24 hours. In addition 10 of 18 animals within the latter group developed an occlusive glomerular lesion, the microscopic features of which were similar to those seen in the generalized Shwartzman reaction. It is suggested that pregnancy in the rat as well as in the rabbit may substitute for the preparatory phase of this reaction.

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Received November 28, 1961. P.S.E.B.M., 1962, v109.

Binding C^{14} Labeled Histamine by Normal Human Serum. (27236)

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It has been observed that normal human serum binds some biologically active amines such as histamine, serotonin, acetylcholine and putrescine(1-3). The amines bound lose their biological activity. The serum constituent responsible for the binding has been identified as a gamma-globulin and given the name plasmapexin I(4). It has also been shown that the binding ability is impaired in the serum of individuals who have shown clinical manifestations of allergy even when these were quiescent. These findings have been confirmed by a number of workers using a variety of technics (reviewed in 5). Kaplan and Davis(6), however, failed to observe the binding of C^{14} labeled histamine by serum using an equilibrium dialysis method. The present paper reports the results of experiments done by a similar technic.

Methods. Partially purified plasmapexin I was prepared from normal human serum. After dilution 1/15 in distilled water, the pH was adjusted to 6.5. The precipitate was discarded and pH reduced to 5.2. The resulting precipitate, containing plasmapexin I, was dissolved in Tyrode's solution and made up to the original volume of serum.

Histamine dihydrochloride labeled with C^{14} in C_2 was used (kindly supplied by Dr. R. W. Schayer and later obtained from The Radiochemical Center, Amersham, England) containing 52 μC per 2 mg with an activity of 11.1×10^8 β/min/2π. Since the counter had an efficiency of about 3% of counts, the real activity of 52 μC of the histamine dihydrochloride was 3.3×10^6 cpm. The solution of labeled histamine was diluted ½ with nonisotopic histamine, adjusted to pH 2.2 and kept at −10°C. Immediately before the experiment the required dilution was made up in Tyrode's solution.

For equilibrium dialysis, 2 ml of a histamine dihydrochloride solution (at concentrations between 1.6×10^{-5} and 2.2×10^{-4} M) were placed in a cellophane bag, together with 1 ml of the plasmapexin I solution containing the same amount of histamine added with 1 ml of Tyrode's solution. Before the experiment, the cellophane was soaked in the histamine solution to eliminate the possibility of small amounts of histamine being adsorbed on it during the dialysis.

At room temperature, the dialysis reaches equilibrium in about 24 hr. Since the amount of protein contained in the bag was small and there was no significant difference in osmotic pressure of the 2 solutions, their volume remained practically constant. After removal of the bag from the outside medium, 1 ml of Tyrode's solution was added to its content and 1 ml of the plasmapexin I preparation was added to the outside compartment. In this fashion, the protein film formed on evaporation of the samples in the planchettes had the same thickness and the same self-absorption.

After counting the radioactivity of the 2 samples, the results were expressed as

$$D = 100\,\frac{I - E}{E}$$

(D = displacement of histamine; I = counts in inner compartment; E = counts in outer compartment).

Results. Five specimens of serum from individuals without clinical allergy were tested. The equilibrium was displaced in all samples toward the plasmapexin-containing compartment (Table I). The displacement was much more marked when low concentrations of histamine were used, suggesting that the amount of histamine bound is limited by the quantity of plasmapexin present.

Table II shows the results obtained with 4 sera taken from allergic subjects. No significant displacement of histamine was noted.

Discussion. The results indicate that displacement of histamine is due to a specific activity of the serum which is impaired in allergic serum. This has now been confirmed in many human sera by pharmacological, chemical and physical methods(5). The results cannot be explained by a Donnan-type equilibrium since total amount of proteins was the same in both normal and allergic sera.

TABLE I. Histamine Binding by Normal Human Sera.

Initial conc. (μg/ml)	Final conc. (μg/ml) I	E	D
3	4.0	2.5	60
6	7.8	4.8	62
20	20.1	17.0	18
30	35.8	29.4	22
40	41.0	36.0	14

TABLE II. Histamine Binding by Serum from Allergic Subjects.

Initial conc. (μg/ml)	Final conc. (μg/ml) I	E	D
3	3	3	0
3	3.1	3.0	+2
6	5.3	5.8	−9
12	12.3	12.0	+2

The discrepancy between these results and those obtained by Kaplan and Davis(6) is difficult to explain because the technical conditions of their experiments have not been completely described. It is possible that the 2 serum specimens used were unable to bind histamine because they had been taken from allergic individuals or because of prolonged storage. However, the results they obtained by pharmacological estimation of histamine suggest that their gamma-globulin did bind the amine. In the presence of the globulin, histamine activity was reduced, to increase again after protein precipitation.

Summary. Histamine binding by normal serum proteins was demonstrated using equilibrium dialysis with C^{14} labeled histamine. The procedure also showed that allergic serum is devoid of this property. These results are in agreement with those obtained by other methods.

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Received November 30, 1961. P.S.E.B.M., 1962, v109.