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ACTION OF ANAPHYLACTIC SHOCK AND ANAPHYLATOXIN ON MAST CELLS AND HISTAMINE IN RATS

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Anaphylactic shock in rats produces disruption of mast cells. These cells are also disrupted when rat mesentery is incubated with antigen *in vitro*. The plasma histamine reaches a maximum about 5 min. after injection of the antigen. Though antihistamines protect rats against anaphylactic shock, they do not prevent mast cell disruption. Previous depletion by compound 48/80 of the histamine bound to the mast cells prevents anaphylactic shock and the increase in plasma histamine. Anaphylatoxin produces no mast cell alterations or plasma histamine increase, and thus does not seem to act as a histamine-releasing agent in rats. Probably nearly all the histamine liberated in anaphylaxis in rats comes from the mast cells.

It is well known that histamine is released from tissues in anaphylaxis. It has recently been shown that most of the tissue histamine is located in the mast cells (Riley and West, 1953; Riley, 1953a), whence it can be displaced by histamine-releasing substances (Riley, 1953b; Mota, Beraldo, and Junqueira, 1953; Mota, Junqueira, Beraldo, and Ferri, 1954; Mota, Beraldo, Ferri, and Junqueira, 1956).

There has been considerable dispute as to whether true anaphylaxis occurs in rats (Dragstedt, 1941). But Halpern, Liacopoulos, and Castillo (1955) have recently shown that it can be regularly induced in rats with bovine serum albumin as antigen, and I have produced it consistently with alum-precipitated horse serum as antigen.

The present experiments were undertaken to study the action of a specific antigen on mast cells and histamine in rats. Since anaphylatoxin is a strong histamine-releasing agent (Rocha e Silva, Bier, and Aronson, 1951), and is able to duplicate the main features of anaphylactic shock in guinea-pigs, its effect on the histamine and mast cells was also investigated in rats.

METHODS

Wistar rats of both sexes weighing 300 to 400 g. were used. The antigen was filtered horse serum stored under aseptic conditions. Immediately before use the serum was precipitated by adding an equal amount of a 2.5% solution of potassium alum. The animals were sensitized with 1 ml. of precipitated serum subcutaneously, and 1 ml. of non-precipitated serum intraperitoneally, given daily for three days. The animals were challenged 18 to 30 days later with 1 ml./100 g. of body weight of horse serum intravenously. As controls, non-sensitized rats were given the same volume of horse serum.

Rat serum anaphylatoxin was prepared as described by Rothschild and Rocha e Silva (1954), and injected intravenously (1 ml./100 g. body weight). The activity of anaphylatoxin was tested by its effect on the isolated guinea-pig ileum.

For microscopical observation of the mast cells, the skin of the snout, the lips, the ears, and the tongue were fixed by injecting fixative through the carotid artery. The mesentery was fixed by immersion, stained, and examined in whole-mount preparations. The fixative was 50% aqueous ethanol containing 10% formaldehyde and 5% acetic acid. Fixation was allowed to proceed overnight, and frozen sections, 50 μ thick, were stained with toluidine blue.

Histamine assays were performed on the atropinized guinea-pig ileum according to Feldberg and Talesnik (1953). All histamine values are given as base.

RESULTS

Effect of Anaphylactic Shock and Anaphylatoxin on Mast Cells.—All 10 sensitized rats injected with the antigen gave reactions 3 min. after the injection, the most common being weakness, prostration, and difficulty in breathing causing retraction of the lower ribs. Scratching was noted in some animals. Most of these signs subsided within 1 hr. and all the animals had recovered

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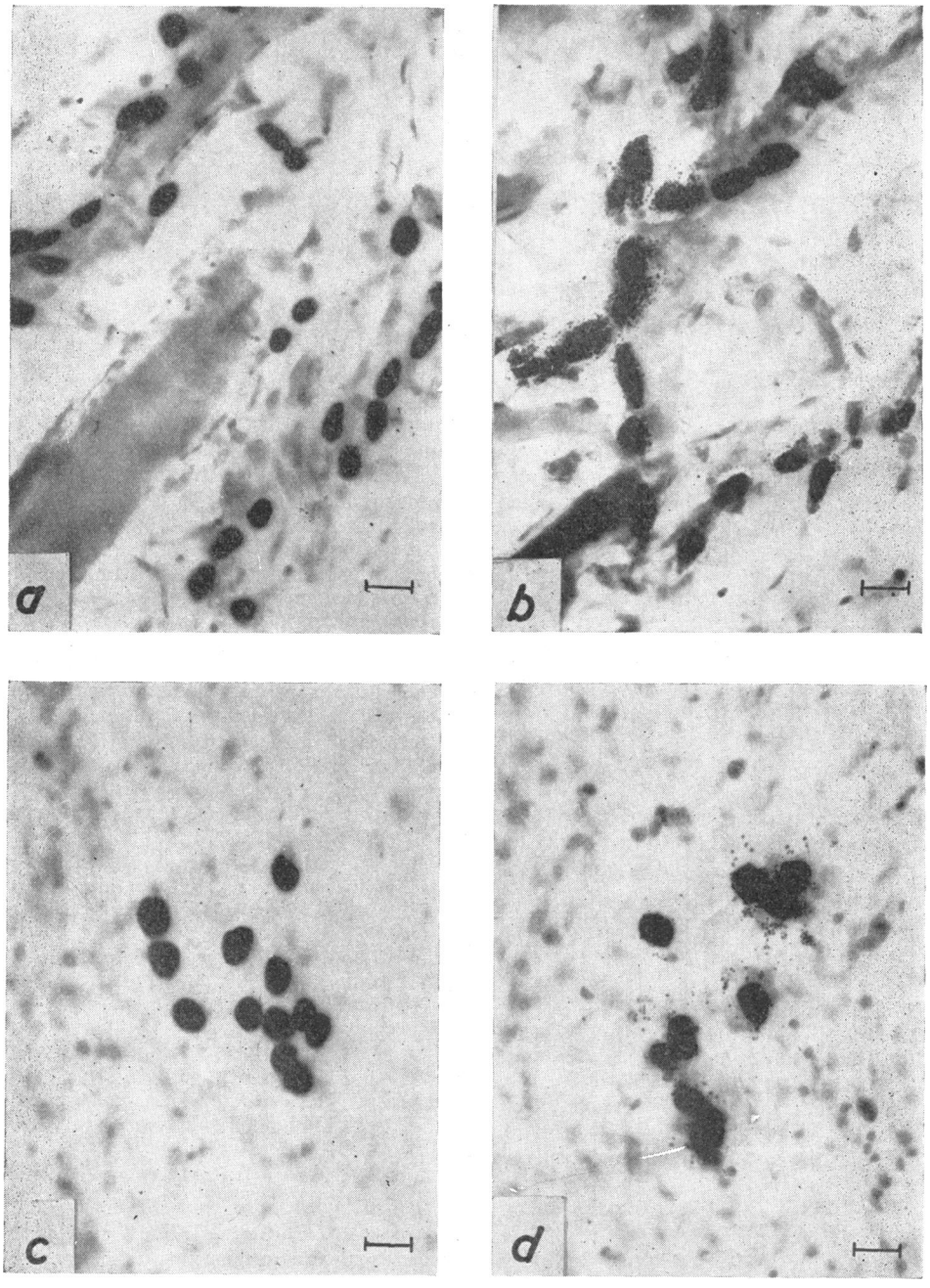

FIG. 1.—(*a*) Skin of a non-sensitized rat injected with horse serum showing normal mast cells. (*b*) Skin of a sensitized rat injected with horse serum showing disrupted mast cells. (*c*) Mesentery of a non-sensitized rat incubated with horse serum showing normal mast cells. (*d*) Mesentery of a sensitized rat incubated with horse serum showing disrupted mast cells. Scale in all photomicrographs = 20 μ.

3 hr. later when they were killed with ether. Although some mast cells kept their usual morphology and the number of altered mast cells varied from animal to animal, microscopical examination of the tissues showed constant and definite alterations of these cells, represented by granule extrusion, very similar to those prompted by the injection of compound 48/80 (Mota *et al.*, 1953). These alterations, present only in the shocked rats, were most conspicuous in the skin (Fig. 1*b*), less so in the tongue, and frequently absent from the mesentery.

None of the 20 rats injected with anaphylatoxin reacted. All of them were killed with ether 3 hr. after the injection, and on microscopical examination no mast cell alterations were observed.

Since adrenalectomy increases the number of reactive mast cells under conditions in which histamine liberation occurs (unpublished observation), anaphylatoxin was also injected to adrenalectomized rats: no mast cell alterations were detected.

In vitro *Experiments.*—Since mast cells of the mesentery react *in vitro* with histamine liberators (Mota *et al.*, 1953; Norton, 1954), similar experiments were conducted with antigen and with anaphylatoxin.

Sensitized rats were anaesthetized with ether and bled from the jugular vein. Pieces of mesentery were carefully dissected out and placed in cold isotonic sucrose. Two or three pieces were then placed in horse serum or rat serum anaphylatoxin for 15 min. at 37°, removed, fixed, stained, and examined. As a control, pieces of mesentery from non-sensitized rats were incubated in the same way with horse serum or non-activated rat serum. Microscopical observation showed mast cell disruption in the mesentery of the sensitized rats (Fig. 1*d*); but no alterations were seen in the mast cells of the non-sensitized controls. In the experiments with anaphylatoxin, in spite of the direct contact of this agent with the tissue, there were no alterations in the mast cells. However, if guinea-pig mesentery is incubated with rat serum anaphylatoxin very conspicuous alterations of the mast cells occur which are similar to those induced *in vitro* by the antigen in tissues of sensitized guinea-pigs.

Effect on Plasma Histamine.—Since blood histamine is increased in guinea-pigs and dogs subjected to anaphylactic shock (Code, 1939), the free histamine level of the plasma of shocked rats was determined.

The rats were lightly anaesthetized by injecting intravenously 2 ml./kg. of a mixture containing 10% of pentobarbitone sodium and 20% of urethane. Blood samples (1 to 2 ml.) were collected by puncture of the jugular vein in a heparin-containing syringe, before, and 3, 5, 10, 15, and 20 min. after the injection of the antigen or anaphylatoxin, and the plasma immediately obtained by centrifugation at 0° and assayed for histamine.

The results of these experiments are shown in Table I. Administration of the specific antigen to sensitized animals led to high plasma histamine levels. The maximum was reached in about 5 min., and then declined so that no histamine was detectable at 20 min. No increase in plasma histamine was detected in rats given anaphylatoxin.

TABLE I

PLASMA HISTAMINE CONCENTRATIONS (μG./ML.) IN SENSITIZED RATS BEFORE AND AFTER INJECTION OF THE SPECIFIC ANTIGEN

Rat	Before Injection	Time After Injection (min.)				
		3	5	10	15	20
1	0·0	0·10	1·50	0·00	0·00	0·0
2	0·0	0·15	0·10	0·10	0·00	0·0
3	0·0	1·00	1·20	1·20	0·05	0·0
4	0·0	0·15	0·05	0·05	0·00	0·0
5	0·0	1·00	1·00	0·50	0·15	0·0
6	0·0	1·00	1·20	0·50	0·10	0·0

Effect of Antihistamines.—In order to see the importance of histamine in the production of anaphylactic shock in rats, the protective effect of antihistamines was tried. From a group of 20 sensitized rats, 10 received intraperitoneally 5 mg. of chlorprophenpyridamine maleate (Alergon), and 1 hr. later all the rats were given antigen. Although all the non-protected rats had severe reactions, the rats pre-treated with antihistamine showed only mild shock. Since adrenalectomy makes rats particularly sensitive to anaphylaxis (Flashman, 1926; Wyman, 1929) these experiments were repeated with adrenalectomized animals. In a group of 10 sensitized and adrenalectomized rats given antigen, 8 died; in a similar group protected with the antihistamine, all survived. However, microscopical examination showed the same mast cell alterations in both groups.

Effect of Previous Treatment with Compound 48/80.—Since repeated injections of compound 48/80 deplete the histamine bound to the mast cells of rats (Mota *et al.*, 1956), it was decided to verify the effect of pre-treatment with compound 48/80 on anaphylactic shock. Thus, from a group of 20 sensitized rats, 10 were given daily intraperitoneal injections of each of the following

doses of compound 48/80: 0.1, 0.1, 0.2, 0.3, 0.4, 0.5, 0.5, 1.0 mg., while 10 others were given corresponding volumes of saline. Twenty-four hours after the last injection, both groups of animals were given antigen. The reactions in the animals treated with 48/80 were minimal, whereas those in the control rats were severe. Furthermore, in another group of 5 rats treated with 48/80, no plasma histamine could be detected after the injection of the antigen.

Discussion

Mast cell alterations in anaphylactic shock were referred to in dogs by Jaques and Waters (1941), and described in guinea-pigs by Mota and Vugman (1956). The modifications presented by these cells in anaphylaxis in rats are more conspicuous and easier to detect than those in guinea-pigs under the same conditions. However, under ordinary conditions of sensitization only in about one-third of the sensitized rats could definite mast cell alterations be found (Mota, 1953). It is interesting to note that only some mast cells present alterations ; the others maintain their usual morphology. We have also observed this after giving small doses of 48/80 to rats. Perhaps these responsive cells represent the source of the mobilizable histamine. It cannot be said whether the anaphylactic reaction takes place in the mast cells themselves, or whether the alterations of the mast cells are a consequence of a reaction taking place elsewhere. However, the hypothesis is tempting that the mast cell might contain antibodies, possibly adsorbed on its surface—which would account for histamine release occurring only when the antigen is applied to the intact cell, as shown by Copenhaver, Nagler, and Goth (1953) and Mongar and Schild (1956). Further experimental work is needed on this point.

It is known that in rats most of the tissue histamine is located in the mast cells or in unknown cells of the wall of the digestive tract (Mota *et al.*, 1956). Thus, both could be the source of the histamine liberated in anaphylactic shock in this species. Rats injected repeatedly with compound 48/80, and thus depleted of histamine bound to the mast cells, are protected from anaphylactic shock, and show no plasma histamine increase ; this strongly suggests that histamine liberated during anaphylactic shock originates in the mast cells.

It has been shown that 5-hydroxytryptamine is a natural constituent of rat mast cells (Benditt, Wong, Arase, and Roeper, 1955), and that substances, such as compound 48/80, that disrupt mast cells (Mota *et al.*, 1953) release 5-hydroxytryptamine from the tissues (Bhattacharya and Lewis, 1956). It is therefore probable that, in anaphylaxis in rats, 5-hydroxytryptamine and other substances are released besides histamine. However, no activity was observed in the plasma of the shocked rats that could not be inhibited by antihistamines. Furthermore, the fact that antihistamines protect rats against anaphylactic shock suggests that histamine is responsible for the major part of the syndrome of anaphylactic shock in this species.

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References

Benditt, E. P., Wong, R. L., Arase, M., and Roeper, E. (1955). *Proc. Soc. exp. Biol., N.Y.*, **90**, 303.
Bhattacharya, B. K., and Lewis, G. P. (1956). *Brit. J. Pharmacol.*, **11**, 202.
Code, C. F. (1939). *Amer. J. Physiol.*, **127**, 78.
Copenhaver, J. H., Nagler, M. E., and Goth, A. (1953). *J. Pharmacol.*, **109**, 401.
Dragstedt, C. A. (1941). *Physiol. Rev.*, **21**, 563.
Feldberg, W., and Talesnik, J. (1953). *J. Physiol.*, **120**, 550.
Flashman, D. H. (1926). *J. infect. Dis.*, **38**, 461.
Halpern, B. N., Liacopoulos, P., and Castillo, C. P. (1955). *C.R. Soc. Biol., Paris*, **149**, 314.
Jaques, L. B., and Waters, E. T. (1941). *J. Physiol.*, **99**, 454.
Mongar, J. L., and Schild, H. O. (1956). Ibid., **131**, 207.
Mota, I. (1953). M.D. Thesis, University of São Paulo, Brazil.
—— Beraldo, W. T., and Junqueira, L. C. U. (1953). *Proc. Soc. exp. Biol., N.Y.*, **83**, 455.
—— —— Ferri, A. G., and Junqueira, L. C. U. (1956). *Ciba Foundation Symposium on Histamine* (p. 47). London: Churchill.
—— Junqueira, L. C. U., Beraldo, W. T., and Ferri, A. G. (1954). *Nature, Lond.*, **173**, 547.
—— and Vugman, I. (1956). Ibid., **177**, 427.
Norton, S. (1954). *Brit. J. Pharmacol.*, **9**, 494.
Riley, J. F. (1953a). *J. Path. Bact.*, **65**, 471.
—— (1953b). *Science*, **118**, 332.
—— and West, G. B. (1953). *J. Physiol.*, **120**, 528.
Rocha e Silva, M., Bier, O., and Aronson, M. (1951). *Nature, Lond.*, **168**, 465.
Rothschild, A. M., and Rocha e Silva, M. (1954). *Brit. J. exp. Path.*, **35**, 507.
Wyman, L. C. (1929). *Amer. J. Physiol.*, **89**, 356.