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HISTAMINE WEAL FORMATION AND ABSORPTION IN MAN

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1 The weal and flare response to intradermal histamine was measured over a range of doses in forearm skin of man.

2 The use of calipers to measure weal thickness was validated by measurement of observer and method errors. Repeated measurements at intervals of 5 min or more compressed the weals by 6% per reading.

3 Flare response reached a maximum at 5 min compared with 20 min for weal thickness and the sensitivity of the flare response was greater than the weal response at lower doses of histamine.

4 Sensitivity, λ was comparable for the measurement of flare, weal diameter and thickness or volume. The advantage of weal thickness was in the measurement of weal formation and resorption.

5 The time course of histamine weal formation and disappearance was measured and found to have a $T_{\frac{1}{2}}$ of 5.4 min and 87 min, respectively.

6 The $T_{\frac{1}{2}}$ of disappearance of comparable 0.9% w/v NaCl solution and serum weals were 18 and 28 min respectively.

7 It is suggested that persistence of histamine weals is due to a vasoactive agent other than histamine.

Introduction

Measurement of the response to intradermal histamine in human skin has been limited to measurement of maximal area of flare and weal. Whilst the extent of a flare can be expressed as area, wealing is a volume response and the additional measurement of weal thickness, from which volume can be calculated, should therefore provide more information. Furthermore, since weal area can only be measured during the short period when the edges are sharp this measurement does not permit the study of rate and duration of the response. We now present a method for measuring weal formation and absorption in man and new data on the skin response to histamine using both this and previous methods.

Methods

Subjects

Nineteen informed healthy volunteers, 15 males and 4 females aged 19 to 56 years, took part in the study. Doses of histamine acid phosphate of 0.1, 1.0, 10, 30 and 90 μg (as histamine base) in 0.1 ml of 0.9% w/v NaCl solution (saline) were injected intradermally into the flexor aspect of the forearm with a 1 ml disposable tuberculin syringe and no. 16 gauge needle. Six subjects had injections with all doses, 13 subjects had injections of 10, 30 and 90 μg only.

Flare

The maximum diameter at right angles to the longitudinal axis of the forearm was measured 5 min after injection. It was not possible to measure the longitudinal diameter as adjacent flares merged in this axis.

Weal

Maximum diameters were measured in the longitudinal axis of the forearm and the diameter at right angles to this at 10 min, ignoring all pseudopodia. Area was calculated from the mean diameter, assuming a circular shape.

Weal thickness was measured with Harpenden calipers at 10, 15, 20, 25, 35, 45, 55, 65, 75 and 90 min in 13 subjects and at 20, 60, 120 and 180 min in 6 subjects, skin thickness also having been measured at the same sites before injection of histamine. One spring was removed from the calipers to reduce tissue compression. To minimize bias, the scale was directed away from the observer and was read only when the

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calipers were in position. Since a fold of skin is measured, the increased thickness due to the weal oedema is half the difference between readings before and after histamine. Weal volume was calculated from the weal area and thickness measured at 20 min.

Saline and serum

In 4 normal subjects the disappearance rate of saline and the subjects' own fresh serum were measured by the same methods immediately and at intervals after intradermal injection of 0.1, 0.3 and 0.5 ml volumes into the flexor aspect of the forearm. These volumes were chosen to give a dermal oedema equivalent to that produced by the histamine weals.

Validation of method

Studies were undertaken to determine observer error and reproducibility. Two observers independently took 6 caliper readings at each of 25 normal uninjected forearm skin sites. The means of the two independent series of measurements of skin thickness was 2.5 ± 0.1 mm (s.e. mean) and 2.6 ± 0.1 mm which were not statistically different. The reproducibility of the reading for each of six observations at the same site was 1.1% s.e. mean for the two observers.

The error from compression of the weal by the calipers was calculated from the decrease in weal thickness produced by their immediate reapplication. In the first experiment histamine acid phosphate in 0.1, 1.0 and 10 μg doses was injected intradermally into the skin of one forearm in five subjects and in both forearms in four subjects and three consecutive caliper readings were taken at 20 min after each injection. The results of this study showed that although the absolute magnitude of the compression of the calipers was to some extent related to the initial volume of weal oedema, and hence dose of histamine, the percentage change was constant for each single reading.

That the magnitude of weal compression was also constant with respect to time of measurement was shown as follows: four weals were induced in four subjects with 1.0 μg histamine and each weal was measured three times in rapid succession at each of 10 min intervals for 60 min. The change in weal size from the first to the second and second to third readings at each 10 min interval was calculated and found to be constant and the overall mean compression for a single reading was 11.4 ± 2.7% regardless of weal size, dose and time of measurement.

Fluid could be seen to reaccumulate spontaneously in these weals. The extent and time course of this return of fluid displaced by the calipers was studied next since if it was little and prolonged, it would reduce the number of observations which could be made of wealing at any one site. To distinguish between weal resorption with time and weal compression by the calipers, changes in weal thickness were measured in five subjects in weals measured at 5, 10, 20 min intervals for 60 min and compared with weals measured only at 60 min. It was found that mean weal compression for measurements taken at intervals of 5 min or more was 5.9 ± 1.5% per reading. Since the decrease in weal size following three readings taken immediately after one another is 11.4 ± 2.7%, rapid restoration of weal oedema must have occurred so that about half is restored within a period of 5 min. Thus the error from repeated readings at 5 to 10 min intervals is not great.

Rate constants

Rate constants were calculated from the equation $y_t = Ae^{K_f t} - Be^{-K_d t}$ (Riggs, 1972) where y_t = value of y at any time t; A = intercept for formation exponential; B = intercept for disappearance exponential; e = base natural logarithm; K_f = rate constant of formation and K_d = rate constant of disappearance.

Results

The response to intradermal histamine is shown in Figures 1 to 3.

Flare

Flare diameter was maximal at 5 min; the response was steepest between 0.1 and 1.0 μg of histamine (Figure 1) and flattened off from 1.0 to 90 μg although it was still approximately linear at the higher dose range; r was 0.72 for the whole of the dose range used, and λ was 0.82. The square of the flare diameter (d^2) was more linear over the whole dose range ($r = 0.60$, $\lambda = 2.6$)

Weal

Weal area The response was approximately linear both above and below 10 μg of histamine, the slope being greater above it. r was 0.73 for the whole of the dose range and λ was 0.79 (Figure 2).

Weal thickness Like area, weal thickness showed a greater incremental response at the higher doses. The sensitivity of the responses was comparable to flare ($\lambda = 0.85$) and the overall r value was 0.72 (Figure 2).

Weal volume The volume response was steepest with doses of 10 μg histamine and above, although as with area, the dose response was approximately linear both above and below this dose ($r = 0.72$) (Figure 2). The sensitivity of the volume response was of the same order as other measurements of weal size, λ being 0.82

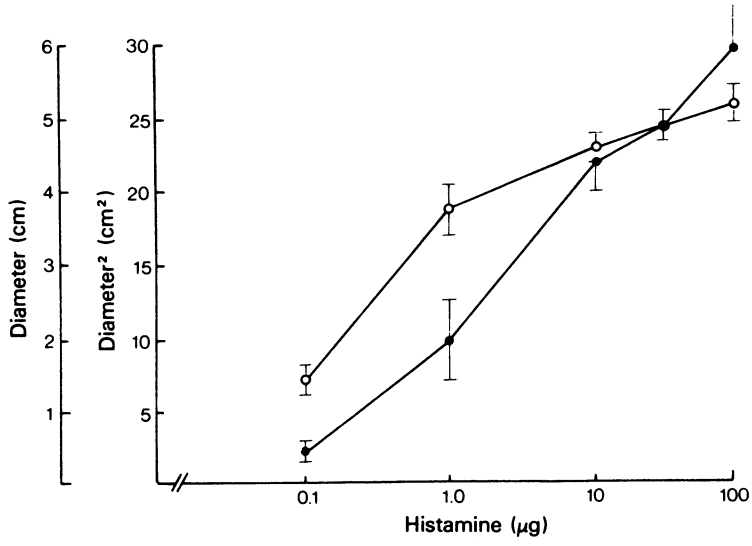

Figure 1 Flare response to intradermal histamine: diameter (○) and diameter^2 (●). Mean values are shown; vertical lines indicate s.e. mean. $n = 6$ at histamine dose 0.1 μg and 1.0 μg; $n = 19$ at histamine dose 10, 30 and 90 μg. For diameter/log dose histamine, $r = 0.72$ $\lambda = 0.82$. For diameter^2/log dose histamine, $r = 0.60$ and $\lambda = 2.6$.

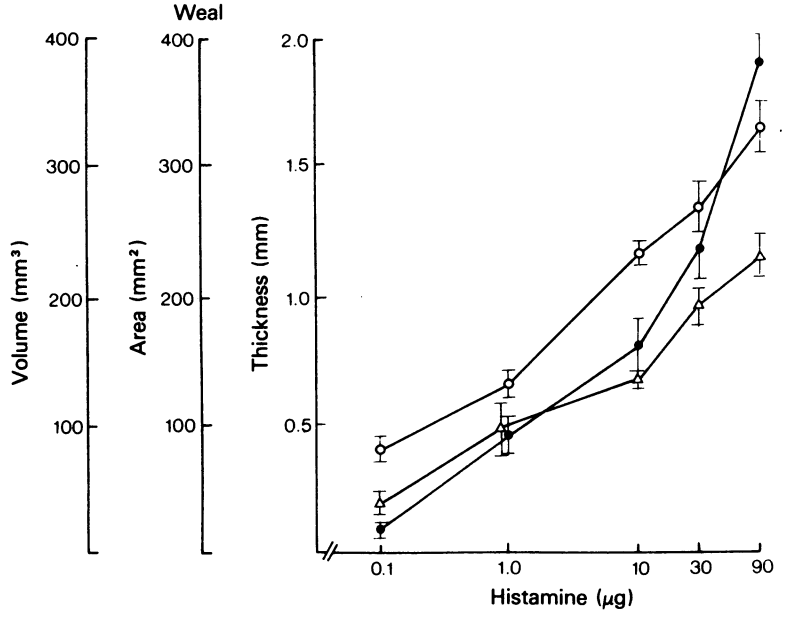

Figure 2 Dose-response of weal area (△), weal thickness (○), and volume (●). Values with s.e. mean (vertical lines) are shown. At doses 0.1 and 1.0 μg histamine base, $n = 6$; at doses 10, 30 and 90 μg histamine base, $n = 19$. For weal area, $r = 0.73$, $\lambda = 0.79$. For weal thickness, $r = 0.72$, $\lambda = 0.85$. For weal volume, $r = 0.72$, $\lambda = 0.82$. The responses of area and thickness are similar but the volume response is steeper, particularly at 10, 30 and 90 μg.

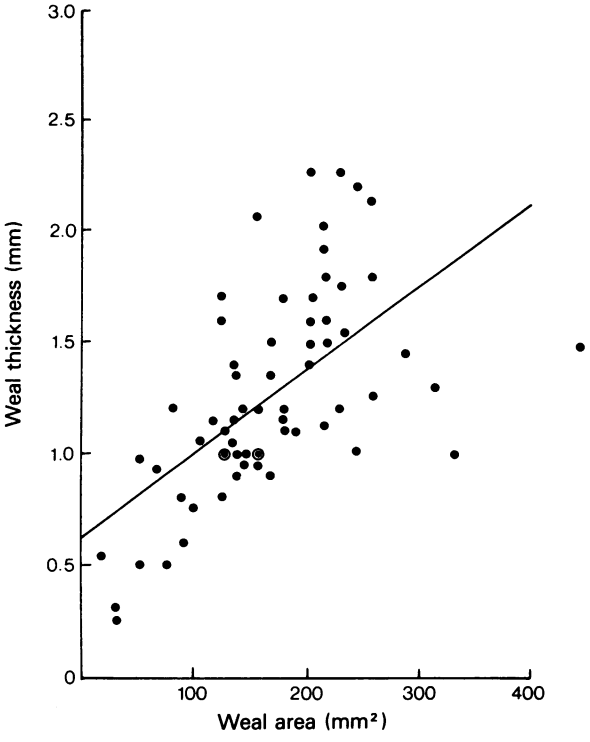

Figure 3 The correlation of weal area and thickness at all doses of histamine; $n = 66$ and $r = 0.57$.

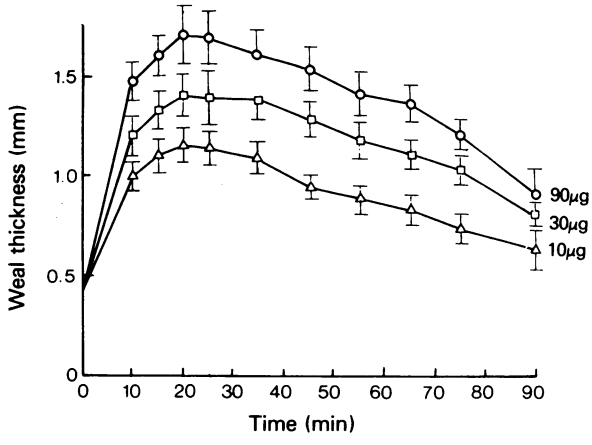

Figure 4 Time course of weal oedema. Weal thickness measured at 5 min intervals from 10 to 25 min, at 10 min intervals from 25 to 75 min and at 90 min. Histamine base: 10 μg (△); 30 μg (□); 90 μg (○). Mean values with s.e. mean (vertical lines) are shown; $n = 13$. The initial thickness was not measured but taken as the mean volume of intradermal injections of 0.05 and 0.9% w/v NaCl solutions established in separate experiments.

compared with $\lambda = 0.85$ for thickness and $\lambda = 0.79$ for area. Although there was an overall correlation between weal thickness and area (Figure 3, $r = 0.57$) this was less good than the dose-response correlation for either area ($r = 0.73$) or thickness ($r = 0.72$). This was due to a subsidiary trend to an inverse correlation between weal area and thickness in some individuals.

Time course of weal formation and resorption The time course of weal formation and resorption is shown in Figures 4 and 5. The magnitude of the weal responses are dose-related; they reach a peak by 15 to 20 min for all doses, after which they decrease in an approximately log-linear fashion over 90 min. The formation and disappearance of the weals calculated from this data are shown in Figure 5, and the corresponding rate constants for weal formation and absorption together with the $T_{\frac{1}{2}}$ calculated from them are given in Table 1. The rate constant of formation and resorption of weals was unrelated to the dose of histamine, although K_d was least at the lowest dose of histamine.

In a smaller series of experiments in six subjects weal disappearance was measured at 20 min intervals for 20 to 180 min over which time weal thickness decreased at a rate comparable to that found in the study of the first 90 min. The slightly slower fall off ($K = -6 \times 10^{-3}$ min^{-1}) was presumably because fewer readings were taken

Resorption of saline and serum To study the extent to which absorption of the histamine weals was due to their water and protein content, the disappearance of weals raised by intradermal injection of saline and serum was measured over a period of 60 min (Figure 6). Disappearance was logarithmic and the rate constants and $T_{\frac{1}{2}}$ of resorption are shown in Table 1. At all volumes of fluid used, saline weals were absorbed faster than serum weals which in turn were absorbed more quickly than histamine weals.

Discussion

The present findings show that whilst conventional methods of measuring weal and flare are adequate over a wide range of doses, the measurement of weal oedema as thickness allows calculation of weal volume and measurement of the time course of weal formation and resorption. Measurement of weal thickness by calipers is rapid and observer error and variance are not great. The main disadvantage of the method is compression of the weal by the calipers. Although this causes a mean shift in weal oedema

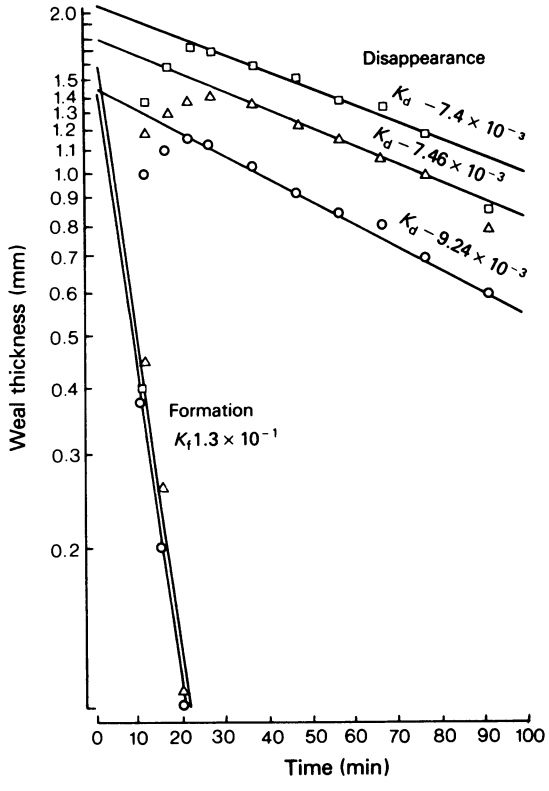

Figure 5 Log plots of weal disappearance and formation rates. Weal disappearance is plotted as the log of the data in Figure 4 and weal formation is calculated during the first 20 min as the difference between log weal thickness and the regression of the log disappearance curve. Symbols as in Figure 4.

fluid of 11.4% per reading the displaced fluid had returned to some extent in the 5 to 10 min left between each reading, giving a mean loss of weal thickness of 5.8% per reading.

A comparison of the various measurements showed that λ for the dose-response for flare diameter and diameter^2, weal area, thickness and volume were comparable for the group as a whole. However, inspection of individual responses showed that weal volume compensates for individual variations in spread of oedema fluid which is a function of the integrity of the dermis. Thus age and certain hormonal changes in the dermis produce larger and flatter histamine weals (Shuster & Scarborough, 1961) and a greater spread of injected fluid. Weals may therefore maintain the same volume by greater thickness and lesser area and *vice versa.*

Measurement of weal area is likewise limited by its spread and can only be measured when the edge is still sharp, usually at 8 to 12 min, which is well before the 20 min when weal oedema is maximal. The present studies also show that flare and weal response are both qualitatively and quantitatively different, the maximal flare response occurring earlier than maximal weal response and the steepest part of the dose-response curve occurring with lower doses of histamine.

The present method has for the first time allowed the measurement of the rates of formation and resorption of histamine weals in human skin and calculation of their rate constants, although compression by the calipers will reduce the accuracy of these calculations. The $T_{\frac{1}{2}}$ for formation is 5.4 min and independent of histamine dose but $T_{\frac{1}{2}}$ for resorption is 75 min for a dose of 10 μg and 93 and 94 min for doses of 30 and 60 μg. Using pontamine blue, Miles & Miles (1952) showed that the effect of histamine on the skin vessels in the guinea-pig was over by 10 to 15 min which compared well with the $T_{\frac{1}{2}}$ we obtained for weal oedema in man. There were no comparable figures for resorption because the dye is fixed in the skin. The studies with saline and serum weals indicate that the slow disappearance of the histamine weals is not a function of their water or protein content since saline weals are absorbed ten times as quickly as serum weals and five times as quickly as histamine weals. Nor is it likely to be due to impaired lymphatic absorption since this is increased by histamine (Lewis & Winsey, 1970).

Persistence of histamine oedema must therefore be due to its continued formation. Since we found weal formation to be complete by 20 min with $T_{\frac{1}{2}}$ of 5.4 min, the vasoactive agent producing this continued vascular permeability cannot be the initial dose of histamine itself. It therefore seems likely that histamine may itself induce formation or liberation of vasoactive materials in the skin. This secondarily released material is unlikely to be histamine since it is known to render the skin refractory to wealing from a second dose (Greaves & Shuster, 1967) and because we have found the disappearance of histamine weals not to be affected by H_1- or H_2-receptor blockers singly or in combination (Cook & Shuster, unpublished observations) in a dose which decreases their formation. We therefore conclude that histamine may induce formation or liberation of other vasoactive materials in the skin. This secondary release of vasoactive materials may explain the limited clinical effect of H_1- and H_2-receptor blockers on histamine wealing even when these are injected directly into the skin in large doses (Cook & Shuster, unpublished observations). To test this hypothesis direct examination of histamine weal oedema for vasoactive agents and a study of the effect of drugs which block other vasoactive agents on the rate of histamine weal resorption

Table 1 The rate constants of disappearance K_d and $T_{\frac{1}{2}}$ are given for different volumes of saline (NaCl) and serum, and for 10, 30 and 90 μg of histamine base in 0.1 ml saline, showing that the disappearance of the histamine weal is much slower than that of saline and serum. $T_{\frac{1}{2}}$ for formation of histamine weals is 5.4 min.

	NaCl (ml)			*Serum (ml)*			*Histamine (μg)*		
	0.1	0.3	0.5	0.1	0.3	0.5	10	30	50
K_d (min^{-1})	-3.8×10^{-2}	-3.74×10^{-2}	-4.15×10^{-2}	-2.35×10^{-2}	-2.82×10^{-2}	-2.44×10^{-2}	-9.24×10^{-3}	-7.46×10^{-3}	-7.4×10^{-3}
$T_{\frac{1}{2}}$ (min)	18	18.5	17	29.5	26	28	75	93	94

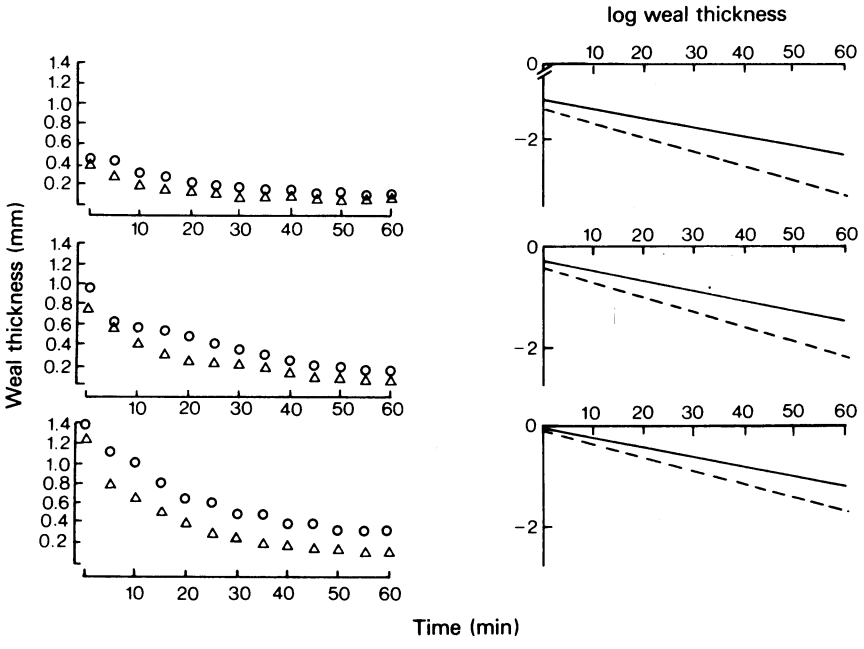

Figure 6 Decrease in serum (○) and saline (△) weals using 0.1, 0.3 and 0.5 ml volumes. The volume of fluid does not significantly influence the rate of absorption but protein content does.

will be required. The development of drugs assayed by the absorption of the histamine weal as well as its formation may improve the treatment of diseases such as urticaria.

References

GREAVES, M.W. & SHUSTER, S. (1967). Responses of skin blood vessels to bradykinin, histamine and 5-hydroxytryptamine. *J. Physiol.*, **193**, 255–267.

LEWIS, G.P. & WINSEY, W.N.P. (1970). The action of pharmacologically active substances on the flow and composition of cat hind limb lymph. *Br. J. Pharmac.*, **40**, 447–460.

MILES, A.A. & MILES, E.M. (1952) Vascular reactions to histamine, histamine-liberator and leukotaxine in the skin of guinea-pigs. *J. Physiol.*, **118**, 228–257.

RIGGS, D.S. (1972). The mathematical approach to physiological problems: a critical primer. Cambridge, Mass: The MIT Press.

SHUSTER, S. & SCARBOROUGH, H. (1961). Senile purpura. *Q. J. Med.*, New Series xxx, **117**, 33–40.

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