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# THE SIMULTANEOUS RELEASE OF HISTAMINE AND A HISTAMINE-DESTROYING FACTOR DURING ANAPHYLAXIS IN RATS

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Mota (1957, 1958) has demonstrated that the concentration of histamine in the plasma of rats is increased during anaphylactic shock. He used alum-precipitated horse serum or *Bordetella* (*haemophilus*) *pertussis* vaccine as adjuvants to aid the development of the supersensitivity. Maruno (1958*b*) has shown that the concentration of histamine in the whole blood of rats previously injected with horse serum alone is greater after challenge with the serum than in control unsensitized animals. The objectives of this study were, first, to confirm the release of histamine during anaphylactic shock in pertussis-vaccinated rats; secondly, to explore the possibility of a simultaneous release of a histamine-destroying factor in such rats as has been shown already for rabbits by Rose & Leger (1952); and, finally, to determine whether release of histamine and a histamine-destroying factor occurred in anaphylactic shock in rats rendered susceptible to sensitization by adrenalectomy instead of by the use of adjuvants.

## METHODS

Healthy white male rats of the Sprague-Dawley strain, weighing 150–250 g, were used in all experiments. The intact animals were sensitized by a single intraperitoneal injection of 25 mg crystalline ovalbumin dissolved in 1 ml. NaCl solution, 0·9 g/100 ml. This was combined in the syringe for injection with 0·5 ml. of a saline suspension of phase-1 *Bordetella* (*haemophilus*) *pertussis* vaccine containing approximately $30 \times 10^9$ killed pertussis organisms. The adrenalectomized rats were sensitized by the subcutaneous injection of 0·5 ml. of sterile horse serum given on four consecutive days without the addition of pertussis vaccine. Rats sensitized to ovalbumin were challenged by the intravenous injection of 3–7 mg of the crystalline protein dissolved in 0·25–0·8 ml. of 0·9 % NaCl solution. One millilitre of the undiluted horse serum was used as the challenging intravenous injection for the adrenalectomized animals.

Anaesthesia was induced by the inhalation of ether or by intraperitoneal injection of pentobarbital sodium 4 mg/100 g body weight. The adrenal glands were removed through a single dorsal mid line incision at the level of the kidneys. The glands were approached

through small incisions just below the margin of the ribs on each side, care being taken to remove each gland without rupture of its capsule. After operation the rats were given 2% glucose in 0·9% NaCl solution to drink.

In the initial phases of the study blood was withdrawn, before and after shock, by cardiac puncture under ether anaesthesia. Later, suspicion was aroused that the trauma associated with cardiac puncture was contributing to the variability of the results. Blood was therefore subsequently withdrawn, under pentobarbital sodium anaesthesia, through a polyethylene catheter placed in the superior vena cava just above the heart by threading it down the jugular vein through a small incision in the neck. In the first and largest group of animals studied blood was withdrawn by both techniques. In the later tests and all those performed on adrenalectomized rats blood was taken via the catheter.

The concentration of histamine in the blood and plasma was determined by a modification of the Barsoum & Gaddum (1935) method (Code & McIntire, 1956) and is expressed in terms of the base throughout. When testing for the destruction of histamine by blood, one sample of blood was extracted immediately after collection and the remainder was set aside for varying periods either at room temperature or at 37–38° C. On some occasions oxygen was passed through the vessel containing the plasma or blood for a brief period before incubation. In some instances histamine was added to the blood before incubation. Occasionally the concentration of histamine in the plasma was determined without extraction by direct addition of the diluted plasma to the segment of guinea-pig ileum used in the assay.

## RESULTS

### *Control experiments on intact and adrenalectomized rats*

The mean concentration of histamine in the blood of sensitized but unchallenged animals was not significantly different from that of rats which had not received sensitizing injections; nor was there any significant difference in blood histamine between animals anaesthetized with ether and those anaesthetized with sodium pentobarbital. The values obtained in the 50 samples of blood withdrawn by both cardiac puncture and venous catheterization from the 50 rats under control conditions in the initial series were combined and gave a mean histamine concentration of $0{\cdot}136 \pm 0{\cdot}010$ $\mu$g/ml. Blood drawn by venous catheterization only, in the later series, gave a much lower mean of $0{\cdot}078 \pm 0{\cdot}008$ $\mu$g histamine/ml. Trauma during cardiac puncture was the likely reason for this difference, for when blood was drawn by cardiac puncture from four rats, first without and then with trauma, the values obtained with trauma were always greater. Likewise, when blood was taken by cardiac puncture or by venous catheterization from alternate rats of similar age, all samples of blood taken by cardiac puncture contained more histamine ($0{\cdot}240 \pm 0{\cdot}034$ $\mu$g/ml.) than those withdrawn through the catheter ($0{\cdot}078 \pm 0{\cdot}008$ $\mu$g/ml.). The mean concentration of histamine in the whole blood of 26 adrenalectomized rats was 0·09 $\mu$g/ml., which was not significantly different from blood of intact rats, withdrawn by the same technique (venous catheterization).

### *Anaphylactic shock*

The concentration of histamine in the blood of intact rats during anaphylactic shock was always greater than that encountered under control conditions (Table 1). During anaphylactic shock the concentration of histamine in blood drawn by cardiac puncture from rats under ether anaesthesia was significantly less than the concentration in blood obtained by venous catheterization from rats under pentothal sodium anaesthesia (Table 1), probably because considerably more time was required to withdraw the blood by cardiac puncture than via the venous catheter.

TABLE 1. Histamine concentration in whole blood of rats during anaphylactic shock

| Conditions | Sensitizing agent | Number of rats | Mean blood histamine ($\mu$g/ml.) |
|---|---|---|---|
| Intact rats | | | |
| Ether anaesthesia (cardiac puncture) | Ovalbumin | 10 | $0{\cdot}733 \pm 0{\cdot}160$ |
| Pentobarbital anaesthesia (venous catheterization) | Ovalbumin | 10 | $1{\cdot}950 \pm 0{\cdot}193$ |
| Adrenalectomized rats | Horse serum | 20 | $0{\cdot}680 \pm 0{\cdot}082$ |

Serial withdrawal of blood samples through the catheter allowed determination of the sequence of the changes in the concentration of histamine in the blood. In the control tests shown in Fig. 1 significant changes did not occur in the concentration of histamine in the blood of unsensitized rats after challenge by egg albumin or in the concentration of histamine in the blood of sensitized rats after challenge by 0·9 % NaCl solution. However, when sensitized rats were challenged with the specific antigen, a decisive increase in the concentration of histamine in the blood always developed with maximal concentrations usually occurring in samples withdrawn from 3–5 min after the challenge (Fig. 1). As is shown in Table 1, the concentration of histamine in the whole blood of adrenalectomized rats during anaphylactic shock ($0{\cdot}680 \pm 0{\cdot}082$ $\mu$g/ml.) always exceeded the range of values of blood drawn from similar rats under control conditions (0·09 $\mu$g/ml.).

### *Distribution of histamine in the blood*

Blood was withdrawn by venous catheterization from a group of sensitized intact rats before and after challenge either with 0·9 % NaCl solution or with the specific antigen. The formed elements of the blood were then separated by centrifugation. In the initial tests centrifugation was done at room temperature. In later experiments the blood was promptly chilled and centrifuged at 0° C. The histamine content of some of the

plasma was tested immediately by adding it directly to the guinea-pig ileum. Usually considerable dilution was required for satisfactory assay. The remainder was subjected to chemical extraction and later assayed for histamine. Small quantities of free histamine, usually < 0·05 μg/ml., were identified regularly in the unextracted plasma of the control rats given 0·9 % NaCl solution and somewhat smaller quantities were identified in extracts of such plasma (Table 2). Plasma from blood which had been promptly chilled and centrifuged at 0° C. after its withdrawal from animals in anaphylactic shock always contained a high concentration of histamine activity. The mean concentration in the plasma of seven rats was 2·36 μg/ml. by direct assay and 1·92 μg/ml. after extraction. Since only small

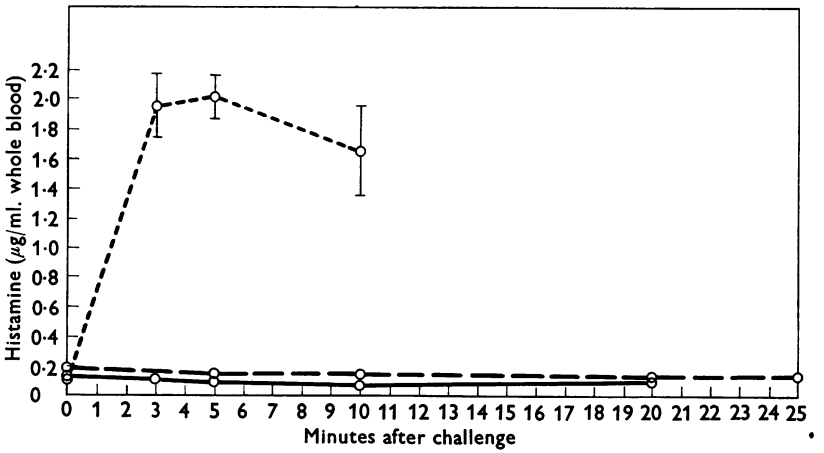


Fig. 1. The concentration of histamine in the blood of sensitized and unsensitized rats after challenge by intravenous injection of a solution of antigen or of 0·9 % NaCl solution; blood withdrawn by venous catheter. —— Sensitized, saline-challenged; – – – unsensitized, antigen-challenged; · · · sensitized, antigen-challenged. Vertical bars show 2 × S.E. of mean.

quantities of plasma were available for extraction, some of the difference may be due to losses sustained during the manipulations involved in the process of chemical extraction.

The distribution of histamine between the plasma and the formed elements was determined in pooled samples of blood drawn under control conditions and during anaphylactic shock. In eight such control collections the mean distribution of the histamine in the blood was 13 % in plasma, 66 % in the white cell layer and 21 % in the red cells. Losses of histamine during the separation were usually less than 20 %. When similar collections of blood from rats in anaphylactic shock were tested, losses of 30–60 % occurred and the plasma, instead of being rich in histamine, often contained no more than samples taken under control conditions. Since such losses

could not be ascribed to the procedures of separation or extraction, suspicion was aroused that an actual destruction of histamine was occurring.

*Release of a histamine-destroying factor during anaphylactic shock*

Blood drawn from animals in anaphylactic shock and allowed to stand undisturbed for 3 hr at room temperature consistently showed a significant (41 %) loss of histamine (Table 3). Control samples drawn from unshocked rats showed little or no destruction of histamine, even when sufficient histamine was added to bring the concentration within the range of that found in the blood in rats during anaphylactic shock (Table 3). Incubating the blood at 37° C increased the destruction of histamine to 54 % in 1·5 hr

TABLE 2. Histamine concentration in plasma of sensitized intact rats

| Conditions | Number of rats | Mean plasma histamine (μg/ml.) Direct assay | Assay after extraction | Difference (%) |
|---|---|---|---|---|
| 0·9 % NaCl injected | 6 | 0·046 ± 0·004 | 0·032 ± 0·003 | 30 |
| Albumin injected | 7 | 2·360 ± 0·175 | 1·916 ± 0·183 | 19 |

TABLE 3. Change in histamine content of blood of rats during incubation

| Number of tests | Condition of animal | Incubation of blood: Temperature (° C) | Incubation of blood: Hours | Histamine added to blood before incubation (μg/ml.) | Change in histamine content after incubation (%) |
|---|---|---|---|---|---|
| 4 | Unsensitized | R | 3 | 2 | + 7 |
| 4 | Sensitized | R | 3 | 2 | − 9 |
| 5 | Shocked | R | 3 | Nil | −41 |
| 7 | Unsensitized | 37 | 1·5 | 2 | − 5 |
| 4 | Sensitized | 37 | 1·5 | 2 | − 3 |
| 9 | Shocked | 37 | 1·5 | Nil | −54 |

*R* = room temperature.

in the blood from animals in anaphylactic shock, but did not increase the tiny quantities disappearing from blood drawn under control conditions. When the plasma of blood from rats in anaphylactic shock was separated and then incubated, the histamine in it was also quickly destroyed, indicating that part or all of the histamine-destroying factor found in the whole blood was present in the plasma fraction.

The results showed that during anaphylaxis in the rat a factor was released which destroyed the histamine liberated during the reaction. The question arose whether histamine added to the blood *in vitro* would be destroyed as effectively. To answer the question blood was drawn from a series of rats in anaphylactic shock and pooled. Samples for histamine determination were taken immediately and the remainder was incubated

at 37° C. for 1·5–3 hr, when further samples were withdrawn for histamine determination. Histamine was then added to the already incubated blood in amounts estimated to bring the concentration to approximately 2–3 μg/ml. and the blood was re-incubated for 1·5–3 hr. The results, which are given in Table 4, demonstrated clearly that the histamine-destroying factor in the blood was equally effective in the destruction of histamine added *in vitro* as it had been in destroying the histamine produced *in vivo* during the anaphylactic shock.

TABLE 4. Destruction of histamine in blood drawn from rats in anaphylactic shock

| | | Before addition of histamine | | After addition of histamine* | | |
|---|---|---|---|---|---|---|
| | | | | | Destroyed after reincubation (%) | |
| Pool number | Number of rats | Blood histamine before incubation (μg/ml.) | Destroyed after incubation† (%) | Blood histamine before reincubation (μg/ml.) | Without aminoguanidine | With aminoguanidine |
| 1 | 2 | 0·83 | 82 | 1·88 | 68 | — |
| 2 | 2 | 1·60 | 87 | 1·92 | 82 | Nil |
| 3 | 4 | 1·65 | — | 3·45 | 50 | 4 |
| 4 | 2 | — | — | 2·92 | 44 | — |
| 5 | 1 | — | — | 3·30 | 10 | — |
| 6 | 2 | 1·25 | 20 | 3·00 | 11 | Nil |
| 7 | 3 | 1·35 | 78 | 2·00 | 68 | Nil |

* Histamine was added to the blood after incubation.
† Incubation at 37° C for 1·5–3 hr.

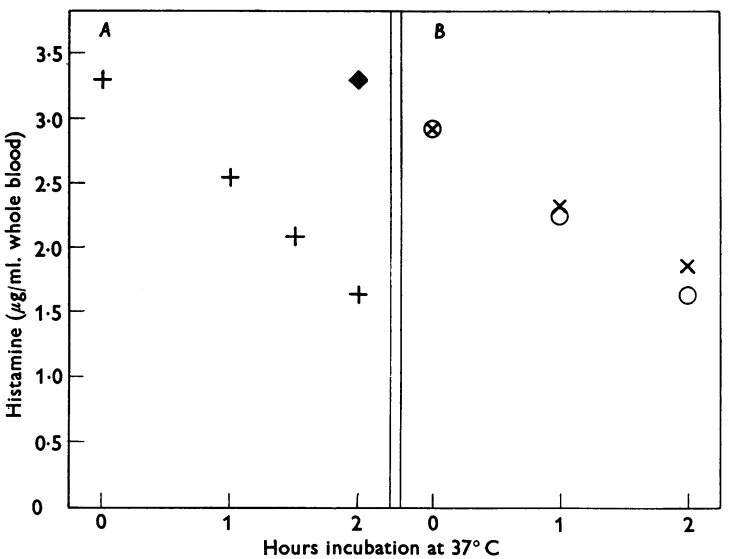


Fig. 2. Destruction *in vitro* of histamine in blood from rats in anaphylactic shock (see text). +, Shock blood+histamine; ◆, aminoguanidine added; ×, shock blood+control blood (1:1)+histamine; ○, shock blood+Tyrode soln. (1:1)+histamine.

Estimates were made next of the rate at which histamine was destroyed in blood drawn from rats in anaphylactic shock. Blood was taken from four rats in anaphylactic shock and pooled. Histamine was added in quantities calculated to bring the total concentration in the pool to between 3 and 3·5 $\mu$g/ml. After withdrawal of control samples, the pooled blood was incubated and samples were withdrawn at 1, 1½, and 2 hr. As is shown in Fig. 2*A*, destruction proceeded in a linear fashion during this period.

Since little or no destruction had occurred in the blood drawn from animals under control conditions, the prospect arose that the blood of healthy rats contained a factor which inhibited the histamine-destroying system. To test this possibility another pooled sample of blood from rats in anaphylactic shock was collected and to one portion of it blood from intact control rats was added, and to another an equal portion of Tyrode solution. The rate of destruction in the two portions of blood was almost identical (Fig. 2*B*). Thus, blood drawn from healthy rats does not contain an inhibitor of the histamine-destroying factor.

Destruction of histamine was usually demonstrable in blood drawn from adrenalectomized rats during anaphylactic shock, but the amounts destroyed during 1·5 hr incubation were generally less than those destroyed in blood from intact rats in anaphylactic shock. For example, in 17 samples of anaphylactic blood from adrenalectomized animals the mean destruction was 32 %, whereas in similar tests on blood from intact rats in anaphylactic shock it was 48 %.

The addition of sufficient aminoguanidine to the blood from animals in anaphylactic shock to produce a concentration of 0·4–0·5 $\mu$g/ml. of blood always completely prevented the destruction of histamine (Table 4; Fig. 2*A*). Since aminoguanidine is known to be an inhibitor of diamine oxidase (Zeller, 1938; Schuler, 1952) our results suggest that this enzyme is responsible for the histaminolytic power of anaphylactic blood.

## DISCUSSION

The mean concentration of histamine in the whole blood of intact male rats which we studied first was 0·136 $\mu$g/ml. when blood was withdrawn by both cardiac puncture and venous catheter. This value is greater than the 0·035 $\mu$g/ml. found by Rose (1938) in male rats but less than the 0·63$\mu$g/ml. reported by Maruno (1958*a*). In our later series, when blood was taken by venous catheter, a mean value of 0·078 $\mu$g/ml. was obtained. This is closer to the values of Rose, who took blood from the inferior vena cava without entering the thorax. Maruno obtained his samples by cardiac puncture. The differences in concentration therefore may be ascribed to differences in technique.

The plasma concentrations of 0·032 and 0·028 $\mu$g/ml. of unchallenged

rats found by us were less than the 0·186 μg/ml. reported in intact rats by Emmelin (1945; cardiac puncture) but comparable to the 0·035 μg/ml. reported by Halpern & Briot (1954; blood from retro-orbital plexus). Once again it seems most likely that the differences were due to differences in the technique employed for obtaining the blood.

Mota (1957, 1958) found that plasma from rats in anaphylactic shock contained increased quantities of histamine activity. He tested the plasma for histamine activity by direct addition to an isolated segment of guinea-pig ileum. Our results confirm his findings. The fact that the active substance survived the chemical extraction employed in this study supports the contention that it is histamine.

The exact role of histamine in the production of the symptoms of anaphylactic shock in rats is not clear. Mota has found, for example, that rats in which the stores of histamine had been depleted by 48/80 show minimal anaphylactic reactions. Sanyal & West (1958*b*), on the other hand, observed that rats in which the histamine stores were depleted by polymyxin B or by whole-body X-ray irradiation showed severe grades of anaphylaxis. The difference may have been associated with the mode of sensitization, for alum-precipitated protein was used as an adjuvant by Mota and *B. pertussis* by Sanyal & West. In addition, depletion of histamine stores is not likely to be the only effect of the agents.

The exact source of the histamine liberated during anaphylactic shock in rats is not known, but the possible sites of origin have been considerably restricted by the recent experiments of Brocklehurst (1960). In his tests the addition of antigen to the fluid perfusing lung, liver, or hind quarters of sensitized rats did not lead to the appearance of histamine in the perfusate. Since Brocklehurst obtained positive results in similar tests of tissues from other species it may be concluded that the lungs, the liver and hind quarters were not the source of the histamine detected in the blood in our study. The small bowel seems the most likely alternative, particularly since this region shows such a striking vascular change during anaphylaxis. The results of Sanyal & West (1958*a*) support such a possibility. They noted a diminution in the concentration of histamine in the jejunum of rats during anaphylactic shock, although, as they pointed out, this may have been due to dilution owing to oedema.

Maruno (1958*b*) has studied rats given repeated injections of horse serum without an adjuvant. In accord with the variable and minimal reactions associated with subsequent challenge with antigen, he obtained variable and minimal changes in the histamine content of the blood. In some of his animals the histamine and histaminase content of some tissues declined after challenge. It is difficult to correlate these results with those obtained in animals showing the severe reactions produced with the aid of adjuvants.

Rose & Leger (1952) found an increase in the histaminolytic power of the serum of rabbits during anaphylactic shock. Our observations in rats are similar. In neither species has the source of the destroying factor been determined.

In general, the degree of anaphylactic shock, the concentration of histamine, and the histamine-destroying power of the blood were less in rats sensitized after adrenalectomy than in rats sensitized with the aid of *Bordetella* (*haemophilus*) *pertussis* vaccine. The differences may have been due to differences in the degree of sensitization or to differences in amount of histamine or histaminase in the tissues after adrenalectomy (Karady, Rose & Browne, 1940; Rose & Browne, 1941; Valette & Huidobro, 1957; Hicks & West, 1958; Bartlet & Lockett, 1959).

## SUMMARY

1. The concentration of histamine in the blood has been estimated under control conditions and during anaphylactic shock in male white rats rendered susceptible to sensitization by the injection of *Bordetella* (*haemophilus*) *pertussis* vaccine and by adrenalectomy.

2. During control studies significant differences in blood histamine were not found between rats anaesthetized with ether and those anaesthetized with pentobarbital sodium; nor were there significant differences in the blood of sensitized and unsensitized animals or of intact and adrenalectomized rats. However, blood drawn by cardiac puncture consistently contained more histamine than blood drawn by venous catheterization.

3. The concentration of histamine in the blood of intact and adrenalectomized animals always increased during anaphylactic shock. During shock free histamine was uniformly detectable in the plasma.

4. During anaphylactic shock in intact and in adrenalectomized rats a histamine-destroying factor, tentatively identified as diamine oxidase (histaminase), was found in the blood.

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