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A SUSPECTED LIPOFUSCIN STORAGE DISEASE OF SHEEP ASSOCIATED WITH INGESTION OF THE PLANT, *TRACHYANDRA DIVARICATA* (JACQ.) KUNTH

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ABSTRACT

NEWSHOLME, S. J., SCHNEIDER, D. J. & REID, CLARE, 1985. A suspected lipofuscin storage disease associated with ingestion of the plant, *Trachyandra divaricata* (Jacq.) Kunth. *Onderstepoort Journal of Veterinary Research*, 52, 87–92 (1985).

Paresis afflicted 85 out of a flock of 770 young Merino ewes kept on old wheat lands in the western Cape during a period of drought. Many of the paretic ewes died. The vegetation was sparse and was dominated by *Trachyandra divaricata*. At necropsy, yellowish-brown discoloration of the grey matter throughout the brain and spinal cord and mild brown discoloration of the liver, renal cortex and lymph nodes were consistently seen. Light microscopical examination revealed abundant, yellowish-brown pigment granules in the cytoplasm of most of the larger neurons. Similar pigment also occurred in some non-nervous tissues. Shrinkage and loss of a few randomly scattered axons were observed in the white matter of the spinal cord in 2 sheep. Histochemical and ultrastructural features of the pigment were consistent with those of lipofuscin.

T. divaricata failed to reproduce the condition when dosed to a sheep, but the paresis and pigmentation shown to be caused by the closely related plant, *T. laxa*, are strikingly similar. *Trachyandra* poisoning appears to be the first documented example in farm animals of an acquired lipofuscin storage disease involving nervous and non-nervous tissues for which a specific plant has been causally implicated.

INTRODUCTION

Outbreaks of a paretic condition in sheep, cattle, horses and swine in South West Africa (S.W.A.) have been described (Grant, Basson & Kidd, 1985) in which abundant, lipofuscin-like pigment accumulated within neurons and in some non-nervous tissues. *Trachyandra laxa* was usually the predominant plant on the farms where outbreaks occurred, and both the paresis and the pigment accumulation were reproduced by dosing this plant to sheep and a horse.

The purpose of this report is to describe clinical and pathological features of an outbreak of paresis in sheep which was associated with ingestion of the plant, *Trachyandra divaricata*.

DESCRIPTION AND DISTRIBUTION OF *TRACHYANDRA DIVARICATA* (JACQ.) KUNTH (LILIACEAE)

Description (Obermeyer, 1962): Plants robust up to 90 cm high (Fig. 1). *Roots* many, not much thickened. occasionally growing to a great depth. *Rhizome* woody, thick, irregular in shape. *Squamae* narrow, tubular, surrounding each leaf- and scape-base separately. *Leaves* linear, up to 100 cm long, 4–12 mm wide, tapering gradually to the apex, flat, glabrous, somewhat fleshy, flexible, erect or usually prostrate, straight or with a lax spiral twist, bright green, occasionally orange at the base. *Inflorescence* stout, usually with accessory branches, divaricately branched; scape 10–50 cm high, stout, glabrous; bracts small, 4 mm long, membranous, widely ovate at the base; pedicels 4–12 mm long. *Flowers* erect, perianth segments 7–12 mm long, white, green-keeled with a yellow dot near the base, spreading, recurved from the middle; stamens yellow in lower half, dimorphous, 3 outer spreading, 3 inner connivent around ovary, spreading and retrorsely scabrid above; ovary with 12 atropous ovules per cell. *Capsule* globose, 12 mm in diameter, slightly inflated, dry or somewhat fleshy, yellowish. *Seeds* 2 mm diameter, smooth, tetrahedral.

Flowering time: Mainly August–September, also throughout the year.

Distribution: South-western to south-eastern Cape Province, also occasional in Namaqualand and Gordonia (Fig. 2). Mainly on sand dunes near the sea.

HISTORY OF OUTBREAK AND CLINICAL SIGNS

During the winter of 1979, a flock of Merino ewes was afflicted with paresis. The flock was kept on old wheat lands in the coastal, sandy area near Darling in the western Cape Province. Since winter rains had been insufficient, the lands had not been resown with wheat for 2 years. During this period, the sheep had grazed the wheat stubble and plants growing on the lands. When the outbreak occurred, drought conditions prevailed. The vegetation was sparse and consisted predominantly of *T. divaricata* (Fig. 3) with some *Cynodon dactylon*. The leaves of many of the *T. divaricata* plants had been eaten (Fig. 4).

In all, 85 out of 770 ewes (11 %) were affected, of which 40 died before the end of August. Affected ewes become recumbent. They were thin but alert, and they ate and drank when food and water were brought to them. They could not rise, and pinch withdrawal reflexes for all limbs were diminished. Surviving ewes were brought to the farm and nursed. Some became able to move on their knees but few recovered, and deaths continued into December 1979. Most of the ewes had 1–3-month-old lambs, but none of these became paretic.

MATERIALS AND METHODS

Three live, 1–2-year-old, paretic ewes and various formalin-fixed tissue specimens collected at necropsy from 4 paretic ewes were sent to the Veterinary Research Institute, Onderstepoort, for pathological examination.

Gross pathology

The 3 live ewes were killed by intravenous injection of pentabarbitone sodium and necropsies were done immediately. Tissue specimens were collected for light and electron microscopy.

Light microscopy

Specimens of brain, spinal cord, liver, lung, kidney, heart, intestine and various skeletal muscles from the 3 ewes were fixed by immersion in buffered 10 % formalin. These specimens and the others received were processed routinely and embedded in paraffin wax. Sections were cut at 4–6 μm thickness and stained with haematoxylin and eosin (HE). Sections from selected paraffin blocks were stained by the periodic acid-Schiff reaction (PAS) (Luna, 1968), Schmorl's method

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FIG. 1 *Trachyandra divaricata*

(Pearse, 1961), a carbol fuchsin method for lipofuscin (Luna, 1968), Lillie's method for melanin (Lillie, 1957), a modified Warthin-Starry method for melanin (Warkel, Luna & Helwig, 1980), luxol fast blue-Holmes silver nitrate (Margolis & Pickett, 1956) and with Berlin blue for iron (Pearse, 1961). Selected sections of brain and spinal cord were deparaffinized, immersed in 10 % hydrogen peroxide solution for 48 h and subsequently stained with HE. Unstained, deparaffinized sections of brain and spinal cord were examined microscopically for fluorescence in transmitted ultraviolet (365 nm) light.

Electron microscopy (EM)

Specimens of spinal cord from 2 of the ewes were collected, diced into 1 mm cubes and fixed by immediate immersion in 4 % glutaraldehyde in Millonig's phosphate buffer at pH 7,3–7,4 (Millonig, 1961) for 3 h. Selected blocks were rinsed with phosphate buffer and post-fixed in 2 % osmium tetroxide, also in the same buffer. Following 2 or more buffer rinses, the blocks were dehydrated in a graded ethanol series, cleared in propylene oxide and embedded in Epon 812 for 48 h at 60 °C.

Thick (1–2 μm) sections were cut and stained with toluidine blue (Trump, Smuckler & Benditt, 1961) for tissue orientations. Relevant blocks were trimmed to size and thin sections were cut, picked up on copper grids and stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). The stained grids were viewed in a Siemens Elmiskop 102 transmission electron microscope.

Dosing trial

A 37 kg Dorper ewe at the Regional Veterinary Laboratory, Stellenbosch, was given consecutive daily oral doses of fresh *T. divaricata* for 5 months. When the plant was collected, it was growing rapidly after good rains. The total dose given was 5,54 kg/kg. At the end of the trial the sheep was killed for necropsy.

RESULTS

Gross pathology

In all the paretic sheep examined, there was moderate to marked yellowish-brown discoloration of the grey matter throughout the brain (Fig. 5) and spinal cord, and mild brown discoloration of the liver, renal cortex and lymph nodes. In 2 of the sheep, the skeletal muscles appeared darker than normal.

Light microscopy

Nervous system: In HE sections of all cases, yellowish-brown pigment granules measuring 0,5–3 μm in diameter were abundant in the perikaryal cytoplasm of most of the larger neurons throughout the brain and spinal cord. In some neurons, the granules were diffusely scattered and in others they formed dense aggregates (Fig. 6) which were situated around the nucleus or in the axon hillock. In a few neurons, occasional, round, eosinophilic bodies were admixed with the granules. The number of granules generally appeared to be related to the size of the neurons, but the cerebellar neurons of Purkinje possessed relatively few granules. Some perineuronal and perivascular glial cells contained a few similar cytoplasmic granules in 2 of the sheep. Similar granules also occurred in the autonomic ganglionic neurons of the gastro-intestinal tract. The granules stained positively with PAS, with Schmorl's method and with the carbol fuchsin method for lipofuscin. They did not

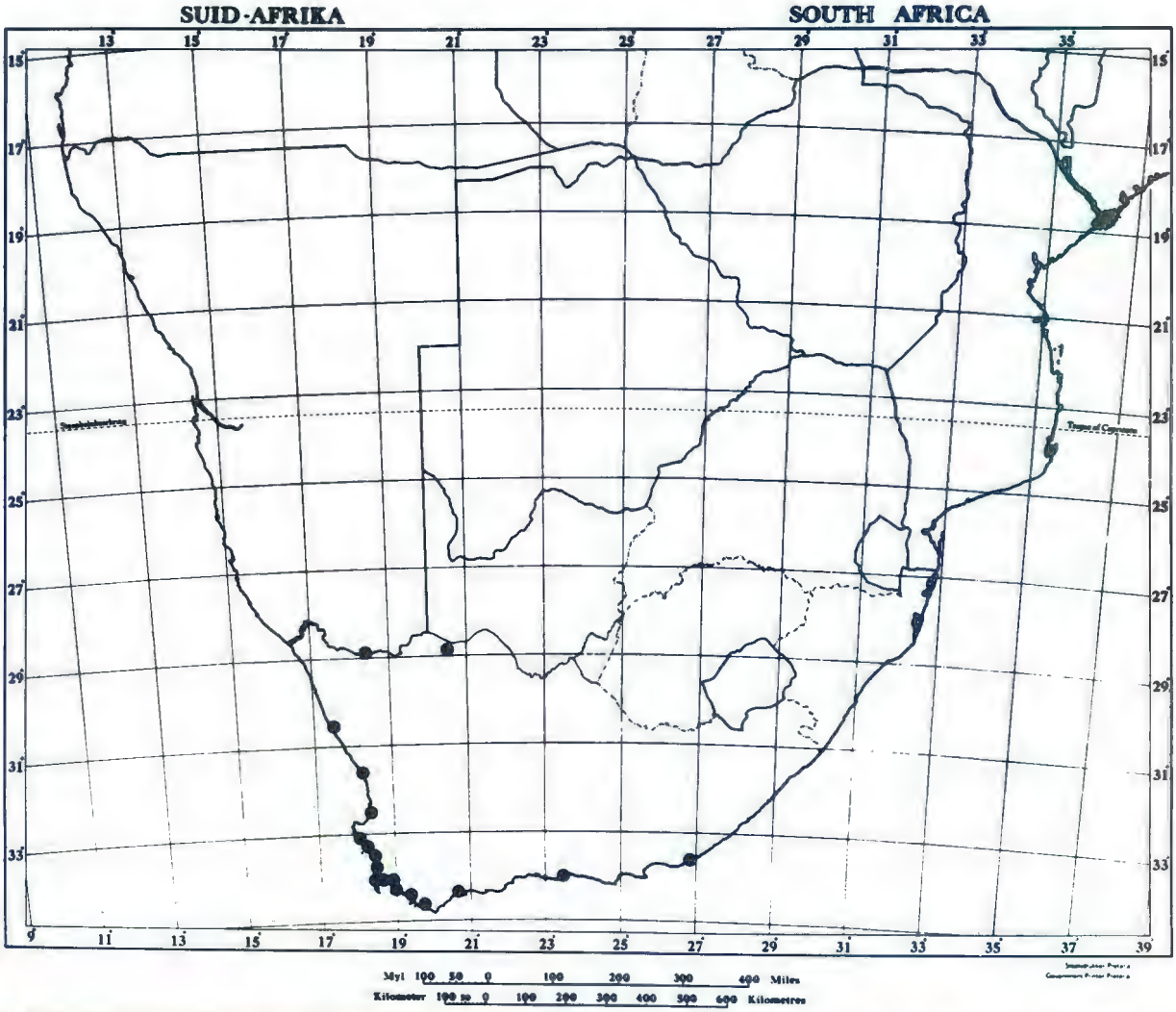

FIG. 2 The distribution of *T. divaricata* in South Africa

FIG. 3 Sparse vegetation, dominated by *T. divaricata*

FIG. 4 Leaves of *T. divaricata* eaten back

stain with Lillie's method for melanin or the modified Warthin-Starry method for melanin, and they were not bleached by hydrogen peroxide treatment. Their reaction to Berlin blue for iron was negative. Fluorescence could not be demonstrated in the granules when examined in ultraviolet light.

In the white matter of the spinal cords of 2 sheep, segments of shrinkage and loss of occasional axons, sometimes with swelling of the surrounding myelin sheath, were observed in sections stained with luxol fast blue-Holmes silver nitrate. Not more than 10 axons so affected were seen in each transverse or paramedian section of spinal cord. Their distribution appeared to be random.

FIG. 5 Diffuse, yellowish-brown discoloration of grey matter of brain

Other tissues: Aggregates of similar granules occurred in hepatic Kupffer's cells and in macrophages in the medulla of lymph nodes, splenic red pulp and pulmonary alveolar walls in all cases. Fewer, more loosely arranged granules were consistently seen in the renal tubular epithelium and adjacent to the nuclei of cardiac and skeletal myocytes. The histochemical reactions of these granules were similar to those of the intraneuronal granules.

Electron microscopy

The perikaryal cytoplasm of many of the ventral horn neurons contained clusters of irregularly shaped, electron-dense bodies measuring 0,5–2,5 μm along their greatest dimension. Variably sized, electron-lucent vacuoles were situated within or at the periphery of some of these bodies (Fig. 7 & 8). Some of the bodies clearly possessed granular and membranous substructures, and a limiting membrane was evident around many of them (Fig. 9). The amount and distribution of the bodies corresponded with those of the granules observed by light microscopy.

Dosing trial

No clinical signs were evident in the sheep throughout the trial period. No macroscopical changes were seen at necropsy, and pigment granules could not be found in the tissues by light microscopy.

Discussion

The available evidence suggests that the paresis and tissue pigmentation in these sheep were related to consumption of *T. divaricata*. When the outbreak occurred, this plant was predominant and much of it had clearly been eaten. The dosing trial with *T. divaricata* failed to reproduce the condition, but the paresis and pigmentation which have been shown to be caused by *T. laxa* (Grant *et al.*, 1985), a closely related plant in S.W.A., are strikingly similar. Further dosing trials using plants at different stages of growth would be necessary to assess the toxicity of *T. divaricata*. The plant used in this trial was growing rapidly after good rains, but the occurrence of the outbreak during a drought suggests that the toxicity of *T. laxa* might be enhanced by drought conditions.

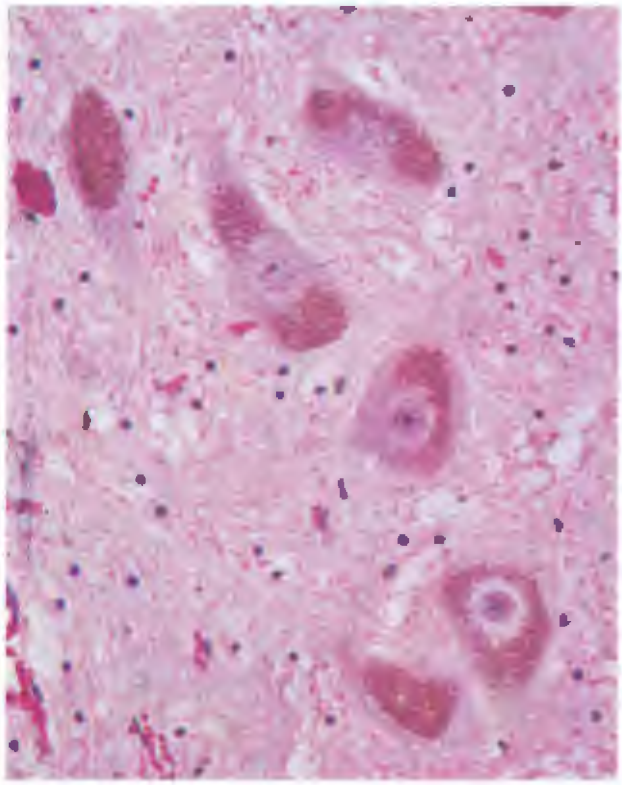

FIG. 6 Abundant, yellowish-brown pigment granules in neuronal cytoplasm; medulla oblongata: HE × 500

The morphological and histochemical features of the intraneuronal pigment are consistent with those of lipofuscin. The possibility of a melanin component has not been excluded, since neuronal melanin and lipofuscin are reportedly similar in their histochemical properties and ultrastructure (Barden, 1969; Roy & Wolman, 1969). Nonetheless, the pigmentation is clearly distinct from that in sheep with chronic *Phalaris* staggers, in which the brain is discoloured bluish-grey and the melanin-like pigment is concentrated in the brain stem and is PAS-negative (Hartley, 1978). The absence of yellow fluorescence in ultraviolet light is unusual for lipofuscin, but has been reported for splenic lipofuscin in mice (Crichton, Busuttil & Price, 1978). It is necessary to determine whether the accumulation of the pigment was abnormal, since intraneuronal lipofuscin accumulation is a consistent feature associated with ageing as is shown by numerous studies, including those of Brody (1960) on human brains, Whiteford & Getty (1966) on dogs and pigs and Reichel, Hollander, Clark & Strehler (1968) and Samorajski, Ordy & Rady-Reimer (1968) on rodents. The relationship of lipofuscin accumulation to ageing has not been clearly defined for sheep, but our own observations of ovine brains, regularly received from various areas of southern Africa, indicate that neuronal lipofuscin is normally scarce in sheep less than 3 years old.

Inherited lipofuscin storage diseases have been reported in various species, including one in sheep (Jolly, Janmaat, West & Morrison, 1980), but reports of acquired lipofuscinosis in farm animals are rare. Neuronal lipofuscinosis has been described in horses with Gomen disease (Hartley, Kuberski, Le Gonidec & Daynes, 1982), for which an environmental cause is suspected but has not been identified. *Trachyandra* poisoning appears to be the first documented example in farm animals of an acquired lipofuscin storage disease involving neurons and other cells for which a specific plant has been causally implicated.

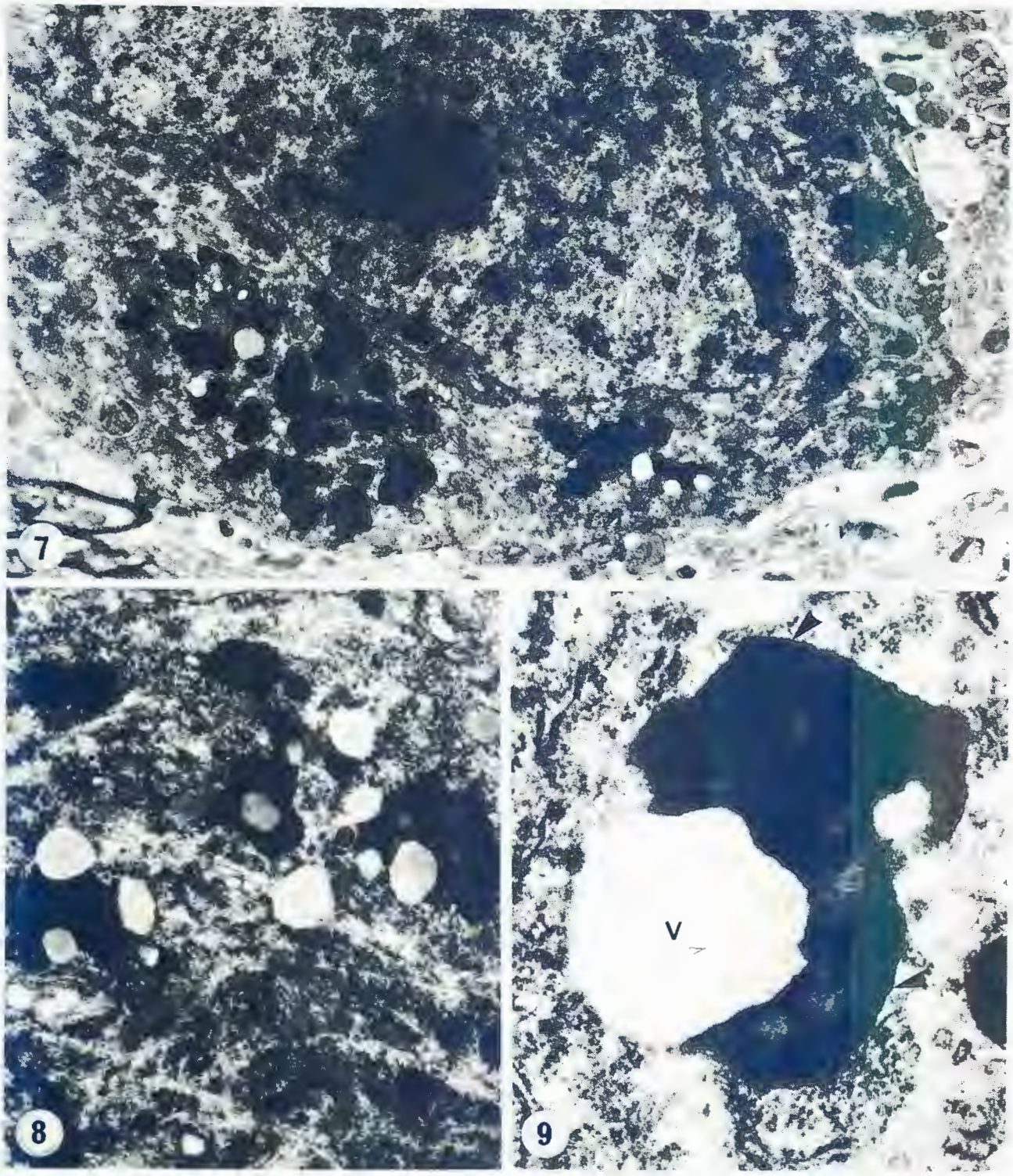

FIG. 7 Clusters of electron-dense bodies; ventral horn cell: EM × 5000

FIG. 8 Electron-dense bodies with vacuoles; ventral horn cell: EM × 10 000

FIG. 9 Electron-dense body with limiting membrane (arrow-heads), granular and membranous substructures and vacuoles (V); ventral horn cell: EM × 40 000

The underlying disorders at the molecular level responsible for the various lipofuscinoses are poorly understood and are probably diverse. Any progress to be made in the pathogenesis of *Trachyandra* toxicosis is likely to await identification of the toxic principle. Whatever the mechanism involved, the degree and extent of neuraxonal injury in these sheep did not seem to us to be sufficient on its own to explain the paresis. The clinical effect, if any, of the pigment in the sheep is open to question. The pigment might reflect an unidentified, sub-lethal cell injury which has other effects on cell function. Alternatively, we find it attractive to speculate that the pigment directly affects neuronal function. Barden (1970) has presented evidence to suggest that neuronal lipofuscin accumulation can depress function of the Golgi apparatus. Progressive decrease in neuronal cytoplasmic ribose nucleic acid content, which has been interpreted to indicate depressed neuronal function, can accompany abundant lipofuscin accumulation (Mann & Yates, 1974).

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