PHOTOSENSITIVITY IN SOUTH AFRICA. IV. PATHOLOGICAL CHANGES IN THE LIVER IN OVINE PHOTOSENSITIVITY CAUSED BY THE PLANT *ASAEMIA AXILLARIS* (THUNB.) HARV. EX JACKSON

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ABSTRACT

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Hepatic lesions in 4 field cases of ovine hepatogenous photosensitization caused by the plant, *Asaemia axillaris* (Thunb.) Harv. Ex Jackson, are described.

The liver was usually swollen, friable and yellowish-brown, with distinct lobulation. Microscopically, the lesions ranged from peripheral coagulative necrosis in 1 animal to others with scattered single cell or small foci of necrosis as well as hepatocellular degeneration and unrest, ductular proliferation, portal fibroplasia and cholestasis.

The liver lesions are compared with those of previously reported experimental cases of *A. axillaris* poisoning in sheep. The significance of zonal necrosis and factors that may have a bearing on their production in different hepatotoxic plant poisonings in sheep and cattle in South Africa are discussed.

INTRODUCTION

There are many hepatotoxic plants in South Africa which cause liver damage in sheep and cattle. Some of these plants are frequently associated with photosensitization (Steyn, 1949; Adelaar, Terblanche, Smit, Naudé & Codd, 1964; Kellerman, Basson, Naudé, Van Rensburg & Welman, 1973). One such plant, *Asaemia axillaris,* has long been known to produce hepatic lesions, sometimes accompanied by icterus and photosensitivity, in sheep (Van der Walt & Steyn, 1939; Steyn, 1949). Nevertheless, information regarding the lesions in the liver with this intoxication is limited, and only Kellerman *et al.* (1973) made mention of the hepatic changes seen in 4 experimentally poisoned sheep.

This study was conducted to evaluate the hepatic lesions of field cases of *A. axillaris* poisoning in sheep. In addition, our findings are compared with the above-mentioned experimentally induced liver lesions with this plant, and other hepatotoxic plants such as *Lasiospermum bipinnatum* and *Athanasia trifurcata*.

HISTORY OF FIELD OUTBREAKS

A farmer in the Kenhardt district, in the north-western Cape Province, reported 4 deaths during October 1977 among a flock of ewes with lambs. Three weeks previously the sheep had reportedly grazed for 1 week in a camp which supported a dense growth of *A. axillaris*. One of the dead animals (Sheep 1) was brought to the regional state veterinarian for necropsy. Formalin-fixed tissues of this sheep were submitted to the Veterinary Research Institute, Onderstepoort, for histopathological examination. Although a diagnosis of *A. axillaris* poisoning was made in this animal, the microscopical lesions in the liver *(vide infra)* differed from those reported earlier by Kellerman *et al.* (1973). It was thus decided to put 8 full-mouth wethers, originating from different farms in the Kenhardt district which are free of *A. axillaris,* on a 4 ha camp with a heavy growth of this plant on the farm where deaths had occurred.

Three days after the sheep had been put into the camp, 2 animals (Sheep 2 and 3) were reported sick, with slight swelling of the lips, but there were no other overt signs of photosensitization. Both sheep were killed for necropsy. Four days later another 2 sheep (Sheep 4 and 5) were reported sick, with swelling of the lips, and, in addition, voiding intensely yellow urine. They were also killed for necropsy. On examination it was found that

Sheep 5 had been suffering from abomasal phytobezoars and was therefore not included in this study. The other sheep which had not become sick were discharged 3 weeks later.

MATERIALS AND METHODS

Necropsies were performed on the sheep that became ill or died during this experiment. Tissues were fixed in 10% buffered formalin and were routinely processed. Sections were cut at 4–6 μm and stained with haematoxylin and eosin (HE). Special stains included Masson's trichrome stain for collagen, Mallory's stain for iron, the Fontana stain for lipofuscin, Wilder's reticulin stain and the periodic acid-Schiff (PAS) stain with and without digestion with diastase for glycogen (Anon., 1968).

RESULTS

Macroscopical pathology

The livers of Sheep 1, 2 and 4 were moderately swollen, friable, and yellowish-brown, with accentuation of the lobulation. This organ, which was more severely affected in Sheep 2 than in the other sheep, showed scattered areas of congestion throughout the parenchyma as well as dark-red zones in most lobules. These gave the organ a mosaic appearance (Fig. 1). The gall bladder in these animals was usually distended with a dark-green bile. Other changes included a slight icterus of the carcasses, swelling, and yellowish-brown discoloration of the kidneys. Apart from slight enlargement of the liver in Sheep 3 no other lesions were seen in it or any other organ.

FIG. 1 Yellowish-brown swollen liver showing congested areas throughout the parenchyma and accentuation of the lobulation

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Microscopical pathology

Liver

Sheep 1: The portal triads showed moderate to marked ductular proliferation and fibroplasia as well as mild polymorphomononuclear cell infiltration and oedema (Fig. 2 & 3). Connective tissue septa bridged adjacent triads. In addition to hepatocellular unrest (depicted by anisocytosis, anisonucleosis, binucleation and an increased mitoses of hepatocytes), most hepatocytes were swollen and revealed cloudy swelling, hydropic degeneration and a mild fatty metamorphosis (Fig. 3). Isolated necrotic liver cells were dispersed throughout the parenchyma (Fig. 3), while patchy areas of necrosis and haemorrhage involved some lobules in certain parts of the liver. Apart from moderate Kupffer cell proliferation and bile stasis, yellowish-brown pigment, which stained positively for lipofuscin, were noted both in Kupffer cells and in a few macrophages in the portal triads.

Sheep 2: Distinct peripheral coagulative necrosis and haemorrhage, bridging adjacent lobules, occurred in the liver (Fig. 4 & 5). A few neutrophils were scattered among the necrotic parenchymal cells, while the sinusoidal lining cells in these affected zones appeared swollen and/or hypertrophied, and were mildly activated. The hepatocytes in the remainder of the lobules revealed cloudy swelling, hydropic degeneration and moderate fatty metamorphosis. The latter was more prominent in the liver cells bordering the zones of peripheral necrosis. Eosinophilic, slightly homogeneous to ground glass-like globules were evident in many of these degenerated hepatocytes. Mitosis of hepatocytes was slightly increased. The bile canaliculi were somewhat dilated, but had an empty appearance. Mild ductular proliferation was the only change in the portal triads.

Sheep 3: The parenchymal changes comprised mild hepatocellular swelling and cytoplasmic vacuolation, the hepatocytes in the peripheral zones of the lobules being particularly affected. Isolated hepatocytes, haphazardly distributed in the parenchyma, showed acidophilic degeneration and necrosis. Other changes included a few small neutrophilic aggregates in a few lobules and lipofuscin pigment in some hepatocytes, Kupffer cells and macrophages in portal triads.

Sheep 4: A notable feature in the liver of this animal was that the portal triads consisted almost exclusively of numerous densely arranged bile ductules, a mild portal fibroplasia and mononuclear cellular infiltration (Fig. 6 & 7). Thin, connective tissue septa often bridged adjacent triads. The parenchyma was not affected by zonal necrosis, but individual necrotic hepatocytes, a few small foci of necrosis or marked hepatocellular ballooning and vacuolation were seen in some lobules. Apart from occasional hepatocytes, which had the ground glass-like appearance of the cytoplasm, the remainder of the liver cells were swollen (cloudy swelling and hydropic degeneration) and showed hepatocellular unrest as well as moderate fatty metamorphosis. The latter was more prominent in the peripheral zones of the lobules. Numerous bile plugs were noted among the degenerated hepatocytes.

Other organs

Nephrosis was present in Sheep 1, 2 and 4. Apart from cloudy swelling and hydropic degeneration of the tubular epithelium, most convoluted tubules in the cortex contained albuminous casts and were slightly dilated. No lesions occurred in the lungs, myocardium, skeletal muscle, spleen, lymph nodes, adrenals, urinary bladder and brain.

DISCUSSION

Although there are many hepatotoxic plants in South Africa (Steyn, 1949; Kellerman *et al.*, 1973), some of which are known to be associated with photosensitization in ruminants, most of them have hitherto received surprisingly little attention. This is especially true of 2 plants, namely, *A. axillaris* and *Lasiospermum bipinnatum*. While *A. axillaris* has been reported to affect only sheep (Van der Walt & Steyn, 1939; Steyn, 1949; Kellerman *et al.*, 1973), *L. bipinnatum* has proved to be toxic in both sheep and cattle (Adelaar *et al.*, 1964; Fair, Tustin & Adelaar, 1970; Kellerman *et al.*, 1973; Thornton, 1977).

The afore-mentioned authors described peracute deaths without any signs of icterus or photosensitization with these intoxications. The latter symptoms are apparently more frequently seen in acute and subacute poisonings with these plants. There is only one report dealing with the liver lesions in sheep poisoned with *A. axillaris* (Kellerman *et al.*, 1973). They described a severe centrilobular hepatic necrosis, involving at least two thirds of most lobules, in one sheep that died peracutely after it had received a single high dose (10 g/kg) of dried *A. axillaris* plant material. However, 2 sheep that were dosed at lower levels (5,0 g/kg and 2,5 g/kg) died acutely after they had become mildly photosensitive. The liver lesions in both sheep comprised prominent periportal fatty changes, midzonal coagulative necrosis and mild centrilobular fatty changes.

Kellerman *et al.* (1973) also reported on the hepatic lesions in one sheep that died after being dosed with toxic plant material of *L. bipinnatum*. The liver lobules revealed a narrow peripheral zone of fatty degeneration, bordered by midzonal coagulative necrosis, while centrizonal hepatocytes were either mildly affected or spared. On the other hand, Adelaar *et al.* (1964) described peripheral necrosis as the most characteristic lesion with this plant in sheep and cattle.

A recent study by Kellerman, Coetzer, Schneider & Welman (1983) of *Athanasia trifurcata* intoxication in sheep, showed that, by varying the dosing regimen, the zonal distribution of necrosis in the liver could be shifted from the periportal zone through the midzonal area to the centrilobular zone of the lobule in different animals.

The aim of this experiment was to study the hepatic lesions of field cases of *A. axillaris* poisoning by allowing sheep to graze freely in a camp with a heavy growth of this plant. In this way certain experimental hazards such as excessively high doses, forced intake, too short or too long intervals between doses, and indigestion disturbances, all of which would seem to have a bearing on the ultimate lesions in the liver, were excluded. A spectrum of lesions was seen in the livers of these animals. One animal showed marked peripheral coagulative necrosis 3 days after it had been put into the camp, while another animal revealed marked ductular proliferation, parenchymal degeneration and scattered single cell necrosis. Other noteworthy changes included anisocytosis, anisonucleosis, increased mitoses and binucleation of hepatocytes, fatty metamorphosis and bile stasis.

Our results and those of Kellerman *et al.* (1973) showed that the liver lesions in *A. axillaris* poisoning could range from distinct zonal necrosis (peripheral, midzonal or centrizonal) to diffuse degeneration and hepatocellular unrest. These changes were often accompanied by ductular proliferation and cholestasis.
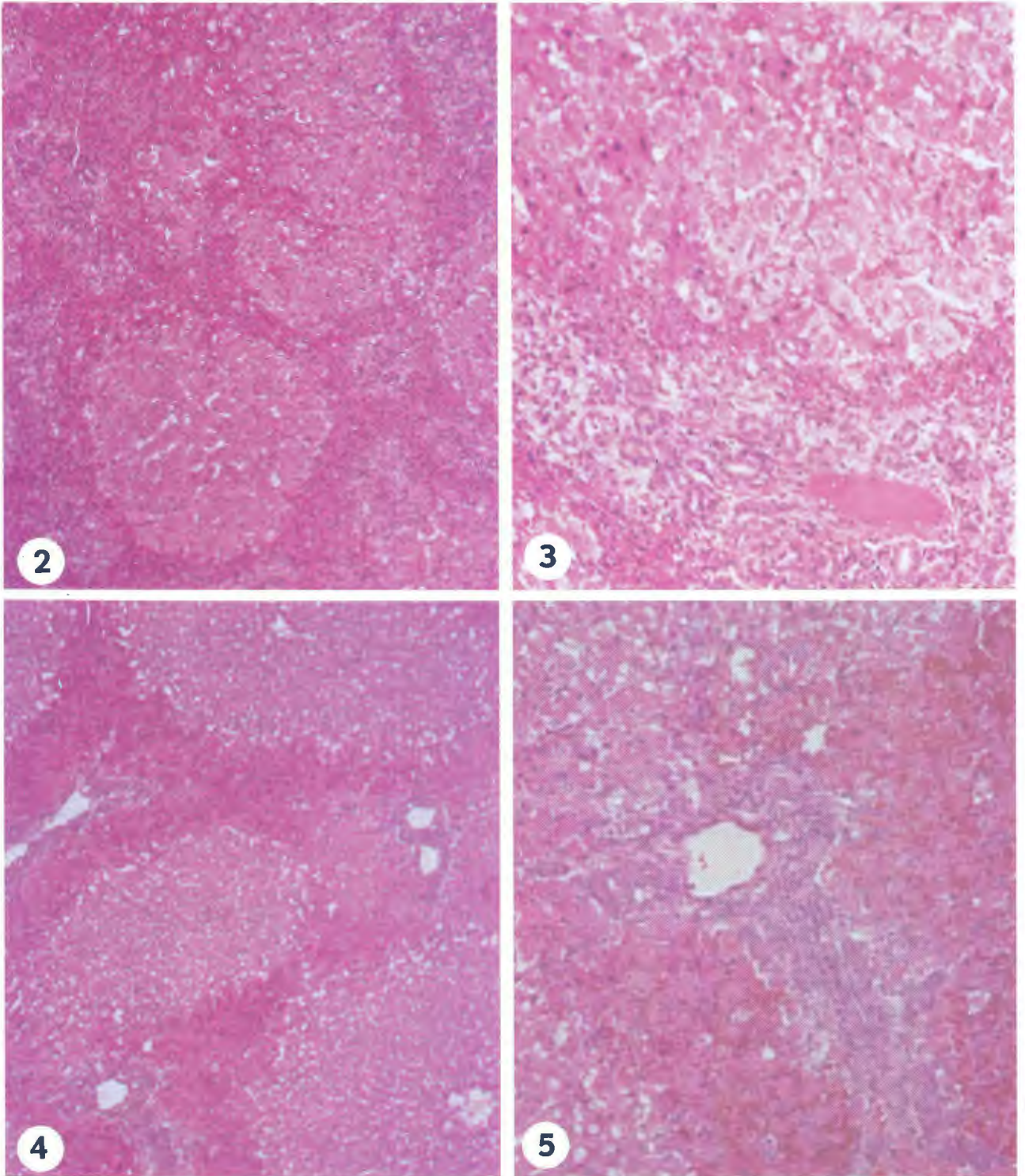
FIG. 2 Moderate to marked ductular proliferation and fibroplasia which bridged adjacent triads: HE × 75
FIG. 3 Portal reactions consisting of moderate ductular proliferation and fibroplasia: HE × 200
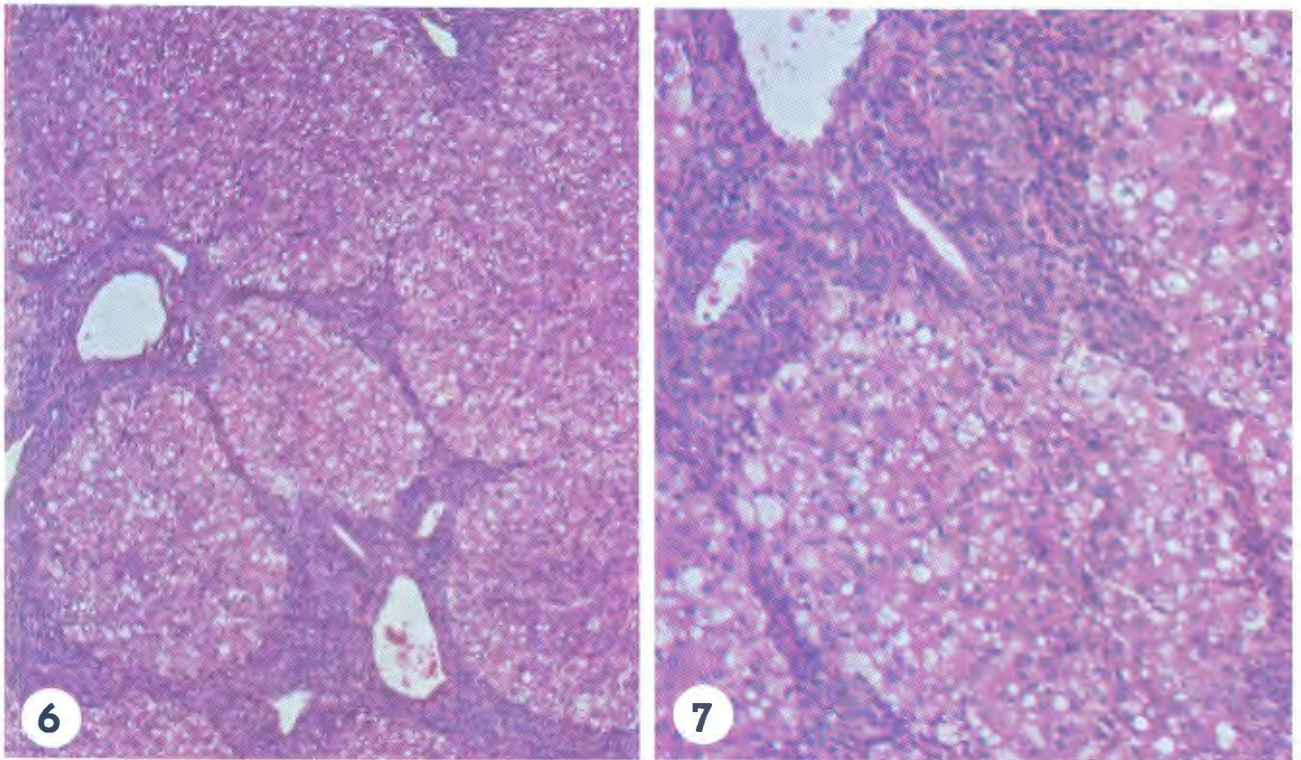FIG. 4 & 5 Peripheral coagulative hepatic necrosis and haemorrhage: HE × 75 and × 200 respectively

FIG. 6 & 7 Portal triads made up almost exclusively of numerous densely arranged bile ductules. Note moderate fatty metamorphosis, specially prominent in the hepatocytes in the peripheral zones of the lobules: HE × 75 and × 200 respectively

It would seem that the dosage and toxic levels of the plant, plus the length and intervals of exposures to its toxic components, might have a bearing on the production of certain lesions in the liver. The significance of zonal necrosis induced by certain hepatotoxic plants, mycotoxins and drugs has received much attention in veterinary and human medicine (Adelaar *et al.*, 1964; Jubb & Kennedy, 1970; Kellerman *et al.*, 1973; McGavin & Knake, 1977; Zimmerman, 1978; Kellerman *et al.*, 1982). These authors indicated that the changes in the liver can apparently not only vary for a specific toxin, but a variety of hepatotoxins can produce very similar lesions. Extreme caution must therefore be taken not to oversimplify the diagnostic significance of these lesions.

Various hypotheses have been put forward to explain the zonal nature of necrosis in livers resulting from different toxins (Jubb & Kennedy, 1970; Kellerman *et al.*, 1973; McGavin & Knake, 1977; Zimmerman, 1978; Kellerman *et al.*, 1982). However, certain questions regarding their pathogenesis remain unanswered. Further research under field and experimental conditions with these plant intoxications are necessary to shed more light on factors that might play a role in their production.

REFERENCES

ADELAAR, T. F., TERBLANCHE, M., SMIT, J. D., NAUDÉ, T. W. & CODD, L. E., 1964. A hitherto unknown poisonous plant, *Lasiospermum bipinnatum* (Thunb.) Druce. Preliminary communication. *Journal of the South African Veterinary Medical Association*, 35, 11–16.

ANON., 1968. Armed Forces Institute of Pathology, Washington. Manual of histologic staining methods. 3rd ed. New York, Toronto, London, Sydney: McGraw-Hill.

FAIR, A. E., TUSTIN, R. C. & ADELAAR, T. F., 1970. Case report. Poisoning of cattle by ganskweek *(Lasiospermum bipinnatum)* (Thunb.) Druce. *Journal of the South African Veterinary Medical Association*, 41, 231–232.

JUBB, K. V. F & KENNEDY, P. C., 1970. Pathology of domestic animals. 2nd ed. Vol. 2 p. 206. New York and London: Academic Press.

KELLERMAN, T. S., BASSON, P. A., NAUDÉ, T. W., VAN RENSBURG, I. B. J. & WELMAN, WILHELMINA G., 1973. Photosensitivity in South Africa. I. A comparative study of *Asaemia axillaris* (Thunb.) Harv. Ex Jackson and *Lasiospermum bipinnatum* (Thunb.) Druce poisoning in sheep. *Onderstepoort Journal of Veterinary Research*, 40, 115–126.

KELLERMAN, T. S., COETZER, J. A. W., SCHNEIDER, D. J. & WELMAN, WILHELMINA G., 1983. Photosensitivity in South Africa. III. Ovine hepatogenous photosensitivity caused by the plant *Athanasia trifurcata* L. (Asteracea). *Onderstepoort Journal of Veterinary Research*, 50, 47–52.

McGAVIN, M. D. & KNAKE, R., 1977. Hepatic midzonal necrosis in a pig fed aflatoxin and a horse fed moldy hay. *Veterinary Pathology*, 14, 182–187.

STEYN, D. G., 1949. Vergiftiging van mens en dier. Pretoria: Van Schaik.

THORNTON, D. J., 1977. Ganskweek *(Lasiospermum bipinnatum)* poisoning in cattle. *Journal of the South African Veterinary Association*, 48, 210–211.

VAN DER WALT, S. J. & STEYN, D. G., 1939. Recent investigations into the toxicity of known and unknown poisonous plants in the Union of South Africa. IX. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 12, 335–366.

ZIMMERMAN, H. J., 1978. Hepatoxicity. New York: Appleton-Century-Crofts.