immunity and the negative results susceptibility may perhaps be gleaned from a comparison of the incidence of experimental IH in the groups of volunteers and the results of the skin test in a similar group chosen at random in institution No. 1. Over a period of 2 years 54 volunteers were exposed and 34 developed clinical or laboratory signs of hepatitis, 8 with jaundice; *i.e.*, 63% were definitely susceptible. Of individuals with specific skin test responses, 33 or 59% were negative and presumably susceptible. Although this appears to be a striking agreement this point requires confirmation by studying the effect of natural or experimental exposure to IH virus in individuals with negative and positive skin tests, respectively.

Summary. A skin test antigen for infectious hepatitis (IH) has been prepared from amniotic fluid of chick embryos infected with IH virus. Thus far, it has given apparently specifically positive skin reactions in all the individuals with past histories of spontaneous or induced IH tested. In contrast, the incidence of positive tests among cases of past serum hepatitis was no greater than that found among an adult group chosen at random.

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In Vitro Histamine Release from Sensitized Rabbit Blood Cells. Evidence Against Participation of Fibrinolysin. (17759)

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A considerable amount of circumstantial evidence has been presented to support the view that the release of histamine associated with anaphylaxis depends upon a proteolytic mechanism. The evidence has been obtained from studies in the dog, guinea pig, and rabbit and some of the studies appear to implicate the plasma protease, fibrinolysin (1-7). The evidence cited is indirect and circumstantial and a more direct type of evidence is desirable.

In this paper are presented the results of our studies on the question of whether fibrinolysin participates in the release of histamine from the blood cells of the sensitized rabbit by antigen, *in vitro*. The availability of (a) crystalline soy bean trypsin inhibitor(8), which is a potent inhibitor of fibrinolysin, and (b) purified bovine fibrinolysin and antifibrinolysin(9,10) offer a direct experimental approach to this problem.

Experimental. The details of rabbit sensitization, exsanguination, the *in-vitro* histamine release reaction, and histamine determination were presented previously(11). Silicone-coated glassware was used for the handling of whole blood and for the histamine release reaction. The authors are grateful to Dr. M. Kunitz for samples of crystalline trypsin and soy bean trypsin inhibitor, STI, and to Eugene C. Loomis for samples of purified bovine fibrinolysin and antifibrinolysin. Some of the STI used was prepared in our own laboratory by the method of Kunitz.

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TABLE I.
Failure of STI to Inhibit Histamine Release from Sensitized Rabbit Blood Cells by Antigen.

Rabbit No.	Histamine in plasma, μg per ml of blood: No antigen	Antigen	STI and antigen	STI level, mg/ml	Inhibition by STI, %
900	.20	1.0	1.1	.4	—12
901	.22	0.78	0.87	.4	—16
7	.05	1.4	1.1	.8	21
8	.13	1.6	2.0	.8	—27
13	.14	2.2	2.4	.8	— 9
14	.10	1.7	1.6	.8	6
19	.13	2.6	1.9	1.6	28
20	.08	0.88	0.88	1.6	0
37	.05	1.1	1.1	1.6	0
38	.11	1.9	1.9	1.6	0

Studies on the rate of histamine release were carried out as follows: Blood and saline solutions were placed in a 37°C water bath and were allowed 15 minutes to reach the temperature of the bath; 25 ml of blood was mixed with 18.8 ml of saline containing (a) no STI and (b) 100 mg of STI. After 5 minutes 12.5 ml of 1:100 egg white in saline was added rapidly with thorough mixing. At 1, 2, 3, 5, and 10 minutes after the addition of antigen, duplicate 4.5 ml aliquots were transferred to conical centrifuge tubes in an ice bath, each tube containing 1 ml of 0.1 M sodium oxalate. The oxalate was used to stop the histamine release reaction immediately(11). The contents of the centrifuge tubes were mixed thoroughly, chilled a few minutes, and centrifuged. The diluted plasma samples were then carried through the histamine purification and determination. The controls on these experiments were blanks at 0 time and 10 minutes, and histamine release by trypsin in the presence and in the absence of STI.

Experiments on the histamine release from normal blood by antigen and plasma from a sensitized animal were performed as follows: To 0.1 ml of blood plasma from a sensitized rabbit either 2.3 ml of saline or 1.3 ml of saline and 1 ml of STI solution (8 mg of STI per ml in saline) were added. One ml of 1:100 egg white in saline was added and the solution was placed in a 37°C water bath for 30 minutes. Two ml of normal rabbit blood at 37° was then added with thorough mixing; the tube was kept at 37° for an additional 10 minutes and then placed in an ice bath. The blood cells were removed by centrifugation and the plasma was analyzed for histamine. The data reported are the averages of duplicate samples. Duplicates ordinarily agreed within ± 10%.

Results and discussion. Our data indicate that the anaphylactic release of histamine from rabbit blood cells *in vitro* does not depend upon the activation of the plasma protease, fibrinolysin. The data of Table I show that a high level of soy bean trypsin inhibitor (STI) fails to inhibit appreciably the release of histamine when antigen is added to blood from the sensitized rabbit *in vitro*. The amount of STI which failed to inhibit the histamine release by antigen (the highest concentration used) is 4 times the quantity which gave a nearly complete inhibition of histamine release by an optimal concentration of trypsin in the majority of cases (Tables II, III, and IV). This is approximately 80 times the amount of STI used by Gerheim and Ferguson(12) to inhibit the fibrinolysin in staphylokinase-activated rabbit plasma.

Plasma samples from the reactions where STI failed to inhibit histamine release by antigen, and from parallel samples without STI, were dialyzed and concentrated and tested for their ability to inhibit histamine release from rabbit blood cells by trypsin. The results (Table II) indicate that after the failure of STI to inhibit histamine release by antigen there is sufficient STI remaining in the plasma to inhibit markedly the histamine release by trypsin. In the

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TABLE II.
Inhibition of Trypsin Histamine Release by STI Remaining in Plasma After Failure to Inhibit Histamine Release by Antigen.

Plasma No. from Table I†	% inhibition of trypsin histamine release*		
	Plasma from reaction without STI	Plasma from reaction with STI	Control STI 0.4 mg/ml
19	2	26	34
20	— 2	36	34
37			
1	21	63	98
2	20	91	99
38			
1	16	95	98
2	53	82	99

* Trypsin conc. was 0.15 mg/ml.
† Plasmas No. 37 and 38 were tested for STI with trypsin reactions on each of two different normal rabbit bloods. Plasmas No. 19 and 20 were tested on a different date and with only one trypsin reaction.

TABLE III.
Histamine Release from Normal Blood Cells by Sensitized Plasma and Antigen.

	Histamine in plasma, μg per ml of blood			
	Rabbit No. 1	Rabbit No. 2	Rabbit No. 3	Rabbit No. 4
Blood + Saline	0.18	0.23	0.21	0.41
" + SP + antigen	1.6	0.94	3.1	2.8
" + " + STI + antigen	1.5	0.98	2.9	2.7
" + trypsin	2.3	1.8	4.1	3.7
" + STI + trypsin	0.29	0.23	0.21	0.38

TABLE IV.
Rate of Histamine Release by Antigen in Presence and in the Absence of STI.*

Reaction time, min.	Histamine in plasma, μg per ml of blood							
	Rabbit No. 233		Rabbit No. 234		Rabbit No. 317		Rabbit No. 318	
	No STI	STI	No STI	STI	No STI	STI	No STI	STI
	With antigen							
1	.58	.62	.24	.28	.26	.43	.16	.20
2	1.3	1.5	.64	.50	.44	.58	.31	.30
3	3.3	3.2	1.9	1.6	.93	.93	.74	.71
5	4.7	5.2	2.6	2.2	1.4	1.5	1.1	1.3
10	6.1	5.4	3.2	3.2	1.8	2.1	1.8	1.4
	Without antigen							
0	—	—	—	—	—	—	.35	—
10	.58	—	.31	—	.42	—	.56	—
	With trypsin							
10	5.0†	1.9†	2.8†	1.0†	2.9	.38	1.80	.41

* Conc. of STI was 1.6 mg/ml in final dilution, or 4 mg/ml of blood in the reactions with antigen; the STI level in the trypsin reactions was 0.8 mg/ml in the final dilution.
† In these experiments the trypsin controls were in the 37° bath for 20 min.

presence of this excess of STI, it is difficult to believe that the plasma protease could in any way participate in the histamine release.

As an answer to this evidence the explanation has been offered that fibrinolysin might release the histamine immediately following or simultaneously with its activation, before STI can inhibit the reaction. To test this

TABLE V.
Histamine Release from Rabbit Blood Cells by Bovine Fibrinolysin, Trypsin, and Antigen.

Rabbit No.	Enzyme conc., mg per ml		Histamine in plasma, μg per ml of blood			
	Fibrinolysin	Trypsin	Controls	With fibrinolysin	With trypsin	With antigen
5	1.6	.10	.12	.37	1.7	—
6	1.6	.10	.11	.14	1.8	—
7	3.2	.15	.05	1.7	2.7	1.4
	4.8			1.5		
8	3.2	.15	.13	.26	2.3	1.6
	4.8			1.5		
13	4.	.15	.14	.28	2.6	2.2
14	4.	.15	.10	.80	2.2	1.7
995	8.	.15	.10	.39	1.8	2.5
996	8.	.10	.05	.38	1.3	.9

TABLE VI.
Effect of Bovine Antifibrinolysin on Histamine Release by Antigen.

Rabbit No.	Histamine released,* μg per ml of blood		Inhibition by antifibrinolysin, %
	No antifibrinolysin	Antifibrinolysin	
217	2.2	2.8	—27
218	2.4	2.8	—17
219	3.9	4.1	— 5
220	3.0	2.3	23

* Histamine released = (histamine in plasma after antigen reaction — histamine in plasma blanks.)

possibility we studied the release of histamine from normal rabbit blood by means of "sensitized plasma" and antigen. As shown in Table III a good release of histamine from rabbit blood cells was obtained when antigen and sensitized plasma were allowed to react for 30 minutes at 37°C prior to their mixing with normal blood. Again the histamine release was not affected by the presence of a high level of STI. Inasmuch as the histamine release from sensitized blood cells is nearly complete in 5 minutes at 37°C (Table IV), the histamine release mechanism must be activated quite rapidly. The 30 minute period at 37° in which sensitized plasma, antigen, and STI are allowed to react should be ample time for any active fibrinolysin to become inactivated by STI. The failure of STI to inhibit the histamine release under these conditions is strong evidence that fibrinolysin has no significant role in the histamine release mechanism under investigation.

Scroggie, *et al.*(13) showed that STI delays the release of histamine from rabbit blood cells by peptone. Our data on the rate of histamine release by antigen in the presence of and in the absence of STI, Table IV, indicate that STI has no effect upon the rate of the anaphylactic release of histamine. This may mean that the mechanisms of peptone and anaphylactic histamine release are dissimilar.

Additional evidence against the participation of fibrinolysin in the *in vitro* anaphylactic histamine release is: (1) the addition of high levels of bovine fibrinolysin to rabbit blood gave a poor and unreproducible release of histamine as compared to the amount released by much lower concentrations of trypsin and by antigen, Table V. The concentrations of fibrinolysin shown in Table V represent the final dilution in the reaction. The highest level used would be 20 mg per ml of blood—an extremely high concentra-

13. Scroggie, A. E., Jaques, L. B., and Rocha e Silva, M., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 326.

tion even through the enzyme may not be 100% pure. (2) Bovine antifibrinolysin, 5 mg per ml of blood, failed to inhibit the histamine release by antigen as shown by the data of Table VI.

The evidence presented here applies only to the mechanism of histamine release from rabbit blood cells by antigen *in vitro* and may have no bearing upon the mechanism of histamine release by antigen in other species. However, in accord with our results is the recent work by Scroggie and Jaques (14), which indicates that in the dog the activated plasma protease is not necessary for the anaphylactic release of histamine in the liver.

The above evidence indicates that any activation of profibrinolysin or any increase in plasma protease activity, which may accompany the *in vitro* histamine release by antigen, is only a coincidental phenomenon and is not a prerequisite for the histamine release.

Summary. The failure of excessive concentrations of soy bean trypsin inhibitor and bovine antifibrinolysin to inhibit the histamine release by antigen and the poor and inconsistent histamine release by bovine fibrinolysin indicate that the *in vitro* anaphylactic release of histamine from rabbit blood cells does not depend upon fibrinolysin activity.

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Release of Adrenocorticotrophic Hormone by Direct Application of Epinephrine to Pituitary Grafts.* (17760)

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It is now known that the injection of epinephrine is followed in normal rats by a fall in the adrenal ascorbic acid(1) and in circulating blood eosinophiles(2). Long(3) has suggested that in many of the conditions associated with an increased degree of activity of the pituitary-adrenal system, a preliminary release of epinephrine is a contributing factor. On the other hand, Sayers and Sayers(4) have found that there is an inverse relationship between the blood levels of adrenocorticotrophic hormone (ACTH) and adrenal cortical hormone (ACH) that determines the relative secretory activity of each gland. They regard the effect of epinephrine as due to its capacity to increase the utilization of cortical hormone and thus reduce its level in the blood traversing the pituitary.

In order to determine whether epinephrine is capable of directly increasing the secretion of ACTH from the anterior pituitary, the following experiment was performed. A group of adult, male, albino rats of the Sprague-Dawley strain, weighing about 250 g each, were hypophysectomized by the parapharyngeal approach, and 3 days later homologous transplants of pituitary tissue from female adult rats of the same strain were inserted into the anterior chamber of the right eyes. Seven rats survived the operations and 6 weeks later all had well vascularized grafts growing in the anterior chamber of the eye. The eosinophile decline in the peripheral blood was selected as an index of adrenal cortical activity

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