

FIRST REPORT OF FACIAL ECZEMA IN SHEEP IN SOUTH AFRICA

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ABSTRACT

MARASAS, W. F. O., ADELAAR, T. F., KELLERMAN, T. S., MINNÉ, J. A., VAN RENSBURG, I. B. J. & BURROUGHS, G. W. First report of facial eczema in sheep in South Africa. *Onderstepoort J. vet. Res.* 39 (2) 107-112 (1972).

The occurrence of facial eczema in sheep in the Republic of South Africa is reported for the first time. The disease in this country is similar to that described in New Zealand and Australia. To date it has only been diagnosed in Merino sheep on artificial pastures in the Humansdorp area of the Cape Province. The fungus *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis was isolated from grass litter in these pastures. One of these isolates was shown to produce sporidesmin and the typical clinical and histopathological signs of facial eczema were reproduced upon dosing this culture to a lamb.

INTRODUCTION

Facial eczema is a photosensitizing disease of sheep and cattle in New Zealand and Australia and is caused by the ingestion of spores of the saprophytic fungus, *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis, containing the hepatotoxic mycotoxin, sporidesmin (Dodd, 1965; Brook & White, 1966; Taylor, 1967; Ciegler & Lillehoj, 1968; Wright, 1968; Brook, 1969).

The causal fungus is a cosmopolitan saprophyte (Ellis, 1960; Dingley, 1962; Gregory & Lacey, 1964). The disease was until fairly recently only known to occur in New Zealand where outbreaks have occurred regularly since the turn of the century (Dodd, 1965). In 1956 the first significant outbreak of the disease reported in any part of the world other than New Zealand occurred in the East Gippsland area of Victoria, Australia (Janes, 1959; Hore, 1960; Flynn, Hore, Leaver & Fischer, 1962; Walsh, 1966). In 1959 a much more serious outbreak occurred in Victoria and an estimated 10 000 sheep died while many thousands more were rendered economically unsound (Flynn *et al.*, 1962; Walsh, 1966). The disease has subsequently also been reported from Western Australia (Gardiner & Nairn, 1962) and New South Wales (Dent & Rofe, 1967). In Texas, *P. chartarum* has been isolated from pastures where photosensitization of cattle occurred, but it has not yet been resolved whether the photosensitivity was caused by sporidesmin or not (Taber, Pettit, Taber & Dollahite, 1968).

This paper reports on the occurrence of field outbreaks of facial eczema in sheep in South Africa, the isolation of the fungus *Pithomyces chartarum* from grass litter in artificial pastures where the outbreaks occurred, and the experimental reproduction of facial eczema in sheep fed a pure culture of this fungus. An account of the morphology of the South African isolates of *P. chartarum* will be published elsewhere (Marasas & Schumann, 1972).

MATERIALS AND METHODS

Isolation of the causal fungus

Dead leaves of *Lolium perenne* L. and *Sporobolus capensis* (Willd.) Kunth. were collected from artificial pastures on two farms in the Humansdorp area during September 1970. Suspected cases of facial eczema had occurred amongst sheep on these pastures. Direct isolations of fungi were made from grass litter by transferring small pieces of leaf tissue to 1,5% malt

extract agar containing 100 mg sodium novobiocin/l and incubating at 25°C. Fungus colonies that appeared on the pieces of tissue were isolated in pure culture.

Culture of the causal fungus

One of the isolates (OP-9) of *P. chartarum* obtained from the grass litter was grown in pure culture on a large scale. In preliminary experiments cultures of this isolate on either potato-carrot broth, bran (Done, Mortimer, Taylor & Russell, 1961) or semi-synthetic broth (Di Menna, Campbell & Mortimer, 1970) incubated at either room temperature or 20°C without near ultra-violet light (NUV) irradiation, produced low yields of sporidesmin as determined by thin layer chromatography. In these experiments, a maximal yield of approximately 0,8 mg sporidesmin per litre was obtained by culturing the fungus in thin layers of semi-synthetic broth in petri dishes (Di Menna *et al.*, 1970) at 20°C in diffuse light.

In the experiment to be described here, the fungus was cultured and irradiated according to the method described by Di Menna *et al.* (1970). Semi-synthetic broth was inoculated with a spore suspension of *P. chartarum* isolate OP-9 and dispensed in 15 ml quantities into disposable plastic petri dishes 8,5 cm in diameter. Cultures were incubated at 20° (\pm 2°)C for 14 days and irradiated for 10 h/d between the 2nd and 14th days of incubation. A total of 130 h NUV radiation was provided by a fluorescent tube (General Electric S40 BLB) giving radiation at 350 nm mounted between two cool white fluorescent tubes (Phillips TL 40W 33RS) 30 cm above the bench.

Extraction, detection and estimation of sporidesmin

Cultures of *P. chartarum* on either potato-carrot broth, bran or semi-synthetic broth were extracted with a mixture of diethyl ether and benzene (90:10) by agitating for 1 hour in a shaker. After separation of the phases two further extractions of the residue were made with diethyl ether.

The combined extracts were dried with anhydrous sodium sulphate, carefully evaporated to dryness and taken up in acetonitrile. Oils and colouring matter were removed by extracting three times with hexane. The acetonitrile fraction was evaporated to dryness and made up to a suitable volume with chloroform.

Silica gel thin layer chromatographic (TLC) plates containing 5% starch were prepared. The chromatogram

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was developed in benzene:ether (60:40) and the plates sprayed with sodium azide-iodine reagent as used by Russell (1960) on paper chromatograms. Sporidesmin appeared as a white spot on a blue background at Rf 0.5. Standards as low as 0.1 µg still gave a distinct white spot.

Sporidesmin added to control medium (potato-carrot broth) and extracted as described above was recovered quantitatively.

A semi-quantitative assay of the toxin in either potato-carrot or semi-synthetic broth was made by TLC and visual comparison with a sporidesmin standard. The standard used was a sporidesmin-benzene solvate which was kindly supplied by Dr. E. P. White via Dr. A. G. Campbell, Ruakura Animal Research Station, Hamilton, New Zealand.

Dosing pure cultures of P. chartarum to a young sheep

Five litres of semi-synthetic broth were inoculated with a spore suspension of *P. chartarum* isolate OP-9. The inoculated medium was dispensed into petri plates, incubated and irradiated as described previously. After an incubation period of 14 days, 1 750 ml of heavily sporulating culture material was harvested and homogenized with 250 ml of water in a Waring blender. A 25 ml aliquot was assayed for sporidesmin content by TLC and found to contain approximately 35 mg sporidesmin/l.

The material was then dosed by stomach tube to a 3-month old, male, Letelle lamb of 15 kg live mass. Initially 1 litre was given, followed by a second and third dose of 0.5 l administered 1.25 h and 17 h later. This is approximately equivalent to 4 mg sporidesmin/kg live mass given in a divided dose of 2 mg/kg (first administration) and 1 mg/kg (second and third administrations).

The lamb was fed on green lucerne and kept in the sun. It was examined daily and routine chemical pathological determinations on the blood were periodically done. Four days after showing signs of photosensitivity the lamb was sacrificed for necropsy. Specimens for histopathological examination were taken from various organs, fixed in 10% formalin, cut in a routine manner and stained with haematoxylin and eosin (HE).

RESULTS

Description of field cases of facial eczema in sheep

The first suspected cases of facial eczema in South Africa were observed during April 1967 in Merino sheep grazing on rye grass-white clover pastures on three coastal farms in the Humansdorp area of the Cape Province. The geographical location of the farms is approximately 34°S, 24°E along the Indian Ocean coast of the Cape Province. The natural vegetation in this area of high and well distributed rainfall is classified as Knysna Forest by Acocks (1953). The soils are generally immature, sandy, podzolic, usually acid and very poor (Van der Merwe, 1962).

Rain had fallen over the artificial pastures on these farms approximately 10 days before the first signs of photosensitivity appeared. In some camps up to 50% of the sheep were affected, but the mortality was low (approximately 10%). Subsequently no further massive outbreaks occurred, but a few cases were observed during September 1970 and August 1971.

Clinical signs: Photosensitivity was manifested by irritation and pruritis. The skin of the face and ears reddened, and these parts later became severely swollen. Hair dropped out of the affected areas and as the disease progressed the superficial layers of the epidermis sloughed

off leaving the deeper layers exposed and covered with scabs. Icterus was not evident in the initial stages but was present in the terminal stages. Temperatures up to 40.5°C were recorded. The sheep were generally lethargic, did not feed normally and tended to lose mass. Dyspnoea and mucopurulent nasal discharges were frequently seen.

Necropsy findings: The carcasses were in poor condition and slightly icteric. The most conspicuous pathological change was in the livers, which showed severe cirrhosis and nodular regeneration. Nephrosis was consistently present.

Histopathological findings: The histopathological specimens examined during the 1967 and 1970 outbreaks were taken mostly from chronic cases. The most important lesions, from a diagnostic point of view, were those encountered in sections of the liver.

Interlobular cirrhosis and bile-duct proliferation were conspicuously present. In some sections the bile-duct proliferation completely dominated the picture and was so severe that it obscured the fibrosis, while in others the connective tissue was rather mature and abundant when compared with the degree of bile-duct proliferation. Some sections revealed the peribiliary fibrosis to be concentrically laid down around some of the medium-sized bile-ducts. A marked leucocytic infiltration occurred in the portal areas, lymphocytes and plasma cells being in the majority. A few bile-ducts were infiltrated by leucocytes. Eccentric subintimal thickening as described by Crawley, Mortimer & Smith (1961) and Mortimer (1963) was seen in some of the blood vessels. Furthermore, connective tissue often encircled islands of proliferating hepatocytes resembling pseudolobulation of the liver. Also present in or near the portal tracts in a high proportion of cases were large macrophages loaded with a finely granular yellowish-brown pigment. The nature of the pigment has not yet been established.

Isolation of fungi

The fungus *P. chartarum* was isolated from the grass litter samples collected on both farms. The identification of one isolate (OP-9), obtained from dead leaves of *Sporobolus capensis* was confirmed (M. B. Ellis, Commonwealth Mycological Institute, Kew, England, personal communication, 1970).

P. chartarum was not dominant on the grass leaves and no spores could be seen upon microscopic examination of the debris. Only a few colonies of *P. chartarum* developed in the large number of plates prepared from each sample. The dominant fungi isolated from the litter were:— *Alternaria alternata* (Fr.) Keissl., *Fusarium roseum* (Lk.) Snyder et Hans., *Stemphylium botryosum* Wallr. and *Colletotrichum graminicola* (Ces.) Wilson.

Climatological data

The climatological data of the Port Elizabeth weather station, which is approximately 160 km from the affected farms, were analysed with the aid of the facial eczema predictor of the New Zealand Department of Agriculture (Crawley & Woolford, 1965).

According to these workers, facial eczema danger periods arise when the grass minimum temperatures are 12.2°C or higher on three successive nights combined with 3.75 mm or more of rainfall. Conditions were very favourable for outbreaks of facial eczema in April 1967 (Table 1).

During the 1970/71 season, climatic conditions were such that an outbreak of facial eczema was again ex-

TABLE 1 Occurrence of potential facial eczema danger periods at Port Elizabeth*

Season	Number of potential danger periods					Total
	Dec.	Jan.	Feb.	March	Apr.	
1966/67	2	2	4	3	5	16
1967/68	1	4	1	3	2	11
1968/69	1	2	0	0	0	3
1969/70	1	2	2	0	1	6
1970/71	1	3	4	3	2	13

*Danger periods determined by the New Zealand Department of Agriculture facial eczema predictor and based on rainfall and grass minimum temperatures recorded at Port Elizabeth Weather Station. A "danger period" arises when the grass minimum temperature is 12.2°C or above on each of three successive nights, combined with 3.75 mm or more of rainfall (Crawley & Woolford, 1965).

pected at Humansdorp during the late summer of 1971 (Table 1). Such an outbreak did not occur, although a few chronically affected sheep were seen on one farm during September 1971. Three possible explanations may be offered for this anomaly:—

- (i) Climatological conditions may have been different at Port Elizabeth where the data were recorded.
- (ii) The pastures at Humansdorp were in excellent condition and consequently there was little dead material for the saprophytic fungus to grow on.
- (iii) Heavy rains during April 1971 (85.3 mm within 4 days) may have washed the toxin-containing spores away or leached the toxin from the spores.

Experimental reproduction of facial eczema

Clinical and chemical pathological findings: The first clinical sign to appear was a severe watery diarrhoea that started on Day 2 and lasted until Day 6. The lamb was treated with an electrolyte solution and a mixture of kaolin and tannic acid. When the diarrhoea had stopped a rumenotoric of green lucerne blended in ruminal fluid was given.

A period of apparent well-being followed until Day 12 when a marked elevation of the serum glutamic oxalacetic transaminase (SGOT) was recorded. No concurrent clinical abnormalities were observed but, as the day was overcast, it was not possible to establish whether the lamb was photosensitive.

The overcast rainy weather persisted until Day 15. During this dull period repeated attempts were made to induce photosensitivity by exposing the lamb to tungsten filamented electric lamps, and black and cold white light irradiation, but these attempts were unsuccessful.

When the weather cleared on Day 15 the lamb was exposed to sunlight for 4.5 h. Extreme photosensitivity resulted. The lamb sought shade and frequently shook its head. The skin of the nose, eyelids and ears became red. This redness was followed by severe swelling of these parts. A coronitis was present, the conjunctiva was mildly icteric and the lamb urinated frequently.

Some remission of signs occurred when the lamb was taken indoors. The swelling subsided but reappeared within 5 min. of re-exposure to sunlight. The icterus and coronitis became progressively worse and on Day 18, when the lamb was sacrificed for necropsy, it still showed signs of photosensitivity and frequent micturition.

The only significant chemical pathological changes observed were elevations of the SGOT and total plasma bilirubin (TBR) levels in the blood (Fig. 1).

Necropsy findings: The carcass was slightly icteric. Localized areas of acute dermatitis with moderate sub-

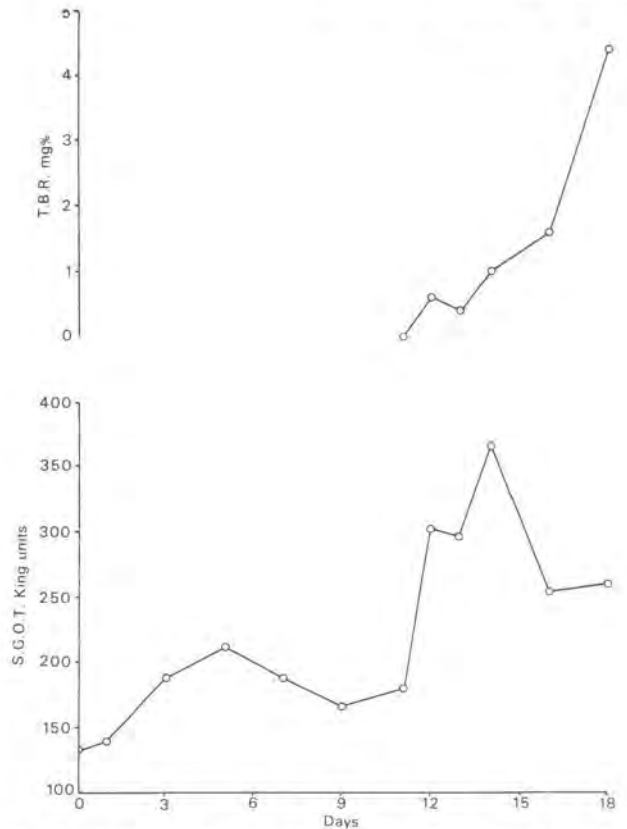


FIG. 1 A graphic presentation of the total plasma bilirubin (TBR) and serum glutamic oxalacetic transaminase (SGOT) levels of the lamb dosed with pure cultures of *Pitheomyces chartarum*.

cutaneous oedema occurred on the ears, face, lips and submandibular region. An acute coronitis was present on all four feet.

The liver was pale yellow-brown in colour with distinct lobulation. It was approximately normal in size and consistency and on the surface a few uneven sunken areas were visible. On cut section some of the bile-ducts were seen to be occluded by olive green plugs. The liver substance contained a number of pinhead-size, dull, greenish to grey foci.

A severe diffuse, necrotic cholecystitis with adhesions to the duodenum and mesentery was present. The wall of the gall bladder was thickened and hardened.

Examination of the urinary bladder revealed a haemorrhagic, ulcerative cystitis with oedema of the wall. The kidneys were tinged greenish brown.

Other features of necropsy were a prominent hydropericardium, slight oedema of the larynx and oesophagus, localized oedema of the duodenal mesentery and slight oedema and pigmentation of the lymph nodes. The spleen, myocardium and endocrines revealed no specific changes.

Histopathological findings: The skin of the affected areas was intensely hyperaemic and oedematous with small haemorrhages in all layers. Necrotic areas with severe neutrophilic infiltration occurred in the epidermis, especially in the *stratum spinosum* and *stratum corneum*.

The liver substance contained several large, incompletely encapsulated foci of coagulative necrosis. The foci were markedly infiltrated by neutrophils. Many hepatocytes were intensely eosinophilic.

In the portal tracts several bile-ducts were necrotic while some were filled with debris. Periductal oedema associated with leucocytic infiltration and concentric

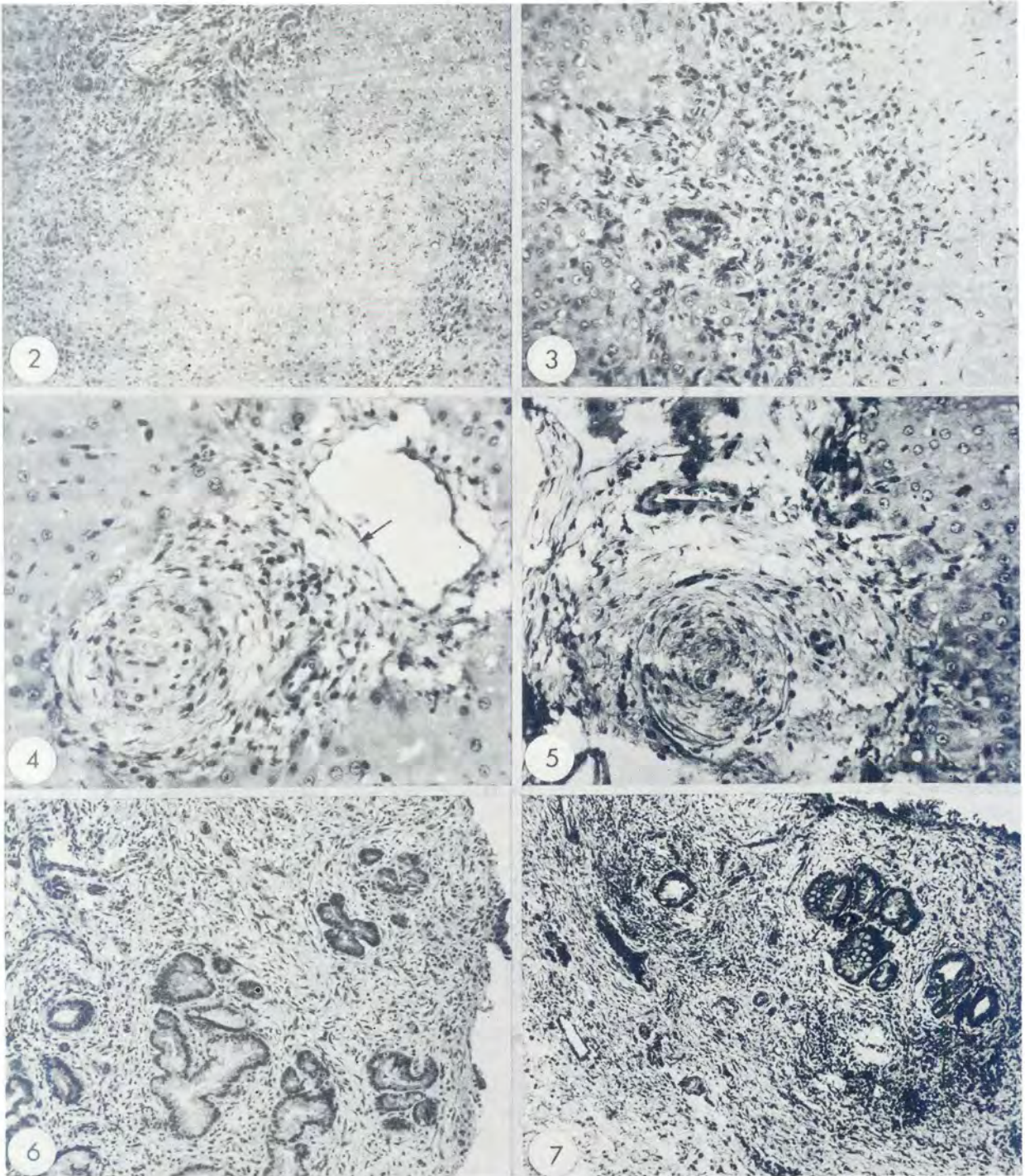


FIG. 2 Focus of partly encapsulated, coagulative necrosis in the liver with slight leucocytic infiltration into the area. Top left hand corner reveals mild bile-duct proliferation. HE \times 75
FIG. 3 Junction of necrotic and normal liver tissue with fibroplasia and mild leucocytic response. HE \times 200
FIG. 4 Occluded bile-duct with concentric fibroplasia around it. In juxta-position there is an eccentric mucoid degeneration below the intima of the blood vessel (arrow). HE \times 200
FIG. 5 Occluded bile-duct with fibroplasia and oedematous changes around it. HE \times 200
FIG. 6 Gall-bladder wall showing loss of epithelium, fibroblast proliferation and mild leucocytic infiltration. HE \times 75
FIG. 7 Wall of the main bile-duct showing changes similar to the above. HE \times 75

fibroplasia was frequently present. Some proliferation of bile-ducts was noted but it was not a conspicuous lesion. In a few blood vessels an eccentric, mucoid, subintimal degeneration was seen to occur in juxta-position to affected bile-ducts.

The wall of the gall bladder was markedly thickened and denuded of epithelium in large areas. Marked fibroplasia and slight oedema associated with moderate infiltration of macrophages and neutrophils were present. The epithelial cells in the invaginations of the mucosa were less affected than those elsewhere.

In the kidneys the epithelium of the proximal convoluted tubules were swollen and the cytoplasm was foamy in appearance. No pigment was observed.

The mucosa of the urinary bladder contained numerous haemorrhages, erosions and ulcers. Slight oedema of the muscular layer, especially around the blood vessels, was present.

DISCUSSION

The outbreaks of photosensitivity in sheep grazing on artificial pastures in the Humansdorp area of the Cape Province was initially diagnosed as facial eczema because of the presence of sporidesmin-producing strains of *P. chartarum* and the similarity of the clinical signs and histopathological findings of the field outbreaks with those of facial eczema in New Zealand (Done, Mortimer & Taylor, 1960; Crawley *et al.*, 1961). Subsequently the typical clinical and chemical pathological syndrome (Mortimer & Taylor, 1962; Done, Mortimer & Taylor, 1962) of sporidesmin poisoning was produced together with typical lesions in the biliary tree, bladder, kidneys, hepatic vessels and skin (Mortimer, 1963) by dosing the local strain of *P. chartarum* to a lamb.

At present facial eczema has been diagnosed only in the Humansdorp area. It is interesting that the affected pastures are reputed to be amongst the longest-established artificial pastures in the eastern Cape Province. The fungus *P. chartarum* has also been isolated from lucerne seed produced in the Cape Province (Marasas & Schumann, 1972), and is probably widely distributed in South Africa. The possibility exists that facial eczema may be, or may become, more prevalent in South Africa than is recognized at present. Consequently a comprehensive study of other syndromes of photosensitivity in sheep in South Africa is currently in progress.

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