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ON THE NATURE OF THE CHEMICAL MEDIATORS INVOLVED IN ANAPHYLACTIC REACTIONS IN MICE

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The effects of mepyramine, promethazine, chlorpromazine and lysergic acid diethylamide have been compared on the capillary permeability changes of the skin, produced by histamine, by 5-hydroxytryptamine and by passive cutaneous anaphylaxis in mice. Promethazine, mepyramine and chlorpromazine can inhibit, in decreasing order of activity, the effect of histamine, whilst lysergic acid diethylamide is inactive. Lysergic acid diethylamide and chlorpromazine are equally potent inhibitors of the action of 5-hydroxytryptamine on the peripheral vascular bed, whilst mepyramine is inactive. Promethazine has intermediate activity. Passive cutaneous anaphylaxis is strongly inhibited by chlorpromazine and by promethazine. Mepyramine and lysergic acid diethylamide, each injected alone, affect only weakly the anaphylactic reaction. However, passive cutaneous anaphylaxis is almost completely abolished by the simultaneous injection of the two last antagonists. It is suggested that the anaphylactic reaction in mice is the result of simultaneous release of both mediators, histamine and 5-hydroxytryptamine, each of them strengthening the effect of the other.

There is general agreement that the anaphylactic reaction is due to a release of endogenous chemical factors. From the considerable accumulation of results, including those originating from this laboratory, it appears that histamine is the only mediator implicated in the dog, the guinea-pig and perhaps in man (Code, 1937; Halpern, 1958; Humphrey & Mota, 1959; Halpern, Liacopoulos & Liacopoulos-Briot, 1959). There is some evidence, however, that histamine is not the sole mediator in other animal species, where it is thought that 5-hydroxytryptamine plays a more or less important role (Fink, 1956; Parratt & West, 1957). In fact, histamine antagonists have little, if any, effect on anaphylaxis in mice (Malkiel & Hargis, 1952), rats (Halpern, Liacopoulos & Perez Del Castillo, 1955; Rowley & Benditt, 1956) and rabbits (Reuse, 1949). On the other hand, reserpine and other agents which deplete 5-hydroxytryptamine stores have been found recently to protect mice against anaphylactic shock (Gershon & Ross, 1962).

It was the aim of these investigations to use various types of antagonists to investigate the nature of the mediators released in anaphylactic reactions in mice, as has been already done with rats and guinea-pigs (Halpern *et al.*, 1959; Craps, 1962). We have selected mepyramine and lysergic acid diethylamide, which are

considered to be specific antagonists respectively of histamine and of 5-hydroxytryptamine. We have also included promethazine and chlorpromazine which antagonize both histamine and 5-hydroxytryptamine (Halpern *et al.*, 1959). From the results it appears that the mechanism of anaphylaxis in mice is rather complex.

METHODS

Mice. Swiss male albino mice, weighing 19 to 27 g, were used.

Antigen. Three-times recrystallized albumen from hens' eggs, prepared according to the method of Kekwick & Cannan (1936), was used.

Antibody. Mouse anti-egg albumen antibody, present in a pool of peritoneal fluid, was prepared according to a slight modification of the method of Munoz (1957). 0.5 ml. of a 1:1,000 dilution of antigen, mixed with an equal volume of complete Freund's adjuvant, was injected intraperitoneally twice with a 3 day interval. The ascitic exudate was collected and pooled beginning 1 month after the first injection. Various pools of peritoneal fluid so obtained contained antibody titres ranging from 1,546 μg to 2,710 μg of antibody-protein-nitrogen per ml. The antibody content was determined by the technique of Heidelberger & Kendall, as described in Kabat & Mayer (1961).

Antagonists of histamine and of 5-hydroxytryptamine. Four antagonists were used: mepyramine, promethazine, chlorpromazine and lysergic acid diethylamide. Each was injected in a volume of 0.5 ml. subcutaneously into the back, 30 min before the challenge with antigen of the passive cutaneous anaphylaxis.

Passive cutaneous anaphylaxis. Passive cutaneous anaphylaxis was induced, with minor changes, as described by Ovary (1958). The ventral surface of the mouse was used instead of the back, since it was found to be slightly more sensitive and to give more clearly defined blue spots after injection of dye. Four areas equidistant from the midline were epilated using quick plucking motions of the thumb and index finger (a fifth midline area was often cleared for a saline control). Epilation was performed the day before the experiment to avoid non-specific irritation and consequent liberation of histamine. The following day, 0.02 ml. containing varying doses of antibody diluted with saline were injected intradermally. Control mice used in each series of experiments received doubling dilutions of antibody ranging from 0.0025 to 0.03 μg of antibody-protein-nitrogen/0.02 ml. (the threshold dose was about 0.003 μg of antibody-protein-nitrogen/0.02 ml.). Antagonist agents were always given 30 min before the antigen. An injection of 0.3 ml. of a mixture of antigen and Evan's blue was given intravenously into the dorsal vein of the penis, 3 hr after sensitization. The mixture contained, in 0.3 ml., 1 mg of egg albumen and 1 mg of Evan's blue. After 30 min, the animals were sacrificed rapidly by a blow on the head, and the results were read from the inner surface of the skin. The minimal concentration of antibody still giving a positive blue spot of about 12 ± 2 mm in diameter was considered to be the threshold. When antagonists were used, the new threshold was calculated as a multiple of the threshold dose found in the control animals.

Sensitivity to histamine. Increasing dilutions of histamine dihydrochloride (Hoffmann-La Roche) in 0.02 ml. of saline were injected intradermally into the abdominal skin, immediately after the intravenous injection of 0.5% Evan's blue solution. After 30 min, the results were read from the inner surface of the skin. The minimum concentration of histamine which gave a definite spot of about 12 ± 2 mm in diameter was taken as the threshold. When antagonists were used, they were injected subcutaneously into the back, 30 min before the injections of histamine. Their power as antagonists is stated in terms of the number of threshold concentrations of histamine, as established in the control, necessary to reveal a definite blue spot.

Sensitivity to 5-hydroxytryptamine. The same technique as that used for histamine was utilized to determine the threshold of activity and the power of the antagonists for 5-hydroxytryptamine.

RESULTS

Histamine and antagonists

The minimum threshold dose of histamine (expressed as base) producing an effect on capillary permeability (spot diameter 12 ± 2 mm) was 0.12 ± 0.02 μg; this mean value correlates well with that obtained by Frick, Stiffel & Biozzi (1962). As shown in Fig. 1, promethazine was the most powerful antagonist of those studied, since a dose of 0.75 mg/kg diminished the cutaneous reactivity to histamine 40-times; a

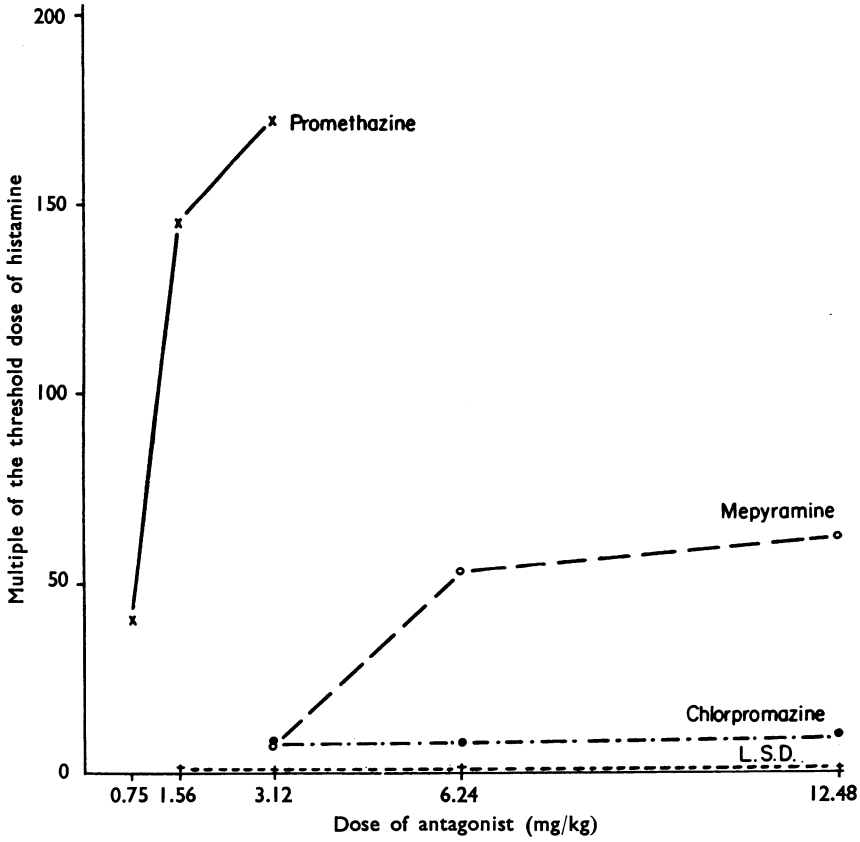

Fig. 1. Changes in the threshold dose of histamine active on capillary permeability after administration of mepyramine, promethazine, chlorpromazine or lysergic acid diethylamide (L.S.D.). Ordinate: multiple of the threshold doses of histamine active in the control mice ($0{\cdot}12 \pm 0.02$ μg of histamine base). Abscissa: dose of antagonists in mg/kg.

dose of 3 mg/kg diminished it more than 160-times. Mepyramine was approximately, weight for weight, one-thirtieth as active as promethazine, and chlorpromazine was less active than mepyramine. Although, at doses of 0.75 mg and 1.5 mg/kg, lysergic acid diethylamide had no antagonistic action, a slight elevation of the threshold dose of histamine occurred with greater doses of lysergic acid diethylamide. A similar observation was made by Halpern *et al.* (1959) and by

Inderbitzin & Craps (1957), and this result is probably due to the central nervous and vascular effects of lysergic acid diethylamide. It is interesting to emphasize that the results obtained in mice with these four antagonists agree essentially with the observations of Halpern *et al.* (1959) in their study on rats.

5-Hydroxytryptamine and antagonists

The average threshold dose of 5-hydroxytryptamine which produced in control mice an effect on capillary permeability (12 ± 2 mm) was 0.0012 ± 0.0002 μg, expressed as base. The results summarized in Fig. 2 indicate that lysergic acid diethylamide was the most powerful antagonist of those studied. A dose of 3 mg/kg raised the threshold of the change in capillary permeability produced by 5-hydroxytryptamine 170-times, and a dose of 6 mg/kg by more than 3,000-times. The activity of

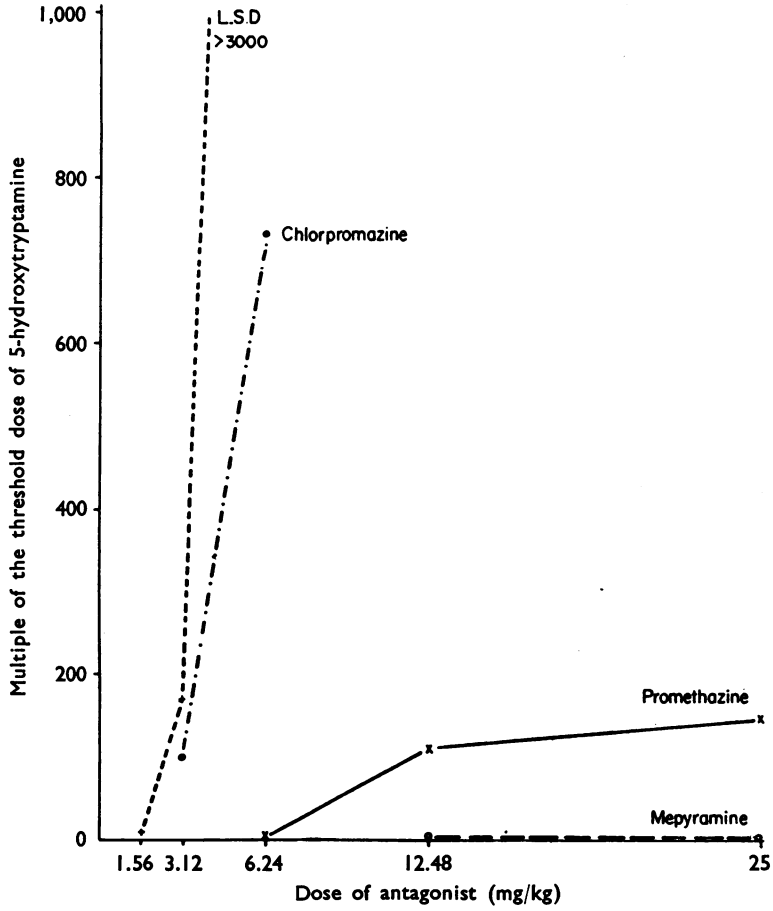

Fig. 2. Changes in the threshold dose of 5-hydroxytryptamine active on capillary permeability after administration of mepyramine, promethazine, chlorpromazine or lysergic acid diethylamide (L.S.D.). Ordinate: multiple of the threshold doses of 5-hydroxytryptamine active in the control mice (0.0012 ± 0.0002 μg of 5-hydroxytryptamine base). Abscissa: dose of antagonists in mg/kg.

chlorpromazine was surprisingly close to that of lysergic acid diethylamide, the threshold dose of 5-hydroxytryptamine in changing the capillary permeability being increased 100-times with 3 mg/kg and 734-times with 6 mg/kg. Promethazine definitely antagonized 5-hydroxytryptamine, but the effect was much less than that of lysergic acid diethylamide or chlorpromazine. Mepyramine was without action. It should be mentioned that very high concentrations of 5-hydroxytryptamine injected intradermally may cause some trivial vascular damage, which is unaffected by the antagonists.

Passive cutaneous anaphylaxis

Striking differences in the activity of the various antagonists were noted with reference to the passive cutaneous anaphylaxis. The results are summarized in Table 1 and schematically represented in Fig. 3. Mepyramine, at doses up to

TABLE 1

INFLUENCE OF CHLORPROMAZINE, PROMETHAZINE, LYSERGIC ACID DIETHYLAMIDE AND MEPYRAMINE ON THE THRESHOLD DOSE OF ANTIBODY NECESSARY TO PRODUCE PASSIVE CUTANEOUS ANAPHYLAXIS IN MICE

The control threshold dose of antibody is arbitrarily adjusted to unity. L.S.D.=Lysergic acid diethylamide

Compound	Dose (mg/kg)	Number of mice	Multiple of the threshold dose
Chlorpromazine	1·56	7	57·5
	3·12	8	292
	6·24	15	>8,437
Promethazine	1·56	7	38
	3·12	13	185
	6·24	10	227
	12·48	14	360
LSD	6·24	18	2·5
	12·48	7	12·5
	25	8	26
Mepyramine	3·12	9	1·64
	6·24	8	2·76
	12·48	8	3·4
	25	15	5·14
Mepyramine + LSD	6·25 + 6·25	10	1,800
Mepyramine + LSD	12·5 + 12·5	6	>4,000

25 mg/kg, increased only slightly the threshold dose of antibody. Lysergic acid diethylamide had a significant action at doses from 12 to 25 mg/kg. On the other hand, promethazine and particularly chlorpromazine were highly potent inhibitors of the anaphylactic reaction in mice. Promethazine, at a dose as low as 1.5 mg/kg, increased the threshold dose of antibody 38-times and, at a dose of 12.5 mg/kg, 360-times. Chlorpromazine was even more effective and, at a dose of 6.25 mg/kg, rendered the small skin vessels practically unresponsive to the anaphylactic reaction.

The very important finding is that when mepyramine and lysergic acid diethylamide were administered simultaneously, there was a considerable mutual reinforce-

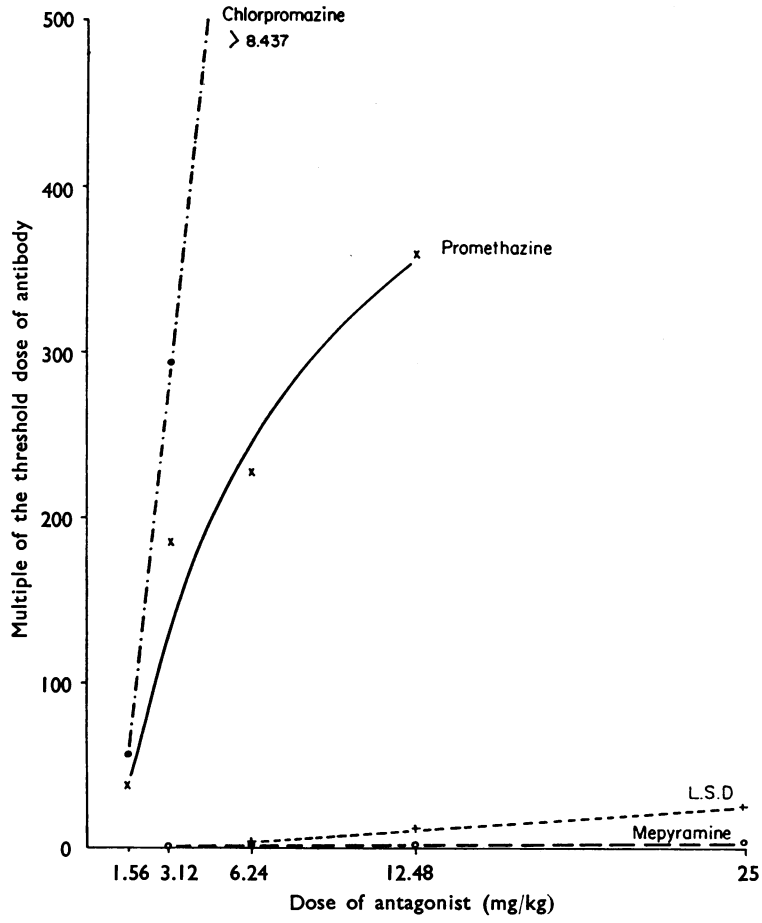

Fig. 3. Passive cutaneous anaphylaxis. Influence of previous treatment with mepyramine, promethazine, chlorpromazine or lysergic acid diethylamide (L.S.D.) on the threshold dose of antibody producing liminal passive cutaneous anaphylaxis (the threshold dose of antibody for controls was 0.003 to 0.005 μg of antibody-protein-nitrogen injected in 0.02 ml.). Ordinate: multiple of the threshold dose of antibody. Abscissa: dose of antagonists in mg/kg.

ment of their ability to inhibit passive cutaneous anaphylaxis in the mouse. Thus, for example, as shown in Fig. 4, the injection of 12.5 mg/kg of mepyramine raised the threshold dose of antibody which increased capillary permeability 3.4-times, and a similar dose of lysergic acid diethylamide 12.8-times. When both substances were injected together at the same doses, a quantity of antibody 4,000-times higher was unable to cause liminal capillary damage.

DISCUSSION

It is obvious from the data reported that anaphylaxis in mice cannot be due solely to the release of histamine. Although we have only investigated the cutaneous anaphylactic reaction, it seems likely that the results with this local reaction can

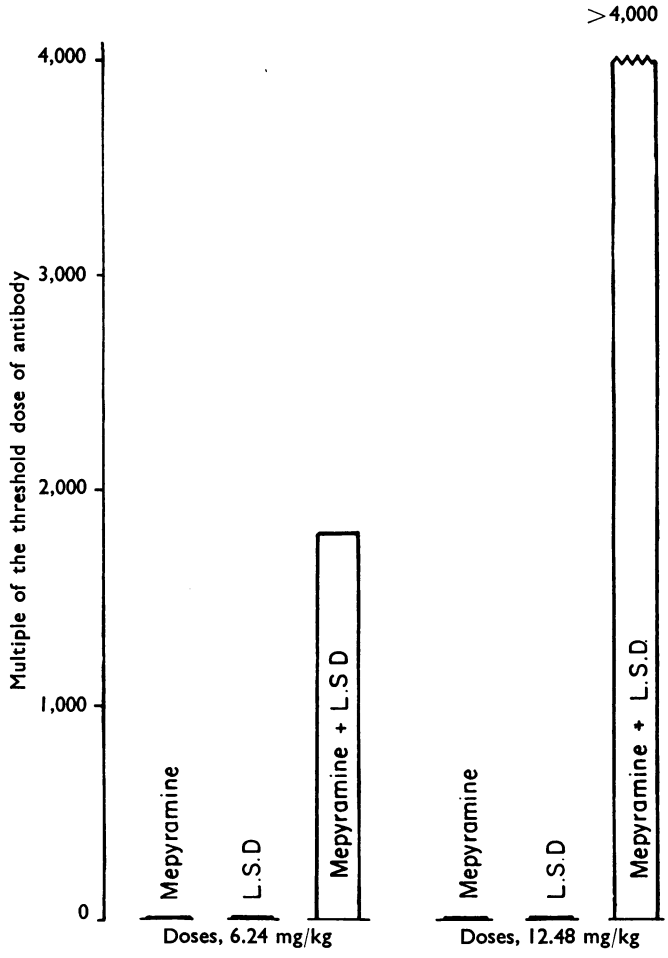

Fig. 4. Passive cutaneous anaphylaxis. Changes in the threshold dose of antibody (0.003 to 0.005 μg of antibody-protein-nitrogen for controls) active on capillary permeability, after the injection of mepyramine alone and lysergic acid diethylamide (L.S.D.) alone, and after the simultaneous injection of both antagonists, at identical doses.

be extended to general anaphylaxis. The sensitivity of the peripheral vessels of the mouse to histamine is greater than that in the guinea-pig or in the rat, since the threshold doses of histamine (expressed as base) which produce the liminal increase of capillary permeability were 0.15 ± 0.02 μg in the mouse, 0.3 μg in the guinea-pig and 0.94 μg in the rat. It is worthwhile to stress that the sensitivity of the capillaries is even greater to 5-hydroxytryptamine than to histamine, the threshold dose being of 0.001 ± 0.0002 μg (as base). It appears therefore that the damaging effect of 5-hydroxytryptamine in the mouse is about 100- to 150-times greater than that of histamine. Similar, although lesser, differences have been found for rats by Sparrow & Wilhelm (1957), Halpern *et al.* (1959) and Craps (1962).

A comparison of the effects of various antagonists on the vascular changes produced by histamine shows that the effectiveness of the antagonists correlates well with their already known pharmacological actions. Lysergic acid diethylamide has a very small effect, which is probably non-specific. Chlorpromazine, which is a weak antagonist of histamine, increases, at a dose of 12.25 mg, the threshold dose of histamine about 10-times. Mepyramine, at a similar dose, raises the threshold dose of histamine about 65-times. The most potent antagonistic action was that of promethazine. These results agree with those of Halpern *et al.* (1959) with rats. They are also consistent with the protective action of these drugs against general histamine toxicity in guinea-pigs, as established by Halpern & Ducrot (1946), Halpern (1947, 1949) and Halpern & Hamburger (1948). Thus mepyramine protects against 220 lethal doses and chlorpromazine against 80 lethal doses of histamine, while promethazine protects against 1,500 lethal doses of histamine. A satisfactory correlation also exists between the activities of the antagonists on other pharmacological effects of 5-hydroxytryptamine and their ability to inhibit the capillary permeability changes produced by this drug. Thus mepyramine has no action, while chlorpromazine and particularly lysergic acid diethylamide are very potent antagonists of 5-hydroxytryptamine. The activity of promethazine is intermediate.

It was important to establish the specificity of the various antagonists in order to interprete the actions of the drugs in a much more complex situation, namely the antigen-antibody reaction, as expressed by the passive cutaneous anaphylaxis. From our results it is obvious that the most effective inhibitors of passive cutaneous anaphylaxis are chlorpromazine and promethazine, while mepyramine and lysergic acid diethylamide were much less effective.

How can one explain these discrepancies ? If histamine were the main mediator, one would expect there to be considerable inhibition by mepyramine, since we have previously shown that mepyramine inhibits strongly the action of histamine on capillary permeability. The same consideration holds for lysergic acid diethylamide if 5-hydroxytryptamine were the main chemical mediator. The obvious conclusion is that neither of these two mediators operates solely. A similar situation has been found by Halpern *et al.* (1959) with certain histamine liberators, dextran, compound 48/80 and 1935 L, which were shown to release in rats both histamine and 5-hydroxytryptamine. Craps (1962) has recently confirmed these findings, and has shown that, while mepyramine or bromolysergic acid diethylamide, administered separately, affects only partially the exudate produced by the various histamine releasing drugs in the rat, full inhibition occurs when the same doses of the antagonists are injected simultaneously. It should be recalled that, in the experiments by Halpern *et al.* (1959) mentioned above, promethazine and chlorpromazine, unlike mepyramine, could inhibit strongly the change in capillary permeability due to the histamine releasing agents.

It is likely that the anaphylactic reaction in mice offers a similar problem: the specific inhibitors possessed only a slight effect on passive cutaneous anaphylaxis of mice, while promethazine and chlorpromazine, which antagonize both histamine and 5-hydroxytryptamine, were very effective. This hypothesis is strongly supported by the fact that the *simultaneous* administration of mepyramine and lysergic acid

diethylamide, in doses which by themselves affected the passive cutaneous anaphylaxis only moderately, caused regularly an almost total inhibition of the phenomenon. Anaphylaxis in mice appears therefore to result from simultaneous release of both mediators, histamine and 5-hydroxytryptamine. It is also apparent that each of these two mediators is strengthening the response to the other. For this reason, a drug which exerts both antagonisms or, even better, a mixture of two specific antagonists, is much more effective than the specific antagonists administered separately.

It is nevertheless acknowledged that no direct proofs are presented here to support the simultaneous release of both histamine and 5-hydroxytryptamine and, to establish this possibility, evidence has still to be provided that a selective depletion of histamine protects the animal against anaphylaxis as does depletion of 5-hydroxytryptamine by treatment with reserpine.

The mepyramine (Neo-antergan), promethazine (Phenergan) and chlorpromazine (Largactil) were graciously supplied by the Société Specia, Paris. The lysergic acid diethylamide was graciously supplied by Sandoz Laboratories, Basel.

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