

## Structural and ultrastructural study of the Meissner plexus in amphibians, *Rana temporaria*

B.E. Fernández Alvarez

Department of Morphology and Cell Biology, Faculty of Medicine, University of Oviedo, Asturias, Spain

**Summary.** A study of the submucous, periglandular and intravillous plexus of the frog has been carried out with light microscopy and conventional electron microscopy.

The existence of a true Meissner plexus, with light microscopy, as well as the existence of sensitive-like structures, with light and electron microscopy have been demonstrated in the frog.

**Key words:** Nerve fibres, Neurons, Morphometry

### Introduction

The submucous plexus is very problematic in amphibians, so Gunn (1951) did not locate it in the intestinal wall, as this lacks neuronal elements, whereas Wong et al. (1971a,b) describe a submucous and a subepithelial plexus in the toad. The confusion is even greater with regard to possible neuronal elements of this plexus in amphibians.

Gunn (1951), using various methods of impregnation, denies the existence of a true Meissner plexus in the frog, finding only an irregular network of nerve bundles consisting of amyelinic fibres lacking neurons.

Wong et al. (1971b), through a histochemical study of AChE activity in *Bufo melanostictus*, establish a submucous plexus and a subepithelial plexus in the esophagus, stomach and intestine.

The submucous plexus has a different structure depending on the regions. Thus, in the duodenum and rectum it is made up exclusively of AChE-positive nerve bundles, while in the stomach and esophagus there are also AChE-positive cells which may be enteric neurons or Schwann cells.

The subepithelial plexus in the esophagus, duodenum and rectum is AChE positive and there are fibres running along the lamina itself which reach the epithelium. In the

stomach, as an isolated case, we found the presence of one AChE-positive cell.

This has prompted us to reexamine this material. We have studied the disposition of the intramural plexus well by light and electron-microscopy and searched for the exact location and morphology of the neurons in these plexuses.

### Materials and methods

This study is based on adult frogs, *Rana temporaria*, of 20 to 30 grammes in weight, collected in the summer, but killed in autumn after anaesthetizing with chloroform. The intestinal tube was extirpated and fixed for light microscopy studies, following the zinc osmium-iodide methodology (Champy-Maillet, 1959), to note nerve fibres and endings; that is, impregnated in block and fixed, followed by paraffin embedding and the obtention of the sections; elimination of paraffin, dehydration and mounting for their later observation. The electron-microscopically studied material was fixed in glutaraldehyde buffered with sodium cacodylate (pH 7.3), perfused through the artery and postfixed in osmium tetroxide.

The morphometric analysis was performed with the image processor Kontron-Videoplan, followed by basic statistic study.

### Results

In describing the vegetative nervous system we have followed the order explained by Cajal in his work: *Histologie du Systeme Nerveux de l'homme et des vertébrés* (1909-1911). In our work this order is observed in Fig. 1. We have limited the present study to the submucous, periglandular and intravillous plexus.

#### *Submucous plexus*

Fibres emerged from the deep muscular plexus which, anastomosing, formed the submucous plexus

(Fig. 2), made up, therefore, of cells and fibres.

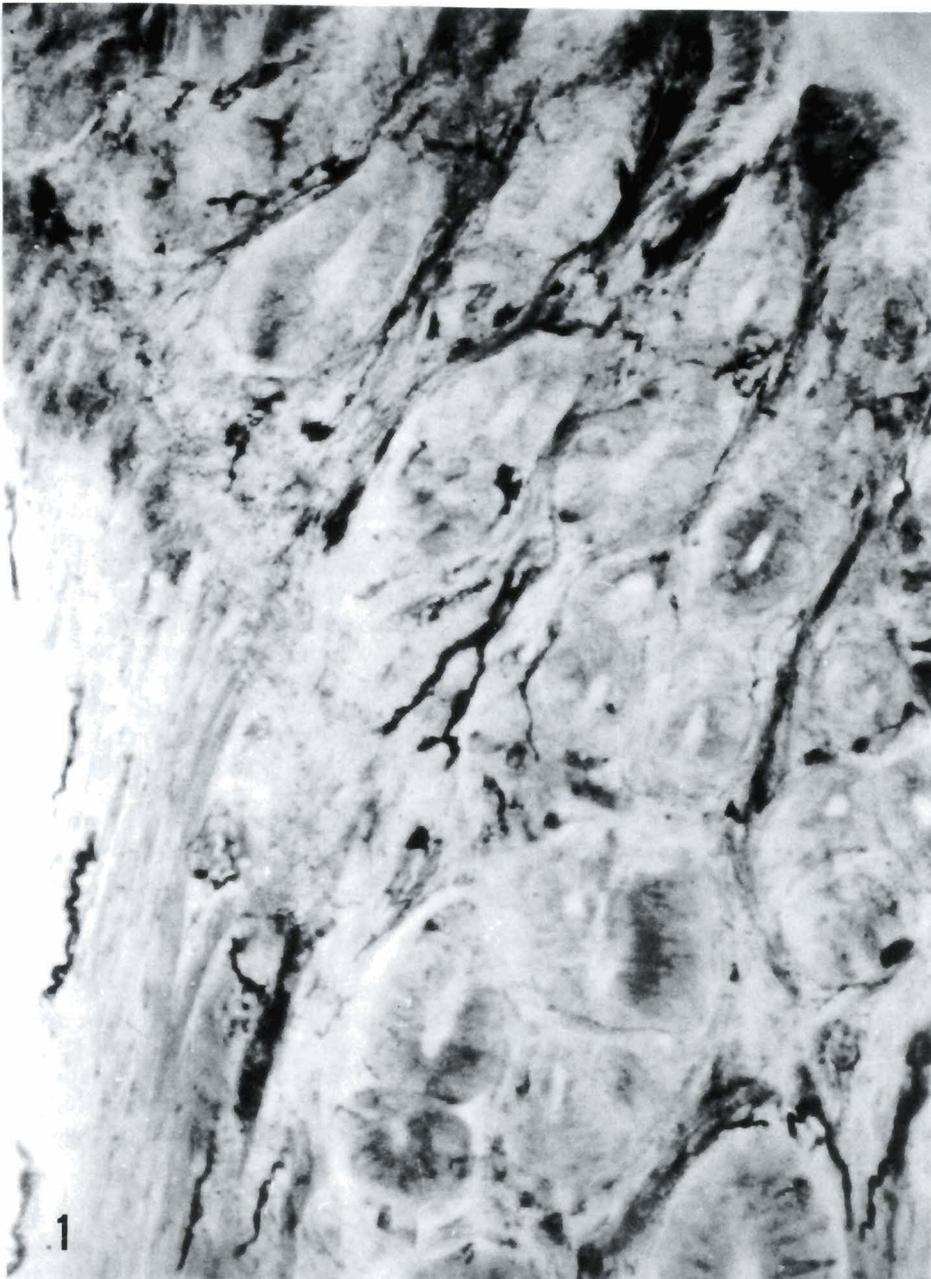
The neurons of this plexus could appear in isolation or as microganglia (Figs. 3-5). Some of the isolated neurons had oval morphology, lacked visible prolongations and showed synapsis images of fibres whose trophic centres are unknown (Fig. 3). They were also seen intercalated in the primary bundles of the plexus (Fig. 4).

The microganglia observed were made up of a small number of neurons (Fig. 5), with their attached somas and with perisomatic synaptic buttons. It was impossible to determine whether we were dealing with extra-

ganglionic fibres or neuron prolongations of the ganglion itself. They were located at the intersections of nerve bundles.

The size of these cells was between 3.01 and 7.85  $\mu\text{m}$  and the mean value was  $4.78 \pm 1.51 \mu\text{m}$ .

The nerve bundles which made up the submucous plexus had different diameters as did their fibres (Fig. 2). These fibres were rippled and had a varicose morphology (Fig. 2). Very thin fibres with thickening were occasionally found, which formed a spiral image (Fig. 6). Certain nerve fibres ended in single thick shape or in plexus shape (Fig. 7).



**Fig. 1.** Transversal section showing the order of the plexuses. Champy-Maillet's osmium-iodide technique. x 125

## *Meissner plexus in the frog*

### *Periglandular plexus*

The general order of this plexus could be seen clearly (Fig. 8). Neuronal elements were found.

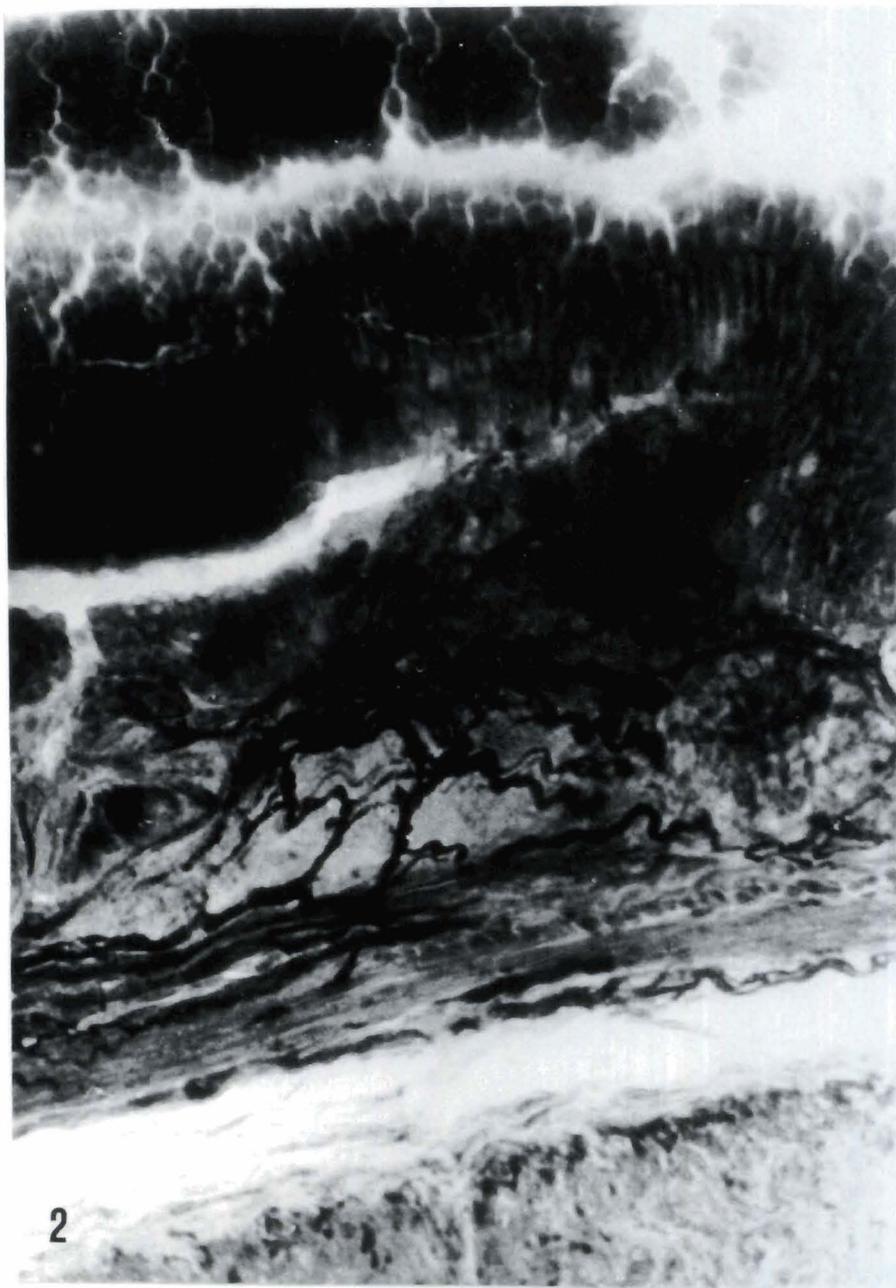
The nerve cells had polygonal or fusiform morphology and sizes between 3.15 and 5.74  $\mu\text{m}$ ; mean value  $3.97 \pm 0.94 \mu\text{m}$ . Sometimes they were star-shaped, with prolongations emerging from the perikaryon. They were of variable thickness and incorporated into the nerve bundles surrounding the acini. Thin varicose fibres could be observed which might contact the neuronal soma (Fig. 9). Sometimes they were inserted into the

thick bundles of the plexus and their expansions were inside the plexus (Fig. 10).

The fibres making up this plexus were grouped in small bundles of variable thicknesses (Figs. 8, 11). In some images fibres could be observed very close to the walls (Fig. 11). At the last, we would show the existence of broader nerve endings which, from their morphology, may be interceptors (Fig. 12).

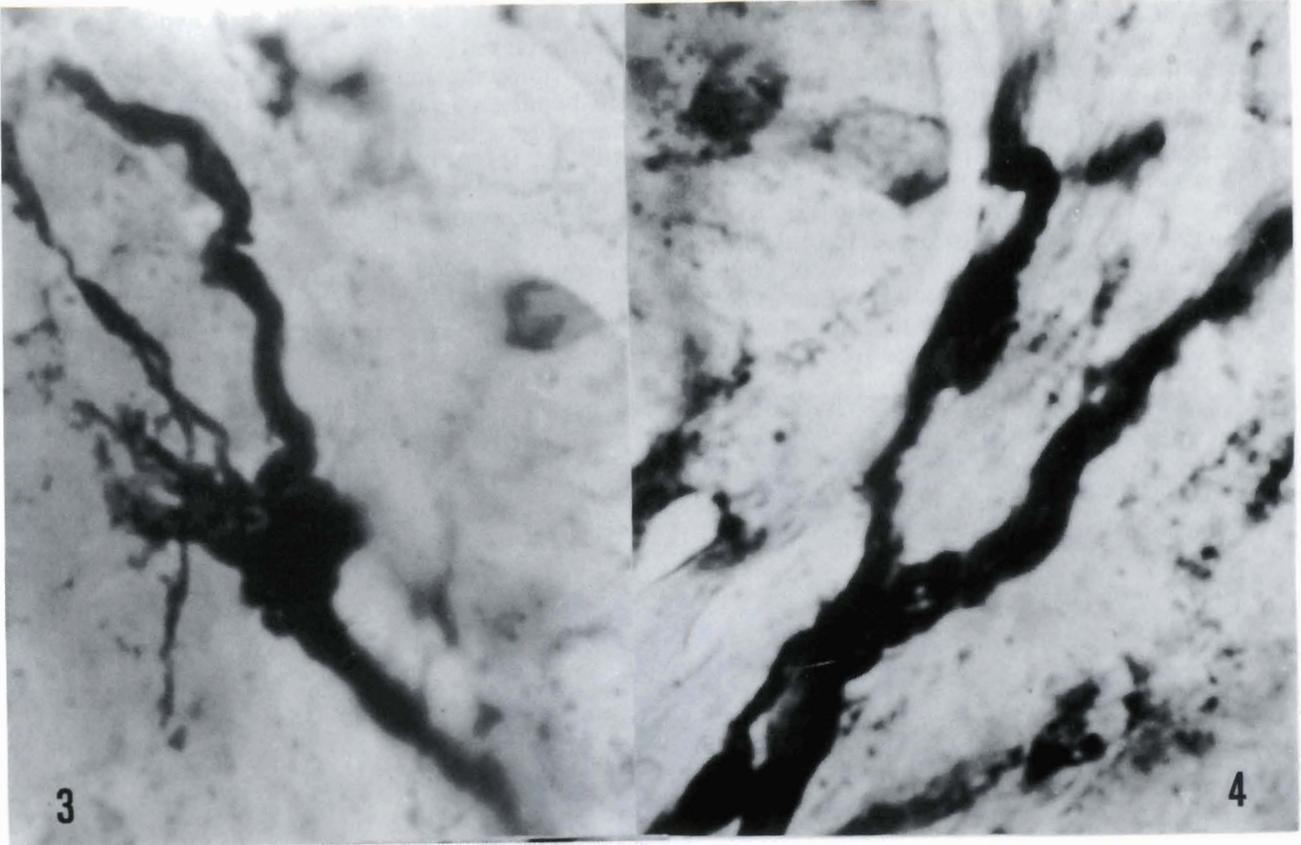
### *Intravillous plexus*

This plexus was a continuation of the periglandular



**Fig. 2.** The submucous plexus. Champy-Maillet's osmium iodide. x 5,000

*Meissner plexus in the frog*



**Figs 3, 4.** Submucous plexus. Isolated neurons. x 5,000

**Fig. 5.** Submucous plexus. Microganglia. x 5,000



### Meissner plexus in the frog

plexus, and the nerve fibres of which it was composed would appear to proceed from the plexus previously described (Fig. 8).

We have found cells like neurons in the interstices of the villusities (Fig. 13). They had a star morphology and their process sit up to the plexus. Their size was between 2.02  $\mu\text{m}$  and 2.80  $\mu\text{m}$  (mean value  $2.27 \pm 0.31 \mu\text{m}$ ).

The nerve fibres next to the vessel-conjunctive axis of the villosity, as in the plexus described previously, had variable thicknesses (Figs. 14, 15). Sometimes, at the basal pole of the villosity, a thin network of varicose fibres was observed and fibres came out which would seem to touch the villosity cells (Fig. 15).

#### Electron microscopy

We shall describe the submucous, periglandular, intravillous and deep muscular plexuses as the continuity of principal plexus or myenteric plexus. Some of these fibres reached the submucosa layer, and were accompanied by their glial covering until their termination. Sometimes, these fibres ran very close to the muscular fibres, which made them homologous with

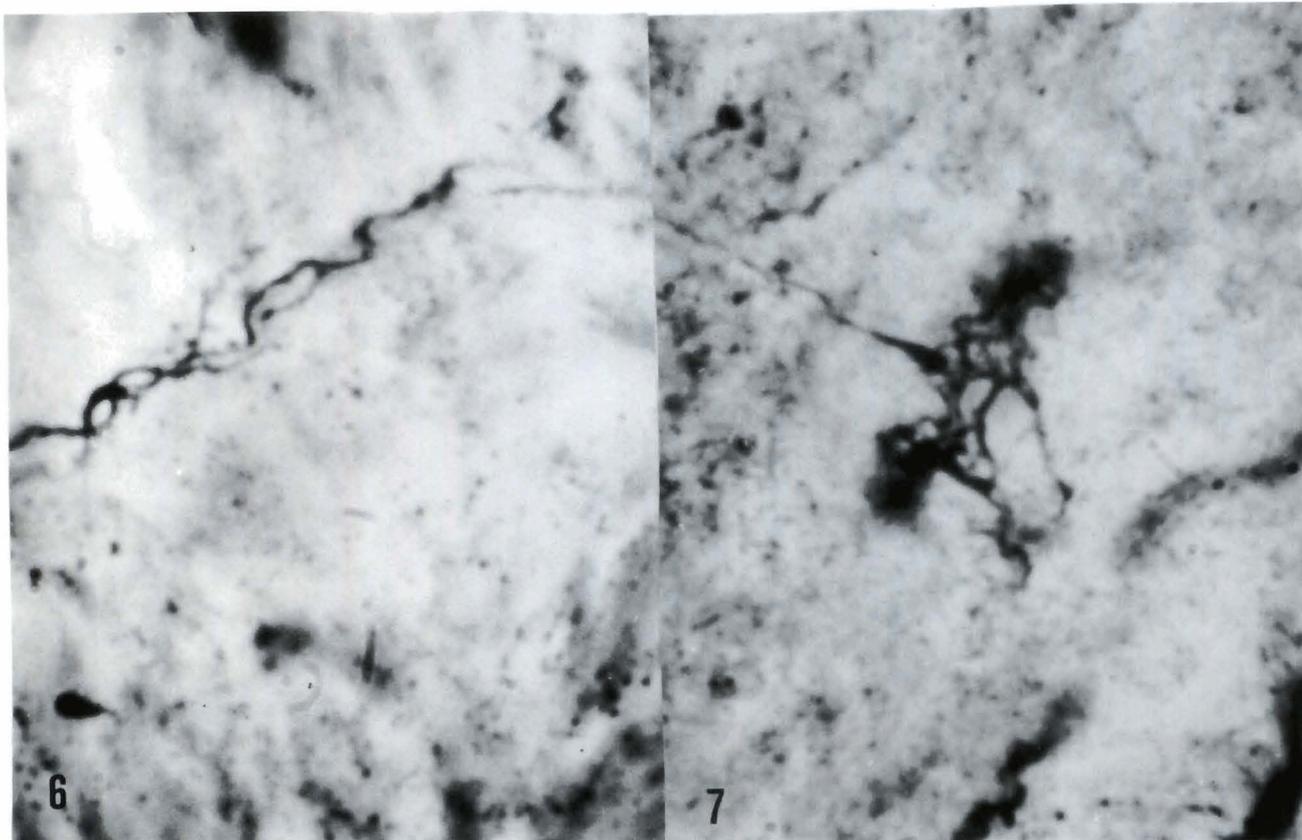
the deep muscular plexus, while in other images they were immersed in the connective tissue, making them a true submucous plexus (Figs. 16-19). The fibres were grouped in bundles containing a variable number of elements. They were surrounded by a glial covering, but on occasions it could be seen that one of these nerve fibres making up the bundle appeared to be directly related to the collagen fibres, with no intervening glial sheath.

The fibres have been grouped into three classes:

- Fibres containing neurotubules and mitochondria.
- Fibres with a control of agranular vesicles.
- Fibres with a control of granular and opaque vesicles.

**Table 1.** Morphometric results of the vesicles.

	MEAN $\pm$ S.D.	MINIMUM	MAXIMUM
Agranular vesicle	568.83 $\pm$ 59.29	427.26	726.11
Granular vesicles and opaque vesicles	1109.79 $\pm$ 229.43	629.96	1730.47



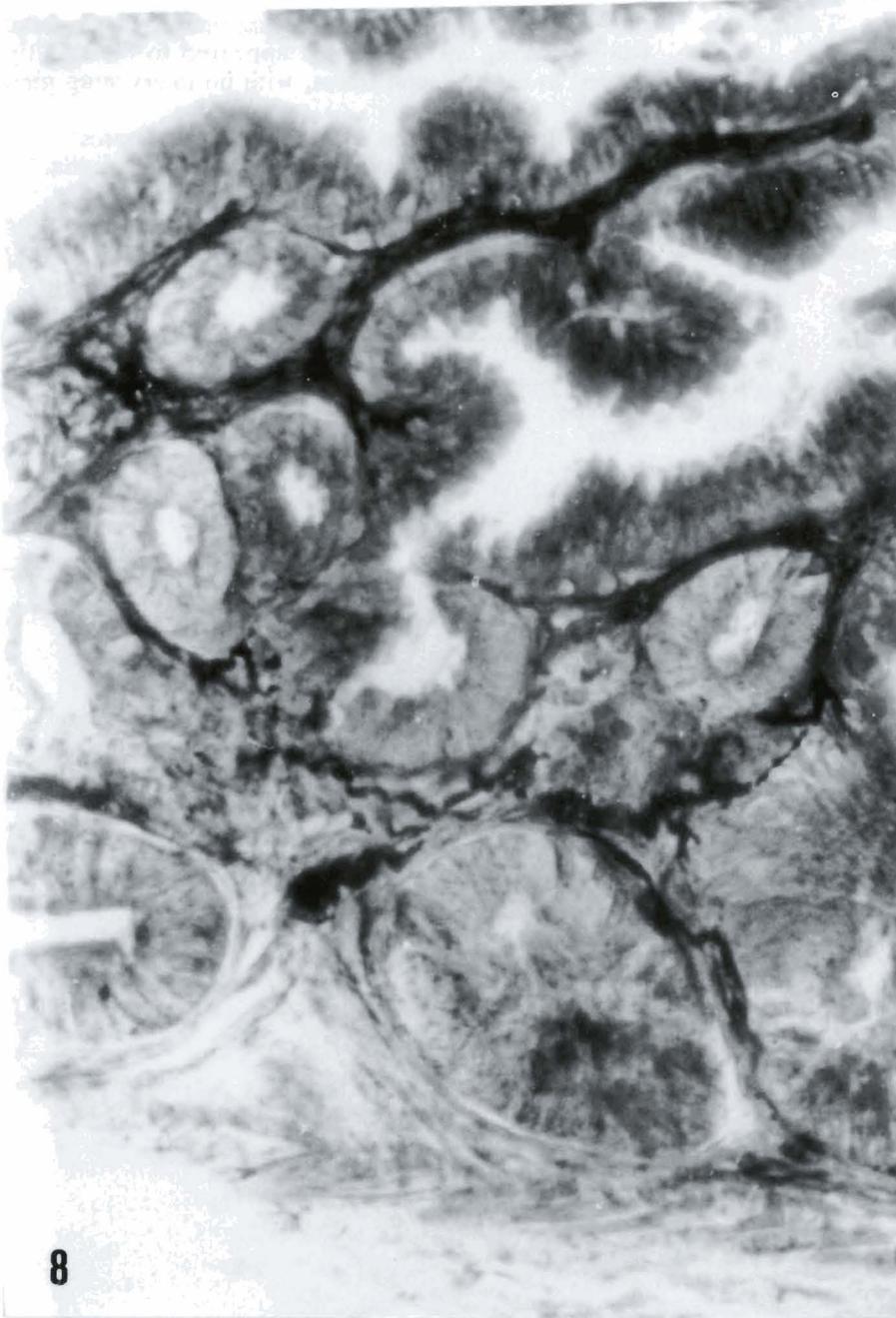
**Fig. 6.** Submucous plexus. The fibres are forming a spiral image. x 5,000

**Fig. 7.** Submucous plexus. Nervous endings. x 5,000

*Meissner plexus in the frog*

In the axoplasm organelles, such as mitochondrias, neurotubules or smooth endoplasmic reticulum could be seen. The vesicle morphometry is seen in Table 1.

A special morphology is observed in the figure 19, which show a nerve fibre containing principally mitochondria, some agranular vesicles and neurotubules.



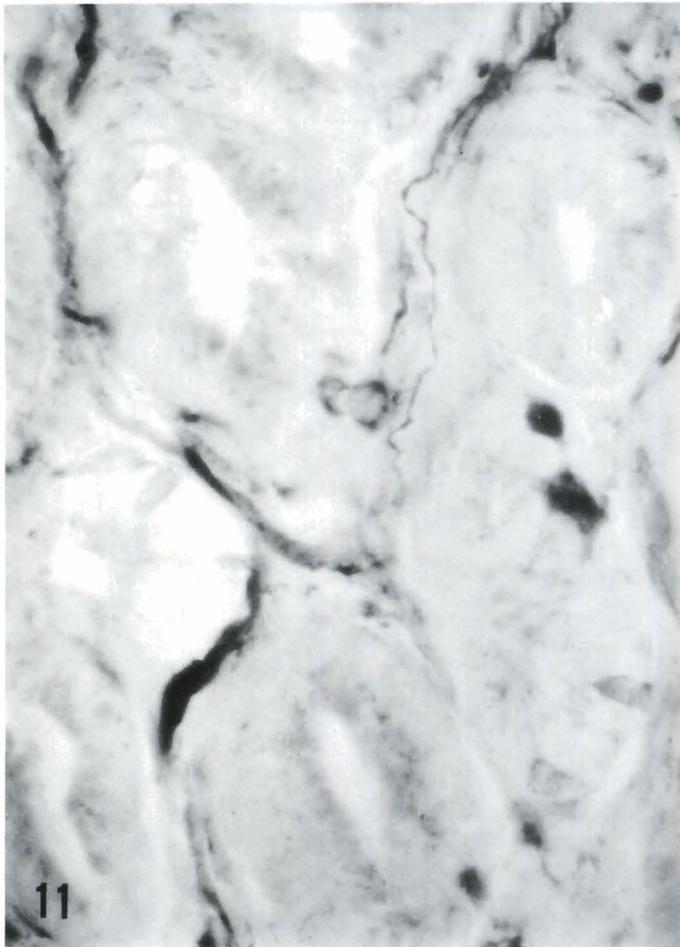
**Fig. 8.** Periglandular plexus. x 2,000

**Fig. 9.** Periglandular plexus. x 5,000

**Fig. 10.** Periglandular plexus. x 5,000

**Fig. 11.** Periglandular plexus. x 2,000

**Fig. 12.** Periglandular plexus. x 2,000



*Meissner plexus in the frog*

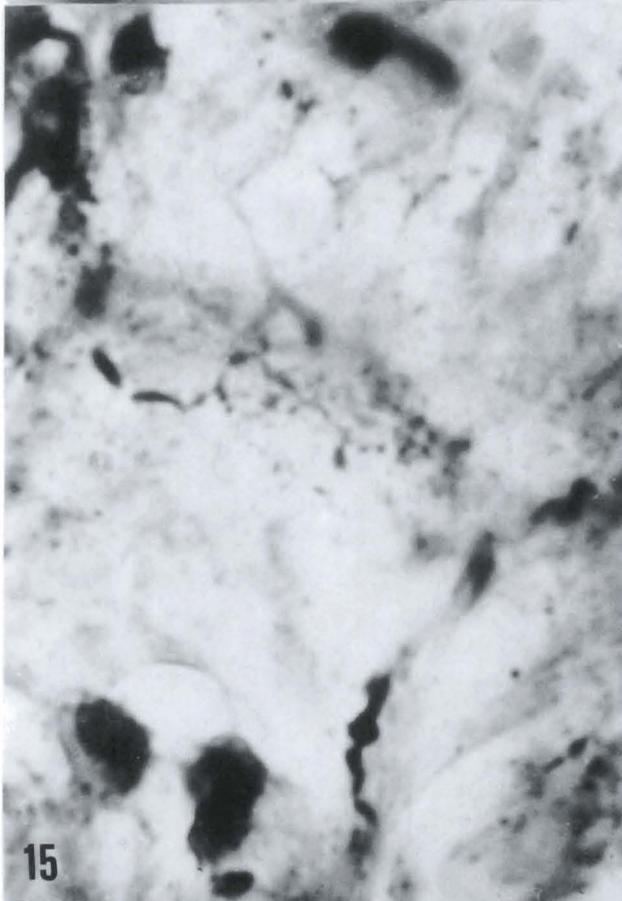
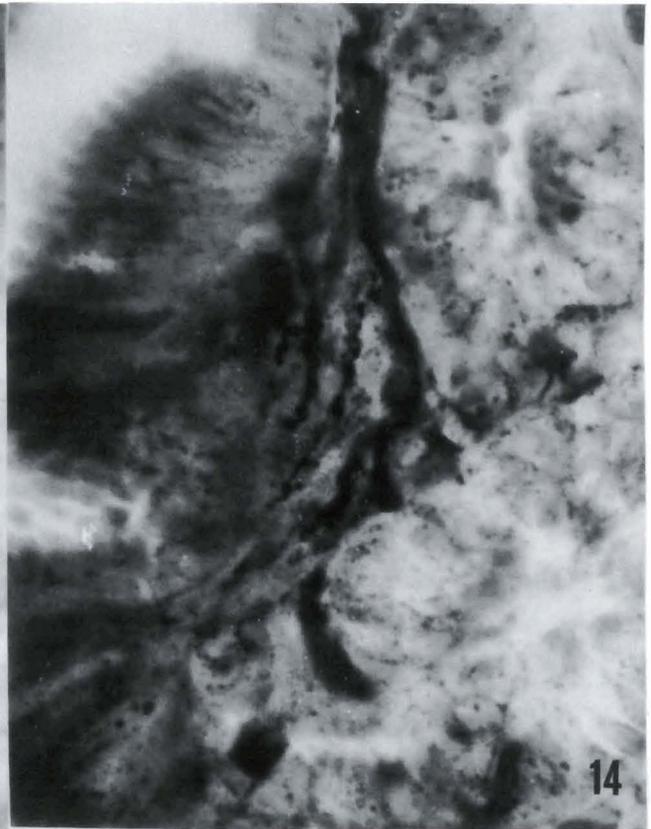
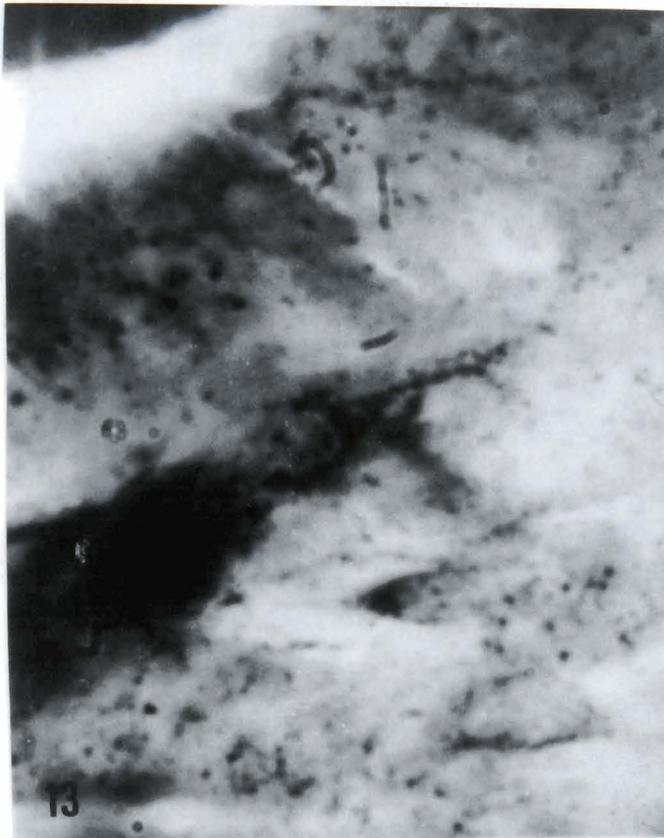
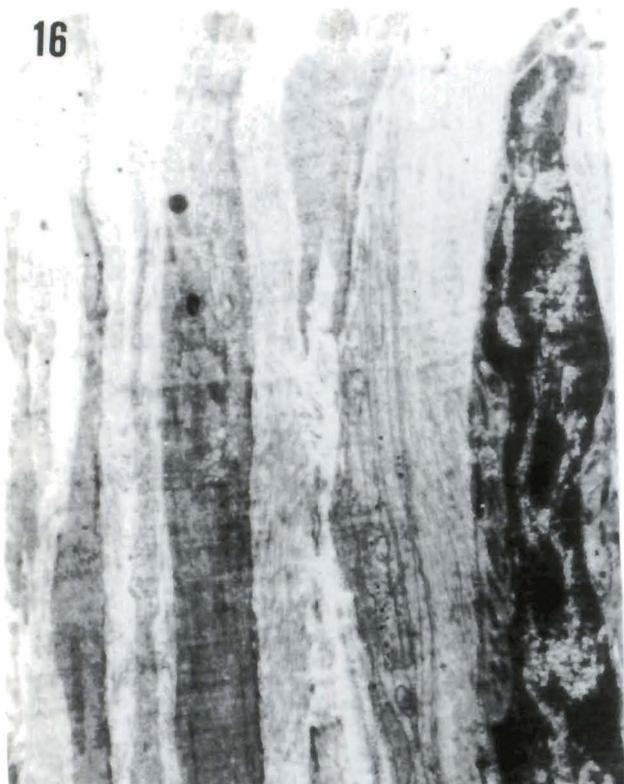


Fig. 13. Intravillous plexus. x 5,000

Fig. 14. Intravillous plexus. x 2,000

Fig. 15. Intravillous plexus. x 5,000

16



It finishes in a nerve bundle in which its accompanying nerve fibres could be observed surrounded by the glial cell. One of these fibres seemed to contact it in a very specific spicule presented by this fibre. This image could represent even a sensitive ending.

#### Discussion

According to our observations and taking as reference another studies (Ramón y Cajal, 1889, 1909; Kuntz, 1922; Bishop et al., 1938; Nicol, 1952; Pick, 1957; Botar, 1966), we have found, in preparations stained employing the osmium-iodide technique, a true Meissner plexus in the frog. In contrast to the opinion maintained by Gunn (1951), the presence of ganglion formations and isolated neurons in close relation with nerve fibres supports our affirmation that, coming from Auerbach's plexus, fibrillar elements branch out into the submucosa, reaching the basal region of the epithelium. For Wong et al. (1971a,b), the submucosa plexus has a different structure according to the region. Thus in the duodenum and rectum it is composed exclusively of AChE-positive nerve bundles, while in the stomach and esophagus there



Fig. 16. TEM. x 10,000

*Meissner plexus in the frog*



Fig. 17. TEM. x 33,500

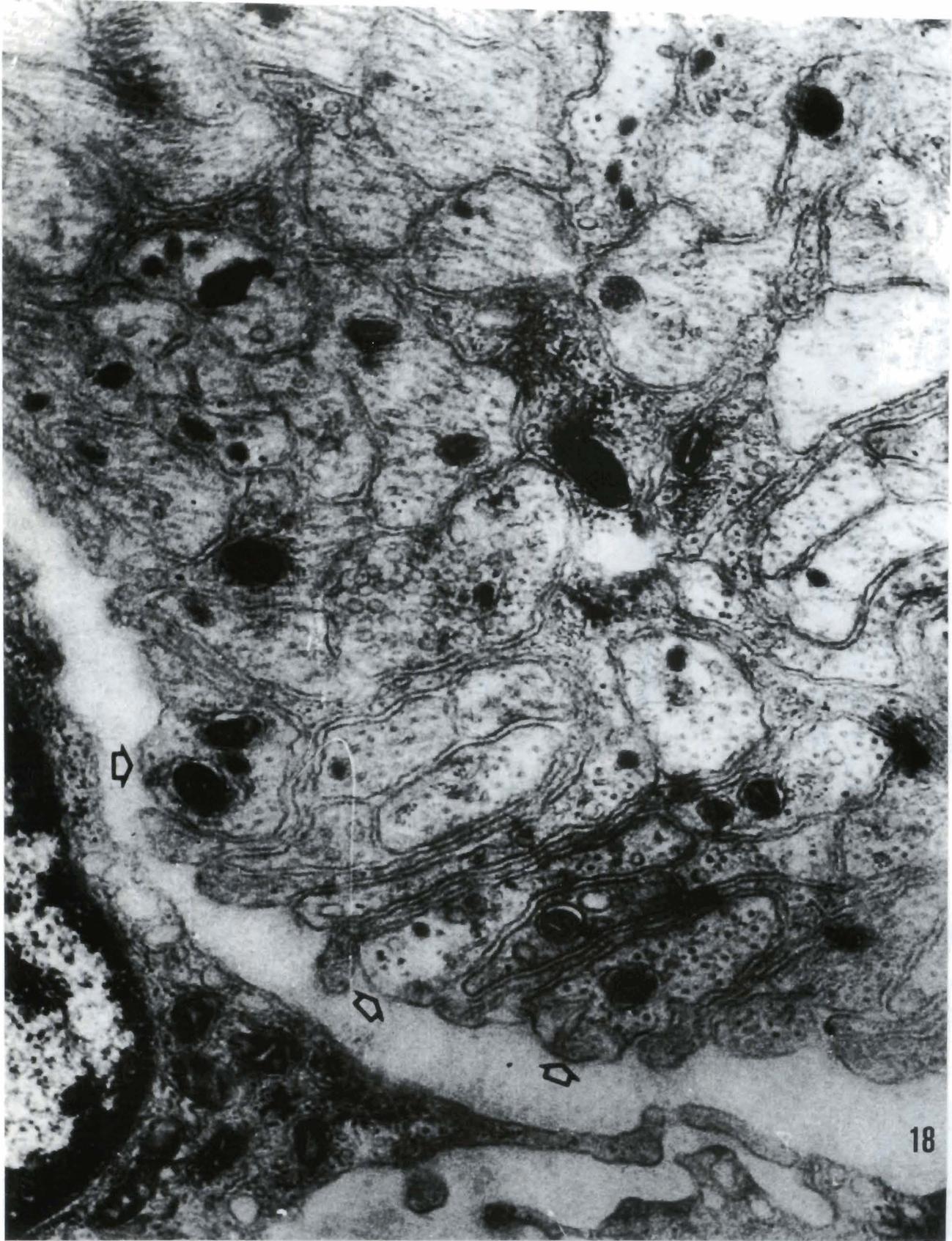


Fig. 18. TEM. x 30,000

*Meissner plexus in the frog*

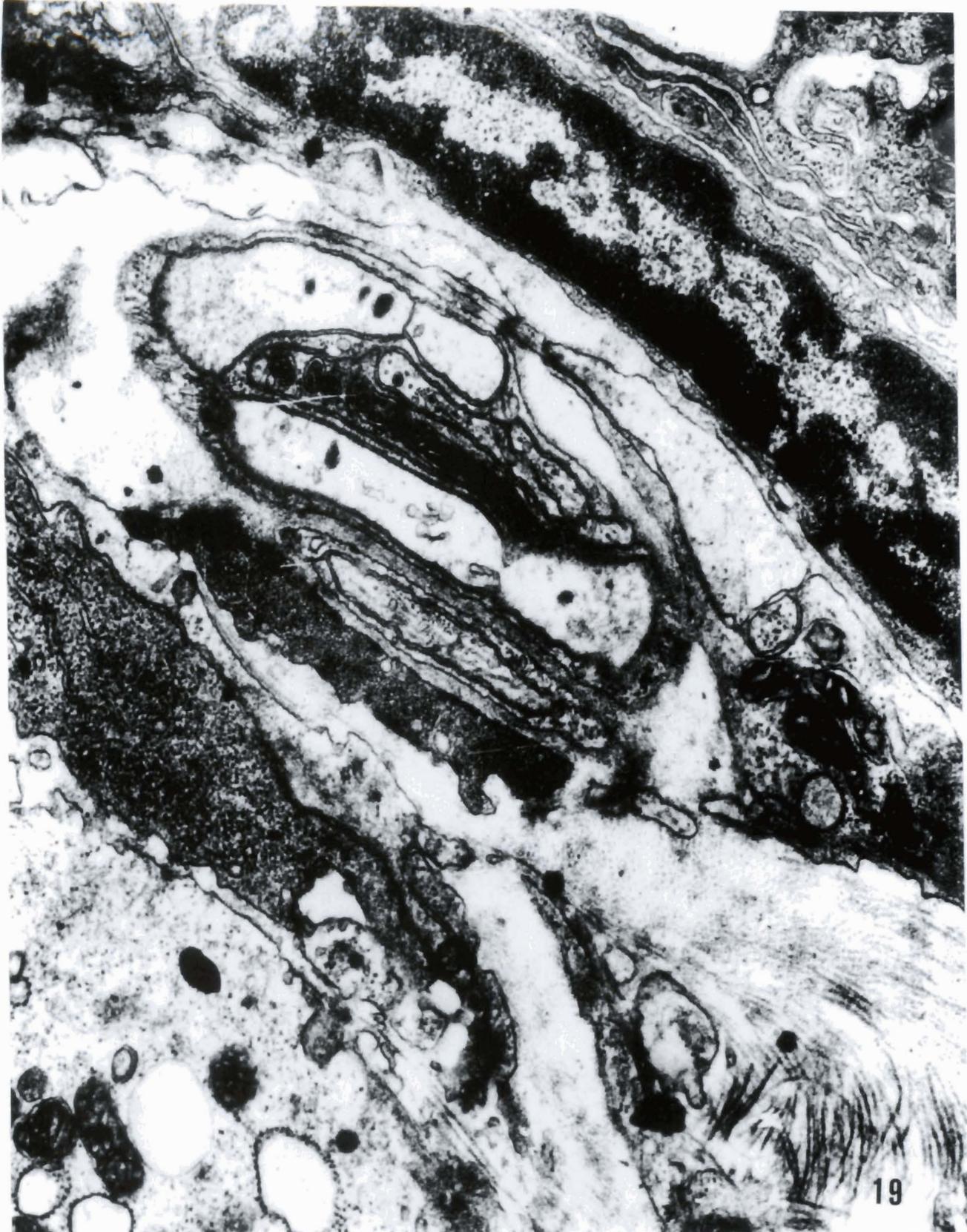


Fig. 19. TEM. x 33,500

### Meissner plexus in the frog

are also AChE-positive cells accompanying these bundles. For the present authors, these cells may be neurons or Schwann cells. In the subepithelial plexus they describe in the esophagus, duodenum and rectum, they find AChE-positive fibres, some of which reach the epithelium. In the stomach, we have the isolated case of an AChE-positive cell (*Bufo melanostictus*). Another cholinergic studies was accomplished in the frog tongue by Kurnova et al. (1986).

Our ultrastructural observations have not revealed the presence of neuronal cells in the pathways examined, although cells corresponding to neuroglia have been visualized (Castelli, 1963; Astudillo et al., 1976). In this respect, our observations coincide with those of other authors (Yamamoto, 1963; Boyd et al., 1964; Botar, 1966; Pick, 1970; Burnstock, 1972, 1975; Winckler, 1973; Taxi, 1975; Gabella, 1976) in the sympathetic ganglia of the frog.

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