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HISTOLOGICAL AND HISTOCHEMICAL RESPONSE OF THE ADRENAL CORTEX IN THE NEWLY HATCHED CHICK TO SOME EXPERIMENTAL CONDITIONS

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Abstract

Some histological and histochemical changes were investigated in the adrenal cortex of the newly hatched chick starved or treated with neurohypophysial substance. Starvation for 6 days caused a significant increase of cortical pale cells in the peripheral region of the adrenal gland. And hypertrophy of this region was apparently noticed. In such glands, moreover, lipid droplets were decreased mainly in the peripheral region. Additionally, alkaline phosphatase, succinic dehydrogenase, glucose-6-phosphate dehydrogenase and 3β -hydroxysteroid dehydrogenase systems in the adrenocortical tissue were all lowered in the whole gland, with most decrease in the inner region. Repeated subcutaneous injection of Pituitan, neurohypophysial substance, in daily doses of 1.5 mg. per chick for 4 days produced an increase of fuchsinophil cells in the whole cortex, associated with a severe accumulation of large lipid droplets. In this case, the enzyme systems were all increased in the inner region, whereas slightly decreased in the peripheral region. These results would seem to support the view that the functional significance of the adrenal cortex in the newly hatched chick was generally agreement with that of the adrenal gland in adult birds.

Previous workers have demonstrated that the constituent of fowl adrenocortical hormone containing corticosterone as the major component is generally similar to that of cortical hormone in the rat^{10,12}). It has been known, however, that hypothalamo-pituitary-adrenal relationships in birds may be differ from those in mammals¹⁴). Practically, when the pituitary is removed in the rat, the adrenocortical tissue degenerates subsequently⁵). In contrast, in the hypophysectomized cockerel a trophic substance continues to stimulate the corticosteroid secretion of the adrenal gland²). RESKO *et al.*¹¹) have pointed out that the neurohypophysial substance injected into intact chicks causes a significant increase in corticosterone concentration in the adrenal venous blood. Accordingly, it is thought that neurohypophysial substance may have a direct or indirect adrenocorticotrophic effect in the fowl.

Moreover, starvation has been considered by several investigators to evoke a demand for increased adrenocortical hormone⁸).

In the present experiment, adrenocortical responses to neurohypophysial stimulation and starvation were investigated in the newly hatched chick. The purpose of this study is to determine whether there is any evidence for histological alteration caused by some increased secretory rates of the adrenal cortex in the immediate post-hatching period.

Materials and Methods

Twenty five newly hatched White Leghorn chicks were used in this study. In an attempt to obtain a possibly uniform material, they were incubated and hatched in this laboratory, and kept in the warm room at 37° during the present experiment. In advance of experiment they were divided into 4 groups

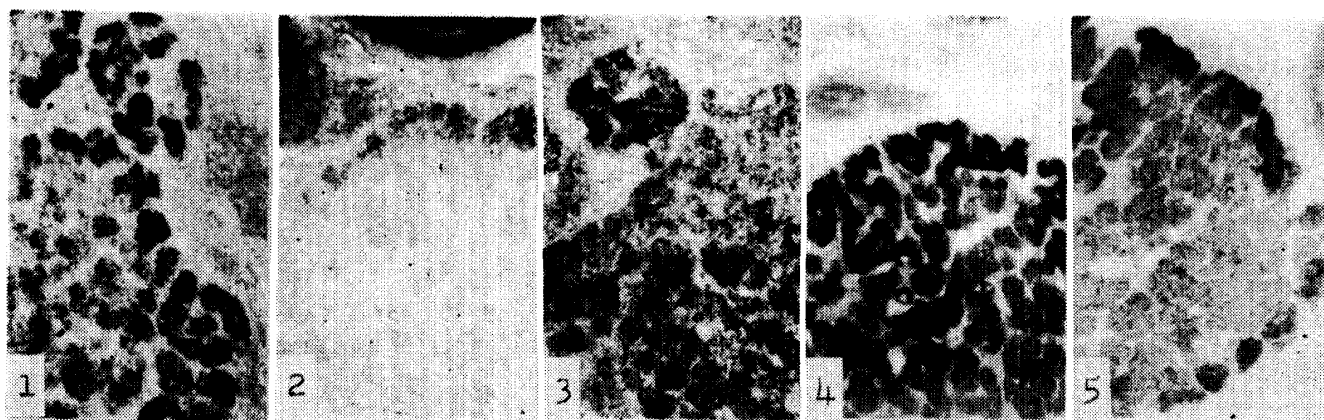
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consisting of 6 chicks, severally. Three groups of them received no food and no drink after hatching, and were sacrificed at 24 hr., 4 days and 6 days after hatching respectively. In preliminary findings, the similarly starved chicks died all at 7 days after hatching. The chicks of remaining group were fed and treated with 3 times of subcutaneous injection of 0.5 mg. of Pituitan, neurohypophysial substance, daily from the period of after hatching to 4 days after. Each chick received a total dose of 6.0 mg. of the drug finally, and sacrificed 2 hr. after the last injection. All birds were killed by decapitation. After that, the adrenal glands were quickly removed and fixed immediately. Histological and histochemical inspections of the adrenal gland were made by the following procedures; ponceau fuchsin-light green stain for histological structure, sudan black stain for lipid concentration, the GOMORI's method⁴⁾ for alkaline phosphatase, the method of NACHLAS *et al.*⁹⁾ for succinic dehydrogenase, the method of ROUDOLF-KLEIN¹³⁾ for glucose-6-phosphate dehydrogenase, and the method of WATTENBERG¹⁷⁾ for 3β -hydroxysteroid dehydrogenase. In all the histoenzymological inspection, parallel sections were incubated in control media lacking substrate.

Results and Discussion

The histological feature of the normal adrenal gland of the newly hatched chick was generally similar to that of the adult gland already described by several workers^{7,15)}. Accordingly, for the explanation of the adrenal structure a brief description may suffice. In the normal adrenal gland of the newly hatched chick, cortical tissues of the peripheral region consisted of pale cells¹⁶⁾ principally as in the adult gland, and obviously separated from the inner region. Cortical tissues in the middle region contained a significant amount of fuchsinophil cell¹⁶⁾, and appeared somewhat darkly. Such middle region was rather extended to inner portion as compared to adult case. Therefore, the central region of this gland was pushed inward and became somewhat indistinct. And such

appearance of the adrenal gland was common to that in the chick fasted for 4 days. In the adrenal gland of the chick starved for 6 days, pale cells of the peripheral region were increased in number and size, and spread over the outer half of the gland. Thereby, fuchsinophil cells were shrunk and tended to be pushed and gathered in the central region of the gland. In contrast, a lot of crushed and wedge-shaped fuchsinophil cells were scattered all over the cortical tissue in the whole gland of the Pituitan-treated chicks. No hypertrophy of pale cells was found out in such gland. These findings indicate that the adrenal cortex in the newly hatched chick has practically similar structure and function to those of the adult gland previously described by the present author⁶⁾. The behavior of adrenal alteration in fowls under various experimental conditions has recently been investigated in this laboratory. And in starved cockerels, pale cells are increased in size and number in the whole gland, particularly in the inner region. In the present case, however, the hypertrophy of pale cells was noticed only in the outer half of the gland. Therefore, the central region seemed incomplete at the period of hatching. The accumulation of fuchsinophil cells in the adrenal cortex is a general feature of the fowl adrenal under neuro-humoral stimulation, according to the investigations in this laboratory. The distribution of lipid droplet in the intact adrenal gland of the newly hatched chick was similar to that of the adult bird, and appeared abundantly in the entire cortical tissue. The lipid droplet was obviously decreased in the whole adrenal cortex of the starved chick. And the lipid depletion was severe in the peripheral region particularly. In the Pituitan-treated chick, lipid concentration was higher than that in intact bird. And the lipid droplets were accumulated densely in the cortical tissue of the whole gland, except the peripheral region containing a moderate amount of lipid droplet. From these results, there seem to definite correlation between the hypertrophy of pale cells and depletion of lipid. The lipid depletion in the adrenal cortex has been considered by several workers to indicate



Figures are parts of the adrenal glands of newly hatched chicks. The darkness exhibits enzymatic activity.

Fig. 1. Alkaline phosphatase in the adrenal gland of a normal chick.

Fig. 2. Alkaline phosphatase in the gland of the chick starved for 6 days.

Fig. 3. Alkaline phosphatase in the gland of the chick treated with 1.5 mg. of Pituitan daily for 4 days.

Fig. 4. Glucose-6-phosphate dehydrogenase in the gland of a normal chick.

Fig. 5. Glucose-6-phosphate dehydrogenase in the gland of the starved chick for 6 days.

a probably increased activity of the tissue. Therefore, it would appear that the starvation causes a stimulation of the cortical tissue in the peripheral region. But the inner region here may be interpreted as possessing less activity. Alkaline phosphatase system in the fowl adrenal is generally detected in the cortical elements and capillary walls. The distribution of this enzyme is somewhat zoned, and dense in the peripheral region of the fowl gland⁶⁾, whereas scant in the corresponding region of the pigeon gland¹⁵⁾. In the intact chick gland this enzyme appeared mainly in the cortical elements, with a high activity in the peripheral region (Fig. 1). In the starved bird, the peripheral region containing a lowered enzyme activity became narrower, and the inner region had a mostly decreased activity (Fig. 2). The enzyme activity in the Pituitan treated bird was rather strong in the inner region, but weak in the peripheral region (Fig. 3). It has been confirmed that the alkaline phosphatase system might be a controlling factor in the transfer of metabolites and electrolytes in particular, since the enzyme increases in all cases where the secretory activity of the adrenal cells is increased and decreased in all cases where the adrenal secretion is slowed. But in the present case, this enzyme activity was not increased in the starved condition considered to induce

a adrenal high activity. Succinic dehydrogenase system was detected in the cytoplasm of cortical cells. This activity was dense in the peripheral region, but slight in the inner region in all cases. The enzyme system in the starved chick gland appeared less activity than that in the control gland. In detail, the activity became weaker in the narrowed peripheral region, and mostly scant in the inner region. In the adrenal gland received Pituitan the activity was generally weaker than that of the controls, but it was somewhat severe in the edge of cortical tissue. The succinic dehydrogenase is a common hydrogenase detected in the adrenal cortex, and shows the existence of a KREBS' cycle, the passway of carbohydrate metabolism involving the steroid biosynthesis. However, in this investigation, the succinic dehydrogenase system was decreased in the adrenal cortex under starved condition considered to induce a cortical high activity. Glucose-6-phosphate dehydrogenase system was densely detected in the whole cortical tissue of the chick adrenal gland. And the most activity of this enzyme was noticed in the peripheral region. Starvation produced a somewhat lower activity of this enzyme in the whole cortex. On the other hand, with Pituitan treatment no change in the distribution of this enzyme system was indicated. A number of experiments have shown that

the synthesis of adrenal corticosteroids from acetate requires reductive energy in the form of NADP-H. And in keeping with this requirement for NADP-H, a high level of glucose-6-phosphate dehydrogenase is commonly observed in adrenal tissues. The existence of a parallelism between the level of this enzyme concentration in the adrenal gland and corticosteroid synthesis is a classical theory in mammals, but it remains unsolved in birds. This hypothesis fails to explain the behavior of glucose-6-phosphate dehydrogenase in the present experiment. 3β -Hydroxysteroid dehydrogenase system was slightly detected in the whole cortical tissue in the normal gland, and it appeared somewhat strongly in the peripheral region (Fig. 4). This enzyme activity in the gland of the starved chick was lowered extremely in the inner region, but remained as a normal level in the peripheral region (Fig. 5). In the Pituitan-treated chick, 3β -hydroxysteroid dehydrogenase activity was rather increased equally in the whole cortical tissue. It has been confirmed that this enzyme system involves the synthesis of most biologically active steroid hormones. The concentration of this enzyme system, therefore, is quite adequate to the evidence of adrenocortical action. However, this enzyme activity in the starved bird was decreased in the inner region of the gland judged histologically to be in high active condition. All through the present study the adrenal cortex of the newly hatched chick exhibited some responses in the histological and histochemical feature to inanition and neurohypophysial substance. And it is noteworthy that the intensities of several enzymes confirmed to have some correlation to steroid biosynthesis agreed with neither the histological changes nor the amount of lipid in the cortical tissue. In the newly hatched chick investigated here, the enzyme systems diminished generally under the adrenocortical conditions indicated to be in high activity by the structural feature

and lipid content. This fact contrasts with the common knowledge of the correlation between the enzymatic intensity and the functional level of tissues. Perhaps this is because the adrenal gland was incomplete at the stage of the immediate post-hatching. It has to be emphasized that the present knowledge on adrenal steroidogenesis is based on experiments involving mammals. Practically, it has been noticed, by SANDOR *et al.*¹⁴⁾, that the course or action of corticosteroid synthesis in birds differs from that in mammals. Further work will be necessary to determine the problem of adrenocortical structure and function in the chick under physiological and experimental conditions.

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ニワトリの初生雛の副腎皮質の組織感応に関する
実験組織学的・組織化学的研究

荻 田 淳

要 約

ニワトリの初生雛の副腎皮質の機能状態を把握するため、白色レグホン種の雛を用い、孵化後より6日間飲食を絶って飢餓状態とした場合、および孵化当日より4日間0.5mgのピツイタン（下垂体後葉物質）を1日あたり3回ずつ皮下注射した場合の副腎について皮質組織の変化を検索した。飢餓状態としたニワトリの雛の副腎では周辺部の皮質組織が pale cell の発達によって拡大し、脂質の減少とともに機能的な高まりが察知された。しかし、alkaline phosphatase, succinic dehydrogenase, glucose-6-phosphate dehydrogenase および 3 β -hydroxysteroid dehydrogenase などはいずれも減少の傾向を示した。ピツイタンを投与した雛の副腎では、皮質組織の全般にわたって fuchsinophil cell の増数や脂質の蓄積が認められたが、各酵素の分布について著明な変化はなかった。これらの結果から、初生雛の副腎皮質は成体のものにくらべてかなり類似した感応を示すが、酵素活性の変化などからみると機能的にはまだ未完成の状態にあるものと考えられる。