Brit. J. Pharmacol. (1952), 7, 298.

THE QUANTITATIVE DETERMINATION OF DIFFUSIBLE HISTAMINE IN BLOOD

BY

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(Received January 18, 1952)

Under physiological conditions most of the histamine in blood is apparently associated with the leucocytes (for references see Guggenheim, 1951), but it is not known in what form the histamine exists. Methods for determining the concentration of histamine in blood generally do not distinguish between histamine which is pharmacologically active and histamine which may be present in inactive combination. When histamine is extracted by chemical means combined histamine may be released (Rocha e Silva, 1944). Furthermore, histamine may be produced by decarboxylation of histidine (Äkerblom, 1941 ; Schmiterlöw, 1949), although Hughes, Salvin, and Wood (1951) have suggested that this source of error is of no practical importance in the method of Barsoum and Gaddum (1935) and its much used modification by Code (1937). Other disadvantages of published methods are the considerable amount of work involved in purifying histamine (Code, 1937 ; McIntyre, Roth, and Shaw, 1947) and the low recoveries (Roberts and Adam, 1950).

We have developed a new method because we wanted to estimate that fraction of histamine in whole blood which could be assumed to be pharmacologically active. That fraction was presumed to be represented by the histamine which was able to diffuse from blood when the blood was dialysed against physiological saline.

Tarras-Wahlberg (1935, 1936) separated histamine from blood by dialysis and Emmelin (1945a) by ultra-filtration. However, Emmelin's method required specially prepared collodion membranes.

We found that histamine in aqueous solution dialysed rapidly through commercial cellophane membranes (Fig. 1). When saline or blood containing histamine was dialysed against an equal volume of saline (see below) the concentration of histamine in the system reached equilibrium in less than one hour. Volume changes due to differences in osmotic pressure were negligible and the dialysate contained half of the original histamine.

The dialysate of blood also contained substances which interfered with the assay of histamine on the isolated ileum of the guinea-pig, and it was necessary to purify the histamine. This was done by means of paper partition chromatography. Dent (1948) and Urbach (1948, 1949) used paper chromatography to isolate histamine qualitatively.

Preliminary experiments showed that when histamine-containing dialysates of blood were acidified with dilute HCl and boiled to dryness, no histamine was lost

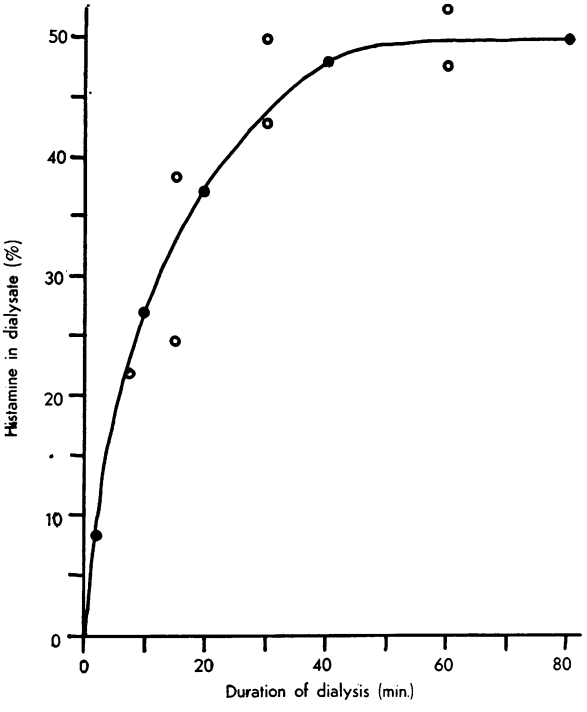

FIG. 1.—Rates of dialysis of histamine. Solid circles : A concentrated aqueous solution of histamine dihydrochloride was dialysed against an equal volume of water. The amounts of histamine which had dialysed were determined gravimetrically on samples of the dialysate. Open circles : Histamine in blood was dialysed against an equal volume of saline, as shown in the first two recovery experiments in the Table.

and none was formed from histidine (Abel and Kubata, 1919 ; Gavin, McHenry, and Wilson, 1933 ; Hughes *et al.*, 1951) or N-acetylhistamine (Roberts and Adam, 1950). Furthermore, no histamine was lost when the dried material was extracted with methanol and the extract purified chromatographically in a butanol-acetic acid system. The developed wet chromatogram showed a band (Rf 0.15–0.25) the opacity of which was distinctly different from that of the rest of the paper. It was found that this band was due to inorganic salts carried over in the methanol extraction. When the dried chromatogram was sprayed with ninhydrin to demonstrate amino-acids (Dent, 1948) and Pauly's diazo reagent to demonstrate histamine and similar compounds (Guggenheim, 1951), it was found that the band containing the inorganic salts also contained all the histamine but no other substance which reacted with these reagents or interfered with the subsequent biological assay. The histamine could be eluted from this band without loss and assayed biologically.

PROCEDURE

A sample of blood (2 ml. to 4 ml.) was taken and semicarbazide immediately added to give a concentration of 1×10^{-5} M in order in inhibit any histaminase present (Mongar and Schild, 1951).

The blood was pipetted into a cellophane bag (20/32 " Nojax " casing made by the Visking Corporation, Chicago) and dialysed against an equal volume of physiological saline in a short, wide test-tube. To ensure good mixing, the tube was stoppered with a rubber bung, clamped to a wheel, and mechanically rotated at about 15 revolutions per min. for 1 hr.

The bag was discarded and the saline solution acidified with 0.1 ml. N-HCl and evaporated to dryness in a bath of boiling water. The residue was extracted three times with about 0.4 ml. methanol, and the extracts were transferred quantitatively to a pencil line drawn on a sheet of Whatman No. 1 filter paper, prepared for chromatography as shown in Fig. 2. The methanolic solution was transferred to the pencil line with a capillary pipette and dried with a current of warm air from a hair-drier. The solution was not allowed to spread more than 0.5 cm. to each side of the line. The transfer of a sample took less than 5 min.

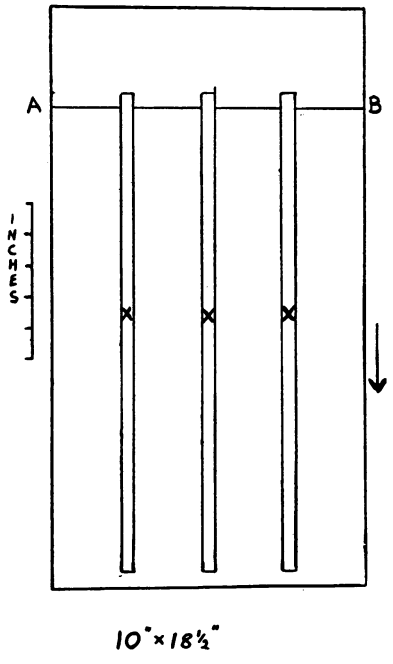

FIG. 2.—A sheet of Whatman No. 1 paper was halved to give two sheets 10 in. × 18½ in. By cutting out the pieces marked X, the paper was divided into four columns, and there was no risk of the substances in one column mixing with those in the next. The extracts of the dialysates were applied to the starting line A–B, and the chromatogram was developed in the direction of the arrow.

The chromatogram was developed overnight at room temperature with a butanol-acetic acid system as the mobile phase (i.e., the butanol phase obtained after mixing 25 vols. of *n*-butanol, 25 vols. of distilled water, and 6 vols. of glacial acetic acid). On the developed, wet chromatogram the opaque band due to inorganic salts was marked. The chromatogram was dried with a current of warm air from a hair-drier until it did not smell of butanol or acetic acid. The marked area was cut into small pieces and briefly shaken in a known volume of Locke's solution. The histamine in this solution was then assayed on the isolated ileum of the guinea-pig, in a bath of 2 ml. capacity. All histamine assays are given in terms of the free base.

RESULTS

A series of recovery experiments were made in order to test the efficacy of the method. Cats were given heparin and bled under nembutal anaesthesia ; semicarbazide was mixed with the blood. Histamine was added to the blood in known amounts and 4 ml. samples were assayed by the method described. As cat blood contained a basal level of histamine (see below) the histamine content of the blood without added histamine was also determined. The results are shown in the Table.

TABLE

RECOVERY EXPERIMENTS TO TEST THE METHOD

All amounts of histamine refer to 4 ml. blood

Dialysis time (min.)	Histamine found in blood only (μg.)	Histamine added (μg.)	Total (μg.)	Total histamine found (μg.)	Recovery (to nearest 5%)	
15	0.02	0.4	0.42	0.2	50	Same experiment
30	0.02	0.4	0.42	0.36	85	
60	0.02	0.4	0.42	0.47	110	
7.5	0.6	0.5	1.1	0.47	45	Same experiment*
15	0.6	0.5	1.1	0.80	75	
30	0.6	0.5	1.1	1.11	100	
60	0.6	0.5	1.1	1.03	95	
60	0.6	0.4	1.0	0.89	90	
60	0.6	0.8	1.4	1.34	95	
60	0.6	1.6	2.2	2.4	110	
60	0.02	0.3	0.32	0.32	100	
60	0.02	0.66	0.68	0.64	95	
60	0.02	0.87	0.89	0.84	95	
60	0.02	0.55	0.57	0.50	90	
60	0.02	1.10	1.12	1.20	105	
60	0.02	0.77	0.79	0.83	105	
60	0.02	0.44	0.46	0.46	100	
60	0.2	1.8	2.0	1.6	80	
60	0.2	4.8	5.0	5.2	105	
60	0.06	0.4	0.46	0.44	95	
60	0.06	0.4	0.46	0.40	85	
Average of recoveries for 60 min. dialysis (16 experiments)					95	

* In this experiment the blood was collected in a vessel containing heparin. The "blood only" figure was abnormally high; this was probably due to histamine in the heparin. In all the other experiments the heparin was injected into the cat 10–20 min. before the blood was taken.

It will be noted that this test of the method depended upon two determinations: (*a*) to find how much histamine was contained in the original sample of blood, and (*b*) to find how much was contained in the blood after a known amount had been added. In view of this procedure, depending upon two assays, the recovery figures were good, the lowest being 80 per cent and the highest 110 per cent.

Histamine concentration in cat's blood

In six cats anaesthetized with chloralose the concentrations of blood histamine were 5.5, 5.0, 3.1, 1.6, 1.2, and 0.9 μg./100 ml. blood. These results agree with those published by Brown, McIntosh, and White (1941), Emmelin (1945b), and Ojha and Wood (1951).

Discussion

Dialysates of blood contained substances other than histamine which acted upon the isolated guinea-pig's ileum. "Slow-contracting substances" were eliminated in the purification. Potassium estimations by means of flame photometry showed that the concentration of K ions in saline dialysates of blood or plasma was 75 μg./

ml., and of haemolysed blood, 110 μg./ml. It was found that any K present with histamine in the solutions used for assay was insufficient to affect the guinea-pig ileum or to alter its sensitivity towards histamine. A disadvantage of dialysis was that only half of the blood histamine was recovered.

Paper chromatography of pure histamine showed that the substance moved in a well-defined band. The *n*-butanol-acetic acid system was chosen because the separation of histamine (Rf 0.15) and N-acetylhistamine (Rf 0.43) was greater than when the *n*-butanol-ammonium hydroxide system of Urbach (1948) was used, and because histamine was stable in the acidic system and could be completely recovered from it. The inorganic salts in the chromatogram did not affect the mean Rf value of histamine.

As little as 1 μg. histamine in 100 ml. blood could be determined. The errors of the estimations in the recovery experiments could be accounted for by the bioassay alone. Recently, several methods have been published for the colorimetric determination of histamine (Camisasca, 1949 ; Madar, Sterne, Rosin, and Frediani, 1950 ; Graham, Lowry, and Harris, 1951). Some of them are claimed to be as sensitive as bioassay. Therefore, the present method could perhaps be improved by using colorimetry instead of bioassay in the estimation of the purified histamine.

Summary

A method is described for the quantitative determination of diffusible histamine in blood. Histamine is separated from blood by dialysis, purified by paper partition chromatography, and assayed biologically.

When histamine was added to blood and assayed by this method, all the histamine was recovered.

We wish to thank Dr. G. S. Dawes for his interest in this work, and one of us (J.R.V.) is grateful to the Medical Research Council for a personal grant.

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ADDENDUM

BY

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In the absence of salts adrenaline and noradrenaline separate clearly from histamine on a paper chromatogram in the butanol-acetic acid system (Born and Vane, 1952); Rf values: histamine 0.15, noradrenaline 0.28, adrenaline 0.36 (Shepherd and West, 1951). With increasing quantities of salts the rate of travel of adrenaline and noradrenaline is decreased. The quantity of salt which is transferred to the chromatogram in solution in methanol, as described by Born and Vane (1952), makes it impossible to free histamine from adrenaline and noradrenaline. If these are present in concentrations sufficient to interfere with the bioassay of histamine the following modification of Born and Vane's procedure is necessary. Acid ethanol is substituted for methanol in order to reduce the amount of salts transferred to the chromatogram, but since histamine itself has a low solubility in ethanol, certain precautions are necessary in order to ensure full recovery of histamine.

After evaporation of the dialysate the residue was extracted with 3×0.4 ml. methanol; 5 ml. acid ethanol (0.1 per cent (v/v) conc. HCl) were added to the extract and the whole was evaporated to about 0.5 ml. on a boiling water bath. The extract was transferred to a 2 ml. tube and the residue washed twice with 0.25 ml. acid ethanol. The extract and washings were combined, cooled to $-10°$ C., and centrifuged. The supernatant fluid was carefully withdrawn and applied to a filter paper and the chromatogram was developed as described by Born and Vane. The salt band was very small or absent, and consequently a control was necessary to indicate the position of the histamine in the chromatogram. A sample of blood was dialysed, extracted, and 5 μg. each of histamine, adrenaline, and noradrenaline were added. After chromatography the control strip was sprayed with a solution of potassium ferricyanide (James, 1948) to locate adrenaline and noradrenaline and with Pauly's reagent to locate histamine.

It was possible by this procedure to recover 100 per cent of added histamine (1 μg. – 0.05 μg./ml.) from dog's blood to which had been added adrenaline and noradrenaline, each at a concentration of 5 μg./ml.

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