

## REGENERATION OF THE VAGUS NERVE AFTER HIGHLY SELECTIVE VAGOTOMY, AN AUTORADIOGRAPHIC STUDY IN THE FERRET STOMACH .

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### دراسة عودة نمو العصب الحائر بعد عملية قطع أفرع العصب الفائقة الانتقاء باستخدام التصوير الاشعاعي الذاتي

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تبحث هذه الدراسة في عودة نمو العصب الحائر بعد عملية قطع أفرع العصب الفائقة الانتقاء في معدة حيوان ابن مقرض وذلك باستخدام التصوير الاشعاعي الذاتي .  
لقد تم فحص جسم المعدة في حيوانات التجارب الحادة باستخدام التصوير الاشعاعي الذاتي فلم يظهر أي ألياف عصبية مضيئة بعد عملية قطع أفرع العصب الحائر الفائق الانتقاء بينما منطقة البواب من المعدة أظهرت عدة ألياف عصبية مضيئة وهذا يثبت ويؤكد أن عملية قطع أفرع العصب الحائر فائق الانتقاء والتي تستخدم كعلاج لقرحة المعدة قد تمت بنجاح وبطريقة سليمة .  
في حيوانات التجارب المزمنة فحص البطن بعد ثلاثة أشهر من عملية قطع أفرع العصب الحائر الفائقة الانتقاء وجد ان هناك نمو نسيجي لأفرع العصب الحائر التي تم قطعها وهذا النمو من الأنسجة وجد ماراً من فوق العقد التي ربطت أثناء العملية لتصل إلى جسم المعدة والفحص النسيجي لهذه الأنسجة وجد أنها أنسجة عصبية . وأيضاً بعد حقن مادة اللوسين المشع في نواه العصب الحائر لحيوانات التجارب المزمنة وبفحص جسم المعدة شوهدت ألياف عصبية مضيئة تغطي جسم المعدة . هذه النتائج تدل على أن ألياف العصب الحائر والتي قطعت أثناء العملية قد نمت وانتشرت لتغذي جسم المعدة .  
هذه الظاهرة لعودة نمو أفرع العصب الحائر بعد قطعه قد تفسر النسبة العالية في رجوع قرحة المعدة بعد العملية الجراحية لقطع أفرع العصب الحائر الفائق الانتقاء وأيضاً قد تضيف تفسيراً إلى أسباب فشل هذه العملية (قطع أفرع العصب الحائر الفائقة الانتقاء) كعلاج لقرحة المعدة في الإنسان .

*Key Words* : Vagal dorsal motor nucleus , Highly selective vagotomy , Vagal regeneration . Autoradiography , Myenteric ganglia .

### ABSTRACT

This study investigates the regeneration of the vagal nerve fibres after highly selective vagotomy in the ferret stomach by using the autoradiographic technique.

Autoradiographic examination of the body of the stomach in the acute experimental animals has failed to show any labelled nerve fibres after highly selective vagotomy while the pylorus has shown many labelled nerve fibres . These observations indicate that the highly selective vagotomy has been performed properly and adequately.

Examination of the abdomen , three months after highly selective vagotomy in the chronic experimental animals showed, regenerated vagal nerve fibres bypassing the vagotomy ligatures to supply the stomach . After injection of these chronic animals with tritiated leucine into the vagal dorsal motor nucleus , labelled nerve fibres were seen in the body of the stomach . This confirmed that the regenerated nerve fibres of the vagus nerve have spread to supply the body of the stomach .

This phenomenon of regeneration of the vagus nerve may explain the high rate of incidence of recurrent peptic ulcer after highly selective vagotomy , and could account for some of the failures of highly selective vagotomy in humans .

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## INTRODUCTION

Vagotomy has undergone a number of modifications for reducing the incidence of recurrent peptic ulcer [1, 2, 3] but the modified operations have shown higher recurrence rates of ulceration [4, 5, 6].

The incidence of recurrent peptic ulcer after vagotomy increases with length of follow up [4, 5].

Reinnervation of the stomach by regeneration of the vagus nerve after vagotomy was observed in dogs and in humans [7]. Similar result have been reached by AL-Muhtaseb [8] in the ferret stomach.

The autoradiographic technique which depends on the axonal transport has been employed recently to investigate the innervation of the gastro-intestinal tract in different species [8, 9, 10].

Therefore the aim of this study is to investigate the regeneration of the vagus nerve in the ferret stomach after highly selective vagotomy by using the autoradiographic technique.

## MATERIALS AND METHODS

Ten adult ferrets were used in this study. The animals were anaesthetized with intraperitoneal injection of 70 mg / kg sodium phenobarbitone. Two of these animals were used as control animals to rule out the possibility of positive and negative chemography.

Four animals were used as acute experimental group, and were immediately injected with tritiated leucine into the vagal dorsal motor nucleus after highly selective vagotomy. The chronic experimental group consisted of four animals which were allowed to survive three months after highly selective vagotomy, and then they were injected with tritiated leucine into the vagal dorsal motor nucleus.

### **Surgery Of Highly Selective Vagotomy**

The ferret was put in the supine position and the abdomen was opened by midline incision.

The vagal trunks around the oesophagus were identified and isolated. By using the surgical microscope all the branches of the ventral and dorsal vagal trunks which supplied the body were cut between two ligatures close to the lesser curvature of the stomach till the incisura angularis. Wide gaps were seen between the two ligatures after cutting. Abdominal wound was then closed in layers and sprayed with antibiotic and plastic dressing.

The abdomens of the chronic experimental animals were reopened after the survival period. The vagotomy ligatures were identified and specimens of the new connections between the cut nerves and the body of the stomach, were taken for histological examination to see if the tissue is neuronal.

The animals were then injected with tritiated leucine into the vagal dorsal motor nucleus to prove by autoradiography whether the vagal nerve fibres regenerated to supply the body of the stomach.

Injection of tritiated leucine into the vagal dorsal motor

nucleus was carried out by using Kopf Stereotaxic Apparatus. About 150 n1 of tritiated leucine were injected by three injections on both sides of the obex which was exposed after opening the atlanto - occipital membrane by using surgical microscope and the micropipette.

The animals were allowed to survive for 48 hours after being injected with tritiated leucine, which is sufficient time for incorporation of tritiated leucine by the vagal nerve cells and also for its transportation by vagal efferent fibres to the stomach. The animals then were sacrificed by perfusion through the heart by 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1M phosphate buffer at pH 7.2 - 7.4. Brain stem, vagus nerve, and stomach were removed and put in a fresh fixative. The stomach was divided into fundus, body, and pylorus by cuts through the greater and lesser curvature into ventral and dorsal surfaces. The body and pyloric parts of the stomach were divided by a line extending from the incisura angularis to the most dependent part of the greater curvature of the stomach (AL-Muhtaseb, 1993) [8]. Each part of the ventral and dorsal surfaces of the body and pylorus of the stomach was divided into six equal areas. Each area of the stomach was cut and sampled for autoradiography. The tissues were dehydrated in graded ethanol, cleared in xylol, infiltrated and embedded in paraffin. Serial sections were made at 10  $\mu$ m. Tissues were then mounted on subbed slides and processed for autoradiography according to Rogers [11] using L4 (Ilford) nuclear emulsion. Tissues were exposed in the refrigerator. The exposure time for the brain stem and vagus nerve was one week, and the exposure time for the stomach was three weeks. After the exposure time, the tissues were developed in Kodak D 19 developer and fixed in 30% sodium thiosulphate. The slides were stained with 1% cresyl fast violet and examined under light and dark - field microscopy using Vickers M 17 microscope.

## RESULTS

Microscopic examination of the vagal dorsal motor nucleus in the medulla oblongata of the control animals showed that there was no positive chemography. Also, the result of negative chemography was negative.

The dorsal motor nucleus of the injected animals was examined microscopically and compared with that of the control animals. Darkfield examination revealed that the dorsal motor nucleus throughout its rostrocaudal extent was heavily labelled with silver grains over the perikaryon of each nerve cell, as well as the vagal efferent fibres arising from it.

All sections of the stomach were examined, with special attention to the preganglionic vagal nerve fibres found in the myenteric ganglia of the ferret. The labelled preganglionic vagal fibres appeared as focal concentration of silver grains distributed into the wall of the stomach forming basket like structures around the somata of the parasympathetic neurons. ( Figs 2, 3 ).

The patterns of distribution of labelled fibers looked the same in the various areas of the stomach, and were consistent in every experiment. The negative areas of the stom-

ach showed many regions of nerve cells in the myenteric ganglia , but no labelled fibers were evident .

Examination of the stomach of the acute experimental animals showed that all areas (1-6) of the ventral and dorsal surfaces of the body of the stomach have failed to show any labelled nerve fibres , while labelled fibres were distributed throughout the ventral and dorsal surfaces of the pyareas (1-6) of the stomach ( Figs. 1 (A) , 2 ) . This result proves that , after highly selective vagotomy , the nerve supply to the body of the stomach is completely deprived of vagal input , while it is preserved for the pylorus of the stomach .

Examination of the abdomen , three months after highly selective vagotomy, bridges of scar tissue were found bypassing the vagotomy ligatures. Histological examination

of this regrown tissue showed that it was normal nerve fibres . Autoradiographic examination of all areas ( 1-6 ) of the ventral and dorsal surfaces of the body of the stomach showed labelled nerve fibres ( Figs. 1 (B) , 3 ) . These labelled nerve fibres were consistent in every experiment . Examination of the ventral and dorsal surfaces of the pyloric areas ( 1-6) of the stomach also showed labelled nerve fibres in all areas similar to those of the acute experimental animals ( Fig 4 ) .

In summary , three months after highly selective vagotomy, reinnervation of the body of the stomach was manifested through the regenerative growth of the vagal nerve fibres which bypassed the vagotomy ligatures to the body of the stomach .

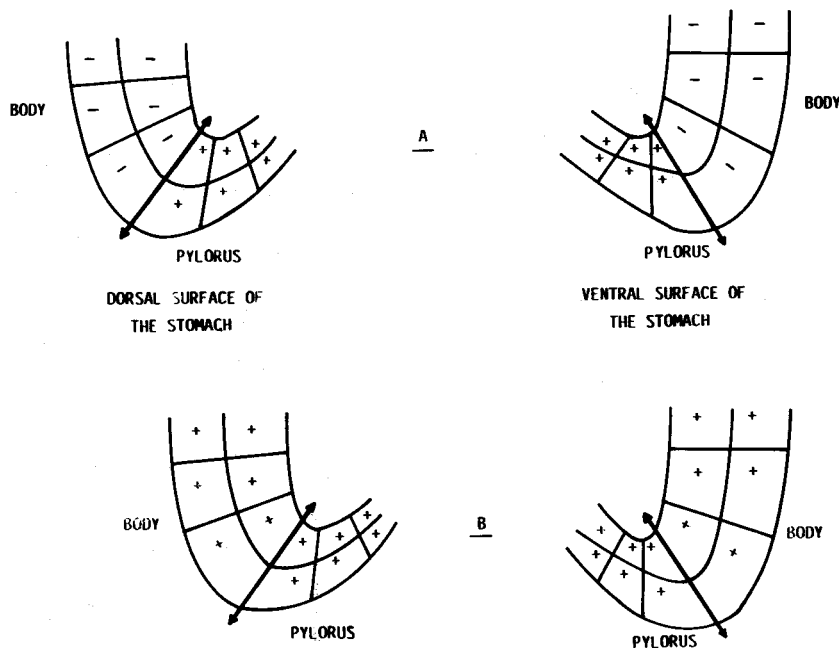


Fig. 1

Fig . 1 : A.Showing the labelled areas of the stomach (+) immediately after highly selective vagotomy .

- All areas of the ventral and dorsal surfaces of the body are not labelled (-) .
- All areas of the ventral and dorsal surfaces of the pylor-

us are labelled (+) .

B. Showing the labelled areas of the stomach (+) three months after highly selective vagotomy .

- All areas of the ventral and dorsal surfaces of the body and pylorus of the stomach are labelled (+) .

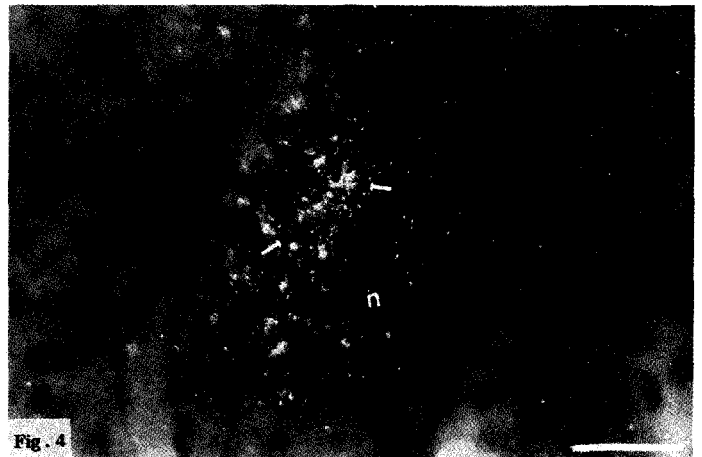
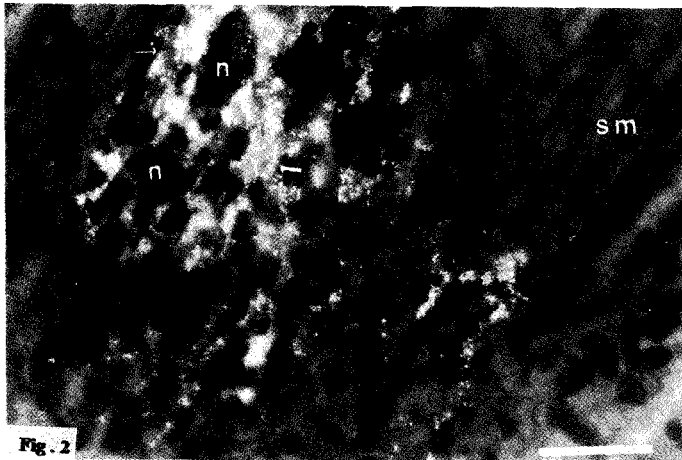


Fig . 2 : Darkfield autoradiograph showing labelled nerve fibres (arrows)of the ventral surface of the pylorus ( area number 1 ) forming circles around the somata of the post-ganglionic neurons within the myenteric plexus , after highly selective vagotomy .

Fig . 4 : Darkfield autoradiograph showing labelled nerve fibres (arrows) of the dorsal surface of the pylorus ( area number 4 ) three months after highly selective vagotomy .

n : nerve cell                      sm : smooth muscle  
 Marker, 25 um

n : nerve cell                      Marker, 25 um

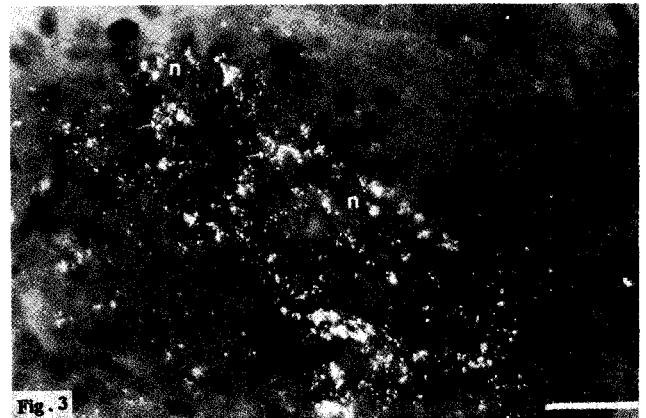
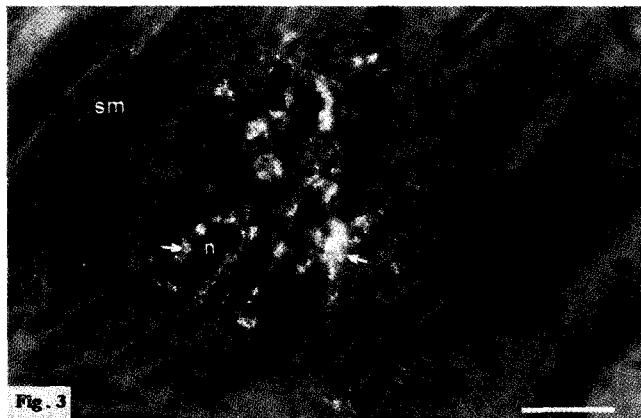


Fig . 3 : Darkfield autoradiograph showing labelled nerve fibres (arrows) of the ventral surface of the (body area number 3) and also of the dorsal surface ( area number 5 ) three months after highly selective vagotomy .

n : nerve cell  
 Marker, 25 um

sm : smooth muscle

### DISCUSSION

The results of this study showed that the body of the stomach of the acute experimental animals was deprived of vagal input while the nerve supply of the pylorus was preserved . Absence of vagal innervation of the body of the stomach indicated that the highly selective vagotomy was performed properly and adequately . This matches the intra-operative testing for completeness of vagotomy [12,13, 14].

Highly selective vagotomy is considered as one of the standard surgical treatments for peptic ulceration [15,1,2,3,16] , but recurrent peptic ulcerations have been reported after highly selective vagotomy and the rate of these

increases considerably with the period of follow up [4,5,6,16,22] .

However , undesirable complications , after vagotomy , such as epigastric fullness , dumping and diarrhoea may be caused by an alteration of normal gastric emptying ; they often disappear or decrease with time[7] . This may be attributed to reinnervation of the areas of the stomach initially deprived of vagal input . Duce and Keen[17] , stated that when an organ is partially denervated the adjacent nerves that survive project some fibres to the denervated areas to compensate for the neuronal loss . Therefore , in this study , after highly selective vagotomy , the vagus nerve regenerated and bypassed the vagotomy ligatures to innervate the body of the stomach . This explains that reinnervation of the stomach may minimise the complications after high-

ly selective vagotomy[7]. Also it explains the recovery of gastric function which has been demonstrated in the ferret stomach after chronic partial vagotomy[18,19].

The results of this study are in agreement with Al - Muhtaseb's[8], " Three months after ventral vagotomy , the ventral trunk regenerated and bypassed the proximal vagotomy ligature to supply the stomach of the ferret ". Regeneration of the vagus nerve has been demonstrated in dogs and man [7] as well as in the gastric wall of the rat seven weeks after highly selective vagotomy [20].

The autoradiographic technique is being increasingly used to investigate the innervation of the gastro - intestinal tract in various species .[8,21.9.10] . Tritiated leucine injected into the vagal dorsal motor nucleus is preferentially taken up only by neuronal perikarya, incorporated into their protein synthesis system and then is transported through the efferent vagal nerve fibres to the axon and nerve terminals in the myenteric ganglia of the stomach [21.9.10] .Other techniques were used to study the innervation of gastro - intestinal tract and recently Berthoud and Bowley [ 23,24 ] used the Dil method to label anterogradely vagal fibres and terminals in the rat gut , they used also the intraperitoneal injection of flurogold to demonstrate the vagal efferent fibers which found restricted to the myenteric plexus , their findings are consistent with the results of this study. In this study , by using the autoradiographic technique , the labelled nerve fibres which were seen in the body of the stomach three months after highly selective vagotomy proved that the regenerated nerve fibres which bypassed the vagotomy ligatures , innervated the body of the stomach or could be due to reactive sprouting of the surviving nerve terminals which may spread from the pyloric areas to denervated areas of the body .

The result of this study may explain that vagal nerve regeneration may account for the recovery of gastric function after vagotomy . Furthermore , the compensatory regeneration of the vagal nerve fibres after highly selective vagotomy may be considered as a probable cause of the high rate of incidence of recurrent peptic ulcer , and this in turn could account for the failure of highly selective vagotomy in humans .

Modification of surgery to prevent regeneration of vagal nerve fibres after highly selective vagotomy could be achieved by putting an insulator in the gap between the proximal and distal ligatures of vagotomy, or could be by suturing the lesser curvature of the stomach to cover the raw areas after vagotomy.

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