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EFFECT OF TEMPERATURE ON THE ANAPHYLACTIC REACTION

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The effect of temperature on the antigen-antibody reaction *in vitro* has been investigated by Mayer & Heidelberger (1942), but no corresponding measurements seem to be available for the anaphylactic reaction. One of us (Schild, 1939) has observed that histamine release in anaphylaxis is greatly reduced at room temperature. In the present experiments this phenomenon was further investigated, and it was found that the anaphylactic histamine release is inhibited by both high and low temperatures. Temperatures which are only slightly above body temperature and which do not affect the viability of cells were found to produce a persistent inhibition of anaphylaxis, which is due to inactivation not of antibody but of some tissue constituent required for the anaphylactic reaction. The effect of temperature on the action of histamine liberators has also been studied. Some of these experiments have already been described (Mongar & Schild, 1955).

METHODS

Histamine release was measured as described in the preceding paper (Mongar & Schild, 1957).

The temperature was controlled by keeping a stoppered 10 ml. beaker containing 1 ml. Tyrode solution and 0·2 ml. tissue in a water-bath which was thermostatically controlled to within 0·1° C.

In order to study the effect of pH on heat inactivation, 0·1 ml. samples of lung tissue were immersed in 5 ml. Tyrode solution, buffered by adding 1 ml. of isotonic sodium phosphate solution of varying pH: the resultant pH was determined with a glass electrode. These samples were heated to 42·5° C for 25 min, neutralized with 0·2 N–NaOH or HCl and the antigen was thus added at 37° C.

In some experiments complement was measured by its effect in producing haemolysis of sheep erythrocytes, as follows. The time was determined for lysis after adding varying amounts of complement-containing guinea-pig serum to the suspension of sensitized red cells. A standard curve was established by plotting time of lysis against concentration of complement. The concentration of complement in heated serum was determined by reference to this.

RESULTS

Effect of temperature on histamine release in anaphylaxis

The histamine-releasing mechanism in anaphylaxis is highly temperature-dependent. A typical curve illustrating the effect of temperature on histamine release from guinea-pig lung is shown in Fig. 1*a*. This has a maximum at body temperature and declines below 35° and above 41° C. It falls off particularly sharply at higher temperatures. Release is abolished below 20° and above 45°.

The curve of spontaneous histamine release in relation to temperature is shown for purposes of comparison (Fig. 1*b*). No appreciable spontaneous histamine release occurs until a temperature of 55° C. is reached. It is clear that the effects of temperature on histamine release in anaphylaxis

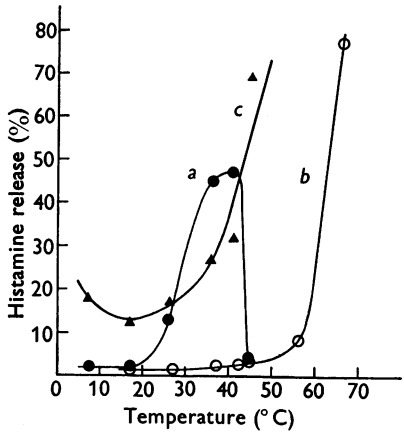


Fig. 1

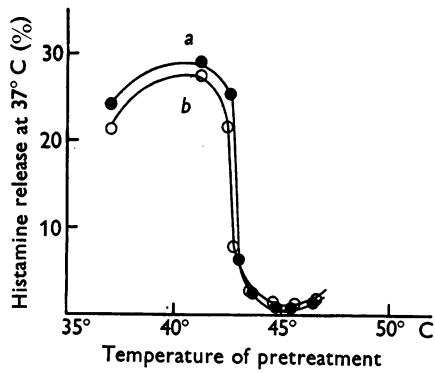


Fig. 2

Fig. 1. Effect of temperature on histamine release. *a*, Tyrode solution + antigen (10^{-3} egg albumin: mean of two experiments); *b*, control (Tyrode alone); *c*, Tyrode + octylamine (2×10^{-4}: mean of two experiments).

Fig. 2. Effect of previous heating on histamine release at 37° C. by antigen from guinea-pig lung. *a*, antigen added immediately after heating; *b*, antigen added 90 min after heating.

TABLE 1. Histamine release by antigen (1 mg/ml.) at various temperatures. The release in two successive 12 min periods has been expressed as a percentage of the total histamine in the tissue

Temp. (° C)	1st release (%)			2nd release (%)			Total release (%)
	Expt. 1	Expt. 2	Mean	Expt. 1	Expt. 2	Mean	
7	0·7	—	1	1·1	—	1	2
17	0·7	—	1	0·7	—	1	2
26	10·8	5·8	8	7·2	2·7	5	13
35½	35	35	35	—	10	10	45
40½	41	34	38	9·8	8	9	47
44½	1·7	2·4	2	1·4	2·2	2	4

are in no way correlated with the effects of temperature on the stability of intracellular histamine.

Table 1 shows the effect of temperature during two successive release periods of 12 min each. In each case histamine release during the second period was less than during the first period. Low and high temperatures thus do not merely cause a delay in histamine release but also produce a decrease of the total quantity released.

Effect of temperature on histamine release by octylamine

The effect of temperature on histamine release by octylamine from guinea-pig lung is shown in Fig. 1*c*. The octylamine curve differs in several respects from the curve for histamine release in anaphylaxis. The main difference is seen above 37° C. when the octylamine curve has a sharp upward trend and the anaphylactic curve an equally sharp downward trend. In this range the octylamine curve parallels the curve for spontaneous release, though at a lower temperature. Below 37° C histamine release by octylamine declines, but not as much as histamine release in anaphylaxis. A further difference is that the releasing activity of octylamine does not cease even at low temperatures.

Heat inactivation of the histamine release mechanism in anaphylaxis

In contrast to the chemical agents studied in the preceding paper, heating produces a persistent inactivation of the anaphylactic mechanism. This is illustrated by two series of experiments in which the samples of sensitized lung were kept for 25 min at temperatures of 41–47° C. The antigen was added at 37° C, either immediately after the heating, or 90 min later during which time the samples were stored at room temperature. The result was the same in both series, as shown by the curves in Fig. 2, which are similar in shape and position. The effects of heat on the anaphylactic mechanism thus persist unchanged for at least 90 min.

The most critical temperature range for heat inactivation of the anaphylactic mechanism is between 42·5 and 43·5° C. In this temperature interval, histamine release was diminished by 90%. No evidence of inactivation was obtained below 42° C: on the contrary there was some evidence of a persisting activation of the anaphylactic mechanism, since in both series of experiments more histamine was released when the lung was kept at 41° C for 25 min and then brought to 37° C than when it was kept at 37° C all the time.

We have carried out a series of more prolonged experiments to study whether heat inactivation is irreversible. Samples of lung tissue were kept for 30 min at 43 or 45° C, then placed in Tyrode solution at 37° C containing 10^{-5} aureomycin, and kept for periods up to 12 hr, when the solution was replaced by one containing antigen (10^{-3} egg albumin) and histamine release was measured. Fig. 3 gives the histamine release expressed as a fraction of histamine release

from controls which were kept at 37° C throughout. At 43° C heat inactivation was incomplete to start with, and the anaphylactic mechanism gradually recovered in about 6 hr. At 45° C inactivation was complete, there was no recovery in 6 hr and only slight recovery of doubtful significance after 12 hr.

Rate of heat inactivation. Varying degrees of inactivation of the anaphylactic mechanism were produced by heating the samples to temperatures of 42·5, 43, 43·5 and 44° C for periods ranging from 5 to 90 min, as shown in Fig. 4. The experiments were carried out on samples from one sensitized lung. After the heating the samples were brought back to 37° C before the antigen was added. The reduction of histamine release after heating, expressed in terms of a control sample kept at 37° C, was taken as an index of heat inactivation of the anaphylactic mechanism.

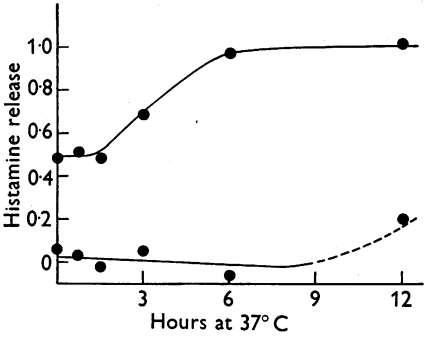


Fig. 3

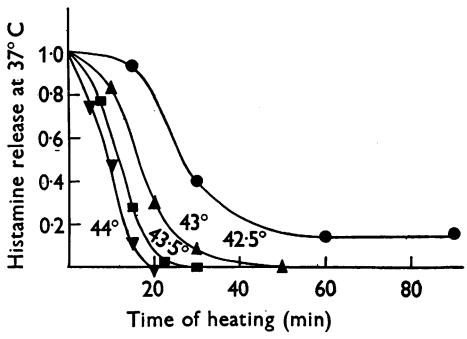


Fig. 4

Fig. 3. Recovery from heat inactivation. Sensitized lung tissue which had been heated for 30 min at 43° C (upper curve) and at 45° C (lower curve) was allowed to recover at 37° C for varying times. Histamine release by antigen was then determined and expressed as proportion of the release from controls which were kept at 37° C throughout.

Fig. 4. Rate of heat inactivation of the anaphylactic mechanism. Samples of chopped lung were heated to different temperatures for varying periods followed by 15 min treatment with antigen at 37° C. Histamine release is expressed as a proportion of the release by unheated controls.

The time of half inactivation at 42·5° C was 27 min; 85% inactivation occurred in 60 min; thereafter the curve seemed to level off with no detectable further inactivation at 90 min. The time of half inactivation at 43° C was 16 min, with complete inactivation at 60 min. Times of half inactivation at 43·5 and 44° C were 12 and 9 min with complete inactivation in 30 and 20 min respectively. In control experiments, samples which were left standing for 90 min at 37° C still released as much histamine as fresh samples.

Effect of pH *on heat inactivation.* The pH is known to affect the temperature of denaturation of proteins and it seemed possible that it would also affect heat inactivation of the anaphylactic mechanism. Samples of chopped sensitized lung were placed in Tyrode solutions of varying pH, and heated

to 42·5° C for 25 min. The samples were then transferred to solutions at pH 8 and 37° C and histamine release by antigen was measured. Control samples were treated in the same way except that they were kept at 37° C. Fig. 5 shows histamine releases from the heated samples expressed as a proportion of the controls. There was a clear effect of pH on heat inactivation; heating to 42·5° C at pH 6·4 produced a more complete inactivation of the histamine release mechanism than heating to the same temperature at a physiological pH. No appreciable effect of pH on heat inactivation was observed in the pH range 7·5–8·4.

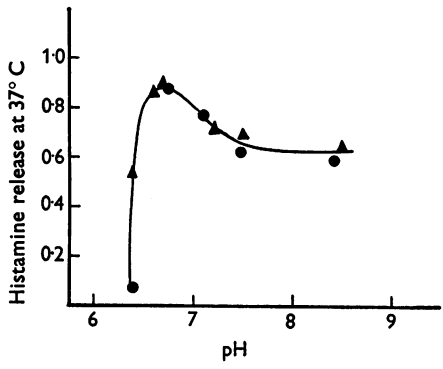


Fig. 5

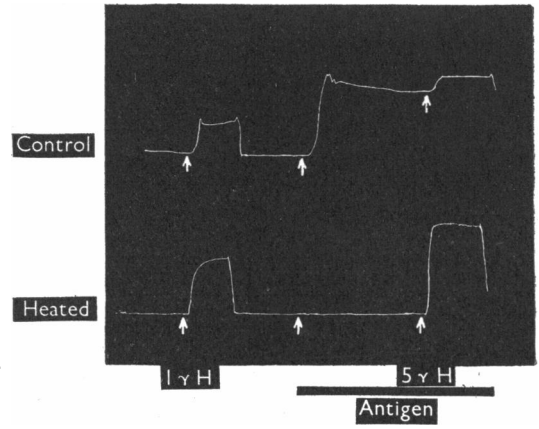


Fig. 6

Fig. 5. Effect of pH on heat inactivation. Samples of lung tissue were heated to 42·5° C for 25 min in Tyrode solution of varying pH: the antigen was then added for 15 min at 37° C and pH 8. Histamine release expressed as a proportion of histamine release by controls submitted to similar pH changes but kept throughout at 37° C; ▲, Expt. 1; ●, Expt. 2.

Fig. 6. Heat inactivation of the anaphylactic reaction in isolated guinea-pig ileum: two preparations from the same sensitized guinea-pig, both suspended in the same bath (100 ml.). Upper tracing, control; lower tracing, preparation heated 2 hr previously to 45° C for 15 min.

Effect of heating on subsequent histamine release by organic bases. Previous heating whilst inactivating the anaphylactic mechanism actually sensitizes the tissues to the action of histamine liberators as shown by the following experiments. Samples of chopped guinea-pig lung tissue were heated to 48° C for 15 min and subsequently treated with either octylamine (0·2 mg/ml.) or 48/80 (1 mg/ml.) at 37° C for 15 min. In two experiments with different guinea-pigs histamine release from the heated lung was 140 and 110% of the controls for octylamine, and 150 and 110% for 48/80. Parallel experiments with antigen gave releases of only 2 and 3% of the controls.

Effect of heat on the anaphylactic reaction of plain muscle

Heat inactivates the mechanism responsible for the anaphylactic reaction of plain muscle in the same way as it inactivates the histamine release mechanism in chopped lung. Temperatures which inactivate the anaphylactic mechanism also affect the contractile function of the cell but only as long as the preparation is kept at the higher temperature. We can thus confirm the finding of Lovatt Evans (1921) that plain muscle heated to 47–49° C loses its contractility, but rapidly recovers from heat paralysis when returned to 37° C.

Fig. 6 shows an experiment illustrating the effect of heat on the anaphylactic reaction. Two pieces of ileum from a sensitized guinea-pig were suspended in a large isolated organ bath at 38° C; one of them had been heated to 45° C, for 15 min, two hours previously. Both preparations responded to a small dose of histamine with a submaximal contraction; a subsequent dose of antigen

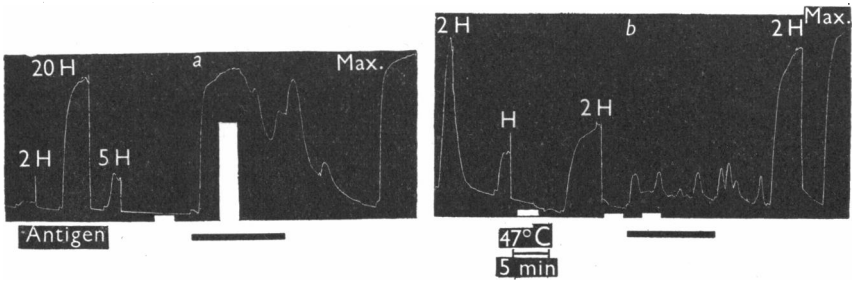


Fig. 7. Heat inactivation of the anaphylactic reaction in the isolated guinea-pig uterus: *a* and *b*, two horns of the same uterus; histamine release indicated by the height of the white columns. Presence of antigen indicated by thick black line. In preparation *a*, which was kept at 37° C throughout, a normal anaphylactic reaction was produced; in preparation *b* the bath temperature was raised to 47° C for a 5 min period 15 min before adding the antigen; although the reaction to histamine is unimpaired by the heat treatment, the anaphylactic reaction is abolished. H = 20 ng/ml.

produced a maximal contraction in the control but no trace of effect in the heated preparation. Finally when a large dose of histamine was given without washing out the antigen, it produced a maximal contraction in both preparations.

Fig. 7 shows a similar experiment involving the two horns of a sensitized uterus, one of which was heated for 5 min to 47° C. In the control preparation (Fig. 7*a*) the antigen produced a typical powerful anaphylactic reaction; in the heat-treated preparation (Fig. 7*b*) it failed to do so; there was only a slight instability of base line which persisted after the antigen was washed out. The reaction to histamine, however, was unimpaired by the previous heating.

The quantity of histamine released by the uterus into the isolated organ

bath during these procedures is indicated by the height of the white columns in Fig. 7. Whereas in the control preparation the histamine content of the bath fluid increased 20-fold following addition of the antigen, in the heat-treated preparation the histamine content did not change. Warming to 47° C by itself did not cause a release of histamine.

Rate of inactivation and recovery. Fig. 8 shows the effect on the anaphylactic contraction of sensitized guinea-pig ileum of previous heating to 43° C. At this temperature inactivation of the anaphylactic mechanism takes place slowly. After 25 min exposure to 43° C the anaphylactic reaction was still maximal. It was reduced after 40 min and almost abolished after 80 min. The preparation was returned to 37° C each time before adding the antigen.

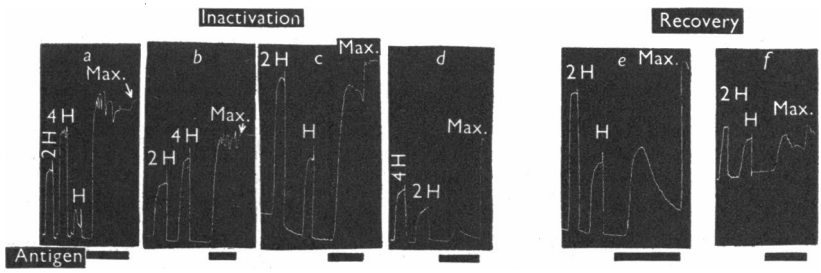


Fig. 8. Inactivation and recovery of the anaphylactic mechanism after heating sensitized guinea-pig ileum to 43° C. These experiments were performed on separate preparations (varying slightly in length) from the same animal; sections *a–d* show gradual inactivation after heating to 43° C for 0, 25, 40, and 80 min respectively; the antigen was added after returning the preparations to 37° C. Sections *e* and *f* show gradual recovery after leaving preparations at 37° C for 80 and 150 min respectively after previous heating for 80 min at 43° C. H, histamine, 5 ng/ml.

After heating for 80 min at 43° C, two of the preparations were allowed to recover at 37° C, one for 1 hr and one for $2\frac{1}{2}$ hr. After 1 hr the first preparation gave a half-maximal anaphylactic contraction; after $2\frac{1}{2}$ hr the other gave a near maximal anaphylactic contraction. In some experiments, in which sensitized guinea-pig ileum was heated to 45° C for 15 min, there was no sign of recovery in 4 hr.

The results of these experiments agree well with those obtained on histamine release from chopped lung under comparable conditions. In both series of experiments inactivation at 43° C was slow, and when heating was interrupted before inactivation was complete, the preparations recovered within a few hours. When complete inactivation was obtained by heating to 45° C there was no recovery in 4–6 hr.

Mechanism of heat inactivation

We have investigated various possible mechanisms by which heat inactivation of anaphylaxis may be brought about. The following alternatives were studied:

(1) denaturation of antibody;
(2) reversible displacement of antibody;
(3) irreversible displacement of antibody; and
(4) inactivation of some tissue constituent other than antibody.

It was shown by Dale (1913) that a sensitized uterus which has been desensitized can be resensitized by incubating it with antibody *in vitro*. It seemed that the technique of passive sensitization *in vitro* would provide a means of studying the effect of heat on both antibody and tissue.

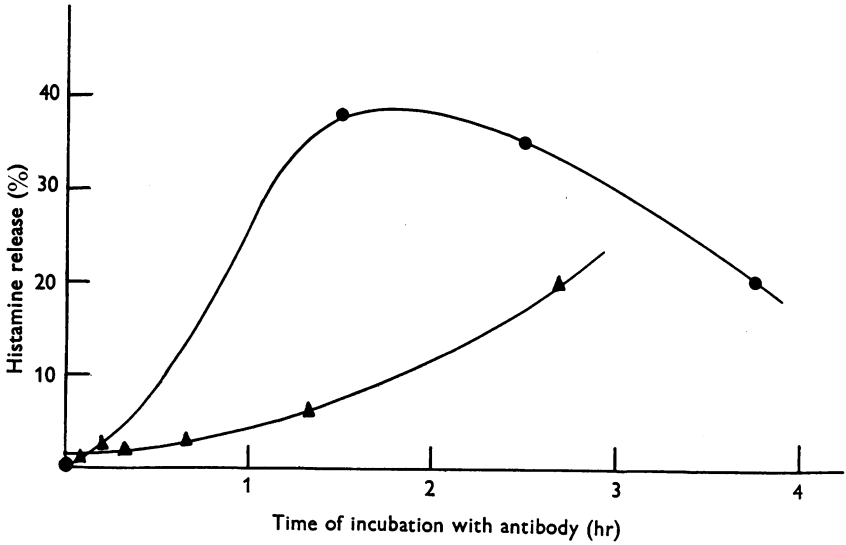


Fig. 9. Passive sensitization *in vitro*. Samples of chopped guinea-pig lung were incubated at 39° C with rabbit anti-ovalbumin serum for varying times and their histamine release with antigen measured: results obtained with two different batches of serum are shown.

Passive sensitization of chopped lung in vitro. As a preliminary we have investigated whether chopped lung could be sensitized *in vitro*; histamine release by antigen provided a measure of sensitization. A series of suspensions of lung particles were placed in Tyrode solution at 39° C containing 5 % rabbit anti-ovalbumin serum. The antigen (10^{-3} egg albumin) was added after various time intervals and histamine release was measured. Passive sensitization occurred rapidly, as is shown for two batches of rabbit serum in Fig. 9. In one instance strong sensitization had occurred in $1\frac{1}{2}$ hr, in the other in $2\frac{3}{4}$ hr.

The quantity of histamine released (38% and 20% of the tissue content) was comparable to that released from actively sensitized tissue.

Failure to inactivate antibody at 45° C. Rabbit anti-ovalbumin serum was heated to 45° for 15 min, and then used to sensitize guinea-pig lung *in vitro*. Its activity in producing passive sensitization was unimpaired and equal to that of unheated serum. In duplicate experiments the percentages of tissue histamine released by antigen from chopped guinea-pig lung incubated at 39° C for 2½ hr with heated and unheated serum, were as follows: heated serum, 65, 74; unheated serum, 63, 72.

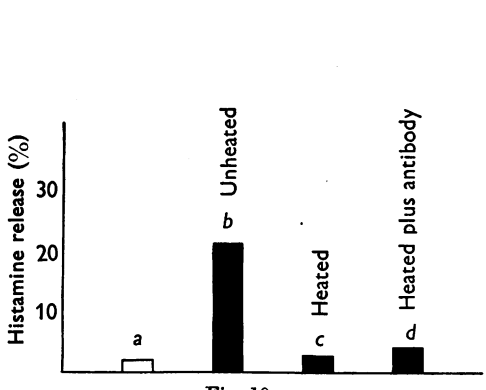


Fig. 10

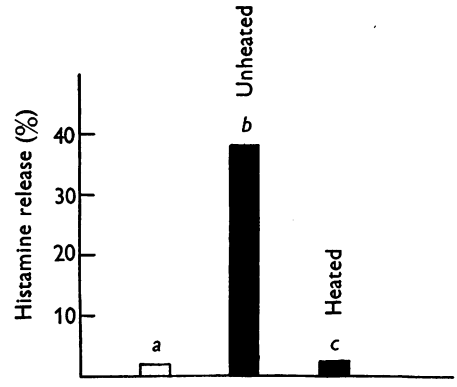


Fig. 11

Fig. 10. Failure to resensitize heated guinea-pig lung tissue. Actively sensitized preparation: histamine release by *a*, Tyrode solution, □; *b*, antigen, ■ (10^{-3} egg albumin for 15 min at 37° C); *c*, antigen after heating tissue to 45° C for 15 min; *d*, antigen after heating and 90 min incubation with antibody.

Fig. 11. Failure to sensitize heated guinea-pig lung tissue. Histamine release from samples incubated for 90 min at 37° C in: *a*, Tyrode solution, □, *b* and *c*, Tyrode solution containing 5% rabbit anti-ovalbumin serum, ■; sample *c* was heated to 45° C for 15 min before incubation with antibody.

Failure to resensitize heated lung. Although antibody is not denatured at 45° C it seemed possible that this temperature would displace antibody from its cellular attachments. If this were the mechanism of heat inactivation of anaphylaxis it should be possible to resensitize passively a sensitized tissue which had been heated. However, this proved impossible, as is shown by the following experiments which were carried out on samples of chopped lung from an actively sensitized animal. One sample was treated with antigen and gave a good histamine release (Fig. 10*b*). A second sample was heated to 45° C for 15 min and then treated with antigen; this gave no significant histamine release (Fig. 10*c*). Another sample was heated to 45° C, incubated at 39° for 90 min with 5% rabbit anti-ovalbumin serum, and then treated with antigen; this gave a very small histamine release compared with normal tissue incubated with antibody (Fig. 10*d*). Thus once the histamine-release

mechanism had been inactivated by heating it could not be appreciably restored by 90 min incubation with antibody.

Failure to detect antibody displaced by heating. The preceding experiment seemed to exclude the possibility of a reversible displacement of antibody by heat, but does not exclude the possibility of an irreversible displacement of antibody. Although in this case the heated tissue could not be expected to take up fresh antibody, sufficient displaced antibody might be present in the surrounding fluid to be detectable by passive sensitization of normal tissue. The following experiment shows that this is not so. Sensitized chopped lung tissue was heated to 45° C for 15 min, in a small volume of oxygenated Tyrode solution (1 ml./g of tissue). This solution was filtered off from the lung tissue and incubated at 39° C for 2 hr with unsensitized chopped lung. When the latter was subsequently tested with antigen, it released no more histamine than lung kept in Tyrode solution. As a control, a sample of the same lung which was incubated with rabbit anti-ovalbumin serum, released 35% of its tissue content of histamine.

Failure to sensitize passively normal heated lung. The following experiments suggest that heat inactivation involves a constituent of normal tissue. Chopped lung from a non-sensitized guinea-pig was heated for 15 min at 45° C and then incubated with rabbit anti-ovalbumin serum at 39° C. After 90 min incubation the antigen was added to the solution, and histamine release measured. Fig. 11 shows the amounts of histamine released in this preparation (*c*), and in an unheated but otherwise similarly treated control preparation (*b*). Whereas the control became strongly sensitized, releasing 38% of its histamine content, the heated lung released no more histamine with antigen than lung which was kept in Tyrode solution (*a*).

The anaphylactic contraction after incubation of normal and heated guinea-pig ileum with antibody. Experiments on the anaphylactic contraction of isolated guinea-pig ileum confirmed the previous results on chopped guinea-pig lung. We found that when guinea-pig ileum is incubated with rabbit anti-ovalbumin serum in Tyrode solution at 39° C, it becomes passively sensitized in 1–3 hr. Fig. 12 shows that a preparation of ileum which was incubated with serum for 1 hr gave a typical anaphylactic response when treated with antigen.

When passive sensitization experiments were carried out on ileum which had previously been heated to 45° C there was no trace of anaphylactic response. Fig. 13 *a–c* shows results of experiments carried out on different intestinal strips from a sensitized guinea-pig. The record at *b* shows that the anaphylactic reaction was abolished by heating the sensitized intestine to 45° C for 15 min and at *c* that it was not restored by incubating the heated preparation with rabbit anti-ovalbumin serum for 2 hr. All preparations responded to histamine, showing that their contractility was undamaged by the heat treatment.

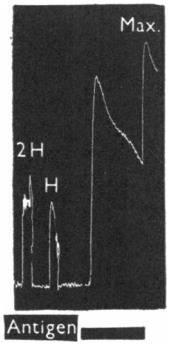


Fig. 12

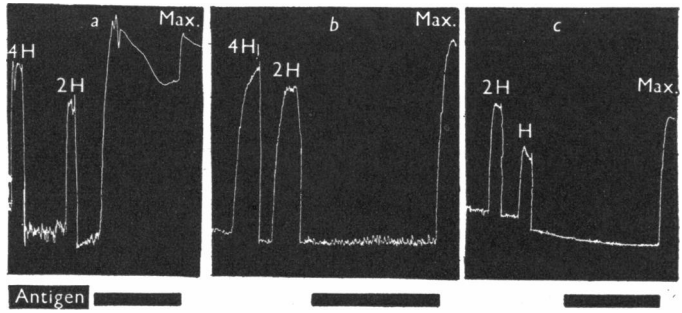


Fig. 13

Fig. 12. Passive sensitization of guinea-pig ileum *in vitro*. Guinea-pig ileum was incubated with rabbit anti-ovalbumin serum in Tyrode solution at 37° C for 1 hr and then suspended in Tyrode solution: addition of the antigen (10^{-3} egg albumin) produced a nearly maximal anaphylactic contraction.

Fig. 13. Failure to resensitize heated guinea-pig ileum. Three strips of ileum from the same actively sensitized animal: *a*, control reaction to antigen (10^{-3} egg albumin); *b*, tissue previously heated to 45° C for 15 min—no anaphylactic contraction; *c*, tissue heated to 45° C for 15 min followed by 2 hr incubation with antibody (5% rabbit anti-ovalbumin serum)—no anaphylactic contraction (no resensitization). H = histamine 10 ng/ml.

Effect of heat on complement

In view of the extraordinary heat lability of the anaphylactic mechanism we have explored the possibility that some factor akin to complement may be involved in the anaphylactic reaction. Although complement is usually stated to be inactivated at 56° C it seemed possible that inactivation might result from prolonged heating at a lower temperature. In order to obtain results comparable to those in anaphylaxis, we have tested the effect of heating complement for 25 min at various temperatures.

Fresh guinea-pig serum was heated, then returned to 37° C, and its lytic effect determined on red cells incubated with rabbit antibody. Fig. 14 shows the degree of inactivation of complement after 25 min heating in the critical temperature range. Complete inactivation occurred at 53° C and no inactivation at 45° C; intermediate temperatures produced incomplete inactivation. The temperature for half inactivation of complement after 25 min heating was 51° C, as compared with 42·6° C. for the anaphylactic mechanism (Fig. 4). There is thus considerable discrepancy between the two inactivation temperatures.

Effect of low temperatures on the anaphylactic reaction

Fig. 1 shows that histamine release in anaphylaxis is inhibited by both low and high temperatures; the mechanism of inhibition by cold is, however,

different from that by heat in that it does not produce permanent effects. A sensitized tissue which has been cooled to 7° C for periods up to 1 hr and then brought back to 37° C, gives a normal histamine release with antigen. The inhibition of histamine release by antigen is seen only when the antigen is added in the cold. The inhibition even at 17° C is complete and thus greater than that achieved with 10 mM phenol.

It was pointed out in the preceding paper (Mongar & Schild, 1957) that phenol, although it inhibits histamine release, does not inhibit the antigen-antibody reaction, so that a sensitized tissue which is treated with antigen in the presence of phenol becomes completely desensitized. It was of interest to find out whether a tissue which is treated with antigen in the cold also becomes desensitized.

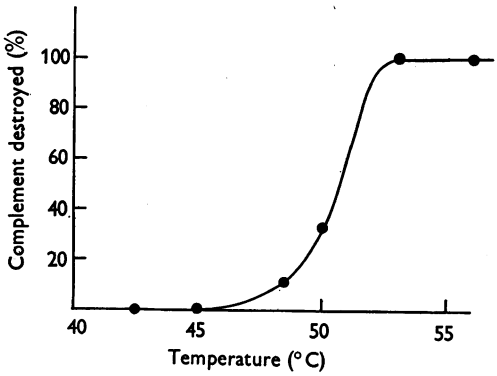


Fig. 14. Heat inactivation of complement. Fresh guinea-pig serum was heated for 25 min to temperatures varying from 42° to 56° C. Serial dilutions of this serum in Tyrode solution were then incubated at 37° C with an equal volume of a 1% suspension of sheep red blood cells plus antibody to test for lytic activity.

In order to test for desensitization, the antigen was added at 17° C and left in contact for various times. The tissue was then warmed to 37° C with the antigen still present and histamine release was measured and compared with control samples to which the antigen was only added at 37° C. Fig. 15 shows an experiment in which the lung samples were treated with antigen in the cold for 15 and 60 min. After warming to 37° C the 15 min sample released almost as much histamine as the control, but the 60 min sample released only 14% of the control. This shows that following a 15 min contact with the antigen at 17° C very little desensitization had taken place, whereas after 60 min the desensitization was well advanced. Thus cold inhibits not only histamine release but also desensitization. But whereas at 17° C the histamine release is completely inhibited the desensitization is only slowed down.

The rate of desensitization at 17° C varied considerably in preparations from different animals. Table 2 shows the degree of desensitization in different experiments. Desensitization has been expressed quantitatively, in terms of histamine release when the tissue was warmed to 37° C, since the more complete the desensitization at 17° C the less histamine can be expected to be released on warming to 37° C.

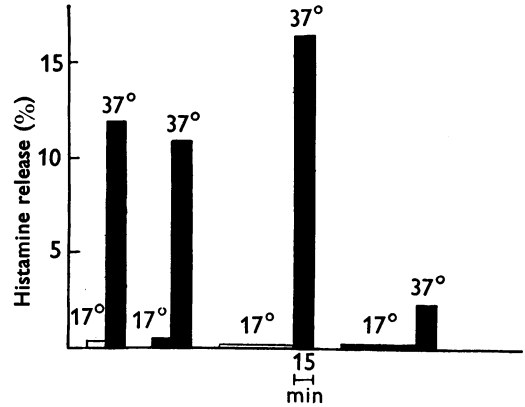


Fig. 15. Effect of cold on desensitization. When antigen is present for 15 min at 17° C there is little desensitization; subsequent histamine release at 37° C is almost as great as with the control: when antigen is present for 60 min at 17° C desensitization is nearly complete; subsequent histamine release at 37° C is 14% of the control. □ Tyrode; ■ Antigen.

TABLE 2. Desensitization at low temperature. Sensitized lung was placed in antigen-containing solution at 17° C for varying times and then warmed to 37° C. Histamine release is expressed as percentage of controls which were treated with antigen only after warming to 37° C. Each experiment was done on tissue from a different guinea-pig

Expt. no.	Min at 17° C	Histamine release (% of control)
2	15	50
3	15	92
4	20	84
5	20	29
1*a*	60	13
1*b*	60	14
2	60	47
3	60	20
6	60	63
2	200	29

Fig. 16 shows an experiment on lung tissue from the same guinea-pig in which the duration of contact with antigen at 17° C was varied. Samples were left in solution of antigen for 0–260 min, then warmed to 37° C, and their histamine release determined. Although the quantity of histamine

released at 37° C becomes less the longer the previous contact with antigen at 17° C, the curve does not tend to zero. The shape of the curve suggests that complete desensitization cannot be achieved at 17° C.

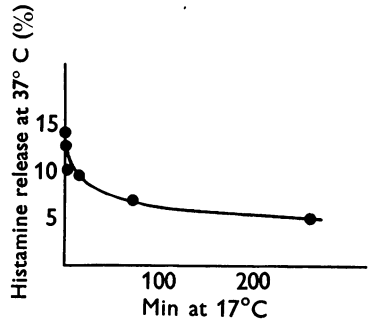


Fig. 16. Effect of time on desensitization in the cold. Samples from the same lung were placed in antigen-containing solution at 17° C for 0–240 min, and then warmed to 37° C for 15 min. Histamine release is given as percentage of tissue content.

DISCUSSION

The limits of temperature within which the anaphylactic reaction can function are remarkably narrow and any departure from the physiological range impairs both histamine release and the anaphylactic contraction. The curve relating histamine release to temperature resembles an enzyme curve, and more recently we have found that the curve relating pH to histamine release also resembles an enzyme curve, but there is no question at this stage of implicating any particular enzyme system in the anaphylactic reaction.

Histamine release in anaphylaxis first increases with temperature and then suddenly falls off. Two opposing factors are probably involved: (1) the effect of temperature on the activity of an enzyme system, and (2) the effect of temperature on its inactivation. The first dominates in the range below 37° C. From the increase of histamine release by antigen with increased temperature, a temperature coefficient for this reaction can be calculated. The Q_{10} is high, of the order of 12, giving an activation energy (μ), as calculated from the Arrhenius equation, of about 45,000 calories. This is a higher value than is found in isolated enzyme systems, which have activation energies up to 25,000 calories (Sizer, 1943).

Heat inactivation. A temperature of 45° C produces a persistent inactivation of the anaphylactic histamine release mechanism. This effect is specific for anaphylaxis and does not extend to histamine release by octylamine. Inactivation does not seem to be due to a denaturation or displacement of antibody. Antibody heated to 45° C fully retains its ability to sensitize tissue passively. The following findings suggest that the effect of heat is unlikely to be due to a loss of antibody from the cell.

(1) Passive sensitization experiments show no evidence that tissue heated to 45° C sheds its antibody.

(2) Heat inactivation when incomplete is spontaneously reversible. This could not be explained if the antibody had been lost from the cell.

(3) A tissue which has been heated to 45° C cannot be passively sensitized. This finding suggests that the effect of heat is on the tissue itself.

Heating probably inactivates some tissue component, possibly the precursor of an enzyme, which is required in the anaphylactic reaction. This heat-labile component is presumably a protein. The temperature coefficient of inactivation of the anaphylactic histamine-release mechanism is of the same order as

TABLE 3. Temperature coefficient of inactivation of anaphylactic histamine release mechanism

Temp. (° C)	Time of half inactivation* (min)	Relative velocity	Log relative velcoity	Difference of log relative velocity
42·5	28·0	1·00	0·000	0·243
43·0	16·0	1·75	0·243	0·124
43·5	12·0	2·33	0·367	0·115
44·0	9·2	3·04	0·482	

Mean = 0·16 per ½° C
≡ 0·32 per ° C

Average temperature coefficient = 2·1 per degree.

* From Fig. 4

TABLE 4

System	Activation energy (μ)	Temperature for half inactivation in 25 min (° C)	Author
Egg albumin	136,000	73	Chick & Martin (1910)
Goat serum complement	199,000	50·6	Famulener & Madsen (1908)
Guinea-pig serum complement	—	51	Present work
Anaphylactic histamine release mechanism	150,000	42·6	Present work

temperature coefficients of protein denaturation. The temperature coefficient of inactivation of the anaphylactic mechanism has been calculated in Table 3 from the rate experiments shown in Fig. 4. It is not constant, but averages 2·1 for 1° C increase of temperature. If only the values for the three higher temperatures which gave complete inactivation are used, the mean coefficient is 1·7. This compares with a temperature coefficient of 1·9 for the coagulation of crystallized egg albumin (Chick & Martin, 1910). The corresponding activation energies (μ) are shown in Table 4; this also includes the μ value for the inactivation of goat serum complement obtained by Famulener & Madsen (1908), which is of the same order of magnitude.

Whilst the temperature coefficient of inactivation of the anaphylactic

mechanism fits in with that of the other two systems, the actual temperature at which inactivation occurs is considerably lower for the anaphylactic mechanism. It is particularly interesting that this temperature is about 8° C lower than that at which complement is inactivated. There is a certain similarity between the action of specific haemolysins and the anaphylactic reaction in that both require, besides antibody and antigen, a heat-labile factor. However, the difference in the inactivation temperature suggests that the heat-labile factor in anaphylaxis, although presumably a protein, is different from serum complement.

Practical implication of heat inactivation. An important practical consideration is whether the temperature threshold for the inactivation of the anaphylactic mechanism can be reduced below 42·5° C. On theoretical grounds no absolute inactivation threshold would be expected. It was pointed out in the classical studies of Chick & Martin (1910) that heat denaturation of proteins is not an instantaneous process but that it occurs at a measurable rate with a very high temperature coefficient. This also seems to apply to inactivation of the anaphylactic mechanism. We have found that whereas at temperatures above 45° C inactivation is very rapid and probably limited only by heat conduction, at lower temperatures it proceeds more slowly; at 42·5° C the time for half inactivation was about 27 min. Our experiments have shown no evidence that temperatures below 42·5° C produce inactivation, but the time of exposure may have been insufficient. We have found in preliminary trials that inactivation of the anaphylactic mechanism *in vivo* occurs at the same temperatures as *in vitro*. The effect of long exposure to temperatures below 42·5° C could thus be investigated in the whole animal.

Another approach is to combine heat treatment with some other treatment which affects the anaphylactic mechanism. We have experimented with a combination of heating and pH changes, and have found that, when tissue was heated to 42·5° C at pH 6·4, the effect of heat was considerably potentiated and the anaphylactic mechanism more completely inactivated than when the same temperature was applied at pH 8. However, no such potentiation was obtained in the physiological range down to pH 7.

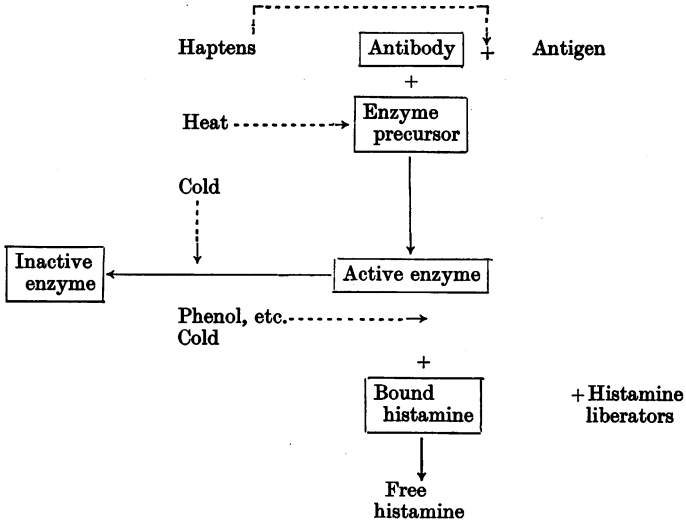
The effect of heat on the anaphylactic mechanism in isolated tissue is not entirely irreversible, and it appears that the less complete the inactivation the greater the chances of recovery. This reversibility does not invalidate the concept of a denaturation reaction, as in the initial stages protein denaturation is not an irreversible process. Since the temperatures used in these experiments affect not antibody but a normal tissue constituent required for anaphylaxis, it is to be expected that in the whole animal the inactivation of the anaphylactic mechanism will eventually be reversed through the normal repair processes of the body. Preliminary experiments indeed suggest that in the intact animal heat inactivation is reversible.

Effect of cold. Cold produces no permanent damage to the anaphylactic mechanism: a sensitized tissue which has been cooled to 17° C and warmed again to 37° C has a normal anaphylactic response. If the antigen is added at 17° C the anaphylactic reaction is apparently completely inhibited as judged by histamine release, but further analysis reveals that the earlier stages of the reaction are not completely inhibited. After rewarming to 37° C, such a tissue proves to be partly desensitized, the degree of desensitization depending on the duration of contact with antigen in the cold. The effect of cold thus resembles that of certain chemical agents such as phenol in inhibiting histamine release in anaphylaxis, but in addition it reduces the rate of desensitization. This does not necessarily mean that the antigen-antibody reaction is inhibited. Mayer & Heidelberger (1942) have measured the rate of the antigen-antibody reaction *in vitro* at 0° C, and have concluded that it is substantially complete within 3 sec. If these results can be applied to our heterogeneous system it would seem probable that the rate of the antigen-antibody reaction is not a limiting factor at 17° C, but that a subsequent reaction is inhibited. In terms of the hypothesis, discussed in the preceding paper (Mongar & Schild, 1957), that the anaphylactic reaction involves the temporary activation of an enzyme system, the effect of low temperature on anaphylaxis could perhaps be explained as follows. When the antigen-antibody reaction takes place at 17° C, the formation or destruction of the active enzyme is slowed down but is not completely inhibited, whereas its action is completely inhibited. This results in a complete inhibition of histamine release in the cold, and a reduced histamine release when the tissue is warmed again, since by then some of the activated enzyme has been destroyed.

Relation to the proteolytic theory. Since the discovery that trypsin releases histamine (Rocha e Silva, 1939), several authors have found that histamine release due to many agents, including antigen, is attended by proteolysis (Ungar, 1947; Humphrey & Jaques, 1955; Haining, 1956). This has led to the suggestion that protein break-down is the cause of histamine release, although it is possible that histamine release and protease activation are parallel phenomena (Ungar, 1956). The action of heat clearly differentiates the enzyme system which we envisage for the anaphylactic histamine release from the proteolytic system envisaged by Ungar. Temperatures above 45° C inactivate the anaphylactic mechanism, but activate the proteolytic mechanism (Ungar & Damgaard, 1954). A further distinction is indicated by the finding that inhibition of the anaphylactic histamine release, whether by heat or by chemical inhibitors, is accompanied by a potentiation of histamine release by organic bases, yet the action of both types of releasers leads to proteolysis. It would seem that proteolysis is a general feature accompanying histamine release due to many different causes, whereas the various inhibitors studied in this work

act on a more specific mechanism which so far has been found to operate only in anaphylaxis.

Mechanism of anaphylaxis. The following scheme, which may be regarded as a working hypothesis, summarizes our views on the mechanism of anaphylaxis:



Combination of antigen with antibody results in the activation of an enzyme system which catalyses reactions leading to histamine release and other effects of anaphylaxis. The activated enzyme is short-lived and in the normal course of events becomes rapidly inactivated. Inhibitors act on different sites. Theoretically they can interfere with the antigen-antibody reaction, with the formation or destruction of the active enzyme or with its action. The most probable site of action of various inhibitors is indicated in the scheme.

SUMMARY

1. The release of histamine in anaphylaxis is maximal at 40° C and is inhibited by high and low temperatures. Raised temperatures produce a persistent inactivation of the anaphylactic mechanism. When plain muscle which has been sensitized to egg albumin is warmed to 45° C for a few minutes and its response to egg albumin tested afterwards at 37° C, histamine release and the anaphylactic reaction are abolished, although the contractility of the muscle is unimpaired. Low temperatures produce no persistent inactivation of the anaphylactic mechanism: if a sensitized tissue is cooled and warmed up again, its anaphylactic reaction is normal, but when the antigen is added at 20° C histamine release is completely inhibited and desensitization is slowed down.

2. The effect of raised temperature on the anaphylactic reaction has been further investigated in experiments in which passive sensitization was produced *in vitro*. A temperature of 45° C does not denature the antibody but inactivates some factor present in normal tissue that is required in anaphylaxis. This factor is presumably a protein, but it is not identical with serum complement.

3. The mechanism of the anaphylactic reaction is discussed in the light of these experiments.

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