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Identification of Histamine in Blood by Paper Chromatography.

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Many experiments have demonstrated that the substance responsible for anaphylactic shock appears in the circulation during this reaction. On the basis of its biological activity, this substance has been identified as histamine. Attempts to provide chemical proof that this substance is histamine have failed because of the small amounts present, the large amounts of interfering substances and the non-specificity of the color reactions generally employed.

In the present study these difficulties have been overcome by a simple purification procedure combined with paper chromatography recently developed for the identification and separation of minute quantities of amino acids[1] and since applied to the identification of a variety of substances.

Method. Paper chromatography consists of the deposition of very small amounts of solution (*i.e.* 0.01 ml) containing a few micrograms of the substances to be separated and identified on a limited area of a filter paper strip. The edge of the strip closest to the point of application of the solution is then suspended from a trough containing a mixture of solvents, one of which is usually water and the other some immiscible liquid, such as phenol, collidine, butanol, etc. The solvent mixture moves down the filter paper strip by capillary action and carries with it the different compounds applied, each at a different rate, presumably according to its individual solubilities in each of the liquids of the solvent mixture. This affords a very effective method of separation and identification of the various components applied to the strip. In adapting these principles to the identification of histamine some modifications have been made. The most important is a procedure for the

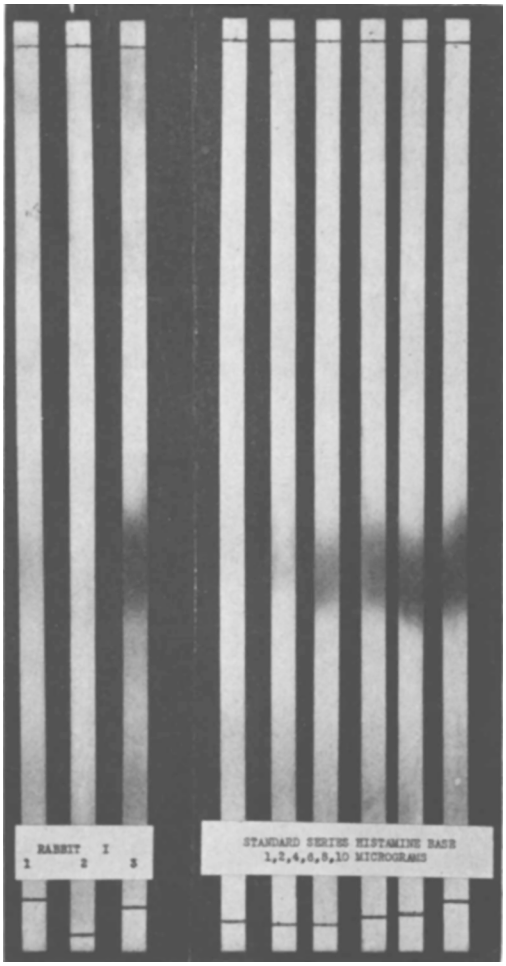

FIG. 1.

Chromatographic Evidence of Histamine Release in Anaphylaxis.

Left to right: (1) Chromatogram of plasma separated from blood of sensitized rabbit. (2) Chromatogram of plasma separated from blood of sensitized rabbit—antigen added to plasma *after* separation. (3) Chromatogram of plasma separated from blood of sensitized rabbit—antigen added to whole blood *before* separation of plasma. (4-9) Chromatograms of graded quantities of histamine.

Upper line—point of application to filter paper strip of butanol extract.

Lower line—point to which solvent mixture advanced at 15 hours.

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[1] Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, 1944, **38**, 224.

application of a relatively large volume of solution containing the histamine to a restricted area of the filter paper strip. For this purpose capillary pipettes with very slow flow rate† in contact with the strips have been employed. The solvent as applied from the pipette is continuously evaporated at the same rate as it is deposited (approximately 1 ml per hour) by means of a hot plate with adjustable temperature control.

In the actual procedure 2-20 ml of plasma are diluted with an equal amount of water and the pH and salt concentration are adjusted according to the procedure used by McIntire[2] for the extraction of histamine with butanol. The histamine is then extracted from the butanol with a small amount of 0.5 N sulfuric acid which is, in turn, brought to the proper salt concentration and pH and re-extracted with approximately 3 ml of butanol. The final butanol extract is then applied to the filter paper strip by means of the previously described pipette. Control strips with known amounts of histamine are prepared in a similar manner. A set of strips is then suspended in a closed chamber from a trough containing butanol saturated with 10% ammonium hydroxide as the solvent mixture. After 12-15 hours the strips are removed, dried and treated with an alkaline diazotized solution of p-bromoaniline employed as a color reagent for histamine by Baraud.[3] Histamine, in excess of 1 μg can be detected as a red band on the strips. Its presence and identity are established by the color of the band and more critically by the position of the colored band on the strips (R_f).‡,1

Results. The method described has been applied to prove the appearance of histamine in plasma when antigen is added (*in vitro*) to the blood of sensitized rabbits. Fig. 1 shows the results of such an experiment. Approximate estimation of the amounts of histamine released is possible by comparison with a standard series of strips (Fig. 1). Histamine has also been identified by this method in the blood of anesthetized dogs undergoing anaphylactic shock. In all experiments simultaneous biological assay of plasma extracts§ on guinea pig ileum showed good agreement with the amounts of histamine found by paper chromatography.

Summary. A method for the chemical identification of histamine in blood has been described and used to prove the appearance of histamine in plasma after addition of antigen (*in vitro*) to the blood of sensitized rabbits and in the blood of dogs undergoing anaphylactic shock.

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2 McIntire, F. C., Roth, L. W., and Shaw, J. L., *J. Biol. Chem.*, 1947, **170**, 537.

3 Baraud, J., Genevois, L., Mandillon, G., and Ringenbach, G., *C. R. Acad. Sci.*, 1946, **222**, 760.

‡ R_f is defined as the ratio of the distance traveled by the substance to the distance traveled by the solvent mixture.

§ Purified by the cotton succinate method, see reference 2.