
原 著

Attitude of Trypsin in Experimental Acute Pancreatitis

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Crystalline Trypsin used in the present experiment was "Trypure" of Novo Industry A/S in Denmark.

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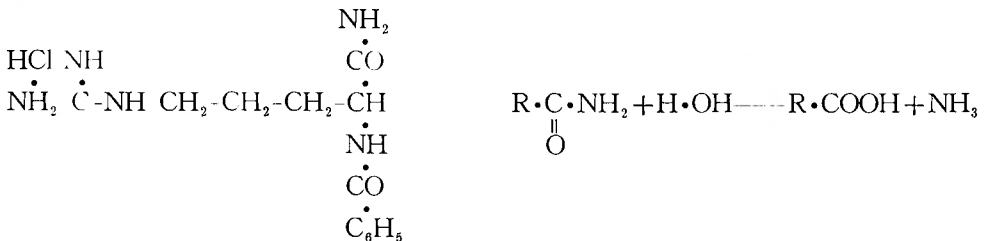
I. INTRODUCTION

Principal situation of acute pancreatitis is, as was clarified by H. CHARI at the beginning of 19th century, nothing but the effect of pancreatic ferment against the pancreatic parenchyma, that is, autodigestion of the pancreas. Experimental and clinical observations on acute pancreatitis have been carried out by HILDEBRAND, OPIE, POLYA, GULEKE, DRAGSTEDT and others. Particularly numerous studies have been accumulated on pancreatic ferments. Most of these are, however, mainly concerned with amylase or lipase, and very few reports are found as to the attitude of trypsin in pancreatitis. This is presumably due to following fact, that is, though there have been many devices for ferment determination using casein, gelatin, fibrin, hemoglobin and many other substrates, neither of these can be deemed as methods for specific determination of trypsin, but that for all the proteolytic ferments including trypsin.

The author of the present paper studied methods of specific determination of trypsin as postulated by BLACKWOOD et al¹⁾, and HAVERBACK et al²⁾, and finally studied and improved the method of NARDI^{3) 4) 5) 6) 7)}, lately reported, in which a specific synthetic substrate of α -benzoyl-1-arginine amide containing amide linkage specifically decomposed by trypsin is used. The author further studied from various aspects the attitude of pancreatic ferment, trypsin, which is one of the problems of pathophysiology in acute pancreatitis, and carried out experiments particularly on the significance of intraperitoneal fluid. Some informations obtained here are to be reported.

II. DETERMINATION OF TRYPSIN

Synthetic substrate of α -benzoyl-1-arginine amide hydrochloride monohydrate (abbreviated to B. A. A. hereafter), which is specifically decomposed by trypsin, was used. This substrate is rapidly decomposed by hydrolytic action of trypsin, producing benzoyl-arginine and ammonia. Here the substrate is decomposed in proportion to the amount of trypsin therein⁸⁾



Ammonia produced was determined according to the method reported by HATANO and KIRITA⁹⁾.

1. Procedure of Determination

Material of 1.0 ml for the determination is mixed with 1.0 ml of B. A. A. solution

of 0.1 M diluted with phosphate buffer at pH 7.8. The mixture is incubated at 25°C for an hour for reaction between trypsin and the substrate. One ml of 1 per cent boric acid solution is put in the inner well of CONWAY'S unit and in the outer well 0.5 ml of the mixture is put and at the opposite side of the outer well 1 ml of saturated potassium carbonate solution, so that these two may not mix together. The wells are tightly closed with glass cover with vaseline on it. Rotating the unit, material and potassium carbonate are well mixed. After left in room temperature for an hour, 0.5 ml of boric acid solution which absorbed ammonia is transferred from the inner well of CONWAY'S unit to a test tube with fitted stopper, and is then subjected to indophenol method (spectrophotometric method). The test tube is immersed in water bath, and 0.05 ml of 0.03 M manganous sulfate solution, 1.0 ml of alkaline phenol reagent, and 0.5 ml of sodium hypochlorite solution are quietly added in this order. The test tube is immediately closed and the content is gently stirred, which is then immersed in boiling water bath for 5 minutes and cooled for 2 minutes in ice water. After let stand in room temperature for 20 minutes for adequate coloration, photometric absorbability is read through a filter of 625 m μ using spectrophotometer. From this absorbability, amount of trypsin is sought on the standard curve drawn according to the determination of purified crystalline trypsin of various concentrations previously known.

For blank determination, distilled water was used, and otherwise the techniques were identical to that above mentioned. For all the determinations including blank, CONWAY'S unit was prepared in duplicate and determination was repeated twice for single material, and average values were taken. When there was a possibility of previously existing ammonia in the material, it was determined and trypsin value was corrected.

2. Serum Trypsin Level in Normal and Simply Laparotomized Dogs

i. Materials and Methods

Adult mongrel dogs weighing about 10 kg were used. Blood was drawn from the femoral vein. As control, simple laparotomy was performed and the pancreas was exposed for several minutes. Blood was taken similarly from the femoral vein after the abdomen was closed. Serum trypsin level was determined in these blood samples.

ii. Results

Normal range of serum trypsin level obtained from 40 normal dogs was between 0 to 1×10^{-4} A. U./ml, 0.5×10^{-4} A. U./ml on the average (A. U. is the abbreviation of Anson Unit).

Determination in 3 dogs of simple laparotomy for control revealed slight change within physiological fluctuation (Tab. 1, Fig. 1).

3. Serum Trypsin level after Intravenous Injection of Crystalline Trypsin

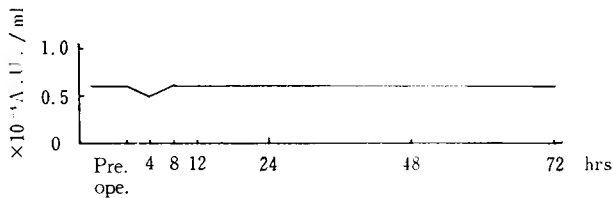
i. Materials and Methods

In 12 adult mongrel dogs weighing about 10 kg, crystalline trypsin of 500, 750, 1000, 1250×10^{-4} A. U./kg each dissolved in 20 ml of saline solution was respectively injected from the femoral vein spending about 5 minutes without anesthesia. After the injection, blood was taken from the femoral vein on another side with the lapse of time and serum trypsin was determined.

ii. Results

Table 1 Serum trypsin level in dogs after simple laparotomy.

dog	hrs	preliminary	4	8	12	24	48	72
	No.	15	0.5	0.4	0.6	0.6	0.5	0.5
	16	0.8	0.5	0.7	0.7	0.8	0.8	0.8
	17	0.4	0.5	0.6	0.5	0.4	0.4	0.4
	mean	0.6	0.5	0.6	0.6	0.6	0.6	0.6

Unit : $\times 10^{-4}$ A.U./ml**Fig. 1** Change in serum trypsin level in control dogs (in mean value)

Serum trypsin level arose markedly showing its peak of 3.6 to 6.4×10^{-4} A.U. ml a few minutes after intravenous injection of trypsin, and data obtained were diversified depending on the amount of trypsin intravenously injected. Intravenously injected trypsin was demonstrated in blood stream as active form for approximately 120 minutes, when the amount of trypsin is as in the present experiment (Tab. 2, Fig. 2).

4. Serum Trypsin Level in Dogs with Acute Pancreatitis

i. Materials and Methods

Acute pancreatitis was produced experimentally in adult mongrel dogs weighing about 10 kg. Namely, the abdomen was opened under ravonal intravenous anesthesia, the pancreas was exposed and the larger pancreatic duct was isolated from the surrounding pancreatic tissue. The autogenous bile of 0.5 ml per kg body weight previously aspirated by gall bladder puncture was rapidly injected from the duct under constant pressure of approximately 150 mmHg, and the pancreatic duct was doubly ligated and cut. Following the bile injection, the entire pancreas showed color of bile and there appeared edema and petechia. Blood samples were taken from the femoral vein.

ii. Results

Serum trypsin level determined with the lapse of time following production of pancreatitis increased gradually or rapidly reaching its peak of 1.2 to 2.5×10^{-4} A. U./ml, 1.8×10^{-4} A.U./ml on the average, approximately 24 hours after the injection, which was then followed by descension. The maximum value corresponds to 2 to 5 times of pre-operative value (Tab. 3, Fig. 3).

5. Summary

As a preliminary experiment, it was studied how much of intravenously injected crystallin trypsin can be demonstrated by the method of determination employed here and how fluctuates active trypsin in peripheral blood, when crystallin trypsin is injected intravenously. Serum trypsin level was in a close correlation to the amount of trypsin injected. It was clarified that active form trypsin can be pursued in serum for approximately 120 minutes as long as such amount of trypsin as used in this preliminary experiment is

Table 2 Serum trypsin level in dogs after intravenous injection of crystalline trypsin.
 1250×10^{-4} A. U./kg (i.p.)

dog	min.	Before inj.	5	10	15	30	60	120
No. 1		0.6	6.8	—	6.2	5.3	4.2	1.7
2		0.5	6.2	—	5.4	5.3	2.8	1.8
3		0.3	6.2	—	5.2	5.0	3.8	1.3
mean		0.5	6.4	—	5.6	5.2	3.6	1.6

1000×10^{-4} A. U./kg (i.p.)

4		0.5	—	5.3	—	5.0	3.2	1.8
5		0.4	—	4.2	—	2.3	2.1	0.7
6		0.7	—	5.5	—	5.0	2.5	1.4
mean		0.5	—	5.0	—	4.1	2.6	1.3

750×10^{-4} A. U./kg (i.p.)

7		0.4	—	4.3	—	2.8	1.7	1.0
8		0.4	—	4.0	—	3.0	1.9	1.1
9		0.5	—	4.7	—	3.2	2.3	1.6
mean		0.4	—	4.2	—	3.0	2.0	1.2

500×10^{-4} A. U./kg (i.p.)

10		0.5	3.9	—	3.2	2.5	1.8	1.2
11		0.2	3.2	—	2.5	2.3	1.2	0.9
12		0.4	3.8	—	2.9	2.8	1.8	1.0
mean		0.4	3.6	—	2.9	2.5	1.6	1.0

Unit : $\times 10^{-4}$ A. U./ml

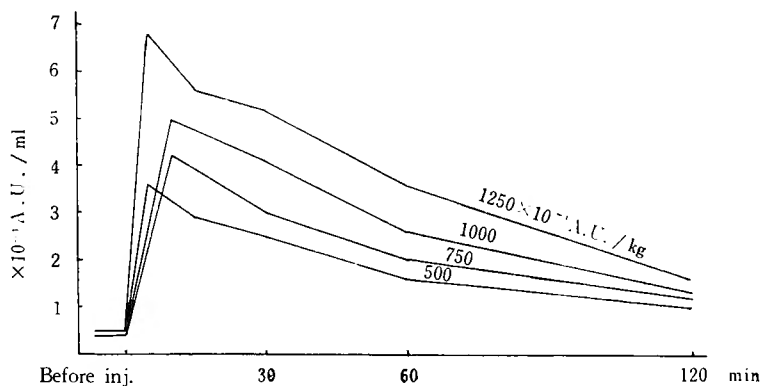
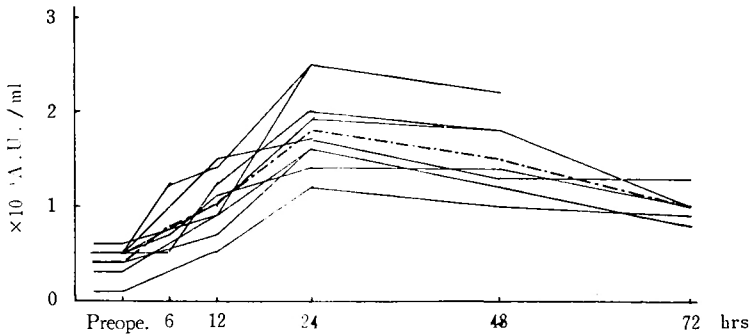


Fig. 2 Change in serum trypsin level in dogs after intravenous injection of crystalline trypsin

Table 3 Serum trypsin level in dogs of experimental pancreatitis.

dog		hrs	Preope.	6	12	24	48	72
No.	21		0.5	—	1.5	1.7	1.3	1.3
	22		0.1	—	0.5	1.2	1.0	0.9
	23		0.5	1.2	1.4	2.5	2.2	—
	24		0.5	0.5	1.2	2.0	1.8	1.0
	25		0.6	—	0.9	1.6	—	—
	26		0.3	—	0.9	2.5	—	—
	27		0.4	—	0.7	1.6	1.2	0.8
	28		0.5	0.7	1.1	1.4	1.4	1.0
	29		0.5	—	1.0	1.9	1.8	1.0
	mean		0.4	0.8	1.0	1.8	1.5	1.0

Unit : $\times 10^{-4}$ A. U./ml**Fig. 3** Change in serum trypsin level in dogs of experimental pancreatitis

concerned.

Serum trypsin level in normal dogs ranged from 0 to 1×10^{-4} A.U./ml, and that of control of simple laparotomy did not exceed this range, its change also being within physiological range reflecting no influence of the false operation.

Serum trypsin level showed its maximum level 24 to 48 hours after production of acute pancreatitis, it being 2 to 5 times of preoperative value. In most occasions, experimental animals died before or at this period. In lethal cases, serum trypsin level showed rapid and prominent increase compared with that in survivors.

It is assumed that the method of determination employed in the present experiment being consisted of "microdiffusion technique of CONWAY and indophenol colorimetry" is far more excellent than the original method of NARDI, in the respects of sensitivity, reproducibility and accuracy.

III. INFLUENCE OF INTRAVENOUS OR INTRAPERITONEAL INFUSION OF CRYSTALLINE TRYPSIN

1. Fluctuation of Arterial and Portal Pressures at Intravenous or Intraperitoneal Infusion of Crystalline Trypsin

i. Materials and Methods

Adult mongrel dogs weighing about 10 kg were used. Arterial pressure was measured by a mercurial manometer connected to the femoral artery, and portal pressure by an aqueous manometer connected to a polyethylene tube which was inserted from the mesenteric vein to the portal trunk. Crystalline trypsin of 1250×10^{-4} A. U./kg was dissolved in saline solution of 50 ml and poured into the peritoneal cavity through a small upper median incision of 3 cm in length. The abdomen was immediately closed with double layer suture and arterial and portal pressures were read. In cases of intravenous injection, crystalline trypsin of 1250×10^{-4} A.U./kg dissolved in 20 ml of saline was slowly injected from the femoral vein spending approximately 5 minutes. Control animals received saline infusion of respectively corresponding amount.

ii. Results

Average levels of arterial and portal pressure changes at intraperitoneal infusion of trypsin in respective 3 animals are represented in Fig. 4. Arterial pressure at intraperitoneal infusion abruptly fell and reached 80 mmHg 6 to 7 minutes after the infusion, which was mostly followed by gradual elevation drawing near the preoperative level 120 minutes after the infusion, although there were slight fluctuations.

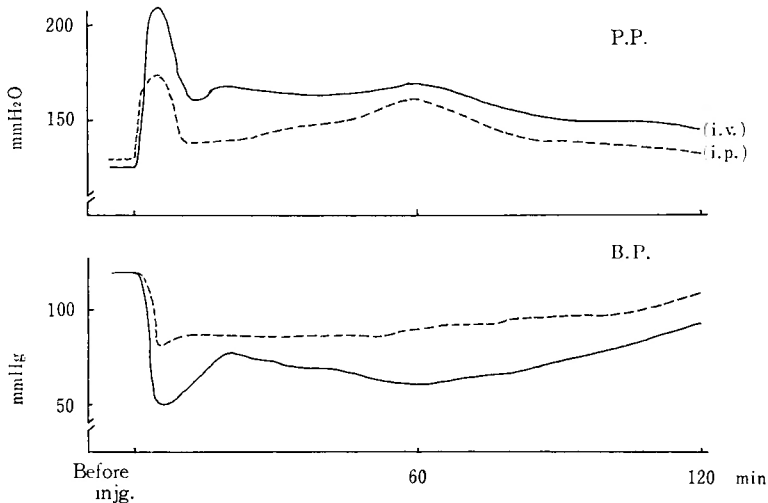


Fig. 4 Fluctuation of arterial and portal pressures after intravenous or intraperitoneal injection of crystalline trypsin. 1250×10^{-4} A. U./ml.

Portal pressure contrariwise arose rapidly immediately after the infusion reaching its maximum level of about 175 mmH₂O 5 to 6 minutes after the infusion, which was followed by a tendency of abrupt fall, although it was sustained in a higher level. The pressure again arose gradually thereafter and it showed tendency of descension towards 60 minutes after the infusion and restored to preoperative level 120 minutes after the infusion.

On the other hand, arterial and portal pressures at intravenous injection showed approximately the same tendency as in intraperitoneal infusion, however, arterial pressure more markedly fell to 50 mmHg at minimum and portal pressure elevated as prominent as 210 mmH₂O at maximum, suggesting more intense effect of intravenous injection of trypsin than intraperitoneal infusion.

In animals of intraperitoneal saline infusion, slight elevation of 10 mmH₂O was observed in portal pressure immediately after the infusion, which was accompanied by no fluctuation in arterial pressure. When saline solution was injected intravenously, no fluctuation could be observed both in arterial and portal pressures.

2. Comparison of Serum Trypsin Level at Intraperitoneal Infusion with Intravenous Injection of Crystalline Trypsin

i. Materials and Methods

Adult mongrel dogs weighing about 10 kg were used. Crystalline trypsin was infused intravenously and intraperitoneally with the same procedure as in the above. Amount of crystalline trypsin was 1250×10^{-4} A.U./kg for intravenous injection and 6250×10^{-4} A.U./kg for intraperitoneal infusion. Trypsin level was determined in the blood taken from the femoral vein.

ii. Results

Serum trypsin level at intravenous injection of crystalline trypsin was as mentioned in II, 3 in the above. The fluctuation of the average value showed maximum level of 6.4×10^{-4} A.U./ml a few minutes after the injection, which was followed by descension thereafter to 1.6×10^{-4} A.U./ml 120 minutes after the injection.

On the other side, serum trypsin level at intraperitoneal infusion of trypsin reached the maximum level of 1.6×10^{-4} A.U./ml and decreased gradually thereafter to become 0.8×10^{-4} A.U./ml 3 hours after the infusion (Tab. 4, Fig. 5).

Table 4 Serum trypsin level in dogs after intraperitoneal infusion of crystalline trypsin.
6250 × 10⁻⁴ A. U./kg (i. p.)

dog	min.	Before inj.	30	60	120	180
No	31	0.5	1.8	1.5	1.2	1.0
	32	0.5	1.5	1.5	1.1	0.8
	33	0.4	1.5	1.3	1.1	0.6
mean		0.5	1.6	1.4	1.1	0.8

Unit : $\times 10^{-4}$ A. U./ml

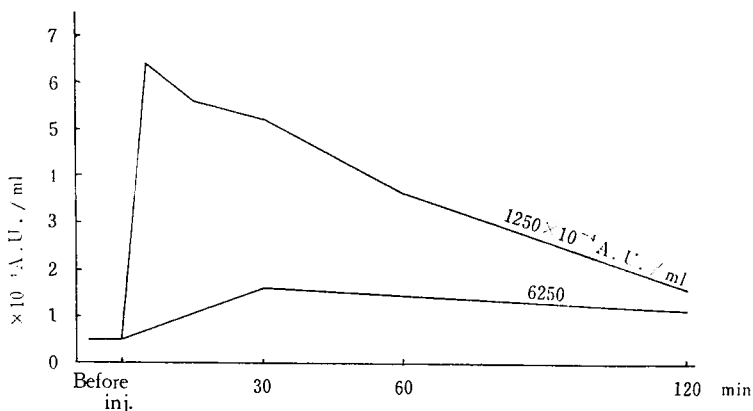


Fig. 5 Change in serum trypsin level after intravenous or intraperitoneal injection of crystalline trypsin (in mean value)

3. Summary

These experiments were carried out in the aim of exploring the difference in fluctuations of arterial and portal pressures and serum trypsin level between intraperitoneal infusion of crystalline trypsin and intravenous injection of it. Arterial pressure fell and portal pressure elevated within a few minutes after infusion of trypsin, however, both of these restored shortly, to some extent, drawing near preoperative level gradually. Changes in arterial and portal pressures were markedly pronounced in intravenous injection than in intraperitoneal infusion of the same dosis of trypsin.

Notwithstanding trypsin was administered 5 times much in intraperitoneal infusion compared with intravenous one, serum trypsin level after the infusion was higher in intravenous injection, being reversed. In other words, elevation of serum trypsin level was conspicuous when trypsin was administered in circulatory system than in peritoneal cavity.

IV. INTRAPERITONEAL FLUID AT ACUTE PANCREATITIS

1. Trypsin Level of Pancreatic Exudate and Peritoneal Fluid

i. Materials and Methods

Acute pancreatitis was produced in adult mongrel dogs weighing about 10 kg. Pancreatic exudate was collected with following procedures. After the procedure of pancreatitis production was accomplished, the pancreasmesenterium around the organ and the small vessels in it were doubly ligated and cut without injuring the capsule of the organ, and the right arm of the pancreas was isolated from the surrounding tissues. The isolated right arm was enclosed with a sac and fixed to the duodenal wall with sutures. A vinyl tube connected to the end of the sac was drained through a small incision in the right abdominal wall to the outside. The left arm of the pancreas was left as it was.

Peritoneal fluid was collected through a rubber drainage from the abdominal cavity (Fig. 6).

ii. Results

Trypsin level in pancreatic exudate was markedly high from the initial stage, being 4 to 5×10^{-4} A.U./ml. The level still arose to the peak of 8 to 12×10^{-4} A.U./ml until 24 to 48 hours after the onset of the disease, and showed as high a level as 6 to 8×10^{-4} A.U./ml 72 hours after the onset (Tab. 5, Fig. 7).

On the other hand, trypsin level in peritoneal fluid was 1.1×10^{-4} A.U./ml on the average 6 hours after the onset, which was followed by maximum value of 2.1×10^{-4} A.U./ml and 1.8×10^{-4} A.U./ml 24 to 48 hours and 72 hours after the onset, respectively. Among these experimental animals, death occurred more frequently towards

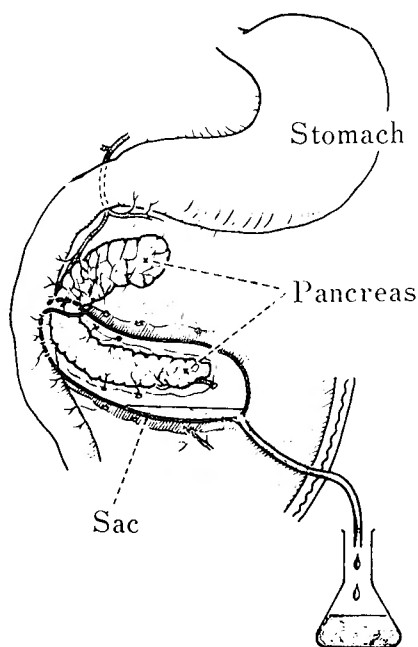


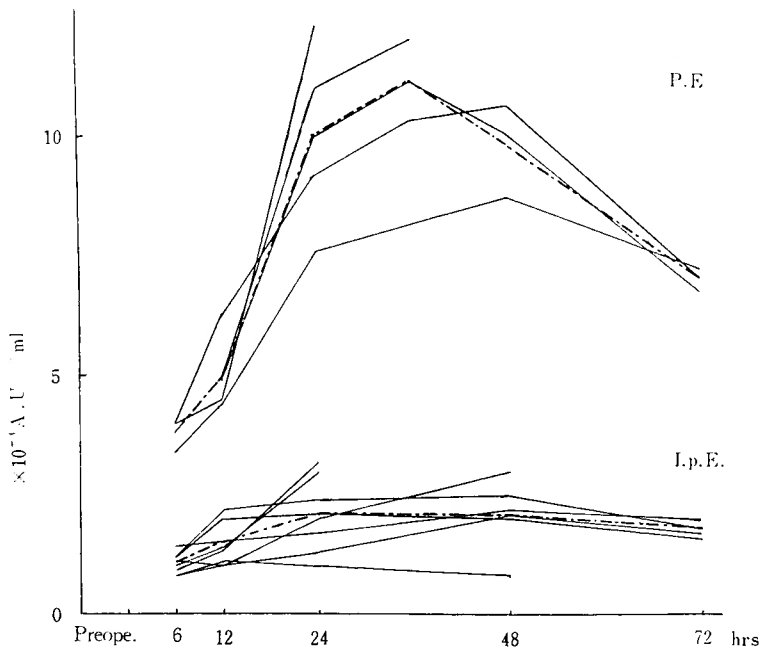
Fig. 6 Method of pancreas exudate collection

Table 5 Trypsin level in pancreatic exudate in dogs of experimental pancreatitis.

dog	hrs	6	12	24	36	48	72
No. 41		—	4.9	11.0	12.0	—	—
42		1.0	6.3	9.2	10.3	10.6	7.0
43		3.4	4.4	7.6	—	8.7	7.2
44		—	5.0	10.0	11.1	10.0	6.7
45		4.0	4.5	12.3	—	—	—
mean		3.8	5.0	10.0	11.1	9.8	7.0

Unit : $\times 10^{-1}$ A. U./ml**Table 6** Trypsin level in intraperitoneal fluid in dogs of experimental pancreatitis.

dog	hrs	6	12	24	48	72
No. 51		1.4	1.5	1.7	2.2	2.0
52		0.8	1.0	1.3	2.1	1.7
53		0.9	1.3	3.2	—	—
54		0.8	1.1	1.0	0.8	—
55		1.0	1.4	3.0	—	—
56		1.2	2.2	2.4	2.5	1.8
57		1.2	2.0	2.1	2.0	1.6
58		1.1	1.0	2.0	3.0	—
mean		1.1	1.5	2.1	2.1	1.8

Unit : $\times 10^{-1}$ A. U./ml**Fig. 7** Change in trypsin level in pancreatic exudate and intraperitoneal fluid in dogs of experimental pancreatitis

the period of maximum trypsin level in those which showed higher and rapid elevation of trypsin level (Tab. 6, Fig. 7).

2. Trypsin Level of Pancreatic Venous Blood and Thoracic Duct Lymph

i. Materials and Methods

Acute pancreatitis was produced in adult mongrel dogs weighing about 10 kg. A polyethylene tube was inserted from the mesenteric vein to the pancreatic vein via the portal vein. The tube was fixed in position and the abdomen was closed. Trypsin level was determined with the lapse of time in pancreatic venous blood taken from this tube.

Thoracic duct lymph was collected with following procedures. The draining of the thoracic duct to the left venous angle was exposed and the thoracic duct was ligated. As the ligated end of the duct was swollen by lymphatic congestion, a small incision was laid on the duct and polyethylene tube of less than 1 mm in caliber was inserted and fixed. Thoracic duct lymph was collected from this thoracic duct fistula¹⁰.

ii. Results

Trypsin level in pancreatic venous blood was determined every two hours postoperatively until 12th hour. The level began to arise 2 to 4 hours after the operation and continued to increase on to reach 2.5×10^{-4} A.U./ml 12 hours after the operation, which is 5 times higher than preoperative level (Tab. 7, Fig. 8).

Trypsin level in thoracic duct lymph also showed approximately the similar tendency to that in pancreatic venous blood, reaching as high a level as 2.2×10^{-4} A.U./ml 12 hours after the operation. However, trypsin level was always higher in pancreatic venous blood than in thoracic duct lymph (Tab. 8, Fig. 8).

3. Serum Trypsin Level in Dogs with Intact Thoracic Duct or Thoracic Duct Fistula

Table 7 Serum trypsin level in dogs of pancreatitis induced with bile-injection, as determined in Vv. pancreaticae.

dog		hrs	Preope.	2	4	6	8	10	12
No.	61		0.6	1.0	1.8	2.1	2.2	2.5	2.5
	62		0.4	1.0	1.6	2.0	2.6	2.5	2.5
	63		0.3	0.8	2.0	1.9	1.9	2.2	—
	64		0.8	1.5	1.5	2.1	2.3	2.3	—
	mean		0.5	1.1	1.7	2.0	2.3	2.4	2.5

Unit : $\times 10^{-4}$ A.U./ml

Table 8 Trypsin level in the lymph of thoracic duct in dogs of pancreatitis induced by bile-injection.

dog		hrs	Preope.	2	4	6	8	10	12
No.	65		0.4	0.8	1.4	1.7	1.7	2.0	2.3
	66		0.3	0.7	1.2	1.6	1.6	2.0	2.1
	67		0.3	1.0	1.3	1.3	1.5	—	—
	68		0.5	0.8	1.5	2.0	2.0	—	—
	mean		0.4	0.8	1.4	1.6	1.7	2.0	2.0

Unit : $\times 10^{-4}$ A. U./ml

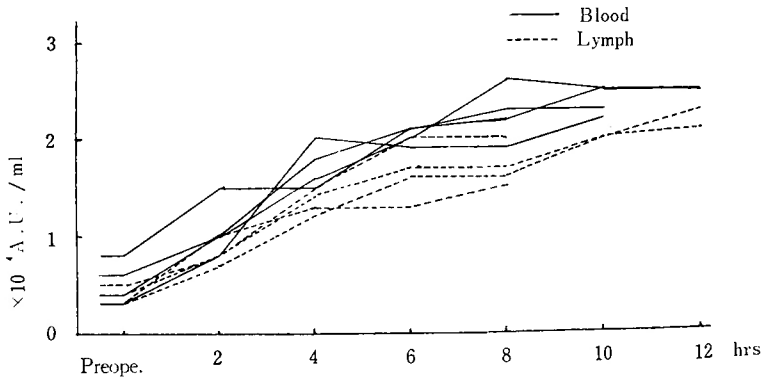


Fig. 8 Change in trypsin level in blood of *Vv. pancreaticae* and lymph of thoracic duct in dogs of experimental pancreatitis

i. Materials and Methods

Acute pancreatitis was produced in adult mongrel dogs weighing about 10 kg and the animals were divided into 2 groups. In one group, the thoracic duct was left intact and in another, thoracic duct fistula was constructed in the similar procedure as described in the above.

ii. Results

Serum trypsin level was determined every 2 hours until 12th hour postoperatively. In animals with the thoracic duct left intact, serum trypsin level showed tendency of elevation 2 to 4 hours after operation, reaching 1.0×10^{-4} A.U./ml 12 hours after operation, which corresponds to 2 times level of preoperative one.

On the other hand, in animals with thoracic duct fistula, serum trypsin level showed no significant fluctuation compared with that in the former group (Tab. 9, 10, Fig. 9).

Table 9 Serum trypsin level after thoracic duct occlusion in dogs of pancreatitis induced by bile-injection.

dog \ hrs		Preope.	2	4	6	8	10	12
No.	71	0.2	0.2	0.2	0.4	0.6	0.6	0.6
	72	0.6	0.6	0.6	0.6	0.7	1.2	1.2
	73	0.7	0.7	0.8	0.8	0.8	1.3	1.4
	74	0.4	0.4	0.5	0.8	0.8	1.0	1.1
	mean	0.5	0.5	0.5	0.7	0.8	1.0	1.1

Unit : $\times 10^{-4}$ A. U./ml

Table 10 Serum trypsin level after release of thoracic duct occlusion in dogs of pancreatitis induced by bile-injection.

dog \ hrs		Preope.	2	4	6	8	10	12
No.	75	0.5	0.7	1.0	1.2	1.2	1.2	1.4
	76	0.1	0.1	0.4	0.5	0.5	0.5	0.7
	77	0.5	0.5	0.5	0.7	0.7	1.0	1.1
	78	0.5	0.1	0.6	0.7	0.7	0.7	0.9
	mean	0.5	0.5	0.6	0.8	0.8	0.9	1.0

Unit : $\times 10^{-4}$ A. U./ml

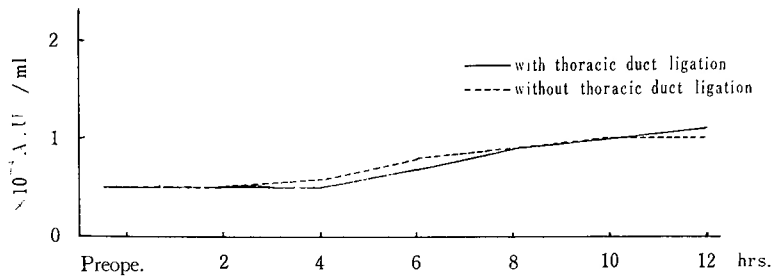


Fig. 9 Comparison of change between serum trypsin level in dogs of experimental pancreatitis with thoracic duct ligation and those without it (in mean value).

4. Serum Trypsin Level in Dogs with and without Drainage of Intraperitoneal Fluid

i. Materials and Methods

Acute pancreatitis was produced in adult mongrel dogs weighing about 10 kg and a rubber drainage was set near the Pancreas, in order to remove intraperitoneal fluid continuously to the outside.

ii. Results

When intraperitoneal fluid was drained to the outside, serum trypsin level gradually arose from 6, 12 to 24 hours after operation, 24th hour maximum level being 1.2×10^{-4} A.U./ml which gradually decreased on to 1.0×10^{-4} A.U./ml on the average 72 hours after operation (Tab. 3, 11, Fig. 10).

Table 11 Serum trypsin level in dogs of experimental pancreatitis with peritoneal drainage.

dog	hrs	Preope.	12	24	48	72
No. 81		0.8	1.2	1.9	—	—
82		0.5	1.7	2.0	1.6	1.1
83		0.6	1.5	2.1	—	—
84		0.2	0.8	1.2	1.1	0.8
85		0.4	1.0	1.4	1.4	—
86		0.5	1.1	1.5	1.2	—
mean		0.5	1.2	1.7	1.3	1.0

Unit $\times 10^{-4}$ A. U./ml

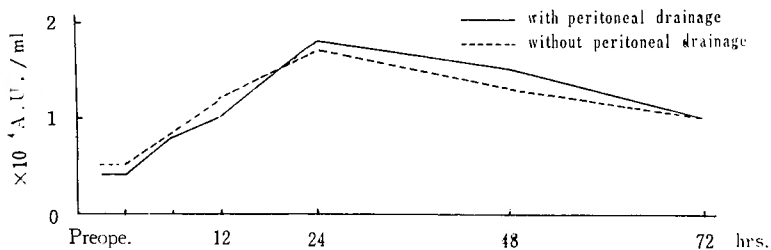


Fig. 10 Comparison of change between serum trypsin level in dogs of experimental pancreatitis with peritoneal drainage and those without it (in mean value).

On the other hand, in the animals without intraperitoneal fluid drainage, serum trypsin level was 1.8×10^{-4} A.U./ml on the average 24 hours after operation, revealing little difference from that in the animals with the drainage.

5. Summary

It is widely admitted that acute pancreatitis is frequently accompanied by intraperitoneal accumulation of bloody fluid, which is more or less invariably observed also in experimental animals. This fluid has been deemed to be consisted of exudate from the pancreas itself and that from the peritoneum¹¹⁾. Hence, trypsin level was determined in the above mentioned 4 groups.

Trypsin level in pancreatic exudate showed rapid elevation as above mentioned reaching the maximum value of 11.1×10^{-4} A.U./ml on the average 36 hours after operation, and intraperitoneal fluid also showed the maximum value of trypsin to be 2.1×10^{-4} A.U./ml 24 hours after operation. Trypsin level is 5 times higher in the former compared with the latter. In other words, it is assumed that trypsin level of the exudate from the pancreas itself is lowered as it mingles and accumulates together with the exudate from the peritoneal surface.

Trypsin level in pancreatic venous blood and thoracic duct lymph showed 2.5×10^{-4} A.U./ml and 2.2×10^{-4} A.U./ml 12 hours after operation, respectively on the average. Namely, trypsin level was constantly higher in the former than in the latter, although slightly.

No significant difference was observed between serum trypsin level in animals with the thoracic duct left opened and the level in those with thoracic duct fistula.

There was no significant difference in serum trypsin level between animals with peritoneal drainage and without it.

V. DISCUSSION

It was postulated by CARVISSAT and KUEHNE in 1867 that pancreatic juice has a proteolytic activity, and the proteolytic component was nominated trypsin by KUEHNE. Since DASTRÉ observed in 1893 that fibrin of thrombus was swiftly digested in plasma of rabbits, it has been generally admitted that proteolytic ferment is contained in serum of various species of animals. DELEZENE and PROZERSKI thereafter reported in 1903 that proteolytic activity of this ferment is augmented when treated with chloroform. It was further clarified that the ferment digests not only fibrin, but gelatin, casein and so on and various devices for determination of this ferment was done and studied using these substrates¹²⁾¹³⁾¹⁴⁾¹⁵⁾. In clinical aspect, GOODPASTURE¹⁶⁾ first observed in 1914 that activity of the proteolytic ferment in serum is strengthened in patients of liver cirrhosis, which was followed by many papers reporting increase or decrease in proteolytic activity of the ferment in various diseases and other conditions¹²⁾¹⁷⁾¹⁸⁾. Actual character of this proteolytic ferment in serum is not clearly understood yet up to date. Although this is called serum trypsin, some differences are observed in its optimal pH and action against various substrates. Accordingly, it is presumed that this ferment contains some essentially different other ferments, though resembling trypsin¹⁹⁾.

However, clinical symptoms seen at acute pancreatitis such as vascular damage of the

pancreatic vessels, shock, alteration of coagulation process, increases in proteolytic activity of serum and decrease in anti-proteolytic activity can be well reproduced, to some extent, experimentally by intravenous injection of crystalline trypsin²⁰), from which it is readily presumed that most part of proteolytic ferment liberated to blood flow at acute pancreatitis is consisted of trypsin.

It is considered that at pancreatic disorders trypsin, one of the pancreatic ferment is liberated to systemic circulation by way of hematogenous and lymphogenous paths as well as other ferments. It must be largely because there has been no identification and discrimination of serum trypsin, which increases in serum due to escape of pancreatic trypsin at pancreatitis, from other proteolytic ferments, that there have been so many discussions on this point without decisive solution.

It is generally understood that special synthetic substrate B. A. A. used in the present experiment is specifically decomposed by trypsin. However, there are assertions that this synthetic substrate is decomposed also by some proteolytic ferments other than trypsin such as chymotrypsin, cathepsin, plasmin, thrombin, papain and bromerin, though there are some differences in the degree of digestion.²¹⁾²²⁾ Accumulated studies have clarified that it is justifiably understood that the synthetic substrate is specifically decomposed by trypsin depending upon an optimal pH, temperature and duration of reaction, and on condition that the reaction be processed in vivo. By the way, MARDI lately insisted little and almost negligible influence of plasmin on this method of determination (Tab. 12)⁶⁾.

Table 12 Action of Proteinases on Benzoyl-L-Arginine Amide

Enzymes	pH	Time (hrs)	Hydrolysis (%)
Trypsin, 0.25mg protein N per cc.	7.8	1	50
		2	64
		20	95
Chymotrypsin, 0.7mg protein N per cc.	7.8	2	1
		19.5	3
Cathepsin	4.0~5.0	—	—
Thrombin (250U.)	9.0	2	10
Plasmin	—	—	—
Papain-HCN	5.0	2	76
		6	96
Bromerin-HCN	5.0	6	45

In trypsin determination in the present experiment, original method of determination of Nardi was modified. In the original method, the titration is performed with 0.01 N HCl against boric acid solution containing indicator, which absorbed ammonia within the inner well of CONWAY'S unit²³⁾. When relatively large amount of ammonia is contained in the material, reading of the terminal point of the titration is comparatively easy since at such occasion the boric acid solution changes into blue in its color and turns into bright red by the titration, and consumption of HCl also being large, technical error can be neglected. Reading of the terminal point is, however, difficult when the amount of ammonia contained in the material is very small. In addition, owing to extremely small consumption of HCl

as 1 to 2 ml, the determination requires well trained skill and there still remains room for subjective factor. On the contrary, the method of ammonia determination reported by HATANO and KIRITA and employed in the present experiment has advantage to the original one in respects of accuracy and sensitivity, permitting no room for subjective factor.

In the experiment of intravenous injection of crystalline trypsin, increase in serum trypsin depended upon the amount of trypsin intravenously injected. However, even when relatively large amount of trypsin was injected intravenously, corresponding increase in serum trypsin could not be observed. This was interpreted to be presumably due to trypsin inhibitor possibly exists in serum.

It has been recognized since early days that there exists some inhibiting substances in serum of animals against the ferments²⁴⁾²⁵⁾²⁶⁾²⁷⁾²⁸⁾²⁹⁾. There have been also many publications on the attitude of trypsin inhibitor at intravenous injection of trypsin²⁴⁾³⁰⁾³¹⁾³²⁾³³⁾. When trypsin is injected intravenously or in occasion of acute pancreatitis, there is a hazard that proteolytic activity of active trypsin appeared in serum destroys important functions of the organism. It is considered that trypsin inhibitor acts upon this point as a defence mechanism, inhibiting the activity of the trypsin. Certain correlation between this inhibitor and trypsin can be readily presumed, which is reported by OBATA in our clinic³⁴⁾. Owing to the effect of the inhibitor, it is only a small part of trypsin intravenously injected that can be demonstrated by the determination. Most part of the trypsin is thought to be inhibited and neutralized immediately. Thus it is assumed that trypsin determined at pancreatic disorders is merely a part of liberated trypsin and unexpectedly large amount of trypsin is emitted.

In the present experiment, it was disclosed that intravenously injected trypsin exists at least for 120 minutes in the blood flow in an active form. Accordingly, it is certain that when pancreatic trypsin escapes continuously into blood flow it can be demonstrated as serum trypsin.

RUSH-CLIFFTON³⁰⁾ demonstrated using substrate of casein in 1952 that serum trypsin level increases in dogs of experimental pancreatitis. In recent years, NARDI observed using substrate of B. A. A. in 1958 an increase in serum trypsin level in patients of pancreatitis and pancreatic cancer, and he further observed that serum trypsin increases in parallel with increase in serum amylase in experimental animals³⁾⁴⁾⁵⁾⁶⁾⁷⁾. ARAKI³⁵⁾, in our country, also observed the same findings using modification of NARDI's method. In the present experiment also, increase in serum trypsin in pancreatitis was observed.

Toxicity of trypsin has been long discussed. As an outstanding feature of acute pancreatitis, not only necrosis of the pancreatic tissue, but also escape of large amount of intensely toxic pancreatic ferment, trypsin is pointed out, and there are many investigators who insist upon toxicity of trypsin as a cause of toxic symptom and lethality³⁰⁾³¹⁾³⁶⁾³⁷⁾³⁸⁾³⁹⁾⁴⁰⁾. On the other hand, there are some who are skeptical to the assertion that alteration of trypsinogen into trypsin plays an important role in pathogenesis of pancreatitis⁴¹⁾.

In the present experiment, however, the higher the trypsin level in pancreatic and peritoneal exudate and in serum and the more rapid the increase in trypsin level, the worse the prognosis was, and early death was frequently observed towards the period of the maximum value of trypsin level. In addition, symptoms and findings of experimental

animals at intravenous injection of trypsin so closely resemble those seen at clinical pancreatitis of serious degree. From this respect also, toxicity of trypsin should be admitted as a factor of lethality of acute pancreatitis.

Determination of serum trypsin level in normal dogs revealed normal range of 0 to 1×10^{-4} A.U./ml, 0.5×10^{-4} A.U./ml on the average, which is interpreted to suggest endocrine secretion of some extent of exocrine substance as was postulated in 1951 by JANOWITZ and HOLLANDER⁴²⁾ that even in normal pancreas small amount of ferment appears in blood flow from the acinar cells through the interstitial spaces. STEIN and POWER et al⁴³⁾ supported in 1956 the existence of veno-interstitial and duct-venous reflux pathways and maintained that these pathophysiological pathways play an important role in liberation of pancreatic ferments into blood flow at pancreatitis.

Concerning the influence of crystalline trypsin on arterial and portal pressures, there was little difference in the tendency of fluctuation in both occasions of intravenous injection and intraperitoneal infusion. However, in quantitative respect, intravenous injection of trypsin of smaller dosis had larger influence on these pressures than intraperitoneal infusion. This is interpreted that even comparatively large amount of active trypsin has less influence on arterial and portal pressures, further, on the whole organism, so long as it exists within the peritoneal cavity than intravenously liberated trypsin of the identical amount, which is assured also from the findings of serum trypsin level at intravenous injection and intraperitoneal infusion of crystalline trypsin.

It is reported that while pancreatic juice which contains activated pancreatic ferment originated from the damaged pancreatic acini exude in the peritoneal cavity being diluted with plasma, peritoneal exudate is produced by secondary subserous neurocapillary lesion or parietal and visceral peritoneum, and intraperitoneal fluid at acute pancreatitis is consisted of the mixture of these two¹¹⁾.

It is said that the former pancreatic exudate contains pancreatic ferment in higher concentration. In the present experiment also, pancreatic exudate collected by the use of sac showed higher trypsin level than intraperitoneal fluid, which may presumably due to dilution with peritoneal exudate and neutralization with the inhibitor while in the abdominal cavity. Although trypsin level was slightly higher in intraperitoneal fluid than in serum, the difference was little, and decrease in trypsin level was slightly delayed in the former than in the latter. Similar finding is reported as to the attitude of amylase that amylase level in intraperitoneal fluid at pancreatitis is higher and persists longer than in corresponding serum⁴⁴⁾⁴⁵⁾⁴⁶⁾. It is interesting that the author of this report insists that valuable diagnostic aids can be obtained in cases of late stadium of acute pancreatitis by utilizing this phenomenon in determining amylase level with simple peritoneal tap.

As the tract of absorption in the serous cavity, capillaries of blood and lymphatic systems (lymphatic space) are pointed out ingeneral as a matter of course, and the lymphatic system is seemed to possess as large a capacity as to correspond to that of blood capillary system. Particularly the abdominal cavity is the site of utmost development of lymphatic apparatus and its absorbability is variously discussed¹⁰⁾⁴⁷⁾. However, it is considered that absorption of ferment from the peritoneal cavity is mostly achieved lymphogenously through the thoracic duct, and scarcely through immediate hematogenous pathway⁴⁸⁾. Problem of

liberation of pancreatic trypsin into blood flow still remains in obscurity and few publications are found except on amylase. It has been only supposed up to date that trypsin also takes the same tract as amylase³⁶⁾⁴⁹⁾. Namely, as to absorption of amylase, opinions do not accord, some insisting that it enters the systemic blood flow chiefly through the portal vein and very little enters by way of thoracic duct and this pattern of absorption is merely secondary one⁴⁹⁾⁵⁰⁾⁵¹⁾⁵²⁾, and some insisting contrariwise that it enters blood flow in most part lymphogenously through the thoracic duct and the remainder immediately enters the blood vessel system⁵³⁾⁵⁴⁾⁵⁵⁾⁵⁶⁾.

Discussing the principal tract of trypsin liberation into blood flow, it should be essential to grasp total amount of trypsin respectively absorbed into the blood vessels and lymphatic system within a certain interval of time. This is achieved by determining trypsin content in serum and thoracic duct lymph and volume of blood and lymphatic flows. It is, however, naturally accepted that the volume of blood flow is obviously larger than that of lymphatic flow. In the present experiment, determination of trypsin level in pancreatic venous blood and thoracic duct lymph at pancreatitis revealed that although the difference between these two was little, the level was invariably higher in the former than in the latter. This finding necessarily leads to the assumption, volume of flows being taken into consideration that trypsin from the pancreas mainly escapes into the portal vein by way of the pancreatic vein. Findings of the experiment in dogs with thoracic duct fistula, that serum trypsin level showed no marked change, also gives basis to this presumption. At this point, it should not be neglected that most part of trypsin hematogenously absorbed drains into the liver through the portal vein.

There was little difference in serum trypsin level between the occasions with and without drainage of intraperitoneal fluid. From this respect also, it is assumed that drainage of intraperitoneal fluid has little significance.

WHIPPLE and GOOD-PASTURE⁵⁷⁾ stated in their article in 1914 that "The peritoneal exudate in acute haemorrhagic pancreatitis contains no toxic substances.--The haemorrhagic peritoneal exudate may be looked upon as having a neutralizing action and appears to benefit rather than injure dogs suffering with acute pancreatitis. Dogs with acute haemorrhagic pancreatitis which are subjected to exploration and removal of peritoneal exudate will appear sicker than control dogs left undisturbed". In short, in acute Pancreatitis, most part of trypsin liberated from the pancreas immediately appears in blood flow and enters the liver through the portal vein. On the other side, trypsin that exude in the peritoneal cavity is only a small part of that liberated from the pancreas. Furthermore, as it is diluted and neutralized while in the peritoneal cavity, encountering the lowering of the activity, drainage of this fluid has little influence on serum trypsin level. Thus from these findings, it is assumed that even if the peritoneal exudate be reabsorbed, it has no influence on the course of acute pancreatitis.

VI. SUMMARY

1) As a method of trypsin determination, Nardi's original method was modified and improved employing the method of ammonia determination reported by HATANO and KIRITA.

2) Intraperitoneal infusion of crystalline trypsin had less influence than intravenous injection of this agent.

3) The peritoneal fluid at acute pancreatitis is the mixture of pancreatic and peritoneal exudates, and its trypsin level had little difference from that of serum, decrease in trypsin level in the peritoneal fluid being delayed than in serum.

4) It is only a small part of trypsin that emigrates in the peritoneal cavity in acute pancreatitis and most of them enters immediately into the systemic circulation.

5) In acute pancreatitis, intraperitoneal drainage has little effect on the change in serum trypsin level.

It was assumed from these findings that intraperitoneal drainage has little significance in acute pancreatitis, and trypsin that enters immediately blood flow and drains into the liver should be rather emphasized.

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和文抄録

実験的急性膵炎に於ける Trypsin の動態

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現在までに Trypsin の膵炎時に於ける様相に就いては文献的にもその数は極めて少ない。これは Trypsin を特異的、直接的に定量しうる方法がなかつた事に原因するものである。著者は、NardiのTrypsin測定法を改良し、犬の膵管内に自家胆汁を注入し、急性膵炎時に於ける Trypsin の動態を実験的に追究し、次の結果を得た。

1) Trypsin 測定法として、波多野・桐田の報告するAmmonia測定法を用いNardi法を改良した。

2) 結晶 Trypsin は腹腔内に注入された場合、静脈内に注入された場合よりも影響が少ない。

3) 急性膵炎時の腹腔内滲出液は、膵滲出液と腹膜よりの滲出液との混合物であり、その Trypsin 値は血

中 Trypsin 値と大差なく、それよりも遅れて減少する。

4) 膵炎時、腹腔中への Trypsin は全体からみて一部にすぎず、大部分は直接総体循環に逸脱する。

5) 腹腔のDrainageを行なつても、血中Trypsin値には余り変化が認められない。

以上の点から、Trypsin に関しては、腹腔滲出液中の Trypsin の血中への移行は膵炎の経過に重大な影響を及ぼすものでなく、むしろ、膵炎発病と同時に血中へ一度に放出されるべき Trypsin の一部分が腹腔中に滲出し、他の滲出液によつて薄められ、貯溜される一現象にすぎないとも考えられる。よつて、直接血行性に移行し肝に流入する Trypsin を重視すべきである。