THE ANAPHYLACTIC REACTION AND TACHYPHYLAXIS TO EGGWHITE IN ALBINO RATS

Ma Pao-Li

From the Department of Pathological Physiology (Head- Corresponding Member of the AMN SSSR A. D. Ado) N. I. Pirogov II Moscow Medical Institute

(Received October 7, 1958. Presented by Active Member of the AMN SSSR N. I. Sirotinin)

White rats are very sensitive to an initial parenteral injection of eggwhite [2, 5, 10, 15]. If 1 ml of the fresh substance is injected intraperitoneally into rats, an edema of the snout, tongue, paws, and clitoris develops. A hyperemia precedes the edema. The edema is reduced by antihistamine preparations [3, 6, 11]. Rats which have survived the anaphylactic reaction develop an insensitive condition, or one of reduced sensitivity to a second injection of considerably larger amounts of eggwhite [2, 15]. The rapid increases in resistance to various bacterial antigens is well known and has been called tachyphylaxis. However, very little work has been done either on the tachphylaxis of rats to eggwhite, or on the increased sensitivity of albino rats to a primary parenteral injection of this substance.

Recently many reports have been published on the part played by the hypophysis and adrenals in allergy and anaphylaxis [1, 4, 8, 9] However, many of these reports are contradictory, and the extent to which the hypophysical-adrenal system is involved is no means clear [7, 16].

The present work represents an attempt to determine the part played by the hypophysis and adrenals in the anaphylactic reaction and tachyphylaxis of albine rats to eggwhite.

METHOD

The experiments were carried out on more than 200 albino rats weighing from 80 to 150 g. Various doses of eggwhite were injected into the femoral vein to provoke the anaphylactic reaction. In acute experiments, records were made of the blood pressure in the carotid artery, and respiration. In order to obtain a more objective estimate of the degree of toxicity of eggwhite to rats, LD_{50} (medial lethal dose) was determined using the method of Reed and Muench [13]. Hypophysectomy was carried out using our modification of Smith's method [17] in which a paratracheal approach was made through the basioccipital bone. Indications that the hypophysis had been completely removed were that there was a twofold increase in the number of eosinophils, and an atrophy of the adrenals (on average 5.1 mg per 100 g weight in the operated animals as compared with 25.7 mg per 100 g weight in the controls). The adrenals were removed from the dorsal side by means of an incision along the ribs. The operations were carried out under ether anesthesia, and the estimation of LD_{50} made in the unanesthetized animals.

RESULTS

1. The anaphylactic reaction to eggwhite. Twenty-four acute experiments on albino rats were carried out. Intravenous injection of small amounts of eggwhite (0.2-1.5 ml per 100 g weight) produced the symptoms similar to those found in anaphylactic shock in sensitized guinea pigs. After 15-20 seconds, an increase in blood pressure of 10-56 mm mercury, and an increased respiration were observed; after 1-1/2 minutes the blood pressure gradually fell, sometimes to zero, but when the dose was not lethal, it recovered quite rapidly (Fig. 1).

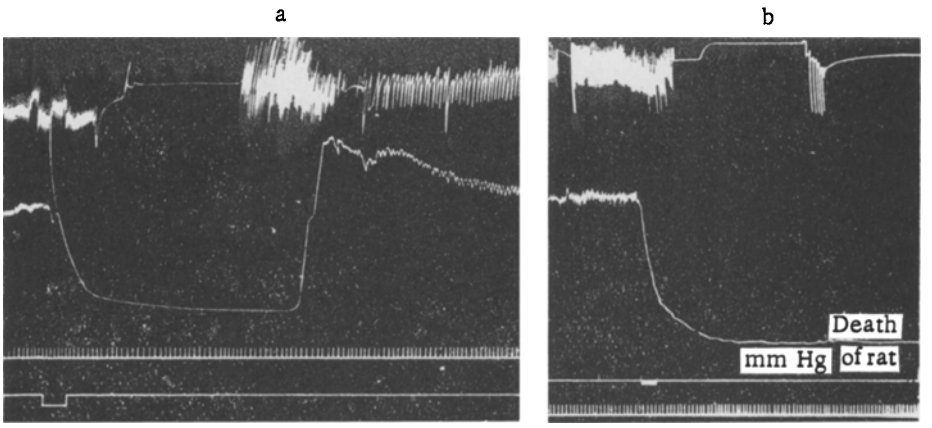

Fig. 1. Changes in the blood pressure and respiration in albino rats given egg-white intravenously. a) intact animal; b) hypophysectomized animal. Curves, from above downwards: respiratory movements, blood pressure, time (2 seconds), stimulus marker – injection of 1 ml of eggwhite into femoral vein.

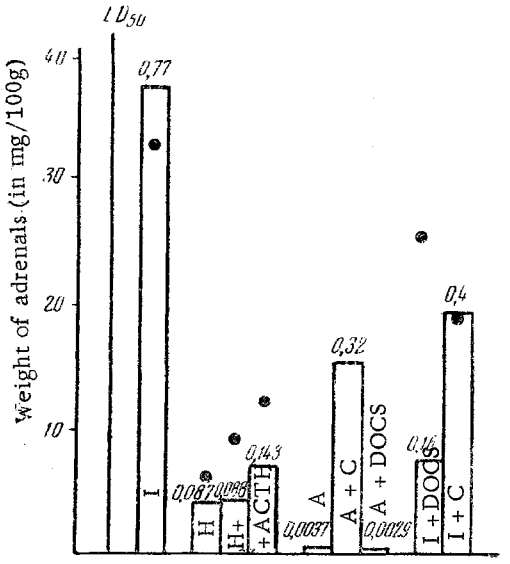

Fig. 2. Value of the LD_{50} for eggwhite and average weight of adrenals in rats in different experiments. Symbols: 1) intact animals; H) hypophysectomized aninals; columns – value of LD_{50}; black dots – average weight of adrenals; ACTH – adrenocorticotropic hormone; A) adrenalectomy; C) cortisone; DOCS desoxycorticosterone.

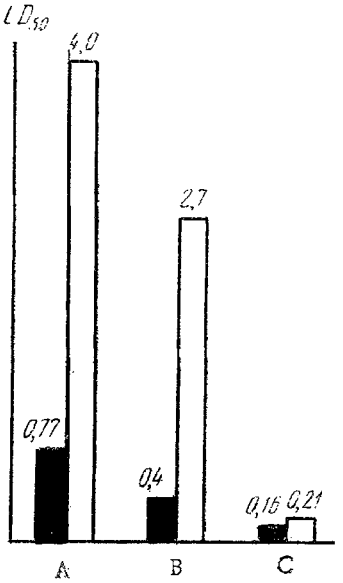

Fig. 3. Value of the LD_{50} in rats for the initial injection of eggwhite, and after tachyphylaxis had developed. Notation: a) intact animals; b) animals receiving cortisone; black columns refer to initial injection of eggwhite; white columns to tachyphylaxis; c) animals receiving desoxycorticosterone.

To determine the LD_{50}, 16 rats were divided into 4 groups of animals of approximately the same weight. Injections were made of eggwhite into the femoral vein, increasing in geometrical progression in the successive groups by a factor of 1.5, so that the amount given were 0.6, 0.9, 1.35, and 2 ml per 100 g weight. Two to three minutes after the injection, a hyperemia of the snout and paws developed, and this was followed by a cyanosis and edema. The hyperemia and edema were best shown in animals given small doses, and the cyanosis best in

those receiving the greatest amounts of eggwhite. After a few seconds, or later, the animals became breathless and restless. After 3-15 minutes, when the shock was severe, they fell on their side and died in convulsions. Quite frequently the first symptoms passed off, and death occurred 30 minutes or even several hours after the injection. Postmortem examination showed severe hyperemia and edema of the intestine. The LD_{50} for these animals was 0.77 ml per 100 g body weight (Fig. 2).

2. The hypophysis and the anaphylactic reaction. In 11 acute experiments, we observed that hypophysectomy increases the sensitivity of rats to eggwhite. After it had been injected intravenously, the blood pressure usually fell rapidly almost to zero, respiration stopped, and the reaction often ended in the death of the rats. Small doses of eggwhite were sufficient to cause death; injection of adrenocorticotropic hormone (ACTH) did not always prevent death (see Fig. 1, b).

To obtain further information on this problem, we determined the LD_{50} in a group of hypophysectomized animals, some of which were subsequently treated with ACTH, while the others received no further treatment. The experiment was performed on the 25-27th day after hypophysectomy. In the operated rats the LD_{50} was 8.8 times less than in healthy animals (0.087).

Different doses of ACTH given at different times to the operated animals did not restore the reduced resistance in the hypophysectomized rats. In those which received 4 units per 100 g body weight of ACTH 7 days before the experiment, the LD_{50} was the same as in the hypophysectomized group. The weight of the adrenals of this group was, on average, equal to 9.1 mg/100 g body weight. In another group of hypophysectomized animals given 1 unit per 100 g of ACTH for 21 days before the experiment, the LD_{50} showed some tendency to increase, although it did not regain the initial value. The weight of the adrenals in this group was also increased, on average to 12 mg per 100 g body weight.

3. The adrenals in the anaphylactic reaction. The adrenals play a far greater part than the hypophysis in the anaphylactic reaction in rats. Removal of the adrenals increases the sensitivity to parenteral injection of eggwhite 200 times; 6 days after the operation, the LD_{50} in these animals was 0.0037 ml per 100 g weight (see Fig. 2).

Injecting 2.5 mg per 100 g body weight of cortisone into the operated animals for 6 days caused a marked increase in resistance (LD_{50}=0.32 ml per 100 g body weight), although it did not return to the original level. A dose of 1 mg per 100 g of desoxycorticosterone (DOCS) for 6 days in the operated group caused a further reduction of the LD_{50} to eggwhite (LD_{50}=0.0029 ml per 100 g body weight; see Fig. 2).

In the next set of experiments, cortisone and DOCS were injected in the same amounts (1 mg per 100 g body weight) into the unoperated group for 7 days. Both substances reduced the rats' resistance to eggwhite; with cortisone the LD_{50} was 0.4 ml, with DOCS it was 0.16 ml per 100 g; see Fig. 2.

4. Tachyphylaxis to eggwhite. In acute experiments, we observed several times that animals which had survived the anaphylactic reaction became more resistant to the same protein subsequently. Tachyphylaxis develops almost immediately after the injection, and is maintained for several days.

In experiments specially designed to induce tachyphylaxis, 24 rats received injections of 0.6 ml per 100 g body weight, which is less than the LD_{50}. After 6 hours, the LD_{50} for the remaining 16 rats was determined. It was found to have a value of 4 ml per 100 g weight, i.e. it was more than five times greater than the original LD_{50} (Fig. 3, a).

The next step was to determine the effect of the adrenal hormones on tachyphylaxis to eggwhite. For this purpose, 2.5 mg per 100 g body weight of cortisone or 1 mg per 100 g weight of DOCS was injected for 7 days into intact rats. Tachyphylaxis was induced by injecting 0.2-0.4 ml of eggwhite. The LD_{50} for a second injection of eggwhite was then determined 6 hours after the first anaphylactic reaction. It was found that animals treated with adrenal cortical hormones developed a tachyphylaxis (Fig. 3, b), while in DOCS treated animals the ability to do so was greatly reduced (Fig. 3, c).

Long ago, Selye [16] showed the effects of hormones of the adrenal cortex and hypophysis on the local manifestation of the anaphylactic reaction in rats (edema, hyperemia). In the present study, besides confirming Selye's results, particular attention has been paid to the general reaction of the rats, which consisted of a change in blood pressure, respiration, and the appearance of tachyphylaxis. For the most part, our results agree with those of Selye: adrenalectomy markedly reduces, and cortisone treatment increases, the rats' resistance to eggwhite; desoxycorticosterone has no definite effect.

In our experiments, hypophysectomy increased the sensitivity to the first eggwhite injection, whereas according to Leger and Masson, there is no alteration in the degree of edema in the hypophysectomized animals. The disagreement is obviously due to the fact that these authors carried out the experiments on the ninth day after the operation, whereas ours were performed later, on the 25-27th day. Different degrees of atrophy of the adrenals can be observed at different times after the operation, and these play an essential part in the anaphylactic reaction of rats to eggwhite. It is important to note also that our method of determining the LD_{50} is more objective than is estimation of the development and intensity of edema. In our experiments, injections of ACTH did not restore the reduced resistance to eggwhite in the hypophysectomized rats. The explanation may be that the dose of hormone was insufficient, or that it was given too late after the operation to prevent atrophy of the adrenals following removal of the hypophysis. The weight of the adrenals in these animals remained 2-3 times below the normal value.

SUMMARY

Anaphylactic shock in rats may be provoked by an initial intravenous injection of fresh eggwhite. Previous administration of cortisone or desoxycorticosterone reduces the animals' resistance to the initial eggwhite injection, while adrenalectomy sharply decreases it.

Cortisone restores the resistance reduced by adrenalectomy, while DOCS produces no appreciable effect.

Resistance to eggwhite is also decreased on the 25th day after hypophysectomy, although to a smaller extent than in adrenalectomy.

Different doses of ACTH employed at various intervals had no visible compensatory effect.

The condition of tachyphylaxis occurred after the first injection of egg white. Preliminary cortisone and DOCS administration more or less preserved the ability to produce a tachyphylactic response.

LITERATURE CITED

[1] B. I. Kadykov, Byull. eksptl. biol. i med., volume 4, No.5, pp. 518-520 (1937).

[2] B. V. Polushkin, in book: Collected Works of the Altai Medical Institute,* (Barnaul, 1957) volume 1, pp. 253-264.

[3] W. G. Clark, E. M. Mackay, Proc. Soc. Exper. Biol. and Med. (1949) v.71, p.86-87.

[4] L. H. Criep, L. D. Mayer, et al., J. Allergy 22,314-329 (1951).

[5] J. Leger, G. Masson, and J. L. Prado, Proc. Soc. Exper. Biol., and Med. (1947) v.64, p.366-370.

[6] J. Leger and G. Masson, Am. J. Med. Sc. (1947) v.214, p.305-307.

[7] J. Leger and G. Masson, Ann. Allergy 6, 131 (1948).

[8] S. J. Malkiel, J. Immunol. 66, 379 (1951).

[9] M. Molomut, J. Immunol 1951 v. 66 p. 379.

[10] J. T. Parker, F. J. Parker, Med. Res., 1924, v.44, p.263-287.

[11] J. R. Parrat, G. B. West, Brit. J. Pharmacol., 1958, v.13, p. 65-70.

[12] W. D. M. Paton, Progress in Allergy, 1958, v.5, p.79-148.

[13] L. J. Reed, H. Muench, Am. J. Hyg., 1938, v.27, p.493-497.

[14] M. Schachter, J. Jalenik, J. Physiol. , 1952, v.118, p.258-263.

[15] H. Selye, Endocrinology, 1937, v.21, p.169-188.

[16] H. Selye, Canad. M. A. J. 1949, v.61, p.553-556.

[17] P. E. Smith, Am. J. Anat., 1930, v.45, p.205-273.

* In Russian.