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THE PATHOLOGY OF EXPERIMENTAL *LASIOSPERMUM BIPINNATUM* (THUNB.) DRUCE (ASTERACEAE) POISONING IN SHEEP. I. HEPATIC LESIONS

M. C. WILLIAMS, Department of Pathology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, Republic of South Africa

ABSTRACT

WILLIAMS, M. C., 1990. The pathology of experimental *Lasiospermum bipinnatum* (Thunb.) Druce (Asteraceae) poisoning in sheep. I. Hepatic lesions. *Onderstepoort Journal of Veterinary Research*, 57, 249–261 (1990).

Key words: plant poisoning; hepatotoxicosis, hepatosis.

Poisoning with the plant *Lasiospermum bipinnatum* was studied in 9 lambs. Intraruminal doses, varying from 1–12 g/kg/day of dried plant, were administered to 8 animals and 1 was fed 2,5 g/kg/day of the material mixed with maize meal for 13 days. Periodic serum analyses were done to monitor liver function. Lambs given 6–12 g/kg/day died or were killed *in extremis*. Clinical signs included progressive anorexia and depression in all these lambs and icterus in 2 animals. Lambs given 1–4 g/kg/day were sacrificed after about 2 weeks. Clinical signs in these animals were minimal or absent. Hepatosis was found in all the lambs, the severity of which correlated with levels of plant administered. Centrilobular necrosis and haemorrhage occurred in 2 of the 4 lambs given high doses; single cell necrosis of hepatocytes was observed with intermediate doses, and diffuse degeneration, which was more severe peripherally, was seen at various doses. In 1 lamb, degeneration was most severe midzonally. Bile ductule epithelial proliferation was observed in 7 of the 9 poisoned animals. Marked hypertrophy of hepatocellular smooth endoplasmic reticulum was seen in 3 lambs given low doses. The hepatic lesions were compared with those reported for poisoning by other hepatotoxic plants belonging to the family Asteraceae and found to be indistinguishable.

INTRODUCTION

Lasiospermum bipinnatum, commonly known as ganskweek, has been reported as a cause of poisoning in ruminants in the field in South Africa on a number of occasions (Walsh, 1909; Adelaar, Terblanche, Smit, Naudé & Codd, 1964; Fair, Tustin & Adelaar, 1970; Thornton, 1977). Adelaar *et al.* (1964) were the first to show that the plant is poisonous to sheep. Four animals were experimentally intoxicated with dried plant material. The lesions are not reported in any detail, but the most striking changes were found in the liver. The authors state that the hepatic lesions varied according to the duration of the illness. In acute cases, diffuse necrosis of the hepatocytes, accompanied by haemorrhage, was observed. Less acute cases showed a constant tendency for the lesions to be confined to the periportal hepatocytes. The latter statement is, however, contradicted by the finding of periportal necrosis in 1 of their experimental sheep which died within 24 h of receiving a high single dose of the toxic material. Raised bilirubin levels were found in 2 poisoned sheep and one of these developed clinical icterus.

Hepatogenous photosensitivity was experimentally induced in a single sheep with *L. bipinnatum* by Kellerman, Basson, Naudé, Van Rensburg & Welman (1973). Microscopic examination of the liver of this animal revealed zonal lesions in the lobules, comprising periportal fatty change, midzonal coagulative necrosis, and a more-or-less normal centrilobular area. These authors also describe fatty change in the myocardium and consider this observation to be of diagnostic significance. In view of the paucity of information on the pathology of *L. bipinnatum* poisoning it was considered that the production of a series of experimentally-induced cases, at varying dose levels, might serve to augment knowledge of the lesions.

MATERIALS AND METHODS

Poisoning with *L. bipinnatum* was induced and studied in 9 sheep. A further 2 animals, to which the plant was not administered, served as controls.

Batches of *L. bipinnatum* in the flowering stage were collected from 2 separate localities in the eastern Cape Province. The first was obtained on the farm "Doornbosch" in the Sneeuberg mountains between Graaff-Reinet and Murraysburg. The plants were harvested in July and were small, prostrate and very woody as a result of the prevailing low temperatures and drought. An outbreak of poisoning by ganskweek has previously been reported in cattle on this farm (Thornton, 1977). The second batch of ganskweek was collected on the farm "Sarnia", near Baroda station, in the Cradock district, in December. The plants were growing in profusion in a slight depression adjacent to an irrigated lucerne field. The plants were lush and stood up to half a metre high with diameters of over one metre.

At both localities whole plants were dug up and their roots manually freed of all adhering soil and clods. The plants were then shade-dried for 2 weeks and the finer stems, bearing leaves and flowers, were separated from the thicker, leafless, woody parts and roots. The latter were discarded and the other portion finely ground in a hammer-mill and stored in brown paper bags until required. Pressed specimens of the flowering plants from both farms were positively identfied as *Lasiospermum bipinnatum* (Thunb.) Druce at the National Herbarium, Brumeria, Pretoria, by a professional staff member.

Eleven weaned Mutton Merino lambs, 4–6 months old and of both sexes, were used in the experiments. The lambs were vaccinated against enterotoxaemia, dewormed with a broad-spectrum anthelmintic and their mass determined before and periodically during the experiments. Large, clearly labelled ear-tags were inserted so as to facilitate identification of the sheep at a distance. The animals were housed under prevailing atmospheric conditions in a concrete pen in which a roofed shelter provided adequate protection from the elements. They were fed *ad libitum* on milled lucerne-hay and drinking water was freely available. A minimum period of 3 weeks of acclimatization was allowed before the commencement of experiments.

The levels and intervals at which plant material was administered to the animals were based on those used in previous experimental work by others (Adelaar *et al.*, 1964; Kellerman *et al.*, 1973). The desired mass of milled plant material was determined by use of a Mettler electronic balance. It was

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TABLE 1 Schedule of administration of milled plant material to lambs

Group	Sheep	Sex	Daily dose (g/kg)	No. of doses	Total dose (g/kg)	Duration of experiment (days)	Outcome	Batch given
High-dose	A	Female	12	2	24	2	Died	Cradock
	B	Wether	8	4	32	5	Killed*	Graaff-Reinet
	C	Wether	8	4	32	5	Died	Cradock
	D	Wether	8	4	32	7	Killed*	Cradock
	E	Wether	6	6	36	7	Died	Cradock
Low-dose	F	Wether	4	12	48	14	Killed	Cradock
	G	Wether	2,5	13	32,5	14	Killed	Graaff-Reinet
	H	Wether	2	14	28	15	Killed	Cradock
	I	Female	1	16	16	17	Killed	Cradock
Controls	J	Wether	–	–	–	7	Killed	–
	K	Female	–	–	–	14	Killed	–

* = *in extremis*

mixed with water in a glass flask at proportions of approximately 1 g of plant material to 10 mℓ of water. Except for the 2 control sheep and sheep G this suspension was then dosed intraruminally to the other sheep by means of a stomach tube. The latter animal was not dosed plant material per stomach tube. It was placed on its own in an adjacent pen and offered a mixture of equal parts of ganskweek and milled lucerne hay. Initially, the animal refused to eat this, but after several hours reluctantly consumed small amounts. On the following day, the lamb was offered the plant material mixed with yellow maize meal in a 1:2 ratio and at a dose rate of 2,5 g of *L. bipinnatum* per kg. This, as well as 12 further daily doses, was readily eaten by the lamb. The sex, dosing regimen, origin of plant material and outcome for each of the animals are given in Table 1.

During the experiments the lambs were observed and inspected at least twice daily and particular attention was paid to their general habitus, appetite, rumen function and appearance of the visible mucous membranes. They were temperatured once a day. Blood was collected at regular intervals into vacuum tubes by jugular vein puncture in order to ascertain and monitor hepatic injury. Serum levels of sorbitol dehydrogenase (SDH) were determined in some sheep and the levels of gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) in all experimental animals. The serum enzyme assays were performed by the staff of the Clinical Pathology Laboratory, Faculty of Veterinary Science, University of Pretoria, using standard methods.

The sheep either died naturally or were sacrificed by exsanguination following induction of barbiturate anaesthesia (Table 1). Necropsies were performed on all of them and the findings recorded. Colour photographs were taken of the most outstanding macroscopic lesions using Ektachrome 100 film.

Samples of the following organs and tissues were collected for light microscopy: liver (left, right and caudate lobes), lung, kidney, myocardium, skeletal muscle, lymph nodes, spleen, brain, adrenal gland, abomasum, small and large intestine and pancreas. Slices not more than 5 mm in thickness were fixed by immersion in 10 % buffered formalin for periods of 48 h–1 week. The fixed tissues were processed routinely, embedded in paraffin wax, sectioned at 4–6 µm and stained with haematoxylin and eosin (HE).

Specimens for electron microscopy were collected as soon after death as possible. In killed animals, this period did not exceed 10 min. Various sites of the livers of most lambs were sampled and in some lambs lung tissue was also sampled. Slices of tissue 1 mm thick were cut, using a sharp blade, and diced into 1 mm cubes. These blocks were fixed by immersion in refrigerated 4 % glutaraldehyde in Millonig's phosphate buffer at pH 7,3–7,4 (Millonig, 1961) for periods of 1–21 days. Selected blocks were rinsed with phosphate buffer and post-fixed in phosphate-buffered 2 % osmium tetroxide. After several buffer rinses, the blocks were dehydrated in a graded ethanol series, cleared in propylene oxide and embedded in LX-112 resin for 48 h at 60 °C. Thick sections (1–2 µm) were cut with glass knives on a Reichert Om U4 ultramicrotome and stained with toluidine blue (Trump, Smuckler & Benditt, 1961). Appropriate smaller areas were selected, using light microscopy, for preparation of thin sections. The selected blocks were trimmed to size and gold to silver sections cut. These were picked up on copper grids, stained for 30 min in saturated aqueous solution of uranyl acetate (Watson, 1958) and for 4 min in 0,2 % lead citrate solution (Reynolds, 1963). The prepared grids were viewed with a Philips 301 transmission electron microscope operated at 80 kV.

RESULTS

Clinical signs

The 5 lambs in the high-dose group received between 6 and 12 g of toxic plant per kg live mass per day and either died naturally or were killed *in extremis* (Table 1). Lamb A, which was given 12 g/kg, died after 2 doses, while in Lamb E, six daily administrations of 6 g/kg proved to be fatal. Progressive depression was evident in all these animals and was manifested as lowered carriage of the head, listlessness and lack of interest in their surroundings. A few of the lambs stood for long periods with lowered heads in a corner of the pen. Anorexia, also progressive, affected every animal in this group. The lambs ate more slowly, later only nibbled at their food, ceased to ruminate and finally showed no interest in food, although they continued to drink water. Rumen movements remained normal in Lambs B, C and E, whereas in A and D rumen stasis was present on the day of death.

The 4 lambs in the low-dose group were given daily doses, ranging from 1–4 g/kg for periods of from 12–16 days (Table 1). Clinical signs were not observed in Lambs G, H and I, and they appeared to be clinically normal at the time they were killed. Lamb F, which received the highest daily dose in this group (4 g/kg) was moderately depressed and ano-

rexic on Day 6. It was not dosed on Day 7 but dosing was resumed the following day, when the animal seemed to be in good health. Mild depression and a slight increase in the rate and depth of respiration were noted on Day 10, but the lamb appeared to be quite well on the subsequent days and was killed on Day 14.

No clinical signs of ill health were observed in the control animals during the experiments.

Chemical pathology

The results of analyses for serum levels of GLDH, GGT and SDH are given in Tables 2, 3 and 4, respectively. Very large increases in GLDH levels were found. In general, the elevations paralleled the level of toxic plant administered. Gamma glutamyl transferase levels followed a similar pattern of increases but these were less marked, while SDH levels were not always consistent with dose levels given.

TABLE 2 Serum glutamate dehydrogenase levels (mI.U./mℓ)

Sheep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
A	6	120							
B	7	11	154	226	145				
C	2	4	6	35	25				
D	12	–	62	–	–	–	303		
E	9	22	15	156	132	152			
F	5	–	64	–	147	–	161	–	75
G	6	–	–	–	–	–	–	–	81
H	5	–	5	–	5	–	4	–	7
I	6	–	7	–	6	–	6	–	5
J	3	–	–	–	–	–	4		
K	8	–	–	–	–	–	–	–	7

– not tested on that day

TABLE 3 Serum gamma glutamyl transferase levels (mI.U./mℓ)

Sheep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
A	24	37							
B	36	35	61	70	72				
C	36	36	43	42	46				
D	31	–	35	–	–	–	70		
E	29	28	39	49	55	61			
F	37	–	36	–	72	–	99	–	72
G	34	–	–	–	–	–	–	–	36
H	28	–	27	–	31	–	30	–	33
I	32	–	32	–	32	–	33	–	33
J	32	–	–	–	–	–	35		
K	34	–	–	–	–	–	–	–	30

– not tested on that day

TABLE 4 Serum sorbitol dehydrogenase levels (mI.U./mℓ)

Sheep	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
B	6	6	22	70	10				
C	6	5	7	12	12				
D	4	–	9	–	–	–	7		
G	3	–	–	–	–	–	–	–	20
J	3	–	–	–	–	–	5		
K	6	–	–	–	–	–	–	–	4

– not tested on that day

Gross hepatic pathology

Hepatosis was the most readily apparent macroscopic lesion and was found in all the sheep given *L. bipinnatum*. The severity of the liver pathology correlated well with the administered levels of plant.

The livers of the lambs in the high-dose group (A–E) showed pathological changes which were essentially similar in appearance (Fig. 1, 2 & 5). Hepatosis was adjudged to be severe in all the sheep in this group except for Lamb C, where only moderate liver lesions were present. In 2 animals (B & D), mild icterus, characterized by pale yellow discoloration of the mucosae, serosae and other body tissues, was present. In the remaining lambs in this group (A, C & E), the carcasses were moderately congested and moderately to severely cyanotic. In all 5 lambs, the livers were slightly to moderately swollen, as judged by rounding of the edges and bulging of the cut surfaces. In general, they were yellow-brown instead of the normal red-brown colour (Fig. 1 & 2). Hepatic surface patterning was much more distinct, the lobules having a red (haemorrhagic) or brown centre, surrounded by a paler tan-coloured peripheral zone (Fig. 5). Friability of the parenchyma was moderately to markedly increased and the consistency was slightly reduced.

In one sheep (D), the gall-bladder wall showed 3 large mucosal haemorrhages about 10 mm in diameter (Fig. 2). These were clearly visible from the serosal surface. Three lambs (B, D & E) had gall-bladders which were moderately distended, with bile of normal appearance, and in 2 animals (A & C) the gall-bladders were normal.

The livers of the low-dose group animals (F–I) had a more varied appearance than those of the high-dose group. The degree of hepatosis was adjudged to be moderate in Lamb F, mild in G and H and very mild in I. In Lamb I, which received the lowest dose of plant (1 g/kg/day) the liver was of normal size, colour and consistency, but there was a slight accentuation of lobulation. In the other 3 animals, the livers were mildly swollen, light brown, slightly more friable and showed distinct lobulation (Fig. 3 & 6). The gall-bladders were normal in all 4 of these sheep.

The livers and gall-bladders of the control animals (J & K) were normal in appearance (Fig. 4).

Light microscopical hepatic pathology

The most obvious lesions in the liver, as determined by light microscopy, are summarized in Table 5. The hepatocyte appeared to be the major target cell in the liver and a variety of pathological changes ranging from degeneration to necrosis, was observed. Hepatic lesions were mildest in the lamb given the lowest daily dose of *L. bipinnatum* (I) and became progressively more severe with increasing dose levels, being most severe in the lamb that received the highest dose rate (A).

Within the liver lobules (in the sense of the "classical" anatomical structure), the hepatocytes showed variable changes, depending on their location within the lobule and the dose rate of toxic plant that had been administed. In 6 lambs (C, D, F, G, H & I), in which degenerated heptocytes predominated over necrotic cells, periportal hepatocytes appeared to be the most severely affected, while cells around the central vein were the least damaged. In the 2 lambs in which hepatocellular necrosis overshadowed parenchymal degeneration (A & B), necrotic hepatocytes were situated centrilobularly and degenerated cells peripherally. In the remaining animal (E), degenerative changes were most severe in the midzonal hepatocytes, less severe around the central vein and mildest in the periportal zone of the lobule.

Although the detailed descriptions given below

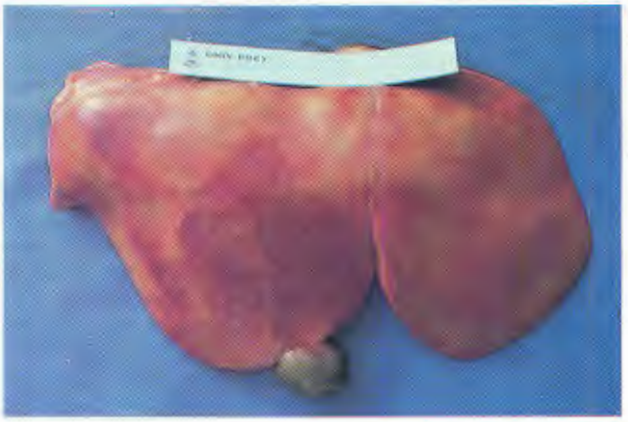

FIG. 1 Lamb A: Liver. Severe hepatosis with widespread, patchy haemorrhage

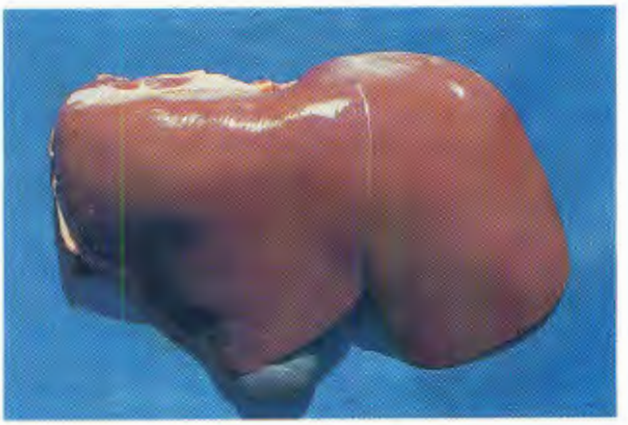

FIG. 4 Lamb K: Liver. Control lamb

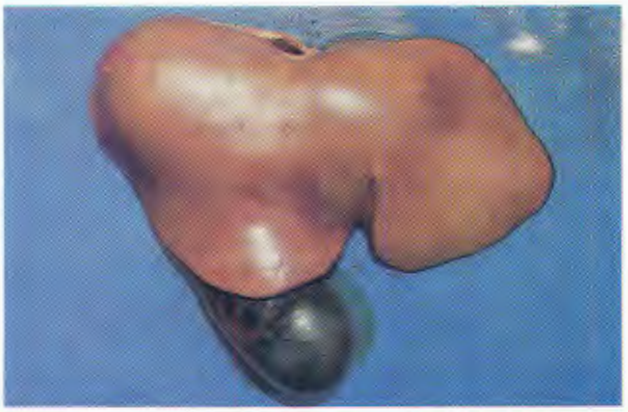

FIG. 2 Lamb D: Liver. Severe hepatosis. Enlarged gall-bladder with focal haemorrhage in the wall

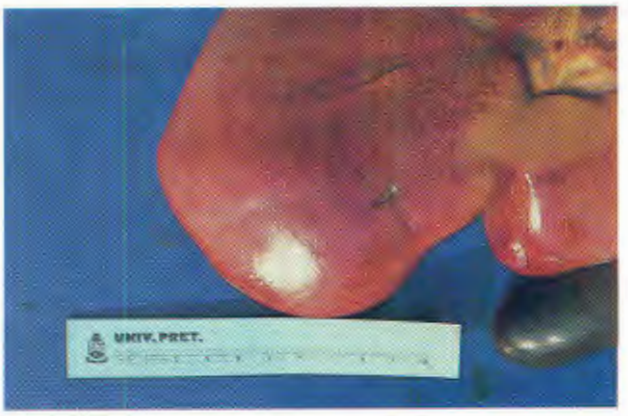

FIG. 5 Lamb A: Liver. Close-up of visceral surface, showing accentuated lobulation

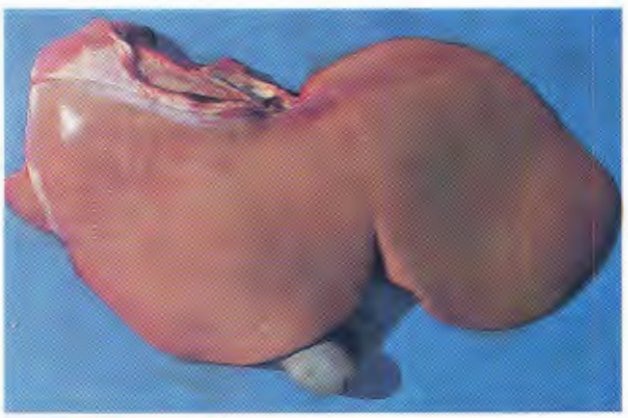

FIG. 3 Lamb F: Liver. Moderate hepatosis

FIG. 6 Lamb F: Liver. Close-up of surface demonstrating very distinct lobulation. Darker brown areas are centrilobular in position

were made from sections cut from samples of the left lobe of the liver, the microscopic lesions in the left, right and caudate lobes were found to be indistinguishable.

In Lamb A, most of the hepatocytes were frankly necrotic, with only a narrow zone of degenerated periportal hepatocytes 3–5 cells wide (Fig. 7). The necrotic cells were shrunken, separated from adjacent hepatocytes and had either a highly eosinophilic appearance (*c.* 30 %) or moderately basophilic tincture (*c.* 70 %). The bluish colour of the latter cells was due to the presence of numerous, very fine, bluish granules (dystrophic calcification) in the cytoplasm.

Nuclei were absent from many of the necrotic cells, but the remainder showed either nuclear ka-

TABLE 5 Principal microscopic liver lesions

Case	Necrosis of hepatocytes			Degeneration of hepatocytes			Proliferation of bile duct epithelium
	Centrilobular	Midlobular	Periportal	Centrilobular	Midlobular	Periportal	
A	+++++	+++++	–	–	–	+++++	++
B	++++	+++++	–	–	–	++++	+++
C	–	–	–	–	+++	++	–
D	++	++	++	+++	++++	+++++	+++
E	++	+++	–	+++	++++	++	+
F	+	++	+	+	+	++	+
G	–	–	–	+	+	++	+
H	–	–	–	+	++	++	+
I	–	–	–	–	+	+	–
J	–	–	–	–	–	–	–
K	–	–	–	–	–	–	–

+++++ very severe
++++ severe
+++ moderate
++ mild
+ very mild
– absent

ryorrhexis or pycnosis. The sinusoids varied in width and were distorted in shape. Hepatic cords were unrecognizable. Severe haemorrhage was present in the centrilobular zone of most lobules, the erythrocytes usually surrounding isolated, necrotic hepatocytes (Fig. 7).

The Kupffer's cells seemed to be more prominent, probably because they were relatively unaffected. The severely degenerated periportal hepatocytes were moderately swollen, with a less dense, feathery cytoplasm. In a few of these cells, the nuclei appeared to be normal but most were variably pycnotic. The hepatic cords and sinusoids in this area were less disrupted than those in the necrotic zone. Mild proliferation of bile ductule epithelial cells was represented by small, scattered clumps or short rows of tightly packed cells in the vicinity of the portal triads.

Occasionally 2 parallel and closely opposed rows of bile ductule cells could be observed emanating from terminal bile ducts. The proliferating cells had ovoid basophilic nuclei and scant, moderately eosinophilic cytoplasms.

Hepatic lesions in Lamb B were similar to those in A but, necrosis was less extensive, the peripheral zone of degenerating hepatocytes wider and bile ductule epithelial proliferation more advanced (Fig. 8). In addition, there was a narrow midzonal area where most of the hepatocytes had disappeared, leaving a network of reticular tissue. Some of the spaces in this framework contained highly eosinophilic, shrunken, necrotic hepatocytes or occasional acidophilic bodies (Fig. 9). Cells with large, round, vesicular nuclei, containing a prominent nucleolus and scant, irregular, basophilic cytoplasm which sometimes contained eosinophilic droplets, were also observed in the midzonal area. Mitotic figures were common in the vicinity of these cells, suggesting that the cells may represent regenerating hepatocytes.

Similar cells were scattered amongst the necrotic hepatocytes in the centrilobular zone. Some neutrophils, many of them necrotic, were seen in the midzonal area. The viable periportal hepatocytes had nuclei which looked normal, but the cytoplasm of most of these cells was markedly swollen and vacuolated. The vacuoles were variable in size and shape, and often peripheral. The remaining cytoplasm consisted of fine eosinophilic granules and globules. Definable sinusoids and hepatic cords were absent in this liver.

Despite the relatively high dose rate (8 g/kg/day), the hepatocytes in the liver of Lamb C showed only degenerative lesions. Vacuoles of varying size and number were present in the hepatocytes and were especially large and numerous in the periportal cells (Fig. 10). The larger vacuoles contained a loosely-woven, pink, fibrillar material.

Lamb D showed marked hepatic lesions, characterized by severe degeneration of the hepatocytes, marked nuclear anisocytosis and mild, scattered, single cell necrosis (Fig. 11). The centrilobular hepatocytes evinced increased cytoplasmic eosinophilia and occasional, small, fat vacuoles. The basophilic clumps of rough endoplasmic reticulum (RER), observed in the livers of control lambs (*vide infra*) were absent from these cells. Midzonal hepatocytes had more acidophilic and granular cytoplasms, with frequent clumping of this material. The periportal hepatocytes showed very marked and variable degenerative lesions, comprising hydropic, fatty and granular types. Frequently, large clumps of eosinophilic material, usually surrounded by a clear halo, were seen in the cytoplasm (Fig. 12). Proliferation of bile ductule cells was more marked in this animal than in any of the other lambs.

In Lamb E, the centrilobular hepatocytes were moderately degenerated, as evinced by increased cytoplasmic eosinophilia and vacuolation, nuclear anisocytosis and cell swelling. There was also scattered single cell necrosis in this zone. The midzonal liver cells were severely degenerated with marked cell swelling, frequent haloed, spherical, eosinophilic bodies in the cytoplasm and very large, vesicular

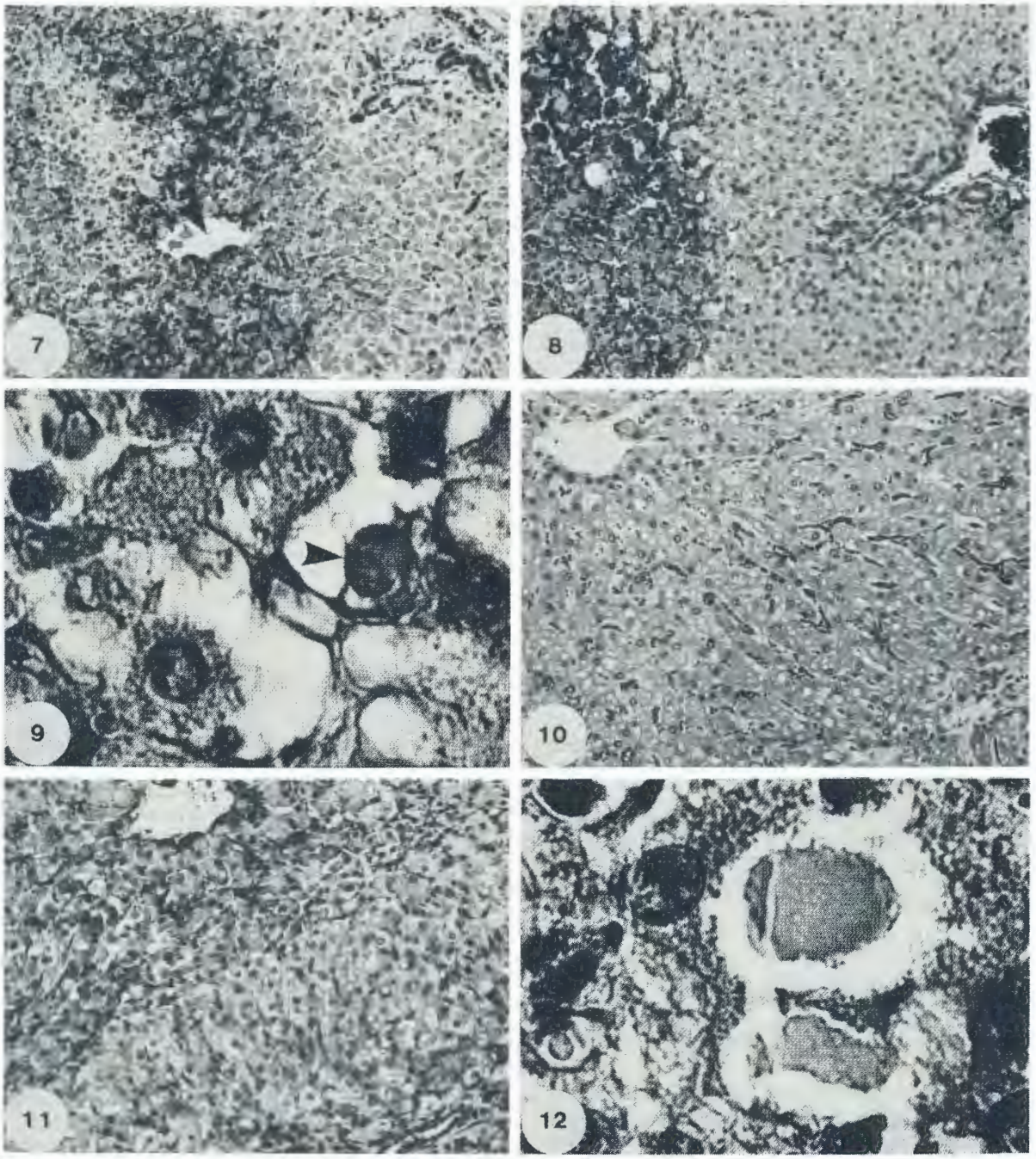

FIG. 7 Lamb A: Liver. Necrosis of hepatocytes and haemorrhage around a central vein. HE × 100

FIG. 8 Lamb B: Liver. Central vein with surrounding necro-haemorrhagic zone and wide band of peripheral degenerated hepatocytes. HE × 100

FIG. 9 Lamb B: Liver. Acidophilic body (arrow) in cytoplasm of hepatocyte in midzonal area of lytic necrosis. HE × 1 000

FIG. 10 Lamb C: Liver. Periportal hepatocytes (lower right) with denser cytoplasm and clearer vacuolation than centrilobular cells. HE × 100

FIG. 11 Lamb D: Liver. Central vein (top) surrounded by hepatocytes with increased cytoplasmic eosinophilia. Peripheral zone of swollen, vacuolated cells. HE × 100

FIG. 12 Lamb D: Liver. Large globules of eosinophilic material surrounded by a clear halo within hepatocytes in the periportal zone. HE × 1 000

nuclei. Moderate single cell necrosis was evident in this area. The periportal hepatocytes showed similar but less severe changes than those in the centrilobular cells.

The livers of Lambs F, G and H had mild lesions. Most of the hepatocytes contained irregular but well-circumscribed, pale orange-pink, homogeneous areas in their cytoplasms. These were particularly large and well-defined in the periportal hepatocytes (Fig. 13). Mild bile ductule epithelial proliferation was present. The centrilobular hepatocytes showed mild cellular swelling. In Lamb F, the hepatocyte nuclei were markedly enlarged, especially in the midzonal and periportal regions of the lobule. Single cell necrosis was observed throughout the lobules but was most striking in the midzonal area in this animal. Numerous, clear, ellipsoidal clefts were observed in the cytoplasm of the centrilobular hepatocytes in Lamb F (Fig. 14).

The only change observed in the liver of Lamb I

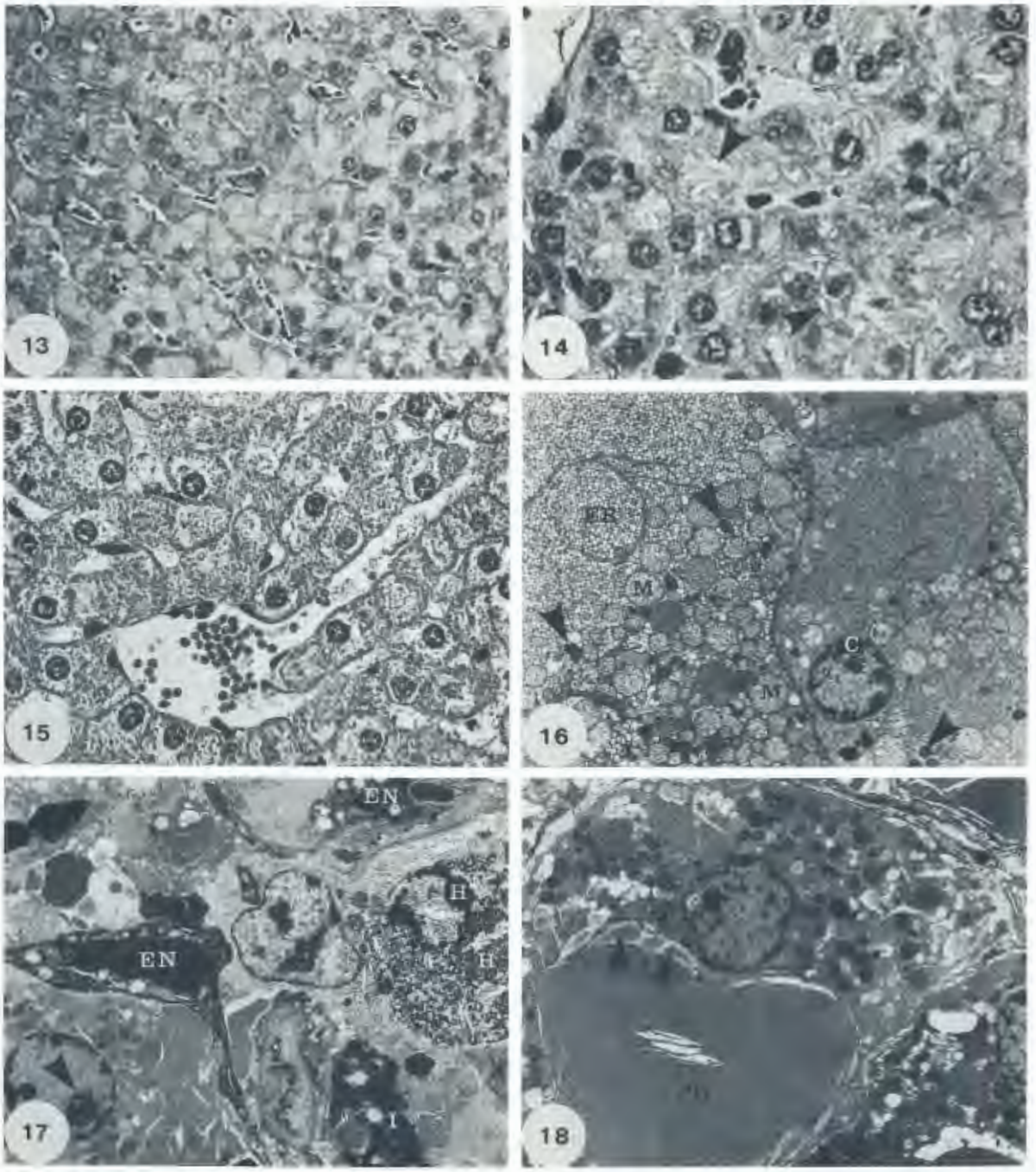

FIG. 13 Lamb H: Liver. Pale homogeneous areas in the cytoplasm of midzonal and periportal hepatocytes representing hypertrophy of smooth endoplasmic reticulum. HE × 200

FIG. 14 Lamb F: Liver. Centrilobular hepatocytes with ellipsoidal cytoplasmic clefts (arrows). HE × 400

FIG. 15 Lamb J: Liver. Centrilobular zone of a control animal. HE × 400

FIG. 16 Lamb B: Liver. Severely degenerated peripheral hepatocytes. Note chromatin margination (C), proliferation and swelling of the endoplasmic reticulum (ER) and concentration of mitochondria (M) and lysosomes (arrows) in one area of the cytoplasm. Sinusoids not visible. × 2 200

FIG. 17 Lamb B: Liver. Necrosis of hepatocytes in centrilobular area. A frankly necrotic hepatocyte can be observed (H) as well as severely degenerated endocytes (EN) and an infiltrating cell (I), which is probably a mast cell. Nucleolar enlargement is present in one hepatocyte (arrow). × 2 200

FIG. 18 Lamb D: Liver. Peripheral hepatocyte containing a large proteinaceous globule (PG). Lipid can be seen accumulating on the periphery of this inclusion (arrows). × 2 800

was a loss of RER clumps from the hepatocyte cytoplasm and very mild, subtle vacuolation of the midzonal and periportal hepatocytes.

The livers of the control lambs (J and K), demonstrated that under the light microscope the hepatocytes had a narrow rim of deeply eosinophilic cytoplasm below the plasmalemma (Fig. 15). The less dense central cytoplasmic core contained scattered, small, irregular, pale blue or bluish-purple patches, shown to be stacked profiles of RER with the electron microscope (*vide infra*). There was very little variation in nuclear size between individual hepatocytes and none between centrilobular cells on one hand and periportal hepatocytes on the other.

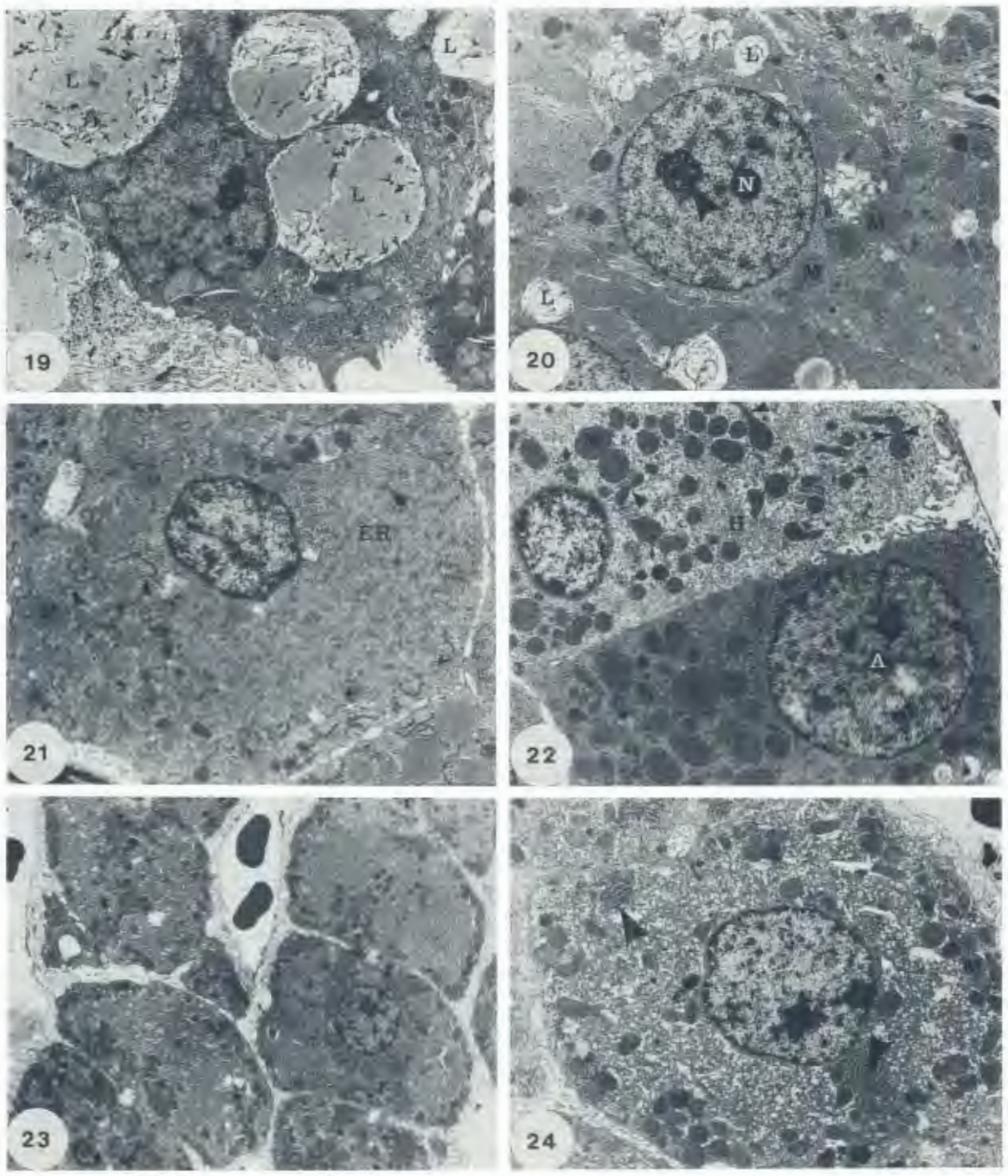

FIG. 19 Lamb D: Liver. Peripheral hepatocyte with several large cytoplasmic lipid droplets (L). × 4 300

FIG. 20 Lamb D: Liver. Hyperactive centrilobular liver cell with enlarged nucleus (N), nucleolus (arrow) and mitochondria (M). Small, developing lipid droplets are also present (L). × 2 800

FIG. 21 Lamb G: Liver. Peripheral hepatocyte showing large area of hypertrophied smooth endoplasmic reticulum (ER) and crowding of other organelles such as mitochondria (M) and single strands of rough endoplasmic reticulum (arrows). × 4 300

FIG. 22 Lamb G: Liver. Generalized hypertrophy of smooth endoplasmic reticulum in one hepatocyte (H) giving it a less dense appearance than the adjacent cell (A). Note also evenly-spread, enlarged mitochondria (M), some of which appear to be undergoing division (arrows). × 4 300

FIG. 23 Lamb J: Liver. Control animal. × 1 800

FIG. 24 Lamb J: Liver. Normal hepatocyte with peripheral mitochondria (M) and stacked profiles of rough endoplasmic reticulum (arrows). × 4 300

Electron microscopical hepatic pathology

Specimens of liver tissue from all the lambs except A, C and K were examined with a transmission electron microscope (EM). The lesions observed could easily be correlated with those seen with the light microscope (LM) but in some instances certain light microscopic findings were clarified with the EM.

In Lamb B the periportal hepatocytes were found to be severely degenerated (Fig. 16), the midzonal liver cells were either degenerated or necrotic and the centrilobular cells were all necrotic (Fig. 17). Nuclear changes observed in degenerated and

necrotic hepatocytes included chromatin and nucleolar margination, nucleolar enlargement, pycnosis and karyorrhexis. Alterations seen in the cytoplasm were: swelling (hydropic degeneration) and proliferation of the endoplasmic reticulum, concentration of the mitochondria and lysosomes at 1 pole of the hepatocyte, mitochondrial swelling (cloudy swelling) and coalesence of some areas of the endoplasm. Many affected mitochondria had lost their internal structure and had become variably more electron-lucent. In some areas of the lobule, the hepatic cords were severely distorted and the sinusoids had collapsed (Fig. 17). Where the sinusoids were still patent, they were filled with fairly dense proteinaceous material. Severely degenerated cells, thought to be endothelial cells, were observed between degenerated and necrotic centrilobular hepatocytes (Fig. 17). Connective tissue in the portal triads was found to be moderately oedematous, something that was not appreciated with the LM.

Many of the hepatocytes in Lambs D and E, especially in the periphery of the lobule, were severely degenerated. A range of cytoplasmic changes were observed, including the formation of large, homogeneous globules of proteinaceous material (Fig. 18) and the accumulation of unsaturated lipid in the form of large cytoplasmic droplets (Fig. 19). Nuclear alterations were also noted in these cells, comprising irregular membranes, nucleolar and chromatin margination and early pycnosis. Hepatocytes closer to the central vein often showed signs of increased metabolic activity as well as early degenerative changes. These cells had large, spherical nuclei with evenly dispersed chromatin and large nucleoli, all of which are associated with cellular hyperactivity. Cytoplasmic changes seen were, mitochondrial enlargement and the formation of small, lipid-containing vacuoles (Fig. 20). In some sections, the sinusoids contained moderate amounts of intraluminal proteinaceous material.

Lesions seen with the EM in Lambs F, G and H were essentially similar. The most striking finding was the presence of large, focal areas of hypertrophied smooth endoplasmic reticulum (SER) in the cytoplasm of, especially, the periportal hepatocytes (Fig. 21). This corresponded to the pale orange-pink areas of cytoplasm seen with the LM (*vide supra*) (Fig. 13). In the affected cells, the remaining organelles were crowded into the residual cytoplasm. The mitochondria appeared to be slightly swollen and the RER occurred as scattered single profiles amongst the vesicular organelles (Fig. 21). In other hepatocytes, hypertrophy of SER was generalized in the cytoplasm, giving these cells a more lucent appearance (Fig. 22). In these pale hepatocytes, as well as in their more opaque neighbours, the mitochondria were more or less evenly distributed in the cytoplasm and were often enlarged (Fig. 22). In the midzonal area of the lobules in Lamb F, single necrotic cells were commonly found.

When examined with the EM, the hepatocytes of Lamb I were found to closely resemble those of the controls (Lambs J and K). Normal hepatocytes are illustrated in Fig. 23 & 24. It can be seen that the mitochondria and lysosomes are mostly located peripherally, just below the cell membrane, and this probably accounts for the eosinophilic rim observed with the LM (Fig. 15). Stacked, scattered profiles of RER (Fig. 24) correspond with the basophilic clumps of material seen in paraffin sections stained with HE. The stacked profiles are, moreover, in sharp contrast to the disorganized single strands of RER observed in Lambs F, G and H (Fig. 21).

Discussion

Hepatic diseases in ruminants which result in the development of icterus and photosensitivity are of considerable economic importance in South Africa (Kellerman & Coetzer, 1985). These conditions can be divided into those which primarily cause hepatocellular degeneration and necrosis and those in which the biliary system is mainly affected (Kellerman & Coetzer, 1985). *L. bipinnatum* poisoning falls into the former group of diseases which, in South Africa, includes poisoning by *Lantana camara* L. (Seawright & Allen, 1972) and some other members of the Verbenaceae (Quinn, 1933), *Asaemia axillaris* (Thunb.) Harv. ex Jackson (Coetzer & Bergh, 1983), *Athanasia trifurcata* L. (Kellerman, Coetzer, Schneider & Welman, 1983), *Microcystis aeruginosa* (Kellerman & Coetzer, 1985), *Phomopsis leptostromiformis* (Kühn) Bubak (Gardiner, 1967), *Nidorella foetida* (Thunb.) DC. (Schneider, Green & Collett 1987), *Hertia pallens* (DC.) Kuntze (Prozesky, Kellerman, Jordaan, Welman & Joubert, 1985), *Pteronia pallens* L.f. (Prozesky, Kellerman & Welman, 1986) and a condition of unknown aetiology known as Stellenbosch photosensitivity (Kellerman & Coetzer, 1985).

Since the characteristic clinical signs in these poisonings are similar (hepatogenous photosensitivity and icterus) emphasis has been placed on the light microscopic hepatic lesion, in particular, as an aid to specific diagnosis. In *L. camara, M. aeruginosa* and *P. leptostromiformis* poisoning and in Stellenbosch photosensitivity the lesion is, in essence, diffuse in the lobule. In *L. bipinnatum, A. axillaris, A. trifurcata, N. foetida, H. pallens* and *P. pallens* poisoning, on the other hand, the microscopic lesion is characteristically zonal. It is probably significant that the latter 6 plants, which tend to cause zonal lesions, all belong to the family Asteraceae.

In the present experiments 9 lambs were poisoned. Ganskweek was dosed to each at varying levels but with a constant interval between administrations (24 h). A variety of lesions were induced in the liver and these were dose-dependent (Table 5).

Generally, in sheep receiving high doses of *L. bipinnatum,* hepatocellular necrosis predominated, whereas parenchymal degeneration was more evident in those given lower doses. Within these 2 groups the severity of necrosis and/or degeneration appeared to correlate positively to the dose level administered.

In those lambs in which necrosis of hepatocytes exceeded degenerative changes there seemed to be a tendency for the necrosis to show a zonal pattern within the lobule, viz. centri- and midlobular. A tendency for zonation of necrosis in the hepatic lobule in sheep has also been noted by others in both natural and experimental cases of *L. bipinnatum* poisoning and in poisoning by other hepatotoxic Asteraceae (Table 6).

Although zonation of necrosis appears to be characteristic for poisoning by hepatotoxic Asteraceae in sheep, no diagnostic importance can be attached to the zone in which necrosis occurs, as this may be centrilobular, midzonal or peripheral in poisoning by ganskweek as well as other hepatotoxic Asteraceae. The factor or factors that may determine which particular zone will be affected are discussed below.

TABLE 6 Distribution of lesions in hepatic lobules in poisoning by hepatotoxic Asteraceae

Plant	Centri-zonal necrosis	Midzonal necrosis	Peripheral necrosis	Single cell necrosis	Diffuse degenera-tion	Centrizonal degenera-tion	Midzonal degenera-tion	Peripheral degenera-tion
L. bipinnatum	10	4 10	1	3 10	6	–	10	10
A. axillaris	4	4	2	2	2	–	–	2 4
A. trifurcata	5	5	5	5	5	–	–	–
H. pallens	7	7	–	7	7	–	–	–
N. foetida	–	9	9	–	–	–	9	9
P. pallens	8	–	–	8	8	–	–	–

1. Adelaar *et al.*, 1964
2. Coetzer & Bergh, 1983
3. Fair *et al.*, 1970
4. Kellerman *et al.*, 1973
5. Kellerman *et al.*, 1983
6. Kellerman & Coetzer, 1985
7. Prozesky *et al.*, 1985
8. Prozesky *et al.*, 1986
9. Schneider *et al.*, 1987
10. Williams, 1990

Scattered, random necrosis of single hepatocytes, which has been referred to by MacSween, Anthony & Scheuer (1979) as acidophilic necrosis, was observed in Lambs D, E & F. Acidophilic necrosis of single cells has also been reported by others in *L. bipinnatum, A. axillaris, A. trifurcata, H. pallens,* and *P. pallens* poisoning (Table 6), again emphasizing the similarity of hepatic lesions in these plant poisonings.

In the 7 lambs that received lower doses of ganskweek, the significant hepatic lesion was diffuse degeneration of hepatocytes which in 5 appeared to be more severe in periportal than centrilobular cells (Table 5). In Lambs C & E, the midzonal liver cells were those most severely affected. Kellerman & Coetzer (1985) noted that in subacute *L. bipinnatum* poisoning diffuse parenchymal degeneration may be found, but they make no mention of these changes being more severe in any particular zone of hepatocytes. The species in which these observations were made and whether they were natural or experimental cases were also not stated. In poisoning by other hepatotoxic Asteraceae, degeneration of hepatocytes was found to be diffuse, midzonal or peripheral (Table 6). As noted above regarding the patterns of necrosis in the lobule, the distribution of degenerative changes in the lobules is likewise of little diagnostic value in distinguishing between poisoning by various Asteraceae.

A characteristic feature of the liver cells ot lambs given the lowest daily doses of plant material (F, G, H & I) was the presence of pale, orange-pink areas of varying size and shape in the cytoplasm (Fig. 13). These changes have not been reported in ganskweek poisoning or in poisoning with other hepatotoxic Asteraceae. The pale areas were most prominent in the periportal hepatocytes and contributed to the impression that periportal parenchymal cells were more severely degenerated than centrilobular hepatocytes. Examination of affected hepatocytes with the electron microscope showed the pale areas to be composed of a mass of proliferating SER which had displaced the other cell organelles (Fig. 21). The crowded organelles in the residual cytoplasm, especially the mitochondria, were responsible for the eosinophilia of these portions of cytoplasm, as observed with the light microscope. Hepatocytes with a similar light microscopic appearance were termed "ground-glass hepatocytes" by Klinge & Bannasch (1968); who attributed the cytoplasmic alteration to marked diffuse hypertrophy of the SER. This hypothesis has been confirmed in ultrastructural studies (Jezequel & Orlandi, 1972).

Detoxification of noxious substances, particularly those absorbed from the gastro-intestinal tract, is an important function of the hepatocyte (MacSween *et al.*, 1979). This ability, especially as regards lipid-soluble drugs, appears to reside largely in the SER, to which large numbers of enzymes are bound, including a mixed-function oxidase (MFO) pathway known as the cytochrome P-450 system (Sodeman & Sodeman, 1979). The P-450 pathway is involved in the metabolism of many drugs and may be utilized in attempts to metabolize the furanosesquiterpene (L.A.P. Anderson, unpublished data, 1989) present in ganskweek. The activity of mixed-function oxidases in the liver is always greatest in the centrilobular hepatocytes and least in the periportal cells (Jubb, Kennedy & Palmer, 1985). This may serve to explain variations in the zonation of hepatic necrosis and degeneration in ganskweek poisoning (Jubb *et al.*, 1985). Allen & Seawright (1973) showed that in poisoning by *Myoporum deserti,* a furanosesquiterpene-containing plant of the family Myoporaceae, the zone of the lobule in which necrosis occurred could be altered by induction or suppression of MFO activity. This is probably the explanation for the variations of zonal necrosis seen in poisoning by hepatotoxic Asteraceae.

The SER hypertrophy observed in the periportal hepatocytes of sheep given the lowest doses of ganskweek probably represent an attempt to produce more MFO. This hypothesis is supported by the observation that the chronic administration of low doses of many drugs causes SER hypertrophy, one of the best known examples being phenobarbitone (MacSween *et al.*, 1979; Sodeman & Sodeman, 1979). In the case of phenobarbitone, however, it is the SER in centrilobular hepatocytes that is hypertrophied (MacSween *et al.*, 1979).

Proliferation of bile ductule epithelium occurs in many forms of liver disease (MacSween *et al.*, 1979) and was present in 7 of the lambs in the current experiment (Table 5). It has been reported by others in experimental ganskweek poisoning (Kellerman *et al.*, 1973; Kellerman & Coetzer, 1985) as well as in poisoning by *A. axillaris* (Coetzer & Bergh, 1983;

Kellerman *et al.*, 1973), *A. trifurcata* (Kellerman *et al.*, 1983), *H. pallens* (Prozesky *et al.*, 1985), *P. pallens* (Prozesky *et al.*, 1986) and *N. foetida* (Schneider *et al.*, 1987).

The observation that cells that appeared to be regenerating immature hepatocytes and which were found in the vicinity of the zone of lysis necrosis in 1 of the lambs given 8 g/kg/day of toxic plant (Lamb B) does not seem to have been made before in *L. bipinnatum* poisoning. The nature of the peculiar ellipsoidal clefts noted in the cytoplasm of centrilobular hepatocytes in Lamb F under the light microscope (Fig. 14) could unfortunately not be determined, as they could not be found in liver specimens examined under the electron microscope. This raises the possibility that they may represent artefacts induced by formalin fixation and this contention is supported by the fact that they were also observed in the hepatocytes of a lamb used as a control in a concurrent but different trial (M. G. Collett, personal communication, 1985).

Haemorrhage from the sinusoids, which accompanied the hepatocellular necrosis that occurred in Lambs A and B in the present study (Fig. 7 & 8), was very similar to that reported by other workers in experimentally-induced ganskweek poisoning in sheep (Adelaar *et al.*, 1964; Kellerman *et al.*, 1973) and in a calf in a field outbreak of *L. bipinnatum* poisoning (Fair *et al.*, 1970). Electron microscopic studies on the liver of Lamb B indicated that haemorrhage probably resulted from severe disruption of the hepatic sinusoids in the necrotic zones (Fig. 17). This may have been due to a direct effect on endocytes lining the sinusoids, as occurs in, for example, poisoning by pyrrolizidine alkaloids (Bull, Culvenor & Dick, 1968). This contention is supported by the finding of what appear to be degenerated endothelial cells with the EM in Lamb B (Fig. 17).

Histological liver lesions not found in the present trials but noted by others in ganskweek poisoning include bile stasis (Kellerman *et al.*, 1973), portal fibroplasia and nuclear pseudoinclusions (Kellerman & Coetzer, 1985).

Hepatosis of variable degree and therefore of variable gross appearance was found in the liver of all the lambs poisoned in this experiment (Fig. 1, 2, 3, 5 & 6). This agrees with previous descriptions of natural and experimental *L. bipinnatum* poisoning (Adelaar *et al.*, 1964; Fair *et al.*, 1970; Kellerman *et al.*, 1973; Thornton, 1977). The most consistent macroscopic feature of the liver in the 9 lambs was accentuation of the surface pattern. This has also been noted in ganskweek poisoning by others (Fair *et al.*, 1970; Kellerman *et al.*, 1973). Invariably the centrilobular areas were darker (red or brown), while the periportal zones were lighter (tan to pinkish-white). The reticulated appearance that this confers on the liver seems to be characteristic of ganskweek poisoning. A similar gross pattern in the liver has been recorded for other hepatic conditions, including poisoning by *A. axillaris* (Kellerman *et al.*, 1973; Kellerman *et al.*, 1983), *A. trifurcata* (Coetzer & Bergh, 1983) and *P. pallens* (Prozesky *et al.*, 1986).

Macroscopic lesions in the gall-bladder, in the form of ecchymoses, occurred in only 1 sheep (D). Oedema of the wall of the gall-bladder has been described in a calf naturally poisoned by *L. bipinnatum* (Fair *et al.*, 1970). It is therefore apparent that severe gross lesions in this structure are unusual in ganskweek poisoning. This may assist in differentiating it from conditions in which marked lesions do occur, such as sporidesmin poisoning (Kellerman & Coetzer, 1985).

The hepatic lesions in *L. bipinnatum* poisoning in sheep show a remarkable similarity to those described in the liver of sheep poisoned by *A. axillaris, A. trifurcata, H. pallens, P. pallens* and *N. foetida* (Coetzer & Bergh, 1983; Kellerman *et al.*, 1973; Kellerman *et al.*, 1983; Prozesky *et al.*, 1985; Prozesky *et al.*, 1986; Schneider *et al.*, 1987). These 6 plants all belong to the family Asteraceae and, although a furanosesquiterpene has only been isolated from *L. bipinnatum*, they probably all possess identical or closely related toxins. This surmise provides an explanation for the very similar hepatic lesions found in all these poisonings. It is remarkable that plants of the Australian genus *Myoporum* (Myoporaceae) also contain furanosesquiterpenes and cause zonal hepatic lesions (Jubb *et al.*, 1985).

Changes in the serum levels of GLDH, GGT and SDH to a greater or lesser extent confirmed and monitored the hepatic injury. Elevation of serum GLDH is practically liver specific, as the GLDH content of the liver is very much higher than that of other large organs and, since the enzyme is localized exclusively in mitochondria, is indicative of severe damage to the hepatocytes (Schmidt, 1979). Doses of ganskweek of between 4 and 12 g/kg/day resulted in markedly raised GLDH and 2,5 g/kg/day in a moderate elevation (Table 2). In the lambs that received the lowest doses (H & I), GLDH remained normal. These results are in accord with the morphological lesions observed in the hepatocytes of the poisoned lambs and support the contention that liver injury in *L. bipinnatum* poisoning is of the cytotoxic type.

A raised GGT serum level indicates that liver injury is of the cholestatic type (Malherbe, Kellerman, Kriek & Haupt, 1977; Schmidt, 1979) and more promptly and more sensitively detects the lower grades of early acute intoxication than any other enzyme (Malherbe *et al.*, 1977). Gamma glutamyl transferase levels were consistently but only moderately elevated in the lambs given doses greater than 4 g/kg/day (Table 3). Cholestatic injury is thus present in ganskweek poisoning, but is of a much lesser order than the cytotoxic component.

Sorbitol dehydrogenase determinations are regarded by some workers as of value in the diagnosis and prognosis of liver disease in ruminants (Shaw, 1974), but it has been pointed out by others that the rise in SDH in liver damage is erratic and slight (Schmidt, 1979). In the present study, serum SDH levels were found to be inconsistent and unreliable as a monitor of hepatic injury. Those obtained in Lambs B and D illustrate this point (Table 4).

The administration, to sheep, of daily doses of higher than 6 g/kg of the dried plant material used in the present experiments proved to be fatal in less than 1 week. This accords well with a previous report in which it was stated that the single acute minimum lethal dose of their batch of *L. bipinnatum* was between 7 and 9,7 g/kg for sheep (Adelaar *et al.*, 1964). Although deaths from natural poisoning by ganskweek have not been documented in sheep (Adelaar *et al.*, 1964), they have been reported in cattle (Fair *et al.*, 1970; Thornton, 1977). Walsh (1909) was the first to give evidence, albeit heresay, that the plant was responsible for deaths in stock but did not mention the species of animal involved. Affected animals may recover or die, the latter no

doubt due to hepatic failure, but no reports regarding morbidity and mortality rates in field outbreaks are available.

Icterus and photosensitivity, resulting from underlying hepatic damage and dysfunction, are rather striking findings in field outbreaks of ganskweek poisoning (Adelaar *et al.*, 1964; Fair *et al.*, 1970; Thornton, 1977) but are not always produced under experimental conditions (Adelaar *et al.*, 1964; Kellerman *et al.*, 1973). No attempt was made in the current study to specifically induce either icterus or photosensitivity. Mild icterus developed in 2 lambs dosed 8 g/kg/day (B & D), but signs of photosensitivity were not observed in these or in any of the other lambs used in the experiments, although they were in an open camp and thus exposed to direct sunlight. Both icterus (Adelaar *et al.*, 1964; Kellerman *et al.*, 1973) and photosensitivity (Kellerman *et al.*, 1973) have been produced experimentally in sheep dosed with *L. bipinnatum*.

Depression and anorexia, both of which were progressive, were major clinical signs observed in fatally-poisoned animals (Lambs A–E) and they also occurred transiently in the lamb given 4 g/kg/day (F). Depression and anorexia have not been noted by other workers, either in outbreaks of ganskweek poisoning (Fair *et al.*, 1970; Thornton, 1977), or under experimental conditions (Adelaar *et al.*, 1964; Kellerman *et al.*, 1973). Digestive disturbances, including ruminal stasis, constipation, tenesmus and colic have been reported (Adelaar *et al.*, 1964; Fair *et al.*, 1970; Kellerman *et al.*, 1973; Thornton, 1977). Stasis of the rumen was observed terminally in only 2 lambs (A & D) in the present investigation, and constipation and tenesmus were not seen. Signs of colic have been reported only in natural cases of ganskweek poisoning in cattle (Fair *et al.*, 1970; Thornton, 1977) but were absent in the lambs studied in the present trial.

Aggressive and wild behaviour has been seen in natural outbreaks of ganskweek poisoning in cattle (Thornton, 1977). None of the animals in the present trial exhibited aggressive or wild behaviour, which, perhaps in the species of animal chosen for the experiment, is not unexpected. Cattle on the other hand frequently manifest aggressive or wild behaviour, following hepatic injury due to a number of toxins, including ganskweek, *Cestrum* spp. and *Senecio* spp. poisoning.

Other clinical signs, reported in the literature in natural and/or experimental *L. bipinnatum* poisoning but not seen in the present cases, include fever (sheep and cattle) (Adelaar *et al.*, 1964; Fair *et al.*, 1970; Thornton, 1977), walking backwards (cattle) (Fair *et al.*, 1970; Thornton, 1977), tachycardia (sheep and cattle) (Adelaar *et al.*, 1964; Fair *et al.*, 1970), haematuria or epistaxis (cattle) (Thornton, 1977) and abortion (cattle) (Thornton, 1977).

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