J. Physiol. (1955) 128, 435–445

THE 5-HYDROXYTRYPTAMINE SHOCK IN THE GUINEA-PIG

By H. HERXHEIMER

From the Surgical Unit, University College Hospital Medical School, London

(*Received* 28 *July* 1954)

5-Hydroxytryptamine (enteramine, serotonin) has been shown to cause contraction of intestinal muscle in a number of species (Erspamer, 1952; Reid & Rand, 1952*a*; Feldberg & Toh, 1953; Robertson, 1953; Sinha & West, 1953). It has a similar effect on the uterus (Erspamer, 1940; Gaddum, 1953; Reid & Rand, 1952*a*) and on the urinary bladder (Erspamer, 1952). As the substance is liberated from the platelets by the antigen–antibody reaction (Humphrey & Jaques, 1953), the possibility exists that it plays an essential part in anaphylactic shock. It seemed, therefore, of interest to investigate its action in the normal and in the sensitized guinea-pig. Some of the results of these investigations have already been published (Herxheimer, 1953*a*, *b*).

METHOD

The inhalation method was used because it provides direct contact between the inhaled substance and the principal shock organ of the guinea-pig, the bronchial mucosa. A 1% (w/v) solution of 5-hydroxytryptamine creatinine sulphate in 5% glycerin was nebulized by compressed air at constant pressure through a commercial nebulizer and driven into the exposure chamber. The method was the same as that used to produce anaphylactic microshock (Herxheimer, 1952). When the chamber was filled, the animals were put in and watched for any signs of shock. If they showed severe dyspnoea, as is seen regularly in the development of histamine, acetylcholine or anaphylactic shock, the time required to reach convulsion point (the preconvulsion time, Herxheimer, 1952) was measured in seconds.

In those experiments in which histamine or acetylcholine were used, these substances were prepared as 0·5 (w/v) histamine phosphate in 50% propylene glycol or as 0·25% (w/v) methacholine in 50% propylene glycol. Anaphylactic sensitization was carried out by injecting intramuscularly 0·7–0·8 ml. of a 5% (w/v) solution of crystalline egg albumin and exposing the animals 21 days later to an aerosol of the same solution. The severity of the sensitization ('microshock') thus produced was measured, as before, by the length of the preconvulsion time.

RESULTS

General effects of 5-hydroxytryptamine (HT) inhalation and injection

The animals show, without exception, the same symptoms as with histamine or methacholine or in anaphylactic shock. After 30 sec or a little later dyspnoea starts, some scratching of the nose may occur and possibly sneezing. Then

the dyspnoea becomes pronounced, and strong abdominal contractions are seen, with pronounced cyanosis of nose and ears. At this stage the animals must be removed from the chamber if histamine, methacholine or an antigen is used. Otherwise the experiment will quickly end with convulsions and death of the animal. With HT, however, death is rare, and convulsions do not always occur. If they do, they do not last long. The animal recovers from them and gets on its feet again, but severe dyspnoea and cyanosis persist and are still present when the exposure is brought to an end after 10–12 min; the animals then regain their normal appearance within 30–120 min. If the same experiment is repeated after 2–3 hr, the sequence of events is similar, but the symptoms take longer to develop. Whilst at the first exposure the preconvulsion time is usually well under 150 sec, it is now 200–300 sec, and if the exposure is repeated after another 2 or 3 hr, it is still longer, possibly in the region of 600 sec; the dyspnoea and cyanosis are not as pronounced as before. If exposures are repeated at daily instead of 2–3 hr intervals, the same increase of the preconvulsion time is observed, but it does not occur if the interval is 4–5 days or longer. There is not much doubt that this is due to tolerance (or tachyphylaxis), which develops quickly.

In a few experiments 20–45 mg of HT was injected peritoneally into guinea-pigs. Within a few minutes the animals started to tremble and became dyspnoeic, some severely. This condition passed off within $\frac{1}{2}$–1 hr. The same happened when 20 mg HT was injected intracardially, but the symptoms developed immediately.

A number of substances were investigated for their possible antagonistic action: the antihistamines mepyramine and promethazine, dihydroergotamine, yohimbine (Shaw & Woolley, 1953), atropine and lysergic acid diethylamide (LSD). These substances were injected intramuscularly 1 hr before the animals were exposed to HT aerosol. Various combinations of atropine and LSD were also used. Some of the results are shown in Table 1, in which the increase of the preconvulsion time under the influence of the antagonistic drug has been compared with the normal HT preconvulsion time obtained before and afterwards, and expressed as a percentage of full protection (Armitage, Herxheimer & Rosa, 1952).

It will be seen that mepyramine (Neo-antergan, Merck) which has very little anti-acetylcholine activity, does not protect significantly against HT, whilst promethazine, which has a pronounced anti-acetylcholine as well as antihistamine activity, does. Dihydroergotamine has little protective action, and only in large doses; yohimbine none.

The only efficient antagonists found were atropine and LSD. A weak protective action is seen with atropine in one experiment with 0·16 mg/kg, with LSD with 0·005 mg/kg. Larger doses (0·65–1·3 mg/kg atropine and 0·05–0·2 mg/kg of LSD) give a high protection of about 60–75 %. A further increase

in the dose of LSD (0·4 mg/kg) gave full protection in some animals, and a high protection in some others (mean 85%). With atropine, it seems as if the maximum protection has been reached with the higher doses used, as was seen previously in antihistamine protection against anaphylactic shock (Armitage *et al.* 1952).

Combined threshold doses of atropine (0·16 mg) and LSD (0·005 mg) (doses which gave a minimum protection in some experiments and none in others) had no protective action. However, when slightly higher doses of both

TABLE 1. Percentage protection to HT shock by atropine, LSD, antihistamines and dihydroergotamine

(* Significant protection.)

Dose (mg)	No. of animals	$\bar{y}$ + S.E. ($\bar{y}$)
	Atropine	
0·16	5	23·4 ± 7·4*
0·16	3	−0·3 ± 12·2
0·32	5	40·2 ± 5·0*
0·32	5	33·4 ± 9·9*
0·65	4	61·5 ± 2·6*
1·3	6	72·2 ± 8·8*
1·3	5	35·6 ± 5·4*
1·3	6	68·0 ± 2·3*
	LSD	
0·001	6	8·7 ±18·0
0·005	5	24·8 ± 7·7*
0·005	5	−2·0 ± 9·9
0·01	6	35·2 ± 9·3*
0·01	4	48·5 ±16·8
0·01	5	7·8 ±13·8
0·02	6	60·5 ±17·1*
0·05	5	70·2 ± 6·8*
0·1	5	68·2 ± 7·3*
0·2	6	77·5 ± 2·7*
0·4	7	86·0 ± 5·1*
0·4	7	84·6 ± 2·7*
	Mepyramine	
1·0	5	27·2 ±10·5
	Promethazine	
1·0	4	60·5 ± 6·0*
3·0	5	68·0 ± 6·2*
	Dihydroergotamine	
0·8	5	39·0 ±21·8
4·0	6	28·7 ±10·7*
	LSD + Atropine	
0·005 + 0·16	6	1·3 ± 7·3
0·005 + 0·16	7	2·4 ±10·3
0·01 + 0·16	4	36·75±28·8
0·01 + 0·32	5	60·0 ± 5·7*
0·01 + 0·32	5	55·6 ± 6·6*
0·01 + 0·65	5	65·2 ±11·7*
0·05 + 0·32	2	33·0 ±37·0
0·05 + 0·65	5	68·4 ± 8·6*
0·1 + 0·65	5	84·8 ± 8·2*
0·2 + 0·65	4	97·75± 2·25*

substances were combined which separately gave a definite protection of 30–48%, the combination gave a protection of 55–60%. Large doses of both substances (0·2 mg LSD + 0·65 mg atropine) gave an almost 100% protection. This shows that the protective action of these two substances is probably additive. If both are combined, a degree of protection is reached which is higher than if each of them is used singly. There is certainly no 'potentiation', as otherwise some protection would have resulted from the combination of subthreshold doses. LSD was also given in large doses (0·1 and 0·2 mg/kg) before anaphylactic microshock. It had no protective effect whatsoever.

Action of HT in man

It has been reported that 1% HT aerosol did not cause bronchial spasm in normal subjects, but in some asthmatic patients (Herxheimer, 1953*b*). Brocklehurst (1953) did not see any spastic effect of HT on the isolated bronchus of non-asthmatic subjects. As it was suspected that the acidity of the HT solution might have an irritant effect on the bronchial mucosa of asthmatic patients, a buffered HT solution with a pH of 7·0 was tried. This did not cause asthmatic attacks in three patients, one of whom experienced an attack with an unbuffered solution. In guinea-pig experiments no difference between the action of buffered and unbuffered HT aerosol was found.

The mutual effect of HT tolerance and desensitization to anaphylactic shock

In the present experiments it has been shown that tolerance to HT can easily be achieved by several exposures following each other at short intervals. By the same method tolerance to anaphylactic shock can be achieved in animals previously sensitized to an antigen (Herxheimer, 1952). This is called desensitization. Both desensitization to an antigen and tolerance to HT are reversible. The former sensitivity is restored within 4–7 days if the animals are not exposed again.

The similarity of both processes suggested a similar mechanism. It was therefore investigated whether tolerance to HT influences anaphylactic shock caused by egg albumin, and whether desensitization to egg albumin influences HT sensitivity. For this purpose the preconvulsion time for HT of 5 groups of 2–6 guinea-pigs and also their preconvulsion times in anaphylactic microshock were determined in 2–3 experiments. The animals were then, by repeated exposures, made tolerant to either HT or to anaphylactic microshock. As soon as this was achieved, those made tolerant to HT were shocked with egg albumin aerosol, and those desensitized to egg albumin were exposed to HT aerosol. The artificial production of tolerance did not prove equally easy in all experiments. In some (Figs. 2, 5) it can be seen that several days of repeated exposures were needed to achieve a high level of tolerance or desensitization.

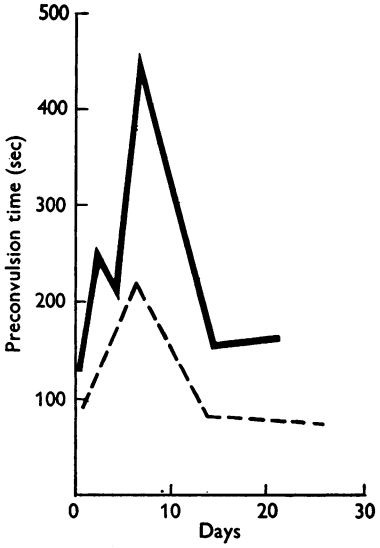

Fig. 1

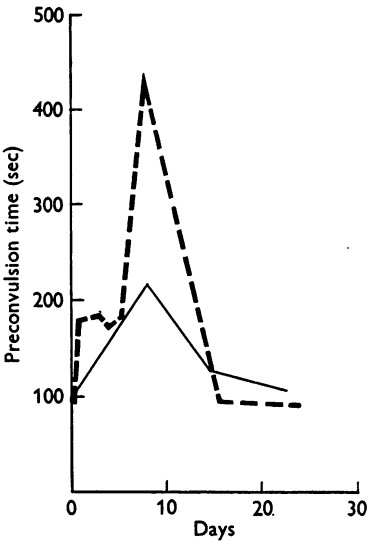

Fig. 2

Fig. 1. Mean of preconvulsion times of six guinea-pigs. Repeated exposures to egg albumin aerosol during 3 consecutive days have led to desensitization on the 6th day of the experiment (——). The HT preconvulsion times have increased and decreased simultaneously (- - -).

Fig. 2. Mean of results from five guinea-pigs. Repeated exposures to HT aerosol during 6 consecutive days have led to tolerance on the 7th day of the experiment (- - -). The egg albumin preconvulsion times have increased and decreased simultaneously (——).

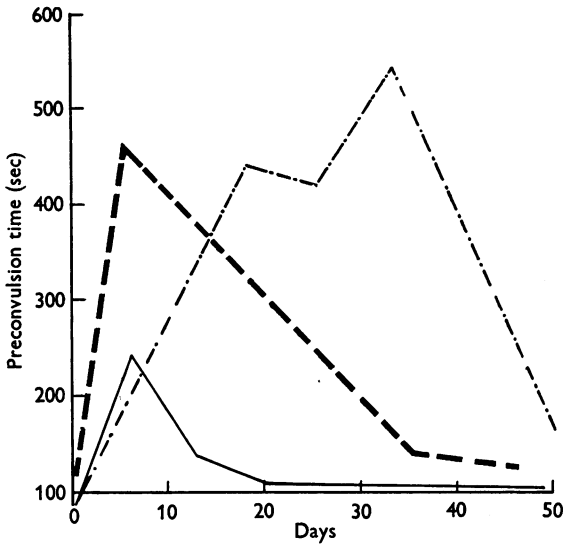

Fig. 3. Mean of results from four guinea-pigs. Repeated exposures to HT aerosol during 4 consecutive days have led to tolerance on the 5th day of the experiment (- - -). The egg albumin preconvulsion times have increased and decreased simultaneously (——). The ACh times have increased and decreased more slowly(-·-·-).

The results show a mutual effect of both procedures: HT tolerance was accompanied by desensitization to egg albumin (Figs. 2–4), and desensitization by HT tolerance (Figs. 1, 5). When after 4–7 days the HT tolerance had disappeared or the animals had resensitized, the corresponding desensitization or HT tolerance had also disappeared. These changes occurred with great regularity and, although the graphs represent the mean preconvulsion times of a number of animals, there was not a single animal in the same group which did not show the same trend. In the experiment shown in Fig. 5 the guinea-pigs were made tolerant to HT twice. In the first instance the response of the sensitivity to egg albumin was pronounced, in the second it was present but

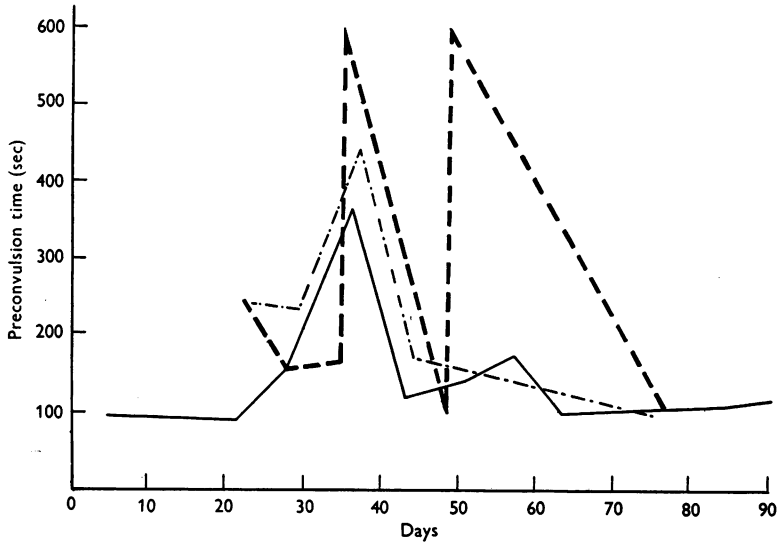

Fig. 4. Mean for ten guinea-pigs. Repeated exposures to HT aerosol before and on the 35th and 48th day respectively have led to tolerance (– – –). The egg albumin preconvulsion times have increased and decreased in the first of the two experiments simultaneously with the HT preconvulsion times. In the second experiment this reaction is less pronounced (——). The ACh preconvulsion times have been followed only during the first experiment (–·–·–).

weak. The increase in the preconvulsion time to egg albumin on the 27th day is believed to be caused by the HT exposure on the preceding night.

The next step was to investigate whether histamine or acetylcholine (ACh) shock would be influenced by HT tolerance or desensitization to anaphylactic shock. In preliminary experiments it had been found that repeated exposure to histamine aerosol caused an increase in the preconvulsion time to this substance, if the exposures were repeated daily over a long period. The increase was somewhat erratic: the preconvulsion times showed a great scatter, and a definite increase would become visible only after some weeks. With ACh such

an increase could not be produced even after many weeks' exposure, and the fluctuation of the preconvulsion times was even greater than with histamine.

The influence of HT tolerance and of desensitization on ACh preconvulsion time is shown in Figs. 3–6. In all experiments the times increase considerably, and in five of the six experiments the increase, after having reached its peak, takes a long time to return to normal. In four experiments (Figs. 3, 6) the base value has not been reached after about 2 and 3 months, when the experiment

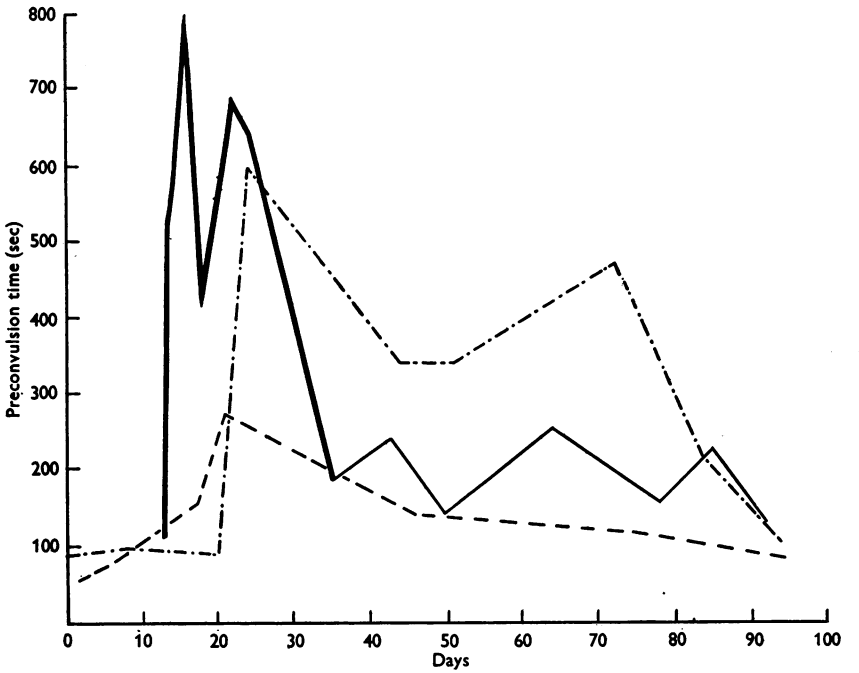

Fig. 5. Mean of four guinea-pigs. Repeated exposures to egg albumin from the 14th to the 22nd day of the experiment have led to desensitization (——). The HT preconvulsion times have increased and decreased simultaneously with the egg albumin times (– – –). The ACh times have increased later and decreased more slowly (–·–·–).

was discontinued. The ACh preconvulsion times therefore take a much longer time to return to normal than is required by HT tolerance or anaphylactic desensitization to disappear. It seems also that the increase in ACh preconvulsion times takes longer to develop than HT tolerance and desensitization to egg albumin. In one experiment (Fig. 5) the ACh preconvulsion time is still normal when desensitization is near its peak and increases only later. In three other experiments (Fig. 6) ACh tolerance is still increasing at a time when the HT or egg albumin times have returned or would have returned to near normal.

Two other experiments in which HT tolerance had the same effect are not included in the figures. A similar effect of egg albumin tolerance on histamine preconvulsion time was seen in one experiment. Four other attempts to reproduce this effect, partly with HT tolerance, partly with egg albumin tolerance, have failed.

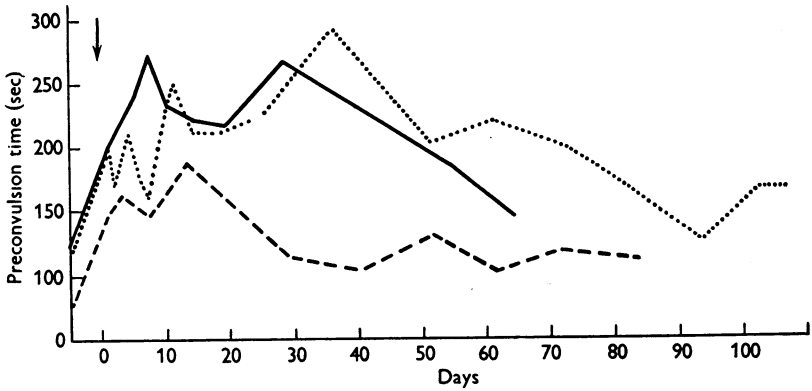

Fig. 6. Three different experiments. On the day marked by arrow desensitization to egg albumin (ten guinea-pigs, ——) and tolerance to HT (twelve guinea-pigs, ... ; or sixteen guinea-pigs, – – –) have reached maximum. The curves represent the preconvulsion times to ACh of these three groups after desensitization and tolerance respectively have been reached.

DISCUSSION

The only obvious effects of HT in the present experiments concern the respiration. As numerous observers have found HT to cause contraction of smooth muscle, it appears likely that the dyspnoea is caused by the contraction of the bronchial muscles. This view is supported by the observation that very similar symptoms are seen with histamine or ACh inhalation, where bronchial constriction is known to be the cause of the dyspnoea. The evidence, however, cannot be regarded as complete without histological pictures of the lungs of guinea-pigs in HT shock. In the dog, Douglas & Toh (1953) observed respiratory stimulation after HT was given intravenously. As their spirometric records show an increase of respiratory volume, and as bronchial obstruction, at least in man, causes a decrease of respiratory volume (Herxheimer, unpublished observations), it is unlikely that in their experiments HT caused bronchial spasm. In the cat, Reid & Rand (1952*b*) have observed broncho-constriction after intravenous injection of 22μg of HT.

Tolerance to HT (tachyphylaxis) on isolated organs has been observed before (Reid & Rand, 1952*a*, *b*; Freyburger, Graham, Rapport, Seay, Govier, Swoap & van der Brook, 1952; Sinha & West, 1953; Gaddum, 1953; Rocha e Silva, Valle & Picarelli, 1953; Gaddum & Hameed, 1954). It should be noted that

tolerance to HT and to the egg antigen can be established easily and within the same period, but that tolerance to histamine is difficult, and to ACh almost impossible to achieve by the same method.

The antagonistic effect of atropine has been noted by Page (1952), Rocha e Silva *et al.* (1953), Robertson (1953) and others; that of lysergic acid diethylamide by Gaddum (1953). The antagonistic action of atropine to HT is much weaker than to ACh, against which small amounts give full protection. The action of atropine in anaphylactic microshock is still weaker than that in HT shock. Armitage *et al.* (1952) found, for instance, the percentage protection given by 0·32 mg/kg atropine in anaphylactic microshock 15%, i.e. negligible, and that of 1·3 mg/kg 44%, whilst in the present experiments the protection against HT, calculated in the same way, was 37 and 58% respectively at these dose levels.

The strongly antagonistic action of LSD to HT is, weight for weight, about tenfold that of atropine. If HT were an essential element in the causation of anaphylactic shock, one would expect LSD to give some protection against it. As there is no evidence of any protection, HT cannot be a direct or main cause of the symptoms of anaphylactic shock.

With this evidence in mind it is difficult to interpret the relationship between HT tolerance and anaphylactic sensitivity which has become apparent in later experiments. In repeated experiments tolerance to HT has increased tolerance to anaphylaxis, and vice versa. Moreover, increased HT tolerance has in every case also increased tolerance to ACh. The reverse could not be shown as we did not succeed in making animals tolerant to ACh. There is also a difference in the time relations. Whereas HT tolerance and anaphylactic sensitivity increase and decrease at about the same time, the increase of ACh tolerance appears to lag behind and returns to normal much later than HT and egg tolerance. The fact that HT tolerance causes ACh tolerance and anaphylactic desensitization, that the latter causes HT tolerance and ACh tolerance, and that atropine antagonizes all three kinds of shock, although to different degrees, suggests a close biological relationship of these processes, possibly a factor common to the three kinds of shock. Such a common factor might be a new substance which is formed, or another, naturally-occurring substance which is formed in excess in the course of the antigen–antibody reaction and also by the HT action, and which has an anti-acetylcholine activity. It seems hardly permissible to speculate further, but it will be necessary to investigate whether tolerance to other substances can be produced with similar effects.

SUMMARY

1. 5-Hydroxytryptamine (HT) aerosol causes a shock syndrome in the guinea-pig similar to that caused by anaphylaxis, histamine and acetylcholine. Tolerance to this effect develops quickly, and fatal HT shock is rare.

2. The strongest antagonists to HT were lysergic acid diethylamide (LSD) and atropine. The antihistamine mepyramine, and yohimbine had no antagonistic action, dihydroergotamine a weak one.

3. LSD had no protective effect in anaphylactic shock. The protective effect of atropine in anaphylactic shock was weaker than in HT shock.

4. When guinea-pigs actively sensitized to egg albumin were desensitized by repeated exposure to the antigen, their tolerance to HT increased. When they were resensitized, their tolerance to HT fell to the previous value. Conversely, if guinea-pigs were made tolerant to HT, they became less sensitive to egg albumin. When they lost their tolerance to HT, their previous sensitivity to egg albumin returned.

5. Development of tolerance to HT and desensitization to egg albumin both caused tolerance to acetylcholine. This tolerance developed slowly and lasted longer than the tolerance to HT and the desensitization to egg albumin.

This work was partly assisted by a personal grant from the Medical Research Council and by a grant for technical assistance from the Asthma Research Council. I am also obliged to Dr P. Armitage for the statistical analysis of the figures, to Dr R. K. Richards, of Abbotts Laboratories, North Chicago, for the supply of hydroxytryptamine, and to Messrs Sandoz for the supply of lysergic acid diethylamide.

Note added in proof

In recent experiments it has been found that nicotine sulphate aerosol caused a shock syndrome similar to that caused by HT. Tolerance developed after repeated exposure and, at the same time, desensitization to anaphylaxis developed, as with HT. Conversely desensitization to anaphylaxis also caused tolerance to nicotine.

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