

## Ultrastructure of the myenteric ganglia in the rumen, reticulum, omasum and abomasum of grey, white and black Karakul lambs

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### ABSTRACT

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Homozygous grey and white Karakul lambs die after they have reached weaning age. This is due to a lethal gene causing digestive disturbances. Previous studies revealed that grey and white lambs have large, milk-filled rumens; the phenomenon was attributed to a significant decrease in the number of myenteric ganglia and neurons in the rumen wall. This study was undertaken to determine whether any morphological differences exist in the ultrastructure of the myenteric ganglia in the forestomach and abomasum of grey, white and black Karakul lambs. Samples of the forestomach and abomasum of grey, white and black Karakul lambs were prepared routinely for electron microscopy and studied with a Phillips electron microscope. No morphological differences could be detected in the structure of the components of the myenteric ganglia in the forestomachs and abomasums of grey, white and black Karakul lambs. It was therefore concluded that the lethal gene in grey and white Karakul lambs results in a paucity of the myenteric ganglia, but does not affect the ultrastructure of these structures.

### INTRODUCTION

Homozygous grey and white Karakul lambs are born apparently normal but, due to a lethal genetic factor, they develop pot-bellies, become weak and emaciated, and die after they have reached weaning age (Nel & Louw 1953). On post mortem examination Nel & Louw (1953) found large, thin-walled rumens and impacted abomasa in affected lambs. Nel (1965) described enlarged forestomachs in newly born grey Karakul lambs and came to the conclusion that digestive tract abnormalities are already present at birth.

Only lambs homozygous for the grey colour are affected. Such animals can be identified at birth by

the lack of pigmentation of the tongue, palate and ears (Nel & Louw 1953).

The enteric nervous system consists of two major plexuses of ganglia and interconnecting nerve fibres, as well as several subsidiary groups of fibres in the circular muscle layer, longitudinal muscle layer, muscularis mucosa and the lamina propria (Richardson 1960; Gunn 1968; Furness & Costa 1980; Gershon 1981). The two ganglionated plexuses are the submucosal plexus which lies in the submucosa and the myenteric plexus which lies between the circular and longitudinal muscle layers of the tunica muscularis (Richardson 1958; Gunn 1968; Gershon 1981). The submucosal plexus controls and coordinates absorptive and secretory functions, bloodflow and contractility of the muscularis mucosa (Cooke 1968). The

myenteric plexus is responsible for the peristaltic movements of the gastro-intestinal system (Richardson 1958; Gershon 1981).

The ganglia of the myenteric plexus contain neurons, supporting cells and profiles of nerve axons (Richardson 1958; Baumgarten, Holstein & Owman 1970; Gabella 1971; Gabella 1972; Cook & Burnstock 1976a; Cook & Burnstock 1976b; Fehér, Csanyi & Vajda 1979; Hoyes & Barber 1980; Gershon 1981). The ganglia are enclosed by a basal lamina and are devoid of blood vessels and connective tissue (Gabella 1972; Gershon & Bursztajn 1978; Gershon 1981). Branched processes of the supporting cells form a blood-myenteric plexus barrier (Gershon & Bursztajn 1978).

The morphology and size of the neurons in the ganglia vary (Gunn 1959; Gabella 1972; Cook & Burnstock 1976a; Fehér *et al.* 1979; Gershon 1981), and the cells display a round to oval nucleus, ribosomes, mitochondria, a Golgi apparatus, a rough endoplasmic reticulum, neurofilaments and neurosecretory vesicles (Palay & Palade 1955; Richardson 1958; Baumgarten *et al.* 1970; Gabella 1972; Cook & Burnstock 1976a; Fehér *et al.* 1979; Gershon 1981).

The supporting cells in the ganglia contain mitochondria, ribosomes, a rough endoplasmic reticulum, a Golgi apparatus and an oval nucleus characterized by deep surface indentations (Gabella 1971; Gabella 1972; Cook & Burnstock 1976b). The cells provide mechanical support and permit sliding of the nervous structures during muscle contraction (Gabella 1971). They also form part of the blood-myenteric plexus barrier (Gershon & Bursztajn 1978).

The axon profiles display mitochondria, neurofilaments and various types of neurosecretory vesicles (Richardson 1958; Baumgarten *et al.* 1970; Gabella 1972; Cook & Burnstock 1976a; Fehér *et al.* 1979; Hoyes & Barber 1980; Gershon 1981).

Studies on the number of myenteric ganglia and neurons (Groenewald & Booth 1992) revealed a paucity of these structures in the affected grey and white Karakul lambs as compared with control black lambs. Since the movement of the gastro-intestinal system is dependent on the normal functioning of the myenteric ganglia (Duncan & Phillipson 1951; Newhook & Titchen 1974; Cooke 1986), the ultrastructures of the myenteric ganglia in the forestomach and abomasum of grey, white and black Karakul lambs were compared to determine whether there were any morphological differences.

## MATERIALS AND METHODS

Five 24-h-old grey, white and black Karakul lambs were slaughtered, and samples were taken from corresponding areas of the rumen, reticulum and oma-

sum. Two additional newborn grey lambs were slaughtered before they had suckled, and matching samples were taken. Grey and white lambs with unpigmented tongues, palates and ears were specifically selected and black lambs were randomly selected.

The samples were rinsed in phosphate-buffered saline (PBS), pH 7.4, to remove the stomach contents. Small blocks of tissue were immersion-fixed in 4% glutaraldehyde in Millonig's phosphate buffer for at least 24 h at 4 °C. The blocks were subsequently rinsed in Millonig's phosphate buffer, post-fixed for 1 h at room temperature in similarly buffered 1% osmium tetroxide and given two final buffer washes. The samples were dehydrated through a graded ethanol series (25%, 50%, 75%, 96% and 100% × 2–10 min per step), cleared in propylene oxide and embedded in Polarbed 812 epoxy resin. Semi-thin sections (0.5 µm) were cut from each block to determine suitable areas for ultra-thin sectioning. Thin sections (0.1 µm) were cut with a diamond knife on a Reichert OmU4 ultramicrotome, stained with uranyl acetate (Watson 1958) (30 min) and lead citrate (Reynolds 1963) (4 min), and examined with a Philips 301 or CM10 transmission electron microscope operated at 80 kV.

## RESULTS

No difference could be detected in the structure of the components of the myenteric ganglia in the rumen, reticulum, omasum and abomasum of grey, white and black Karakul lambs.

In all instances the myenteric ganglia were separated from the muscle layers by a connective tissue layer (Fig. 1). The ganglia consisted of neurons of various sizes, supporting glial cells and profiles of nerve axons (Fig. 1). Virtually no intercellular space was evident between the various cell types, and the interior of the ganglia was totally avascular (Fig. 2). No perineurial or endoneurial sheaths surrounding the axons in the ganglia could be demonstrated. However, bundles of axons outside the ganglia were surrounded by an endoneurium as described for the peripheral nervous system (Fig. 9 and 10). The endoneurium consisted of the basal lamina of the Schwann cells and a layer of predominantly longitudinally orientated, fine collagen fibres (Fig. 9 and 10).

There was considerable variation in the size of the neurons in the ganglia studied (Fig. 1). The neurons had a large, often eccentrically placed, round to oval nucleus with one or two prominent nucleoli (Fig. 2). The nucleoplasm was finely granular with a few condensations of chromatin attached to the nuclear envelope (Fig. 2). Rough endoplasmic reticulum was abundant in the cytoplasm (Fig. 3), while smooth endoplasmic reticulum was present in lesser quantities.

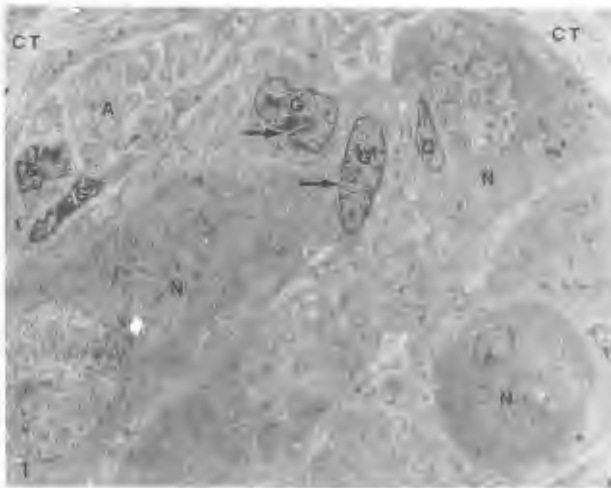


FIG. 1 A myenteric ganglion in the reticulum of a grey lamb. The connective tissue layer surrounding the ganglion (CT), neurons (N) of various sizes, glial cells (G) and profiles of axons (A) are shown. Note the indentations (arrows) in the electron dense nuclei of the glial cells (G) (2200 x)

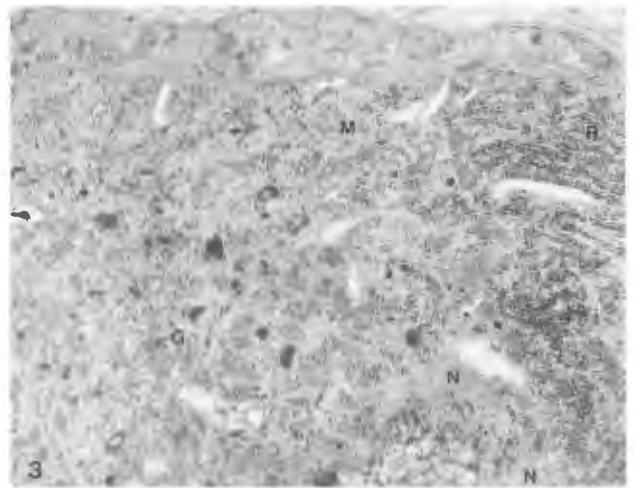


FIG. 3 A neuron in the myenteric ganglion in the rumen of a black lamb. Abundant rough endoplasmic reticulum (R) is evident. Note the mitochondria (M), Golgi apparatus (G) and neurofilaments (N) (7500 x)

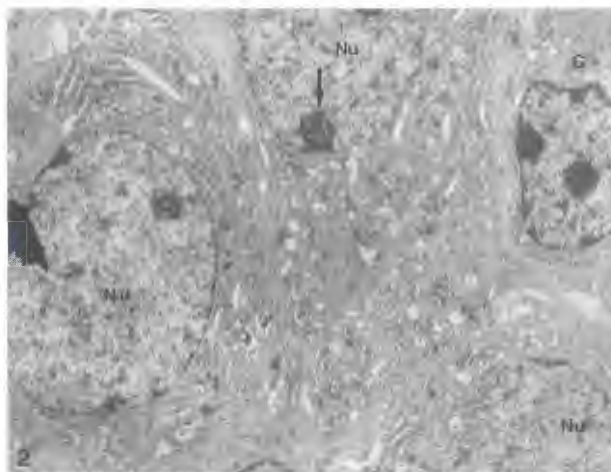


FIG. 2 A myenteric ganglion in the rumen of a black lamb. The nuclei of the neurons (Nu) are large and oval shaped with one to two nucleoli (arrow). Ribosomes, rough endoplasmic reticulum and mitochondria are visible in the cytoplasm of the glial cell (G). Note the absence of blood vessels in the ganglion (4300 x)

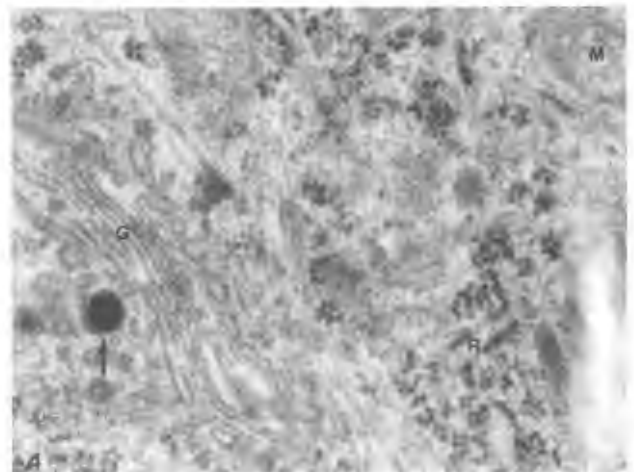


FIG. 4 A neuron in the myenteric ganglion in the reticulum of a grey lamb. Mitochondria (M), a Golgi apparatus (G) and rough endoplasmic reticulum (R) are visible. Note the small granular secretory vesicle (arrow) (43000 x)

Numerous ribosomes were scattered throughout the cytoplasm (Fig. 5). Many small mitochondria and a Golgi apparatus were apparent (Fig. 4 and 6). Microtubules (Fig. 7) and bundles of neurofilaments (Fig. 3) were scattered in the cytoplasm. Neurosecretory vesicles in the neurons were few in number (Fig. 4, 6 and 7) but were demonstrated in all the neurons studied. Small and large granular vesicles were evident (Fig. 7). Some axon profiles lay against the neurons (Fig. 8).

The supporting cells of the ganglia resembled the astroglia of the central nervous system (Fig. 1).

They almost completely separated the neuronal elements from the surrounding connective tissue. In the ganglia, the nuclei of the glial cells appeared to outnumber the nuclei of the neurons (Fig. 1). The bodies of the glial cells were generally smaller than those of the neurons (Fig. 1). They frequently gave off long processes, making it difficult to ascertain the limits of the cell bodies. The nucleus was generally oval in shape and deeply indented (Fig. 1). The nuclei of the glial cells were more electron-dense than those of the neurons (Fig. 1). The glial cell cytoplasm was rich in ribosomes, rough endoplasmic reticulum and

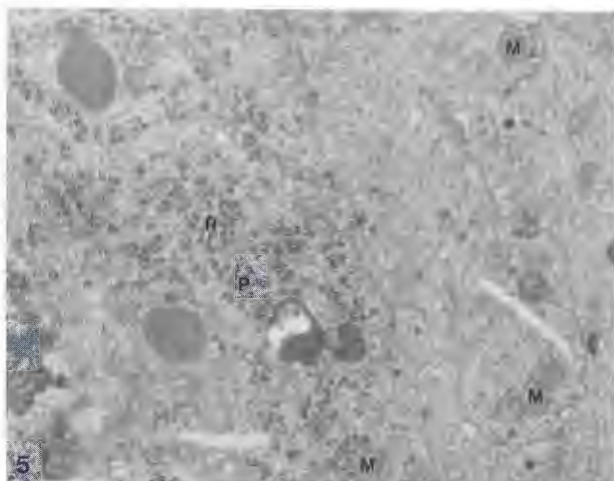


FIG. 5 A neuron in the myenteric ganglion in the omasum of a black lamb. Mitochondria (M), polyribosomes (P) and rough endoplasmic reticulum (R) are apparent (18000 x)

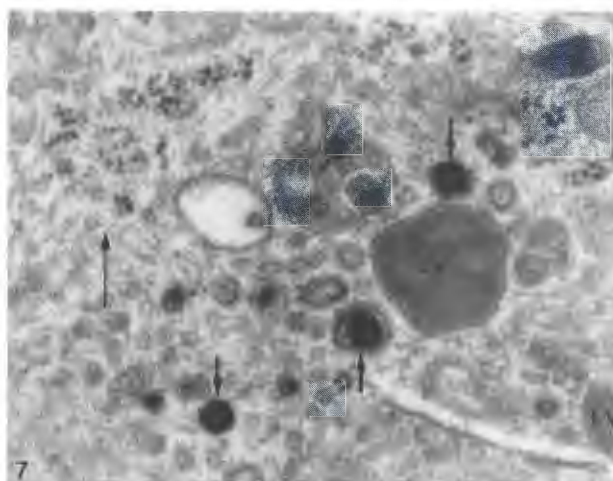


FIG. 7 A neuron in the myenteric ganglion in the reticulum of a grey lamb. Small (short arrows) and large (LV) granular vesicles are present. Note the microtubules (long arrow) in the cytoplasm (43000 x)

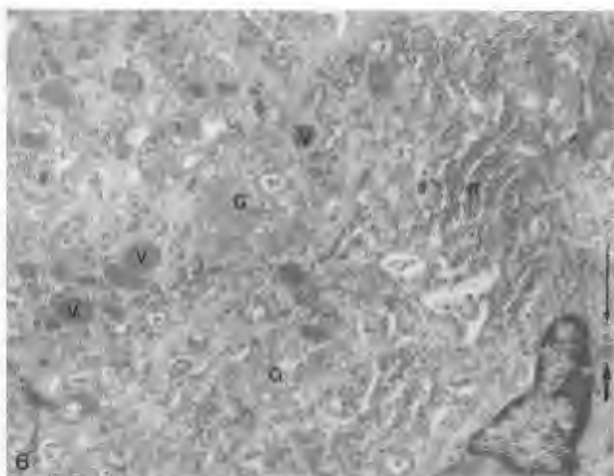


FIG. 6 A neuron in the myenteric ganglion in the rumen of a black lamb. Note the neurosecretory vesicles (V). Rough endoplasmic reticulum (R) and a Golgi apparatus (G) are evident (9800 x)

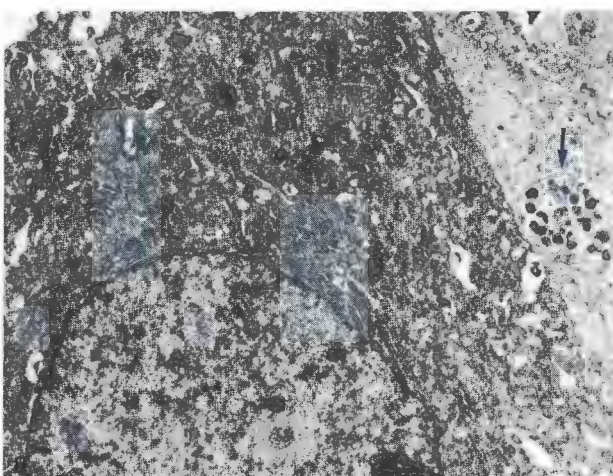


FIG. 8 A myenteric ganglion in the omasum of a white lamb. A profile of an axon with neurosecretory vesicles (arrow) lies against the neuron (7500 x)

oval mitochondria, and displayed a well developed Golgi apparatus (Fig. 2 and 6).

Numerous profiles of nerve axons were evident in all the ganglia studied (Fig. 1). These profiles contained mainly microtubules (Fig. 11), neurofilaments (Fig. 10), mitochondria (Fig. 9) and some coated vesicles (Fig. 13). The majority of axon profiles showed a number of neurovesicles (Fig. 11). Three different types of neurovesicles could be identified in the axons, namely:

- Small, granular vesicles (85 nm) containing very dense granules in the centre of the vesicle, the shape varying from round to oval (Fig. 11 and 12).

- Small, agranular vesicles (80 nm) with an electron-dense membrane and a light staining core (Fig. 12).
- Large, granular vesicles (195 nm) also containing a dense granular core, vesicle shape varying from round to elongated (Fig. 12 and 14).

## DISCUSSION

The general organization of the myenteric ganglia in the three groups of lambs studied, was not dissimilar to that described in various species by other authors (Richardson 1958; Baumgarten *et al.* 1970; Gabella 1971; Gabella 1972; Cook & Burnstock 1976a;

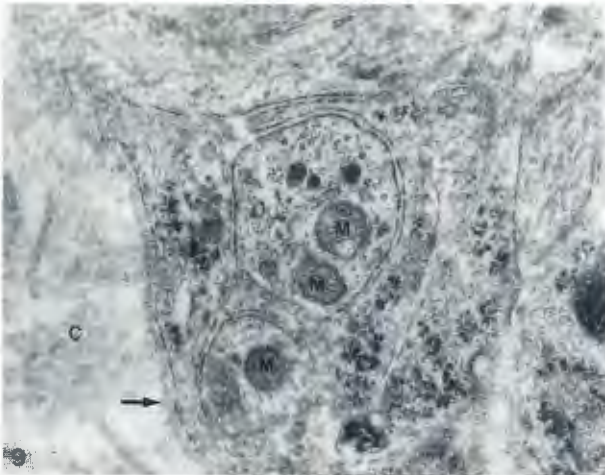


FIG. 9 Axon profiles in the myenteric plexus in the rumen of a black lamb. Mitochondria (M) can be identified. The endoneurium formed by the basal lamina of the Schwann cell (arrow) and fine collagen fibres (C) are visible (36000 x)

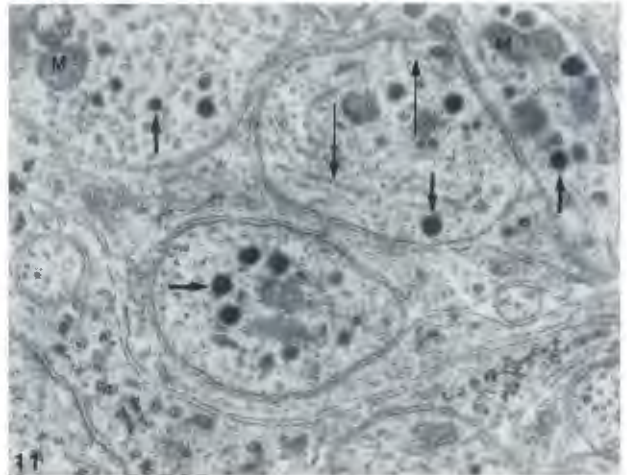


FIG. 11 Axon profiles in the myenteric ganglion in the reticulum of a grey lamb. Mitochondria (M), small granular vesicles (short arrows) and microtubules (long arrows) are evident (28000 x)

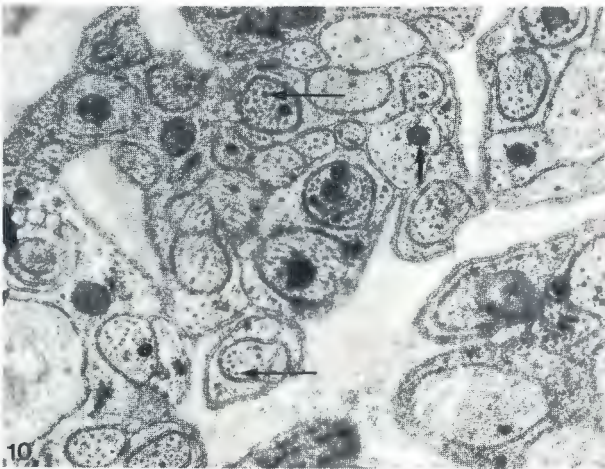


FIG. 10 Axon profiles in the myenteric plexus in the rumen of a grey lamb. Note the mitochondria (short arrow) and neurofilaments (long arrows) (18000 x)

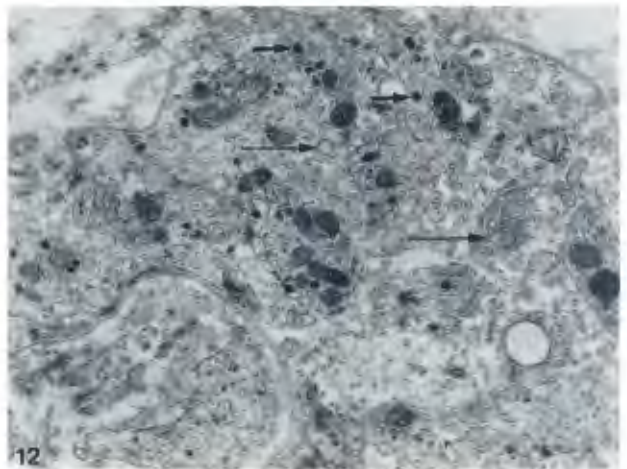


FIG. 12 Axon profiles in the myenteric ganglion in the rumen of a white lamb. Note the large granular (V), small granular (short arrows) and small agranular (long arrows) vesicles (13000 x)

Cook & Burnstock 1976b; Fehér *et al.* 1979; Hoyes & Barber 1980; Gershon 1981). The compactness of the structure, the absence of blood vessels and connective tissue, and the virtual absence of intercellular spaces were reminiscent of the parenchyma of the central nervous system rather than that of other autonomic ganglia.

Gershon & Bursztajn (1978) described a blood-myenteric plexus barrier formed by a sheath of supporting cell processes which prevent capillaries from entering the ganglion. In this study no blood vessels could be detected in the ganglia, thus confirming the findings of Gershon & Bursztajn (1978). These

authors also reported impermeable junctions that prevent the passage of tracers between endothelial cells of myenteric capillaries, and postulated that the blood-myenteric plexus barrier might be functionally analogous to that of the blood-brain barrier. As the myenteric blood vessels were not studied, these results could not be confirmed.

The absence of connective tissue in the myenteric ganglia, described by various authors (Gabella 1972; Gershon & Bursztajn 1978; Gershon 1981), was confirmed in this study. No endoneurium or perineurium could be detected in the ganglia. However, nerve fibres lying outside the ganglia were surrounded by a typical endoneurium consisting of the

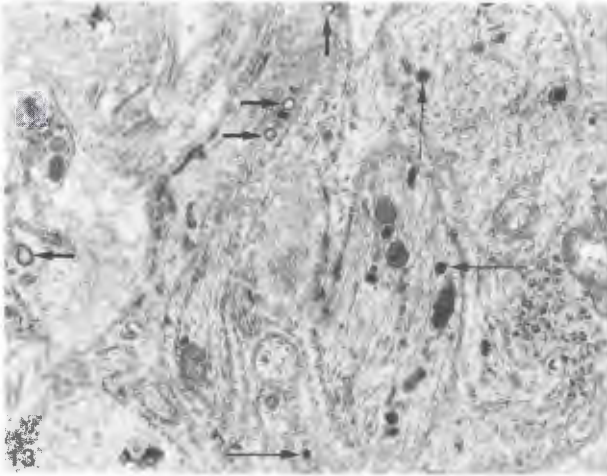


FIG. 13 Axon profiles in the myenteric ganglion in the reticulum of a white lamb. Small granular (long arrows) and coated (short arrows) vesicles are evident (18000 x)

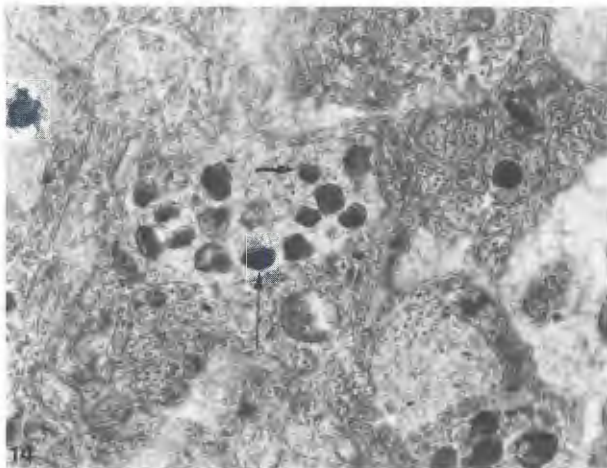


FIG. 14 Large granular vesicles in axon profiles in the omasal myenteric ganglion of a white lamb. The shape of the vesicles varies from round (short arrow) to elongated (long arrow) (18000 x)

basal lamina of the Schwann cell and fine collagen fibres as described by Krstić (1984).

The variation in the morphology and size of the neurons in the myenteric ganglia described by Gunn (1959), Gabella (1972), Cook & Burnstock (1976a), Fehér *et al.* (1979) and Gershon (1981) was also observed in this study. Small, medium and large neurons were generally distinguished. The organelles found in the neurons of the ganglia studied, were similar to those described by Palay & Palade (1955), Richardson (1958), Baumgarten *et al.* (1970), Gabella (1972), Cook & Burnstock (1976a), Fehér *et al.* (1979) and Gershon (1981). However, very little smooth endoplasmic reticulum could be detected in the samples. Gabella (1972) stated that this organ-

elle is not "uncommon". In other studies (Baumgarten *et al.* 1970; and Fehér *et al.* 1979) no mention is made of smooth endoplasmic reticulum in neurons.

While there was no difference in the number of neurosecretory vesicles in the myenteric ganglia of the three groups of lambs examined in this study, there were fewer vesicles than were reported in the adult guinea-pig (Baumgarten *et al.* 1970; Gabella 1972; Cook & Burnstock 1976a; Gershon 1981), man, the rhesus monkey (Baumgarten *et al.* 1970) or the cat (Fehér *et al.* 1979). This phenomenon might be due to the fact that the lambs were only 24 h old when the samples were taken. The fine granular nucleoplasm with chromatin condensations attached to the nuclear envelope found in the neurons of Karakul lambs have also been described in the guinea-pig (Gabella 1972). These observations are in contrast to the findings of Baumgarten *et al.* (1970) who found no chromatin condensations in the neurons of the rhesus monkey, guinea-pig or man.

The supporting cells in the myenteric ganglia have been described as glial cells (Gabella 1971; Gabella 1972; Gershon 1981) or Schwann cells (Richardson 1958; Cook & Burnstock 1976b), while Baumgarten *et al.* (1970) refers to them as both glial and Schwann cells. According to Gershon (1981) the supporting cells resemble the glial cells of the central nervous system more than they do the Schwann cells of the peripheral nervous system. Despite the difference in names these cells all display similar morphological characteristics. In this study the general structure of the glial cells with its organelles confirms the results of other authors (Richardson 1958; Baumgarten *et al.* 1970; Gabella 1971; Gabella 1972; Cook & Burnstock 1976b; Gershon 1981).

Small agranular, small granular and large granular vesicles have been reported in axonal profiles by various authors (Richardson 1958; Baumgarten *et al.* 1970; Gabella 1972; Cook & Burnstock 1976a; Fehér *et al.* 1979; Hoyes & Barber 1980; Gershon 1981). All three types of vesicles were observed in this study. There was no difference in the number of the various vesicles in the three groups of lambs studied, but there seemed to be fewer vesicles present than are described in the adult guinea-pig (Baumgarten *et al.* 1970; Gabella, 1972; Cook & Burnstock 1976a; Hoyes & Barber 1980; Gershon 1981) or cat (Fehér *et al.* 1979). This is probably due to the fact that the lambs were only 24 h old when the samples were taken.

Oki and Daniel (1977) studied the effects of vagotomy on the gastric neuronal apparatus of the dog stomach and found that presynaptic nerves and some large motor neurons underwent degeneration after vagotomy. Since no degenerative changes of any of the nerve fibres or neurons could be detected in this study, it can be assumed that the vagal nerve supply to the stomachs is sufficient.

It is concluded that despite the paucity of myenteric ganglia and neurons in the grey and white lambs, there is no difference between the ultrastructure of these structures and of those in normal control lambs.

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