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PHOTOSENSITIVITY IN SOUTH AFRICA. II. THE EXPERIMENTAL PRODUCTION OF THE OVINE HEPATOGENOUS PHOTOSENSITIVITY DISEASE GEELDIKKOP (*TRIBULOSIS OVIS*) BY THE SIMULTANEOUS INGESTION OF *TRIBULUS TERRESTRIS* PLANTS AND CULTURES OF *PITHOMYCES CHARTARUM* CONTAINING THE MYCOTOXIN SPORIDESMIN

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ABSTRACT

KELLERMAN, T. S., VAN DER WESTHUIZEN, G. C. A., COETZER, J. A. W., ROUX, CECILIA, MARASAS, W. F. O., MINNE, J. A., BATH, G. F. & BASSON, P. A., 1980. Photosensitivity in South Africa. II. The experimental production of the ovine hepatogenous photosensitivity disease geeldikkop (*Tribulosis ovis*) by the simultaneous ingestion of *Tribulus terrestris* plants and cultures of *Pithomyces chartarum* containing the mycotoxin sporidesmin. *Onderstepoort Journal of Veterinary Research*, 47, 231–261 (1980).

The mycoflora of toxic pastures were surveyed during a number of outbreaks of ovine hepatogenous photosensitivity in South Africa. Pure cultures of several isolates were dosed to sheep, but only those of *Pithomyces chartarum* and *Myrothecium verrucaria* proved to be toxic.

Photosensitization was induced in sheep by dosing them with cultures of a *P. chartarum* isolate (GA10) obtained from *Tribulus terrestris* plants collected during an outbreak of geeldikkop in the Karoo. Thus for the first time a mechanism whereby *T. terrestris* plants can contribute to the causation of ovine hepatogenous photosensitivity was demonstrated.

When cultures of GA10 equivalent to approximately 0,75—4,0 mg/kg sporidesmin were dosed at Onderstepoort Veterinary Research Institute to Highveld and Karoo sheep on a diet of lucerne, facial eczema was produced. Dosing the same cultures at levels equivalent to *c.* 1,0 mg/kg of sporidesmin in the Karoo resulted in lesions characteristic of both facial eczema and geeldikkop. Typical hepatic lesions of geeldikkop could be elicited by dosing GA10 at levels equivalent to *c.* 0,25— 0,7 mg/kg of sporidesmin to Karoo sheep grazing on predominantly *T. terrestris* pastures in the Karoo. In the latter experiment geeldikkop was induced in the sheep on *T. terrestris* pastures, while those receiving identical doses on veld with little *T. terrestris* developed facial aczema.

Geeldikkop, therefore, can be brought about by the ingestion of *T. terrestris* plants together with toxic cultures of *P. chartarum*. The plant appears not only to act as a vehicle for ingestion of spores, but also to interact with sporidesmin to induce lesions typical of geeldikkop, whereas sporidesmin alone results in facial eczema. Indications are that it can enhance the ability of sporidesmin to cause photosensitivity or, possibly, vice versa.

The histopathological findings of these experiments are described in detail.

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Resumé

PHOTOSENSIBILITÉ EN AFRIQUE DU SUD. II. LA PRODUCTION EXPÉRIMENTALE DE LA MALADIE DE PHOTOSENSIBILITÉ HEPATOGÈNE OVINE (TRIBULOSIS OVIS) *PAR L'INGESTION SIMULTANÉE DE PLANTES DE* TRIBULUS TERRESTRIS *ET DE CULTURES DE* PITHOMYCES CHARTARUM *CONTENANT LA MYCOTOXINE SPORIDESMINE*

Les mycoflores de pâturages toxiques ont fait l'objet d'une enquête pendant un certain nombre d'éruptions de photosensitivité hepathogène ovine en Afrique du Sud. Des cultures pures de plusieurs isolats furent administrées au mouton, mais seulement celle de Pithomyces chartarum *et de* Myrothecium verrucaria *se révélèrent toxiques.*

La photosensibilité fut produite chez le mouton en l'administrant avec des cultures d'un isolat de P. chartarum (*GA*10) *obtenu de plantes de* Tribulus terrestris *ramassées pendant une éruption de* Tribulosis ovis, *dans le Karoo. Pour la première fois, donc, un mécanisme par lequel des plantes de* T. terrestris *peuvent contribuer à la causation de la photosensibilité hepatogène ovine a été démontré.*

*Quand des cultures de GA*10 *équivalentes à approximativement* 0,75–4,0 *mg/kg de sporidesmine furent administrées, à l'Institut de Recherche d'Onderstepoort, à des moutons du Highveld et du Karoo s'alimentant sur une diète de luzerne, l'eczema facial se produisit. L'administration de cultures similaires à des niveaux equivalents à* c. 1,0 *mg/kg de sporidesmine, dans le Karoo, causa des lésions caractéristiques d'eczema facial et de* Tribulosis ovis. *Des lésions hépatiques typiques de* Tribulosis ovis *purent être mises à jour en administrant GA*10 *à des niveaux équivalents à* c. 0,25–0,7 *mg/kg de sporidesmine à des moutons du Karoo paturant sur des patures à prédominance de* T. terrestris *du Karoo. Dans la dernière expérience la* Tribulosis ovis *fut produite chez les moutons des pâtures à* T. terrestris, *tandis que ceux recevant des doses identiques sur des champs avec peu de* T. terrestris *révélèrent de l'eczema facial.*

La Tribulosis ovis *peut donc être produite par l'ingestion de plantes de* T. terrestris *avec des cultures toxiques de* P. chartarum. *La plante ne semble pas seulement agir comme piège à spores et comme véhicule pour l'ingestion des spores, mais aussi inter-agir avec la sporidesmine pour produire des lésions typiques de* Tribulosis ovis *tandis que la sporidesmine seule résulte en eczema facial. Il existe des indices que la plante peut accroître l'aptitude de la sporidesmine à causer la photosensibilité ou peut-être vice versa.*

Les lésions histopathologiques de ces expériences sont décrites en détails.

General introduction

Description of the disease

Geeldikkop is a seasonal photosensitivity disease of sheep and goats grazing on predominantly *Tribulus terrestris* ('dubbeltjie') pastures in the Karoo. The plant is a nutritious annual herb that sporadically becomes toxic under certain conditions, e.g. when wilted during hot, dry spells following summer rains. In a severe outbreak of the disease hundreds of thousands of sheep living in an area covering about one third of the country can be affected.

No other syndrome of ovine photosensitization, apart possibly from facial eczema in New Zealand, can match it in the magnitude of the outbreaks or in the extent of the damage wrought.

The photosensitivity is of a secondary or hepatogenous type (Rimington & Quin, 1934; Clare, 1952, 1955) associated with the presence of cholesterol-like crystals in the hepatocytes, Kupffer cells and within the bile duct system. Also associated with it are hepatocytic degeneration, scattered individual hepatocyte necrosis, Kupffer cell activation and pigmentation, portal fibroplasia and bile duct proliferation (Theiler, 1918; Van Tonder, Basson & Van Rensburg, 1972; Kellerman, Basson, Naudé, Van Rensburg & Welman, 1973).

Some theories on the aetiology of geeldikkop

(a) *The role of* Tribulus terrestris: Previously, geeldikkop was experimentally reproduced by feeding *T. terrestris* plants to sheep (Theiler, 1918; Quin, 1928, 1929; Van Tonder *et al.*, 1972; Bath, Van Tonder & Basson, 1978), but other similar trials were often unsuccessful and positive results were obtained only in endemic areas during outbreaks of the disease. Recently, however, Bath *et al.*, (1978) reproduced geeldikkop by dosing a sheep with *T. terrestris* plants kept frozen for 6 weeks after they had been collected in a toxic camp on a farm 100 km away.

The fact that *T. terrestris* sporadically becomes toxic under specific climatic conditions led to the speculation that the wilted plant could sometimes produce a labile hepatotoxin (Theiler, 1918; Quin, 1928, 1929; Van Tonder *et al.*, 1972; Bath *et al.*, 1978). While bearing in mind that such a toxin could possibly have been too labile for extraction with the methods then used, or that non-toxic *T. terrestris* may have been extracted, it is nevertheless true that no toxin capable of causing geeldikkop has ever been isolated from *T. terrestris* (Brown, 1968). Recently G. F. Bath (unpublished data, 1979) induced geeldikkop with lyophilized alcoholic extracts of toxic *T. terrestris*, but the toxic principle(s) has not yet been identified.

Finally, if *T. terrestris* was the only cause of geeldikkop, it would be difficult to explain how a disease indistinguishable from it such as dikoor (*Panicum* photosensitization) can occur in places where *T. terrestris* is absent (Quin, 1928; Steyn, 1928; Rimington & Quin, 1934).

(b) *The role of selenium:* In an effort to answer some of these questions, Brown and his co-workers studied the role of selenium in the aetiology of the disease (Brown & De Kock, 1959; Brown & De Wet, 1962; Brown, 1962, 1963, 1964, 1968). They regarded geeldikkop and enzootic icterus as being different manifestations of the same disease entity (Brown & De Wet, 1962; Brown, 1962, 1963, 1966).

The underlying cause of the disease was thought by these workers to be a subclinical chronic selenosis that disrupted enzyme systems, notably those connected with the selective permeability of cell membranes and the glycolytic cycle (Brown, 1962, 1963, 1968). The theory was supported by evidence such as the demonstration of potentially dangerous amounts of selenium in plants from geeldikkop and enzootic icterus-prone areas (Brown & De Wet, 1962), by the fact that sheep from such areas had higher levels of selenium in certain body tissues than those from control areas (Brown, 1968) and by the relationship that could be observed between the selenium content of the veld and the incidence of the disease (Brown & De Wet, 1962; Brown, 1968). The haemolytic component of the syndrome was considered to be an auto-immune reaction to aberrant selenoprotein antigens formed in the bodies of affected sheep (Neethling, Brown & De Wet, 1968). Recently Bath (1979) reported that enzootic icterus was a form of copper poisoning.

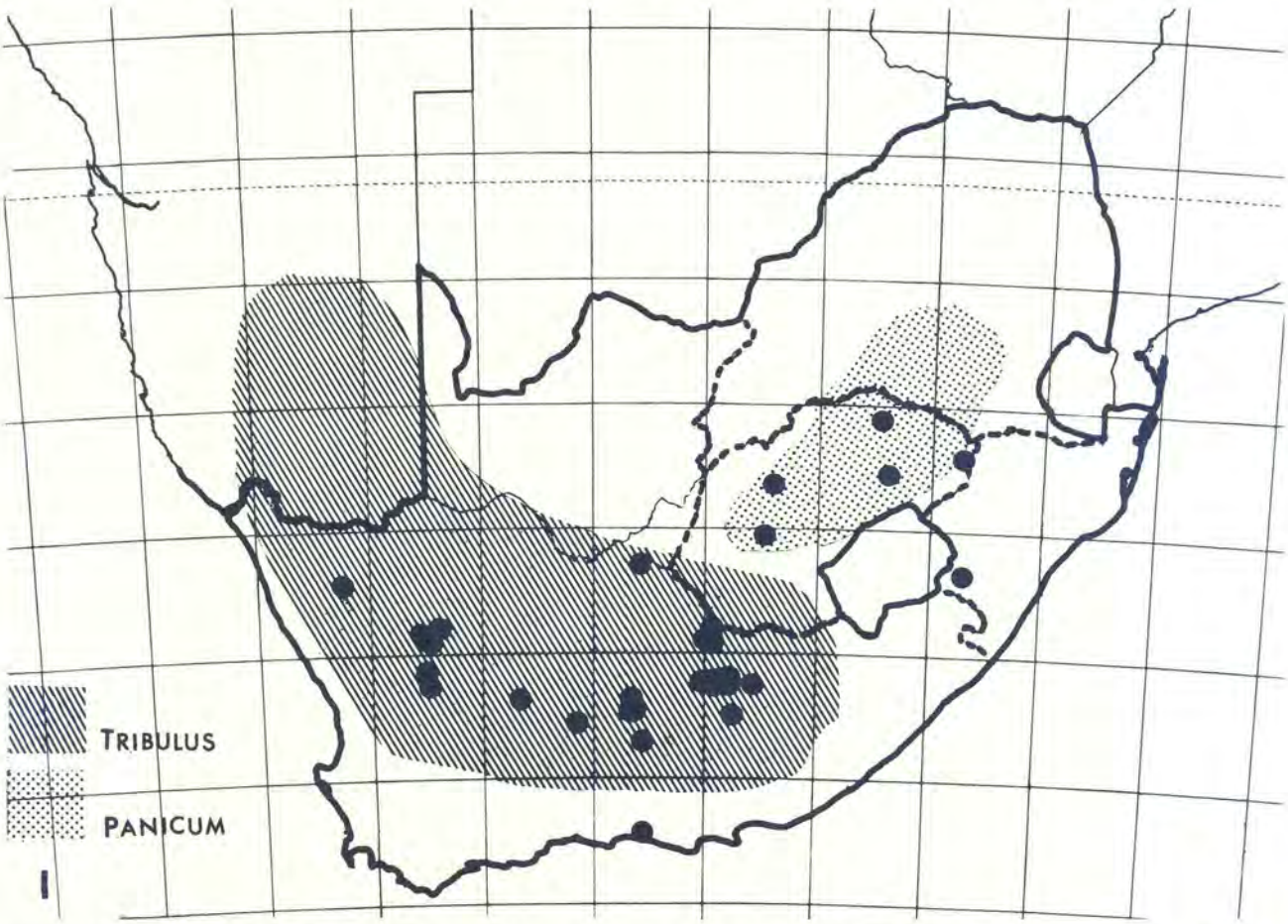


FIG. 1 Distribution of some of the outbreaks of geeldikkop (*Tribulosis ovis*) and dikoor (*Panicum* photosensitivity) investigated during this study

(c) *The role of fungi:* For many years there has been speculation that fungi might be involved in the aetiology of geeldikkop, Theiler (1918) being amongst the first to make observations in this connection. He reported the isolation of a new species of *Calletodridium* (probably a misprint for *Colletotrichum*) growing parasitically on *T. terrestris* plants on a farm in the Orange Free State where previously geeldikkop had been experimentally reproduced by feeding *T. terrestris* to sheep. When similar dosing trials carried out with diseased *T. terrestris* plants failed, he attributed the nontoxicity of the plant either to its mature growth stage or to other factors such as the fungal infection. Later Steyn (1928) speculated that dikoor (*Panicum* photosensitization), a disease closely related to or perhaps identical with geeldikkop (Quin, 1928, 1930, 1933; Van Tonder *et al.*, 1972), might be caused by smut-infected *Panicum* grass. Although feeding trials with smutted *Panicum* heads from a field where dikoor had occurred failed to induce photosensitivity in sheep, Steyn (1928) deserves credit for being the first person to postulate that ovine photosensitization may be caused by a fungus.

Mycotoxicological research into the aetiology of ovine photosensitization in South Africa was given fresh impetus by reports from New Zealand (Percival, 1959a, 1959b; Thornton & Percival, 1959; Thornton & Ross, 1959) that facial eczema was a mycotoxicosis. Brown (1964) reported negative results from the dosing of sheep with 23 different fungi, including *Alternaria, Curvularia, Fusarium, Helminthosporium, Hendersonia, Myrothecium, Penicillium* and *Rhizopus,* isolated from *T. terrestris.* The fungi were "grown on a variety of media, including minced *T. terrestris,* and under a variety of conditions". None of the fungi, however, produced symptoms of geeldikkop. Subsequently, Gouws (1965) reported on another mycotoxicological investigation into the aetiology of geeldikkop, this time carried out by the National Chemical Research Laboratory, CSIR. with *T. terrestris* plants and litter collected from farms in the Victoria West district, Cape Province (CP), and the Bethuli district, Orange Free State (OFS), where geeldikkop had occurred. Microscopic examination of these plants revealed that *Alternaria* spores commonly occurred on the leaves and twigs, and the majority of the 130 pure cultures of fungi obtained from this material were *Alternaria tenuis.* The remaining isolates "consisted of a few unidentified *Helminthosporium* (=*Bipolaris*) and *Stemphyllium* species and an assortment of mucors, aspergilli and penicillia". In view of the preponderance of dematiaceae, 28 of these cultures were selected for oral toxicity tests in day-old chicks. The fungi were cultured on maize and the fungus-infested maize meal incorporated into chicken mash. Three species, *A. tenuis, Helminthosporium* (=*Bipolaris*) sp. and *Stemphyllium* sp., were toxic to chicks. In subsequent toxicity tests it was established that 2 of these isolates, *A. tenuis* and *Helminthosporium* (=*Bipolaris*) sp., were also toxic to day-old ducklings, newly weaned rats, and Merino sheep. In the sheep dosing experiment, one sheep which received maize inoculated with *A. tenuis* died after 24 days and another which received maize inoculated with *Helminthosporium* (=*Bipolaris*) sp. died after 15 days. None of the treated sheep developed

pathological signs of geeldikkop, but necrosis in many tissues was seen on post-mortem examination. It was subsequently found that 2 of the fungi used in these experiments produced known mycotoxins, namely, the *Helminthosporium* (=*Bipolaris*) sp., sterigmatocystin (Holzapfel, Purchase, Steyn & Gouws, 1966), and the strain of *A. tenuis*, tenuazonic acid (Dr I. F. H. Purchase, CSIR, personal communication, 1972).

The object of the current investigation was to survey the mycoflora of pastures during outbreaks of ovine photosensitivity and to assay the hepatotoxicity of various isolates by dosing cultures of them to sheep.

PART I

Dosing trials with pasture fungi collected during outbreaks of ovine hepatogenous photosensitivity

Materials and Methods

Collection of samples for survey purposes

Samples consisting of either living *T. terrestris* plants, dead Karoo plants, wheat or oat litter in cultivated lands or dead grass leaves in planted pastures were collected in camps where outbreaks of photosensitivity in sheep had occurred. The samples of plant material were kept as free of soil as possible and placed in new paper bags.

An intensive mycological investigation of the mycoflora of a reaped wheat land was carried out on a farm near Heilbron, OFS. Samples of wheat stubble and grass leaves (*Panicum coloratum* and *Eleusine africana*) were collected once a week at 6 sites in a wheat field that was mechanically reaped during the first week of December, 1971. The sampling was continued until the land was ploughed in February, 1972. The 6 subsamples of wheat stubble collected each week were thoroughly mixed and the fungi isolated by the dilution plate method. The samples of grass leaves were treated similarly.

Procedures for isolation of fungi

Samples of living and/or dead plant material collected in camps where outbreaks of photosensitivity in sheep occurred, were examined directly for the presence of fungal spores. In addition, fungi were isolated from these materials by the following 3 isolation methods.

Humid chamber method: Small pieces of plant material were placed on sterile, moistened filter paper discs in Petri dishes and incubated at 25 °C for 2–5 days. Fungal colonies developing on the pieces of tissue were then isolated in pure culture.

Direct plating method: Small pieces of plant material were aseptically plated onto the surface of solidified agar (*vide infra*) in Petri dishes and incubated at either 18, 25 or 37 °C. Colonies of fungi that grew out of the pieces of tissue were then isolated in pure culture.

Dilution plate method: Samples of plant material, consisting either of living leaves or dry straw, were cut into small pieces. Dilution series ($1:10^3$—$1:10^6$) were prepared from aqueous suspensions of the shredded plant material (5 g in 250 mℓ of sterile water shaken for 10 minutes on a wrist-action shaker). From each dilution 1 mℓ aliquots were pipetted into sterile Petri dishes, mixed with melted agar (*vide infra*) and the plates incubated for 7 days at either 18, 25 or 37 °C. Colonies that could not be identified directly were isolated in pure culture for identification purposes.

Media for culturing the fungi

For the isolation and identification of fungi, the following culture media were used: Malt extract agar (MEA) containing 1,5% malt extract (Anon., 1960), occasionally with 100 mg/ℓ of sodium novobiocin added to inhibit bacterial growth (Butler & Hine, 1958); Czapek agar (CA) with tergitol to suppress growth of Mucorales prepared as described by Steiner & Watson (1965), and potato-carrot agar (PCA) (Anon., 1960).

Bulk cultures for dosing to sheep were grown on these same media but without the addition of sodium novobiocin in (MEA) and tergitol in the CA. In addition, the following media were also used for the preparation of bulk cultures: Lucerne meal agar (LMA), 100 g of lucerne meal and 20 g of agar per ℓ of distilled water; potato-carrot broth (PCB) prepared as described by Done, Mortimer, Taylor & Russel (1961); semisynthetic broth (SSB) as described by Di Menna, Campbell & Mortimer (1970); distributed in 20 mℓ quantities in plastic Petri dishes under intermittent near-ultra-violet light irradiation for production of sporidesmin as described by Marasas, Adelaar, Kellerman, Minne, Van Rensburg & Burroughs (1972).

Maize: 200 g of yellow maize kernels and 120 mℓ of distilled water per 1 ℓ glass fruit jar and autoclaved for 20 minutes at 121 °C.

Bran: 100 g of bran in 80 mℓ of distilled water per 1 ℓ glass fruit jar, stirred, left overnight and autoclaved for 60 minutes at 121 °C.

Natural hay media: Lucerne hay, *T. terrestris* plants or wheat straw were soaked overnight in water placed in 1 ℓ glass fruit jars and autoclaved for 90 minutes at 121 °C.

Stock cultures of fungi were lyophilized or maintained on slants of 1,5% malt extract agar in McCartney bottles at 18 °C. Inoculum was prepared by transferring mycelium and/or spores from stock cultures to the surface of either 1,5%, MEA or PCA in 1 ℓ Erlenmeyer flasks (100 mℓ agar/flask). The flasks were incubated at either 18, 25 or 37 °C until profuse sporulation occurred. These spores were harvested in sterile water containing a drop of Teepol*, and the resulting spore suspension used to inoculate various culture media.

Following inoculation of the various substrates with the fungi to be tested, the cultures were incubated under various conditions (Appendix 5). Cultures were incubated in the dark in standard incubators at either 20, 25, 28 or 37 °C. Some cultures were irradiated with white fluorescent light (Atlas Daylight 40W tubes) in a Conviron incubator** in which fluctuating day and night temperatures could be obtained. Near ultraviolet light radiation (NUV) was provided by fluorescent tubes (General Electric S40 BLB) giving radiation at 350 nm mounted between 2 cool white fluorescent tubes (Phillips TL 33RS) at a distance of 30 cm above bench height (Marasas *et al.*, 1972). These cultures were irradiated in a room with temperature control (±2 °C) in which fluctuating day and night temperatures could be obtained. Some cultures were exposed to natural temperature fluctuations by incubating them either in a glasshouse at the Veterinary Research Institute (VRI), Onderstepoort, or in the field at Heilbron, OFS (Appendix 5).

* Shell Chemicals, South Africa (Pty) Ltd
** Controlled Environments, Canada

TABLE 1 Toxicity of fungal cultures to sheep

Culture No.	Fungus	Dosage rate		Route stomach tube s.t. ruminal fistula r.f.	Mass of sheep (kg)	Origin of sheep	Result
		Quantity	Days				
1	*Pithomyces karoo*	20 ℓ	9	s.t.	26	Karoo	Neg.
2	*P. karoo*	20 ℓ	9	s.t.	18,5	Karoo	Neg.
3	*P. karoo*	6 ℓ	2	s.t.	31	Karoo	Neg.
3	*P. karoo*	5 ℓ	2	s.t.	31	Karoo	Neg.
4	*P. karoo*	3,6 kg	2	r.f.	32,5	Karoo	Neg.
4	*P. karoo*	3,6 kg	2	r.f.	38	Barkly East	Neg.
5	*P. karoo*	1,6 kg	2	r.f.	39	Barkly East	Neg.
5	*P. karoo*	1,7 kg	2	r.f.	31,5	Karoo	Neg.
6	*P. karoo*	8 ℓ	2	s.t.	31,5	Karoo	Neg.
7	*P. karoo*	5,2 ℓ	2	s.t.	40	Karoo	Neg.
8	*P. karoo*	11 kg	2	r.f.	26	Highveld	Neg.
9	*P. karoo*	4 ℓ	1	s.t.	31,5	Karoo	Neg.
10	*P. karoo*	3 ℓ	1	s.t.	29	Karoo	Neg.
11	*P. karoo*	6 kg	2	r.f.	38	Karoo	Neg.
12	*P. karoo*	3,2 ℓ	2	s.t.	34,5	Karoo	Neg.
14	*Tiarosporella graminis* var. *karoo*	9 kg	18	r.f.	36	Karoo	Neg.
15	*T. graminis* var. *karoo*	5,8 kg	15	r.f.	33,5	Karoo	Neg.
17	*Ulocladium chartarum*	12 kg	17	r.f.	23	Highveld	Neg.
18	*Epicoccum nigrum*	8,4 kg	13	r.f.	23	Highveld	Neg.
19	*Cladosporium* sp.	7,1 kg	13	r.f.	23,5	Highveld	Neg.
20	*Cladosporium* sp.	4,1 kg	1	r.f.	23,5	Highveld	Neg.
21	*C. macrocarpum*	4 kg	4	r.f.	14,5	Highveld	Neg.
21	*C. macrocarpum*	12 ℓ	6	s.t.	14,5	Highveld	Neg.
23	*Aspergillus fumigatus*	0,5 kg	1	r.f.	31	Karoo	Neg.
24	*A. fumigatus*	5,4 ℓ	1	s.t.	31	Karoo	Neg.
26	*Myrothecium verrucaria*	7,9 ℓ	2	s.t.	38	Highveld	Fatal gastritis
26	*M. verrucaria*	8,4 ℓ	57	s.t.	47	Highveld	Mass loss
72/1	*Alternaria* sp.	7,2 ℓ	4	s.t.	27	Highveld	Neg.
72/7	*Phaeoramularia kellermaniana*	18 ℓ	5	s.t.	39	Highveld	Neg.
72/5	*Phoma sorghina*	10,5 ℓ	4	s.t.	43	Highveld	Elevation SGOT*
72/11	*P. sorghina*	17,2 ℓ	9	s.t.	—	Highveld	Elevation SGOT

* Serum glutamic oxaloacetic transaminase

Maintenance and dosing of experimental animals

Plant material was milled or cut into small fragments by hand for dosing per ruminal fistula or stomach tube to sheep. Cultures on artificial media were homogenized in a Waring blender and then administered per stomach tube. In some instances sheep from the Karoo, and, in others, from the Transvaal Highveld or Barkly East, were used as test animals (Table 1).

The sheep were fed on green lucerne, examined daily and kept in the sun. Periodically all, or some, of the following chemical pathological determinations were done on the blood: γ-glutamyl transpeptidase (γ-GT), serum urea nitrogen, serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase, bilirubin, total plasma proteins, glucose, serum calcium, sodium and potassium. At necropsy specimens were taken from various organs, fixed in 10% formalin, processed in a routine manner and stained with haematoxylin and eosin (HE).

Results

The details of the fungi isolated from the various substrates, etc., are given in Appendices 1–4.

A total of 12 species were cultured (Appendix 5) and tested for toxicity by dosing them to sheep (Table 1). In the case of 3 species, namely, *Pithomyces karoo, P. chartarum* (see Marasas *et al.*, 1972 for toxicity data) and *Cladosporium macrocarpum,* more than one isolate were tested. With the exception of *P. chartarum* (Table 2) from Humansdorp (Marasas *et al.*, 1972), the only other toxic fungus to be isolated was *Myrothecium verrucaria.* High doses of culture material of the latter fungus resulted in acute deaths from haemorrhagic abomasitis and low doses resulted in a loss in mass (Table 1). The findings on the toxicity of *M. verrucaria* are in accord with those of Di Menna & Parlé (1970) and Di Menna (1971).

Discussion

The first reports on the mycoflora of pasture plants associated with ovine photosensitivity in South Africa were those of Theiler (1918) and Steyn (1928). Subsequently, lists of fungi isolated from *T. terrestris* plants collected in pastures where outbreaks of geeldikkop had occurred were published by Brown (1964) and Gouws (1965). Following the discovery of *P. chartarum* and the diagnosis of facial eczema in sheep grazing rye grass/white clover pastures in South Africa (Marasas *et al.*, 1972), surveys were also made of the mycoflora of wheat field debris (Papendorf & Jooste, 1974; Jooste, 1976) and of *Panicum* leaves and litter (Eicker, 1976). Recently, in a study of the aerospora above an *Eragrostis* pasture on the Highveld of South Africa (Van der Merwe, Eicker, Marasas & Kellerman, 1979), several fungi isolated from South African pastures during outbreaks of ovine photosensitivity were described as new species, viz: *Pithomyces karoo* (Marasas & Schumann, 1972), *Phaeoramularia kellermaniana* (Marasas & Bredell, 1974), *Drechslera multiformis* (Jooste, 1975), *Tiarosporella graminus* var. *karoo* and *T. tritici* (Sutton & Marasas, 1976).

In the current investigation the mycoflora of pastures were studied during 17 widespread outbreaks of ovine photosensitivity (Appendix 1). The fungal flora found in different localities are not strictly comparable, as a variety of methods of isolation were used and specific identifications were not made in many genera, e.g. *Alternaria, Cladosporium, Phoma* and *Fusarium.* Moreover, until the aetiology of both geel-

dikkop and dikoor is known and it can be proven to the contrary, the possibility cannot be excluded that, as in the case of facial eczema, intoxicated sheep may appear to be normal for a considerable time before signs of photosensitivity appear. The species of fungi isolated during an outbreak may consequently be quite different from those present at the time of intoxication, which might have occurred several weeks before. An attempt was made to solve this problem by sampling the Heilbron *P. coloratum* pastures at weekly intervals before any case of photosensitivity occurred (Appendices 3 & 4). Unfortunately, only one sheep became photosensitive during the survey and consequently the mycoflora found could not be correlated with the onset of photosensitization. Although the findings of this survey are similar in most respects to those of Eicker (1976), *P. chartarum* was not found at Heilbron. It would appear that mycological surveys of pastures before cases of photosensitivity occur hold the most promise of discovering the causal fungus or fungi.

The current survey revealed that, although the mycopopulation of the litter was higher than that of the leaves, few qualitative differences could be observed between the two. The dominant fungi on both substrates were *Phoma* spp., particularly *Phoma sorghina*. These results are in agreement with those reported in New Zealand (Di Menna & Parlé, 1970; Di Menna, 1971) and in South Africa by Eicker (1976). Although all the fungi found on the litter were also recovered from the grass leaves (Appendices 4 & 5), the reverse was not always true, since *Stagonospora* sp., *Hendersonia* sp., *Myrothecium varrucaria* and *Staurонema* sp. were isolated only from the leaves. This indicates that some of the fungi isolated from the grass leaves are probably parasites or phylloplane saprophytes. It is clear, however, that the spores of saprophytic fungi growing on the wheat debris could adhere to the grass leaves and hence be ingested by stock.

The dilution plate method used in the mycological survey at Heilbron failed to reveal any major qualitative or quantitative changes in the mycoflora during the period of survey (Appendices 3 & 4). Nevertheless, such changes can and do occur very rapidly in the field, as happened after rain during the first week of December, when sooty, sporulating colonies of fungi soon became evident on the wheat stubble, particularly at the nodes. These fungal mats consisted mainly of 5 species: *Cladosporium macrocarpum*, *Cladosporium* sp., *Alternaria alternata, Epicoccum nigrum* and *Phoma sorghina*.

The survey, as a whole (Appendix 1), showed that 4 genera, namely, *Alternaria, Cladosporium, Fusarium,* and *Phoma* represented by several species, could be found in all 17 localities. Since it was not possible to do comprehensive specific identifications in these groups, it is not known whether the same species of each genus were present at all the various localities or not.

Some fungi such as *Tiarosporella graminis* var. *karoo* (Sutton & Marasas, 1976) were isolated only in the Karoo (Appendix 1). *Phaeoramularia kellermaniana* (Marasas & Bredell, 1974) and *Ulocladium chartarum* occurred only on cultivated lands. An interesting finding was that the species *Pithomyces karoo* (Marasas & Schumann, 1972) was isolated from both living and dead plant material at 3 different localities in the Karoo, as well as from *T. terrestris* plants used in a successful induction of geeldikkop at Middelburg (Cape) (Van Tonder *et al.*, 1972) and from oat litter in the cultivated land at Heilbron.

PART II

The experimental reproduction of a hepatogenous photosensitivity syndrome indistinguishable from geeldikkop by dosing cultures of a Karoo isolate of *Pithomyces chartarum* to sheep

Introduction

Since the dosing trails described in Part I failed to reveal the presence of pasture fungi capable of causing ovine hepatogenous photosensitivity, i.e. apart from *P. chartarum* (Marasas *et al.*, 1972), a new approach to the investigation had to be found. For instance, in the design of the new trials, cognizance had to be taken of the fact that, although many photosensitivity diseases were suspected of being mycotoxicoses, only 3 toxic fungi, namely, *P. chartarum* (Thornton & Percival, 1959), *Phomopsis leptostromiformis* (Brash, 1943; Bennets, 1957; Gardiner, 1967a, 1967b), and *Penicillium veridicatum* (Budiarso, Carlton & Tuite, 1970) have ever been shown to cause photosensitivity (Marasas & Kellerman, 1978). The possibility of *P. veridicatum* being involved in the aetiology of geeldikkop was discounted on the grounds that the photosensitivity induced by it had been limited to experimentally-fed rats. Of the 2 fungi that could cause natural outbreaks of ovine hepatogenous photosensitivity, *P. leptostromiformis* was considered unlikely to be responsible for geeldikkop as the pathological changes (Gardiner, 1967a, 1967b) were obviously different, natural outbreaks of lupinosis had always been associated with lupine plants, and photosensitization was seldom observed in this disease (Gardiner, 1967a; Van Warmelo, Marasas, Adelaar, Kellerman, Van Rensburg & Minne, 1970; Kellerman *et al.*, 1973; Marasas & Kellerman, 1978). The remaining fungus, *P. chartarum,* was the only one known to be capable of causing outbreaks of ovine hepatogenous photosensitivity on a scale similar to that of geeldikkop. Consequently, despite the fact that *P. chartarum* was not known to occur in the Karoo and that pithomycotoxicosis (or facial eczema) is usually associated with rye grass-clover pastures, and notwithstanding the differences in pathology between the 2 diseases (*vide infra*), it was resolved to investigate the possibility that *P. chartarum* was responsible for geeldikkop. The diagnosis of facial eczema at Humansdorp (Marasas *et al.*, 1972) and near Johannesburg (Van der Westhuizen, Kellerman, Roux & Ehret, unpublished data, 1976), was an additional incentive for carrying out this investigation.

1. Isolation of *P. chartarum* from pastures in South Africa

Materials and Methods

The methods used in this experiment were essentially the same as those described in Part I.

Method of isolation

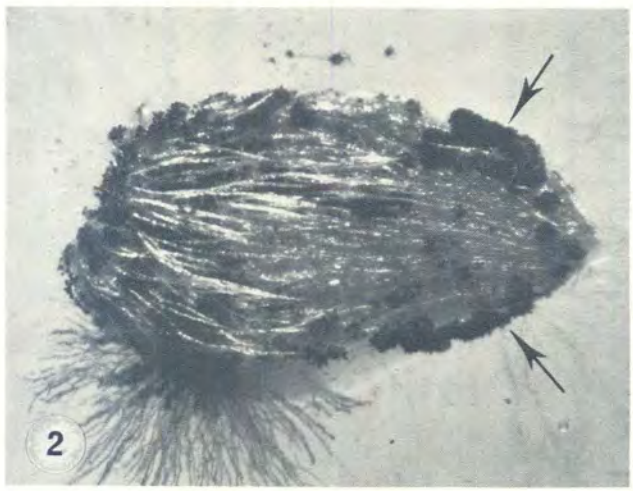
Samples of *T. terrestris* plants, various grasses and other plants from pastures and pasture plant debris were collected from 20 different localities. Out of 134 samples collected, 116 originated from pastures on which ovine hepatogenous photosensitivity had occurred (Table 2). Leaflets from these specimens were placed on PCA plates in clear plastic or high silica glass Petri dishes and incubated at 20 °C with mixed daylight/NUV illumination for 7–20 days. The leaflets were then examined at 40× magnification with a stereomicroscope and spores from sporulating colonies of *P. chartarum* (Fig. 2) were picked off with sterilized needles and transferred to fresh PCA and MEA plates for further incubation.

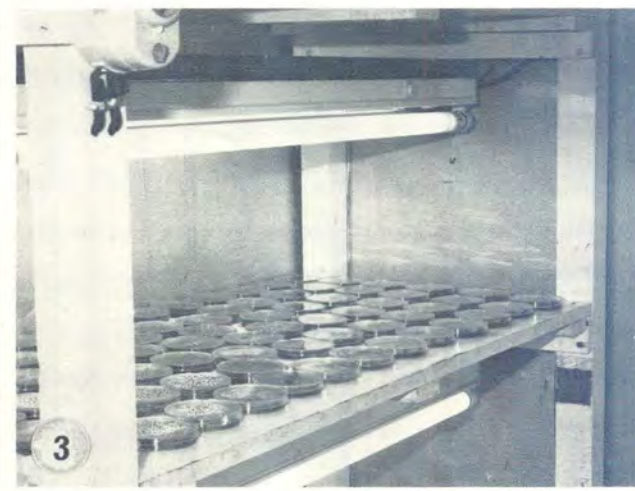
Sporidesmin production

Isolates of *P. chartarum* were tested for their ability to produce sporidesmin by growing each isolate on SSB in plastic Petri dishes. Each of 10 dishes per isolate containing 20 mℓ of medium was incubated with NUV illumination for 21 days. at 20 °C. The contents of 4 Petri dishes of each isolate were then homogenized in a blender and an estimation made of their sporidesmin content.

Estimation of the sporidesmin content of cultures

Five mℓ quantities of *P. chartarum* cultures were shaken up in 200 mℓ of ether for 30 min. The resultant extracts were evaporated, taken up in 10 mℓ of chloroform and spotted on silica gel plates containing 1,5% starch. The chromatograms (Fig. 4) were developed, and the sporidesmin content of the cultures were assayed as described by Marasas *et al.*, (1972). (A summary of the findings is given in Table 2.)





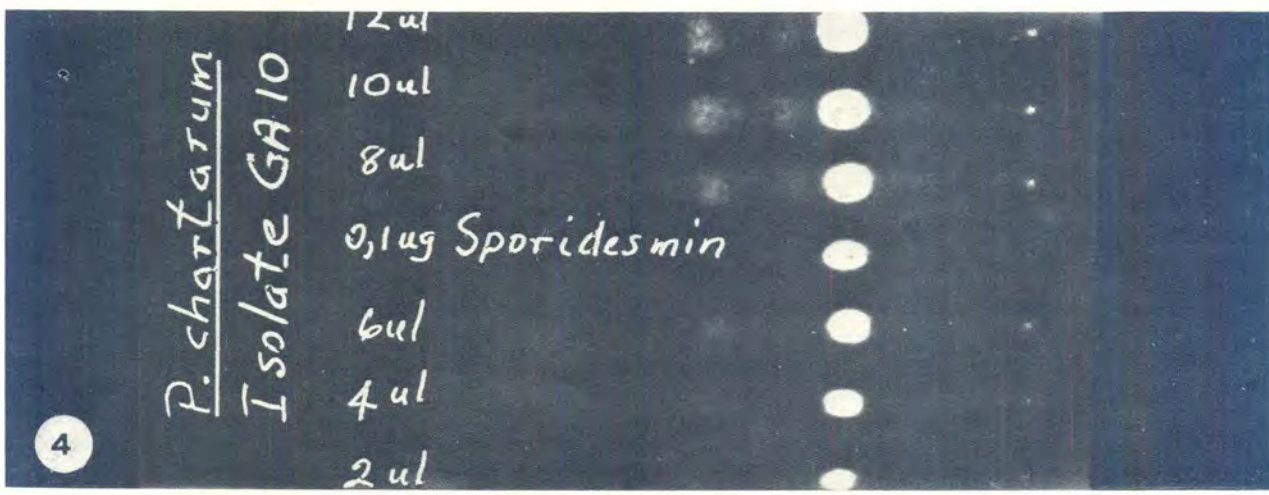


FIG. 2 Sporulating colonies of *P. chartarum* on a leaflet of *T. terrestris* from a toxic pasture after 7 days of incubation on PCA: ×50
FIG. 3 *P. chartarum* being cultured on semi-synthetic broth at 20 °C. Note the near ultraviolet light irradiation
FIG. 4 Thin layer chromatograph of sporidesmin in a culture of GA10
FIG. 5 A lamb (Sheep 1) being dosed with cultures of isolate GA10
FIG. 6 Photosensitivity (Sheep 3)

TABLE 2 The localities, substrate, number of samples examined, number of isolates of *P. chartarum* obtained and assayed for sporidesmin and their highest yield in pure culture

Locality	Substrate	Collection date	No. of samples	Isolates of *P. chartarum*	Isolates assayed	Number positive	Highest yield mg/ℓ
Aberdeen, C.P.	*Tribulus terrestris**	1972	6	2	2	1	0,2
	*T. terrestris**	1976	4	4	4	1	50,0
Carnarvon, C.P.	*T. terrestris**	1978	14	54	43	4	4,0
	Pasture debris*	1978	5	5	5	0	—
Cradock, C.P.	Pasture debris*	1977	5	16	—	—	—
Clocolan, O.F.S.	*Cynodon dactylon**	1978	1	1	1	0	—
De Aar, C.P.	*T. terrestris**	1976	15	5	5	0	—
		1976	6	0	—	—	—
Delmas, Tvl.	*Eragrostis curvula**	1973	1	4	4	0	—
	Niderella sp.*	1973	1	1	1	0	—
	Panicum sp.*	1973	1	2	2	0	—
Gobabis, S.W.A.	*Helichrysum* sp.	1975	1	3	1	0	—
Grootfontein, S.W.A.	*T. terrestris**	1973	1	1	1	0	—
	*T. terrestris**	1974	1	31	31	1	1,0
Humansdrop, C.P.	*Sporobolus capensis**	1970	1	1	1	1	35,0
	*Lolium perenne**	1970	1	2	2	1	6,0
Jansenville, C.P.	*T. terrestris**	1979	2	35	0	—	—
Johannesburg, Tvl.	*L. perenne**	1976	7	51	25	17	5,0
Kroonstad, O.F.S.	*T. terrestris**	1978	2	0	0	—	—
	*Panicum laevifolium**	1978					
Magaliesburg, Tvl.	*P. laevifolium**	1979	5	12	0	—	—
Middelburg, C.P.	*T. terrestris**	1976	9	1	1	0	—
	Pasture debris	1977	1	3	3	1	2,0
	Eragrostis lehmaniana	1978	1	2	0	—	—
	Melica decumbens	1978	1	1	1	1	7,0
	*T. terrestris**	1979	5	8	0	—	—
	Pasture debris*	1979	4	1	0	—	—
Murraysburg, C.P.	*T. terrestris**	1979	1	1	0	—	—
Piketberg, C.P.	*C. dactylon**	1975	2	18	15	13	60,0
Pretoria, Tvl.	*Cenchrus ciliaris*	1975	15	48	11	0	—
Richmond, C.P.	*T. terrestris**	1976	4	1	1	0	—
	*T. terrestris**	1979	2	3	0	—	—
	Pasture debris*	1979	1	1	0	—	—
Stellenbosch, C.P.	*L. perenne**	1975	5	13	6	2	0,4
	*L. perenne**	1978	2	5	0	—	—
Underberg	*Plantago* sp.*	1972	1	1	1	0	—

* Material examined was associated with photosensitivity in animals grazing the pasture

Results

From the results summarized in Table 2, it is evident that *P. chartarum* is widely distributed in southern Africa. The fungus was repeatedly isolated from *T. terrestris* as well as from other pasture plants, particularly when collected in camps where geeldikkop had occurred.

Less than one quarter of the 167 isolates assayed for sporidesmin were capable of producing this toxin in measurable quantities in culture. Toxigenic strains, therefore, appear to occur less frequently than non-toxigenic ones. Also, the ability of the toxigenic isolates to produce sporidesmin in pure culture varied more than a hundred fold. While the majority of toxigenic isolates yielded approximately 1 mg/ℓ of sporidesmin, only 3 isolates yielded more than 30 mg/ℓ. One of these was isolated from *T. terrestris* from the Karoo, another came from the cooler Humansdorp area, and the third from the south-western Cape Province which has a Mediterranean climate. All the toxigenic isolates were obtained from material that had been associated with outbreaks of photosensitization in farm animals. Some non-toxigenic isolates, however, also originated from this material.

These results further indicate that the method of direct plating of leaflets of *T. terrestris* and pasture debris on agar plates, followed by direct stereomicroscopic examination of the material for the presence of colonies of *P. chartarum*, is most suitable for the detection and isolation of this fungus on this material. The colonies did not spread onto the medium but remained confined to the plant material.

Discussion

P. chartarum occurs in the cool, relatively moist climate of the coastal belt, in the hot, semi-arid climate of the Karoo, in the warm temperate grasslands of the Transvaal Highveld and in the savannah of north-eastern South West Africa. This species is therefore adapted to growth under a wide range of conditions of temperature, moisture and substrate.

The association of *P. chartarum* with *T. terrestris* is an important finding which has not previously been reported. The occurrence of strains of this fungus capable of producing sporidesmin on *T. terrestris* in localities where photosensitization has occurred is of great significance as an indication of its possible role in the aetiology of geeldikkop.

2. The dosing of pure cultures of *P. chartarum* isolate GA10 to sheep

EXPERIMENT 1

Materials and Methods

Dosing regimen

In the first part of the experiment, cultures of this isolate were dosed per stomach-tube to locally bred Merino sheep at VRI, Onderstepoort (Table 3).

Sheep 1, a milk-tooth ewe lamb of 10 kg live mass, was dosed (Fig. 5) once with an amount of culture

material equivalent to approximately 2,0 mg/kg of sporidesmin, and twice with the equivalent of *c.* 1,0 mg/kg/day. An amount of culture material corresponding to *c.* 4 mg/kg was thus given to it in 3 days. It became photosensitive on the 10th day and was euthanized for necropsy on the 18th day.

Sheep 2, an 18 kg milk-tooth ram, was given culture material equivalent to *c.* 1,0 mg/kg of sporidesmin on 3 successive days, i.e. a total of *c.* 3,0 mg/kg. After becoming mildly photosensitive on the 9th day it died on the 20th day.

Sheep 3, a milk-tooth ram of 13 kg, was given the equivalent of *c.* 0,5 mg/kg, 1,5 mg/kg and 1,0 mg/kg, i.e. a total of *c.* 3,0 mg/kg of sporidesmin in 3 days. On the 10th day it was obviously photosensitive (Fig. 6) and on the 16th day it died.

Sheep 4, a ram lamb of 14 kg, was dosed with the equivalent of *c* 0,25 mg/kg/day for 8 successive days, which, in all, amounted to *c.* 2,0 mg/kg of sporidesmin. It died on the 12th day without having shown obvious signs of photosensitivity.

Sheep 5, a 13 kg milk-tooth ram, was treated as above. It was euthanized on the 12th day, also without becoming photosensitive.

Sheep 6, a 14 kg male lamb, was given *c.* 0,25 mg/kg/day of sporidesmin on 4 successive days, i.e. culture material equivalent to *c.* 1,0 mg/kg. It was euthanized *in extremis* on Day 12 without having become photosensitive.

Sheep 7, a 14 kg male lamb, also received the equivalent of *c.* 1,0 mg/kg of sporidesmin in doses of *c.* 0,125 mg/kg/day, 3 times per week (Mon., Wed. and Frid.). The course of 8 doses was completed in 16 days. On the 17th day it became photosensitive and on Day 46 it was euthanized for necropsy.

Results

The results are summarized in Table 3.

Clinical signs

At the above levels of dosing, some sheep died without becoming photosensitive (Table 3). Those that survived long enough to become photosensitive showed typical signs of hepatogenous photosensitivity, namely, avoidance of sunlight, inflammation of the facial skin and oedema of the eyelids, muzzle and ears. Depending on the length of exposure, the skin of the affected parts became parchment-like and sloughed off. The photosensitivity was invariably accompanied by icterus and coronitis. A number developed diarrhoea of sufficient severity to warrant treatment with electrolyte solutions, while some urinated frequently, indicating that the urinary bladders were also affected.

Chemical pathology

The activity of γ-GT and GOT was elevated in the serum. This was accompanied, in the longest lived cases, by the elevation of total bilirubin in the blood and the retention of phylloerythrin.

Pathology

Gross pathology.—In addition to the usual signs of photosensitization such as dermatitis, coronitis and icterus of varying degrees, lesions were seen in the livers of all the sheep.

The livers were usually normal or slightly larger than normal, yellow-brown in colour and distinctly lobulated. The surfaces were often uneven and frequently pitted with small shallow depressions. A few greyish-white foci, a few millimetres in diameter, were distributed throughout the parenchyma in the majority of cases. In addition, larger foci, measuring several centimetres in diameter and accompanied by fibrinous perihepatitis, were seen in Sheep 2. Occasionally, sparsely distributed, minute, yellow-green discolorations were evident both on the surface and in the substance of the livers. On cut surfaces the portal tracts were conspicuous and in the majority of cases the walls of the larger bile ducts were thickened by periductal fibrosis. Some of these bile ducts were plugged with inspissated bile and sometimes the bordering parenchyma was bile stained. No changes were present in the gall bladders of Sheep 6 and 7, but in the others lesions varying from oedema and haemorrhages to necrosis and erosion of the mucosa were visible.

TABLE 3 Dosing trials with cultures of a *P. chartarum* isolate (GA10) obtained from *T. terrestris* plants collected during an outbreak of ovine photosensitivity at Aberdeen in the Karoo. The trials were done at the VRI, Onderstepoort, using locally bred lambs

Sheep			Dosing regimen				Result
No.	Live mass (kg)	Age*	mg/kg/day × n**	Period over which dosing occurred (Day 0–Day n)	Total dosed mg/kg	Duration of experiment (days)	
1	10	m–t	2,0 × 1 1,0 × 2	0–2	4	18	Photosensitive. Euthanized
2	18	m–t	1,0 × 3	0–2	3	20	Mildly photosensitive. Died
3	13	m–t	0,5 × 1 1,5 × 1 1,0 × 1	0–2	3	16	Photosensitive. Died
4	14	m–t	0,25 × 8	0–7	2	12	Died
5	13	m–t	0,25 × 8	0–7	2	12	Euthanized *in extremis*
6	14	m–t	0,25 × 4	0–3	1	12	Euthanized *in extremis*
7	14	m–t	0,125 × 8	0–16	1	46	Photosensitive. Euthanized

* m–t=milk tooth

** mg/kg/day × n=Estimated intake of sporidesmin in mg/kg live mass/day × doses. The sheep were dosed either daily or 3 times per week, except on week-ends or public holidays

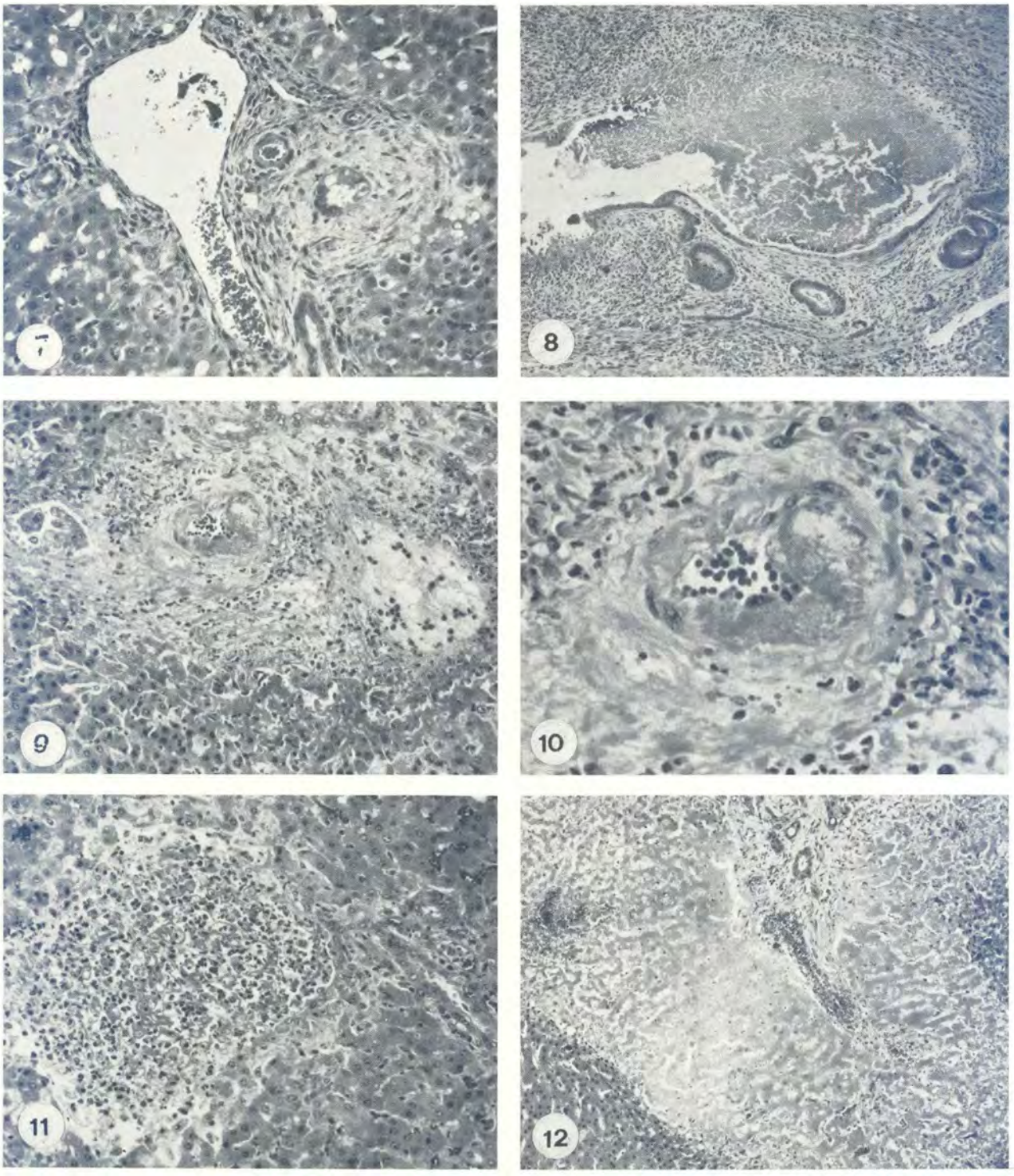


FIG. 7 Moderate portal reaction with pronounced periductal fibrosis and necrosis of the bile duct epithelium: HE×200
FIG. 8 Severe necrosis of a larger bile duct and surrounding tissue: HE×75
FIG. 9 Necrosis of a bile duct with thickening of the blood vessel wall in juxtaposition to it: HE×200
FIG. 10 Conspicuous thickening and necrosis of a blood vessel wall: HE×500
FIG. 11 Parenchymal infarct near portal triad. Note leakage of bile between the necrotic hepatocytes and polymorphonuclear cells: HE×200
FIG. 12 Infarct involving several contiguous lobules: HE×75

The kidneys of all the sheep were affected to some extent and showed changes such as enlargement, mottled discoloration, minute whitish spots in the cortex (tubular dilatation) and oedema of the pelvis. The wall of the urinary bladders of Sheep 1–6 were thickened and oedematous, and the mucosal surfaces blemished by haemorrhages and grey-brown eroded areas. No lesions were noticed in the bladder of Sheep 7.

The only other notable lesions were gastro-intestinal stasis, ascites, hydrothorax and hydropericardium.

Histopathology.—The histopathological lesions in the livers did not deviate from those described for facial eczema by Mortimer (1963) and Mortimer, White & Di Menna (1978). All the livers showed a moderate to marked portal reaction (Fig. 7) which comprised moderate bile duct proliferation and fibrosis

with slight mononuclear cell infiltration (lymphocytes, plasma cells and pigment-laden macrophages). The fibrosis was particularly pronounced around the bigger bile ducts and larger blood vessels (Fig. 7). Some bile ducts were necrotic (Fig. 7), were infiltrated with poly morphonuclear cells and were often associated with leakage of bile into the surrounding tissue. In advanced cases the bile ducts were completely obliterated with scar tissue.

The larger bile ducts, not associated with portal triads, were severely affected (Fig. 8), their lumina being often completely or partially occluded by plugs composed of inspissated bile, cellular debris and inflammatory cells. In these cases almost the entire wall of the bile ducts was necrotic, and periductal fibrosis and proliferation of granonulati tissue were particularly pronounced (Fig. 8 & 9). The ductal necrosis was so severe in some cases that it intruded into the adjacent granulation tissue. Bile often leaked into the surrounding tissue, causing necrosis of blood vessels (Fig. 9 & 10), sometimes with formation of thrombi or thickening of blood vessel walls in juxtaposition to the affected bile ducts (Fig. 10). As a result of these biliary and vascular changes, parenchymal infarcts were formed (Fig. 11). The infarcts, usually small, affected only segments of individual lobules, but in Sheep 2 they were much larger and involved numbers of contiguous lobules (Fig. 12). Apart from this, the parenchyma was minimally affected, having only minor changes such as cloudy swelling, hydropic degeneration and some fatty metamorphosis. The Kupffer cells were moderately activated and many contained yellow-brown pigments.

Bile stasis was not a conspicuous feature of the histopathology of the sheep in Experiment 1.

The histopathological lesions in the gall bladders of Sheep 1–5 included necrosis of the mucosa and part of the submucosa, necrosis of some blood vessels, and oedema. The eroded areas were frequently bile stained and infiltrated with neutrophils.

In the kidneys degenerative changes such as cloudy swelling and hydropic degeneration, sometimes with necrosis of individual tubules, were evident mainly in the cortex. The only other noteworthy changes were tubular dilatation, the presence of albuminous and bile casts, and yellow-brown pigmentation of the epithelium.

The urinary bladder of Sheep 7 was not affected, but in all the other sheep the following lesions were present: focal or diffuse necrosis of the mucosa and submucosa, frequently accompanied by vasculitis, haemorrhage, oedema and slight fibroplasia. Often the necrotic mucosa was stained with bile and infiltrated by neutrophils.

Discussion

Birefringent crystals were absent from the livers of all of the sheep. The microscopic picture tallied with that of facial eczema (Mortimer, 1963; Mortimer *et al.*, 1978) and could not be confused with geeldikkop (Theiler, 1918; Brown, Le Roux & Tustin, 1960; Van Tonder *et al.*, 1972).

The significance of this trial (with respect to the aetiology of geeldikkop) was that here, for the first time, a mechanism had been demonstrated by which *T. terrestris* could possibly cause ovine hepatogenous photosensitization.

EXPERIMENT 2

Experiment 2 was designed to determine whether the histopathological picture of facial eczema (as produced in Experiment 1) could be modified to resemble more closely that of geeldikkop, by varying the dosing regimen, diet, age and origin (Highveld or Karoo) of the experimental sheep.

Materials and Methods

Dosing regimen

(a) In the first part of this trial, sheep from Middelburg in the Karoo were dosed with cultures of the Karoo isolate (GA10) of *P. chartarum* immediately after their arrival at the VRI, Onderstepoort (Table 4).

Sheep 8, a full-mouth ewe of 44 kg live mass, was dosed culture material equivalent to *c.* 0,5 mg/kg of sporidesmin, followed by *c.* 0,125 mg/kg/day on the 3rd and 5th days. Thus a total of *c.* 0,75 mg/kg of sporidesmin was given to it in 5 days. On the 14th day it became photosensitive and on the 19th day it was euthanized for autopsy.

Sheep 9, a milk-tooth wether of 31 kg, received the equivalent of *c.* 0,25 mg/kg of sporidesmin on 4 consecutive days. As no response was elicited, an additional dose of *c.* 0,25 mg/kg was given to it on the 14th day. The sheep duly became photosensitive on the 31st day and was allowed to recover. After an interval of 116 days after the last dosing (i.e. on Day 130 of the experiment), the sheep was dosed again. This time it received *c.* 0,125 mg/kg daily for 5 days, after which dosing was interrupted for 2 days, and resumed at the same level for 3 days (Days 137, 138 and 139). In all, it was given *c.* 2,25 mg/kg of sporidesmin in 139 days. On the 140th day it died without having become photosensitive for a second time (Table 4).

Sheep 10, a milk-tooth wether of 33 kg, was dosed daily with *c.* 0,125 mg/kg of sporidesmin-equivalent for 5 consecutive days (Days 0–4). Thereafter, it was dosed 6 times more, i.e. on Days 6, 8, 11, 14, 16 and 18. On the 31st day it became photosensitive and was allowed to recover. No culture was administered from Days 18–130. Dosing was then resumed at *c.* 0,125 mg/kg daily for 5 days (Days 130–134), followed by a single dose on Day 137. The sheep became severely icteric and was euthanized *in extremis* on Day 144. A total of *c.* 2,125 mg/kg of sporidesmin divided into 17 doses was therefore administered to it during the 144 days of the trial (Table 4).

(b) Six Karoo sheep were dosed 3 times per week with cultures of GA10 at the Regional Veterinary Laboratory (RVL), Middelburg. Sheep 11–14 were on predominantly *T. terrestris* pastures and Sheep 15 and 16 on the natural veld containing almost no *T. terrestris* (Table 5).

Sheep 11, a full-mouth wether of live mass 32 kg, was kept on predominantly *T. terrestris* pastures and dosed with culture material equivalent to *c.* 0,25 mg/kg $\times$ 2 and *c.* 0,125$\times$3 in 9 days. A further dose equivalent to *c.* 0,125 mg/kg was given to it on the 16th day. The total amount of culture material received by this sheep was equivalent to *c.* 1,0 mg/kg sporidesmin divided into 6 doses and administered over 16 days. On the 27th day it became mildly photosensitive and on the 28th day it was euthanized for necropsy. A month after the experiment with Sheep 11 had ended, 2 more sheep (12 and 13) were placed on the *T. terrestris* pasture and dosed.

TABLE 4 Dosing trials with cultures of a *P. chartarum* isolate (GA10) obtained from *T. terrestris* plants collected during an outbreak of ovine photosensitivity at Aberdeen in the Karoo. The trials were conducted at the VRI, Onderstepoort, with sheep from the Karoo

Sheep			Dosage regimen				Result
No.	Live mass (kg)	Age*	Sporidesmin mg/kg/day × n**	Period over which dosing occurred (Day 0–Day n)	Total sporidesmin mg/kg	Duration of experiment (days)	
8	44	f–m	0,5 × 1 0,125 × 2	0–5	0,75	19	Photosensitive. Euthanized
9	31	m–t	0,25 × 4 0,25 × 1 0,125 × 8	0–3 14 130–139	2,25	140	Photosensitive. Died
10	33	m–t	0,125 × 5 0,125 × 6 0,125 × 6	0–4 6–18 130–137	2,125	144	Photosensitive. Euthanized

* f–m=full mouth
m–t=milk tooth
** mg/kg/day × n=Estimated intake of sporidesmin in mg/kg live mass/day × doses

TABLE 5 Dosing trials with cultures of a *P. chartarum* isolate (GA10) obtained from *T. terrestris* plants collected during an outbreak of ovine photosensitivity at Aberdeen in the Karoo. The trials were carried out at the Regional Veterinary Laboratory, Middelburg (C.P.), with Karoo sheep

Sheep			Dosing regimen				Grazing	Result
No.	Live (kg)	Age*	Sporidesmin mg/kg/day × n**	Period over which dosed (Day 0–Day n)	Total sporidesmin *c.* mg/kg	Duration of experiment (days)		
11	32	f–m	0,25 × 2 0,125 × 3 0,125 × 1	0–9 16	1,0	28	*T. terrestris*.....	Photosensitive
12	36	6T	0,5 × 1 0,125 × 5	0 3–12	1,125	24	*T. terrestris*.....	Photosensitive
13	39	6T	0,125 × 8	0–17	1,0	24	*T. terrestris*.....	Photosensitive
14	34	6T	—	—	—	—	*T. terrestris*.....	Not affected
15	41	f–m	0,25 × 2 0,125 × 3	0–9	0,875	28	Veld+*T. terrestris*....	Photosensitive
16	39		—	—	—	—	Veld+*T. terrestris*....	Not affected

* f–m=full mouth
6T=six tooth
** mg/kg/day × n=Estimated intake of sporidesmin in mg/kg/day × doses

Sheep 12, a 6-tooth wether of live mass 36 kg, was given the equivalent of *c.* 0,5 mg/kg of sporidesmin followed by 5 doses of *c.* 0,125 mg/kg (Days 3, 5, 7, 10 and 12), a total dose of *c.* 1,25 mg/kg of sporidesmin divided into 6 doses over 12 days. Sheep 12 became mildly photosensitive on the 17th day and was euthanized for necropsy on the 24th day.

Sheep 13, a 6-tooth wether of live mass 39 kg, was given the equivalent of *c.* 1,0 mg/kg of sporidesmin in 8 doses of *c.* 0,125 mg/kg each over 17 days. On the 17th day it became photosensitive, and on Day 24 it was euthanized.

Sheep 14, a full-mouth wether of live mass 34 kg, received no culture material, but otherwise received the same treatment as Sheep 11. It remained healthy throughout the experiment.

Sheep 15, a mature wether of 41 kg, penned on natural pasture with some *T. terrestris*, received the equivalent of *c.* 0,25 mg/kg×2 and *c.* 0,125 mg/kg× 3, a total of *c.* 0,875 mg/kg of sporidesmin divided into 5 doses over 9 days. On the 23rd day it became photosensitive and was euthanized for necropsy on Day 28.

Sheep 16, a full-mouth wether of 39 kg, was treated in the same manner as Sheep 15 except that it was not dosed. It showed no ill effects.

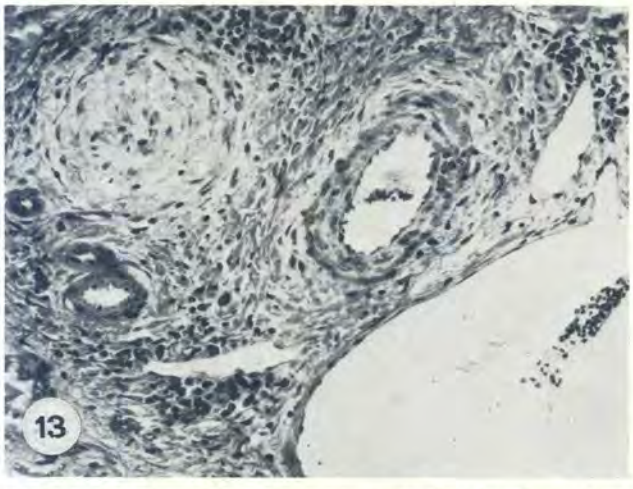
Results

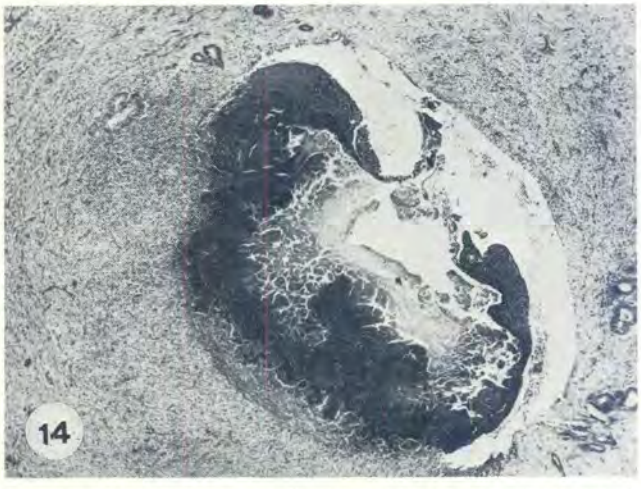
Clinical signs and chemical pathology

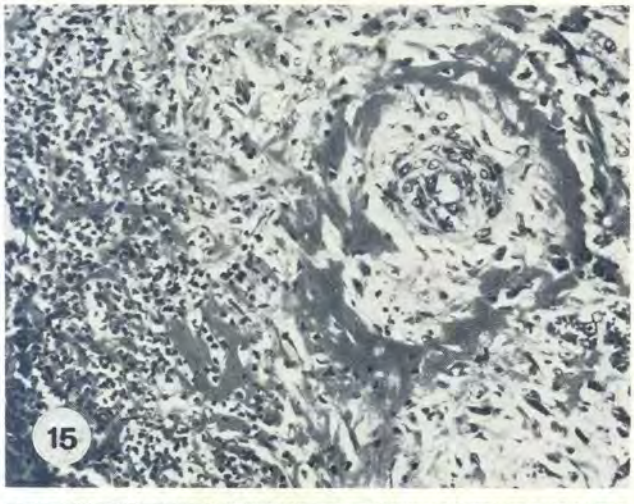
None of the sheep developed diarrhoea. Apart from this the clinical signs and chemical pathological changes of the sheep dosed in Experiment 2 were similar to those described in Experiment 1.

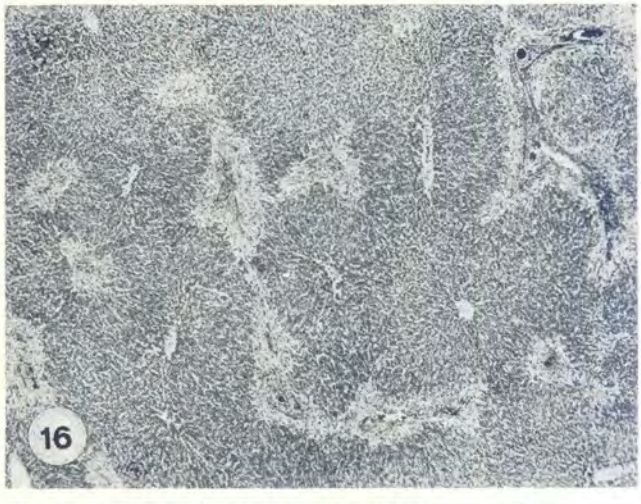
Pathology

Gross pathology.—All the dosed sheep had lesions in the livers which ranged from a slight brown discoloration, mild accentuation of the portal tracts and some thickening of bile ducts (Sheep 8), to more pronounced changes such as conspicuous portal fibrosis, nodular regeneration, and marked thickening of bile ducts (Sheep 10). In addition, bile stasis and bile thrombi were seen in many cases. The gall bladder of Sheep 8 appeared to be normal, but those of the others were affected to a greater or lesser extent by changes ranging from the presence of cloudy white areas on the mucosa (Sheep 12) to thickening of the walls coupled with haemorrhages and erosions in the mucosae. Erosions and haemorrhages were present also in the urinary bladder of all the sheep except Sheep 8 and 9, which were normal. Nephrosis was a common finding and some sheep had gastro-intestinal stasis as well.









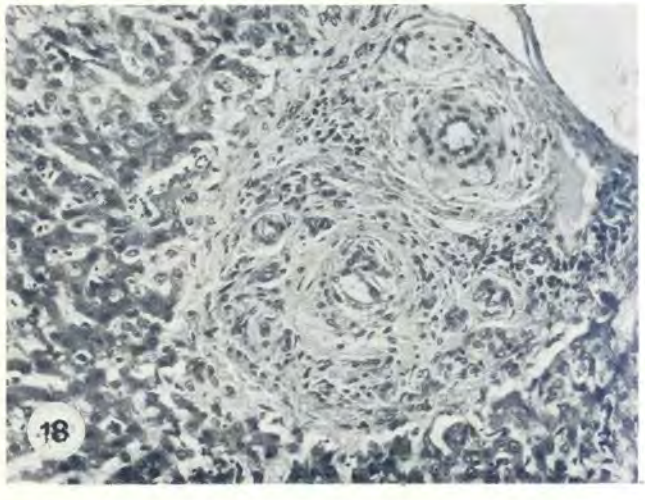


FIG. 13 Replacement of the entire bile duct with scar tissue: HE×200
FIG. 14 Pronounced necrosis of a larger bile duct and periductal granulation tissue: HE×30
FIG. 15 Perivascular oedema in the vicinity of a necrotic bile duct: HE×200
FIG. 16 Note severe portal reaction in Sheep 11: HE×30
FIG. 17 Severe portal reaction associated with pseudolobulation in the liver: HE×75
FIG. 18 Marked portal fibrosis. Note cholesterol-like clefts in bile duct: HE×200

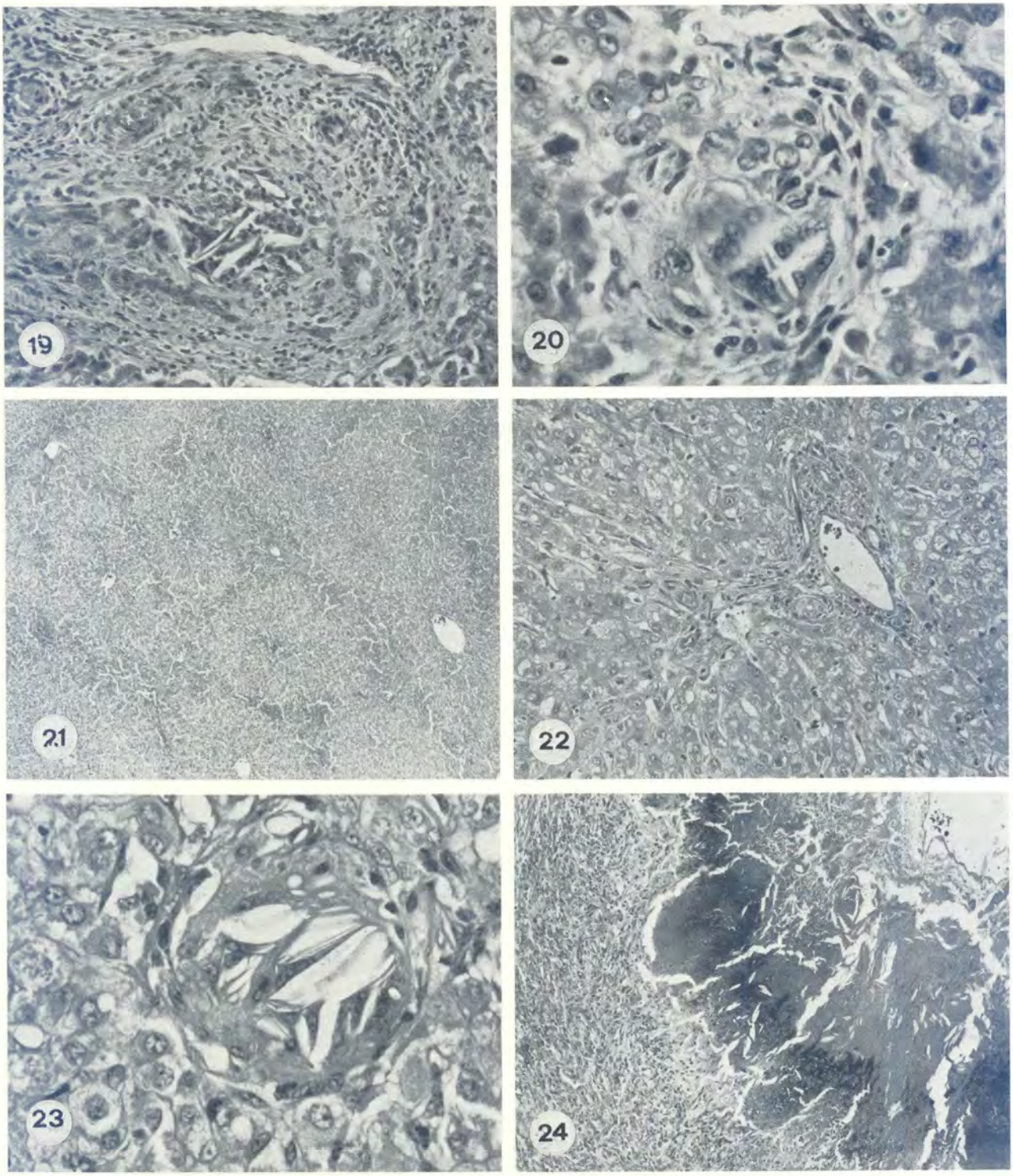


FIG. 19 Portal reaction with severe fibroplasia, moderate mononuclear cell infiltration, distortion of the bile ducts and many crystals in and around ducts: HE×75
FIG. 20 Crystals in a bile duct: HE×500
FIG. 21 Note mildness of the portal reaction in Sheep 12: HE×30
FIG. 22 Portal triad showing only a slight fibroplasia: HE×75
FIG. 23 Distortion of a bile duct with many crystals: HE×500
FIG. 24 Pronounced necrosis of larger bile duct. Note crystals between cellular debris: HE×75

A small area of degenerative change encompassing the caudate lobe in the liver of control Sheep 16 which grazed on the natural veld was an interesting lesion. Apart from this area the consistency and colour of the liver was normal. No obvious lesions were noticed in the liver of the *T. terrestris* control (Sheep 14).

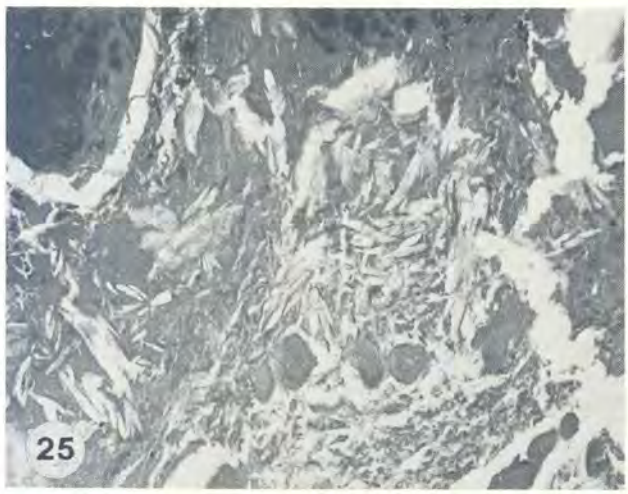
Histopathology

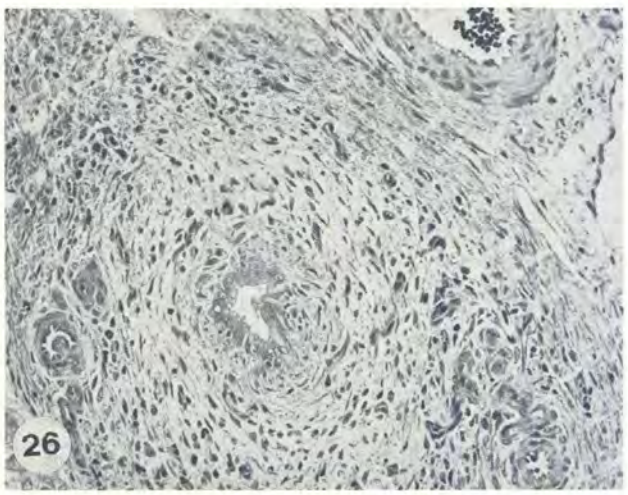
(a) *Karoo sheep dosed with cultures of GA*10 *at Onderstepoort.*—Sheep 9 was too decomposed for histopathological examination. The liver lesions of Sheep 8 corresponded to those described in Experiment 1. Especially conspicuous in this case was the replace-

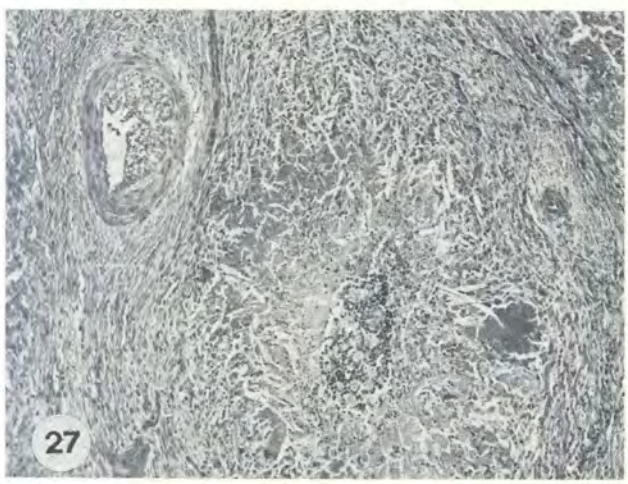
ment of entire bile ducts by scar tissue (Fig. 13) and necrosis of the larger bile ducts, sometimes associated with periductal and perivascular oedema (Fig. 14 & 15). In Sheep 10 the usual histopathological picture of facial eczema in the liver was distorted by additional lesions such as the replacement of groups of lobules by proliferating bile ducts, connective tissue, and pigment-laden macrophages. Disseminate centrilobular eosinophilic necrosis was evident in parts of the liver.

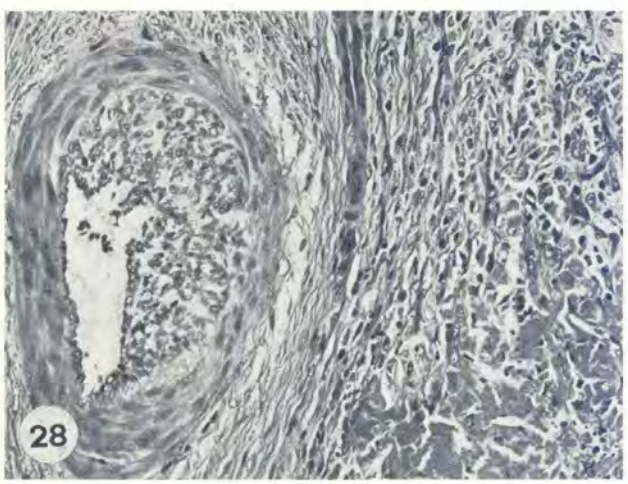
(b) *Karoo sheep dosed with cultures of GA*10 *at the RVL, Middelburg*.—In view of the importance of the histopathology in the diagnosis of ovine hepatogenous photosensitization, the principal features of each sheep will be discussed separately.

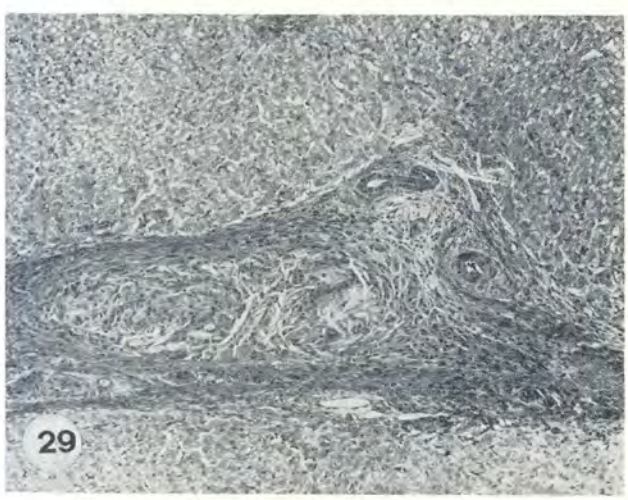
Sheep 11.—The most conspicuous change in the liver of Sheep 11 was a fairly severe portal reaction (Fig. 16), consisting of moderate fibrosis accompanied by bile duct proliferation and some necrosis of the biliary











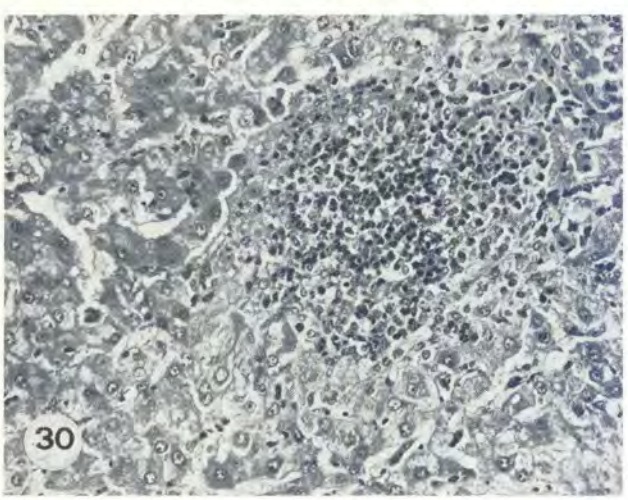


FIG. 25 Many crystals between cellular debris in necrotic bile duct: HE×200
FIG. 26 Prominent periductal fibrosis: HE×200
FIG. 27 Necrotic bile duct and subintimal thickening of the blood vessel wall in juxtaposition to it: HE×75
FIG. 28 Acentric subintimal thickening of the blood vessel wall in the vicinity of a necrotic bile duct: HE×500
FIG. 29 Portal tract. Bile duct containing crystals and conspicuous fibrosis around many of the bile ducts: HE×75
FIG. 30 Small periportal necrotic foci infiltrated by many inflammatory cells: HE×200

epithelium. These changes were sufficiently severe in places to form pseudolobules (Fig. 17). Crystals were seen in and around a few of the bile ducts (Fig. 18, 19 & 20) and many of the portal tracts were infiltrated by moderate numbers of mononuclear cells and a few pigment-laden macrophages (Fig. 19).

The changes in the parenchyma consisted of cloudy swelling, hydropic degeneration and necrosis of a few individual hepatocytes. Mild Kupffer cell proliferation had taken place and many of these cells as well as a few hepatocytes contained yellow-brown pigments.

The epithelial cells of the convoluted tubules of the cortex of the kidneys showed relatively mild degenerative changes (cloudy swelling and hydropic degeneration) and some contained yellowish-brown pigments. Often the tubules in the cortex were moderately dilated and a fair number contained albuminous or bile casts. No lesions were observed in the urinary bladder.

Sheep 12.—The portal reaction was similar to that of Sheep 11, but it was much milder; in fact, the exceptional mildness of the portal reaction (Fig. 21 & 22) was one of the principal features of this case. Crystals were again present in and around the bile ducts of a small percentage of triads (Fig. 23).

In contrast to the generally mild portal reaction, some of the larger bile ducts were severely necrotic and partially blocked with plugs of inspissated bile, cellular debris and large numbers of crystals (Fig. 25). Severe periductal and perivascular fibrosis was seen around some of the bigger ducts and vessels (Fig. 26). Another noteworthy lesion was the thickening of the walls of 2 blood vessels in juxtaposition to affected bile ducts (Fig. 27 & 28). This lesion appeared to be the chronic stage of the subintimal lesions described in facial eczema. The architecture of a small number of triads appeared to be disturbed (Fig. 29).

The parenchyma, generally speaking, was minimally affected, showing as it did, mild degenerative changes, necrosis of a few individual hepatocytes and infrequently occurring small periportal necrotic foci (Fig. 30).

Lesions in the gall bladder were similar to but more severe than those of Sheep 11. In addition, the wall of the bladder was thickened as a result of oedema and fibrosis of the submucosa. Areas of necrosis with haemorrhage and neutrophil infiltration were present on the mucosal surface.

Sheep 13.—The portal reaction was similar to but slightly more severe than that of Sheep 12 (Fig. 32). Again, pronounced periductal and perivascular fibrosis was noticeable around the larger ducts and vessels. Crystals of varying sizes and shapes (cigar, rod, and filamentous with sharp points) were present in or around some of the bile ducts (Fig. 33, 34 & 35). Uneven proliferation and necrosis of the ductular epithelium were commonly associated with these crystals. Frequently it even appeared as though the epithelium was actively proliferating on their surfaces. Sometimes bile leaked out of affected ducts into the surrounding tissue, causing necrosis and oedema of the triads as well as focal necrosis of the bordering hepatocytes (Fig. 35).

The changes in the parenchyma were similar to those already described for Sheep 11 and 12.

Lesions akin to those of Sheep 11 and 12 were noticed in the gall bladder, kidney and urinary bladder. In addition, the blood vessels in the submucosa of the urinary bladder revealed fibrinoid degeneration and necrosis (Fig. 36).

Sheep 15.—A moderate to severe portal reaction, similar to that of the other dosed sheep, was present (Fig. 39). Other prominent changes included the presence of many macrophages (in the portal triads) and Kupffer cells filled with greyish-brown pigments, and crystals in a few of the bile ducts (Fig. 40). The usual minimal changes were present in the parenchyma of the liver, the kidneys were mildly affected in the way previously described, no lesions were evident in the urinary bladder and the gall bladder was too autolyzed for examination.

Sheep 14.—A small focal lesion was observed in the otherwise normal tissue of the liver of this control animal (Fig. 37). This lesion consisted of a severe portal reaction, which included bile duct proliferation, portal fibrosis and mononuclear cell infiltration interspersed with many macrophages containing yellowish-brown or grey-brown pigments. A notable feature was the presence of a fair number of crystals in and around some of the bile ducts in the affected area (Fig. 38). No lesions were seen in the other organs.

Sheep 16.—The pathological changes in the liver of this control animal were limited to a localized area encompassing the caudate lobe. The lesions consisted of a severe portal reaction, which comprised bile duct proliferation, fibrosis (Fig. 41) especially around the larger bile ducts and blood vessels, and infiltration by a few macrophages containing yellow-brown pigments. A number of crystals were present in the bile ducts (Fig. 42). The epithelial cells of some bile ducts were necrotic and in others the biliary epithelium had undergone such marked proliferation that it appeared to be composed of giant cells.

Discussion

The pathological changes in the livers of sheep affected with facial eczema (Done, Mortimer & Taylor, 1960; Crawley, Mortimer & Smith, 1961; Mortimer, 1963) and geeldikkop (Theiler, 1918; Brown *et al.*, 1960; Van Tonder *et al.*, 1972) are well known. According to Van Tonder *et al.*, (1972) the typical billiary and vascular necrosis seen in facial eczema is absent in geeldikkop and the characteristic microscopic lesion of geeldikkop, which distinguishes it from other disease entities such as facial eczema and enzootic icterus, is the presence of polarizing, crystalloid, cholesterol-like material in the hepatocytes, Kupffer cells and the bile ducts, the latter being frequently occluded by it. Theiler (1918) referred to them as 'tyrosin crystals' but their exact composition has never been established. Limited chemical analyses have shown that the crystals do not consist of cholesterol, cholic acid, sodium glycocholate or sodium taurocholate (Dr L. A. P. Anderson, VRI, Onderstepoort, personal communication, 1978). According to Smith, Jones & Hunt (1972), birefringent crystals can result from the precipitation of insoluble cholesterol-like salts during cholestasis. The experimental production of crystalline precipitates in bile canaliculi by the administration to rats of monohydroxy bile salts is reported by Miya, Richardson, Mayr & Javitt (1977) and the mechanism by which these precipitates develop is discussed by Schaffner & Popper (1969).

Although the presence of hepatic birefringent crystals is not a feature unique to geeldikkop, it is rarely seen in other conditions. In Texas, crystals have been reported in ovine *Agave lechuguilla* (Mathews, 1938) and *Nolina texana* (Mathews, 1940) poisoning, and in New Zealand in sheep suffering from a hepatogenous photosensitivity syndrome caused by intoxication with

Panicum miliaceum (Clare, 1952). The only other ovine disease in which crystals occur is dikoor (*Panicum* photosensitization). This disease differs from geeldikkop only in severity, dikoor purportedly being a milder syndrome (Van Tonder *et al.*, 1972).

Birefringent crystals have never been described in facial eczema either in New Zealand (Mortimer *et al.*, 1978), in natural outbreaks of the disease in South Africa, or in sheep dosed with the Humansdorp isolate (OP 9) of *P. chartarum* (Marasas *et al.*, 1972).

Seen against this background, the demonstration of crystalloid material in the livers of sheep suffering from experimentally induced facial eczema in the Karoo is of considerable importance. Moreover, the significance of this finding is heightened by the fact that the sheep were dosed with cultures of a strain (GA10) of

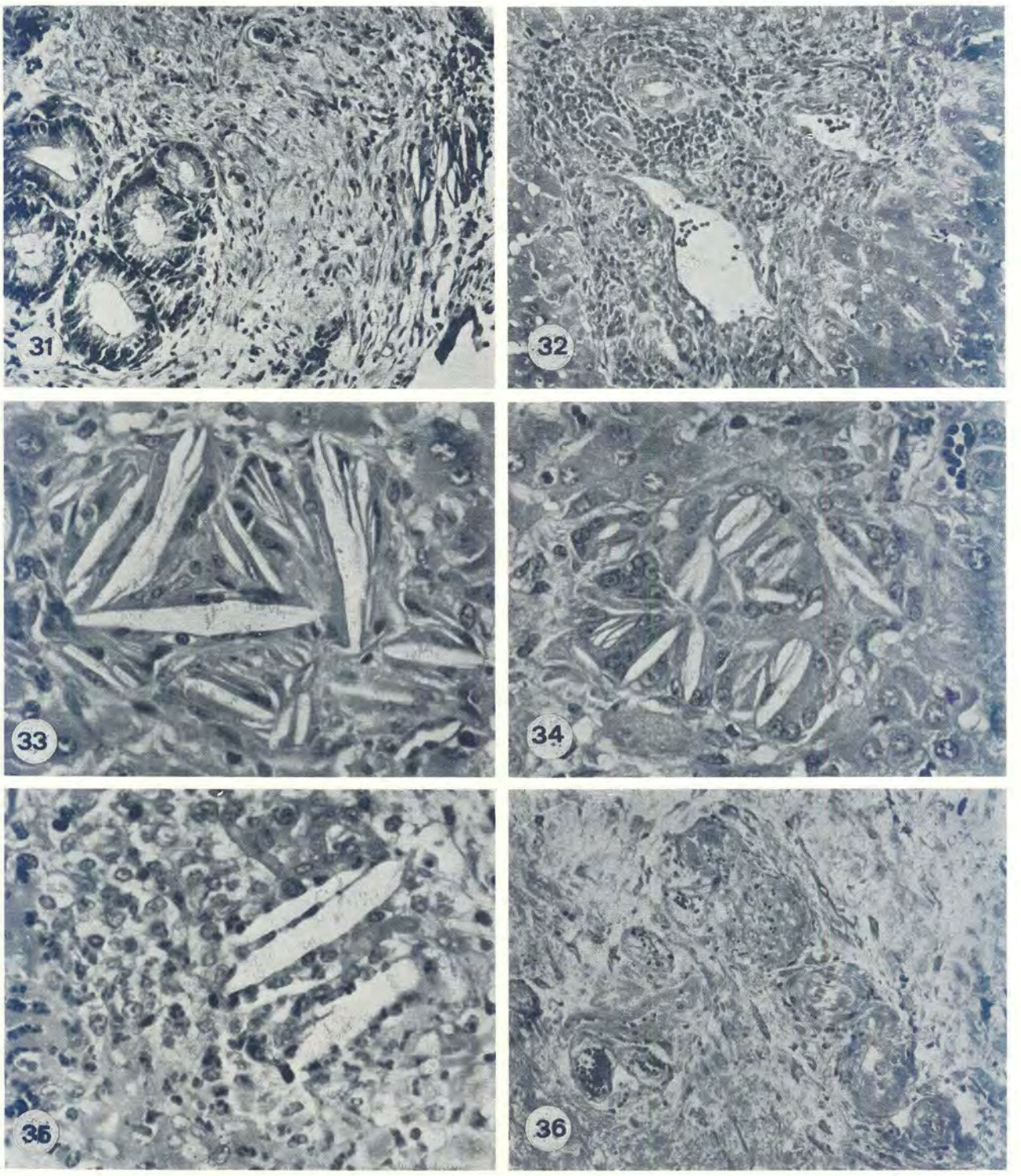
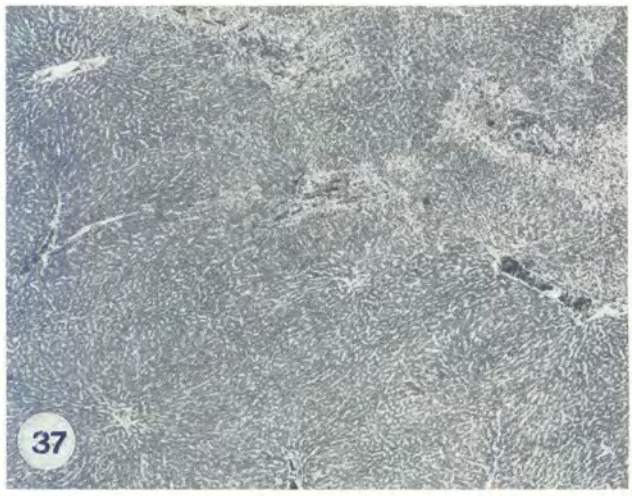
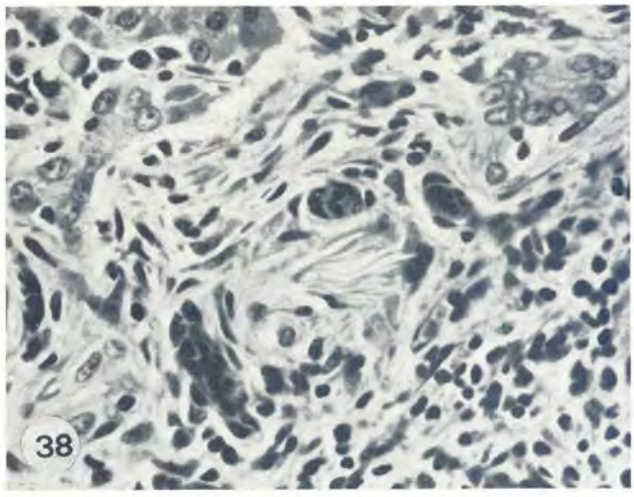
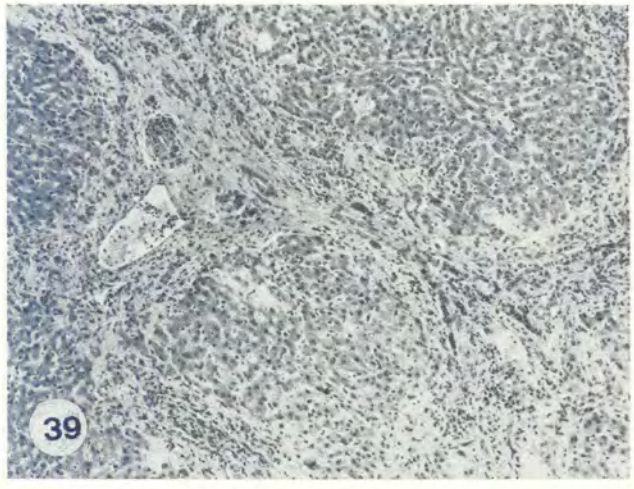
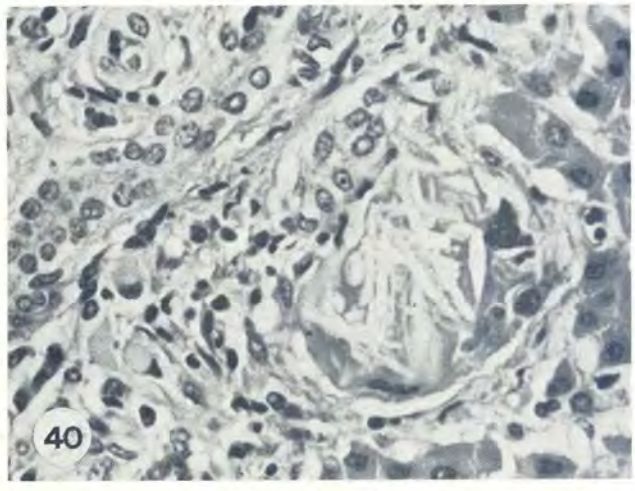


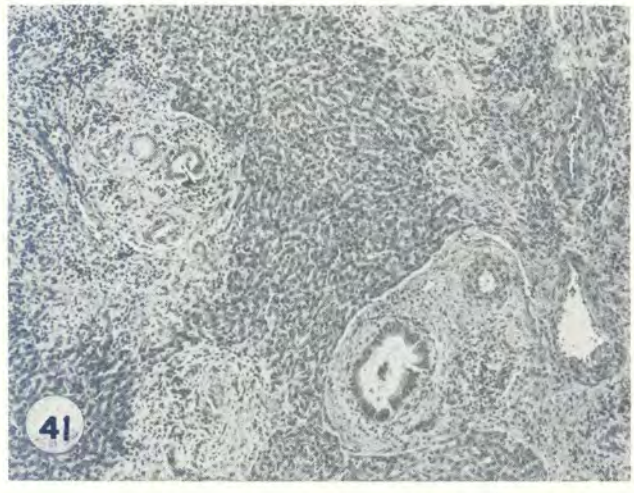
FIG. 31 Crystals in mucosa of gall bladder: HE×200
FIG. 32 Portal triad with many mononuclear cells and a moderate fibroplasia: HE×200
FIG. 33 & 34 Distorted bile ducts containing many crystals of varying size and shape. Note uneven proliferation of the bile duct epithelium in Fig. 34: HE×500
FIG. 35 Leakage of bile from a bile duct containing crystals into the surrounding tissue. Note parenchymal necrosis, oedema and polymorphonuclear cell infiltration in the necrotic area: HE×500
FIG. 36 Urinary bladder. Fibrinoid degeneration and necrosis of the blood vessels in the submucosa: HE×200











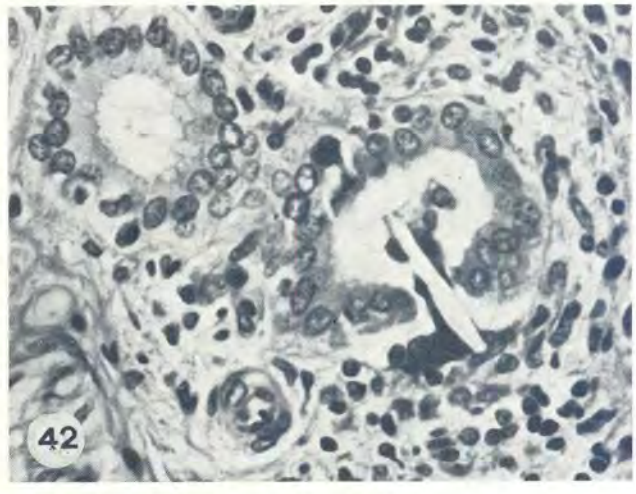


FIG. 37 Focal area with severe portal reaction in top right corner: HE×30
FIG. 38 Note fibroplasia, mononuclear cell infiltration and bile duct containing crystals: HE×500
FIG. 39 Severe portal reaction resulting in pseudolobulation: HE×75
FIG. 40 Bile duct blocked with crystals. Note pigment-laden macrophages in the portal triad: HE×500
FIG. 41 Very pronounced portal reaction: HE×75
FIG. 42 Rod-shaped crystal accompanied by uneven epithelial proliferation in a bile duct: HE×500

P. chartarum isolated from *T. terrestris* associated with an outbreak of geeldikkop. If the crystals were indeed induced by the culture, the question arises whether the ability to form them was a unique property of the Karoo isolate. All the evidence indicates otherwise, however, as crystals were not formed in the livers of either Karoo or Highveld sheep dosed with GA10 at Onderstepoort. Since Karoo sheep at the VRI, Onderstepoort, failed to develop crystals it was surmised that the formation of crystals was dependent on an unknown environmental factor(s) in the Karoo, and that this factor was probably *T. terrestris*.

Van Tonder *et al.* (1972) observed that the crystalloid material in geeldikkop was located in hepatocytes, Kupffer cells and bile ducts. It was not known whether

the more limited distribution of crystalloid material (i.e. confined to the portal tracts and bile ducts in these cases) was a significant difference or not. Neither was it known whether the crystalloid material in the experimental animals differed from that of geeldikkop, as the chemical and physical properties of the crystalloid material in geeldikkop had never been defined.

One of the most interesting aspects of the entire investigation was the occurrence of crystals in both control animals. Crystalloid material was seen in a focal lesion (thought to be either parasitic in origin or a biliary infarct) in the otherwise normal liver of the *T. terrestris* control (Sheep 14). The presence of crystals in such a focal lesion suggested that more than one agent, acting in conjunction with *T. terrestris*, could be responsible for their formation. A localized area of portal change was also observed in the caudate lobe of the veld control (Sheep 16). In this case, apart from the localized distribution and more severe nature of the lesions, they were similar to those of the dosed sheep in all respects, including the presence of crystals.

The possibility now had to be considered whether the sheep in the experiment had suffered from subclinical geeldikkop. Militating against this theory were the normal levels of γ-GT activity in the sera of the control sheep, which virtually precludes the possibility of a subclinical attack having taken place during or even some weeks before the experiment (Malherbe, Kellerman, Kriek & Haupt, 1977). Moreover, it would be very unusual for the lesions of geeldikkop to be locally distributed as in Sheep 16. The fact that geeldikkop had not been reported at Grootfontein in 7 years and that no sheep on the property or its environs had become clinically affected with geeldikkop during the season further reduced the probability of the experimental sheep having been subclinically affected with the disease.

When the crystals are considered in conjunction with the other liver lesions, the following tentative conclusions can be drawn:

Although the livers of Sheep 11 and Sheep 15 contained elements of the pathological changes usually associated with geeldikkop (e.g. crystals), the histopathological picture as a whole resembled that of facial eczema rather than of geeldikkop. This opinion was based on the relative severity of the portal reaction which resulted in the formation of pseudolobules in some places (Sheep 11, Fig. 17). In the liver of Sheep 12 the histopathological characteristics of both diseases were more or less equally balanced. This could be seen from the infrequently occurring subintimal lesions in the blood vessels (Fig. 28) and the relatively severe fibrosis around some of the large bile ducts (Fig. 26), as in facial eczema. These were coupled with a mild portal reaction in the majority of triads (Fig. 21 & 22) and crystals in some bile ducts and portal tracts (Fig. 23), as in geeldikkop. Some larger bile ducts were also necrotic (Fig. 24), a feature of facial eczema, but contained many crystals (Fig. 24 & 25), a feature of geeldikkop.

The 4th sheep (Sheep 15) had a moderate to severe portal reaction and crystals, but the severe reaction in the larger bile ducts and the subintimal thickening of the blood vessels were absent. In this sheep it therefore appeared that the emphasis of the liver lesions had shifted slightly in favour of geeldikkop.

As can be expected in facial eczema, gall and urinary bladder lesions were present in Sheep 10, 12 and 15. Crystals in the mucosa and submucosa of the affected gall bladders, however, were a unique finding. Perhaps it is pertinent to point out that gall and urinary bladder lesions were not invariably present in sheep dosed with cultures of GA10.

EXPERIMENT 3

From these trials it appeared probable that lesions indistinguishable from those of geeldikkop could be induced in the livers of sheep in the Karoo by judiciously dosing them with cultures of isolate GA10. A new series of trials was therefore designed to test this hypothesis.

Materials and Methods

Dosing regimen

In the foregoing experiments the sheep were dosed with sufficient culture to elicit a clinical response, i.e. either photosensitivity or death. This meant that relatively high doses of culture (equivalent to *c.* 0,75—*c.* 4,0 mg/kg of sporidesmin) had to be given. In the new trial, however, the dosage rate was reduced to a more realistic level so that, as in a natural outbreak, only a proportion of the flock would become photosensitive. The sheep were dosed with a culture of GA10 equivalent to the estimated amounts of sporidesmin ingested during natural outbreaks of facial eczema in New Zealand, namely, 0,4–0,7 mg/kg of sporidesmin (Mortimer *et al.*, 1978; White, Mortimer & Di Menna, 1978). In addition to this, a new, supposedly subclinical dosing level, equivalent to *c.* 0,25 mg/kg of sporidesmin, was introduced. By dosing them at these levels we intended to induce more meaningful pathological lesions.

A flock of 36 full-mouth Merino ewes (average live mass 42 kg) and 34 milk-tooth Merino wethers (average live mass 25 kg) was divided into 2 unequal groups (Table 6). The smaller group was penned on a pasture composed almost exclusively of *T. terrestris*, which was occasionally irrigated, and the other group on non-irrigated veld with almost no *T. terrestris*. Both groups were placed in their respective camps 1 week before the experiment started in order to allow them to adapt themselves to the grazing.

(a) *Sheep on predominantly* T. terrestris *pastures* (*Table* 6). Four lambs were dosed with cultures equivalent to *c.* 0,25 mg/kg of sporidesmin, 3 ewes with the equivalent of *c.* 0,4 mg/kg, 1 lamb and 2 ewes with *c.* 0,5 mg/kg, and 1 lamb and 2 ewes with *c.* 0,7 mg/kg. One sheep in each of the subgroups *c.* 0,4–0,7 mg/kg received half the dose on 2 consecutive days; the others were given a single dose.

Four lambs and 5 ewes were left undosed as controls but otherwise they were treated exactly like the dosed sheep.

The sheep that reacted were necropsied within 3 days of becoming photosensitive and those that did not become photosensitive on the 22nd day.

In this experiment 13 sheep were dosed and 9 were kept as controls.

(b) *Sheep on veld with almost no* T. terrestris (*Table* 6). Five lambs and 2 ewes received culture material equivalent to *c.* 0,25 mg/kg of sporidesmin; 3 ewes the equivalent of *c.* 0,35 mg/kg; 3 lambs and 4 ewes the equivalent of *c.* 0,4 mg/kg; 2 lambs and 4 ewes the equivalent of *c.* 0,5 mg/kg; and 3 lambs and 1 ewe the equivalent of *c.* 0,7 mg/kg. Four sheep in the *c.* 0,4 mg/kg subgroup and 2 sheep in the *c.* 0,5 mg/kg subgroup were given a divided dose (Table 6). One of the sheep (V24) in the *c.* 0,5 mg/kg subgroup disappeared without trace on the 15th day.

Nine lambs and 12 ewes in the flock remained undosed as controls. The 2 sheep that reacted were necropsied within 2 days of becoming photosensitive; the rest, when the trial was terminated (Day 25).

Altogether 27 sheep were dosed and 21 were kept as controls.

Results

Clinical signs

All the sheep that reacted clinically (Table 6) had typical signs of hepatogenous photosensitivity as described before. The sheep on *T. terrestris* became photosensitive on the specified days after dosing: Sheep T2 on the 12th day, Sheep T4 on the 11th day (*c.* 0,25 mg/kg subgroup); Sheep T21 on the 11th day, Sheep T23 on the 12th day (*c.* 0,5 mg/kg subgroup); Sheep T31 on the 11th day, Sheep T33 on the 14th day (*c.* 0,7 mg/kg subgroup). One control became photosensitive on the 7th day, i.e. 14 days after being placed on the *T. terrestris* pasture. Altogether 6 out of 13 dosed sheep and 1 out of 9 controls on *T. terrestris* became photosensitive.

Two out of the 27 dosed sheep on the veld, namely, V26 and V38, became photosensitive on the 24th and 23rd days respectively. They received culture material the equivalent of *c.* 0,5 mg/kg and *c.* 0,7 mg/kg of sporidesmin respectively.

None of the 21 controls reacted.

Chemical pathology

The γ-GT activity in the sera of the dosed sheep on *T. terrestris* was elevated, the greatest elevation being recorded in those that became photosensitive (156–402 mIU/mℓ). The activity in the subclinical cases varied between 69–151 mIU/mℓ. Apart from the one control sheep that became photosensitive, none of the undosed sheep showed conspicuous changes in their γ-GT activity.

In the veld trial, the γ-GT activity of the dosed sheep was noticeably elevated in Sheep V19, V26, V30, V36 and V38 (107–348 mIU/mℓ). No conspicuous changes occurred in the γ-GT activity of the control sheep.

Pathology

(i) *Gross pathology*

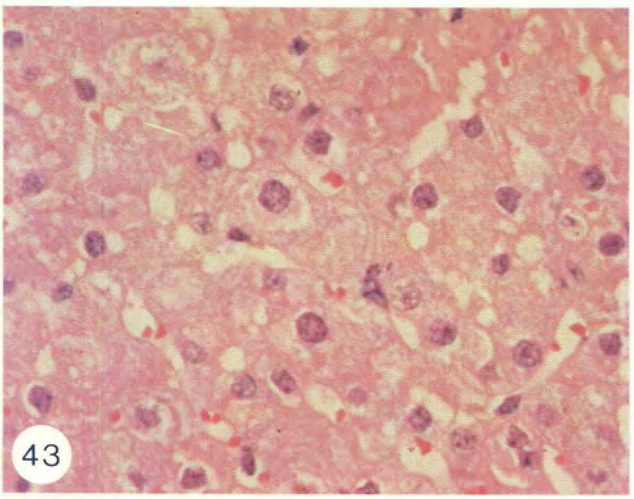
(a) *Sheep on predominantly* T. terrestris *pastures.* Generally speaking the pathological changes in the livers of the sheep that became photosensitive were relatively inconspicuous. The livers were usually either normal in size or slightly swollen, with normal consistency. They were yellowish-brown to khaki-brown in colour and the lobulation was accentuated. In a few instances, at the higher doses, the larger bile ducts traversing the liver were slightly more prominent than normal. The *ductus cysticus* and frequently the bigger bile ducts contained a fine chalky white deposit (crystals) that oozed out with the bile when the liver was incised. The wall of the *ductus cysticus* was invariably oedematous and sometimes slightly thickened, and large quantities of the fine crystalline deposit were visible on the mucosal surface.

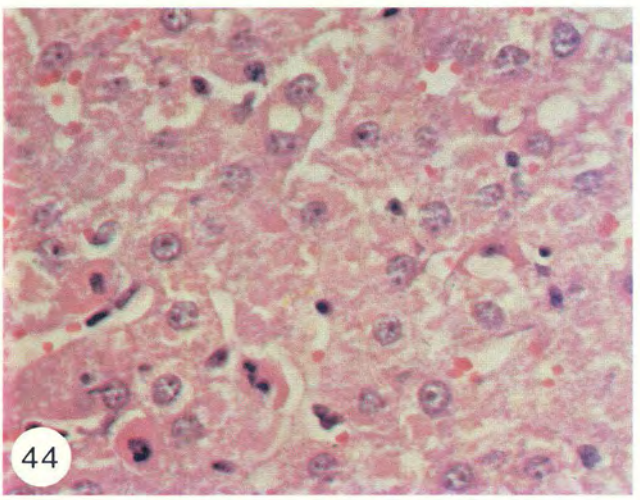
In addition to the above changes, shallow, focal or localized, yellowish-brown sunken areas of varying size were often seen in the liver, especially in the caudate lobe or near to it. The lobulation in these focal areas was much more prominent than in other areas. Indications of parasitic infestation (such as localized

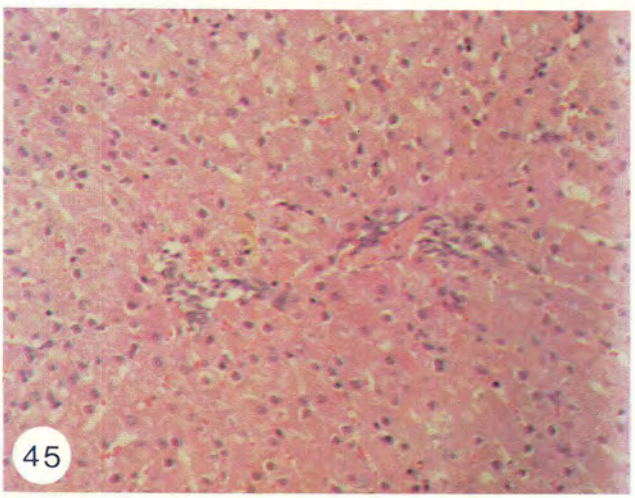
TABLE 6 Dosing trials in sheep at Middelburg (CP) with cultures of *P. chartarum* isolate GA10 at levels estimated to cause facial eczema in New Zealand

	Dosing regimen Sporidesmin equivalent (mg/kg)															
	0,25 Sheep		0,35 Sheep		0,4 Sheep		0,5 Sheep		0,7 Sheep		Control Sheep					
	Age	No.	Age	No.	Age	No.	Age	No.	Age	No.	Age	No.	Age	No.	Age	No.
T. terrestris..	L	T1	—	—	A	T11	**L**	**T21**	**L**	**T31**	L	T41	L	T51	L	T61
T. terrestris..	**L**	**T2**	—	—	A	T12	A	T22	A	T32	A	T42	A	T52	A	T62
T. terrestris..	L	T3	—	—	A	T13*	**A**	**T23***	**A**	**T33***	A	T43	A	T53	**L**	**T63**
T. terrestris..	**L**	**T4**	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Veld.....	L	V5	A	V37	L	V14	A	V24	L	V34	L	V44	L	V54	L	V64
Veld.....	L	V6	A	V39	L	V15*	A	V25	L	V35	L	V45	L	V55	L	V65
Veld.....	L	V7	A	V40	L	V16*	**A**	**V26***	L	V36	L	V46	L	V56	L	V66
Veld.....	L	V8	—	—	A	V17	A	V27*	**A**	**V38**	A	V47	A	V57	A	V28
Veld.....	L	V67	—	—	A	V18	L	V29	—	—	A	V48	A	V58	A	V68
Veld.....	A	V10	—	—	A	V19*	L	V30	—	—	A	V49	A	V59	A	V69
Veld.....	A	V10	—	—	A	V20*	—	—	—	—	A	V50	A	V60	A	V70

L=Lamb; A=Adult; **T2**=Bold figures indicate photosensitive sheep; *=Divided dose







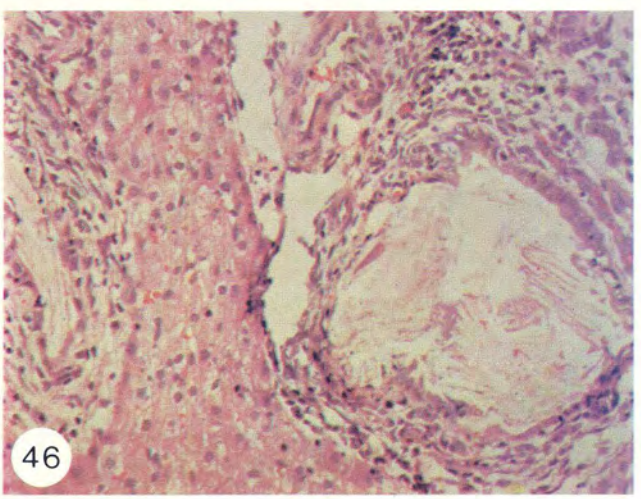
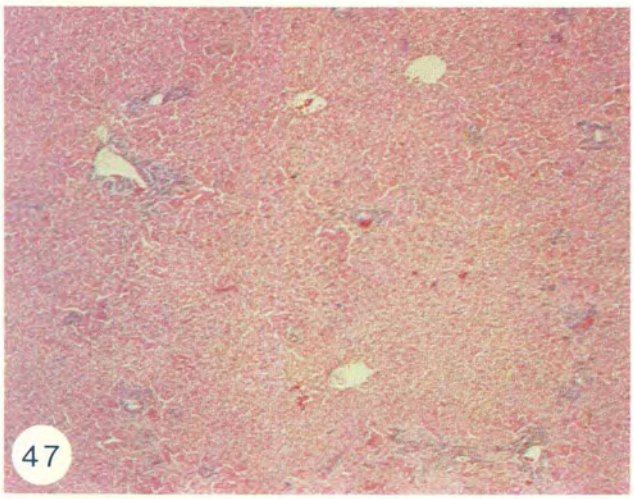
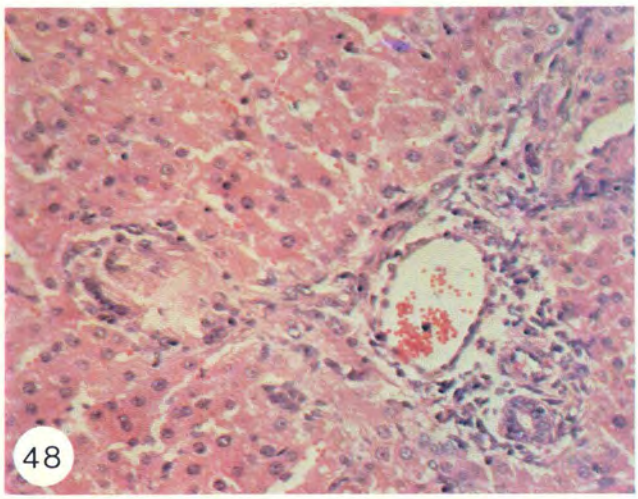
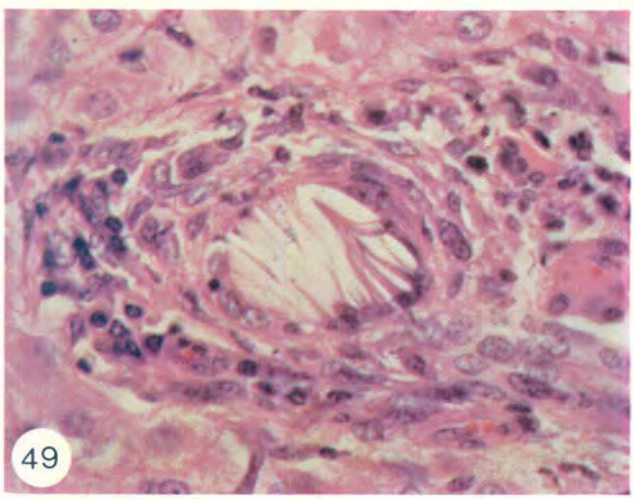
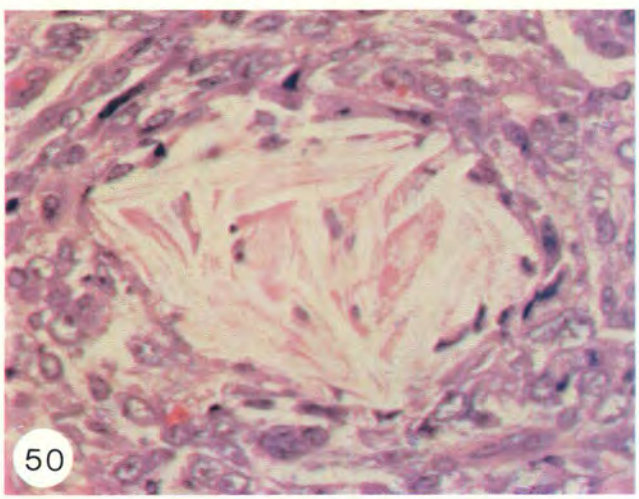


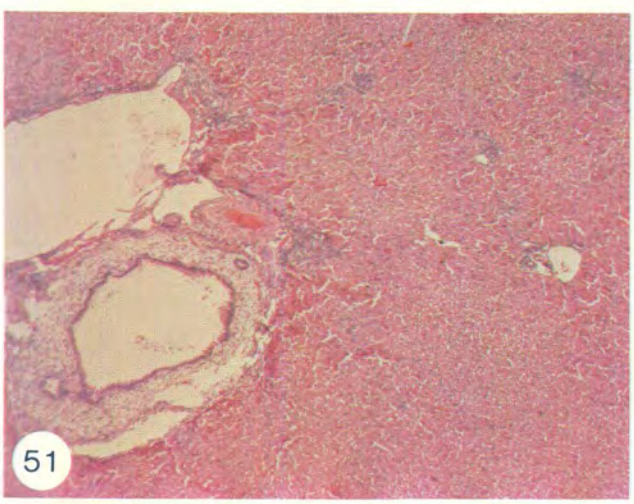
FIG. 43 & 44 Marked degenerative changes of the parenchyma in the liver of Sheep T4: HE×500
FIG. 45 Note that the portal triads are minimally affected: HE×200
FIG. 46 Major bile duct blocked with crystals: HE×200











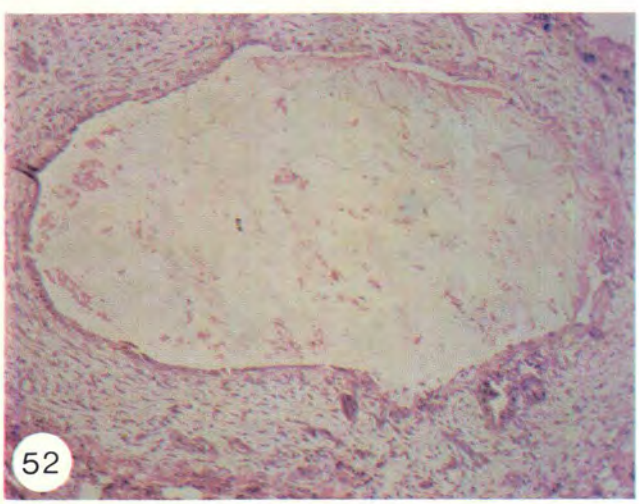


FIG. 47 General appearance of the liver in Sheep T33. Note moderate portal reaction: HE×30
FIG. 48 Moderate portal reaction. Bile duct containing crystals: HE×200
FIG. 49 & 50 Bile ducts almost completely blocked with crystals. Note degenerative and necrotic changes of the lining epithelium in Fig. 50: HE×500
FIG. 51 Slight fibroplasia around larger bile duct: HE×30
FIG. 52 *Ductus cysticus* containing many crystals. Moderate periductal oedema and fibrosis: HE×75

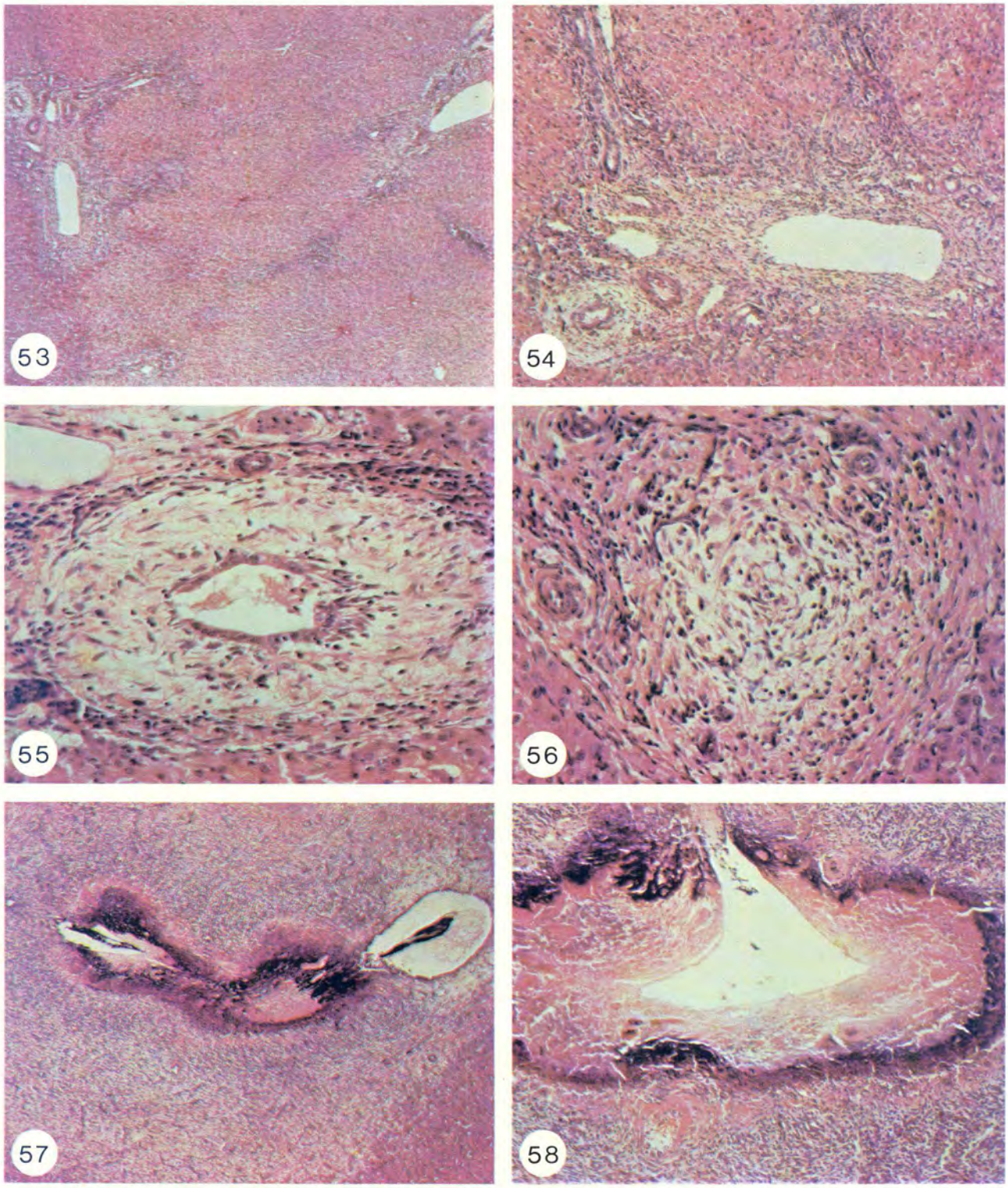
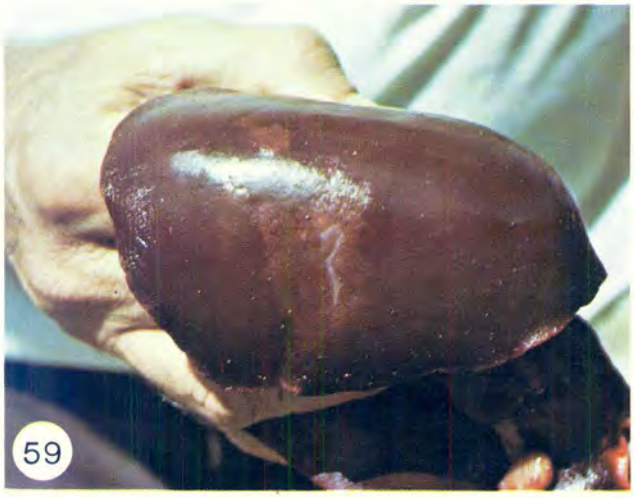
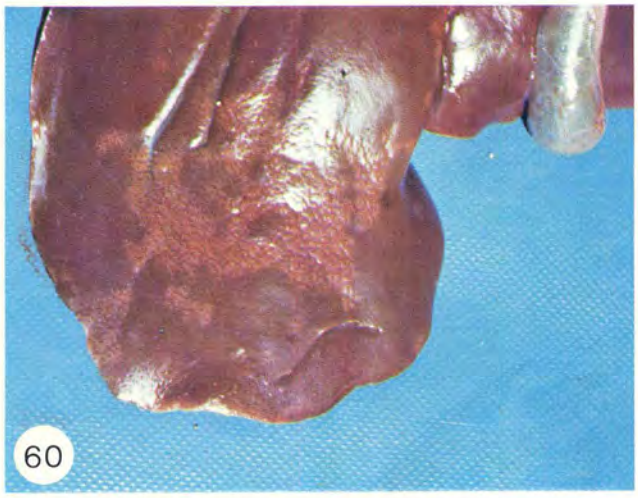
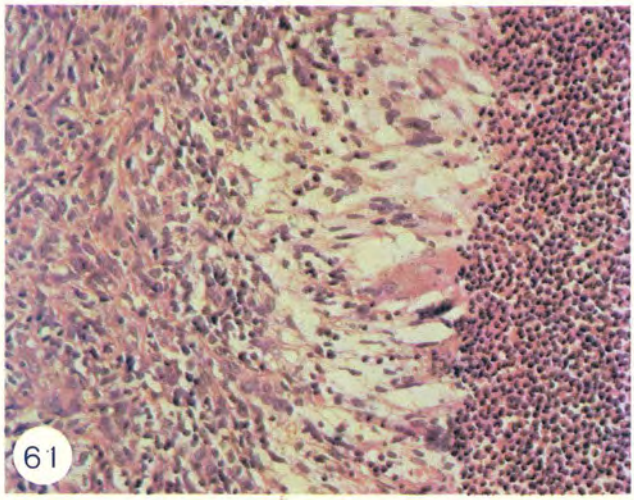
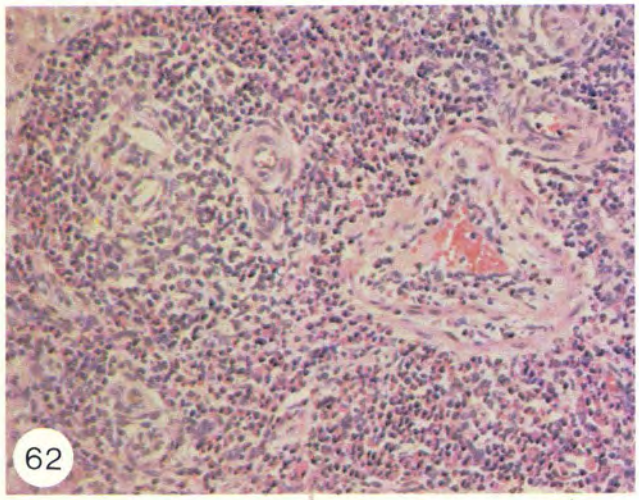


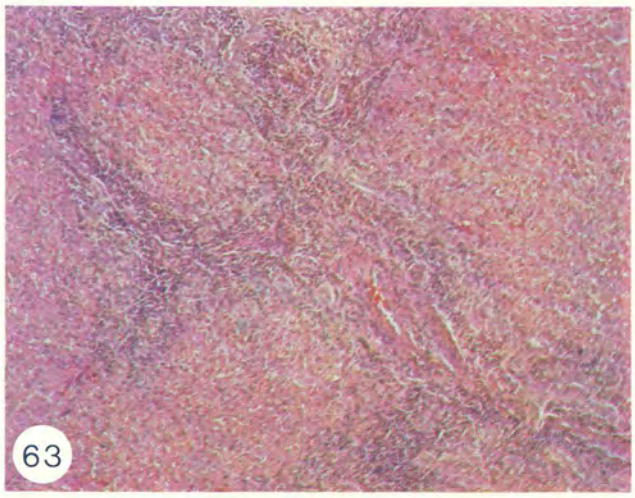
FIG. 53 Severe portal reaction in Sheep V38: HE×30
FIG. 54 Portal triad with severe fibroplasia and a moderate bile duct proliferation and mononuclear cell infiltration: HE×200
FIG. 55 Pronounced periductal fibrosis: HE ×500
FIG. 56 Replacement of bile duct with scar tissue: HE×500
FIG. 57 Larger bile duct showing severe necrosis and periductal granulation tissue proliferation: HE×30
FIG. 58 Note thickening of blood vessel wall in juxtaposition to necrotic bile duct: HE×75











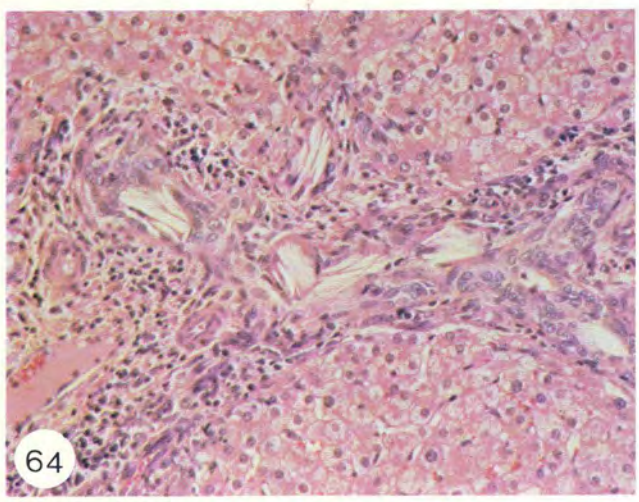


FIG. 59 & 60 Focal yellowish-brown sunken areas in control Sheep T51. Note migratory parasitic tract in focal area in Fig. 59
FIG. 61 Typical parasitic granuloma with central area of necrosis, surrounded by palisade epitheloid cells, giant cells, many mononuclear cells and eosinophils: HE×75
FIG. 62 Portal triad infiltrated with many mononuclear cells and eosinophils: HE×75
FIG. 63 Pronounced portal reaction in focal lesion: HE×75
FIG. 64 Cholesterol-like clefts in many of the bile ducts: HE×200

fibrous adhesions, migratory tracts and nodules) were commonly seen in the livers, and some of the focal areas were definitely associated with these parasitic lesions.

The walls of the gall bladders were sometimes slightly oedematous and the mucosal surfaces were covered with a fine crystalline deposit. The viscosity of the bile varied from watery to fairly thick, while the bile itself was dark green in colour and usually contained copious amounts of the crystalline deposit.

In addition to the liver lesions, the usual signs of hepatogenous photosensitivity were present, namely, photodermatitis, coronitis, icterus and nephrosis. The kidneys were swollen, discoloured (yellowish-brown to greenish-brown), and contained minute cysts in the cortex. No urinary bladder lesions were evident.

Apart from the focal lesions the parenchyma of the subclinical cases were normal. It is interesting to note that the *ductus cysticus* and bigger bile ducts almost invariably contained whitish crystals. Sometimes these ducts were oedematous and appeared to be somewhat thickened.

The control animal that became photosensitive had lesions indistinguishable from those of the 4 dosed sheep that became photosensitive at the higher dosage levels. None of the other controls had lesions of geeldikkop. Focal lesions (*vide infra*) similar to those of the dosed sheep were present in all but 2 of them.

(b) *Sheep on veld grazing with almost no* T. terrestris. The liver of Sheep V38 that became photosensitive at the *c.* 0,7 mg/kg level was yellowish-brown, the lobulation was distinct, and the larger bile ducts were markedly thickened and bile-stained. These changes, especially the thickening of the bile ducts, were not as marked in Sheep V26 that received *c.* 0,5 mg/ kg.

Many of the subclinical cases had no lesions in their livers, while others had lesions (as described above) ranging in degree from very mild to fairly severe.

Focal hepatic lesions similar to those of the sheep on *T. terrestris* were present, but they occurred less frequently and were shallower.

The control sheep had no lesions of facial eczema. Indications of parasitic infestations were present in both the dosed and undosed sheep but yellowish-brown sunken areas were not seen in the controls.

None of the sheep on the veld pastures had lesions in their urinary and gall bladders.

(ii) *Histopathology*

(a) *Sheep on predominantly* T. terrestris *pastures.* All the sheep that became photosensitive on *T. terrestris* had typical lesions of geeldikkop (Brown *et al.*, 1960; Van Tonder *et al.*, 1972). At the lowest dosing level (Sheep T2 & T4) the lesions closely resembled those described by Brown *et al.* (1960). The parenchymal changes in them were rather prominent, while the portal tracts were only minimally affected (Fig. 43). Almost all the hepatocytes showed degenerative changes of some kind. The cells were swollen; the cytoplasm which was granular and slightly vacuolated sometimes contained well demarcated eosinophilic globules of varying sizes and shapes (eosinophilic splashing) (Fig. 44). A few necrotic hepatocytes were scattered here and there throughout the lobules. Some hepatocytes contained yellowish-brown pigments, the Kupffer cells were moderately activated, and small neutrophilic foci were seen in the sinusoids and around necrotic hepatocytes. Crystals were present in a few hepatocytes and in the Kupffer cells of Sheep T4, and these cells were frequently associated with focal areas of prominent reticuloendothelial cell proliferation or infiltration. The portal reaction, at this level of dosing, was so mild as to be almost imperceptible. The *ductus cysticus* and many of the larger bile ducts were partially or completely blocked with crystals (Fig. 45 & 46). At the points of blockage the lining epithelium of these ducts were flattened, atrophic and sometimes necrotic. These changes were often accompanied by mild to moderate periductal fibroplasia and oedema, and sometimes mild cholangitis.

The remainder of the sheep that became photosensitive, namely, Sheep T21, T23, T31, T33, had the typical lesions of geeldikkop described by Theiler (1918) and Van Tonder *et al.* (1972). Parenchymal changes similar to those in the previous sheep were present, but the changes were less pronounced, and in Sheep T31 crystals were seen in some of the Kupffer cells and hepatocytes. In contrast to those in the sheep on the *c.* 0,25 mg/kg level, the portal reactions were relatively more severe (Fig. 47 & 48). These changes consisted of moderate bile duct proliferation, some fibroplasia and slight mononuclear cell infiltration (Fig. 48). Fairly often the triads were mildly oedematous and were infiltrated by a few neutrophils and eosinophils and varying numbers of macrophages laden with homogenous khaki-brown pigment. Many bile ducts were blocked with crystals (Fig. 49 & 50). Fine eosinophilic granules were attached to the surfaces of the crystals of some ducts. The accumulation of crystals resulted in distortion of the ducts and uneven proliferation of the ductular epithelium (resembling giant cells), occasionally accompanied by necrosis of the biliary epithelium (Fig. 48–50). Bile sometimes leaked from these ducts into the surrounding tissue, causing necrosis of small groups of periportal hepatocytes. The larger bile ducts and *ductus cysticus* were not seriously affected (Fig. 51), though they were frequently oedematous and blocked with crystals (Fig. 52).

The portal reactions in lamb T31 were more severe than those of the other sheep dosed with *c.* 0,7 mg/kg of sporidesmin. All the photosensitive sheep had lesions in their kidneys similar to those described in Experiments 1 & 2.

Lesions varying in intensity from very mild to severe were noticed in the subclinical cases. Below *c.* 0,5 mg/kg the livers were minimally affected except for crystals in the larger bile ducts and *ductus cysticus,* and the slight biliary changes associated with these crystals. At higher dosage rates the lesions varied from very mild to more severe than those of the sheep that became photosensitive. In one subclinical case that received *c.* 0,7 mg/kg sporidesmin (Sheep T32), the larger bile ducts and *ductus cysticus* were severely necrotic and contained many crystals. It should be emphasized that crystals were invariably present in the *ductus cysticus* and sometimes in the larger bile ducts of all the dosed sheep.

Half of the sheep that became photosensitive on *T. terrestris,* namely, Sheep T4, T31, T33, manifested focal microscopic areas of necrosis and desquamation of the lining epithelium of the gall bladder. This necrosis was most prominent in Sheep T31 where it extended into the submucosa. In the other sheep that responded clinically, namely, Sheep T2, T21, T23, only slight neutrophil infiltration and oedema of the submucosa were present, but no necrosis of the lining epithelium. Crystals were often associated with the necrotic areas in all the photosensitive sheep. Apart

from Sheep T32, which had typical microscopic areas of focal necrosis in the lining epithelium, none of the other subclinical cases showed lesions in the gall bladder.

One of the controls (Sheep T63) had liver lesions similar to those of the sheep that became photosensitive at the higher (*c.* 0,5–0,7 mg/kg) levels of dosing, but the *ductus cysticus* contained no crystals. Focal areas of necrosis were also evident in the lining epithelium of the gall bladder of this sheep. Apart from the local lesions (*vide infra*), which at this stage are not regarded as adequate evidence of subclinical geeldikkop, no other lesions were seen in the livers of the control sheep.

(b) *Sheep on veld with almost no* T. terrestris. The 2 sheep that became photosensitive on the veld had lesions of facial eczema as described in Experiment 1 and by Done *et al.* (1960); Crawley *et al.* (1961); Mortimer (1963); Mortimer *et al.* (1978) and Marasas *et al.* (1972). The lesions in Sheep V38 that received *c.* 0,7 mg/kg included the following: moderate to severe portal reaction (Fig. 53 & 54); pronounced periductal fibrosis (Fig. 55); obliteration of bile ducts with scar tissue (Fig. 56); necrosis of larger bile ducts with concomitant periductal proliferation of granulation tissue (Fig. 57); and necrosis and thickening of blood vessel walls in juxtaposition to necrotic bile ducts (Fig. 58).

Sheep V26, that became photosensitive at the lower dosing level (*c.* 0,5 mg/kg), had relatively milder lesions. The portal reactions were more moderate; the periductal fibrosis was still prominent, but the larger bile ducts, which were surrounded by extensive granulation tissue, were not conspicuously necrotic.

Focal areas of necrosis of the lining epithelium of the gall bladder was present only in Sheep V38.

The lesions in the kidneys of Sheep V26 and V38 were similar to those of the positive *T. terrestris* cases.

The effect on the livers of the non-photosensitive dosed sheep ranged from no lesions at the *c.* 0,25 and *c.* 0,35 mg/kg levels to fairly severe lesions at higher doses. It is interesting to note that many sheep in the subgroups receiving *c.* 0,4–0,7 mg/kg had no lesions in the livers, while some individuals in the same subgroups were fairly severely affected.

None of the control sheep had lesions of facial eczema or geeldikkop.

Signs of subclinical enzootic icterus

Signs of subclinical enzootic icterus were seen in some of the adult sheep (both dosed and control) grazing on *T. terrestris* and the veld. These signs included the presence of macrophages containing homogeneous khaki-brown pigments in the portal triads, slight to moderate bile duct proliferation and fibrosis in many triads, megalocytosis of scattered hepatocytes, and pigmentation of some hepatocytes and Kupffer cells.

The severity of the histopathological lesions was positively correlated with the liver copper values listed in Table 7.

No relationship could be seen between liver copper values and the activity of γ-GT in the sera.

Focal hepatic lesions

Focal lesions similar to those observed in the controls of Experiment 2 were present in many of the sheep in Experiment 3 grazing on *T. terrestris*, both the dosed and control sheep being equally affected. These focal lesions could often be directly associated with the granulomatous reaction to parasites (probably *Cysticerci*) and their migratory tracts (Fig. 59 & 60), while in other instances the evidence of parasitic involvement was either less direct or absent. The indirect evidence consisted of eosinophil and polymorphonuclear cell infiltration, the presence of pigment-laden macrophages, and certain changes involving the portal tracts. Some bile ducts in all of the focal areas invariably contained crystals.

TABLE 7 The liver copper values (wet basis) of sheep in Experiment 3

Age	Sheep No.	Copper ppm	Age	Sheep No.	Copper ppm	Age	Sheep No.	Copper ppm
L*	T1	26,0	L	V5	77,0	A	V38	120
L**	T2	3,0	L	V6	43,0	A	V39	234
L	T3	23,0	L	V7	37,0	A	V40	65
L	T4	—	L	V8	32,0	L	V44	42
A	T11	138,0	A	V9	194,0	L	V45	46
A	T12	30,0	A	V10	—	L	V46	50
A	T13	51,0	L	V14	28,0	A	V47	495
L	T21	3,0	L	V15	52,0	A	V48	269
A	T22	3,0	L	V16	32,0	A	V49	255
A	T23	104,0	A	V17	366,0	A	V50	100
L	T31	13,0	A	V18	44,0	L	V54	72
A	T32	46,0	A	V19	164,0	L	V55	65
A	T33	3,0	A	V20	245,0	L	V56	70
L	T41	35,0	A	V24	59,0	A	V57	309
A	T42	31,0	A	V25	130,0	A	V58	71
A	T43	108,0	A	V26	370,0	A	V59	290
L	T51	57,0	A	V27	284,0	A	V60	112
A	T52	27,0	A	V28	46,0	L	V64	34
A	T53	362,0	L	V29	36,0	L	V65	27
L	T61	25,0	L	V30	37,0	L	V66	49
A	T62	74,0	L	V34	37,0	L	V67	53
L	T63	2,0	L	V35	24,0	A	V68	100
			L	V36	145,0	A	V69	544
			A	V37	120,0	A	V70	55

* L=Lamb
** A=Adult

The granulomatous parasitic lesions each comprised a central necrotic area with neutrophil and eosinophil infiltration, surrounded by palisade epithelioid cells, giant cells and many mononuclear cells and eosinophils (Fig. 61). In the areas bordering on the granulomatous lesions, the very prominent portal reaction consisted of severe bile duct proliferation and marked fibrosis, frequently accompanied by pronounced mononuclear cell and eosinophil infiltration (Fig. 62). In some instances eosinophils were seen within the bile ducts and blood vessels. Crystals were always present in some of the bile ducts (Fig. 63 & 64). The parenchyma in these focal or localized areas was atrophied, degenerative and stained less intensely with H & E than the rest of the liver. In addition, the Kupffer cells were moderately activated, and neutrophilic foci, which were sometimes associated with small necrotic areas, were encountered.

Focal lesions were seen in the livers of both the dosed and control sheep grazing on the veld, but their incidence appeared to be much lower in the veld sheep and a striking dissimilarity in the latter group was the absence of crystals. The severity of the portal lesions in the veld controls were less marked than those in the dosed sheep.

Discussion

A notably larger proportion of dosed sheep became photosensitive on *T. terrestris* than on the veld (cf. 6 out of 13 with 2 out of 27). Moreover, 2 out of 4 sheep on *T. terrestris* became photosensitive at the *c.* 0,25 mg/kg level (cf. 0 out of 7 on the veld), a dose considerably smaller than that which would normally be expected to cause such a reaction. Since the morbidity at this level of dosing was fairly high (50%), the estimated amount of sporidesmin ingested by sheep in many natural outbreaks of geeldikkop, in which the morbidity is usually below this figure, could be considerably less, probably in the order of 0,15 mg/kg. Thus, assuming that subclinical geeldikkop was not present in the experimental flock, it would appear that *T. terrestris* can, in some inexplicable manner, enhance the ability of sporidesmin to cause photosensitivity. Conversely, if *T. terrestris* can induce photosensitivity on its own without the aid of sporidesmin, the reverse would also be true; in that case, sporidesmin would enhance the ability of *T. terrestris* to cause photosensitivity.

Perhaps the most significant finding of the entire investigation was that all the sheep that became photosensitive on *T. terrestris* had typical histopathological lesions of geeldikkop in their livers. The only exception was that in some of them the periductal fibrosis around a few of the larger bile ducts was slightly more pronounced than that usually seen in natural geeldikkop. The spectrum of histopathological lesions in the affected sheep ranged from mainly parenchymal changes in the livers as reported by Brown *et al.* (1960) to the more typical portal reactions described by Theiler (1918) and Van Tonder *et al.* (1972). On the other hand, the macroscopic and histopathological lesions of the sheep that became photosensitive on the veld were typical for facial eczema. This experiment, in which geeldikkop and facial eczema were separately produced by the administration of identical doses of a *P. chartarum* culture to sheep grazing respectively on *T. terrestris* and veld, demonstrated that *T. terrestris* interacts with sporidesmin to induce lesions of geeldikkop, while sporidesmin alone results in facial eczema. At present the interaction between *T. terrestris* and sporidesmin cannot be fully explained.

Another effect of *T. terrestris* appeared to be the shortening of the latent period usually associated with sporidesmin, i.e. the time between ingestion of the toxin and the manifestation of photosensitivity. The dosed sheep on *T. terrestris* became photosensitive between the 11th and 14th days, while those on the veld reacted only on the 23rd and 24th days. Moreover, the latent period on *T. terrestris* appeared to be independent of dose as can be seen from the fact that one of the first sheep to become photosensitive received the equivalent of only *c.* 0,25 mg/kg of sporidesmin. Although these periods are probably too variable to be used as an exclusive parameter for distinguishing between the 2 diseases, it is nevertheless noteworthy that in geeldikkop Van Tonder *et al.* (1972) reported latent periods as short as 3–5 days in sheep fed on *T. terrestris*.

Some doubt may be cast on the validity of this experiment by the fact that one of the *T. terrestris* control sheep developed geeldikkop. Although the control could conceivably have contracted the disease as a result of the ingestion of toxic *T. terrestris per se*, or *T. terrestris* contaminated with toxic spores of *P. chartarum*, the results of this experiment are valid because 6 out of 13 of the dosed sheep developed geeldikkop as opposed to 1 out of 9 of the controls, a disparity too large to be reasonably attributed to chance.

Both the dosed and control sheep on *T. terrestris* had localized areas of severe portal reaction with eosinophil infiltration and crystals in the bile ducts. The portal reaction in these areas was more prominent than that encountered in geeldikkop. Similar lesions, but without crystals, were encountered in the livers of the dosed sheep in the veld. In the veld controls, on the other hand, these lesions occurred only infrequently and were milder in nature. Although the aetiology of these localized portal lesions is not clear in some instances, such changes could be brought about by migratory parasites. However, when *T. terrestris* is ingested, crystals frequently appear concomitantly with these lesions.

Not all types of liver damage in sheep grazing on *T. terrestris* in the Karoo lead to the formation of crystals, as they appear to form only in response to specific types of liver injury. The specific nature of the requirements for crystal formation is underlined by the fact that 9 lambs running with the *T. terrestris* group, but dosed with *Senecio retrorsus* (pyrrolizidine alkaloids) or made photosensitive by the administration of either *Phomopsis leptostromiformis* cultures or dried *Microcystis toxica* (alga), showed typical lesions of the respective intoxications in their livers, without crystals (Coetzer & Kellerman, unpublished data, 1979). Perhaps it is significant that these hepatotoxins primarily affect the hepatocytes and not the biliary system as in the case of intoxication with sporidesmin.

General discussion and summary

In this investigation the mycoflora of toxic pastures were studied during several outbreaks of ovine hepatogenous photosensitivity. Some of the fungi isolated were cultured in various ways (Table 5) and dosed to sheep, but only *P. chartarum* and *M. verrucaria* proved to be toxic.

Species of *Pithomyces* in South Africa have been reported only fairly recently (Marasas & Schumann, 1972), but the results presented in the present paper show that at least one species, *P. chartarum*, is quite widely distributed geographically in South Africa on

various hosts and substrates. These include a number of very important plants in natural grazing as well as in artificial pastures. Of the two species recorded in these studies, *P. chartarum* was isolated more frequently and in greater numbers from pasture plant hosts than *P. karoo*. It was also recorded by Eicker (1976), Bezuidenhout (1977) and Van der Merwe *et al.* (1979) in grass pastures in South Africa and by Ellis (1960, 1971) on a wide range of substrates elsewhere. In New Zealand, *P. chartarum* constituted only 1% of fungal isolates from pastures even during a severe outbreak of facial eczema (Di Menna & Parlé, 1970). This accords with the findings of this study that *P. chartarum* was only a minor component of the mycoflora of the plants and litter examined here. Another noteworthy point was that both toxigenic and nontoxigenic strains of *P. chartarum* were frequently isolated from the same material and that the non-toxigenic strains predominated. This situation is the opposite to that pertaining in New Zealand where, according to the relevant literature, non-toxigenic strains apparently occur rather infrequently (Di Menna & Parlé, 1970). It has not been demonstrated, however, that strains of *P. chartarum* which do not produce sporidesmin in culture on SSB are also non-toxigenic in the field.

Ovine hepatogenous photosensitivity was induced in sheep by dosing them with cultures of a *P. chartarum* isolate (GA10) obtained from *T. terrestris* collected during an outbreak of geeldikkop at Aberdeen in the Karoo. Thus, for the first time, a mechanism was demonstrated by which *T. terrestris* could cause photosensitivity.

At VRI, Onderstepoort, this isolate induced only facial eczema in both Karoo and Highveld sheep grazing on lucerne. In the Karoo, on the other hand, at approximately similar doses, Karoo sheep grazing on *T. terrestris* pastures developed lesions with features of both facial eczema and geeldikkop. When the level of sporidesmin was reduced to the estimated amounts ingested during natural outbreaks of facial eczema in New Zealand (*c.* 0,5–0,7 mg/kg), Karoo sheep grazing on *T. terrestris* in the Karoo developed geeldikkop. At the same time similarly dosed sheep grazing on veld with almost no *T. terrestris* developed facial eczema.

It is also interesting to note that sheep grazing on *T. terrestris* contracted geeldikkp at supposedly subclinical doses (*c.* 0,25 mg/kg) of sporidesmin. This, together with the fact that more sheep became photosensitive on *T. terrestris* than at comparable doses on the veld, indicated that *T. terrestris* plants could enhance the ability of sporidesmin to cause photosensitivity or, possibly, vice versa.

From the results of these trials it would appear that *T. terrestris* plays a complex role in the aetiology of the disease. Like rye grass in facial eczema, it appears to act as a vehicle for the ingestion of spores, but it has additional functions, e.g. *T. terrestris* can interact with sporidesmin in some way to induce lesions of geeldikkop, whereas sporidesmin alone results in facial eczema.

At this stage it is not clear by what mechanism the ability of sporidesmin to cause photosensitivity is enhanced or the interaction between the plant and mycotoxin takes place. According to Mortimer *et al.* (1978) the lesions of facial eczema in the bile ducts progress from necrosis of the epithelium to necrosis of the entire wall, followed by severe periductal proliferation of granulation tissue and fibrosis, culminating in the occlusion of some bile ducts with cellular debris and/or scar tissue. The latent period is the time required for the above-mentioned lesions to develop. In Experiment 3 this period was considerably shorter in the sheep that contracted geeldikkop (11–14 days) than in those that developed facial eczema (23–24 days). The disparity in the latent periods may possibly be explained in the following way. In facial eczema it is primarily the biliary tree that is affected (Mortimer *et al.*, 1978), but in geeldikkop the typical biliary and vascular changes of facial eczema are absent and parenchymal changes are present. It would therefore appear that in geeldikkop other mechanisms are in operation, 2 of these conceivably being the parenchymal changes and the blockage of bile ducts with crystals. The clearest evidence of the possible existence of the latter mechanism was found in the sheep on *T. terrestris* that became photosensitive at the lowest dosing level (*c.* 0,25 mg/kg). In these sheep (Sheep T2 & T4) the portal reaction was too slight to account for the retention of phylloerythrin and subsequent photosensitization. Despite being intoxicated with sporidesmin, the occluding mechanism for facial eczema described by Mortimer *et al.* (1978) was absent. Clearly, some other occluding mechanism was in operation here and the only one demonstrable was obstruction of major bile ducts with crystals.

The chemical nature of the crystals is unknown, but since they are apparently not composed of common bile salts such as cholesterol, cholic acid, sodium glycocholate or sodium taurocholate (Dr L. A. P. Anderson, 1978, VRI, Onderstepoort, personal communication) the possibility exists that they are the product of hepatocytes (whether aberrant or normal) and components contributed by *T. terrestris*. These components may well vary in *T. terrestris* according to locality, growth stage and physiological condition of the plant. The occurrence of geeldikkop, therefore, might be linked with the status of the crystal-promoting factor(s) in the plant. Research must now be done to establish the composition of the crystals and the requirements for their formation in the body.

It is not yet known at what stage of the latent period the crystals are formed, but if this happens early on, it would explain the shorter latent periods noticed in geeldikkop. Other features distinguishing geeldikkop from facial eczema such as the absence of pronounced necrosis of major bile ducts etc., may also be explainable in the light of the early occlusion of bile ducts with crystals. Research is urgently required on the role of birefringent crystals in the pathogenesis of geeldikkop.

The crystals in the localized lesions of the control sheep grazing on *T. terrestris* are regarded as evidence of the presence of a crystal-promoting factor in the plant.

The possibility that *P. chartarum* is involved in the aetiology of dikoor and that *Panicum* grasses contain a similar factor(s) for the formation of crystals should be investigated.

Finally, it must be pointed out that geeldikkop is not facial eczema, but a more complex disease that can be caused by the simultaneous ingestion of sporidesmin and *T. terrestris* plants. The fact that sporidesmin has been incriminated in the aetiology of geeldikkop does not of course preclude other hepatotoxins (acting in conjunction with *T. terrestris*) from performing a similar function. The plant itself may possibly produce such a toxin, and further research is indicated to exclude or prove such a possibility.

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APPENDIX 1

Fungi isolated from various substrates collected in camps where outbreaks of photosensitivity in sheep occurred

Locality	Farm No.	*Acremoniella verrucosa*	**Acremonium* spp.	**Acrophialophora levis*	**Alternaria* spp.	**A. zinniae*	*Arthrinium* sp.	**Ascochyta hordei*	*Aspergillus candidus*	**A. fumigatus*	**A. nidulans*	**A. niger*	**A. ochraceus*	*A. terreus*	*Botryodiplodia* sp.	*Camarosporium* sp.	*Chaetomium* spp.
Colesberg	A				+												
	B				+												
Conway	C				+												
	D				+												
	E				+												
Hopetown	F	+			+										+	+	+
Cradock	G				+												
Middelburg (Cape)	H				+			+			+						+
	I			+	+	+		+		+	+	+	+				
Hofmeyr	J				+						+						+
Gouda	K				+				+	+		+		+			+
Heilbron	L	+	+		+		+	+		+							+
Bloemfontein	M		+		+			+									
Harrismith	N		+		+												
Calvinia	O				+						+						
Underberg	P		+		+							+					
Humansdorp	Q		+		+							+					
Total		2	5	1	17	1	1	4	1	3	4	4	1	1	1	1	5

* Taxa isolated from *Tribulus terrestris*

APPENDIX 1 (*continued*)

Fungi isolated from various substrates collected in camps where outbreaks of photosensitivity in sheep occurred

Locality	Farm No.	*Chaetopyrena penicillatum*	**Cladosporium* spp.	**C. macrocarpum*	*Colletotrichum graminicola*	*Coniochaeta* sp.	*Coniothyrium* sp.	*Curvularia* spp.	*C. senegalensis*	**Diplodia* spp.	**Drechslera* spp.	**D. australiensis*	*D. bicolor*	**D. multiformis*	*Embellisia chlamydospora*	**Epicoccum nigrum*	**Fusarium* spp.
Colesberg	A		+			+										+	+
	B		+			+											+
Conway	C		+								+						+
	D		+							+							+
	E		+							+							+
Hopetown	F	+	+				+	+		+	+						+
Cradock	G	+	+					+		+	+					+	+
Middelburg (Cape)	H		+														+
	I		+							+		+				+	+
Hofmeyr	J		+	+							+						+
Gouda	K		+								+						+
Heilbron	L		+	+			+	+	+	+	+		+			+	+
Bloemfontein	M		+	+						+	+			+		+	+
Harrismith	N		+	+	+						+					+	+
Calvinia	O		+							+	+				+		+
Underbreg	P		+				+	+			+						+
Humansdorp	Q		+	+	+											+	+
Total		2	17	5	2	2	3	4	1	7	9	1	1	1	1	7	17

* Taxa isolated from *Tribulus terrestris*

APPENDIX 1 (*continued*)

Fungi isolated from various substrates collected in camps where outbreaks of photosensitivity in sheep occurred

Locality	Farm No.	*F. acuminatum	*F. avenaceum*	*F. chlamydosporum	*F. culmorum*	*F. moniliforme	*F. equiseti*	*F. equiseti* var. *langipes*	*Gelasinospora calospora*	*Geotrichum* sp.	*Gliocladium roseum*	*Gonatobotrys simplex*	*Hendersonia* sp.	*Leptosphaerulina australis*	*Macroventuria anomochaeta*	*Mucor* spp.	*Myrothecium verrucaria*
Colesberg	A			+									+			+	
	B																
Conway	C																
	D																
Hopetown	E																
Cradock	F														+		
Middelburg (Cape)	G													+			
	H			+		+										+	
Hofmeyr	I	+															
Gouda	J	+				+										+	
Heilbron	K	+		+	+	+		+		+		+	+			+	+
Bloemfontein	L	+															
Harrismith	M	+														+	
Calvinia	N																
Underberg	O	+	+		+						+					+	+
Humansdorp	P				+		+		+							+	+
	Q																
Total		6	1	3	3	3	1	1	1	1	1	1	2	1	1	7	3

* Taxa isolated from *Tribulus terrestris*

APPENDIX 1 (*continnued*)

Fungi isolated from various substrates collected in camps where outbreaks of photosensitivity in sheep occurred

Locality	Farm No.	*Paecilomyces* spp.	*Penicillium* spp.	*Pestalotia* sp.	*Phaeoramularia kellermania*	*Phoma* spp.	*P. sorghina*	*Pithomyces chartarum*	*K. karoo*	*Ramichloridium schulzeri*	*Rhizoctonia solani*	*Rhizopus* spp.	*Scopulariopsis* sp.	*Spegazzinia tessarthra*	*Stagonospora* sp.	*Stauronema* sp.	*Stemphylium botryosum*
Colesberg	A					+			+		+						+
	B					+											
Conway	C					+											
	D					+											
Hopetown	E		+	+		+			+								+
Cradock	F					+											+
Middelburg (Cape)	G					+											
	H		+			+			+		+	+					
Hofmeyr	I		+			+						+					+
Gouda	J		+			+						+		+			+
Heilbron	K	+	+			+	+		+	+	+	+	+		+	+	
Bloemfontein	L					+	+										
Harrismith	M				+	+	+										
Calvinia	N																
Underberg	O		+		+	+	+					+					
Humansdorp	P		+			+	+	+		+		+			+		+
	Q					+		+				+					
Total		1	7	1	2	17	4	2	4	2	3	7	1	1	2	1	6

* Taxa isolated from *Tribulus terrestris*

APPENDIX 1 (*continued*)

Fungi isolated from various substrates collected in camps where outbreaks of photosensitivity in sheep occurred

Locality	Farm No.	*Stilbella* sp.	**Thielavia terricola*	**Tiarosporella graminis* var. *karoo*	*T. tritici*	*Torula herbarum*	**Trichoderma viride*	*Ulocladium chartarum*	**Sterile fungi*
Colesberg	A			+			+		+
	B			+			+		+
	C			+			+		
Conway	D								+
	E								+
Hopetown	F	+		+			+		+
Cradock	G			+		+			+
Middelburg (Cape)	H								+
	I		+	+			+		+
Hofmeyr	J							+	+
Gouda	K		+				+	+	
Heilbron	L				+		+		
Bloemfontein	M								
Harrismith	N								+
Calvinia	O							+	
Underberg	P					+	+		+
Humansdorp	Q						+		+
Total		1	2	6	1	2	8	3	12

* Taxa isolated from *Tribulus terrestris*

APPENDIX 2

Fungi isolated from various substrates by the dilution plate method

Fungi	Incubation temperature (°C)	Propagules/g wet mass (× 10^4)				
		Bloemfontein Wheat	Bloemfontein *T. terrestris*	Swinburne Oats	Calvinia Wheat	Underburg Lolium
Acremonium spp.	18	3				20
Alternaria spp.		133	4,3		10	
Ascochyta hordei		13				
Cladosporium spp.		40		15		13
C. macrocarpum			1,0			
Diplodia sp.			0,6			
Drechslera multiformis			0,3			
Fusarium spp.		30	1,3	35		96
Epicoccum nigrum		46	0,3			
Mucor spp.						3
Penicillium spp.					15	
Phaeoramularia kellermaniana					5	
Phoma spp.		150	1,3	135	35	136
Sterile fungi					5	
Total (× 10^4)		415	9,1	185	70	268
Acremonium spp.	25	63				
Alternaria spp.		230	2,0		20	
Aspergillus nidulans					10	
Cladosporium spp.		6		5		10
Coniothyrium sp.						20
Curvularia sp.						5
Diplodia sp.					10	
Embellisia chlamydospora					10	
Epicoccum nigrum		6		30		
Fusarium spp.		53	0,5	115		40
Mucor spp.						5
Myrothecium verrucaria						15
Penicillium spp.					10	
Phaeoramularia kellermaniana					10	
Phoma spp.		93	2,5	335	30	215
Ramichloridium schulzeri						10
Ulocladium chartarum					10	
Sterile fungi						5
Total (× 10^4)		451	5,0	485	110	325

APPENDIX 3

Mycoflora of wheat stubble, Rusthof, Heilbron[a]

Fungi	Incubation temperature (°C)	Propagules/g wet mass (× 10⁴)[b] Sampling date (1971–1972)						Total	Percentage
		Dec. 23	Dec. 29	Jan. 5	Jan. 13	Jan. 20	Jan. 27		
Alternaria spp.	18	40	125	75	15	40	80	375	20,8
Ascochyta sp.			20	15	20	3	6	64	3,5
Cladosporium spp.		30	50	55	85	24	43	287	15,9
Fusarium spp[d]			5	5		16		26	1,4
Phoma spp.		240	110	255	105	167	166	1 043	57,7
Sterile			5		3	3		11	0,6
Total (× 10⁴)		310	315	405	228	253	295	1 806	
Alternaria spp.	25	223	153	100	63	70	43	652	43,0
Ascochyta sp.			13	13	13		6	45	3,0
Cladosporium spp.		30	6	6	23	13	6	84	5,5
Drechslera spp.			3					3	0,2
Epicoccum nigrum		6	10					16	1,1
Fusarium spp.[d]		13		6	3	10	10	42	2,8
Phoma spp.[c]		66	90	143	116	106	143	664	43,8
Ramichloridium schulzeri							6	6	0,4
Sterile							3	3	0,2
Total (× 10⁴)		338	275	268	218	199	217	1 515	

[a] Samples consisted of wheat straw and stubble that remained in the ground and were collected in a wheat field which was reaped between 3rd and 10th December 1971
[b] Each figure represents the average of three plates prepared from a dilution of 1:10⁵
[c] Predominantly *P. sorghina*
[d] Including *F. acuminatum*, *F. culmorum* and *F. equiseti* var. *longipes*

APPENDIX 4

Mycoflora of *Panicum coloratum* and *Eleusine africana* leaves, Rusthof, Heilbron[a]

Fungi	Incubation temperature (°C)	Propagules/g wet mass (× 10⁴)[b] Sampling date (1971–1972)						Total	Percentage
		Dec. 23	Dec. 29	Jan. 5	Jan. 13	Jan. 20	Jan. 27		
Alternaria spp.	18	1,3	3,3		2,0			6,6	6,6
Ascochyta sp.			1,3	2,6	1,5	1,0	0,5	6,9	6,9
Cladosporium spp.		0,6	2,6	2,3	1,0	0,3	2,0	8,8	8,8
Fusarium spp.[d]				0,3	0,5		0,5	1,3	1,3
Phoma spp.[c]		8,0	7,1	13,0	9,5	24,0	11,5	73,1	73,1
Stagonospora sp.		0,6		0,3				0,9	0,9
Sterile		1,0	0,3				1,0	2,3	2,3
Total (× 10⁴)		11,5	14,6	18,5	14,5	25,3	15,5	99,9	
Alternaria spp.	25	3,0	10,3	4,0	1,0	0,3		18,6	18,9
Ascochyta sp.		0,3		1,5	0,3		0,3	2,4	2,4
Cladosporium spp.		0,3			0,3	0,3		0,9	0,9
Drechslera spp.				0,5				0,5	0,5
Epicoccum nigrum		0,6	1,3			0,3	0,3	2,5	2,5
Fusarium spp.[d]						0,3	0,6	0,9	0,9
Hendersonia sp.					0,3			0,3	0,3
Myrothecium verrucaria							0,3	0,3	0,3
Phoma spp.[c]		5,0	6,3	15,0	13,6	17,3	11,6	68,8	70,0
Ramichloridium schulzeri							0,6	0,6	0,6
Stagonospora sp.		0,3				0,3		0,6	0,6
Stauronema sp.							0,6	0,6	0,6
Sterile				1,0			0,3	1,3	1,3
Total (× 10⁴)		9,5	17,9	22,0	15,5	18,8	14,6	98,3	

[a] Samples consisted of a mixture of leaves of *P. coloratum* and *E. africana* plants sprouting in a wheat field which was reaped between 3rd and 10th December 1971
[b] Each figure represents the average of three plates prepared from a dilution of 1:10⁴
[c] Predominantly *P. sorghina*
[d] Including *F. acuminatum*, *F. culmorum* and *F. equiseti* var. *longipes*

APPENDIX 5

Preparation of fungal cultures for dosing to sheep

Culture No.	Fungus	Isolate source	Culture medium	Amount	Container	Incubation temperature	Incubation period (days)
1	*Pithomyces karoo*	*Gnidia polycephala*, Colesberg	Potato-carrot broth	26 ℓ	Erlenmeyer flasks	20 °C	29
2	*P. karoo*	*G. polycephala*, Colesberg	Potato-carrot broth	21 ℓ	Erlenmeyer flasks	28 °C	29
3	*P. karoo*		Malt extract agar	15,5 ℓ	Roux flasks	20 °C	66 } 71
						28 °C	5 }
4	*P. karoo*	*G. polycephala*, Colesberg	Bran	3,6 kg	Fruit jars	20 °C	59 } 64
						28 °C	5 }
5	*P. karoo*	*G. polycephala*, Colesberg	Bran	3,6 kg	Fruit jars	Glasshouse	59
6	*P. karoo*	Oat stubble, Heilbron	Malt extract agar	6,25 ℓ	Roux flasks	25 °C	44
7	*P. karoo*	Oat stubble, Heilbron	Malt extract agar	18,3 ℓ	Glass Petri dishes	25 °C	21
8	*P. karoo*	Oat stubble, Heilbron	Lucerne hay	5 kg	Fruit jars	Field-Heilbron	22
9	*P. karoo*	Oat stubble, Heilbron	Semi-synthetic broth	5 ℓ	Glass Petri dishes	25 °C	18
10	*P. karoo*	*Rhigozum trichotomum*, Hopetown	Semi-synthetic broth	3 ℓ	Glass Petri dishes	25 °C	18
11	*P. karoo*	*R. trichotomum*, Hopetown	*T. terrestris* hay	6 kg	Fruit jars	25 °C	21
12	*P. karoo*	*Tribulus terrestris*, Middelburg (Cape)	Potato-carrot agar	5 ℓ	Plastic Petri dishes	25 °C+NUV[a]	14
13	*P. karoo*	*T. terrestris, Middelburg* (Cape)	Malt extract agar	12 ℓ	Glass Petri dishes	Day: 25 °C+NUV[a] Night: 10 °C	14
14	*Tiarosporella graminis* var. *karoo*	*Eriocephalus* sp., Colesberg	Maize	22 kg	Fruit jars	28 °C	21
15	*T. graminis* var. *karoo*	*Eriocephalus* sp., Colesberg	Maize	2 kg	Fruit jars	28 °C	21 } 35
						Glasshouse	14 }
16	*Ulocladium chartarum*	Oat litter, Middelburg	Semi-synthetic broth	5 ℓ	Plastic Petri dishes	25 °C+NUV[a]	15
17	*U. chartarum*	Wheat litter, Gouda	Lucerne hay	5 kg	Fruit jars	Field-Heilbron	22
18	*Epicoccum nigrum*	Wheat stubble, Heilbron	Wheat straw	7 kg	Fruit jars	Field-Heilbron	14
19	*Cladosporium* sp.	Wheat stubble, Heilbron	Wheat straw	5 kg	Fruit jars	Field-Heilbron	14
20	*Cladosporium* sp.	Wheat stubble, Heilbron	Malt extract agar	4 ℓ	Glass Petri dishes	25 °C	14
21	*Cladosporium macrocarpum*	Wheat stubble, Heilbron	Malt extract agar	7 ℓ	Glass Petri dishes	25 °C+NUV[a]	17
22	*C. macrocarpum*	Wheat stubble, Heilbron	Malt extract agar	15 ℓ	Glass Petri dishes	Day: 22 °C+FL[b] Night: 18 °C	18
23	*Aspergillus fumigatus*	*T. terrestris*, Middelburg (Cape)	*T. terrestris* hay	0,5 kg	Fruit jars	37 °C	12
24	*A. fumigatus*	*T. terrestris*, Middelburg (Cape)	Lucerne meal agar	4 ℓ	Glass Petri dishes	37 °C	3
26	*Myrothecium verrucaria*	Wheat stubble, Heilbron	Malt extract agar	12 ℓ	Glass Petri dishes	18 °C+FL[b]	11
72/1	*Alternaria* sp.	Wheat stubble, Heilbron	Semi-synthetic broth	13 ℓ	Glass Petri dishes	Day: 25 °C+NUV[a] Night: 10 °C	14
72/7	*Phaeoramularia kellermaniana*	Wheat stubble, Calvinia	Malt extract agar	3 ℓ	Glass Petri dishes	Day: 25 °C+FL[a] Night: 10 °C	14
72/5	*Phoma sorghina*	Wheat stubble, Heilbron	Potato-carrot agar	12 ℓ	Glass Petri dishes	20 °C+NUV[a]	15
72/11	*P. sorghina*	Wheat stubble, Heilbron	Potato-carrot agar	15 ℓ	Plastic Petri dishes	20 °C+NUV[a]	17

[a] NUV=Cultures irradiated with near ultraviolet light (350 nm) for 12 h/d
[b] FL=Cultures irradiated with white fluorescent light for 12 h/d

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