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AMAUROSIS IN SHEEP RESULTING FROM TREATMENT WITH RAFOXANIDE

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ABSTRACT

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Amaurosis occurred in sheep on various farms in the Republic of South Africa after treatment with rafoxanide. Histopathological examination revealed a *status spongiosus* of varying severity in the central nervous system in all the cases, having a predilection for certain areas such as the periventricular area of the lateral ventricles, optic tracts, lateral geniculates and optic fasciculi. The retina was the only ocular tissue affected and lesions observed in the retina included necrosis of nerve cells in the ganglionic layer. In chronic cases of amaurosis this layer showed a complete absence of nerve cells. The possible pathogenesis of the lesions and their differentiation from those found in certain plant toxicoses are discussed.

Résumé

AMAUROSE OVINE CONSÉCUTIVE AU TRAITEMENT À LA RAFOXANIDE

Dans diverses fermes de la République sud-africaine, des moutons qui avaient été traités à la rafoxanide ont été atteints d'amaurose. L'examen histopathologique a mis en évidence dans tous les cas un status spongiosus *de gravité variable dans le système nerveux central, avec une préférence pour certaines régions telles que l'aire périventriculaire des ventricules latéraux, les tractus optiques, les geniculi latéraux et les faisceaux optiques. Le seul tissu oculaire qui fût atteint était la rétine et les lésions qu'on y a remarquées incluaient la nécrose de cellules nerveuses dans la couche ganglionnaire. Cette couche était complètement dépourvue de cellules nerveuses dans des cas d'amaurose chronique.*

On discute les hypothèses relatives à la pathogénèse de ces lésions et leur différenciation d'avec celles qui accompagnent certaines toxicoses d'origine végétale.

INTRODUCTION

In 1969, Mrozik, Jones, Friedman, Schwartskopf, Schardt, Patchett, Hoff, Yakstis, Riek, Ostlind, Plisher, Butler, Cuckler & Campbell announced the discovery of a new fasciolicidal compound, rafoxanide [3,5-diiodo-3′-chloro-4-(p-chlorophenoxy-salicylanilide], one of the halogenated salicylanilides.

Since its appearance and general use, rafoxanide has proved to be an effective fasciolicide in both sheep and cattle (Snijders, Horak & Louw, 1971; Horak, Snijders & Louw, 1972; Boray, Wolff & Trepp, 1973). Boray (1971) emphasized that its anthelmintic efficacy was dependent on the age and susceptibility of the host.

Toxicity trials proved rafoxanide to be a safe drug (Sutherland & Batty, 1971; Snijders *et al.,* 1971; Mrozik *et al.,* 1969; Guilhon, Jolivet & Barnabé, 1971; Boray, 1971). According to Mrozik *et al.* (1969), sheep not infested with liver fluke survived treatment at a dosage of 200 mg/kg, while death occurred in sheep with natural liver fluke infestation when treated at the same dosage. Some of the non-infested sheep treated with a dosage of 200 mg/kg and some of the infested sheep that received dosages of 100 mg/kg or more showed signs of blindness. These authors made no mention of lesions in the eyes or central nervous system (CNS) that could explain the visual impairment.

Brown, Rubin, Hite & Zwickey (1972) reported that Washko & Norcross (1968), using a single dose of 100 mg/kg of rafoxanide in sheep and multiple doses of 250 mg/kg in the rat, found vacuoles in the white matter of the brain and opacity of the lens. Blindness and vacuolation of the CNS were briefly described in sheep with another halogenated salicylamide, clioxanide, in Australia (O'Brien, 1970). The fact that no detailed report on the lesions caused by rafoxanide in sheep were to be found in the literature and the absence of any references to retinal changes motivated the present study.

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HISTORY

Cases of blindness in sheep following the administration of rafoxanide have been under investigation since 1972. Thirteen animals showing amaurosis have been submitted to Onderstepoort since 1972. Six of the 7 owners could not recall the exact dose they had given to the sheep, although it was apparent that an overdose of rafoxanide had been used in every instance. One of the animals examined originated from a flock of 30 animals that had been treated with a dosage of 20 ml/50 kg, the average mass of the sheep. On the day following the treatment all the sheep showed inappetence. All except 2 animals recovered and both of these showed complete blindness 2 days after treatment. The 2 affected sheep were autopsied 7 days after treatment when it became clear that there was no improvement. Amaurosis with marked mydriasis 2–3 days after treatment with rafoxanide was the most prominent clinical observation made by all the owners. In 1 of the animals submitted for examination, the marked mydriasis was accompanied by slight ataxia of the hindquarters. Blindness was permanent since 3 of the animals with amaurosis kept under observation for 2½ years showed no improvement. In the flocks investigated, the incidence of amaurosis varied from single animals to 10% of the flock.

MATERIALS AND METHODS

Eleven sheep and the brains of 2 other sheep were submitted for examination. The brains were fixed *in toto* before coronal sectioning and selection of blocks for histological examination and specimens from various other organs were collected in 10% buffered formalin, except the eyes which were suspended in unbuffered 10% formalin. Tissue blocks were embedded in paraffin wax, sectioned at 3–5 μm thickness and stained with haematoxylin and eosin (HE). The special staining techniques employed were those of Van Gieson (Lillie, 1954), periodic acid Schiff reaction (PAS), (Pearse, 1961), Luxol fast blue (Margolis & Pickett, 1956), Mallory's phosphotungstic acid haematoxylin (Anon, 1960), Holzer's (Anon, 1960), Dahl's (Pearse, 1961), Luxol fast blue-Holmes' (Margolis & Pickett, 1956) and Sudan black (Lillie, 1954).

Results

Gross pathology

No gross lesions were observed in any of the sheep apart from bulging of the optic fasciculi around the circumference of the optic foramen in 3 of the animals.

Microscopic pathology

Brain

The most prominent lesion in the brains examined was a bilateral *status spongiosus* unaccompanied by any inflammatory reaction in the white matter affecting different areas of the brain (Fig. 1). Vacuoles in the areas of *status spongiosus* varied considerably in size, from 7–75 μm, and some were even larger. Luxol fast blue-Holmes' stained preparations showed the vacuoles to be transected by thin strands of material but none of the other special staining techniques employed showed up any other material within the cavities.

Three of the brain specimens showed dilated perivascular spaces, while the neuropil around the bloodvessels was sieve-like in appearance. Neurones in the various parts of the CNS in all the specimens examined were unaffected, but glia cells with enlarged eosinophilic cytoplasm in the areas showing *status spongiosus* were present in 3 of the animals.

The extent of the spongy lesions varied considerably in different animals. They were particularly prominent in 7 of the brain specimens examined but only mild in 6 others. Spongy lesions showed a predilection for the periventricular areas (Fig. 1) which included the *hippocampus* (*fimbria* and *alveus*), *corpus callosum, fornix* and *septum pellucidum*. Other areas in which lesions occurred less commonly were the optic radiation, lateral and medial geniculate and optic tracts. In pronounced cases the entire white substance of the brain was affected, although the extent of the lesion varied in the different areas.

Optic fasciculi

Marked lytic necrosis with mobilization of gitter cells and severe myelin loss were present in 1 animal. Spongy lesions of varying severity, without gitter cell reaction, were seen in the optic fasciculi of 5 of the animals (Fig. 3). They were more prominent at the periphery of the optic fasciculi and were probably due to swelling and pressure against the optic foramen. Areas showing lytic necrosis had a moth-eaten appearance (Fig. 4). One animal only had no lesions in the optic fasciculi.

Spinal cord

Spongy lesions similar to those in the brain were present in the spinal cord of 8 of the 12 cases from which this structure was available for examination (Fig. 2). The dorsal tracts were the areas most often affected except in 1 animal where the entire peripheral zone of white matter showed a narrow band of *status spongiosus*. Although representative samples of the whole spinal cord of only 3 animals were examined, it appeared that lesions were not present at all levels of the spinal cord and that the lumbar region was the area most consistently affected.

Spinal nerves

Spongy lesions corresponding to the lesions observed in the brain were present in 1 animal only (Fig. 2).

Eyes

The retinas of the eyes of 6 of the 7 animals available for examination, were the only intra-ocular tissues affected. No lesions at all were present in one animal. Lesions observed in the eyes of the 3 sheep autopsied $2\frac{1}{2}$ years after treatment showed a complete absence of nerve cells in the ganglionic cell layer of the retina (Fig. 7). In one of these chronic cases the fact that the inner and outer nuclear layers appeared less dense was apparently due to a diminished cellular content. In 2 animals autopsied approximately 2–6 weeks after treatment, vacuoles of different size containing an eosinophilic material were seen in the cytoplasm of the neurones in the ganglionic cell layer. Neurophagocytosis was prominent and in focal areas of the retina there seemed to be an increase of glial cells (Fig. 6). Oedema, seen as the accumulation of homogeneous eosinophilic material between the neuropil in the retina, severe haemorrhages and congestion were present in an animal examined 2 days after dosing (Fig. 8). Vacuoles, as previously described, were present in the cytoplasm of odd neurones in the ganglionic cell layer. A prominent feature in the retina of this case was the presence of numerous necrotic neurones in the ganglionic cell layer (Fig. 9). The entire cytoplasm of these dead cells consisted of an eosinophilic amorphous material. Nuclei of the necrotic neurones commonly exhibited pyknosis and karyorrhexis, while in some the fading of the nucleus gave evidence of chromatolysis. Only a vague outline of the nucleus or no evidence of it at all was present in these instances. A few of the necrotic neurones were infiltrated or surrounded by neutrophils. Some of the glia cells in the ganglionic layer showed enlarged cytoplasms. Small purplish granules of different size, probably representing nuclear debris, were irregularly scattered between the dead neurones.

Lens

There were no lesions present in the 2 lens specimens available for examination.

Discussion

Status spongiosus, microscopically characterized by vacuoles in the white and/or grey matter which gives the tissue its spongy or sieve-like appearance, may be found in a variety of conditions and therefore does not represent a specific CNS lesion (Jellinger & Seitelberger, 1970). Spongy changes without primary loss of neural elements may be due to swollen astrocytes (Klatzo, 1967), swollen astrocytic processes (Klatzo, 1967), vacuolation of oligodendroglia (Luse & Harris, 1960), intramyelin vacuoles (Suzuki & Kikkawa, 1969), intra-axonal vacuoles (Hirano, Levine & Zimmerman, 1967), distended extracellular spaces (Bogaert & Bertrand, 1949, cited by Jellinger & Seitelberger, 1970) or the result of several of these (Adachi, Wallace, Schneck & Volk, 1966). Suzuki & Kikkawa (1969) stated that intramyelinic vacuoles induced by cuprizone could be explained by a metabolic disturbance of the oligodendroglia which showed vacuoles in their cytoplasm. At the light microscope level, *status spongiosus* is similar in appearance in many of these instances (Suzuki & Kikkawa, 1969). The exact location of the vacuoles cannot be determined accurately at this level, but it appeared to be within the myelin sheaths in the present material studied.

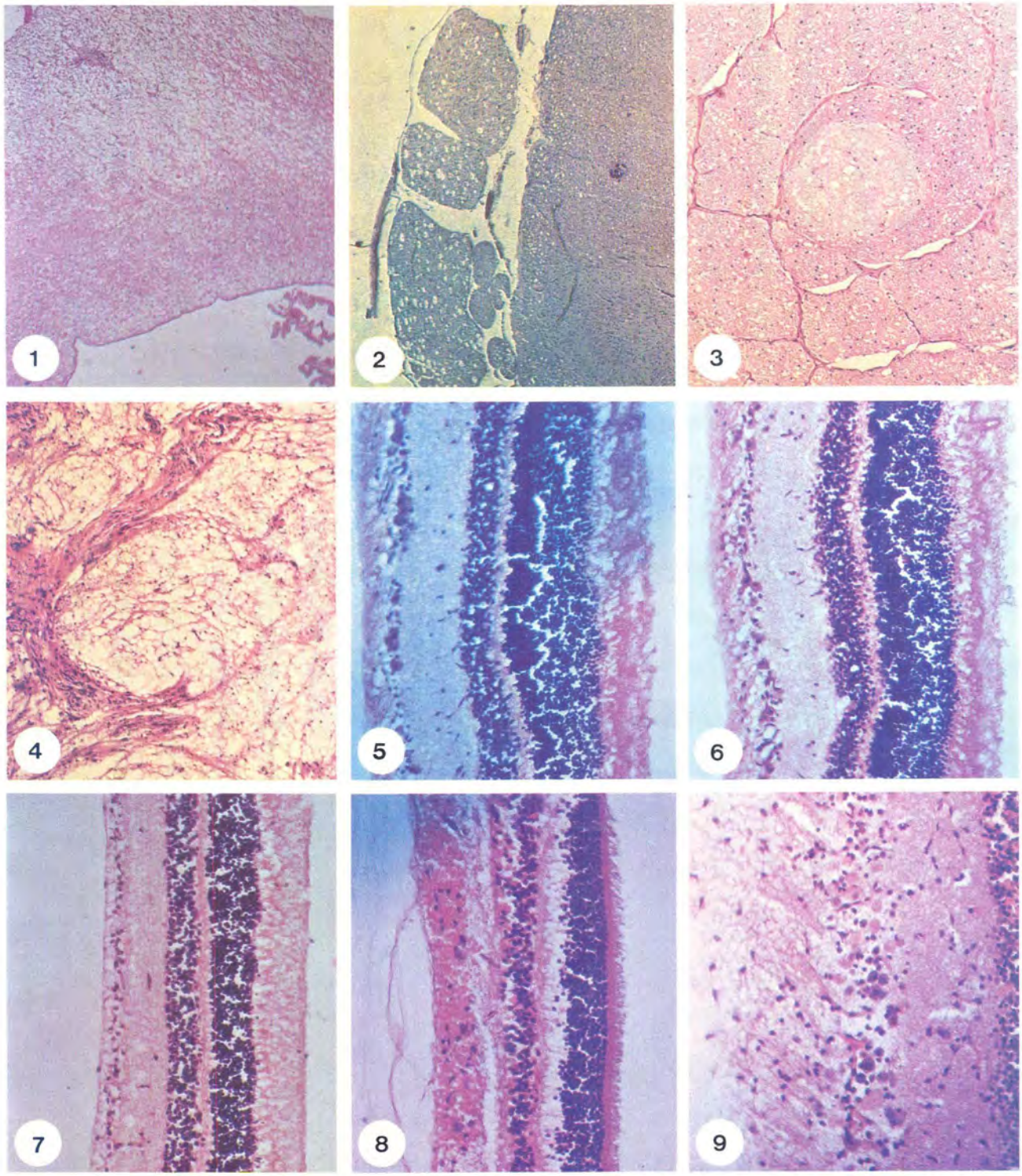

FIG. 1 Periventricular area (*corpus callosum*) with severe *status spongiosus*. HE × 75
FIG. 2 Spinal cord and spinal nerves showing mild *status spongiosus*. HE × 75
FIG. 3 Optic fasciculus with central area showing lytic necrosis. HE × 75
FIG. 4 Optic fasciculi showing severe lytic necrosis. HE × 75
FIG. 5 Normal retina. HE × 200
FIG. 6 Retina of sheep autopsied approximately 4 weeks after treatment with rafoxanide. Note increase of glia cells in the ganglionic cell layer. HE × 200
FIG. 7 Retina of sheep autopsied 2½ years after treatment with rafoxanide. Note absence of neurons in the ganglionic cell layer. HE × 200
FIG. 8 Severe haemorrhages and congestion in the retina of a sheep autopsied 2 days after administration of rafoxanide. HE × 200
FIG. 9 Retina of the same animal shown in Fig. 8. Note swelling of neurons and gliosis in the ganglionic cell layer. HE × 500

According to Adachi *et al.* (1966), histochemical studies showed that activity of adenosine triphosphatase (ATPase) within mitochondria of astrocytic processes was decreased in areas showing spongy lesions. Jellinger & Seitelberger (1970) reported that a membrane-bound ATPase, activated by Na^+ and K^+, plays an important role in the active transport of ions and water across cell membranes. In 1965 Torack (cited by Jellinger & Seitelberger, 1970) observed that membrane-bound ATPase, when inhibited, evoked a form of brain oedema. Adachi & Volk (1968) mentioned the possibility that enzyme defects, especially ATPase in astrocytes and basement membranes of cerebral blood vessels, might play an important role in the pathogenesis of *status spongiosus* in infancy.

In 1973, Sanderson (cited by Yorke & Turton, 1974) proved that many fasciolicidal or anti-cestode anthelmintics act by uncoupling electron transport-linked phosphorylation and in doing so, inhibit the uptake of oxygen at high levels. Earlier, Miert & Groenewald in 1969 (cited by Yorke & Turton, 1974) used mammalian mitochondria in experiments and came to the same conclusion. As rafoxanide was the most potent inhibitor of ATPase production in experiments carried out by Yorke & Turton (1974), it is possible that the *status spongiosus* observed in the CNS of the sheep examined may be due to ATPase inhibition by this drug.

The high incidence of spongy lesions in the nerve tracts involved in the conduction of light impulses in the cases reported here was of considerable interest. Those included in these pathways were the optic fasciculi, optic chiasma, optic tracts, lateral geniculate and optic radiation, all of which showed pronounced lesions. Papilloedema, associated with increased cerebro-spinal fluid pressure, spongy changes of the periventricular white matter, *corpus callosum*, *fornix*, periphery of the optic chiasma and perivascular areas throughout the CNS in dogs after experimental treatment with rafoxanide was reported by Brown *et al.* (1972).

The blindness observed in the sheep could be due to the lesions in the retina or optic nerves, or to the spongy lesions in the CNS. Unfortunately no definite correlation between the severity of the eye and brain lesions could be made as the eyes of the animals showing mild spongy lesions were not available for examination. To the best of our knowledge this is the first documentation of lesions in the retina of sheep exposed to rafoxanide. Brown *et al.* (1972) described slight bilateral equatorial lenticular degeneration (cataract) in 11 of the 12 dogs given rafoxanide and superficial retinal oedema and haemorrhages in others. According to these authors, Washko & Norcross in 1969 observed lens opacities in sheep dosed with a single dose of 100 mg/kg of rafoxanide.

Liver fluke-infested sheep are more susceptible to rafoxanide than normal animals (Mrozik *et al.*, 1969). Non-infested sheep tolerated rafoxanide at a dosage rate of 200 mg/kg. Death occurred in some animals naturally-infested with liver fluke at the same dosage rate. One sheep inoculated with 1 000 metacercariae died after treatment with rafoxanide at 100 mg/kg. In contrast Boray (1971) reported a maximum tolerated dose of 45 mg/kg (recommended dosage 7,5 mg/kg, Horak, Snyders & Louw, 1972). Campbell, Ostlind & Yakstis (1970) proved that the formulation of rafoxanide also influenced its toxicity. In the present cases under review, however, it became clear that faulty handling of the drug, over-estimation of the animal's body mass, the use of faulty drenching equipment and failure to read the directions on the label were the main causes of poisoning.

In Southern Africa amaurosis is a feature of various plant poisonings. According to Steyn (1949), *Brassica* spp. and *Ornithogalum* spp. can give rise to blindness in sheep but the lesions caused by these plants which may be responsible for amaurosis have not yet been documented. *Helichrysum argyrosphaerum* (Basson, Kellerman, Albl, Von Maltitz, Miller & Welman, 1976), when ingested in large quantities by sheep, causes *status spongiosus* of the brain, spinal cord, optic fasciculi and nerves, followed by paralysis. The lesions in this condition resemble those of rafoxanide in many respects and should be kept in mind as an important differential diagnosis since various species of *Helichrysum* are widely distributed in South Africa. The lesions present in the eye in rafoxanide toxicity, however, differ from the lesions described in *H. argyrosphaerum* poisoning. Necrosis commencing in the layer of rods and cones and extending to the inner nuclear layer was observed in the latter condition, but no lesions were present in the ganglionic cell layer. In comparison, the ganglionic layer of the retina was most severely affected with rafoxanide intoxication and no changes were present in the layer of rods and cones. Cataracts were observed 2–3 months after the initial outbreaks of *Helichrysum* poisoning under natural conditions but this lesion could not be reproduced experimentally (Basson *et al.*, 1976). No cataracts were found in sheep poisoned with rafoxanide under field conditions.

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