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The Schultz-Dale response of the longitudinal muscle strip preparation of guinea-pig ileum

M. MAUREEN DALE AND LUCILLA ZILLETTI*

Department of Pharmacology, University College London

Summary

1. The mast-cell distribution in the various layers of the ileum has been described.

2. Histamine-content, anaphylactic histamine-release and the anaphylactic dose-response curve of the full-thickness ileum and of the longitudinal muscle strip have been measured and compared.

3. Tetrodotoxin 10^{-7} g/ml had no obvious effect on the anaphylactic dose-response curve of either preparation. This suggests that the plexus is not of any great importance in the Schultz-Dale reaction.

4. Exposure of the longitudinal muscle strip to octylamine 10^{-3} g/ml for 1 min reduced the mast cell content by 95–100%. After this treatment the dose-response curve to antigen was eliminated, although the muscle still responded to small doses of histamine and to anaphylactic mediators. Pre-treatment of antibody with octylamine did not impair passive sensitization and subsequent response to antigenic challenge. This suggests that the classical Schultz-Dale reaction in the strip is mediated mainly by mast cells, and possibly other cells, and is probably not due to a direct effect of the antigen-antibody reaction on the smooth muscle.

5. The typical three-phase anaphylactic response (quick contraction, quick relaxation, slow contraction) of full-thickness ileum is discussed and compared with the predominantly two-phase response of the longitudinal muscle strip. No evidence was found for the release of a relaxation-factor. It is suggested that the initial fast phase may be due to mediators released from mast cells among the longitudinal muscle fibres, and the sustained contraction to a second wave of mediators reaching the longitudinal muscle from deeper layers of the ileum.

Introduction

The Schultz-Dale reaction of the guinea-pig ileum was one of the earliest manifestations of *in vitro* anaphylaxis to be described (Schultz, 1910). But it has always been a puzzle because it has not really fitted into the pattern of *in vitro* anaphylaxis as seen in other tissues. In general it is believed that during anaphylaxis one of the main occurrences is the combination of antigen with antibody on the surface of

* Present address: Department of Pharmacology, University of Florence, Italy.

mast cells, with subsequent release of histamine and other substances. But although the ileum is one of the tissues consistently rich in histamine (Paton, 1958) and is a very sensitive anaphylactic organ, readily giving very dramatic responses to small doses of antigen, the histamine-release recorded during anaphylaxis has been very low. This was first reported by Schild (1939). Mongar & Schild in 1952 recorded only 2% release from the ileum as compared with 36% from aorta, 31% from uterus, and 10% from lung. Hawkins & Rosa (1956), Boreus & Chakravarty (1960) and Brocklehurst (1960) also recorded very low releases from this tissue.

In most sensitized tissues a decrease in mast cell content also accompanies the manifestations of anaphylaxis (Mota & Vugman, 1956 ; Mota, 1959), but this has not appeared to be so with the ileum (Boreus & Chakravarty, 1960).

In fact, recent work by Alonso-de Florida, del Castillo, Garcia & Gijon (1968) on denervated diaphragm has again raised the possibility previously discussed by Schild (1964) that the Schultz-Dale response may be due to the direct effect of the antigen-antibody reaction on the muscle cells.

Another possible explanation for the anomalous nature of anaphylaxis in the ileum is that, unlike the lung, aorta, uterus, etc., it contains highly complex intrinsic nerve plexuses. Early workers had believed that these neuronal networks were involved in the anaphylactic response (Nakamura, 1941 ; Danielopolu, Rudeman & Simionescu, 1948 ; Geiger & Alpers, 1959).

The actual Schultz-Dale response of the ileum is mainly, if not entirely, due to the outer longitudinal muscle coat. It is possible to remove viable strips of this muscle and use it separately for Schultz-Dale experiments. This longitudinal muscle strip preparation (Ambache, 1954 ; Rang, 1964 ; Paton & Zar, 1968) provides a much simpler and more logical system than full-thickness ileum for the investigation of the mechanism of the Schultz-Dale reaction.

Methods

Anaphylactic tests

Details of the sensitization of guinea-pigs and the performance of the anaphylactic tests have been given previously (Dale, 1965 ; Dale & Okpako, 1969), the only difference in the present study being that the immersion fluid used was Krebs solution with the following composition (mM): NaCl 113, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25, glucose 11·5. The antigens used were egg albumin in active anaphylaxis experiments and dinitrophenyl-human-serum albumin in passive anaphylaxis experiments. The longitudinal muscle strip was prepared as described by Paton & Zar (1968) and Ambache (personal communication). In all anaphylactic tests the response of the tissue to 10 ng/ml and 20 ng/ml of histamine was assessed both at the beginning and at the end of the experiment, and in the experiments on plexus and tetrodotoxin the responses to nicotine (10 μg/ml) and DMPP (10 μg/ml) were also tested. At the end of every experiment histamine 1 μg/ml or acetylcholine 1 μg/ml was administered in order to measure the maximum contraction.

All drug concentrations refer to the final concentration of the salt in the bath, with the exception of histamine, where the concentrations are expressed as base.

The Schultz-Dale reaction is expressed as a percentage of the maximum possible contraction.

Histological preparations

Tissue for mast cell studies was fixed in Mota's fixative (Mota & Vugman, 1956) and stained with 1% toluidine blue. Tissue for studies on the nerve plexus was stained supravitally in 0·1% methylene blue in oxygenated Krebs solution. For mast cell counts, three pieces of muscle strip were used for each concentration of drug, the tissue being spread out gently on a glass slide. Each piece was divided into two, one half being exposed to the drug and the other half serving as control.

Assay of histamine

Histamine was extracted by the modified method of Feldberg & Paton (1951) and assayed, with a 2+1 design, on guinea-pig ileum as previously described (Giotti, Guidotti, Mannaioni & Zilletti, 1966).

Drugs

Histamine acid phosphate, acetylcholine chloride, nicotine hydrogen tartrate, atropine sulphate, 1,1-dimethyl 1-4-phenylpiperazinium iodide (DMPP), mepyramine maleate, octylamine (Koch-Light Ltd.), tetrodotoxin citrate (Koch-Light Ltd.), neostigmine methylsulphate, adrenaline hydrogen tartrate.

Results

Relevant histological features of the ileum

In terms of overall bulk, the longitudinal muscle forms a relatively small proportion of the total thickness of the ileum. The distribution of mast cells between the various layers of the ileum was determined by counts on serial sections. The number of mast cells per section varied from 50 to 200, but the proportion found in each layer was surprisingly constant. The percentages, with standard errors, were

Longitudinal muscle	2·3% ±0·3%
Circular muscle	4·7% ±0·5%
Mucosal area	93·1% ±0·7%

These figures show that most of the mast cells lie in the mucosal area. There are nevertheless fairly large numbers of mast cells in the longitudinal muscle. When this muscle layer is spread on a slide, like mesentery, it can be fixed and stained for mast cells. There is great variation from animal to animal, but in general the following pattern of mast cell distribution occurs in the outer layers:

(i) One plane of mast cells is associated with the longitudinal muscle and usually oriented in the same direction as these fibres (see Plate 1a).

(ii) Another plane is closely associated with the cellular elements of Auerbach's plexus. This can be seen in Plate 1, where a preparation stained supravitally with methylene blue to demonstrate the plexus (b) may be compared with a similar preparation stained with toluidine blue to show the mast cells (c).

(iii) A third plane lies in close association with the circular muscle, the cells again oriented in the direction of the muscle fibres (Plate 1d).

Although most tissues from sensitized guinea-pigs manifest a decrease in mast cell content after exposure to antigen, this had not appeared to be the case with the

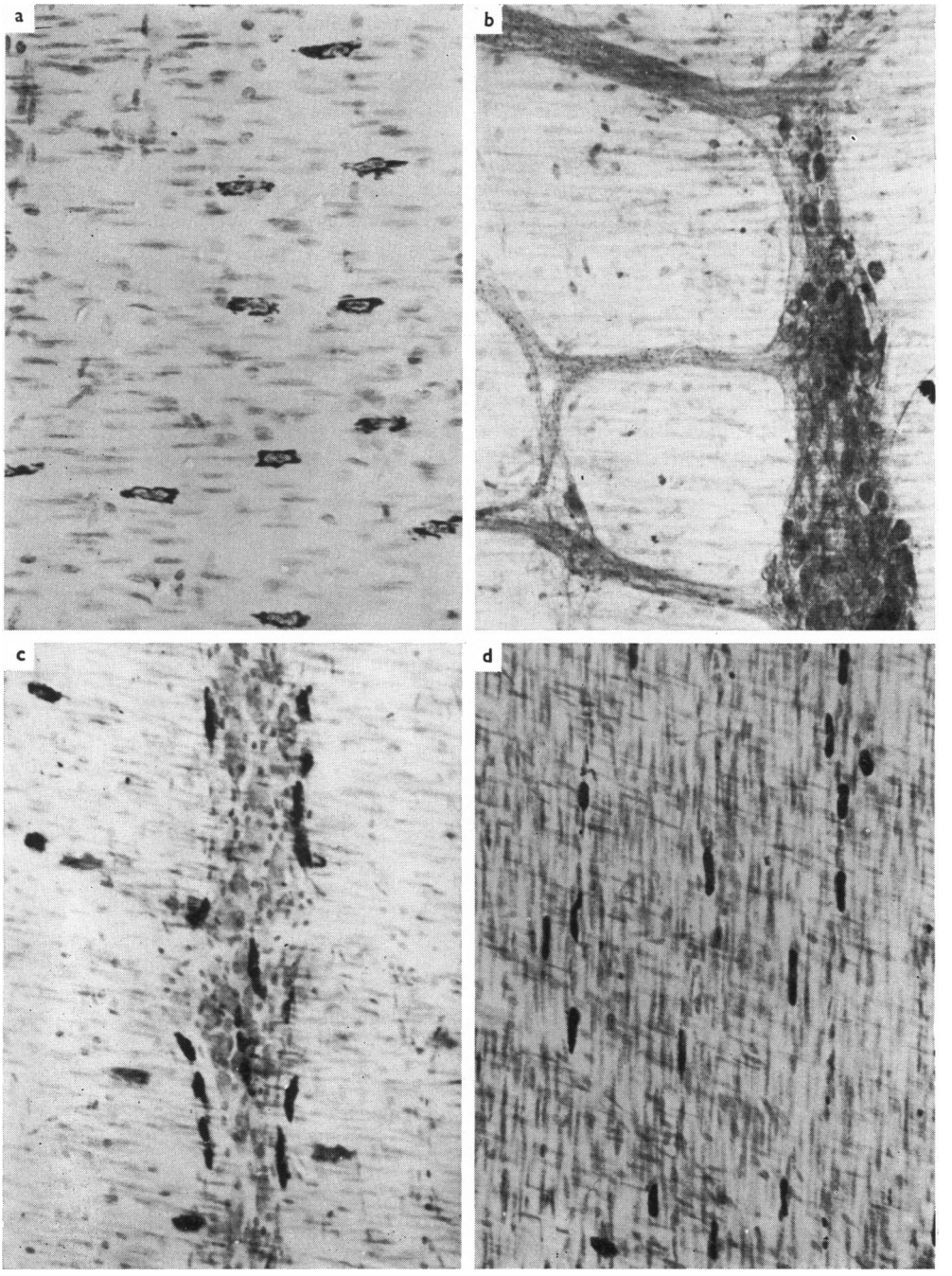

PLATE 1. Photographs of spreads of the muscle coats of guinea-pig ileum (×360). (a) Outer, longitudinal muscle coat, stained with toluidine blue 1%. The plane of the muscle fibres is horizontal and mast cells can be seen oriented with the muscle fibres. (b) Outer longitudinal muscle coat and Auerbach's plexus, stained supravitally with methylene blue to show neuronal elements. (c) Outer longitudinal muscle coat and Auerbach's plexus stained with toluidine blue to show mast cells, visible as dark dots, associated with the cellular elements of the plexus. (d) Circular muscle layer stained with toluidine blue to show a further plane of small, elongated mast cells lying between the bundles of muscle fibres, which are running vertically.

ileum. In the present study, however, with longitudinal muscle strips, a decrease in mast cell content with increasing doses of antigen was readily apparent (Fig. 1). It can be seen that even after high doses of antigen some mast cells remain.

Histamine-content and histamine release

The histamine content of full-thickness ileum was found to be 20·7 μg/g wet weight (S.E. $\pm$1·6) and that of the longitudinal muscle strip 8 μg/g (S.E. $\pm$1·9). The percentage of histamine released by antigen was, however, significantly greater in the strip. Challenge with egg albumin 10^{-3} g/ml elicited a release of 18·7% (S.E. $\pm$2·3) of the histamine content of the strip and 4·8% (S.E. $\pm$2·1) of the histamine content of the ileum. In earlier experiments (in which we used fewer samples and for which we had not completely standardized the technique used) there was considerable variability in histamine-release and on one occasion we obtained as much as 39% histamine-release from the longitudinal muscle. The variability may have been due, in part, to seasonal variations or to differences between different batches of guinea-pigs.

Anaphylactic dose-response curve

The anaphylactic dose-responses of both full-thickness ileum and the longitudinal muscle strip are shown in Fig. 2. The records represent the cumulative effect of

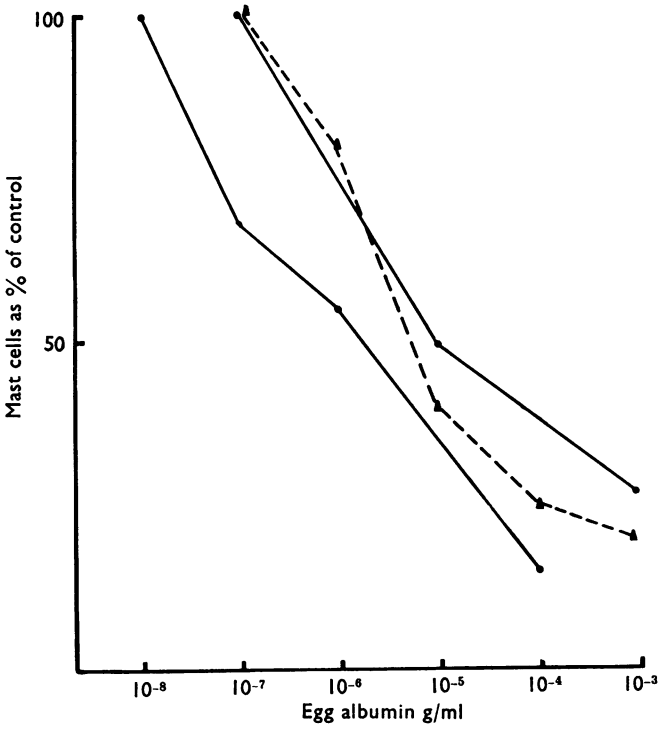

FIG. 1. Dose-response curve for mast cell decrease with antigen. The continuous lines give the results of two experiments with ileal longitudinal muscle strips. Each point represents the mean results of three separate preparations of strip. For each preparation the mast cell count was expressed as a percentage of an adjacent control strip. Results reported by Mota (1959) with mesentery (broken line) are included for comparison.

successive increasing doses of antigen in each individual preparation and thus show desensitization in the higher concentrations. We felt justified in using this approach because it was shown by Okpako (1967) that the dose-response curve obtained in this way was not significantly different from that obtained when the response to each dose is elicited from a fresh preparation. In most of the previous studies of factors affecting the Schultz-Dale response, near maximal doses of antigen have been used. We felt that in a response as complex as the anaphylactic reaction it would be of more value to examine the effect of potential antagonists on the dose-response curve.

Role of Auerbach's plexus in the Schultz-Dale response

It has been reported that, in smooth muscle, effects produced by nerve stimulation are abolished by tetrodotoxin (Kao, 1966 ; Gershon, 1967). In the present study, this substance in a concentration of 5×10^{-7} g/ml eliminated the response of the muscle to nicotine 10^{-5} g/ml and to DMPP, 10^{-5} g/ml, in both whole ileum and strips, while leaving the response to histamine unaffected. The anaphylactic dose-response curve was not significantly altered in the presence of tetrodotoxin (Fig. 3). In another experiment the anaphylactic dose-response curve was found to be the same in both innervated longitudinal muscle strips and strips which had been denuded of plexus by the technique described by Paton & Zar (1968).

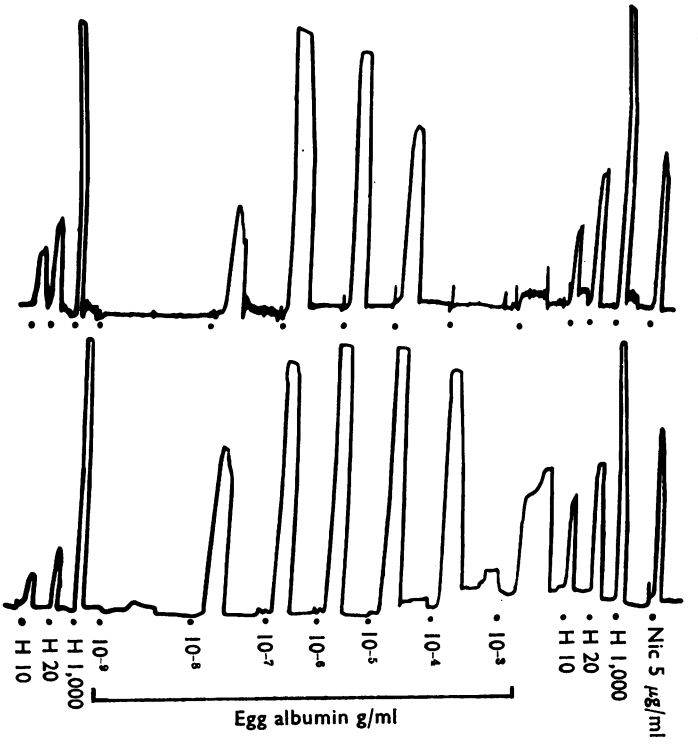

FIG. 2. Anaphylactic contractions to successive increasing concentrations of antigen in the longitudinal muscle strip (upper record) and full-thickness ileum (lower record). Each dose of antigen was left in the bath for 3 min before being washed out. H, Histamine, the concentration being given in ng/ml. Nic, Nicotine.

Role of the mast cells in the Schultz-Dale response

Mota (1959) had reported almost complete elimination of mast cells from mesentery with octylamine. In the present study we found that exposure of the longitudinal muscle strips to octylamine 10^{-3} g/ml for 1 min decreased the mast cell count by 95–100% as compared with adjacent control strips (Fig. 4). The histamine content of the strips was reduced from 5·9 to 2·6 μg/g wet weight by octylamine, but there was no significant change in the histamine content of full-thickness ileum. After exposure to octylamine 10^{-3} g/ml for 1 min the longitudinal

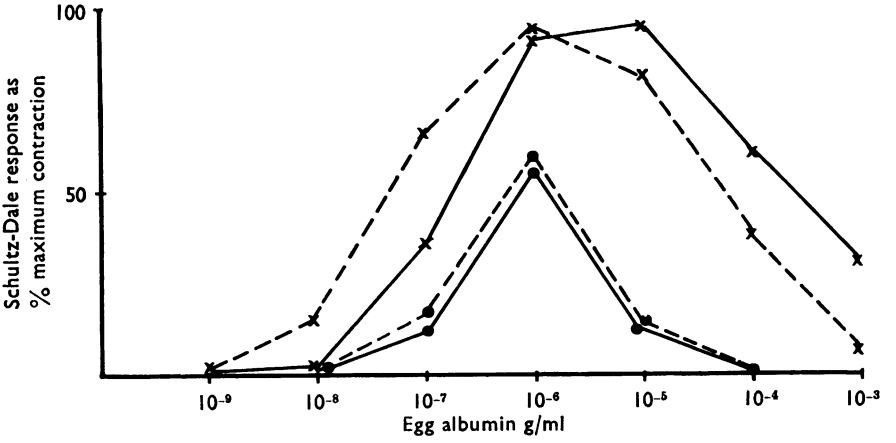

FIG. 3. Anaphylactic dose-response curves in the presence of tetrodotoxin 10^{-7} g/ml (continuous lines), as compared with the dose-response curves in control preparations (dotted lines) ×, Whole-thickness ileum; ●, longitudinal muscle strip.

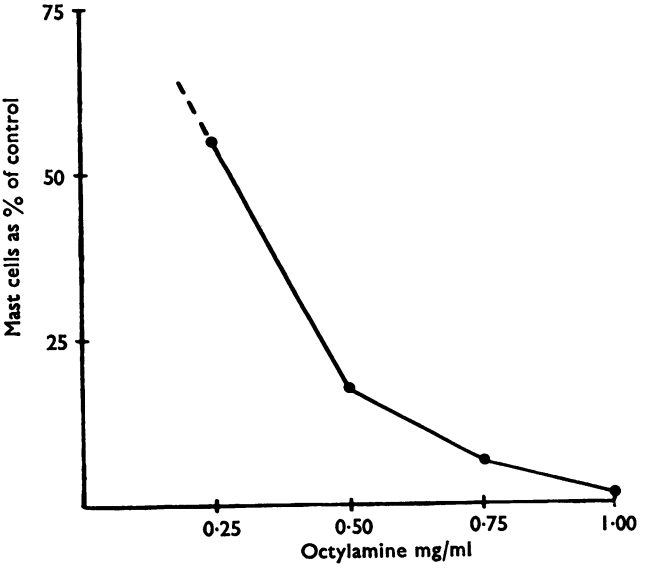

FIG. 4. Dose-response curve for mast cell decrease with octylamine in the longitudinal muscle strip. The mast cell content for each strip exposed to octylamine is expressed as a percentage of the count in an adjacent control strip. Each point represents the mean of three strips from each of two guinea-pigs.

muscle strip still gave a contractile response to small concentrations (5–10 ng/ml) of histamine but gave no response to antigen (Fig. 5). The anaphylactic response of the whole ileum was reduced but not abolished.

When, after octylamine treatment, the longitudinal muscle strips were challenged with single very high doses of antigen (egg albumin 10^{-3} g/ml, which usually produces a maximum contraction), small responses of about 6% of the maximum contraction were sometimes seen. This was felt to be due to the fact that in some strips 1–5% of the mast cells may remain after exposure to octylamine.

In other experiments the strips were exposed to octylamine and then challenged with a high dose of antigen, egg albumin 10^{-3} g/ml. Virtually no contraction occurred. But when sensitized tissue, rich in mast cells (for example, mesentery),

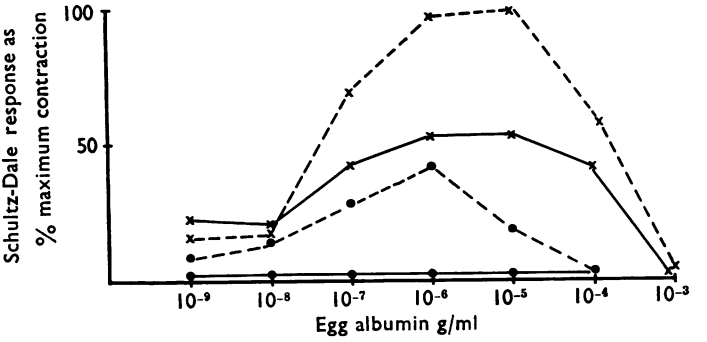

FIG. 5. Anaphylactic dose-response curves after pretreatment with octylamine 1 mg/ml for 1 min (continuous lines) as compared with the dose-response curves of control preparations (dotted lines). ×, Whole-thickness ileum ; ●, longitudinal muscle strips.

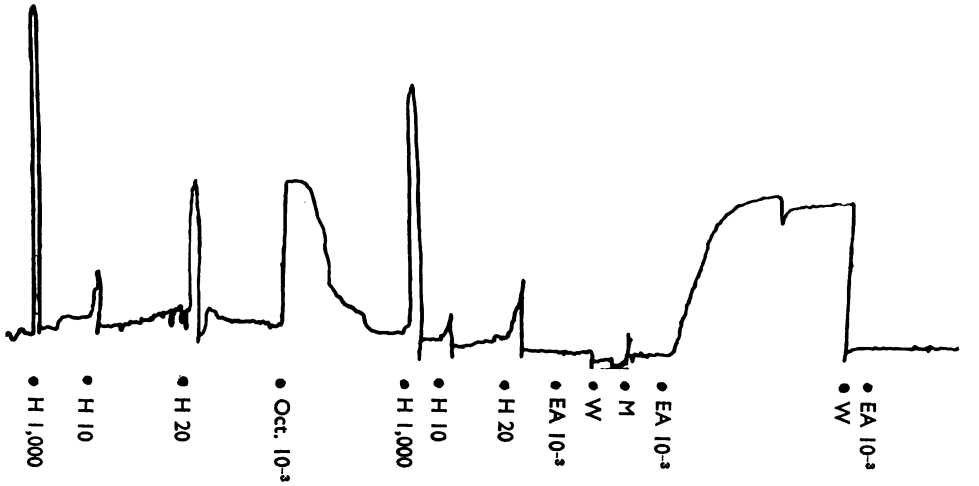

FIG. 6. Effect of challenging the longitudinal muscle strip with antigen in the presence of sensitized mesentery, after the anaphylactic response of the strip had been eliminated by prior treatment with octylamine. H, Histamine (concentrations expressed as ng/ml); Oct, octylamine (g/ml); EA, egg albumin; W, wash. Mesentery added to bath at M and left in during subsequent 15 min challenge with egg albumin.

was added to the bath, and the challenge with egg albumin was repeated, an anaphylactic response invariably occurred, demonstrating that after octylamine treatment the muscle was still capable of responding to the mediators of anaphylaxis (Fig. 6).

One possible explanation for the results with octylamine is that the octylamine inhibits the anaphylactic reaction by interfering with the active sites of antibodies which may be fixed on the smooth muscle cells. To test this, passive sensitization of strips was carried out with antibody which had been pretreated with octylamine 10^{-3} g/ml. The anaphylactic response of such strips was no different from that of strips sensitized with untreated antibody (Fig. 7).

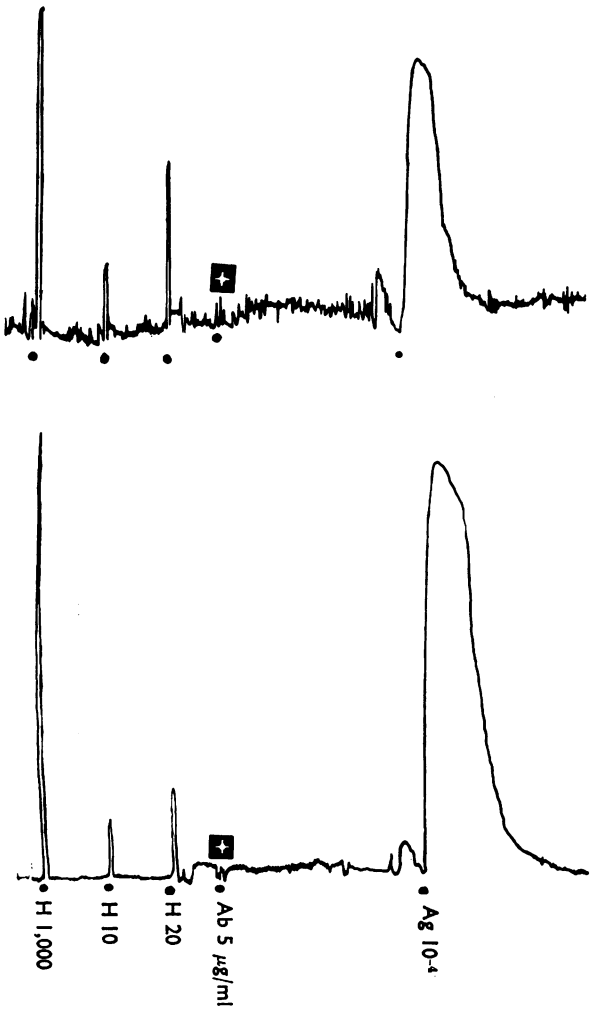

FIG. 7. Anaphylactic responses to antigen in the longitudinal muscle strip after passive sensitization with purified γ_1 antibody (+). In the lower record the antibody (Ab) had been pretreated with octylamine 1 mg/ml for 1 min and then dialysed against Krebs solution for 12 h. Ag, Dinitrophenyl-human-serum albumin ; H, histamine, the figures denoting concentration in ng/ml.

Sequence of events in the anaphylactic contraction in the ileum

The form of the Schultz-Dale response of full-thickness ileum is constant and very typical. There are three phases—an initial very fast contraction, followed by a partial relaxation, followed by a further long-sustained contraction. Hawkins & Rosa (1956) reported that the first quick response, but not the sustained contraction, could be eliminated by an antihistamine. The implication was that the first contraction was due to histamine and the second possibly due to other substances. The Schultz-Dale reaction of the longitudinal muscle strip comprises virtually only the first two phases (Fig. 8). On Hawkins & Rosa's interpretation the longitudinal muscle strip could be manifesting only the histamine-mediated response. When the effect of mepyramine 10^{-7}M was tested, however, the results were as follows: the mean anaphylactic contraction of control strips was 97% (standard error 1·1%) and that of the mepyramine-treated strips was 17% (standard error 4·3%). In only four of the thirteen mepyramine-treated strips was there no response at all to antigen. On this evidence one could not say categorically that the response of the longitudinal muscle strip was due to histamine and only histamine.

One explanation for the short-lived anaphylactic contraction of the longitudinal muscle preparation is that a " relaxation-factor " might be released. Anaphylactic

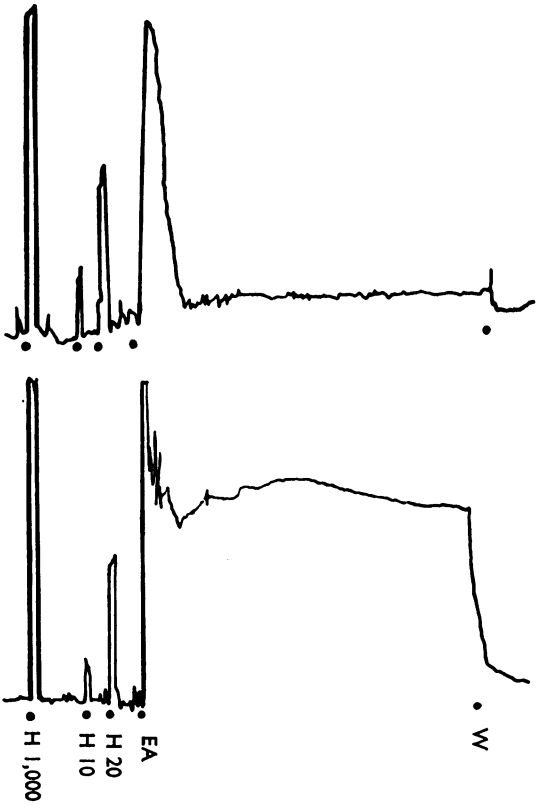

FIG. 8. Anaphylactic responses to 15 min challenge with single large doses of antigen egg albumin 10^{-8} g/ml (EA) in the longitudinal muscle strip preparation (upper record), and full-thickness ileum (lower record). H, Histamine, the figures denoting concentration in ng/ml; W, wash.

relaxation of guinea-pig tracheal muscle has been reported by Alonso-de Florida & Cordoba (1965). Holman & Hughes (1965) have considered the possible existence of intrinsic inhibitory neurones in Auerbach's plexus which could release a transmitter producing relaxation.

We tested whether a " relaxation-factor " was implicated in the second phase of the Schultz-Dale reaction by adding the immersion fluid of sensitized strips challenged with antigen to normal strips in which the tone has been increased with neostigmine $2{\cdot}5 \times 10^{-8}$ g/ml in Krebs solution containing mepyramine. In five sets of experiments we were unable to obtain any convincing evidence of a " relaxation-factor " produced by antigen, although a dose-response relaxation could be obtained with adrenaline.

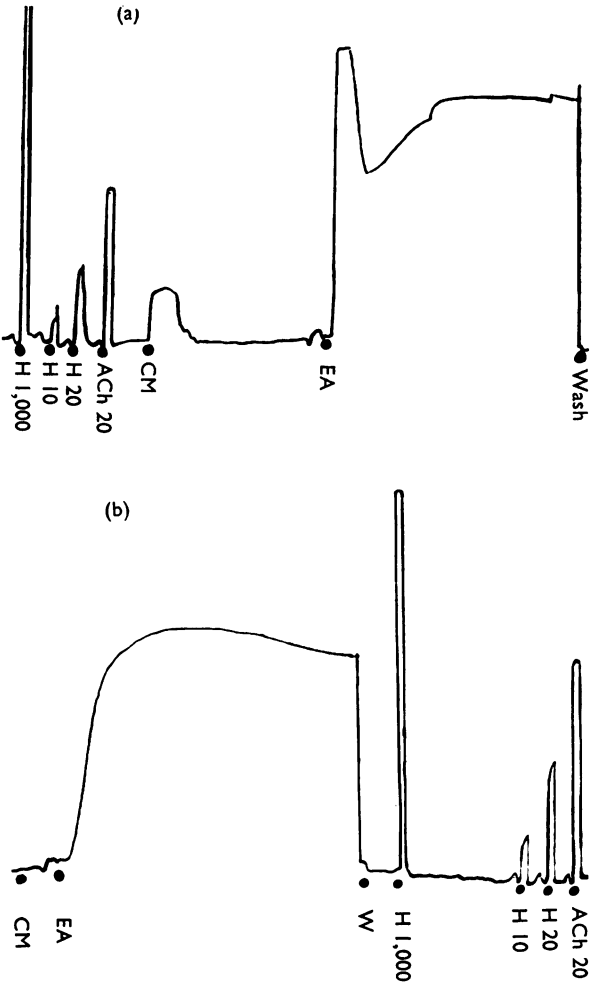

FIG. 9. Anaphylactic responses of longitudinal muscle strips to 15 min challenge with single doses of egg albumin 10^{-3} g/ml (EA). For the first anaphylactic response, EA in record (a), the preparation consisted of sensitized strip, with circular muscle+mucosa loose in the bath, added at CM. For the second anaphylactic response in record (b) the preparation consisted of desensitized longitudinal muscle strip with circular muscle+mucosa loose in the bath. H, Histamine ; ACh, acetylcholine, the concentrations being given in ng/ml ; W, wash.

In the longitudinal muscle preparation, high doses of antigen (10^{-3} g/ml) produced as much relaxation in the presence of tetrodotoxin, 10^{-7} g/ml, as in control strips. This suggested that if any inhibitory neurones were present they were contributing as little to the anaphylactic response as the other elements of the plexus (Dale & Zilletti, 1969).

If there is no relaxation-producing mediator, a possible explanation for the short-lived initial contraction of ileal muscle is that it is due to mediators released only from the small proportion of mast cells lying amongst the longitudinal muscle fibres. This would produce a very high concentration at the site of release, but as the gradient between these sites and the rest of the bath fluid would be very steep, the concentration would fall rapidly and the muscle would relax. The sustained contraction could be due to a second wave of mediators released from other sites deeper in the ileal tissue. To test this we compared the anaphylactic responses to egg albumin 10^{-3} g/ml in the following two preparations.

(*a*) Sensitized longitudinal muscle strip set up for recording, with sensitized mucosa + circular muscle present in the bath.

(*b*) Desensitized longitudinal muscle strip set up for recording, with sensitized circular muscle + mucosa present in the bath.

The main difference between these two preparations is that in (*a*) there are sensitized mast cells *in* the longitudinal muscle, whereas in (*b*) there are none.

Preparation (*a*) consistently gave the classical three-phase Schultz-Dale reaction (Fig. 9a), whereas preparation (*b*) gave only the slow sustained contraction (Fig. 9b).

Discussion

When the longitudinal muscle strip preparation, instead of full-thickness gut, is used for an analysis of the Schultz-Dale reaction, ileal tissue is not as different from other tissues in its responses as it has previously appeared to be. In the present study the percentage of histamine released from the strip by antigen was comparable with that released from lung, and there was a dose-response decrease in mast cells with increasing concentration of antigen comparable with that found in the mesentery.

It had been considered that the intrinsic neuronal networks might be involved in the Schultz-Dale response. Autonomic modulation of anaphylactic histamine release has been suggested by Giotti *et al.* (1966) for guinea-pig heart, and the moderating effect of adrenergic factors on anaphylactic bronchoconstriction in the guinea-pig *in vivo* can be seen from the work of Collier & James (1967). But from the results obtained in the present study it appeared that Auerbach's plexus was not essential for, and probably not implicated in, the Schultz-Dale response, either as regards the release of acetylcholine and of other postulated transmitters (Ambache & Freeman, 1968) which might contribute to the contractile phase of the response, or as regards a "relaxation-factor". This conflicts with the interpretation of the mechanism of the reaction put forward by Geiger & Alpers (1959).

Another question considered in this study was to what extent the mast cells were involved in the anaphylactic response of ileal smooth muscle. We came to the conclusion that the Schultz-Dale response in the longitudinal muscle strip depended mainly on the mast cells or other mediator-releasing cells and that a direct effect of

the antigen-antibody reaction on the muscle was probably not involved. This conflicts with the opinion of Alonso-de Florida *et al.* (1968) based on work on the sensitized denervated guinea-pig diaphragm. Using intracellular electrodes they recorded changes of potential in this tissue when antigen was added by microtap. They interpreted their findings to mean that the antigen-antibody reaction was having a direct effect on the muscle, pointing out that the time course of the changes of potential favoured this mechanism rather than the alternative possibility that the depolarization was due to mediators released from mast cells. However, the *contraction* of the sensitized denervated diaphragm produced by antigen was apparently inhibited by an antihistamine (Alonso-de Florida *et al.*, 1965). In this connexion it is interesting to note that the tendinous part of guinea-pig diaphragm is immensely rich in mast cells, and also that during an anaphylactic response to antigen the diaphragm releases a greater proportion (43%) of its histamine than almost any other tissue in the guinea-pig (Mongar & Schild, 1952).

It is possible that the mechanism of the anaphylactic reaction is different in ileal smooth muscle and denervated skeletal muscle. Further work is needed to clarify the issue.

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