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Electron microscopic and autoradiographic analysis of the distribution of the vagus nerve in the ferret stomach

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التحليل المجهري الدقيق والإحصائي لتوزيع العصب الحائر في جدار معدة إبن مقرظ بإستخدام التصوير الإشعاعي الذاتي

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في هذه الدراسة تم حقن اللوسين المشع في النواة الحركية للعصب الحائر بعد القطع الحاد والمزمن لأحد العصبين (العصب الحائر الأمامي أو الخلفي).

إن الطريقة التي استخدمت لأخذ عينات من المعدة وتطبيق إختبار (χ^2) في تحليل الصور المجهرية الإلكترونية المشعة والتي تظهر الأعصاب مضيئة، يثبت أنها موزعة توزيعاً متجانساً في منطقتي جسم وبواب المعدة، كذلك أن تطبيق إختبار (χ^2) أظهر أن الحبيبات المشعة المضيئة للأعصاب غير معتمدة على المنطقة المختارة من المعدة.

التحليل الإحصائي لتوزيع العصب الحائر الأمامي والخلفي يؤكد أن السطح الأمامي لجسم المعدة يستمد أعصابه من العصب الأمامي وأما السطح الخلفي لمنطقتي جسم وبواب المعدة فيستمد أعصابه من العصب الخلفي أما السطح الأمامي لمنطقة البواب فيستمد أعصابه من العصبين الأمامي والخلفي.

بالنسبة لإعادة نمو وتوزيع العصب الحائر الأمامي والخلفي بعد القطع المزمن لأحد العصبين، أظهر التحليل الإحصائي نقص واضح في عدد الألياف العصبية المضيئة في مناطق السطح الخلفي لجسم المعدة وتفسير هذا يعود للأنتشار البسيط للعصب الحائر الأمامي والذي إنتشر ليغذى منطقة صغيرة على سطح المعدة الخلفي بينما إنتشر العصب الحائر الخلفي ليغذي جميع مناطق السطح الأمامي للمعدة. إن اختلاف نسبة إنتشار العصب الحائر الأمامي والخلفي قد يكون له علاقة بوظيفة المعدة الإفرازية خاصة إذا كان العصب الحائر الأمامي هو الأكثر فاعلية من ناحية إفراز المعدة.

التحليل الإحصائي لنتائج هذه الدراسة قد يفسر فقدان الحركة الدورية للمعدة ووظائفها بعد القطع الحاد للعصب الحائر، كذلك إن نمو وإنتشار العصب الحائر بعد القطع المزمن للعصب قد يفسر عودة المعدة لوظيفتها الطبيعية.

إن نتائج هذه الدراسة أيضاً أضافت معلومات عن توزيع العصب الحائر الأمامي والخلفي قد تفيد في العلاج الجراحي لقرحة المعدة، والإنتشار الواسع للعصب الحائر الخلفي يوضح سبب العملية المستخدمة في علاج قرحة الإثني عشر والتي يقطع بها العصب الحائر الخلفي بالإضافة إلى قطع طولي مصلى عضلى (Sero myotomy).

Key words : Vagal dorsal motor nucleus, Truncal vagotomy, Myenteric ganglia, Axon profile analysis

ABSTRACT

In this study, tritiated leucine was injected into the vagal dorsal motor nucleus after acute and chronic partial vagotomy. The method of sampling of the stomach, application of χ^2 test and the analysis of the electron microscopic autoradiographs revealed that the distribution of silver grains over the axon profiles were uniformly distributed over the body and pyloric areas of the stomach. Also a χ^2 test showed that the number of grains is independent of the area chosen. Statistical analysis of the distribution of the dorsal and ventral vagus nerves confirmed that the ventral surface of the body of the stomach is innervated by the ventral vagus nerve while the dorsal surface of the body and pylorus is innervated by the dorsal vagus nerve and the ventral surface of the pylorus is innervated by both ventral and dorsal vagus nerves. Statistical analysis of the redistribution of the dorsal and ventral vagus nerves after chronic partial vagotomy showed a significant drop in the number of labeled nerve fibers in the areas of the dorsal surface of the body of the stomach, because the ventral vagus nerve had spread to supply just a small area on the dorsal surface of the stomach. On the other hand, the dorsal vagus nerve spread to supply all areas of the ventral surface of the stomach. However, the degree of territorial had spread in the dorsal and ventral vagus nerves may be correlated with secretomotor changes, especially if the ventral nerve is predominantly secretomotor. The statistical analysis of the results could also explain that the functional loss of gastric motility after acute partial vagotomy and the gastric recovery after chronic partial vagotomy. Furthermore, the results may add more information on each vagus nerve which is important in vagotomy treatment of peptic ulceration, so the recent operation of dorsal truncal vagotomy and seromyotomy which is used in treatment of chronic duodenal ulcer is explained by the spread of dorsal vagus nerve to supply all areas of the stomach after chronic ventral vagotomy.

INTRODUCTION

The ferret is increasingly used as an experimental animal and is well suited to the investigation of the vagus nerves. The histology, anatomy and physiology of the vagus nerve and stomach are very similar in the ferret and man [1,2,3,4,5,6]. This animal is carnivores and can be bred easily in laboratory conditions and maintained on a pelleted diet.

The distribution of the vagus nerves in the wall of the ferret stomach has been studied by the method of partial acute vagotomy and light autoradiography [1], as well as the redistribution of each vagus nerve in the ferret stomach has been studied three months after unilateral abdominal vagotomy and by using the autoradiographic technique at light microscopic level. The results showed differences in the distribution and redistribution of the ventral and dorsal vagus nerves after chronic partial vagotomy, and no explanation was given for that difference. Furthermore, the results of anatomical and physiological changes after partial vagotomy were in contrast to each other [4,5,6], and no explanation was given to that also. Surgical treatment of peptic ulcer has undergone a number of modifications of vagotomy aimed at reducing the incidence of complications by leaving more fibers of the vagus nerve intact [7,8,9,10], but no satisfactory explanations was given. Therefore the aim of this study is to confirm the previous results of the distribution of each vagus nerve in the wall of the ferret stomach by using the technique of autoradiography at electron microscopic level, also to investigate the redistribution of the ventral and dorsal vagus nerve after chronic partial vagotomy. However, statistical analysis will be used to compare the distribution and redistribution of the ventral and dorsal vagus nerve after acute and chronic partial vagotomy, which may add more information on the distribution and redistribution of each vagus nerve. The statistical analysis may add more explanation on the modification of surgery as treatment of peptic ulceration.

Table 1

Type of animal	no. of animals	injection of tritiated leucine	vagotomy
I control group	2	not injected	no vagotomy
	+		
	2	injected	no vagotomy
II Acute experimental group	3	injected	ventral vagotomy
	+		
	3	injected	dorsal vagotomy
III chronic experimental group	3	injected after 3 months	ventral vagotomy
	+		
	3	injected after 3 months	dorsal vagotomy

Materials and Methods

16 adult ferrets of both sexes weighing 600-1500 gm were used in this study. The animals were divided into three main groups, see Table 1.

The animals were anaesthetized with intraperitoneal injection of 70 mg/kg sodium phenobarbitone. The procedure of vagotomy and injection of tritiated leucine into the vagal dorsal motor nucleus were done according to the previous study of Al-Muhtaseb [1]. The animals were allowed to survive for 48 hours following injection of tritiated leucine, which is enough time for the incorporation of tritiated leucine by the vagal nerve cells and for its transportation by the efferent vagal fibers to the stomach. Then they were perfused by 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1M phosphate buffer at pH 7.2-7.4 through the heart.

Brain stem and vagus nerve were removed and processed for light autoradiography according to Rogers [11], sections were stained with 1% cresyle violet and examined with a vickers M17 microscope. The stomach was rapidly dissected out and divided into fundus, body and pylorus and by cuts through the greater and lesser curvature, it was divided into ventral and dorsal surfaces. Each part of the ventral and dorsal surfaces of the body and pylorus of the stomach was divided into equal size areas for sampling purposes, Al-Muhtaseb [1]. From the ventral and dorsal surfaces of the body of the stomach, areas 4 and 6 were taken for the EM autoradiographic study, and also areas 2,4 and 6 were taken from the ventral surface of the pylorus, see Fig. 1 in Al-Muhtaseb [1].

The areas of the stomach were each cut into small pieces, less than $1mm^2$ and processed for electron microscopic autoradiography. The tissues were stored overnight in buffer at 4^0 c in refrigerator, then were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for one hour at 4^0 c The tissues were then dehydrated in graded ethanol and embedded in araldite. Five blocks from each area of the stomach were taken randomly and semi thin sections of 1 (m thickness were cut and stained with toluidine blue and then examined with the light microscope to identify the myenteric ganglia in the section.

Ultrathin sections were then cut and stained with 3% uranyl acetate followed by lead citrate. All the sections were then coated with a thin layer of carbon film by evaporation and mounted on small corks using perforated double sided cellotape. The cork-mounted grids were then coated with a thin monolayer of L4 (Ilford) nuclear emulsion using the loop method William [12] and then

were exposed for three months in refrigerator at 4^0 c. After the exposure period, the coated grids were developed in Kodak D19 developer, and fixed in freshly prepared 30% sodium thiosulphate. The grids were washed, dried and examined using Philips 200 transmission electron microscope.

Electron micrographs were taken and the structures of the myenteric ganglia of each area of the stomach were photographed and recorded at a constant magnification 25,000 with a special attention given to the labeled structures, especially the axon profiles. The magnification was just high enough to make the smallest axons visible, also high enough to make the silver grains visible. A series of 20 micrographs were analyzed from each area of the stomach by counting the number of grains over the axon profiles (A) which are the only relevant structure expected to be labeled. Also the number of grains over the other structures (B) other than the axon profiles were counted in the same micrographs.

Table 2

Acute Ventral Vagotomy

Stomach areas		Animal Serial No.	Ventral Surface					Dorsal Surface				
			A	B	χ^2_A	χ^2_B	%	A	B	χ^2_A	χ^2_B	%
Body	area no. 4	1						131	16	**21.72***	**0.45**	**88**
		2						115	11			
		3						116	16			
		Total						**362**	**43**			
	area no. 6	1						59	10			
		2						88	11			
		3						100	16			
		Total						**247**	**37**			
Pylorus	area no. 2	1	161	21	**5.81**	**6.22**	**89**					
		2	151	23								
		3	128	13								
		Total	**440**	**57**								
	area no. 4	1	130	15								
		2	102	12								
		3	167	20								
		Total	**399**	**47**								
	area no. 6	1	131	16								
		2	128	21								
		3	113	13								
		Total	**372**	**50**								

* means statistical significance at 5% level of significance

RESULTS

Microscopic examination of the dorsal motor nucleus of the injected animals compared with that of the control animals showed that dorsal motor nucleus throughout its rosto-caudal extent was heavily labeled with silver grains over the perikaryon of each nerve cell, as well as the vagal efferent fibers arising from it. In the control animals examination of the dorsal motor nucleus and stomach showed that there was neither positive nor negative chemography, Al-Muhtaseb [1]. Also autoradiographic examination of the myenteric ganglia of the control animals at electron microscopic level shown labeled preganglionic vagal fibers distributed into all areas of the stomach. The patterns of distribution of labeled fibers were similar in all acute and chronic experimental animals and in all areas of the stomach.

1. Distribution of the dorsal vagus nerve in the wall of the stomach

Electron Microscopic (EM) examination of the ultrathin sections of all areas of the body and pyloric regions of the stomach after acute ventral vagotomy showed that labeled nerve fibers of the dorsal vagus nerve were distributed uniformly over the areas of the dorsal surface of the body and pyloric areas of the stomach, see Table 2 and Fig. 1.

Table 3

Acute Doral Vagotomy

Stomach areas		Animal Serial No.	Ventral Surface					Dorsal Surface				
			A	B	χ^2_A	χ^2_B	%	A	B	χ^2_A	χ^2_B	%
Body	area no. 4	1	87	17	**0.002**	**0.195**	**87**					
		2	82	12								
		3	104	14								
		Total	**273**	**43**								
	area no. 6	1	84	10								
		2	85	12								
		3	105	17								
		Total	**274**	**39**								
Pylorus	area no. 2	1	91	14	**4.108**	**1.41**	**87**					
		2	101	11								
		3	125	28								
		Total	**317**	**53**								
	area no. 4	1	112	11								
		2	103	15								
		3	138	25								
		Total	**353**	**51**								
	area no. 6	1	90	15								
		2	82	9								
		3	131	18								
		Total	**303**	**42**								

2. Distribution of the ventral vagus nerve in the wall of the stomach

EM examination of all areas of the body and pyloric regions of the stomach after acute dorsal vagotomy showed that labeled nerve fibers of the ventral vagus nerve were distributed uniformly over the areas of the ventral surface of the body and pylorus, see Table 3 and Fig. 2.

From the above results of the acute experimental animals, it is concluded that the ventral surface of the body of the stomach is innervated by the ventral vagus nerve while the dorsal surface of the body and pylorus is innervated by the dorsal vagus nerve. The ventral surface of the pylorus is innervated by both vagus nerves.

Table 4

Chronic Ventral Vagotomy

Stomach areas		Animal Serial No.	Ventral Surface					Dorsal Surface				
			A	B	χ^2_A	χ^2_B	%	A	B	χ^2_A	χ^2_B	%
Body	area no. 4	1	129	13	**2.46**	**1.515**	**92**	213	13	**28.3***	**1.35**	**92**
		2	126	11				131	11			
		3	135	14				134	8			
		Total	**390**	**38**				478	32			
	area no. 6	1	102	7				89	19			
		2	124	11				118	12			
		3	123	10				120	11			
		Total	**349**	**28**				327	42			
Pylorus	area no. 2	1	136	13	**1.96**	**0.5**	**92**					
		2	134	12								
		3	99	11								
		Total	**369**	**36**								
	area no. 4	1	131	11								
		2	144	12								
		3	132	16								
		Total	**407**	**39**								
	area no. 6	1	133	6								
		2	147	16								
		3	116	11								
		Total	**396**	**33**								

3. Redistribution of the dorsal vagus nerve 3 months after ventral vagotomy

EM examination of all areas of the body and pyloric regions of the stomach after chronic ventral vagotomy showed that the dorsal vagus nerve had spread to innervate all areas of the ventral surface of the body, see Table 4 and Fig. 3.

Table 5

Chronic Dorsal Vagotomy

Stomach areas		Animal Serial No.	Ventral Surface					Dorsal Surface				
			A	B	χ^2_A	χ^2_B	%	A	B	χ^2_A	χ^2_B	%
Body	area	1	76	9	**8.9***	**0.023**	**90**	85	11			**87**
	no.	2	78	12				94	15			
	4	Total	**154**	**21**				**179**	**26**			
	area	1	100	12								
	no.	2	111	10								
	6	Total	**211**	**22**								
Pylorus	area	1	98	15	**3.095**	**0.78**	**90**					
	no.	2	86	9								
	2	Total	**184**	**24**								
	area	1	110	12								
	no.	2	125	14								
	4	Total	**235**	**26**								
	area	1	96	11								
	no.	2	90	9								
	6	Total	**186**	**20**								

*Note that the third animal died during operation.

4. Redistribution of the ventral vagus nerve 3 months after dorsal vagotomy

EM examination of all areas of the body and pyloric regions of the stomach after chronic dorsal vagotomy showed that the ventral vagus nerve invaded area 4 of the dorsal surface of the body close to lesser curvature, see Table 5 and Fig. 4.

By comparing the results of the acute ventral vagotomy with chronic ventral vagotomy (Table 2 vs. Table 4), the results showed a significant increase in the percentage of the grains over the axon profiles in the areas of the pylorus (p-value = 0.0041), this is due to spread of the dorsal vagus nerve to supply the dennervated areas of the pylorus of the stomach after ventral vagotomy.

Also the results showed a significant increase in the number of grains over the axon profiles in the areas of the body (p-value = 0.0047), this is due to spread of the dorsal vagus nerve to supply all areas of the ventral surface of the body which was previously innervated by the ventral vagus nerve.

By comparing the results of the acute dorsal vagotomy with chronic dorsal vagotomy (Table 3 vs. Table 5), the results showed no significant increase in the percentage of grains over the dorsal areas of the body of the stomach (p-value = 0.0668), because after the dorsal vagotomy the ventral vagus nerve invaded a small area on the dorsal surface of the stomach close to the lesser curvature.

Also the results showed a significant increase in the

percentage of labeled axon profiles over the pyloric areas of the stomach (p-value = 0.025), because after dorsal vagotomy the ventral vagus nerve spread by reactive sprouting to supply the pyloric areas of the stomach.

By comparing the results of the chronic ventral vagotomy with chronic dorsal vagotomy (Table 4 vs. Table 5), the results showed no significant drop in the number of labeled axons in the areas of ventral surface of the body of the stomach (p-value = 0.3372), because the dorsal vagus nerve spread to supply the dennervated areas of the ventral surface of the body after ventral vagotomy but not with the same efficiency.

Also, the results showed no significant drop in the number of labeled axons in the areas of the pylorus of the stomach (p-value = 0.1841), because the pyloric areas innervated by both ventral and dorsal vagal nerves after vagotomy, so the survived vagal nerves spread to supply the dennervated areas of the pylorus .

By comparing the results of the chronic ventral vagotomy with chronic dorsal vagotomy (Table 4 vs. Table 5), the results showed significant drop in the number of labeled axons in the areas of the dorsal surface of the body of the stomach (p-value = 0.0228), because after the chronic dorsal vagotomy the ventral vagus nerve spread to supply a small area of the dorsal surface of the stomach close to the lesser curvature, while the dorsal vagus nerve spread to supply all areas of the ventral surface of the stomach.

A χ^2 goodness of fit test for tables 2-5, shows that number of grains are uniformly distributed over the body and pyloric areas of the stomach. Also this test shows that the number of grains is independent of the area chosen, except for dorsal surface of A of tables 2,4,5 because the area of the stomach (area 4) which is close to the lesser curvature of the stomach, which is also close to the vagal nerves that could be innervated by more vagal branches.

DISCUSSION

Distribution of the dorsal and ventral vagus nerves in the wall of the ferret stomach have been studied at the electron microscopic level. Our findings are in agreement with Asala and Bower [4], Berthoud [13], and also confirmed and completed Al-Muhtaseb [1] results who used the same technique but at light microscopic level.

Statistical analysis of the distribution of the vagus nerves showed that the areas of the stomach which are innervated by the dorsal vagus nerve is larger than that are innervated by the ventral vagus nerve after acute partial vagotomy. These results explained why there is greater functional loss of gastric motility after dorsal trunk vagotomy [6]. Statistical analysis of the redistribution of the ventral and dorsal vagus nerves after chronic partial vagotomy showed a significant drop in the number of labeled axon profiles in the areas of the dorsal surface of the body of the stomach, because the ventral vagus nerve had spread to supply just a small area on the dorsal surface of the stomach, while the dorsal vagus nerve had spread to supply all areas of the ventral surface of the stomach. These results explained the recovery of gastric motility after chronic partial vagotomy in the ferret [5] and also the increase in the intragastric pressure after acute ventral vagotomy [6]. The differences in the redistribution of the ventral and dorsal vagus nerve after chronic partial vagotomy are clear, but a definite reason for that difference has not been established. It is possible that the sprouting and spreading of the vagus nerve into dennervated areas could be related to secretomotor function. So the degree of territorial spread in the dorsal and ventral vagus nerves may be correlated with secretomotor changes, especially if the ventral vagus nerve is predominantly secretomotor. Surgical treatment of peptic ulcer has undergone a number of modifications of vagotomy aimed at reducing the incidence of complications by leaving more fibers of the vagus nerve intact [7,8,9,10], but that surviving branches were able to re-innervate the glandular tissue [1], which is the probable cause of recurrent ulceration. However, the

operation of dorsal truncal vagotomy and seromyotomy [8], which is used to treat chronic duodenal ulcer is explained by the results of this study. Analysis of the results showed that the spreading of the dorsal vagus nerve is larger than the spreading of the ventral, where it spreads to supply all areas of the stomach after chronic ventral vagotomy. Therefore, the operation depends on cutting the dorsal vagus nerve only.

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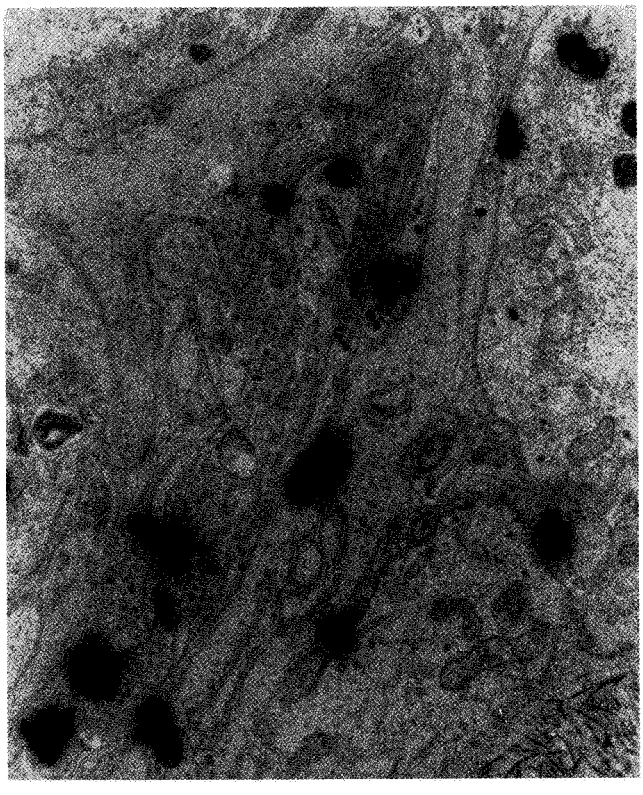

Fig. 1:

Electron microscopic autoradiography from area number 4 of the dorsal surface of the body of the stomach after performing acute ventral vagotomy showing silver grains overlying the axon profiles in the myenteric ganglia of the ferret stomach.

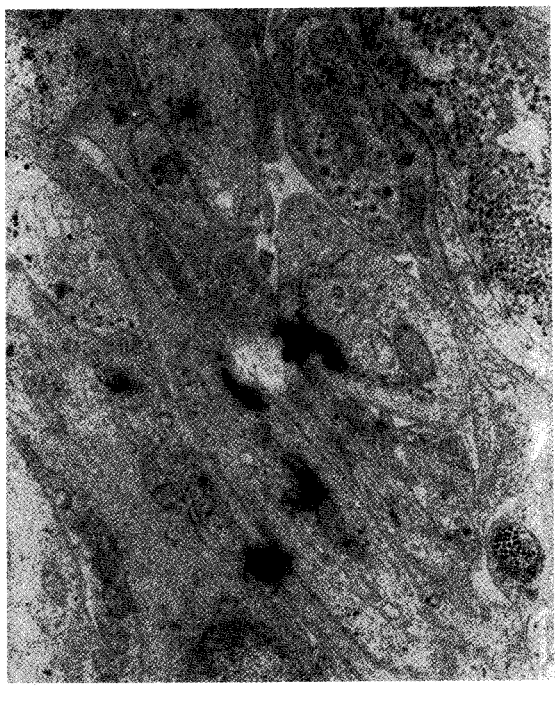

Fig. 2:

Electron microscopic autoradiography from area number 6 of the ventral surface of the body of the stomach after performing acute dorsal vagotomy showing silver grains overlying the axon profiles in the myenteric ganglia of the ferret stomach.

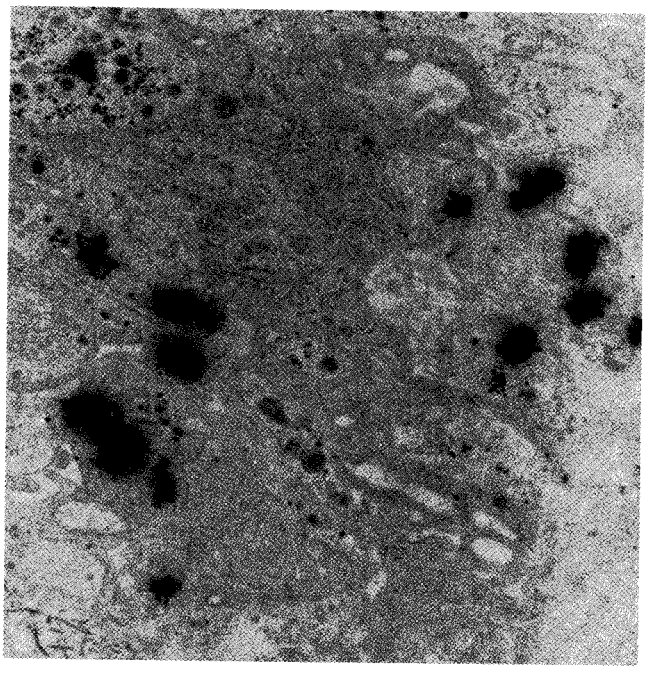

Fig. 3:

Electron microscopic autoradiograph from area number 6 of the ventral surface of the body of the stomach three months after performing acute ventral vagotomy showing silver grains overlying the axon profiles in the myenteric ganglia which was originally unlabeled.

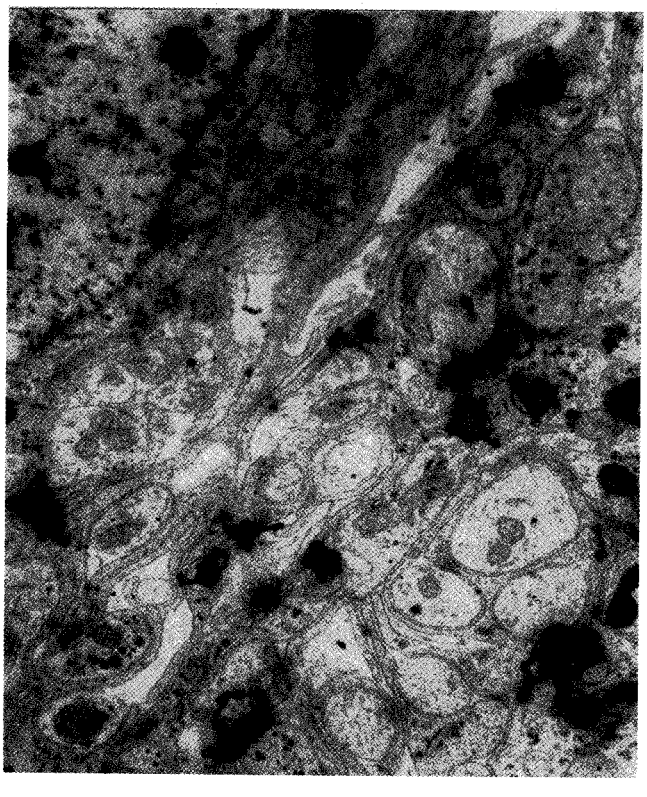

Fig. 4:

Electron microscopic autoradiograph from area number 4 of the dorsal surface of the body of the stomach three months after performing dorsal vagotomy showing silver grains overlying the axon profiles in the myenteric ganglia which was originally unlabeled.