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# The fiber composition of the abdominal vagus of the rat

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**Summary.** The present study provides a LM and EM inventory of the fibers of the rat abdominal vagus, including dorsal and ventral trunks and the five primary branches. Whole mounts (n=15) were prepared to characterize the branching patterns. A set of EM samples consisting of both trunks and all branches (i.e. dorsal and ventral gastric, dorsal and accessory celiac, and hepatic) were then obtained from each of six additional animals. A complete cross-sectional montage (×10000) was prepared from each sample. All axons were counted, and >10% of them were evaluated morphometrically.

The means of unmyelinated axon diameters for each of the five branches were similar (0.75–0.83 μm). However, the shapes of the fiber size distributions, as summarized by their skew coefficients, revealed that the two gastric branches differed significantly from the two celiac branches; furthermore, the hepatic size distribution differed from all others. Most of the myelinated fibers (85%) in all branches were <2.6 μm in diameter and had sheath widths between 0.1 and 0.5 μm. The gastric branches, however, also contained a few larger myelinated fibers with sheath widths as great as 0.85 μm. Whole mounts revealed fibers which were not of supradiaphragmatic orgin within all five vagal branches; these adventitial bundles were traced along the perineurium between adjacent branches. The sum of the fibers in the five branches (26930) was 21% more than the number counted in the parent trunks (22272); this excess probably reflects the adventitial fiber content. The whole mounts also showed that a large and regularly positioned paraganglion was associated with the dorsal branches.

The structural profiles observed (i.e. unmyelinated and myelinated fibers size distributions, presence of extrinsic fascicles, glomus tissue content, etc.) differentiate the vagal branches into three morphologically distinct sets: a gastric pair, a celiac pair, and a hepatic branch. The fiber counts, when considered with observations of the numbers of efferents and adventitial fibers in the nerve, suggest that the percentage of efferent fibers is much higher than in all the widely accepted estimates found in the literature: efferent fibers may represent over a quarter of the total number of fibers.

**Key words:** Autonomic nervous system – Enteric nervous system – Paraganglia – Parasympathetic – Sympathetics

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## Introduction

The vagus nerves contain sensory and motor fibers that mediate vital digestive reflexes and influence ingestive behavior. The vagi enter the abdomen as two trunks coursing on the esophagus. These trunks, the dorsal and ventral, traditionally have been recognized as dividing into four or five (depending on species) distinct primary branches at the subdiaphragmatic esophageal level (see Fig. 1a). These branches are fundamental organizational elements of the parasympathetic nervous system, insofar as they are reflected in CNS topographic representations of the vagus. The preganglionic neurons within the dorsal motor nucleus are organized into spatially discrete longitudinal columns, each corresponding to a different abdominal branch (Fox and Powley 1985; Powley et al. 1987). In the case of sensory fibers, the different branches project to different regions of the nucleus of the solitary tract (Altschuler et al. 1989; Norgren and Smith 1988). Furthermore, physiological studies have indicated that the different branches mediate different functions. For instance, the gastric branches control stomach acid secretion; the hepatic branch has been shown to influence the motility of the gall bladder and biliary tract, and the motility of the distal intestine and colon is mediated by the celiac branch(es) (e.g., Griffith 1969, *human*[1]).

Little is known about the structure of the branches themselves. Very few analyses which might indicate specializations or complexities of the system have been performed. No study has inventoried both trunks and their derivative abdominal branches form the same tissue samples. To date, only the abdominal vagal trunks (Asala and Bower 1986, *ferret*; Gabella and Pease 1973, *rat*; Mei et al. 1980, *cat*;) and the hepatic branch of the rat (Kohno et al. 1987; Prechtl and Powley 1987) have been examined with electron microscopy, and these studies have confirmed the inadequate resolving power in the earlier light microscopic studies. Moreover, the fiber counts provided by these recent studies underscore the inter-individual variability of vagal structure and thus the need to compare the branches within the same individual.

One question that remains to be resolved is which symmetries and similarities occur among the branches. The vagi, like the viscera they innervate, do not show an uncomplicated symmetry such as that observed in the bilateral

[1] Wherever appropriate, comparisons involving animals other than rats include the name of the animal with the citation

innervation of the skeletal musculature. Nonetheless, the bilaterally symmetrical columns of preganglionic somata that form the vagus nerves may jointly provide the same functional innervations (e.g. motor, secretomotor, chemoreceptive, etc.) to different sides or segments of a viscus. At least this is the case with the gastric innervation of the rat. Its stomach has symmetrical sides that originate from left and right portions of the developing alimentary canal, with each side innervated primarily by the ipsilateral vagus (see Fox and Powley 1985; Legros and Griffith 1969). What has not been clear, however, is whether or not the hepatic and celiac branches of the ventral vagus have partially or completely symmetrical counterparts or homotypes in some or any of the dorsal vagal branches.

Another unresolved issue is the question of the number or percentage of sensory and motor fibers in the vagus and in each of its branches. So far this issue has been addressed for the vagi only at the level of the diaphragm and only in species other than the rat (Agostoni et al. 1957, *cat*; Evans and Murray 1954, *rabbit*). These early light microscopic studies suggested the widely cited conclusion that the abdominal vagus is more than 90% afferent, a conclusion that is difficult to reconcile with the cell counts from recent retrograde tracer studies (see Discussion).

## Materials and methods

### *Whole mounts*

Osmium-stained whole mounts of the abdominal vagi were prepared from 15 adult male Sprague Dawley rats (Harlan Industries, IN, USA). A tissue block including the upper abdominal organs and vagi was removed from each animal and exposed to osmium tetroxide vapors until the nerve fiber detail was legible. The nerves were then dissected from the viscera, dehydrated, cleared and mounted on slides (see Prechtl and Powley 1986, 1987, 1988). To minimize visceral fat which can obscure the nerves, the body weights of eight animals were reduced according to the protocol of Richter and Rice (1942) prior to sacrifice.

### *EM montages*

*Tissue preparation.* The vagi of 12 additional adult male Sprague Dawley rats ($183 \pm 4$ g body weight; all values are given as means and standard errors of the means) were prepared for electron microscopy, and six of these specimens were eventually analyzed (see *Sampling* below). To minimize fat depots, the animals were gradually reduced to approximately 70% of their initial body weight (Richter and Rice 1942).

After the deeply anesthetized (sodium pentobarbital, 80 mg/kg) animal was laparotomized, a tissue block containing the viscera and vagi was promptly (< 30 sec) excised and submerged in a large beaker of oxygenated Ringer's solution (120 mM NaCl, 7.5 mM KCl, 24 mM $NaHCO_3$, 0.6 mM $MgCl_2$, 1.9 mM $CaCl_2$, 11 mM D-glucose, 2.8 mM HEPES, pH 7.4, $O_2$95% + $CO_2$5%) for an initial trimming. The esophageal adventitia was incised from the diaphragm to the lesser curvature and then separted from the esophagus with all of the nerves and vessels preserved intact (Fig. 1b). The specimen was pinned to an optically clear silicone rubber wafer (Sylgard, Dow Corning Corp Midland, MI, USA), and all of its vagal components were identified. The specimen was then immersed in agitated fixative (3% glutaraldehyde in 2 mM $CaCl_2$, 60 mM $H_2O_2$, 100 mM Na cacodylate, pH 7.4) for 1–2 h and then in the same fixative without $H_2O_2$ for 1 h according to the protocol of Peraccia and Mittler (1972). The tissue was subsequently treated with 2% osmium tetroxide in 100 mM Na cacodylate buffer at pH 7.2 for 2 h followed by 2% uranyl acetate in 60 mM maleate buffer at pH 5.0 for 1.5 h. After graded dehydration in chilled methanol (4° C), the specimens were unpinned, transferred to room temperature acetone, and infiltrated with Epon.

*Electron microscopy.* Thin sections (80–95 nm) were collected on single slotted grids, stained with lead citrate, and examined with a Philips EM400 electron microscope at 80 kV. Complete montages of each specimen were produced by exposing plates at ×2750 and enlarging them to ×10000. For calibration, every montage included an exposure of a diffraction grating replica (Pelco, 21600 ln/cm).

### *Quantiative methods*

All LM measures were made with camera lucida (X100–X200) in combination with a digitizing tablet and computed with the Bioquant System IV Analysis Program.

For EM analyses, each montage was covered with acetate sheeting, and a marker was used to divide the nerve into *subfascicles.*[2] The subfascicles were identified as groups of fibers separated by endoneurial cell processes or intraneural space with collagen fibrils. Most of the subfascicles so defined would probably correspond to the endoneurial tubes described by Sunderland (1980), but others may have represented fiber groupings within endoneurial tubes. All of the axons in each subfascicle were counted and simultaneously marked.

For the sample of unmyelinated fiber morphometric measurements, a rectangular array of points (16 pts/mm$^2$)

[2] The terms currently used to characterize the vagi are based on dissections and do not adequately apply to the finer structure visible at the light microscopic level. In particular, structures that have been known as the subdiaphragmatic vagal branches in the rat (Fig. 1a) often consist of several individually ramifying groups of fibers. Although the common terms "branch", "ramus", and "fascicle" are used interchangeably in some applications, in the present study they are used more restrictively. The classically recognized primary divisions of the vagi are still referred to as branches, but the term ramus is used especially to refer to each of the two or more individual nerves which often compose a branch.

In addition to the rami, much smaller nerves separate from, or course with, the abdominal vagi. The smaller nerves (approx. 13–30 μm in width) typically contain less than 100 fibers and the term fascicle is used to designate them. The term subfascicle is used to describe an intraneural group of axons delineated by endoneurial cell processes and wide margins of intraneural space. The term adventitial fibers adopts the usage established by Evans and Murray (1954, *rabbit*) to refer to fibers in the vagus which do not originate in the cervical tenth cranial nerve but enter the vagus at some level distal to the nodose ganglion. The term adventitial bundle is used to distinguish groups of adventitial fibers visible in nerve whole mounts which appear to enter the vagal branches from the abdomen.

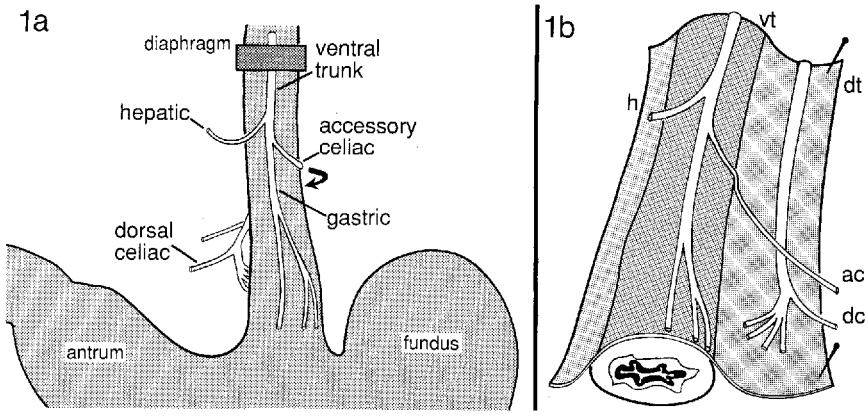


**Fig. 1 a, b.** Gross anatomy of the abdominal vagal branches in the rat. **a** Schematic of vagal branches on the abdominal esophagus. **b** Schematic illustrating in vitro dissection method. *Abbreviations: ac*, accessory celiac branch; *dc*, dorsal celiac branch; *dt*, dorsal vagal trunk; *h*, hepatic branch; *vt*, ventral vagal trunk

was superimposed on each subfascicle in the nerve cross section and the outer limit of the axon with its center closest to a given point was traced on an overlying sheet of acetate. If the orientation of an axon was oblique to the plane of section as judged by features of its cytoskeleton, the next closest axon was sampled instead. The areas and inner perimeters of the axon tracings were evaluated with an OASYS Image Analyzer (LeMont Scientific, State College, Pennsylvania, USA; Linescan Program). The program calculated the perimeter of each axon by selecting 16 perimeter points and determining the distances between them with the Pythagorean equation. The perimeter and area measurements were converted to perimeter-derived (p-) and area-derived (a-) diameters, which equalled the diameters of circles having the same perimeters or areas. Unless otherwise specified, all diameters given for unmyelinated axons are based on perimeters.

For myelinated fibers, the inner and outer boundaries of the myelin sheath were traced. All the myelinated axons were traced, except those sectioned obliquely or at paranodes or Schmidt-Lanterman incisures. The image analyzer was used to evaluate sheath width, inner sheath perimeter and area-equivalent total fiber diameter. To facilitate comparison with the studies available on myelinated fibers, area-derived diameters are given in the graphs.

The fiber size distributions were compared with the statistical programs of the SAS Institute INC (Cary, NC, USA). Skew coefficients, the third moments about the means of the standardized distributions, were calculated by the SAS Univariate procedure. Nonparametric ANOVAs were performed by transforming the data into ranks according to the protocol of Conover and Iman (1981). The Tukey HSD procedure (see Winer 1971) was used for comparing means.

*Specimens sampling.* A source of potential error was the relatively common occurrence of one or more of the vagal trunks or branches appearing as a furcation of multiple rami rather than a coherent bundle. Because a goal of the present study was a complete inventory of the fibers in the abdominal vagus, these split-branch cases were more difficult to analyze – they increased the probability of not observing all relevant axons in an EM reconstruction. To minimize this source of error, the group of 12 animals was initially prepared, and six specimens with sets of relatively coherent primary branches were selected for full analysis. Count/recount and measure/remeasure reliability checks fell within $1 \pm 0.2\%$.

## Results

### *Whole mounts*

*Branch anatomy of the abdominal vagus.* A simple configuration of the vagal trunks and branches in which most or all of the branches occurred as single coherent rami (as illustrated in Fig. 1) was the most common pattern observed. Nonetheless, a number of more complex ramifications did occur. The trunk and individual branches of the ventral vagus evidenced more complex ramifications in 20%–47% of the specimens. The dorsal trunk and its branches were typically still more complex. The celiac division was comprised of $4.8 \pm 0.4$ bundles which ramified from the dorsal vagal trunk within a rostro-caudal zone of 0.3–1.1 mm. The dorsal gastric branch was formed by the division of the residual vagal trunk into $2.8 \pm 0.2$ bundles at a rostro-caudal level approximately 0.3 mm distal to the separation of the first celiac bundle.

*Esophageal fascicles.* In each specimen, in addition to the primary bundles, two–four smaller fascicles ($20 \pm 1$ μm in diameter) separated from either side of the ventral trunk at sequential levels. Because at least most of these distinctively myelinated fascicles appeared to innervate the esophagus and none were observed to project to alternative targets, they are described here and in following sections as esophageal fascicles. In contrast to the trunk itself, the fascicles contained a relatively high concentration of myelinated fibers. The fascicles repeatedly divided on the esophagus, and virtually all of the finer twigs penetrated the esophageal serosa. Similar fascicles, $3.9 \pm 2.5$ in number and $18 \pm 9$ μm in width, also branched from or coursed in close association with the dorsal vagal trunk.

*Adventitial bundles.* In addition to the fibers which descend into the abdomen by way of the thoracic vagi, presumably from the brainstem or nodose ganglion, we also found fiber bundles in the hepatic (see Prechtl and Powley 1987) and celiac branches that projected to or from a gastric branch (Fig. 2c). These adventitial fiber bundles were observed in 74% of the hepatic-gastric bifurcations and in 80% of the accessory celiac-gastric segments. They were always seen as coherent bundles of unmyelinated fibers which coursed along and within the edge of the adjoining branches (as in Fig. 2c). They could be followed distally for hundreds of microns to a few millimeters in each branch. Although we were unable to clearly identify these bundles in all of

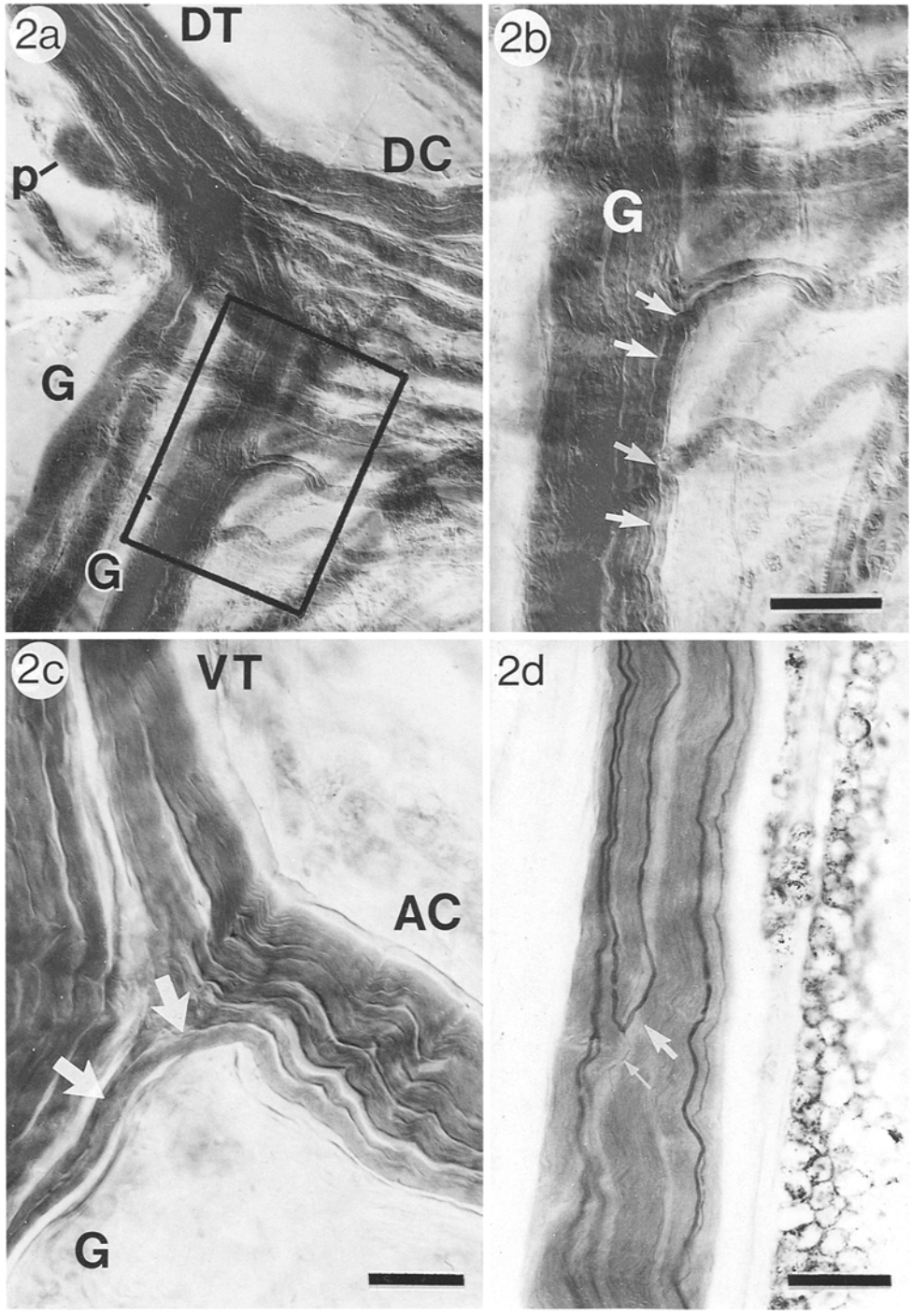


**Fig. 2a–d.** Fiber components of the abdominal vagi. **a** In this specimen the dorsal vagal trunk (*DT*) divides into the dorsal celiac branch (*DC*) and two gastric branch stems (*G*). Distal to the dorsal celiac branch (lower right side of panel) numerous bundles are shown coursing through the celiogastric bifurcation. The celiogastric paraganglion (*p*) occupies its characteristic location – opposite to the dorsal celiac branch. Nomarski optics, width of inset = 180 μm. **b** The *inset* in panel **a** is shown enlarged in panel **b**; the *arrows* indicate adventitial fiber components coursing between the gastric and celiac branches. Nomarski optics, scale bar = 100 μm. **c** Whole mount of the accessory celiac branch (*AC*) showing that, although most of its fibers are derived from the ventral trunk (*VT*), a small bundle of fibers (*arrows*) courses from, or to, the gastric branch (*G*). Nomarski optics, scale bar = 50 μm. **d** Whole mount of the ventral vagal trunk showing a recurrent myelinated fiber (*arrow*). The palely-stained paths coursing in parallel with the nerve fibers are blood vessels. Scale bar = 100 μm

the specimens, this failure may well reflect the problems with non-optimal osmication or positioning of the whole mounts, and they may have been nevertheless present. None of the individual myelinated fibers discernable at the LM level (see below) were ever observed in these bundles.

When the hepatic or celiac branch was composed of a series of two or more rami, the bundle of adventitial fibers was detected in the more caudal one. There was no indication of sizeable bundles communicating between non-contiguous branches (i.e. between the hepatic and accessory celiac). The hepato-gastric bundle amounted to 15–35% of the hepatic branch width, and the celio-gastric bundle was 12–30% of the accessory celiac branch width.

As in the case of the ventral vagal branches, the celiogastric bifurcation of the dorsal vagus contained bundles that did not appear to originate in the supradiaphragmatic vagus. These adventitial bundles coursed through the celiogastric bifurcation within the various celiac and gastric bundles (Fig. 2b, white arrows). The more caudal (lower part of panel) bundle in Figure 2b appears to lack fibers of supradiaphragmatic vagal origin and to be entirely adventitial.

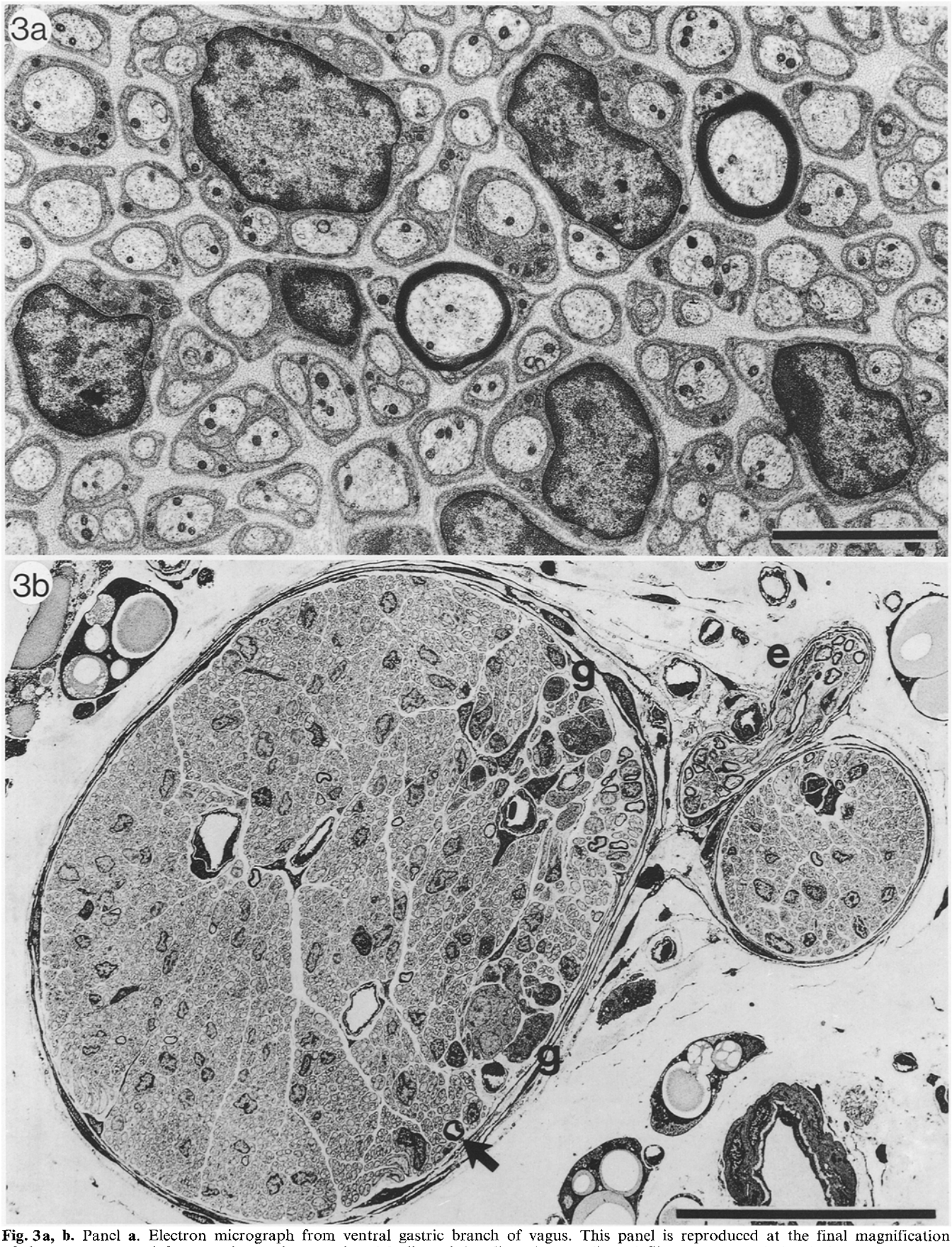


**Fig. 3a, b.** Panel **a**. Electron micrograph from ventral gastric branch of vagus. This panel is reproduced at the final magnification of the montages used for counting and measuring. Myelinated (n = 2) and unmyelinated fibers occur, and the darker Schwann cell cytoplasm engulfing the unmyelinated axons is apparent. Scale bar = 2 μm. Panel **b**. Micrograph of the ventral gastric branch. The section contains glomus tissue (*g*) in separate zones. Also shown is a ramifying esophageal fascicle (*e*) adjacent to a ventral gastric twig that presumably innervates the gastric fundus. A thickly myelinated fiber in the main branch is indicated by an *arrow*. Scale bar = 50 μm

**Table 1.** Numbers of fibers in the vagal branches

| Vagal nerve | Unmyelinated fiber number | Percentage of combined | Myelinated fiber number | Percentage of of combined |
|---|---|---|---|---|
| Ventral | | | | |
| Ventral trunk | 11275 ±466 | 82 ±3 | 52 ±6 | 114 ±12 |
| Hepatic branch | 2936[a] ±257 | 23 ±2 | 10 ±2 | 27 ±8 |
| Accessory celiac | 2893[a] ±278 | 21 ±2 | 6 ±2 | 9 ±4 |
| Ventral gastric | 7445[b] ±314 | 56 ±3 | 31 ±3 | 68 ±8 |
| Dorsal | | | | |
| Dorsal trunk | 10880 ±628 | 83 ±3 | 65 ±9 | 134 ±17 |
| Dorsal celiac | 4201[c] ±320 | 32 ±1 | 2 ±1 | 5 ±1 |
| Dorsal gastric | 9355[b] ±352 | 68 ±1 | 51 ±4 | 95 ±1 |

The means of the fiber counts (±SEMs) for unmyelinated and myelinated fibers are given in first and third columns. In the first column, the means of branches with different superscript letters are significantly different ($Ps < 0.05$). For each of the specimens the numbers of fibers in the primary branches (ventral or dorsal vagus) were combined and then the branches and the trunk were expressed as a percentage of the contained total. The means of the percentages of the combined are given for both unmyelinated (second column) and myelinated fibers (fourth column)

*Errant myelinated fibers.* Sixty-seven percent of the whole mount specimens were stained sufficiently lightly so that individual myelinated fibers could be traced in the vagus for many millimeters or even for the entire length of the specimen, thorax to stomach. In 50% of such specimens, one or more myelinated fibers descended to below the diaphragm, some as far caudal as the gastric branch, and then pivoted 180° to return up the thoracic vagus (Fig. 2d). In some instances two or more such fibers descended and returned in parallel. Other vagrant fibers reversed direction but did not return to the thoracic vagus. For example, in one specimen a myelinated fiber was traced down the ventral gastric branch and then back up and into the accessory celiac branch. In another specimen, a fiber coursed in the accessory celiac branch half-way around the esophagus to the region of the dorsal vagus, and then returned to the ventral vagus to enter the gastric branch. The fibers showing recurrent paths were typically larger than the majority of abdominal vagal fibers.

*Nodular paraganglia.* Small clusters of glomus tissue were found in the whole mounts (as well as sections – cf Fig. 3b) at all levels of the ventral vagus. They were generally observed near bifurcations and less often in the vagal trunk rostral to the hepatic bifurcation. In particular, the hepatic and gastric branches contained large paraganglia which produced conspicuous thickenings of the nerve or took the form of a nodule on one side of the nerve. Nodular paraganglia were also found in the more distal twigs of the gastric branch. Such paraganglia were not found on the esophageal segment of the accessory celiac branch, nor on the vagal trunk rostral to the accessory celiac branch.

The celio-gastric bifurcation of the dorsal vagus was always associated with a large nodular paraganglion. It contained hundreds of glomus cells as well as two–seven neuronal somata and measured 86±6 μm in width and 171±10 μm in length. Moreover, the paraganglion had a relatively constant location on the side of the dorsal trunk: close to the esophageal wall, opposite the celiogastric bifurcation (labelled p in Fig. 2a). This celio-gastric (C-G) paraganglion was traversed by three to six distinct nerve bundles. Analyses of embedded and sectioned C-G paraganglia showed that this structure had an average volume of $113 \pm 21 \times 10^4$ μm$^3$. Higher power electron micrographs from thin sections of the same specimens were used to decisively identify the glomus cells.

### *EM analysis of fiber content*

*Numbers of fibers in the ventral vagus.* Table 1 summarizes the average fiber content for each branch. The ranges of the various totals of unmyelinated fibers per branch, the animal-to-animal variability, and the proportions among branches can be extracted from Fig. 4a in which the individual branch totals are plotted and connected by a line for each animal.

The ventral vagal trunk contained 9634–12480 unmyelinated axons and 31–65 myelinated axons. The percentage of myelinated fibers varied from 0.3–0.6%.

The number of fibers in the hepatic branch averaged approximately one-fourth (26±4%, see Table 1) the number of fibers in the ventral trunk, although the range of proportions varied from 18–43%. In terms of fiber number, the animal with the largest ventral trunk (# 4) had the smallest hepatic branch (see Fig. 4a), and the one with the smallest vagal trunk (# 8) had the largest hepatic branch; however, the negative correlation between the

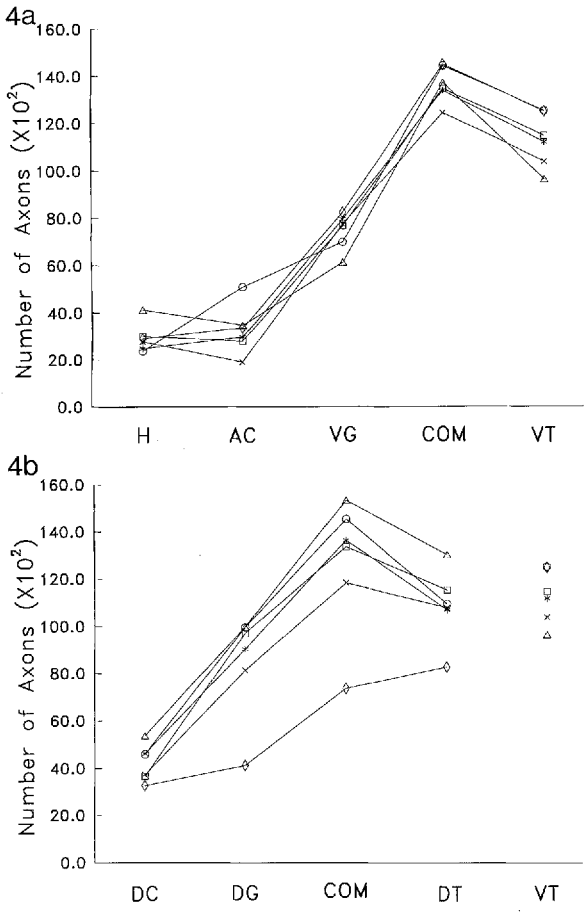


**Fig. 4a, b.** Panel **a**. Proportions of fibers in the ventral branches. Each animal is represented by a different symbol type. Each symbol indicates the number of fibers in an individual animal for a given branch or trunk specimen (*abscissa labels*) or combination of specimens. The symbols are connected by lines in order to compare the quantities of fibers within and among individual animals. *H*, hepatic branch; *AC*, accessory celiac; *VG*, ventral gastric; *COM*, the three branch totals combined; *VT*, ventral trunk. *asterisk*, specimen # 3; *circle*, # 4; *square*, # 6; *triangle*, # 8; *diamond*, # 10; *cross*, # 11. Panel **b**. Numbers of unmyelinated fibers in the dorsal branches. Each symbol indicates the number of fibers in an individual animal for a given branch specimen (*abscissa labels*) or combination of specimens. *DC*, dorsal celiac branch; *DG*, dorsal gastric branch; *COM*, the two branch totals combined; *DT*, dorsal trunk. The corresponding data for the ventral trunk (*VT*) are given for direct comparison. Symbol assignments are the same as in Panel **a**

trunk and branch counts failed to reach significance ($r = -0.70$, $p > 0.12$, $n = 6$).

The number of fibers in the accessory celiac branch (Fig. 4a) also amounted to about one-fourth ($28 \pm 3\%$) of those in the ventral trunk and did not differ statistically from the hepatic branch ($p > 0.05$) in this respect. The accessory celiac branch had the smallest number of myelinated fibers; two of the samples contained no myelinated fibers at all. Its mean percentage of myelinated fibers, 0.1%, was significantly lower ($p < 0.05$) than that of either of the other ventral vagal branches, each of which approximated 0.4%.

The ventral gastric branch contained $66 \pm 3\%$ of the number of fibers in the ventral trunk and a relatively large but variable number of myelinated fibers. The gastric branch of animal # 10 was sampled more distally than the others and included two rami, one of which was an esophageal fascicle (*e* in Fig. 3b). The eight myelinated fibers in this esophageal fascicle represent 24% of all its fibers; this percentage of myelinated fibers is approximately 60 times greater than that found in the branches. The larger bundle (Fig. 3b) is only 0.3% myelinated. The ventral gastric branch specimen shown in Fig. 3b also contains a single myelinated fiber with an exceptionally thick sheath (indicated with a thick arrow). This same kind of axon was observed in five of the six ventral gastric branch specimens and could even be identified in whole mount preparations of the gastric branch.

As Fig. 4a suggests, the combined branches (hepatic, accessory, and gastric) contained $2360 \pm 346$ more unmyelinated axons than the parent ventral trunk ($P < 0.01$). The number of fibers in each branch and in the ventral trunk expressed as a mean percentage of the combined branch number is given in Table 1. In the case of the myelinated fibers, the sum in the individual branches versus the number in the trunk, however, was irregular and not reliably different than the count for the trunks.

*Numbers of fibers in the dorsal vagus.* The counts for the dorsal trunk and its derivative branches as well as the combined totals of dorsal celiac and gastric branches for each of the individual specimens are given in Fig. 4b. The average fiber counts for the dorsal vagus are summarized in Table 1. As Fig. 4b indicates, the dorsal gastric branch of one specimen (# 10) had fiber counts which were more than two standard deviations below the mean. Moreover, this specimen had conspicuously distorted axons in all of its bundles. The data on this specimen's fiber content are given in Fig. 4b, but they are not included in the means of Tables 1 or 2.

The dorsal vagal trunk contained 8277–13027 unmyelinated fibers and 42–95 myelinated fibers. Its percentage of myelinated fibers ranged from 0.4–0.9 and averaged $0.6 \pm 0.06\%$. Table 1 shows that the dorsal trunk contained 83% of the sum of unmyelinated fibers found in the dorsal celiac and gastric branches combined; if the data from animal # 10 are excluded (see above), the combined count is statistically greater than that of the dorsal trunk ($P < 0.05$). In contrast, the dorsal trunk contained 34% more myelinated fibers than the combined dorsal celiac and gastric branches.

The mean number of fibers in the dorsal celiac branch (4201) was greater than that of the hepatic or accessory celiac branches but less than either gastric branch ($Ps < 0.05$). The dorsal celiac branch had extremely few myelinated fibers, less than three per specimen, and its percentage of myelinated fibers was significantly smaller than those of all other subdiaphragmatic branches, exept the (accessory or) ventral celiac.

The mean number of fibers in the dorsal gastric branch (9355) did not differ from that of the ventral gastric branch, but was statistically greater than those of the hepatic and celiac branches ($Ps < 0.05$).

*Unmyelinated axon size distributions of the ventral vagus.* The ventral trunk and its derivative branches all had axons which ranged from 0.1 μm to approximately 1.8 μm in diameter. With the exception of one hepatic branch specimen, all of the size distributions were positively skewed. Furthermore, all of the distributions differed significantly from nor-

**Table 2.** Fiber size distributions of the vagal branches

| Vagal branch | Unmyelinated | | Myelinated | |
|---|---|---|---|---|
| | p-Diameter (μm) | Skewness | Axon (μm) | Total fiber (μm) |
| Ventral trunk | 0.76 ±0.02 | 0.38 ±0.04 | 1.47 ±0.07 | 2.15 ±0.09 |
| Hepatic branch | 0.83[a] ±0.02 | 0.05[a] ±0.02 | 1.37 ±0.07 | 1.96 ±0.08 |
| Accessory celiac | 0.77[a] ±0.02 | 0.38[b] ±0.08 | 1.24 ±0.03 | 1.90 ±0.09 |
| Ventral gastric | 0.77[a] ±0.02 | 0.61[c] ±0.04 | 1.45 ±0.05 | 2.08 ±0.06 |
| Dorsal trunk | 0.72 ±0.01 | 0.48 ±0.05 | 1.53 ±0.05 | 2.31 ±0.10 |
| Dorsal celiac | 0.75[a] ±0.02 | 0.37[b] ±0.08 | 1.68 ±0.52 | 2.38 ±0.65 |
| Dorsal gastric | 0.72[a] ±0.02 | 0.64[c] ±0.03 | 1.40 ±0.08 | 2.12 ±0.08 |
| D. esophageal fascicles | 0.99[b] ±0.06 | – – | 1.56 ±0.11 | 2.18 ±0.08 |
| V. esophageal fascicles | 0.93[b] ±0.03 | – – | 1.50 ±0.11 | 2.07 ±0.15 |

The first, third, and fourth columns contain the means (±SEMs) of the fiber size distributions for unmyelinated (col. 1) and myelinated fibers (cols. 3 and 4). The *p-diameter* statistics are perimeter-derived diameters. The myelinated *axon* data are derived from the cross-sectional areas of myelinated axons without their sheaths. And the *total fiber* statistics are based on area of the myelinated fiber including its sheath. The second column contains the mean skew cofficient for the trunks and branches. The size distribution means for branches in the first and second columns which have different superscript letters are significantly different ($Ps < 0.05$)

mal distributions of the same means and standard deviations (Kolmogorov-Smirnov test, $P's < 0.01$). Table 2 lists the mean diameters ± SEMs for axons of each of the branches. The graphs in Fig. 5b, 5c, and 5d are comparably scaled and include the axon size distributions of the ventral vagal branches.

The ventral trunk size distribution most closely resembled that of the gastric branch, to which it contributed at least 45% of its fibers. Although none of the branches had many unmyelinated axons greater than 1.6 μm in diameter, the parent trunk had virtually none. Of the three primary ventral branches, the hepatic branch appeared as the most distinctive. Its mean axon diameter was considerably larger than the others (Table 2), but the comparison did not reach significance ($P > 0.07$). The mean skew coefficient of the hepatic branch size distribution ($S = 0.05$), however, was significantly smaller ($P < 0.05$; trunk- $S = 0.37$, accessory- $S = 0.38$, gastric- $S = 0.61$). As illustrated in Fig. 5b, almost one-third (29%) of the hepatic branch axons were greater than 1.0 μm in diameter, but only 15% of the accessory branch axons were that large.

The size distribution of the ventral gastric branch fell between those of the accessory celiac and hepatic branches. The mean skew coefficient of the gastric branch was significantly greater ($S = 0.61$, $Ps < 0.05$) than that of the other ventral branches.

*Unmyelinated axon size distributions of the dorsal vagus.* Figure 5a, 5c, and 5d presents the fiber size distributions of the dorsal vagal nerves and for comparisons includes the distributions of the (putatively) homotypic ventral vagal nerves.

The mean diameter of dorsal vagal trunk axons was significantly smaller than that of ventral vagal trunk fibers ($0.72 \pm 0.01$ versus $0.76 \pm 0.02$, $P < 0.05$). The axonal size distributions of the two vagal trunks, however, did not differ in skewness ($P > 0.16$; see Fig. 5a).

The mean diameter of axons in the dorsal celiac branch (Table 2) did not differ from those of the other branches, but its axon size distribution as summarized by the mean skew coefficient ($+0.37 \pm 0.08$, Table 2) differed from those of all the other subdiaphragmatic branches ($P < 0.05$), except the accessory celiac ($+0.38 \pm 0.08$, see Fig. 5d).

The dorsal gastric branch had a similarly small mean axon diameter ($0.72 \pm 0.01$). The axon size distribution of the dorsal gastric branch was highly skewed ($+0.64 \pm 0.03$, Table 2) and differed from all the subdiaphragmatic branches ($P < 0.05$) except the ventral gastric branch ($+0.61 \pm 0.0$, see Fig. 5c).

*Esophageal fascicles*

In addition to the primary subdiaphragmatic branches, four abdominal esophageal fascicles associated with the dorsal vagus, and four from the ventral vagus, were also analyzed. The esophageal fascicles sampled contained 18–48 fibers each, 8–16% of which were myelinated (see Fig. 6a). Myelinated fiber populations of the esophageal fascicles were distinguished by their small sizes and thin shealths (described below). As indicated in Table 2, the unmyelinated

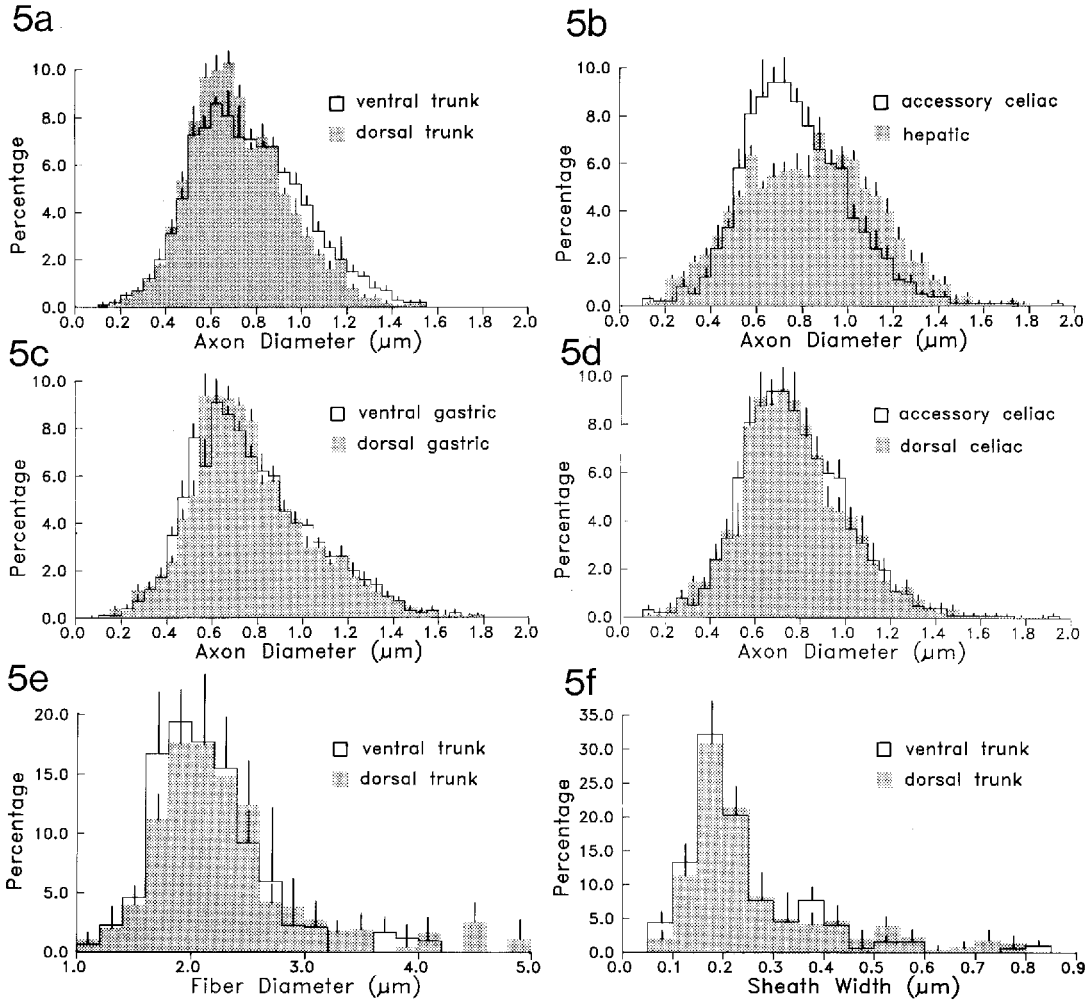


**Fig. 5a–f.** Fiber size spectra of the abdominal vagal trunks and branches. Panel **a**. Mean distribution of ventral and dorsal trunks. Panel **b**. Hepatic and accessory celiac distributions compared. Panels **c** and **d** compare fiber size spectra of putatively homotypic branch pairs. Panel **c**: Ventral and dorsal gastric branches. Panel **d**: Dorsal celiac and accessory celiac distributions. Panels **e** and **f** summarize myelinated fiber size distributions. Panel **e**: Averaged fiber size (sheath+axon) distributions of the vagal trunks. Panel **f**: Averaged distributions of myelin sheath widths for the vagal trunks

fibers in these fascicles were considerably larger ($P<0.05$) than the axons of the dorsal vagal branches; their means were also significantly greater than those of the ventral vagal branches. Skew coefficients were not calculated because the fascicles provided too few sampled axons to produce continuous size distributions.

In addition to these esophageal fascicles, fascicles of entirely unmyelinated fibers coursed with the abdominal vagi. These stained only lightly with osmium and were difficult to identify and trace in whole mounts. One type of unmyelinated fascicle distinguished itself by the large size of its axons with a mean diameter of $1.43\pm0.3$ μm, with several axons greater than 2 μm in diameter. These fascicles of large fibers were always found on the side of the associated branch, attached to its perineurium (Fig. 6b, see also fascicle labelled "X" in Fig. 7 of Prechtl and Powley 1987).

*Myelinated fiber populations*

Forty-five percent of the myelinated fibers of the two trunks had axolemma diameters (i.e. axon without sheath) within the size range of many unmyelinated fibers (i.e. between 0.7 and 1.4 μm, a-diameters). The axolemma size distributions did not show any extreme differences in shape from distributions based on total fiber size (axon+sheath).

Figure 5e shows the myelinated fiber size distributions for the ventral and dorsal trunks. Most (85%) of the myelinated fibers in the trunks were small (i.e. <2.6 μm in diameter) and the majority of myelinated fibers of the branches were similarly distributed. In particular, the gastric branch distributions closely resembled those of the vagal trunks; 91% of their fibers were less than 2.6 μm in diameter, and they had an equally broad range of sizes, including fibers greater than 4.0 μm in diameter. Although 91–92% percent of the myelinated fibers in the hepatic and accessory celiac branches were also small (<2.6 μm), these branches lacked fibers greater than 4.0 μm. Only four myelinated fibers were found in the dorsal celiac specimens; one of them was 4.1 μm in diameter and the rest were small (<2.0 μm). The esophageal fascicles distinguished themselves by containing only the smaller type of myelinated fibers (99% of the fibers <2.8 μm).

The results on myelin sheath measurements appeared to parallel those on fiber size. Eighty-one percent of the

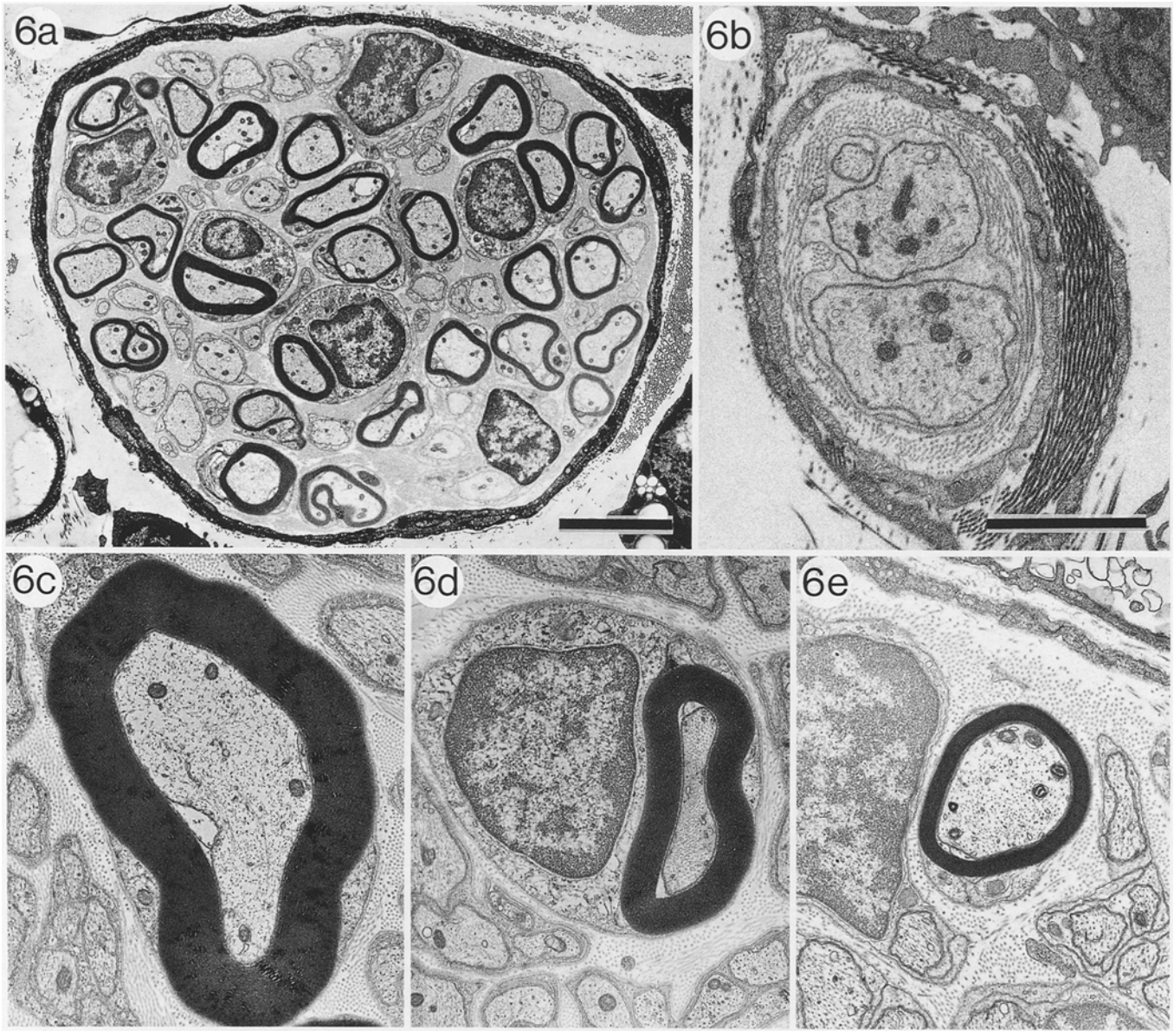


**Fig. 6a–e.** Associated fascicles and fibers of the abdominal vagus. Panel **a**. An esophageal fascicle sampled at a rostral level of the abdominal esophagus, within about 50 μm of a dorsal vagal trunk. scale bar = 4 μm. Panel **b**. Fascicle of large unmyelinated fibers found on the perineurium of a dorsal gastric branch specimen. Note that one of its axons is considerably larger than the myelinated axons in panels **d** and **e** which are at the same magnification. scale bar = 2 μm, same for panels **c, d, e**. Panel **c**. Large thickly myelinated fiber; its sheath width is 0.78 μm. Panel **d**. Fiber with an intermediately thick sheath (0.4 μm). Panel **e**. Thinly myelinated fiber (sheath width = 0.24 μm)

fibers in both trunks had sheath widths less than 0.35 μm, but the range was broad with some as great as 0.85 μm (Fig. 5f).

Most of the myelinated fibers in the primary branches (mean 85 ± 5%) and in the esophageal fascicles (96%, Fig. 6a) also had thin sheaths (i.e. < 0.35 μm, see Figs. 6a and 5f). Only the gastric branches contained the full range of sheath widths found in the parent trunks (Fig. 5f), including sheaths from 0.5–0.85 μm in width (Fig. 6c).

*Intraneural organization*

In each specimen examined, the fibers within the vagus were segregated in zones delineated by the extended processes of endoneurial cells and by conspicuous margins of intraneural space and collagen fibrils. Some of the branch specimens, and four of the five single ventral trunks, had their fiber contents divided by a continuous septum of connective tissue. In the absence of such septa, however, it was not possible to reproducibly define the subfascicles of a given specimen. Nevertheless, we used these subfascicles as the sampling units for the morphometric analyses. Subsequently, a Kruskal-Wallis one-way analysis of variance was used to test whether or not mean axon diameters from the various subfascicles differed more than would be expected by chance. Fifteen of the 18 branch specimens did show significant differences with most type I error probabilities (p) less than 0.001.

## Discussion

### *Whole mounts*

In addition to providing the observations necessary to design the present EM analyses, the whole mount study provided new information on several aspects of vagal organization.

An observation relevant to the design and interpretation of functional studies on the vagal branches is the presence within the vagi of subdiaphragmatic adventitial (i.e. not of tenth cranial nerve origin) fibers. We had previously observed these fibers in one of the abdominal branch points (Prechtl and Powley 1987), but the present experiment indicates that they are found in all the primary branches. After the vagus is sectioned below the nodose ganglion, a procedure which separates all the sensory and motor fibers from their cell bodies, not all of the fibers degenerate. The surviving fibers amount to 5–10% of the unoperated vagus and have been called "adventitial" (Agostoni et al. 1957, *cat*; Evans and Murray 1954, *rabbit*; Kemp 1973, *dog*). These axons have been regarded as sympathetic, and the finding of catecholaminergic fibers in the vagus, which degenerate after truncal vagotomy (Ahlman et al. 1979, *dog*; Murobayashi et al. 1968, *cat* and *dog*), is consistent with this interpretation. In contrast, however, the adventitial fibers described here are not of supradiaphragmatic origin, rather they course within and between the subdiaphragmatic vagal branches.

The complexity of the abdominal vagi is also exemplified by the recurrent fibers that were observed. In several cases, myelinated fibers were traced in and out of plexuses formed by multiple rami of a single primary branch. More impressive, however, were the instances in which myelinated fibers made 180° hairpin turns to return rostrally to the thorax, or to enter an alternative branch. These redirected fibers did not meander (Fig. 2d); they followed orderly paths from one zone or branch to another.

The whole mount analysis indicates that in only about half of the animals are each of the primary branches formed by a single coherent ramus as schematized in Fig. 1. The complexities of the celio-gastric ramification and the convolutions involving the splitting of primary branches into multiple rami have not been fully appreciated in earlier descriptions of the rat, in part because of the limitations of the traditional gross dissection and light microscopic techniques previously employed. The structure of the celio-gastric bifurcation of the dorsal vagus matches that reported by Boekelaar (1985) and is comparable to that observed in larger animals (Kemp 1973, *dog*; Mackay and Andrews 1983, *ferret*; Mitchell 1940, *human*).

Several observations from the whole mounts suggest that when multiple rami did occur at the site of a branch, they were simply different groupings of the same axons which normally constituted the given branch. When multiple rami occurred at a branch site, the individual elements were smaller in size. Accordingly, for example, a hepatic branch specimen which had two ramifications contained the usual number of fibers for that branch (2% below the mean). Additionally, in most cases, the multiple rami presumably representing a single primary branch would commonly anastomose or exchange communicating fascicles as the bundles coursed centrifugally.

**Table 3.** Proportions of fiber types in the vagal branches

| Vagal nerve | Fiber totals | Motor fibers | Adventitial fibers | Sensory fibers |
|---|---|---|---|---|
| Ventral | | | | |
| Ventral trunk | 11327 | 28% | – | 72% |
| Hepatic branch | 2946 | 7% | 20% | 73% |
| Accessory celiac | 2899 | 11% | 20% | 69% |
| Ventral gastric | 7476 | 36% | 16% | 48% |
| Dorsal | | | | |
| Dorsal trunk | 10945 | 26% | – | 74% |
| Dorsal celiac | 4203 | 9% | 28% | 63% |
| Dorsal gastric | 9406 | 26% | 13% | 61% |

The first column contains the averaged total of unmyelinated and myelinated fibers. Motor fiber percentages are calculated with the counts of retrogradely labelled cells (Fox and Powley 1985) and are given in the second column. The third column contains the estimates of subdiaphragmatic adventitial fiber content. The dorsal gastric branch estimate does not include adventitial fibers contributed by caudal celiac bundles. Parity is assumed between the hepatic and accessory celiac branches. In the fourth column the sensory fiber percentages are based on the fiber totals minus the motor and adventitial fiber estimates

### *Electron microscopy*

The EM inventory obtained for the rat subdiaphragmatic vagus suggests several conclusions. To facilitate the consideration of these points, Table 3 lists the combined (unmyelinated and myelinated) mean fiber counts for each of the trunks and branches. The table also includes motor fiber content estimates based on counts of retrogradely labelled efferents (Fox and Powley 1985). The adventitial fiber estimates included are inferred from the greater number of fibers in the branches than in their parent trunks. The number of sensory fibers estimated by the residuals would be a few percent too high if the rat abdominal vagus includes other adventitial fibers of supradiaphragmatic origin and/or efferents originating in the nucleus ambiguus.

*Unmyelinated Fiber Content.* The mean number of axons counted in the ventral trunk (11275) appears greater than that reported in an earlier study on the rat (i.e. 9489 fibers; Gabella and Pease 1973), but the ranges of the two samples overlap considerably, and their means do not differ significantly ($P > 0.08$). The substantial variance in both studies is typical of fiber content analyses of both somatic and visceral nerves. With the differences in fixation and morphometry (perimeter- versus area-derived measurements) taken into consideration, the size distribution of unmyelinated fibers in the present experiment is also similar to the range reported by Gabella and Pease (0.25–1.0 μm, 1973), although a small but considerable number of axons with

diameters less than 0.25 μm was observed in the present study.

Estimates from counts of dorsal motor nucleus cells retrogradely labelled by applying tracer to the ventral trunk (Fox and Powley 1985) indicate that 28% of the fibers in that trunk are preganglionic motor neurons (Table 3). This percentage is about three times the amount which is repeatedly cited in the secondary literature. The possible reasons for this discrepancy are discussed below. A much smaller number of medullary cells are found labelled in and around the nucleus ambiguus, although these are likely the source of some of the myelinated fibers, particularly those in the esophageal fascicles (see Bieger and Hopkins 1987 and Dahlqvist et al. 1986).

The number of fibers found in the hepatic branch in this study (2936 ± 257) compares quite closely with the result from our previous study of six different specimens (2887 ± 287, Prechtl and Powley 1987). Kohno et al. (1987) reported a smaller mean number of fibers (2072 ± 43) in the hepatic branch in an EM study based on ten Wistar rats. The finding that only about 200 cells in the dorsal motor nucleus accumulate retrograde labelling after tracer treatment of the hepatic branch (Fox and Powley 1985; Norgren and Smith 1988) implies that, among the subdiaphragmatic branches, the hepatic branch has the smallest proportion (7%, Table 3) of motor fibers.

The hepatic axon size distribution was the most symmetrical of any of the branch fiber distributions (Fig. 5b), and it had the largest mean axon diameter (0.83 μm). The differences in area-derived mean diameters between the present and our previous study (0.72 versus 0.66 μm; Prechtl and Powley 1987) fall short of statistical significance and could be related to differences in the fixation method (i.e. intravascular versus immersion).

Until reported by Sawchenko and Gold in 1981, the "accessory" or "ventral" celiac branch was unrecognized. The ventral vagus of the rat, like that of other mammals investigated, was considered to have only hepatic and gastric branches and no regularly occurring celiac branch (see Legros and Griffith 1969). A few authors have reported a small branch from the anterior vagus to the celiac ganglion via the left gastric artery in humans (McCrea 1924; Mitchell 1940; Ruckley 1964). But in a recent and more systematic study, Mackay and Andrews (1983) found such an anterior celiac branch in only 6 out of 31 cadavers. These same authors also examined 23 ferrets and found no evidence of a ventral celiac branch. In every rat we examined, either by serial-reconstruction (Prechtl and Powley 1985) or by nerve whole mounts, we found an accessory celiac branch. Cell counts in the dorsal motor nucleus after retrograde labelling indicate that 11% of the fibers in this branch are motor (Table 3).

Over half of the fibers in the ventral vagal trunk course caudally on the esophagus to become the ventral gastric branch. Accordingly, the ventral trunk and gastric branch have similar mean axon diameters, although they differ in skew coefficients. This difference appears because the ventral gastric branch reliably has a slightly greater proportion of its fibers below 0.8 μm, which results in a smaller median. The ventral gastric branch, when treated with retrograde tracer, labels approximately 2700 cells in the dorsal motor nucleus (Fox and Powley 1985) and therefore has an efferent content of at least 36% (Table 3).

In each specimen the sum of the counts of the individual branches of the ventral vagus was about 2400 fibers greater (2360 ± 366) than the number of fibers in the parent trunk (Table 1, Fig. 4a). If this surplus exclusively represents the adventitial bundles, then the same fibers would have been counted twice in the communicating branches and would actually amount to about 1200. If divided equally, the result would be 600 adventitial fibers for hepatic and accessory celiac branches each, i.e. 20% (Table 3) of each branch. This extrapolated amount would fit reasonably well with the coarse estimates of the adventitial bundle dimensions obtained from whole mounts. Collateralization of axons near the branch points could also contribute to the combined branch totals, and this possibility can not be ruled out here.

Another finding partially characterizing the adventitial fiber content was that all the branches had unmyelinated axons in the large size range of 1.6–1.8 mm (p-diameters), but the ventral trunk rostral to its abdominal furcation did not. These particularly large fibers probably form a portion of the adventitial fiber content. Consistent with this interpretation is the observation that 10 days after ventral truncal vagotomy (unpublished pilot experiment, n = 1), some conspicuously large fibers were found among the surviving population in the ventral gastric branch. A systematic sampling was not performed, but some of the fibers measured had diameters greater than 1.7 μm. This apparent size of some of the adventitial fibers and their characteristic lack of myelination would be consistent with the conclusion(s) that they are axons of sympathetic postganglionics, processes from the enteric nervous system, and/or neurites originating from the neurons located in paraganglia.

The dorsal celiac branch had about one-third more fibers than the hepatic or accessory celiac branches, but unlike any of the other subdiaphragmatic vagal components, its unmyelinated fiber size distribution closely matched that of the accessory celiac branch. Moreover, like the accessory celiac, the dorsal celiac branch had very few myelinated fibers.

The unmyelinated fibers of the dorsal gastric branch differed both in number and size distribution from those of all of the subdiaphragmatic branches except the ventral gastric branch. Another feature the dorsal gastric branch shared exclusively with the ventral gastric branch was that it contained one to three exceptionally large axons with sheath widths as great as 0.85 μm. Over 90% of the gastric branch specimens (dorsal and ventral) had such thickly sheathed axons, and none of the other branch specimens did.

The fiber counts indicated that the gastric branches were composed of over half of all abdominal vagal fibers. In the only other fiber count study involving gastric branches, a similar interpretation was reached (Kemp 1973, *dog*). This result would also parallel the finding that over 80% of all of the efferents in the abdominal vagus project through the gastric branches (Fox and Powley 1985). In human dissection studies, however, the posterior gastric branch is consistently described as receiving only one-third of the fibers of the dorsal trunk, with the remainder of the axons passing into the posterior celiac branch (Loeweneck 1969; Mitchell 1940). This finding may represent a genuine species difference in fiber distribution (and may suggest that in man both complements of celiac axons are fused into a common branch), or it may simply signify that in these dissection studies the more compact gastric branch appears smaller

than the broad fan of sheathed fascicles that form the human celiac branch. Moreover, the celiac branch designated in these studies is known to contain sympathetic components (McCrea 1924; McSwiney 1931).

In general, the distributions of sheath widths suggest at least three different groups of myelinated fibers with thin (0.1–0.3 μm), intermediate (0.3–0.5 μm), and thick (>0.5 μm) sheaths. By far, most of the fibers in any of the branches had thin sheaths, and the peak percentages of their size distributions centered around 0.15 or 0.2 μm.

Figure 5e shows that the myelinated fibers range from 1.0–4.0 μm in total fiber diameter (area-derived measure) and most of them are less than 2.8 μm. This distribution matches that reported by Fahrenkamp and Friede (1987) on the rat, and is also very similar to the distribution reported on the abdominal vagus of the pigeon (Schwaber and Cohen (1978). The distributions of myelinated abdominal vagal fibers in the cat (Agostoni et al. 1957; Mei et al. 1980) and the ferret (Asala and Bower 1986) are also similar but shifted a little to the right (larger fibers); all of the fibers are less than 6 μm and most are between 2 and 4 μm.

*Homotypy*

The fiber counts and size distributions for both unmyelinated and myelinated fibers establish that the branching pattern of the vagus does not represent a random assorting of axons. The vagi ramify so as to distribute characteristic axonal subpopulations into their appropriate branches. More specifically, the structural profiles (i.e. unmyelinated and myelinated fiber size distributions, presence of extrinsic fascicles, glomus tissue content, etc.) differentiate the vagal branches into three distinct sets. The ventral and dorsal gastric branches do not differ from each other in terms of structural profiles, whereas they do differ from all the other branches in terms of these patterns. The ventral (accessory) and dorsal celiac branches are also similar to each other on numerous measures, but they are very different from the hepatic and gastric branches, suggesting that the celiacs form a second bilaterally symmetrical or homotypic pair of branches. The hepatic branch has a unique structural profile distinguishing it from either that of the gastrics or that of the celiacs. Moreover, the argument that the primary vagal branches in the rat constitute two symmetrical pairs as well as one unpaired branch is complemented by the evidence of symmetrically and spatially distinct central maps of efferent somata (Fox and Powley 1985) and afferents (Norgren and Smith 1988) in the medulla oblongata. The hypothesis that the dorsal celiac branch is (at least partially) the right homotype of the accessory celiac branch also receives support from the fact that they both project to the same regions of the small and large intestine (Berthoud and Powley, unpublished; Boekelaar 1985; Carlson et al. 1989; Ferenci et al. 1989).

*Afferent efferent fiber ratio*

In earlier LM studies, motor (supranodosal) vagotomies were performed and surviving fibers were counted in order to infer the number of degenerated motor fibers in the trunks. Despite the large variance in these studies, the typical finding, or rather interpretation, was that less than 10% of abdominal vagal fibers had degenerated, and hence that the vagus nerve was almost entirely sensory in function. This estimate has been widely reported (e.g. Agostoni et al. 1957, *cat*; Evans and Murray 1954 rabbit; Kemp 1973, *dog*) and incorporated into numerous texts, influencing assumptions about the neural control of the viscera. The fiber counts of the abdominal vagal trunks in the present EM study (total 22300 fibers), together with the observation that abdominal retrograde tracers label about 6000 neurons in the dorsal motor nucleus (Fox and Powley 1985; see also Lu and Sakai 1984a, 1984b), imply that at least 27% of these fibers are preganglionic efferents. Comprable motor estimates can also be obtained by quantifying the cell loss that occurs in the dorsal motor nucleus of the vagus after subdiaphragmatic vagotomy (Fox and Powley 1984, *rat*; Kitchell et al. 1973, *calf, goat, sheep*; Mitchell and Warwick 1955, *rhesus monkey*).

Assuming that all the surviving fibers in the vagus were sensory, the difference between the total number of fibers and the motor estimate would yield a sensory estimate. In other species, however, 5–10% of the abdominal fibers have a non-vagal supradiaphragmatic source. If such extravagal fibers are also present in the rat vagi, then the actual motor/sensory ratio would be even higher than 27:73. Furthermore, for estimating more accurate motor/sensory ratios for the different branches, it is necessary to consider not only the numbers of efferents, but also the contribution of the abdominal adventitial bundles described in the present report. For example, in the extreme cases (ventral gastric – cf. Table 3) the motor/sensory ratio approaches parity when the adventitial fibers are considered.

Although the original motor/sensory estimates were obtained with light microscopy, a similar inference (<10% motor) has also been obtained in one EM study (Asala and Bower 1986, *ferret*). These authors, however, acknowledge the difficulty of reconciling this result with the cell counts of retrogradely-labelled motor neurons in the same species. We suspect that their motor/sensory ratios based on motor vagotomies are inaccurately low because some of the reactive Schwann cell processes present during degeneration were mistaken for surviving axons. After supranodosal or infranodosal vagotomy in the rat, numerous small rounded profiles are found in the abdominal vagi (unpublished observation; also see McDonald 1983 and Payer 1979). These Schwann cell processes can be only barely distinguished from small axons by their slightly different concentration of intermediate filaments (Tohyama et al. 1983).

*Physiological implications*

The present inventory of vagal fibers has implications for experiments on the digestive, ingestive, and metabolic functions of the nerve. First, this analysis suggests that, contrary to the prevailing view, the vagus contains a sufficient percentage of motor fibers to support some discrete abdominal reflexes. While postganglionic and enteric motor neurons vastly outnumber the vagal preganglionics, one can not assume that vagal mechanisms must, because of a severe constraint imposed by motor fiber number, mediate only diffuse systemic reflexes such as those traditionally assigned to the sympathetic division of the autonomic nervous system. The possibility of discrete visceral functions being accessible to vagal preganglionics would seem all the more plausible if it is assumed that these efferents contact Lang-

ley's "mother cells" or "command neurons" (Wood 1987) within the gut wall.

Furthermore, the different motor:sensory ratios of the branches (with corrections for adventitial fibers) indicate not only that these branches express distinctive and specialized functions, but also that they vary substantially in their functions. The gastric branches, with their (approximately) 1:2 ratios, would be expected to mediate proportionately more and/or more punctate efferent functions than the hepatic branch with its 1:10 ratio.

The nonrandom distributions of fiber sizes in the vagal subfascicles and adventitial bundles and the sorting of specific fiber sizes into the different branches both suggest the relevance of attempting to correlate these structural groupings with specific immunohistochemical patterns and with physiological specializations.

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