THE PATHOLOGY OF *CESTRUM LAEVIGATUM* (SCHLECHTD.) POISONING IN CATTLE

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ABSTRACT

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The clinical features and pathological findings of 6 steers drenched with dried plant material of *Cestrum laevigatum* are described. Doses ranging from 0,5 to 10 g/kg/day were given intraruminally for 1 to 38 days.

Animals that received 5 to 10 g/kg/day showed nervous signs including ataxia, muscle tremors, hypersensitivity and intermittent chewing. Clinical signs in the steers which received 0,5 to 4 g/kg/day were mild.

High doses induced moderate to severe hepatosis characterized by centrilobular to midzonal coagulative necrosis, haemorrhage and congestion. At lower rates only mild hepatic lesions, characterized by disappearance of hepatocytes and collapse of the reticulin stroma in the centrilobular areas were evident. Ultrastructural changes were primarily limited to the hepatocytes and comprised degeneration, necrosis and fatty change. Degeneration and necrosis of endothelial cells and disruption of sinusoidal walls were occasionally observed.

INTRODUCTION

Cestrum laevigatum (Schlechtd.), commonly known as Ink-berry, is a perennial shrub or tree belonging to the Solanaceae. The plant is native to South America and was introduced into southern Africa as evergreen ornamental shrubs, hedges and sometimes as windbreaks in gardens (Steyn, 1934). It has spread rapidly in South Africa and is now a proclaimed weed. *Cestrum laevigatum* is common in the moister eastern parts of the country and is especially abundant on the slopes and in the gulleys of the Chase Valley on the outskirts of Pietermaritzburg (Thorburn, 1934; Vahrmeyer, 1981).

There is a paucity of published information on *C. laevigatum* poisoning in animals. Mention is made by Steyn (1934) who cited Chase (1903), Hutcheon (1903) and Walsh (1909) as stating that *C. nocturnum* is poisonous to cattle in South Africa. It appears that the plant was incorrectly identified and that the poisonings should have been attributed to *C. laevigatum* (Steyn, 1934). He was, however, not able to induce poisoning by dosing various growth stages of the plant to sheep and rabbits.

Thorburn (1934) incriminated *C. laevigatum* as the cause of Chase Valley disease which had occurred near Pietermaritzburg for many years. The disease was produced experimentally in 12 cattle and the macroscopical liver lesions were described as acute inflammation and fatty degeneration to cirrhosis of the liver and oedema and petechia in the wall of the gall-bladder. The histopathological changes were not reported. Similar lesions were produced in sheep and goats by dosing plant material, but the plant was not toxic when given to the horse, pig, rabbit, fowl and guinea-pig.

After the initial studies by Thorburn (1934), more than 30 years elapsed before Döbereiner, Tokarnia & Canella (1969) described the pathology of *C. laevigatum* poisoning in 21 natural bovine cases in Brazil. The most significant gross lesions were evident in the liver, which had a nutmeg appearance on cut surface. In some cases the wall of the gall-bladder was oedematous and contained haemorrhages. Microscopically, the changes in the liver were char-acterized by centrilobular necrosis as well as congestion and haemorrhage. Experimental feeding of the plant to yearling calves produced clinical signs and lesions comparable to that of the field cases.

In the past 10 years, *C. laevigatum* has from time to time been associated with liver damage in cattle in South Africa, although these reports usually remained unconfirmed (N. J. Coetzee, State Veterinarian, Bloemfontein, personal communication, 1988). Poisoning also occurs inland along rivers such as the Vaal, where the banks may be heavily infested with the plant (T. S. Kellerman, Veterinary Research Institute, Onderstepoort, personal communication, 1991).

Two other *Cestrum* spp., namely *C. aurantiacum* (Shone & Drummond, 1965; Mugera & Nderito, 1968) and *C. parqui* (Lavers, 1953; McLennan & Kelly, 1984) are also primarily hepatotoxic and produce clinical signs and lesions similar to those reported with *C. laevigatum* poisoning. *Cestrum parqui* is found in South Africa (Watt & Breyer-Brandwyk, 1962), but poisoning by it has not been reported. *Cestrum diurnum*, on the other hand, contains a dihydroxyvitamin D_3-glycoside and causes a disease in cattle in North America similar to vitamin D intoxication (Hughes, McCain, Chang, Haussler, Villareale & Wasserman, 1977).

The purpose of this report is to describe clinical and pathological findings in cattle dosed with *C. laevigatum* plant material at different doses and intervals.

MATERIALS AND METHODS

Plant material

Young shoots of *C. laevigatum* were collected during July and August 1985 in the Chase Valley near the Allerton Regional Veterinary Laboratory, Pietermaritzburg. The plants were dried in the shade, ground to a coarse powder in a hammer-mill, and stored at −10 °C until required.

Experimental animals and dosing procedures

Seven Hereford steers, aged 2,5 to 3 years, which had not previously been exposed to the plant were used in the experiments. During the day the animals were kept in the sun in pens and at night they were stabled. The animals were fed pellets (Onderste-

TABLE 1 Dosing regimen of *C. laevigatum* plant material to steers

Group	Steer	Initial body mass (kg)	Dose (g/kg × *n)	Dosing regimen			Duration of experiment (Day 0 to termination)	Fate of animal
				Days on which dosed	Total dose (g)	Total dose (g/kg)		
High dose	1	289	10 × 1	0	2 890	10	1	Died
	2	255	8 × 1	0	2 040	8	2	Euthanized
	3	292	5 × 2	0–1	2 929	10	3	Euthanized
Low dose	4	115	4 × 1	0	460	4	32	Euthanized
	5	285	2,5 × 4	0–3				
			0,5 × 16	16–31	5 130	18	32	Euthanized
	6	300	0,5 × 22	0–21				
			1,0 × 17	22–38	8 400	28	39	Euthanized
Control	7	317	—	—	—	—	39	Euthanized

*n = days

poort formulated ration) and milled lucerne hay (*Medicago sativa*) *ad libitum*. Drinking water was available at all times.

Milled plant material was administered to 6 steers at different dosage levels (0,5 to 10 g/kg/day) and for varying periods of time (1 to 38 days) (Table 1). The plant material was mixed with water and dosed intra-ruminally by means of a stomach tube. One steer that did not receive plant material served as a control (Table 1). During the trials a detailed clinical examination was carried out daily on each animal.

Clinical pathology

Venous blood was collected from each animal on Day 0, at least twice weekly during the course of the experiment as well as just prior to death. The serum activities of the following enzymes were determined at 25 °C using Boehringer Mannheim test kits: lactate dehydrogenase (LD; EC 1.1.1.27), aspartate transaminase (AST; EC 2.6.1.1), creatine kinase (CK; EC 2.7.3.2.) and gamma-glutamyltransferase (GGT; EC 2.3.2.2.).

Macroscopical pathology

With the exception of Steer 1, which died naturally after dosage of plant material, all the animals were killed by exsanguination after an intravenous overdose of pentabarbitone sodium. A detailed necropsy was performed on each animal and tissue specimens were collected for light and transmission electron microscopy. In the steers that were euthanized, specimens of the liver were placed in fixative (*vide infra*) within 10 min of death of the animals.

Microscopical pathology

Samples of the liver (from at least three different parts), gall-bladder, spleen, kidneys, pancreas, adrenal glands, rumen, abomasum, small and large intestines, lymph nodes, myocardium, skeletal muscles, lungs, brain and spinal cord were fixed by immersion in 10 % neutral buffered-formalin for 3–7 days. Tissue blocks were processed routinely, embedded in paraffin wax, cut at 4–5 μm and stained with haematoxylin and eosin (HE). Sections of liver from selected paraffin blocks were stained by a modified Masson's trichrome method (Luna, 1968), Gomori's reticulin impregnation (GRI) (Pearse, 1961), Schmorl's method (Pearse, 1961), Berlin blue for iron (Pearse, 1961), Von Kossa (Pearse, 1961) and the periodic acid-Schiff reaction (PAS) (Luna, 1968).

Transmission electron microscopical pathology

Specimens of liver (from at least two different sites) from each animal were diced into 1 mm cubes and fixed by immediate immersion in 2,5 % glutaraldehyde in 0,1 M sodium cacodyalate buffer at pH 7,3 to 7,4 (Hayat, 1970) for 8 to 10 h. Selected blocks were rinsed with phosphate buffer and post-fixed in 2 % osmium tetroxide, also in the same buffer. Following two or more buffer rinses, the blocks were dehydrated in a graded ethanol series, cleared in propylene oxide and embedded in Polarbed 812 for 24 h at 60 °C.

Thick (1–2 μm) sections were cut with glass and diatome diamond knives on a Reichert Ultracut ultramicrotome and stained with toluidine blue (Trump, Smuckler & Benditt, 1961) for tissue orientations. Relevant blocks were trimmed to size and thin sections were cut, picked up on copper grids and stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). The stained grids were viewed in a Jeol 1200 EX transmission electron microscope.

RESULTS

Clinical signs

Marked clinical signs were evident in the steers of the high-dose group. Steer 1 was depressed on Day 1 and slightly ataxic manifested as posterior weakness (swaying of hindquarters) and a change in gait (short steps with the hind feet wide apart). These signs were accompanied by a lack of appetite, profuse salivation, tachycardia, ruminal atony and frequent micturition. The animal died on that same day.

In Steer 2 depression, anorexia, generalized muscle tremors, ruminal stasis and soft faeces were evident on Day 1. At times there were signs of abdominal pain (grinding of the teeth, groaning and kicking at the abdomen). Prior to euthanasia on Day 2, the steer went into lateral recumbency, and foamed at the mouth. The animal appeared hypersensitive as evidenced by a severe blinking reflex when tapped lightly on the head.

Steer 3 showed anorexia, listlessness and voided soft faeces. On Day 2 it was reluctant to stand and was unsteady on its feet. The following day pronounced nervous signs were evident. The steer was standing with the fetlocks in flexion and the hind legs tucked under the body. Signs of incoordination developed which included scuffing of the feet, knuckling over at the phalangeal joints and apparent difficulty in walking backwards. Occasionally it bumped clumsily against objects. Later, there was foaming at the mouth and signs of abdominal pain. At times it showed intermittent chewing movements, had a bewildered facial expression and walked about drunkenly. The steer was destroyed on Day 3.

Steers in the low-dose group developed only mild clinical signs. Steer 4 appeared slightly dull on Day 4, but thereafer was normal. Steer 5 showed depression, inappetance, salivation, scuffing of the feet and weakness of the hindquarters as well as polypnoea and tachycardia on Day 4. Marked rises in the serum activity of AST occurred on that day and dosing was stopped. Dosing was resumed on Day 16 (Table 1). The animal appeared in good health until euthanized on Day 32.

On Days 23 to 26, Steer 6 ground its teeth, developed ruminal stasis and voided dry faeces, sometimes covered with mucous. The animal appeared to be clinically normal on subsequent days until it was killed on Day 39.

Clinical signs were not manifested by Steer 7 during the course of the experiment.

Clinical pathology

The serum activities of the enzymes AST and LD in the steers of the high dose group were markedly elevated. Activities rose rapidly up to 30-fold after 24 h. In Steer 3, although gradually decreasing after 72 h, the activities reached 2 to 3 times predosing levels prior to euthanazia. In Steers 4 and 5, peak activities of AST and LD of up to 10-fold were reached after 96 h. The activities then diminished and reached predosing levels by Day 25. In Steers 1–5, elevations in GGT activity were generally similar to those for AST and LD but of lower magnitude, while the CK activities remainded normal throughout the trial.

Activities of AST, LD, GGT and CK were not increased in Steers 6 and 7.

Macroscopical pathology

Liver: In Steers 1 to 3 the liver was moderately swollen and deep red to bluish-red. The lobulation was markedly accentuated, each lobule having a dark red centre surrounded by a pale almost yellowish zone, giving the organ a mosaic appearance (Fig. 1). In Steer 1 focal, dull greyish-yellow, ill-defined patches were noticed subcapsularly on the visceral surface of the left lobe, while in other areas there were numerous petechial haemorrhages under the capsule. The livers of Steers 1 & 2 on cut surface oozed copious amounts of blood and the parenchyma had a friable consistency. The gall-bladder walls of all three were mildly to moderately oedematous and in Steer 3 contained numerous petechiae and ecchymoses (Fig. 2). In Steer 1 the gall-bladder

FIG. 1 Distinct lobulation of the liver in Steer 2 caused by congested and haemorrhagic centrilobular areas surrounded by paler peripheral zones.
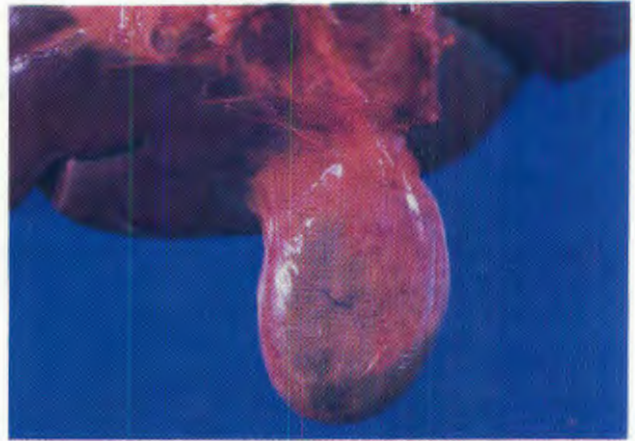

FIG. 2 Numerous petechiae and ecchymosis are present in the wall of the gall-bladder in Steer 3.

contained light orange-yellow bile. The hepatic lymph nodes in the 3 steers were mildly to moderately oedematous and in Steer 2 marked oedema about the hilus of the liver was present.

Mild hepatic changes were the only lesions evident in Steers 4 to 6. The liver in Steer 4 was slightly enlarged, bluish-grey, showed distinct lobulation, and had a normal consistency. In Steers 5 and 6 the lobulation was slightly accentuated and the consistency somewhat increased. The gall-bladder in the 3 animals was normal.

Other organs: Steer 2 showed a mild hydrothorax and hydropericardium, and the trachea contained a small amount of white froth. In Steer 3, petechiae and ecchymoses occurred in the subcutaneous and intermuscular tissues particularly over the sternum, shoulders and hindquarters; subpleurally over the greater vessels and ribs; subendocardially in the papillary muscles; subepicardially in the coronary grooves; peritracheally; perioesophageally; perirenally and subperitoneally; and in the mesenterium and omentum. Endo- and epicardial, pleural and mesenteric haemorrhages were noticeable in Steer 2. The mucosa of the abomasum in Steers 1 and 2 was mildly congested and in Steer 1 contained scattered petechiae. The peripheral lymph nodes in Steer 3 were swollen and moist.

There were no significant lesions in the control steer.

Microscopical pathology

Liver: The liver lesions in Steer 1 comprised coagulative necrosis of hepatocytes of almost entire lobules, sparing only a narrow rim of degenerated hepatocytes on the periphery of the lobules (Fig. 3). Moderate congestion and some haemorrhages were noticable in the necrotic areas. Most parenchymal cells at the periphery of the lobules showed acidophilic degeneration: they were shrunken, assumed a rounded or angular outline and were separated from neighbouring liver cells. The deeply acidophilic cytoplasm frequently contained variably sized pink to bright eosinophilic, hyaline globules and pale, well-circumscribed, finely granular oval areas (Fig. 4). The nuclei of these cells were pycnotic and showed clumping and margination of chromatin. Hepatic cords throughout the organ were disrupted. Condensation of reticular fibres in the necrotic areas was demonstrated with GRI. Moderate numbers of neutrophils were distributed among the degenerated and necrotic liver cells in some lobules. The inter-
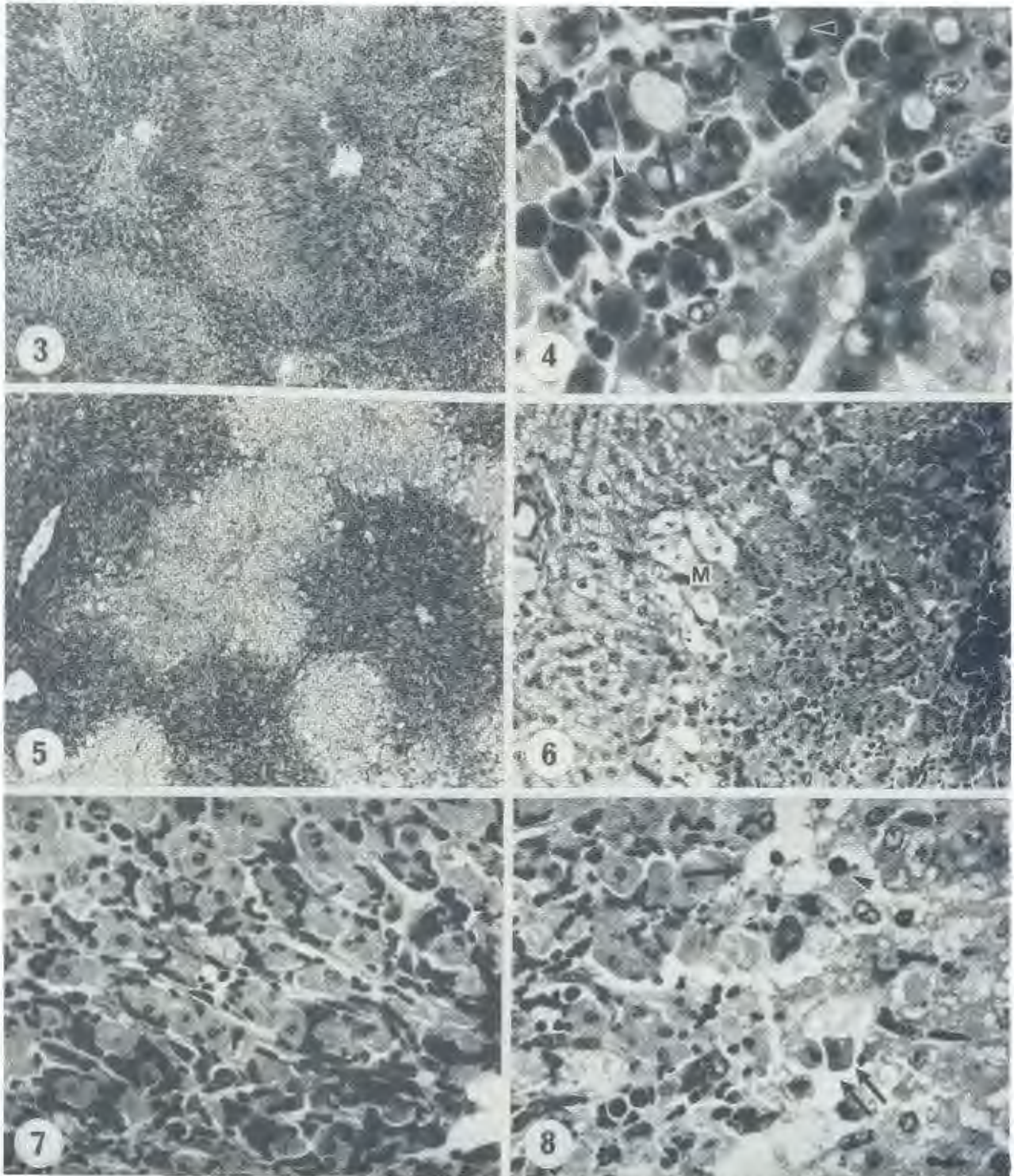
FIG. 3 Steer 1. Extensive hepatocellular necrosis of the centrizonal and midzonal areas and a narrow rim of degenerated hepatocytes at the periphery of the lobule. HE × 40

FIG. 4 Steer 1. Acidophilic degeneration of hepatocytes in the periphery of the lobule. Affected cells are dark staining with pycnotic nuclei and contain eosinophilic globules (arrow heads) and well circumscribed, finely granular, oval areas (arrow). HE × 700

FIG. 5 Steer 2. Markedly accentuated lobulation due to centrilobular necrosis, haemorrhage and congestion. HE × 40

FIG. 6 Steer 2. Each lobule can be subdivided into 3 zones: a centrilobular zone of coagulative necrosis, haemorrhage and congestion (at right); a middle zone of ballooning degeneration and lytic necrosis (M); and a peripheral zone of fatty degeneration (at left). HE × 200

FIG. 7 Steer 2. Coagulative necrosis of hepatocytes adjacent to central vein. HE × 300

FIG. 8 Steer 2. Ballooning degeneration of hepatocytes in midzonal area (arrow). Note necrotic cells (double arrows) and hyaline globule (arrow head) in this zone. Peripheral parenchymal cells show fatty degeneration (at right). HE × 300
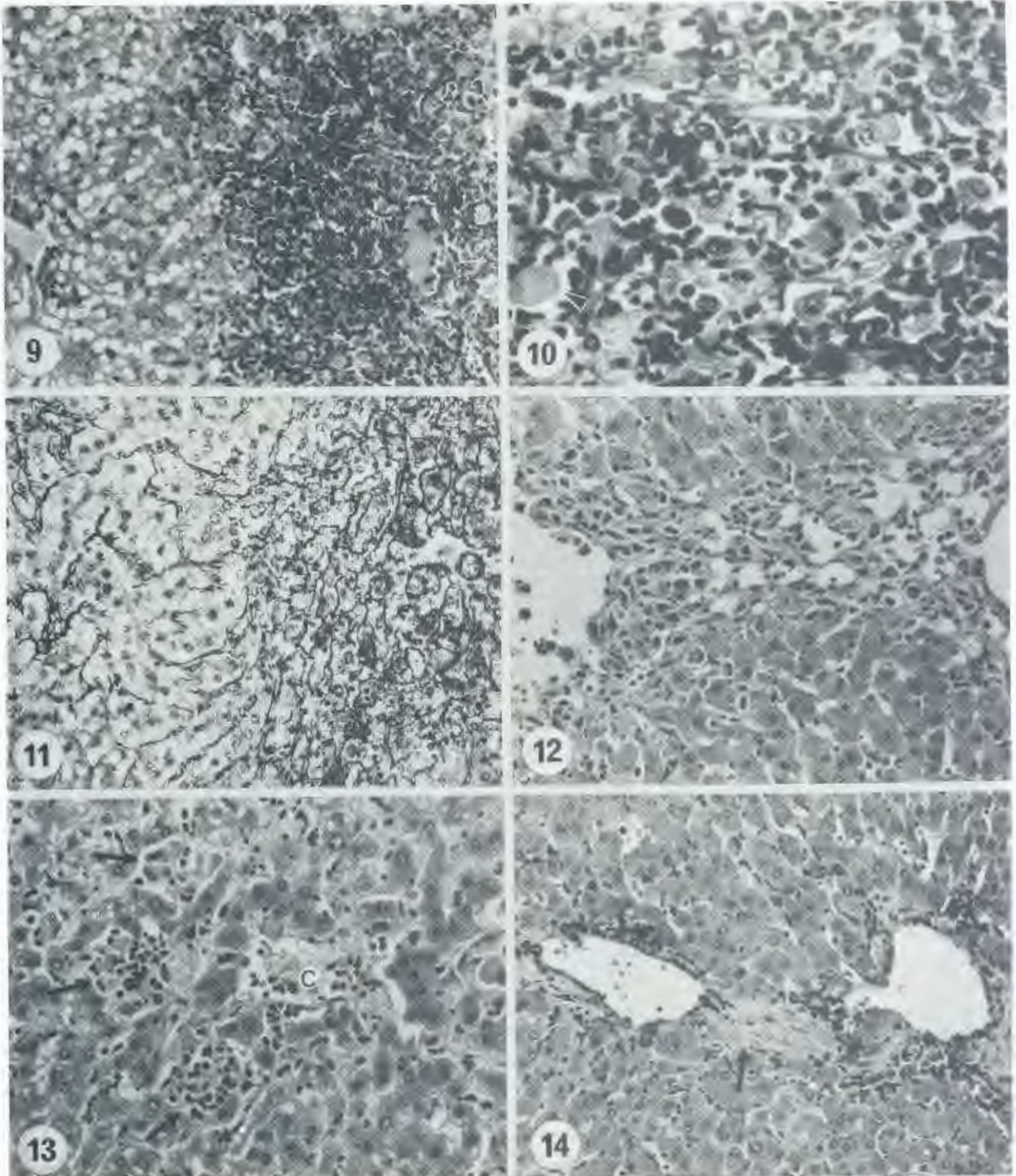
FIG. 9 Steer 3. Centrizonal hepatocellular necrosis, congestion and haemorrhage (at right) with fatty degeneration of the remaining hepatocytes (at left). HE × 200

FIG. 10 Steer 3. Centrilobular hepatocytes are necrotic (arrow heads). Sinusoidal walls and lining cells are prominent, and centrilobular zone is infiltrated by macrophages (arrows). HE × 400

FIG. 11 Steer 4. Pronounced condensation of reticulin framework around the central vein (at right). GRI × 200

FIG. 12 Steer 4. Two central veins are linked by collapsed stroma in which dilated sinusoids are discernible. Affected area is infiltrated by macrophages and lymphocytes. HE × 300

FIG. 13 Steer 5. Small groups of macrophages admixed with lymphocytes and plasma cells are evident in centrilobular area (arrows) (C, central vein). HE × 300

FIG. 14 Steer 6. Centrilobular fibrosis and joining of central veins by bundles of collagen (arrow). HE × 300
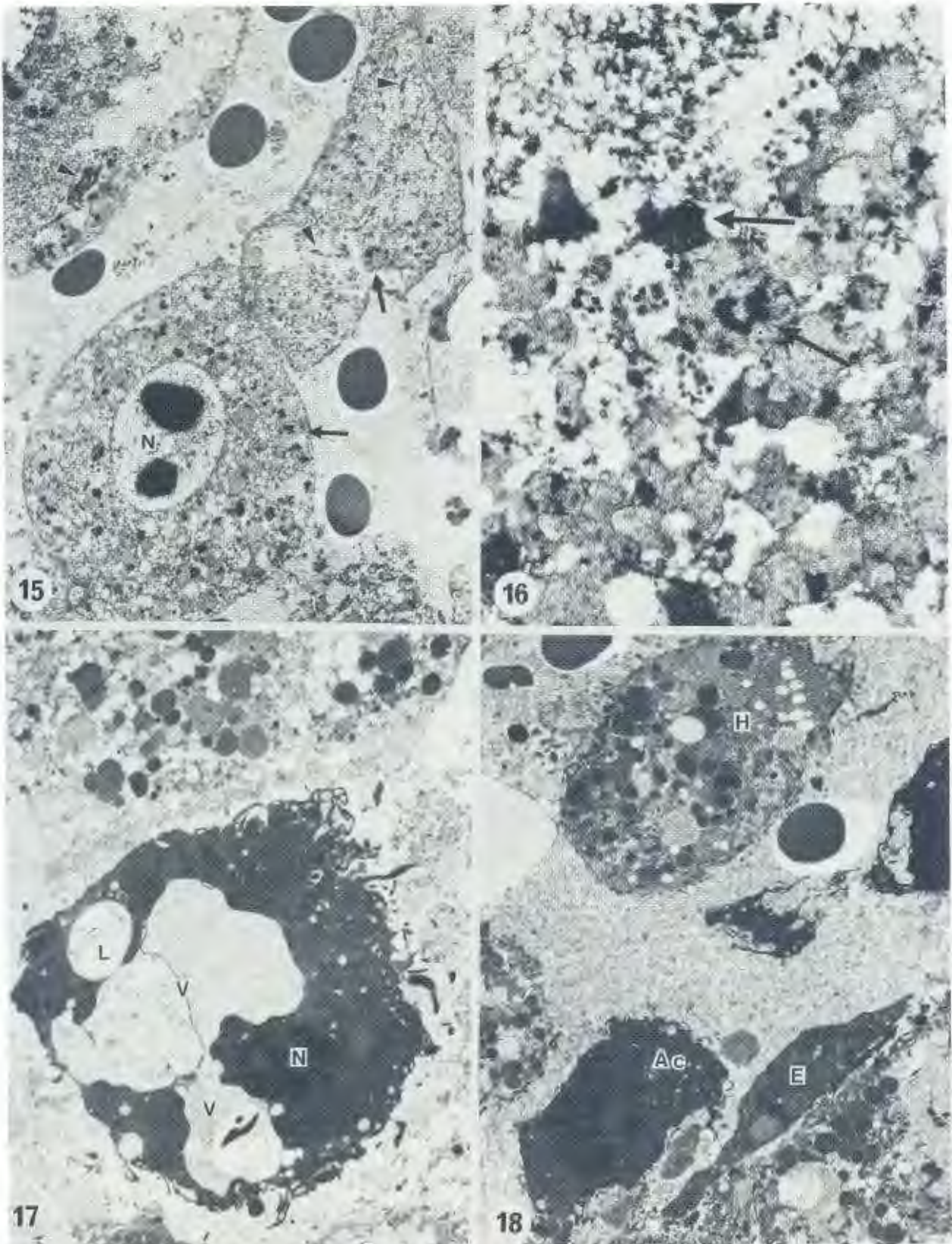
FIG. 15 Steer 1. Necrotic centrilobular hepatocytes contain fibrin (arrow heads). Note mineralization of mitochondria (arrows) and condensation of chromatin (N). × 4000

FIG. 16 Steer 1. Mineralization of mitochondria in necrotic hepatocyte (arrows). × 13 500

FIG. 17 Steer 1. Acidophilic degeneration of hepatocyte at the periphery of a lobule. The nucleus is pycnotic (N) and the cytoplasm stains darkly and contains vacuoles (V) and a lipid globule (L). × 4500

FIG. 18 Steer 1. Acidophilic degeneration (Ac) and necrosis of hepatocyte (H) and necrosis of endothelial cell (E) in the peripheral zone of the lobule. × 4000

stitium of most portal tracts was mildly oedematous.

Three distinct zones of degeneration and necrosis of hepatocytes could be distinguished in the lobules of Steer 2 (Fig. 5 & 6). The centrilobular areas showed coagulative necrosis, haemorrhage and congestion, bridging adjacent lobules (Fig. 7). Necrotic liver cells usually had an intense eosinophilic appearance, but in c. 20 % of the cells the cytoplasm was faintly bluish due to the presence of numerous fine basophilic granules indicative of mineralization. Small numbers of neutrophils and macrophages were distributed among the necrotic cells and in the sinusoids. This area was bordered by a middle zone of hepatocytes showing ballooning degeneration and lytic necrosis (Fig. 6 & 8). The affected hepatocytes were severly swollen, had indistinct cell membranes and most of the cytoplasm was replaced by large vacuoles giving the cell a rarified appearance. The nuclei were pycnotic or showed lytic changes. Large spaces, lined by a network of reticular tissue remained where hepatocytes had disappeared (Fig. 8). These spaces sometimes contained necrotic hepatocytes, cellular debris or bright eosinophilic hyaline globules. The third zone comprised the hepatocytes at the periphery of the lobules which revealed severe fatty change. The reticulin network in the centrilobular areas was often condensed, while disruption of the reticulin occurred in the midlobular areas (Fig. 8). Portal triads showed mild fibrosis and focal infiltrates of mononuclear cells, mainly lymphocytes and plasma cells.

In Steer 3 most of the hepatocytes in the centrilobular area were necrotic and lysed, leaving an irregular and collapsed network of reticular fibres (Fig. 9–11). Faint basophilic stippling of the cytoplasm was evident in some of these cells. Variable numbers of macrophages, lymphocytes and neutrophils infiltrated the areas of collapsed stroma (Fig. 10). The macrophages contained pinkish granular cytoplasmic debris and frequently also small amounts of haemosiderin and lipofuscin. These cellular infiltrations as well as moderate hypertrophy of sinusoidal lining cells gave the centrilobular areas a more cellular appearance than normal. Hepatocytes in the remainder of the lobules showed prominent fatty degeneration. Mild fibroplasia and focal aggregates of mononuclear cells were evident in the portal triads.

In Steer 4 necrosis, followed by lysis of hepatocytes in the centrilobular areas resulted in collapse of the reticulin stroma (Fig. 12). Dilated sinusoids, surrounded by fine trabeculae of immature connective tissue, traversed the affected areas. These sinusoids anastomosed and often communicated with central veins. Single or small groups of hepatocytes occasionally became entrapped in the connective tissue. Central veins were usually dilated; some also contained slender bundles of collagen in their walls. Moderate numbers of lymphocytes and macrophages and a few neutrophils infiltrated the affected areas. Hepatocytes throughout the remainder of the lobules showed cloudly swelling.

In Steers 5 and 6 hepatocytes throughout the lobules showed mild hydropic degeneration, anisokaryosis and frequently binucleation. Scattered foci of hepatocytes especially in the centrizonal areas were replaced by poorly defined groups of pigmented macrophages admixed with lymphocytes and a few plasma cells (Fig. 13). The macrophages contained large amounts of lipofuscin and haemosiderin. Kupffer cells were mildly hypertrophic and frequently contained similar pigment. Central veins in some lobules were duplicated and adjacent veins in Steer 6 were sometimes linked by bundles of collagen (Fig. 14).

Other organs: the wall of the gall-bladder in Steers 1 to 3 was moderately oedematous and in Steer 3 some subserosal petechial and ecchymotic haemorrhages were present. In Steers 1 to 3 tubular epithelial cells in the cortex of the kidneys were mildly to moderately degenerated and showed prominent fatty changes. Other lesions in the three steers included mild to moderate oedema of the brain involving particularly the cerebral grey matter; and necrosis of lymphocytes in the germinal centres of the lymph nodes, spleen and Payer's patches.

Transmission electron microscopical pathology

Hepatocytes in the centrilobular and midlobular areas in Steer 1 were necrotic. The cells were shrunken, microvilli were absent and the intercellular spaces were often widened. Focal necrosis of the cytoplasm of severely affected hepatocytes was evident and these cells sometimes contained strands of fibrin (Fig. 15). Occasionally pieces of necrotic cytoplasm were shed from the periphery of the cells. Degenerated and necrotic hepatocytes showed loss of internal structure, mineralization of mitochondria, depletion of glycogen, swelling of the endoplasmic reticulum and the presence of variably sized lipid globules in the cytoplasm (Fig. 16). The parenchymal cells at the periphery of the lobules showed acidophilic degeneration; they were detached from adjoining liver cells and the cytoplasm was shrunken (Fig. 17 & 18). There was increased electron density of the cytoplasm obscuring organelles, the presence of large irregular vacuoles containing granular material and lipid droplets. Nuclear changes included disorderly clumped or condensed chromatin, absence of nucleoli, pycnosis and occasionally fragmentation of the nuclei.

In Steers 2 and 3 there was destruction of liver cell plates in the centrilobular areas and the hepatocytes were either severely degenerated or necrotic (Fig. 19 & 20). The spaces of Disse and intercellular spaces were widened and contained numerous red blood cells, sequestered portions of necrotic hepatocytes as well as scattered neutrophils (Fig. 20).

Dilation of the rough endoplasmic reticulum was a feature of the ballooned hepatocytes at the periphery of the lobules in Steer 2 (Fig. 21). Dilated cisternae of endoplasmic reticulum often occupied most of the cytoplasm of the affected cells, displacing other organelles to the margins of the cell. Some cisternae were devoid of any contents, but others contained finely granular or floccular, mildly electron-dense material. Nuclear changes accompanying the ballooning degeneration included focal dilation of the perinuclear cisternae, clumping and margination of chromatin and pycnosis (Fig. 21). The most prominent change in the remainder of the hepatocytes in Steers 2 and 3 comprised the accumulation of numerous lipid droplets in the cytoplasm (Fig. 22).

The sinusoidal walls in Steers 1 to 3 were generally well preserved even in areas bordered by adjacent necrotic hepatocytes. In some areas in Steer 1, however, the sinusoidal walls were not clearly delineated, while in Steers 2 and 3 they were often mildly thickened (Fig. 20). Sinusoids and spaces of Disse frequently contained abundant fine granular material (probably plasma) and fragments of necrotic parenchymal cells. Endothelial cells occasionally
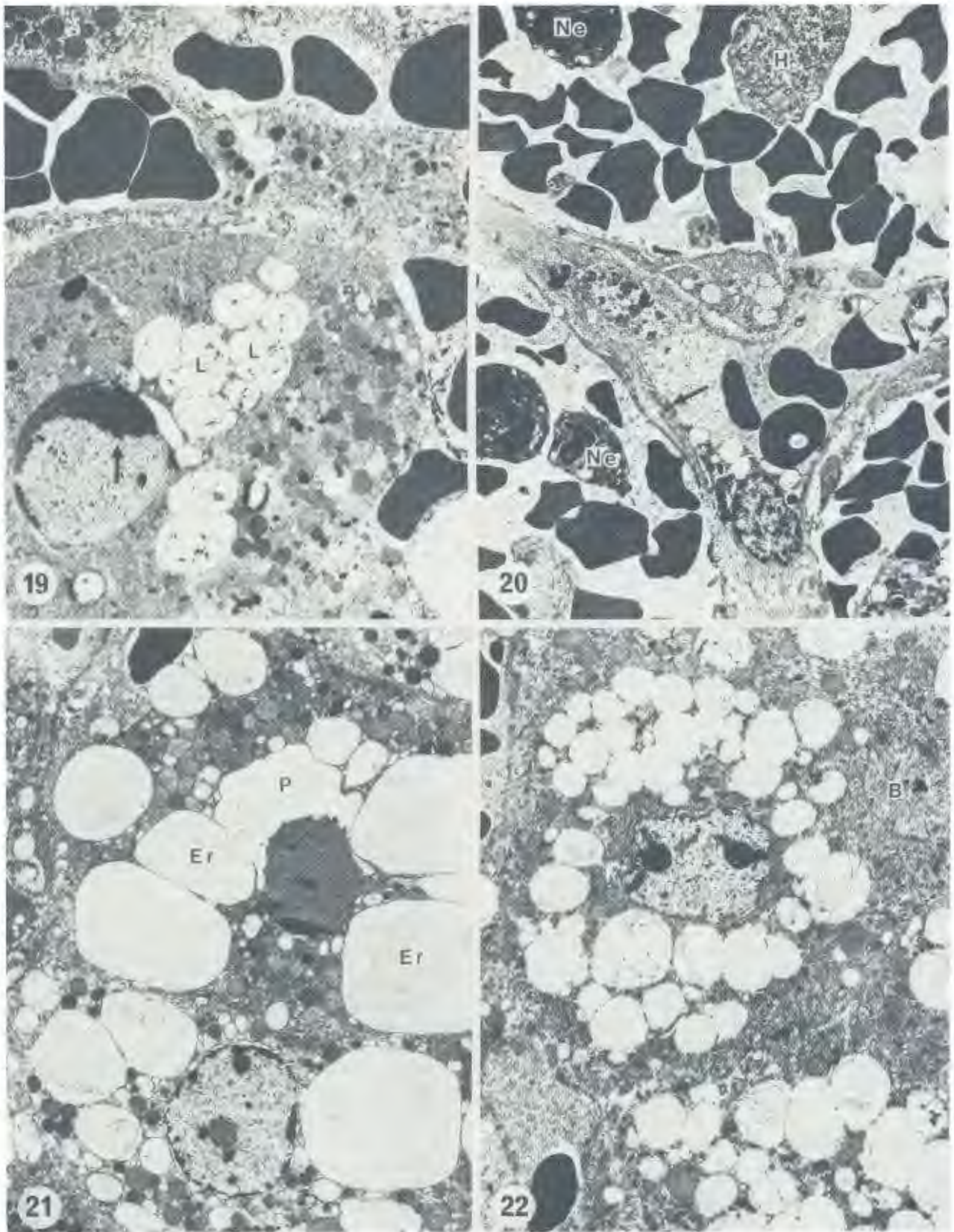
FIG. 19 Steer 2. Necrotic centrilobular hepatocyte showing margination of chromatin (arrow) and lipid globules (L) in the cytoplasm. ×
4000.

FIG. 20 Steer 2. Large perisinusoidal spaces which contain necrotic parenchymal cells (H), neutrophils (Ne) and erythrocytes are evident.
The sinusoidal walls appear thickened in several places (arrows). × 3000.

FIG. 21 Steer 2. Ballooning degeneration of two hepatocytes in the midlobular area. There is marked dilation of the endoplasmic
reticulum (Er) and perinuclear cisternae (P). The upper nucleus is pycnotic and shows margination of chromatin. × 4000

FIG. 22 Steer 3. Hepatocyte at the periphery of a lobule containing numerous liped globules. Bile canaliculus (B) appears normal. × 4000

were necrotic (Fig. 18) while others revealed small lipid globules and lipofuscin granules in their cytoplasm.

Kupffer cells in Steer 1 appeared unaffected. In the centrizonal areas in Steers 2 and 3, Kupffer cells displayed evidence of phagocytosis. The cytoplasm was enlarged and usually contained lipid globules and several lysosomes and phagosomes filled with lipofuscin pigment and electron-dense masses of digested material. Some lysosomes contained membranous whorls.

Only mild degenerative changes were evident in the hepatocytes in Steers 4 to 6. Some hepatocytes had a less dense or lighter appearance which was associated with a relative decrease in smooth endoplasmic reticulum. Sinusoids were congested or empty, and in some areas erythrocytes filled the spaces of Disse. Red blood cells sometimes replaced the liver cell cords. Bundles of collagen fibres were noticeable in the spaces of Disse in some of the sections.

There were no significant microscopical and ultrastructural lesions in the liver of the control animal.

DISCUSSION
Based on the pathological findings in the present study of poisoned cattle, it is clear that *C. laevigatum* contains a substance that acts primarily on the liver. Lesions in the livers of the animals in the high-dose group comprised centrizonal necrosis and haemorrhage, which sometimes extended into the midzonal areas. Similar lesions were evident in sheep that were dosed with *C. laevigatum* plant material (Van der Lugt, 1990). Centrilobular necrosis has previously been described with *C. laevigatum* (Döbereiner et al., 1969), *C. parqui* (McLennan & Kelly, 1984; Kudo, Kelly & Oelrichs, 1978) and *C. aurantiacum* (Mugera & Nderito, 1968) poisoning in ruminants.

Centrilobular hepatic necrosis is a relatively common finding in the livers of sheep and cattle in South Africa (Kellerman, Coetzer & Naudé, 1988). Several hepatotoxic plants are especially important in this respect. Centrilobular necrosis has been associated with poisoning by *Senecio* spp. in ruminants as well as with plants which belongs to the family Asteraceae, namely, *Asaemia axillaris* (Coetzer & Bergh, 1983), *Athanasia trifurcata* (Kellerman, Coetzer, Schneider & Welman, 1983), *Hertia pallens* (Prozesky, Kellerman, Jordaan, Welman & Joubert, 1985), *Lasiospermum bipinnatum* (Williams, 1985), *Pteronia pallens* (Prozesky, Kellerman & Welman, 1986) and *Nidorella foetida* (Schneider, Green & Collett, 1987) in sheep.

The nature, distribution and extent of the liver lesions caused by plants of the Asteraceae depend on several factors including the levels and intervals of dosing and toxicity of the plant (Kellerman et al., 1983; Williams, 1985). High doses of plant material cause distinct zonal necrosis (peripheral, midzonal or centrizonal), while the lesions induced by lower doses range from diffuse degeneration and hepatocellular unrest (depicted by anisocytosis, anisonucleosis, binucleation and increased mitoses of hepatocytes) to scattered single cell or focal necrosis. Different patterns of zonal necrosis could seemingly not be induced in acute poisonings with either *Cestrum* spp. or *Senecio* spp. even by varying the dosing regimen.

Hepatic lesions in sheep and cattle similar to those described for acute *C. laevigatum* poisoning may also be caused by intoxication with the blue-green alga *Microcystis aeruginosa* (Jackson, McInnes, Falconer & Runnegar, 1984; Galey, Beasley, Carmichael, Kleppe, Hooser & Haschek, 1987). Other causes of centrilobular necrosis include anaemic conditions, e.g. haemonchosis, anaplasmosis and babesiosis; anoxia as a result of acute heart failure and shock; and Rift Valley fever, particularly in cattle (Kellerman et al., 1988).

The toxic principle(s) in *C. laevigatum*, *C. parqui* and *C. aurantiacum* have not been identified. According to Steyn (1934), Wehner stated that the fruit, leaves and bark of *C. laevigatum* contained a saponin as well as an amorphous, bitter substance he called cestrumid. Canham & Warren (1950a) reported the presence of saponins in extracts of *C. laevigatum* which on hydrolysis yielded 2 sapogenins identified as gitogenin and digitogenin. They claimed to have produced lesions typical of Chase Valley disease and attributed these to the haemolytic action of the isolated saponins, but no details were given. The same workers also claimed to have isolated gitogenin and digitogenin from *C. parqui* (Canham & Warren, 1950b). Lopez, Keeler, Sharma & Shupe (1984) studied the toxicity of several fractions obtained from *C. parqui* extracts in mice and concluded that saponins and various cardiac glycosides acounted for most of the toxic effects of this plant. On the other hand Kudo et al. (1978), regarded the saponins and glycosides not to be the active principles of *C. parqui* and induced centrilobular liver necrosis in mice and sheep with a chromatographically pure, highly water-soluble, but as yet unidentified toxin.

The mechanism of action of the toxic principle(s) of *C. laevigatum* is not known. Most lipid-soluble drugs and toxins are metabolized by the mixed function oxidase (MFO) system, a chain of enzymes that includes NADPH cytochrome C reductase and cytochrome P450. The oxidases convert lipophilic compounds into more polar and water-soluble metabolites that are more easily excreted via the bile or urine (Swick, 1984). Hepatocytes in the periacinar zone of the hepatic acinus, which roughly corresponds to the centrilobular area of the classic lobule, have an abundance of smooth endoplasmic reticulum which contains the MFO system. This explains the relative susceptibility of the periacinar (centrilobular) hepatocytes to injury by toxic metabolites formed during detoxification of chemical agents (Rappaport, 1979). If the toxin of *Cestrum* spp. is indeed highly water-soluble, as reported by Kudo et al., (1978), it follows that one would not expect the MFO system to be involved in its metabolism, yet the pattern of necrosis in poisoning with *Cestrum* spp. is consistently centrilobular. An explanation for this finding is likely to follow once the toxic principle(s) has been identified.

Induction of cytochrome P450 and other microsomal enzymes is reflected in proliferation of the smooth endoplasmic reticulum (SER) (Phillips Poucell, Patterzon & Valencia, 1987). Proliferation of SER was absent in the animals in our experiment, especially of those in the low-dose groups receiving plant material for up to 38 days. This findings support the contention that the toxic substance of *C. laevigatum* is water-soluble and therefore not metabolized by the SER.

Fatty degeneration of hepatocytes in the periportal areas was particularly prominent is Steers 2 and 3. According to Zimmerman (1978), fatty degeneration induced by most hepatotoxins is the result of impaired egress of triglycerides from liver cells. This may follow deficient or defective apoprotein synthesis; impaired assembly of triglycerides, phospholipids and an apoprotein to form a complex; or alteration of the plasma membrane leading to interference with the movement of very low density lipoproteins out of hepatocytes.

Ultrastructural observations in this study confirmed that the toxic substance of *C. laevigatum* primarily affects hepatocytes. Injury to hepatocytes was of the cytotoxic type (Zimmerman, 1978), and changes in the bile secretory apparatus or biliary retention indicative of cholestasis were not evident. The most conspicuous hepatocytic changes included prominent nuclear changes, cytoplasmic disintegration and fragmentation, dilation of the endoplasmic reticulum and fatty degeneration.

Ballooning degeneration (hydropic degeneration) of hepatocytes in the midlobular zone was marked in Steers 2 and 3. This type of degeneration is one of the hallmarks of acute viral hepatitis in man (Ishak, 1976; Bianchi, Zimmerli-Ning & Gudat, 1979) though it may be found in many other conditios such as those caused by drugs and other toxins (Zimmerman & Ishak, 1979). Dilation of the endoplasmic reticulum is probably a consequence of impaired intracellular control of water and ions (Phillips *et al.*, 1987). Ballooned hepatocytes may recover completely, or undergo more profound degenerative changes leading eventually to lytic necrosis and disappearance ("drop-out") of the affected cells (Bianchi *et al.*, 1979). As opposed to what occurs in ballooning degeneration in which cell water content is increased, the main event in acidophilic degeneration (Steer 1) is the loss of intracellular water, although it is generally believed that plasma membrane injury is the underlying defect in both types of degeneration (Phillips *et al.*, 1987).

Haemorrhagic diathesis, characterized by widespread haemorrhages especially in the subcutis and on the serous membranes, was found in Steer 3. Other workers have reported on the occurrence of haemorrhages in various tissues in acute poisoning with *Cestrum* spp. (Thorburn, 1934; Mugera & Nderito, 1968; Döbereiner *et al.*, 1969; McLennan & Kelly, 1984). The mechanism of haemostatic disturbance which occurs during these intoxications remains unproven. McLennan & Kelly (1984) found increased prothrombin times in cattle experimentally poisoned by *C. parqui* and speculated on the role of diminished hepatic synthesis of coagulation factors or disseminated intravascular coagulation (DIC) in the pathogenesis of the haemorrhagic diathesis. Release of tissue thromboplastin by the necrotic hepatocytes and inadequate removal of activated coagulation factors by the affected liver may contribute to the development of DIC (Martinez & Palascak, 1982). Haemorrhage and oedema of the gall-bladder wall commonly occurs in acute, fatal hepatotoxic conditions (Kelly, 1985), includig *C. laevigatum* (Thorburn, 1934), *C. parqui* (McLennan & Kelly, 1984) and *C. aurantiacum* (Mugera & Nderito, 1968) poisonings in ruminants. These lesions are indicative of haemodynamic disturbances in the liver (Kellerman *et al.*, 1988) and may also be related to a direct effect by toxic metabolites excreted in the bile (Kelly, 1985).

In the original studies of Chase Valley disease, Thorburn (1934) mentioned cirrhosis in some of his experimental cattle, but a histopathological description of it was not given. The presence of cirrhosis, however, was not supported by other studies on *Cestrum* spp. poisoning (Lavers, 1953; Mugera & Nderito, 1968; Döbereiner *et al.*, 1969; McLennan & Kelly, 1984). Cirrhosis in man has been attributed to exposure to a number of hepatotoxins and to drug-induced hepatic injury. It may be a sequel to the subtle injury of prolonged exposure to hepatotoxins or may follow subacute hepatic necrosis. Rarely it may follow a single episode of necrosis (Zimmerman & Ishak, 1979). In the current experiment cirrhosis was not induced in cattle either by a single sublethal dose of plant material or by exposure to low doses of toxic material for periods up to 38 days.

The brain oedema in Steers 1 to 3 may explain the neurological signs, including ataxia, staggering, muscle twitching and hypersensitivity. McLennan & Kelly (1984) reported brain oedema in a bovine animal which died of *C. parqui* poisoning, while nervous signs of a similar nature in cattle have been reported in poisonings by *C. laevigatum* (Thorburn, 1934; Döbereiner *et al.*, 1969), *C. parqui* (Lavers, 1953; McLennan & Kelly, 1984) and *C. aurantiacum* (Mugera & Nderito, 1968). Aggression has been described in cattle with *C. laevigatum* (Thorburn, 1934; Döbereiner *et al.*, 1969) and *C. parqui* (Lavers, 1953) poisonings, but was not noticed in animals in the current study.

Apart from the liver, the enzymes AST and LD in ruminants are also found in substantial quantities in other tissues particularly in cardiac and skeletal muscles. On the other hand, CK is almost entirely limited to cardiac and skeletal muscles (Doxey, 1983; Doxey, 1984; Coles, 1986). In the absence of histological evidence of injury to the myocardial and skeletal muscles and with normal serum values of CK, the increased activities in AST and LD therefore reflect the severity of the liver damage (Coles, 1986). Aspartate transaminase and LD are located in the cytosol and mitochondria of hepatocytes, and consequently circulating levels increase when hepatocytes undergo degeneration or necrosis thus releasing the enzyme (Doxey, 1983). In hepatic conditions these enzymes are therefore indicative of hepatocellular injury and subsequent altered plasma membrane permeability (Duncan & Prasse, 1977).

Gamma-glutamyltransferase is a membrane-bound enzyme in hepatocytes and in bile ductular epithelium (Schmidt & Schmidt, 1984). Increases in the activity of GGT is most commonly used as an indicator of cholestasis in chronic liver disease (Malherbe, Kellerman, Kriek & Haupt, 1977; Doxey, 1983; Coles, 1986). In acute toxic liver damage the serum enzyme pattern is extremely variable as it results from various factors including disturbed cellular permeability, inhibition of protein synthesis and cholestatic injury to hepatocytes superimposed one upon the other (Schmidt & Schmidt, 1984).

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