

QUANTITATIVE STUDIES IN SKIN TESTING

V. THE WHEALING REACTIONS OF HISTAMINE AND RAGWEED POLLEN EXTRACT

HENRY H. SWAIN,* M.S., M.D., AND ELMER L. BECKER,** PH.D., M.D.

CHICAGO, ILL.

IT WAS propounded in 1927, by Lewis,¹ that histamine is of importance in the mediation of the immediate-wheal type of response in human skin. He showed that the reaction of skin to intradermally injected histamine is a triple response. First seen is a local red reaction caused by local arteriolar dilatation. Next to be observed is the surrounding red flare, mediated by an axon reflex. Finally there appears a pale, firm, sharply demarcated, round or oval wheal, caused by the extravasation of fluid and plasma protein through the hyperpermeable capillary wall.

Lewis showed that this triple response is seen whenever the skin comes in contact with any of a wide variety of noxious stimuli of intermediate intensity—heat, cold, stroking, peptone, nettle stings, etc. He postulated that each of these agents is capable of causing the tissues to release histamine or some histamine-like substance.

The specific allergic skin responses differ from the whealing caused by heat, cold, stroking, etc., in that an antigen-antibody reaction is involved. Only those subjects who harbor the specific antibody show the triple response when exposed to a given antigen.

Katz² in 1942 proved that histamine is actually released during the cutaneous reaction to specific antigens. He demonstrated that the amine is present in the exudate of a blister after the injection of an antigen to which the subject is sensitive. Nilzen³ showed that after whealing caused by mechanical stimulation there is a decrease in the amount of extractable histamine in the skin.

Nilzen also studied the blood levels of extractable histamine in patients suffering from chronic urticaria. He found that the level is low during acute attacks and rises during recovery. The probable explanation for this finding lies in the work of Dragstedt⁴ who showed that free histamine disappears rapidly from the blood. Rosenthal and Tabor⁵ and more recently Urbach⁶ have shown that the amine is conjugated to form acetyl histamine, 4 (beta-acetyl aminoethyl) imidazole, which is excreted in the urine.

The evidence that histamine is released during the cutaneous whealing reaction is convincing, but there remains the question of whether it is the only pharmacologically-active substance involved in the reaction. This is a question which will be considered later in the light of the present investigation.

This study was made possible by the financial assistance of Parke, Davis & Company, Detroit.

*Department of Pharmacology, University of Illinois College of Medicine.

**Allergy Unit & Dept. of Bacteriology, University of Illinois College of Medicine. Present address: Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C.

The work of Schloss,⁷ Alexander, Harter, and McConnel,⁸ Bowman,⁹ and, more recently, Becker and Rappaport¹⁰ has shown that differences exist in the skin sensitivity to allergens at different sites, and, moreover, that these differences follow a regular pattern. For instance, Becker and Rappaport examined the forearms of ragweed-sensitive subjects and found that constant doses of antigen cause larger wheals on the ulnar aspect than on the radial aspect. Likewise sites near the cubital fossa are more reactive than sites near the wrist.

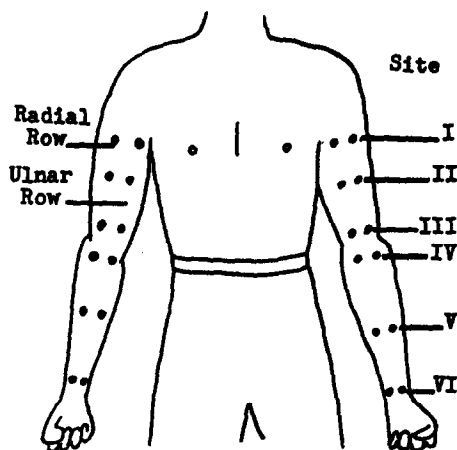


Fig. 1.—Position of injection sites for histamine on arm and forearm.

Differences in site sensitivity are open to at least two different interpretations: it is possible that the antigen causes the release of different amounts of histamine at different sites, or there may be a difference in the whealing activity of the skin at the various sites to rather constant amounts of released histamine. Bowman⁹ and, more recently, Wiseman¹¹ believed that they could demonstrate a difference between the site-response to histamine and the site response to a specific allergen. This evidence would favor the former alternative. The present authors examined the site-response of the skin of the upper extremity to constant doses of histamine, and compared the results with the site-response to constant doses of ragweed pollen extract, as determined by Becker and Rappaport.

To determine whether or not other substances besides histamine are active in mediating the allergic wheal reaction, the investigators studied the effect of dosage upon wheal diameter. The dose-response curve for histamine was determined, and this was compared with the dose-response curve obtained by Becker and Rappaport for ragweed extract. It was noted that the curves for histamine and the specific antigen are of the same general type. Therefore appropriate subjects were tested simultaneously with graded doses of both histamine and ragweed pollen extract.

There is a large amount of variation encountered in skin testing. The data therefore were submitted to statistical analysis to measure the various factors

affecting wheal diameter and to determine the accuracy of the techniques employed.

METHODS AND RESULTS

Part I. Histamine Site-Response.

Subjects: A group of 23 adult human volunteers were chosen without regard to age, sex, race, or ragweed sensitivity.

Solution: Crystalline histamine dihydrochloride (Hoffmann LaRoche) was dissolved in distilled water containing sufficient sodium hydroxide to render the solution neutral (pH 6.5 to 7.0), and sufficient sodium chloride to make it isotonic. The solution was sterilized by passage through a Seitz filter. In strength it contained one part of free histamine base per 10,000 parts of solution. A fresh solution was prepared for each day's injections.

Sites: Six radial and six ulnar sites were tested on the flexor aspect of each upper extremity. The sites in each row were numbered from proximal to distal,

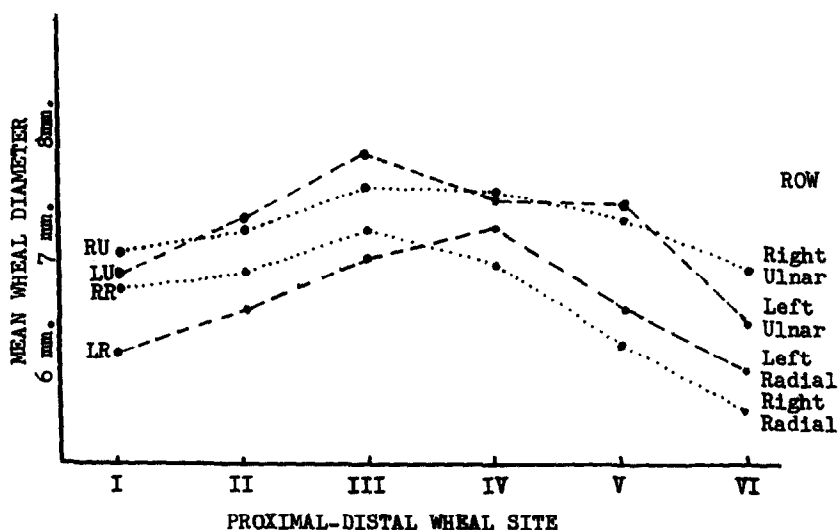


Fig. 2.—The change of responsiveness to histamine with the position of the injection site on arm and forearm.

as shown in Fig. 1, and were located as follows: I, 2 cm. below insertion of pectoralis major muscle; II, midway between I and III; III, 1.5 cm. above cubital crease; IV, 2.5 cm. below cubital crease; V, midway between IV and VI; VI, 2 cm. above proximal carpal crease.

Technique: The histamine solution was injected at the 24 sites with a 1 c.c. syringe and a No. 27 short-bevel needle. The needle tip was placed into the corium of the skin, and the solution was injected so as to produce a bleb approximately 2 mm. in diameter. After 15 minutes the diameters of the resultant wheals were measured with vernier calipers to tenths of millimeters. In the case of oval wheals, the longer diameter was measured. Pseudopods on wheals were excluded from measurement. The results of the 24 tests on each of 23 subjects were plotted and submitted to an analysis of variance,¹² con-

TABLE I

SOURCE OF VARIANCE	D.F.	SUM OF SQUARES	MEAN SQUARE	F	(1% F)	P
Subjects	22	205.18	9.32	11.9	(1.88)	< 1%
Positions	5	77.11	15.42	19.7	(3.06)	< 1%
Rad. vs. Ul.	1	53.54	53.54	68.3	(6.70)	< 1%
Left vs. Rt.	1	0.06	0.06	0.07	(6.70)	> 5%
1st Order Interactions	165	146.15	0.88	1.14	(1.32)	> 5%
Error	357	280.03	0.78			
Total	551	762.07				

sidering as sources of variation (a) subjects, (b) positions I to VI, (c) radial vs. ulnar aspect, and (d) right vs. left extremity. The error term in the analysis of variance was considered a measure of the accuracy of the technique.

Results: The mean wheal diameters at the 24 sites are shown in Fig. 2. A summary of the analysis of variance of the data is shown in Table I.

Fig. 2 and Table I taken together indicate that:

1. Individuals differ significantly in their reactivity to histamine, as would be expected.
2. There is a statistically significant difference among the six sites in each row, those sites at the cubital region being most reactive to histamine, and those at the wrist least reactive.
3. Sites in the ulnar rows are significantly more reactive than corresponding sites in radial rows.
4. There is no significant difference between corresponding sites on the left and right extremities.
5. These four sources of variation are relatively independent of one another in their effects upon the mean wheal diameter, as is shown by the insignificant value for F for the first order interactions.

The sources of variation studied here for histamine showed a pattern similar to that of corresponding sources of variation when Becker and Rappaport studied the site differences in the whealing of ragweed pollen extract.

Part II. Histamine Dose-Response.

Subjects: A group of 54 human volunteers from the out-patient population of the University of Illinois Allergy Clinic were employed in this portion of the investigation. They were chosen without regard for age, sex, race, type of allergic complaint, or specific sensitivity, with the exception that subjects under ten years of age were avoided.

TABLE II

	SUBJECT 1 RIGHT ARM	NO. 1 LEFT	NO. 2 RIGHT	NO. 2 LEFT	NO. 3 RIGHT	NO. 3 LEFT
I	D	A	C	F	E	B
II	F	E	A	B	D	C
III	C	B	F	D	A	E
IV	A	D	E	C	B	F
V	B	C	D	E	F	A
VI	E	F	B	A	G	D

Solutions: A stock solution of histamine dihydrochloride was prepared to be sterile, isotonic, neutral in reaction (pH 6.5-7.0), and in strength one part free histamine base per 100 parts of solution. Successive quartile dilutions were prepared from this stock solution by serial passage of 1 c.c. of solution into 3 c.c. of sterile isotonic saline. The six solutions so prepared had strengths as follows:

A, 0.01 (stock solution)	D, 0.000156
B, 0.0025	E, 0.000039
C, 0.000625	F, 0.0000096

Sites: Six sites were employed on each upper extremity of the subjects in the dose-response determinations. These six sites were located in a line along the mid-flexural surface of the extremity, as indicated in Fig. 3. The six dilutions of histamine were distributed among the six sites by means of a Latin square randomization, so that site differences might be eliminated. A different column of the Latin square was used for each extremity, and a new square was generated from a table of random digits for each three subjects (each six extremities). A sample Latin square is shown in Table II.

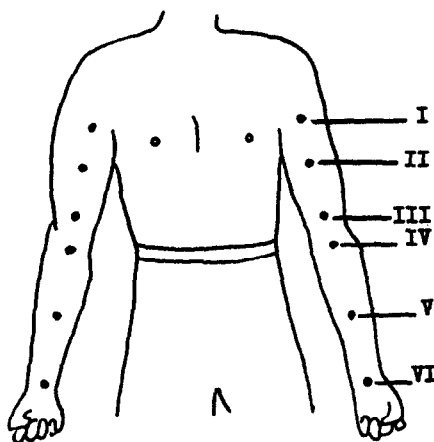


Fig. 3.—Position of injection sites for ragweed on arm and forearm.

Technique: The six dilutions of histamine were placed in six syringes fitted with No. 27 short-bevel needles. The dilutions were injected intradermally at the indicated sites, and after fifteen minutes the longest diameters of the resultant wheals were measured. The mean wheal diameter for each dilution was calculated, and the results were plotted.

Results: Fig. 4 is a semilogarithmic plot of the mean wheal diameter against the logarithm of the concentration of histamine injected. The equation for the best straight line through these points is

$$Y = 2.51 X + 18.65$$

where Y is the mean wheal diameter and X is the logarithm of the histamine concentration. The standard error of the observed points from this equation is 0.299 mm.

Fig. 5 is a log-log plot of the mean wheal diameter logarithm against the histamine concentration logarithm. The equation for the best straight line through these points is

$$Y = 0.1131 X - 1.3742$$

where both X and Y are logarithms. The standard error of the observed values from this equation is 0.255 mm. Comparing this standard error with the value of 0.299 mm. for the semilog plot, it is apparent that the fit of points to one plot is not remarkably better than to the other.

Becker and Rappaport found that their ragweed data could be fitted to a dose-response curve of this log-log type. Thus the two substances appear to behave in the same general manner under the transformation of dose response. In order to test more rigidly the similarity between the dose-response curves, the technique was repeated, measuring the response to both histamine and ragweed extract in the same subjects at the same time.

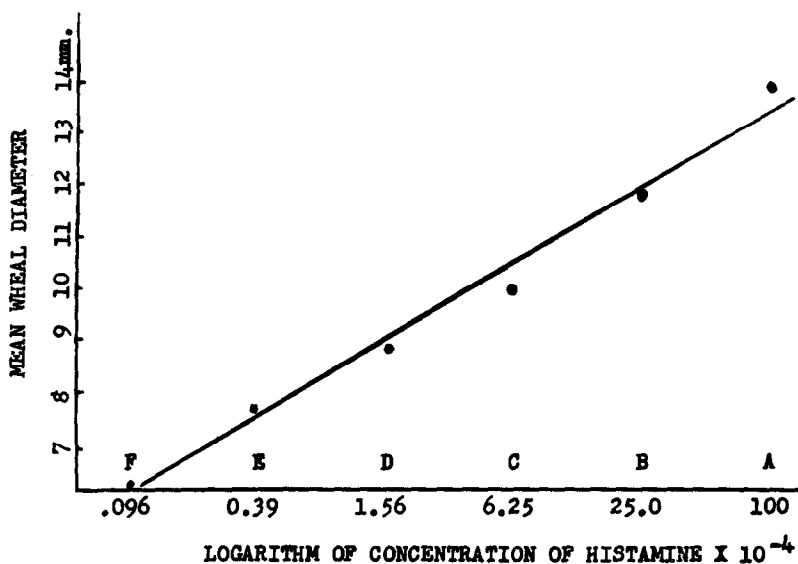


Fig. 4.—The relationship between the logarithm of histamine concentration and mean wheal diameter.

Part III. Histamine-Ragweed Dose-Response Comparison.

Subjects: A group of 24 volunteers from the University of Illinois Allergy Clinic were used in this portion of the project. Of necessity, all subjects were ragweed-sensitive.

Solutions: The histamine solutions employed were identical to those used in Part II. The ragweed solutions were prepared by quartile dilution from a stock solution of 10 per cent aqueous ragweed pollen extract which contained 1.5 mg. of nitrogen per c.c. The resultant solutions were sterile, isotonic, neutral in reaction, and had strengths as follows:

Stock, 0.10 (not injected)	C, 0.00039
O, 0.025 (not injected)	D, 0.000096
A, 0.00625	E, 0.000024
B, 0.00156	F, 0.000006

Sites: The sites used were the same as in Part II.

Technique: Each subject received injections of ragweed extract in one extremity and injections of histamine in the other. The solutions were alternated from right to left and left to right extremities on consecutive subjects. A Latin square was used to randomize the injection sites. The same pattern was used for both histamine and ragweed injections in a given subject so that he received corresponding doses at corresponding sites on the two extremities. A new Latin square was generated for each six subjects.

The diameter of each histamine wheal was plotted against the diameter of the corresponding ragweed wheal. On the same plot, the mean diameter for each of the six histamine dilutions was plotted against the corresponding ragweed dilution mean diameter.

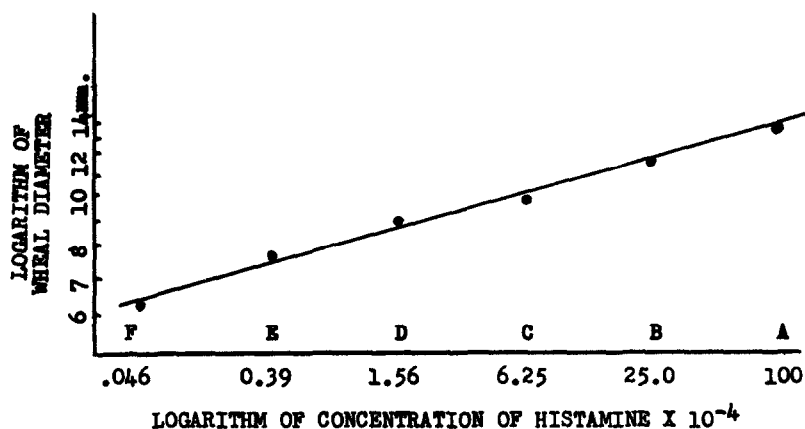


Fig. 5.—The relationship between the logarithm of histamine concentration and logarithm of wheal diameter.

Results: Fig. 6 shows the diameter of each of 144 histamine wheals plotted against the diameter of the corresponding ragweed wheal. Also shown are the mean values for the six dilutions employed. The straight line whose slope = 1 represents a theoretical axis upon which all points would fall if ragweed = histamine and the data were collected without error.

From Fig. 6 we see that:

1. Mean values for the six dilutions closely approximate the theoretical axis.
2. Individual values show considerable variation. (The coefficient of correlation, r , for the array is + 0.589.)

The lack of perfect correlation may be caused by the variability of response to the liberated histamine or may have its explanation in the liberation of some substance other than histamine which is at least in part responsible for the whealing reaction. By the statistical technique of analysis of covariance we can test the hypothesis that the variation in the histamine wheal diameters is sufficiently large to account for the variation seen in the ragweed wheal diameters. Table III is a summary of the analysis of covariance of these data.

TABLE III

SOURCE OF VARIATION	D.F.	Sh ²	Shr	Sr ²	SUM OF SQUARES	D.F.	MEAN SQUARE
Total	143	2744.85	1693.46	3007.47	1962.68	142	
Dilutions	5	1207.10	1049.53	866.48			
Error	138	1537.75	643.93	2140.99	1871.35	137	13.66
					91.33	5	18.26

$F = 18.26/13.66 = 1.34$
 (5% F = 2.88)
 P > 5%

The small value for F indicates that there is no significant difference between the behavior of the two substances. The answer to the statistical question is, "Yes, the variation in the histamine data is sufficient to account for the variation encountered in the ragweed data." Within the limits of the techniques employed, there is no apparent need to postulate the existence of wheal-mediating substances other than histamine.

DISCUSSION

The results obtained in this study must be viewed in the light of the several serious limitations inherent in the quantitative skin testing procedures employed. First is the large amount of variation encountered in the data. To a considerable extent this variation arises from the mechanical difficulty of injecting a small constant amount of solution to a constant depth into the skin.

A second limitation is the flatness of the dose-response curve. As the concentration of whealing agent is increased 1,000 times, the wheal diameter increases by a factor of two. This means that wheal diameter is not a particularly sensitive tool for measuring amounts of apparent histamine.

The third limitation of the method is the narrow range over which wheal diameters can be studied accurately. Wheals less than 4 mm. in diameter are difficult to measure precisely. Wheals larger than 12 or 15 mm. in diameter are prone to develop pseudopods, and this introduces an additional source of error. Furthermore, with higher concentrations of a specific antigen there is an increasing chance of inducing a constitutional reaction in the subject. It is altogether possible that certain differences, now hidden, would become apparent if the range of study could be extended. As Clark¹³ has pointed out, curves of radically different types may be indistinguishable if viewed over narrow ranges only.

The limitations imposed by the narrow range of study and the flatness of the dose-response curve make it especially important that the variation caused by mechanical factors such as injection technique be reduced to an absolute minimum. This was attempted by the use of statistical analysis on samples containing a large number of individuals.

These statistical techniques relate the magnitude of observed differences to the magnitude of the standard error of the method. With a smaller standard error, finer differences can be detected. Since standard error is an inverse function of the square root of the number of items in the sample, standard error can be made smaller by employing larger samples. But to thus increase the accuracy

of the present investigation by any significant amount would require prohibitively large samples. For example, to increase by a factor of ten the accuracy of Part I of this study would require a sample of 55,200 skin wheals.

Within the limits of the techniques employed, the site response to ragweed pollen extract in ragweed-sensitive subjects and the site response to histamine were indistinguishable. The same differences in site reactivity which Becker and Rappaport¹⁰ showed in their ragweed studies were found in this study of histamine. Not only is there a qualitative similarity, but to a certain extent there is a quantitative parallelism as well. Becker and Rappaport found that the decreased reactivity of the radial aspect as compared with the ulnar aspect was equivalent to a 50 per cent decrease in the strength of the solution employed. In this histamine study, the corresponding decrease in reactivity was equivalent to a 43 per cent weaker solution.

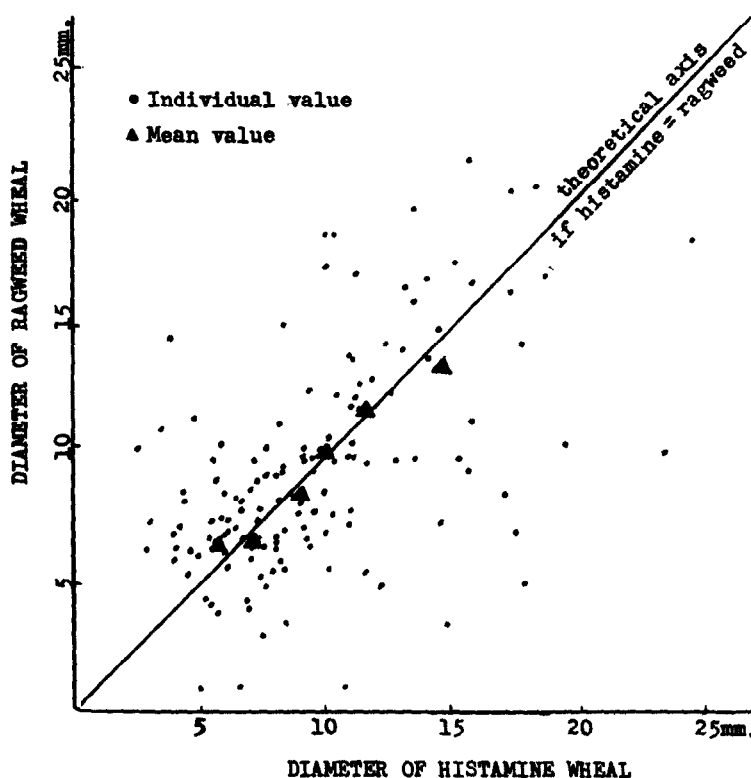


Fig. 6.—The relationship between histamine and ragweed response.

In the ragweed study Site VI was less reactive than Site IV by an amount equivalent to a 55 per cent decrease in solution strength. In this study the site reactivity decrease from IV to VI was equivalent to a 60 per cent weaker solution.

Thus we conclude that since the same site differences are found to histamine as to ragweed injection that the antigen-antibody portion of the wheal mechanism is not implicated in the site difference but rather that it

must be inherent in the differences of the skin itself. We believe that there is a difference in the whealing activity of the skin at various sites to rather constant amounts of released histamine.

Our conclusions are at variance with the findings of Bowman⁹ and more recently Wiseman.¹¹ Wiseman stated, "The author was able to confirm the findings of Bowman who showed that responsiveness to histamine increased as tests were introduced progressively down the arm, whereas responsiveness to atopens diminished under the same circumstances."

We must point out that in studies involving small numbers of individuals, apparent differences must be very large to be significant. We can, of course, say nothing concerning Wiseman's conclusions as to the relative hyperactivity of the crease area since that area was not tested in our work.

In Part III of this study, the dose-response curves of histamine and ragweed extract were compared. The mean difference between histamine and ragweed wheal diameters is of little interest because the experiment was so designed that this mean difference was as close to zero as possible. Of greater interest is the question of whether there is in the ragweed data a component of variation not present in the histamine data. If such an additional component were present, it would suggest the presence of additional whealing substances. When the technique of analysis of co-variance was used to answer this question, no such component was found.

Thus the findings of this study lend support to the idea that histamine is the mediating substance in the cutaneous whealing caused by a specific antigen in appropriately sensitive subjects. From these findings there is no evidence that there are other pharmacologically-active substances mediating the reaction. But the error of the method is sufficiently large that the presence of other active agents cannot be definitely excluded. All that can be said of them is that if they are present, their effect is small with respect to the error of the measuring procedure.

SUMMARY

1. Variations in the site response to constant doses of intradermally injected histamine were observed. These differences closely paralleled those seen previously in the site response of ragweed-sensitive subjects to intradermally injected ragweed pollen extract.
2. It is concluded that the antigen-antibody portion of the wheal mechanism is not implicated in the site differences, but rather it must be inherent in the differences of the skin itself.
3. Within the range studied, the dose response of histamine approaches linearity on both logarithmic and semi-logarithmic plots.
4. The variation seen in the histamine dose-response data is sufficient to account for the variation encountered in the ragweed dose-response data.
5. Within the limits of the techniques employed, there is no evidence that there are other active substances besides histamine mediating the cutaneous whealing reaction induced by a specific antigen.

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