

Failure to elicit histamine eosinophilotaxis in the skin of atopic man. Description of an improved technique

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Confirmation of reports that histamine is eosinophilotactic was unsuccessful in a series of blind studies in atopic man. Two methods were used, one of which provided greater uniformity in the distribution of cells, better cellular morphology, and greater ease of interpretation than methods described hitherto. It was concluded that histamine is not eosinophilotactic.

The often-quoted eosinophil-attracting properties of histamine have been a subject of controversy. Archer¹ reported that eosinophils were drawn to skin sites in ponies injected with histamine intracutaneously in a concentration of 0.5 mcg. per milliliter. The response increased as the concentration was raised, reaching a peak at 100 mcg. per milliliter, with a fall in response as the concentration was raised to 1,000 mcg. per milliliter; a second rise occurred, peaking at 5,000 to 10,000 mcg. per milliliter, and finally the response fell at 20,000 mcg. per milliliter. Eidinger and colleagues,² applying a drop of histamine in a concentration of 100 mcg. per milliliter to a denuded skin site in man, reported an eosinophilotactic response in 9 out of 14 atopic subjects but no response in nonatopic subjects. The peak response occurred 2 to 4 hours after application. Feinberg and Feinberg³ injected histamine intracutaneously and later denuded the area and collected the exudate. They reported eosinophilotactic responses to histamine in atopic and, to a lesser degree, in nonatopic subjects. There was some response to saline as well, although this varied markedly.

In contrast to these reports, Litt,⁴ in well-controlled studies in guinea pigs, reported failure of histamine to attract eosinophils when injected intra-abdomi-

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nally, intracutaneously, or into footpads. Likewise, Cohen and Sapp⁵ obtained no response when histamine was injected into the skin of guinea pigs. Finally, Fowler and Lowell⁶ failed to elicit eosinophilotactic responses at superficially denuded skin sites with 100 to 10,000 mcg. of histamine per milliliter in atopic subjects.

These contradictory reports prompted a fuller investigation of the eosinophilotactic properties of histamine. Two methods were used in the present study. The first, Method I, was that originally described by Rebeck and Crowley⁷ and adopted by Eidinger and colleagues² and Fowler and Lowell.⁶ The second, Method II, was developed in an effort to enhance the eosinophilotactic response, to improve the quality of the preparations, and to increase the flexibility of the procedure. Both methods were applied in attempts to elicit eosinophilotactic responses to histamine. These attempts consistently failed, although no difficulty was encountered with either method in eliciting responses with allergenic extracts in suitably selected subjects.

MATERIALS AND METHODS

Histamine and allergenic extracts were diluted in 0.85 per cent NaCl solution to make the concentrations indicated in Table I. The concentration of histamine is expressed as the base in micrograms per milliliter and that of allergenic extracts as weight of dry defatted pollen per volume of diluent.

Twelve subjects were selected in whom a diagnosis of atopic disease had been made, based on the presence of seasonal rhinitis, asthma, or both and intense immediate-type skin reactions (wheal and flare) on scratch testing. One additional subject exhibited skin reactivity to *Ascaris* antigen in a concentration of 0.4 γ N per milliliter or higher intracutaneously. All exhibited some degree of eosinophilotactic response when the appropriate allergenic extract was applied for approximately 20 hours to a superficially denuded skin site.

Coverglass preparations were air-dried, and the adherent cells were stained with Wright's stain and mounted on labeled glass slides. In order to remove bias, the labels were subsequently covered and the slides coded by another individual prior to reading. Preparations were scanned under lower power magnification and sites were selected in which the cells were distributed in a monolayer and in which staining allowed recognition of various cell types. The number of eosinophils among 1,000 cells was recorded.

Method I was that of Rebeck and Crowley⁷ as modified by Eidinger and colleagues.²

Method II was similar to Method I in the preparation of the skin site. However, instead of a coverglass, a piece of filter (pore size = 0.2 μ)* slightly larger than the denuded area was laid on the site, upon which was then placed a pledget of absorbent cotton big enough to take up and hold about 0.35 ml. of an aqueous solution. The object was to permit transfer of fluid from the cotton to the denuded surface and at the same time to retain the cells at the skin site. The filter and cotton pledget were then enclosed in a chamber (Fig. 1) to give

*Millipore Filter Corporation, New Bedford, Mass.

Table I. Eosinophils per 1,000 white cells in coverglass preparations

Subject	Saline			Histamine			Allergenic extract		
	Interval (hours)	Eosinophils per 1,000 cells	Concentra- tion (mcg./ml.)	Interval (hours)	Eosinophils per 1,000 cells	Concentra- tion (mcg./ml.)	Type and concentration (w/v)	Interval (hours)	Eosinophils per 1,000 cells
Method I									
M. Doy.	4	1	20	20	1		Rag. 1:10	20	354
K. C.	4	2	100	4	0				
	20	3	100	4	7		Rag. 1:10	20	24
J. F.	20	0					Tim. 1:10	20	224
			20	20	2				
			100	20	1				
J. M.	20	6	200	20	2		Rag. 1:10	20	80
			20	20	0				
			200	20	0				
L. S.	4	0	100	4	9		Tim. 1:10	20	125
	20	0	100	20	0				
J. Mc.	4	0	100	4	0		Rag. 1:10	20	275
	20	0	100	4	0				
M. Don.	20	1	200	20	3		Rag. 1:10	20	319
Method II									
L. M.	4	0	10	4	0		Tim. 1:10,000	20	42
	20	0	10	20	1				
			100	4	1				
			100	20	6				
			1,000	4	2				
			1,000	20	3		Rag. 1:1,000	20	98
J. B.	4	0	10	4	0				
	20	0	10	20	0				
			100	4	0				
			100	20	0				
			1,000	4	1				
			1,000	20	0				
R. F.	4	0	10	4	0		Rag. 1:100	20	65
	20	1	10	20	0				
			100	4	0				
			100	20	0				
			1,000	4	0				
			1,000	20	0				
D. M.	20	0	10	20	0		Rag. 1:100	20	70
			100	20	0				
			1,000	20	2				
R. B.	10	1	10	20	1		Tim. 1:1,000	20	140
			100	20	0				
			1,000	20	7				
A. F.	20	0	10	20	0		Asc. 10 γ N/ml.	20	38
			100	20	0				
			1,000	20	6				

Rag. = ragweed pollen extract; tim. = timothy pollen extract; and asc. = *Ascaris lumb.* extract.

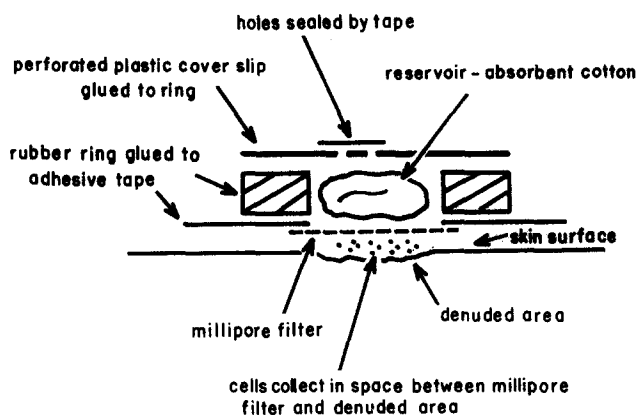


Fig. 1

Schematic drawing of chamber used in Method II.

protection and to minimize the loss of fluid and evaporation. The chamber was constructed of a $\frac{3}{4}$ inch rubber-hose washer fastened with a thermoplastic glue* to a $1\frac{1}{2}$ by $1\frac{1}{2}$ inch piece of surgical tape.† The floor of the chamber was then cut out. A plastic coverglass was glued to the upper surface of the washer to form a roof in which two small holes were made. With a syringe and needle, 0.35 ml. of the test solution was injected through one of the holes, the other hole permitting escape of displaced air. The holes were then sealed with cellophane tape. At the end of the period of exposure, the chamber and filter were removed and the cells sampled once, or repeatedly if desired, by lightly pressing a coverglass to the denuded area. The coverglass was then dried and stained.

RESULTS

At 20 to 24 hours, Method II gave a copious exudate consisting predominantly of polymorphonuclear cells which, when distributed in a monolayer, were well stained and readily identified. This cellular response differs from that seen in our preparations made by Method I and in descriptions by others in that large mononuclear cells, apparently macrophages, were either lacking or present only in small numbers.

A total of 71 preparations were made. The number of eosinophils among 1,000 cells counted is shown in Table I. Among 19 control sites with saline the range was 0 to 6 eosinophils, with a mean of 0.8 and a median of 0. With histamine in concentrations ranging from 10 to 1,000 meg. per milliliter sampled at 4 or at 20 hours, the range was 0 to 9 eosinophils with a mean of 1.4 and a median of 0. In contrast to this, tests with specific allergenic extracts gave a range of 24 to 354 eosinophils with a mean of 143 and a median of 98. These results are shown in a composite plot in Fig. 2 in which the ordinate gives the number of eosinophils per 1,000 leukocytes.

*Thermogrip Hot Melt Glue, United Shoe Machinery Corp., Reading, Pa.

†Band-Aid Clear Tape, Johnson and Johnson, New Brunswick, N. J.

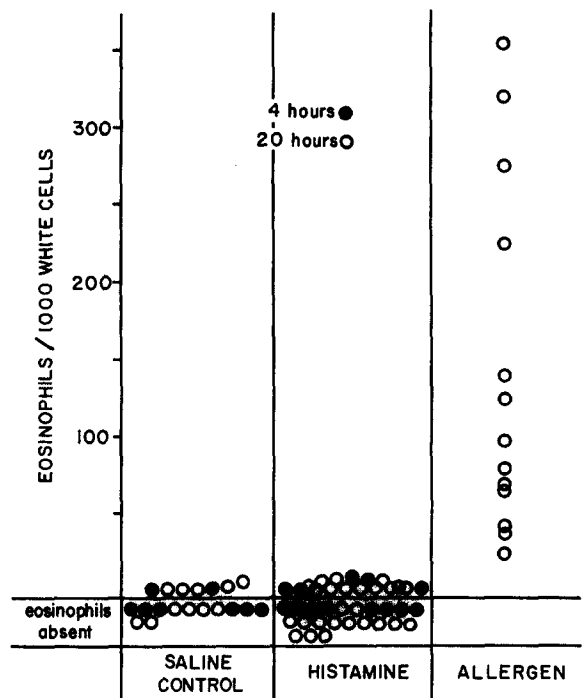


Fig. 2

DISCUSSION

The traditional central position of histamine as the mediator of allergic responses would place it high on the list of substances suspected of mediating any of the components of this response, and it was logical to examine a possible role for histamine in the accumulation of eosinophils at the site of allergic reactions in man and animals. Since definition of this role would appear to require only rather simple experimental techniques, it is surprising that so profound a division of opinion should have developed. Since the studies reported here conspicuously failed to demonstrate an eosinophilotactic role for histamine, the description of such a role by others requires explanation. We have only two to offer. First is the bias that might favor one more role for the most venerable of chemical mediators. We attempted to avoid any bias either for or against by reading all the preparations blind. Second is the tendency for dead or dying cells to take on a pinkish hue when stained with Wright's stain. Such cells are much more commonly seen with Method I, and neutrophilic polymorphonuclear cells may at times appear remarkably granular. Thus in color and in granularity these cells may superficially resemble eosinophils. Identification of such cells as neutrophils rather than eosinophils is possible if true eosinophils are present in the preparation or if a positive control preparation is available for comparison. It will then be seen that the granules of the eosinophils are larger, better defined, and stain more intensely. We were especially impressed

with the intensity of nonspecific pink staining in some preparations made with aspirin in aspirin-sensitive subjects in whom it was finally shown that eosinophilotaxis was not elicited. In addition to the intense pink color, large size, and clarity of their granules, most eosinophils have a bilobed nucleus.

In Method II, the distribution of the cellular exudate on the coverglass tended to be much more uniform than that seen in Method I, and enumeration of cell types in different areas of the preparation gave similar results. The cells appeared to be intact, and identification could therefore be made with much greater certainty. Possessing as it did a reservoir, Method II permitted more prolonged exposure of the denuded site to histamine. By analogy with eosinophilotaxis by allergens, where dilutions of extract as great as 1:1,000,000 have given specific eosinophilotactic responses,⁸ Method II might have been expected to succeed in demonstrating an eosinophilotactic role for histamine where Method I failed. However, the data (Table I) do not suggest that more prolonged contact with histamine was helpful in eliciting evidence for eosinophilotaxis, and since readings were made both at 4 hours and at 20 hours, it seems unlikely that insufficient time for accumulation of eosinophils can explain the negative findings.

Three experiments with the histamine liberator 48/80 likewise failed to give an eosinophilotactic response. Although 48/80 was not studied in detail, the negative results suggest that endogenous, like exogenous, histamine is not eosinophilotactic.

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