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A QUANTITATIVE STUDY OF REPEATED ANAPHYLACTIC REACTIONS IN ISOLATED TISSUES

BY

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The well-established principle of quantitative biological investigations, based upon the comparison of successive responses in the same animal or tissue, is not readily applicable to anaphylactic reactions. Each anaphylactic reaction in a sensitized animal induces a condition in which responses to subsequent doses of antigen are modified (Besredka, 1907; Anderson & Rosenau, 1908; Weil, 1913), thus rendering direct comparison impossible. This induced state of densitization has been shown to apply similarly to anaphylactic reactions *in vitro* (Schultz, 1910; Dale, 1913).

In a recent paper, Hicks & Leach (1963) have shown that the repeated administrations of the same dose of antigen in the same animal at regular intervals results in a series of anaphylactic responses, the severity of which decreases exponentially. The rate of desensitization was related to the dose of antigen used. In the whole animal this finding is of interest with regard to the development of more reliable quantitative evaluation of anaphylactic reactions. A similar investigation has therefore been undertaken into the anaphylactic desensitization of isolated guinea-pig tissues.

METHODS

Sensitization of animals. Virgin female albino guinea-pigs (Dunkin Hartley strain), 300 to 500 g body weight, were sensitized by a single intraperitoneal dose of horse serum (2 ml./kg). All animals were used 21 to 24 days after sensitization.

Production of anaphylactic responses in isolated smooth muscle preparations. Anaphylactic responses to doses of antigen in isolated sensitized uterus and ileum preparations were recorded by the method of Schultz (1910) and Dale (1913) in an organ-bath containing oxygenated Tyrode-Ringer solution at 37° C. Doses of antigen were added to the bath and remained in contact with the tissue for 15 min, being then replaced by three changes of bath fluid. The maximal response of the muscle, obtained during the first 5 min of the reaction, was recorded. This procedure was repeated at 20-min intervals until no further responses to the antigen were observed. Adjacent segments of the same tissue were similarly treated using different doses of antigen, and the experiment was repeated on tissue from several guinea-pigs.

Release of smooth muscle-stimulating substances during anaphylactic reactions in sensitized lung tissue. Anaphylactic release of smooth muscle-stimulating substances from isolated sensitized lung tissue was determined according to the method of Nicoll & Campbell (1940) and Campbell & Nicoll (1940). Segments of nonsensitized guinea-pig ileum were maintained in an organ-bath of oxygenated Tyrode-Ringer solution at 37° C. Portions of lung tissue weighing approximately 1 g were isolated from freshly killed sensitized guinea-pigs, and perfused free of blood with warmed Tyrode-Ringer solution. These portions of sensitized lung tissue were placed in the organ-bath with the nonsensitized guinea-pig ileum. Addition of antigen to

the bath fluid produced a release of smooth muscle-stimulating material from the lung tissue, revealed by a slow, sustained contraction of the ileal muscle. The same dose of horse serum antigen was administered to the bath at 30-min intervals, remaining in contact with the tissue for 20 min. Before each addition of antigen the bath fluid was changed three times and the ileum was allowed to relax to its former resting level. Other portions of tissue from the same lung were similarly treated using different doses of antigen, and the experiment was repeated on tissue from several guinea-pigs.

RESULTS

Desensitization in isolated preparations of guinea-pig uterus or ileum

When the same dose of horse serum antigen was repeatedly administered at 20-min intervals to isolated segments of either sensitized guinea-pig ileum or uterus, a series of responses was obtained which successively decreased in magnitude. An illustration of a

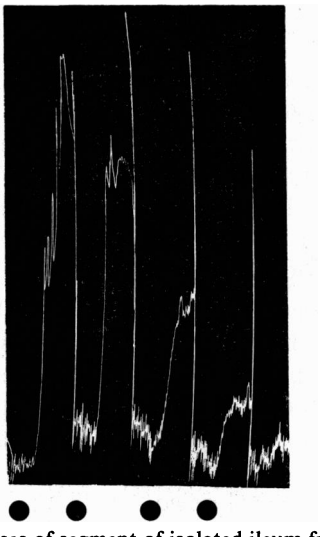

Fig. 1. Kymograph record of responses of segment of isolated ileum from a guinea-pig sensitized to horse serum. At the dots, horse serum (0.4 ml./100 ml.) was added to the bath at 20-min intervals.

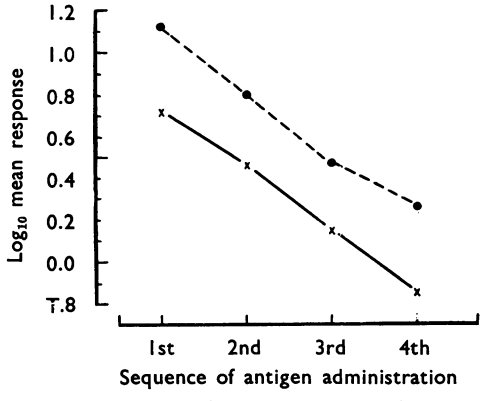

Fig. 2. Curves showing mean heights of successive responses to antigen, on log scale, plotted in order of succession. Both curves express responses to repeated administrations of horse serum antigen (0.4 ml./100 ml.), ●– – –● denoting responses of guinea-pig ileum, and ×——× of guinea-pig uterus.

typical series of reactions is shown in Fig. 1. When the heights of contraction are plotted in their order of sequence a curve is obtained which appears to follow an exponential pattern. This curve is transformed to a straight line when the log of the response is plotted (Fig. 2).

Results from groups of tissue segments treated with different doses of antigen are shown in Table 1. It can be seen that, when higher doses of antigen are used, the extent of this desensitization effect increases. The degree of desensitization may be assessed from the negative slope of the straight lines obtained when the log of successive responses to the same dose are plotted in their order of sequence. Values so obtained can be related to the dose of antigen (Fig. 3).

TABLE 1

SUCCESSIVE RESPONSES OF SENSITIZED SMOOTH MUSCLE TO REPEATED DOSES OF ANTIGEN *IN VITRO*

Responses are expressed as mean heights of contractions in cm, and as the relationship of antigen dose to the negative slopes of desensitization curves (means and standard errors). Antigen doses (I, II, III and IV) are successive

Test preparation	Dose of antigen (ml./100 ml.)	No. of tissue segments	Height of contraction (cm) after antigen doses I	II	III	IV	Negative slope of desensitization curves
Guinea-pig ileum	0·1	4	7·0	6·1	4·9	3·4	0·30±0·05
	0·2	6	5·5	3·9	3·1	1·5	0·46±0·09
	0·4	5	13·1	6·1	2·9	1·8	0·95±0·24
	0·8	4	8·2	3·1	1·1	0·4	1·27±0·09
	1·0	4	6·3	2·2	0·8	—	1·32±0·06
Guinea-pig uterus	0·1	3	7·1	5·1	4·5	—	0·28±0·16
	0·2	3	13·6	8·4	5·6	3·9	0·51±0·20
	0·4	4	5·1	2·9	1·3	0·7	0·83±0·08
	0·8	2	5·6	2·1	0·8	—	1·12±0·13
	1·0	3	6·0	1·5	0·3	—	1·74±0·24

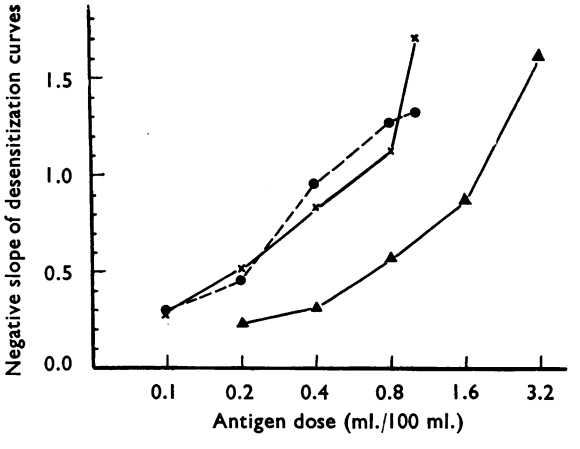

Fig. 3. Curves relating doses of horse serum antigen to negative slope of desensitization curves. ●——● denotes results obtained using guinea-pig ileum, ×——× guinea-pig uterus, and ▲——▲ results from sensitized guinea-pig lung by the method of Campbell & Nicoll (1940). Horse serum antigen dose is expressed as ml./100 ml. in the organ-bath.

This relationship between successive responses to repeated administrations of the same dose of antigen applied to results from both uterus and ileum preparations at all doses of antigen investigated. These results suggest that the progress of desensitization of the isolated smooth muscle follows an exponential pattern.

Desensitization produced in isolated sensitized guinea-pig lung

The severity of the successive anaphylactic reactions in isolated sensitized lung resulting from repeated administrations of the same dose of antigen was indirectly evaluated from the induced responses of nonsensitized guinea-pig ileum. When the same dose of horse serum was administered to the bath at 30-min intervals a successively decreasing series of contractions of the ileum muscle were recorded. As in the previous experiments, when the magnitude of response was plotted linearly or logarithmically in order of sequence, either curves which appeared to be exponential or straight lines were obtained. Results of several experiments using different doses of antigen are shown in Table 2. It may be seen that a greater desensitization effect is produced when higher doses of antigen are used. Evaluation of the desensitization from the negative slope of desensitization curves may be related to antigen doses (Fig. 3).

TABLE 2

SUCCESSIVE RESPONSES OF NONSENSITIZED GUINEA-PIG ILEUM PRODUCED BY ACTIVE MATERIALS RELEASED FROM SENSITIZED GUINEA-PIG LUNG AFTER REPEATED ADMINISTRATIONS OF ANTIGEN

Responses are expressed as mean heights of contractions in cm, and as the relationship of antigen dose to the negative slopes of desensitization curves (means and standard errors). Antigen doses (I, II, III and IV) are successive

Dose of antigen (ml./100 ml.)	No. of tissue segments	Height of contractions (cm) after antigen doses				Negative slope of desensitization curves
		I	II	III	IV	
0·2	3	4·1	3·4	2·9	3·3	0·23±0·10
0·4	5	3·5	3·2	2·3	1·8	0·31±0·04
0·8	4	6·9	4·1	2·6	2·3	0·56±0·07
1·6	4	3·7	1·5	0·9	—	0·87±0·18
3·2	5	6·6	1·9	0·5	—	1·6 ±0·21

From the similarity of these results to those observed using sensitized ileum or uterine tissue, it may be deduced that a similar pattern of desensitization occurs with the release of smooth muscle-stimulating substances to repeated doses of antigen.

DISCUSSION

Many attempts have been made to overcome the difficulties caused by desensitization effects in the quantitative evaluation of anaphylactic reactions *in vitro* or to employ this phenomenon as an indication of the severity of such reactions. Such attempts have not been completely successful as the exact nature of the mechanism of desensitization has not been fully appreciated.

In 1913, Dale enunciated the principle that the dose of antigen which produces a maximal anaphylactic response of plain muscle completely desensitizes it to any further doses of antigen. This principle forms the basis of " end point " methods of assays which depend

upon the concentration of antigen necessary to produce a maximal reaction in sensitized tissue (Moody, 1940; Winter, 1945). Most interest, however, has been applied to attempts to produce multiple successive anaphylactic responses of a comparable nature. Walzer & Grove (1925), Kendall & Shumate (1930) and Kendall (1930) showed repeated contractions of sensitized guinea-pig intestinal strips resulting from repeated administrations of antigen. The qualitative nature of the process of desensitization was thus demonstrated but the quantitative aspects of it were not fully investigated. Alberty (1953a, b) confirmed the possibility of producing repeated anaphylactic responses to successive administrations of the same dose of antigen, and devised a system of ascending and repeated dosage for producing multiple reactions. The size of any reaction produced by this system does not appear to be precisely predictable.

The results of the present investigation show that the responses of guinea-pig isolated sensitized tissue *in vitro* to repeated administrations of the same dose of antigen decrease each time in an exponential manner. The degree of desensitization may be assessed on the basis of the slopes of the desensitization curves and can be related to the dose of antigen. Quantitative results with regard to the process of desensitization can therefore be obtained which are very similar to those for bronchoconstrictor responses of the guinea-pig *in vivo* (Hicks & Leach, 1963). This similarity is revealed in all three tissues used in this investigation, but quantitative differences between each tissue exist with regard to the relationship of the dose of antigen to the effect. It can be stated that the process of desensitization for anaphylactic reactions of the guinea-pig tissue follows the same essential pattern *in vivo* and *in vitro*.

These results also reveal the difficulties involved in attempts to produce multiple anaphylactic reactions which are comparable on a simple quantitative basis. This would involve the prediction of a system of dosage, which must take into account the relationship between antigen dose, response of target organ, degree of desensitization produced by each reaction, and the modification of the response by previous reactions, together with the inherent variability in and between animal tissues. In addition there is a finite limit to the materials available for the production of anaphylactic reactions beyond which complete desensitization must occur. The quantitative value of this approach is therefore doubtful.

The results quoted in this paper do, however, provide a means of predicting the relative sizes of anaphylactic responses to successive administrations of the same dose of antigen. Either this information or the relationship between the antigen dose and the degree of desensitization could, with suitable experimental design, form a basis for precise investigation into some aspects of anaphylactic reactions. A valuable point with regard to the utilization of this information experimentally is that it depends upon the use of submaximal doses of antigen, measured in terms of submaximal responses; whereas difficulty may arise in the use of " end-point " methods for investigating tissue sensitization due to the possibility of maximal reactions occurring in one or more of the components of the anaphylactic response. It is also a valuable point that the desensitization effect is independent of the size of the tissue employed. In the case of experiments based upon comparison of single Schultz-Dale responses, the control of the size of tissue is of particular importance and difficulty. In view of the probable complexity of the active material released in such responses the frequent use of comparison with standard doses of histamine must be considered of doubtful validity.

SUMMARY

1. Desensitization produced by repeated administrations of the same dose of antigen has been investigated in isolated preparations of uterus, ileum and lung from guinea-pigs sensitized to horse serum (single intraperitoneal dose, 2 ml./kg) for 21 to 24 days.

2. Repeated administrations of antigen at constant time intervals produces a series of anaphylactic responses, which decrease with successive doses in an exponential pattern.

3. When the logs of heights of contraction are plotted in sequence a straight line is produced (desensitization curve).

4. The negative slope of the desensitization curves may be related to the dose of antigen.

5. It is suggested that this approach may form the basis for reliable quantitative investigation of anaphylactic reactions *in vitro*.

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