STUDIES ON THE RELATIONSHIP OF 5-HYDROXYTRYPTAMINE AND THE ENTEROCHROMAFFIN CELL TO ANAPHYLACTIC SHOCK IN MICE*

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Plate 37

(Received for publication, September 22, 1961)

The mechanism that underlies anaphylactic shock in mice has not, as yet, been determined. The organ or organs most concerned are not known. Nor has there been a definite identification of a chemical mediator.

It is doubtful that histamine plays a significant role in bringing about the reaction. Mice are extremely insensitive to histamine (1–3) and much less than a toxic amount of histamine is present in the normal mouse (1). In addition, antihistamine drugs, which completely protect guinea pigs, do not protect the mouse from anaphylactic shock (3). These drugs also fail to influence the contraction, *in vitro*, of uterine strips from sensitized mice, in response to antigen (Schultz-Dale reaction, 4), though they do antagonize the effects of histamine. The uterus is also relatively insensitive to histamine.

Since the release of histamine probably does not play a significant role in mouse anaphylaxis, much attention has recently been directed to the possibility that 5-hydroxytryptamine (serotonin) might mediate the reaction. *In vitro* studies, using the Schultz-Dale reaction as an indicator of anaphylaxis, have provided some of the evidence implicating serotonin. The mouse uterus is much more sensitive to serotonin than to histamine, and the contraction that usually follows administration of antigen is blocked by antimetabolites of serotonin, such as lysergic acid diethylamide and reserpine (5). Prior treatment of mice with these compounds or with other antimetabolites of serotonin (6–8) protects mice against lethal anaphylactic shock. In addition, serotonin has been shown to be liberated from rabbit (7), though not mouse (10) platelets during anaphylactic shock. Finally, serotonin administration to mice (11) and to guinea pigs (12) mimics anaphylaxis.

Serotonin release, however, has not definitely been shown to be the cause of anaphylactic shock in mice. Analyses of the serotonin content of blood and tissues of animals dying in anaphylactic shock did not reveal significant differences from similar analyses in control animals (10). This finding does not eliminate serotonin as a possible toxic agent in mouse anaphylaxis. There is a wide variation in the normal serotonin content of tissues among individual mice, so that lethal amounts of serotonin could con-

* This research was supported by the United States Public Health Service Grant B-1523 (C2).

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ceivably be released at particularly effective sites and still go undetected by analyses of blood and tissues in many animals (13). Still, the evidence which implicates serotonin is indirect. It comes mainly from *in vitro* studies, and from studies using serotonin antimetabolites.

There is a considerable amount of evidence that indicates that the enterochromaffin (argentaffin) cells of the gastrointestinal tract represent a serotonin depot. Serotonin has been identified in high concentration in tissues rich in these cells (14, 15). Tumors (carcinoid) of enterochromaffin cells contain large amounts of serotonin (16). The histochemical reactions of these cells are similar to those of serotonin models (17, 18). Reserpine causes a release of serotonin from the gut which parallels a depletion of enterochromaffin substance (18). Lillie, however, pointing to the negative reaction shown by these cells for indoles, maintained that serotonin is unlikely to be the enterochromaffin substance (19). Other investigators have attributed the absence of an indole reaction to formalin fixation (17, 18).

In the present study several lines of evidence are presented which support the hypothesis that release of serotonin is causally related to mouse anaphylaxis. Substances which cause the depletion of serotonin stores render mice refractory to anaphylactic shock. Serotonin-depleting procedures also cause a depletion of enterochromaffin substance, a depletion which is also observed after anaphylactic shock. Further, the amount of enterochromaffin substance present correlated well with the susceptibility of mice to anaphylactic shock. Finally, the behavior of mice undergoing anaphylactic shock is similar to that following procedures known to release serotonin.

Materials and Methods

Animals.—A strain of mice derived originally from the DBA/2 and the BALB/c (Bar Harbor) and bred for 4 years in this laboratory was used. The animals were equally divided between the sexes and averaged 22 gm in weight.

Antigen.—Three antigens, diphtheria and tetanus toxoids, and pertussis vaccine combined (DPT) were used to sensitize and to challenge mice. Aluminum phosphate adsorbed product (tri-immunol) was obtained from Lederle Laboratories. Fluid DPT (tridipigen) was obtained from Eli Lilly and Company and material of the same lot number was used throughout.

Method of Sensitization.—Animals were sensitized by three subcutaneous injections of DPT, all of 0.1 cc. The first injection was of the aluminum phosphate adsorbed product. The second and third sensitizing injections were of fluid DPT. The second injection followed 2 weeks after the first and the third injection was given 1 to 2 weeks after the second. Anaphylactic shock was produced by intravenous challenge with fluid DPT exactly 1 week following the third sensitizing dose. The antigen used for challenge was diluted to maintain a constant ratio of volume to weight of mouse. A dose of antigen, 0.03 cc/gm, which was larger than the maximal amount of antigen used for challenge was shown to be non-toxic to a control series of thirty non-sensitized mice. Deaths following a challenge dose of antigen were therefore considered to be due to anaphylactic shock and were counted for 24 hours after the injection of DPT.

Drugs and Chemicals.—All drugs were dissolved in physiological saline unless otherwise noted.

1. 5-Hydroxytryptamine creatinine sulfate (Sigma Biochemical Co., St. Louis) was given

to mice by intravenous injection *via* the tail vein. Amounts given are expressed as mg/kg, but concentrations were chosen to maintain a constant ratio of volume to weight of mouse.

2. Reserpine (obtained through the courtesy of Ciba Pharmaceutical Co., Summit, New Jersey) was given intraperitoneally in doses of 5 mg/kg.

3. β-TM10 (SKF 6890, 2-(2-(6-dimethylphenoxy)propyl) trimethyl ammonium chloride monohydrate, obtained through the courtesy of Smith, Kline and French Laboratories, Philadelphia) was given subcutaneously, in four doses of 150 mg/kg each, over a period of 3 days. The final dose was given 1 hour prior to challenge with DPT.

4. Norepinephrine (Winthrop Laboratories, New York) was given subcutaneously in a dose of 0.01 mg in 0.5 cc solution immediately following challenge with DPT.

5. L-α-methyl dopa (3,4-dihydroxyphenyl-l-α-methylalanine, generously supplied by Merck Sharp & Dohme, West Point, Pennsylvania) was suspended in a solution containing 75 mg/cc of polyvinylpyrrolidinone (Matheson, Coleman and Bell, Cincinnati) in physiological saline. Polyvinylpyrrolidinone was included in order to provide a sustained release of l-α-methyl dopa from its site of subcutaneous injection (20). L-α-methyl dopa was given in a regimen of nine injections, 400 mg/kg each, at 6 hour intervals. Challenge with DPT followed the final injection by 1 to 2 hours.

6. Histamine phosphate (Nutritional Biochemicals Corp., Cleveland) was given in a dose of 750 mg/kg intravenously.

7. Compound 48/80 (Burroughs, Wellcome Research Laboratories, Tuckahoe, New York) was given in a dose of 50 mg/kg intravenously.

8. JB 516 (catron, β-phenyl isopropyl hydrazine HCl, generously supplied by Lakeside Laboratories, Milwaukee) was given intraperitoneally in a dose of 60 mg/kg.

9. 5-Hydroxytryptophane (Sigma) was given intraperitoneally in a dose of 50 mg/kg.

Histological Technique.—Tissues were fixed in 10 per cent formalin at pH 4.5 for at least 48 hours. The tissues were then washed in water, dehydrated in ethanol, cleared in cedarwood oil and benzene, and embedded in paraffin. Enterochromaffin cells were demonstrated by the methenamine silver method of Gomori and Burtner cited in reference 21 and the ferric ferricyanide method of Lillie and Burtner (22). The two methods showed approximately the same numbers of cells on alternate serial sections.

EXPERIMENTAL OBSERVATIONS

Effects of the Antigen.—Pertussis vaccine has been shown to increase the susceptibility of mice to serotonin, to histamine, and when combined with other antigens, to anaphylactic shock (2, 3, 11, 24–26). While in some strains of mice an increased susceptibility to histamine has not followed administration of pertussis vaccine (27), no strains have been reported, prior to this study, in which there has not been an increase in sensitivity to serotonin. Since this study is concerned with the toxicity of serotonin and its relation to anaphylactic shock, the effect of the antigens used on the toxicity of serotonin was ascertained.

The toxicity of various doses of serotonin was determined in a series of nonsensitized control mice and in animals sensitized to DPT. Table I shows that the lethality of serotonin did not differ significantly between the two groups, the 95 per cent confidence limits of the LD_{50} overlap. The antigens used in sensitizing this strain of mice, therefore, did not alter the sensitivity of these animals to serotonin.

Effect of Reserpine on Anaphylactic Shock.—Reserpine has been shown to

cause the release and subsequent depletion of stores of serotonin and catechol amines (28) and to protect mice from anaphylactic shock (6, 7). As seen in Table I, reserpine does not alter the toxicity of serotonin. The following experiments were designed therefore, to investigate the means whereby reserpine is able to prevent lethal anaphylaxis and, in particular, whether its ability to deplete serotonin or catechol amines is responsible.

Animals were sensitized to DPT and separated into several groups. The lethality of various doses of DPT was determined in a control group of mice receiving no additional treatment. The lethality of DPT was then determined for experimental mice receiving various treatments in addition to challenge with DPT. Comparisons were made with the control groups and the effects of these treatments on anaphylactic shock were thereby assessed.

TABLE I

Effect of Sensitization to DPT and the Administration of Reserpine on the Lethality of Serotonin

Treatment	No. of animals	LD_{50}* expressed as mg/kg serotonin given intravenously	95 per cent confidence limits of LD_{50}
Control mice—not sensitized to antigen (DPT)	79	245	219–275
Mice sensitized to antigen (DPT) challenged with serotonin 1 week after third sensitizing dose	78	245	221–272
Mice sensitized to antigen (DPT) and given reserpine 48 and 24 hours prior to challenge with serotonin	58	260	229–296

* Determined by the method of Litchfield and Wilcoxon (23).

Three groups of mice received reserpine. One group was given two doses of reserpine 48 and 24 hours prior to challenge with DPT. Another group received one dose 48 hours prior to challenge. The third group received one dose 30 minutes before challenge with DPT.

The results, which are summarized in Table II, confirm that reserpine does protect against anaphylactic shock. It does not do so, when it is given shortly before challenge with DPT. Reserpine has essentially disappeared from tissues 24 hours after injection (28). Mice are therefore protected from anaphylactic shock after the administration of reserpine at a time when the drug itself is no longer present in the animal. Moreover, there is a time delay after the injection of reserpine before the protective effect develops. With respect to these characteristics the protective action of reserpine parallels its amine-depleting and sedative actions. Thus, it is probable that the ability of reserpine to prevent lethal anaphylaxis is owing to an indirect, delayed action of reserpine, such as amine depletion, rather than to a direct effect of the drug itself.

In order to examine the possible role that catechol amine depletion might play in the prevention of anaphylactic shock, β-TM10 and norepinephrine

were given to mice. TM10 has been shown to deplete catechol amines from the adrenal medulla (29) and to prevent the release of catechol amines from sympathetic nerve endings upon stimulation of the nerve (30). β-TM10, a congener of TM10, has been shown to have the same sympatholytic effects as TM10 (but is devoid of muscarinic side effects) (30). As can be seen in Table II, β-TM10 did not significantly protect mice from anaphylactic shock. Norepinephrine, however, given at the time of challenge, did afford a slight but

TABLE II

Effect of Reserpine, β-TM 10 and Norepinephrine on Anaphylactic Shock

Treatment	No. of animals	Deaths/No. of animals in group at various doses of antigen (DPT) in sensitized mice, *cc/gm*					LD_{50}* with 95 per cent confidence limits
		0.002	0.004	0.012	0.020	0.028	
Control	71	0/11	4/15	10/15	15/15	15/15	0.0077 (0.0054–0.0116)
Reserpine, 48 and 24 hrs. prior to chellenge	60		0/15	0/15	0/15	0/15	—
Reserpine, 48 hrs. prior to challenge	40			0/12	5/28		—
Reserpine, 30 min. prior to challenge	43		5/12	12/15	16/16		0.0052 (0.0034–0.0075)
β-TM 10 for 3 days prior to challenge	60		2/15	11/15	14/15	15/15	0.0090 (0.0050–0.0162)
Norepinephrine at time of challenge	30			2/15‡	10/15§		—

* Determined by the method of Litchfield and Wilcoxon (23).

‡ $0.05 >$ p-compared by means of a $(Chi)^2$ test with control results.

§ $0.02 >$ p.

significant degree of protection. It has already been reported that epinephrine protects mice from anaphylactic shock (7). Catechol amines may therefore be looked upon as protective substances in the case of mouse anaphylaxis. Thus, the depletion of these amines by reserpine would be more likely to enhance rather than to prevent anaphylactic shock. The finding that β-TM10 does not protect against anaphylactic shock supports this view.

Effect of L-α-*Methyl Dopa on Anaphylactic Shock.*—L-α-methyl dopa inhibits the decarboxylation of aromatic amino acids (31, 32). It will therefore prevent the synthesis of serotonin from its precursor 5-hydroxytryptophane by inhibiting the enzyme, 5-hydroxytryptophane decarboxylase (32–34). If it is given over a period of time, substantial depletion of stores of serotonin gradually

results (34). It will also deplete catechol amines. In these ways L-α-methyl dopa resembles reserpine. The two drugs do not resemble each other, however, in chemical structure or in the mechanism by which they cause serotonin depletion. Reserpine produces serotonin depletion most probably by causing the release of bound serotonin to the free form from which it may subsequently be metabolized (28).

As can be seen in Table III, L-α-methyl dopa protects mice from anaphylactic shock when it is given over a period of time long enough to produce a depletion of stores of serotonin (34). These two drugs therefore, L-α-methyl dopa and reserpine, which are known to be similar only in their ability to deplete amines, both protect against anaphylactic shock. Reserpine, which is a more potent serotonin depletive than L-α-methyl dopa (28, 34) provides greater protection.

Enterochromaffin Cells and Anaphylactic Shock.—The presence of granules of enterochromaffin substance is necessary for the histochemical demonstration

TABLE III

Effect of L-α-Methyl Dopa on Anaphylactic Shock

Dose of antigen (DPT)	Lethality of challenge with antigen deaths/No. of animals in group		
	α-Methyl Dopa	Control	P*
cc/gm			
0.020	5/15	15/15	$0.001 > p$
0.012	4/15	10/15	$0.05 > p$

* Determined by $(Chi)^2$ test.

of the enterochromaffin cells. Therefore a loss of enterochromaffin substance is reflected in histological slides as a loss of cells. The number of duodenal enterochromaffin cells was therefore determined in control sensitized mice, in mice subjected to anaphylactic shock, and after various other procedures. No attempt was made to grade depletion of enterochromaffin substance within individual cells, so that only the loss of enough enterochromaffin substance to prevent the staining of cells could be detected.

A great deal of variation in the number of enterochromaffin cells along the gastrointestinal tract was observed. However, there was a uniform distribution of cells in the segment of duodenum between the pyloric sphincter and the entrance of the pancreatic duct. The number of cells in this region was also relatively constant between animals and this was seen to be a zone of high concentration of the cells. A 2 mm section of duodenum from this region was always selected for histochemical examination. The duodenum was oriented so that serial cross-sections could be cut. 250 sections, 8 micra in thickness, were cut, enterochromaffin cells demonstrated, and the number of cells in every fifth section was counted. Results are summarized in Table IV.

The number of enterochromaffin cells was greatly diminished in mice after anaphylactic shock, indicating release and depletion of enterochromaffin substance (Figs. 1 and 2). Reserpine, as expected (18), depleted the cells, but, unexpectedly, serotonin did also. Depletion of these cells seems to be a specific response since the number of cells was not significantly diminished after several non-specific stressful procedures. These included the subjection of mice to cold, fatigue, and drowning, by first storing them at 5°C for 2 hours, and then forcing them to swim in water at that temperature until they fatigued and drowned. Mice were also given lethal doses of histamine and 48/80, the latter a substance that causes the degranulation of mast cells with a consequent release of serotonin and histamine from these cells (35, 36).

TABLE IV

Enterochromaffin Cell Counts

Treatment	Mean No. of enterochromaffin cells counted in samples of 50 sections	95 per cent confidence limits of the mean	Estimated No. of enterochromaffin cells in whole 2 mm of duodenum	No. of animals counted
Control	15,204	16,907–13,501	76,019	10
Anaphylactic shock	4,948	5,875–4,021	24,738	5
150 mg/kg serotonin	8,496	11,460–5,532	42,482	5
5 mg/kg reserpine in two doses 24 hrs. apart	7,814	7,872–7,756	39,073	3
750 mg/kg histamine	15,983	16,976–14,990	79,916	4
Cold, fatigue, and drowning	12,871	14,142–11,600	64,355	3
50 mg/kg 48/80	15,365	20,197–10,533	76,827	3

The Effect of Enterochromaffin Cell-Depleting Substances on Anaphylactic Shock.—Both serotonin and reserpine cause enterochromaffin cell depletion. Reserpine can protect mice from lethal anaphylactic shock. Therefore, in order to study the possible relationship between enterochromaffin cell depletion and protection against anaphylactic shock, the effect of serotonin on lethal anaphylaxis was determined. In addition, the time course of the recovery of enterochromaffin cells after reserpine injection, was correlated with the return of susceptibility to anaphylactic shock. Results are summarized in Table V.

Sensitized mice were challenged with DPT 3 and 4 hours after having received an intravenous dose of serotonin. The dose of serotonin, 150 mg/kg, was sublethal (no deaths in 15 sensitized mice), but was able to cause enterochromaffin cell depletion. After 3 hours, as can be seen in Table V, prior treatment with serotonin provided good but only partial protection against anaphylactic shock. After 4 hours, however, protection was complete.

48 hours after a single injection of reserpine, the number of enterochromaffin cells is low and animals are resistant to anaphylactic shock. After 108 hours,

however, when the enterochromaffin cell counts had returned to about 80 per cent of normal, the ability of treated mice to undergo anaphylactic shock had similarly returned. A correlation has been demonstrated therefore between numbers of enterochromaffin cells and susceptibility of mice to anaphylactic shock.

The Effect of Sublethal Doses of Antigen on the Toxicity of Serotonin. The observations that sublethal doses of serotonin were able to cause the depletion of enterochromaffin cells and to protect mice from anaphylactic shock, seem

TABLE V

Enterochromaffin Cell Counts and Susceptibility to Anaphylactic Shock

Treatment	Time between first treatment and subsequent challenge with antigen	Mean No. of argentaffin cells in 50 section samples	Deaths/No. of animals in group at two doses of antigen (DPT) in sensitized mice, *cc/gm*	
			0.012	0.020
	hrs.			
Control	—	15,204 (10)*	10/15	15/15
150 mg/kg serotonin	3	8,496 (5)	3/15	
150 mg/kg serotonin	4	8,496 (5)	0/15‡	0/15‡
5.0 mg/kg reserpine	48	9,114 (3)	0/12	5/28‡
5.0 mg/kg reserpine	72	—		7/15
5.0 mg/kg reserpine	108	12,806 (5)		22/26§
5.0 mg/kg reserpine followed after 48 hrs. by 1-α-methyl dopa	113			4/15§

* Numbers in parentheses refer to the number of animals in the group for which the mean was determined.

‡ Compared by means of a $(Chi)^2$ test with the results obtained from control animals. $(0.001 > p)$

§ These results were compared with each other by means of a $(Chi)^2$ test. $(0.001 > p)$

at first, to conflict with the serotonin-release hypothesis of anaphylaxis. It was of interest, therefore, to determine whether the toxicities of serotonin and antigen are additive in sensitized mice, as would be predicted from the serotonin-release hypothesis.

A sublethal dose of antigen, 0.002 cc/gm, (see Table II), was given to sensitized mice. 30 minutes later they were challenged with serotonin. The results of this experiment, summarized in Table VI, show that the injection of antigen prior to a dose of serotonin does increase the toxicity of the serotonin. A summation between the lethality of antigen and serotonin can be demonstrated therefore, though we have only been able to observe this when the administration of antigen precedes that of serotonin. It has been reported (7) that prior treatment of mice with 5-hydroxytryptophane, the precursor of serotonin,

potentiates passive anaphylaxis, and that administration of serotonin or 5-hydroxytryptophane abolishes the protection afforded by reserpine against passive anaphylaxis.

The Effect of L-α-*Methyl Dopa on the Return of Susceptibility to Anaphylactic Shock after an Injection of Reserpine.*—48 hours after a single injection of reserpine, mice were placed on a 9 dose regimen of L-α-methyl dopa. The animals were then challenged with DPT 55 hours after the first injection of L-α-methyl dopa, and 113 hours after the injection of reserpine. As can be seen from Table V, L-α-methyl dopa prevented the return of susceptibility of the mice to anaphylactic shock, which ordinarily follows 108 hours after injection of reserpine. The lethality of DPT remained at about the same level that would be expected 48 hours after a single dose of reserpine when administration of L-α-methyl

TABLE VI

The Effect of Prior Treatment with Antigen on Serotonin Lethality

Treatment	Total No. of animals	LD_{50}* in mg/kg of serotonin with 95 per cent confidence limits
Control—mice sensitized to antigen (DPT).....	78	245 (221–272)
0.002 cc/gm DPT given to sensitized mice 30 min. prior to challenge with serotonin.....	72	190 (176–205)

* Determined by the method of Litchfield and Wilcoxon (23)

Note: The ratios of the components, in the case of animals receiving DPT and serotonin, to the independently effective doses (LD_{50} of each drug given alone) total approximately to unity (serotonin, 190/245 = 0.775; DPT, 0.002/0.0077 = 0.259; Sum = 1.034). This indicates that the toxicity of serotonin and DPT is additive.

dopa was begun. Thus, since L-α-methyl dopa is an inhibitor of serotonin synthesis, this finding may be taken as evidence that serotonin resynthesis is necessary, after its depletion by reserpine, for the induction of anaphylactic shock.

Behavioral Aspects of Anaphylactic Shock.—The behavior of mice undergoing anaphylactic shock was studied and compared with that of animals subjected to known serotonin-releasing procedures. The behavior of the animals used in this experiment, during anaphylactic shock, was similar to that which has been described in the literature as early as 1911 (37). As a means of facilitating description, the behavioral pattern may be divided into three stages. For 2 to 7 minutes following challenge with antigen there is no change in the behavior of mice. Therefore, though changes do occur in the vasculature of the ears at this time (38), this stage of anaphylaxis was called the latent phase. The mice then pass through a brief period of hyperactivity, not more than 1 minute in length, marked by scratching of the nose (which swells), biting of the anus,

and hyperexcitability to auditory and tactile stimuli. This period ushers in the second, or quiet phase, of anaphylactic shock. The animals lie prone, or huddle, and rarely move unless disturbed. Breathing appears to be difficult and at first is rapid and shallow, but becomes deep, forced, and very slow. The fur is ruffled. When forced to move, the animals often have an unsteady gait, and some weaken and appear to be unable to support their own weight. Diarrhea is almost always seen. This quiet phase of anaphylactic shock is very variable in length, and while rarely as brief as 4 minutes, it usually lasts for 10 to 20 minutes, and sometimes for several hours. After sublethal doses of antigen this phase tends to be long and animals lie quietly for up to 24 hours. The third, or lethal phase of anaphylactic shock is one of prostration. Breathing is irregular and gasping in nature. Animals become unresponsive even to painful stimuli. Body temperature has fallen and is low. The mice then characteristically roll over onto their sides, show some convulsive kicking movements, and stop breathing. The heart continues to beat for some time after breathing has ceased.

The second, or quiet, phase of anaphylactic shock resembles the sedated behavior of mice following the injection of reserpine. Thirty animals given reserpine, 5 mg/kg, each day for 3 days, were deeply sedated but could be roused. They showed an unsteady gait and a fall in body temperature. Diarrhea was prominent. Since evidence exists, indicating that the sedative effects of reserpine may be traced to serotonin release (39), the behavioral similarity between anaphylactic shock and reserpine treatment was investigated further.

It has been reported (40) that animals pretreated with a monoamine oxidase inhibitor and then given reserpine, become agitated rather than sedated. These mice mimic the agitated behavior of mice given 5-hydroxytryptophane, which penetrates into the brain and is decarboxylated to serotonin. This agitation is also accentuated if a monoamine oxidase inhibitor is given before 5-hydroxytryptophane (41, 42). Both of these procedures can be interpreted as productive of a high concentration of free serotonin in the brain (42, 43). The effect of a potent monoamine oxidase inhibitor, JB 516 (44), on anaphylactic shock was therefore determined.

JB 516, given to 60 sensitized mice, 1 to 2 hours before challenge with DPT, did not have any effect on the lethality of anaphylactic shock, but it did produce a profound change in the behavioral picture. The latent phase of anaphylactic shock existed as before, but the quiet phase was abolished. Rather, a picture of continuing agitation and excitement was seen. Eyes were open wide, the animals were hyper-responsive to tactile and auditory stimuli, and the fur was ruffled. Body temperature had risen. The mice eventually came to assume a characteristic posture with all four legs rigidly extended away from the body. The animals moved their heads rapidly from side to side and a generalized muscular tremor was seen. Periodically some of the animals vocalized and jumped up and down. Thirty sensitized mice given JB 516 alone, sometimes

became mildly hyperactive, but never showed the pattern of agitation which accompanied JB 516 and anaphylactic shock. Death of mice given JB 516 before a dose of antigen came suddenly, with no preceding phase of prostration. The agitated animals became rigid, ceased breathing, and rigor mortis followed rapidly, often within a minute. An identical pattern of behavior, marked by the characteristic posture, was observed in thirty animals receiving reserpine, and in thirty animals receiving 5-hydroxytryptophane 1 hour after a dose of JB 516. In these cases also, body temperature rose.

The behavioral pattern of mice undergoing anaphylactic shock bears some striking similarities, therefore, to that of mice treated with reserpine. In both cases, there is produced a period of sedation and a fall in body temperature. When treatment follows administration of a monoamine oxidase inhibitor, in both cases again, agitation replaces sedation, and a fall in body temperature is replaced by a rise.

DISCUSSION

Reserpine protects mice from anaphylactic shock (6, 7) and also releases serotonin and catechol amines (28). We have confirmed the ability of reserpine to protect mice and have found that the protection is maximal when the drug is given 2 days and again 1 day prior to challenge with antigen, but is nonexistent when reserpine is given 30 minutes prior to challenge. The protection by reserpine is therefore a delayed phenomenon and is probably not due to a direct action of the drug itself. It seems likely that the delay may be needed for the slow process of amine depletion. β-TM10, a member of a series of drugs which prevent the release of catechol amines from sympathetic nerves and deplete catechol amines from the adrenal medulla (29, 30), did not protect mice from anaphylactic shock. Since, in addition, epinephrine (7), and norepinephrine do provide protection, it does not seem likely that catechol amine depletion by reserpine could be responsible for the ability of reserpine to protect mice against anaphylactic shock.

L-α-methyl dopa, a drug which will, like reserpine, cause the depletion of serotonin, but which does so by a different mechanism also protects against anaphylactic shock. It prevents serotonin synthesis by blocking the decarboxylation of 5-hydroxytryptophane (32–34). Reserpine interferes with serotonin binding by tissues (28) leading to the release of serotonin and its subsequent metabolism by monoamine oxidase. Since these two drugs have little in common besides the ability to cause amine depletion, yet both protect mice from anaphylactic shock, it seems reasonable that the common action would probably be responsible for the protection in both cases. In addition, since catechol amine depletion is unlikely to provide protection, these findings may be taken as evidence that the presence of serotonin is necessary for anaphylactic shock to occur.

The enterochromaffin cell population of the mouse duodenum was studied because there is a considerable amount of evidence (14–18) indicating that these cells represent a serotonin depot. Enterochromaffin substance was severely depleted by anaphylactic shock. Nonspecific stresses, such as lethal histamine, or 48/80 administration, or the subjection of mice to cold, fatigue, and drowning did not release enterochromaffin substance. This finding is consistent with the hypothesis that serotonin is released from these cells in anaphylactic shock.

Reserpine, which protects against anaphylactic shock and releases serotonin, also depletes enterochromaffin substance. When, after the injection of reserpine, the number of enterochromaffin cells is low, as after 48 hours, mice are refractory to anaphylactic shock. However, as the enterochromaffin cell population recovers from the injection of reserpine, as it has largely done after 108 hours, the susceptibility of mice to anaphylactic shock similarly returns. Analyses of intestinal serotonin have also returned nearly to control levels at this time (14, 28). The protection from anaphylactic shock may be maintained, however, if animals are prevented by L-α-methyl dopa from resynthesizing serotonin. The state of the enterochromaffin cell population thus seems to correlate well with the susceptibility of mice to anaphylactic shock.

It was unexpectedly found that serotonin, like reserpine and anaphylaxis, causes the depletion of enterochromaffin substance. If the enterochromaffin cells do contain serotonin, then serotonin must be viewed as being able to cause its own release and depletion. Consistent with this view is the fact that sublethal doses of serotonin protect mice from anaphylactic shock. While incomplete protection follows 3 hours after an injection of serotonin, complete protection is seen after 4 hours. Most likely, time is needed for serotonin metabolism, after the injection of serotonin, before protection can be afforded. This finding at first seems to conflict with the hypothesis that anaphylactic shock is due to serotonin release. It would seem that if serotonin were the cause of anaphylactic shock, then doses of serotonin should add to endogenous serotonin, released by antigen, thereby increasing, rather than decreasing the susceptibility of mice to anaphylactic shock. The resolution of this apparent paradox was thought probably to lie in the timing of the administration of the drugs. When doses of antigen precede doses of serotonin, the effects are additive and the toxicity of the serotonin is enhanced as would be expected if antigen causes the release of serotonin. But when serotonin was given before a dose of antigen, and time was permitted for its breakdown, enterochromaffin cell depletion occurs, and we may postulate therefore that a depot of serotonin no longer remains to be released by antigen. Consequently, when the drugs are given in this way serotonin protects against anaphylactic shock.

Since enterochromaffin substance is released in anaphylactic shock, two independent lines of inductive evidence are thus brought together. One indicates that serotonin is the chromogenic material in the enterochromaffin cell,

and the other indicates that serotonin is released and plays a role in anaphylactic shock. Both lines of evidence are supported by this study, and indeed each now provides support for the other. As is always the case in an inductive argument, however, uncertainty remains. Future studies of a more direct nature will be necessary, but the definite establishment of one of these points will provide evidence in favor of the other.

There is a strong similarity between the behavior of mice given reserpine, and those undergoing anaphylactic shock. Both groups of animals show sedation during at least one phase of treatment, and both show a fall in body temperature. More importantly however, the effect of monoamine oxidase inhibition on anaphylactic shock is also similar to the effect of monoamine oxidase on reserpine treatment. In both cases depression is replaced by agitation. In both cases a fall in body temperature is replaced by a rise. The agitated behavior that follows monoamine oxidase inhibition in both cases is also similar to that seen following monoamine oxidase inhibition and the administration of 5-hydroxytryptophane, which raises brain serotonin levels. It has been proposed that sedation after reserpine administration is due to serotonin release (28, 39), and that the agitation that follows, when reserpine is combined with monoamine oxidase inhibitor, is due to the resultant high concentration of free serotonin in the brain (42, 43). Therefore, the similarity between the behavioral effects of anaphylactic shock and reserpine raises the possibility that serotonin release is a common feature, responsible for the effects in both cases. These behavioral findings, when reviewed along with the other evidence presented above, support the hypothesis that serotonin release is causally related to anaphylactic shock.

SUMMARY

Protection against anaphylactic shock in mice by reserpine has been shown to be a delayed phenomenon, probably not dependent upon a direct effect of reserpine. The release and depletion of catechol amines by reserpine show little likelihood of being responsible for protection because these substances are in themselves protective against anaphylactic shock, while β-TM 10, a drug which interferes with their release is not. Since L-α-methyl dopa and reserpine both deplete serotonin, and since both protect against anaphylactic shock, it is proposed that serotonin depletion is responsible for the protection.

Enterochromaffin substance is depleted in anaphylactic shock. It is also depleted by reserpine and serotonin, both of which protect against anaphylactic shock when given prior to challenge with antigen. The amount of enterochromaffin substance seems to correlate with susceptibility to anaphylactic shock.

The behavior of animals undergoing anaphylactic shock and the effect of shock on body temperature is similar to the effects on behavior and temperature

of treatment with reserpine, which is known to release serotonin. The effect of monoamine oxidase inhibition on animals undergoing anaphylactic shock is also similar to the effect of monoamine oxidase inhibition on animals given reserpine.

These results are consistent with the views that the release of serotonin is causally related to anaphylactic shock in mice and that serotonin is accumulated in the chromogenic material of the enterochromaffin cell.

The authors would like to express their appreciation to Dr. Roy Swan for his advice, encouragement, criticism, and his assistance in revising the manuscript. They wish also to express their gratitude to Miss Vira Levytska for her able technical assistance.

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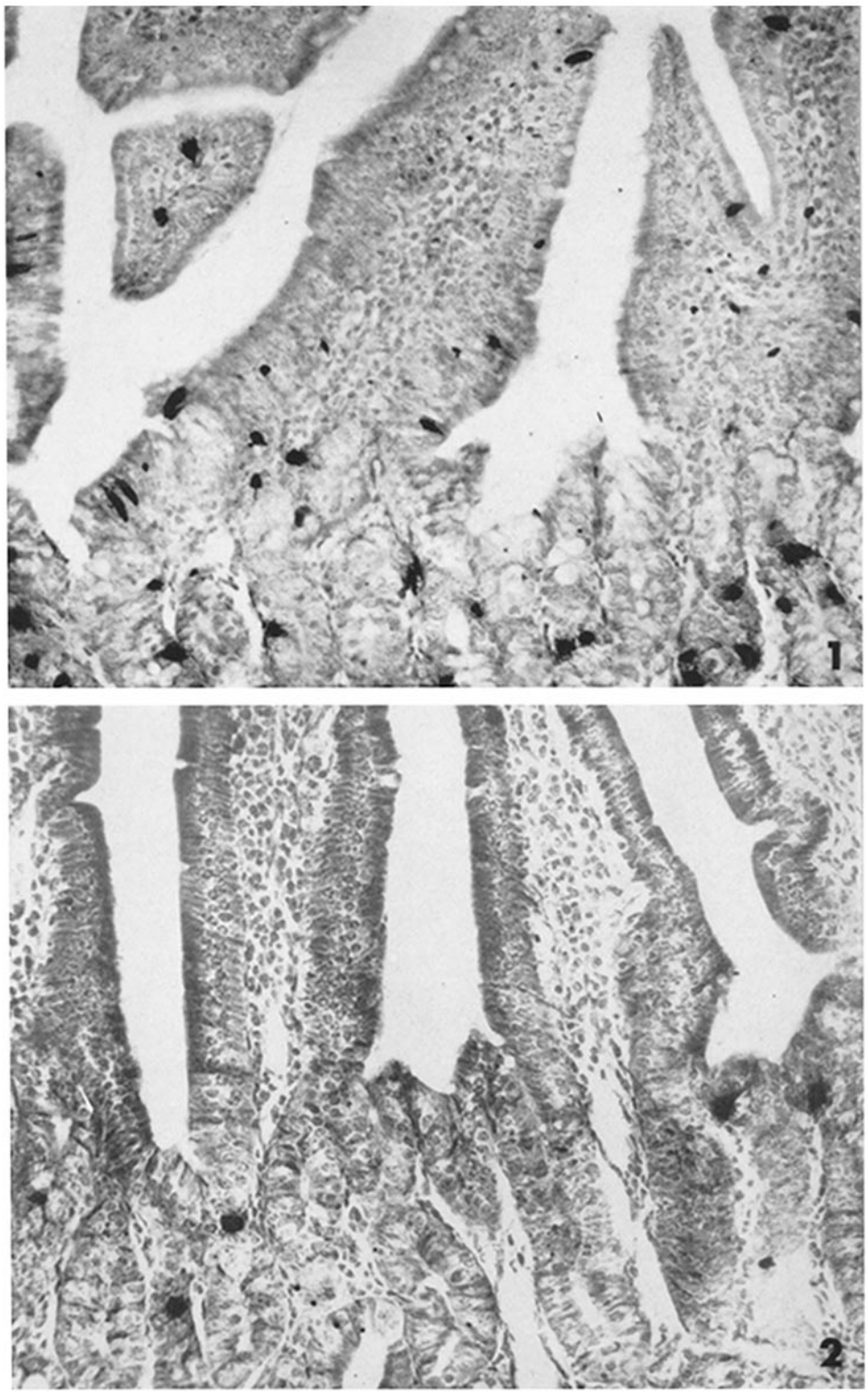
EXPLANATION OF PLATE 37

FIG. 1. A low power micrograph of a portion of the duodenum of a normal mouse. The enterochromaffin cells are stained black. Methenamine silver method, neutral red counterstain. 8 micra section thickness. × 300.

FIG. 2. A lower power micrograph of a portion of the duodenum of a mouse subjected to anaphylactic shock. The enterochromaffin cells, stained black, are few in number. Methenamine silver method, neutral red counterstain. 8 micra section thickness. × 300.



(Gershon and Ross: 5-hydroxytryptamine in mouse anaphylaxis)