RESEARCH PAPERS

THE ANTI-ANAPHYLACTIC ACTIVITY OF ETHANOLAMINE AND CHOLINE

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Ethanolamine, *N*-methylethanolamine, *N*-dimethylethanolamine and choline have anti-anaphylactic activity in actively sensitised guinea-pigs. Administered alone their activity is slight, but they considerably potentiate the limited protection afforded by mepyramine. Ethanolamine has been shown to inhibit the release of the slow reacting substance of anaphylaxis in guinea-pig lung subjected to anaphylaxis *in vitro*.

Various authors have described an anti-allergic factor in peanut oil, lecithin fractions obtained from soya beans, and egg yolk (Coburn and Moore, 1943; Coburn, Graham and Haninger, 1954; Long and Martin, 1956). This factor has been identified as *N*-2-hydroxyethyl-palmitamide (Kuehl, Jacob, Ganley, Ormond and Meisinger, 1957) and shown to suppress passive joint anaphylaxis in the guinea-pig (Ganley, Grassle and Robinson, 1958). These last authors also stated that ethanolamine, *N*-methylethanolamine, *N*-dimethylethanolamine and choline were active in the same test. It has since been reported that *N*-2-hydroxyethyl-palmitamide and ethanolamine hydrochloride suppress passive anaphylaxis in the mouse (Ganley and Robinson, 1959) and that ethanolamine, *N*-dimethylethanolamine and choline diminish anaphylactoid oedema induced in rats (Cronheim and Toekes, 1959).

Ethanolamine and its *N*-methyl derivatives became the subject of anti-anaphylactic studies in this laboratory when it was noted that ethanolamine and choline salts of glycyrrhetinic acid had greater activity against Arthus reactions in the guinea-pig than that reported for the disodium salt of glycyrrhetinic acid hydrogen succinate by Brown, Christie, Colin Jones, Finney, MacGregor, Morrison Smith, Smith, Sullivan, Tarnoky, Turner, Watkinson and Wotton (1959). In the present study, ethanolamine and its *N*-methyl derivatives were examined as antagonists of anaphylaxis induced in actively sensitised guinea-pigs. In this species, both histamine and the slow reacting substance of anaphylaxis (SRS-A) are liberated from sensitised lung by antigen (Brocklehurst, 1953; 1955; 1956; 1960). The experiments were designed to show inhibition of the effects of histamine and SRS-A and also changes in the amounts of these substances released during anaphylaxis.

METHODS

Anaphylactic Shock in vivo

Guinea-pigs of either sex weighing 200 to 250 g. were obtained from Mr. Donald Harrodine of March, Cambs, and sensitised to commercial

egg albumin (G. T. Gurr, London) by the subcutaneous injection of 100 mg. in 1 ml. normal saline. They were fed on Diet 18 pellets (Oxo Ltd.) and received 50 mg. of ascorbic acid each morning in drinking water contained in amber glass bottles. Overnight the animals were given tap water.

Three weeks after the sensitising dose of antigen, the animals were subjected to anaphylactic shock by the technique developed by Herxheimer (1952). Each animal was placed in a 10 in. diameter glass vessel and exposed to an aerosol of antigen. The aerosol was produced by applying air at a pressure of 5 lb./sq. in. to a Riddostat inhaler (Riddell Products Ltd., London) containing a 5 per cent w/v solution of egg albumin in distilled water. The animal was removed from the aerosol at a point immediately before the onset of convulsions. This point was usually characterised by a powerful convulsive expiration and contraction of the abdominal muscles. The time in seconds required to reach this point was termed the "collapse time" and assumed to be similar to the "pre-convulsion time" described by Herxheimer (1952). Confirmation that shock had occurred was obtained by listening for coughs and examining the retinae for signs of central cyanosis. If the shocked animal was not removed from the aerosol at its collapse time, it was likely to die almost immediately. After the collapse time had been measured, a mixture of 95 per cent oxygen and 5 per cent carbon dioxide was applied to severely distressed animals. In this way, animals were saved which otherwise might have died, and overestimation of the collapse time avoided.

After the first exposure to antigen, more antibody is formed in the animal and this newly-formed antibody can be removed by a second exposure to antigen. Hence, the amount of antibody formed, the severity of the shock, and magnitude of the collapse time all depend on the interval between successive exposures. In the present experiments, the time interval for each animal which gave approximately the same collapse time for every re-exposure to antigen was 7 days. The collapse time obtained under these conditions was designated the "normal collapse time".

Increase in the collapse time following treatment with a drug indicates a protective effect. In measuring the protective effect of a number of antihistamine drugs, Armitage, Herxheimer and Rosa (1952) compared a preconvulsion time obtained on the day of treatment with the mean of two preconvulsion times obtained for exposures to antigen made several days before and on several days after drug treatment. The interval between these successive antigen exposures was usually 3 or 5 days. A percentage protection was calculated according to the formula: per cent protection $= 100\ (1 - C/T)$, C = mean of two normal preconvulsion times, and T = preconvulsion time observed on day of treatment. When results were calculated in this way, 100 indicated complete protection (T = 00), and 50 indicated tolerance of twice the normal amount of antigen (T = 2C). In these experiments protection has been expressed as T/C, which is referred to as the "protection ratio", and corresponds approximately to the number of lethal doses of antigen which a protected animal can tolerate.

Anaphylactic Shock in vitro

Anaphylactic shock was induced in intact guinea-pig lungs undergoing perfusion through the pulmonary artery with Tyrode solution at 37° as described by Brocklehurst (1960). The perfusate was collected for 30 minutes after antigen administration, centrifuged to remove blood cells, and then examined for histamine and SRS-A.

Estimation of Histamine and SRS-A in Perfusates From Anaphylactic Guinea-pig Lung

These were performed on guinea-pig ileum suspended in 2 ml. of aerated Tyrode solution at 37° as described by Brocklehurst (1960).

Results of *in vivo* Experiments

Normal Sensitivity of the Animals

A group of nine animals was exposed to antigen at weekly intervals for 3 weeks. The mean of the last two of these three exposures for each animal in the group is given as the normal collapse time in Table I. This

TABLE I

THE SENSITIVITY TO ANTIGEN (EXPRESSED AS A PROTECTION RATIO) OF A GROUP OF NINE GUINEA-PIGS EXPOSED TO AEROSOLISED ANTIGEN AT WEEKLY INTERVALS

Animal No.	Normal collapse time in sec.	Protection ratio									
1	100	1·22	1·76	0·99	1·05	4·00	1·06	2·35	1·23	1·12	0·85
2	130	1·27	0·83	0·89	0·96	0·65	1·29	0·73	0·57	0·59	0·49
3	89	1·27	1·07	1·04	0·90	0·76	0·83	0·90	0·94	0·97	0·65
4	90	0·88	0·73	1·21	0·99	0·35	1·14	1·11	1·77	2·56	1·83
5	103	1·20	0·89	1·02	1·04	0·91	1·40	0·91	0·97	1·43	1·39
6	107	0·88	0·67	0·75	0·75	0·44	1·34	0·62	0·51	0·84	0·60
7	83	0·95	0·97	1·16	0·91	1·23	1·37	1·58	1·19	1·53	1·51
8	110	1·21	0·55	0·70	0·55	0·58	0·51	0·83	0·54	0·68	0·81
9	143	1·07	0·91	1·01	0·92	0·68	0·98	0·88	0·94	1·50	1·17
Mean		1·11	0·93	0·97	0·89	1·07	1·10	1·10	0·98	1·25	1·03
S.D.		0·03	0·12	0·03	0·02	1·28	0·09	0·28	0·16	0·36	0·22

S.D. = Standard deviation

Table shows the results of exposing these animals to antigen at weekly intervals for ten consecutive weeks. Their tolerance to antigen is expressed as a protection ratio calculated from their normal collapse times. It can be seen that in any one week the mean protection ratio for the whole group does not vary outside the limits 0·89 $\pm$0·02 to 1·25 $\pm$ 0·36. The week to week variation of any individual animal is, however, larger.

The Protection Afforded by Antihistamines

The results obtained by Armitage, Herxheimer and Rosa (1952) show that antihistamines afford a definite but limited protection against aerosol induced anaphylactic shock. In fact, none of the antihistamines which they investigated gave a protection substantially greater than that represented by a protection ratio of 4. This was verified in a group of nine animals exposed to antigen at weekly intervals in which the protection

afforded by various doses of mepyramine, administered intramuscularly as the maleate 1 hr. before antigen exposure, was examined on alternate weeks. The results are shown in Fig. 1. It can be seen that doses in the range 1 to 3 mg./kg. afford a maximum protection. The value of the

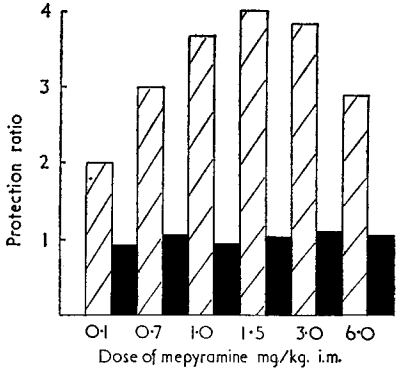

FIG. 1. The protective effects of various doses of mepyramine determined in a group of 9 animals exposed to aerosolised antigen at weekly intervals. Shaded columns show the effects of mepyramine. The figures indicate the dose in mg./kg. mepyramine base administered as mepyramine maleate intramuscularly 1 hr. before antigen exposure. Solid columns indicate the effect of exposing the animals without pretreatment on alternate weeks.

protection ratio is close to 4. Lower or higher doses afford less protection. These findings agree with those of Armitage and others (1952) for mepyramine maleate.

The Constancy of Antihistamine Protection

A further group of nine animals was exposed weekly to antigen for eleven consecutive weeks. On alternate weeks the protection afforded

TABLE II

THE PROTECTIVE EFFECT OF MEPYRAMINE (EXPRESSED AS A PROTECTION RATIO) IN A GROUP OF NINE GUINEA-PIGS EXPOSED TO AEROSOLISED ANTIGEN AT WEEKLY INTERVALS

Animal No.	Normal collapse time in sec.	Protection ratio										
		N	T	N	T	N	T	N	T	N	T	N
41	130	1·12	8·78	1·22	8·02	1·22	3·96	1·14	5·22	1·16	3·83	1·11
42	90	1·05	7·23	1·05	2·73	1·05	3·20	0·97	4·03	0·95	4·04	0·99
43	180	1·04	8·19	0·96	3·93	0·99	4·43	1·00	3·69	1·17	5·10	1·21
44	124	1·10	5·09	1·14	3·88	1·19	4·00	1·19	4·03	1·21	4·05	1·24
45	130	1·12	6·12	0·91	6·62	1·00	4·70	1·03	4·25	1·08	4·00	1·10
46	90	1·02	3·73	1·09	4·07	1·09	3·83	1·22	6·51	1·38	4·11	1·38
47	94	0·93	3·63	1·11	4·68	1·17	7·75	1·18	3·95	1·25	4·00	1·29
48	88	1·05	4·04	1·03	3·96	1·09	4·02	1·06	4·20	1·05	4·17	1·15
49	90	0·98	3·81	1·05	4·02	1·06	4·18	1·06	4·29	1·11	3·92	1·14
Mean		1·04	5·62	1·06	4·66	1·09	4·45	1·09	4·46	1·15	4·14	1·19
S.D.		0·004	4·13	0·008	2·59	0·007	1·70	0·008	0·77	0·015	0·14	0·013

S.D. = Standard deviation

N = exposure without drug pre-treatment. T = exposure after 1 mg./kg mepyramine base administered as mepyramine maleate intramuscularly one hr. before exposure to antigen.

by 1 mg./kg. of mepyramine was examined. The results are given in Table II. For the whole group of nine animals the mean protection ratio calculated for that dose of mepyramine ranged from 4·14 ± 0·14 to 5·62 ± 4·13.

The Activity of the Ethanolamine-choline Series

The limited protection afforded by mepyramine to guinea-pigs undergoing anaphylactic shock under the conditions of these experiments might be due to the inability of mepyramine to protect animals from bronchoconstriction induced by SRS-A. It was, therefore, decided to investigate

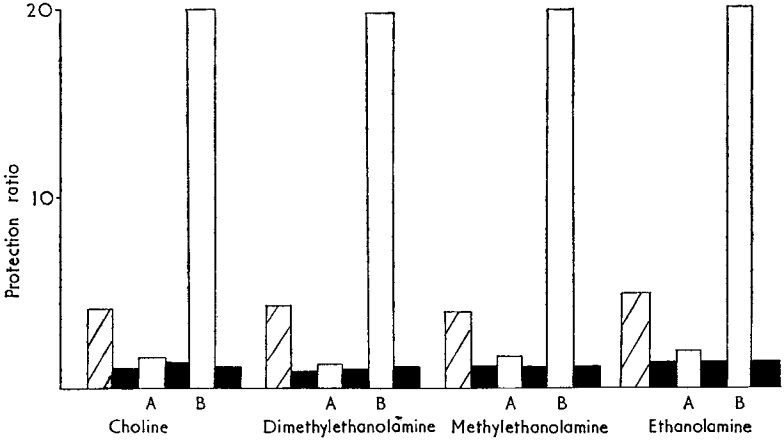

FIG. 2. The protective effects of choline, dimethylethanolamine, methyl ethanolamine and ethanolamine in groups of 9 guinea-pigs exposed to aerosolised antigen at weekly intervals. The shaded columns show the effect of 1 mg./kg. mepyramine base. The open columns show the effect of test material. A shows the effect of test material administered alone at 20 mg./kg. B shows the effect of test material at 20 mg./kg. administered simultaneously with 1 mg./kg. mepyramine base. The solid columns show the effect of exposing the animals without pretreatment on alternate weeks. All substances were administered intramuscularly one hr. before exposure to antigen. Mepyramine was administered as the maleate and other materials as hydrochlorides.

the ethanolamine-choline series both alone and simultaneously with a maximum protection dose of mepyramine, in the expectation that an SRS-A antagonist would potentiate the limited protection afforded by mepyramine alone. The results of the first experiments are shown in Fig. 2.

Protection ratios up to a value of 20 were determined. No attempt was made to estimate protection ratios of a higher order because of the time involved in making the observations in groups of nine animals. For an animal having a normal collapse time of 3 min., a protection ratio of 20 indicates tolerance to antigen of 1 hr. None of the four substances had much activity when given alone, but all of them potentiated the action of mepyramine. In a further experiment using promethazine as the antihistamine a similar result was obtained. Peak antihistamine

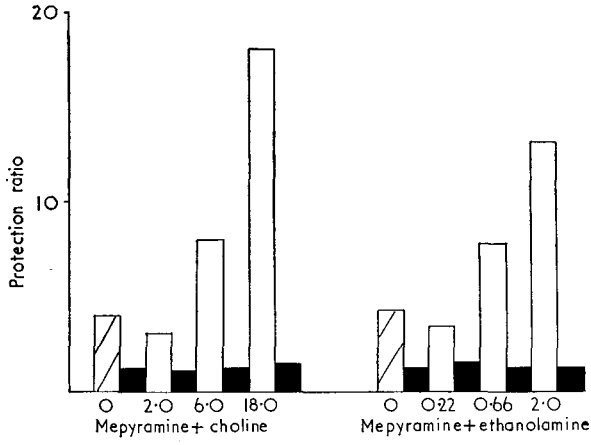

FIG. 3. The effect of various doses of ethanolamine and choline when administered simultaneously with mepyramine to groups of 9 guinea-pigs exposed to aerosolised antigen at weekly intervals. The shaded columns show the effect of 1 mg./kg. of mepyramine. The open columns show the effect of administering test material simultaneously with 1 mg./kg. of mepyramine. Figures indicate dosage of test material in mg./kg. Solid columns show the effect of exposing the animals to pretreatment on alternate weeks. All substances were administered intramuscularly 1 hr. before exposure to antigen. Mepyramine was administered as the maleate, ethanolamine as the hydrochloride and choline as the chloride.

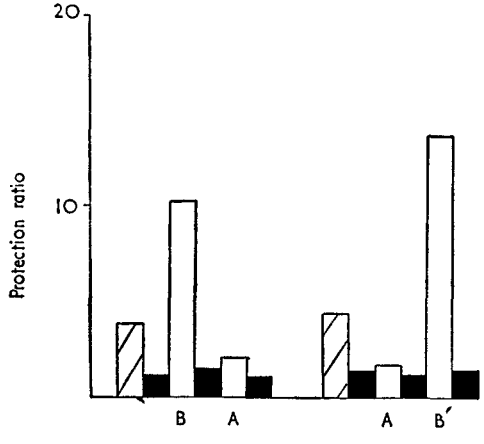

FIG. 4. The effect of orally administered ethanolamine on two groups of 9 animals exposed to aerosolised antigen at weekly intervals. The shaded columns show the effect of 1 mg./kg. mepyramine given intramuscularly. The open columns show the effects of ethanolamine, A shows the effect of administering 500 mg./kg. of ethanolamine orally. B is the effect of 1 mg./kg. mepyramine administered intramuscularly at the same time as 500 mg./kg. of ethanolamine orally. All drugs were administered 1 hr. before exposure to antigen. The solid columns record the effects of exposing the animals to antigen without pretreatment on alternate weeks.

activity observed with a group of nine animals corresponded to a protection ratio of 4·00 ± 0·21 and was achieved with 0·25 mg./kg. promethazine, administered as the hydrochloride 1 hr. before exposure to antigen. Protection ratios of 20 were observed after the simultaneous administration of promethazine 0·25 mg./kg. and ethanolamine 20 mg./kg.

Using two groups of nine animals a quantitative comparison was made of the ability of ethanolamine and choline to potentiate mepyramine. The results are shown in Fig. 3. Ethanolamine had between six and nine times the activity of choline.

The activity of ethanolamine after oral administration was then examined. The results are shown in Fig. 4 from which it can be concluded that orally administered ethanolamine potentiates the effect of intramuscularly administered mepyramine.

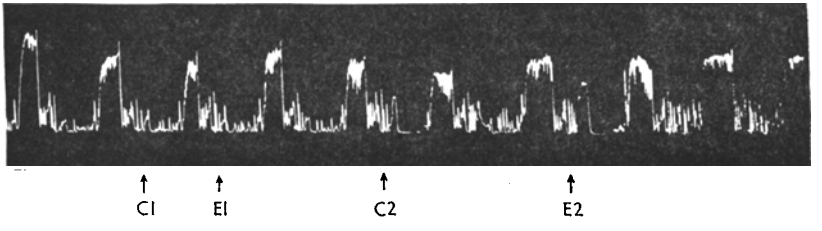

FIG. 5. The effect of ethanolamine and choline on the isolated guinea-pig ileum at 37° in a bath of 2 ml. capacity containing Tyrode with mepyramine and atropine each at a concentration of 10^{-6} g./ml. Unlabelled contractions were produced by SRS-A in Tyrode perfusate from sensitised guinea-pig lung shocked *in vitro*. C1 = Choline 100 μg./ml. E1 = Ethanolamine 100 μg./ml. C2 = Choline 1 mg./ml. E2 = Ethanolamine 1 mg./ml. Choline and ethanolamine were administered as chloride and hydrochloride respectively and left in contact with the tissue for 30 sec. They were removed from the bath before eliciting the next contraction. Spasmogen contact time = 2 min. Dose interval = 5 min.

RESULTS OF *in vitro* EXPERIMENTS

Pharmacological Antagonism of Histamine and SRS-A

The results of the above experiments suggested that ethanolamine had pharmacological actions antagonistic to SRS-A rather than histamine. Tests using the isolated guinea-pig ileum showed that neither ethanolamine nor choline in both concentrations up to 1 mg./ml. antagonised histamine. Fig. 5 shows that neither substance antagonised SRS-A.

Inhibition of SRS-A Release During Anaphylaxis

It seemed possible that ethanolamine inhibited histamine or SRS-A release or both during anaphylaxis. Experiments were made using lungs from groups of four sensitised animals which were perfused and shocked *in vitro*. Comparisons were made between the histamine and SRS-A content of perfusates derived from shocked but otherwise untreated animals (controls) and shocked animals previously treated with ethanolamine. In all, four such experiments were made using a total of 32 animals.

Each experiment with eight animals (four controls) was made in a single day. The histamine and SRS-A assays were made with freshly prepared perfusate, since SRS-A is not stable. The time factor thus precluded the use of more elaborate experimental designs for the assay procedures. The results of one typical experiment are given in Figs. 6 and 7.

Fig. 6 compares the histamine equivalent of perfusate P4 from a control animal with perfusate Q4 from an animal which had received three daily doses of 200 mg./kg. ethanolamine (given intramuscularly as the hydrochloride) on the days immediately preceding anaphylactic shock. Perfusate P4 in a 1 in 200 dilution and Perfusate Q4 in a 1 in 200 dilution gave responses approximately equivalent to 0·002 μg. histamine per ml. (H) indicating that pretreatment with ethanolamine did not inhibit histamine release during subsequent anaphylaxis.

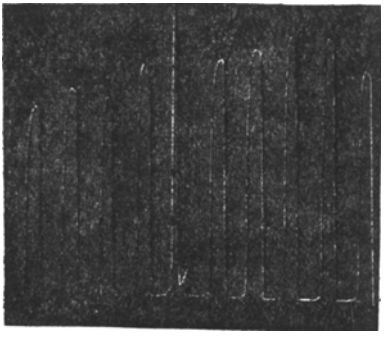

FIG. 6. An approximate estimate of the histamine content of perfusates P4 and Q4. Guinea-pig ileum. 37°. Tyrode. 2 ml. bath. H = Histamine 0·001 μg./ml. P4/200 = Perfusate P4 at a 1 in 200 dilution. Q4/200 = Q4 at a 1 in 200 dilution. Q4/100 = Perfusate Q4 in a 1 in 100 dilution followed immediately by a wash. For the origins of perfusates P4 and Q4 see text. Spasmogen contact time = 30 sec. (except Q4). Dose interval = 3 min.

Fig. 7 shows an experiment in which the same two series of perfusates (P and Q) were compared for SRS-A content on the same piece of isolated guinea-pig ileum. The tissue was first tested for histamine sensitivity (H, 2H and H/2) and then mepyramine and atropine were added to the Tyrode solution. Ten min. later histamine blockade was demonstrated by adding a large dose of histamine (100H) to the bath. The response induced by changing the Tyrode in the bath (T) is shown for comparison. Then, whereas the activity of the Q perfusates from animals pretreated with ethanolamine was observed to be negligible, the activity of the P perfusates from control animals was found to be appreciable. It was concluded that ethanolamine is capable of inhibiting the release or formation of SRS-A, or both, in guinea-pig lung undergoing anaphylaxis.

DISCUSSION

Since an antigen-antibody reaction in sensitised guinea-pig lung leads to the liberation of histamine and SRS-A, both of these substances can be

expected to contribute to the syndrome of anaphylaxis observed in a sensitised animal inhaling an aerosol of the specific antigen. Under these conditions antagonism of the released histamine by pretreatment with mepyramine or promethazine affords only a limited degree of protection. The increased measure of protection observed after the simultaneous administration of an antihistamine and ethanolamine might therefore

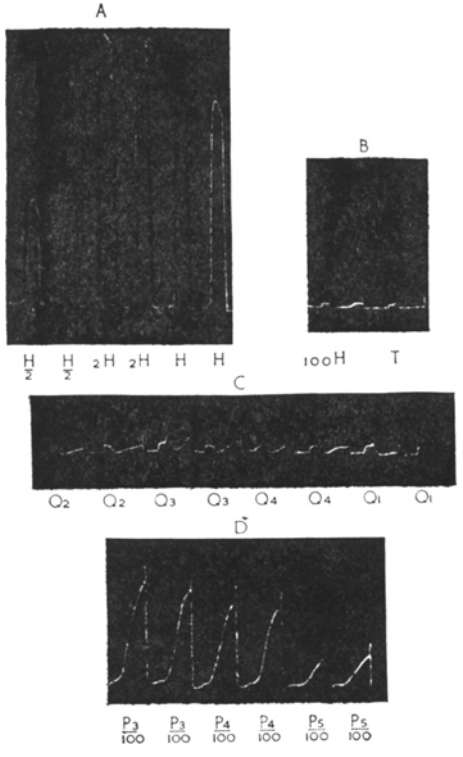

FIG. 7. A comparison of the SRS.A contents of two series of anaphylactic guinea-pig lung perfusates (P and Q). Guinea-pig ileum 37° 2 ml bath. Tyrode solution. Between A and B mepyramine and atropine at concentrations of 10^{-6} g./ml. were added to the wash fluid. H = Histamine 0·001 μg./ml. T = response to changing Tyrode in bath. Pl/100, Q1/100, etc. = responses to 1 in 100 dilutions of perfusate. For origins of the perfusates see text. Dose interval = 3 min. before adding mepyramine and atropine to the Tyrode but 5 min. thereafter. Spasmogen contact time = 30 sec. before mepyramine and atropine and 60 sec. thereafter.

indicate protection from the pharmacological actions of both histamine and SRS-A. Exploration of such a hypothesis led to the observation that pretreatment with ethanolamine could inhibit the release of SRS-A in sensitised guinea-pig lungs subjected to anaphylaxis *in vitro*. However, the dose of ethanolamine required in these experiments was appreciably greater than that required to potentiate mepyramine in animals subjected to anaphylaxis *in vivo*. This apparent anomaly is worthy of comment.

In the *in vivo* experiments it has been demonstrated that a single intramuscular dose of as little as 2 mg./kg. can significantly potentiate 1 mg./kg. of mepyramine. In the *in vitro* experiments reported here three daily doses of 200 mg./kg. of ethanolamine were necessary before inhibition of SRS-A release could be demonstrated unequivocally. There are a number of possible explanations. Under the *in vivo* shock conditions, it can be calculated from the amount of antigen released as aerosol per minute and the volume of air containing antigen which is breathed by the experimental animal per minute, that the amount of antigen actually inhaled during the normal collapse time is about 1 or 2 μg. Because of antigen losses on the walls of the apparatus and in the upper parts of the respiratory system of the guinea-pig, exact quantitative determination of the dose of antigen reaching the lungs is difficult. The estimation has been attempted unsuccessfully by other authors (see references in Winter and Flataker, 1955). By comparison, the minimum amount of antigen (1 mg.) necessary for the liberation of histamine and SRS-A in the *in vitro* experiments is large. In addition, the route of administration was different. In one experiment antigen was administered via the air passages and in the other by the capillaries. Whereas in the first, administration was virtually continuous and ceased on reaching a predetermined end-point, in the other experiment antigen probably reached its reaction site in a sudden high concentration. There are thus a number of reasons why the dose of ethanolamine used in the *in vivo* experiments is not the same as that used to establish its mechanism of action.

The mechanism whereby ethanolamine inhibits the release of SRS-A is not yet known. However, the metabolic fate of ethanolamine has previously been studied in rats by Stetten (1941), Pilgeram, Gal, Sassenrath and Greenberg (1953), and Pilgeram, Hamilton and Greenberg (1957). Studies with ^{15}N- and ^{14}C-labelled ethanolamine have shown that it is rapidly incorporated into tissue phospholipids either as ethanolamine itself or after conversion to choline or serine. The remainder is metabolised to carbon dioxide and urea. The observed inhibition of SRS-A release during anaphylaxis in sensitised guinea-pig lung is thus most likely related to an effect of ethanolamine on phospholipid synthesis or phospholipid turnover in that tissue. This possibility is now the subject of further investigations.

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