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Studies on the Pathogenesis of Postvagotomy Ulcer of the Stomach

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Vagotomy is at present widely used as the surgical therapeutic procedure for peptic ulcer. Since Dragstedt and Owens initially tried bilateral truncal vagotomy for the patients with duodenal ulcer in 1943, many investigators have studied the changes of gastric secretion and motility due to vagotomy, clinically and experimentally. Moreover, after Holle described selective proximal vagotomy in 1964, the validity of vagotomy for peptic ulcer was appreciated[15].

On the other hand, the reduction in gastric mucosal blood flow by vagotomy is well known. It can easily be assumed that vagotomy, which by nature reduces gastric acid and pepsin secretion, should affect the gastric defensive system considerably.

The lysosomes are cell organelles which are limited by a membrane and contain a number of enzymes that are active at acid pH. Originally, they have been designated as suicide bags, and it has been thought that the freeing and activation of the acid hydrolases play as a trigger in the process of the necrosis of the living cells. And there are some other evident functions e. g., digestion and lysis of dead cells, engulfing extracellular materials, referred to as heterophagy, and autodigestion at the partial breakdown of the structure of the cytoplasm called autophagy. In vivo, shock, ischemia, and administration of the bacterial endotoxin may increase the permeability of the lysosomal membrane, allowing the enzymes to escape and become activated[3].

Therefore, in order to clarify how the reduction in gastric mucosal blood flow by vagotomy affect the releasing of the lysosomal enzymes, the author investigated the changes of cathepsin and β-glucuronidase activities that are due to vagotomy. On the other hand, the change of hexosamine contents in the gastric mucosa due to vagotomy was determined, which is thought to be a constituent of gastric mucosubstance, one of the defensive factors against acid-pepsin digestion. And the author also investigated the influence of vagotomy on the experimental gastric ulcer. Satisfactory results were obtained as described below.

Material and Methods

Experiment I. Influence of vagotomy on the healing of the experimental gastric ulcer.

Key words : postvagotomy ulcer, lysosomal enzyme, cathepsin, β-glucuronidase, hexosamine
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Ten adult mongrel dogs, weighing 8-17 kg, were used. They were put on a fast for about 24 hours before each experiment, and were then anesthetized with sodium pentobarbital. An endotracheal tube was inserted immediately after the induction of anesthesia and was connected to the respirator. They were divided into 2 groups according to the types of operation.

Vagotomy-Ulcer group (5 dogs) : Bilateral truncal vagotomy and HEINEKE-MIKULICZ type of pyloroplasty were performed, and simultaneously, 1 ml of 40% acetic acid was injected into the anterior wall of the angular incisure (acetic acid ulcer).

Non-Vagotomy-Ulcer group (5 dogs) : HEINEKE-MIKULICZ type of pyloroplasty alone was performed, and similarly acetic acid ulcer was produced.

The stomach was resected 2 weeks after each operation, and the area of the ulcer was measured. Histologically, the healing index of the ulcer was calculated on the preparation of Hematoxylin-Eosin stain[14] (Fig. 1).

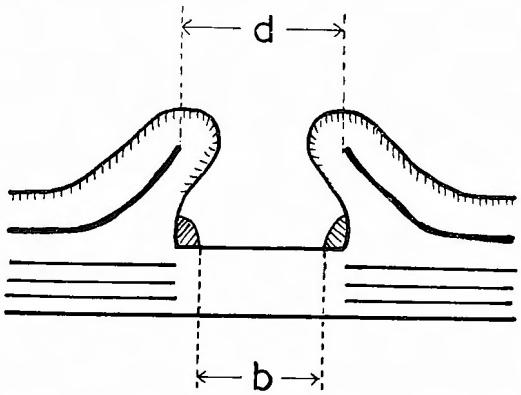


Fig. 1. Histological measurements and healing index.
d Distance of the ruptured muscularis mucosae.
b Length of the base of the ulcer.
The healing index (HI) $= \left(1 - \frac{b}{d}\right) \times 100$.

Experiment II. The changes of lysosomal enzyme activities due to vagotomy.

1) Assays of catheptic activities.

Thirty-seven adult mongrel dogs, weighing 7-21 kg, were used, and divided into 4 groups according to the types of vagotomy.

SPV group (7 dogs) ; selective proximal vagotomy was performed. SV group (5 dogs) ; selective gastric vagotomy with HEINEKE-MIKULICZ type of pyloroplasty was performed. TV group (5 dogs); bilateral truncal vagotomy with HEINEKE-MIKULICZ type of pyloroplasty was performed. Control group (sham-operated group, 5 dogs) ; the branches of the left gastric vessels were ligated carefully at their entrances into the gastric wall, not to impair the vagus nerves.

The stomach of each group was resected 2 weeks after operation and the mucosa was gathered and divided into 3 gastric portions ; the lesser curvature of the corpus, the greater curvature of the corpus, and the antrum. Catheptic activities of these mucosal tissues were determined. In order to study periodical changes of mucosal catheptic activities after SPV, 3 additional groups of SPV were produced as follows ; SPV-4-week group, SPV-6-week group, and SPV-8-week group, and the catheptic activities were determined at 4, 6, and 8 weeks after SPV.

Catheptic activity assays were determined by UCHINO's method[33]. Mucosal extract was produced in an ice bath as shown in Table 1, to which gelatin solution was added as substrate solution. This reaction mixture was incubated for 24 hours at 37° C. An increase of amino

Table 1. Technical procedure for the determination of catheptic activity

1. Enzyme solution
   a) Gastric mucosa, 10~30g
   b) Homogenize in a Waring blender after adding 3 volumes of glycerine water (1 : 1), while being kept cold, and mixed with 1/6 volume of toluene……4 C, 24 hours
   c) Centrifuge at 3000RPM for 15 minutes and filtered.
   d) The filtrate was used as the test material.
2. Substrate solution : Pure gelatin, 4% buffer solution.
3. Buffer solution : Mc ILVAINE's citrate buffer solution, pH 4.4
4. Reaction mixtures :

	The principal reaction mixture		The contrasted reaction mixture			
	I_0	I_{24}	II_0	II_{24}	III_0	III_{24}
Enzyme solution	2	2	2	2		
Glycerine water					2	2
Substrate solution	10	10			10	10
Buffer solution	10	10	20	20	10	10
Distilled water	1	1	1	1	1	1
(cysteine)	1	1	1	1	1	1
Toluene	1	1	1	1	1	1

(Unit : ml)

5. SOERENSEN's formoltitration.
   Enzyme Activity = $(I_{24}-I_0)-\{(II_{24}-II_0)+(III_{24}-III_0)\}$

acids which had been produced by degradation due to enzymatic reaction were measured by means of Soerensen's formoltitration. The activities of cathepsin was indicated by the volumes of N/10 NaOH solution used for titrating neutralization. And simultaneously, the reaction mixtures added by cysteine were also studied, which activates the cathepsin C activity peculiarly.

2) Assays of β-glucuronidase activities.

Fifteen adult mongrel dogs, weighing 7-21 kg, were used, and divided into the following 3 groups; SPV group (5 dogs), TV group (4 dogs), and Control group (6 dogs).

Two weeks after operation, β-glucuronidase activity of the mucosa of the lesser curvature of the corpus was assayed by means of p-nitrophenyl glucuronide colorimetry[22]. Tissue extract was obtained in the same manner as the assay of catheptic activity, and p-nitrophenyl glucuronide was used as the substrate. After the incubation of the reaction mixture for 4 hours at 37 C without deproteinization, transparent yellowish tone of p-nitrophenol which was produced by degradation due to enzymatic reaction was determined by colorimetry under an alkaline condition (Tab. 2).

Experiment III. Assays of hexosamine contents in the gastric mucosa.

Twelve adult mongrel dogs, weighing 9-14 kg, were used, and divided into 3 groups

Table 2. Technical procedure for the determination of ß-glucuronidase activity

1. Reaction mixture

	S(principal reaction mixture)	B(blank)
Acetic acid buffer	0.7	0.7
Substrate solution	0.1	
Enzyme solution	0.2	0.2

incubate for 4 hours at 37°C

Acetic acid buffer : 0.1M, pH 4.0

Substrate solution : 0.1M p-nitrophenyl glucuronide solution

Alkali reagent : 200ml of glycine buffer added by 50ml of 0.5 N NaOH

2. Add 2.0ml of alkali reagent to each tube after adding 0.1ml substrate solution to the blank tube.
3. Read optical density at 400mμ on a spectrophotometer
4. Standard curve was made by using p-nitrophenyl solution.

(4 dogs to every group) ; SPV group, TV group, and Control group.

Hexosamine contents in the mucosa of the lesser curvature of the corpus were assayed by means of the modified BOAS' method, 2 weeks after operation[4,11]

As shown in Table 3, the gastric mucosa was gathered in an ice bath and washed with iced saline solution, and preserved in acetone for 24 hours at 4°C. After that the mucosal tissue was homogenized in a Waring blender, and was defatted and dehydrated. Then the mucosal tissue was dried completely in a vacuum desiccator.

One hundred mg of these prepared materials was hydrolyzed and filtered.

Table 3. Technical procedure for the determination of hexosamine content in the gastric mucosa

0.2ml of each filtrate added with 0.8ml of H_2O

| 2ml of 2N HCl

Neutralization

| 1 drop of 1% phenolphthalein
| 4 N NaOH drop by drop until it turns red
| 0.5 N HCl, until the color disappears
| 1ml of 2% acetylacetone reagent

Boiling water bath, at 89-92°C for 45 minutes

| 2.5ml of ethylalcohol after cooling at room temperature

Mixture

| 1 ml of EHRLICH's reagent

Mixture

| total volume of sample makes up to exactly 10 ml with ethylalcohol

Reading optical density at 530 mμ on a spectrophotometer

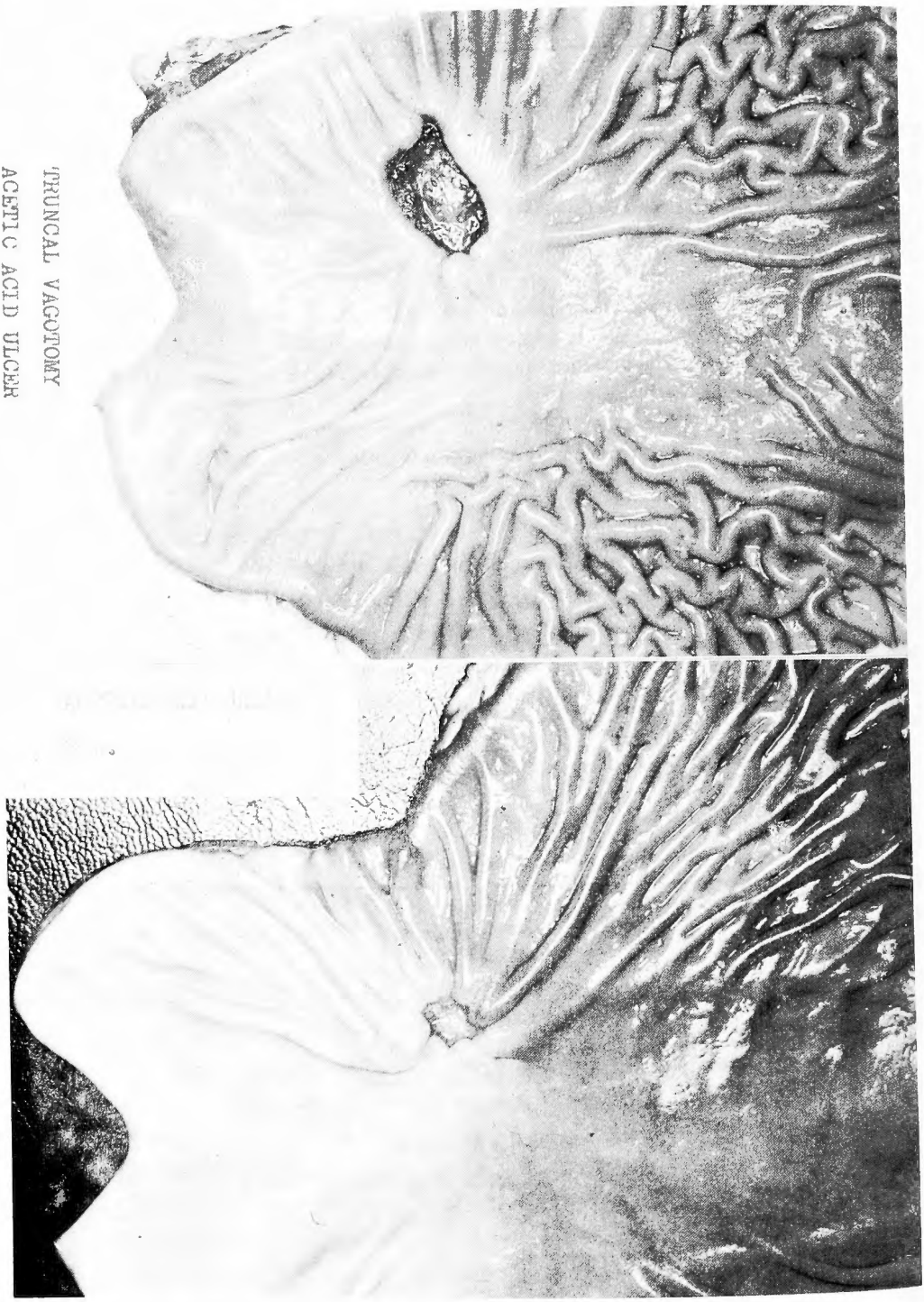
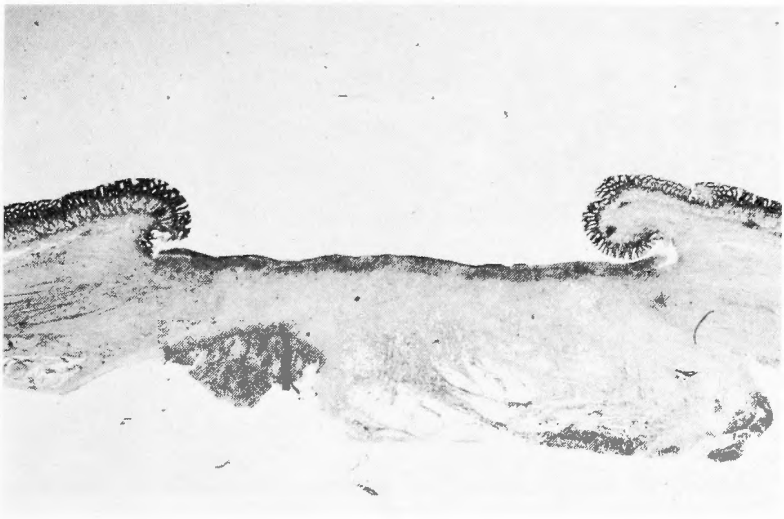
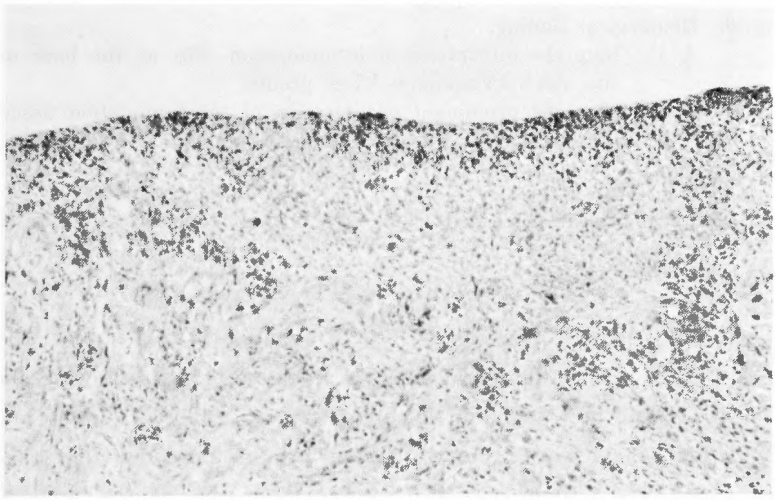


Fig. 2. Acetic acid ulcer 2 weeks after each operation.
A. Vagotomy-Ulcer group Note the huge ulcer.
B. non-Vagotomy-Ulcer group : Note the favorable protuberance of mucosa and the prominent convergence of the mucosal folds.



A



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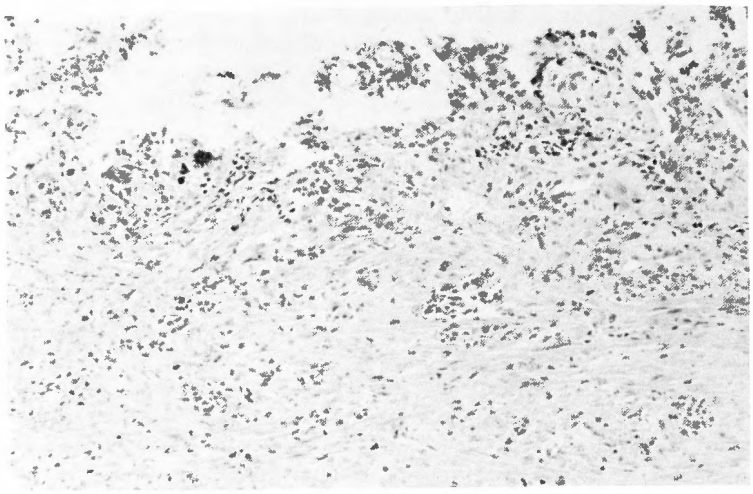
C



D

Fig. 3. Histological findings.
A, B Note the infiltration of inflammation cells at the base of the ulcer. (Vagotomy-Ulcer group)
C, D : Note the prominent proliferation of the granulation tissue and capillary infiltration. (non-Vagotomy-Ulcer group)

Hexosamine contents in these filtrates were determined according to the modified BOAS' method (Tab. 3).

Results

Experiment I. The areas of gastric ulcers 2 weeks after operation are shown in Table 4. The mean area of the non-Vagotomy-Ulcer group was $43.8 \pm 6.6 mm^2$ and that of the Vagotomy-Ulcer group was $135.0 \pm 3.8 mm^2$. These results indicated that the ulcer of the Vagotomy-Ulcer group was 3 times or more greater than that of the non-Vagotomy-Ulcer group 2 weeks after operation ($p<0.01$) (Fig. 2, Fig. 3).

Meanwhile, histologically, the distance of the segregated muscularis mucosae at the ulcer site (d) and the length of the base of the ulcer (b) were measured, and the healing indices were calculated (Fig. 1). The healing index of the non-Vagotomy-Ulcer group was 20.65 ± 3.62, and that of Vagotomy-Ulcer group was 6.20 ± 0.12 (Tab. 4). Distinctly, the healing index of the Vagotomy-Ulcer group indicated a lower value than that of the non-Vagotomy-Ulcer group ($p<0.01$).

Microscopically, at the base of the ulcer, prominent proliferation of the granulation tissue and capillary infiltration were seen in the non-Vagotomy-Ulcer group, but in the Vagotomy-Ulcer group the granulation was scarce (Fig. 3).

Experiment II.

1) Catheptic activities.

Catheptic activities of the mucosa of the lesser curvature of the corpus 2 weeks after

Table 4. Measurements of acetic acid ulcer

		area (mm^2)	d (mm)	b (mm)	Healing Index
Non-vagotomized group	1	31	9.3	6.8	26.9
	2	62	9.2	7.8	15.2
	3	38	9.2	7.4	26.9
	4	44	8.8	7.6	13.6
	$\bar{x}$	43.8			20.65
	SD	13.3			7.25
	SE	6.6			3.62
Vagotomized group	1	153	12.8	12.0	6.3
	2	117	12.3	11.5	6.5
	3	122	10.0	9.4	6.0
	4	148	18.9	17.4	6.0
	$\bar{x}$	135.0			6.2
	SD	7.5			0.24
	SE	3.8			0.12

various methods of vagotomy are shown in Table 5 and Figure 4. The value of each group was as follows; In the Control grou p0.296 $\pm$0.023, in the SPV group 0.414$\pm$ 0.020, in the SV group 0.392$\pm$0.008, and in the TV group 0.405$\pm$0.013. Each vagotomy group indicated higher value than that in the control group ($p<0.01$), but there was no significant difference among three vagotomy groups.

And there was a similar appreciable tendency in the reaction mixture without cysteine activation. In the next, the gastric mucosa was

Table 5. Catheptic activities of the corpus mucosa ; in relation to the methods of vagotomy

(N/10 NaOH ml)

Control			SPV		SV		TV	
		cysteine (−)		cysteine (−)		cysteine (−)		cysteine (−)
	0.222	0.203	0.394		0.366	0.264	0.417	0.307
	0.298	0.248	0.385		0.388	0.258	0.444	0.306
	0.273	0.202	0.432	0.364	0.417	0.325	0.394	0.320
	0.354	0.255	0.416	0.256	0.396	0.319	0.366	0.366
	0.331	0.255	0.478	0.301	0.394	0.263	0.405	0.287
			0.326	0.262				
			0.469	0.354				
$\bar{x}$	0.296	0.233	0.414	0.307	0.392	0.286	0.405	0.317
SD	0.051	0.028	0.052	0.050	0.018	0.033	0.029	0.030
SE	0.023	0.012	0.020	0.022	0.008	0.015	0.013	0.013

divided into 3 portions, the lesser curvature of the corpus, the greater curvature of the corpus, and the antrum. And the mucosal catheptic activities of each portion was compared between the Control and SPV groups. The catheptic activities of the lesser and greater curvatures of the corpus in each group indicated similar values, but those in the SPV group were higher than in the Control group. On the other hand, catheptic activities of the antral mucosa showed lower values in both groups (Tab. 6).

Concerning the catheptic activities of the antral mucosa, the enzyme activities indicated high values only in the TV group in which the denervation was thought to extend to the

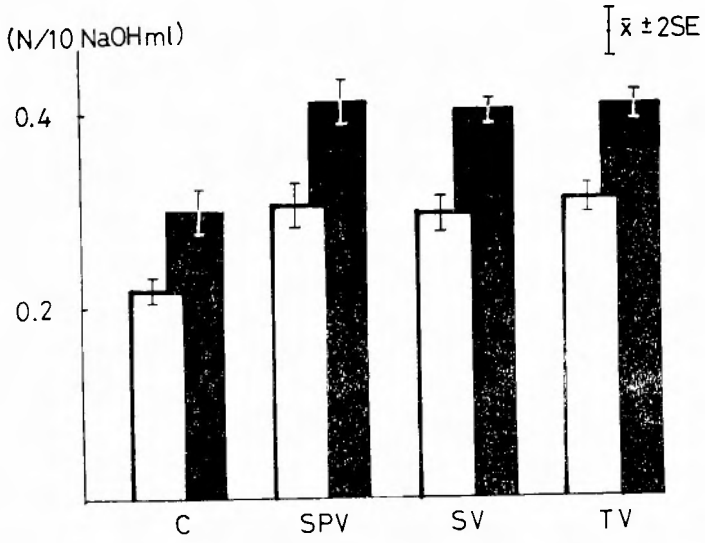


Fig. 4. Catheptic activities of the corpus mucosa after various methods of vagotomy. Solid bar graph showing the reaction mixture added with cysteine and white bar graph without cysteine. C ; control group, SPV ; selective proximal vagotomy group, SV ; selective vagotomy group, TV ; truncal vagotomy group. Each vagotomy group indicated higher activities than the control group ($p < 0.01$), but there was no significant difference among three vagotomy groups, and there was a similar tendency in the reaction mixture, with cysteine or without cysteine.

Table 6. Catheptic activities in comparison with each gastric portion (N/10 NaOH ml)

	Control			SPV		
	lesser curvature	greater curvature	antrum	lesser curvature	greater curvature	antrum
	0.222			0.394	0.425	
	0.298		0.132	0.385		
	0.273	0.251	0.182	0.432		0.168
	0.354	0.320	0.289	0.416		0.156
	0.331	0.339	0.150	0.478	0.403	0.253
				0.326		0.134
				0.469	0.380	0.271
x̄	0.296	0.303	0.188	0.414	0.403	0.196
SD	0.051	0.046	0.070	0.052	0.023	0.061
SE	0.023	0.027	0.035	0.020	0.013	0.027

Table 7. Catheptic activities of the antral mucosa

(N/10 NaOH ml)

	Control	SPV	TV
	0.132	0.168	0.269
	0.182	0.156	0.323
	0.289	0.253	0.205
	0.150	0.134	0.269
		0.271	0.254
$\bar{x}$	0.188	0.196	0.264
SD	0.070	0.061	0.042
SE	0.035	0.027	0.019

ml, in the SPV group 1518.6±53.5 u/10ml, and in the TV group 1570.5 ±59.1u/10ml. Enzyme activities in vagotomized groups were significantly higher than those in Control group.

Experiment III. Hexosamine contents

Hexosamine contents in 10mg of dry weight of the corpus mucosa 2 weeks after operation are shown in Table 10, and Figure 8 ; in Control group 285.95±14.46 μg/10 mg, in SPV group 238.20±3.93 μg/10mg, and in TV group 217.98

antral region (Tab. 7) (Fig. 5). As to the periodical changes of mucosal catheptic activities of the lesser curvature of the corpus after SPV, they continued to show high value until 6 weeks after operation, but 8 weeks after operation they indicated similar values to those in the control group (Tab. 8.) (Fig. 6).

2) β-glucuronidase activities.

β-glucuronidase activities in the mucosal extract of the stomach 2 weeks after operation are shown in Table 9, and Figure 7 ; In the Control group 1155.7±113.4 u/10

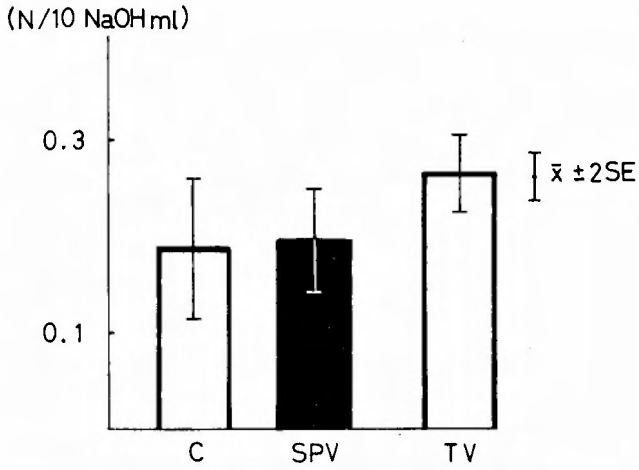


Fig. 5. Catheptic activities of the antral mucosa after various methods of vagotomy. Only in the TV group, the activities indicated high value ($p<0.01$).

Table 8. Catheptic activities of the corpus mucosa; in relation to the postoperative period

(N/10 NaOH ml)

	Control		SPV (2W)		SPV (4W)		SPV (6W)		SPV (8W)	
cysteine	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
	0.222	0.203	0.394		0.435	0.301	0.398	0.302	0.277	0.206
	0.298	0.243	0.385		0.397	0.260	0.366	0.352	0.303	0.225
	0.273	0.202	0.432	0.364	0.392	0.362	0.392	0.301	0.304	0.137
	0.354	0.255	0.416	0.256	0.389	0.257	0.374	0.283	0.337	0.253
	0.331	0.255	0.478	0.301	0.408	0.268	0.394	0.302	0.273	0.241
			0.326	0.262						
			0.469	0.354						
$\bar{x}$	0.296	0.233	0.414	0.307	0.404	0.290	0.385	0.308	0.299	0.212
SD	0.051	0.028	0.052	0.050	0.019	0.044	0.014	0.026	0.026	0.046
SE	0.023	0.012	0.020	0.022	0.008	0.020	0.006	0.012	0.011	0.020

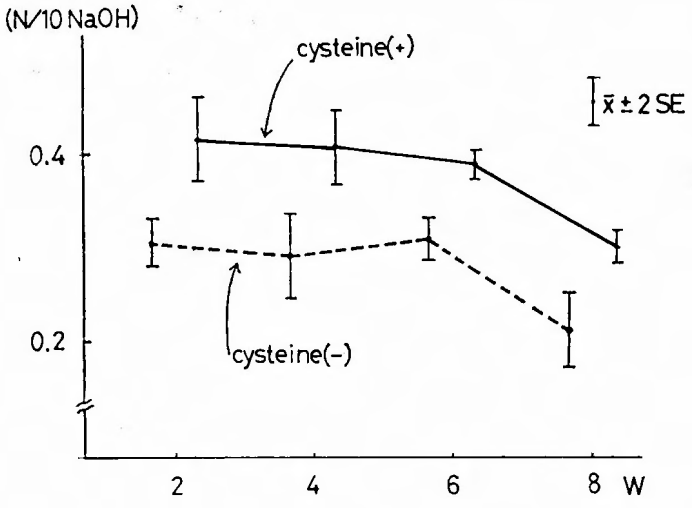


Fig. 6. Changes of catheptic activities of the corpus mucosa after SPV. The activities remained at a high value until 6 weeks after operation ($p<0.01$).

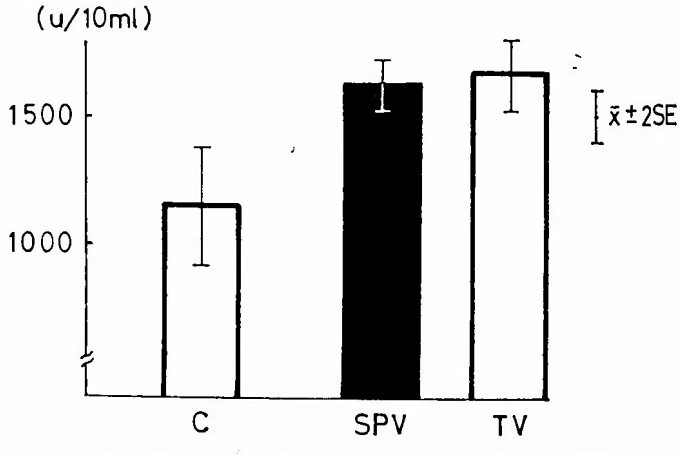


Fig. 7. β-glucuronidase activities of the gastric mucosa after various methods of vagotomy. The activities in vagotomized groups indicated a high value than the control group ($p<0.05$).

Fig. 7

Table 9. β-glucuronidase activities of the gastric mucosa

(u/10ml)

	Control	SPV	TV
	994	1531	1406
	1025	1500	1657
	1594	1593	1563
	1400	1641	1656
	1046	1328	
	875		
x̄	1155.7	1518.6	1570.5
SD	277.8	119.7	118.2
SE	113.4	53.5	59.1

Table 10. Hexosamine contents in the gastric mucosa

(μg/10mg)

	Control	SPV	TV
	312.5	245.0	212.5
	259.4	243.8	240.6
	309.4	235.9	221.9
	262.5	228.1	196.9
x̄	285.95	238.20	217.98
SD	28.92	7.85	18.27
SE	14.46	3.93	9.14

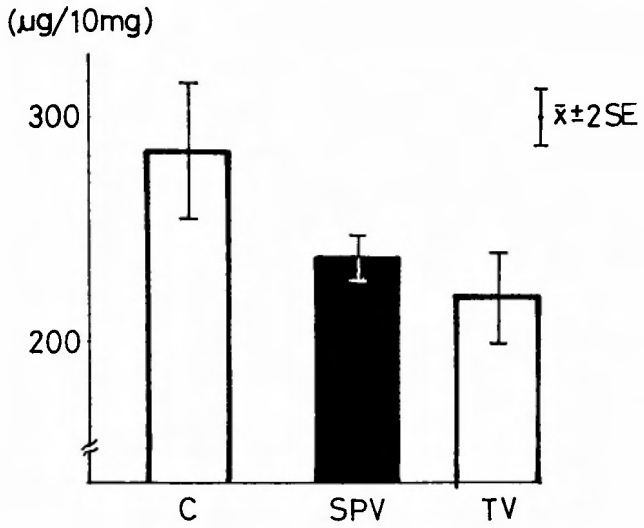


Fig. 8. Hexosamine content values in the gastric mucosa after various methods of vagotomy. The vagotomized groups indicated lower contents than the control group ($p<0.05$, $p<0.01$).

± 9.14 μg/10mg. Two vagotomized groups had lower contents than non-vagotomized control group ($p<0.05$).

Discussion

The vascular architecture of the gastric mucosa has been precisely investigated by BARCLAY, BENTLEY, BARLOW, and others[1,2,25], by using microangiographic techniques. According to these investigators, the gastric wall is supplied by vessels branching off from the arterial chains at the lesser and greater curvatures. Perforating the muscular layers, they ramify into a rich submucosal plexus. From this arterial net, mucosal arteries arise, and anastomosing partly below and partly above the muscularis mucosae, they finally divide into slender arterioles, perpendicularly perforating the mucosal membrane. Between these mucosal arterioles, a very rich capillary network is spun. The venous return is collected from this network and empties into straight mucosal veins perpendicularly penetrating the mucosal depth. These are drained finally by submucosal veins. And arteriovenous anastomoses were demonstrated in the submucosal layer which were under the control of autonomic nervous system. It was shown that they were closed upon vagal stimulation and opened upon sympathetic stimulation. It is a very interesting problem how the vagotomy affects the blood flow of gastric mucosa which have a peculiarity on vascular pattern like this.

As to the change of total gastric blood flow due to truncal vagotomy, PETER, SHIMAZU, et al.[27,32]. reported 35-50% reduction in acute experiments. Under histamine stimulation, ISHII recognized remarkable reduction of total gastric blood flow up to 76% due to vagotomy[16]. As to gastric mucosal blood flow, NAKAYAMA, ISHII, et al. reported that about 52%

reduction under histamine stimulation by vagotomy[21] They reported that the reduction in gastric mucosal blood flow was induced immediately by vagotomy and maintained for 3 months after nerve section. NYLANDER and OLERUD demonstrated that vagotomy induced ascending mucosal arterioles empty into the venous system at the mucosal surface directly without an intervening capillary net, and the occurrence of mucosal arteiovenous communications was thus established[24].

According to the aforementioned previous investigations, it was surely thought that vagotomy reduced the gastric mucosal blood flow. How this reduction in the mucosal blood flow affects the healing of the gastric ulcer, or how it affects the activities of the lysosomal enzymes which are activated under hypoxic state, are very important problems.

There are many methods for producing the chronic gastric ulcer, e. g. histamine ulcer, cinchophene ulcer, clamping-cortisone ulcer, formaline ulcer, gastric mucosa resection ulcer, acetic acid ulcer, etc., but there is no satisfactory method that can produce a similar ulcer as found in human ulcers.

Since acetic acid ulcer is produced by such a nonphysiological manner as an injection of acetic acid into the gastric wall or a coating of acetic acid to the gastric serosa, this method cannot be a way that pursues the pathogenesis of ulcer. But its histological findings after the occurrence of the ulcer quite resembles the human chronic ulcer, so it can be a suitable method for the assessment of the healing state of the ulcer[26].

According to this report, as to ulcer areas and healing indices, significant prolongation of the ulcer healing was shown in Vagotomy-Ulcer group in comparison with in non-Vagotomy-Ulcer group. Although from the viewpoint of offensive factors, it is beyond doubt that the non-Vagotomy group maintains much higher acid secretion than the Vagotomy group, there is significant prolongation of ulcer healing in the vagotomized group. These events imply that vagotomy plays some adverse roles in the genesis from occurrence of the ulcer to healing.

TORIUMI, et al. investigated the effect of vagotomy on ulcer healing using the acetic acid ulcer technique in rats, and reported the similar prolongation of ulcer healing due to vagotomy[34].

The lysosomes were discovered and designated by means of biochemical methods by de DUVE in 1955[10]. In the following year, NOVIKOFF, et al. presumed that the organelles containing electromicrographically dense bodies composed of lysosomes[23]. In the 1960's, by OGAWA, et al., histochemical and electromicrographical investigations of the lysosomes had been continued, and recently, the lysosomes draw great attention from the viewpoint of pathology and clinical medicine. The lysosomes are distributed over almost all of the constitutional cells with the exception of red cells, which have been designated as suicide bags originally, and containing about 40 acid hydrolytic enzymes. These acid hydrolases are said to be freed and activated by an increase of the permeability of the lysosomal membrane. In vivo, shock, ischemia, and the administration of the bacterial endotoxin may increase the permeability of the lysosomal membrane[19].

CLERMONT and WILLIAMS reported that experimentally acid phosphatase activity was increased in the serum and lymph during endotoxin, hemorrhagic, or cardiogenic shock, but which was inhibited by the administration of prednisolone, the stabilizer of the lysosomal membrane[5)6)7)35)]. MACHIEDO, et al. also recognized the efficacy of prostaglandin E_1 in experimental hemorrhagic shock which was thought to be a stabilizer of the lysosomal membrane as well. And they emphasized the important role of lysosomal enzymes in the pathogenesis of circulatory shock[18)].

TAKAHASHI reported that in clinical cases the catheptic activity in the intact part of the gastric wall of gastric ulcer patients was specifically enhanced in comparison with duodenal ulcer or gastric cancer. And experimentally, he had induced gastric ulcer by histamine-incjection in dogs, and recognized significantly higher activity of cathepsin in the gastric wall of the gastric ulcer group than those in nonulcer or normal control group, and concluded that concerning the pathogenesis of gastric ulcer the localized mucosal change played an important role, as compared with duodenal ulcer[33)].

According to the investigation of the stress and serotonin ulcer, KIRA recognized that in stress ulcer, the lysosomal enzyme, N-acetyl-β-glucosaminidase was activated in the superficial layer of the gastric mucosa accompanied with localized hypoxia. On the other hand, in the stress-serotonin group the circulatory disturbance attained a deeper layer and acid phosphatase activity was augmented in the lamina propria mucosae[17)].

FERGUSON, et al. recognized that in rats, plasma cathepsin D activity increased in the experimental ulcer induced by serotonin injection[12)].

Cathepsin is the protein splitting enzyme discovered by SALKOWSKI in 1890. There are 5 isomers of cathepsin, A, B, C, D, and E, due to SH-dependency, optimum pH, and substrate specificity. A ; optimum pH is 5.7 and cysteine is not necessary for activation, B ; optimum pH is 5.3 and cysteine is indispensable for activation, C ; optimum pH is 5.0-6.0, some degree of activation is shown without cysteine but with cysteine activation is enhanced, D ; optimum pH is 3.5, there is no effect with cysteine, E ; optimum pH is 2.5, no effect with cysteine[31)].

In the author's investigation, as the reaction mixture was rectified at pH 4.5, cathepsin C and D were assayed chiefly. And since the enzyme activity in the reaction mixture added by cysteine indicated higher value than that without cysteine, it was obvious that the enzyme reaction in this experiment was due to cathepsin.

From the result of the author's experiment, the catheptic activity had been increased by vagotomy, and it was thought that vagotomy induced the gastric mucosa more friable. There was no rise in catheptic activity of the antral mucosa in SPV group in which the vagal innervation to the antrum was preserved, and this result indicated that the rise in catheptic activity was precisely related to vagotomy.

The problem of how long the reduction in the gastric mucosal blood flow due to vagotomy maintained was controversial. DELANEY reported that its reduction was not maintained for more than 4 weeks[8)], but NAKAYAMA, et al. reported that its reduction was maintained

for at least 3 months after nerve section[21]. In this experiment, the catheptic activity 8 weeks after vagotomy indicated similar value to that in the control group. But concerning the problem whether this event reflects the improvement of gastric mucosal blood flow, further studies are necessary.

Beta-glucuronidase is a lysosomal enzyme as well, and was thought to be related to the hydrolysis of mucosubstances like other glycosidases[13]. Considering that a part of gastric mucosal barrier consists of mucosubstances, the rise of β-glucuronidase activity in gastric mucosa by vagotomy is very interesting. Mucosubstances are formed chiefly by acid mucopolysaccharide, mucoprotein, and glycoprotein. These mucosubstances all contain hexosamine, so in order to study wholly the state of mucosubstances in the gastric mucosa, the investigation of hexosamine assay is desirable.

KIRA recognized the disappearance of the stromal mucopolysaccharide in the lamina propria mucosae in the histochemical investigation of stress-serotonin ulcer[17].

WISE and BALLINGER clarified the 75% reduction in gastric soluble mucus secretion and the marked reduction in mucosal contents of gastric mucus constituents ; hexose, hexosamine, sialic acid, and fucose, by vagotomy in mongrel dogs[36].

In the author's experiment, a reduction in the hexosamine content attended with a rise of β-glucuronidase activity in gastric mucosa by vagotomy was recognized. It is obvious that these changes with a rise of cathepsin activity take a part in the prolongation of ulcer healing.

RITCHIE described that ischemia itself impaired the gastric mucosal barrier[29,30]. And he recognized that topical administration of 2-4-dinitrophenol, a labilizer of the lysosomal membrane, disrupted the mucosal barrier[28].

Formerly, according to DRAGSTEDT's description, it was thought that after truncal vagotomy for duodenal ulcer, gastric ulcer seldom occurred due to the hypergastrinemia caused by the stagnation in the antrum[9].

NAGAO, et al. reported the case in which three years after SPV for duodenal ulcer, an ulcer had occurred at the corpus of the stomach[20].

Based on the author's experimental observations the activities of lysosomal enzymes, chiefly cathepsin, were enhanced and mucosal hexosamine contents were reduced by vagotomy, along with the prolongation of the healing of the experimental gastric ulcer.

Conclusions

The influence of vagotomy upon ulcer healing and the change of the activities of lysosomal enzymes due to vagotomy were assessed, and the conclusions described below were obtained.

1) Vagotomy induced the prolongation of the healing of acetic acid ulcer from the viewpoint of the area and the healing index.

2) Catheptic activity assay of the corpus mucosa showed higher value in all vagotomized groups than in control group. Catheptic activity of the antral mucosa was low by nature,

but by truncal vagotomy it had risen, though in SPV and control groups it had maintained low values.

As to the periodical changes, the rise of this enzyme activity was maintained for 6 weeks.

3) Beta-glucuronidase activity assay indicated a high value in vagotomized groups the same as that in catheptic activity.

4) The hexosamine contents in the gastric mucosa was reduced in each vagotomized group.

From these results, as to the pathogenesis of postvagotomy ulcer, the rise of activities of lysosomal enzymes, chiefly cathepsin, and the reduction in hexosamine contents in the gastric mucosa are thought to play important roles.

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和文抄録

迷切後胃潰瘍の成因に関する研究

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迷切の塩酸およびペプシン分泌に対する抑制効果は明らかにされているが，一方迷切が胃粘膜の防御機構にどのような影響をあたえるかに関しては不明な点が多く，さらに迷切後胃潰瘍の成因も解明されていない．そこで実験的に，胃潰瘍を作成し，その治癒に対して迷切が，いかなる影響をあたえるかを検討した．また迷切による胃粘膜のライソゾーム酵素の活性の変化，および胃粘膜ヘキソサミンの含量の変化をも検討した。

実験1：雑種成犬を使用し，迷切潰瘍群（幹迷切に幽門形成を加え，胃角部に酢酸潰瘍を形成），非迷切潰瘍群（幽門形成を行い，胃角部に同様に潰瘍を形成）の2群を作成し，2週後に胃の潰瘍面積を計測し，また組織学的に，潰瘍治癒係数を算出した．

面積は，迷切潰瘍群では135.0±3.8mm²，非迷切潰瘍群では43.8±6.6mm²であり，迷切群は非迷切群に比して3倍以上の面積の広さを示した．治癒係数は，迷切潰瘍群では6.20±0.12，非迷切潰瘍群では，20.65±3.62であり，明らかに迷切群の方が低値を示した．

実験2：雑種成犬に各種迷切を行い，2週後に胃粘膜を採取し，胃体部および胃幽門洞部の粘膜カテプシン活性をゼラチンを基質として Soerensen のフォルモール滴定法で測定した．またβ-グルクロニダーゼ活性を p-ニトロフェニール・グルクロナイド比色法によって測定した．

胃体部粘膜カテプシン活性を中和滴定に要した N/10 NaOH 量で示すと，対照群では0.296±0.023，SPV 群では0.414±0.020，SV 群では0.392±0.008，TV 群では0.405±0.013であり，各迷切群はいずれも対照群に比して高値を示した．しかしながら各迷切群相互の間には有意の差は認めなかった．幽門洞部粘膜のカテプシン活性を対照群，SPV 群および TV 群について比較してみると，除神経が幽門洞部にもおよんでいると考えられる TV 群においてのみ活性が高値を示した．

胃体部粘膜のカテプシン活性を経時的にみてみると，術後6週までは高値を示したが，8週になると対照群と同様の値を示した．

術後2週のβ-グルクロニダーゼ活性は，対照群では1155.7±113.4u/10ml，SPV 群では1518.6±53.5u/10ml，TV 群では1570.5±59.1u/10ml であり，やはり迷切群において有意に活性が上昇していた．

実験3：各種迷切後2週の胃体部小彎側粘膜のヘキソサミン含量を Boas の変法によって測定した．対照群では285.95±14.46μg/10mg，SPV 群では238.20±3.93μg/10mg であり，TV 群では217.98±9.14μg/10mg であり，両迷切群は対照群に比して有意に低い含有量を示した．

迷切は実験潰瘍の治癒を明らかに遷延せしめた．また低酸素状態で活性化される酸性水解酵素であるライソゾーム酵素，特にカテプシンの活性は迷切によって増強した。胃粘膜の Barrier を構成すると考えられるムコ物質の構成成分であるヘキソサミンの胃粘膜含量は迷切によって減少した．このような事実から迷切はその発生に局所素因がより関与しうる胃潰瘍の発生を促し，その治癒を遷延せしめるものと考えられる．