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# The topography, architecture and structure of the enteric nervous system in the jejunum and ileum of cattle

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ABSTRACT

To date, there appear to have been no detailed and clear descriptions of the nerve plexuses and their subdivisions in the intestine of cattle. In this study, the enteric nervous system in the jejunum and ileum of 12 1-y-old calves was examined using neurofilament protein and vasoactive intestinal peptide immunohistochemistry in wholemounts and paraffin sections combined with staining of paraffin and historesin sections with haematoxylin and eosin. The main organisation of the plexuses was similar to that of the pig, horse and man with external and internal submucous plexuses being morphologically distinct, with further subdivisions of the internal submucous plexus into the external and internal subplexuses. However, in contrast to pig, horse and man, the submucous layer was firmly attached to the inner circular muscle layer. The myenteric plexus was well developed with large ganglia, and primary and secondary nerve strands. Its main axis was oriented parallel to the outer longitudinal smooth muscle; large ganglia and primary nerve strands fused to form complex ganglia, and 2 types of tertiary nerve strands were observed. Antibodies to neurofilament proteins and vasoactive intestinal peptide revealed adendritic, pseudouniaxonal or multiaxonal type II neurons only in the myenteric and submucous plexuses. This appears to be the first report of the identification of isolated uniaxonal, multidendritic type IV neurons in the mucous pericryptal plexus. The new information presented here provides further evidence for the existence of anatomical and functional differences between the external and internal submucous plexuses and for supporting the nomenclature proposed earlier.

*Key words*: Neurofilament proteins; vasoactive intestinal peptide; small intestine.

## INTRODUCTION

The enteric nervous system (ENS) has for a long time been recognised as consisting of 2 ganglionated plexuses: the myenteric plexus located between the circular and longitudinal smooth muscle layers and the submucous plexus in the submucous layer (Furness & Costa, 1980). However, in recent years it has been established that the submucous plexus comprises 2 different ganglionated plexuses, the internal submucous plexus (ISP) and the external submucous plexus (ESP) (Scheuermann et al. 1987*b*; Messenger & Furness, 1990; Timmermans et al. 1992; Pearson, 1994; Balemba et al. 1998). The ISP is located adjacent to the mucosa and is proposed to be primarily concerned with the control of transepithelial ion transport, mucosal blood flow and immune reactions (Surprenant, 1994). The ESP, on the other hand, is thought to be a 'relay station' between the ISP and the myenteric plexus, and between the ISP and the prevertebral ganglia as well as playing a motor role for the inner circular smooth muscle (Timmermans et al. 1992). Understanding the structural organisation of the ENS is therefore of importance, especially in relation to the role of the ENS in the control and regulation of the various activities that are carried out in the gut during normal and pathological conditions.

Various techniques including routine histological staining, immunohistochemistry and scanning electron microscopy have been employed to study the

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organisation of the enteric plexuses of pig, man and horse (Mannl et al. 1986; Scheuermann et al. 1987*a*, *b*; Stach, 1989; Holy & Burnstock, 1989; Timmermans et al. 1990; Brehmer et al. 1994; Pearson, 1994; Balemba et al. 1998), but the morphological descriptions of the submucous plexus are not fully concordant. While it is generally accepted that the boundary between ESP and ISP is the vascular arcades (Scheuermann et al. 1987*a*, *b*; Brehmer et al. 1994; Pearson, 1994; Balemba et al. 1998), other authors have reported a third intermediate plexus in the submucous layer of the colon of the pig (Gunn, 1968), opposum (Christensen & Rick, 1987) and man (Hoyle & Burnstock, 1989). It is therefore not yet clear whether the ESP and ISP should be considered as 2 different plexuses. In addition, their appropriate nomenclature has yet to be agreed upon (Scheuermann et al. 1987*b*; Hoyle & Burnstock, 1989; Pearson, 1994; Balemba et al. 1998). There are also contradictory reports on the organisation of the ESP and ISP in relation to the vascular arcades and the lymphoid follicles in the region of the gut with Peyer's patches (Krammer & Kühnel, 1993; Lowden & Heath, 1994; Balemba et al. 1998). In addition, studies on the structural organisation of the enteric nervous system have centred mainly on the guinea pig, pig, horse, man and very little in cattle. In the calf, the 2 submucous plexuses were reported to be well differentiated (Mannl et al. 1984). However, studies which are based on the use of wholemounts and a comparable topographic, architectural and structural description to that of the ENS in the intestine of the guinea pig (Messenger & Furness, 1990), the pig (Scheuermann et al. 1986, 1987*a*, *b*; Balemba et al. 1998) and horse (Pearson, 1994) appear not to have been undertaken.

The objective of this study was to investigate the topography, architecture and structure, and types of neurons in the ENS of cattle by combining immunohistochemical and routine histological methods so as to give a more elaborate understanding of the composition and interrelationships of the ENS of cattle and to comment on the ENS nomenclature.

## MATERIALS AND METHODS

### *Animals, sampling and processing*

Male calves aged 1 y (n = 12) of Jersey and Friesian–Boran cross breed were used. Calves were euthanised and within 20–25 min of death, the abdomen was opened to expose the stomach and intestines. Tissue segments $\sim 3$ cm long were collected from the proximal, middle and distal parts of the jejunum and ileum, and immediately immersed in 0.1 M phosphate buffered saline (PBS) solution of pH 7.3 and processed for fixation in 4.5% neutral buffered formaldehyde as described by Balemba et al. (1998). Tissues for embedding (paraffin and historesin) were fixed for 7 d at room temperature and those for microdissection were fixed for 48 h.

The myenteric and submucous wholemounts were teased apart by separating the outer longitudinal from the inner circular smooth muscle. Both types of wholemount were then re-pinned with the inner circular muscle uppermost and remnants of the inner circular smooth muscle layer were peeled off to enable visualisation of the plexuses. Embedding, sectioning and staining of historesin and paraffin sections, and staining for vasoactive intestinal peptide (VIP) and neurofilament protein (NF)-like immunoreactivities (IR) by the 2 step indirect streptavidin-ABComplex/HRP immunoenzymatic method was performed as described by Balemba et al. (1998). Staining for NF and VIP-like IR was undertaken on wholemounts and in 25 μm paraffin sections, and haematoxylin and eosin (H & E) was used to stain 5 μm paraffin and 2.5 μm historesin sections. The sources and dilutions of the reagents used are given in the Table. The counting of immunoreactive neurons in the ganglia was performed using the $\times 20$ objective and the criteria described by Scheuermann et al. (1986, 1987*a*, *b*) were used to classify the nerve strands in the wholemounts.

## RESULTS

### *General*

The NF and VIP-like IR revealed ganglia, nerve fibre strands and neurons in the ENS of cattle both in wholemounts and paraffin sections. However, topographic, architectural and structural features, as revealed by ganglia and nerve strands and structural features on neuronal surfaces in wholemounts, were best demonstrated by staining for NF-like IR (Figs 1*a*, *b*, *c*, 2*a*, 4*a*). Paraffin sections stained by H & E (Figs 2*b*, 3, 4*b*) and those stained for the VIP-like (Fig. 5*a*) and NF-like IR (Fig. 5*b*) complemented the topographic, architectural and structural features revealed by wholemounts.

### *Myenteric plexus*

The myenteric plexus was found to be always overlying the outer longitudinal smooth muscle when visualised from the mucosal side of wholemounts stained for NF and VIP-like IR. The plexus showed variability in the size and structure of the ganglia.

Table. *Reagents, dilution, and source*

| Reagent | Dilution | Cat. no | Source |
|---|---|---|---|
| Monoclonal rabbit anti swine-VIP | 1:1400 | 8084-4 | Dr Fahrenkrug, Bispebjerg, Denmark |
| Pooled monoclonal mouse antihuman neurofilament proteins | 0.069444 | 168 | Immunotech, France |
| Biotinylated affinity isolated swine antirabbit immunoglobulins | 1:500 | E0353 | Dako, Denmark |
| Biotinylated affinity isolated rabbit antimouse immunoglobulins | 1:500 | E0464 | Dako, Denmark |
| Streptavidin-ABComplex/HRP | 1:500 | K0377 | Dako, Denmark |

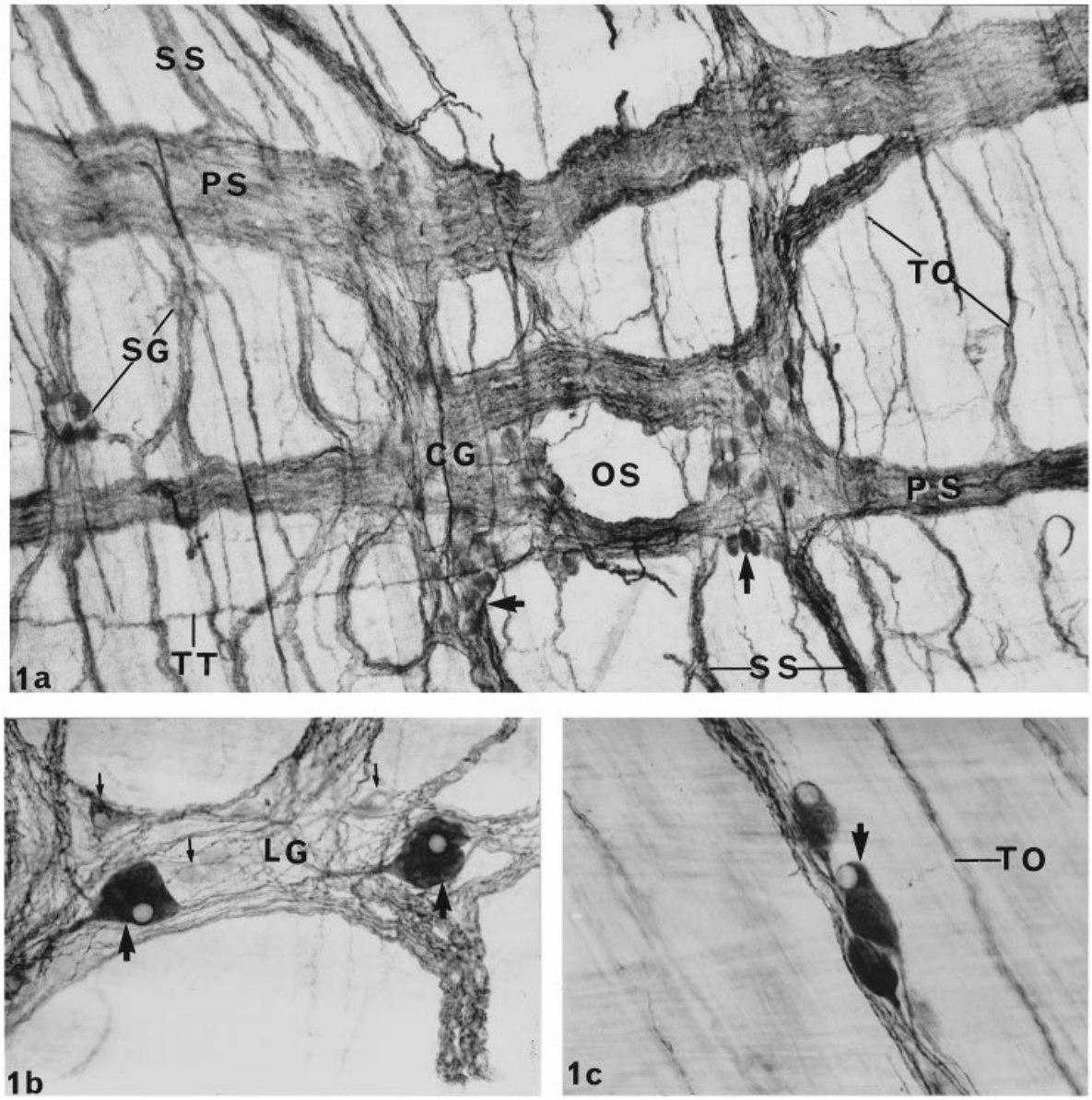


Fig. 1. NF-like IR in the myenteric plexus in wholemount preparation from the jejunum and ileum of cattle. (*a*) NF-like IR in the myenteric plexus from the jejunum of cattle, viewed from the mucosal side. Complex ganglia (CG) surround open spaces (OS) within the meshwork of the complex ganglia. Small ganglia (SG), primary nerve strands (PS), secondary nerve strands (SS), type I (TO) and type II (TT) tertiary strands and clusters of NF-like IR type II neurons (arrows) can be seen. The outer longitudinal smooth muscle layer in the background is almost out of focus. ×250. (*b*) Large ganglion (LG) of the myenteric plexus from the ileum of the calf. Note the variability in size and reactivity of the NF-like IR neurons. Two intensely stained neurons (large arrows) are distinctly larger than the small neurons (small arrows). Large neurons are principally adendritic, pseudouniaxonal to multiaxonal with peripherally located nuclei. They appear to be situated close to the surface of the ganglion compared with the smaller neurons, some of which are situated deep in the ganglion. ×700. (*c*) Type II neurons (arrow) in a secondary nerve strand and type I (TO) tertiary nerve strands nearby. These are large, ovoid, isolated, principally adendritic, uniaxonal neurons with peripherally located nuclei. ×550.

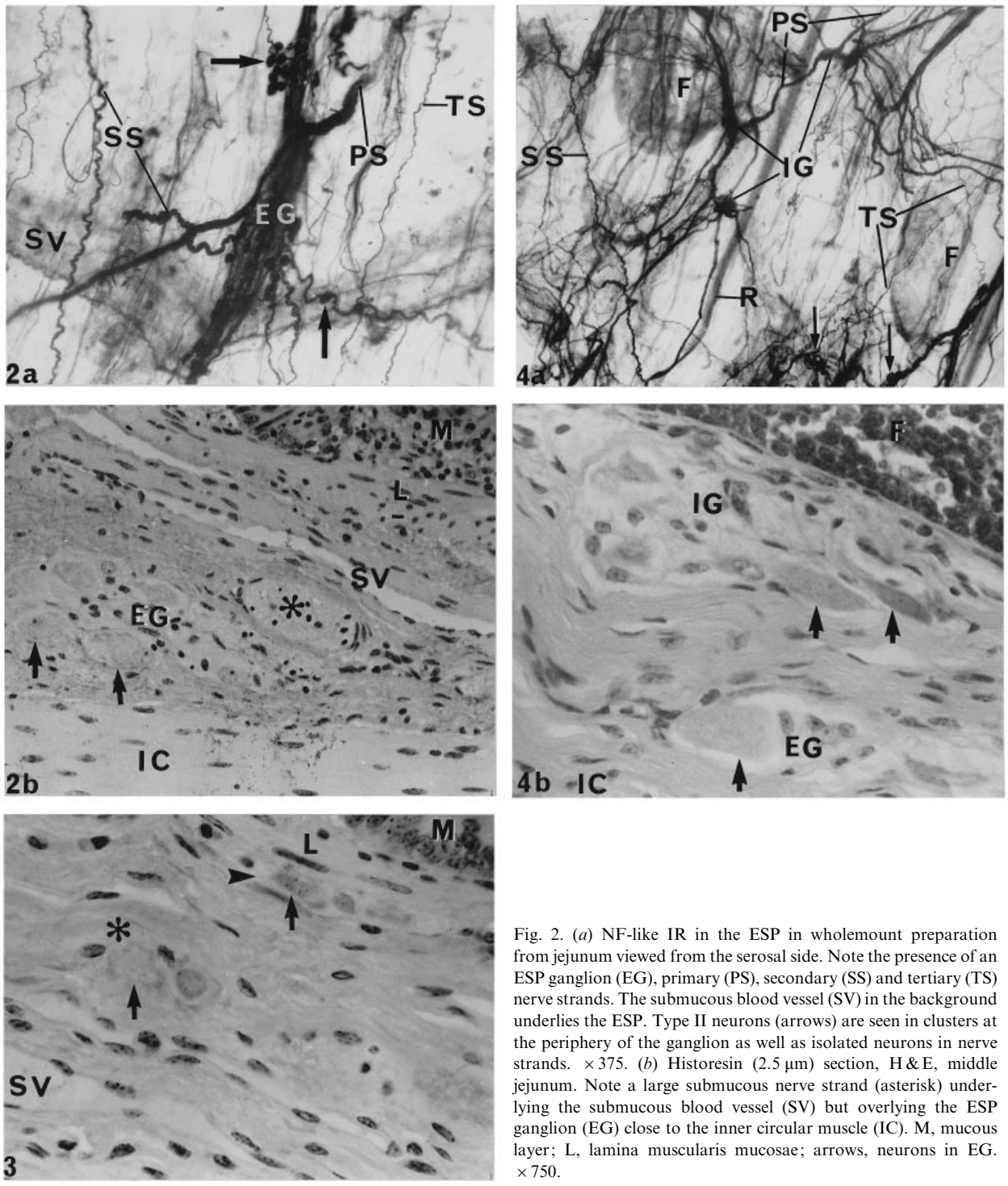


Fig. 2. (*a*) NF-like IR in the ESP in wholemount preparation from jejunum viewed from the serosal side. Note the presence of an ESP ganglion (EG), primary (PS), secondary (SS) and tertiary (TS) nerve strands. The submucous blood vessel (SV) in the background underlies the ESP. Type II neurons (arrows) are seen in clusters at the periphery of the ganglion as well as isolated neurons in nerve strands. ×375. (*b*) Historesin (2.5 μm) section, H & E, middle jejunum. Note a large submucous nerve strand (asterisk) underlying the submucous blood vessel (SV) but overlying the ESP ganglion (EG) close to the inner circular muscle (IC). M, mucous layer; L, lamina muscularis mucosae; arrows, neurons in EG. ×750.

Fig. 3. H & E paraffin section (5 μm) from the proximal jejunum. Ganglia of the ISP are at 2 different topographic levels. The internal ISP ganglion (ISPi) (arrowhead) is small and is situated adjacent to the laminar muscularis mucosae (L). The external ISP ganglion (ISPe) (asterisk) is larger and overlies a blood vessel (SV) of the submucous vascular arcades. Neurons (arrows) are variable in size, being smaller in the ISPi than in the ISPe. ×1800.

Fig. 4. (*a*) NF-like IR in the ISP in a wholemount of the ileal submucous layer viewed from the serosal side. Note the ISP ganglia (IG), primary (PS), secondary (SS) and tertiary (TS) nerve strands. ISP ganglia underlie the base of the follicles (F). Immunoreactive neurons (arrows) at the lower right are small in size and not easily detectable. Remnants of muscle fibres (R) of the lamina muscularis mucosae are recognised in the background. ×375. (*b*) A 5 μm H & E paraffin section from the distal ileum. The ISP ganglion (IG) underlies the base of the follicle (F). The ESP ganglion (EG) is also underlying the Peyer's patches follicle (F) but is situated closer to the inner circular muscle layer (IC). The 2 ganglia are clearly situated at 2 different topographic levels. Neurons (arrows) in ISP are smaller than those of the ESP. ×1800.

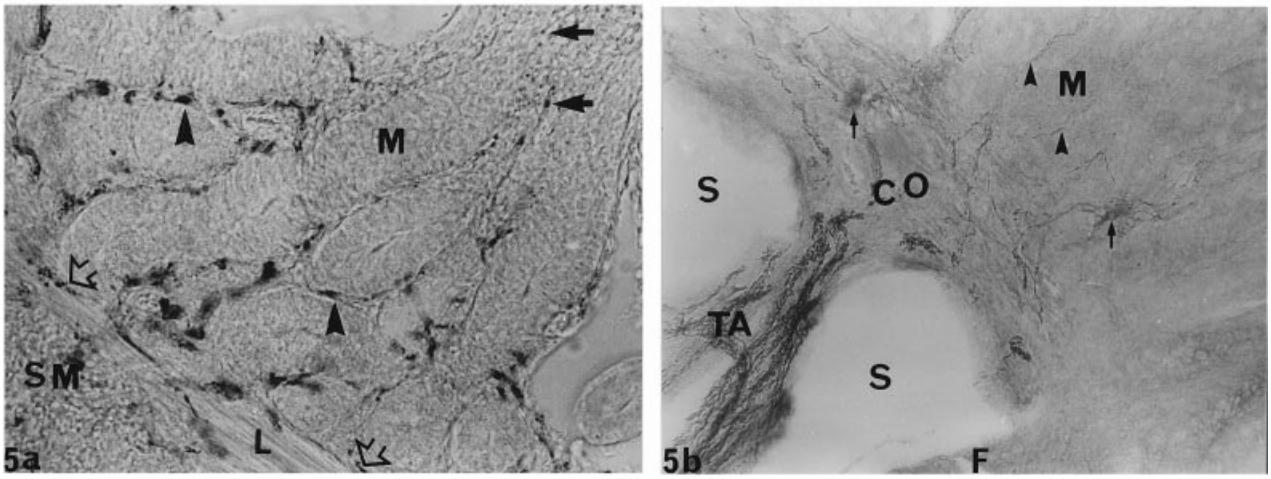


Fig. 5. (*a*) VIP-like IR in a 25 μm paraffin section. Note the nerve fibre varicosities of the external proprial (open arrows), pericryptal (arrowheads) and the villous plexuses (arrows). The mucous layer (M) with crypts, lamina muscularis mucosae (L) and submucous connective tissue (SM) can be seen. × 750. (*b*) NF-like IR in a 25 μm paraffin section showing IR in nerve fibres of the pericryptal plexus (arrowheads) in the mucous layer (M), traffic areas (TA) and corona (CO) in the follicles (F) of Peyer's patches. Two IR neurons (arrows) in the mucosa are multiaxonal and multidendritic (type IV) neurons. The spaces (S) are artifacts due to the detachment of the follicles from the connective tissue in the traffic areas and the lamina muscularis mucosae. × 750.

Ganglia were complex, large or small (Fig. 1 *a*, *b*). Complex and large ganglia, and primary and secondary nerve strands were clearly visible macroscopically. Apparently, complex ganglia were formed by fusion of either large ganglia or large and small ganglia making it difficult to determine the boundaries of individual component ganglia. There was always an open space in a mesh at the centre of the complex ganglia (Fig. 1 *a*). Large ganglia were ovoid or elongated. Complex and large ganglia were interconnected mainly by primary (internodal) and secondary nerve strands (Fig. 1 *a*, *b*). The main axis of the complex and large ganglia, and primary strands was oriented parallel to the underlying longitudinal muscle layer whereas secondary strands were mainly oriented perpendicular to the main axis of the longitudinal muscle layer, complex and larger ganglia and the primary strands.

Tertiary nerve strands, which were the smallest sized strands, either interconnected ganglia, primary strands or secondary strands and were differentiated into 2 types (TO, TT). Type 1 tertiary strands (TO) were large, short and abundant while type 2 tertiary strands (TT) were few, slender, elongated and more wavy (Fig. 1 *a*). Type 2 strands interconnected distant ganglia, and were oriented perpendicular and parallel to the axis of the complex and large ganglia, and to the primary nerve strands respectively, and most of them emerged from, and terminated at, the periphery of ganglia where NF-like IR neurons formed clusters. Secondary and tertiary nerve strands crossed either over or under the ganglia and primary nerve strand meshwork making the entire mesh appear interwoven. Small ganglia and isolated extraganglionic cell bodies were also identified within the secondary and type 1 tertiary nerve strands and in the intersections of these strands with the primary strands and their ganglia (Fig. 1 *a*, *c*). The tertiary nerve strands of the myenteric plexus joined a fine and delicate network of nerve fibres of the tertiary plexus and both gave rise to fine nerve strands that coursed into the outer longitudinal smooth muscle layer. Clusters of NF-like IR neurons were observed at the periphery of the ganglia. The NF-like IR neurons differed in size and shape. They were adendritic, pseudouniaxonal or multiaxonal, and ovoid or elongated in shape with peripherally located nuclei.

### *External submucous plexus* (*ESP*)

Viewed from the serosal surface, the ESP was situated in a more dense submucous connective tissue (SM) on the inner side of, and close to, the inner circular smooth muscle layer above the submucous vascular arcades. Both the ESP ganglia and interconnecting nerve strands varied in size and appeared to be situated at different topographic levels (Fig. 2 *a*). Large ganglia were oval to elliptical in shape, while smaller ganglia (not shown) were oval to round. Large ganglia and their associated primary strands formed the major axis of the ESP and gave rise to secondary and tertiary interconnecting nerve strands. The small ganglia were observed at the intersections between the primary and secondary, and between secondary and tertiary strands. The meshwork of the small ganglia, secondary and tertiary nerve strands was within the

meshwork of the large ganglia and primary nerve strands. Many tertiary nerve strands from the ESP coursed into and then parallel to muscle fibres of the inner circular muscle. These strands and those interconnecting the ESP to the myenteric plexus firmly attached the ESP to the inner circular muscle layer. Many tertiary nerve strands and some secondary nerve strands made connections between the ESP and ISP. Large nerve fibres were mainly observed between the submucosal vascular arcades on the luminal side and the ESP on the serosal side (Fig. 2*b*). The NF-like IR neurons were similar to those recorded in the myenteric plexus. Although they were observed in the entire ganglion, many neurons formed clusters at the periphery, particularly at the intersections of the ganglia and emerging nerve strands. Isolated extraganglionic neurons were observed in secondary and tertiary nerve strands.

*Internal submucous plexus (ISP)*

Wholemounts and paraffin sections both revealed the ISP meshwork to be situated between the submucous vascular arcades on the outer side and lamina muscularis mucosae on the inner side. The ISP ganglia and nerve strands varied in size and were situated at different topographic levels (Figs 3, 4*a*). Large ganglia were ovoid to polygonal and were situated close to the submucosal vascular arcades and were described as the external ISP ganglia. The smaller rounded ganglia, on the other hand, were situated close to the lamina muscularis mucosae and were described as the internal ISP ganglia (Fig. 3). The entire ISP meshwork, however, was firmly attached to the lamina muscularis mucosae by interganglionic nerve strands and nerve fibres interconnecting the ISP to the mucosal plexuses. The larger ISP ganglia constituted a broader mesh whereas smaller ganglia were organised in narrow meshes. Compared with the myenteric plexus, the ISP meshwork exhibited no major orientation to the lamina muscularis mucosae, inner circular smooth muscle or intestinal villi-crypt axis. The NF-like IR neurons were morphologically similar to those recorded in the myenteric plexus and the ESP but were smaller in size ($\sim$ 1.5–2 times smaller) than those in the ESP and the myenteric plexus.

*Internal submucous plexus (ISP) in Peyer's patches*

In the Peyer's patches, as in other parts of submucous layer the ESP and ISP were situated at different topographic levels and were still clearly separated by the submucous vascular arcades (Fig. 4*a*, *b*). The ISP formed a continuous mesh of 3 ganglionated subplexuses around the follicles. From the serosal surface, the first and outermost ISP subplexus was situated between the base of the follicles and the submucous vascular arcades, the second was in the interfollicular region (traffic areas) and the third was situated in the corona. Ganglia and neurons in the first 2 subplexuses were larger than those in the third subplexus. Comparing the meshworks of the 3 ganglionated plexuses, the ESP constituted the widest and most irregular nerve meshwork while the ISP formed the most narrow-meshed plexus.

*Enteric nervous system in the lamina propria*

Four plexuses were identified in the lamina propria and were best revealed by VIP-like IR (Fig. 5*a*). These were the external propria plexus, which overlay the lamina muscularis mucosae (L) and the internal propria plexus (not shown) located at the base of the intestinal villi. Others were the pericryptal plexus which surrounded the intestinal crypts and the villous plexus which was located around the core of the villi. One striking feature was the observation of very few uniaxonal multidendritic NF-like immunoreactive solitary neurons in the pericryptal plexus in paraffin sections (Fig. 5*b*). These neurons gave rise to nerve fibres towards the serosal and the luminal surfaces of the lamina propria.

*Vasoactive intestinal peptide*

As mentioned earlier, morphological features revealed by VIP-like IR were similar to those revealed by NF-like IR which showed them much more readily. The VIP-like IR varicosities were abundant in the 3 ganglionated plexuses as well as in the mucous layer (M) and the inner circular muscle layer. In the plexuses, especially the ganglia, VIP-like immunoreactive nerve fibre varicosities varied in size (not shown). Large, medium and small sized varicosities were identified. While small and medium sized varicosities were observed mainly in nerve fibres, large, intensely staining varicosities appeared chiefly around neurons.

The VIP-like IR neurons were ovoid or elongated in shape with peripherally located nuclei and pseudouniaxonal to multiaxonal. These neurons were numerous in the ISP, moderate in number in the ESP and scarce in the myenteric plexus. The NF-like IR neurons were more abundant in the ESP (30–60 cells per ganglion), less so in the ISP (2–25 cells per ganglion) and fewer (0–15 cells per ganglion) in the

myenteric plexus. In all 3 plexuses, both VIP and NF-like IR neurons in one ganglia varied in size. Larger neurons were peripherally located, while smaller neurons were mainly located towards the centre (Figs 1*a*, *b*, 2*a*, 4*a*).

## DISCUSSION

### *Microdissection*

Wholemounts from the submucous layer are usually prepared by freeing the submucous layer from the underlying circular muscle without much damage to the ESP (Gunn, 1968; Stach, 1989; Messenger & Furness, 1990; Pearson, 1994; Balemba et al. 1998). In this study, the microdissection of the jejunal and ileal submucous wholemounts was performed by teasing the submucous layer and inner circular muscle layer from the outer longitudinal muscle followed by careful removal of individual muscle fibres by using very fine forceps. It appears that in cattle the ESP is firmly attached to the circular muscle layer by connective tissue and nerve strands, supporting an earlier report by Gunn (1968) that ease of microdissection differs between mammals. Ileal tissues, however, were easier to dissect than jejunal tissues.

### *General*

Our findings on the topography, architecture and structure as well as the morphological features of the NF and VIP-like IR neurons in the myenteric, ESP and ISP plexuses, variability in the size and shape of ganglia and nerve strands and size of neurons, the tertiary aganglionic plexus and nerve plexuses in the lamina propria including solitary neurons in the PCP are roughly comparable to those described in the pig (Scheuermann et al. 1986, 1987*a*, *b*; Timmermans et al. 1990; Krammer & Kühnel, 1992; Balemba et al. 1998), horse (Pearson, 1994), man (Hoyle & Burnstock, 1989), guinea pig (Messenger & Furness, 1990; Llewellyn-Smith et al. 1993) and in calves (Mannl et al. 1984). The difficulties in the delineation of boundaries of the complex ganglia, and large ganglia particularly at the intersections with the primary nerve strands in the myenteric plexus are similar to those observed in horse (Pearson, 1994). The finding of well defined nerve fibre strands between the submucous vascular arcades and the ESP is in agreement with earlier observations in histological sections of calf (Mannl et al. 1984). These fibres could be the ESP interganglionic nerve strands observed at different levels. The recorded variation in the number and size of neurons between the 3 plexuses is in accordance with the earlier observation in calves (Mannl et al. 1984) and in pig (Timmermans et al. 1990; Balemba et al. 1998).

### *Myenteric plexus*

The meshwork of complex and larger ganglia and the primary and secondary nerve strands in the myenteric plexus in jejunum and ileum of cattle was roughly comparable to that of the pig (Scheuermann et al. 1986; Balemba et al. 1998), horse (Pearson, 1994) and other mammals (Gunn, 1968). It was comparably larger and clearly visible macroscopically after staining for NF-like IR. Furthermore, the meshwork of secondary and tertiary nerve strands was more interwoven and complex than that observed in the pig. In addition, the main axis for the orientation of the myenteric plexus differed between the species, supporting the report on variation pattern amongst species by Furness & Costa (1987). In the pig, it is oriented perpendicular to that of the outer longitudinal smooth muscle layer (Scheuermann et al. 1986) whereas in cattle it is oriented parallel to the outer longitudinal smooth muscle layer. Fusion of ganglia to form large complex ganglia was observed in the myenteric plexus, whereas this feature was recorded in the ISP in the pig (Scheuermann et al. 1987*a*). In addition, the tertiary nerve strands were categorised into 2 types: the type 1 tertiary nerve strands which were large, short and abundant and type 2 tertiary nerve strands which were slender, elongated, more wavy, fewer and interconnected areas of aggregates of the NF-like IR neurons between distant ganglia; they were usually oriented parallel to the main axis.

### *Submucosal plexuses*

The present findings in cattle showed clear morphological and functional differences between the ESP and ISP, providing additional data to support an earlier proposition (Scheuermann et al. 1987*a*, *b*; Brehmer et al. 1994; Pearson, 1994; Balemba et al. 1998) for subdivision of the submucous plexus into the ESP and ISP by using the submucous vascular arcades as a topographic landmark both in areas with and without the follicles of Peyer's patches. The study also provided further evidence for the topographic subdivision of the ISP ganglia into small sized inner ISP ganglia (the internal ISP ganglia), which appear directly apposed to the lamina muscularis mucosae, and the larger ganglia (external ISP ganglia) which are situated closer to the submucous vascular arcades. In

the Peyer's patches, ISP surrounded the follicles as a continuous mesh of 3 ganglionated subplexuses. The functional significance of this organisation is, however, not known.

Although the findings on the ESP and ISP in the Peyer's patches are similar to those of the pig (Balemba et al. 1998), VIP-like IR neurons could not be demonstrated in the ISP subplexus in the corona of the calf, which is in contrast to that found in the pig. There is, therefore, a need for using other ENS markers to study the neurochemical composition and morphological features of the ISP complexity around the follicles in order to provide a better understanding of the interactions between the ENS and cellular elements in the follicles and, hence, more insight into the role of ENS in immune responses.

Morphologically, the VIP and NF-like IR neurons were similar to type II neurons recorded in the intestine of the pig (Scheuermann et al. 1987*c*; Stach, 1988; Krammer & Kühnel, 1992), thus giving further weight to the observations made in the pig (Stach et al. 1990) that type II neurons are present in all ganglionated plexuses, being frequently seen when using antineurofilament proteins. In the pig, the antineurofilament protein antibody revealed types IV and VI neurons mainly in the myenteric plexus (Balemba et al. 1998). Although the same antibody was used in the present study, the NF-like IR did not show type IV neurons in the myenteric and submucosal plexuses. The reason for the observed difference is uncertain. However, the findings are suggestive of species variation in the composition of neurons among the plexuses. The finding is however in agreement with the findings by Brookes et al. (1991) in the guinea pig and Krammer & Kühnel (1992) and Balemba et al. (1998) in the pig that in the gastrointestinal tract, some neurons in the ganglia cannot be stained by antibodies to neurofilament protein. The NF-like IR neurons among the myenteric plexus, ESP and ISP varied in size. Additionally, in all 3 plexuses, the NF-like IR neurons in a single ganglion varied in size, large neurons being predominantly situated peripherally. A similar observation to the latter was reported in the pig by Mannl et al. (1986) and Timmermans et al. (1990).

There have been terminological controversies with different names applied to the 2 submucous plexuses (Furness & Costa 1987; Scheuermann et al. 1987*b*; Hoyle & Burnstock, 1989; Timmermans et al. 1992; Pearson, 1994). As the organisation of the ENS in the jejunum and ileum of cattle was in many ways similar to that in the small intestine of the pig, horse and man, we have in the present study given further support to the nomenclature proposed by Pearson (1994) and Balemba et al. (1998), which is clear as it is based on the topographic location of the plexuses.

### *Mucous layer plexuses*

The organisation of the mucous layer plexuses was similar to that reported in the pig (Balemba et al. 1998). Isolated neurons have been reported earlier in the mucous layer (Furness & Costa 1987; Balemba et al. 1998). These neurons in the mucous layer in the jejunum of the pig were positive for VIP-like IR (Balemba et al. 1998) whereas, in the present study in cattle, VIP-like IR did not show mucosal neurons. Instead, staining for NF-like IR revealed uniaxonal, and multidendritic type IV neurons in the PCP in the mucous layer which is a new finding. The functional significance of the presence of isolated neurons in the PCP requires investigation.

The observation that the VIP-like IR varicosities were more abundant in the mucous layer, and in the inner circular smooth muscle layer compared with the outer longitudinal muscle, and those on VIP-like IR neurons in the submucous plexus, supports the findings of Timmermans et al. (1990) in the pig. Three types of VIP-like IR varicosities were recorded in the present study. A comparable observation which led to the proposition that there might be more than one type of VIP-containing nerves with variability in their possible functions has been reported in the human respiratory tract (Laitinen et al. 1985).

### *Conclusions*

In conclusion, the present observations in cattle, although with overall similarities, differed from those in the guinea pig, pig, horse and man in the following features. (1) the submucous layer was firmly attached to the inner circular muscle layer; (2) neurofilament protein-like IR revealed clearly the elaborate meshwork of the ganglia and nerve strands and that of the ganglionated plexuses and enabled the identification of 2 types of tertiary nerve strands in the myenteric plexus; (3) the major axis of the myenteric plexus was oriented parallel to the inner circular muscle layer, thus showing species variation; (4) large nerve fibre strands were observed between the ESP and the submucous vascular arcades; (5) staining for NF-like IR revealed uniaxonal and multidendritic type IV neurons in the pericryptal plexus whereas staining for VIP-like IR did not show neurons in the mucous layer; (6) the NF-like IR revealed type II neurons in the myenteric, external and internal submucous

plexuses which differs from the pig in which the same antibody has been reported to show types IV and VI neurons.

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