Arch Jap Chir 46 (5), 530~550, Sept., 1977

Reconstruction of the Posterior Celiac Vagal Branch Function by Autogenous Nerve Grafting as a Countermeasure Against the Postoperative Sequelae after Thoracic Esophagectomy

by

Masaaki Nagashima

The 2nd Surgical Division, Yamaguchi University School of Medicine (Director : Prof. Dr. Koichi Ishigami)

Received for publication May 12, 1977

Various types of postoperative sequelae may occur after thoracic esophagectomy because bilateral vagal trunks must be cut off owing to the anatomical relationship among the vagus nerve, the esophageal nerve plexus and the thoracic esophagus. It is presumable that the dysfunctions of the posterior celiac branch of the vagus which controls the pancreas, the biliary tract, the small intestine and the proximal part of the large intestine may be one of the factors of such postoperative sequelae.

Then as a countermeasure against these postoperative sequelae after thoracic esophagectomy, the author tried to reconstruct the functions of the posterior celiac vagal branch

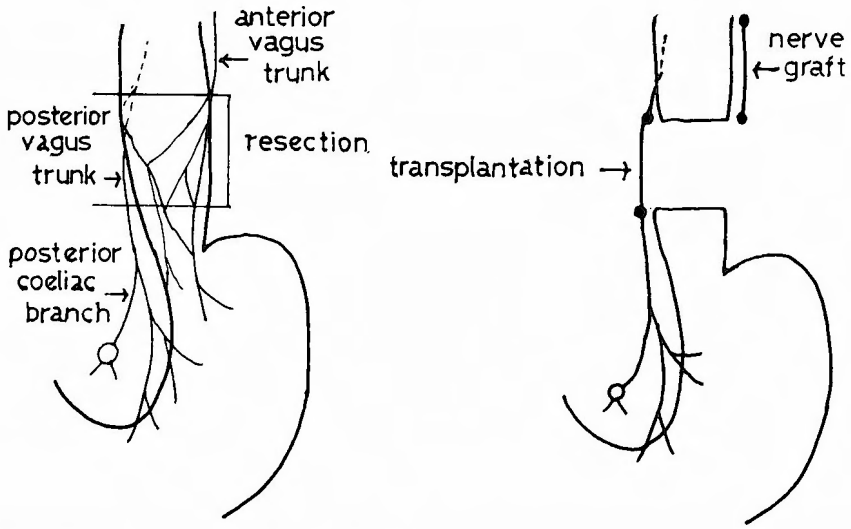

Fig. 1.

Key words Posterior celiac vagal branch, Regeneration, Nerve grafting, Thoracic esophagectomy, Serotonin (5-HT)

Present address : The 2nd Surgical Division, Yamaguchi University School of Medicine, Ube, Yamaguchi, 755, Japan.

by transplanting the autogenous nerve graft removed from the left vagal trunk into the resected defect of the right posterior vagal trunk in dogs (Fig. 1).

Impressed by the suggestion of Emeritus Professor KIMURA of Kyoto University[31], ISHIGAMI[22)24)] invented a new method of operation, total gastrectomy with preservation of the hepatic and posterior celiac vagi, to cope with postoperative sequelae following total gastrectomy. And then, ISHIGAMI[25)] and FUCHIMOTO[15)] reported that the preservation of the hepatic and posterior celiac vagi during the operative procedure of total gastrectomy may contribute to lessen the occurrence of postoperative complaints, such as diarrhea, weightloss, reflux esophagitis, etc., to help keep digestion and absorption of fat and protein, carbohydrate metabolism, functions of the liver and gallbladder, etc. in good condition and to improve the postoperative nutritional status.

In 1870 PHILLIPEUX and VULPAIN[46)] reported that the transplanted nerve graft can introduce the regenerating nerve fibers over the defected gap of the nerve. The author, first of all, replaced the defect of the right posterior trunk of the vagus with the nerve graft and studied whether the posterior celiac branch of the vagus would be able to regenerate histologically or not. But it is questionable whether the histological regeneration of the vagi would be in accordance with its functional recovery or not. Then the author stained cholinesterase, lytic enzyme of acetylcholine in the vagus which is thought to be involved in humoral transmission of the vagus, and studied whether the humoral transmission is present or not, along the regenerated nerve fiber.

STRAUSS[51)] and TOBE[58)] had reported that electric stimulation of the vagi in dogs and rats resulted in a highly significant increase in plasma 5-HT levels in portal vein blood. In order to prove the recovery of the function of the posterior celiac vagal branch, in dogs, the author cut the right posterior vagal trunk proximal to the nerve graft, electrically stimulated the distal cut end, and examined whether plasma 5-HT levels in portal vein blood increase or not. With these methods, the author has proved that the autogenous nerve grafting would improve the posterior celiac vagal dysfunction after thoracic esophagectomy.

1. Materials

Fifty-three mongrel dogs of both sexes weighing 6 to 21 kg were used in these experiments. Under intubation anesthesia with Ketalar and Nembutal, they were operated upon and examined. Forty-three of them were used for main experiments and others for control experiments (Fig. 2). Forty-three dogs were divided into four groups. In group 1, after right-side thoracotomy, the right posterior vagal trunk was cut off and resutured. In group 2, after right-side thoracotomy and laparotomy, the right posterior vagal trunk and the posterior celiac branch were cut off and resutured. In group 3, after right-side thoracotomy, the right posterior vagal trunk was cut off and resutured at two points. And in group 4, after right-side thoracotomy and laparotomy, autogenous transplantation of the graft removed from the left vagal trunk into the defect of the right posterior vagal trunk

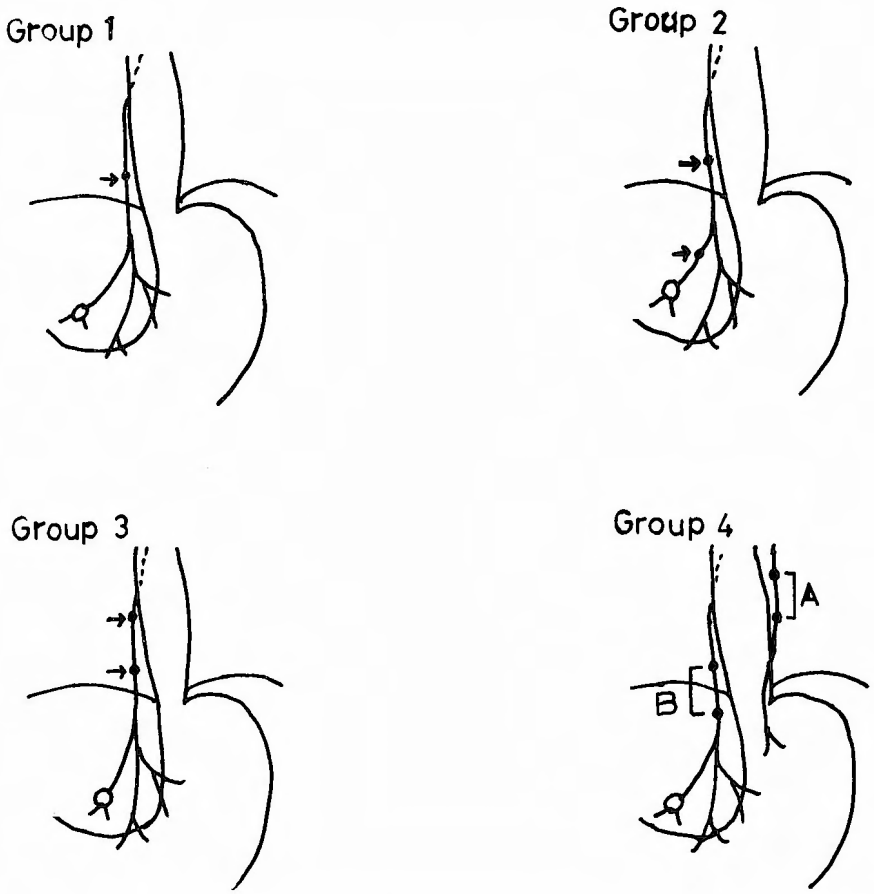

Fig. 2. Group 1 : Cutting off and resuturing of the posterior vagal trunk after right-side thoracotomy
Group 2 : Cutting off and resuturing of the posterior vagal trunk and the posterior celiac branch after right-side thoracotomy and laparotomy
Group 3 : Cutting off and resuturing at the two points of the posterior vagal trunk after right-side thoracotomy
Group 4 : Taking off the nerve graft from the anterior vagal trunk (A) and transplanting into the defect of the posterior vagal trunk (B) after right-side thoracotomy and laparotomy

and additional pyloroplasty were performed. In these dogs, the regeneration of the nerve was observed. Seventeen of these dogs died in the early postoperative period and could not be examined. On the contrary, the other twenty-six dogs could be fed for 10 to 533 days after operation and then examined.

For each nerve suture, using atraumatic needle with 6-0 nylon suture having been reported lesser tissue reactions, 2 to 3 interrupted sutures were performed. While nerve suturing, special attention was given to the nerves to avoid rotation and twisting, and both stumps of the nerve were tied together so as to joint relatively loosely.

During the postoperative course, the dogs received 1 gm of A-B penicillin a day for about 7 days by intramuscular injection to prevent bacterial infection. The dogs suffering from dehydration because of vomiting and other causes, on every such occasions, received physiological saline or 5 % glucose solution by intravenous drip infusion or subcutaneous injection.

2. Methods

In order to observe the nerve regeneration histologically, the author used the silver staining method by BODIAN[5)33)] and the cholinesterase staining method by KARNOVSKY[30)]. In order to examine the functional regeneration of the nerve, the vagal release of serotonin under electrical stimulation was used. Under intubation general anesthesia, thoracotomy and laparotomy were performed on dogs. In dogs of groups 1 and 3, the left vagal trunk was divided. And then, in dogs of groups 1, 3 and 4, right posterior vagal trunk was divided 3 cm proximal to the suture site. The distal part of the divided nerve was mounted by a clip electrode 2 cm proximal to the suture site and stimulated with 6 volt at 20 pulses/sec. for 4 m sec. by Saneisokuki's 3F31 type electrostimulator. Blood samples of the portal vein were obtained prior to, 5,15 and 30 min. after stimulation with a heparinized syringe through siliconized polyethylene tube which had already been introduced into the splenic vein. Plasma 5-HT was extracted from each blood sample by ÜDENFRIEND's method[59)] and measured by Hitachi-512 type fluorescence spectrophotometer.

As control experiments, in ten dogs without the previous operation after the division of the left vagal trunk, the right posterior vagal trunk was divided and its proximal and distal portions were electrically stimulated, respectively, under the same condition as the above description. The group stimulated at the distal side was named group 5 and those at the proximal side was named group 6.

Silver staining was performed in 15 cases. Both silver and cholinesterase stainings were performed in 2 cases. Silver and cholinesterase stainings, and measurement of plasma 5-HT were performed in 8 cases. Silver staining and measurement of plasma 5-HT were performed in one case. The measurement of plasma 5-HT only was performed in 10 cases. Using the above methods, 36 mongrel dogs including the control groups were examined.

3. Results

1) *Silver staining*

Removed nerves were fixed in 10% neutral formalin solution and embedded in paraffin and cut into slices, 10 μ in thickness. Bodian's silver staining for the nerve fibers was performed on paraffin sections. When both silver and cholinesterase were stained with a nerve, 10 % neutral formalin solution with $CaCl_2$ at the rate of 1 % was used for fixation. But no difference was observed as to the effect of silver staining between 10 % neutral solution with and without $CaCl_2$.

Silver staining was performed in 26 cases ; 4 cases in group 1, 3 cases in group 2, 13 cases in group 3 and 6 cases in group 4 (Table 1).

Table 1.

Group	1	2	3	4
Number of the operated cases	8	5	16	14
Number of the cases alive over ten days after operation	4	5	15	6
Number of the nerve stained cases	4	3	13	6
Mean alived term of the nerve stained cases (day)	123	261	104	266
Length of the nerve graft (cm)		8.6	3.6	4.2
Distance between resuturing point and diaphragm (cm)	3.6	6.7	2.4	1.4
Distance between resuturing point and celiac ganglion (cm)		2.6		3.3
Number of histologically completely regenerated cases	3	3	8	4
Regenerating rate of the incompletely regenerated cases (mm/day)	0.8		0.7	
Number of the cholinesterase stained cases	3		4	3
Number of the 5-HT examined cases	3		3	3
Number of the histologically and functionally regenerated cases	3		3	3

The average survival time in group 1 was 123 days, in group 2, 261 days, in group 3, 104 days and in group 4, 266 days.

The average distance between resuturing point of the nerve and diaphragm in group 1 was 3.6 cm, in group 2, 6.7 cm, in group 3, 2.4 cm and in group 4, 1.4 cm. The average distance between resuturing point of the nerve in the abdominal cavity and diaphragm in group 2 was 2.6 cm and in group 4, 3.3 cm. The average length of the nerve graft in group 2 was 8.6 cm, in group 3, 3.6 cm and in group 4, 4.2 cm.

Three cases in group 1, 3 cases in group 2, 8 cases in group 3, and 4 cases in group 4 showed complete regeneration over the whole length of the nerve fibers in the silver stained specimens. The rate of regeneration in the incompletely regenerated cases in group 1 was 0.8 mm per day and in group 3 was 0.7mm per day.

i) Findings of the nerve fibers proximal to the suture site

Retrograde degeneration and the appearance of macrophage were noted in both of the completely and incompletely regenerated cases. But these findings were noted more often in the incompletely regenerated cases in a short postoperative period and rarely noted in the completely regenerated cases in a long postoperative period (Fig. 3) (Fig. 4). The regenerated fibers were also more abundant in the completely regenerated cases than in the incompletely regenerated cases. Near the suture site, products of the retrograde degeneration of the nerve, thickly regenerated fibers, proliferation of the Schwann cells, etc. were mixed.

ii) Findings near the suture site

There were ramification, aberration and retroflexion of the nerve fibers, the figures of

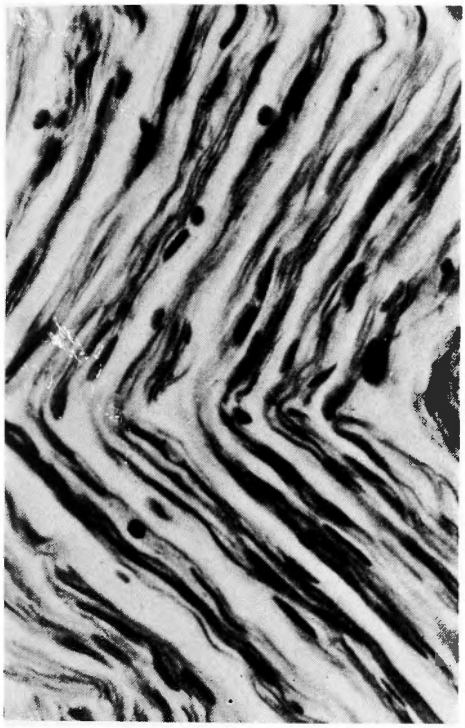

Fig. 3. Two cm proximal to the resuturing point.
One hundred and nineteen days after operation.
Group 1
Bodian's silver staining

Fig. 4. Two cm proximal to the proximal anastomosis.
Five hundred and thirty three days after operation.
Group 4
Bodian's silver staining

regenerating fibers being surrounded by the suture, tissue reactions, scar formation, etc. near the suture site (Fig. 5). And the findings that revealed the suture may prevent the pass of the regenerating fibers or the regenerating fibers are running to the distal side bypassing the suture were also noted. In contrast to the above findings, in the portion apart from the suture, regenerating fibers were running straightforward to the distal side. The regenerating fibers distal to the suture were more numerous but thinner as compared with those proximal to the suture (Fig. 6). In the cases that had two sutured sites such as groups 2, 3 and 4, near the distal suture site, proliferations of the connective tissue were occasionally noted and the regenerating fibers were scarce in number as compared with those near the proximal suture site.

iii) Findings of the nerve graft and the portion between two points of resuture

In the cases of the incomplete regeneration, the Wallerian degeneration, thin fibers, macrophage, cell infiltration, proliferation of Schwann cells, products of the degeneration, etc. were mingling in the nerve graft and the portion between two points of resuture (Fig. 7).

The more the postoperative periods elapsed, the lesser these findings were observed. Regenerating fibers which were ramified and aberrative near the suture site were running

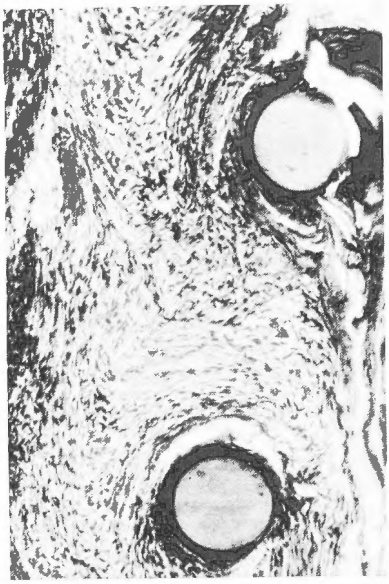

Fig. 5. Anastomotic region.
Four hundred and seventy six days after operation.
Group 4
Bodian's silver staining.

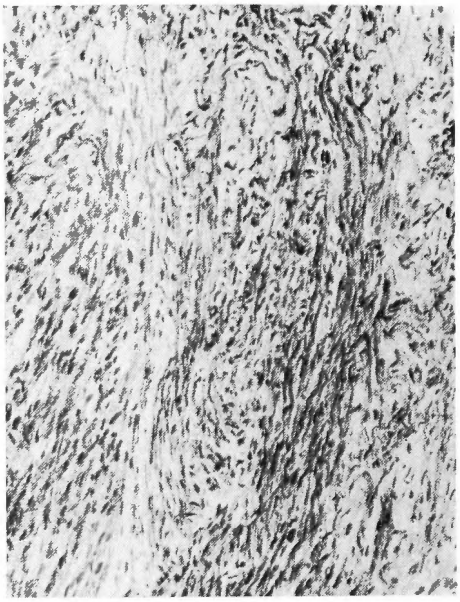

Fig. 6. Anastomotic region.
Two hundred and ninety eight days after operation.
Group 4
Bodian's silver staining.

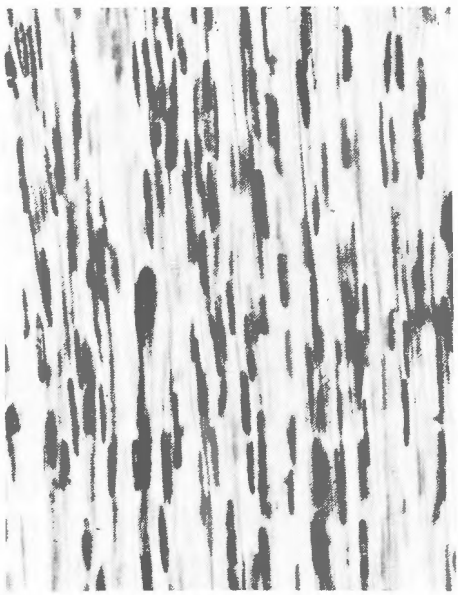

Fig. 7. One and a half cm distal to the proximal anastomosis of the nerve graft.
Two hundred and fifty one days after operation.
Group 4
Bodian's silver staining.

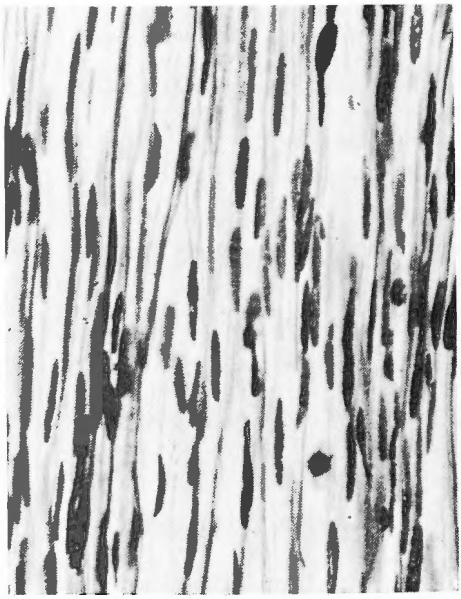

Fig. 8. Two cm distal to the proximal anastomosis of the nerve graft.
Five hundred and thirty three days after operation.
Group 4
Bodian's silver staining.

Fig. 9. One and a half cm proximal to the distal anastomosis of the transplanted nerve graft.
Four hundred and seventy six days after operation.
Group 4
Bodian's silver staining.

Fig. 10. Three cm distal to the distal anastomosis of the trasplanted nerve graft.
Four hundred and seventy six days after operation.
Group 4
Bodian's silver staining.

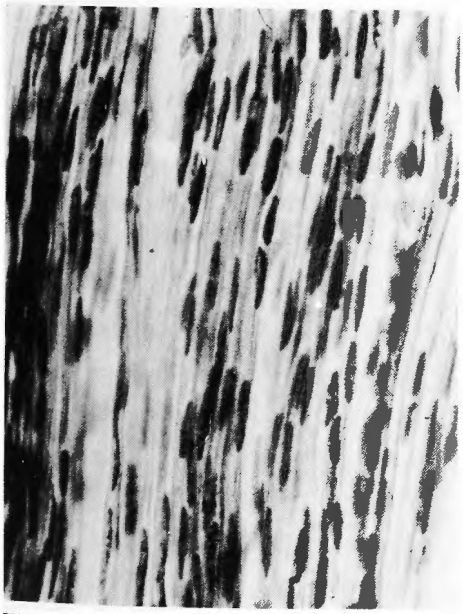

Fig. 11. Four cm distal to the distal anastomosis of the transplanted nerve graft.
Two hundred and fifty one days after operation.
Group 4
Bodian's silver staining.

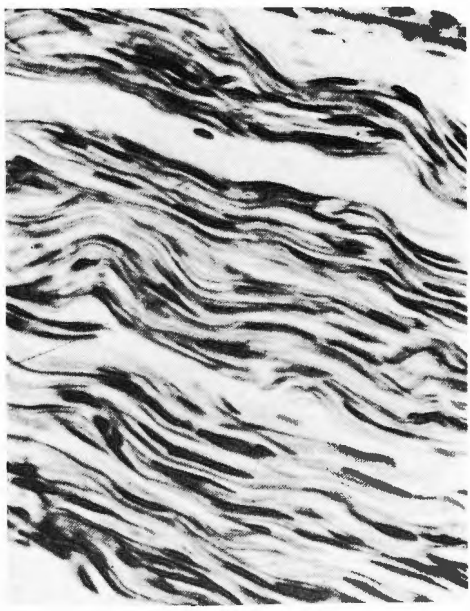

Fig. 12. Two and a half cm distal to the resuturing.
One hundred and nineteen days after operation.
Group 4
Bodian's silver staining.

straightforward orderly and ribbonlike on the distal side (Fig. 8).
In the cases in which the regeneration was completed, products of degeneration and macrophage were also noted in some parts (Fig. 9).

iv) Findings of the portion distal to the distal suture site

In the completely regenerated cases, regenerating fibers were running perfectly to the distal part straightforward and orderly, and Schwann cells were also running regularly to the distal part (Fig. 10) (Fig. 11) (Fig. 12).
But the fibers were thin and loose. Macrophages, products of degeneration, etc. were partially noted in them. In the incompletely regenerated cases, degenerations and macrophages were noted more intensely than in the proximal part.

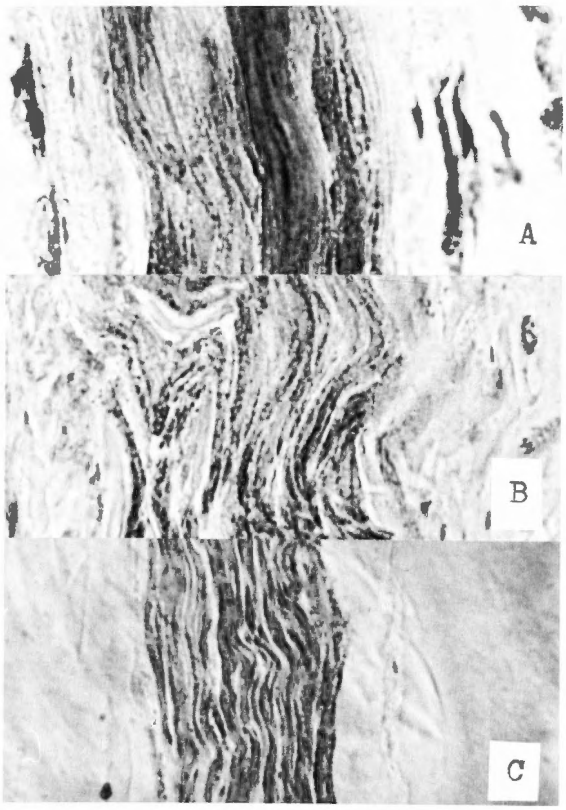

Fig. 13. Four hundred and seventy six days after operation.
Karnovsky's cholinesterase staining.
Group 4.
A) One cm proximal to the proximal anastomosis.
B) One and a half cm proximal to the distal anastomosis of the transplanted nerve graft.
C) One cm distal to the distal anastomosis.

2) *Cholinesterase staining*

The removed nerves were fixed overnight in cold 10% formalin solution containing $CaCl_2$ at the rate of 1%, cut in a cryostat, 10μ in thickness and stained by KARNOVSKY's method for cholinesterase. The nerves whose cholinesterase were stained indicated the histochemical findings of probable regeneration because they had elapsed the period during which histological regeneration had already accomplished. Comparing the proximal portion proximal to the suture site, the suture site and the portion distal to the suture site with each other, these three portions indicated almost equal activity of cholinesterase (Fig. 13).
But in the nerve bundle, the extension with high activity of cholinesterase decreased gradually in conformity with the distance from the proximal portion. Based on these results, it could be assumed that histological and histochemical regeneration agree with each other.

3) *Findings of plasma* 5-*HT measurement*

After electric stimulations were given

to the right posterior vagal trunk, the levels at 5 minutes, 15 minutes and 30 minutes of plasma 5-HT were compared with 0 minute' levels. Plasma 5-HT levels before the stimulation were 0 minute' levels.

In three dogs of group 1, plasma 5-HT levels after stimulation elevated from 20 to 52.3% (Fig. 14). In three dogs of group 3, it elevated from 33.3 to v109.6% (Fig. 15). In three dogs of group 4, it ele - ated from 53.4 to 67.4% (Fig. 16). In five dogs of group 5, it elevated from 22.2 to 118.1% (Fig. 17). vIn five dogs of group 6, it indicated little or no ele - ation and it rather dropped (Fig. 18).

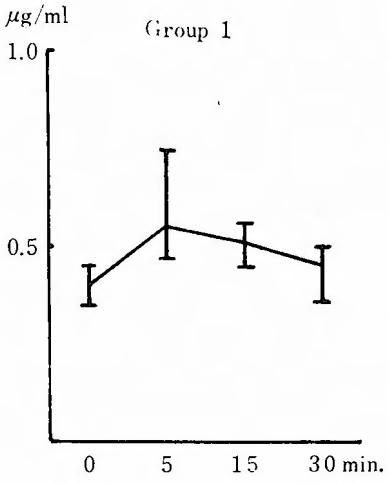

Fig. 14.

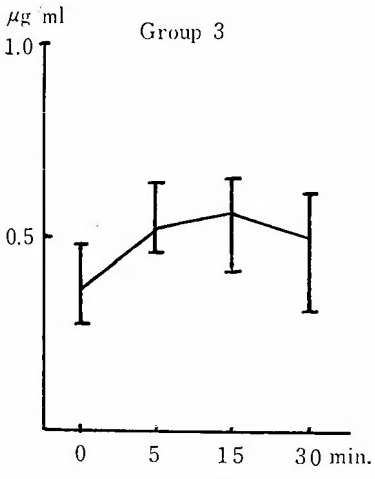

Fig. 15.

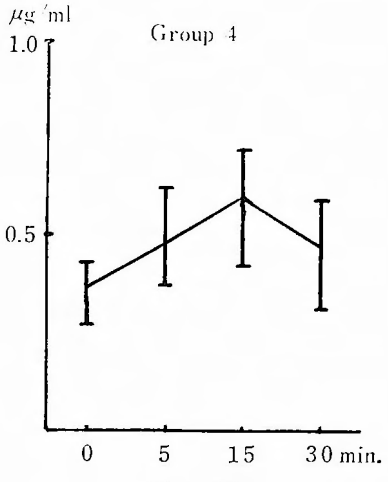

Fig. 16.

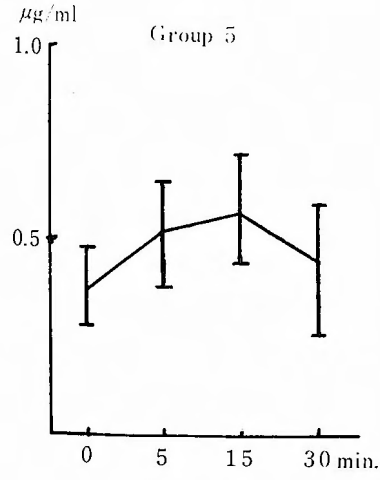

Fig. 17.

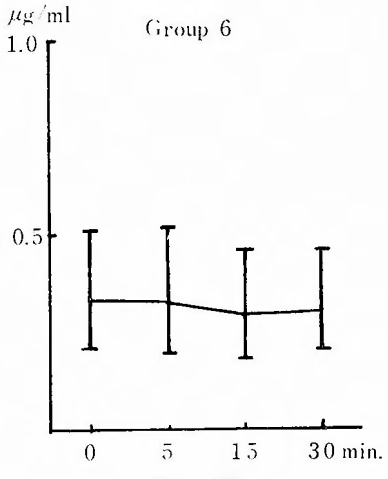

Fig. 18.

Discussion

Both vagus nerves emerge from their pulmonary plexuses behind the right and left lung roots, respectively, and each trunk divides into two to four branches which run obliquely and closely along the anterior and posterior surfaces of the esophagus. These branches connected with each other form network-like esophageal nerve plexus. These numerous nerve fibers emerging from this plexus join again to form the single, double or rarely triple trunks of the anterior and posterior vagus nerves, respectively. Usually, the left vagal trunks pass anterior, and the right posterior, to the esophagus two to six cm above the diaphragm. Because thoracic esophagectomy is accompanied with bilateral truncal vagotomy depending upon these anatomical relationships, the patients must suffer postoperatively from abdominal discomfort and pain, sensation of abdominal distention, sudden onset of diarrhea, disturbance of digestion and absorption, etc. It is probable that the dysfunction of the posterior celiac vagal branch which controls the pancreas, the biliary tract, the small intestine and the proximal part of the large intestine may be one of the factors of such postoperative sequelae.

Previously, impressed by the suggestion of Emeritus Professor KIMURA of Kyoto University, ISHIGAMI[22)24)31)] invented a new operative procedure which is called "total gastrectomy with preservation of the hepatic and the posterior celiac vagi", as a countermeasure against sequelae after total gastrectomy. After investigation of the effect of his new procedure from many viewpoints, he reported that the new procedure might reduce the postoperative complaints, such as diarrhea, reflux esophagitis, anorexia, weightloss, etc. and help keep digestion and absorption of protein and fat, glucose metabolism, functions of the liver and the gall bladder, etc. in good condition, and improve the postoperative nutritional state[15)25)]. And ISHIGAMI concluded that the largest factor for the long-term dysfunction would result from bilateral truncal vagotomy at the esophageal reconstruction by the KIRSCHNER-NAKAYAMA type of gastric tube, then he invented a new procedure[26)], esophago-gastrostomy with additional vagal implantation into the wall of the gastric tube, and he reported that this procedure would improve the nutritnalio conditions after esophageal reconstruction[25)]. SHILS[50)] reported that two patients whose esophagus were resected with vagus nerve being preserved had good absorption of fat and no anorexia after operation. Based on these viewpoints, as a method to improve the abolition of the function of bilateral vagi which are divided at the thoracic esophagectomy, the author tried to reconstruct the functions of the posterior celiac branch of the vagus by the autogenous nerve transplantation. That is to say, the author tried to reconstruct the functions of the posterior celiac vagal branch by transplanting the autogenous nerve graft removed from the left vagal trunk into the resected defect of the right posteror vagal trunk in dogs. The author examined the vagal regeneration morphologically and functionally, and investigated from various viewpoints.

1) *Changes of the divided nerve*

It is well known that if the nerves divided, degeneration such as the Wallerian degeneration, retrograde degeneration, transneuronal degeneration, etc. may occur.

i) the Wallerian degeneration[62]

In 1805, WALLER described the degeneration arisen in the distal nerve separated from nerve cell. The periods that the Wallerian degeneration begins to appear in the myelinated axon have many differences such as within 2 minutes[66] to 48 hours later[61] among many reporters. Also in the unmyelinated axon, its differences were reported such as from 11 to 22 days later[13], from 2 to 4 days later[39], etc. by each reporter. It is reported that the myelin sheath begins to degenerate after 1 to 12 hours[34] or after 6 days[12]. Many investigators reported that the degeneration of the axon began more quickly than that of myelin sheath[12][19][39][45]. According to the author's investigation, in some cases the degeneration had not been completed 2 weeks after operation.

ii) Retrograde degeneration

It is the degeneration caused in the nerve cell side of the nerve fiber by axonal division. CAJAL[10] said that its extent does not run over one segment of the Ranvier's node, but BLÜMCKE[4] said its extent runs over from the first, the second to the third Ranvier's node. In the author's investigations, such findings were noted.

iii) Transneuronal degeneration

It is said that in almost all the nerve degeneration, its extent is limited within an injured neuron, but occasionally the degeneration runs over the synapse. The author did not observe such findings.

2) *Regeneration of the nerve*

It has been an established, a well-known theory that if there are pathways for the nerve fibers outgrowing from proximal side after the products of the degenerated nerves disappeared, the nerve fibers may regenerate running through the pathways. It is thought that Schwann cells play the role of the pathways for introducing the regenerating nerve fibers.

In 1870, PHILLIPEAUX and VULPAIN[46] pointed out that the nerve graft can introduce the regenerating nerve fibers through the defected parts. The autograft, homograft and heterograft are used for nerve grafting. In the cases of nerve grafting with homograft or heterograft, there are big difficulties, the same as other visceral transplantations, such as immunity and foreign body reactions. Autogenous nerve grafting has the advantage of overcoming these difficulties.

BALLANCE and DUEL[2] used the autogenous nerve grafting for the facial palsy. Many successful cases have been reported such as BUNNELL and BOYES' report[8], etc. SEDDON[18] indicated that the autogenous nerve grafting is the best method. In the cases that had undergone the autogenous nerve grafting for general peripheral nerve injuries, there was a drawback: this procedure causes new sacrificial dysfunctions at the part where the nerve graft was removed. But in the author's experiments of transplantation, there is an advantage : this procedure does not cause new dysfunctions owing to the nerve grafting, because the left vagal trunk must be divided accompanied with thoracic esophagectomy.

The periods necessary for the nerve regeneration depend upon the distance between the injured part and the effector organ, speed of regeneration and the condition of the

pathways of the regenerating nerves. SUNDERLAND[52] named the time from nerve injury to the beginning of recovery of the palsied nerve as the latent period, and he divided it as follows : (i) initial delay : the term from the nerve injury until the regenerating axon begins at the distal end of injured nerve, (ii) intermediate delay : the term from the period when the regenerating axon has just run over the injured site to its arrival at the distal end, (iii) terminal delay : the term from the arrival of the regenerating axon at the distal end to the biginning of functional recovery. Many investigations concerning initial delay have been reported. CAJAL[10] reported it as more than 7 days, GUTMANN, et al.[18] as 7.27 days, ARAKAWA[1] as about 10 days, YAMAMOTO[67] as 8.1 days after funicular suture, etc. The author, regarding it as about 10 days, continued to perform the further experiments.

It is thought that the method of suturing, material of suturing, inflammation, scar formation, blood supply, etc., are involved in the effective factors for the growth of the regenerating axon. In this experiment, it was thought that the operative detriment, particularly in group 4, management of the postoperative nutrition and prevention of the complications were important factors for the regeneration. YAMAMOTO[67] reported about the method of nerve suturing that funicular suture was much better than the epineurial suture used formerly. In this study, 2 or 3 interrupted sutures were used for the anastomosis of the nerve.

Recently, the nerves have been sutured densely under the operating microscope. In this study, the operating microscope was not used, because the suturing must be performed in the deep part of thoracic cavity, and the operative field moved with respiration. It is said that the lesser the foreign body reaction caused by the material of nerve suturing is, the better the result is, and nylon suture is now the best[28),29)].

The atraumatic needles with 6-0 nylon suture were used in this study. It is said that blood supply for the nerve grafting site is very important for the nerve regeneration. In order to keep blood supply for the nerve graft in good condition, blood supply near the injured part must not be reduced. The author payed attention to preserving the nutritional blood vessels of the surroundings as far as possible at the time of nerve division. Regenerating nerve fibers run through the Schwann tube as the guiding path. Its speeds differ among all reporters. GUTMANN, et al.[18] reported its speed as 2.0 mm/day in the fibular nerve of the rabbits, GOLSETH[17] as 2.02 mm/day in the sciatic nerve of the cats, TASAKA[54] as 2.38 mm/day in the sciatic nerve of the rabbits, SHIBAGAKI[49] as 1.4 mm/day, YAMAMOTO[67] as from 1.1 to 2.9 mm/day. In the author's cases which died or were sacrificed at half way of the regeneration, its speed ranged from 0.7 to 0.9 mm/day. The results were worse than other reporters. It is thought to be due to the fact that many specimens were taken in the early postoperative period, and the suturing procedure and its technique were not so excellent. These must be investigated further.

3) *Morphological and functional regeneration of the nerves*

When the anatomical regeneration of the nerves is completed, it is important to know

whether it means the functional regeneration or not. Many opinions have been reported about this point. Complete functional regeneration will be accomplished, when the regenerating nerve fibers arrive at the target organ which they innervated previously. However, as a rule, it won't to be useful for functional regeneration that the nerve fibers which are functionally different arrive at the target organ.

As a problem of orientating the direction for the arrival of the regenerating fibers to the target organ, CAJAL[10] and FORSSMAN[14] insisted on the neurotropism theory, and said that when a chemical substance is released from the Schwann tube and then the regenerating axon will be introduced selectively into the objective tube. But WEISS, et al.[64),65] said that the regenerating fibers take the direction by contacting with many kinds of substances on the pathway.

In accord with that, NAKAI[38] explained that the growing end of the axon has the ability to select each substance autonomously. However, NOMURA[43] said that even if the growing end of axon can differentiate Schwann cells from other cells, it would not know the direction of Schwann tube, and said that the functional connection of the regenerating axon with the target organ depends upon true chance as CAIRNS and YOUNG[9] reported. By means of the suturing or the transplantation of nerve, many successful cases of the functional recovery have been reported. It is thought that 80% of the functional connections of the regenerating fibers with the target organ are sufficient to accomplish the clinically satisfactory recovery of the function. The reason why the connections of the regenerating fibers with the target organ amount to as much as 80% in spite of such a rare occurrence as depending upon true chance, NOMURA[42] indicated, as HOLMES and YOUNG[21] reported, that because many regenerating fibers are noted in a Schwann tube, the probability that the Schwann tube may contain the nerve fibers which can agree with a target organ may be high. In this study, the figure that many regenerating fibers are running in a Schwann tube was noted. NOMURA[43] described that the axon of the proximal stump must regenerate branching into scores of branches, in order to introduce many regenerating fibers into a Schwann tube, and the axon must increase in number to score hundreds times more of the axons. The author also noted such figures in the observation near the anatomosed part. It is natural that the more the regenerating fibers arrive at the target organ, the better the functional regeneration is accomplished. But it is a troublesome problem in the nerve grafting that there are two suture sites. That is to say that the regeneration is easy at the proximal suture site because after initial delay, the period when the regenerating fibers begin to enter into the nerve graft is earlier than the period that the connective tissues enter into the nerve graft. But NOMURA[40),41] and IYO[27] indicated that at the portion distal to the distal suture site, the number of the regenerating fibers decreased, because the passing of the regenerating fibers were interfered by the phenomenon that at the distal suture site the connective tissue had proliferated before the regenerating fibers arrived at the distal suture site passing through the nerve graft. Consequently, LEWIS[35] and BSTEH, et al.[7] described that it is better that the previous distal suture be removed and sutured

again predicting the period when the regenerating fibers arrive at the distal suture site. After the distal suture site which has many connective tissues has been removed, the regenerating fibers which have arrived at the distal suture site are given a new motif such as cutting of the axon and then the fibers are branched out into many branche and are regenerated. Furthermore, the number of the fibers which are running to the distal side have increased and are still in good condition for their progress. BJÖRKESTEN[3] and SEDDON, et al.[48] said that the removal and resuturing are not always necessary. In this study, it was not performed. In clinical cases, it is a future problem because the detriment such as laparotomy must be performed again.

4) *Functional regeneration of the nerve*

Many kinds of methods have been used to prove the functional regeneration of the nerve. The author tried to examine the cholinesterase activity of the nerve and the vagal release of plasma 5-HT in order to prove the vagal regeneration.

i) Examination of the cholinesterase activity

The acetylcholine-cycle theory in the transmission of the nerve excitation has been proposed by NACHMANSOHN, et al. [37]. The acetylcholine metabolism is thought to progress as follows : After the inactive acetylcholine which is stored in the nerve membrane is activated sometimes by active electric current, the activated acetylcholine produces the membrane potential on the nerve membrane, and the membrane potential, as the action potential acts on the next part, then the nerve membrane returns to the former condition preparing for the next excitation. At that time, acetylcholine is split by cholinesterase. In order to supply the inactive acetylcholine, acetylcholine is synthesized from choline and acetic acid by cholineacetylase and at the same time inactivated. That is to say, it is an essential condition for cholinesterase to distribute in the nerve fibers in order that acetylcholine may become involved in the condition of stimulation in the nerve fibers. UONO [60] verified the existence of cholinesterase activity in the vagus and sciatic nerves.

It is said that the cholinesterase is useful for the examination of the acetylcholine metabolism because it is more stable than acetylcholine, and if the tissue is kept at low temperature, the activity of the cholinesterase in the tissue will not decrease.

Within the body, two sorts of cholinesterase are present. One of them is called true cholinesterase distributing in the nervous system, muscle end-plate, erythrocytes and sperm. The other is called pseudocholinesterase distributing in the liver, atrium, intestinal mucosa and serum. Many descriptions have been reported that within the nerve the cholinesterase activity is found in the border between the axon membrane or the axon and Schwann cell[6),20),36)]. It can be said that if the nerve has normal structure and conductive function, its cholinesterase activity may be positive. Based on this idea, the author examined the cholinesterase activity in order to prove the histochemical regeneration of the nerve graft and its conductive function. In the cases in which histological regenerations were accomplished, cholinesterase activity was observed at the site of the nerve suture, its proximal and distal sides. Its activity at the portion distal to the suture site was noted to be almost

of the same degree as that at the suture site and its proximal site. In the group of the autogenous nerve grafting the same findings were also observed.

ii) Vagal release of serotonin

Serotonin is the active amine distributing widely from the gastric mucosa to the anal mucosa of mammalian and is produced in the enterochromaffin cells. It is recognized as one of the gastrointestinal hormones. Serotonin has been known to internally secrete with receiving the adequate intraluminal stimulation to the gastrointestinal tract[32),44),47),55),57)].

Serotonin is also secreted directly by the vagal stimulation, that is to say, the vagal release of 5-HT is present.

STRAUSS[51)] reported that after the stimulation of the canine's vagus, plasma 5-HT of the portal vein blood increased.

Moreover, TOBE, et al.[56)58)] reported that serotonin in the gastroduodenal tissues increased after vagotomy and after the electric stimulation to the distal side of the divided vagus, plasma 5-HT of the portal vein increased, and at the same time, serotonin in the gastrointestinal tissue decreased. On the other hand, DRAPANAS[12)] reported that serotonin fluorescence in the tissue decreased after vagotomy. In our laboratory, ISHIGAMI[23)], FUCHIMOTO[16)] and WAKABAYASHI[63)], in a series of clinical and experimental studies, examined the relationship between the occurrence of the dumping syndrome and the vagus, and noted that in the group in which the celiac vagi was preserved, the dumping syndrome occurred more frequently than in the total vagotomised group, and in the dumping syndrome cases, at the seizure or at the time of dumping test, plasma 5-HT levels of the portal vein blood increased. They emphasized the relationship between the celiac vagal branch and the release of 5-HT. SUZUKI[53)] also described that the vagotomy suppressed the release of serotonin. The author, presuming that the vagus is related to the release of 5-HT based on these series of reports, predicted that when the regeneration of the transplanted vagus is accomplished, plasma 5-HT of the portal vein blood will be increased by the electric stimulation. In groups 1, 3 and 4 of the experimental dogs of this investigation, plasma 5-HT levels of the portal vein blood in each group increased after the electrical stimulation of the portion proximal to the suture site, the peaks of the levels were by 52.3%, 109.4%, and 67.4%, respectively. It was assumed that the sutured nerves had been regenerated functionally down to the peripheral end. On the other hand, in group 6 as a control study, no increase of plasma 5-HT was noted. This fact suggest paradoxically, that the vagal release of 5-HT occurs under the condition that the vagus is connected with the gastrointestinal tract. These results show that the serotonin measurement of the portal vein blood after the electrical stimulation is adequate to prove the regeneration of the vagal function.

Conclusion

1. Many kinds of postoperative dysfunctions may occur by bilateral truncal vagotomy accompanied with thoracic esophagectomy. It is presumable that the dysfunctions of the posterior celiac branch of the vagus may be one of the factors of such postoperative sequelae.

The author tried to reconstruct the functions of the posterior celiac vagal branch by transplanting the autogenous nerve graft removed from the left vagal trunk into the resected defect of the right posterior vagal trunk in dogs. As a method of nerve suture, 2 or 3 interrupted sutures through the all layers were performed macroscopically.

2. The experiments of the transplantation were performed in 14 dogs. Four of them lived from 251 to 533 days and their regenerative conditions were investigated. As for control experiments, in 8 dogs the division and resuture of the right posterior vagal trunk were performed, in 16 dogs the division and resuture at two points of the right posterior vagal trunk, and in 5 dogs the division and resuture at each point of the right posterior vagal trunk and the posterior celiac vagal branch. They were also investigated.

3. As for method for the observation of the nerve regeneration, histological observation with BODIAN's silver staining of the nerve fibers and histochemical observation with KARNOVSKY's cholinesterase staining of the nerve were used. And furthermore, plasma 5-HT levels of the portal vein blood were measured after the electrical stimulation of the portion proximal to the nerve suture site, and vagal release of 5-HT was investigated.

4. One of 4 cases in the transplanted group, that survived a long term, showed complete regeneration of the nerve by histological observation only. The other three cases showed complete morphological and functional regeneration by above three methods.

In these cases, the posterior celiac vagal branch showed the same degree of the cholinesterase activity throughtout the whole length of the right posterior vagal trunk and the posterior celiac branch of the vagus nerve ; and after the electrical stimulation, an average 59% increase of plasma 5-HT in the portal vein blood was noted.

5. The above-mentioned results of experiments showed that it is possible to regenerate the posterior celiac branch histologically, histochemically and functionally, by the autogenous transplantation of the vagus, and to minimize the dysfunction of the posterior celiac vagal branch caused by truncal vagotomy accompanied with thoracic esophagectomy.

Acknowledgement

Grateful acknowledgement is made to Prof. Dr. KOICHI ISHIGAMI for his kind guidance and criticisms throughout this investigation and to Dr. KOSAKU WATANABE, the vice-director of Ube-Kosan Central Hospital, for his kind support.

The abstract of this paper was presented before the 5th Meeting of the Japanese Society of Vagotomy, Ube, Feb. 15, 1976, the 51st Chugoku-Shikoku Regional Meeting of the Japanese Association for Surgery, Hiroshima, Sept. 25, 1976 and the 10th General Assembly of the Japanese Society of Gastroenterological Surgery, Kobe, July 14, 1977.

References

1) Arakawa, Y. · Experimental studies on the peripheral nerve suture——The part of Schwann cells in neural scar. J Juzen Med Soc **74** : 121-136, 1966.
2) Ballance, C. & Duel, A. B. The operative treatment of facial palsy. Arch Otolaryng (Chicago) **15** 1-70, 1932.
3) Björkesten, G. Clinical experiences with nerve grafting. J Neurosurg **5** : 450-463, 1948.

4) Blümcke, S., et al., : Axoplasmic alterations in the proximal and distal stumps. Acta Neuropath **7**: 44-61, 1966.
5) Bodian, D. A new method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat Rec **65** : 89-97, 1936.
6) Brzin, M.: The localization of acetylcholinesterase in axonal membranes of frog nerve fibers. Proc N A S **56**: 1560-1563, 1966.
7) Bsteh, F. X. : Experimentelles zur Frage der zweizeitigen Nerveninterplantation. Zbl Neurochir **13** 23-28, 1953.
8) Bunnell, S. & Boyes, J. H. : Nerve grafts. Am J Surg **44** : 64-75, 1939.
9) Cairns, H. & Young, J. Z. : Treatment of gunshot wounds of peripheral nerves. Lancet **27** : 123-125, 1940.
10) Cajal, S. R. Y. : Degeneration and regeneration of the nervous system. Trans. and Ed. May, R. M., I and II, Oxford Univ Press London, 1928.
11) Cravioto, H. : Wallerian degeneration : Ultrastructural and histochemical studies. Bull Los Ang Neurol Soc **34** : 233-253, 1969.
12) Drapanas, T., et al. Experiences with surgical management of acute gastric mucosal hemorrhage. Ann Surg **173** 628-640, 1971.
13) Fisher, E. R. & Turano, A. : Schwann cells in Wallerian degeneration. Arch Path **75** : 517-527, 1963.
14) Forssman, J. : Zur Kenntniss des Neurotropismus. Beitr Path Anat All Path **27** : 407-430, 1900.
15) Fuchimoto, T., et al. . Total gastrectomy with preservation of the hepatic and posterior celiac vagi——a countermeasure against postvagotomy syndrome following total gastrectomy. Rinsho Geka **26** : 1023-1029, 1971.
16) Fuchimoto, T. : The vagus and dumping syndrome. The autonomic nervous system **12** : 123-130, 1975.
17) Golseth, J. G. & Fizzel, J. A. Electromyographic studies on cats after section and suture of the sciatic nerve. Am J Physiol **150** : 558-567, 1947.
18) Gutmann, E., et al. The rate of regeneration of nerve. J Exper Biol **19** 14, 1942.
19) Hallpike, J. F. & Adams, C. W. M.: Proteolytic enzymes in myelin breakdown. Neuropath Pol **7** · 225-231, 1969.
20) Hirano, H. & Ogawa, K. : Ultrastructural localization of cholinesterase activity in nerve endings in the guinea pig heart. J electro Micr **16** : 313-321, 1967.
21) Holmes, W. & Young, J. Z. : Nerve regeneration after immediate and delayed suture. J Anat **77** : 63-96, 1942.
22) Ishigami, K. : Total gastrectomy and vagus nerve. Arch Jap Chir **39** 1-2, 1970.
23) Ishigami, K. Dumping and afferent loop syndromes and countermeasure against them. Geka Chiryo **24** 649-665, 1971.
24) Ishigami, K. : Operative method of total gastrectomy with preservation of the hepatic and posterior celiac vagi. Geka Shinryo **14** · 1469-1474, 1972.
25) Ishigami, K. : Various problems in vagotomy in the operation of the upper digestive tract, especially total gastrectomy with preservation of the hepatic and posterior celiac vagi and intrathoracic esophagogastrostomy with implantation of the vagus nerves into the wall of gastric tube. Geka, **34** : 1122-1131, 1972.
26) Ishigami, K. Operative met hod for cancer of the esophagogastric region. Geka Shinryo **14** 558-562, 1972.
27) Iyo, A.: Experimental studies on nerve suture, especially on distribution and structure of nerve fibers in suture lines. The central Japan J Orthopeadic & Traumatic Surgery **10** : 522-540, 1967.
28) Kageyama, N., et al. Successful peripheral nerve regeneration after homografting. Brain and Nerve **18**: 351-358, 1966.
29) Kaihatsu, N. : Experimental studies on the allotransplantation of the vagal nerve, with special respect to lung transplantation. Shikoku Acta Medica **28** : 130-142, 1973.
30) Karnovsky, M. & Roots, L. : A "direct coloring" thiocholine method for cholinesterases. J Histochem & Cytochem **12** : 219-221, 1964.
31) Kimura, C., et al. Total gastrectomy with preservation of the hepatic and posterior celiac vagi.

The 10th Kinki Regional Meeting of the Japanese Society for Surgery, 1968.
32) Kobayashi, S. & Fujita, T. Emiocytic granule release in the based granulated cells of the dog induced by intraluminal application of adequate stimuli. In Gastro-Entero-Pancreatic endocrine system. In cell biological approach (ed. by Fujita, T.) Igaku-Shoin, Tokyo, 1973.
33) Lee, G. & Luna, H. T. Further studies of Bodian's technique. Am J Med Technol **30** : 355-362, 1964.
34) Lee, J. C. Electron microscopy of Wallerian degeneration. J Comp Neurol **120** 65-74, 1963.
35) Lewis, D. Some peripheral nerve problems. Boston med Surg J **188** : 975-984, 1923.
36) Lorenzo, A. J. D., et al. : Fine structural localization of acetylcholinesterase in single axons. J Ultrast Res **28** : 27-40, 1969.
37) Nachmansohn, D. & Machado, A. L. : The formation of acetylcholine. A new enzyme : "Choline Acetylase" J Neurophysiol **6** : 397-403, 1943.
38) Nakai, J. : Experimental study on the neurotropism. The 15th Nippon Igakukai Sokai Gakujutsu Shukai Kiroku **5** : 710-711, 1959.
39) Nathaniel, E. J. H. & Pease, D. C. Degenerative changes in rat dorsal roots during Wallerian degeneration, J Ultrast Res **9** 511-532, 1963.
40) Nomura, S. & Toda, N. Experimental studies on nerve grafts. The central Japan J of Orthopaedic & Traumatic Surgery **10** : 1054-1064. 1965.
41) Nomura, S. : Operative method for the peripheral nerve injury. Seikeigeka **18** : 1242-1254, 1967.
42) Nomura, S. : Operative method for the peripheral nerve injury. Noshinkei Gaisho **2** : 41-57, 1970.
43) Nomura, S. Nerve grafting and regeneration. Degeneration and regeneration in nervous system. Igaku Shoin Ltd., Tokyo, 1 edit, 365-386, 1975.
44) O'Hara, R. S., et al. Serotonin release mediated by intraluminal sucrose solutions. Surg Forum **10** : 215-218, 1959.
45) Ohmi, S. : Electron microscopic study on Wallerian degeneration of the peripheral nerve. Z Zellforsch **54** 39-67, 1961.
46) Phillipeux, J. M. & Vulpain, A. . Note sur des essais de greffe d'un troncon de nerf lingual entre les deux bouts du nerf hypoglosse, Apres excision d'un segment de ce dernier nerf. Arch de Physiol Norm et Pathol **3** 618-620, 1870.
47) Resnick, R. H. & Gray, S. J. : Chemical and histologic demonstration on hydrochloric acid induced release of serotonin from intestinal mucosa. Gastroenterol **42** : 48-55, 1962.
48) Seddon, H. J. Nerve grafting. J B J S **45**-B : 447-461, 1963.
49) Shibagaki, E. The influence of nerve suture on the recovery of intrinsic muscle function after the peripheral nerve section at different level. J of the Japanese orthopaedic Association **43** : 1045-1055, 1969.
50) Shils, M. E. : The esophagus, the vagi and fat absorption. Surg Gynec & Obstet **132** : 709-715, 1971.
51) Strauss, R. J., et al. Plasma 5-hydroxytryptamine and plasma 5-hydroxyindoleacetic acid levels after vagal stimulation. J Surg Res **12** : 334-340, 1972.
52) Sunderland, S. : Nerves and nerve injuries. E & S. Livingstone Ltd, 1968.
53) Suzuki, K. : Autonomic nerve and upper digestive tract. The autonomic nervous system **12** : 215-217, 1975.
54) Tasaka, H. . Electromyographic study of the peripheral nerve palsy. J Kyoto Prefect Med Univ **63** 95-111, 1958.
55) Tobe, T. Biogenic amines in the human G. E. P. endocrine system as studied by fluorescence histochemistry. In gastro-entero-pancreatic endocrine system. A cell biological approach. (ed. by Fujita.) Tokyo, Igaku Shoin 1973.
56) Tobe, T., et al. : Vagal release of 5-HT. 5th World Congres of Gastroenterology, Mexico 1974.
57) Tobe, T. Enterochromaffin cells and carcinoid syndrome. Jap J Clin Med **32** 745-750, 1974.
58) Tobe, T., et al. · The vagus and serotonin. The autonomic nervous system **12** : 115-118, 1975.
59) Üdenfriend, S., et al. : Assay of serotonin and related metabolites, enzymes and drugs. In D. Glick(ed.) Methods of Biochemical Analysis, p. 95. Wiley (Interscience), New York, 1958.
60) Uono K. The enzymes involving the autonomic nerve transmitters. Jap J Clin med **16** : 943-972, 1958.

61) Vial, J. D. : The early changes in the axoplasm during Wallerian degeneration, J. Biophys. Biochem Cytol **4** : 551–555, 1958.
62) Waller, A. : Experiments on the section of the glossopharygeal and glossal nerves of the frog and observation of the alterations produced thereby in the structure of their primitive fibers. Phil Trans Roy Soc London **140** : 423–429, 1850.
63) Wakabayashi, N. · Studies on total gastrectomy with preservation of the hepatic and the posterior celiac vagi, especially the relation of the hepatic vagi to gallbladder function and the posterior celiac vagi to dumping syndrome. Arch Jap Chir **42** : 211–228, 1973.
64) Weiss, P. . In vitro experiments on the factors determining the course of the outgrowing nerve fiber. J Exp Zool **68** 393–448, 1934.
65) Weiss, P. & Taylor, A. C.: Further experimental evidence against "Neurotropism" in regeneration. J Exp Zool **95** : 233–257, 1944.
66) Williams, P. L. & Hall, S. M. Chronic Wallerian degeneration——an in vivo and ultrastructural study. J Anat **109** : 487–503, 1971.
67) Yamamoto, K. : A comparative analysis of the process of nerve regeneration following funicular and epineurial suture for peripheral nerve repair. Arch Jap Chir **43** : 276–301, 1974.

和文抄録

食道切除術後機能障害の対策としての自家神経移植による迷走神経後腹腔枝機能の再建

山口大学医学部外科学教室第2講座(指導：石上浩一教授)

長　島　正　明

胸部食道切除にさいして，迷走神経，さらに食道神経叢の胸部食道に対する解剖学的関係から，両側迷走神経が切断されるために，術後に各種の機能障害が発生する．これには膵，胆道，小腸および大腸の一部を支配している迷走神経後腹腔枝が切断されて，その機能が脱落することが一因と考えられる．著者はこのような，胸部食道切除後の機能障害に対する対策として，実験的に雑種成犬において，右後迷走神経幹切除欠損部に，左迷走神経幹から採取した自家神経片を移植し，中枢側から神経線維を再生せしめることによって，後腹腔枝機能を再建する試みを検討したので，その成績を報告する．本実験は，全麻下に開胸・開腹を行い，左迷走神経片を右後迷走神経幹欠損部へ移植し，神経線維の再生を待って，再生状態の観察を行った．実験犬の数は53頭で，本実験の移植群は14頭，そのうち4頭が251—533日間生存し，観察を行うことができた．また対照予備実験として，右後迷走神経幹の切断・再縫合群の8例，2カ所における切断・再縫合群16例，右後迷走神経幹および後腹腔枝の切断・再縫合群5例を作成し，並行して観察した．観察方法は移植部を含む神経に対して，神経線維鍍銀染色，神経コリンエステラーゼ染色を行い，同時に vagal release of serotonin の存在に着目し，移植・縫合部より中枢の迷走神経幹を電気刺激して，門脈血中セロトニン定量を行い，神経再生が機能的に移植部から後腹腔枝へと行われているかを調べた．対照・予備実験群および本実験群ともに，形態学的再生の起こっているものでは，電気刺激後の門脈血中セロトニンは上昇し，形態学的再生と機能的再生は一致すると考えられた．すなわち，長期生存の移植群4例のうち3例につき，前記3方法で観察すると，再生神経線維は移植部を越えて末梢の後腹腔枝まで再生し，コリンエステラーゼ染色では，移植部より中枢から末梢まで同程度の活性を示していた．また電気刺激後には，73%の門脈血中セロトニン上昇を認めた．また対照の無処置犬10頭で，右後迷走神経幹を切断し，5頭づつにそれぞれ中枢側および末梢側断端を刺激すると，門脈血中セロトニンは，前者では上昇せず，後者では他の再生した群と同様に上昇を認めた．これは門脈血中セロトニン上昇は，迷走神経による場合，腸管と迷走神経が結合された状態で生ずると考えられ，再生状態の観察方法として有意義であった．これらの成績から，食道切除に伴う胸部迷走神経切断による後腹腔枝機能の脱落症状に対する対策として，迷走神経自家移植により，迷走神経後腹腔枝を組織学的，組織化学的および機能的に再生せしめうることを実験的に証明した．