

ASCORBIC ACID CONTENT IN THE ADRENALS OF CHICK EMBRYOS RECEIVING INJECTIONS OF ACTH AND INSULIN

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It has been found that a great variety of agents capable of causing a state of stress may also produce stimulation of the secretory activity of the adrenals. This increased secretion invariably leads to a lowering of the reserves of cholesterol and ascorbic acid in the adrenals [10,18]. Most of the facts concerning the ascorbic acid content of the adrenals have been obtained in guinea pigs and rats. There is little information on the changes in the content of this substance in other species of animals, including birds. Moreover, most of the facts relate to the postembryonic period of development. Investigations on young birds and chickens [3,6,7,15] have shown that, despite marked hypertrophy of the adrenals, the ascorbic acid concentration in the glands does not fall during the action of various factors. Data relating to the ability of the embryonic adrenals to lower their reserves of ascorbic acid during a period of increased secretion are very few [8,14], and nothing whatever is known about birds in this respect.

The object of the present study was to investigate the effect of ACTH and insulin on the ascorbic acid content of the adrenals of chick embryos.

METHOD

Experiments were carried out on chick embryos of the White Leghorn breed. Eggs weighing from 52 to 60 g were incubated. Embryos were taken for investigation after the 13th day. The ascorbic acid content of the adrenals was determined by the method of Roe and Kuether [17], and wood charcoal (clarifying, mark A) was used for oxidation. ACTH and insulin were injected intravenously [2] in a dose of 0.05 and 0.043 unit/g body weight. Material was taken 1,3,6,12,24, and 48 h after injection of the hormones in the case of ACTH, and 3,5,6, and 12-14 h after injection of insulin. Besides the determination of the weight of the adrenals and the total ascorbic acid content, the concentration of ascorbic acid per 100 mg of tissue also was calculated.

Altogether 60 experiments were performed, in each of which the ascorbic acid content of the adrenals of 12 embryos was determined (6 control and 6 experimental). Experiments also were conducted on chicks between the ages of 3 and 13 days. The doses of ACTH and insulin injected into the chicks varied from 2 to 4 units. On the day of the experiment the chicks took no food but were allowed water. The birds were killed by decapitation.

RESULTS

After an interval of 1.5 h, injection of ACTH into the embryos caused a fall of 14-27% in the ascorbic acid content of the adrenals by comparison with the control level. A similar pattern was observed after 3 h (Fig. 1). Besides a lowering of the reserves of the vitamin, its concentration in the adrenals also fell. The more marked fall in the ascorbic acid concentration in the glands of the embryos aged 15 days or more was caused by the fact that, 3 h after injection of ACTH, the weight of the adrenals in the embryos of this age was greater than in the controls. It should be noted that the reaction of the adrenals to injection of ACTH into 18-day embryos was less marked than in embryos of the 13th-17th days of incubation. In the adrenals of the chicks, in contrast to those

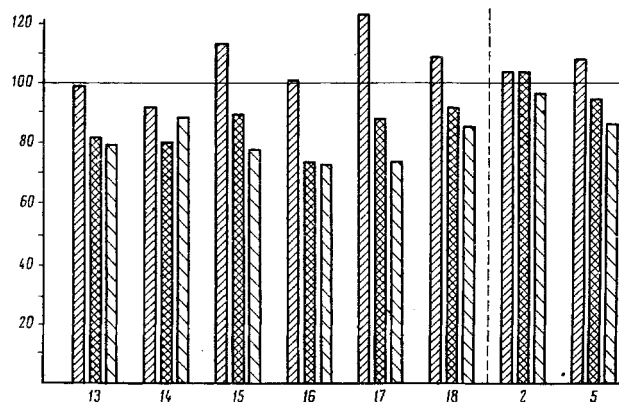


Fig. 1. Content of ascorbic acid in adrenals and their weight 3 h after injection of ACTH. Along the axis of ordinates—changes observed in experimental series by comparison with controls (in %); along the axis of abscissas—age of embryos (in days). Horizontal line—control level. The vertical broken line conventionally denotes the period of hatching. Column with close shading—weight of adrenals; column with cross-shading—content of ascorbic acid; column with wide shading—concentration of ascorbic acid.

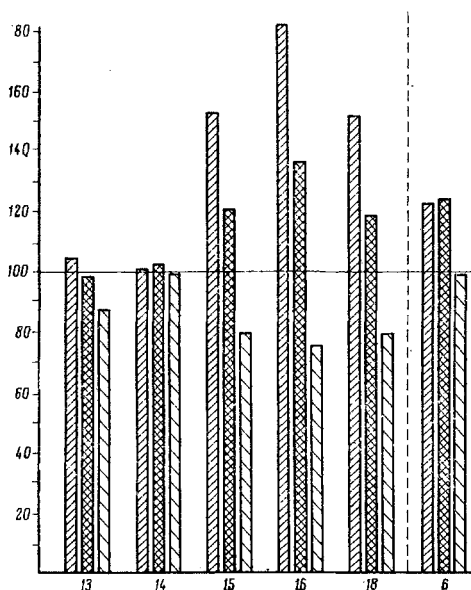


Fig. 2. Ascorbic acid content and weight of adrenals 6 h after injection of ACTH. Legend as in Fig. 1.

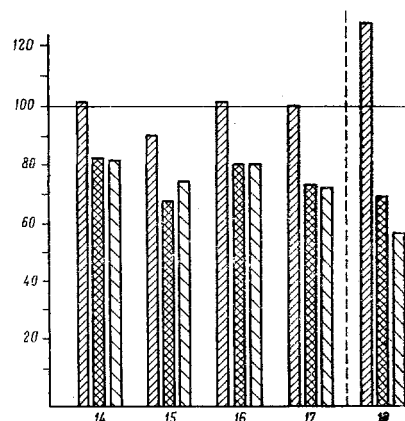


Fig. 3. Ascorbic acid content and weight of adrenals 3.5 h after injection of insulin. Legend as in Fig. 1.

of the embryos, after injection of ACTH no decrease could be observed in either the total content or the concentration of ascorbic acid. No reaction was seen in the chicks even when the dose of hormone injected was increased from 2 to 4 units.

The results obtained in these two series of experiments agreed mainly with the results of the investigations conducted in mammals. A characteristic fall in the content and concentration of ascorbic acid 1.5–3 h after injection of ACTH was observed in the adrenals of newborn rats [1,4,13] and of rat fetuses [8,14]. The results of our experiments, like the data given in the literature, showed that the reaction of the glands to injection of ACTH observed in developing chick embryos was absent in the young chicks. Only in one investigation [19] was a decrease in the ascorbic acid content of the adrenals observed in White Leghorn cocks 12 h after the injection of ACTH.

Various explanations are offered in the literature for the somewhat exceptional reaction of the adrenals of young birds to conditions of stress—the absence of a decrease in the ascorbic acid level. In particular, the importance of the initial concentration of the vitamin for manifestation of this effect (about 400 mg%) is stressed [20], as also is the age factor [15]. In recent years information has been obtained concerning the role of the sac of Fabricius in birds [16]. It has been shown that in young chicks from which this sac has been removed, injection of ACTH is followed by a decrease in both the content of ascorbic acid (by 49-41%) and its concentration in the adrenals (by 57-45.8%). It may be assumed that in embryos, in which this sac has not become completely formed, it has no significant effect on the reaction under investigation. Indications of the comparatively later development of the sac of Fabricius in chick embryos are also given in the literature [11].

Six hours after the injection of ACTH the ascorbic acid concentration in the adrenals of the 15-18-day embryos was low as before (Fig. 2). The ascorbic acid concentration in the 13-14-day embryos and chicks was indistinguishable from normal. It is noteworthy that 6 h after injection of the hormone, parallel with the increase in weight of the adrenals, their ascorbic acid content also rose. However, the increase in its content in the embryos was relatively much smaller than the increase in the weight of the glands. A similar increase in the content of the vitamin 6 h after injection of ACTH has also been observed in growing rats [12].

The increased ascorbic acid content of the adrenals over the normal level was still present in our experiments 12 h after injection of the hormone. The weight of the glands varied within normal limits. These changes, admittedly to a less marked degree, were also observed 24 h after injection of ACTH. It is natural to suggest that in the adrenals stimulated by injections of ACTH, side by side with the process of cell division, synthesis of ascorbic acid also takes place. This suggestion is made more likely by the fact that chicks are among the animals capable of synthesizing endogenous ascorbic acid. The above changes in the weight of the adrenals and in the total content and concentration of ascorbic acid in the glands disappeared completely 48 h after injection of ACTH.

The action of insulin on the adrenals of birds has been studied less intensively than the action of ACTH. In our experiments the changes in the ascorbic acid content of the embryos following injection of insulin were basically the same as the changes caused by ACTH. For example, 3.5 h after injection of insulin into embryos of 14-17 days of incubation, the ascorbic acid concentration and its total content in the adrenals were consistently lowered (Fig. 3). The fall in the ascorbic acid concentration in the embryos was mainly brought about by a change in its total content, for the weight of the glands had not yet increased after this time interval. In the 10-day old chicks, injection of insulin (4 units) was accompanied, as in the embryos, by a decrease in the content and concentration of ascorbic acid in the glands. It should be noted that the same dose of hormone caused no visible changes in the content and concentration of ascorbic acid in the adrenals after 1.5-2 h. This result is in full agreement with the findings of other authors [6], who found no change in the ascorbic acid content in 6-week old chicks 2 h after injection of insulin. A fall in the ascorbic acid content of the adrenals has been described in mammals (rats) after the injection of insulin [5].

The changes observed 3.5 h after the injection of insulin were still present 6 h after injection. At this time, however, a difference was observed between the reaction of the 13-14 day embryos and the embryos of later periods of incubation. In the early stages of incubation (13-14 days) the injection of insulin into the embryos caused, besides a decrease in the ascorbic acid content, changes in the weight of the adrenals. In consequence, the concentration of the vitamin in these embryos showed little change, and remained practically at the control level. Conversely, in embryos of the 15th-17th days of development, the ascorbic acid concentration was considerably reduced (25-37%). The most marked effect after 6 h was observed in the 12-day old chicks. The fall in the ascorbic acid content in this experiment was 32%, whereas its concentration fell by a greater degree—by 53%. At this time interval after the injection of insulin, the weight of the adrenals also was increased in some experiments.

As after injection of ACTH, 12-14 h after the injection of insulin the ascorbic acid content of the adrenals increased both in the 15-18-day embryos and in the young chicks. However, because of the corresponding increase in the weight of the adrenals, the ascorbic acid concentration fell very slightly. The changes in the concentration and content of ascorbic acid, and also in the weight of the glands in the 13-14-day embryos after 12-14 h were analogous to those observed 6 h after injection of insulin. The decrease in the weight of the adrenals of the embryos in the early periods of incubation may be interpreted as the result of a delay in the development of the organ caused by hypoglycemia. This explanation is confirmed by data showing the delay in the general growth of embryos during administration of insulin, especially in the early stages of development. It is evident that in embryos of a comparatively early age all the links of the anti-insulin mechanism capable of counteracting the hypoglycemia are not yet established.

The discovery that the content of ascorbic acid is increased 12-14 h after injection of insulin into older embryos and young chicks is confirmed by the results of experiments conducted on adult rats [9]. This increase, as in the experiments with ACTH, probably reflects the process of synthesis of the vitamin, taking place in the adrenals.

Hence, the embryonic adrenals are capable of lowering the reserves of ascorbic acid after injection of ACTH and during "stress" caused by the injection of insulin. The cause of the different reaction of the adrenals of young chicks after hatching in response to the injection of insulin and ACTH has not been fully explained. It may be postulated that the mechanism by means of which the decrease in the ascorbic acid content in the adrenals is brought about is different in respect of these two hormones.

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