Chromatographic Demonstration of Histamine Release in Anaphylactic and Trypsin Shock. (17520)

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A large body of evidence has accumulated to indicate that histamine is released in anaphylactic shock and similar reactions in amounts adequate to account for many features of these phenomena.(1-4) The methods employed to ascertain the presence of histamine in such phenomena were based mostly on the biological activity of this substance. Convincing chemical proof has been lacking since adequately sensitive and specific chemical methods to detect small amounts of histamine in the presence of other substances have been developed only very recently.(5,6) A preliminary report has described the identification of histamine released from sensitized rabbit blood cells *in vitro* by paper chromatography(7) and further proof for the specificity of the method has been presented.(8) The purpose of this communication is to present more complete details as to the method and to report the results obtained by its use in anaphylactic and trypsin shock in dogs.

Method. Blood samples (40 ml) were collected in heparinized or oxalated tubes from dogs undergoing shock. The samples were centrifuged and the plasma (approx. 20 ml) separated. Each plasma sample was diluted with an equal volume of distilled water in a shell vial and 3 g of a salt mixture (consisting of 62.5 g anhydrous Na_2SO_4 and 10 g $Na_3PO_4 \cdot H_2O$) was added for each 10 ml of the diluted plasma, according to the procedure of McIntire.(9) To each 20 ml of diluted plasma 10 ml of butanol were added to extract the histamine and the samples were shaken in a mechanical shaker for 30 minutes. The aqueous, protein and butanol layers were separated by centrifugation. The butanol layer was drawn off and placed into a clean shell vial. The butanol extraction was repeated and all butanol extracts from each plasma sample were combined. (An increase in the amount of butanol was not found to improve the histamine extraction significantly, with amounts of plasma up to 20 ml. Increasing the number of butanol extractions, similarly, had no appreciable effect on the recovery of histamine when added to control plasma.)

To the combined butanol extracts were added 5 ml of 0.5 N H_2SO_4. The tube was shaken for 10 minutes, centrifuged briefly and the aqueous bottom layer transferred to a small glass-stoppered vial by means of a suction-bulb pipette.(9) The butanol solution was again extracted with 2 ml of H_2O in a similar manner and this second aqueous layer combined with the acid.

The combined H_2SO_4 and H_2O extracts were neutralized with 10% NaOH in a small glass-stoppered vial and 1.5 g of the previously mentioned salt mixture and 2.5 ml of butanol were added. After shaking the vial for 20 minutes, the layers were separated by brief centrifugation and the butanol layer transferred with the suction-bulb pipette to an applicator pipette in contact with the filter paper strip* which was stretched horizontally

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* The filter paper used in this and previous work was supplied by the Eaton-Dikeman Co., Mount Holly Springs, Pa., in convenient rolls, $\frac{5}{16}$" wide.

TABLE I.
Histamine Content (μg Base per 20 ml) of Dog Plasma Before and During Anaphylactic Shock.

Dog. No.	Degree of shock	By paper chromatography		By bioassay	
		Before	During	Before	During
1	Fatal	0	>10	<1	50
2	,,	0	>10	<1	48
3	,,	0	>10	<1	16
4	Mild	0	0	<1	< 1
5	Fatal	0	>10	<1	28

TABLE II.
Histamine Content (μg Base per ml) of Dog Plasma Before and During Trypsin Shock.

Dog No.	Dose trypsin, mg/kg	Degree of shock	By paper chromatography		By bioassay	
			Before	During	Before	During
1	50	Mild	0	>3<5	Not done	
2	100	Fatal	0	>10	<1	38
3	100	,,	0	0	<1	<1
4	100	,,	0	>10	Not done	
5	100	,,	0	>10	,,	,,
6	100	,,	0	>10	,,	,,

over a hot plate (temperature at level of strips 70-70°) as previously described.(10) The extraction of the aqueous layer was repeated with 1 ml of butanol which was added to the first butanol extract in the pipette.

After application of the final butanol extract to the paper strip, the strips from both control and shock samples of blood were stapled to narrow cardboard at intervals of ½ cm. Chromatography with butanol saturated with 10% NH_4OH, and color development with diazotized p-bromoaniline was carried out as described in a previous communication.(8) Histamine, when present in amounts exceeding 2-3 μg, made its appearance on the strips as a red band at an R_f value of approximately 0.56. Control strips to which known amounts of histamine had been applied were run simultaneously. This permitted a rough approximation of the amount of histamine in the 20 cc plasma samples.

Anaphylactic shock. Blood samples were collected from 5 dogs sensitized to horse serum both before and 1-3 minutes after i.v. injection of the antigen. The release of histamine as shown by chromatography and bioassay† is summarized in Table I. One of the experiments is illustrated in Fig. 1.

Trypsin shock. Six dogs were injected i.v. with crystalline trypsin (Armour). Blood samples were taken before and during shock. The results are summarized in Table II. One of the experiments is illustrated in Fig. 2.

Release of histamine in dogs shocked with peptone could not be demonstrated by chromatography since the amounts of peptone required to produce severe or fatal shock (1 cc of 20% Bacto-Protone (Difco)/kg) interfered markedly with the chromatographic procedures. This was in part due to the interference of peptone with proper separations on the chromatograms, as well as to the presence of substances in the peptone solutions which give a strong color reaction with the diazotized p-bromoaniline used as the color reagent on the chromatograms. Attempts to separate these interfering substances by either electrodialysis or repeated butanol extractions of peptone solutions resulted in fractions which did not produce shock in the dogs. Addition of small amounts of peptone solutions to normal rabbit blood, however, caused release of histamine into the plasma which could be demonstrated by the chromatographic procedure.

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† All bioassays were kindly performed by Dr. Lorenzo Giscafré and Dr. Wilson T. Beraldo.

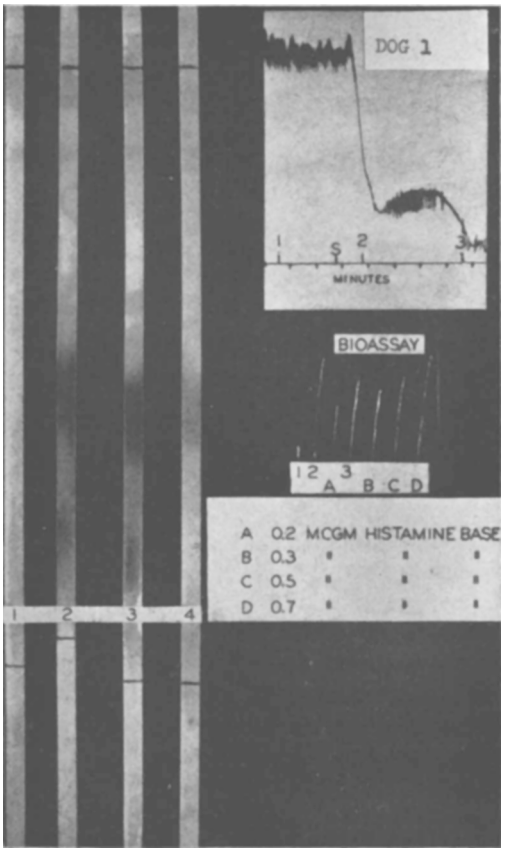

FIG. 1.

Paper chromatograms, blood pressure record and bioassay from a dog during anaphylactic shock.

1, 2 and 3 on B.P. record signify blood samples drawn S—antigen injection.

1, 2 and 3 on chromatograms and bioassay record correspond to the respective blood samples.

4 on chromatograms represents 10 μg pure histamine base chromatographed as control. Note the much heavier bands of histamine on strips 2 and 3 at the same location and absence on strip 1. The substance(s) on strip 2 and 3 responsible for the dark bands below the histamine bands have not been identified.

A, B, C, D on bioassay record represent standard amounts of histamine added to the muscle bath for calculation of the amounts of histamine in the plasma samples.

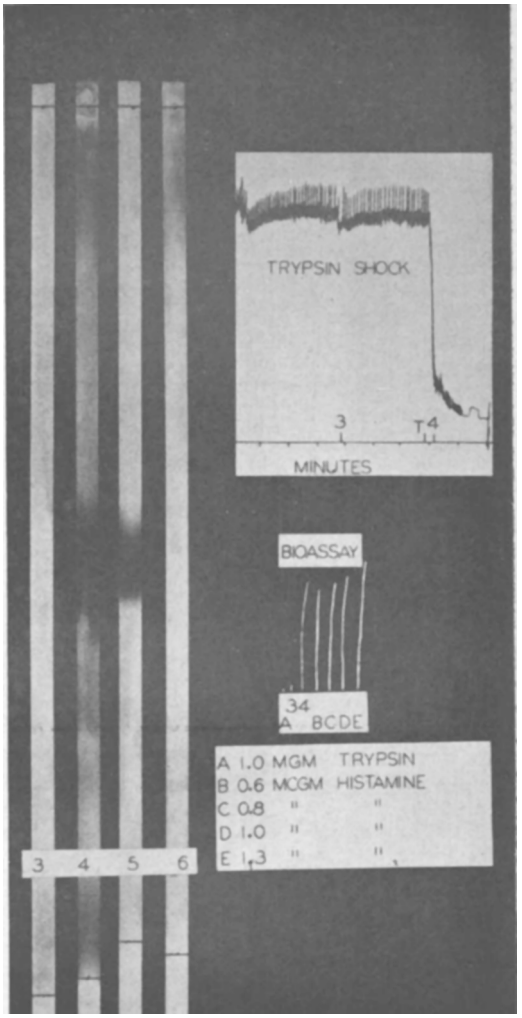

FIG. 2.

Paper chromatograms, blood pressure record and bioassay from a dog during trypsin shock.

3 and 4 on B.P. record signify blood samples drawn T—trypsin (100 mg/kg) injection.

3 and 4 on chromatograms and bioassay record correspond to the respective blood samples.

5 on chromatograms represents 10 μg pure histamine chromatographed as control.

6 on chromatograms represents 40 mg trypsin chromatographed as control.

A on bioassay record represents a trypsin control.

B, C, D, E on bioassay record represent standard amounts of histamine added to the muscle bath for calculation of the amount of histamine in the plasma samples.

Discussion. The experiments described identify histamine liberated during anaphylactic and trypsin shock in dogs. While the chromatographic method as employed routinely in this study does not permit exact quantitation, a better estimation of the amount of histamine can be achieved by chromatographing a series of control strips with graded amounts of histamine along with aliquots of the sample. For example—ali-

quots of the first butanol extract of the shock plasma obtained from one of the dogs were chromatographed with a standard series of histamine samples. A butanol sample containing 7.8 μg histamine (calculated on the basis of bioassay of the plasma) showed a chromatogram falling between standard chromatograms of 5 and 10 μg histamine and corresponding most closely to the 7 μg standard as judged by an unbiased observer. The chromatographic method with a limit of sensitivity of 2-3 μg per sample is obviously inferior to bioassay in this respect but is superior so far as specificity is concerned. The demonstration of histamine release in amounts roughly equivalent to those observed in the anaphylactic experiments in a high percentage of the trypsin shock experiments confirms an earlier report from this laboratory(3) but is in contrast to a more recent report, also from this laboratory.(11) We are not at present prepared to explain the discrepancy.

Summary. A method for the chemical identification of histamine in blood by paper chromatography is described in detail and experiments are presented to show the release of relatively large amounts of histamine in dogs undergoing anaphylactic and trypsin shock. Peptone, when injected into dogs in amounts sufficient to produce severe shock, interfered with the identification of histamine which could, however, be found on the chromatograms after addition of small amounts of peptone to rabbit blood (*in vitro*).

The author wishes to express his appreciation to Dr. Carl A. Dragstedt for many helpful suggestions.

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Received November 1, 1949. P.S.E.B.M., 1949, v72.

Inhibition of Hyaluronidase by Aromatic Compounds. (17521)

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The aim of our experiments was to find substances inhibiting the action of hyaluronidase. Repeating Guerra's work,(1) we(2) confirmed his observations that this enzyme was inhibited by sodium salicylate. Prior to this, hyaluronidase was found to be inhibited by heparin, gastric mucin, pseudo-globulin,(3) morphine,(4) hyaluronic acid,(5) estrogens(6) and, of course, enzyme inactivators. These experiments were carried out *in vivo*. By studying the effect on the area of spread in the skin, it was found that ascorbic acid,(3) glycerin,(7) triacetin,(7) arsenious oxide,(7) lecithin,(8) peptones,(8) urethane, sodium diazobenzene sulfonate,(9) hirudin,(7) human urine(10) and diazotized proteins(11) enhance this enzymatic action. Obviously, these lists fail to reveal chemical structures which are essential for anti-hyaluronidase activity. We, therefore, undertook to study a series of simple chemically related compounds and used the turbidimetric method described by Kass and Seastone.(12)

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