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REPEATABLE 'MICROSHOCKS' OF CONSTANT STRENGTH IN GUINEA-PIG ANAPHYLAXIS

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Fatal anaphylactic shock can be produced by the reaction between widely differing amounts of antigen and antibody. It may be assumed that the lethal effect is the same whatever the extent of the reaction beyond a necessary minimum of antigen and antibody. Most poisons act in a similar way: they produce the same lethal effect, whether the minimum lethal dose is used or multiples of it. If their lethal effect is to be counteracted by an antidote, the dose of the antidote must be varied according to that of the poison. A dose counteracting the minimum lethal dose could not be expected to prevent death from double the lethal dose. Such a relationship between poison and antidote may also be suspected for anaphylactic shock, whatever its mechanism. If the reaction of antigen and antibody produces a substance or substances causing the shock, the amount of these substances will vary with the number of antigen and antibody units involved. We should expect that the dosage of a counteracting or protecting substance must also vary accordingly. It follows that experiments which end in lethal anaphylactic shock have little quantitative meaning, as the lethal shock may be produced by widely differing amounts of shock-causing agent.

As Kabat & Landow (1942) have shown, it is possible to produce mild anaphylactic shock by reducing the amounts of antigen used. Humphrey (1951) has tried to distinguish degrees of non-lethal shock according to the severity of the symptoms. The method described here goes further and consists in the production of non-lethal shock of a definite intensity. It is assumed that shocks of the same intensity are induced by an approximately constant amount of antigen-antibody reaction. Protective drugs can therefore be tested quantitatively.

METHOD

In order to prevent a lethal effect the shock substance is introduced as an aerosol. Guinea-pigs are injected intramuscularly 21 days beforehand with 0·7 ml. of a 5 % solution of crystalline egg albumin. The exposure to aerosol of

the same solution is carried out in a glass case measuring 24 by 12 by 12 in. which is open on one side and resembles an aquarium. It is placed open side down on a smooth surface covered by rubber. A hole in this surface is connected by tubing to a commercial nebulizer filled with the antigen. The aerosol is created by compressed air which drives the liquid through the nebulizer and the tubing into the glass case. The amounts of antigen introduced are very small. They act on the surface of the most sensitive organ of the guinea-pig—the bronchi—and the effect becomes visible after a very short time, 15–60 sec. The shock symptoms are the usual ones, but instead of appearing in quick succession with a fatal outcome, their severity increases gradually as more and more antigen is inhaled. The first sign is usually a change in respiration. The abdominal muscles are used on both sides and the abdominal walls are drawn in by the respiratory effort. These movements gradually become stronger. They are sometimes interrupted by a sneeze: the respiration may then become slower and deeper, or more rapid and shallow; in the latter case the whole animal takes part in the movement which can be observed best by watching the head move rapidly to and fro. At this stage the animal must be taken out. Otherwise gasping for air, convulsions, passing of urine, cyanosis and possibly death will follow in quick succession. Individual differences are great. In some guinea-pigs the period between the appearance of deep abdominal contractions and convulsions is short, in others longer. Some always sneeze early, some do not sneeze at all. The moment at which the respiratory distress is so great that convulsions may occur is called the 'convulsion point', and the duration of the period of exposure to the aerosol the 'preconvulsion time'.

The decision whether convulsion point has been reached is difficult and subjective. For this reason in all our experiments two observers were present who had gained a long experience. The difficulty arises mainly with the long preconvulsion times. If the animal is very sensitive and heavy breathing occurs after an exposure of 60–100 sec, convulsions will follow usually within a few seconds, and the possible error is small. If the preconvulsion time becomes longer, the stage of severe dyspnoea also may last longer and the possible error increases. If severe dyspnoea begins after 400 sec or later, it is sometimes uncertain whether convulsions will follow at all. In these cases preconvulsion time means the period necessary to produce severe dyspnoea.

If a sensitized animal is exposed to the shock-producing aerosol for the first time, its reaction may occur very rapidly, the first signs appearing after 15–60 sec and convulsion point being reached a few seconds later. If it is exposed again at daily intervals its reactions occur less rapidly. The preconvulsion time increases quickly by 30–50 % or more from exposure to exposure. This is presumably due to desensitization, and if this process of daily exposure is continued, the animal will be practically desensitized (i.e. convulsion point

can no longer be reached) after four to six exposures. If now an interval of at least 8–10 days is interposed, reformation of antibodies will take place and the resensitized animal will again show a preconvulsion time of 80 sec or less.

If, instead of at an interval of 8–10 days, exposures take place at an interval of 2–7 days, an intermediate stage between desensitization and resensitization is reached; some reformation of antibodies has taken place, and the preconvulsion time will be between 80 and 130 sec. On this level it can be kept approximately constant by adapting the interval to the individual reaction of the guinea-pig.

RESULTS

Table 1 shows the change in preconvulsion time in nine guinea-pigs exposed once on each of 3 consecutive days. The animals, nos. 54, 58 and 60, show some irregular variations, but for all animals the times show an increase. Table 2 shows repeated exposures at 2–3 hr intervals; here desensitization is almost complete after the fourth exposure. Twenty-four hours later, it has not progressed further. On the contrary, in animal no. 126, some resensitization has already begun. After 8 days, resensitization appears to be complete.

Table 3 shows the maintenance of a satisfactory constant preconvulsion time at 2–3-day intervals, and the variations one is likely to find. Table 4 shows a few values obtained for the protective action of promethazine (phenergan). For this purpose the mean preconvulsion time in the exposures before and after the experiment with promethazine have been compared with the preconvulsion time during it.

DISCUSSION

Aerosolized inhalants were first used by Kallós & Pagel (1937). Ratner & Gruehl (1931) have used dry finely powdered substance for inhalation in a similar way. The use of inhaled aerosols for repeated mild shocks does not appear to have been tried before.

The method is based on immunological facts which have been well known for many years: (1) after an incomplete shock the guinea-pig remains shock-refractory for some time, and (2) this refractoriness disappears later. These phenomena have been ascribed to desensitization and resensitization. If an intermediate stage between the two exists it must be theoretically possible to maintain a constant state in which partial or mild shock of a certain degree can be produced and repeated. We have found this intermediate stage and have been able to vary the intensity of the attack by varying the interval between exposures. We have found it a practical method of producing mild shock of constant intensity and we regard this kind of shock as a 'microshock' (Urbach & Gottlieb, 1946). As would be expected, the method has its advantages and disadvantages. The advantages are its repeatability and its resemblance to human asthma which probably is no coincidence. The disadvantage is the variability of the figures obtained. This is due partly to errors inherent

TABLE 1. Increase in preconvulsion time when microshocks were given at daily intervals

Animal no.	18. vi. 51 (sec)	19. vi. 51 (sec)	20. vi. 51 (sec)
53	45	57	80
54	70	105	100
56	60	75	75
58	55	85	80
60	55	45	65
66	55	70	85
67	50	75	95
68	70	80	115
69	55	70	90
Mean	57	64	78

TABLE 2. Increase in preconvulsion time when microshocks were given at intervals of several hours

Animal no.	11.20 a.m.	2.00 p.m.	4.15 p.m.	6.15 p.m.	24 hr later	8 days later
123	70*	150	360	680†	660†	58
124	80*	127	385	630†	660†	55
125	85*	95	555	650†	640†	80
126	61*	210	450	375	150‡	60

* In this experiment 1·6 % egg albumin was used, otherwise 5 %.

† Convulsion point could not be reached; symptoms of moderate dyspnoea were present but did not increase in severity.

‡ Convulsed.

TABLE 3. Relative constancy of preconvulsion time when microshocks were given at intervals of 2–3 days

Animal no.	23. vi. 51	25. vi. 51	27. vi. 51	30. vi. 51	2. vii. 51
53	80	85	75	95	95
54	105	105	135	95	110
56	80	100	80	90	75
66	100	120*	140*	110*	100
67	125	90	130	100	110
69	120	85	115	130	130
Mean	103	97	112	103	103

* Convulsed.

TABLE 4. Protective action of promethazine (phenergan). The influence of increasing doses of this substance on the preconvulsion time

Dosage (mg/kg)	No. of animals used	Mean pre-convulsion time before and after phenergan (sec)	Preconvulsion time under the influence of phenergan (sec)
0·01	5	108	103
0·025	6	108	140
0·05	9	109	144
0·1	8	133	262
0·1	8	136	288
0·25	7	107	542*
0·5	7	133	479†
0·75	6	123	521
1·5	7	161	556
3·0	7	125	376†

* One animal did not show severe dyspnoea. The preconvulsion time has been ignored in this average.

† Two animals did not show severe dyspnoea. Their preconvulsion time has been ignored in the average.

in the method of assessing convulsion point. They can be compensated by increasing the number of observations. The other cause of variability lies in the differing individual reactions of the guinea-pigs, some of which will vary in their reaction from day to day whilst others will remain fairly constant. These variations remain even if other errors are excluded from the technique. The nebulizer used must remain the same because of its individual characteristics; the nebulizing pressure must remain constant; animals which tend to be excited and breathe abnormally before the exposure must be excluded, for, with a greater volume of ventilation, they would be exposed to a greater amount of inhalant. Some animals become so conditioned to the procedure that they start panting immediately they are put into the inhalation box. They cannot be used for experiments. Another point is that sooner or later the spontaneous desensitization which begins several months after the sensitizing injection gradually increases the preconvulsion time so much that the animal ceases to be useful for timed experiments.

SUMMARY

1. In guinea-pigs sensitized to egg albumen mild shock is produced by inhalation of antigen aerosol. By ending the exposure just before the convulsion stage fatal shock is avoided. The period of exposure to the antigen aerosol is termed the preconvulsion time.

2. If the animal is gradually desensitized by rapidly repeated exposures, the preconvulsion time becomes longer. If the time between exposures is increased, the preconvulsion time becomes shorter because resensitization takes place.

3. For each animal an interval between exposures can be found after which the preconvulsion time remains approximately constant.

4. The method has been found useful for testing quantitatively the action of substances counteracting or enhancing anaphylactic shock.

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