

5-HYDROXYTRYPTAMINE AND HISTAMINE AS MEDIATORS
OF THE VASCULAR INJURY PRODUCED BY AGENTS
WHICH DAMAGE MAST CELLS IN RATS*

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PLATES 18 AND 19

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No single agent has been found which accounts for the hyperemia and increased vascular permeability associated with many common reactions to injury. Histamine appears to be important in some reactions, but there is abundant evidence that it is not the sole active agent, and in many reactions may not be the major factor. The following experiments demonstrate that the edema produced in the rat by ovomucoid and three other agents which damage mast cells is mediated by the combination of 5-hydroxytryptamine and histamine, or closely related substances.

In previous studies (1) of the acute vascular reaction produced by ovomucoid in rats it was shown that the edematous reaction in the skin was related to the distribution and concentration of mast cells in this tissue. Histamine was found to be associated with the mast cells and was shown to be released from tissues by ovomucoid. These parallelisms suggested that the histamine released might be a major factor in the production of hyperemia and edema in the rat. However, a direct correlation was not observed between the quantity of histamine released and the amount of edema produced. This finding suggested that another agent or agents present in skin might be involved in the production of edema.

The occurrence of material with the biological and chemical characteristics of 5-hydroxytryptamine in rat skin and its association with mast cells (2) induced us to test synthetic 5-hydroxytryptamine for its capacity to produce increased vascular permeability and edema. When injected subcutaneously, 5-hydroxytryptamine was found to be an exceedingly potent agent in this respect. This raised the question of whether 5-hydroxytryptamine, operating alone or in combination with histamine, might not mediate the edema pro-

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duced by ovomucoid and other agents which damage mast cells. The work which follows provides evidence that the two substances, acting together, do in fact produce the vascular response and that 5-hydroxytryptamine, or a closely related substance, is the major active component.

Material And Methods

The substances injected to produce edema were: ovomucoid, dextran, 48/80, testis extract, 5-hydroxytryptamine, and histamine.¹ The concentrations of histamine and 5-hydroxytryptamine are recorded as micrograms of free base per milliliter while the concentrations of the other agents producing edema are recorded as micrograms of dry material per milliliter. These agents were diluted in fresh 0.86 per cent NaCl and this diluent was used for control injections.

The edema-producing substances were injected into the paws of rats. Each paw was injected subcutaneously, using a tuberculin syringe and a 27 gauge long-bevel needle. The needle was inserted between the 3rd and 4th digits to approximately the midpoint of the dorsum; 0.05 ml. was injected into the forepaw and 0.10 ml. into the hind paw. The fore and hind paws on one side of the rat were used to measure the edema produced by a test agent; thus each rat was used to measure the edema produced by two agents or one agent and a saline control. Injections were rotated so that equal numbers of determinations for each agent were made on the right and left sides of rats.

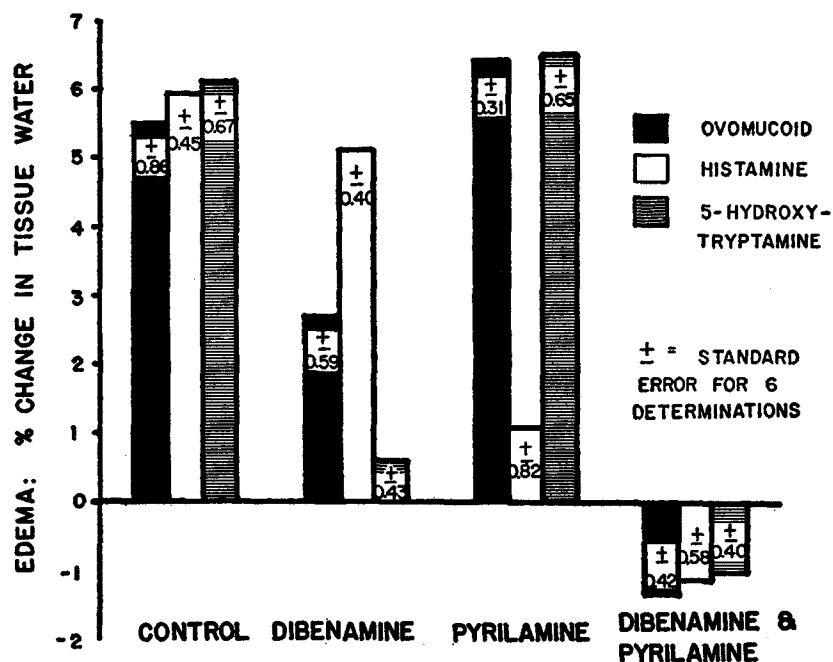
Each rat was injected intravenously with 2 mg. of Evans blue in 0.5 ml. of saline immediately prior to subcutaneous injections. Since Evans blue is adsorbed to plasma proteins, the blue staining of edematous tissues indicated increased vascular permeability to plasma proteins as well as to water (3). 3 to 5 minutes after injection of appropriate concentrations of the edema-producing agents, bluing of the wheal occurred. By 30 minutes there was maximum swelling and deep bluing of the entire dorsum of the foot that persisted for 4 or more hours. Injection of saline produced little or no bluing. The gross swelling from injection of saline subsided in about 2 hours; the edema was measured at this interval after challenge.

To measure the edema produced by subcutaneous injections, the tissue water in paw skins was determined. The full thickness dorsal paw skins, from the wrist and ankle to the interdigital spaces, were carefully removed from anesthetized and exsanguinated rats. The fore and hind paw skins from one side were combined for each determination. Each sample, fore and hind paw skin, was weighed and dried to constant weight at 100°C. for 5 days. Paw skin 2 hours after saline injection contained about 75 per cent water; paw skin with maximum edema contained about 85 per cent water. In Text-figs. 1 and 2 the edema is recorded as the percentage change in tissue water between paw skins injected with saline and paw skins injected with edema-producing agents. The changes in tissue water were computed from an average value for paws injected with saline and an average value for paws injected with an edema-producing agent.

¹ Ovomuroid was prepared as previously described (1). Dextran with a mean molecular weight of 80,000, the type used as a "plasma expander," was kindly supplied by Commercial Solvents Corporation, Terre Haute, Indiana. 48/80, the formaldehyde polymer of *p*-methoxyphenethylmethylamine, was kindly supplied by Burroughs-Wellcome Co., Tuckahoe, New York. Testis extract was a preparation (8 HY 11) used in previous studies: Benditt *et al.* *Proc. Soc. Exp. Biol. and Med.*, 1951, 77, 643. Synthetic 5-hydroxytryptamine creatinine sulfate was supplied through the courtesy of Dr. George Berryman, Abbott Laboratories, Inc., North Chicago. Histamine dihydrochloride was purchased from Eastman Kodak Co., Rochester.

In repeated experiments the gross response, bluing and edema, graded from 0 to 4+, correlated well with water determinations. Invariably a 2+ gross response equalled a 2 to 3 per cent increase in tissue water and a 4+ gross response equalled a 5 per cent or more increase in tissue water. Therefore in several experiments gross grading of the response was used to measure the reaction.

Mast cells were examined in histologic sections of the injected paw skins. The tissues were fixed in ethanol (80 per cent) and neutral formaldehyde (4 per cent), imbedded in paraffin, and sectioned at 5 μ . Toluidine blue, 0.1 per cent in 0.01 M acetic acid with 50 per cent ethanol V/V was used for staining mast cells.

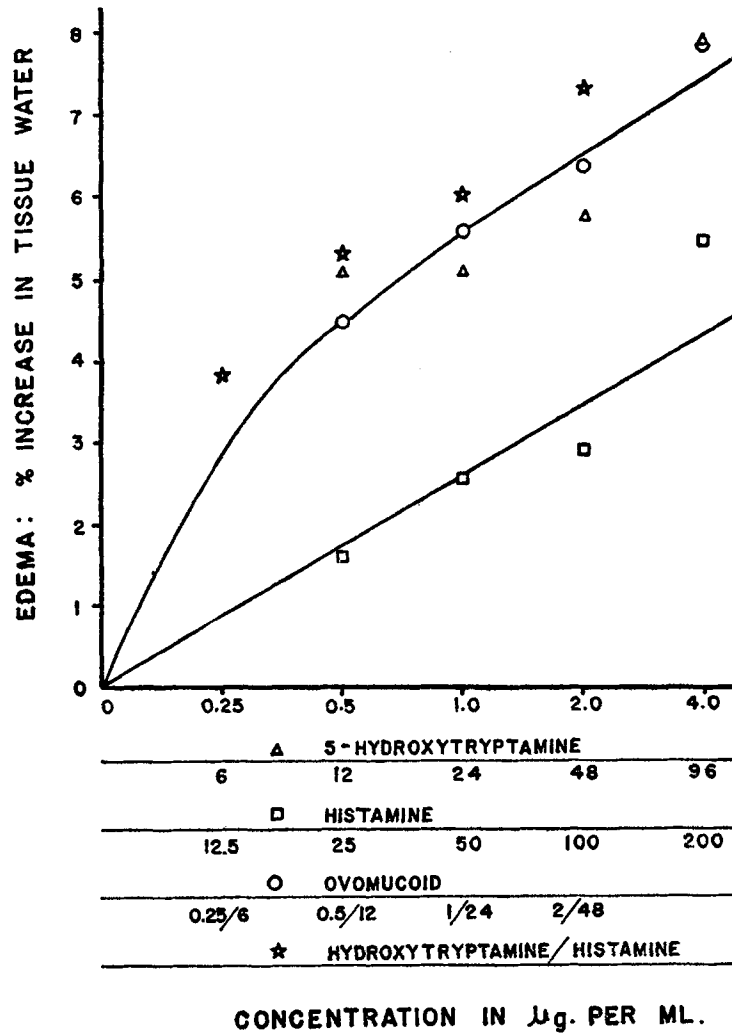


TEXT-FIG. 1. Comparison of the edema produced by ovomucoid 50 μ g./ml., histamine 200 μ g./ml., and 5-hydroxytryptamine 1 μ g./ml., and also the inhibition of edema produced by these agents with dibenamine 8 mg./kg. and/or pyrilamine 4 mg./kg.

Drugs used to inhibit edema were injected intravenously. Pyrilamine maleate (pyrilamine) was dissolved in saline; Dibenamine hydrochloride (dibenamine) was dissolved 100 mg. per ml. of diluting fluid; immediately before use the stock solution of dibenamine was diluted in saline to give the required concentration.² Drug doses were computed as milligrams per kilogram of body weight. Concentrations of dibenamine and pyrilamine were adjusted so that equal volumes were given for all drug doses. Control rats received saline and/or diluting fluid

² Pyrilamine maleate (also known as neoantergan) was kindly supplied by Merck and Co., Rahway, New Jersey. Dibenamine hydrochloride was kindly supplied by Smith, Kline, and French Laboratories, Philadelphia, the stock-diluting fluid formula recommended by them had the following composition: ethanol 48.5 ml., propylene glycol 48.4 ml., and concentrated HCl 0.2 ml.

diluted in saline. Dibenamine (or the control injection) was given 15 minutes, and pyrilamine (or the control injection) was given 10 minutes before local injections of the edema-producing



TEXT-FIG. 2. Edema produced by 5-hydroxytryptamine and histamine alone and in combination compared with the edema produced by ovomucoid. Each point is the average of 4 determinations.

agents. These time intervals were selected after preliminary experiments demonstrated, that pyrilamine inhibition of the edema produced by histamine subsided in about 1 hour, and that dibenamine inhibition of the edema produced by 5-hydroxytryptamine was maximum at 15 minutes, though active for more than 4 but less than 24 hours after intravenous injection.

Young adult, female, Sprague-Dawley rats weighing 160 to 190 gm. and maintained on

Purina chow and water *ad libitum* were used. Rats were anesthetized with ether for all injections.

EXPERIMENTAL OBSERVATIONS

Preliminary Experiments.—

In a series of preliminary experiments, concentrations of each agent were found which would produce edema of equal severity with about a 5 per cent increase in tissue water in the paw skins; the concentrations were: ovomucoid 50 $\mu\text{g./ml.}$, dextran 50 $\mu\text{g./ml.}$, histamine 200 $\mu\text{g./ml.}$, and 5-hydroxytryptamine 1 $\mu\text{g./ml.}$ Testis extract 150 $\mu\text{g./ml.}$ produced an equivalent increase in tissue water, but the edema was more diffuse, presumably owing to the presence of hyaluronidase in the extract. Each agent produced a gelatinous edema similar to that found in rats injected intravenously with ovomucoid (1). In Fig. 1 the gross edemas produced by ovomucoid, histamine, and 5-hydroxytryptamine are compared with the response produced by saline.

It was found that dibenamine 5 mg./kg. regularly inhibited the edema produced by 5-hydroxytryptamine 1 $\mu\text{g./ml.}$, and pyrilamine 2.5 mg./kg. regularly inhibited the edema produced by histamine 200 $\mu\text{g./ml.}$ In the following experiments the doses of drugs were: dibenamine 8 mg./kg. and pyrilamine 4 mg./kg. These doses were used to insure adequate drug levels and were not apparently toxic. Edema produced by histamine and edema produced by 5-hydroxytryptamine could be clearly differentiated by the use of these antagonists: Dibenamine did not affect the edema produced by histamine and pyrilamine did not affect the edema produced by 5-hydroxytryptamine. It was found that dibenamine partially inhibited but pyrilamine did not reduce the edema produced by ovomucoid.

These results suggested that the edema produced by ovomucoid is mediated in a large measure by 5-hydroxytryptamine; however, the results did not exclude histamine as a possibly active agent in the response. In the following two experiments the relative participation of 5-hydroxytryptamine and histamine in the edematous response to ovomucoid injection was examined.

Inhibition of Ovomucoid-Produced Edema by Dibenamine and Pyrilamine.—

The experiment was designed to test the effects of dibenamine and pyrilamine, alone and in combination, on the edema produced by ovomucoid, by 5-hydroxytryptamine, and by histamine. The concentrations of the three edema-producing agents were selected to produce an equivalent edematous response, characterized by approximately a 5 per cent increase in paw skin water.

Forty-eight rats were divided into four groups: One group of 12 control animals was treated with saline and the dibenamine diluting fluid; the other groups of 12 animals each were treated with: (a) dibenamine, (b) pyrilamine, and (c) dibenamine and pyrilamine respectively. Each

rat was used to measure the response produced by two agents (or one agent and saline); thus, the response to each of the three edema-producing agents and saline was measured six times in different rats in each group.

The results, presented in Text-fig. 1, show the changes in tissue water produced by 5-hydroxytryptamine, histamine, and ovomucoid in the four groups of rats. The changes in tissue water were computed from an average base line value derived from the 24 determinations on saline-injected paws in the four groups. There were no statistically significant differences in tissue water for saline-injected paws among the four treatment groups. The data clearly demonstrate that ovomucoid did not produce edema in rats treated with both drugs. They also show that: (a) the selected doses of the three agents produced an equivalent edematous response in the control animals, (b) dibenamine inhibited the response to 5-hydroxytryptamine without affecting appreciably the edema produced by histamine, (c) pyrilamine inhibited the edema produced by histamine without affecting the edema produced by 5-hydroxytryptamine, (d) dibenamine alone partially inhibited and pyrilamine alone did not affect edema produced by ovomucoid, and (e) neither 5-hydroxytryptamine nor histamine produced edema in rats treated with both drugs.

These results suggest that edema produced by ovomucoid is mediated in a large part by 5-hydroxytryptamine and/or related substances blocked by dibenamine. However, it is not apparent from these data why pyrilamine alone did not partially inhibit edema produced by ovomucoid. In the following experiment a reasonable explanation for this finding is given.

Edema Produced by 5-Hydroxytryptamine and Histamine, alone and in Combination, Compared with the Edema Produced by Ovomuroid.—

The possible roles of 5-hydroxytryptamine and histamine in the edema produced by ovomucoid were examined by comparing the edema produced by these two agents, alone and in combination, with the edema produced by ovomucoid. It was assumed that the quantities of 5-hydroxytryptamine and histamine or related substances "released" by ovomucoid were directly related to the quantity of ovomucoid injected. It was further assumed that these agents were "released" in approximately the ratio they were present in the subcutaneous areolar tissue of the skin of rat feet. Assays showed about 1 part of 5-hydroxytryptamine to 16 to 26 parts of histamine in this tissue (2).

Each of four serial twofold dilutions of (a) 5-hydroxytryptamine alone, (b) histamine alone, (c) 5-hydroxytryptamine and histamine combined in the ratio of 1:24, and (d) ovomucoid was tested in four rats; saline was tested in 16 rats. The ranges of concentrations tested were selected to give from just measurable to near maximum edema.

The results are plotted in Text-fig. 2. On the abscissa 1 $\mu\text{g./ml.}$ of 5-hydroxytryptamine was set coincident with 50 $\mu\text{g./ml.}$ of ovomucoid since these

concentrations were shown previously to produce an equivalent edematous response. The scale for histamine and the mixture of histamine and 5-hydroxytryptamine was then set by the ratio derived from the assays. Each point is the average of four determinations. One curve is fitted to the points for histamine and the other to the points for ovomucoid.

It is apparent that within the dose range selected: (a) there is increasing edema with increasing concentrations of the agents, (b) histamine is a much less potent edema-producing agent than 5-hydroxytryptamine, (c) histamine added to 5-hydroxytryptamine increases only slightly the edema produced by 5-hydroxytryptamine and, (d) the form of the dose response curve for the edema produced by ovomucoid is very similar to that produced by 5-hydroxytryptamine alone or combined with histamine. These findings are consistent with the suggestion that edema produced by ovomucoid is mediated in a large measure by the action of 5-hydroxytryptamine or a related substance. Since histamine combined with 5-hydroxytryptamine in the ratio used produced only slightly greater edema than 5-hydroxytryptamine alone, one would not expect an appreciable reduction in edema by an antihistaminic drug.

If the data are examined in another way, it is apparent that ovomucoid releases histamine as well as 5-hydroxytryptamine (or related substances inhibited by pyrilamine and dibenamine) in about the ratio of 1:24. In Text-figs. 1 and 2, 50 $\mu\text{g./ml.}$ of ovomucoid produced about a 5.5 per cent increase in tissue water; 0.5 $\mu\text{g./ml.}$ of 5-hydroxytryptamine combined with 12 $\mu\text{g./ml.}$ of histamine produced a similar rise in tissue water. If both histamine and 5-hydroxytryptamine were released by 50 $\mu\text{g./ml.}$ of ovomucoid, then on blocking the 5-hydroxytryptamine component one might expect a residual edema equal to that produced by 12 $\mu\text{g./ml.}$ of histamine. In the first experiment, Text-fig. 1, and in two other experiments not given in detail here, the increase in tissue water produced by 50 $\mu\text{g./ml.}$ of ovomucoid in dibenamine treated rats ranged from 1.2 to 2.5 per cent. This closely approximates the increase in tissue water, 1.8 per cent, produced by 12 $\mu\text{g./ml.}$ of histamine administered alone.

The Effects of Ovomuroid, 5-Hydroxytryptamine, and Histamine on Mast Cells and the Effect of Dibenamine and Pyrilamine on Mast Cell Damage.—

Since the edema produced by 5-hydroxytryptamine, closely resembled that produced by ovomucoid, it was desirable to investigate its effects on mast cells. The effects of ovomucoid and histamine on mast cells were also examined. It was of interest to determine whether the inhibition of the ovomucoid response by pyrilamine and dibenamine affected mast cell damage.

Two doses of each agent were tested: ovomucoid 50 and 1000 $\mu\text{g./ml.}$, histamine 50 and 1000 $\mu\text{g./ml.}$, and 5-hydroxytryptamine 1 and 20 $\mu\text{g./ml.}$ The lower concentration was selected to produce a 3 to 5 per cent increase in tissue water and the high concentration to produce

maximum edema, or about a 10 per cent increase in tissue water. Saline and each dose of the three agents were tested in two untreated rats and in two rats treated with both dibenamine and pyrilamine. Rats were sacrificed 2 hours after challenge and each full thickness paw skin was obtained for histologic section. Since both fore and hind paws were injected, sections of four paws injected with each agent were evaluated. The microscopic evidence of edema and the degree of mast cell damage with spill of mast cell granules into surrounding tissue were graded separately. The findings for each group of four paws injected with the same substance were comparable.

In untreated rats saline produced a slight edema and a few damaged mast cells, presumably due to the trauma of injection and removal of the skin. Neither concentration of 5-hydroxytryptamine or of histamine produced greater mast cell damage, than saline, though the edema produced by the higher concentrations was severe. The lower dose of ovomucoid produced minimal but distinct mast cell changes in excess of the controls, while the higher dose produced wide spread mast cell damage.

In animals treated with dibenamine and pyrilamine prior to injection of any of the injurious agents there was no edema. Following injection of saline and either concentration of 5-hydroxytryptamine or histamine the observed changes were similar to those in untreated rats. Following injection of either concentration of ovomucoid there was distinct and substantial mast cell damage with spill of granules into the surrounding tissue; in the absence of edema this appeared even more marked than in the untreated rats. Photomicrographs of some representative sections, shown in Figs. 2 to 5, demonstrate the principal findings.

The different sites of action of ovomucoid and of histamine or 5-hydroxytryptamine are clearly shown by the difference in effects on mast cells. Damage to mast cells with spill of granules into the surrounding tissue is not secondary to the production of edema. Furthermore, the inhibition of edema produced by ovomucoid is not mediated through the prevention of mast cell damage, but rather through the effects of the antagonists on "released" 5-hydroxytryptamine and histamine.

The Mechanism of Edema Production by 48/80, Dextran, and Testis Extract.—

Under similar conditions the mode of action of 48/80, dextran, and testis extract were compared with ovomucoid. The effects of these agents on mast cells was determined for two concentrations of each agent. One was the concentration which produced about a 5 per cent increase in tissue water and the other was twenty times this concentration. It was observed that each agent produced mast cell damage with spill of granules into the surrounding tissue similar to that produced by the comparable concentration of ovomucoid.

The effect of dibenamine and pyrilamine as antagonists for the edema produced by these mast cell-damaging agents was tested. The concentration of

each injurious substance was that which was found to produce about a 5 per cent increase in tissue water. Dextran 50 $\mu\text{g./ml.}$, 48/80 5 $\mu\text{g./ml.}$, testis extract 150 $\mu\text{g./ml.}$, and ovomucoid 50 $\mu\text{g./ml.}$ were each tested in four control rats and in four rats treated with: (a) dibenamine, (b) pyrilamine, or (c) dibenamine and pyrilamine. The edema and bluing were graded in the

TABLE I
The Effect of Dibenamine and Pyrilamine Alone and in Combination on the Gross Edema Produced by the Local Injection of Dextran, 48/80, Testis Extract, and Ovomuroid

Treatment	Gross edema			
	Dextran 50 $\mu\text{g./ml.}$	48/80 5 $\mu\text{g./ml.}$	Testis extract 150 $\mu\text{g./ml.}$	Ovomucoid 50 $\mu\text{g./ml.}$
Dibenamine*	1	2	1	1
	<i>2+‡</i>	2	2	1
	2	2	<i>2+</i>	2
	2	3	2	2
Pyrilamine*	4	4	4	3
	<i>4+</i>	4	4	4
	4	<i>4+</i>	<i>4+</i>	4
	4	4	4	4
Dibenamine and pyrilamine	0	0	0	0
	<i>0</i>	0	<i>0</i>	0
	0	0	0	0
	0	0	0	0
Diluting fluid§ and saline	4	4	4	3
	<i>4+</i>	4	4	3
	4	<i>4+</i>	<i>4+</i>	4
	4	4	4	4

* Dibenamine 8 mg./kg. and pyrilamine 4 mg./kg.

‡ The numbers in columns are the recorded responses for individual rats; the italicized number is the average response for four rats, to the nearest whole number.

§ Dibenamine diluting fluid diluted in saline.

gross. The results, presented in Table I, show that the drugs affected similarly the edema produced by these four agents. Dibenamine alone partially inhibited, pyrilamine alone had no effect, and the two drugs together completely inhibited the edema produced by each agent. Histologic sections of the paw skins showed that the drugs, administered in combination did not prevent mast cell damage. The results indicate that the mechanism of edema production was similar for these four mast cell-damaging agents.

DISCUSSION

The experiments described demonstrate clearly that several agents which damage mast cells in the rat release a substance in addition to histamine. This substance behaves *in vivo* like 5-hydroxytryptamine. To this we can now add the following ancillary evidence (2): Mast cells prepared from peritoneal washing of rats contain a material which is indistinguishable from 5-hydroxytryptamine by its biological and chemical properties and this substance is present in rat skin and subcutaneous tissues in proportion to the mast cell content of these tissues. Histamine is also found in these isolated mast cells in the quantity expected from measurements of the volume of mast cells and the concentration of mast cells in skin. The ratio of 5-hydroxytryptamine to histamine ranged between 1:16 and 1:26 by the two methods of estimation used. The combined evidence makes it very likely that 5-hydroxytryptamine is the edema-producing agent released by substances which damage mast cells.

Tryptamine also produces edema and increased capillary permeability when injected subcutaneously in rats. On a weight basis it requires 10 times as much tryptamine as 5-hydroxytryptamine to cause equivalent edema. This is in keeping with the relative reactivity of these two substances in other biological systems (4), and suggests that a 5-hydroxytryptamine receptor site is involved (5). Tyramine in concentrations up to 1000 $\mu\text{g./ml.}$ did not produce increased vascular permeability in rats (6).

Dibenamine is best known for its adrenergic blocking properties (7); its antagonism to 5-hydroxytryptamine has been more recently described (5). It seems unlikely from evidence now available that inhibition of edema by dibenamine is related to its adrenergic blocking activity. We have found that neither adrenalin nor noradrenalin in concentration up to 1000 $\mu\text{g./ml.}$ injected subcutaneously produces edema. In the rat the edema produced by egg white is partially inhibited by both adrenalin and noradrenalin (8). Consequently, if the adrenergic blocking effects of dibenamine were involved, one might reasonably expect enhancement of edema rather than inhibition.

Pyrilamine is a highly specific and potent antihistaminic agent (9) and for this reason was selected for our studies. We have found that pyribenzamine does not inhibit the edema produced in the rat by 5-hydroxytryptamine; its antihistaminic activity in the rat is similar to pyrilamine but it is less potent than pyrilamine on a weight basis. It is of interest that doses of pyrilamine of 2 mg. or more/kg. regularly potentiate slightly the edema produced by 5-hydroxytryptamine or the mast cell-damaging agents. This coincides with an observation that pyrilamine potentiates the 5-hydroxytryptamine induced contraction of the cat's nictitating membrane (10).

The doses of dibenamine and pyrilamine were higher than the minimum necessary for the inhibition of edema produced by the mast cell-damaging

agents. If the dose of each drug is decreased to one-half that used in the present experiments there is complete inhibition of the local edema produced by the subcutaneous injection of ovomucoid or the severe generalized edema produced by the intravenous injection of ovomucoid.

The capacity of 5-hydroxytryptamine (and tryptamine) to produce increased vascular permeability and edema has not been previously described. The site and mechanism of this action are not yet evident. It is known that 5-hydroxytryptamine is present in the nervous system (11), that it has a competitive action with acetylcholine on the molluscan heart (12), and that it initiates nerve reflexes (13). These actions suggest that 5-hydroxytryptamine may be another neurohumor. In this regard it has been reported recently that the edema produced on the palmar surface of the hind feet of rats by testis extract is inhibited by procaine block or section of the sciatic nerve (14). However, in our hands, nerve section or block does not inhibit the edema produced by 5-hydroxytryptamine or ovomucoid. It is of interest that neither acetylcholine nor carbamylcholine-chloride in doses up to 1000 $\mu\text{g./ml.}$ produce edema in rats' feet and that atropine in full therapeutic doses does not inhibit the edema produced by mast cell-damaging agents (15).

Erspamer presented evidence for an antidiuretic action of 5-hydroxytryptamine in rats and suggested that the site of action was in the kidney (16). It is significant that he found the antidiuretic action to be several times more effective following subcutaneous than following intraperitoneal or intravenous injection. Computations from our data suggest that there would be sufficient local loss of water in subcutaneous tissue with the doses of 5-hydroxytryptamine he used to account for the reported reduction in urine output.

It has been suggested that 5-hydroxytryptamine liberates histamine (17). Our experiments do not bear this out: There are no apparent mast cell-damaging effects with large doses of 5-hydroxytryptamine; the edema produced by histamine can be completely inhibited without affecting the edema produced by 5-hydroxytryptamine. Furthermore we have not found following repeated local injections of 5-hydroxytryptamine the refractory state seen following repeated injections of ovomucoid (1) or other known "histamine releasers" (18).

The *in vivo* assay methods used in these studies have potential value for assessing the actions of both edema-producing agents and drug antagonists. For example, using this method we have shown that chlorpromazine and phenergan are potent 5-hydroxytryptamine antagonists as well as antihistaminics (19).

The capacity of 5-hydroxytryptamine to produce increased vascular permeability upon local injection in certain sites in the rat has been demonstrated. It will be remarkable if this action of 5-hydroxytryptamine and its association with mast cells is unique to the rat. The present studies offer no explanation

of the mechanism of mast cell damage produced by substances of such widely differing origin and apparent composition as ovomucoid, crude testis extract, dextran, and 48/80.

SUMMARY

Each of the four agents, ovomucoid, dextran, 48/80, and testis extract, when injected beneath the skin of the dorsa of the paws of rats produces a local vascular injury characterized by a protein-rich edema. Each agent also produces damage to mast cells.

Either 5-hydroxytryptamine or histamine produces a response similar in the gross to that elicited by the agents which damage mast cells; however, neither of these two agents produces mast cell damage. On a weight basis 5-hydroxytryptamine is a much more potent edema-producing agent than histamine.

The edema-producing action of 5-hydroxytryptamine can be differentiated from the similar action of histamine by the use of specific antagonists; dibenamine is a 5-hydroxytryptamine antagonist and pyrilamine a histamine antagonist.

The edema produced by the mast cell-damaging agents is partially inhibited by dibenamine but is not diminished by pyrilamine. It is completely inhibited by treatment of rats with both drugs.

The drugs which inhibit edema do not prevent mast cell damage by ovomucoid, dextran, 48/80, or testis extract.

The observations are consistent with the hypothesis that agents which damage mast cells, "release" both 5-hydroxytryptamine and histamine and that in the rat the edema associated with mast cell damage is mediated largely by 5-hydroxytryptamine.

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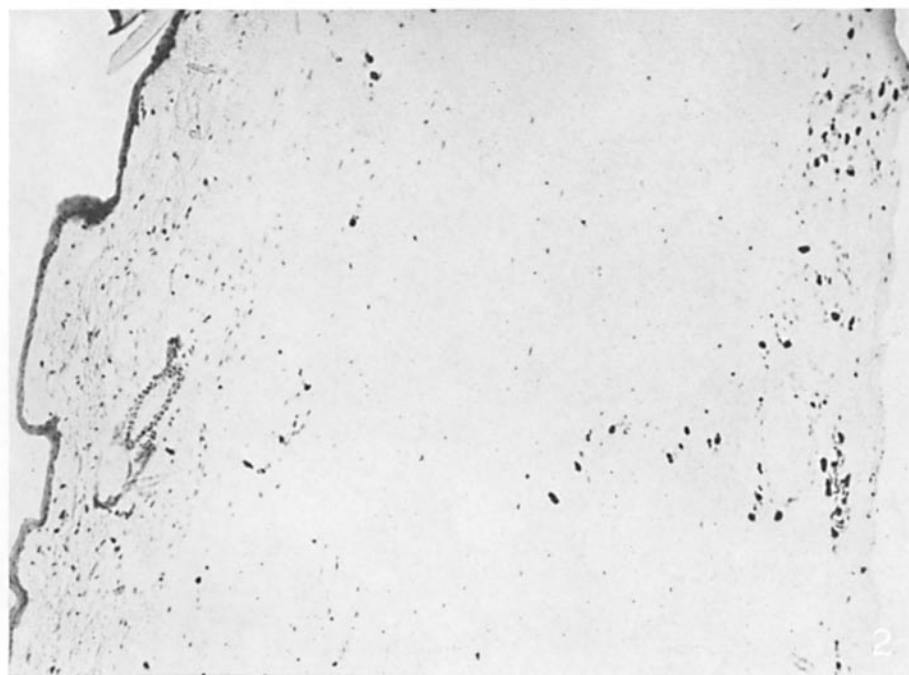
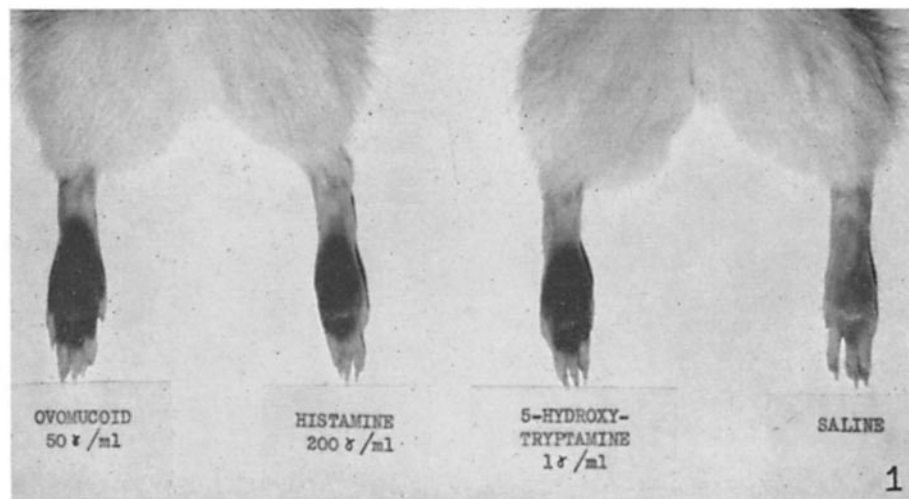
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EXPLANATION OF PLATES

PLATE 18

FIG. 1. Gross edema and bluing 2 hours after subcutaneous injection of ovomucoid, histamine, 5-hydroxytryptamine, and saline respectively into the paws of two normal rats. Each rat was injected intravenously with 2 mg. of Evans blue just prior to local injections.

FIG. 2. Section of rat paw skin stained with toluidine blue for demonstrating mast cells. Paw skin of a normal rat injected subcutaneously with 5-hydroxytryptamine 20 $\mu\text{g./ml}$. It shows severe edema. $\times 103$.



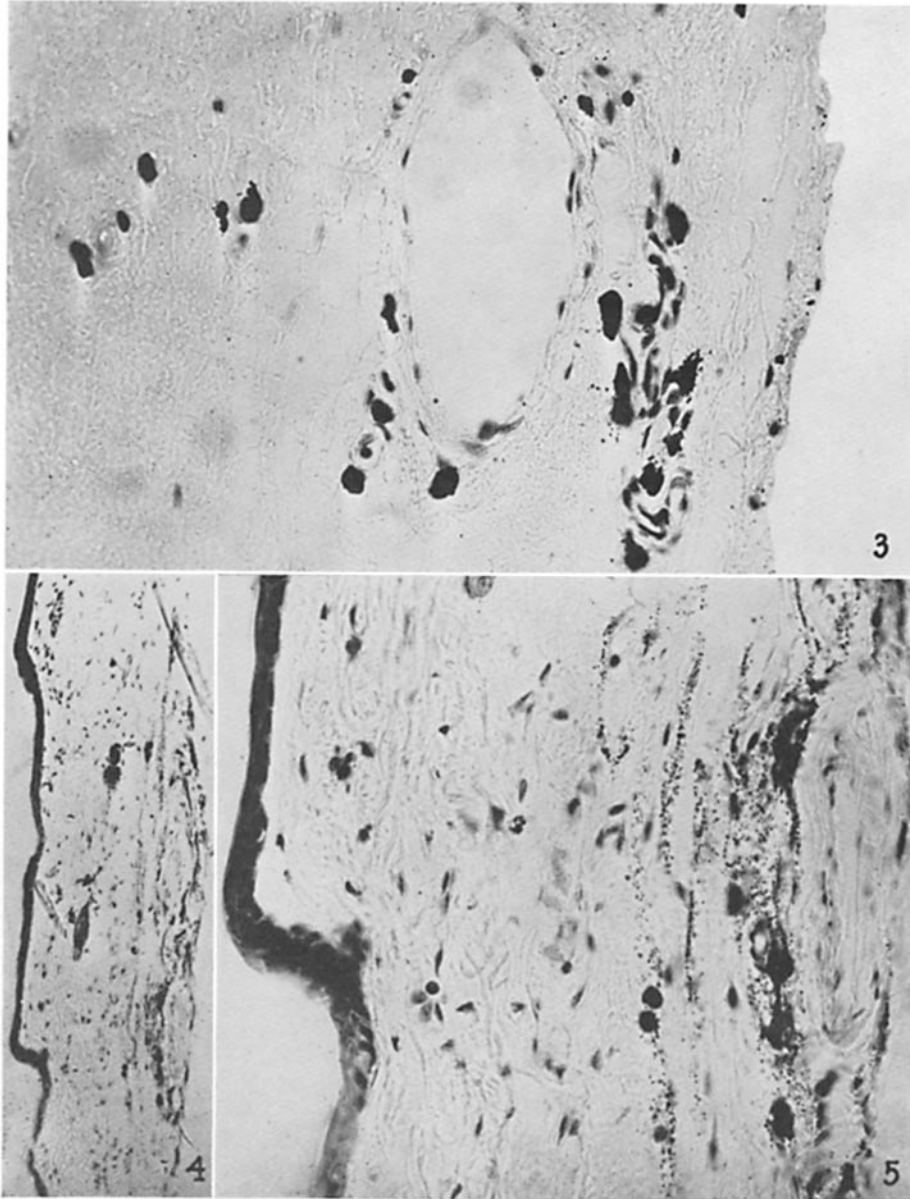
(Rowley and Benditt: 5-hydroxytryptamine and histamine and vascular injury)

PLATE 19

FIG. 3. Sections of rat paw skin stained with toluidine blue for demonstrating mast cells. Same as Fig. 2. Shows edema and intact or only slightly damaged mast cells. $\times 360$.

FIG. 4. Paw skin of a rat treated with dibenamine and pyrilamine and injected subcutaneously with ovomucoid 1000 $\mu\text{g.}/\text{ml.}$ $\times 96$.

FIG. 5. Same as Fig. 4. Shows no edema and marked widespread disruption of mast cells. $\times 360$.



(Rowley and Benditt: 5-hydroxytryptamine and histamine and vascular injury)