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QUANTITATIVE EVALUATION OF GUINEA-PIG ANAPHYLAXIS IN VIVO

BY

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Two methods for recording bronchoconstrictor responses to antigen injected into sensitized guinea-pigs have been used to show that the dose/response relationship is a normal pharmacological one characterized by a sigmoid curve. Studies of desensitization indicate that the progressive decrease in sensitivity to repeated administrations of the same dose of antigen follows an exponential pattern. It is suggested that this approach might form a basis for reliable quantitative investigations of anaphylactic phenomena.

The methods of quantitative evaluation of anaphylaxis in guinea-pigs all suffer in that they are concerned with indirect systemic effects rather than the primary anaphylactic response; for example the quantal evaluation of mortality (Coulson & Stevens, 1949), the semi-quantitative "scoring" method (Humphrey, 1951) and the preconvulsion-time measurements described by Herxheimer (1952). These methods may be subject to wide quantitative variation which would be eliminated if, in the evaluation of guinea-pig anaphylaxis, the primary response of the main target organ (the bronchial smooth muscle) were measured directly.

A method for the assessment of guinea-pig anaphylaxis has therefore been investigated in which the bronchoconstrictor response has been recorded and related to the dose of antigen administered. Further quantitative information has been sought from an investigation of the mechanism of desensitization.

METHODS

Female albino guinea-pigs (Duncan Hartley Strain, War Department, Porton Down), of 300 to 400 g body weight and sensitized 21 days earlier to horse serum (2 ml./kg, intraperitoneally), were anaesthetized with either pentobarbitone sodium (60 mg/kg) or urethane (1.5 g/kg).

Drugs and challenging doses of antigen were administered intravenously through a cannula introduced into an external jugular vein, and washed in with 0.5 ml. of heparinized saline. The bronchiolar tone, as indicated by the "resistance" to positive pressure inflation, was determined using either the constant pressure method described by Konzett & Rössler (1940) or the constant volume method of Dixon & Brodie (1903). Artificial ventilation was maintained throughout the experiment from a miniature Starling Ideal Pump, and the pressure recording system was attached to a side-arm of the tracheal cannula. The stroke of the ventilation pump was adjusted to produce a volume of 1 ml. of air per 100 g body weight plus an amount equivalent to the dead space of the apparatus, at a rate of 36 strokes/min. Using the Konzett & Rössler technique a pressure of 8 cm of water gave optimal results.

A typical record of the severity of the bronchoconstriction produced in response to horse serum antigen is shown in Fig. 1. Its measurement may be attempted in two ways: (i) by measuring the maximal increase in bronchiolar constriction, as indicated by the highest excursion of the pressure-recording lever; or (ii) by assessing the "integrated" increase in bronchiolar tone during the complete period of the anaphylactic response, expressed as the increased area traversed by the recording lever above the continuation of the level of the control record.

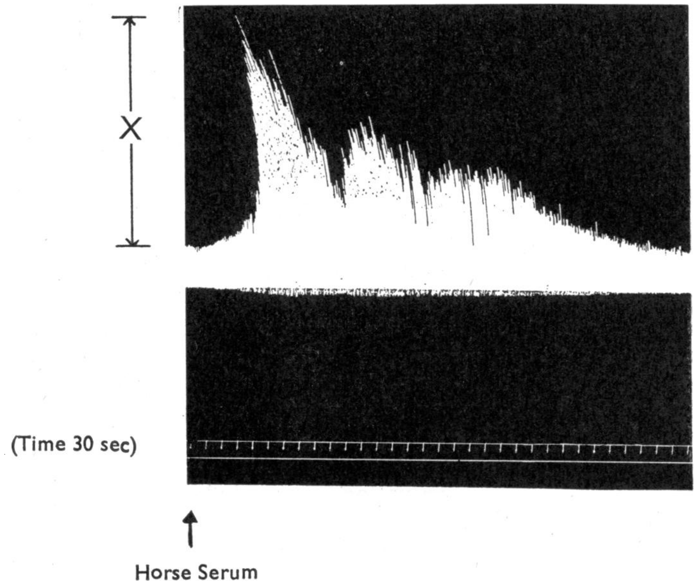

Fig. 1. Bronchoconstriction produced by injection of antigen into sensitized guinea-pigs (method of Konzett & Rössler, 1940), to show method of evaluating increased resistance to inflation. At the arrow, 0.8 ml./kg of horse serum antigen was injected intravenously. X=decrease in tidal volume due to maximal increase in bronchiolar constriction.

A comparison of the two methods from forty records showed a correlation coefficient of 0.992 (39 d.f.) and, for simplicity, the method (i) using the maximal excursion of the lever was chosen for all further work. The severity of a bronchoconstriction has been assessed as the percentage decrease in tidal volume, calculated from the increase in amplitude of the kymograph record during bronchoconstriction (X) and the maximal increase in excursion (Y) when the bronchi are totally occluded by clamping the tracheal bifurcation (Fig. 2).

RESULTS

Relationship of the bronchoconstrictor response to the dose of antigen

The degree of bronchoconstriction produced in response to a dose of horse serum antigen was determined for each animal in a group of uniformly sensitized guinea-pigs. The mean values from groups receiving different doses of antigen, either with sodium pentobarbitone or with urethane anaesthesia, are shown in Table 1,

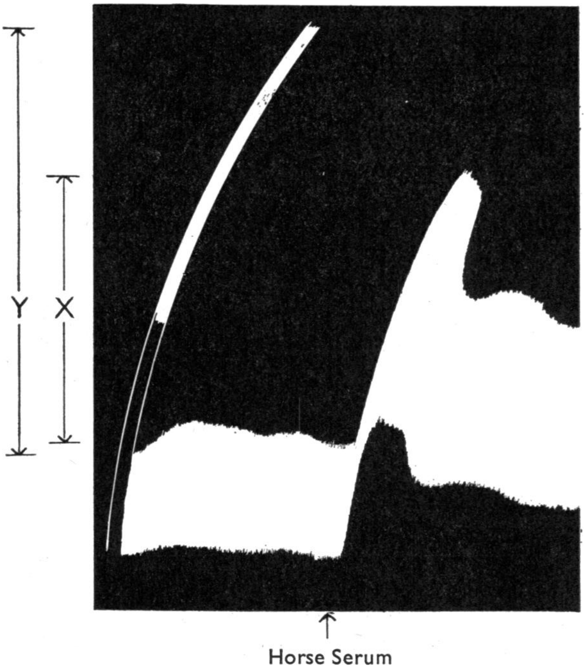

Fig. 2. Bronchoconstriction produced by injection of antigen into sensitized guinea-pigs (method of Dixon & Brodie, 1903) to show method of assessing severity of increased resistance to inflation. At the arrow, 0.8 ml./kg of horse serum antigen was injected intravenously. X= decrease in tidal volume due to maximal increase in bronchiolar constriction. Y=maximal excursion produced with total bronchial occlusion. Bronchoconstriction is assessed as the % reduction in respiratory volume, or 100 X/Y.

TABLE 1

BRONCHOCONSTRICTOR RESPONSES OF SENSITIZED GUINEA-PIGS PRODUCED BY DIFFERENT DOSES OF ANTIGEN

Tidal volumes are mean percentage decreases with standard errors

	Constant volume method		Constant pressure method	
Dose of antigen (ml./kg)	Decrease in tidal volume (%)	No. of animals	Decrease in tidal volume (%)	No. of animals
Pentobarbitone anaesthesia				
0·1	17·6±1·4	7	19·95±2·3	5
0·2	21·2±3·3	3	29·6 ±2·2	4
0·4	50·5±1·3	3	57·9 ±1·6	5
0·8	69·8±3·1	4	83·5 ±1·22	6
1·0	76·5±2·7	4	84·25±1·76	2
Urethane anaesthesia				
0·1	20·5±3·1	5	23·3 ±5·2	3
0·2	21·2±2·9	5	25·6 ±1·72	4
0·4	55·4±1·7	5	62·1 ±4·16	4
0·8	73·8±4·5	6	89·2 ±1·82	4
1·0	80·0±3·3	3	85·6 ±7·87	3

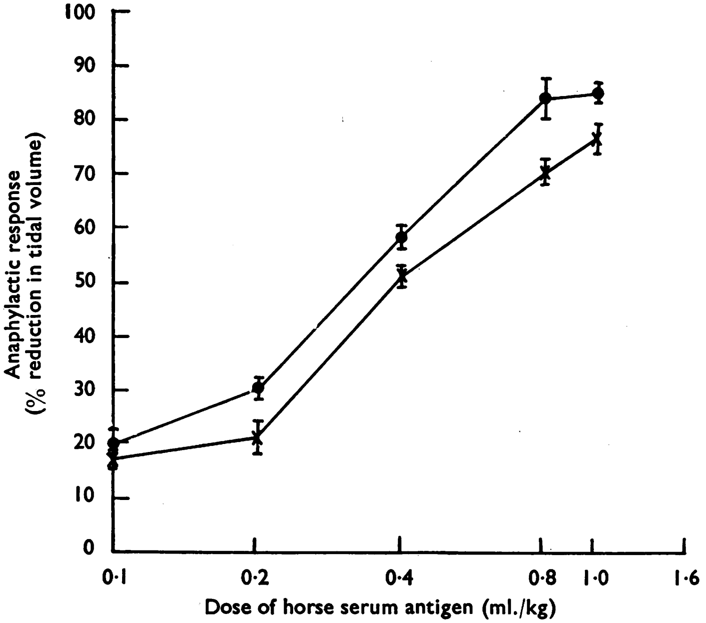

Fig. 3. Relationship of dose (ml./kg) of horse serum antigen (abscissa) to severity of anaphylactic response (ordinate) in guinea-pigs anaesthetized with sodium pentobarbitone (60 mg/kg). ● —— ● method of Konzett & Rössler (1940). x —— x method of Dixon & Brodie (1903).

and a typical log dose/response curve from these results is seen in Fig. 3. It can be clearly seen that graded responses are obtained with different dose levels. The sigmoid appearance of the curves can be confirmed by conversion of the percentage values of the bronchoconstrictor response to probits, which reveals a straight-line relationship. It is therefore possible to relate the severity of submaximal anaphylactic responses to the dose of antigen.

Dose-effect relationship in the progress of anaphylactic desensitization

Desensitization was obtained by giving the same dose of antigen repeatedly to the same animal at 30 min intervals, and the severity of each response was assessed. Results from five groups of animals, treated with different dose levels of antigen, are summarized in Table 2, and illustrated for a given antigen dose series in Fig. 4.

These results show that the severity of the anaphylactic response decreases with successive doses of antigen, and that the extent of this decrease depends on the dose of antigen. When the degree of bronchoconstriction is plotted linearly in order of sequence, as in Fig. 5, a curve is obtained which is transformed to a straight line when the log of the size of response is plotted against its order of sequence.

This result suggests that, for a given dose of antigen, the progress of desensitization follows an exponential pattern ; the straight-line relationships seen for each dose level of antigen (Fig. 6) add further support to this contention. The degree of desensitization, determined from the slopes of the curves shown in Fig. 6, can be seen to increase with larger doses of antigen.

TABLE 2

SUCCESSIVE BRONCHOCONSTRICTOR RESPONSES PRODUCED BY REPEATED DOSES OF ANTIGEN

Tidal volumes are mean percentage decreases

Anaesthetic	Group	No. of animals	Dose of antigen (ml./kg)	Decrease in tidal volume (%) 1st response	2nd response	3rd response	4th response
Method of Dixon & Brodie (1903)							
Pentobarbitone	1	7	0·1	17·6	15·5	12·7	—
	2	3	0·2	21·2	15·6	12·0	—
	3	3	0·4	50·5	27·1	14·9	—
	4	4	0·8	69·8	26·7	8·6	—
	5	4	1·0	76·5	16·3	6·1	—
Urethane	1	5	0·1	20·5	16·5	14·2	11·4
	2	5	0·2	21·2	16·9	13·3	10·9
	3	5	0·4	55·4	26·0	13·9	7·2
	4	6	0·8	73·8	23·5	7·4	7·0
Method of Konzett & Rössler (1940)							
Pentobarbitone	1	5	0·1	20·0	17·1	14·9	12·0
	2	4	0·2	29·6	22·9	18·9	15·3
	3	5	0·4	57·9	29·4	14·7	9·2
	4	6	0·8	83·5	28·3	8·67	—
	5	2	1·0	84·3	21·9	6·7	—
Urethane	1	3	0·1	23·3	20·0	16·9	—
	2	4	0·2	25·6	20·4	13·7	—
	3	4	0·4	62·1	33·4	21·5	—
	4	4	0·8	89·2	27·7	9·8	—

A comparison of the residual responses was made by expressing each subsequent response as a percentage of its antecedent. The results of such calculations (Table 3) reveal a close similarity between the percentage values of successive anaphylactic responses to the same dose of antigen, as would be expected in a series in which there was an exponential relationship. The values for the residual responses indicate that there is a constant proportional decrease in each successive anaphylactic response to the same dose of antigen, and that larger doses of antigen produce smaller residual responses.

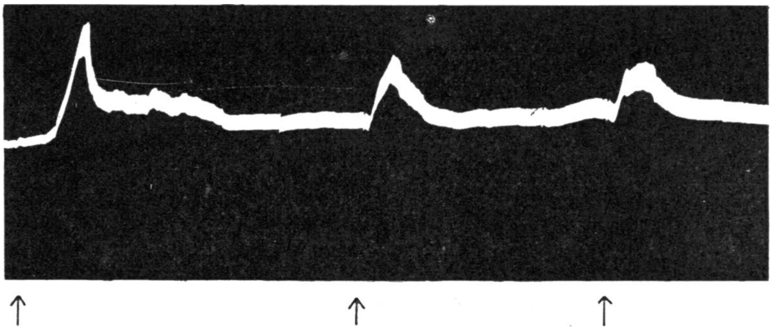

Fig. 4. Bronchoconstriction produced by injection of antigen into sensitized guinea-pigs (method of Dixon & Brodie, 1903). The effect of repeating the dose of antigen at 30 min intervals on the anaphylactic response. 0.2 ml./kg of horse serum antigen was injected intravenously at the arrows.

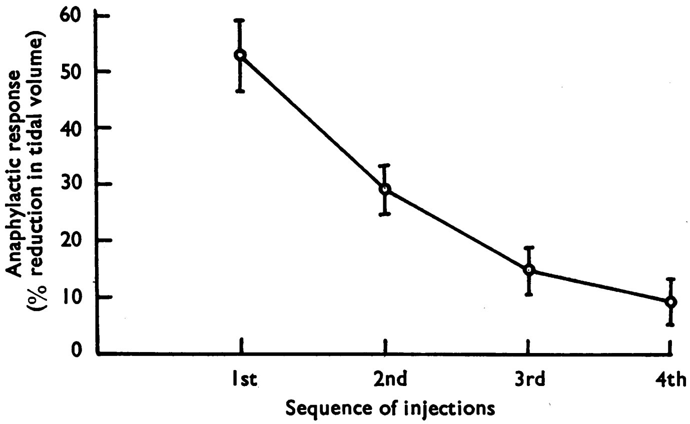

Fig. 5. Relationship between the order of sequence of injections of horse serum antigen (0.4 ml./kg) given at 30 min intervals (abscissa) and the mean severity of the anaphylactic responses produced in a group of five guinea-pigs (ordinate).

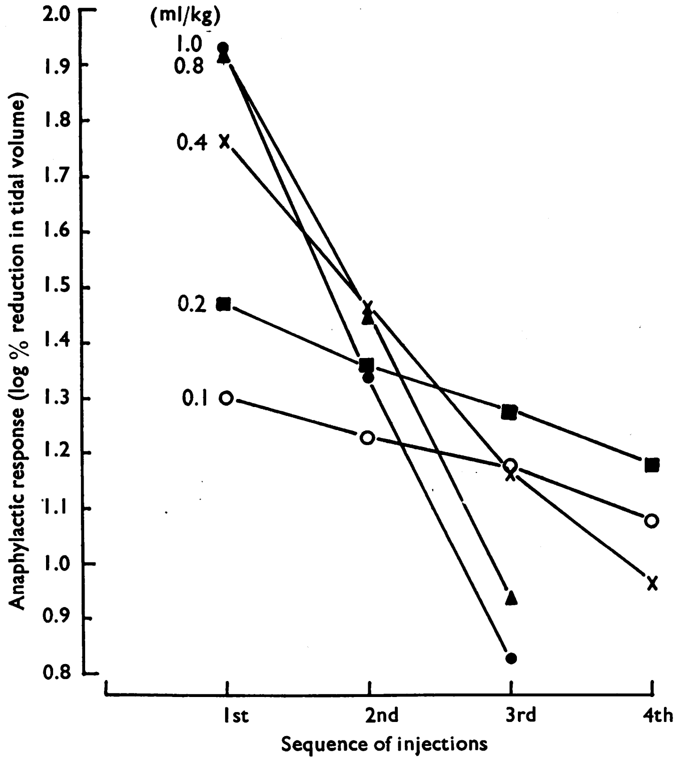

Fig. 6. Relationship between the order of sequence of injections for different dose levels of horse serum antigen given at 30 min intervals (abscissa) and the log of the severity of anaphylactic response produced in guinea-pigs (ordinate). o —— o 0.1 ml./kg, ■ —— ■ 0.2 ml./kg, x —— x 0.4 ml./kg, ▲ —— ▲ 0.8 ml./kg and ● —— ● 1.0 ml./kg of horse serum.

TABLE 3

SUCCESSIVE ANAPHYLACTIC BRONCHOCONSTRICTOR RESPONSES PRODUCED BY REPEATED DOSES OF ANTIGEN

Subsequent responses are expressed in terms of the antecedent response, and the values for three successive responses also given as means with standard errors

Anaesthetic	Group	No. of animals	Dose of antigen (ml./kg)	Mean % relationship of successive responses: 2nd/1st	3rd/2nd	4th/3rd	Mean	*d.f.*
Method of Dixon & Brodie (1903)								
Pentobarbitone	1	7	0·1	88·4	82·3	—	85·6±3·32	12
	2	3	0·2	74·0	78·0	—	75·8±2·0	5
	3	3	0·4	54·2	52·8	—	53·5±2·1	5
	4	4	0·8	37·6	35·4	—	36·6±0·8	7
	5	4	1·0	22·8	36·2	—	29·5±3·5	7
Urethane	1	5	0·1	79·1	89·4	82·3	83·6±2·9	14
	2	5	0·2	80·5	79·3	82·6	80·8±4·8	14
	3	5	0·4	47·6	53·3	52·1	51·0±2·7	14
	4	6	0·8	33·3	27·1	33·3	30·7±6·3	17
Method of Konzett & Rössler (1940)								
Pentobarbitone	1	5	0·1	88·6	82·0	87·1	85·0±4·5	10
	2	4	0·2	77·8	80·7	78·7	79·1±2·9	10
	3	5	0·4	49·4	51·2	57·3	51·7±1·6	9
	4	6	0·8	33·8	30·4	—	32·2±1·5	9
	5	2	1·0	26·2	28·5	—	26·9±2·6	3
Urethane	1	3	0·1	86·5	84·2	—	82·9±1·9	5
	2	4	0·2	80·7	76·0	—	78·7±2·9	7
	3	4	0·4	54·5	65·5	—	60·0±3·6	7
	4	4	0·8	30·0	37·1	—	34·1±2·2	7

DISCUSSION

Previous investigations of guinea-pig anaphylaxis by Koessler & Lewis (1927) and more recently by Friebel & Basold (1953) and by Alberty (1959) have employed methods of recording bronchoconstrictor responses, but no quantitative evaluation was made. Ideally, quantitative studies should relate the severity of the anaphylactic reaction to the dose of antigen, and this approach has formed the basis of several investigations utilizing more indirect effects, notably by Kabat & Landau (1942), Hoene, Coutu, Horava, Procopio, Robert & Salgado (1952) and Coulson & Stevens (1949). The interpretation of such work was, however, open to some doubt since there has been some confusion as to the exact nature of the antigen dose/response relationship. This doubt arose from speculation that anaphylaxis was an "all-or-none" phenomenon, which received support from the work of Dragstedt (1943). The position was largely clarified by Carr (1954) who concluded that the available evidence pointed towards a graded dose/response relationship, and he suggested that Dragstedt's results could be explained on the basis that the doses of antigen used were sufficiently large to produce maximal responses. Herxheimer (1952) criticized methods based upon lethal anaphylactic effects for similar reasons, that the doses required were too high. Using a balanced spirometer, Carr & Curry (1956) demonstrated a graded relationship between dose of antigen and degree of bronchoconstriction, and their work is confirmed by the results of this investigation. These results indicate that the relationship of log dose of antigen to bronchoconstrictor response suggests a normal pharmacological relationship characterized by a sigmoid curve.

This relationship has been demonstrated by both methods of recording, but some differences between the two methods in the slope of the dose-response curves were noted, the curves obtained by the constant pressure method being the steeper. This difference was not, however, great and might be ascribed to slightly greater lung inflation pressures developed on the Dixon & Brodie system. Responses recorded by the Konzett & Rössler method show a tendency to persist, especially when the intensity of the reaction is severe, and may require a transient increase in pressure to reverse them (Collier, Holgate, Schachter & Shorley, 1960). The greater pressure in the Dixon & Brodie system may in some measure overcome this tendency, since it was found that the incidence of persistent bronchoconstriction was much less using this method. It would suggest that this method is of greater value in providing a continuous record of the course of the anaphylactic responses in animals anaesthetized with either urethane or sodium pentobarbitone.

It has long been known that repeated doses of antigen produce a state of desensitization (Besredka, 1907; Anderson & Rosenau, 1908; Weil, 1913). Studies of desensitization were used by Winter (1945) to provide an indication of the severity of anaphylaxis, but the quantitative basis of this phenomenon has been generally neglected. Attempts to overcome the desensitization effect and to produce a number of identical anaphylactic responses in the same animal have been made for the purpose of investigating possible anti-anaphylactic drugs. Alberty (1959) produced multiple responses of somewhat variable severity by a system of ascending repeated dosage, while Herxheimer (1952) achieved constant strength responses by considerably prolonging the interval between the repeated doses of antigen. The experiments in our investigation were concerned with the quantitative relationship between the severity of responses produced by successive doses of antigen. They were attempted in order to produce a series of predictable responses in the same animal. The results show that when the same dose of antigen is repeatedly administered to a sensitized guinea-pig at 30 min intervals responses are obtained which regularly decrease by the same proportion. As the proportional decrease is greater with larger doses of antigen, the most likely explanation of the decrease is that the desensitization phenomenon is the result of depletion of available active material or fixed antibody. This agrees with a similar suggestion made by Paton (1961).

These results appear to have some similarity with those reported by Rocha e Silva & Rothschild (1956) for the desensitization of guinea-pig ileum to the effects of anaphylatoxin, in which experiments the progress of desensitization was quantitatively linked to depletion of histamine. However, the fact that these workers obtained a linear decrease in the size of successive responses contrasts with the exponential relationship reported in this paper. This result suggests a difference in the mechanisms whereby antigen and anaphylatoxin release pharmacologically active materials.

The information obtained in this investigation may be compared to the work of Stresemann (1961) who showed that the repeated exposure of sensitized guinea-pigs to aerosols of antigens resulted in progressively decreasing sensitivity. An exponential relationship is indicated by the straight lines obtained by plotting the log of sensitivity values against the time sequence of successive administrations of aerosol.

It is suggested that the information obtained regarding the progress of anaphylactic desensitization could form a basis for useful quantitative investigation of this type of reaction.

REFERENCES

ALBERTY, VON J. (1959). Versuche über den Anteil von Histamin am anaphylaktischen Asthma. *Int. Arch. Allergy*, **14**, 162–204.

ANDERSON, J. F. & ROSENAU, M. J. (1908). Further studies on anaphylaxis. *J. med. Res.*, **19**, 37–66.

BESREDKA, A. (1907). Comment peut-on combattre l'anaphylaxie ? *Ann. Inst. Pasteur*, **21**, 950–959.

CARR, E. A. (1954). Drug allergy. *Pharmacol. Rev.*, **6**, 365–424.

CARR, E. A. & CURRY, C. F. (1956). Comparative effects of Compound 48/80, histamine and antigen, and the relation between challenging dose of antigen and anaphylactic response in guinea-pigs sensitised to egg albumin. *Int. Arch. Allergy*, **8**, 271–283.

COLLIER, H. O., HOLGATE, J. A., SCHACHTER, M. & SHORLEY, P. G. (1960). The bronchoconstrictor action of bradykinin in the guinea-pig. *Brit. J. Pharmacol.*, **15**, 290–297.

COULSON, E. J. & STEVENS, H. (1949). Quantitative studies in anaphylaxis. I. Influence of age and body weight of guinea-pigs on the sensitising and shocking dose. *J. Immunol.*, **61**, 1–10.

DIXON, W. E. & BRODIE, T. G. (1903). Contributions to the physiology of the lungs, Part I. The bronchial muscles, their innervation, and the action of drugs upon them. *J. Physiol. (Lond.)*, **29**, 97–173.

DRAGSTEDT, C. A. (1943). Relation of dose of antigen to degree of anaphylactic shock in dogs. *J. Immunol.*, **47**, 505–506.

FRIEBEL, H. & BASOLD, A. (1953). Über das steuerbare Meerschweinchenasthma. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmakol.*, **217**, 13–20.

HERXHEIMER, H. (1952). Repeatable microshocks of constant strength in guinea-pig anaphylaxis. *J. Physiol. (Lond.)*, **117**, 251–255.

HOENE, R., COUTU, L., HORAVA, A., PROCOPIO, J., ROBERT, A. & SALGADO, E. (1952). Influence of ACTH on anaphylactic shock in guinea pigs. *J. Allergy*, **23**, 343–351.

HUMPHREY, J. H. (1951). Effect of cortisone upon some experimental hypersensitivity reactions. *Brit. J. exp. Path.*, **32**, 274–283.

KABAT, E. A. & LANDAU, H. (1942). Quantitative study of passive anaphylaxis in the guinea pig. *J. Immunol.*, **44**, 69–74.

KOESSLER, K. K. & LEWIS, J. H. (1927). Determination of bronchospasm in the guinea pig. *Arch. intern. Med.*, **39**, 163–181.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchial muskulatur. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmakol.*, **195**, 71–74.

PATON, W. D. M. (1961). A theory of drug action based on the rate of drug-receptor combination. *Proc. roy. Soc. B*, **154**, 21–69.

ROCHA E SILVA, M. & ROTHSCHILD, A. M. (1956). Experimental design for bioassay of a material inducing strong tachyphylactic effect (anaphylatoxin). *Brit. J. Pharmacol.*, **11**, 252–262.

STRESEMANN, E. (1961). Desensitisation in the guinea-pig by anaphylactic microshock repeated at different intervals. *J. Physiol. (Lond.)*, **159**, 384–390.

WEIL, R. (1913). Studies in anaphylaxis. *J. med. Res.*, **28**, 243–285.

WINTER, L. B. (1945). Influence of duration of sensitisation on anaphylaxis in the guinea-pig. *J. Physiol. (Lond.)*, **104**, 71–83.