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PHOTOSENSITIVITY IN SOUTH AFRICA. 1. A COMPARATIVE STUDY OF *ASAEMIA AXILLARIS* (THUNB.) HARV. EX JACKSON AND *LASIOSPERMUM BIPINNATUM* (THUNB.) DRUCE POISONING IN SHEEP

T. S. KELLERMAN[1], P. A. BASSON[1], T. W. NAUDÉ[1], I. B. J. VAN RENSBURG[1] and WILHELMINA G. WELMAN[2]

ABSTRACT

KELLERMAN, T. S., BASSON, P. A., NAUDÉ, T. W., VAN RENSBURG, I. B. J. & WELMAN, WILHELMINA G., 1973. Photosensitivity in South Africa. 1. A comparative study of *Asaemia Axillaris* (Thunb.) Harv. Ex Jackson and *Lasiospermum Bipinnatum* (Thunb.) Druce poisoning in sheep. *Onderstepoort J. vet. Res.* 40 (3), 115-126 (1973).

Experimental evidence is presented to confirm field observations that *Asaemia axillaris* (Thunb.) Harv. ex Jackson and *Lasiospermum bipinnatum* (Thunb.) Druce cause ovine hepatogenous photosensitization.

The two plants have fairly distinct distributions. While *Lasiospermum* is most prevalent in the eastern Karoo, *Asaemia* is commonest in the west and, except perhaps in the southern central Karoo, they seldom occur together. The locality of an outbreak should therefore be taken into account when making a diagnosis.

Peripheral fatty changes and midzonal necrosis of liver lobules were seen in both *Asaemia* and *Lasiospermum* poisoning. *Asaemia* poisoning could, however, be distinguished from *Lasiospermum* poisoning by the presence of centrilobular fatty degeneration and the absence of haemorrhages and bile pigmentation.

Although it may be difficult to distinguish between the two types of plant poisoning they are easily differentiated from geeldikkop (a major photosensitizing disease of the Karoo), which lacks a zonal pattern of hepatocytic degeneration and necrosis. Moreover, the crystalloid material characteristic of geeldikkop is not encountered in *Asaemia* or *Lasiospermum* poisoning.

INTRODUCTION

Plants that cause hepatogenous photosensitivity abound in South Africa. Steyn (1949) cites the following examples: *Tribulus terrestris* L.; *Brachiaria* and *Panicum* grasses; *Lantana camara* L.; *Lippia pretoriensis* Pears., *L. rehmannii* Pears., *L. javanica* (Burm. f.) Spreng. (= *L. asperifolia* A. Rich.); *Lupinus* spp.; *Asaemia axillaris* (Thunb.) Harv. ex Jackson, *Trifolium pratense* L., and the alga *Microcystis toxica* Stephens. Recently the causal fungus of facial eczema, *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis, was added to the list (Marasas, Adelaar, Kellerman, Minne, Van Rensburg & Burroughs, 1972).

The most important of these syndromes, geeldikkop, occurs in the Karoo where a severe outbreak can incapacitate more than 500 000 sheep (Steyn, 1949). Geeldikkop has been experimentally reproduced by feeding *Tribulus terrestris* plants to sheep (Theiler, 1918; Quin, 1928, 1929; Van Tonder, Basson & Van Rensburg, 1972) but such trials were not always successful (Quin, 1933a; Brown, 1968). Moreover, geeldikkop could be reproduced in this way only in endemic areas during outbreaks of the disease. At such times factor(s) appear to be present that render *Tribulus* plants toxic.

The sporadic nature of geeldikkop outbreaks, and difficulties encountered in reproducing it with *T. terrestris*, led to speculation that the plant was transiently toxic when climatic or physiologic conditions favoured synthesis of a toxin (Theiler, 1918; Quin, 1928, 1930, 1933a; Quin & Rimington, 1935; Brown, 1959b; Van Tonder *et al.*, 1972). Another theory is that *Tribulus* plants are periodically contaminated by mycotoxin-containing spores, cf. ryegrass leaves in the case of facial eczema (Brown, 1959a; Brown & De Wet, 1962; Kellerman & Marasas, unpublished data). Van Tonder *et al.*, (1972) conceded that either possibility could explain the aetiology of geeldikkop.

Although *Tribulus* plants contain nitrates (Rimington & Quin, 1933), saponins and sapogenins (Enslin & Wells, 1956; De Kock & Enslin, 1958), none of these compounds cause typical signs of geeldikkop (Brown, 1968). The significance of minerals (notably selenium and copper) in the aetiology of geeldikkop and enzootic icterus was extensively reported on by Brown in numerous publications (Brown & De Kock, 1959; Brown, 1962, 1963; Brown & De Wet, 1962; Brown, 1964, 1968). The literature on geeldikkop was reviewed by Brown (1959a, 1968).

Outbreaks of photosensitivity (dikoor) amongst sheep grazing *Panicum* grasses on cultivated lands were described by Steyn (1928) and Rimington & Quin (1937). The similarity between geeldikkop and dikoor was noted by Quin (1928, 1930, 1933a) but he considered the latter a milder disease (Quin, 1928). This was confirmed by Van Tonder *et al.*, (1972). Dikoor has not been experimentally reproduced and it is not clear whether the two conditions are separate entities.

Lippia rehmannii, *L. pretoriensis* and *Lantana camara* can cause hepatogenous photosensitivity when dosed to sheep (Quin, 1933b; Steyn & Van der Walt, 1941). The active principles are pentacyclic triterpene acids. The structures of the icterogenic agents, i.e. icterogenin and 22 β-angeloyloxyoleanolic acid, were determined by Barton & De Mayo (1954) and Anderson, De Kock & Enslin (1961). A β-orientated hydroxyl group (preferably 3 β-OH) on the A-ring of the molecule, as well as a 22 β-angeloyloxy side chain were shown to be essential for icterogenic activity (Brown, Rimington & Sawyer, 1963; Brown & Rimington, 1964). The toxicity of *Lippia* plants was related to factors such as pruning and weather (Roets,

[1]Veterinary Research Institute, Onderstepoort
[2]Botanical Research Institute, Private Bag X101, Pretoria
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1937). In nature cattle alone seem to be affected as no outbreaks have been reported in sheep (Steyn, 1949).

Water contaminated with the alga *M. toxica* can induce severe liver damage, icterus and photosensitivity in domestic animals (Steyn, 1943, 1944, 1945, 1949; Stephens, 1949). The alga is most common in dams and pans of the north-eastern Orange Free State and south-eastern Transvaal (Stephens, 1949). Two toxins are thought to be involved: phycocyan, a fluorescent pigment that produces photosensitivity (Steyn, 1945) and an alkaloid that causes liver damage (Louw, 1950). More recently compounds such as substituted purines and cyclic polypeptides have been isolated from other algae (Anon., 1967) but these do not cause hepatogenous photosensitivity.

Lupines are grown in the winter rainfall areas of the Cape Province. The disease lupinosis is a mycotoxicosis (Gardiner, 1966) and is caused by *Phomopsis leptostromiformis* (Kühn) Bubák according to Van Warmelo, Marasas, Adelaar, Kellerman, Van Rensburg & Minne, 1970. The notable lesions are liver damage and icterus (Groenewald, Smit & Adelaar, 1954; Gardiner, 1967) and although photosensitivity can be present, particularly amongst cattle (Gardiner 1967), it is not a constant feature of the syndrome in South Africa.

Trifolium pratense and other clovers are cultivated only to a limited extent in the sheep-rearing areas of South Africa and hence do not contribute greatly to the problem of photosensitization.

The only syndromes of photosensitivity that occur concurrently with geeldikkop (and can be clinically confused with it) are *Lasiospermum bipinnatum* (Thumb.) Druce and *Asaemia axillaris* poisoning.

L. bipinnatum poisoning has long been known from field observations to cause ovine hepatogenous photosensitivity in the eastern Karoo but feeding trials at Onderstepoort produced only hepatic damage and icterus (Adelaar, Terblanche, Smit, Naudé & Codd, 1964). Subsequently a field outbreak of liver damage and photosensitivity amongst cattle in the Orange Free State was attributed to poisoning with this plant (Fair, Tustin & Adelaar, 1970).

A. axillaris is another plant known from field observations to cause photosensitivity of sheep (Steyn, 1949; D. G. Steyn, Pretoria, personal communication, 1972). Van der Walt & Steyn (1939) described an experimental case of *A. axillaris* poisoning in a sheep where once again liver damage and icterus, but not photosensitivity, were produced.

The present investigation confirms field observations that photosensitivity is a manifestation of *A. axillaris* and *L. bipinnatum* poisoning. The lesions of *A. axillaris* poisoning are described and compared with those of geeldikkop and *L. bipinnatum* poisoning.

This study was undertaken in the hope of making some contribution towards the definition of ovine photosensitization syndromes in South Africa.

Description and Distribution of Plants

Family: Compositae
Name: *Asaemia axillaris* (Thunb.) Harv. ex Jackson
Synonyms:
1. *Tanacetum axillare* Thunb.
2. *Stilpnophyton axillare* (Thunb.) Less.
3. *Asaemia axillaris* (Thunb.) Harv.
4. *Pteronia geigerioides* Muschler ex Dinter

Common name: Vuursiektebossie

Description: A much branched, glabrous, woody shrublet up to 50 cm high and 1 m in diameter. *Root* thick and woody. *Stems* pale yellowish, ending in spines. *Leaves* opposite, linear, connate and sheathing at the base, up to 30 mm long, *c.* 1 mm wide. *Flower-heads* small, solitary, sessile or subsessile, axillary, homogamous, few to several-flowered, discoid, with bisexual fertile yellow or whitish florets. *Involucre* ovoid, 5 to 7 mm high and 4 to 5 mm broad, shorter than the florets; bracts imbricated, in 4 to 5 rows, varying from ovate and oblong to linear, hard, sometimes with membranous or serrate margins, glabrous. *Receptacle* flat, naked. *Corolla tube* tubular, usually slightly widening upwards, glabrous; lobes 5, lanceolate, sometimes cucullate at the apex. *Anthers* linear to oblong-linear, obtuse, with a lanceolate or ovate appendage. *Ovary* oblong in outline, 2 to 4 angled, glabrous; style terete, slightly swollen at the base; style-branches linear, truncate. *Achenes* cuneate, sub-compressed, smooth, pappus none. *Distribution:* The distribution of *A. axillaris* is illustrated in Fig. 1. The plant, which is endemic to Southern Africa, has been recorded in the following areas:

South West Africa: Maltahöhe, Keetmanshoop.
Cape Province: Namaqualand, Kenhardt, Prieska, Van Rhynsdorp, Calvinia, Williston, Carnarvon, De Aar, Hanover, Aberdeen, Willowmore.

Acocks (1953) regards *A. axillaris* as a typical species of the Upper or Typical form of the Western Mountain Karoo. It is also a typical species of the Central Lower Karoo and penetrates into the False Arid Karoo. The flowering time is from August to May, especially during October and November.

The only other species in this endemic genus is *A. inermis* Phill., which occurs in the Laingsburg, Ceres, Van Rhynsdorp and Moorreesburg districts of the Cape Province. This species differs from *A. axillaris* in being spineless. The vuursiektebossie can be distinguished from other Karoo bushes by its spines and sessile flowerheads.

Habitat: Vuursiektebossie is common in the vicinity of dams, vleis, pans, rivers, hollows and on alluvial flats. It often grows in depressions where water collects on irrigated lands. The plant becomes common in low-lying places, on trampled-out veld and calcarious, brackish or red sandy loam soils.

The following description of *L. bipinnatum* is given by Adelaar *et al.* (1964).

Family: Compositae
Name: *Lasiospermum bipinnatum* (Thunb.) Druce
Synonyms: *Lidbeckia bipinnata* Thunb.
Lasiospermum radiatum Trevir
Common Names: Ganskweek, gansbossie

Description: Decumbent to erect, herbaceous perennials, up to 40 cm high, with stout, woody, rhizomes. *Stems* numerous, seldom branched, arising from the crown of the rhizome, decumbent to ascending, terete, striate, glabrous. *Leaves* alternate, crowded at base, bipinnatisect, up to 10 cm long, 2 to 4 cm wide, green glabrous on both sides, lobes apiculate. *Capitula* solitary, terminal on long, ascending seldom branched peduncles up to 30 cm long, with membranous, entire to sparsely dentate bracts, capitula more or less disc-shaped, 3 to 3,5 cm in diameter, ray-florets white to pale purplish pink reflexed with age, disc-florets yellow. *Involucre* somewhat discoid; bracts in 3 rows, more or less elliptic, green, with membranous margins, spreading as the fruits mature. *Achenes c.* 5 mm long, covered with yellowish hairs, which gives a woolly appearance to the flowering head as the fruits mature; pappus none.

Distribution: The distribution of *L. bipinnatum* is illustrated in Fig. 2. The plant occurs in the south-eastern, southern and western Transvaal, Orange Free State, Griqualand West, south western, central and eastern Cape and Lesotho, but it has not been recorded in Natal.

Habitat: Ganskweek prefers vleis but can grow almost anywhere. It has been found on mountains, on flats, alongside roads and in backyards. The plant also grows well on sweet veld where the soil pH is high. *L. bipinnatum* can bloom throughout the year but it flowers most commonly in summer.

TOXICITY TRIALS

1. *Asaemia axillaris*

Materials and methods

Early fruiting stages were collected at Calvinia late in February 1972, dried in the shade and railed, loosely packed in jute bags, to Pretoria. The material was stored in a well ventilated shed for some weeks before it was used.

Preparatory to dosing, the woody stems were separated from the soft debris (twigs, fruits, leaves and fragments of bark) by beating the bags with sticks. The soft debris was milled to a fine powder for dosing per stomach tube to sheep as described under Results (Table 1).

The sheep were fed on green lucerne, kept in the sun and examined daily. Periodically the following standard chemical pathological determinations were done on the blood:– serum urea nitrogen, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, bilirubin (only when serum was yellow), total plasma protein, glucose, serum calcium, sodium and potassium. At necropsy specimens were taken from various organs, fixed in 10% formalin, cut in a routine manner and stained with haematoxylin and eosin (HE). Some frozen sections were stained with oil red-O (ORO).

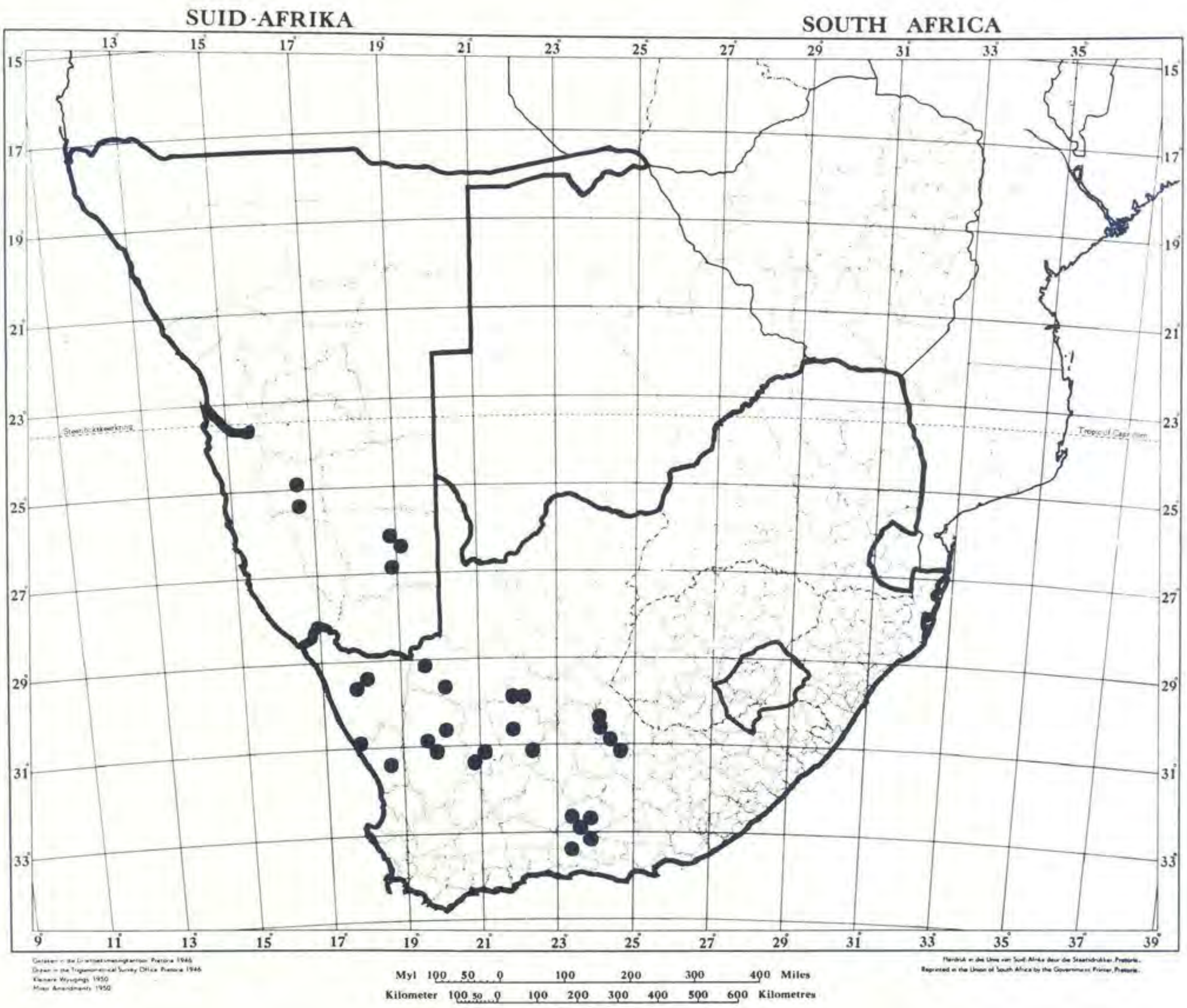

FIG. 1 The distribution of *Asaemia axillaris*

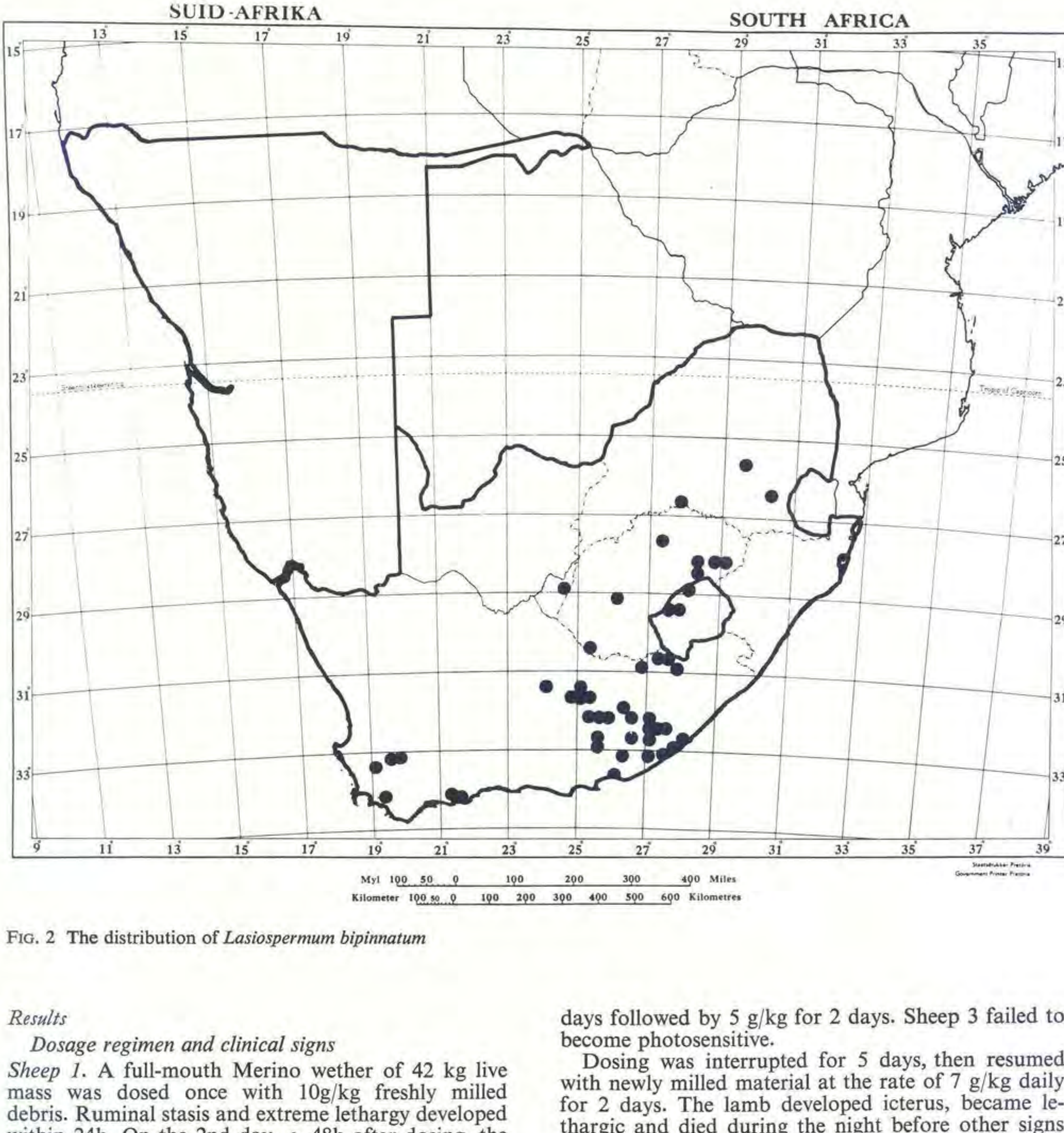

FIG. 2 The distribution of *Lasiospermum bipinnatum*

Results

Dosage regimen and clinical signs

Sheep 1. A full-mouth Merino wether of 42 kg live mass was dosed once with 10g/kg freshly milled debris. Ruminal stasis and extreme lethargy developed within 24h. On the 2nd day, *c.* 48h after dosing, the sheep was destroyed *in extremis.* It was found lying in lateral recumbency with pronounced icterus, mild tachycardia and dyspnoea. No signs of photosensitivity were observed.

Sheep 2. A milk-tooth Merino wether of 24 kg live mass received 5 g/kg of soft debris daily for 4 days. On the 4th day severe hepatogenous photosensitivity set in. The lamb was icteric, lethargic, and constantly sought shade. Coronitis was present on all four feet and the lips, ears and face were grossly swollen. After exposure to solar radiation for 3h, the lamb was moved to a stable where it died 15h later.

Sheep 3. The same debris given to Sheep 1 the previous week was dosed to a milk-tooth Merino wether of 25 kg live mass at the rate of 2,5 g/kg daily for 7 days followed by 5 g/kg for 2 days. Sheep 3 failed to become photosensitive.

Dosing was interrupted for 5 days, then resumed with newly milled material at the rate of 7 g/kg daily for 2 days. The lamb developed icterus, became lethargic and died during the night before other signs could be observed.

Sheep 4. A six-tooth Merino wether of 30 kg live mass received 3,5 g/kg of newly milled material daily for 6 days. The sheep developed ruminal stasis, pronounced icterus, mild coronitis and lethargy on the 7th day. The skin of the nose was slightly reddened and on the 8th day mild submandibular oedema (bottle jaw), that lasted for a day, was observed. Recovery was rapid. When the sheep was slaughtered on Day 14 the habitus was normal but icterus and coronitis were still present. The photosensitized areas of skin on the nose formed crusts that sloughed off.

Chemical pathology

The most conspicuous chemical pathological

FIG. 3 *Asaemia axillaris*

changes in the four sheep were marked concurrent elevations of the serum oxalacetic transaminase (SGOT) and total bilirubin (TBR) levels of the blood (Table 1).

Necropsy findings

Peracute poisoning (*Sheep 1*): No signs of photosensitivity were noticed on the head and hoofs. The carcase was mildly icteric, generally congested and cyanotic. A moderate ascites, mild hydropericardium, severe hydrothorax and marked oedema of both mediastinum and diaphragm were present. The liver was slightly enlarged, severely congested and reduced in consistency. After bleeding out it was a pale yellow-greyish brown colour with accentuated lobulation. On close inspection the lobules appeared to be discoloured, being greyish white along the periphery and khaki brown at the centre. The liver was severely infested with *Stilesia hepatica*. The gall-bladder was well filled with a foetid brownish green fluid. In addition, numerous dull greyish white areas of necrosis, a few millimetres in diameter, were encountered in the mucosa. The myocardium, which was a pale biscuit colour, had a diffuse parboiled appearance. Impaction of the caecum and colon and slight enterorrhagiae were noticed. The lungs were congested but not conspicuously oedematous. Petechial haemorrhages and oedema occurred bilaterally in and around the nerves of all the limbs, especially along the sciatic nerve and brachial plexus.

Acute poisoning (*Sheep 2 and Sheep 3*): The two acute cases revealed mild or moderate icterus and localized lesions of photosensitization (erythema and oedema) of the head and hooves. In one sheep the lymph nodes of the head were swollen. The liver was slightly enlarged, very fatty in appearance with reduced consistency and very distinct dull greyish lobulation. Some areas had a slight greenish centrilobular discolouration. The volume of bile was about normal and its colour a greenish khaki. The lungs were markedly oedematous and congested. A mild hydro-

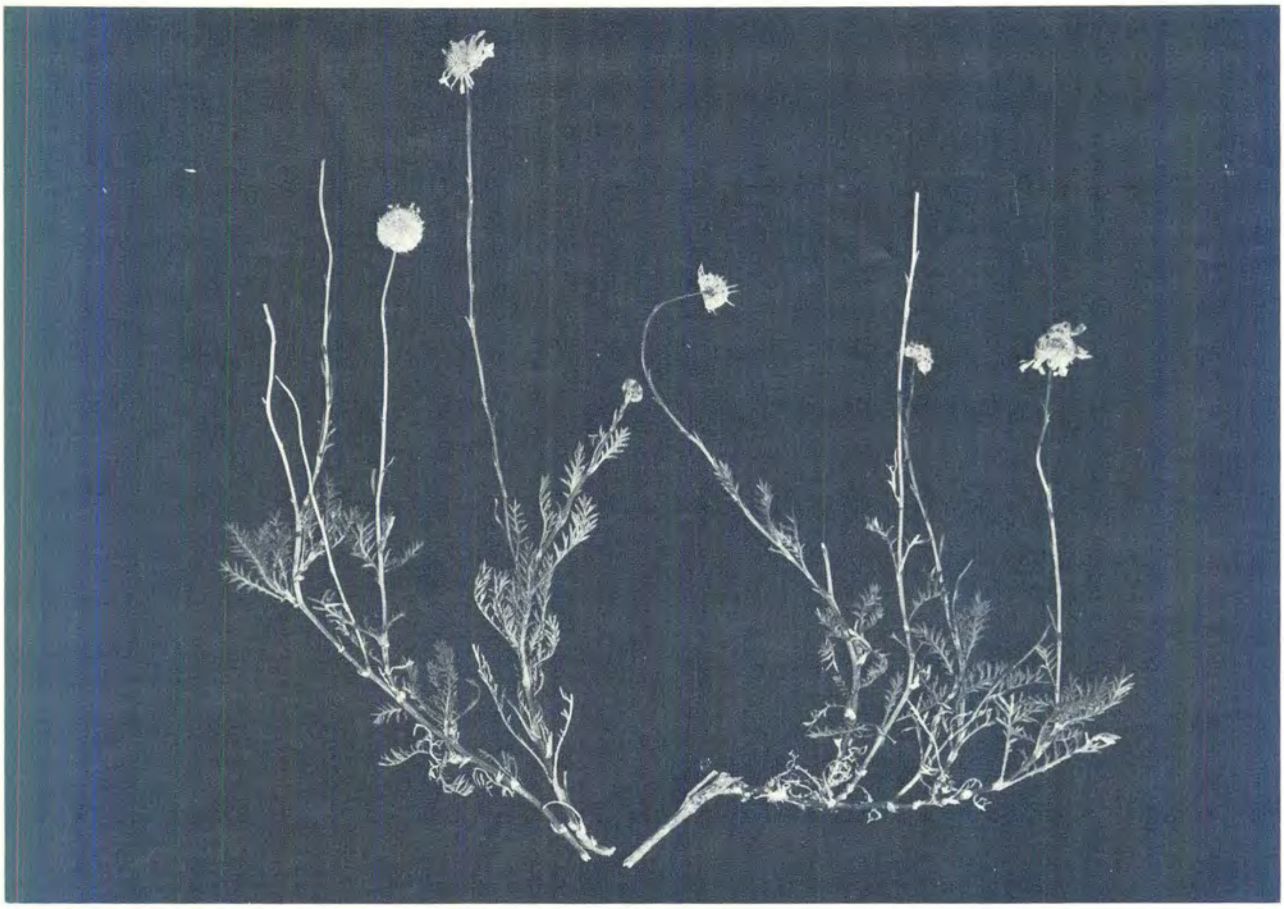

FIG. 4 *Lasiospermum bipinnatum*

TABLE 1 Changes in serum glutamic oxaloacetic transaminase (SGOT)* and total bilirubin (TBR) levels in the blood of sheep dosed with *A. axillaris* and *L. bipinnatum* plants.

Days	*A. axillaris*												*L. bipinnatum*		
	Sheep 1			Sheep 2			Sheep 3			Sheep 4			Sheep 5		
	Dose g/kg	SGOT KU	TBR mg%	Dose g/kg	SGOT KU	TBR mg%	Dose g/kg	SGOT KU	TBR mg%	Dose g/kg	SGOT KU	TBR mg%	Dose g/kg	SGOT KU	TBR mg%
1	10,0	86	—	5,0	106	—	2,5	100	—	3,5	119	—	1	152	
2		319	0,4	5,0	106	—	2,5	119	—	3,5				152	—
3		267	1,2	5,0	207	2,9	2,5	100	—	3,5					
4				5,0	276	5,0	2,5	100	—	3,5	131	—		99	—
5							2,5			3,5					
6							2,5	100	—	3,5					
7							2,5				232	6,5		131	—
8							5,0								
9							5,0	138					2		
10														146	—
11								100	—						
12															
13														132	—
14								100	—		152	5,3			
15							7,0	188	—						
16							7,0	226							
17															
18														139	—
19															
20															
21													4	124	—
22														254	6,7
23														311	8,2
24															

*SGOT in King Units (KU)

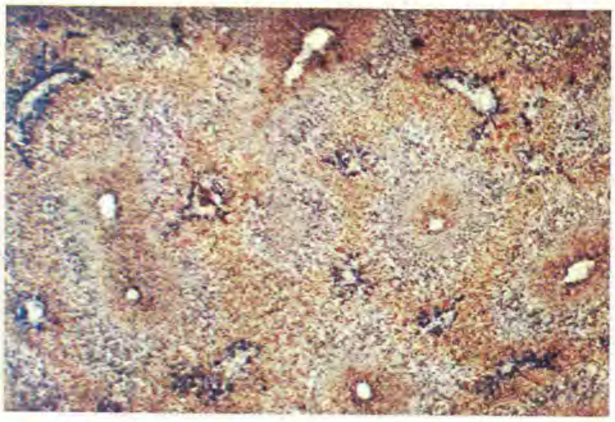

FIG. 5 Acute ovine *Asaemia* poisoning (low magnification). Note the zonal distribution of fatty degeneration in the liver lobules

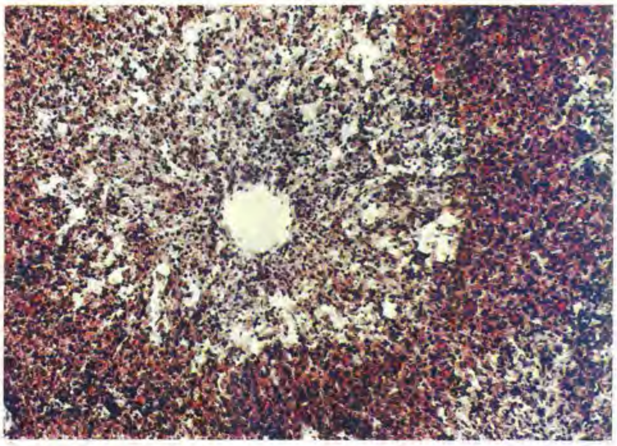

FIG. 6 Acute ovine *Asaemia* poisoning (high magnification)

pericardium (25 ml), slight to mild hydrothorax and very mild ascites were present. The mediastinum, perirenal tissue and mesenterium were mildly oedematous and petechial haemorrhages and oedema occurred bilaterally in and around the nerves of all the limbs, as described in the case of Sheep 1. Many subepicardial petechiae were present. The myocardium had a diffuse parboiled appearance and the kidneys showed mild pallor. The adrenal cortex was either fatty or slightly enlarged. The content of the large intestine was fairly solid and drier than normal. In the one case where sufficient urine could be obtained for examination with a test tape (Hemacombistix)* small quantities of sugar and protein were recorded. The pH of the urine was 6,0.

Subacute poisoning (*Sheep 4*): This sheep, which was slaughtered, revealed only mild lesions. The icterus seen during the first few days of the disease was absent at autopsy. The photosensitized areas of skin were scabby or contained crusts that sloughed off. A red band (*c.* 1 cm wide) of coronitis was present on the lateral surfaces of all hoofs but the medial aspects were not always affected. There was a slight hydropericardium. The liver had a light brown colour and its lobulation was accentuated. No lesions were detectable in either the gall or urinary bladders, except for a few tiny petechiae in the latter. The bile had a medium chlorophyl green colour and the urine pH was 6,5.

Histopathological findings

Peracute case (*Sheep 1*): *Liver:* The most conspicuous feature was severe centrilobular necrosis involving at least two thirds of most lobules (Fig. 5, 6, & 7). Along the periphery there remained a narrow zone of vacuolated hepatocytes that reacted positively for lipids with ORO stain. The necrotic areas of adjacent lobules were often confluent, leaving only small islets of viable cells around the portal triads (Fig. 7). Since necrosis was confined to hepatocytes, Kupffer cells and endocytes appeared to be relatively prominent (Fig. 8). Mild bile duct proliferation and round cell infiltration were observed in the portal areas (Fig. 5 & 7).

Gall-bladder: Large areas of mucosa were severely necrotic with neutrophil infiltration into the submucosa and muscular layers. Large basophilic areas, which appeared to be an admixture of nuclear contents and cytoplasm, were also observed (Fig. 9).

Myocardium: Severe intermuscular haemorrhage occurred in muscle fibres below the endocardium and some subendocardial fibres showed cloudy swelling. In addition, small ORO positive vesicles were seen in fibres throughout the myocardium, but the vesicles were not numerous or conspicuous.

Diaphragm: The muscle layers were mildly oedematous.

Kidney: The cytoplasm of the convoluted tubular epithelium was slightly granular in appearance. A few hyaline droplets were infrequently seen in a small number of these cells.

Lymph nodes: No lymph nodules were observed but large monocytes were abundant in the medullary sinusoids.

Spleen: Once again no lymph nodules were present but the organ was severely congested.

Other tissues: No distinct lesions of photosensitization were noticed in the skin and hoofs. Mild haemorrhages occurred around the major nerve tracts.

Acute poisoning (*Sheep 2 and Sheep 3*): *Liver:* Each lobule had a very characteristic zonal pattern of lesions (Fig. 5). The portal areas revealed mild to moderate bile duct proliferation, while the hepatocytes had a prominent peripheral vacuolated zone of fatty changes, followed by a middle zone of prominent coagulative necrosis and lastly a vacuolated centrilobular area with mild fatty changes. These three zones were approximately equal in width but the severity of the specific changes varied in the two animals. In the most severe case (Sheep 3) necrosis was more prominent and the centrilobular fatty degeneration was either absent or very mild, being partially replaced by the necrosis and varying types of degeneration. The middle zone in Sheep 2 was only partially necrotic and on the periphery a small number of individual necrotic hepatocytes were seen. Bile pigmentation or lipofuscinosis was not observed but very mild megalocytosis was present in the peripheral zone of Sheep 3. Some nuclei in this zone also contained lipid material. In both animals mitotic figures were noticed, some of which appeared to be abortive.

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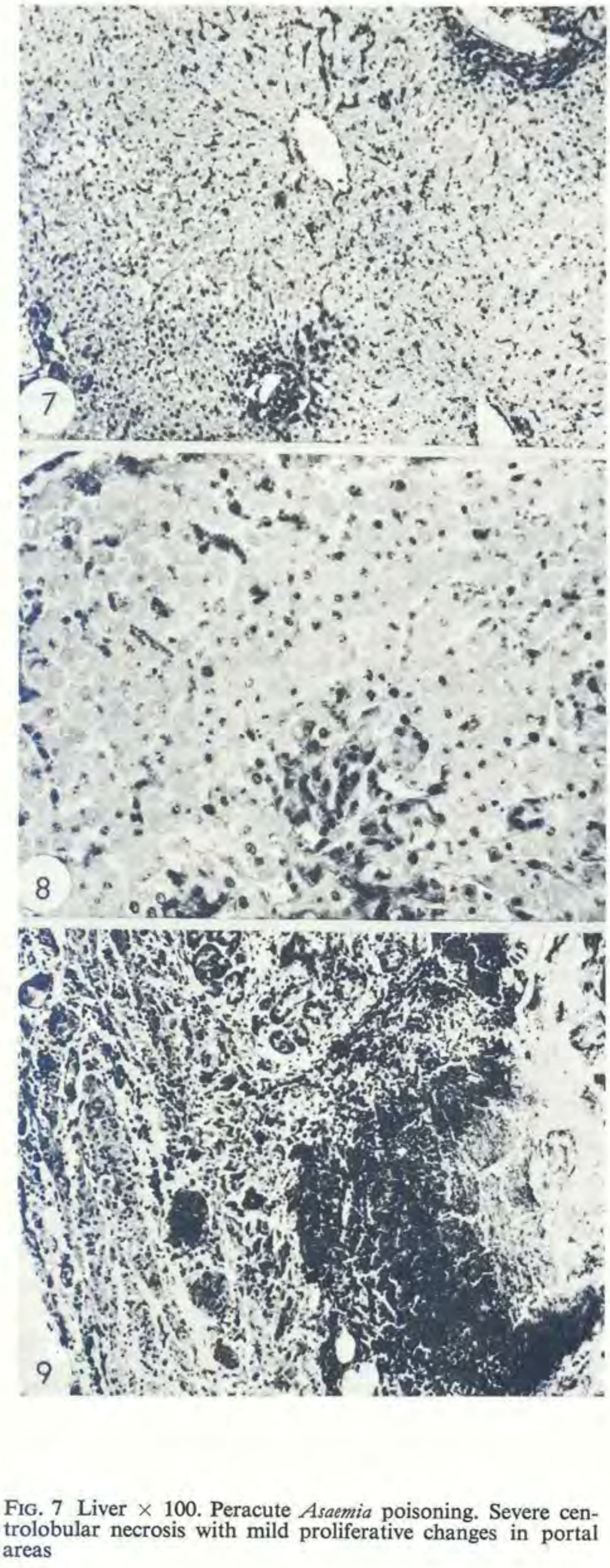

FIG. 7 Liver × 100. Peracute *Asaemia* poisoning. Severe centrolobular necrosis with mild proliferative changes in portal areas

FIG. 8 Liver × 250. Few viable cells round portal triad with severe necrosis towards central vein in left top corner

FIG. 9 Gall-bladder × 100. Gall-bladder wall revealing necrosis and inflammatory response

Kidney: Moderate to severe fatty nephrosis was present in the cortex and medullary rays.

Myocardium: The myocardial fibres contained small vesicles which proved to be positive for lipids with ORO stain. This change was only noticeable under high magnification (oil immersion) and proved to be fairly diffuse. A few foci of Zenkers' degeneration, as well as localized areas of cloudy swelling were seen in Sheep 3.

Spleen: The splenic corpuscles were small and in one case (Sheep 3) contained karyorrhectic material. Moderate to marked pigmentation was present.

Lymph nodes: Congestion and a few haemorrhages were noticeable. The follicles in Sheep 3 were small and atrophic.

Skin and hoofs: Lesions typical of photodynamic dermatitis were present in Sheep 2. The lesions consisted of congestion, haemorrhages, oedema, a mild infiltration of neutrophils, localized necrosis of the epidermis and dermal papillae and vascular necrosis. These changes were most pronounced in the skin of the nasal area. The papillae in the hoof, especially the coronary papillae, were congested, oedematous and contained a small number of neutrophils. Both the skin and hoof of Sheep 3 revealed congestion and oedema only.

Lungs: Prominent congestion and oedema were present. The oedematous fluid in Sheep 3 was more copious and proteinaceous than in Sheep 2.

Other tissues: The nerves were surrounded by haemorrhages and the hypophysis was congested and mildly oedematous.

Subacute poisoning (*Sheep 4*): No fatty changes or necrosis were present in the *liver*, but mild hydropic changes were still evident on the periphery of lobules. The portal areas revealed mild bile duct proliferation, very mild fibroplasia, mild peripheral oedema and foci of very mild mixed-cell reaction. Several small foci of mononuclear infiltration and a few foci of fibrosis were found in the *myocardium*. These lesions, however, were very mild. Prominent acanthosis, mild hyperkeratosis, moderate oedema and fibroplasia (in the *stratum papillare*) were present in the *skin*. The skin surface was covered by a thick necrotic crust and the intima of several vessels was markedly proliferated. Neutrophils were still in evidence. The papillae in the *hoof* were congested, oedematous and contained mild haemorrhages.

2. *Lasiospermum bipinnatum*

Materials and methods

The experimental procedures were essentially similar to those of Trial 1.

Shade dried, flowering *L. bipinnatum* plants picked in March 1971 at Middelburg (C.P.) were milled *en toto* and dosed per stomach tube to a sheep at Onderstepoort as described below.

Results

Clinical signs

Sheep 5. A milk-tooth Merino wether of 27 kg live mass received 1 g/kg on Day 1, 2 g/kg on Day 9, and 4 g/kg on Day 21.

The sheep did not respond to the first two administrations of *L. bipinnatum* material. Early on the 2nd day after the last administration mild icterus was observed (Table 1). Thereafter the condition of the sheep rapidly deteriorated. The icterus became more pronounced, mild coronitis was present, ruminal movements ceased and the animal constantly sought shade. On the 3rd day (*c.* 52 h after dosing) the sheep died.

Chemical pathology

Once again the most conspicuous change in the blood chemistry was a concurrent elevation of the SGOT and TBR, which occurred when clinical signs were evident (Table 1).

Necropsy findings

The carcase was markedly icteric. Haemorrhages were present in the pericardium, epicardium (pronounced), pulmonary artery, lymph nodes (mild), gall-bladder (mild), subcutis and fascia. The lungs were prominently oedematous and congested. Mild oedema occurred in the mediastinum, perirenal area and subcutis. A slight hydropericardium was present. The liver had a yellowish copperbrown colour with distinct dull grey lobulation. The dull grey mozaic pattern of the lobules was often turned a reddish colour by mild haemorrhages. The colour of the centrilobular areas varied from reddish-brown to yellowish-brown. Liver consistency was decreased. The myocardium had a diffuse parboiled appearance and a suspected mild nephrosis was present. Acute mild lesions of photosensitization were evident on the hoofs, nasal area, lips and eyelids. Small haemorrhages and even some free blood were visible in the vicinity of the nostrils. The large intestine was mildly impacted.

Histopathological findings

Liver: Examination of lobuli revealed a narrow peripheral vacuolated zone of fatty degeneration followed by a midzonal area of coagulative necrosis (with some kariorrhexis) and a centrilobular area which was either mildly affected or spared. Bile pigment had accumulated in the bile canaliculi, Kupffer cells and hepatocytes. Some of the necrotic material in the Kupffer cells was mineralized. Mild haemorrhages were present and a few neutrophils were seen in the portal areas. Another feature was mild proliferation of the bile ducts.

Kidneys: Mild cloudy swelling and vacuolar (fatty) degeneration were present in the cortex and outer zone of the medulla. Pigmentation was not obvious and cystic changes were absent.

Myocardium: The changes recorded in the myocardium were increased eosinophilia, vesiculation (fatty degeneration), rarefaction and necrosis of myocardial fibres, and mild haemorrhage. These changes were more advanced than those seen in cases of *Asaemia* poisoning.

Other organs: The lungs were congested and oedematous. Both the *skin* and *hoofs* had early lesions of photosensitization which included congestion, mild oedema, haemorrhages and vascular necrosis. The wall of the *pulmonary artery* contained a few prominent haemorrhages and the *adrenal cortex* was moderately fatty.

DISCUSSION

The various syndromes of hepatogenous photosentivity cannot be distinguished by outward appearances alone as the *ante mortem* signs are similar. A distinction can, however, usually be made between *Asaemia* and *Lasiospermum* poisoning on the basis of the geographical distribution of the plants. *A. axillaris* is almost entirely confined to the western Karoo while *L. bipinnatum* occurs most commonly in the eastern Karoo and, except perhaps in the central southern Karoo, the plants are seldom found together (Fig. 1 and 2). Geeldikkop occurs throughout the Karoo and can be confused with both *A. axillaris* and *L. bipinnatum* poisoning.

The most reliable diagnosis of these conditions is based on pathological findings. Diffuse fatty degeneration and accentuated lobulation of the liver are necropsy features common to lupinosis, *Asaemia* and *Lasiospermum* poisoning. However, in our experience photosensitivity is extremely rare in lupinosis; when present it is usually indicative of *Asaemia* or *Lasiospermum* poisoning. Moreover, in *Lasiospermum* poisoning the liver is haemorrhagic and bile-stained. However, neither the pitted areas overlying obliterated bile ducts in the liver nor the cystic nephrosis that is characteristic of geeldikkop, dikoor and facial eczema (Van Tonder *et al.*, 1972), are seen in the entities discussed above. The gall-bladder and urinary bladder lesions commonly found in facial eczema are also lacking. [The gall-bladder lesions seen in the experimental case of peracute *Asaemia* poisoning (Sheep 1) were not a constant finding and can probably be attributed to the very high dose given.]

Microscopically the hepatic lesions of acute *Asaemia* poisoning vary considerably from those of geeldikkop, dikoor and facial eczema. The circular, zonal distribution of fatty degeneration and necrosis is not a feature of the latter diseases. Furthermore, the typical crystalloid material of geeldikkop (Van Tonder *et al.*, 1972) and the biliary necrosis and vascular lesions of facial eczema (Mortimer, 1963) are lacking. Pigmentation and Kupffer cell activation are not present in *Asaemia* poisoning. However, subacute cases of the latter may be difficult to differentiate from mild atypical cases of geeldikkop, dikoor and other hitherto unclassified cases of photosensitivity found in South Africa. In such cases the only feature which would distinguish geeldikkop and dikoor is the presence of typical polarizing, crystalloid material or clefts left by the material after sections are processed. This would necessitate examination of several liver sections from different animals. Characteristic lesions in other tissues, however, should be of considerable assistance in making a diagnosis. In the case of either *Asaemia* or *Lasiospermum* poisoning such lesions are fatty changes in the myocardium, the absence of cystic changes in the kidneys and the normal appearance of the mucosa of the urinary bladder.

Although peripheral necrosis was reported to be the most characteristic hepatic lesion in *Lasiospermum* poisoning (Adelaar *et al.*, 1964), the present experimental case showed some similarities to *Asaemia* poisoning since peripheral fatty changes and midzonal necrosis were present in both. Bile pigmentation and mild haemorrhages, however, were only evident in *Lasiospermum* poisoning. Fatty degeneration of the myocardium and kidneys occurred in both conditions but the myocardial necrosis was more prominent in *Lasiospermum* poisoning. Generally speaking, therefore, it is evident that the spectrum of lesions in these two entities may overlap.

In the case of lupinosis (Van Warmelo *et al.*, 1970) the fatty metamorphosis is usually, but not invariably, more diffuse than in *Asaemia* or *Lasiospermum* poisoning. In addition megalocytosis occurs in lupinosis. Hyaline droplet degeneration may be present in lupinosis and pigmentation is prominent, especially in subacute and chronic cases. Fibroplasia is, in this instance, the most prominent lesion in the portal areas and overshadows the bile duct proliferation.

The peculiar zonal hepatic changes of *Asaemia* poisoning are difficult to explain but may be due to a combination of a direct toxic action on the liver (peripheral and midzonal lesions) and indirect effects as a result of myocardial involvement (centrilobular lesions).

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