

Enzymological Studies on Pathogenesis of Gastric Ulcer and Studies on the Causes of the Bleeding from Gastric Ulcer

by

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Part. 1 Enzymological studies on the pathogenesis of gastric ulcer

INTRODUCTION

The cause of gastric ulcer hasn't been clarified completely as yet. It is generally accepted that the changes in the gastric wall, which were caused by the acid-peptic digestion at the site of the regional circulatory disturbance, should gradually develop into chronic gastric ulcer. However, it is well known that the auto-tissue protein is not digested by the acid-peptic action in its living condition.

HENNING once pointed out that cathepsin, auto-tissue protein splitting enzyme, which is activated by hypoxia resulting from the circulatory disturbance and decomposes the protein in the weak acid reducing system, should play an important role in the occurrence of initial mucosal changes in gastric ulcer. The author has determined quantitatively the cathepsin activities in the gastric and duodenal walls of gastric ulcer, gastric cancer and duodenal ulcer in the clinical cases or in the experimentally induced histamine-ulcer in dogs.⁸⁾ On the other hand, the author has also investigated histochemically the distribution of cathepsin activities in the gastric wall of gastric ulcer.

Chapter 1. The distribution of cathepsin activities in the gastric walls of gastric ulcer

(I) Method of experiment

(1) Experimental material

The gastric walls of gastric ulcer in the clinical cases and in the experimentally induced histamine-ulcer in adult mongrel dogs ranging in weight from 7 to 15kg were used.

(2) Method inducing the experimental gastric ulcer⁴⁾

WANGENSTEEN, O. H. and others have succeeded in inducing erosion, acute ulcer and chronic ulcer in the gastric and duodenal walls of dogs and other animals by continuous intramuscular injections of histamine in beeswax.

(a) Method of preparing 1% oily histamine-injection

One g of histamine dihydrochloride $C_5H_9N_3 \cdot 2HCl$ was dissolved in 0.5 cc of distilled water to obtain its saturated solution. This solution was emulsified by shake after mixing with 97 cc of sesame-oil in the Japan pharmacopoeia, and 20 cc of emulsion, Emazol No. 410. Ten cc of this mixture was kept in each sterilized vial for the experimental use.

(b) Method of injection

In regard to the amount of 1% oily histamine-injection, YÖ²⁰⁾ (1954) reported that

injection of 2 mg per kg of body weight showed more effective stimulation in the gastric secretion than that of 4 mg per kg, and that the gastric secretion decreased following the administration of the latter amount of histamine because the dogs were emaciated by severe side effects.

Therefore, the former amount of histamine was injected into either side of the hips of hungry dogs alternatively every day. Then, the author made the dogs go without food for four hours at least.

WANGENSTEEN, O. H. and Yō reported that erosion and acute ulcer in gastric walls were induced when the histamine was administered in a relatively short period. As acute ulcer in early stages was used for the experimental purpose, and the histamine-injection was repeated for about seven days.

(3) Method of taking out pieces of tissue

As shown in Fig. 1, five small pieces of tissue were cut off from the pyloric and cardiac portions along the greater and lesser curvatures of the resected gastric wall of gastric ulcer. The author has made the cathepsin-staining sections from them.

(4) TAKAMATSU's staining method for cathepsin

The cathepsin-staining was carried out by means of TAKAMATSU's method, as shown in Tab. 1. Pieces of tissue were frozen by acetone-dry ice, fixated, dehydrated at a temperature under 10°C immediately after being cut off, and embedded in soft paraffine with the melting point of 48° C to 52° C to prevent cathepsin from decomposing. Solution for the

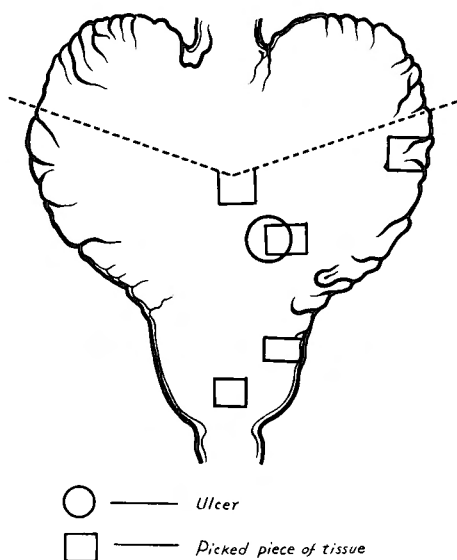


Fig. 1 Method of picking pieces of tissue

Tab. 1 TAKAMATSU's staining method for cathepsin

- (1) Pieces of fresh tissue are frozen by acetone-dry ice.
- (2) Fixate and dehydrate in acetone-alcohol solution at a temperature under 10°C for 48 hours.
- (3) Clear in xylene.
- (4) Embed in soft paraffine at a temperature under 53°C and harden at 4°C.
- (5) Cut sections 4~6 μ .
- (6) Attach section to slide.
- (7) Dehydrate.
- (8) Remove paraffine in xylene.
- (9) Remove xylene in 99% alcohol.
- (10) Dehydrate.
- (11) Immerse for 24 hours in a prepared substrate mixture at 37°C.
- (12) Wash in water.
- (13) Dehydrate in alcohols.
- (14) Clear in xylene.
- (15) Mount in balsam.

Tab. 2 Composition of substrate solution

gelatine	1 g
0.1% methylene blue solution	2 cc
distilled water	200 cc

The reaction of this mixture is rectified to pH 4.5 by adding N/10 HCl or NaOH solution.

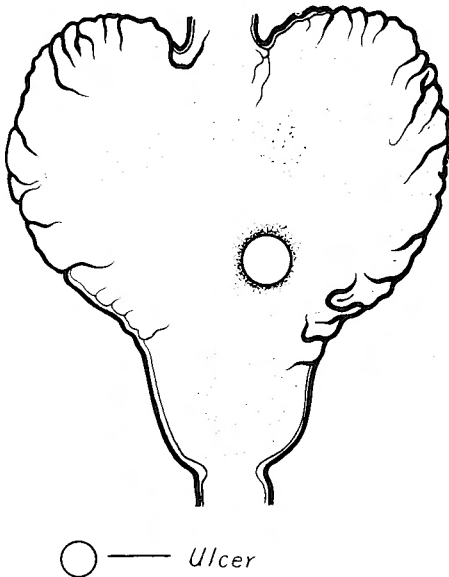


Fig. 2 Expected distribution of cathepsin in the lesion of gastric ulcer

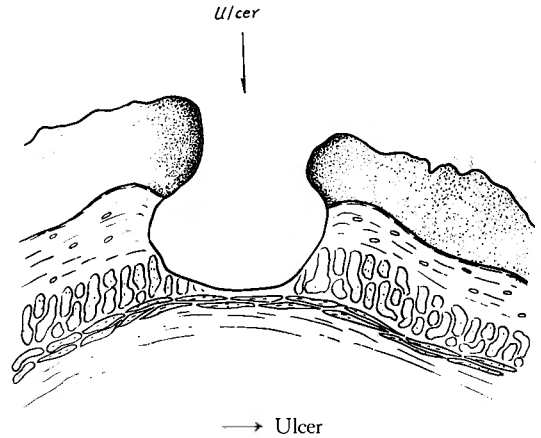


Fig. 3 Expected distribution of cathepsin in the lesion of gastric ulcer

staining, as shown in Tab. 2, contains gelatine and 0.1% methylene-blue solution. When this solution comes into contact with the tissues, gelatine is decomposed into the lower molecules by the proteolytic action of cathepsin. Thus, methylene-blue combining with decomposed gelatine becomes isolated and the richer a part is in cathepsin activity, the more deeply it is stained by methylene-blue.

(II) Experimental results

The histochemical preparations of cathepsin showed that (1) in all of them only the mucous membrane was stained deeply, so that the distribution of cathepsin activities there were dense. To the contrary, other parts were hardly stained, where its activities were scanty. (2) Staining density was nearly identical in every portion of the mucous membrane and no deep staining in the area of gastric ulcer was demonstrated. Cathepsin-staining of normal gastric wall is shown in Photo 1.

Chapter 2. Measurement of cathepsin activity in the gastric and duodenal walls

(I) Experimental method

(1) Experimental material

The gastric walls of gastric ulcer, duodenal ulcer, gastric cancer and other diseases in the clinical cases, and the gastric and duodenal walls in the experimentally induced histamine-gastric ulcer in dogs and normal gastric and duodenal walls of dogs were used.

(2) Experimental method

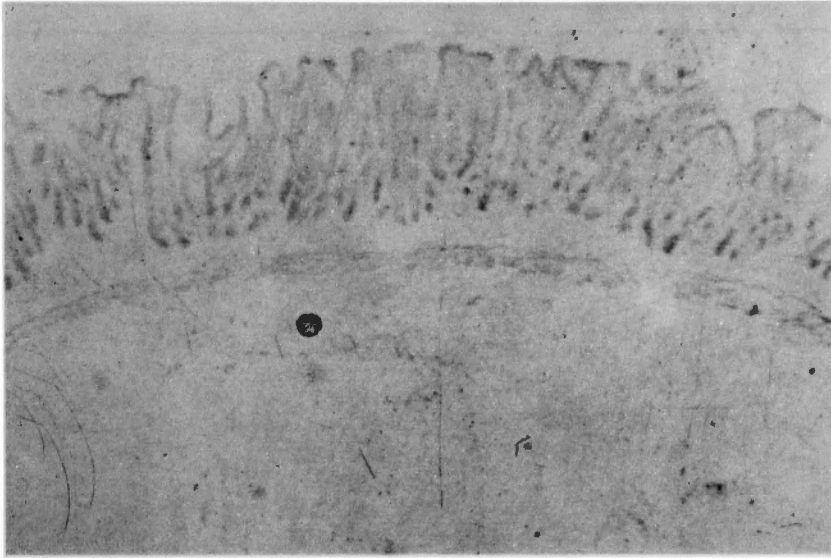


Photo 1 Histochemical appearance of cathepsin in normal gastric wall

(a) Enzyme solution. As shown in Fig. 4, about 30g of the gastric wall was taken out, cut into pieces, and homogenized while being kept cold, in a Waring blender, after adding 3 volumes of glycerine water (1:1). The homogenate was mixed with 1/6 volume of toluene, put into the refrigerator for 24 hours and filtered. The filtrate was used as the test material. Enzyme solution was also prepared from the total length of duodenum in the same manner.

(b) Substrate solution. Pure gelatine was used by dissolving it in a buffer solution at the rate of 4%.

(c) Buffer solution. MC ILVAINE's citrate buffer solution (pH 4.0~4.5) was used.

(3) Method of measurement

Each solution was mixed at the rate, as shown in Tab. 3. I (A and B) was the principal reaction mixture. With II (C and D) and III (E and F), the acidity increase due to self-digestion of enzyme and the substrate itself were measured. After the reaction was rectified to pH 4.5, they were incubated for 24 hours at 37° C, and 5.0 cc of the reaction mixtures were drawn out with a pipette before and after the incubation to which 1 % Phenol-phthalein was added as an indicator and according to SOERENSEN's formol-titration, their acidities were measured. If I_0 , II_0 and III_0 show 0 hour values and if I_2

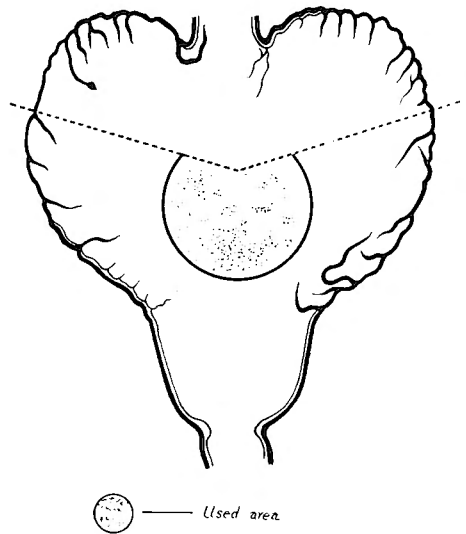


Fig. 4 Area used for the determination of catheptic activity

Tab. 3 Method of making the reaction mixtures

Composition	The principal reaction mixture		The contrasted reaction mixture			
	I		II		III	
	A	B	C	D	E	F
Enzyme solution	2	2	2	2		
Substrate solution	10	10			10	10
Buffer solution	10	10	20	20	10	10
Distilled water	1	1	1	1	1	1
Glycerine water					2	2
Toluene	1	1	1	1	1	1

(Unit : cc)

II₂₄ and III₂₄ show 24 hour values, enzyme activity is calculated by means of the following formula.

Enzyme activity = (I₂₄-I₀) - {(II₂₄-II₀) + (III₂₄-III₀)}

(II) Experimental results

Catheptic activities measured in clinical cases are shown in Tab. 4. It is worthy of note that the cathepsin activity in the gastric wall of gastric ulcer was specifically enhanced, as compared with those of gastric cancer and duodenal ulcer, and as shown in Tab. 5, those of the experimentally induced histamine-gastric ulcer in dogs were also specifically enhanced, as compared with those of non-histamine-ulcer group and normal group.

Among the catheptic activities in the duodenal walls of the histamine-ulcer, non-histamine-ulcer and normal group, no remarkable differences were demonstrated.

Tab. 4 Catheptic activities (Clinical cases)

No.	Age	Sex	Clinical diagnosis	Histological diagnosis	Gastric acidity before operation		Catheptic activities (N/10 NaOHcc)
					Total acidity	Free acidity	
1	37	♂	gastric ulcer	ulcer	45	35	0.279
2	65	♂	gastric ulcer	ulcer	85	68	0.248
3	11	♂	gastric ulcer	ulcer			0.255
4	52	♂	gastric ulcer	ulcer			0.219
5	47	♂	gastric cancer	adenocarcinoma	25	13	0.050
6	46	♀	gastric cancer	carcinoma simplex			0.067
7	61	♂	gastric cancer	adenocarcinoma	- 8	- 10	0.168
8	61	♀	gastric cancer	adenocarcinoma	7	0	0.128
9	61	♂	gastric cancer				0.121
10	32	♂	duodenal ulcer		129	95	0.143
11	28	♀	duodenal ulcer		81	48	0.113
12	25	♂	duodenal ulcer		70	61	0.129
13			duodenal ulcer				0.121
14			duodenal ulcer				0.117
15	29	♂	duodenitis		44	32	0.140
16	26	♂	pyloric syndrome	inflammation			0.132

Tab. 5 Catheptic activities (Dogs)

No.	Color	Sex	Weight of body (kg)	Duration of histamine injection (days)	Occurrence of ulcer	Catheptic activities (N/10 NaOHcc)	
						Stomach	Duodenum
1	brown	♀	8	7	+	0.233	—
2	brown	♂	10	6	—	died	—
3	white	♂	9	6	—		
4	white	♀	9	7	—	0.196	0.141
5	dark brown	♂	9	7	+	0.214	0.170
6	brown	♀	11	8	—	—	—
7	brownish white	♀	12	0	—	0.157	0.155
8	brown	♂	13	0	—	0.132	0.170
9	white	♂	10	0	—	0.151	0.139

In No. 9 cytochrome c (20mg/day) and FAU (10mg/day) were injected for four days.

DISCUSSION

The theories¹¹⁾ concerning the pathogenesis of gastric ulcer may be classified roughly into two groups, in which local or general factors are taken into consideration, respectively. The theories, in which such causes, as digestion, circulatory disturbance, gastritis, infection, mechanical stimuli etc., are taken into consideration, belong to the former, and those, in which such causes, as nervous or endocrine imbalance, nutritional imbalance, allergy etc., are taken into consideration, belong to the latter. However, no theory can clarify the cause of the gastric ulcer by itself. It is generally accepted that as a result of acid-peptic digestion on locus minoris resistentiae in the gastric mucosa, which is induced by the combination of several factors, the gastric ulcer should be induced and its healing should be retarded by mechanical and chemical stimuli, finally developing into chronic ulcer. However, JOHN HUNTER and others mentioned that the living gastric wall is not digested by the acid-peptic action, and further, although the action of pepsin disappears in the reaction under pH 4~5, the acidity of gastric juice is not always high, but often low in the gastric ulcer. Therefore, digestive action of the acid-pepsin is not thought to be the definitive factor in its occurrence.

Cathepsin¹⁾²⁾³⁾⁷⁾¹⁴⁾⁶⁾, is the auto-tissue protein splitting enzyme discovered by SALKOWSKI in 1890, and at first it had been so named and studied in detail by WILLSTÄTTER & BAMANN in 1929. This enzyme is contained within every tissue of animal, especially abundant in liver, spleen, kidney and gastric mucous membrane, is the mixture of many peptidases and proteinases, and its optimal pH ranges from 4.0 to 5.0. According to WALDSCHMIDT and LEITZ, cathepsin is in a non-activated state in normal tissues, but will be activated by the stoppage of the living phenomenon of the tissue, leading to autolysis. KANZAKI has maintained that cathepsin is perfectly active even in a living body, and under the influence of the environment according to the living phenomenon, functions either decomposingly or synthetically. Furthermore, so far as the physical nature of the environment stays within a definite scope, it does not function decomposingly and, being apparently inactive, will function decomposingly in an abnormal condition, beyond the normal scope. Some scholars had already suggested that cathepsin in the gastric mucosa should

play an important role in autolysis of gastric wall, and therefore, it should be the principal cause of gastric ulcer. TAMESUE¹⁷⁾ (1934), examining the autolysis of gastric mucosa-gruel in the clinical cases, maintained that the autolysis of gastric mucosa was carried out by catheptic enzyme, and therefore catheptic action should play an important role in the developmental mechanism of gastric ulcer. On the other hand, HENNING⁵⁾ (1956), considering an important role of cathepsin in the occurrence of initial mucosal changes in gastric ulcer, has maintained that autolysis of tissues will be brought about, when the gastric cathepsin is activated by the circulatory disturbance, inclining to the reduction side of local oxidized reduced electric potential and the lowering of local pH because of the accumulation of lactic acid and other oxides. MATSUO⁹⁾ (1936) also supported the above-mentioned theory. As this theory is not yet demonstrated in the real developmental process of gastric ulcer, the author has investigated histochemically the distribution of catheptic activities in the gastric ulcer by means of TAKAMATSU's method. At first, the author had expected that cathepsin should show the dense distribution in the lesion of gastric ulcer, as shown in Figs. 2 and 3, especially in its initial mucosal changes. However, no definitely localized increased distribution of cathepsin activities was demonstrated in the lesion of gastric ulcer. It showed the uniform distribution throughout the gastric mucosa.

As the circulatory disturbance may be induced by the changes of the mucosal capillary which are concerned with such general factors as the imbalance of nervous or endocrinological control, allergy etc., the circulatory disturbance in gastric mucosa and the occurrence of gastric ulcer always should be multiple. However, the fact is contrary to the above.

Therefore, it is necessary to presume other factors which produce the circulatory disturbance or the decline of resistance in the localized area in the gastric mucosa. After investigating in detail the structure of gastric blood-vessel, NOGAKI had clarified that there are the spiral and rope-like tortuous arteries in the mucosa and submucosa of adult stomach, and that they are more frequently demonstrated in the cases of gastric ulcer, and moreover, distribute in the predilection site for gastric ulcer. From this point of view, he inferred that they should play an important role in the occurrence and delayed healing of gastric ulcer, by disturbing the regional circulation.

Öi¹³⁾ has also mentioned that the junction between the non-parietal cell area and the parietal cell area is an embryologic, congenital juncture and corresponds to locus minoris resistentiae. From this point of view, it is supposed as follows :

Although the distribution of cathepsin activities in the gastric wall is diffuse and uniform, initial localized mucosal changes in the gastric wall are brought about by activated catheptic activities, induced by the marked local circulatory disturbance, originating in the congenitally specific construction of blood vessel in the predilection site for gastric ulcer, in which, of course, locus minoris resistentiae at the junction area also should be concerned.

Matsuo¹⁰⁾ had measured catheptic activities in every portion of the digestive tract. The author has measured the catheptic activities of the gastric walls of the peptic ulcer in the clinical cases and compared them with those in gastric cancer and duodenal ulcer. On the other hand, having compared those of histamine-ulcer in dogs with those of normal

stomach, it has been demonstrated that the cathepsin activity of the gastric wall of gastric ulcer was specifically enhanced. Although it may be too soon to conclude that cathepsin should be concerned with the occurrence of peptic ulcer, the above-mentioned results confirm the author's conclusion.

CONCLUSION

It is generally accepted that the most important peptic-ulcer-abetting agency is the acid-peptic action of gastric juice. However, it is well known that the living auto-tissue protein is not digested by the acid-peptic action. From this point of view, the author supposed that cathepsin, auto-tissue protein splitting enzyme, which is activated by hypoxia resulting from the circulatory disturbance and functions in the weak-acid reducing system, should play an important role in the occurrence of initial mucosal changes in gastric ulcer. Therefore, histochemical and quantitative investigations of cathepsin activity in the gastric wall of gastric ulcer in the clinical cases and in the experimentally induced histamine ulcer in dogs were carried out.

The results obtained are as follows :

(1) In the clinical cases, the catheptic activity in the gastric wall of gastric ulcer was specifically enhanced, as compared with those of gastric cancer and duodenal ulcer, etc..

(2) The catheptic activity in the gastric ulcer induced by histamine-injection in dogs was specifically enhanced, as compared with those of non-histamine-ulcer group and normal group etc..

(3) Even in a comparatively fresh gastric ulcer, cathepsin distribution in the gastric wall is not specifically dense in the lesion of gastric ulcer, but unexpectedly, it was uniform throughout the gastric wall.

From these results, it is supposed that although the catheptic distribution in the gastric wall, especially dense in mucosa, is diffuse and uniform, and that the initial localized mucosal changes in the gastric wall are caused by the enhanced catheptic activities which are induced by the marked local circulatory disturbance, originating in the congenitally specific construction of blood vessel in the predilection site for gastric ulcer, with which "locus minoris resistentiae in the junction" also should be concerned, and moreover, the change should develop into gastric ulcer.

Part 2. Studies on the cause of the bleeding from gastric ulcer

INTRODUCTION

Regarding the pathogenesis of massive bleeding from gastric ulcer, the following questions still remain unsettled.

(1) Although it seems plausible that the first to fall a victim to the digestive action of gastric juice should be the vein with its thin wall, followed later by the artery, why should gastric hemorrhage be usually of arterial origin ?

(2) It is accepted that the bleeding from gastric ulcer occurs mostly in the domain of A. gastrica sinistra, but why so ?

Regarding these questions, up to the present, various experimental discussions and theories have been proposed, but they have not yet been clarified completely. Therefore, the author has carried out the following investigations concerning them.

Chapter 1. Why should the bleeding from gastric ulcer occur mostly in the domain of *A. gastrica sinistra*?

Section 1. Measurement of blood pressure in *A. gastrica sinistra et dextra* and *A. gastroepiploica sinistra et dextra*

In regard to the reason why the bleeding from gastric ulcer occurs mostly in the domain of *A. gastrica sinistra*, it is supposed that the blood pressure in *A. gastrica sinistra* should be relatively high because the course of this artery is shortest among the four gastric arteries originating in *A. coeliaca* or aorta. Therefore, the author has measured the blood pressure in four gastric arteries in dogs and compared with each other.

(I) Experimental method

Adult mongrel dogs ranging in weight from 10 to 24 kg, put under intravenous general anesthesia with Nembutal (per kg 25mg), were used. From mercury manometers with polyethylene-tubes, 40 cm in length, were prepared as an experimental apparatus. Two cc of POLYRAN-meito INJ. (dextran sulfate) was used as an anticoagulant. The tips of these tubes were inserted into the proximal origins of the second branches of *A. gastroepiploica dextra* and *A. gastrica dextra et sinistra*, and the proximal origin of the first branch of *A. gastroepiploica sinistra* from the peripheries of each artery respectively using four mercury manometers connected respectively with the lumens of four arteries, their blood pressure was simultaneously measured.

(II) Experimental results

The blood pressures measured in *A. gastrica sinistra et dextra* and *A. gastroepiploica sinistra et dextra* are shown in Tab. 6.

Tab. 6 Blood pressure in gastric arteries in dogs

No.	Color	Sex	Weight of body	Blood pressure in gastric arteries (mmHg)			
				<i>A. gastrica sinistra</i>	<i>A. gastrica dextra</i>	<i>A. gastroepiploica sinistra</i>	<i>A. gastroepiploica dextra</i>
1	brown	♂	13	98	96	84	84
2	white	♂	24	150	148	144	144
3	dark brown	♀	21	146	148	140	138
4	brown	♂	13	134	134	128	128

(1) The blood pressures in *A. gastrica sinistra et dextra* were somewhat higher than those of *A. gastroepiploica sinistra et dextra*.

(2) The blood pressures in the arteries of the same name on both sides showed nearly the same values.

(III) Summary

A. gastrica sinistra et dextra and *A. gastroepiploica sinistra et dextra* are very small in diameter, but *A. gastroepiploica dextra* is relatively thick. Although a large dog, 24 kg in weight, and the polyethylene tube, about 1 mm in inside diameter, were used, the insertion of tubes into three gastric arteries was very difficult and took considerable time.

Therefore, the results obtained mean only the relative values. Eventually, as shown in Tab. 6, it was not demonstrated that the blood pressure in *A. gastrica sinistra* was the highest among them and that the blood pressure in *A. gastroepiploica sinistra*, having

the longest inside diameter, was especially high.

Chapter 2. Why should the bleeding from gastric ulcer be usually of arterial origin?

Regarding the reasons why the bleeding from gastric ulcer is usually of arterial origin, difficulty in thrombus-formation etc., in connection with blood pressure and blood flow, is also considered. However, the author, supposing that it is due to the difference of the chemical composition between arterial and venous walls, has carried out the following experiments. Of course, regarding the blood vessel-system in which the gastric bleeding originates, there are intramucosal capillary vessels in gastritis, submucosal blood vessel net in shallow gastric and duodenal ulcer, small blood vessels which pass through the wall along the lesser curvature of the stomach, especially the posterior wall a little proximal from pars angularis and the posterior muscle layer of duodenum, in chronic ulcer, the arteries outside the gastric wall and the blood vessels in such parenchymatous organs as liver, pancreas etc. and others. But the blood vessel system outside the gastric wall was used as the following experimental object.

Section I. Resistance of proteins purified from the arterial and venous walls against the acid-peptic digestion

(I) Experimental method

(1) Purifying method of proteins composing the arterial and venous walls

The arterial and venous walls of an adult dog were taken out to the greatest extent and each one was cut into pieces and homogenized while being kept cold in a Waring blender, after adding 2 volumes of distilled water. The homogenate was kept in a refrigerator for 24 hours, and the supernatant fluid was separated by 2000 R. P. M. of centrifugation for 20 minutes.

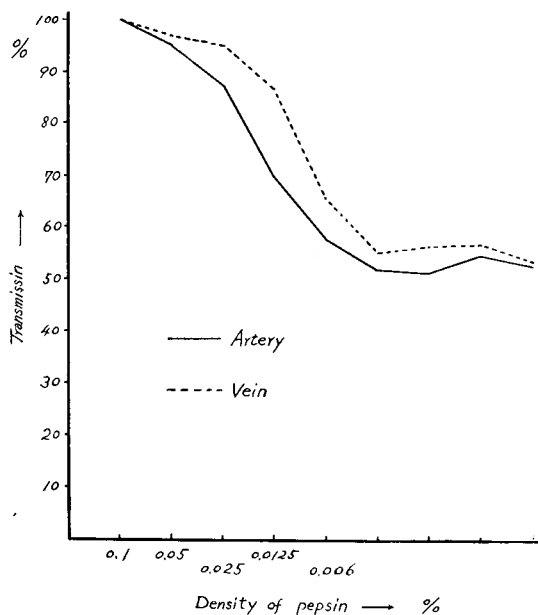


Fig. 5 Resistance of proteins purified from the arterial and venous walls against the acid-peptic digestion (Case 1)

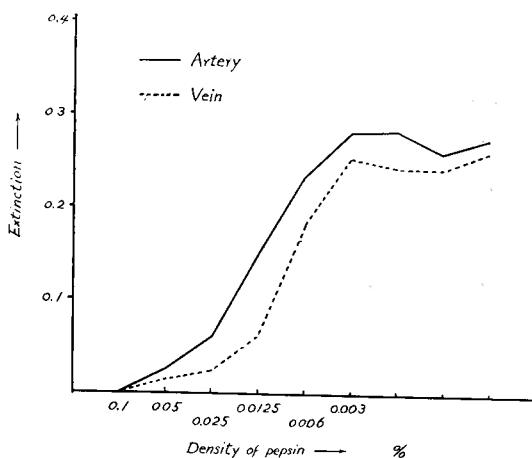


Fig. 6 Resistance of proteins purified from the arterial and venous walls against the acid-peptic digestion (Case 1)

When this fluid was made into 70% alcohol solution by adding 99 % alcohol, sedimentation of protein was brought about. This protein, filtered by the negative suction, and freed of water and fat with pure alcohol and ether, was used as experimental material.

(2) Measurement of resistance of proteins against the acid-peptic digestion

Immediately after 0.03 g of protein was dissolved in 10 cc of N/10 NaOH solution,

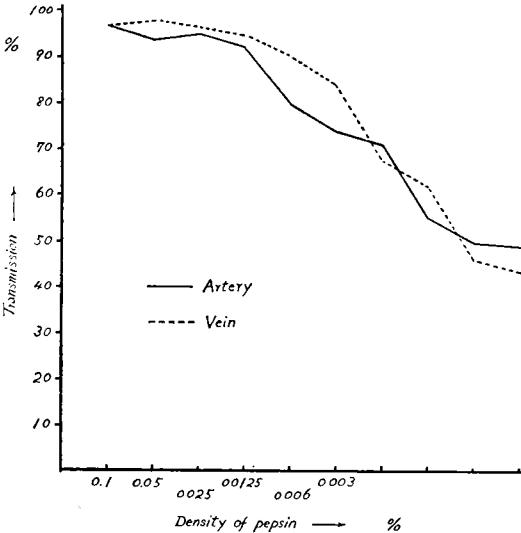


Fig. 7 Resistance of proteins purified from the arterial and venous walls against the acid-peptic digestion (Case 2)

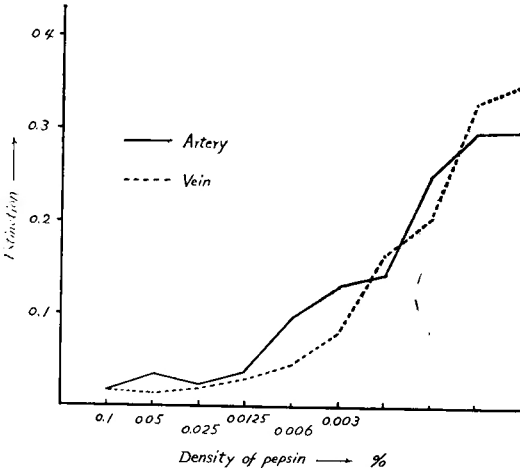


Fig. 8 Resistance of proteins purified from the arterial and venous walls against the acid-peptic digestion (Case 2)

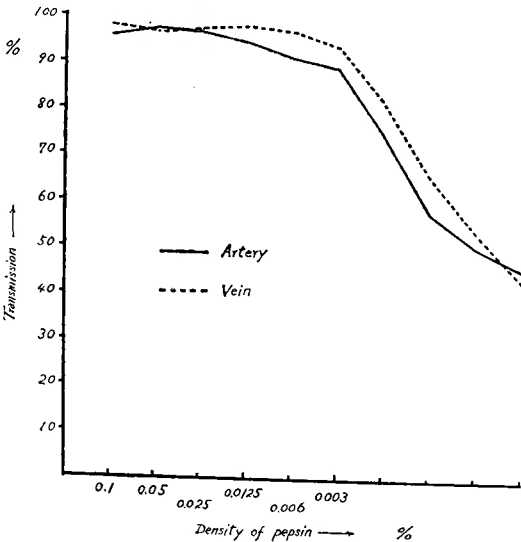


Fig. 9 Resistance of proteins purified from the arterial and venous walls against the acid-peptic digestion (Case 3)

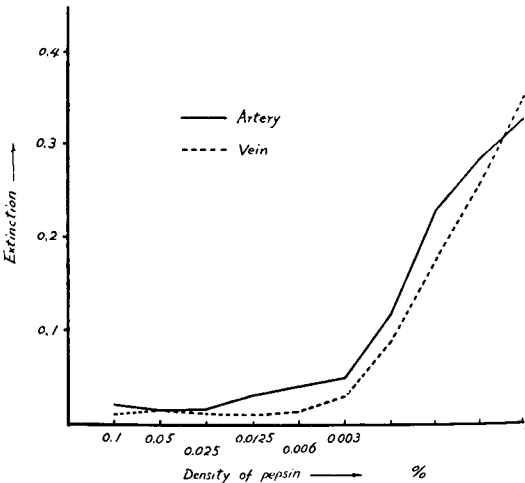


Fig. 10 Resistance of proteins purified from the arterial and venous walls against the acid-peptic digestion (Case 3)

20 cc of N/10 HCl solution was added. Two cc of protein, taken with a pipette from each test tube and added to pepsin (pepsin ($\times 2$) N. B. C. made in U. S. A.) solution in various densities, was rectified to pH 1.6 and incubated for 30 minutes at 38°C. After adding 0.5 cc of 10 % sulfosalicylic acid solution, white turbidity produced by the non-decomposing protein was determined colorimetrically with 640 m μ wave length of monochromatic light from the tungsten light source.

(II) Experimental results

The extent to which protein of the blood vessel was digested by the pepsin solution of various densities was measured colorimetrically and shown by extinction and transmission in Figs. 5 to 10. From these results, the resistance of proteins purified from the arterial and venous walls against the acid-pepsin showed no significant difference between them.

Section 2. Changes of the arterial and venous walls caused by the acid-peptic digestion

The histological changes, which the arterial and venous walls had suffered from the acid-peptic digestion, were compared with each other.

(I) Experimental method

Lienal artery and vein in dogs cut into sections of about 1 to 1.5 cm in length and both their ends were ligated with silk threads. They were steeped in 3 % pepsin solution at pH 1.4, and incubated for definite hours at 38°C. And then hematoxyline-eosin staining sections were made of them.

(II) Experimental results

In Photo 2, the histological appearances of A. et V. lienalis which were steeped in 3% pepsin solution at pH 1.4 for 5 hours at 38° C, are shown. In the venous wall, all the layers were digested by the acid-peptic action and only a part of the wall enclosed by connective tissue remained in its original shape. To the contrary, in the arterial wall,



Photo 2 Histological appearances of lienal artery and vein steeped in 3% pepsin solution at pH 1.4 and incubated for 5 hours at 38°C.



Photo 3 Histological appearances of lienal artery and vein incubated for 6 hours under the same condition as in Photo 2.

the adventitia and most of the media were digested, but all the layers were not broken by the acid-peptic action. In Photo 3, the histological appearances of lienal artery and vein, which were incubated for 6 hours under the same condition as in Photo 2, are shown. Lienal artery, which previously was free of the breakdown of all the layers, was digested completely. From these results, it was demonstrated that the arterial wall was more difficult to be digested than the venous wall when they were subjected to the acid-peptic action. However, the difference in the resistance between arterial and venous walls is thought to be due to the difference in their thickness, especially of media.

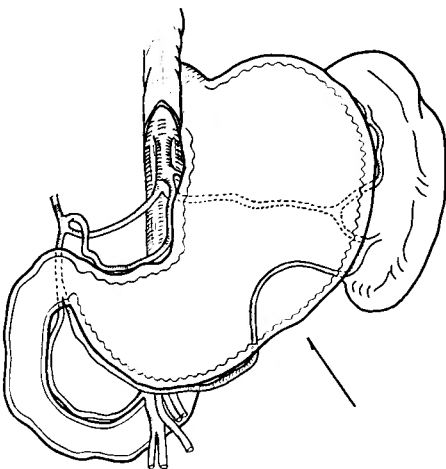


Fig. 11 The main trunk of *A. gastroepiploica sinistra* was introduced into the gastric cavity without disturbing the blood stream through it.

Section 3. Changes of the arterial and venous walls caused by gastric juice.

Although the experiment in Section 2 was carried out *in vitro*, the following experiment was carried out *in vivo* with the same purpose.

(I) Experimental method

Adult mongrel dogs ranging about 10 kg in weight put under intravenous general anesthesia with Nembutal (per kg 25 mg), were used. *A. et V. gastroepiploica sinistra* were isolated 8 to 10 cm in length and introduced into the gastric cavity without disturbing the blood stream through it, passing through the incised wound which was made at the anterior gastric wall, in parallel with and along the greater curvature, as illustrated in Fig. 11. The spleen was sutured to the surface of the stomach to prevent the pull on the embedded blood vessels. These blood

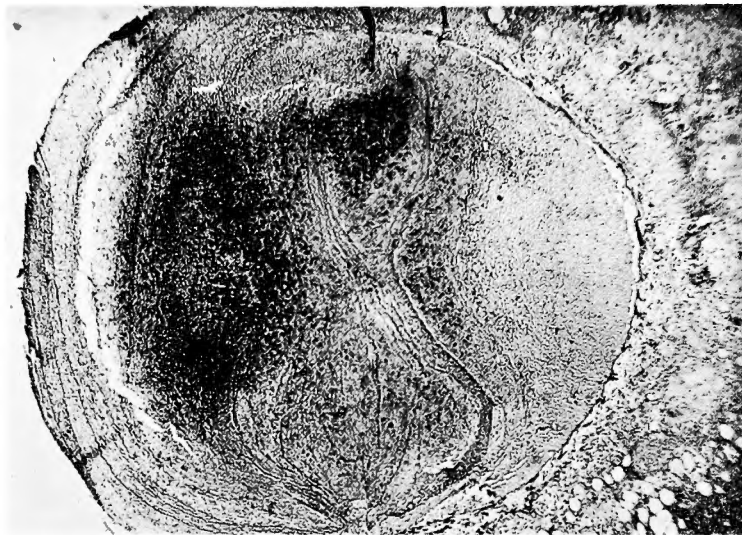


Photo 4 Histological appearance of *V. gastroepiploica sinistral* introduced into the gastric cavity without disturbing the blood stream through it.



Photo 5 Histological appearance of *A. gastroepiploica sinistral* introduced into the gastric cavity without disturbing the blood stream through it (Artery of Case 1).

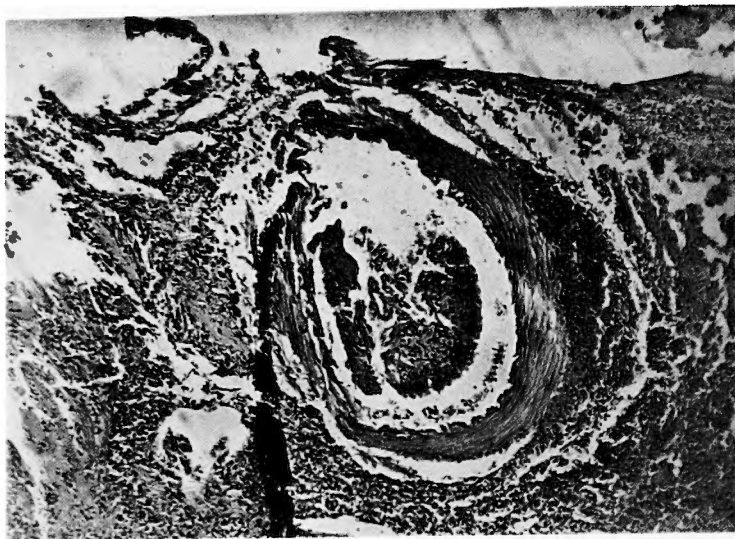


Photo 6 Histological appearance of A. gastroepiploica sinistra introduced into the gastric cavity without disturbing the blood stream through it (Artery of Case 2).



Photo 7 Histological appearance of A. gastroepiploica sinistra introduced into the gastric cavity without disturbing the blood stream through it (Artery of Case 3).

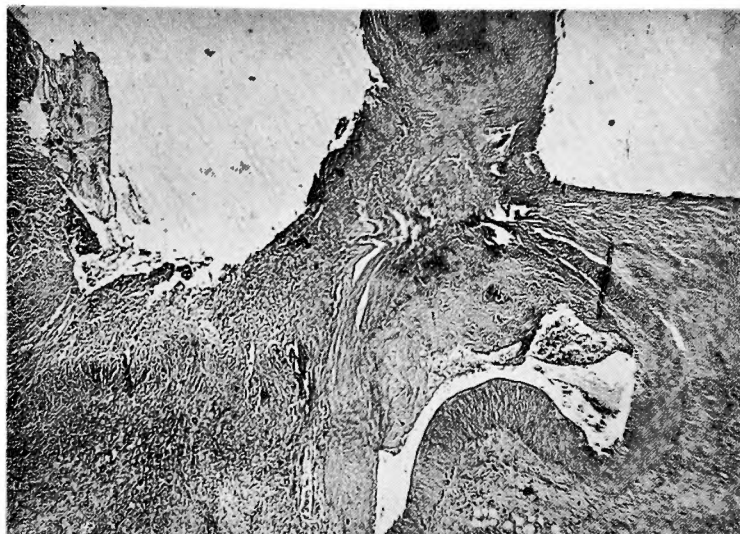


Photo 8 Histological appearance of artery protruding beyond the bottom of the bleeding gastric ulcer in clinical case.

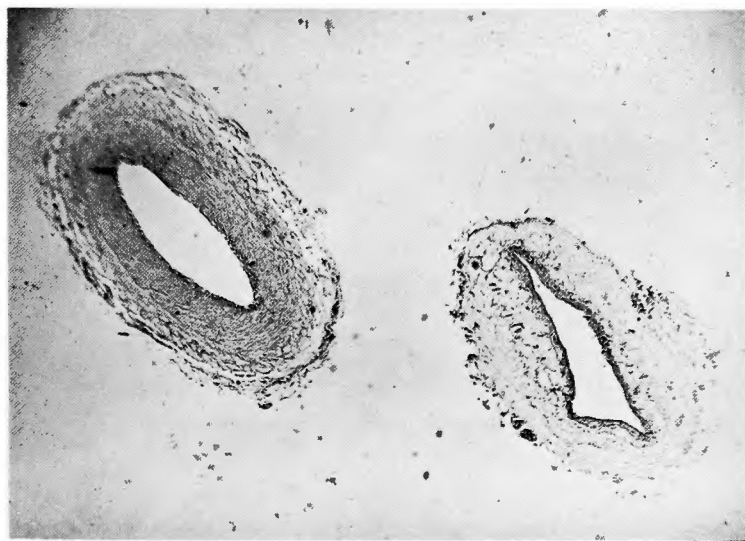


Photo 9 Histological appearances of lienal artery and vein steeped in 3% pepsin solution at pH 4.5 and incubated for 24 hours at 38°C.

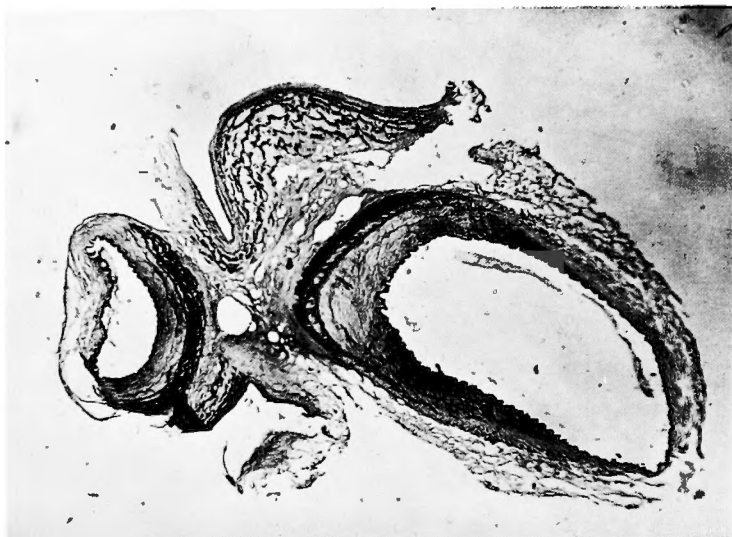


Photo 10 The elastic fiber staining, by means of Weigert's method, made from A. et V. gastropiploica sinistra used in Section 3.

vessels, exposed to the gastric juice in the gastric cavity of living dogs for 48 hours, were taken out and hematoxyline-eosin staining sections were made from them.

(II) Experimental results

(1) The histological appearance of vein.

As shown in Photo 4, nearly similar changes were found in every cases. Namely, the outer layers of the venous wall, which were exposed to gastric juice, had disappeared so completely that they became no more discernible. And they were replaced with several layers of firm white thrombus, which had been formed in correlation with red thrombus filling up the lumen of vein and seemed to strengthen the venous wall.

(2) The histological appearance of artery.

As shown in Photo 5, the outer layers of the arterial wall, which were exposed to gastric juice, were digested completely as well as vein, and they were replaced with relatively firm white thrombus. On the other hand, as shown in Photo 6, almost all of the three layers of half the circumference of the arterial wall were digested and there was only a little lumen of artery. In the center of the lumen, there was the isolated red thrombus, but tendency to the thrombus-formation in the lumen was poor. In Photo 7, almost all the layers of half the circumference of the arterial wall were digested and there was only a little lumen similar to Photo 6.

In the lumen, red thrombus was formed in contact with the outside wall, but such tendency was very poor, as compared with vein. These changes are very similar to the vascular changes seen in the lesion of bleeding gastric ulcer in clinical cases, as shown in Photo 8.

Section 4. The changes of arterial and venous walls subjected to the catheptic digestion

The changes of arterial and venous walls caused by cathepsin should not be over-

looked.

(I) Experimental method

Lienal artery and vein in dogs were steeped in the solution (pH 4.5) without pepsin and incubated for 24 hours at 38°C, and then hematoxyline-eosin staining sections were made of them.

(II) Experimental results

Because the time of cathepsin action was limited to a very short period, no observable histological changes were found in these sections and therefore, no noticeable difference between artery and vein was found.

Section 5. The histological changes of arterial and venous walls caused by the combination of cathepsin and pepsin actions

In this experiment, the author observed the histological changes caused by the combination of pepsin action from the outside of vascular walls and intracellular cathepsin action.

(I) Experimental method

Lienal artery and vein were steeped in 3% pepsin solution at pH 4.5 and incubated for 24 hours at 38°C, and then hematoxyline-eosin staining sections were made of them.

(II) Experimental results

These histological appearances are shown in Photo 10. Their changes were generally poor, as compared with those in Section 2, as effective pH of solution did not correspond to the optimal pH of pepsin.

Regarding the arterial wall, the histological changes could not be found, but the whole layers of adventitia were edematous. Regarding the venous wall, the whole layers of adventitia and most of media were edematous. Comparing both of them to each other, it is possible to say that the changes of artery was poorer than that of vein similar to Section 2.

Section 6. Cathepsin staining of arterial and venous walls

Comparing the cathepsin activities distributing in the arterial and venous walls by dying-density, the author tried to clarify the influence of digestive action of cathepsin on the blood vessels.

As arterial and venous walls become in contact with arterial or venous blood, respectively, which have different oxygen contents, it is expected that contents of cathepsin, which are influenced by the local oxidized-reduced electric potentials, should differ.

(I) Experimental results

As in both lienal artery and vein, cathepsin in vascular walls was hardly dyed by means of TAKAMATSU's method, and the distribution of cathepsin activity could not be compared between arterial and venous walls.

Section 7. The histological changes of the elastic fibers in arterial and venous walls under different conditions

The arterial and venous walls are different in their contents of elastic fibers which might show some resistance to the digestive action of gastric juice, therefore, the digestive degree of their elastic fibers was compared with each other following the digestion by gastric juice.

(I) Experimental method

The elastic fiber staining sections by means of WEIGERT's method were made from A. et V gastroepiploica sinistra, used in Section 3.

(II) Experimental results

The histological appearance of artery.

As shown in Photo 10, firm internal and external elastic plates formed the circle-like layer in the normal area of artery. The elastic fibers also existed in media intermingled with the muscle tissue. On the other hand, in the area which was digested by gastric juice, only internal elastic plate scarcely remained and other elastic fibers had completely disappeared.

The histological appearance of vein.

In the normal area, there were very fine elastic fibers which showed circle-like distribution in media and elastic fibers which were scattered roughly in adventitia. On the other hand, the elastic fibers had completely disappeared in the area that was digested by gastric juice. From these histological appearances, it was demonstrated that the arterial wall could be exempted from the digestion by the gastric juice owing to its internal elastic plate, while the venous wall was destroyed completely.

DISCUSSION

Regarding the reason why the bleeding gastric ulcer occurs very frequently in the domain of A. gastrica sinistra, it has been mentioned that A. gastrica sinistra branches out from A. coeliaca immediately after the latter branches out from aorta, or that⁶⁾ its blood pressure is the highest as it has the largest lumen among all the gastric arteries. TAKASE⁴⁾, measuring the blood pressure in A. gastrica sinistra with venous catheter, mentioned that it is higher than that in A. radialis. The author, measuring the blood pressure in four gastric arteries in adult dogs, couldn't demonstrate that the value in A. gastrica sinistra was especially higher than the values in other arteries, at least. On the other hand, NAGAO³⁾, investigating the relation between the bleeding blood vessel and the area of ulcer, mentioned that gastric ulcer occurs most frequently in the lesser curvature, especially in the area governed markedly by A. gastrica sinistra. However, A. gastrica sinistra should not be taken into serious consideration as the bleeding blood vessel, because not only A. gastrica sinistra distributes in the so-called domain of A. gastrica sinistra, but A. gastroepiploica dextra et sinistra also distribute relatively densely there, according to the investigation by stain-injection into the arteries. From the result of the author's investigations in dogs, it is very likely that other factors might be concerned with the bleeding mechanism in gastric ulcer.

Further investigation was necessary to clarify this point of view. Since HUNTER, various researches have been undertaken to investigate the digestive effect of gastric juice on the living tissue, but experiments have been carried out to determine the effect of gastric juice on blood vessels concerning the causes of gastric hemorrhage. DRAGSTEDT mentioned that the hind leg of frog which was allowed to hang into the gastric juice in the flask gave the following results: the skin was digested after 2 hours, the fascia after 2 hours, next the muscle and finally the blood vessels. JELLINEK, H. and others in 1958 first investigated in vivo the effect of gastric juice on blood vessels. In their experiments,

aorta abdominalis, inferior vena cava and lienal artery and vein had been introduced into the gastric cavity in dogs to let them be exposed to gastric juice, and then the dogs had died from the bleeding by arterial perforation. It had been demonstrated that their histological changes had been remarkable in artery, but very slight in vein. They concluded that the venous wall had stronger resistance to gastric juice than the venous wall. However, as it is the gastric blood vessels which really cause the bleeding from gastric ulcer, the author introduced A. et V. gastroepiploica, which were isolated most easily among gastric arteries and developed relatively well into the gastric cavity, and reinvestigated the results obtained by JELLINEK, H. and others. The results were shown in Chapter 2; that is, venous wall always disappeared in its whole layers, while in 2 cases of arterial wall, although their whole layers were subjected to total necrosis, their lumens were preserved. From these results, it is impossible to demonstrate that the resistance of vein is greater than that of artery and also, regarding the thrombus formed in the lumen, it was generally thicker and firmer in vein than in artery. Of course these results should be elucidated in part from the point of the velocity of blood flow through them. On the other hand, several experiments were carried out in vitro, keeping pace with the above-stated experiments, but the author couldn't obtain the results necessary to clarify the difference between artery and vein, except for the result which made clear that the resistance of arterial wall against the acid-peptic digestion is greater than that of venous wall. Generally, when the acid-peptic digestion acts on the vascular wall from outside, coagulative factors are activated, thrombus is formed and moreover, fibrine adhering to the inner surface of vascular wall protects it, while the adhered fibrine is melted by plasmin, which is produced when plasminogen is activated by fibrinolytic phenomenon, and thrombus-formation is disturbed. It is understandable that when hemostatic balance, which regulates harmonically these two mechanisms, happens to be broken and leans to one side, hemorrhage will occur or thrombus will be formed. Because the arterial wall has thick media and is rich in firm elastic fibers, as judged by common sense, the resistance against the digestion by gastric juice is more or less greater than the venous wall, but thrombus-formation in the artery is poorer than in the vein. Further, this poor thrombus on the arterial wall is liable to be torn off and removed under high blood pressure. Therefore, it is well accepted that arterial bleeding will occur, when hemostatic balance is broken due to these reasons. On the other hand, because the venous wall is properly protected by firm thrombus, although it is completely digested by acid-peptic action, and moreover because venous blood pressure is lower, it is likely that venous bleeding does not occur. Furthermore, it is also rightly presumed that the reflexive contraction of collateral arteries due to the thrombus-formation in certain arteries plays a role in the occurrence of the necrosis in local tissue and therefore, would function as an important promotive cause of bleeding.

CONCLUSION

Up to the present, regarding the massive bleeding from gastric ulcer, the following questions still remain unsettled: why it occurs most frequently in the domain of A. gastrica sinistra, and why it is of arterial origin. The author has carried out the following experiments to solve these questions.

(a) The blood pressure of four gastric arteries were compared among one another.

(b) The resistances of arterial and venous walls and proteins purified from them against the acid-peptic digestion were compared to each other.

(1) Among four gastric arteries, the blood pressures in *A. gastrica sinistra et dextra* were somewhat higher than those in *A. gastroepiploica sinistra et dextra*, but those in the arteries of the same name on both sides showed nearly the same values.

It was not demonstrated that the blood pressure in *A. gastrica sinistra* was the highest among them. Therefore, it is supposed that the factors other than the blood pressure will be also concerned, regarding the question as to why the bleeding from gastric ulcer is frequent in the domain of *A. gastrica sinistra*.

(2) Regarding the resistance of the proteins purified from arterial and venous walls against the acid-peptic digestion, no remarkable difference was demonstrated between them.

(3) With *A. et V. gastroepiploica sinistra* introduced into the gastric cavity passing through the gastric wall without disturbing the blood stream through it, the histological changes by the digestive action of gastric juice were investigated and it was demonstrated that the changes of the venous wall were a little more remarkable, and that the venous wall always disappeared, while thrombus-formation in vein was more remarkable, as compared with that in the artery.

(4) With pieces of lienal artery and vein, the histological changes caused by the acid-peptic digestion were investigated. The changes of vein was a little more remarkable, and it required a longer period to cause the complete digestion of the arterial wall, as compared with the venous wall.

(5) Autolysis of arterial and venous walls by catheptic action was not demonstrated within 24 hours. Although the author tried to stain cathepsin in blood vessels, he failed because the distribution of cathepsin activities are probably scarce in amount.

Finally, it was demonstrated that the resistance of the proteins purified from the arterial and venous walls against the acid-peptic digestion were not different between them and that the resistance of the arterial wall to the gastric juice was greater than the venous wall, because the former has the thick media and is rich in firm elastic fibers, but that the thrombus-formation in the artery is poorer than in the vein. And the author wishes to presume that the arterial bleeding would easily occur, because poor thrombus on the inner surface of arterial wall is liable to be torn off. It is also presumed that the reflexive contraction of collateral arteries due to the thrombus-formation in certain arteries, plays a role in the occurrence of the necrosis in local tissue and therefore, would function as an important promotive cause of bleeding.

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REFERENCES

Part 1.

- 1) Akabori, S. : Chemistry of proteins. Kyoritsu Shuppan Co., Ltd, 3 : 291, 1955.
- 2) Baldwin, E. : Dynamic aspects of biochemistry. Iwanamishoten, 76, 1954.

- 3) Fruton, J. & Simmonds, S. : General Biochemistry. Iwanamishoten, **2** : 515, 1956.
 - 4) Hay, L. J., Varco, R. L., Code, C. F. & Wangenstein, O. H. : The experimental production of gastric and duodenal ulcer in laboratory animals by the intramuscular injection of histamine in beeswax. Surg. Gynec. & Obst., **75** : 170, 1942.
 - 5) Henning, H. : Lehrbuch der Verdauungskrankheiten. Stuttgart. 1956.
 - 6) Hirahara, K. : Studies on cathepsin. Med. J. Shinshu Univ., **4** : 529, 1959.
 - 7) Ishikawa, H. : Experimental studies on the pathophysiological significance of cathepsin activity. Jikken-shokaki-byo-gaku, **15** : 697, 765, 1940.
 - 8) Kataoka, N. : The vasculological study on the experimentally produced gastric ulcer by means of the plastic injection method. Nihon-geka-hokan, **29** : 293, 1960.
 - 9) Matsuo, I. : The significance of cathepsin in the cause of gastric and duodenal ulcer. Jikken-shokaki-byo-gaku, **12** : 923, 1937.
 - 10) Matsuo, Y. : Experimental studies on the causes of reflux esophagitis, with especial emphasis on the significance of esophageal catheptase, bile and bacterial infection. Nihon-geka-hokan, **28** : 2006, 1959.
 - 11) Maruta, K. : A view of results on the causes of gastric ulcer. Shinshu-igaku-zasshi, (I) **6** : 457, 1957., (II) **7** : 9, 1958.
 - 12) Nogaki, S. : The stereopathological studies of gastric blood vessel system by means of the plastic cast method. Gihu-idai-kiyo, **8** : 234, 1960.
 - 13) Ōi, M. : Gastric and duodenal ulcer. Nihon-geka-zensho, **19** : 366, 1957.
 - 14) Onoyama, M. : Studies on tissue protease. Acta schol. med. Univ. in Kyoto, **27** : 76, 1949.
 - 15) Salkowski, Z. : Z. Kl. Med., **17** : 77, 1890.
 - 16) Takamatsu, H. : The histochemical studies on the protease. Nihon-byori-gakkaishi, **43** : 519, 1954.
 - 17) Tamesue, H. : Chemical mechanisms on the occurrence of gastric ulcer. Fukuoka-ikadaigaku-zasshi, **27** : 1065, 1934.
 - 18) Waldschmidt-Leitz, E., A. Schäffner, I. J. Bek & E. Blum : Z. Physiol. Chem., **188** : 17, 1930.
 - 19) Willstätter, R., & E. Bamann. : Z. Physiol. Chem., **180** : 127, 1928.
 - 20) Yō, M. : Influence of the continuous histamine injection on the gastric secretion, gastric mucosa, perietal cell and glomerulus in parietal cell. Tokyo-jikei-idai-zasshi, **70** : 486, 1954.
- Part 2.
- 1) Jellinek, H. : A comparison of the experimental results for gastric hemorrhage of arterial origin with resection and autopsy material. Surg. Gynec. & Obst., **107** : 495, 1958.
 - 2) Matsuoka, M. : Interpretation of blood coagulation. Nihon-ishikai-zasshi, **51** : 693, 1964.
 - 3) Nagao, H. : Treatment of the bleeding gastric ulcer. Geka-chiryō, **9** : 185, 1963.
 - 4) Takase, H. : Studies on the bleeding gastric ulcer. Nihon-gekagakkai-zasshi, **63** : 941, 1962.
 - 5) Takeshige, Y. : The structure of artery and the distribution of the nutritive blood vessels of artery. The saishin-igaku, **18** : 745, 1963.
 - 6) Tame, T. : The distribution of gastric arteries from a clinical view-point. Nihon-geka-hokan, **7** : 306, 1940.

和文抄録

胃潰瘍の発生病理に関する酵素化学的研究
および胃潰瘍性出血の成因に関する研究

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第1編 胃潰瘍の発生病理に関する
酵素化学的研究

胃潰瘍の発生に関しては終局的には酸ペプシンによる消化が主役を演ずると考えられているが、ペプシンや酸には生活胃壁を消化する力はなく、少なくともその最初の粘膜変化には血行障害に伴う低酸素症によつて賦活され弱酸性で作用する組織自解酵素のカテプシンが重要な役割をもつと考え、臨床例および成犬に作製したヒスタミン潰瘍胃において、胃のカテプシン染色およびカテプシン能測定を行なつて次のような成績を得た。

(1) 臨床例において胃潰瘍胃のカテプシン能は他の胃癌胃、十二指腸潰瘍胃等のそれらに比して著しく増強していた。

(2) 成犬に作製したヒスタミン潰瘍胃のカテプシン能も成犬の正常胃や胃潰瘍陰性例のそれらに比して著しく増強していた。

(3) 比較的新鮮な胃潰瘍胃においてもカテプシンの胃壁における分布は潰瘍の周辺において特に密であるということではなく、予想に反してむしろびまん性に一樣な分布状態を示していた。

以上の成績から胃潰瘍胃の胃壁、ことに粘膜においてはカテプシンの分布量が大であるが、更に胃潰瘍の好発部位において、先天的に存在する血管構築上の特殊性から局部的に著しい血行障害を生じて低酸素症を招来し、それによつてカテプシンが賦活されると、継ぎ目の抵抗減弱部位という因子も加わつて、局所的な初期の粘膜変化が起り、潰瘍へ進展していくものと理解される。

第2編 胃潰瘍性出血の成因に関する
研究

従来胃潰瘍性出血に関して、(a) 左胃動脈領域になぜ多いか、(b) なぜ動脈性出血であるか、ということが疑問とされてきた。私はこの疑問を少しでも解決す

る目的で、成犬を使つて、(a) に対しては胃に分布する4動脈の血圧を測定して比較検討し、又(b)に対しては動静脈壁およびそれらより精製した蛋白質の酸・ペプシン消化に対する抵抗性を比較検討した。

(1) 胃に分布する4つの動脈の中、左・右胃動脈の血圧は左・右胃大網動脈の血圧よりやや高い値を示したが、左・右胃動脈は常にほぼ同じ値を示し、左胃動脈の血圧が最も高いことはなかつた。従つて胃潰瘍出血が左胃動脈領域に多いことに関しては血圧以外の要因も関係しているものと推定される。

(2) 動静脈壁よりそれぞれ精製した蛋白質の酸・ペプシン消化に対する抵抗性については両者の間に著しい差を認めなかつた。

(3) 血流を保つたままで胃腔内に縫埋した左胃大網動静脈について、胃液の消化作用による組織学的変化を追求したところ、静脈においてやや強い変化を示し、常にその壁は消失していたが、血栓形成は静脈においてより著明におこつていた。

(4) 切除した脾動静脈について、酸・ペプシンの消化作用による組織学的変化を追求したところ、その変化は静脈においてやや強く、動脈壁が消化破かいされるまでには静脈壁よりやや時間的のおくれを示した。

(5) カテプシン作用による動静脈壁の自家消化作用は24時間以内には認められなかつた。又動静脈壁のカテプシン染色を行なつたが、その分布量が少ないためか、染色することが出来なかつた。

以上によつて動静脈壁はそれらの構成蛋白質の酸・ペプシン消化に対する抵抗性においては差異がなく、又動脈は厚い中膜や強固な弾性線維を有するために、静脈より胃液に対する抵抗性が大であるが、血栓形成に関しては静脈より劣弱であり、且つこの劣弱な血栓は優勢な動脈圧によつて剝離除去されるために、動脈性出血を引き起すものと理解したいのである。また動脈特有の血栓による側副動脈の反射性収縮も、局所の組織壊死に役割を演じ、出血への促進的要因となるであろう。