bearing gastric cancer did not differ significantly from those of 11 patients having benign gastric lesions. These results are in contrast to those found in animal experiments, and are in contrast to those obtained with certain other liver function tests which appear to be impaired in patients with gastric cancer.

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Activation of Serum Protease in Peptone Shock.*

A. EVELYN SCROGGIE,† L. B. JAQUES, AND M. ROCHA E SILVA.

From the Department of Physiology, University of Saskatchewan, Saskatoon, Canada, and Department of Biochemistry and Pharmacodynamics, Instituto Biologico, São Paulo, Brazil.

A mechanism for the release of histamine and heparin in anaphylactoid conditions has been previously postulated by one of us.[1] According to this theory, the anaphylotoxins are released as a direct result of the activation of a serum protease which has been described in the literature as "plasma trypsin,"[2] "lytic factor,"[3] "plasmin,"[4] "tryptase"[5] and "fibrinolysin."[6] The activation of this protease in peptone shock is indicated by an increase in the fibrinolytic activity of the blood taken from dogs in the severe stages of shock, the fibrinolysis being observed in the tubes of the protamine titration test for heparin.[7] Heparin has been shown to have an inhibitory effect on "plasma trypsin."[2] Hence the addition of protamine to the blood, as in the protamine titration test for heparin, provides conditions under which the activation of the serum protease may be demonstrated by the resultant fibrinolysis.

Fibrinolytic activity of the blood has been observed by Nolf[8] after the injection of peptone into the liverless and the anterior dog. Since the anterior dog has its inferior vena cava and thoracic aorta ligated just above the diaphragm, thus excluding the liver from the circulation, and since in the dog most of the heparin released comes from the liver, the anterior dog provides conditions *in vivo* similar to those provided *in vitro* by an excess of protamine. The anterior dog was used in our experiments to study the fibrinolytic activity of the blood in peptone shock. A second system which has been studied is the release of histamine from the leucocytes of the rabbit on the addition of peptone. The activity of the serum protease has been investigated by the use of the trypsin inhibitor isolated from unheated soya bean flour, which has been shown to be an inhibitor of the serum protease.[9]

Materials.

Soya Bean Trypsin Inhibitor (S.B.I.): We are indebted to Dr. M. Kunitz for a sample of this material recrystallized 5 times.

Peptone. Difco proteose-peptone.

Heparin (1000 units/ml) and *Protamine* (Salmine hydrochloride) were supplied by the Connaught Laboratories, University of Toronto.

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† Scholar of the National Council of Jewish Women of Canada for Research on Blood Plasma, University of Saskatchewan.

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4 Christensen, L. R., *J. Gen. Physiol.*, 1945, **28**, 363.

5 Ferguson, J. H., *Science*, 1943, **97**, 319.

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TABLE I.
Fibrinolysis of Blood of Anterior Dog in Peptone Shock.

Protamine (mg per ½ ml blood)	Minutes after peptone injection									
	0*		2		6		18		30	
	Hr	Units†	Hr	Units	Hr	Units	Hr	Units	Hr	Units
.100	4	2	1	4	2	3	2	3	2	3
.050	24‡	0	2	3	2	3	2	3	4‡	1
.025	”	0	2	3	2	3	2	3	4‡	1
.012	”	0	2	3	2	3	4‡	1	∞	0
.000	”	0	2	3	2	3	24	0.5	∞	0
Control	∞	0	2	3	2	3	24	0.5	∞	0
Total lytic activity		2		19		18		11		5

* After operation and before peptone injection.
† Arbitrary units, total lysis in 1 hr given the value 4, in 2 hr, 3, in 4 hr, 2, in 24 hr, 0.5, and partial lysis in 4 hr given the value 1.
‡ Only partial lysis (at least half the clot) in the time stated.

Fibrinogen. Armour Fraction I. *Thrombin.* Lederle's "Hemostatic Globulin."

Peptone Shock in the Anterior Dog. The dogs were anesthetized with sodium pentobarbital, arranged for recording carotid blood pressure and prepared as anterior animals according to Nolf.[8] Injections of peptone were made into one of the external jugular veins and blood samples drawn from the other into a syringe rinsed with saline but containing no anticoagulant. The fibrinolytic activity of this blood was determined by adding it to a series of protamine tubes, the same as used in the protamine-titration test for heparin. A typical determination of fibrinolytic activity is shown in Table I. The fibrinolytic activity of each blood sample is expressed as the sum of the arbitrary units of activity assigned to each tube of the protamine series according to the length of time required for the lysis of the blood in that tube. The experiment recorded here shows the typical changes in fibrinolytic activity after peptone injection, an immediate increase followed by a slower decrease.

On the injection of peptone the blood pressure fell quite markedly in all cases but it was found that a concentration of 300 mg of peptone per kg was necessary to produce a prolonged fall in blood pressure and evidence of a prolonged activation of the serum protease.

To investigate the effect of the S.B.I. on the fibrinolysis in other experiments, part of each blood sample was added to the S.B.I. solution and pipetted to the protamine series after the control. Since the S.B.I. has some anticoagulant activity[9] a drop of thrombin was added to each tube of both control and S.B.I. series. The inhibitory effect of the S.B.I. on the fibrinolysis is well illustrated in Table II, where moderate lytic activity is completely inhibited by concentrations of S.B.I. as low as 0.34 mg per ml of blood.

In one experiment, S.B.I. (20 mg/kg) was injected into the anterior dog 2 minutes before the injection of the peptone. The injection of the S.B.I. had no effect on the blood pressure nor any effect on the fall in blood pressure after the injection of peptone. However, as shown in Fig. 1, there was a marked decrease in the degree of fibrinolysis. Also the preparation survived more than twice as long as a number of similar preparations subjected to the injection of peptone without previous treatment with S.B.I.

Since the platelets and leucocytes are believed to be involved in the production of peptone shock,[1] counts of the cellular elements were made during the experiments and are given in Table III. Platelets and leucocytes were greatly reduced after the injection of peptone, this reduction being accompanied by a marked agglutination of the platelets. The platelets and leucocytes decreased to the same level (approximately 25,000/mm^3 for platelets and 2,000/mm^3 for leucocytes) regardless of the dose of peptone, the initial

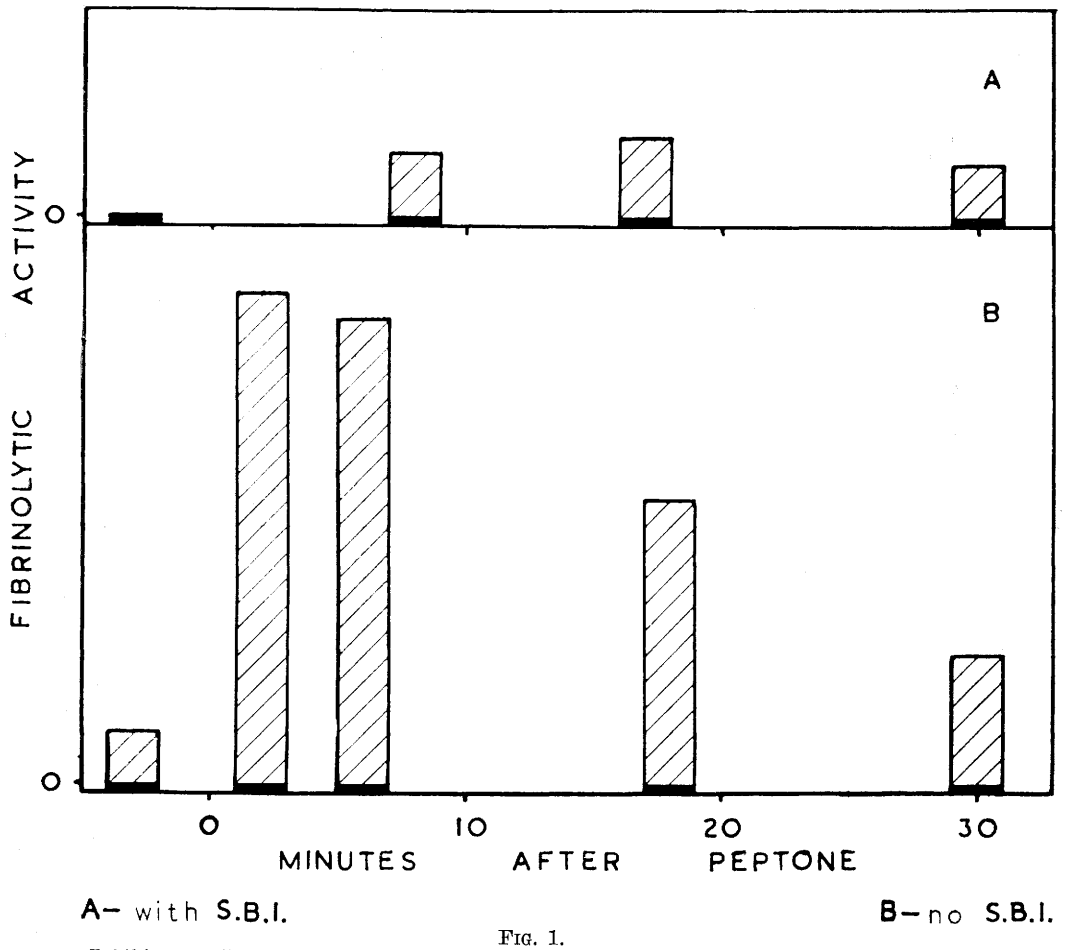

FIG. 1.
Inhibitory Effect of S.B.I. *in vivo* on Fibrinolysis. A—Anterior dog injected with S.B.I. (20 mg/kg) 2 minutes before the injection of peptone (300 mg/kg). B—Anterior dog injected with peptone (300 mg/kg).

level of the cells, the previous injection of the S.B.I., or the extent of the fibrinolysis produced.

The Effect of Protamine on Fibrinolysis. In peptone shock in the intact dog, the addition of protamine to the blood enables fibrinolysis to be observed. This could be due to the neutralizing effect of protamine on heparin, since heparin has been shown to antagonize the serum protease.[2] However, the fact that fibrinolysis occurred first in the tube with the highest concentration of protamine, regardless of the quantity of heparin present, indicated that protamine might have some other action promoting fibrinolysis as well as that of neutralizing heparin. The proteolytic activity of the blood is thought to be inhibited normally by a proteolytic inhibitor which is present in serum, so that activation of the protease occurs when the inhibitor is removed. Protamine might act by competing with the free enzyme for

TABLE II.
Effect of S.B.I. on Fibrinolysis in Blood of Anterior Dog after Peptone.

S.B.I. (mg/cc blood)	Lytic activity of control blood	Lytic activity with S.B.I.
1.7	24	0
0.83	16	0
0.67	14	0
0.34	14	0
0.17	14	2

TABLE III.
Platelet and Leucocyte Count of the Anterior Dog in Peptone Shock.*

No. of dog	Dose of peptone (mg/kg)	Pre-operative		Before peptone		After peptone		
		Leucocytes /mm³	Platelets × 1000/mm³	Leucocytes /mm³	Platelets × 1000/mm³	Time min.	Leucocytes /mm³ × 1000	Platelets × 1000/mm³
3	150	12,100	419	10,300	440	6	2.2	28 (72%)†
						20	5.2	70 (50)
						35	4.6	69 (51)
4	200	17,600	258	11,300	276	6	2.4	26 (68)
						19	2.9	25 (30)
						58	7.5	85 (15)
						10	5.4	38 (33)
6	300	8,400	225	4,400	229 (9%)	5	2.0	19 (73)
						37	1.2	14 (45)
8‡	300	5,100	445	5,100	426 (6%)	8	2.0	22 (39)
						26	2.4	52 (42)
	300					11	2.5	35 (7)
10	300	15,900	481	11,200	471 (5%)	4	5.4	104 (76)
						30	3.7	19 (41)
						60	4.7	68 (33)

* Leucocyte and platelet counts have been adjusted to the red cell count in the normal sample.
† Figures in parentheses refer to the percentage agglutination of the platelets.
‡ Dog injected with 20 mg S.B.I. per kg 2 minutes before injection of peptone.

combination with the inhibitor released during the process of activation.

To study the effect of protamine on fibrinolysis, a lytically active dog serum was prepared by treatment with chloroform.[10] This "chloroform serum" was used to lyse a standard fibrin clot, the lysis being inhibited by fresh dog serum which contains the natural inhibitor of the serum protease. The effect of protamine was tested on this lytic system, which, like the blood from the anterior dog in peptone shock, lacks the complicating factor, heparin.

In one experiment, a constant amount of protamine (300 μg) was incubated at 37.5°C with varying dilutions of the "inhibitor serum" for 5 minutes before the addition of the "chloroform serum" and clotting elements (0.2 ml of a 1:10 dilution of thrombin and 0.5 ml of 0.3% fibrinogen). Fig. 2 shows that for each dilution of the "inhibitor serum" the clot with protamine lysed before its control.

In another experiment, different amounts of protamine were incubated at 37.5°C with a constant "inhibitor serum" dilution for 5 minutes before the addition of the "chloroform serum" and clotting elements. Table IV A shows that increasing the concentration of the protamine in the clot increased the

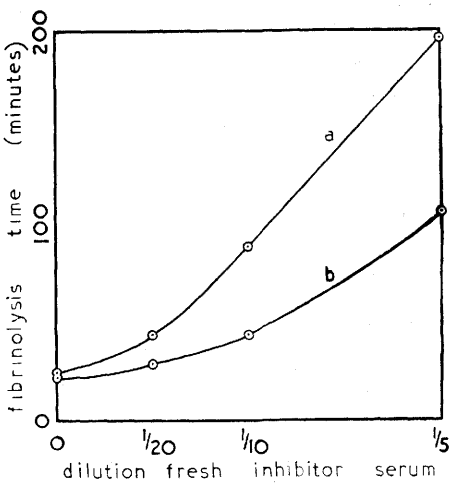

FIG. 2.

Effect of Protamine on Fibrinolysis. a (no protamine)—inhibitor serum + saline incubated at 37°C for 5 minutes, then 0.1 ml of chloroform serum, 0.2 ml of 1:10 thrombin and 0.5 ml of 0.3% fibrinogen added. b (+ protamine)—inhibitor serum + protamine incubated at 37°C for 5 minutes before the addition of chloroform serum + thrombin + fibrinogen.

[10] Christensen, L. R., *J. Bacteriol.*, 1944, **47**, 471.

TABLE IV.
Effect of Protamine and S.B.I. on Fibrinolysis.*

A			B	
Protamine (μg)	Inhibitor serum (1/5 dilution) (ml)	Lysis time (min)	S.B.I. (mg)	Lysis time
—	—	27	—	6.5 min
30	—	27	3.0	(no lysis in 12 hr)
—	.1	216	2.0	" " " " "
300	.1	138	1.0	" " " " "
30	.1	206	0.1	" " " " "
3	.1	2166	0.01	(lysis within 12 hr)
			0.001	" " " "

* Standard fibrin clot containing 0.2 ml of a 1:10 dilution of hemostatic globulin and 0.5 ml of 0.3% fibrinogen (Armour Fraction I).
A—Total volume 1.5 ml containing 0.4 ml chloroform serum.
B—Total volume 1.4 ml containing 0.1 ml chloroform serum.

speed of lysis. S.B.I. added to a lytically active clot, inhibited the lysis, as shown in Table IVB. Tagnon[9] has reported similar observations.

Effect of S.B.I. on release of histamine from rabbit blood by peptone. Blood was taken into heparin from rabbits by cardiac puncture. Part of the blood was incubated for 5 minutes at room temperature with S.B.I. (0.5 mg per ml of blood) while 2 controls were incubated with saline. To the blood + S.B.I. and to one of the controls, peptone was added (15 mg per ml of blood) while to the other control saline was added. The peptone used had been freed of histamine by treatment with permutit.[11] The mixtures were incubated for various times and histamine extractions performed on their plasmas by the method of Code.[12] The assay on the guinea pig ileum showed that in a number of experiments with an incubation time of 15 minutes, there was an inhibition by S.B.I. of the release of histamine by peptone. Little inhibition was demonstrated by the S.B.I. with an incubation time of 30 minutes.

The effect of time of incubation on the release of histamine is shown in Fig. 3. Three mixtures, a and c containing blood + saline and b containing blood + S.B.I., were incubated for 5 minutes at room temperature. Peptone was added to a and b and saline to c, the concentrations of S.B.I. and peptone

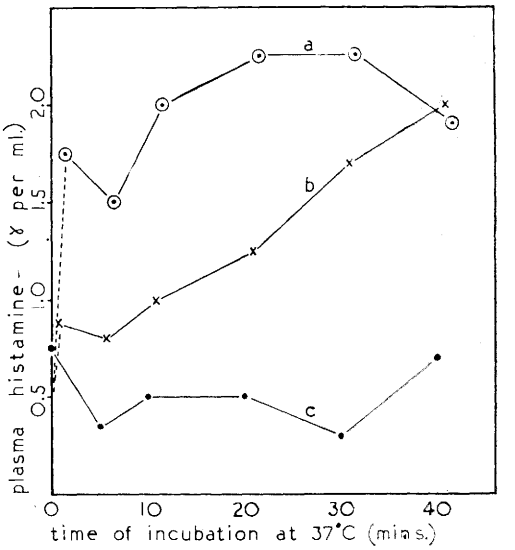

FIG. 3.

Effect of S.B.I. on the Release of Histamine from Rabbits' Blood by Peptone. a—Blood + Peptone. b—Blood + S.B.I. + Peptone. c—Blood + Saline.

in the blood being the same as above. From these mixtures incubated at 37°C, samples were taken at various times and packed in snow until all the samples were taken. These were centrifuged simultaneously and their plasmas extracted for histamine. With saline alone added to the blood, plasma histamine remained constant, the variation shown in the samples indicating the degree of accuracy of the method used ($\pm$0.2 μg histamine). With peptone alone added to the blood, the release of histamine initially was rapid, slowing to

11 Gotzl, F. R., and Dragstedt, C. A., *J. Pharm. and Exp. Ther.*, 1941, **74**, 33.

12 Code, C. F., *J. Physiol.*, 1937, **89**, 257.

a plateau in about 20 minutes. When the blood had been incubated with S.B.I. previously, the addition of peptone was not followed by a rapid increase in plasma histamine. However, the S.B.I. did not completely inhibit the release of histamine, which proceeded at a slow rate.

Discussion. As shown above, we have found that S.B.I. inhibits fibrinolysis due to the serum proteolytic enzyme activated by chloroform, while protamine inhibits the natural serum inhibitor of this enzyme. Since S.B.I. inhibited the lysis occurring in peptone shock in the anterior dog, while the addition of protamine increased the lysis, it is evident that the fibrinolysis due to peptone is due to the activation of the plasma enzyme. S.B.I. inhibited the release of histamine by peptone from rabbit blood cells. This suggests that activation of serum protease is a step in the release of histamine by rabbit blood cells. Unfortunately sufficient S.B.I. was not available for the corresponding experiment in the dog to test the effect on liberation of histamine by body cells.

The action of the strongly basic protamine on fibrinolysis of both blood and fibrin clots lends support to the hypothesis of Ferguson[5] and others that the inhibitor of the plasma proteolytic enzyme is a polypeptide with acidic groups analogous to those of heparin or that heparin is a prosthetic group for this inhibitor.

The strikingly uniform reactions of platelets and leucocytes, as far as their counts are concerned, despite wide variation in the fibrinolytic effect produced, would indicate that these cell elements are neither concerned with the production of, nor affected by, the activation of the serum protease. However, there is some evidence that this may not be the case. Smears from the cut edge of fragments of lung tissue, prepared according to the technique described by Rocha e Silva[13] showed that when fibrinolysis was marked, disintegration of the platelet clumps in the lung vessels had proceeded to a degree where only dark-staining granules remained as remnants of the disintegrated platelets. When fibrinolysis was weak or absent, the disintegration of the platelets in the clumps was very slight or absent. Thus it appears that although the counts of circulating platelets and leucocytes may not indicate any relationship to fibrinolysis, the smear technique for examining clumps of platelets and leucocytes may indicate the true relationship of these cell elements to fibrinolysis and other results of the injection of peptone.

Summary. The relationship of the serum protease to the action of peptone was investigated using soybean trypsin inhibitor and protamine. Soybean trypsin inhibitor inhibited fibrinolysis by chloroform serum (activated serum protease). It inhibited the fibrinolysis occurring in peptone shock in the anterior dog and also the release of histamine from rabbit blood cells *in vitro* on the addition of peptone. Protamine accelerated fibrinolysis in the anterior animal and also lysis by the serum protease in the presence of the natural serum protease inhibitor.

We wish to acknowledge the hospitality of Dr. C. H. Best in the Department of Physiology, University of Toronto. We are indebted to Dr. E. Fidlar for the platelet counts.

[13] Rocha e Silva, M., Porto, A., and Andrade, S. O., *Arch. Surg.*, 1946, **53**, 199.