STUDY OF THE PATHOLOGY OF THE OVINE HEPATOGENOUS PHOTOSENSITIVITY DISEASES, FACIAL ECZEMA AND GEELDIKKOP (*TRIBULOSIS OVIS*), WITH SPECIAL REFERENCE TO THEIR PATHOGENESIS

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ABSTRACT

COETZER, J. A. W., KELLERMAN, T. S., SADLER, W. & BATH, G. F., 1983. Photosensitivity in South Africa. V. A comparative study of the ovine hepatogenous photosensitivity diseases, facial eczema and geeldikkop (*Tribulosis ovis*), with special reference to their pathogenesis. *Onderstepoort Journal of Veterinary Research*, 50, 59–71 (1983).

The subject of this study was the pathological and scanning electron microscopical changes in the biliary systems of sheep suffering from facial eczema or geeldikkop (*Tribulosis ovis*), or made photosensitive by ligation of the common bile duct. While an obliterative cholangitis is responsible for the retention of phylloerythrin in facial eczema, the occlusion of bile ducts with crystalloid material (microliths) appear to perform a similar function in geeldikkop. The similarities and differences between the 2 diseases are discussed in the light of their pathogenetic mechanisms.

INTRODUCTION

Ovine hepatogenous photosensitivity in South Africa can be induced by several hepatotoxic plants, an alga, *Microcystis toxica* (Steyn, 1949; Van Tonder, Basson & Van Rensburg, 1972; Kellerman, Basson, Naudé, Van Rensburg & Welman, 1973; Kellerman, Coetzer & Schneider, 1983; Coetzer & Bergh, 1983) and 2 fungi, namely, *Phomopsis leptostromiformis* (Van Warmelo, Marasas, Adelaar, Kellerman, Van Rensburg & Minné, 1970; Marasas, 1978) and *Pithomyces chartarum* (Marasas, Adelaar, Kellerman, Minné, Van Rensburg & Burroughs, 1972; Kellerman, Van der Westhuizen, Coetzer, Roux, Marasas, Minné, Bath & Basson, 1980). Apart from facial eczema, which is caused by *P. chartarum*, the other hepatogenous photosensitivity syndromes in sheep develop as a result of hepatocellular damage. Sporidesmin, the mycotoxin produced by *P. chartarum*, primarily affects the biliary system, causing an obliterative cholangitis that culminates in retention of the photodynamic agent, phylloerythrin (Mortimer, White & Di Menna, 1978).

Recently Kellerman *et al.* (1980) demonstrated that the ovine hepatogenous photosensitivity disease geeldikkop (*Tribulosis ovis*), can be experimentally produced by the simultaneous ingestion of toxic cultures of *P. chartarum* and the plant *Tribulus terrestris*. Although facial eczema and geeldikkop have some characteristics in common, it would seem, from pathological observations, that their pathogenetic mechanisms differ. Our aim in this communication is to shed more light on a mechanism(s) that might play a role in the development of photosensitization in geeldikkop, and to compare it with that reported in facial eczema.

MATERIALS AND METHODS

Specimens for light and ultra microscopy were collected from the sheep that developed geeldikkop or facial eczema in a previous experiment (Kellerman *et al.*, 1980), as well as from 7 sheep that became photosensitive on wilted *T. terrestris* after being dosed with 0,5 mg/kg sporidesmin in a subsequent trial. Segments of the extra- and intrahepatic bile ducts, the *ductus cysticus* and gall bladders of these animals and 21 control sheep were fixed in 10% buffered formalin, embedded in paraffin wax and processed routinely for light microscopy. In addition to haematoxylin and eosin, special stains were applied to selected sections.

For scanning electron microscopy (SEM) the ducts were sliced transversely or split longitudinally into 1–4 mm blocks and fixed in 4% buffered glutaraldehyde.

The crystalloid material suspended in the bile of some geeldikkop sheep was allowed to sediment out, the sediment being stored in analar ethanol at 4 °C. The glutaraldehyde fixed tissues and the crystalloid material were prepared for SEM as follows:

They were post-fixed in 2% osmium tetroxide for 1 h and then dehydrated in an alcohol series. The alcohol was cleared with an alcohol:amylacetate solution (1:1) followed by critical point-drying of the specimens with CO_2. The material was then coated with gold, using a sputter coater and viewed at 30 Kv with a ISI 100 scanning electron microscope.

Three sheep were anaesthetized with pentobarbitone sodium and their common bile ducts ligated as described by Quin (1933). The sheep were then fed on green lucerne, placed in the sun, and examined daily. Two of the 3 sheep were necropsied 12 days after the operation and the other after 7 days.

RESULTS

The pathology, with particular reference to the liver lesions of experimentally induced cases of geeldikkop and facial eczema, has been reported on elsewhere (Kellerman *et al.*, 1980). In the present study the biliary changes in these sheep, their controls, as well as those that were made photosensitive by ligation of the common bile duct were examined in greater detail.

Geeldikkop

Gross pathology: In the sheep that became photosensitive after receiving low levels of sporidesmin (0,25 mg/kg), the extra- and intrahepatic bile ducts, the *d. cysticus* and the loose connective tissue about the hilus of the liver were usually mildly oedematous. Although the affected ducts were slightly thickened, their walls were still partially translucent, making it possible to discern chalky-white sediment (needle-point to almost pin-head in size) in their lumina. Small aggregates of this sediment lodged at numerous locations in the extrahepatic bile ducts and *d. cysticus* (Fig. 1). On palpation of the ducts these aggregates moved about freely in the bile.

In the sheep that were dosed with higher levels (*c.* 0,5–0,7 mg/kg) of sporidesmin the larger bile ducts were sometimes very oedematous and moderately thickened, making it difficult or impossible to discern the sediment through their walls.

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Received 21 October 1982—Editor

On cut section, the chalky-white sediment oozed with the bile from the intra- and extrahepatic bile ducts and the *d. cysticus*, all the mucosal surfaces of which were covered with copious amounts of this material. The impression was gained that some of the larger bile ducts were occluded, to varying degrees, by the sediment. The fine particles were often suspended in the bile or deposited on the mucosal surface of the gall bladder. Sometimes the viscosity of the bile was increased.

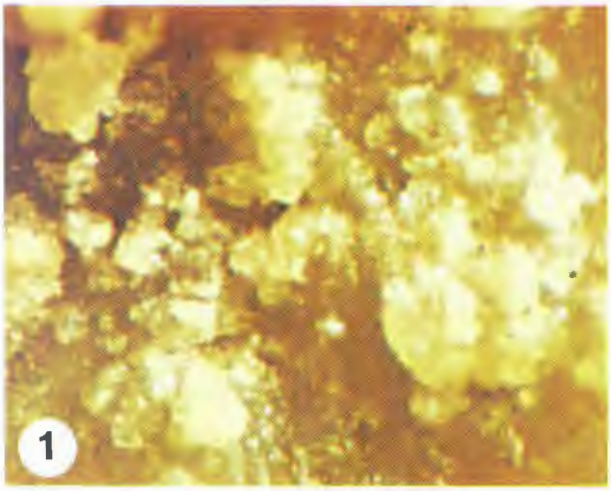
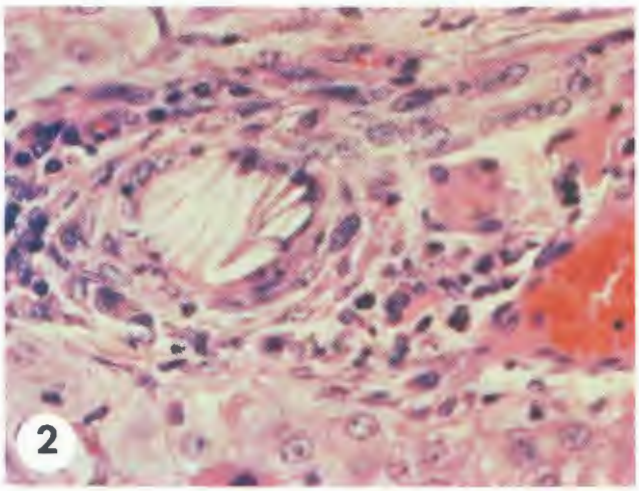
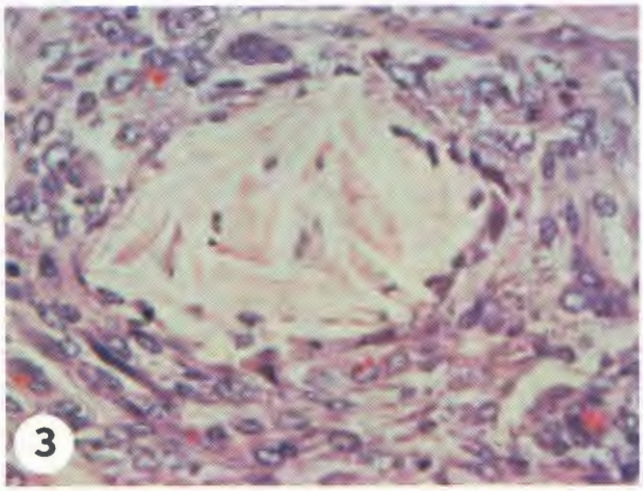


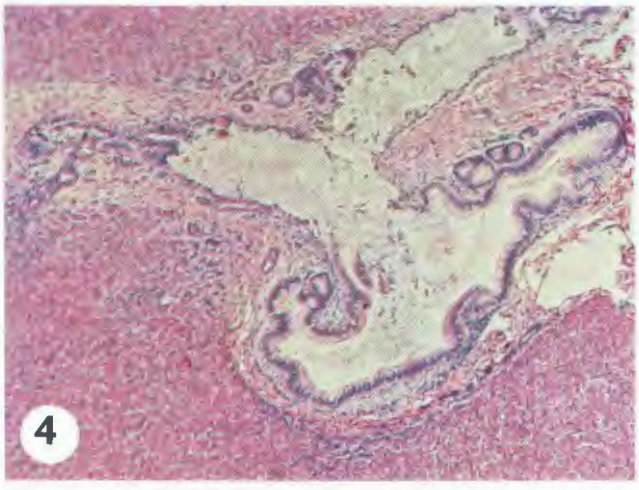
FIG. 1 Extrahepatic bile duct: copious amount of crystalloid material on mucosal surface

Light microscopy: The most conspicuous feature was the accumulation of crystalloid material in the portal bile ducts (Fig. 2), in the larger intra- and extrahepatic bile ducts (Fig. 3–5), and in the *d. cysticus*. The lumina of some of these ducts, especially those in the larger bile ducts and the *d. cysticus* were often partially or almost entirely occluded with crystalloid material. A mild to moderate periductal lamellar fibrosis was associated with these occlusions, and the ducts appeared to be somewhat dilated. At the sites where crystalloid material had accumulated, the lining epithelium revealed degenerative and necrotic changes or appeared to be flattened and atrophic (Fig. 5). The morphology of the bile ducts and ductules in the portal triads was often distorted by the presence of the material and the uneven proliferation (sometimes resembling giant cells) of epithelium.

Scanning electron microscopy: The amount of crystalloid material within the extrahepatic bile ducts and *d. cysticus* varied at different locations along their lengths, and also between animals. While the lumina in some segments could be almost entirely obliterated by crystalloid material (Fig. 6), at other points along the same ducts they might be sparsely distributed, or even absent. The morphology of the microliths varied considerably, from needle-like spicules or pleomorphic plates to more bizarre forms (Fig. 7). The majority of these microliths, however, were plate-like with sharp or round edges (Fig. 8–11). As a result of the pleomorphism of the plate-like microliths it was impossible to calculate their precise







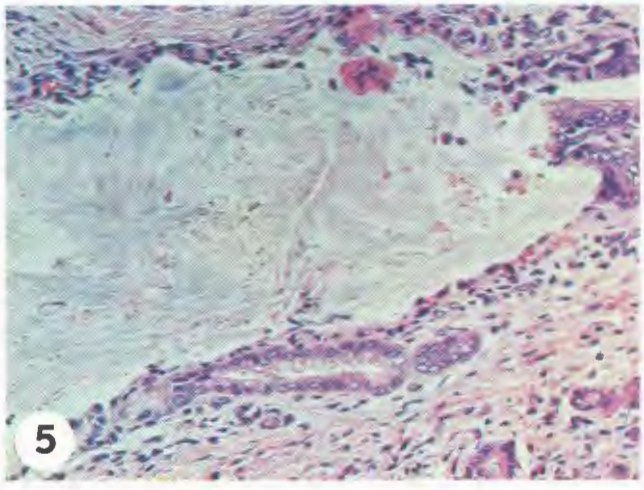


FIG. 2 & 3 Occlusion of portal bile ducts with crystalloid material: HE × 500
FIG. 4 Crystalloid in a large intrahepatic bile duct: HE × 75
FIG. 5 Accumulation of crystalloid material in a large intrahepatic bile duct. Note atrophic, degenerative and necrotic changes in the lining epithelium: HE × 200

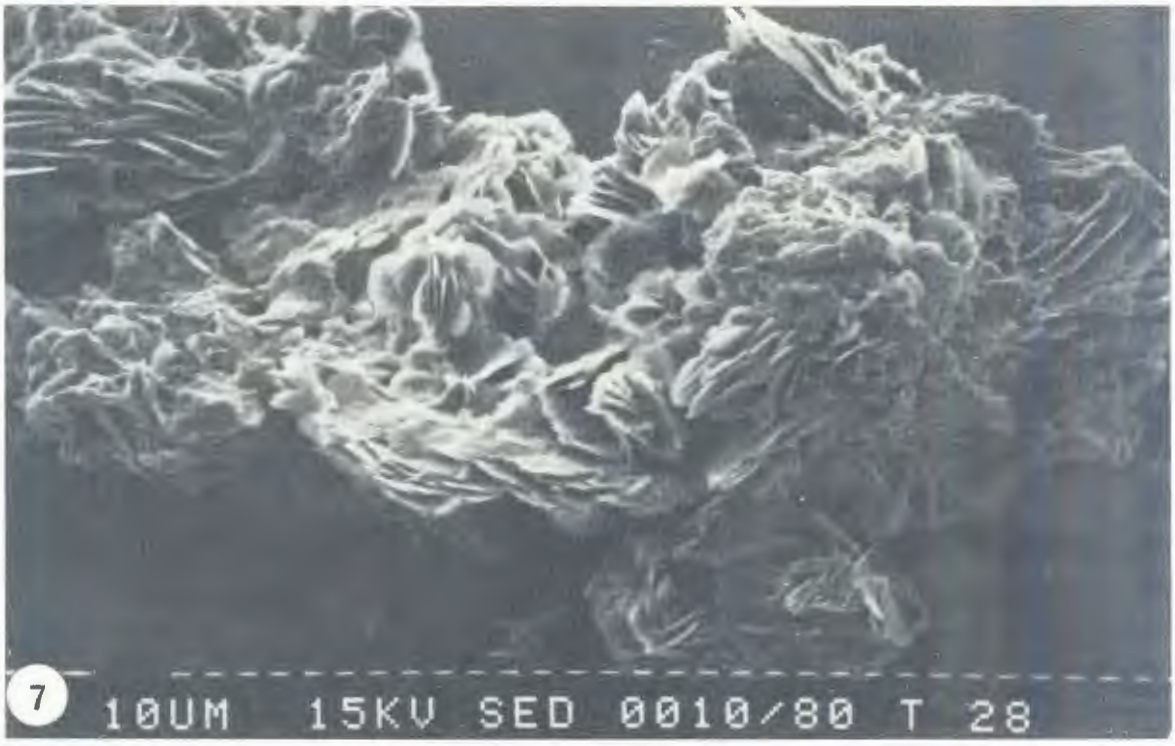


FIG. 6 Extrahepatic bile duct occluded by crystalloid material: × 160
FIG. 7 & 8 Microlith composed of pleomorphic plates and bizarre plate-like structures: × 320 and × 640 respectively

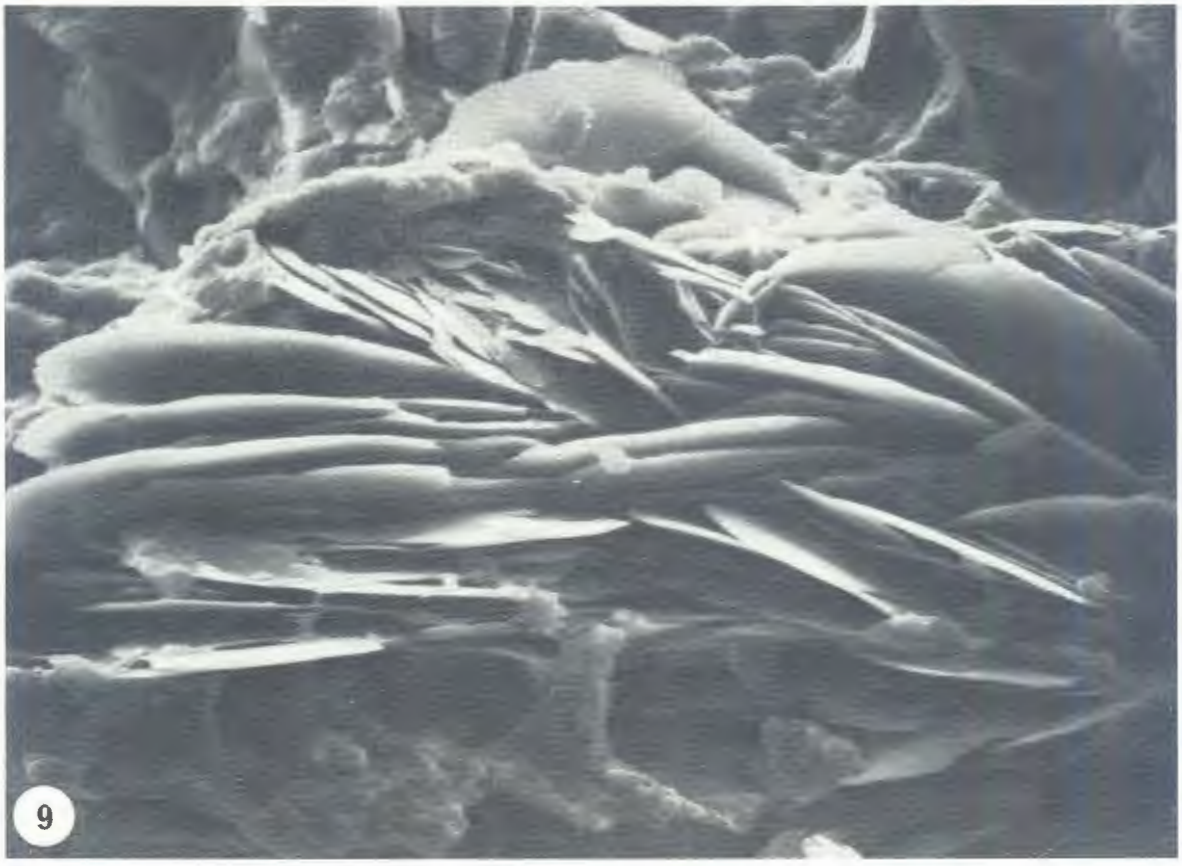
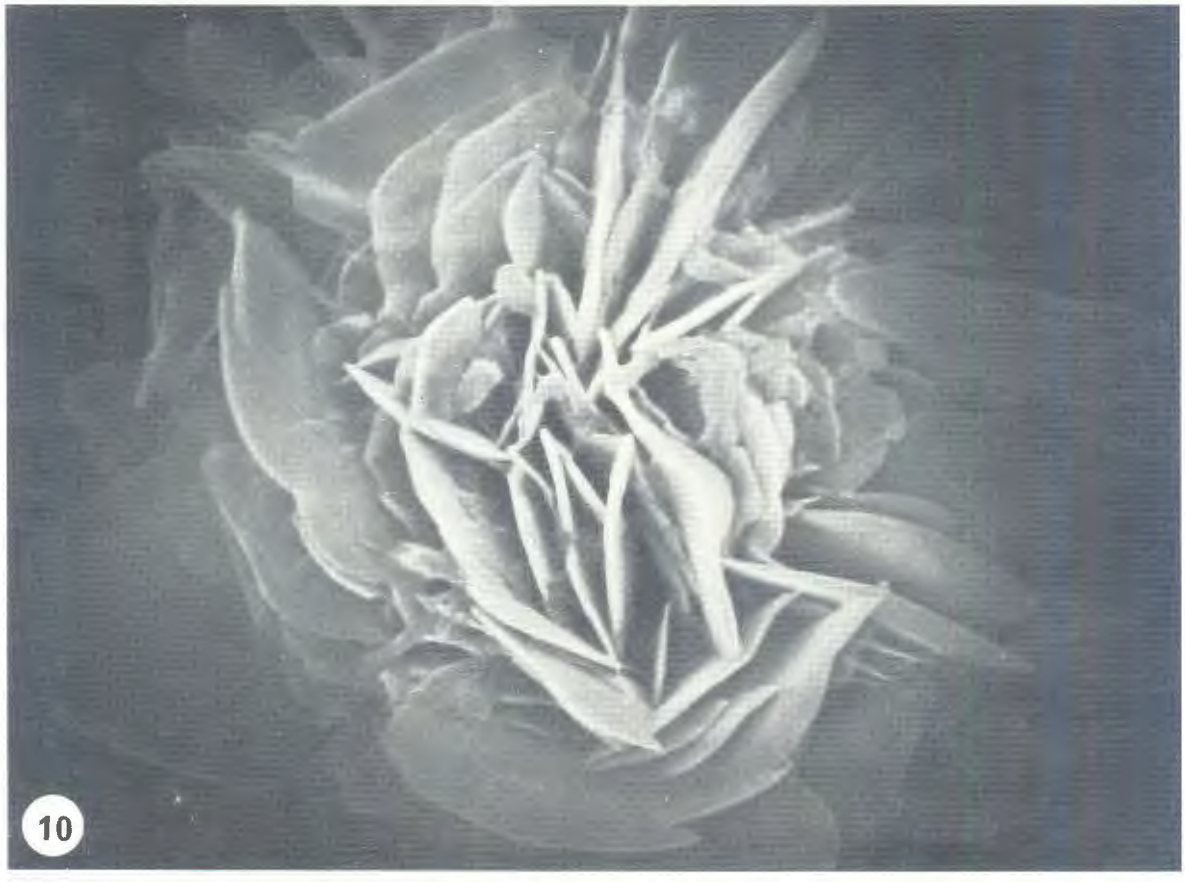


FIG. 9–11 Plate layered upon one another in different pattern: × 1280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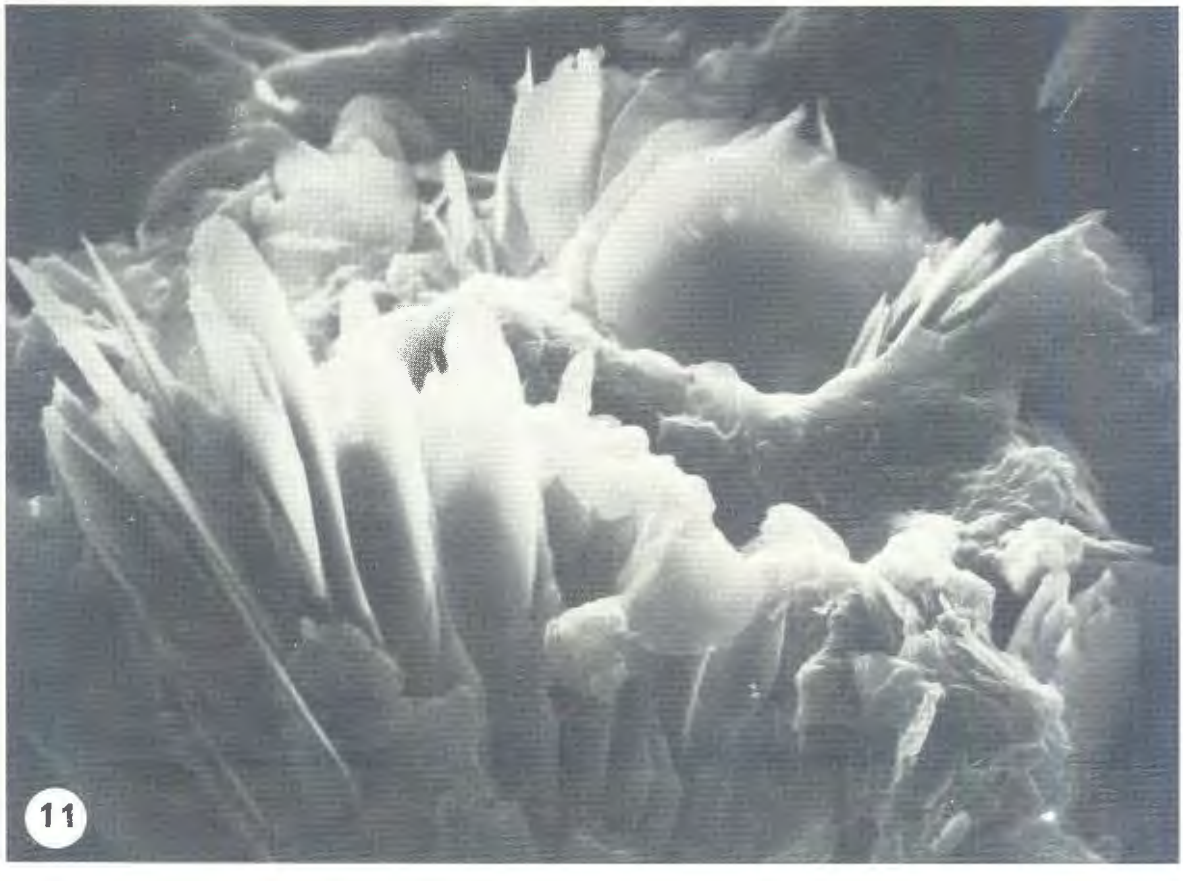
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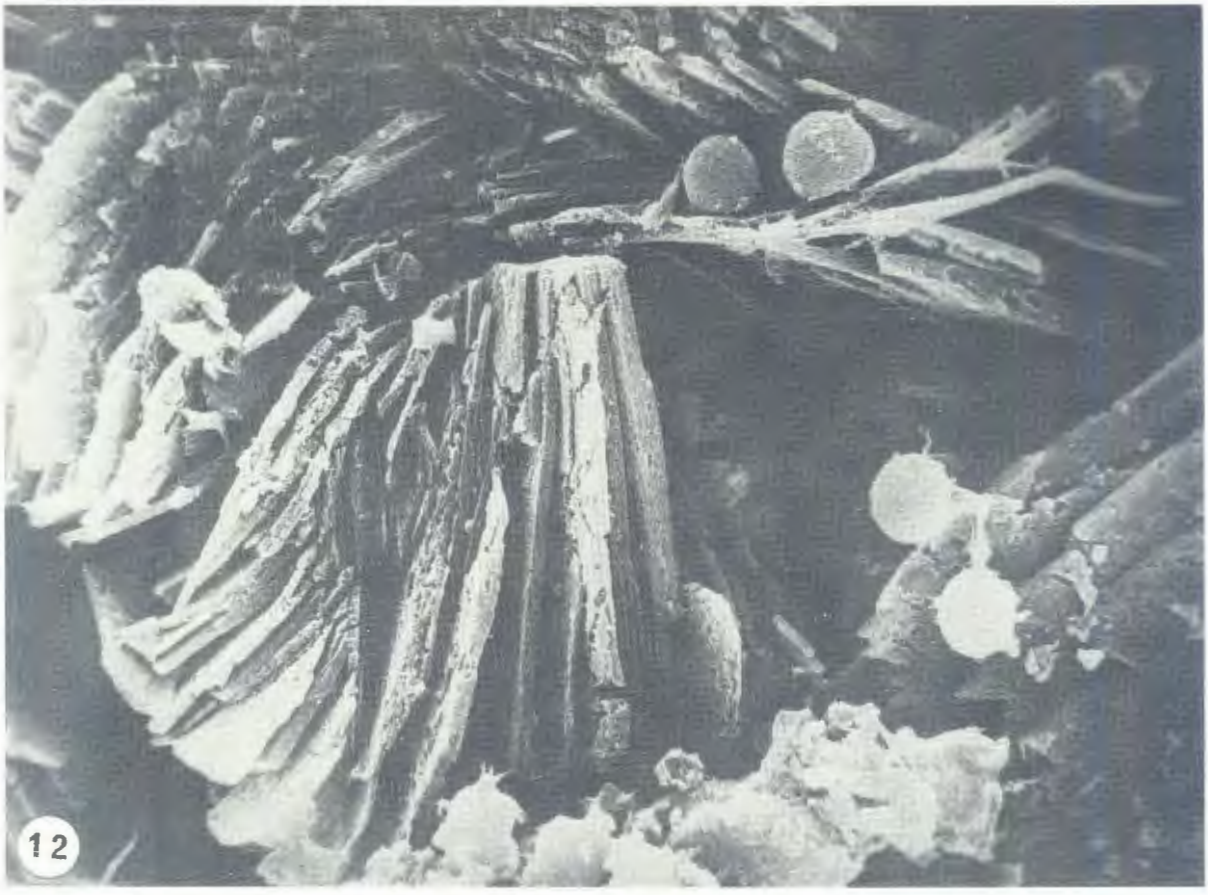


FIG. 12 The surfaces of the plates appeared eroded and marked with small pits and cracks: × 1800

size, but generally speaking they were *c.* 0,5 µm thick and *c.* 2–20 µm wide. Their surfaces were often slightly eroded and irregular, and marked with small pits and cracks (Fig. 12). Fine fibrillar material (mucous) and/or small globular structures (5–7 µm in size) adhered to some of the microliths or were dispersed between them (Fig. 13 & 14). The globular structures were often connected to one another by short, sparsely distributed, surface processes (Fig. 14).

The mucosal surfaces were affected to varying degrees along the length of the ducts, the lining epithelium being most severely affected where the microliths aggregated. Here swelling and partial or complete loss of microvilli, sometimes accompanied by separation and sloughing of isolated groups of epithelial cells, were evident. A few red blood cells and some inflammatory cells were often seen in these denuded areas. Also occasionally present were similar mucosal lesions, not associated with microliths.

Greater or lesser amounts of crystalloid material could be found in the bile of the gall bladder (Fig. 15).

Facial eczema

Gross pathology: The macroscopical changes in the liver, with particular reference to the biliary lesions of sheep affected with facial eczema, have been thoroughly investigated (Mortimer, 1963; Marasas *et al.*, 1972; Mortimer *et al.*, 1978; Kellerman *et al.*, 1980). These workers concluded that the lobulation was accentuated by the presence of the moderate to severe portal reaction. The walls of the larger bile ducts (both intra- and extrahepatic) as well as the *d. cysticus*, were often markedly thickened and oedematous (Fig. 16), while in some of them the lumen was partially or almost totally obliterated by scar tissue. On cut section, the inner surfaces of these ducts sometimes appeared to be bile-stained.

The gall bladder wall was often oedematous, while the mucosa frequently contained multifocal haemorrhages, erosions or ulcerations. Copious amounts of mucous were usually present in the bile or adhered to the mucosal surface of the affected parts.

Light microscopy: The biliary tree was the structure primarily affected. A moderate to severe portal fibroplasia and bile duct proliferation were seen in most livers (Fig. 17), the fibrosis being mostly arranged in concentric patterns around the bile ducts of the portal triads and the larger bile ducts traversing the parenchyma (Fig. 17 & 18). In general, the mucosa of the major intra- and extrahepatic bile ducts and the *d. cysticus* was more regularly affected with degeneration and/or necrosis than the smaller bile ducts or ductules in the triads. The ductal necrosis was often associated with marked periductal granulation tissue proliferation and fibrosis (Fig. 19). In some instances, the lumina were entirely replaced by scar tissue (Fig. 20). The above changes, together with necrotic debris and inspissated bile, culminated in the partial or complete occlusion of the affected ducts. Vascular lesions, which included fibrinoid degeneration and necrosis of the blood vessel wall in juxtaposition to the affected duct, sometimes accompanied the changes in the biliary system.

The wall of the gall bladders was often oedematous and, in some instances, showed multifocal erosions, ulcerations or haemorrhages.

Scanning electron microscopy: Save for the absence of crystalloid material, the SEM changes in the bile ducts and *d. cysticus* were very similar to those of geeldikkop. Where the bile ducts were necrotic, the microvilli were lost and the epithelium was desquamated, exposing the submucosa (Fig. 21).

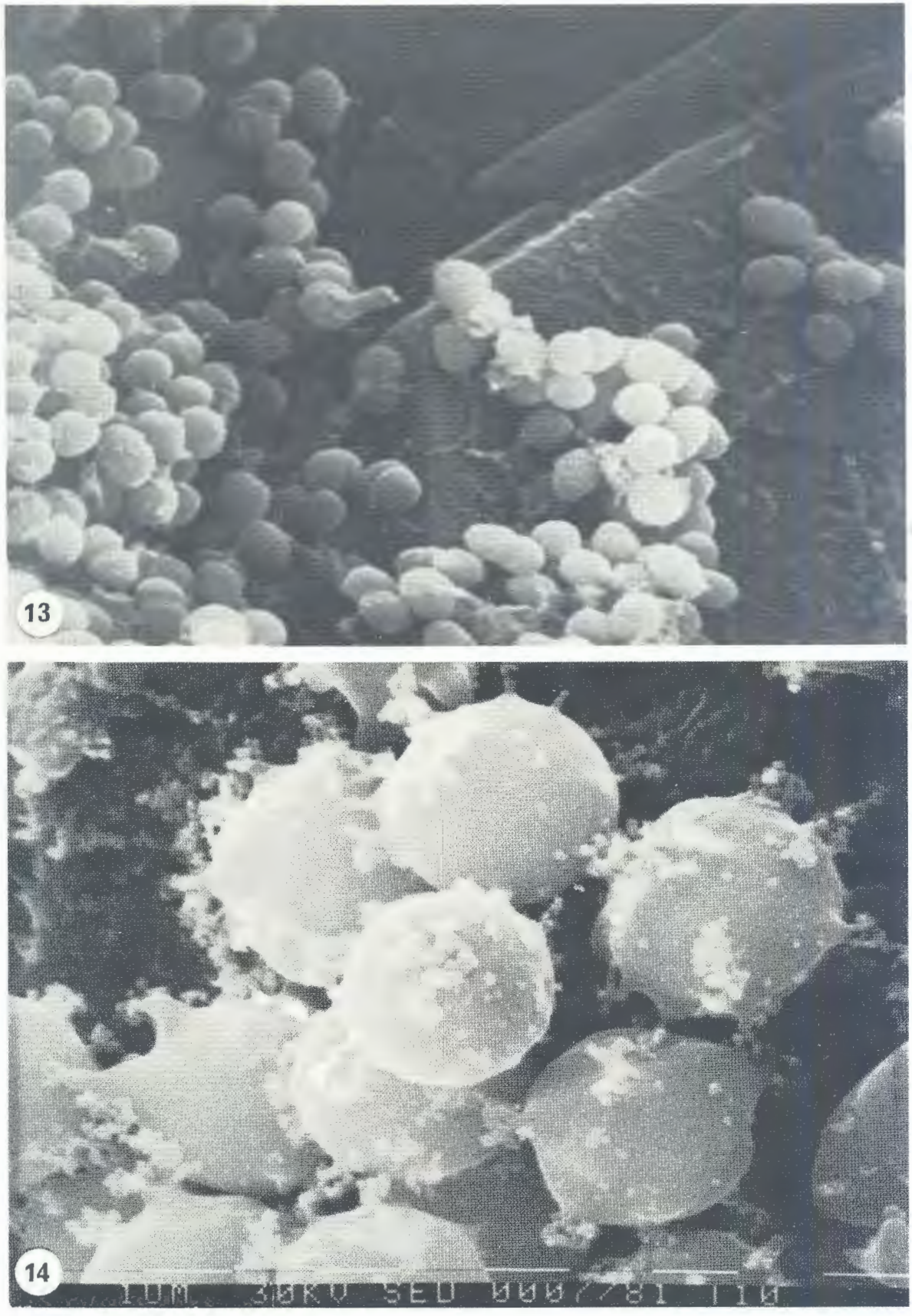


FIG. 13 & 14 Fine fibrillar material and/or small globular structures, adhering to some of the microliths: × 10 000 and N 20 000 respectively

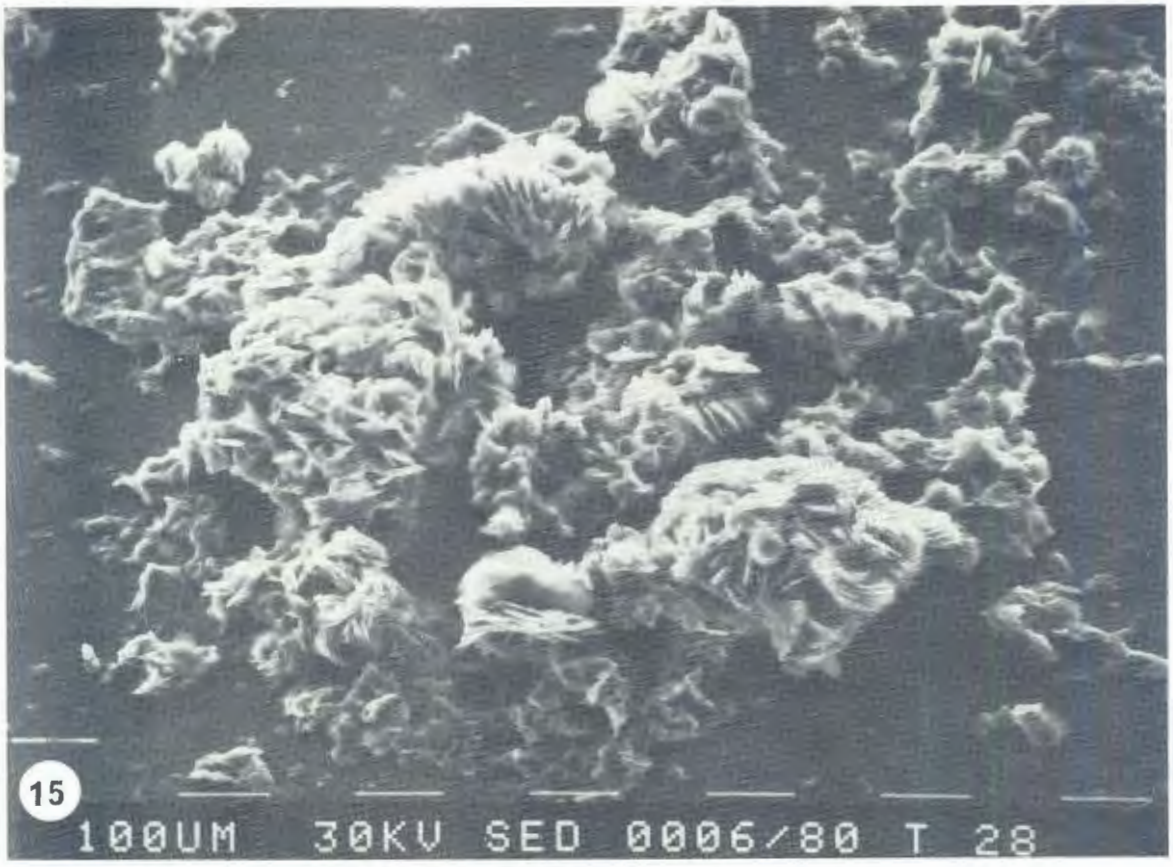


FIG. 15 Crystalloid material in bile in gall bladder: × 160

Control Sheep

The macroscopical and microscopical appearance of the bile ducts was essentially normal. Scanning electron microscopic observations showed that the mucosal surface of the extrahepatic bile ducts and *d. cysticus* were made up of either longitudinal and transverse folds or convolutions covered with epithelium (Fig. 22 & 23). The luminal surface of the lining epithelial cells possessed microvilli (Fig. 24) which were sometimes interspersed with isolated or small groups of cilia arranged in rows. The latter became more numerous in the *d. cysticus* (Fig. 25). In some areas the cilia were so abundant and tall that they intertwined. Occasionally, the microvilli were partially covered with a slightly granular material, thought to be residual glycocalix.

Fine fibrillo-granular deposits of varying size were seen on the lining epithelium. Only a few of these small, almost round globules (5–7 μm in diameter) with a smooth surface were aggregated. The deposits were interpreted as biliary debris containing mucin, cellular debris and bile. In addition, electron-dense amorphous aggregates of inspissated bile were noted in some of the bile ducts. However, no crystalloid material occurred in any of the ducts studied.

Ligation of common bile duct

Gross pathology: Two of the 3 experimental sheep became photosensitive 2 days after ligation and the other on the 3rd day. All the animals developed photodermatitis, coronitis and icterus. The 2 sheep that became photosensitive first where necropsied on the 12th day and the other on Day 7.

The livers of these animals were normal in size and light brown in colour. In one of them the Glisson's capsule was thickened and had an irregular appearance, with numerous fibrin strands adhering to it. Apart from the liver lesions, the adrenals were swollen and a nephrosis was invariably present. Other lesions included peritonitis and ascites, the fluid having a high protein content and a yellow colour.

Light microscopy: Although the microscopical changes in the liver of the sheep necropsied on Day 7 were more pronounced, similar lesions occurred also in the livers of the other two. The most conspicuous changes consisted of mild to marked lamellar fibrosis and oedema around many of the bile ducts in the portal triads and larger bile ducts traversing the parenchyma (Fig. 26–28). This was accompanied by mild to moderate bile duct proliferation and infiltration of a few mononuclear cells, some of which contained lipofuscinous pigment in the portal areas. A number of the bile ducts appeared to be dilated, and contained bile.

Diffuse vacuolar degeneration of the parenchyma was evident in all 3 of the livers. Bile pigment was present in some of the affected hepatocytes, as well as in Kupffer cells. Eosinophilic, slightly homogeneous to ground glass-like globules and anisonucleosis occurred in many of the degenerated liver cells. Isolated necrotic hepatocytes were scattered throughout the parenchyma, while periportal foci of hepatocellular necrosis (bile infarcts) were evident in 2 livers (Fig. 26, 27, 29). The foci were usually heavily infiltrated with neutrophils and pigment-laden macrophages. In some foci the necrotic tissue was bile stained or else the bile was dispersed among the cellular debris.

In 2 livers the Glisson's capsules were prominently thickened due to fibroplasia and oedema, with fibrin adhering to the outer surface. Numerous bile pigment-laden cells were noted in the thickened capsule.

FIG. 16 Marked thickening of extrahepatic bile ducts. Note inspissated bile in affected bile ducts

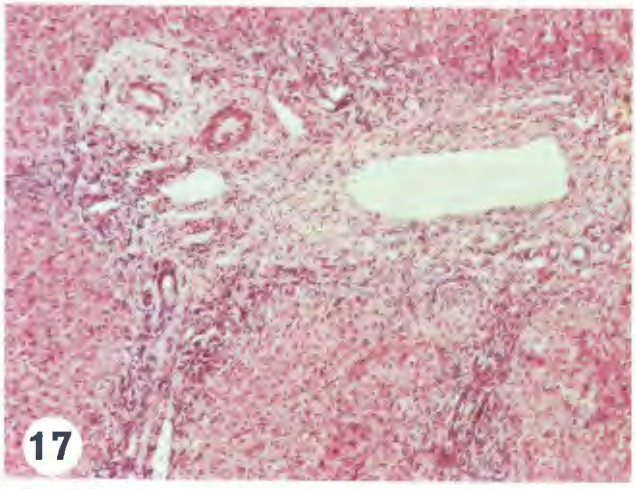
DISCUSSION

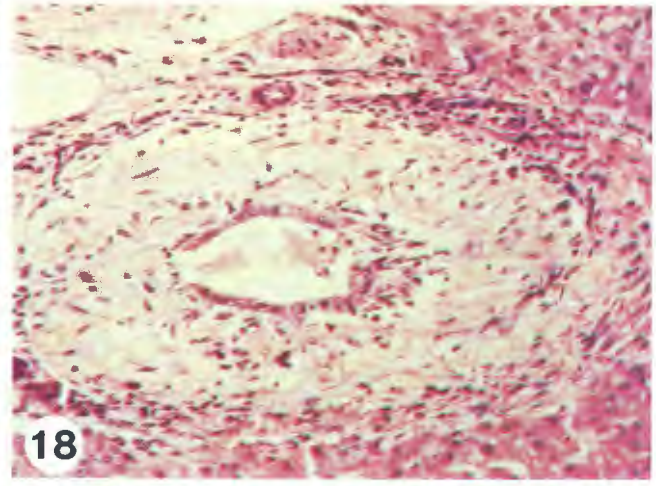
Ovine hepatogenous or secondary photosensitization is brought about by the action of certain plant poisons or mycotoxins on either the parenchyma and/or the biliary system. In both instances the damage is of such a nature that phylloerythrin, a photodynamic agent normally rapidly excreted via the liver, is retained.

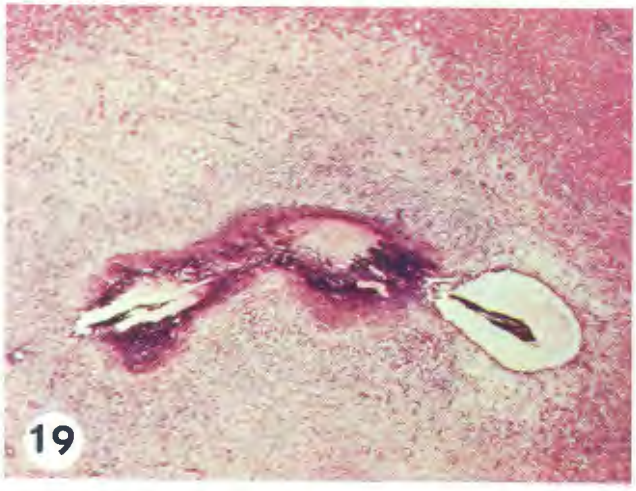
Plants such as *Asaemia axillaris* (Kellerman *et al.*, 1973; Coetzer & Bergh, 1983), *Lasiospermum bipinnatum* (Kellerman *et al.*, 1973) and *Athanasia trifurcata* (Kellerman *et al.*, 1983) involve primarily the liver parenchyma. The hepatic lesions in poisoning with these plants have been shown to range from different patterns of zonal necrosis in some animals to diffuse parenchymal degeneration in others.

In both facial eczema and geeldikkop the biliary tree is the structure primarily affected. The lesions in the case of facial eczema, which consist of necrosis and periductal fibrosis plus scarring of intra- and extrahepatic bile ducts, culminate in obliteration of the ducts and retention of phylloerythrin. The occluding mechanism in geeldikkop, on the other hand, appears to be the blockage of especially the larger bile ducts with crystalloid material. This material aggregates, often in plates, to form dense casts or microliths, which can ultimately impede the excretion of phylloerythrin.

In facial eczema the latent period (the time between ingestion of the toxin and the manifestation of photosensitivity) correlates with the time required for the obliterative cholangitis to develop. This latent period of roughly 10–30 days is often longer than that recorded in geeldikkop (Kellerman *et al.*, 1980, Van Tonder *et al.*, 1972) which can be as short as 2 days (Bath & Kellerman, unpublished data, 1980). It is now believed that the sudden blockage of the bile ducts with microliths early on in the development of geeldikkop may explain the short latent period usually seen in this disease.







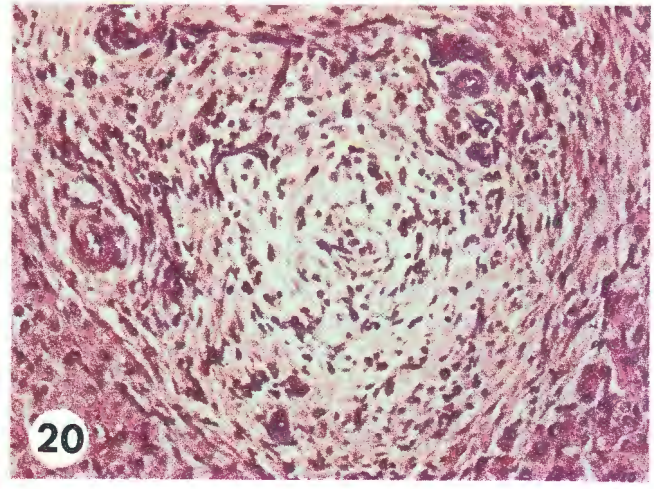


FIG. 17 Portal triad with severe fibroplasia and a moderate bile duct proliferation: HE × 200

FIG. 18 Pronounced concentric lamellar fibrosis around a bile duct: HE × 500

FIG. 19 Larger intrhepatic bile duct showing necrosis and preiductal granulation tissue proliferation: HE × 30

FIG. 20 Replacement of bile duct with scar tissue: HE × 500

Apart from the different occluding mechanisms operating in facial eczema and geeldikkop, it has been shown that sheep can be made photosensitive from about 2–3 days after ligation of the common bile duct (Quin, 1933; Ford, 1976).

Obstruction of especially the larger bile ducts by whatever mechanism, whether by necrosis and scarring, as in facial eczema, by microliths, as in geeldikkop and dikoor (=*Panicum* grass photosensitivity) (Kellerman *et al.*, 1980) or by ligation of the common bile duct, results in very similar lesions in the liver. The most important change is concentric, lamellar fibrosis around the bile ducts, which is an indication of obstructive cholangitis. Other changes include bile duct proliferation, oedema and fibroplasia in the portal triads, foci of periportal hepatocellular necrosis (bile infarcts), and diffuse parenchymal degeneration.

The degree of portal reaction does not always vary significantly among sheep affected with facial eczema, geeldikkop and dikoor. In each of these syndromes the severity of the portal reaction ranges from mild to severe. Factors that may play a role in this respect seem to be either the degree and duration of occlusion or whether major or smaller tributary branches of the biliary tree are affected. In facial eczema necrosis of the entire walls of major bile ducts is often accompanied by pronounced periductal fibrosis and/or granulation tissue proliferation. Since sporidesmin, which acts on the bile duct epithelium, is cleared from the body in the short period of about 24 h (White, Mortimer & Di Menna, 1978), it would seem that the initial damage wrought by the mycotoxin is exacerbated by the irritant effect of bile.

In geeldikkop, where the crystalloid material in the bile ducts may damage the mucosa mechanically and give rise to pressure atrophy and/or spotty degeneration and necrosis of the lining epithelium, the lesions never progress to the advanced stage seen in facial eczema.

Crystalloid material in bile ducts, Kupffer cells and hepatocytes, however, is not unique to geeldikkop; it occurs also in dikoor, a syndrome indistinguishable from geeldikkop. Although rarely seen in other hepatic conditions in ruminants, crystalloid material has also been reported in hepatogenous photosensitization in Texas induced by the plants *Agave lechuguilla* (Mathews, 1938) and *Nolina texana* (Mathews, 1940).

Kellerman *et al.* (1980) demonstrated that *T. terrestris* can interact with sporidesmin to induce lesions of geeldikkop, while sporidesmin alone results in facial eczema. The specific requirements for crystalloid formation in the liver are not clear, but it would seem that they do not form in response to all types of liver damage in sheep grazing on wilted *T. terrestris* in the Karoo. In a limited experiment, sheep, running on *T. terrestris* and dosed with *Senecio retrorsus*, or made photosensitive by the administration of either *P. leptostromiformis* cultures or dried *Microcystis toxica*, showed typical lesions of the respective intoxications without crystalloid material (Coetzer & Kellerman, unpublished data, 1979). Only those given sporidesmin had geeldikkop.

The chemical nature of the crystalloid material is not known, but it is apparently not composed of common bile salts such as cholesterol, cholic acid, sodium glycocholate or sodium taurocholate (Dr L. A. P. Anderson, VRI, Onderstepoort, personal communication, 1978). The crystalloid material contributed by *T. terrestris* and the factor(s) responsible for its formation may vary according to locality, growth stage and physiological

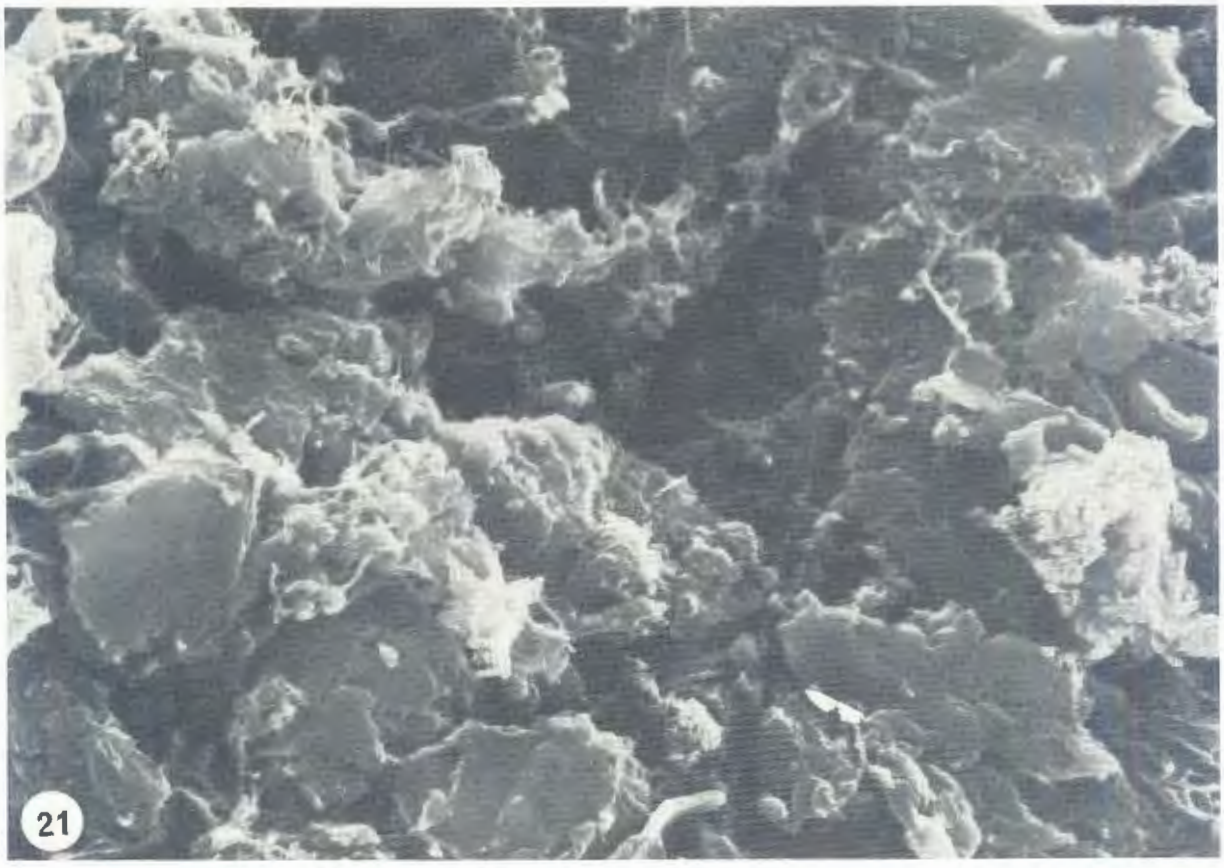


FIG. 21 Extrahepatic bile duct of a sheep affected with facial eczema. Note loss of microvilli and desquamation of necrotic epithelium. exposing the submucosa: × 10 000

condition of the plant. The occurrence of geeldikkop, therefore, seems to be linked with the status of the crystallogenic factor(s) in the plant.

Just as cholesterol gallstones form in laboratory animals and man with high cholesterol intakes, it appears that *T. terrestris* constitutes a lithogenic diet, responsible for saturation and supersaturation of the bile with a crystalloid factor(s) or their products. These crystalloid factors can conceivably precipitate spontaneously; however, experimental results have indicated that the formation of microliths can be triggered by low levels of sporidesmin, thereby enhancing the ability of *T. terrestris* as well as of sporidesmin to cause photosensitivity.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation of the assistance given by the Department of Hepatic Pathology and the Photography Division of the Armed Forces Institute of Pathology, Washington DC 20306 in the preparation of the sections and the photographs respectively, and their gratitude to Mrs R. Coetzer for typing the manuscript.

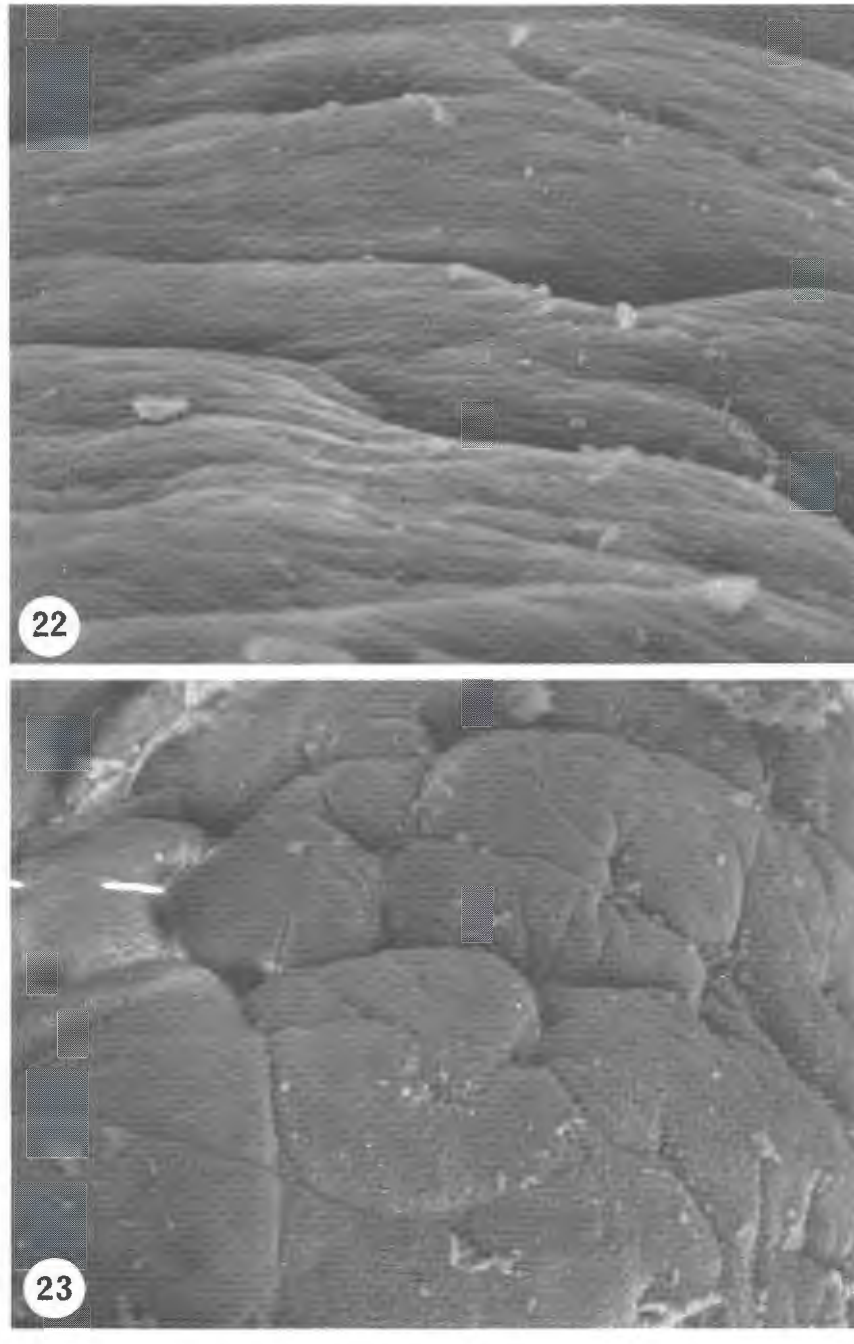


FIG. 22 & 23 Mucosal surface of extrahepatic bile duct. Note longitudinal and transverse folds covered with epithelium: × 640

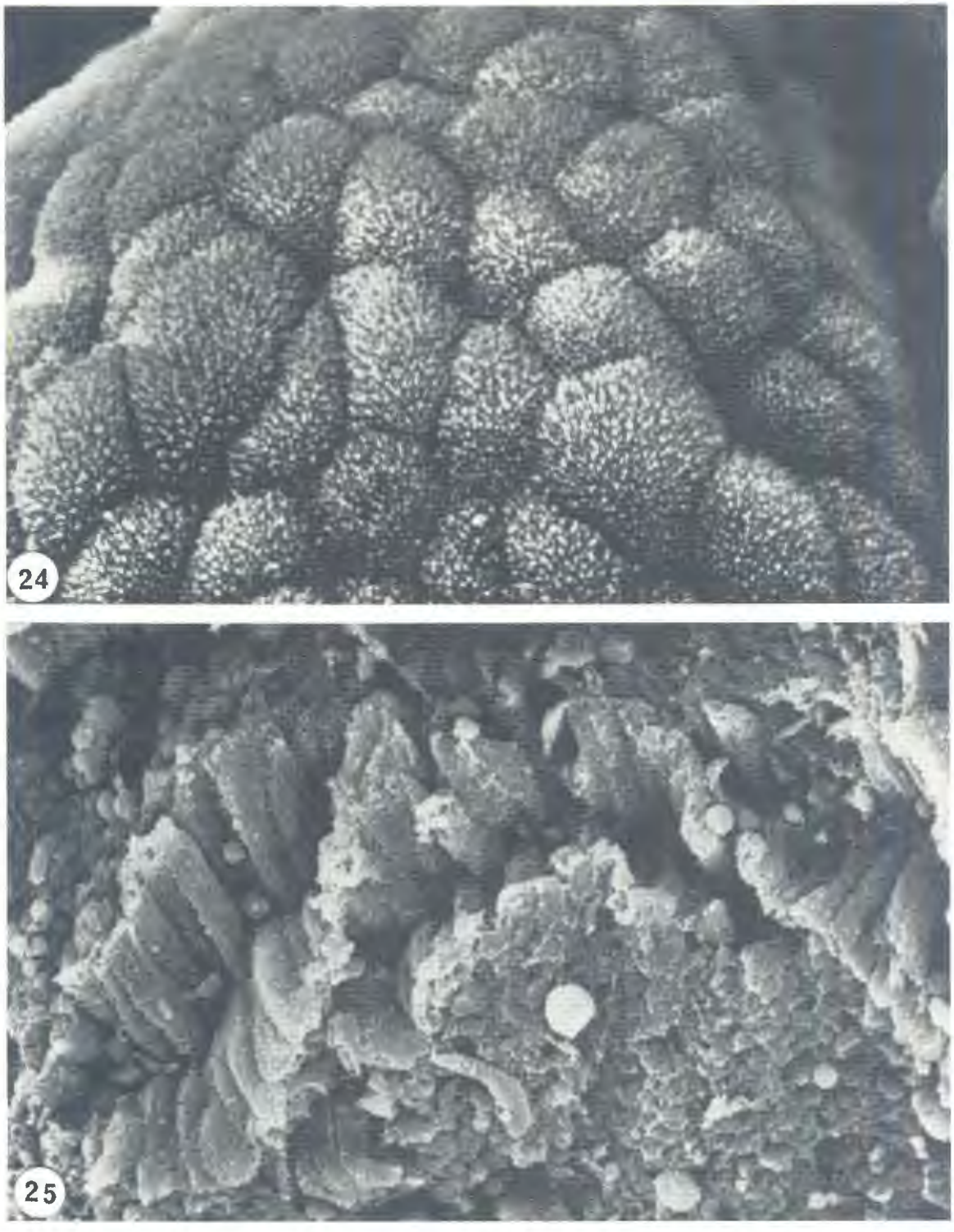


FIG. 24 Lining epithelium of extrahepatic bile duct. Note numerous microvilli: × 1280
FIG. 25 Cilia arranged in rows in *ductus cysticus:* × 2500

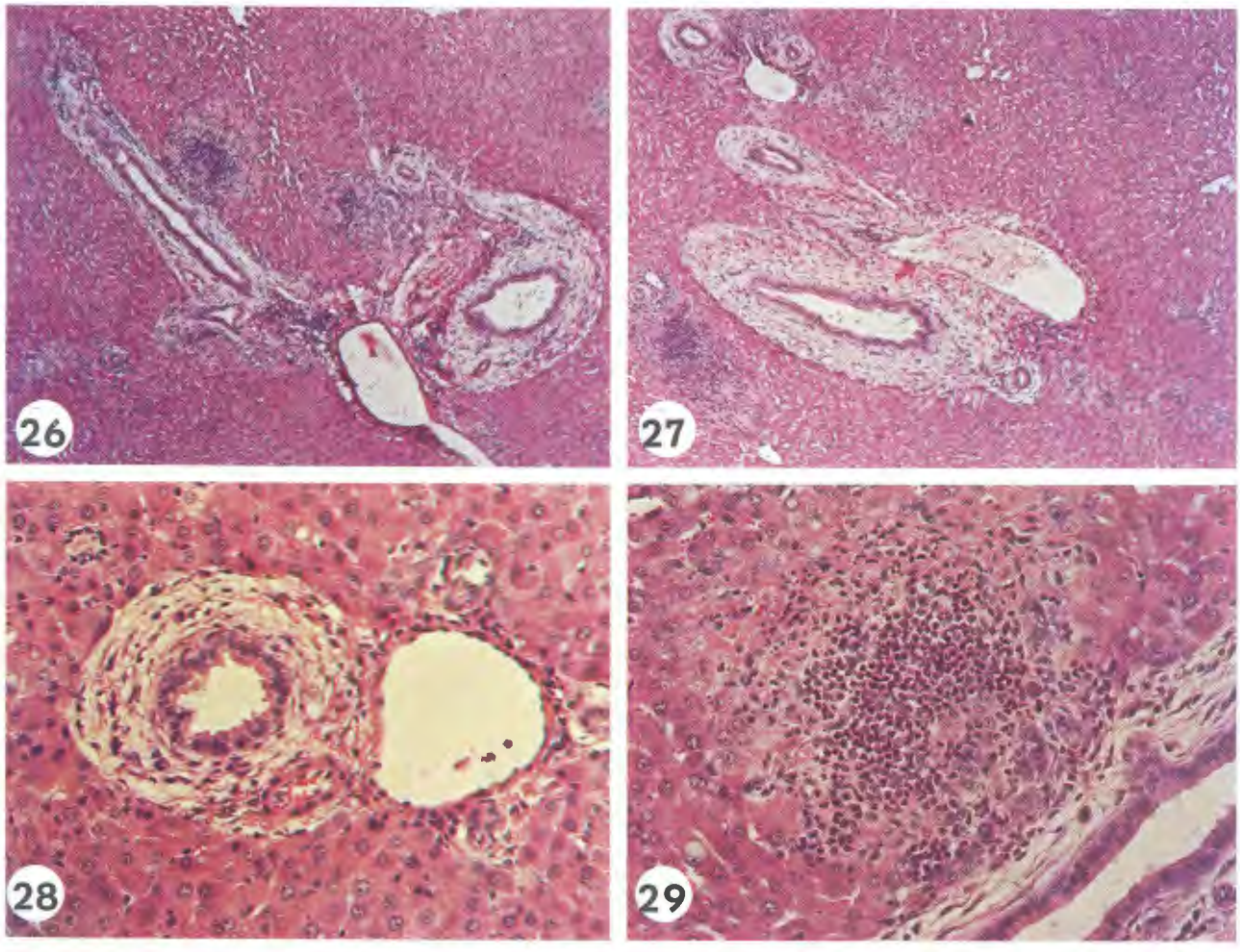


FIG. 26 & 27 Bile duct ligation in sheep. Prominent concentric lamellar fibrosis and oedema around many of the bile ducts. Note periportal foci of hepatocellular necrosis (bile infarcts). HE × 30

FIG. 28 Concentric lamellar fibrosis and oedema around a bile duct: HE × 200

FIG. 29 Periportal focus of hepatocellular necrosis infiltrated with many neutrophils: HE × 200

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