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Recovery of Anaphylactic Sensitivity in the Guinea-pig Ileum after Desensitization

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Summary. The recovery of anaphylactic sensitivity of the ileum after desensitization has been investigated, and the dose–response and time–response relationships described. An attempt has been made to investigate the mechanism of recovery of sensitivity.

INTRODUCTION

During the course of an investigation into the use of anaphylactic tests in tumour immunology studies it was noted that the guinea-pig ileum after desensitization frequently recovered sensitivity to the concentration of antigen previously given (Dale, 1965). Anaphylactic reactions are in the main considered to be acute dramatic episodes which, if they are not fatal, leave the animal (or tissue) refractory. The fact that in the ileum at least the whole process could occur again within a short period of time seemed to warrant further attention. This phenomenon of resensitization or recovery from desensitization was therefore further investigated.

MATERIAL AND METHODS

Male Hartley guinea-pigs were sensitized with one injection of twice-crystallized ovalbumen, 250 μg in Freund's incomplete adjuvant intramuscularly. After 6 weeks they were killed and used for Dale–Schultz experiments. Short strips of ileum were set up in 2-ml isolated organ baths in oxygenated Tyrode's solution. Contractions were recorded with isotonic frontal levers. After it had been established that the ileum was sensitive to low doses of histamine (10–20 ng/ml) the tissue was challenged with a selected concentration of egg albumin and the Dale–Schultz reaction recorded. The antigen was washed out and readministered repeatedly till the tissue was desensitized, usually after two doses (Fig. 1). The ileum was then left undisturbed, but continuously perfused with warm Tyrode's solution, for a varying period of time (the 'resensitization interval') and finally was challenged again with the same dose of antigen to assess the return of anaphylactic sensitivity (Fig. 1). This latter response is referred to as the 'second response' or 'recovered response'. Responses are expressed as a percentage of the maximum possible histamine contraction.

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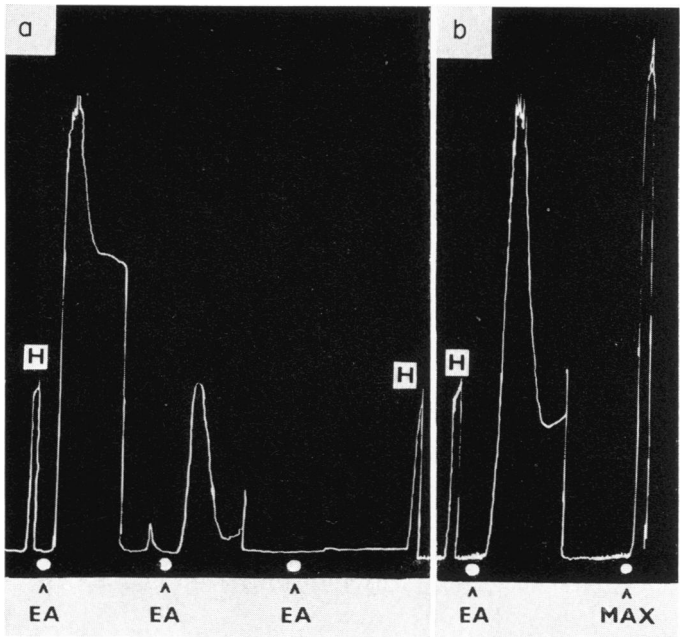

FIG. 1. Anaphylactic responses of ileum to egg-albumin 10^{-6} g/ml (EA). Contact time: 3 minutes. (a) Initial response with subsequent desensitization. (b) Recovered response 3 hours later. H, Histamine 10 ng; MAX = the maximum contraction of the ileum.

RESULTS

GENERAL CHARACTERISTICS OF THE RECOVERED RESPONSE

Variability of the recovered response

In several experiments the recovered responses with various concentrations of antigen were measured in a large number of strips (six to ten) after a uniform time interval, under standard conditions, and in each experiment the standard errors of the six to ten responses to each dose were measured. Recovered responses were consistently obtained but were variable in size. The distribution of the responses appeared to be homoschedastic and the mean standard error was 5·1. It was considered, therefore, that it would not be possible to detect factors which produced a subtle change in recovery of anaphylactic sensitivity.

The dose–response relationship (Fig. 2)

The dose–response curve for the Dale–Schultz reaction obtained with first challenge with antigen has been plotted using separate strips of ileum for each reading at each concentration. The results show the usual sigmoid type of curve (curve ○ in Fig. 2). A concentration of EA of 10^{-6} or more usually produces a maximum contraction of the ileum. The dose-response curve for the final challenge, after 3 hours resensitization, is bell-shaped, the recovered responses to the high concentrations 10^{-5}, 10^{-4}, 10^{-3} becoming progressively smaller (curve × in Fig. 2).

The curve for the second challenge after 6 hours is much the same but the peak is broader

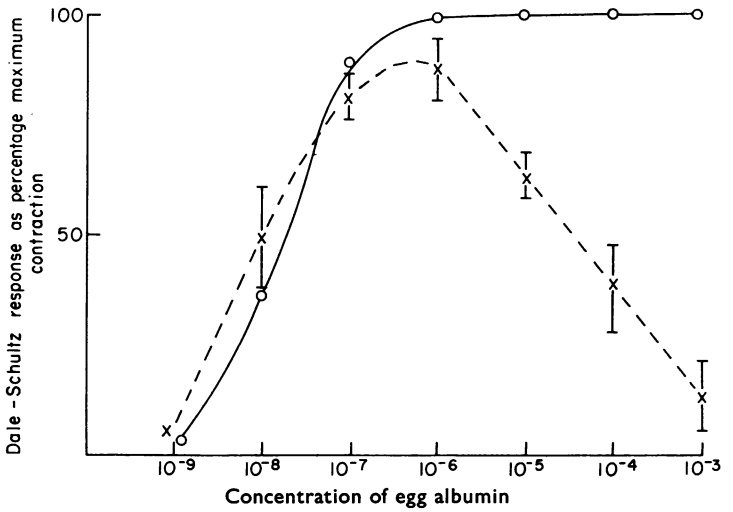

FIG. 2. Dose–response curves of ileum to egg-albumin. O, Responses on first challenge; ×, recovered responses with standard errors.

and the descending limb is shifted to the right—the higher concentrations giving rather larger responses, e.g. the results from the batch of animals from which Fig. 2 was taken showed recovered responses of 100 per cent to 10^{-5}, 50 per cent to 10^{-4} and 24 per cent to 10^{-3} after a 6-hour resensitization interval.

There is a great deal of variation between individual animals and between different batches of animals. In Fig. 2 it can be seen that the standard errors for the recovered responses are very large. In poorly sensitized animals both curves are shifted to the right and the bell-shaped curve is altogether smaller.

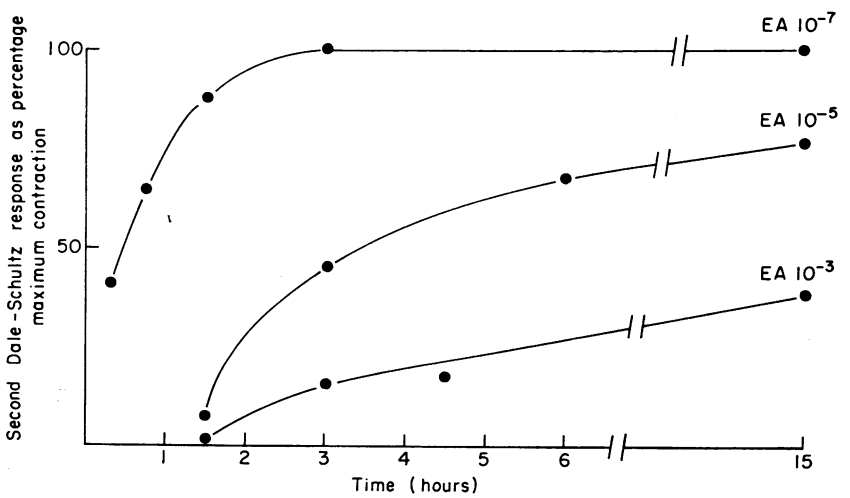

FIG. 3. The time–response relationship of the recovery of anaphylactic sensitivity. Only the recovered responses ('second response') are given. Two or more separate strips of ileum used for each point on the graph.

The time–response relationship (Fig. 3).

The return of anaphylactic sensitivity varies with time. After desensitization to 10^{-7} EA the ileum usually starts responding again to this same concentration within 15 minutes and by 3 hours the response is of the same magnitude as the initial Dale–Schultz reaction. The response to higher doses returns more slowly.

Recovery of sensitivity with small doses of antigen was so rapid that desensitization could not easily be achieved if the interval between repeated additions of antigen was prolonged. An example of this is shown in Fig. 4. When EA 10^{-7} was given repeatedly at 3-minute intervals (a), the response diminished rapidly. When the same dose was given at intervals at 45 minutes (b), the ileum continued to respond to the challenge. In other experiments, contractions of approximately constant size were obtained for up to ten applications of the same dose of antigen.

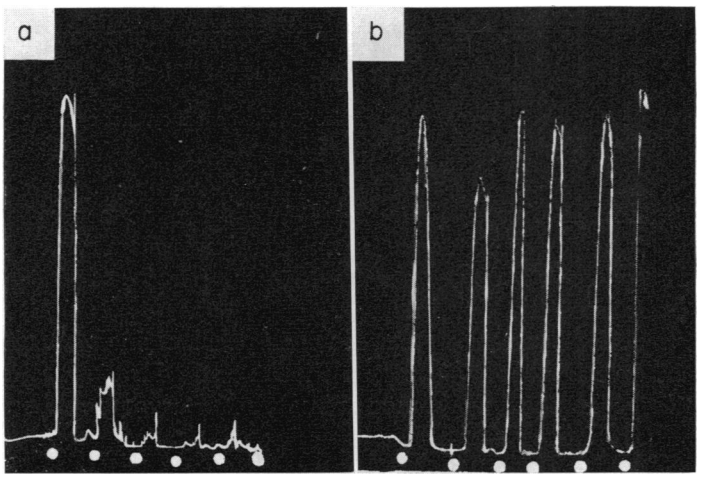

FIG. 4. Anaphylactic contractions of guinea-pig ileum to egg-albumin 10^{-7} g/ml (EA) using a contact time of 1 minute. Each administration of EA shown by white dot. (a) Three-minute intervals between doses of EA. (b) Forty-five-minute intervals between doses of EA.

Recovery of the anaphylactic response in passively sensitized tissue

This was investigated using purified guinea-pig γ_1-globulin in high concentrations (50 μg/ml) for passive sensitization. There was a reasonable return of anaphylactic sensitivity, a mean recovered response of 33 per cent of maximum contraction being obtained on final challenge using 2 μg/ml of the antigen. (It was not possible to compare these responses with the responses of actively sensitized ileum because in the latter case there is no information about the degree of sensitization.)

EXPERIMENTS IN WHICH CONDITIONS DURING THE RESENSITIZATION INTERVAL WERE VARIED (SEE TABLE 1 AND FIG. 5)

In each of these experiments two sets of strips were used: (i) control strips which were bathed in oxygenated Tyrode's solution at 37° during the resensitization interval, and (ii) test strips which were subjected to some modification of these conditions (alteration of temperature, anoxia, etc.) during the resensitization interval. In all these experiments *all*

challenges with antigen were made while the strips were bathed in oxygenated Tyrode's solution at 37°. In each experiment, additional strips were set up and subjected to the relevant modification of resensitization conditions (low temperature, anoxia, etc.) for 3 or 4½ hours first before being challenged with antigen for the first time, to ascertain that the altered conditions did not affect the ability of the ileum to give an anaphylactic response *per se*. The results are given in Table 1. For some of the modifications only a very few experiments were carried out. It was realized that subtle changes would not be detected in so variable a system and so if no appreciable difference between test and control strips was seen after the first few attempts, no further experiments were done.

The effect of low temperature during resensitization (Fig. 5)

In several experiments, some strips of ileum, after challenge and desensitization with various concentrations of antigen were switched to cold Tyrode's solution and kept at a low temperature throughout the resensitization interval (either 3 or 4½ hours). They were then warmed up to 37° and when the histamine responses were back to normal they were challenged a second time. The strips kept at low temperature gave appreciably smaller Dale–Schultz responses on second challenge. Control loops subjected to the same temperature changes and then challenged for the first time showed no reduction in anaphylactic response. The results of one such experiment are given in Fig. 5. The effect is rather more marked at higher concentrations.

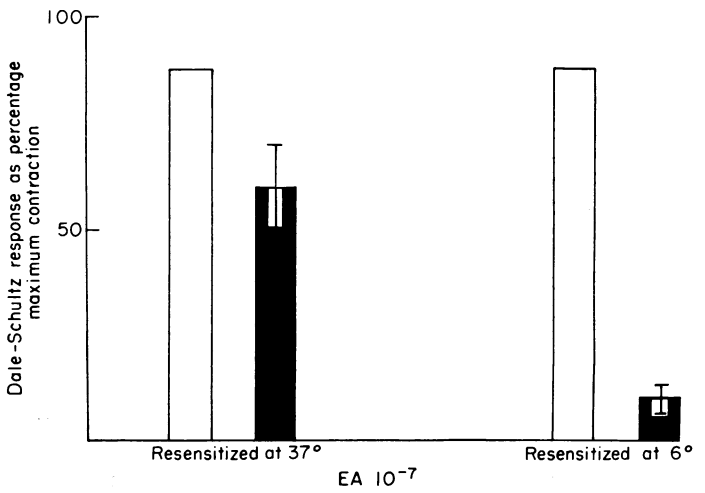

FIG. 5. The effect of low temperature during resensitization. Open columns, initial responses; solid columns, recovered responses. Control strips of ileum shown on the left. Test strips, kept at 6° throughout the 3-hour resensitization interval, shown on the right.

The effect of malonate (see Table 1)

Sodium malonate in a concentration (20 mM) sufficient to inhibit competitively succinic acid dehydrogenase in the Krebs cycle was added to the perfusing Tyrode's solution during the resensitization interval. The results were variable (the standard errors were very large) but showed no consistent significant effect of malonate.

TABLE 1

RESULTS OF EXPERIMENTS MODIFYING CONDITIONS DURING THE RESENSITIZATION INTERVAL

Modification of conditions for *test* strips	Concentration of antigen (mg/ml)	Response to first challenge as percentage maximum contraction	Recovered response as percentage maximum (standard errors in parentheses)	
			Control strips	Test strips
O_2-lack (nitrogen bubbled through bath)	10^{-5}	96	83 (6)	86 (9·6)
Glucose-lack	10^{-7}	96	35 (6·1)	23 (3·1)
Sodium malonate (20 mM in Tyrode's solution)	10^{-5}	99	16	16·5 (3·1)
Puromycin 10^{-4} M 1 hour before challenge and throughout resensitization interval	10^{-4}	94	58	61
Chloramphenicol, 50 μg/ml for 1 hour before experiment and throughout resensitization interval	10^{-4}	95	61 (12)	37 (4·8)
Chloramphenicol, 200 μg/ml for 1 hour before experiment and throughout resensitization interval	10^{-4}	96	40 (2·8)	36 (4·8)
Actinomycin-D for 1 hour before and throughout resensitization interval	10^{-4}	100	52 (17·2)	57 (9·1)

The effect of glucose-lack (see Table 1)

Test strips of ileum were bathed in glucose-free Tyrode's solution during the resensitization interval. There was no significant difference in the response of these loops and the response of the control loops to second challenge.

The effect of oxygen-lack (see Table 1)

Nitrogen was bubbled through the baths of test loops. There was no difference between test and control loops in the response to the second challenge with antigen.

The effect of a combination of oxygen-lack and glucose lack

Test strips exposed to nitrogenated glucose-free Tyrode's during the resensitization interval seemed to lose vitality and manifested an overall reduction in contractible response, but taking this into account (i.e. expressing the response as a percentage of the reduced maximum response) did not give a significantly reduced anaphylactic response to the second challenge.

The effect of substances believed to interfere with protein synthesis (see Table 1)

Three antibiotics believed to interfere with protein synthesis (Puromycin, Chloramphenicol, Actinomycin-D) were tested using concentrations which had been shown to affect synthesis of globulins, etc., in similar *in vitro* systems (Uhr, 1963; Ambrose and Coons, 1963; Smiley, Heard and Ziff, 1964; Strander, 1966; Vasquez and Monroe, 1967). There was no direct effect on the smooth muscle and no significant effect on recovery of sensitivity was seen.

EXPERIMENTS IN WHICH CONDITIONS DURING FIRST CHALLENGE WERE MODIFIED

The effect of phenol during first challenge with antigen (Fig. 6)

Test strips were challenged with a high dose of antigen, EA 10^{-4}, in the presence of phenol 20 mM in Tyrode's solution. No Dale–Schultz reaction took place but the tissues tested immediately after washing out the phenol were found to be desensitized to EA 10^{-4}. Control strips were challenged with EA 10^{-4} in Tyrode's until desensitized. All strips were bathed in oxygenated Tyrode's at 37° during the resensitization interval and then challenged again with EA 10^{-4} in the usual manner. There was no significant difference between the recovered responses of the test and control strips (Fig. 6).

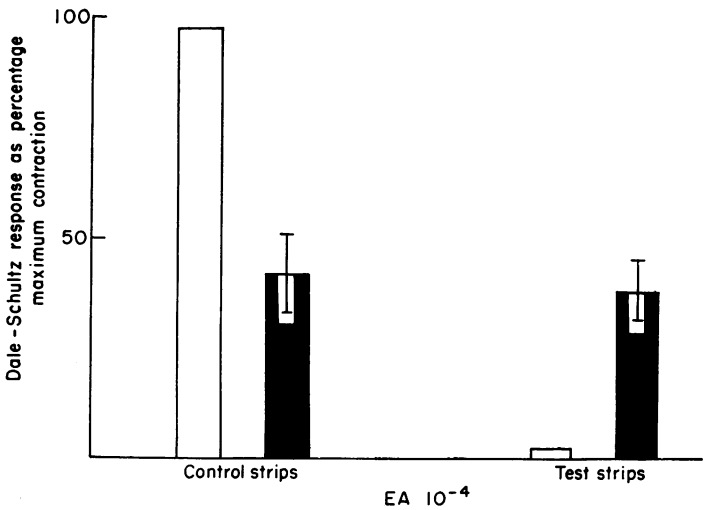

FIG. 6. The effect on resensitization of phenol during initial challenge with antigen. The test strips were challenged with EA 10^{-4} in the presence of phenol 20 mM. All strips were washed in Tyrode's for 4½ hours and then challenged again with EA 10^{-4}. Open columns, initial responses; solid columns, recovered responses.

The effect of calcium-lack during the first challenge with antigen (Fig. 7)

Test strips were challenged in calcium-free Tyrode's which contained NaEDTA, 0·5 mM. No response occurred. The strips were then changed to normal Tyrode's solution for 3–4½ hours washing and then final challenge. Control strips were treated in the usual way. There was no significant difference between the recovered responses of test and control strips. The possibility was considered that in these experiments the strips had not become completely equilibrated with calcium-free Tyrode's solution. Further experiments were done in which the test strips were left for 2 or more hours in calcium-free Tyrode's solution containing NaEDTA 0·5 mM before and after the first challenge, and were changed to normal Tyrode's solution about one hour before the second challenge. In these strips the final maximum response of the tissue to histamine was decreased, indicating a depression of contractility of the muscle. The recovered anaphylactic response as a percentage of this altered 'maximum' response was significantly greater than the response of the control strips.

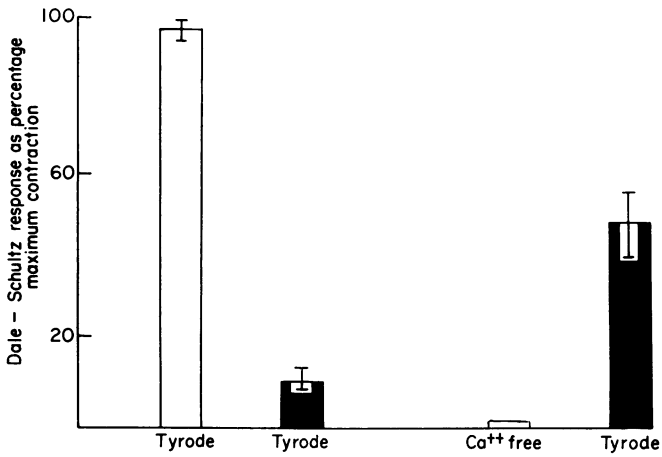

FIG. 7. The effect on resensitization of calcium-lack during the initial challenge with EA 10^{-4}. Test strips of ileum on right. Control strips on left. Open columns, initial responses; solid columns, recovered responses. Resensitization interval: 4½ hours.

We found that tissue after challenge in calcium-free Tyrode's solution could be changed to ordinary Tyrode's solution without producing an anaphylactic reaction. There was some increase in muscle tone on changing the fluid but this was not very different from that obtained with unchallenged strips or unsensitized strips and did not in our opinion constitute a Dale–Schultz reaction. This is in conflict with the findings of Huidobro and Valette (1960).

DISCUSSION

There are several possible explanations for the recovery of the response of the ileum to the desensitizing dose of antigen.

It is conceivable that resensitization is due to release and/or synthesis of new antibody, i.e. that the first *in vitro* contact with antigen stimulates local antibody release from and/or synthesis in the lymphoid tissue in the ileum and that the later Dale–Schultz response is a result of the reaction of antigen with this newly available antibody. With sufficiently sensitive systems, *in vitro* release of antibody has been demonstrated within 15–75 minutes (Jerne and Nordin, 1963; Zaarlberg, 1964; Dutton, 1967). Askonas and Humphrey (1958a, b) demonstrated *in vitro* synthesis in isolated guinea-pig tissues within the 1st hour or so. It seemed that it could be possible for new antibody release or synthesis to occur in guinea-pig ileum in the observed resensitization interval. If such synthesis or release of antibody is involved, there should be no recovery of anaphylactic sensitivity in passively-sensitized ileum. When this was put to the test, however, we obtained good recovery of response with this preparation (see also, Swineford and Reynolds, 1951). It was, therefore, apparent that new synthesis or release of antibody was not a prerequisite for resensitization.

If new antibody is not formed or released, one must assume that the antibody which reacted with antigen to give the first response becomes available again. One possible explanation of desensitization and subsequent resensitization is as follows: when antigen combines with cell-fixed antibody, the formed complex is biologically active for a short

time but the molecules remain combined for much longer. This may interfere with further anaphylactic reactions by blocking the available antibodies—desensitization. In time the complex dissociates leaving the antibody free to react again—resensitization.

However, dissociation, by itself, though probably a necessary condition for resensitization, does not constitute a sufficient explanation of it. Availability of antibody would be of no use unless the tissue had a reasonable potential for anaphylactic response. This condition of being 'anaphylactically competent' at the time of final challenge, could be fulfilled in several different ways:

(1) It is possible that the tissue has an unlimited capacity for anaphylactic response. This would imply either that the store of cellular materials used during the anaphylactic reaction (enzymes, histamine, etc.) was practically inexhaustible or else that the antibody was fixed to smooth muscle and the antigen–antibody reaction could stimulate contraction without the intervention of a mediator such as histamine.

(2) It is possible that intracellular components used up during the reaction are reconsituted—the resensitization interval representing the time necessary for such reconstitution. This hypothesis would explain the shapes of the dose–response and the time–response curves of the recovered response: the smaller the dose used the less reconstitution necessary and the more rapid the recovery (Fig. 2), and the more complete the recovery within the time periods used for the dose–response curve (Fig. 3). The larger the dose the more reconstitution necessary and the slower and more incomplete the recovery (Figs. 2 and 3).

(3) It is possible that the anaphylactic resources and the tissue are neither unlimited nor replaceable within the time period of the experiment, but that the resources are not completely depleted on first challenge. This hypothesis also provides an explanation of the shape of the dose–response curve. Small dose of antigen would utilize very little of the store of anaphylactic components of the tissue, and as soon as there had been sufficient dissociation of antigen–antibody complexes the tissue would be able to respond vigorously again. Larger doses would deplete considerably more of the components and recovery would be of lesser degree (Fig. 2). [The slower rate of recovery with larger doses of antigen (Fig. 3) could also be due to the formation of different and more stable complexes with different ratios of antigen to antibody.]

The first possibility—that the tissue had an unlimited capacity for anaphylactic response seemed to be unlikely and was not tested. Histological data did not appear to accord with a hypothesis of a virtually inexhaustible supply of the materials used in the response. On the other hand a hypothesis of a direct effect of the antigen–antibody reaction on smooth muscle cells would not easily explain the character of the dose–response and time–response curves unless one postulated profound and prolonged fatigue of the muscle after high doses of antigen or else attributed the slow recovery after high doses purely to slow dissociation.

The second possibility—reconstitution of intracellular components—was investigated (Table 1 and Fig. 5). It was considered that any reconstitution would be very likely to involve an energy-utilizing process. Consequently, factors which interfere with energy utilization might interfere with resensitization. Further, reconstitution of the anaphylactic enzyme system might well involve protein synthesis. Inhibitors of protein synthesis might, therefore, inhibit resensitization. But the experiments in which O_2 and glucose were excluded, or Krebs' cycle inhibitors used, were all negative, as were those with inhibitors of protein synthesis (see Table 1). These results imply that an active process of reconstitution

is not involved, or else that, if an active process *is* involved, it is singularly invulnerable to interference with the, admittedly, crude techniques used. The decreased recovery noted when resensitization was carried out in the cold could be held to support the possibility of an active process of reconstitution being involved. But the low temperature might also decrease recovery by decreasing dissociation of antigen and antibody.

The possibility next considered was that after the first response there was some remaining anaphylactic potential in the tissue which would determine whether a second response could take place and how large it would be. For the purpose of investigation it was assumed that the Schild–Mongar scheme for anaphylactic release of histamine was applicable (Fig. 8), i.e. that a three stage process occurs involving:

(1) The initial antigen–antibody reaction.

(2) A by-pass system—possibly involving the activation of a labile temperature-dependant mechanism which could be enzymic.

(3) Release of pharmacologically active products.

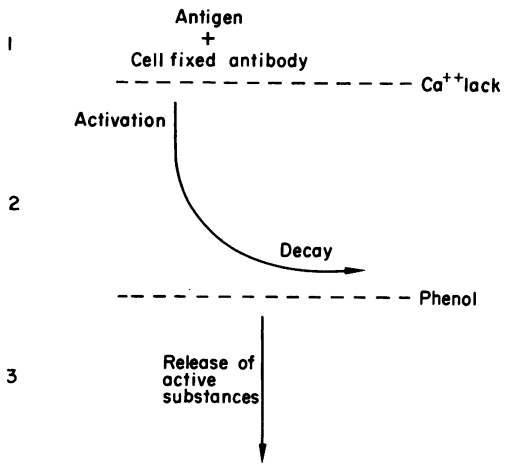

FIG. 8. Postulated reaction scheme for anaphylactic histamine release (Mongar and Schild, 1962).

Accepting for the moment that when a tissue recovers sensitivity, stage one has been reversed, i.e. that there has been dissociation of antigen and antibody, the question posed was: which of the latter two stages is the limiting factor in resensitization? Mongar and Schild (1962) had suggested that the inhibitory action of phenol was due to uncoupling of stages 2 and 3, and that calcium lack probably uncoupled stages 1 and 2 (see Fig. 8). The effect of these factors on resensitization was tested. The results of experiment with phenol (see Fig. 6) indicate that whether all three stages go to completion (as in the control loops) or only two go to completion (as in the test loops), the recovery of the anaphylactic response is the same. In other words even if there is no utilization of the store of releasable active products—there is no increase in the recovered response. This implies that it is not stage 3 but stage 2, the by-pass stage which is of particular importance in determining the magnitude of the second response. If this is so then factors which uncouple stages 1 and 2 and allow only the antigen–antibody reaction to occur should result in an appreciably larger response on final challenge. And, in the main, in experiments C2 in which first

challenge of the test strips took place in calcium-free Tyrode's solution, the recovered response of these strips was increased as compared to the controls. But these results should be interpreted with great caution because at the time of final challenge the tissue of the test strips showed some decrease in vitality and contractility and the 'recovered responses' had, therefore, to be expressed in terms of the *reduced* maximum response to histamine.

Although some resynthesis of cellular components has not been completely excluded it would appear that the most likely explanation for the recovery of the anaphylactic response is that there is dissociation of the antigen–antibody complex allowing a further antigen–antibody reaction to take place. The *magnitude* of the recovered response could well depend on the potential remaining in the tissue, for carrying on the second, 'by-pass', stage of the anaphylactic process.

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