

RELEASE OF HISTAMINE IN THE BLOOD OF RAGWEED-SENSITIVE INDIVIDUALS

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A FEW investigators have reported that the exposure of sensitized blood cells to a specific antigen results in the liberation of histamine into the plasma. This reaction may be elicited by adding homologous antigen either to whole blood of actively sensitized animals¹⁻³ or to whole blood obtained from patients with allergic symptoms known to be due to an antigen such as that present in ragweed pollen.⁴

Katz,⁵ in summarizing his studies of histamine release, indicated that the reaction was dependent upon exposure of sensitized blood cells to the specific antigen involved. The reaction took place readily when the blood cells were freed of plasma and suspended in saline solution before being exposed to the antigen, indicating that the reaction was strictly a cellular one, not mediated by any substance present in the plasma.

Previous investigators of the histamine release phenomenon have employed the biologic method of histamine assay. Graham and associates⁶ have developed a chemical method of histamine assay which is dependent upon the formation of a colored conjugation compound of dinitrofluorobenzene and histamine, the concentration of which can then be determined spectrophotometrically. This technique was employed in our studies. By the method, amounts of histamine added to plasma are recovered within a range of plus or minus 10 per cent.

METHODS

Venous blood was obtained from proved ragweed-sensitive patients (hay fever, asthma) who were free of symptoms at the time and who, at the time of obtaining the first blood sample, had not been exposed to ragweed pollen for five months. All patients except one were receiving periodic hyposensitizing injections of ragweed extract, several of them having received injections as recently as one week prior to the time blood was obtained. In five, it was possible to obtain a second blood sample during the fourth week of the ragweed pollen season, when all five were having mild symptoms of pollinosis. All blood specimens were collected in syringes wet with a solution containing 10 mg. of sodium heparin per milliliter. Approximately 50 ml. of blood were obtained from each individual and divided into two equal parts. To one portion was added 0.25 ml.

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of ragweed extract containing 2,500 protein nitrogen units of ragweed antigen. The other remained untreated. Both portions were then incubated in a water bath at 37° C. for 20 minutes. After these procedures on the whole blood of four individuals, the blood was assayed for histamine. A blank containing 0.25 ml. of ragweed extract, 1 ml. of 4 M. sodium acetate, and 5 ml. of 5 per cent trichloroacetic acid, the protein precipitant used in this method, was run with each pair of determinations. Results are listed in Table I.

TABLE I. HISTAMINE DETERMINATIONS OF WHOLE BLOOD PLUS ANTIGEN

PATIENT	BLOOD HISTAMINE	BLOOD (PLUS RAGWEED) HISTAMINE
F. T.	130	136
B. G.	129	133
J. M.	130	112
W. H.	113	128

Figures represent micrograms of histamine per liter of whole blood.

Treated in the same manner as those appearing in Table I, the remaining ten pairs of blood samples were centrifuged, the plasma removed, and the histamine content of the plasma determined. Results appear in Table II.

TABLE II. HISTAMINE DETERMINATIONS OF THE PLASMA FROM WHOLE BLOOD PLUS ANTIGEN

PATIENT	PLASMA FROM BLOOD WITHOUT RAGWEED ANTIGEN	PLASMA FROM BLOOD PLUS RAGWEED ANTIGEN
M. J.	4	10
G. C.	3	34
L. H.	4	63
C. St.	4	35
R. D.	5	131
R. C.	6	44
D. P.	3	23
L. M.	3	19
J. J.	2	29
C. Sc.	3	9

Figures represent micrograms of histamine per liter of plasma.

TABLE III. CONTROL DETERMINATIONS ON THE BLOOD PLASMA OF FIVE NONSENSITIVE INDIVIDUALS

PATIENT	PLASMA FROM BLOOD WITHOUT RAGWEED ANTIGEN	PLASMA FROM BLOOD PLUS RAGWEED ANTIGEN
J. N.	5	7
A. B.	24	17
R. E.	3	6
R. D.	4	7
H. I.	7	8

Figures represent micrograms of histamine per liter of plasma.

During the ragweed season blood was obtained from five of the ragweed-sensitive individuals whose blood histamine release had been determined a few months previously. All individuals had been under regular hyposensitizing treatment in the interval between the two determinations as well as for a variable period prior to that. All had shown a good response to therapy.

In Table IV, some figures shown in Table II are again presented for comparison.

TABLE IV. COMPARATIVE DETERMINATIONS OF PLASMA HISTAMINE VALUES BEFORE AND DURING THE POLLEN SEASON

PATIENT	PLASMA FROM BLOOD WITHOUT RAGWEED ANTIGEN		PLASMA FROM BLOOD PLUS RAGWEED ANTIGEN	
	March	September	March	September
C. S.	4	4	35	54
R. C.	7	7	44	58
D. P.	3	7	23	62
L. M.	3	4	19	68
J. J.	2	13	29	75

Figures represent micrograms of histamine per liter of plasma.

DISCUSSION

When the results of histamine determinations of the whole blood of ragweed-sensitive individuals are examined (Table I), it is apparent that there is no significant variation in the "blood" and "blood plus ragweed" figures. Total blood histamine is largely in ratio to the total leukocyte count. One does not increase total blood histamine appreciably by adding antigen to a sensitive individual's blood. In these four pairs of determinations the largest increase, of 15 μg per liter, is but 13 per cent. The lack of significance of this increment is emphasized by the large and constant increase in the value of plasma histamine found when blood of sensitive individuals is incubated with antigen. The percentile increase varies from 180 to 2,520 per cent, with an average increase of over 900 per cent. By coincidence, 900 per cent is also the figure given by Katz and Cohen⁴ as the greatest plasma histamine increase obtained in two of ten sets of determinations they selected as examples. The amount of histamine released in these two instances was 27 and 45 μg per liter. Table II and Table IV show that the largest amount released in our experiments was 126 μg per liter, and in seven instances was over 50 μg per liter. Our values were, therefore, generally higher, for reasons not determined but probably inherent in the methods used. Since the blood of non-allergic individuals showed no significant histamine release (Table III) when treated with antigen, compared with the blood of those who had hay fever, it is apparent that the release phenomenon depends upon allergic sensitivity.

It has been reported that erythrocytes as well as white cells participate in the histamine release reaction, but to what extent each cell type contributes its histamine is not known. One might assume that cellular types high in histamine contribute the most. From this viewpoint, figures arrived at in the histamine assay of red and various white blood cell elements are important. With the dinitrofluorobenzene technique, the approximate contribution of various blood elements to the total blood histamine has been determined as follows⁷:

Erythrocytes	20 per cent
Neutrophils	50 per cent
Basophils	15 per cent
Eosinophils	1 per cent
Platelets	3 per cent
Lymphocytes	1 per cent
Plasma	4 per cent

With the chemical method, the average total blood histamine value of 122 μg per liter is high when compared with values obtained by biologic assay.⁸ This may be due to the fact that chromogens other than histamine are detected by the dinitrofluorobenzene method. The color-producing substance or substances present in human erythrocytes are not altered by exposure to histaminase and are, therefore, not histamine. If one determines only the chromogenic fraction of human whole blood which is acted upon by histaminase, an average normal value of 82 μg per liter is found.⁹ This figure more nearly approaches values obtained by the biologic method.

In our determinations, occasionally more than 60 per cent of the whole blood histamine, calculating from the average normal value of 122 μg per liter, has been released into the plasma after addition of antigen. The neutrophils, containing 50 per cent of the total blood histamine, must have contributed, and probably the basophils and other white cell elements as well. The blood samples obtained during the fourth week of the ragweed season, from patients with symptoms of ragweed pollinosis, showed a greater histamine release (300 per cent increase) when compared with nonseasonal values. Five of the seven histamine release values exceeding 50 μg per liter were in the coseasonal group. The number of determinations made is so far too small to permit any valid conclusions. One may speculate that since plasma histamine values remain about the same in and out of the ragweed season, the increased amount of histamine released during the ragweed season is quickly removed from the plasma, and either the blood cells, for reasons unknown, give up their histamine more readily during the season, or the number of leukocytes in the blood is greater at this time. During the ragweed season, total white blood cell counts were done on the samples obtained and these were within normal limits. Unfortunately, total white counts were not performed on the samples obtained previously. Regardless of the fundamental cause of greater histamine release during the ragweed season, it is possible that the increased liberation of histamine in coseasonal samples may offer an explanation of the higher incidence of constitutional reactions during the ragweed season when patients are being hyposensitized on conventional perennial treatment schedules.

CONCLUSIONS

1. An appreciable release of histamine into the blood plasma was detected when whole blood of a clinically sensitive individual was incubated with ragweed antigen.
2. No such increase appeared in unseparated whole blood, similarly treated.
3. The discrepancy is attributed to a transfer to the plasma of histamine contained in blood cells.
4. Greater release of histamine occurred in blood samples taken during the ragweed season than in those taken preseasonally.

REFERENCES

1. Katz, G.: Histamine Release from Blood Cells in Anaphylaxis in Vitro, *Science* **91**: 221, 1940.

2. Dragstedt, Carl A., DeArellano, M. R., and Lawton, A.: The Relationship of Histamine to Anaphylaxis in the Rabbit, *Science* **91**: 617, 1940.
3. Rose, B., and Browne, J. S. L.: Studies on the Release of Histamine from the Blood Cells of the Rabbit by the Addition of Horse Serum or Egg Albumin in Vitro, *J. Immunol.* **41**: 403, 1941.
4. Katz, G., and Cohen, S.: Experimental Evidence for Histamine Release in Allergy, *J. A. M. A.* **117**: 2, 1782, 1941.
5. Katz, G.: The Role of Blood Cells in the Anaphylactic Histamine Release, *J. Pharmacol. & Exper. Therap.* **72**: 22, 1941.
6. Graham, H. T., Lowry, O. H., and Harris, F. B.: Microchemical Determination of Histamine in Biological Fluids, *J. Pharmacol. & Exper. Therap.* **101**: 15, 1951.
7. Graham, H. T., Wheelwright, F., Parish, H. H., Jr., Marks, A. R., and Lowry, O. T.: Distribution of Histamine Among Blood Elements, *Federation Proc.* **2**: 350, 1952.
8. Code, C. F., and MacDonald, A. D.: The Histamine-like Activity of Blood, *Lancet* **2**: 730, 1937.
9. Graham, H. T.: Unpublished data.