J. Anat. (1989), **166**, pp. 7–22
With 7 figures
Printed in Great Britain

Neuronal populations in the submucous plexus of the human colon

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(*Accepted* 18 *January* 1989)

INTRODUCTION

The submucous plexus of the gastrointestinal tract has been observed and studied since the mid-nineteenth century (Meissner, 1857; Henle, 1871; Drasch, 1881), and its neurocytoarchitecture investigated at intervals during more recent years (Schabadasch, 1930*a*, *b*; Ikeda, 1957; Schofield, 1960, 1968; Gunn, 1968; Stach, 1972, 1975, 1977*a*, *b*; Wilson, Furness & Costa, 1981*a*, *b*; Mannl, Popischil & Dahme, 1984; Scheuermann & Stach, 1984; Scheuermann, Stach & Timmermann, 1984). However, much remains to be learned about its structure and function.

From histological and physiological studies, the submucous plexus has been shown to be involved in control of contractile activity of the muscularis mucosae and smooth muscle within villi, coordination of intestinal motility and control of electrolyte and ion transport across the epithelial border; it has also been shown to contain sensory, integrative and motor elements (Bülbring, Lin & Schofield, 1958; Hukuhara, Yamagami & Nakayama, 1958; Hukuhara & Miyake, 1959; Schofield, 1968; Hirst, Holman & McKirdy, 1975; Hirst & McKirdy, 1975; Stach, 1977*c*; Nozdrachev, 1980; Nozdrachev, Kachalov & Sanin, 1981; Keast, Furness & Costa, 1985*a*, *b*; Stach & Scheuermann, 1985; Bridges, Rack, Rummel & Schreiner, 1986; Goerg, Roux, Wanitschke & Meyer zum Büschenfelde, 1986; Furness & Costa, 1987). Although the submucous plexus is considered to be a single entity, it is made up of separate ganglionated plexuses (Schabadasch, 1930*a*, *b*; Ohkubo, 1937; Rintoul, 1960; Gunn, 1968; Stach, 1977*a*, *b*; Scheuermann & Stach, 1984). As early as 1881 Drasch observed "If you can set up different optical cross-sections, you become aware that the ganglia lie at different heights in the submucosa. Those lying deeper are always connected with those lying higher." Differentiation of plexuses within the submucosa has been determined in several species, including rodents, rabbits, ruminants, carnivores and primates. Generally speaking, the separation is more easily observed in the large intestine and in larger animals, probably because of a greater thickness of the submucosa.

In the literature a controversy has developed over the years as to the nomenclature of the plexuses within the submucosa. The reasons for the uncertainty of the correct naming of the component plexuses have been detailed (Furness & Costa, 1987); however, no suggestion as to what would be a suitable nomenclature was then made, other than that the entire neural networks of the submucosa be called Meissner's plexus or the submucous plexus. In this communication, the name Meissner's plexus will be applied to the plexus lying adjacent to the muscularis mucosae and the name Henle's plexus will be applied to the plexus lying close to the circular muscle. This

terminology was adopted by Schabadasch (1930*b*) and has been widely used even though it is historically slightly incorrect; it is simple and avoids cumbersome Latin terminology such as *plexus submucosus internus* (Meissner's plexus) and *plexus submucosus externus* (Henle's plexus). Similar terms were the cause of much of the confusion originally.

The work described in this paper was undertaken in order to determine whether or not the submucous plexus of the human colon contains a number of ganglionated plexuses, and whether the tiered arrangement of ganglia seen in cross-sections through the gut represents a tiered arrangement of separately identifiable plexuses. Using a stain for NAD-dependent dehydrogenases (NADH-diaphorase), nerve cell bodies were visualised. The distribution of nerve cells across the submucosa was examined and areas of cell bodies were measured to see if there were different size-populations in different regions of the submucous plexus. The number of cells per ganglion and conformations of ganglia were also studied.

MATERIALS AND METHODS

Tissue was obtained from uninvolved areas of distal sigmoid colon which was removed during extirpation of rectal carcinoma or villous adenoma (one patient). Tissue was obtained from 6 females aged 38–71 years (mean 52 years) and 6 males aged 26–90 years (mean 59 years).

Samples, the whole thickness of the colonic wall, approximately 1 cm^2, were fixed in 4% (w/v) paraformaldehyde in 0·1 M phosphate-buffered saline (PBS) (pH 7·3) for 90–120 minutes. After washing in PBS (3 × 10 minutes), tissues for cryostat sections were placed in 7 % sucrose in PBS overnight at 4 °C. Tissues for wholemount preparations were kept in PBS. Sections (5–10 μm thick) were cut either transversely or longitudinally to the axis of the viscus. Wholemount preparations were made of the submucosa. The sample was pinned onto a wax tray with the mucosa uppermost, and the mucosa and submucosa together were separated from the muscle coat by lifting a corner and gently peeling it away with the aid of forceps and small scissors (2·5 cm blade). The muscular coat was removed, the submucosa and mucosa were pinned into the wax with the submucosa uppermost and the submucosa was dissected from the muscularis mucosae in a similar fashion. The freed submucosa was gently stretched and pinned out onto silicone rubber. Using a pair of fine dissecting scissors (3 mm blade), an upper and then a middle lamina were dissected free, leaving a lower lamina on the silicone rubber. These laminae corresponded to levels of the submucosa adjacent to the circular muscle, adjacent to the muscularis mucosae and at a level in between. The separated laminae were pinned out onto silicone rubber for staining.

Staining

Sections and wholemounts were stained for NADH-diaphorase in order to visualise nerve cell bodies. Tissues were incubated in PBS (pH 7·3) containing NADH (1 mmol/l) and nitroblue tetrazolium (0·6 mmol/l) for 30–45 minutes or 45–60 minutes, for sections and wholemounts respectively. The degree of staining was controlled visually. As the tissue had been fixed, it was not necessary to stop the reaction quickly. The tissues were dehydrated through graded alcohols, cleared with Histoclear (National Diagnostics) and mounted in D.P.X. (BDH).

Statistical analyses

For all statistical tests, probability levels of less than, or equal to, 5% were taken to be significant. Frequency-distributions were compared using chi-squared tests, and

Table 1. *Thickness of layers of the wall of human distal sigmoid colon*

Layer	Thickness (μm)	Thickness (%)
Longitudinal muscle	616	(41·1)
Circular muscle	1360	(38·6)
Submucosa	1126	(29·3)
Muscularis mucosae	53	(28·1)
Mucosa	670	(34·2)

Values given are mean (coefficient of variation) from 12 patients. The coefficient of variation (standard deviation/mean) is expressed as a percentage. Measurements were made from sections of tissue fixed in a contracted state. The longitudinal muscle measurements did not include taeniae.

differences between means were compared using analysis of variance. Areas of cell bodies were estimated using a graticule fitted into the eyepiece of the microscope. The calibration of the graticule was such that the smallest marked division was 11·6 or 18·25 μm (depending on objective) and the unit square was 134·26 or 333 μm respectively. Cells were measured according to the number of squares they occupied or their linear dimensions. In constructing histograms of cell body areas, bin widths of 60 μm^2 were chosen because this was the approximated size of error in measuring cell areas, and then a moving average with a width of two bins was taken to provide the final histogram. This process was carried out in order to smooth the errors of estimation of cell body area. For the frequency-distribution of cells per ganglion, the bin widths of the histogram were based on a Fibonacci sequence. (A Fibonacci sequence is one in which a given term is obtained from the sum of the previous two terms.) The Fibonacci sequence is a natural growth sequence and rises less steeply than an integral logarithmic progression.

OBSERVATIONS

In the sections, ganglia were seen in the myenteric plexus and at several levels within the submucosa. There was a layer of ganglia which lay close to the circular muscle (i.e. of Henle's plexus) and a layer of ganglia which lay close to the muscularis mucosae (i.e. of Meissner's plexus). Between these two plexuses ganglia lay at various levels (Fig. 1). In wholemount preparations, ganglia interconnected by fibre tracts were seen in all three separated laminae.

From sections, the thicknesses of components of the gut wall, including the submucosa, were measured (Table 1). The distribution of ganglia across the submucosa was observed (Fig. 2), normalising the thickness of the submucosa to 100%. The ganglia of Meissner's and Henle's plexuses lay predominantly within 10%, approximately 100 μm, of either the muscularis mucosae or circular muscle respectively. Of the 170 ganglia that were counted, 52·5% lay between 11% and 60%, i.e. 120–800 μm, away from the muscularis mucosae, with a mean position $\pm$S.E. of 34·6 $\pm$ 1·70%. Assuming that the distribution of ganglia of Meissner's plexus would be similar to that of Henle's plexus, then the vast majority of this 52·5% of ganglia might belong to a separate plexus.

Areas of nerve cell bodies in the submucous plexus were estimated, and neurons from the myenteric plexus were also measured for comparison (Table 2). Nerve cell bodies of the myenteric plexus were typically much larger than those found in ganglia

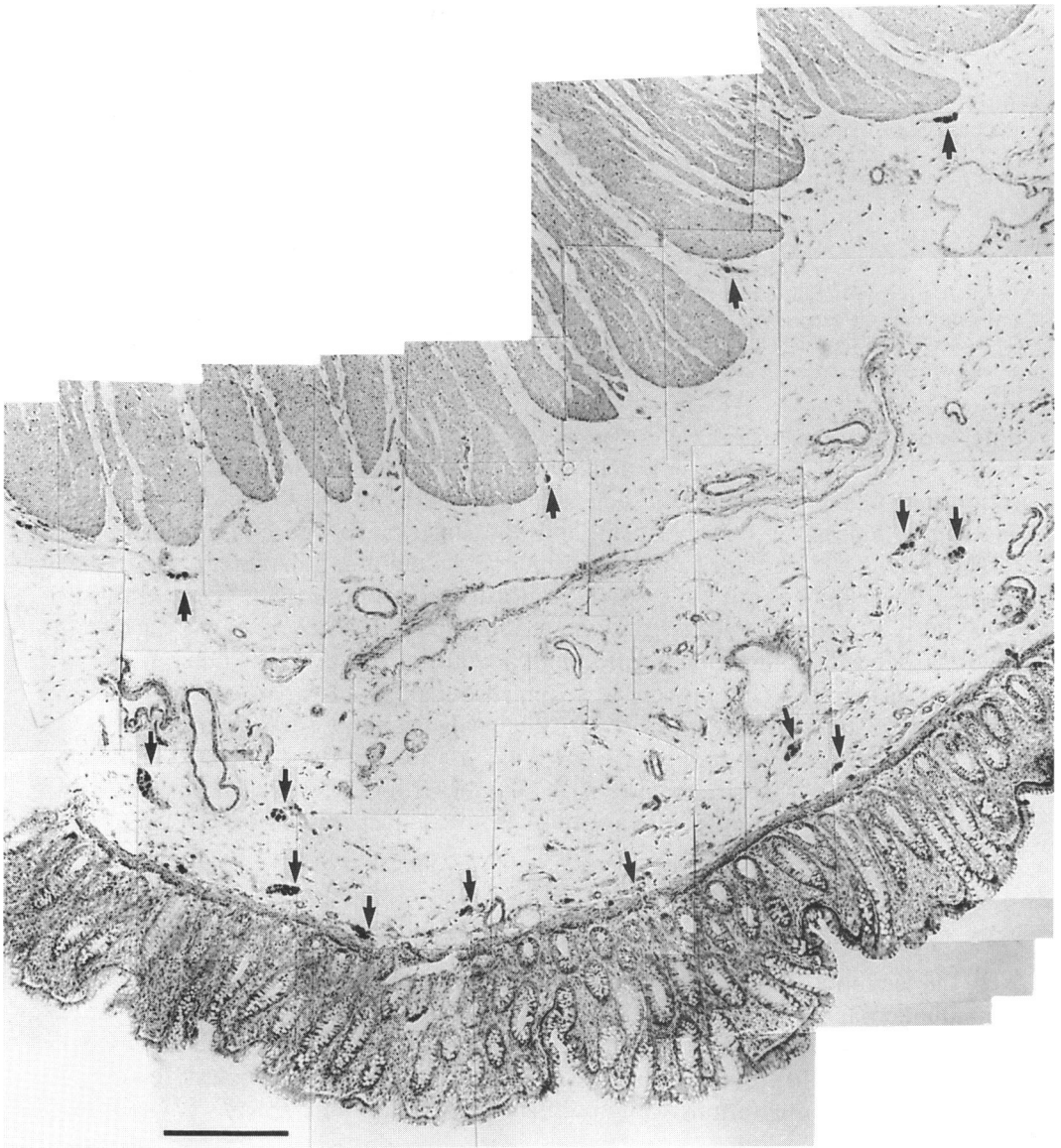


Fig. 1. Composite photomicrograph of a transverse section through part of the human distal sigmoid colon showing the inner margin of the circular muscle layer towards the top of the picture, the submucosa, muscularis mucosae and mucosa. Some ganglia of the submucous plexus lying at various levels, close to the circular muscle, close to the muscularis mucosae or in between, are indicated by arrows. Section stained for NADH-diaphorase. Scale bar represents 610 μm.

in the submucosa. Within the submucous plexus, although the range of cell body area was much the same in each plexus or region, the mean cell body area of neurons of Henle's plexus was greater than that of Meissner's plexus or of neurons from the intermediate region ($P < 0{\cdot}001$ in both cases). There was no significant difference between mean cell size in Meissner's plexus and cells from the intermediate region. Analysis of the frequency-distribution of cell body areas (Fig. 3) revealed that in Henle's plexus only 15·1% (of 99 cells) had an area less than 180 μm^2, while in the

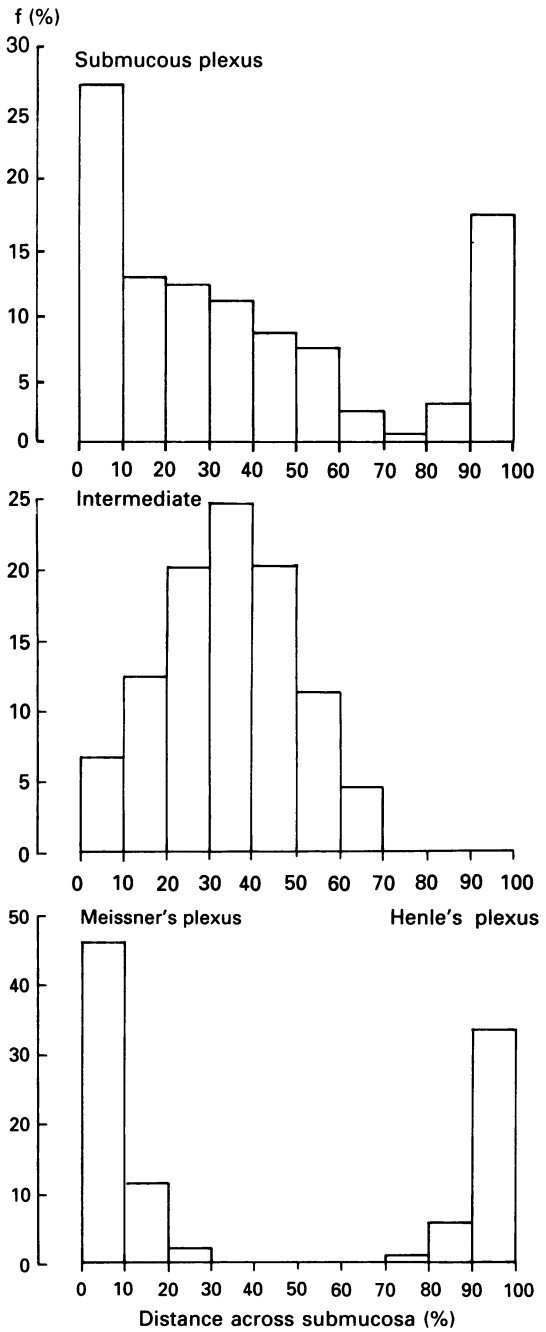


Fig. 2. Distribution of ganglia across the submucosa. Abscissae show the distance across the submucosa normalised to 100%, with 0% representing the border with the muscularis mucosae and 100% the border with the circular muscle. In the upper panel the distribution of 170 ganglia is shown. Approximately half (89) of these ganglia lay between 11% and 60% with a mean position $\pm$ standard deviation of 34·6 $\pm$ 16·04%. The middle panel shows the predicted distribution of these 89 ganglia assuming that they would be distributed normally. The lower panel shows the result of subtracting the middle panel from the upper panel, revealing the distribution of ganglia from Meissner's plexus and Henle's plexus separated from an intermediate layer.

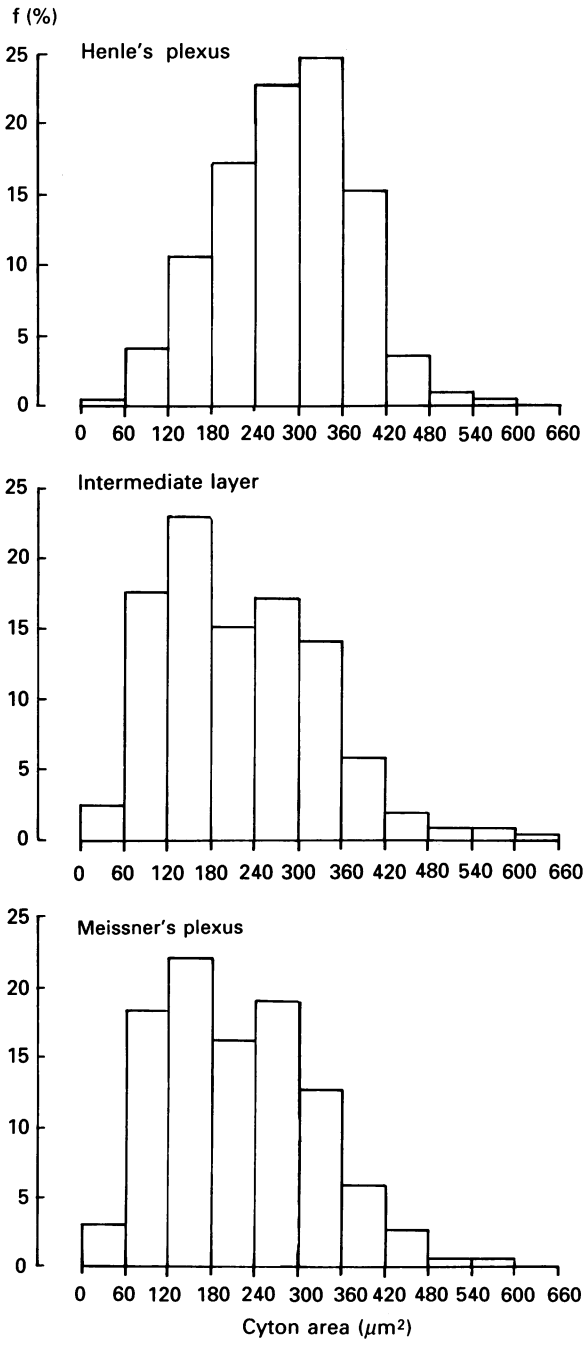


Fig. 3. Frequency-distribution of sizes of nerve cell bodies (cyton area) in regions of the submucous plexus of human distal sigmoid colon. The areas of 99, 102 and 96 nerve cell bodies were measured in Henle's plexus, the intermediate layer and Meissner's plexus respectively. There was no significant difference between the distribution of the intermediate layer and Meissner's plexus but that of Henle's plexus significantly differed from either of the other two divisions ($P < 0{\cdot}001$, chi-squared test, both cases).

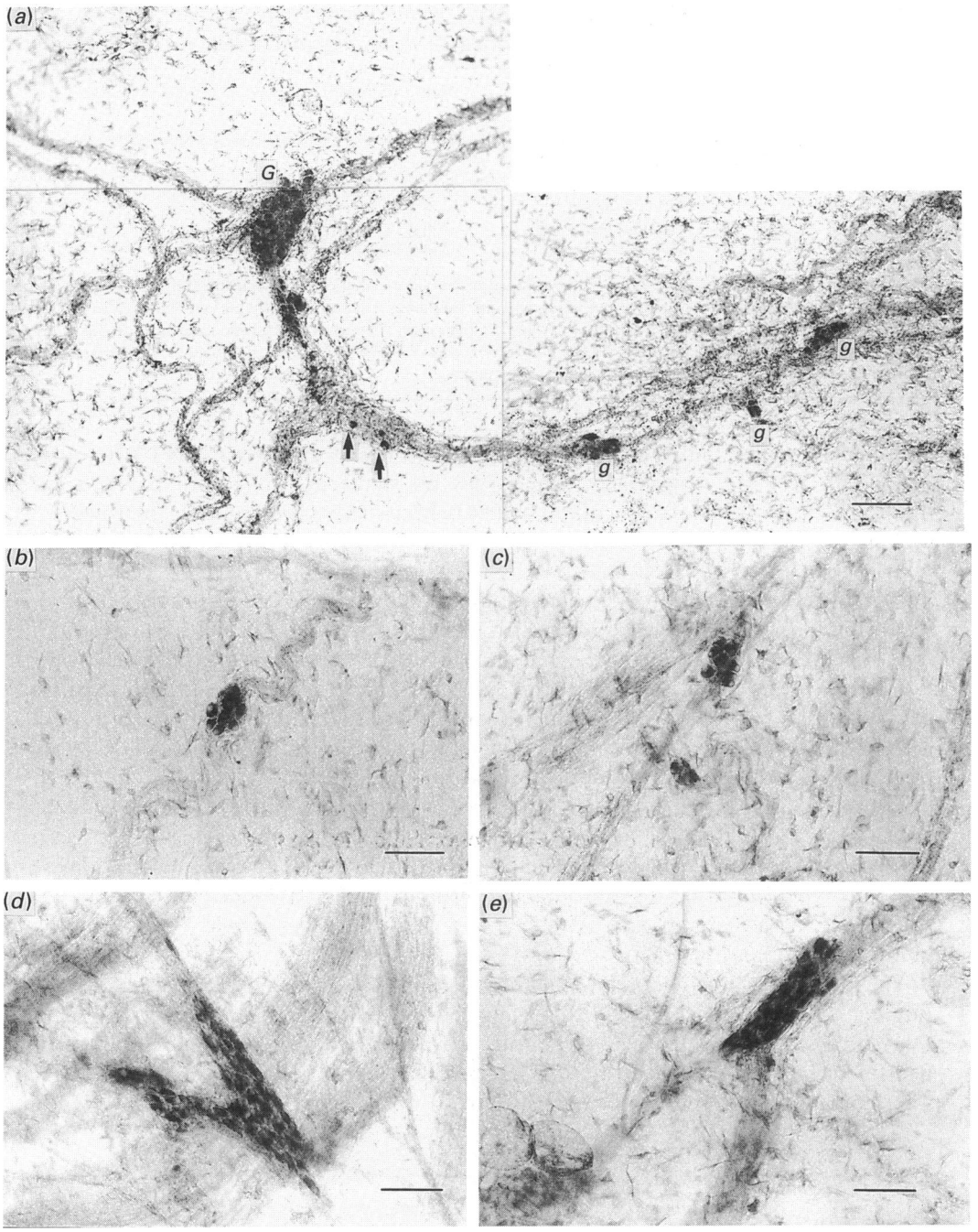


Fig. 4 (*a–e*). Ganglia and fibre tracts in separated layers of the submucous plexus of human distal sigmoid colon. (*a*) Composite photomicrograph of an area of plexus isolated from a region adjacent to the muscularis mucosae (Meissner's plexus). A large ganglion (G) lies at the junction of four fibre tracts as does a much smaller ganglion lying close by. The large fibre tract which runs from left to right connects some small ganglia (*g*) and contains dispersed neurons (arrows). Other fibre tracts can be seen which run for several mm without the presence of single neurons or ganglia. Scale bar represents 200 μm. (*b–e*) Low power photomicrographs of ganglia in isolated preparations of the middle region of the submucous plexus; in (*b*) a Type T ganglion is illustrated and in (*c*) a Type N and a Type D are shown. (*d*) and (*e*) show Type N ganglia. Note the appearance of neurons of differing sizes within a ganglion. Scale bars in (*b–e*) are 100 μm.

Table 2. *Area of nerve cell bodies in ganglia of the myenteric plexus and divisions of the submucous plexus of human distal sigmoid colon*

Plexus	Nerve cell body area (μm^2)	
Myenteric plexus	443 ± 51·1	(25)
Submucous plexus		
Henle's plexus	326 ± 9·4	(99)
Intermediate layer	266 ± 11·7	(102)
Meissner's plexus	244 ± 10·1	(96)

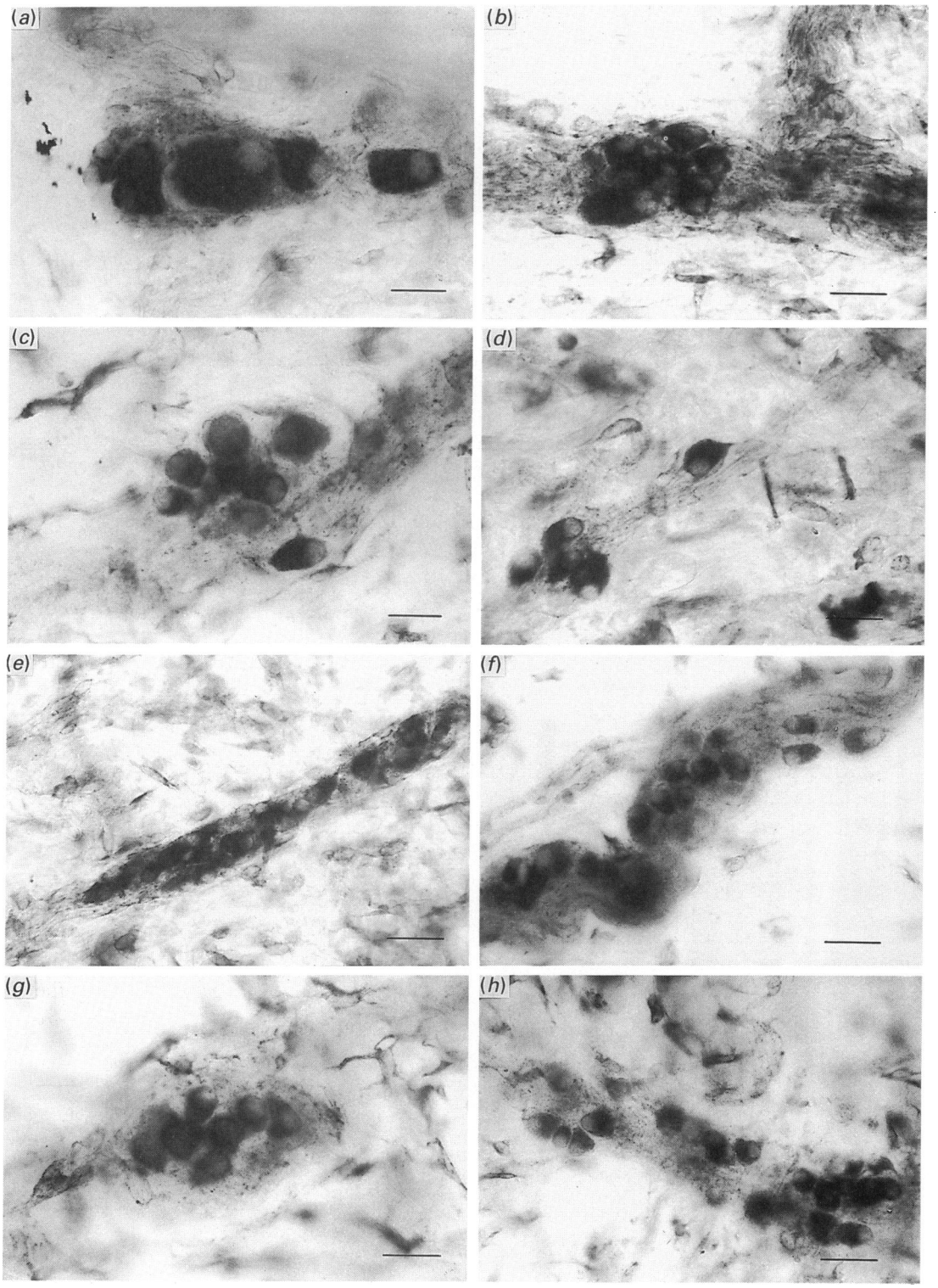
Values given are mean ± standard error (n). Measurements were made from sections stained for NADH-diaphorase. The mean nerve cell body area for the myenteric plexus was significantly greater than for any other region ($P < 0.05$). The mean cell body area of Henle's plexus was significantly larger than that of the other divisions of the submucous plexus ($P < 0.001$) and there was no significant difference between mean areas of cell bodies in the intermediate layer and Meissner's plexus.

intermediate region and Meissner's plexus the equivalent figures were 43·1 % (of 102) and 43·2 % (of 96) respectively. There was no significant difference between the frequency-distribution of areas of cell bodies in Meissner's plexus and the intermediate region.

In wholemount preparations of laminae corresponding to Henle's plexus, the intermediate region and Meissner's plexus, ganglia had recognisably different conformations; these fell into six categories which were found useful for subsequent analysis (Figs. 4–6). Ganglia were seen at clearly defined junctions or intersections of two or more fibre tracts (Type N, for node), or in a more amorphous group (Type G) with no clear passage of fibre tracts. Ganglia embedded within fibre tracts were designated Type T (for tract) and those which were displaced and lay alongside a fibre tract were called Type S (for side). All these ganglia tended to be roughly circular or oval. A less frequent arrangement was for the cell bodies within a ganglion to be arranged in a linear fashion (Type R, for ribbon) and the last type, also rare, was seen when ganglia lay apparently at the end of a fibre tract (Type D, for drumstick). These ganglion types could be divided into two groups: GN and TSRD, because TSRD ganglia are all variants of ganglia related to a single fibre tract while Types G and N involve more than one tract.

The distinction of ganglion types within plexuses and regions was observed, and the number of visible cell bodies within ganglia was counted (Tables 3 and 4; Fig. 7). During these measurements, lone neurons were deemed to be ganglia containing one neuron. The frequency-distribution of ganglia within Henle's plexus and the intermediate region were very similar (Table 3) with Type T accounting for 63–64 %

Fig. 5 (*a–h*). Different types of ganglia from three different layers within the submucous plexus of the human distal sigmoid colon. (*a–e*) Ganglia from isolated preparations of the layer of the submucous plexus adjacent to the circular muscle (Henle's plexus). Note, in the Type G ganglion in (*a*), a very large neuron of a size that is not seen in ganglia from other layers of the plexus. (*b*) and (*d*) are variants of Type T ganglia, associated with a single fibre tract, not located at a node, while (*c*) is a Type S ganglion. Note the association of apparently single neurons with the ganglia in (*c*) and (*d*). A Type R ganglion is shown in (*e*). The ganglion in (*f*) is also Type R, but from the intermediate layer of the submucous plexus. Ganglia of Types D and T, from the layer adjacent to the muscularis mucosae (Meissner's plexus), are shown in (*g*) and (*h*) respectively. Note that cell bodies from ganglia associated with the plexus by the circular muscle are typically larger than those of ganglia from other layers. Also note that ganglia contain cells of various sizes, and that the Type D ganglia appear to be encapsulated. Scale bars represent 25 μm in (*a–d*), (*g*) and (*h*), 40 μm in (*e*) and (*f*).


(a)
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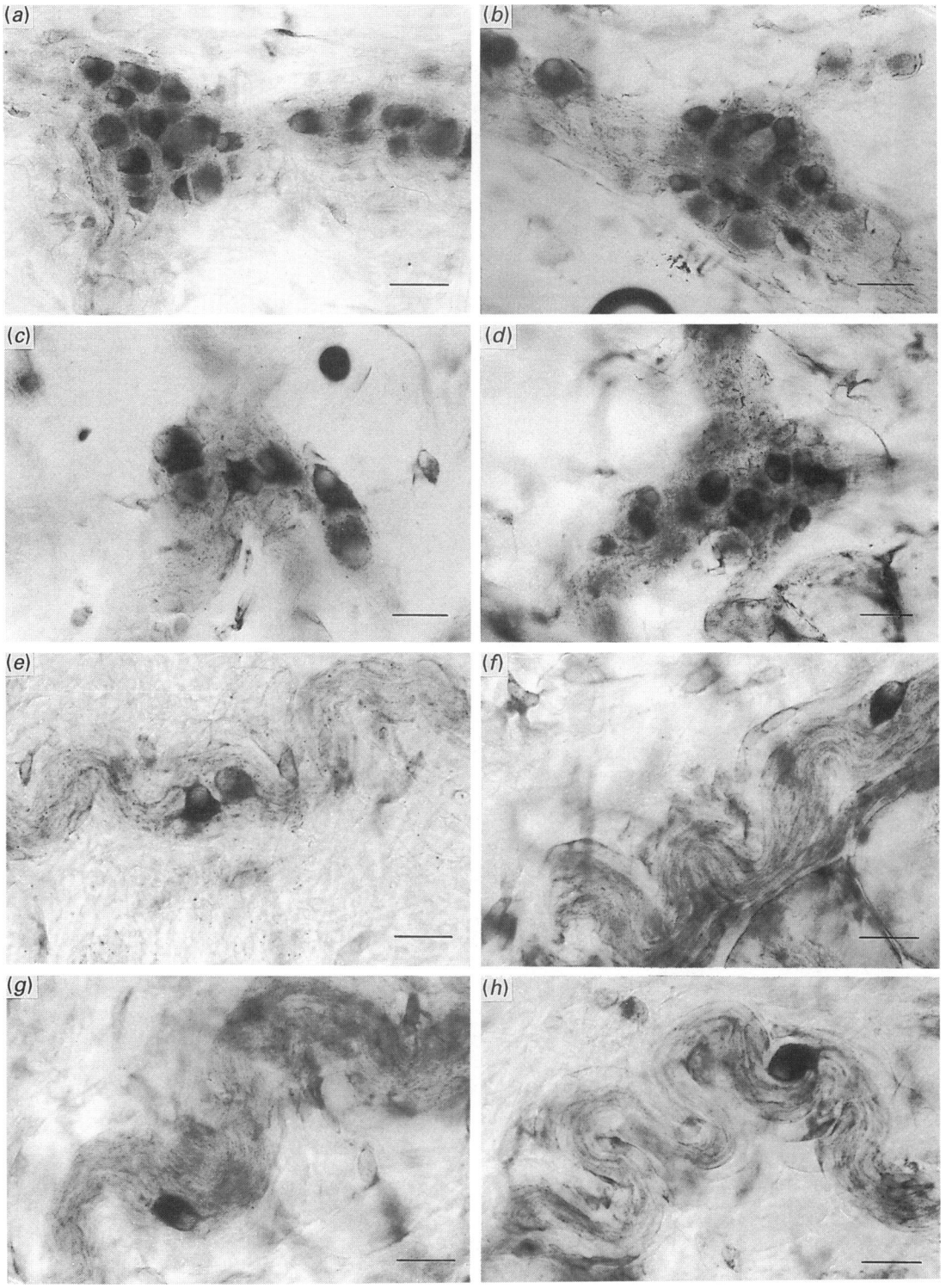
(a)
(b)
(c)
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(f)
(g)
(h)

Table 3. *The occurrence of ganglia of different types in divisions of the submucous plexus of human distal sigmoid colon*

Ganglion type	Henle's plexus (n = 306)	Intermediate layer (n = 190)	Meissner's plexus (n = 277)
G	8·8	11·6	9·7
N	16·9	16·4	35·8
T	64·0	63·5	49·0
R	3·9	1·1	1·4
S	5·5	4·3	2·9
D	1·0	3·2	1·1

Values are the number of a particular ganglion type per total number of ganglia, expressed as a percentage. 'n' is the number of ganglia observed, in wholemounts of divisions of the submucous plexus stained for NADH-diaphorase. The frequency-distributions of types of ganglia were not significantly different between Henle's plexus and the intermediate layer, but were significantly different between Meissner's and Henle's plexuses ($P < 0{\cdot}01$) and between Meissner's plexus and the intermediate layer ($P < 0{\cdot}001$), using chi-squared tests.

Table 4. *Number of cells per type of ganglion in divisions of the submucous plexus of human distal sigmoid colon*

Ganglion type	Henle's plexus	Intermediate layer	Meissner's plexus
G	11±1·5 (29)	9±0·9 (22)	8±1·2 (29)
N	10±0·8 (56)	12±1·4 (32)	11±0·7 (97)
T	5±0·4 (189)	6±0·4 (120)	6±0·5 (136)
R	11±2·1 (12)	16±6·0 (2)	9±3·2 (4)
S	4±0·9 (17)	5±0·8 (8)	5±1·4 (8)
D	5±0·9 (3)	5±1·2 (6)	10±1·9 (3)

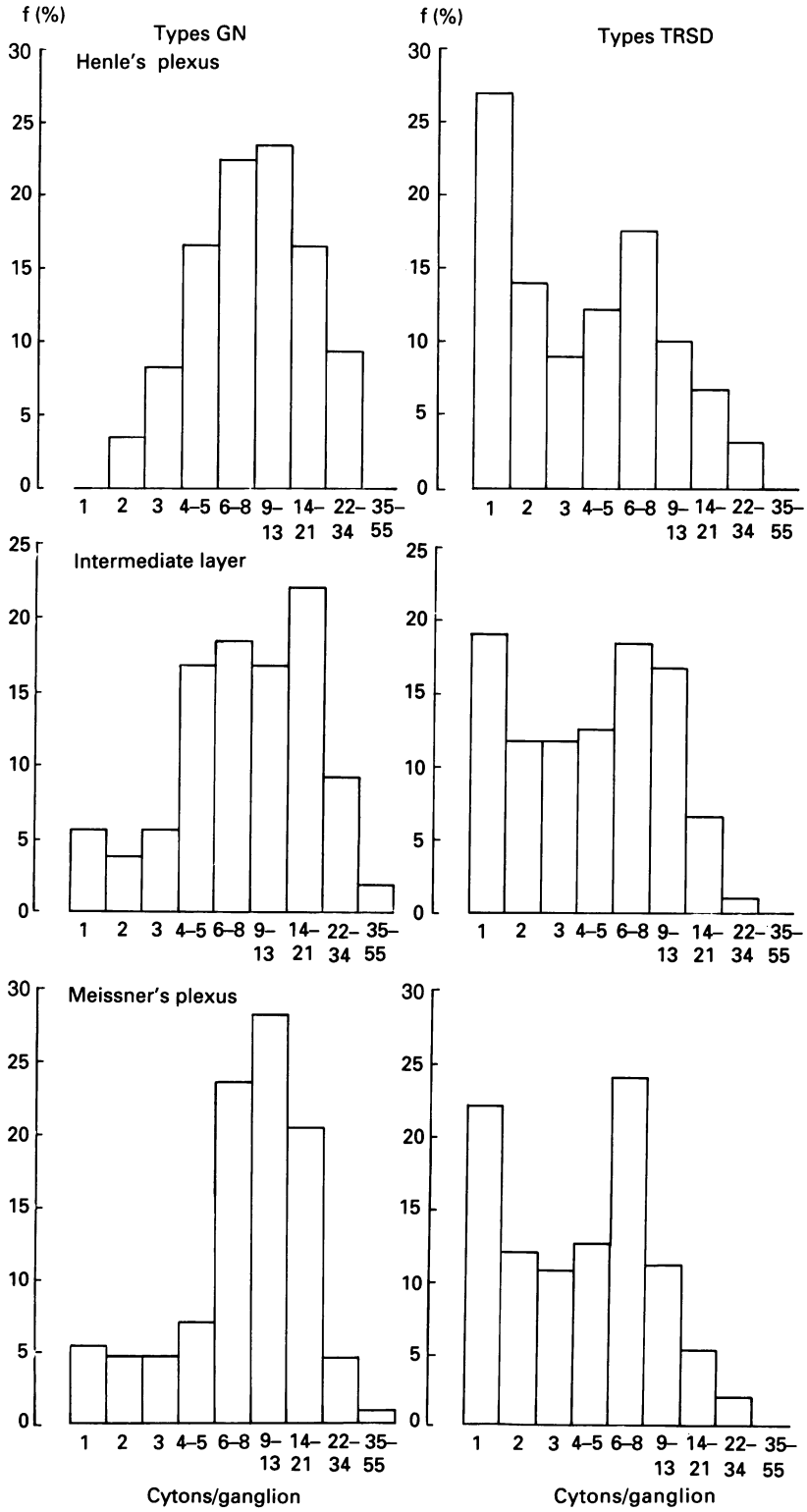
Values are mean ± standard error (n) of number of cells counted in a given type of ganglion. Observations were made from wholemount preparations of divisions of the submucous plexus stained for NADH-diaphorase.

of all ganglia, Type G and N accounting for 25–28 % and Types SRD accounting for approximately 10 %. The frequency-distribution of ganglion types of Meissner's plexus was different, with just under 50 % Type T, 45·5 % Type G or N and approximately 5 % Types SRD.

DISCUSSION

From the observations reported here it is evident that, besides their different anatomical locations, Henle's plexus and Meissner's plexus contain several distinguishing features. In the former there was a lack of small neurons and a greater

Fig. 6 (*a–h*). Ganglia and single neurons from different layers within the submucous plexus of the human distal sigmoid colon. (*a–d*) Type N ganglia, lying at nodes created by two or more fibre tracts. (*a*) and (*b*) are from the layer of plexus adjacent to the circular muscle (Henle's plexus). Note that ganglia are composed of cells of many sizes and that these Type N ganglia are closely associated with other ganglia. (*c*) and (*d*) are from the middle layer and from a region adjacent to the muscularis mucosae (Meissner's plexus) respectively. (*e*) Two closely associated neurons forming the smallest possible ganglion in a fibre tract; (*f–h*) are single neurons in fibre tracts, all from the plexus adjacent to the circular muscle (Henle's plexus). As in Figures 4 and 5, ganglia contain neurons of different size and morphology, and nerve cell bodies from ganglia associated with Henle's plexus tend to be larger than other nerve cell bodies. Scale bars represent 40 μm in (*a*) and (*b*), and 25 μm in (*c–h*).


f (%)
Types GN
Henle's plexus
Types TRSD
Intermediate layer
Meissner's plexus
0
5
10
15
20
25
30
1 2 3 4–5 6–8 9–13 14–21 22–34 35–55
Cytons/ganglion

preponderance of large neurons, there were fewer ganglia at intersections or junctions of fibre tracts and, in these particular types of ganglia, there were more likely to be three or more neurons.

From the observation that Henle's plexus lay almost totally within the 20% of the region of the submucosa at the margin of the circular muscle, yet Meissner's plexus apparently extended some 70% of the thickness of the submucosa from the muscularis mucosae, it has to be considered whether or not the neurons in the latter domain constitute more than one plexus, with Meissner's plexus actually occupying only 20% of the thickness. The observed distribution of ganglia was markedly different from that in the opossum colon where approximately 90% of the ganglia of Meissner's and Henle's plexuses lie within 20% of the thickness of the submucosa from the muscularis mucosae and circular muscle respectively (Christensen & Rick, 1987). The distribution in the human colon could be due to the presence of a third plexus, and it is noteworthy that when a population of ganglia with an assumed normal distribution about a mean of 35% was removed from the pool, the remaining ganglia from Henle's and Meissner's plexuses had identical distributions from their respective margins. Also, a third plexus has been described in the pig large intestine which has the same location with respect to Henle's and Meissner's plexuses as seen here, and was called the intermediate plexus (Gunn, 1968). In wholemounts of laminae of the submucosa, prepared from Henle's plexus, Meissner's plexus and the intermediate region, untidy arrangements of ganglia were seen, connected by fibre tracts. Quantitative measurement of meshwork sizes was not attempted because of differences in degree of stretch which would arise during preparation, although it is appreciated that there are differences between different layers (Gunn, 1968; Stach, 1977*a*, *b*). The fibre tracts could be followed for long distances before encountering junctions with other tracts, or ganglia. The preparations from each layer gave the impression of there being three separate plexuses. However, this would not be a substantive reason for proposing the existence of a separate plexus. Even though wholemounts could be made, they were still only viewed in effectively two dimensions.

Although different populations of nerve cells, based on size of nerve cell body, were identified in Henle's plexus and Meissner's plexus, no such differences were seen between Meissner's plexus and the intermediate layer, implying an homogeneous population. There were differences between the occurrences of certain types of ganglia between Meissner's plexus and the intermediate layer, with more ganglia associated with branching fibre tracts and fewer with single fibre tracts, than there were in the intermediate region. This was the only difference, other than anatomical location, found between these two neural networks.

The functional significance of either a very thick Meissner's plexus or a separate plexus lying above it is unclear. Most of the sections were from material fixed in a contracted state, and it is possible that the apparent depth of Meissner's plexus represents an accommodation of the nerve network, which would be much flatter when stretched. *In vivo*, the colonic circumference will vary from 10 cm to 25 cm

Fig. 7. Frequency-distribution of number of neurons per ganglion (cytons/ganglion) of either Types GN or Types TRSD in divisions of the submucous plexus of human distal sigmoid colon. For a given division, the distribution of the types GN differed significantly from the Types TRSD ($P < 0{\cdot}001$). The frequency-distribution of types of ganglia differed significantly between Meissner's plexus and both other divisions ($P < 0{\cdot}001$) but there was no significant difference between those of Henle's plexus and the intermediate layer. Numbers of ganglia counted were 85, 54 and 126 Type GN, and 21, 136 and 157 Type TRSD in Henle's plexus, the intermediate layer and Meissner's plexus, respectively. Chi-squared tests were used.

depending on its state of contractility. However, in preparations which were stretched prior to being fixed, substantial numbers of ganglia were still found in the intermediate region of submucosa.

The structure of the human colonic submucous plexus differs greatly from that of the guinea-pig ileum although the mean maximum diameter of guinea-pig nerve cell bodies is $19{\cdot}4 \pm 0{\cdot}8$ μm ($n = 30$) with a range of 11·5–29 μm (Wilson *et al.* 1981*a*) and these values are broadly similar to the human values. However, the numbers of neurons per ganglion differs, for in the guinea-pig there were most commonly either 4–8 or 20–30 (Wilson *et al.* 1981*a*) whereas in the human colon, at any level, lone neurons or ganglia containing 6–8 neurons were the commonest amongst TRSD types, and in GN types the most common number of neurons per ganglion was from 9–21. As in the guinea-pig large intestine (Gabella & Trigg, 1984), the mean of the nerve cell body areas in the submucous plexus was smaller than that of the myenteric plexus, but in the human colon the mean of the nerve cell body areas was larger than that in the corresponding plexuses of the guinea-pig (Gabella & Trigg, 1984).

Until more information is available concerning the physiology of the submucous plexus and until unique functions can be attributed to these components, it will not be possible to state clearly the exact nature of the construction of the submucous plexus. At a light microscope level the next step would be to examine the chemical coding of neurons in the submucous plexus and, in particular, to look for expression of neuropeptides and transmitter substances by neurons, a differential localisation of which would be important in helping to determine the structure and function of the submucous plexus. Only a few immunocytochemical studies have been carried out so far on the human colon, including the neuronal localisation of vasoactive intestinal polypeptide, substance P, bombesin, enkephalin, somatostatin, neuropeptide Y and galanin (Ferri *et al.* 1982, 1983; Keast, Furness & Costa, 1985*c*; Hoyle & Burnstock, 1989). The description of different sizes of nerve cell bodies and different arrangements of ganglia may eventually be correlated to different neuropeptides and transmitter substances.

SUMMARY

The submucous plexus of the human distal colon was studied in order to determine whether or not it contains two or more ganglionated plexuses which can be separately identified. Nerve cells were visualised in sections through the wall of the distal colon, and in wholemount preparations of laminae from the submucous plexus by staining for NADH-diaphorase activity. The submucous plexus appeared to contain three identifiable plexuses: Henle's plexus was located adjacent to the circular muscle layer, Meissner's plexus was located adjacent to the muscularis mucosae and a third intermediate plexus was found which lay closer to the muscularis mucosae than to the circular muscle. In Henle's plexus, there were fewer smaller neurons than in the other plexuses: 15·1% had an area less than 180 μm^2, while in the intermediate plexus and in Meissner's plexus the equivalent figures were 43·1% and 43·2% respectively. In Meissner's plexus, approximately half the ganglia were associated with single fibre tracts and half with two or more fibre tracts, but in the intermediate plexus and Henle's plexus, approximately three quarters of the ganglia were associated with single fibre tracts and the remaining quarter with multiple tracts.

Dr G. Gabella (University College London) is thanked for his critical appraisal of the manuscript and J. A. Fowler, M.A. (St Joseph's Hospital, London) is gratefully thanked for assistance in the translation of the German papers cited.

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