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Histamine-Induced Increase in Fibrinolytic Activity.* (28977)

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There appears to be a possible relationship between blood fibrinolytic activity and endogenous histamine release in a wide variety of conditions. In humans, following various forms of shock there is increased fibrinolytic activity(1) and depletion of cellular histamine(2). In dogs, endotoxin injection results in increased fibrinolytic activity(3) and plasma histamine(4). In mice increased tissue histidine decarboxylase activity follows stress, including endotoxin and epinephrine injection which tend to increase fibrinolytic activity(5).

To study the relationship between histamine and fibrinolysis we have determined fibrinolytic activity of blood in dogs following intravenous injection of histamine.

Materials. (1) Histamine phosphate USP (Burroughs Wellcome), dissolved in pyrogen-free water to a concentration of 0.5 mg/ml, or histamine phosphate USP (Abbott), 1 mg/ml in isotonic saline; (2) Bovine fibrinogen (Behringwerke, Marburg/Lahn, Germany); (3) Bovine Thrombin Topical (Parke-Davis), 50 NIH units/ml in buffer; (4) Bovine chloroform-activated plasmin (Parke-Davis), 10 Loomis units/ml in buffer; (5) Veronal-HCl-NaCl buffer, pH 7.35, according to Owren(6).

Methods. Histamine phosphate was given intravenously in a single dose (50-200 μg/kg) to 17 healthy mongrel dogs (14-23 kg) of either sex without anesthesia or sedation.

The euglobulin fraction of plasma was precipitated from a mixture of 1 ml plasma with 15 ml distilled water by adjusting the pH to 5.7 with 1% acetic acid solution. The euglobulin fraction was isolated by centrifugation and resuspended in one ml of buffer. All these steps were carried out at 4°C.

Fibrinolytic activity of the euglobulin fraction was determined on standard bovine fibrin plates, prepared according to a modification of the method of Astrup and Müllertz (7). Four and a half ml of bovine fibrinogen solution, 240 mg% in buffer, were added to 4.5 ml of 0.05 M $CaCl_2$ solution and the mixture clotted in a standard Petri dish with 0.1 ml thrombin solution. One drop (30 lambda) of euglobulin sample was placed on the fibrin layer. After incubation at 37°C for 20 hours the area of the lysed zone, expressed as the product of two perpendicular diameters, was measured. For each sample 3 separate determinations were made and the mean area determined.

Euglobulin lysis times were performed in 3 experiments. A volume of 0.9 ml euglobulin solution was clotted by addition of 0.1 ml thrombin solution and the time required for complete lysis at 37°C was recorded.

The antiplasmin activity of the plasma stored at 4°C for 24 hours was determined as follows. Two-tenths ml of plasma was mixed with 0.8 ml plasmin solution. After incubation of this mixture for 1 hour at 37°C, the residual fibrinolytic activity was determined on standard fibrin plates.

Glutamic-oxalacetic transaminase (GOT) in plasma was determined with the Sigma Chemical Co. reagents.

Protein content of the euglobulin fraction

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TABLE I. Effect of Intravenous Injection of Histamine Phosphate on Fibrinolytic Activity of Blood.

Dose of histamine (μg/kg)	No. of exp	Control 1	Control 2	5 min.‡	10 min.	20 min.	30 min.
				Fibrinolytic activity of euglobulin fraction as mm² of lysis (mean ± S.E.)			
50	5	364 ± 26	361 ± 33	578† ± 76	492† ± 34	408 ± 26	368 ± 29
100	7	312 ± 28	316 ± 30	603† ± 42	514† ± 18	433* ± 21	375 ± 28
200	5	343 ± 44	343 ± 40	710† ± 36	570† ± 22	480† ± 36	427* ± 24
				Fibrinolytic activity of plasma as mm² of lysis (mean and range)			
50	3	0	0	37 (9–72)	5 (0–12)	0	0
100	5	0	0	19 (9–30)	2 (0–9)	0	0
200	4	0	0	26 (6–56)	12 (0–49)	0	0
				Euglobulin lysis time in min.			
50	1	80	82	12	18	30	45
100	1	45	45	5	8	14	20
200	1	105	100	10	25	50	55

* Mean significantly different from control means at 5% level.
† Mean significantly different from control means at 1% level.
‡ Time after injection of histamine.

was determined by the biuret method(8).

At the start of each experiment, a control blood sample was obtained. Isotonic saline, in an amount equal to the volume of histamine solution to be administered (0.75-8.9 ml) was injected intravenously. Five minutes later, a second control blood sample was collected and the histamine phosphate solution was injected. Blood was obtained 5, 10, 20 and 30 minutes after injection of histamine. Nine volumes of blood were mixed immediately with 1 volume 0.129 M trisodium citrate solution and centrifuged. Citrate concentrations were adjusted according to hematocrit so that all plasma samples contained equal amounts of citrate.

Statistical analysis. For determination of the significance of differences between the activity of pre- and postinjection samples, the standard error of the difference between any 2 of the means of serial samples was calculated as $\frac{s_e^2}{2}$ where s_e^2 is the mean square of the error given by the analysis of variance of the data. Use was made of Dunnett's multiple comparisons test for testing a number of treatment (postinjection) means against the mean of the two control (preinjection) values(9).

Results. None of the dogs had serious side effects after injection of histamine. "Flushing" over the exposed part of the skin occurred following injection of histamine (200 μg/kg) in 2 of 5 dogs.

Table I summarizes results of the study of fibrinolytic activity. A significant increase in fibrinolytic activity of the euglobulin fraction of the plasma occurred in all 17 dogs following intravenous injection of histamine, regardless of the dose. This increase was maximal in blood samples collected 5 minutes after injection. Comparison of the effects obtained with the 3 doses of histamine indicates that the extent and duration of the fibrinolytic effect are dose dependent. The fibrinolytic activity of whole plasma on the fibrin plate was much less than the activity of the euglobulin fraction prepared from the same plasma. Plasma collected 5 minutes after injection exhibited a slight activity on the fibrin plate; occasionally there was some effect with the 10 minute samples. The euglobulin lysis time was shortened after injection of histamine. The shortest lysis time was obtained with the euglobulin fraction of blood samples collected 5 minutes after injection but englobulin lysis was still accelerated 30 minutes after injection.

The level of GOT in the plasma rose consistently following injection of histamine (Table II). However, it attained its maximal increase later than did the fibrinolytic activ-

TABLE II. Effect of Intravenous Injection of Histamine Phosphate on Level of GOT in Plasma.

Dose of histamine (μg/kg)	No. of exp	Control 1	Control 2	5 min.‡	10 min.	20 min.	30 min.
				GOT level in Karmen units per ml of plasma (mean ± S.E.)			
50	5	15.4 ± 3.0	15.6 ± 2.6	17.4 ± 3.2	21.0* ± 3.3	23.8† ± 4.0	22.4† ± 4.1
100	7	17.6 ± 3.2	17.4 ± 3.1	20.4 ± 3.5	25.4* ± 4.0	25.7* ± 5.1	24.3* ± 5.1
200	4	9.0 ± 2.0	8.5 ± 1.9	12.5 ± 3.6	15.7* ± 3.0	21.7† ± 4.2	21.0† ± .9

* Mean significantly different from control means at 5% level.
† Mean significantly different from control means at 1% level.
‡ Time after injection of histamine.

ity and there was little or no diminution during the further period of observation.

The antiplasmin content of the plasma and protein content of the euglobulin fraction were not altered after histamine injection.

As a further control, histamine was added *in vitro* to the whole blood or plasma of a normal dog to final concentrations of 0.5-50 μg histamine per ml. Fibrinolytic activity of the euglobulin fraction isolated from these samples was not different from the activity of the euglobulin fraction isolated from blood or plasma to which a corresponding volume of isotonic saline had been added. Application of histamine phosphate on the fibrin plate did not produce fibrinolysis.

Discussion. Our results demonstrate that intravenous injection of histamine results in a prompt increase in blood fibrinolytic activity. It is possible that hypotension is the mechanism by which histamine increases fibrinolytic activity, since a number of other drugs with hypotensive properties enhance fibrinolysis(10). On the other hand, histamine may have a more specific action. Histamine, as opposed to catecholamines, has been shown to have a direct injurious effect on cells of vascular tissues(11). These tissues (especially small veins) contain abundant fibrinolytic activity(12). Damage of these cells by histamine could release proteolytic materials into the circulation.

Increase in fibrinolytic activity resulting from direct cellular damage by histamine might be accompanied by increased blood levels of other intracellular enzymes. GOT is a widely distributed intracellular enzyme which is released upon cellular damage(13). The relatively slow gradual increase of GOT following histamine injection is in marked contrast to the rapid appearance and decline of blood fibrinolytic activity. There are a number of possible explanations. There might be proteolytic breakdown of transaminase immediately after injection of histamine; or the increase in fibrinolytic activity might be neutralized very rapidly by inhibitors in plasma whereas there are no plasmatic inhibitors for GOT; or, finally, increase in fibrinolytic activity could be independent from the release of intracellular enzymes such as GOT. Regardless of its mechanism of action, the potential effect of endogenous histamine on blood fibrinolytic activity must be considered in a wide variety of conditions such as various forms of stress, shock and after epinephrine injection.

Summary. Intravenous injection of histamine phosphate in the dog in a dosage of 50-200 μg/kg body weight increased the fibrinolytic activity of the plasma and of the euglobulin fraction of the plasma. The plasma antiplasmin and protein content of the euglobulin fraction were not altered. The level of GOT in the plasma increased after injection of histamine but the rise appeared later than the rise in fibrinolytic activity.

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Egg Infectivity Assay of Trachoma Virus.* (28978)

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The report by T'ang *et al*(1) on successful isolation of trachoma virus in fertile chicken eggs has been confirmed(2). Growth of trachoma virus in the laboratory has contributed greatly to advancement of trachoma research. Before a virus can be studied in detail, a quantitative technique for its assay is necessary. Titration of trachoma virus in egg yolk sac is the only generally applicable method of assay. There are varying reports on reliability of yolk sac titration. Sowa and Collier (3) obtained irregular results and concluded that infectivity cannot be stated in terms of an egg 50% lethal end point. Jawetz and Hanna(4) reported that reliable and reproducible infectivity assays were usually obtained with an exception of seasonal insusceptibility of eggs during late summer and fall. Litwin(5) has stated that 50% lethal dose in eggs gives relatively poor estimates of total growth of trachoma. Based on experience with isolation of trachoma virus(6,7) where detection of virus depends on finding elementary bodies (EB) in smears of yolk sac membranes, usefulness of an egg-infectious titration based on demonstration of EB has been evaluated.

Materials and methods. Virus strains. Two strains of trachoma virus, TW-1 and TW-3 (formerly called TW-10 and TW-29), representing Taiwan immunological prototypes(8) and one, Bour(9), supplied by Dr. E. Jawetz were used in these studies.

Embryonated eggs. White shell eggs 50 ± g from White Leghorn chickens of the same stock fed on an antibiotic free diet were used exclusively. All eggs were incubated at 38°C before use.

Preparation of virus pools. Virus dilutions estimated to kill embryonated eggs in 7-9 days in 0.2 ml were inoculated into yolk sacs of 6- or 7-day-old embryos. Eggs were incubated at 35°C with air sac in upright position and candled daily. When about ⅓ had died, yolk sacs of remaining eggs were aseptically harvested. Attached yolk material was stripped away with forceps. Further removal of yolk was accomplished by placing harvested material at 4°C for ½ hour and then blotting on absorbent filter paper. Yolk sacs were pooled, weighed, and 20% suspension made

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