Electron microscopy of gastric mucosal innervation in rats

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Summary. Electron microscopy of the rat stomach has shown vagal innervation of gastric epithelial cells with contact points. Unmyelinated axons of diameter 0.06 and 0.20 µm were demonstrated passing in the connective tissue between epithelial cells.

There is disagreement regarding the innervation of mammalian gastric mucosa as studied by conventional light microscopy[1-3] using a methylene blue stain. The purpose of this study was to identify the innervation of the epithelial cells in the rat gastric mucosa by electron microscopy. This supplements our earlier findings of nerve branches from the vagus passing into the gastric epithelial layer using the methylene blue stain[4].

Materials and methods. 10 normal male Wistar rats were starved overnight, anaesthesized with ether and identical areas of the stomach wall was dissected from the fundus. Minimum of 7 pieces of gastric fundus was processed for electron microscope. The specimens were fixed in 2% Glutaraldehyde (M/15 Sorensen's phosphate buffer pH 7.35–7.5); and stained with 2% osmium tetroxide and lead citrate. The sections were examined using a Philips 209 electron microscope.

Results. Electron microscopy showed that in all of these animals the nerve fibres in the mucosa were unmyelinated. The axons were located in the connective tissues between

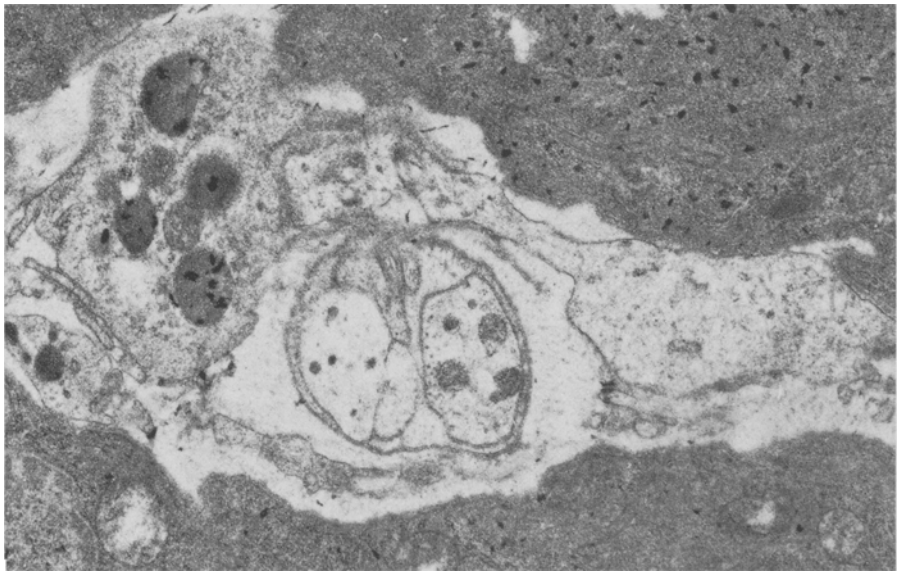

Fig. 1. A small nerve bundle running in the connective tissue between gastric epithelial cells. 3 axons are contained in the cytoplasm of 1 Schwann cell. × 16,800.

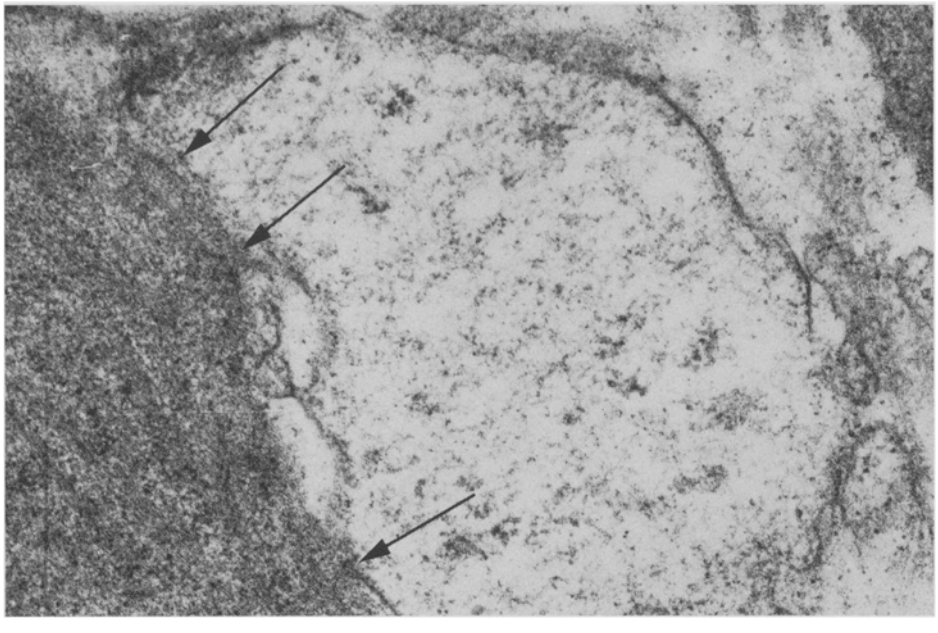

Fig. 2. Zones of contact (arrowed) between axon and an epithelial cell. × 60,000.

epithelial cells (figure 1). In 3 animals, contacts were found between axons and parietal cells (figure 2).

The dimensions of the cross sections of the axons were measured on the electron micrographs of 3 animals. From these, 20 axons were measured. There was a range of axon diameter between 0.06 and 0.20 µm. These were the smallest and largest diameters found. The nerves at the points of contact were of roughly the same diameter as axons in the nerve trunk and no evidence of expansion or narrowing was observed. The illustrated axon at a contact point was 0.11 µm in diameter (figure 2). At a point of contact, there was a very narrow gap between the axonal membrane and the epithelial cell membrane (figure 2). Neither membrane showed any specialized feature. No synaptic vesicles were observed. The width of the gap varied between 6 and 14 Å with a mean width of 11 Å. In the illustrated example there are, however, short areas in which no gap can be found and the cell membranes may be fused.

Discussion. In the methylene blue preparations, nerves were identified clearly in the muscle coat in all 10 animals, in the muscularis mucosae in 7 and in the mucosa in 3 animals. A number of these fibres could be followed to the parietal cells[4]. However, it was not possible to determine the exact termination points using these methylene blue preparations.

These electron microscopical studies have shown that the parietal cells and nerve fibres have points of contact, but in these areas there were no specialized functional structures, no synaptic vesicles and no mitochondria. However, points of contact between axons and gastric epithelial cells have not been as clearly shown before it, in fact, similar observations have been made before. In the innervation of vascular smooth muscle, physiologists occasionally discussed 'en passant' synapses, or points of contact between fine axons and the muscle cell membrane. The functional significance of these has not been elucidated, and as with our findings, it cannot be stated that they were, in fact, synapses of functional importance. The intention in illustrating these was to record their presence so that the matter can be pursued in future experiments. These findings will now allow us to study the changes which occur after vagotomy regarding vagal nerve degeneration and possible regeneration, for if this occurs clinically, it may be an important cause of recurrent peptic ulceration after vagotomy.

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The effect of dietary amino acids on the growth of tumors[1]

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Summary. In C3H mice, a direct dose response relationship between tumor growth and dietary amino acid is seen for fibrosarcoma and mammary carcinoma, extending over a range the lower limit of which is defined by the minimum amino acid requirements, and the upper limit by the amino acid level found in most stock diets.

Inhibition of the incidence and growth of malignant tumors has been reported in animals fed diets deficient in protein or in essential amino acids[3-16]. In these studies, most test diets produced impairment of body growth, although a critical level of moderate phenylalanine restriction was found which slightly inhibited tumor growth without affecting host weight[11]. In some studies, the casein content of the 'control' diet varied from 12 g/100 g diet[11] to 18%[6], 20%[15] and 28%[12]. In other studies, the 'control' diet was the standard mouse or rat chow usually containing protein in excess of 20%[3-5,7,8,10,13-16]. Thus the above-mentioned reports deal only with the effect of dietary amino acid restriction on tumor growth, comparing an amino acid deficient diet with a 'control' diet containing an arbitrary amount of amino acid, usually in excess of minimum requirements. This report describes the influence of dietary

Effect of 4 weeks dietary treatment in 6-week-old C3H/CrlBR male mice

Dietary treatment	Body weight (g)		Food intake (g food/ mouse/24 h)	Relative spleen weight[c]	Total serum protein (g/100 ml)	Serum albumin (g/100 ml)	Blood leucocytes (cells/mm^3)	Neutrophils (cells/mm^3)	Lympho-cytes (cells/mm^3)
	Initial weight[a]	Final weight (%)[b]							
Purina	22.2±0.4	122.9±2.6	3.53±0.16	52.0±2.1	5.35±0.14	3.74±0.1	5908±833	1980±186	3750±182
Diet 1	21.2±0.3	120.8±2.0	3.24±0.15	45.6±5.0	5.39±0.22	3.92±0.12	4895±809	1482±170	3240±175
Diet 2	21.3±0.5	112.1±2.7[d]	3.20±0.19	39.5±1.5[e]	4.77±0.21	3.63±0.18	4134±673[d]	1177±106[e]	2851±118[e]
Diet 3	22.0±0.2	123.0±3.0	3.30±0.17	49.3±4	5.43±0.23	3.80±0.14	5230±780	1700±112	3400±178

[a]) Mean of 10 mice ± SEM. [b]) Percentage of initial weight. Superscripts indicate statistically significant difference from the mean for the mice fed Purina lab chow: [d]) $p < 0.02$; [e]) $p < 0.01$ (Student's t-test).

[c]) $\frac{\text{Spleen weight}}{\text{Body weight}} \times 10^{-4}$.