STUDIES ON SERUM PROTEOLYTIC ENZYME INHIBITION

Effect of Tissue Destruction, Cortisone Acetate, and Splenectomy on the Serum Trypsin Inhibitor*,‡

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The allergic response in tissue has been ascribed by Ungar and Damgaard (1) to an initial release of a proteolytic enzyme with the subsequent elaboration of histamine and heparin. The proteolytic enzyme in the leukocyte also is thought to contribute to the inflammatory response in tissue (2). Since the serum has marked inhibitory power against the action of leukoprotease, trypsin, plasmin, and chymotrypsin (3), the inhibitory substance of serum may control to some extent the activity of the proteolytic enzymes associated with inflammation.

Many investigators (4–6) have described an increase of the serum trypsin inhibitor in pathological conditions associated with tissue destruction. Such an elevation has been interpreted as a non-specific indication of disease and of the same clinical significance as an elevated fibrinogen concentration. Melchoir and Sliwinski (6) in experiments on rats have been unable to demonstrate a rise in the serum inhibitor level when extensive tissue destruction had occurred through starvation of the animals. However, a fall in the level of the inhibitor after hypophysectomy was noted. Recent studies have shown that the inhibitory power of blood (7, 8) and urine (9) is elevated in conditions not associated with tissue destruction such as exposure to cold, the administration of adrenocorticotrophic hormone, and in postoperative states as has been shown to be associated with the elevation of the hyaluronidase inhibitor (10). Ungar *et al.* (11) found that in rabbits given cortisone there was a rise in the rate of inhibition of trypsin by the animals' sera but total serum inhibitor values were normal. However, the increased rate could not be elicited in cortisone-treated animals following splenectomy. The authors interpreted these findings as demonstrating that the inhibitory power of serum was mediated through the spleen.

In an attempt to define further the role of the serum inhibitor in inflammation, serial studies of the serum trypsin inhibitor were performed under conditions of tissue destruction by a chemical irritant, irradiation, and thermal

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injury, following splenectomy, and after the administration of cortisone acetate and egg albumin.

Materials and Methods

Trypsin.—Tryptar (Armour and Company, Chicago, Lots 43409 and 54011). was used This was diluted in 0.0025 N HCl to 1 mg./ml. as a stock solution and was kept at 5°C. The stock solution was subsequently diluted for use so that 1.0 ml. when incubated for 20 minutes in 1 ml. of 1.0 per cent casein gave an optical density of 600 at 280 mμ in the Beckman ultraviolet spectrophotometer. The amount of tryptar used was between 10 and 30 gamma per ml. depending upon its activity.

Casein.—Borden's vitamin-free casein diluted to 1.0 per cent in phosphate buffer, pH 7.6, was used.

Serum.—Blood was obtained by femoral puncture and allowed to clot before removing the serum. The serum was stored at 20°C.

Turpentine-Falba Mixture.—A suspension of equal parts by volume of commercial turpentine and falba (Pfaltz and Bauer, Inc., New York) was used. This was warmed slightly prior to use in order to facilitate the injection. All injections were given into the deltoid muscle.

Trypsin Inhibition.—Kunitz's (12) method and calculation for the determination of the serum trypsin inhibition were used and the results are expressed in terms of per cent inhibition per 0.01 ml. of serum. The studies of the serial sera of the same animal were all done on the same day to avoid the daily variation in the method and in the activity of the tryptar. All determinations were run in duplicate.

X-Radiation.—Monkeys were irradiated in pairs in ¾ inch plywood restraining boxes at 200 PKV and 20 ma. at distances of 168 cm. and 175 cm. measured to the middle of the animals. Radiation was from above and boxes rested on the concrete floor. The total filtration using a Machlett FCX tube (Machlett Laboratories, Springdale, Connecticut) was ½ mm. copper and 2 mm. of aluminum. Dose rates in air were determined using a Victoreen 25 r medium energy chamber (Victoreen Instrument Co., Cleveland) placed in the positions to be occupied by the animals. Direct radiation was determined as 4.3 R.P.M. at 175 cm. and 4.7 R.P.M. at 168 cm. Each animal was given a dose calculated to be 400 r.

Precipitins.—Precipitates were read in 3 to 4 cm. pyrex tubes which had a diameter of 3 mm. and were sealed at one end. Serum was introduced into such tubes with a fine glass pipette and was individually overlaid by saline and 0.005 per cent solution of egg albumin. The presence of a precipitate at the interphase was determined by comparing with saline control against a standard light source.

Egg Albumin.—A 10 per cent solution of powdered egg albumin (J. T. Baker & Co., Phillipsburg, New Jersey) in 0.9 per cent NaCl in distilled water was sterilized by passing through a Seitz filter and given intravenously using sterile precautions.

Animals.—*Macacus rhesus* monkeys were used in all experiments. The monkeys' weights varied between 2 and 4 kilograms. All had negative tuberculin tests prior to use. In some instances the monkeys were used for more than one experiment.

Statistical Evaluation.—The statistical studies were done using the Student t test. The probabilities are listed.

RESULTS

1. Normal Variations (Table I):

To determine the effect of repeated bleedings on the behavior of the serum trypsin inhibitor, three normal monkeys were bled at intervals of 3, 8, and 17 days.

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The determinations of the serum inhibitor showed moderate variations between animals but no statistically significant change from the initial values associated with the bleedings.

TABLE I

Values of Serum Trypsin Inhibitor in Per Cent Inhibition per 0.01 ml. of Serum in Normal Animals Following Repeated Bleedings

Time of bleeding	Animals			
	8-25	E 1	M 2	P
days				
1	27.3	39.3	44.7	
3	35.7	33.3	49.7	0.50
8	38.0	34.0	50.0	0.50
17	34.0	34.0	52.6	0.50

TABLE II

Serum Trypsin Inhibitor Values in per Cent Inhibition per 0.01 Ml. of Serum Following the Injection of Turpentine-Falba into the Left Deltoid

Period	Animals					
	5-34*	5-35*	5-36*	8-25‡	8-35‡	P
Control	51.4	40.0	42.0	54.9	55.2	
	Injection of turpentine-falba IM					
8 hrs.	53.5	50.5	41.0	58.2	57.5	0.20
24 "	60.0	55.5	50.0	52.8	58.7	0.10
48 "	70.0	80.6	65.6	76.5	60.3	0.01
4 days	69.0	74.5	50.0	80.6	73.0	0.01
6 "	—	—	—	6.1	70.6	
7 "	60.5	60.0	—	—	—	
12 "	—	—	—	70.6	65.6	

* Animals were given 0.3 cc. of turpentine-falba mixture.

‡ Animals were given 0.5 cc. of turpentine-falba mixture.

2. *Turpentine-Falba Injection (Table II)*:

To determine the response of the serum trypsin inhibitor to a chemical irritant, five normal animals were injected intramuscularly into the left deltoid with a suspension of the turpentine-falba.

The local reaction consisted of swelling, tenderness, and voluntary splinting of the entire extremity for 24 to 48 hours. This reaction gradually subsided without suppuration so that full activity of the extremity had returned in 1 week. The serum trypsin inhibitor determinations were obtained before and after injection. These determinations demonstrate a rapid increase in the serum

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trypsin inhibitory power evident 24 hours after the injection of turpentine-falba suspension. The greatest inhibitory activity was found 48 hours after injection following which there was a slow decline toward normal values.

TABLE III

Serum Trypsin Inhibitor in per Cent Inhibition per 0.01 ml. of Serum and White Blood Cell Counts per ml. Following 400 r Total Body Irradiation

On the 8th day post radiation two animals (5-34 and 5-18) were given 0.5 cc. of turpentine-falba suspension.

Period	Animals								
	M 2		E 1		5-34		5-18		P
	Per cent inhibition	WBC $\times 10^3$	Per cent inhibition	WBC $\times 10^3$	Per cent inhibition	WBC $\times 10^3$	Per cent inhibition	WBC $\times 10^3$	
−4 days	54.9	12.0	53.1	7.6	55.4	12.5	57.7	13.6	
−1 day	53.0	14.5	51.3	12.1	53.0	14.5	55.1	11.3	
0 days	Total body irradiation (400 r)								
2 hrs.	50.2	3.1	51.2	3.5	54.6	26.2	56.5	3.2	0.50
1 day	55.2	4.5	58.1	3.5	55.1	4.1	59.2	5.8	0.05
2 days	56.4	2.7	59.1	2.1	56.1	4.4	60.2	4.8	0.05
3 "	55.2	2.9	56.6	3.1	56.0	3.9	58.9	2.4	0.01
7 "	56.5	2.3	57.4	1.6	54.9	2.1	57.3	3.1	0.02
8 "					Turpentine-falba injection IM				
8 hrs.	59.6	2.5	58.0	1.8	58.7	3.0	58.5	9.9	
9 days	60.6	1.4	58.1	1.2	59.9	1.7	59.9	3.6	
10 "	58.8	3.4	58.1	2.2	72.8	1.2	70.7	2.3	
12 "	61.6	2.0	58.7	1.8	73.7	1.1	68.9	1.9	
14 "	62.8	—	62.1	—	76.1	—	64.7	—	
20 "	64.1	2.0	61.0	1.7	75.4	0.7	64.9	10.1	
31 "	—	4.4	—	3.9	—	4.8	—	10.1	
49 "	—	11.9	—	6.9	—	8.9	—	17.1	

3. X-Radiation (Table III):

The response of serum trypsin inhibitor was studied following total body irradiation. Four animals were each given 400 r total body irradiation and serial determinations of the serum inhibitor and total white blood cell count were done.

Following the exposure to the irradiation there was a prompt and extensive drop in the white blood cell count evident in 3 of the 4 animals 2 hours after the irradiation and in all animals in 24 hours. The counts remained low for the subsequent 30 days. The trypsin inhibitor values showed a slight increase over the control period. Although the increase in activity was significant, the elevation was of only slight magnitude when contrasted with the response following the chemical irritant found in the previous experiment.

Eight days after irradiation 2 animals (5-34 and 5-18) were given an injection of 0.5 cc. of the turpentine-falba suspension. The injection produced the

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inflammatory reaction previously described. Serial studies of the serum inhibitor demonstrated an elevation comparable to that seen in the control

TABLE IV

Serum Inhibitor Values in per Cent Inhibition per 0.01 ml. of Serum Following Necrotization of the Deltoid Muscle in an Area 10 × 2 cm. by Electrocautery under Nembutal Anesthesia

Period	Animals			
	E 1	M 2	8-25	P
Control	49.5	56.0	53.3	
"	51.0	61.0	55.3	
	Thermal injury			
1 day	59.0	61.0	58.0	0.50
3 days	55.5	62.0	58.0	0.20
8 "	59.0	56.5	56.5	0.50
13 "	60.0	58.2	56.5	0.50

TABLE V

Serum Trypsin Inhibitor Values in per Cent Inhibition per 0.01 ml. of Serum Following 19 Days of Daily Injections of 25 mg. of Cortisone Acetate per Kilogram of Body Weight

Period	Animals				
	T-6	T-8	M-2	E-1	P
Control	61.1	49.2	48.3	42.6	
0 days	Cortisone acetate 25 mg./kg./day intramuscularly				
2 days	67.0	54.8	52.3	47.4	0.01
5 "	68.3	56.7	55.0	53.6	0.01
9 "	72.1	71.7	67.0	63.7	0.01
12 "	75.5	81.6	80.3	74.8	0.01
16 "	68.3	72.6	75.2	73.0	0.05
19 "	67.0	66.1	63.4	65.1	0.05
	Cortisone discontinued				
19½ days	62.2	61.2	61.3	66.2	0.01
20 "	62.0	57.2	61.7	58.2	0.05
21 "	63.0	61.0	59.4	58.2	0.05
23 "	63.2	57.1	55.8	54.8	0.05
27 "	64.3	51.8	53.2	50.6	0.05
33 "	63.4	52.2	50.8	48.5	0.05
43 "	68.3	48.5	54.0	45.4	0.20

animals (Table II). The white cell count was transiently elevated at 8 hours in one animal (5-18) but the subsequent counts were low.

4. Thermal Injury (Table IV):

To determine the effect of tissue destruction by thermal injury on the serum trypsin inhibitor, the left deltoid muscle of each of three animals was necrotized by an electrocautery

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in an area of 10 × 2 cm. (about the area of induration produced with the turpentine-falba injection). This was performed through a surgical incision under nembutal anesthesia.

The necrotizing procedure caused only a slight tissue reaction with local induration. There was no edema and there was little voluntary splinting. Serial studies of the trypsin inhibitor following this type of tissue destruction failed to demonstrate any significant change.

TABLE VI

Serum Inhibitor Values in per Cent Inhibition per 0.01 ml. of Serum Following Splenectomy with Subsequent Daily Injections of Cortisone Acetate

Period	Animals			
	T 8	T 24	8-25	P
Control	40.4	40.2	68.8	
0 days	Splenectomy			
2 "	47.2	48.6	77.1	.01
4 "	55.9	68.0	83.7	.10
10 "	45.0	60.7	76.1	.20
14 "	42.2	67.4	77.9	.50
17 "	Cortisone acetate—25 mg./kg./day			
18 "	69.6	67.1	87.3	.02
20 "	85.7	83.7	98.0	.05
24 "	93.5	75.2	83.9	.10
26 "	97.5	72.8	84.1	.20
31 "	83.2	58.0	74.0	.20
33 "	84.8	66.0	70.4	.20
	Cortisone discontinued			
37 days	67.4	—	70.6	—

5. Cortisone Acetate Administration (Table V):

To determine the effect of cortisone acetate on the behavior of the serum trypsin inhibitor, four animals were given daily intramuscular injections of cortisone acetate (Merck & Co., Inc. Lot 01C 3062D) in a dose of 25 mg. per kilogram per day for 19 days. The injection site was rotated through all extremities.

The inhibitor titers rose promptly in all animals and were maintained at high levels. The values returned to normal upon discontinuing the hormone.

6. Splenectomy and Cortisone Acetate (Table VI):

Since Ungar (11) and his associates failed to demonstrate any change in the inhibitory activity of serum following the administration of cortisone in splenectomized rabbits, a comparable study was undertaken.

Three monkeys were splenectomized under nembutal anesthesia. 17 days after operation, all animals were given daily intramuscular injections of cortisone acetate (Merck & Co., Inc., Lot 01C 3063D and 01C 3065D), in a dose of 25 mg. per kilogram of body weight for 21 days.

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The serial serum trypsin inhibitor values showed a marked and significant elevation for 4 days following the operation and then fell toward preinjection values. The daily administration of cortisone acetate beginning on the 17th postoperative day was followed by a prompt rise in the titer of the serum inhibitor. The inhibitor values remained significantly elevated for 3 days during the administration of cortisone acetate. Subsequent values, despite continued cortisone administration, were erratic and not statistically significant.

TABLE VII

Serum Trypsin Inhibitor Values in per Cent Inhibition per 0.01 ml. of Serum Following the Injection of Turpentine-Falba Suspension during Prolonged Cortisone Administration

Probabilities are based upon a comparison of values obtained before and after the injection of the irritant.

Period	Animals			
	T-25	T-27	T-28	P
Control	54.6		64.6	
"	57.0	63.1	60.7	
	Cortisone acetate—25 mg./kg./day			
2 days	71.9	64.6	82.7	
7 "	78.5	83.8	97.3	
12 "	75.7	78.9	84.4	
14 "	71.2	77.7	84.7	
16 "	74.0	79.9	75.9	
20 "	72.8	73.8	76.3	
22 "	74.1	74.4	78.6	
27 "	72.4	78.4	80.3	
28 "		Turpentine-falba injection		
8 hours	66.2	71.1	76.0	0.05
29 days	69.1	68.5	77.9	0.20
30 "	75.9	78.4	75.4	0.50
32 "	80.7	76.8	76.4	0.50
34 "	75.0	82.8	83.0	0.05
	Cortisone discontinued			
36 days	68.9	74.6	70.6	0.20

7. Injection of Turpentine-falba during the Administration of Cortisone Acetate (Table VII):

In order to assess the influence of the chemical irritant on the serum trypsin inhibitor during cortisone acetate therapy, four animals were given daily intramuscular injections of cortisone acetate (Merck & Co., Inc. Lot 01C 3062D) in a dose of 25 mg. per kilogram of body weight per day for 34 days. On the 28th day of therapy all animals were given an intramuscular injection of 0.5 cc. of the turpentine-falba mixture.

The resulting inflammation did not appear in the gross different than responses seen previously in control animals. The study of the serum trypsin

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inhibitor showed the prompt initial rise previously encountered after cortisone therapy. The greatest increase was encountered at 8 days, then the values declined slightly on subsequent determinations.

The response to the chemical irritant was irregular. There was a slight but significant fall 8 hours after the injection but the subsequent values showed no statistical significant increase until 6 days after the injection. Upon discontinuing the cortisone, the inhibitory activity fell promptly.

TABLE VIII

Inhibitor Values in per Cent Inhibition per 0.01 ml. of Serum Following the Injection of a 0.5 cc. of Turpentine-Falba 48 Hours after the Withdrawal of Cortisone Acetate

Probabilities are based upon a comparison of the values before and after the injection of the irritant.

Period	Animals				
	8-25	5-34	5-35	5-18	P
Control	51.1	50.9	51.0	53.1	
"	49.3	51.0	51.2	52.5	
Cortisone acetate—25 mg./kg./day					
2 days	57.4	57.8	56.2	56.8	
5 "	—	62.2	57.6	67.3	
9 "	—	63.4	65.6	67.4	
11 "	69.8	73.0	69.4	72.7	
15 "	67.7	72.4	72.7	72.6	
17 "	Cortisone discontinued				
19 "	62.2	71.5	65.1	67.3	
Turpentine and falba injection					
8 hours	57.6	63.4	61.7	63.1	0.02
20 days	58.8	62.7	80.6	60.7	0.50
21 "	73.6	59.5	78.3	74.5	0.50
23 "	70.8	68.5	76.4	70.6	0.20
27 "	58.8	60.9	67.0	58.5	0.20
33 "	51.8	53.5	60.0	55.8	0.05
43 "	60.0	50.0	53.9	54.5	0.001

8. Cortisone Acetate Withdrawal Followed by the Injection of Turpentine-Falba Suspension (Table VIII):

To determine the behavior of the serum trypsin inhibitor to a chemical irritant when substitution therapy with cortisone acetate was suddenly withdrawn, four monkeys were injected intramuscularly with cortisone acetate in a daily dose of 25 mg. per kilogram of body weight for 17 days. The cortisone was then stopped and 48 hours after the last dose, all animals were given 0.5 cc. of the turpentine-falba suspension intramuscularly.

The gross inflammatory reactions appeared similar to those occurring in untreated animals. The trypsin inhibitor response was again erratic as in the

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previous experiment. There was a slight initial fall at 8 hours, but subsequent values were never significantly higher than the preinjection values. It should be emphasized that the trypsin inhibitor values were elevated prior to the injection of the chemical irritant.

9. Injection of Foreign Protein (Table IX):

Four animals were injected intravenously with a 10 per cent solution of egg albumin in a dose of 1 gm. per kilogram body weight. Serial determinations of the serum trypsin inhibitor and precipitins were performed.

TABLE IX

Serum Inhibitor in per Cent Inhibition per 0.01 ml. of Serum Following the Intravenous Injection of Egg Albumin in a Dose of 1 gm. per Kilogram of Body Weight

The precipitins (Ppt) obtained are indicated in the parentheses.

Period	Animals								P
	6-95		6-98		7-03		M-1		
	Per cent inhibition	(Ppt)	Per cent inhibition	(Ppt)	Per cent inhibition	(Ppt)	Per cent inhibition	(Ppt)	
Control	37.0	(0)	37.0	(0)	56.5	(0)	53.5	(0)	
"	36.5	(?)	42.5	(0)	53.0	(0)	54.0	(0)	0.50
1 day	Egg albumin—1 gm./kg./body weight/intravenously								
4 days	38.5	(0)	52.0	(0)	52.1	(1+)	56.0	(0)	0.50
10 "	39.0	(1+)	42.0	(±)	62.5	(1+)	57.0	(1+)	0.02
14 "	46.0	(1+)	43.0	(1+)	62.0	(1+)	62.0	(1+)	0.05
16 "	43.5	(1+)	43.5	(±)	60.0	(1+)	55.0	(2+)	0.02
19 "	42.0	(1+)	46.5	(?)	61.5	(?)	58.0	(?)	0.02
25 "	45.0	(±)	48.5	(?)	57.5	(±)	77.0	(1+)	0.20

The inhibitory power of the serum increased slightly in studies done on the 10th, 14th, 16th, and 19th day after the injection of the egg albumin. Although the rise in the inhibition values was statistically significant, the magnitude of the change was small. Precipitins were demonstrable in all animals.

DISCUSSION

The nature of the serum proteolytic enzyme inhibitor of serum is not known.

Jensen *et al.* (8) consider the trypsin and plasmin inhibitor to be identical. Shulman (13) indicates that the serum chymotrypsin and trypsin inhibitor can be separated from the plasmin inhibitor. Jacobsson (14) using zone electrophoresis in a block of filter papers describes two trypsin inhibitors of serum, one traveling with the $alpha_1$ proteins and the other with the $alpha_2$ proteins. These, he states, represent 85 per cent of the inhibitor activity of serum but $alpha_1$ inhibitor has more activity than $alpha_2$ in a ratio of 9:1. Further, he found that although both have antitrypsin activity only

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the inhibitor in the alpha$_2$ protein–inhibited plasmin. Inhibitory activity of other enzymes by the partially purified plasmin inhibitor of Loomis (15) and the trypsin inhibitor of Laskowski (16) has not been reported.

Therefore, in measuring the trypsin inhibition as reported herein, it is probable that both plasmin and trypsin inhibitors are being measured.

Because the behavior of the serum trypsin inhibitor resembles superficially that of the hyaluronidase inhibitor (10), it might be argued that they are identical inhibitors but are measured by different functions.

However, the serum proteolytic enzyme inhibitor is relatively heat-stable at 56° for 30 minutes, losing only 10 per cent of its activity whereas the hyaluronidase inhibitor is completely destroyed at this temperature (17). Further, the partially purified inhibitor obtained by the method of Peanasky and Laskowski (18) exhibited no demonstrable inhibitory effect against hyaluronidase when studied in this laboratory by the turbidimetric method (19). In most clinical studies the two inhibitors behave in a similar fashion. However, in pregnancy the serum trypsin inhibitor is regularly elevated and falls promptly after termination of pregnancy (20), whereas the hyaluronidase inhibitor remains normal prepartum and becomes elevated in the postpartum period (10).

An increase of the serum trypsin inhibitor activity has been associated with tissue destruction and correlated with the sedimentation rate (4, 21). However, in the studies presented herein there was no change in the serum inhibitor following thermal injury and only a slight increase accompanying immunization and irradiation. Although the amount of tissue destruction might be too limited to activate the inhibitor, the drop in the white blood cell count following irradiation was prompt and extensive. In contrast the inflammatory reaction following the chemical irritant, the surgical trauma of splenectomy and the cortisone acetate administration were all associated with a pronounced rise in the inhibitor values. Further, in the experiments in which there had been prolonged substitution therapy with cortisone in an attempt to create adrenal insufficiency, the response to the chemical irritant did not produce significant elevation of the trypsin inhibitor. Obviously the response is difficult to evaluate since the preinjection inhibitor level of the cortisone treated animals was elevated prior to the injection of the turpentine-falba suspension. However, these experiments suggest that the activation of the trypsin inhibitor may be in part mediated by the adrenal rather than by a product of tissue destruction. Unfortunately all experiments on adrenal insufficiency produced by surgical adrenalectomy were difficult to evaluate because of the regular mortality within 24 hours after the injection of the chemical irritant.

This relationship between the serum trypsin inhibitory activity and tissue destruction suggests a tissue source for the inhibitor.

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MacFarlane and Biggs (22) have estimated the inhibitory activity in a number of organs, especially the lungs, adrenal, and kidney. However, in extensive studies of homogenates of various monkey tissues no inhibitory activity against trypsin could be demonstrated. Muscle tissue from the site of the turpentine-falba injection and normal tissue incubated with hydrocortisone also failed to show inhibition although such a procedure by itself did not inactivate trypsin inhibitor in control studies.

The studies of Ungar and Damgaard (11) indicated that there was no increase in the total activity of the trypsin inhibitor following the administration of cortisone but they did demonstrate that the rate of inhibition was increased and in splenectomized animals no increase in rate of inhibition could be demonstrated.

We were unable to confirm these findings in monkeys as all normal and splenectomized animals had a significant increase in the inhibitor activity of serum following the administration of cortisone.

The role of the serum trypsin inhibitor in inflammation is not known. The inhibitor has been shown to be effective against leukoprotease (23). This suggests that it controls in part the extent of the inflammatory reaction. Ungar's postulation (1) that the proteolytic enzyme is initially elaborated in the allergic reaction suggests that the anergy following operation and during cortisone therapy may be due to the activation of the serum inhibitor. The clinical observation that patients treated with cortisone control infections poorly might also be associated with a failure in the response of the inhibitor such as was seen in these animals which were given substitution therapy.

Because of the close correlation between the sedimentation rate and the proteolytic enzyme inhibitor, it is of interest to postulate that this relationship is due to the effect of the enzyme-inhibitor complex on the circulating fibrinogen. MacFarlane and Biggs (22) suggest that the extensive fall in fibrinogen in 5 hours in chloroform poisoning (24) might be due to proteolysis from plasmin after inactivation of the inhibitor by chloroform since the profound depletion is more than can be accounted for by mere failure in regeneration (25). It is conceivable that the relationship between the sedimentation rate and the inhibitor is due to the activity of the serum proteolytic enzymes on fibrinogen as controlled by the serum inhibitor.

SUMMARY

Serial studies of the serum levels of trypsin inhibitor have been performed on monkeys following injection of turpentine-falba, total body irradiation, thermal injury, splenectomy, administration of cortisone acetate and egg albumin. Following the injection of turpentine-falba mixture there was a prompt elevation in the trypsin inhibitor power of the serum. A similar response accompanied the administration of cortisone acetate. The response to cortisone was not inhibited by removal of the spleen. Animals premedicated with cortisone

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acetate for 3 and 4 weeks showed no statistically significant change in the level of the serum inhibitor following injection of the turpentine-falba.

Total body irradiation and immunization with egg albumin were accompanied by a very slight rise in the inhibitory power of the serum despite a prompt and extensive drop in the total white blood cell count in the irradiated animals, and development of precipitins in all of the immunized animals. The significance of these findings is discussed.

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BIBLIOGRAPHY

1. Ungar, G. and Damgaard, E., *J. Exp. Med.*, 1955, **101**, 1.
2. Opie, E. L., *Physiol. Rev.*, 1922, **2**, 552.
3. Grob, D., *J. Gen. Physiol.*, 1943, **26**, 405, 423, 431.
4. Shulman, N. R., *J. Exp. Med.*, 1952, **95**, 605.
5. Ratnoff, O. D., *Johns Hopkins Hosp. Bull.*, 1951, **88**, 304.
6. Melchior, J. B. and Sliwinski, R. A., *Cancer Research*, 1954, **14**, 677.
7. Clifton, E. E., *J. Lab. Clin. Med.*, 1952, **39**, 105.
8. Jensen, H., Chamovitz, D. L., Volkringer, E., Gray, E., Gosney, J. E., and Kocholaty, W., *Blood*, 1953, **8**, 324.
9. Dillard, G. H. L., *J. Lab. Clin. Med.*, 1950, **36**, 266.
10. Glick, D., *J. Mount Sinai Hosp.*, 1950, **17**, 207.
11. Ungar, G., and Damgaard, E., *J. Exp. Med.*, 1951, **93**, 89.
12. Kunitz, M., *J. Gen. Physiol.*, 1947, **30**, 291.
13. Shulman, N. R., *J. Exp. Med.*, 1952, **95**, 593.
14. Jacobsson, K., *Scand. J. Clin. Lab. Inv.*, 1955, suppl. 14, 57.
15. Loomis, E. C., Ryder, A., and George, C., Jr., *Arch. Biochem.*, 1949, **20**, 444.
16. Laskowski, M. and Laskowski, M., Jr. *Advances Protein Chem.*, **9**, 203.
17. Dorfman, A., Ott, M. L., and Whitney, R., *J. Biol. Chem.*, 1948, **174**, 621.
18. Peanasky, R. J., and Laskowski, M., *J. Biol. Chem.*, 1953, **204**, 153.
19. Tolksdorf, S., McCready, M. H., McCullagh, D. R. and Schwenk, E. *J. Lab. Clin. Med.*, 1949, **34**, 74.
20. Gräfenberg, E., *Münch med. Woch.*, 1909, **56**, 702.
21. Raab, W., *Z. ges. exp. Med.*, 1935, **96**, 60.
22. MacFarlane, R. G., and Biggs, R., *Blood*, 1948, **3**, 1167.
23. Grob, D., *J. Gen. Physiol.*, 1949, **33**, 103.
24. Whipple, G. H., and Hurwitz, S. H., *J. Exp. Med.*, 1911, **13**, 136.
25. Whipple, G. H., *Am. J. Physiol.*, 1914, **33**, 50.

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