THE HISTAMINE-HEPARIN COMPLEX

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Some factors concerned in the formation *in vitro* of a complex between the base histamine and the acid heparin have been studied. More histamine is contained in the complex when precipitation is carried out in acid medium in the presence of adenosine triphosphate. Although the staining properties and morphological characters of the complex resemble those of natural mast cell granules, it has not been possible to release most of the histamine from the synthetic granules without dissolving them. 5-Hydroxytryptamine, noradrenaline and adrenaline failed to form complexes with heparin and did not alter the formation of the complex of histamine and heparin.

IN recent years, tissue mast cells of mammals have been shown to contain both heparin and histamine[1,2]. Although there is a close relationship between the heparin content of a particular tissue and the amount of histamine that can be extracted from it, it is still uncertain how the histamine is held in these cells. Experiments have therefore been made to study the affinity of heparin for histamine under varying conditions. Since 5-hydroxytryptamine (5-HT) has been suggested as a constituent of mast cells in certain species[3], the affinity of heparin for 5-HT has also been studied. A preliminary note on this work has already been reported[4].

METHODS

The following drugs were dissolved in normal saline. Ox heparin, 20 mg./ml., histamine base, histamine acid phosphate, histamine dihydrochloride, 5-HT creatinine sulphate, noradrenaline and adrenaline acid tartrates, and adenosine triphosphate (ATP), each 1 mg./ml. After mixing equal volumes of one of the amines and of heparin, the pH value of the mixture was determined and adjusted if necessary. Ethanol was then added to give a final concentration of 70 per cent (v/v). At other times, adenosine was included, and occasionally acetone was used for precipitation instead of the alcohol. The precipitate of heparin was separated by centrifugation, dissolved in normal saline and assayed for its amine content. The histamine content of both the complex and the supernatant was estimated on the isolated atropinised guinea pig ileum, checking the specificity of the response with mepyramine (10^{-7}). The 5-HT content was estimated on the isolated atropinised uterus or colon of a rat in oestrus, using 2-bromolysergic acid diethylamide to check specificity. The adrenaline content was measured on the blood pressure of an atropinised cat before and after dibenamine (10 mg./kg.).

In a few experiments, the precipitate was shaken with toluidine blue (0·1 per cent, w/v) in 70 per cent (v/v) ethanol, washed with 70 per cent

ethanol, and then spread on a microscope slide and covered with Xam neutral mountant.

For paper chromatographic studies, the chief solvent system used was 70 per cent (v/v) ethanol adjusted to pH 6·0. This pH value was chosen as more histamine was removed by the precipitate of heparin at acid pH values than at alkaline pH values. Other solvent systems used included *iso*propanol : water : acetic acid (53 : 46 : 1) and *n*-propanol : water (3 : 1). Spots each of 0·01 and 0·05 ml. of the mixture of amine and heparin were applied to Whatman No. 1 paper and ascending chromatograms run for 6 hours. The developing agents used were Pauly's reagent for histamine, Ehrlich's reagent for 5-HT, potassium iodate for noradrenaline and adrenaline, and toluidine blue for heparin. The intensities of the colours which developed were compared with those of known amounts of standard amine solutions. In some experiments, the active areas were eluted from the chromatograms with water and tested biologically.

RESULTS

Heparin-Histamine Complex

Heparin formed complexes with all three histamine preparations. However, about twice as much histamine was removed from the solution when the acid phosphate or the dihydrochloride was used (67–70 per cent) than when the base was tested (36 per cent). Table I shows the mean

TABLE I

THE DISTRIBUTION PER CENT OF HISTAMINE IN THE SUPERNATANT AND IN THE PRECIPITATE OF HEPARIN WHEN ETHANOL IS ADDED TO THE MIXTURE OF HISTAMINE, 1 mg., AND HEPARIN, 20 mg., WITH AND WITHOUT ADENOSINE TRIPHOSPHATE, 1 mg.

Histamine preparation	With adenosine		Without adenosine	
	Supernatant	Precipitate	Supernatant	Precipitate
Base	70	30	64	36
Acid phosphate ..	10	90	33	67
Dihydrochloride ..	10	90	30	70

results of 18 experiments, and also records the results when adenosine triphosphate was included in the mixture. The presence of ATP allows more histamine to be removed from the mixture by the heparin when one of the histamine salts was used, but not with the base. In all experiments, the complex of heparin and histamine base was much finer than that of heparin and one of the histamine salts. Repeated washing of the complexes with ethanol or acetone failed to remove the histamine, but they were readily soluble in water, normal saline or weak acid, giving solutions with biological effects corresponding to those of the respective amine and free heparin.

The combination of heparin and histamine took place at all temperatures used (between 4 and 40°), the amount of histamine removed being fairly constant. But the pH value of the mixture before precipitation was significant, as more histamine was associated with the complex if the solution was acid. The optimal pH value was about 6·0 (Fig. 1). This

complex stained with toluidine blue and resembled in appearance and size natural mast cell granules isolated from mouse connective tissue[5]. In the solvent system of 70 per cent ethanol adjusted to pH 6·0, the mixture

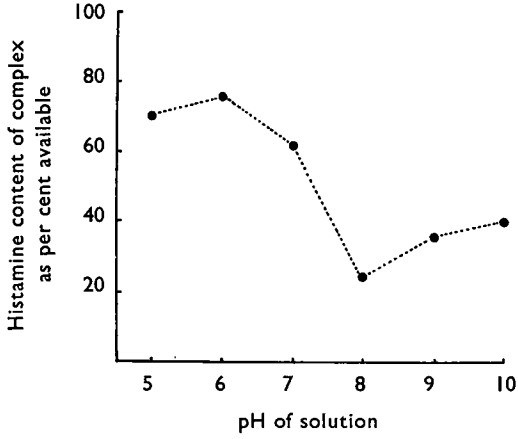

FIG. 1. The effect of pH on the histamine content of the heparin-histamine complex, formed by adding ethanol to a mixture of heparin, 20 mg., and histamine acid phosphate, 1 mg. Maximal removal of histamine from the solution occurs at pH 6·0.

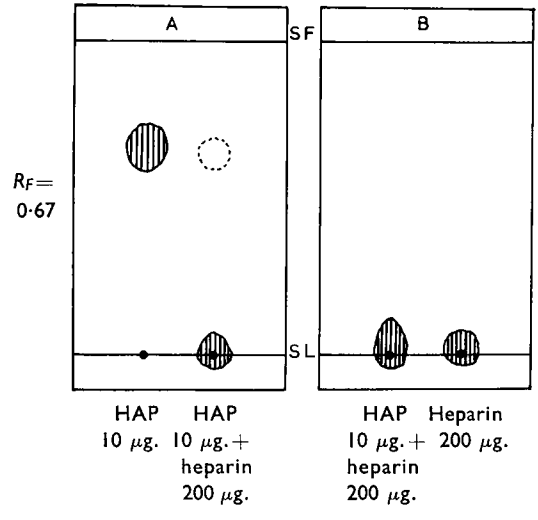

FIG. 2. Chromatographic separation of the heparin-histamine complex. Solvent is 70 per cent ethanol (pH 6·0). Ascending paper chromatograms 6 hour contract. Histamine (HAP) developed by Pauly reagent (A), heparin by aqueous toluidine blue (B). Note the two spots for histamine in the mixture of histamine and heparin, the stronger being at R_F value = 0. SL = starting line. SF = solvent front.

of heparin and histamine gave two spots for histamine, one at R_F value of 0·68 and one at 0. Histamine alone gave one spot at R_F 0·67, and heparin one at R_F 0. On elution, about 70 per cent of the total histamine

was found to be located with the heparin, the remainder running to the spot corresponding to free histamine (see Fig. 2).

Complexes of Heparin with Other Bases

When 5-HT, noradrenaline, or adrenaline was used instead of histamine, the heparin precipitate contained less than 1 per cent of the respective amine. The chromatographic behaviour of the mixtures in 70 per cent ethanol (pH 6·0) also resembled that of the respective constituents. When each of these three amines was added to the heparin and histamine mixture before precipitation with ethanol, there was no alteration in the composition of the complex with histamine.

Complexes of Histamine with Other Substances

When hog mucin was used in place of heparin, no histamine was attached to the precipitate of this mucopolysaccharide formed by the addition of ethanol. The histamine liberator, compound 48/80, failed to displace histamine from its complex with heparin, but when it was added to the mixture of heparin and histamine before the addition of ethanol the uptake of histamine was reduced from 72 per cent at pH 6·0 to 53 per cent. In a similar manner, the amino acid, lysine, slightly reduced the uptake of histamine by heparin.

Discussion

The results show firstly that histamine forms a complex *in vitro* with heparin, and secondly that the morphological characters and staining properties of the complex resemble those of naturally occurring granules. Its formation appears to be specific as noradrenaline, adrenaline and 5-HT all fail to combine with heparin under similar experimental conditions. Further, it can be shown that, when precipitated heparin is shaken with these amines, only histamine is taken out of solution. These results have been obtained with heparin of ox origin, and it is possible that different conclusions might be reached when heparin of a different source is used.

The preparations of heparin and histamine in the complex (namely, 20 parts to 1 part respectively) are similar to those found in extracts of tissues known to be rich in mast cells[6]. Yet it has not been possible so far to release most of the histamine from the synthetic complex except by dissolving it. Certain basic amino acids like lysine displace histamine from its attachment *in vivo* to the acidic residue of heparin by some kind of cation exchange[7], yet they fail to do so from the synthetic complex *in vitro*. Likewise, compound 48/80 is not effective in this respect. Both lysine and compound 48/80 slightly reduce the uptake of histamine by heparin when present in the mixture before precipitation[8], but further work is needed to determine the optimal conditions for this action.

In the presence of adenosine triphosphate, more histamine is removed from the mixture by heparin especially when the pH of the solution is around 6·0. In the tissues, biologically active amines like noradrenaline, adrenaline, 5-HT and histamine are associated with adenosine triphosphate which assists in their binding by providing energy bonds. It is

possible that natural mast cell granules use adenosine triphosphate in this manner and this would explain why its presence causes more histamine to be present in the synthetic complex. Adsorption of the histamine to the surface of the precipitated heparin is unlikely since a good adsorbent like kaolin removed not only histamine, but also noradrenaline, adrenaline and 5-HT, from solutions of these amines.

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