

The binding of histamine by normal and allergic human serum

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The binding of histamine by sera obtained from normal and allergic subjects was studied in vitro with biological and chemical techniques. The difficulties encountered in the biological measurement of serum histaminopexy were overcome, though only partially, by using the optimal concentrations of histamine and serum and introducing a control. Serum histaminopexy was not impaired in all cases of allergic asthma but was abolished in all severe cases of drug hypersensitivity. The dialysis and electrophoresis techniques failed to measure serum histaminopexy. The uptake of ^{14}C -histamine and/or its metabolites by serum increased after fractionation and incubation at 37°C . The mechanism of the biological reaction of serum histaminopexy was discussed in the light of these findings.

Mackay and associates¹ observed the presence of an antihistamine factor in guinea pig serum which decreases the response of the isolated ileum to histamine and is readily activated by dilution. However, these authors used the term histamine binding, since they could not recover the free histamine from the fraction G2 α -histamine complex by the usual extraction process with hydrochloric acid.

Parrot and Laborde² used the term histaminopexy to denote the immediate drop in the biological activity of histamine when mixed with dialyzed human or guinea pig serum and tested on the isolated guinea pig ileum. In 1964 Parrot and associates³ reported that histamine is not enzymatically destroyed by normal human serum but bound to a γ -globulin named plasmapexine 1, calcium ions being essential for the binding. According to these authors, 1.0 ml. of normal human serum can bind as much as 10 to 25 μg histamine dihydrochloride. These observations were confirmed biologically^{4, 5} and chemically.^{6, 7}

However, the binding of histamine by normal human serum, as demonstrated biologically and chemically, has always been subject to many controversial re-

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ports. The biological method of histaminopexy was reported to be inconsistent and nonreproducible.⁸⁻¹⁰ It was not possible to demonstrate the binding of histamine by normal human serum, different fractions of γ -globulins, plasmapexine 1, albumin and Cohn fractions II and V with equilibrium dialysis and gel-filtration techniques.^{11,12}

Recently, a specific fraction which induced histaminopexy was separated from normal human serum.¹³ The mechanism of the reaction was not fully investigated. However, enzymic destruction of histamine was ruled out, and the possibility that histamine was bound by the specific fraction was forwarded.

The present work was undertaken to study the pharmacologic method for measuring serum histaminopexy and to investigate whether serum histaminopexy is impaired in allergic patients. The binding of histamine by normal human serum and its specific fraction has also been tested with the use of dialysis and electrophoresis techniques.

METHODS

Blood was taken by venipuncture from donors attending the Blood Transfusion Service and from patients in the Medicine and Therapeutics Wards, Dundee Royal Infirmary and Maryfield Hospital. The blood was incubated for one hour in a water bath at 37° C., and the serum was separated by centrifugation at 1,210 g for 10 minutes. The serum was kept at 4° C. and used within 48 hours. In a few experiments, the serum was stored in the deepfreeze for a few months.

Measurement of serum histaminopexy

The method used is a modification of that of Laborde and associates.¹⁴ The control and test sera were dialyzed separately in Visking dialysis tubing, grade 24/32, against 250 volumes of saline for 24 hours at 4° C. Allowance was made for any change of volume of the serum during dialysis. Into two 10 ml. volumetric flasks, 0.5 ml. of either the test or control sera were diluted to about 4 ml. with Tyrode's solution. Five milliliters of a histamine dihydrochloride solution (2 μ g per milliliter) was added and each flask made up to 10 ml. A segment of guinea pig ileum was suspended in a 20 ml. organ bath under a tension of 1 to 2 G. Tyrode's solution containing atropine sulfate, 1×10^{-7} , used and the temperature was maintained at 37° C.

The contractions of the ileum produced by a standard histamine solution were then compared with 0.2 to 0.3 ml. of both the control serum-histamine and the test serum-histamine mixtures. The nonbound histamine in the serum-histamine mixture was estimated by a simple comparison assay, and the apparent reduction in the biological activity of histamine was expressed as a percentage of the true value. In some experiments, the 7S γ -globulin, separated as will be described, was tested on the isolated ileum in the automatic apparatus of Boura and associates.¹⁵

Fractionation of normal human serum

The method of Guirgis¹³ was used. Briefly, 5.0 ml. of normal human serum was chromatographed on a 2 $\times$ 15 cm. diethylaminoethyl (DEAE)-Sephadex

column. Stepwise elution was performed with the following successive buffers: 0.005M, pH 7.0; 0.02M, pH 6.3; 0.05M, pH 5.9; and finally 0.4M, pH 5.2. Protein solutions, having an optical density of more than 0.1 at 280 m μ and eluted by the same buffer, were pooled together into a main fraction, adjusted to a fixed volume, and dialyzed. The first fraction contained 7S γ -globulin, and the fourth fraction induced histaminopathy.¹³

Dialysis of ¹⁴C-histamine-human serum mixtures

¹⁴C-Histamine dihydrochloride (specific activity 116 μ c per milligram), 3.78×10^5 d.p.m. in 0.01 ml. solution, was added to three 1.0 ml. portions of normal human serum and to three 2.0 ml. portions of the pooled fourth fraction. The mixtures were incubated for 10 minutes at room temperature, 24 hours at room temperature, and for 24 hours at 37° C. A drop of chloroform was sometimes added to the mixtures to prevent any possible bacterial growth. Each mixture was put into a Visking dialysis bag and dialyzed against 900 ml. saline for 24 hours. The control was made by adding the same amount of radioactive histamine to saline and dialyzing it under the same conditions as in the test samples. After dialysis, a 0.1 ml. portion from each bag was mixed with 0.5 ml. hyamine hydroxide and the mixture was warmed to 60 to 70° C. for a few minutes. The mixture was then dissolved in 10 ml. of a solution of liquid scintillation medium consisting of 2.0 Gm. of 2,5-diphenyloxazole (PPO) and 50 mg. dimethyl POPOP-1, 4-bis-2(4-methyl-5-phenyloxazolyl)-benzene in 500 ml. of toluene. The radioactivity was measured on a Nuclear-Chicago scintillation counter. Calculations were:

1. When serum was used:

$$\begin{aligned} \text{uptake} &= \text{c. p. m. in test} - \text{c. p. m. in saline control} \\ &\times \frac{100}{\text{efficiency}} \times \frac{1.0}{\text{volume serum counted (0.1 ml.)}} \\ &\text{d.p.m. per 1.0 ml. serum} \end{aligned}$$

2. When the fourth fraction was used:

$$\begin{aligned} \text{uptake} &= \text{c. p. m. in test} - \text{c. p. m. in saline control} \\ &\times \frac{100}{\text{efficiency}} \times \frac{\text{volume of fourth fraction}}{\text{volume of fraction counted} \times \text{volume of serum fractionated (5.0 ml.)}} \\ &\text{d.p.m. per 1.0 ml. serum} \end{aligned}$$

Electrophoresis of ¹⁴C-histamine-serum mixtures

¹⁴C-Histamine dihydrochloride containing 1.89×10^6 d.p.m. was added to 0.5 ml. of normal human serum. The control was made by adding the same amount of radioactive histamine to 0.5 ml. saline. After incubation for 10 minutes at room temperature, 10 μ l of the test and control mixtures were applied on a cellulose acetate paper 5×20 cm. and subjected to electrophoresis with tris-maleate buffer, pH 7.4. After 3 hours of electrophoresis, each strip was dried between two filter papers and scanned for radioactivity in a Nuclear-Chicago strip counter. The same strip was then stained, washed, and dried with the use of the method of Padmore and Emmet.¹⁶ The concentrations of the protein fractions were then measured in arbitrary units in a Beckman Analytrol electrophoresis scanner.

RESULTS**Effect of serum on the isolated ileum and histamine response**

Undiluted human serum caused a slow contraction of the isolated ileum (Fig. 1), due to the release of the slow-reacting substance of Schachter.¹⁷ The onset and extent of the reaction was dependent on the concentration of the serum. The effect of the serum on the ileum was not affected by atropine or mepyramine.

Five per cent normal human serum had no effect on the isolated ileum (Fig. 1). However, when histamine was mixed with the dilute serum, partial antagonism of histamine (histaminopexy) was observed on the ileum (Fig. 1). The final concentrations of histamine and serum in the organ bath were found to be critical, being optimal at 0.010 to 0.015 μ g of histamine dihydrochloride per milliliter and 0.0005 to 0.00075 ml. serum per milliliter. The dose-response curves for histamine and histamine-serum mixtures converged more as higher concentrations were used (Fig. 2).

During the cold period of the year, usually extending from the end of October until the beginning of March, histaminopexy was difficult to demonstrate. It was also observed that the sensitivity of the ileum to histamine at this period was decreased. In these cases the serum was kept in the deepfreeze for later testing. After storage the percentage of histaminopexy of sera obtained from normal subjects was within the normal range.

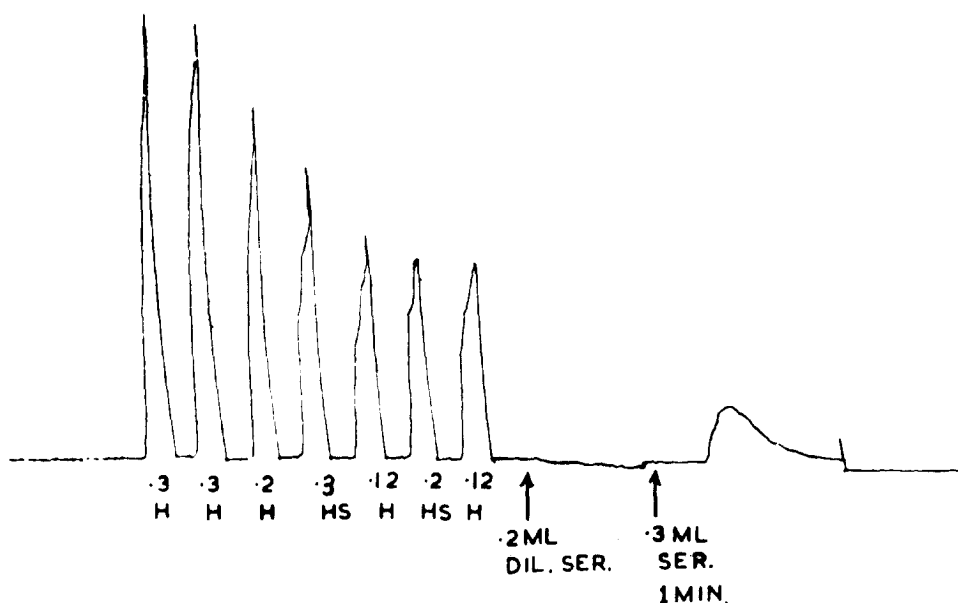


Fig. 1

Tracing to show histaminopexy of normal human serum on the guinea pig ileum. HS is a histamine-serum mixture consisting of 5.0 per cent serum and 1.0 μ g per milliliter histamine dihydrochloride. H is a standard histamine solution, 1.0 μ g per milliliter. Doses in milliliters were added to a 20 ml. organ bath. At the first arrow, 0.2 ml. of 5.0 per cent solution was added. After washing, 0.3 ml. of undiluted serum was added at the second arrow and left for one minute.

The effect of 7S γ -globulin on the isolated ileum and histamine response

When 2.0 mg. of γ -globulin, separated from human serum by chromatography on DEAE-Sephadex column was added to a 20 ml. organ bath, a slow contraction of the ileum occurred. However, when the γ -globulin was diluted to the same final concentration and tested in the organ bath, rapid and marked stimulation of the ileum occurred. In the automatic apparatus of Boura and co-workers,¹⁵

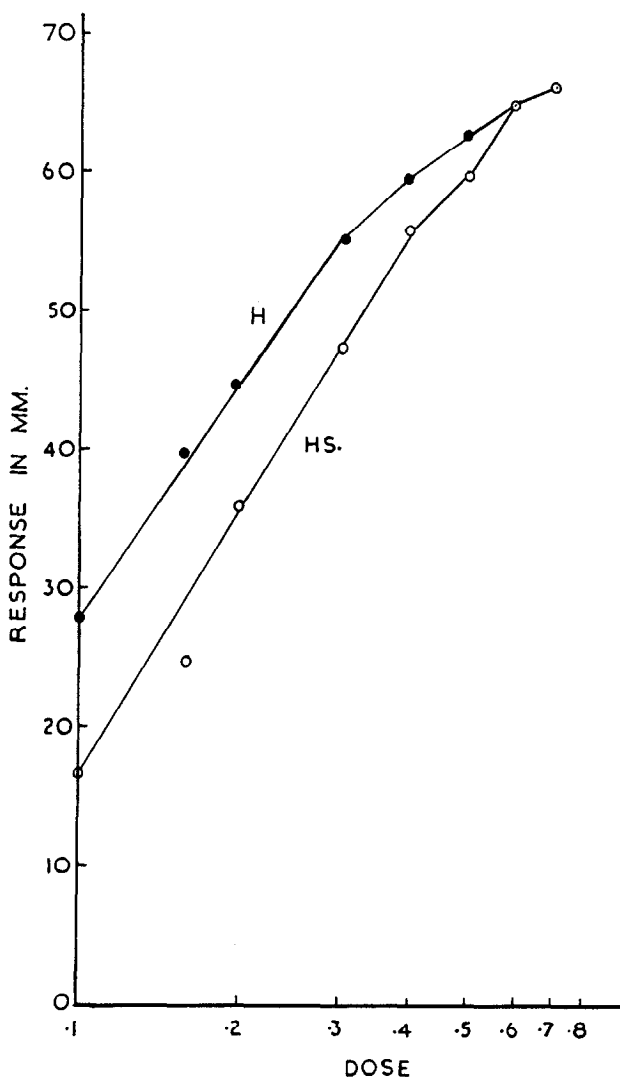


Fig. 2

Graph to show dose-response curves for histamine and histamine-serum mixtures on the guinea pig ileum. The histamine and serum concentrations are the same as in Fig. 1. The doses were plotted on a logarithmic scale.

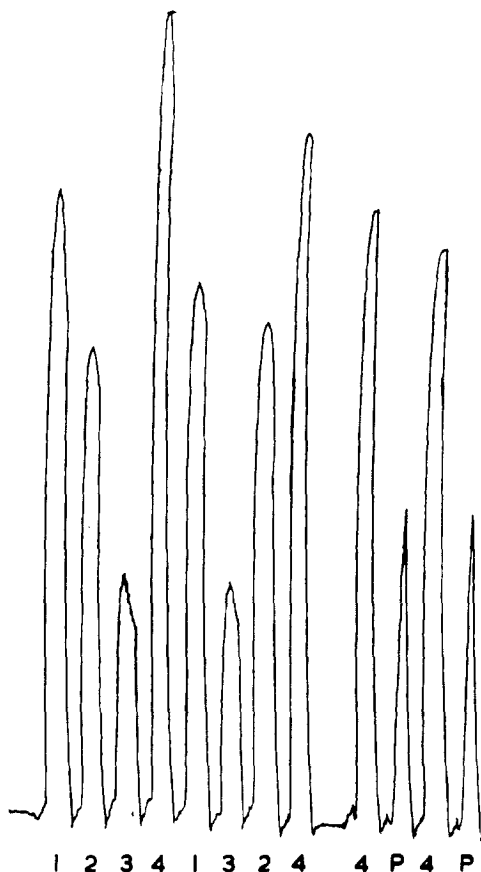


Fig. 3

Tracing to show that the γ -globulin separated on DEAE-Sephadex column and tested in the automatic apparatus of Boura and associates¹⁵ stimulated the ileum and potentiated the histamine response. 1, 0.02 μ g per milliliter histamine; 2, 0.016 μ g per milliliter histamine; 3, 0.01 μ g per milliliter histamine; 4, 0.01 μ g per milliliter histamine in 0.1 mg. per milliliter of γ -globulin; P, 0.01 mg. per milliliter of γ -globulin. As can be seen, the ileum became less responsive to the standard histamine after γ -globulin.

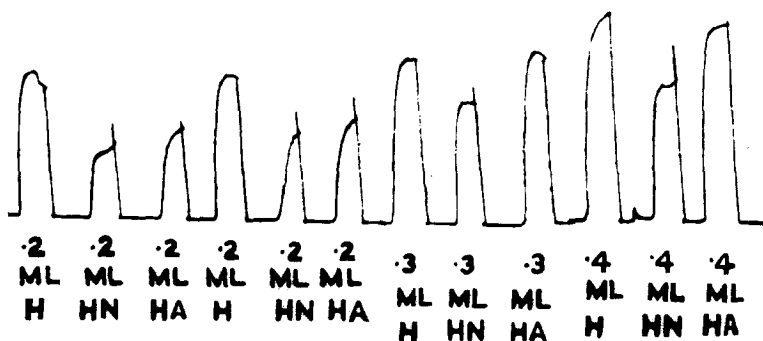


Fig. 4

Tracing to show serum histaminopexy of one subject suffering from hypersensitivity reaction to chloramphenicol. The conditions are similar to that in Fig. 1. H is a standard histamine dihydrochloride solution, 1.0 μ g per milliliter; HN is a histamine-normal serum mixture of the same histamine concentration and containing 5.0 per cent normal serum; HA is a histamine-allergic serum mixture of the same histamine concentration and containing 5.0 per cent allergic serum. As can be seen, with 0.2 ml. of the histamine-serum mixtures, the difference in the histaminopexy between normal and allergic serum was small.

the γ -globulin fraction (0.1 mg. per milliliter) stimulated the ileum, potentiated the histamine response, and caused tachyphylaxis (Fig. 3).

Serum histaminopexy in allergic patients

Because of the difficulties encountered in the measurement of serum histaminopexy, it was found necessary to compare the histamine-normal serum mixture serving as a control and the histamine-test (in this case allergic) serum mixture against the standard histamine solution on the same piece of ileum and under the same conditions of sensitivity and percentage of maximal contraction of the ileum.

It was observed that with small concentrations of histamine dihydrochloride, 0.01 μ g per milliliter, the difference in the percentage of serum histaminopexy between normal and allergic patients disappeared (Fig. 4).

Allergic asthma. Eleven serum samples obtained from 7 patients with allergic asthma during their asthmatic attacks showed the following results:

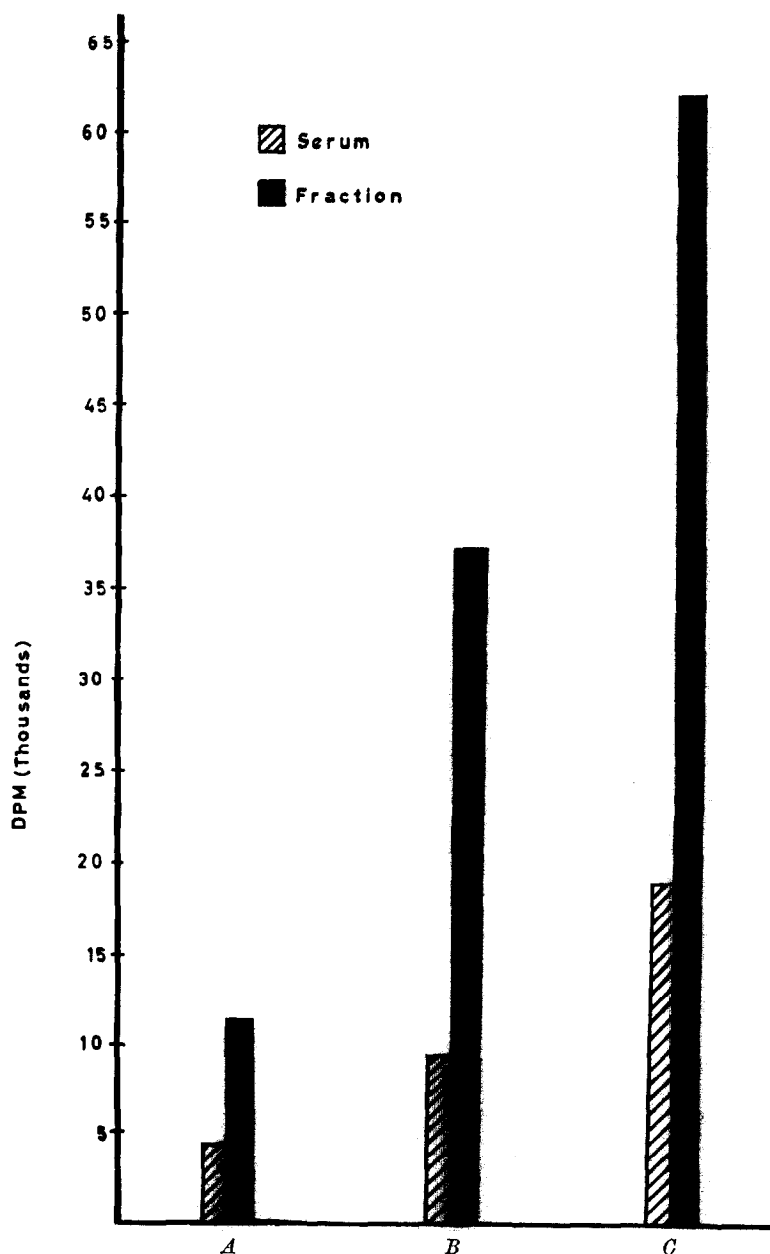
1. Four blood samples taken daily from a patient within a week showed histaminopexy percentages of 15, 21, 8, and 10, with a mean of 13.
2. Two patients showed normal histaminopexy of 37, 30, and 38 per cent.
3. The histaminopexy percentages for the remaining cases were 10, 0, 9, and 5.
4. The percentage of histaminopexy in 11 serum samples, taken from normal subjects and serving as controls, ranged from 20 to 45 with a mean of 31 and standard deviation of 5.1. The difference between the control and allergic patients is significant, $P < 0.01$.

Drug hypersensitivity. Blood was taken from 5 subjects with severe drug hypersensitivity while the clinical manifestations of the reaction were at their peak and before the administration of any drug (Table I). There was no de-

Table I. *The cases of severe drug hypersensitivity studied*

<i>Case No.</i>	<i>Sex</i>	<i>Age (yrs.)</i>	<i>History</i>
1	Male	27	Admitted to hospital with cystitis and urethritis; large urinary bladder stone; developed bronchitis; treated with chloramphenicol; urticarial rash and large swellings over legs, chest, and arms; tracing shown in Fig. 4.
2	Male	30	Shwartzman reaction with a necrotizing process in the neck; papilledema; infection coupled with hypersensitivity reaction to antibiotic?
3	Female	28	Acute reaction to antitetanus serum; generalized urticaria.
4*	Male	35	Absent reflexes; dorsal column degeneration; histamine given subcutaneously as a test for stomach acidity; severe shock.
5	Male	27	Essential hyperglyceridemia; diabetes treated with chlorpropamide; developed urticarial rash; drug withdrawn and patient improved.

*This case may well be an exaggerated pharmacological response to histamine.

**Fig. 5**

Graph to show the uptake of ^{14}C -histamine and/or its metabolites by serum and by the fourth fraction eluted from DEAE-Sephadex column. The ^{14}C -histamine was mixed with serum and with its fraction and incubated. *A*, 10 minutes' incubation at room temperature; *B*, 24 hours' incubation at room temperature; and *C*, 24 hours' incubation at 37°C .

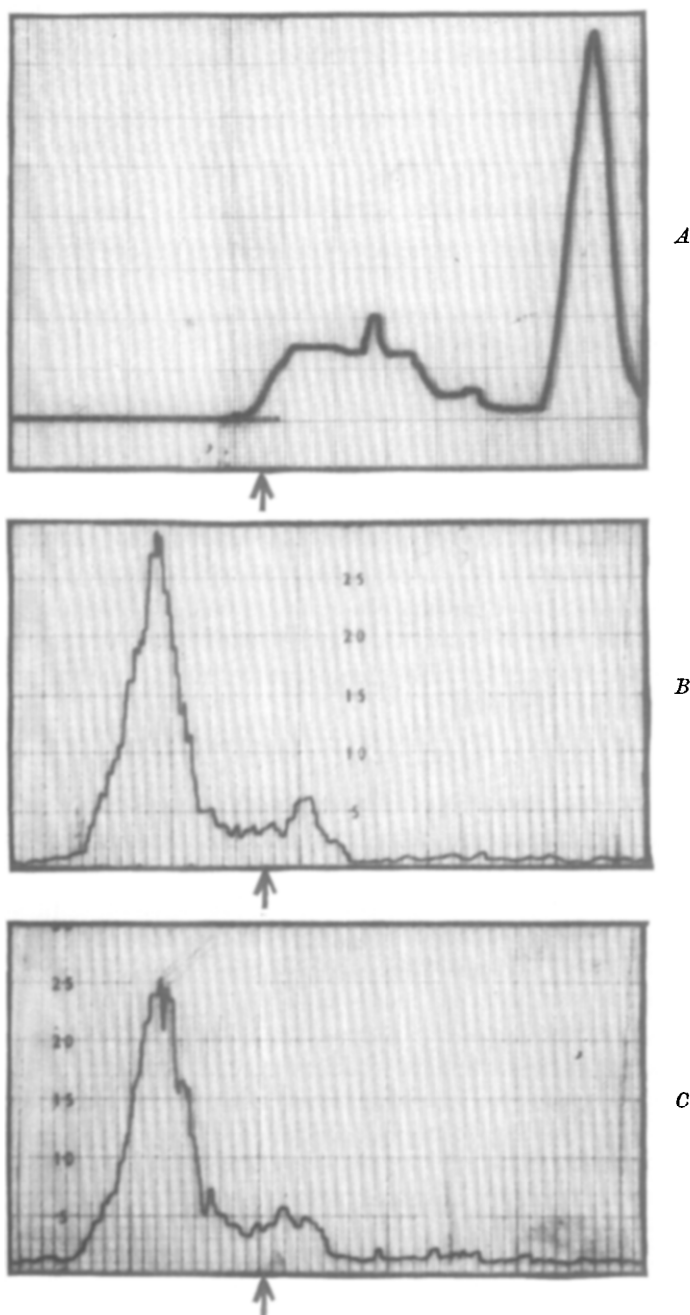


Fig. 6

^{14}C -Histamine dihydrochloride containing 1.89×10^6 d.p.m. was added to 0.5 ml. normal human serum and to 0.5 ml. saline. Ten microliters of each mixture was applied on a cellulose acetate paper, 5×20 cm., and subjected to electrophoresis with the use of tris-maleate buffer, pH 7.4. *A*, electrophoresis strip of ^{14}C -histamine-serum mixture scanned for protein concentrations in a Beckman Analytrol; *B*, electrophoresis strip of ^{14}C -histamine-serum mixture scanned for protein concentrations in a Nuclear-Chicago paper scanner for radioactivity; *C*, electrophoresis strip of ^{14}C -histamine-saline mixture scanned for radioactivity. As can be seen, no ^{14}C -histamine moved in association with the serum proteins.

tectable serum histaminopexy in the 5 cases. The percentage of histaminopexy in sera taken from normal subjects serving as control subjects was 25. The difference between the normal and drug-hypersensitive subjects was highly significant, $P < 0.001$.

Dialysis

Fig. 5 shows the effect of time and temperature on the amount of ^{14}C -histamine and/or its metabolites bound by normal serum and its fraction after 24 hour dialysis. Each result is a mean of not less than 3 experiments. When 10^{-3}M disodium salt of ethylenediaminetetraacetic acid (EDTA) was incorporated into the incubation mixture, no detectable effect on the amount bound by serum was observed.

Electrophoresis

Preliminary results showed that both ^{14}C -histamine and serum proteins moved toward the anode when barbitone buffer, pH 8.6, was used. To overcome this difficulty, the barbitone buffer was replaced by tris-maleate buffer, pH 7.4. The free histamine then moved toward the cathode while the serum proteins moved toward the anode. Fig. 6 shows that after 10 minutes of incubating the ^{14}C -histamine-serum mixture at room temperature, no histamine was bound and carried by the serum proteins.

DISCUSSION

There is a strong probability that the antihistamine activity of guinea pig serum reported by Mackay and associates¹ and the histaminopexy of Parrot and Laborde² are manifestations of the same phenomenon.

The cause of the reported inconsistency in the measurement of serum histaminopexy in normal and allergic subjects^{8, 10} can be attributed in part to: (1) the use of unbalanced concentrations of histamine and serum; (2) the presence of a smooth muscle-stimulating factor in serum; (3) the failure of the isolated ileum to measure histaminopexy during the cold period of the year, possibly related to certain endocrinological changes occurring in the guinea pig at this period of the year; (4) the lack of a significant difference in the percentage of serum histaminopexy between normal and allergic patients with use of low concentrations of histamine; and (5) the absence of adequate control serum.

In the present work we have partially overcome these difficulties by using the optimal concentrations of histamine and serum and introducing a positive control serum.

It was interesting to notice that, on testing the prediluted 7S γ -globulin fraction, the onset, rate, and extent of the stimulation of the isolated ileum was markedly increased, as distinct from the direct addition of the serum or its fraction to a 20 ml. organ bath. Predilution of the γ -globulin prior to its application on the isolated ileum may have activated the kallikrein enzyme system.^{18, 19} The smooth muscle-stimulating property is thus enhanced, and histaminopexy is obscured. This may explain the failure of previous authors^{8, 10} to measure serum histaminopexy on the automatic apparatus.

Though the number of allergic patients studied was small, it was evident

that histaminopexy was not reduced in all. There was also a fairly wide variation from day to day in the percentage of histaminopexy in the same allergic patient. The difference in serum histaminopexy, however, between the normal and the drug-hypersensitive subjects was clear-cut. According to Gordon,²⁰ there is a greater tendency for individuals who are truly atopic and/or who are from families that have atopy to develop drug hypersensitivity. Further work is in progress to assess whether serum histaminopexy was already impaired in these patients before the occurrence of the drug-hypersensitivity reactions. It is worth noting that no increase in the blood histamine concentration was found in allergy and drug hypersensitivity.^{12, 21} A disturbance in histamine metabolism in allergic asthma²² may account for the impairment of histaminopexy in our patients.

It is doubtful whether there was any enzymic destruction of histamine during the reaction of histaminopexy as this was demonstrated biologically within 2 minutes of mixing the histamine with normal serum.^{2, 3, 13} These results are in agreement with the findings of Lindell and Viske.²³ In order to interpret the 20 to 45 per cent serum histaminopexy as a binding reaction, a corresponding percentage of binding of histamine by serum should be demonstrated chemically. However, the dialysis experiments carried out within 10 minutes of mixing ¹⁴C-histamine with the serum showed that 1.0 ml. serum binds the equivalent of 4,000 d.p.m. Seemingly, this amount of ¹⁴C-histamine was too small to be detected when 0.01 ml. of ¹⁴C-histamine-serum mixture was subjected to electrophoresis, and no evidence of binding was demonstrated with the use of this technique.

There are three possible explanations to account for the failure of the chemical techniques to demonstrate a percentage of uptake of histamine similar to that found biologically.

First, the forces involved in the attachment of histamine to serum are probably electrostatic. The histamine, therefore, is unavailable biologically, but not reactive chemically.

Second, either the naturally occurring amines, e.g., histamine, or certain proteins in serum interfere with the binding of the exogenous histamine by the specific binding factor. In the biological measurement of serum histaminopexy, a great dilution factor occurs by testing the histamine-serum mixture in the organ bath. Thus the effect of the interfering substances is abolished, and binding of the histamine by the binding factor occurs. Such a possibility is supported by the observation that 1.0 ml. of human serum shows a threefold increase in its uptake of ¹⁴C-histamine after separation of the binding fraction from human serum. With further purification of this fraction, a correlation between the amount bound in the chemical technique and the amount bound biologically could be achieved.

Third, there is an antihistamine-like substance in normal human serum. In appropriate dilution, the substance partly antagonizes the histamine effect on the isolated ileum.

Though it is difficult at the present time to assess with certainty the mechanism of serum histaminopexy, the first possibility seems the most likely. It was therefore considered valid in the present investigation to define histaminopexy as histamine binding, and the two terms were used interchangeably.

The irreversible uptake of ¹⁴C-histamine by serum or its binding fraction

after incubation is most probably different from the immediate nonenzymic biological reaction of histaminopexy. The uptake of ^{14}C -histamine by serum was not reduced by EDTA, an observation which throws doubt on the specificity of the uptake. Since the uptake is a function of time and temperature, enzymic reactions in the histamine and/or serum proteins during the incubation period cannot be excluded.

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