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Inhibitory Effect of Heparin upon Histamine Release by Trypsin, Antigen, and Proteose.*

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Anaphylactic, trypsin, and peptone shock are closely related phenomena. The prominent symptoms are similar and the intravenous injection of antigen, trypsin, or proteose into intact animals has been shown to result in the liberation of significant amounts of histamine.[1-3] It has also been shown that the addition of antigen, trypsin, or proteose to rabbits' blood (rendered incoagulable with heparin) leads to the release of histamine from cells to plasma.[4-9] It is possible that the mechanisms of the release are the same in each case. A study of the effect of various agents in inhibiting or augmenting the release of histamine by the above substances suggests itself as a means of determining whether similar mechanisms are involved. Inasmuch as rabbits' blood can be divided into samples which can be considered identical in every respect, this tissue seems to provide an ideal medium for such studies.

It has been shown that heparin is capable of inhibiting the proteolysis of various substrates by trypsin,[10,11] and we have found that heparin antagonizes the toxic effects of trypsin in intact animals.[12] Consequently the present experiments were performed to determine whether heparin has an inhibitory effect upon the release of histamine by trypsin, and if so, to examine the effect of heparin upon the release of histamine by antigen and by proteose.

Experimental. Blood was obtained from unanesthetized rabbits by cardiac puncture. Sufficient heparin‡ was added to this blood to prevent clotting, a concentration of 0.02%. Each blood sample was divided into 3 tubes. Saline was added to the first tube; specific antigen, trypsin, or proteose was added to the second tube; and specific antigen, trypsin, or proteose plus additional heparin, a final concentration of 0.12%, was added to the third tube. The tubes were carefully inverted a few times and then immediately centrifuged for 5 min in an angle centrifuge. The supernatant plasma was pipetted off and assayed for its histamine equivalent content against standard histamine solutions on either the guinea pig intestine or the blood pressure of an etherized-atropinized cat. The plasma samples were extracted in most cases by Code's modification of the method

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‡ Connaught, Lederle, and Roche-Organon heparin have all been employed with equal results.

TABLE I.
Effect of Excess Heparin upon the Release of Histamine from Cells to Plasma in Rabbit's Blood.

Rabbit No.	Histamine releasing agent	Plasma histamine (micrograms of histamine base/cc)		
		I	II	III
1	Trypsin	0.51	1.45	0.51
2	"	0.20	0.72	0.29
3	"	0.09	0.42	0.29
4	"	0.00	0.72	0.36
5	"	0.27	1.52	1.30
1	Proteose	0.45	1.20	0.21
2	"	0.29	0.80	0.27
3	"	0.82	1.60	0.80
4	"	1.33	2.66	<0.53
5	"	0.51	0.72	<0.26
6	"	1.54	1.86	0.52
7	"	0.40	3.09	0.24
8	"	1.54	3.09	0.53
1	Antigen	0.27	4.26	0.48
2	"	2.02	3.41	2.24
3	"	3.52	7.24	2.66
4	"	2.39	3.46	1.33

Column I: Plasma obtained from weakly heparinized bloods (0.02%).
Column II: Plasma obtained from weakly heparinized bloods (0.02%) to which a histamine releasing agent: antigen (egg white, 4.7 mg/cc of blood), peptone (Bacto-protone, 4.7 mg/cc of blood), or trypsin (crystalline trypsin, 0.4-0.6 mg/cc of blood) was added.
Column III: Plasma obtained from strongly heparinized bloods (0.12%) to which identical amounts of the above releasing agents were added.

of Barsoum and Gaddum.[13] In a few cases the unaltered plasmas were tested directly on the atropinized cat.

For the anaphylactic experiments, rabbits were sensitized to powdered egg white by repeated injections and a sufficient quantity of 10% solution was added to the blood to make the final concentration 4.7 mg/cc. For the peptone experiments, a sufficient quantity of a 10% solution of Bacto-Protone (acidified, shaken with permutit, filtered and neutralized) was added to the blood to make the final concentration 4.7 mg/cc. For the trypsin experiments, sufficient crystalline trypsin (Plaut) was added to the blood to make the final concentration 0.4-0.6 mg/cc. The results are shown in the accompanying table.

Discussion. It is apparent that increased amounts of heparin inhibit the release of histamine from cells to plasma whether this is brought about by trypsin, proteose, or specific antigen. The results thus far do not permit a precise statement as to the concentration of heparin required to exhibit this inhibiting effect, but they indicate that it must be considerably in excess of that required to prevent coagulation.

There are a number of reports in the literature regarding the effect of heparin upon anaphylactic shock in intact animals. Some workers have obtained marked inhibiting effects while others have obtained none. It seems probable from the present results that the negative experiments may have been due to the employment of an ineffective concentration of heparin.

The parallel effect of heparin in the 3 cases suggests that the mechanisms of histamine release may be the same in each case, that is to say, it is possible that some protease activity may be responsible for the histamine release in anaphylactic and peptone shock.

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