3β-Hydroxysteroid Dehydrogenase Activity in the Adrenals of Normal and Hypophysectomized Chick Embryos[1] (36644)

L. V. Domm and G. C. Ericson

Department of Anatomy, Loyola University Stritch School of Medicine, Maywood, Illinois 60153

Studies on the avian adrenal have revealed a dependence on the anterior pituitary during the embryonic period. Adrenal weights and ascorbic acid content have been reported to be lower than normal in hypophysectomized chick embryos (1, 2). The cortical cords of normal embryos comprise 44% of the adrenal, those of hypophysectomized ones 31% and those of hypophysectomized ones with pars distalis grafts 43% (3). Hypophysectomized chick embryos show a delay in the development of cortical cords and a decrease in adrenal free cholesterol (4).

The enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) is involved in one of the early stages of steroid hormone biosynthesis, namely, the oxidation of Δ^5-3β-hydroxysteroids to Δ^5-3-ketosteroids, and is present in all known steroid producing tissues. The histochemical demonstration of 3β-HSD activity can be regarded as evidence of steroid biosynthesis. 3β-HSD activity in the rat adrenal has been reported to be under the influence of the pituitary (5).

The purpose of this investigation was to study the activity, time of appearance and distribution of 3β-HSD in the adrenals of normal, hypophysectomized and hypophysectomized chick embryos receiving pituitary transplants and exogenous ACTH.

Methods. Four groups of embryos of the white Leghorn fowl were studied. The first consisted of normal embryos, the second of embryos hypophysectomized by partial decapitation (6) at stages 10 or 11 (33–45 hr), the third of 8.5 day hypophysectomized embryos which received single, whole adenohypophyses from 17 day donor embryos transplanted to the chorioallantoic membrane and the fourth of hypophysectomized embryos which received 1.0 IU of porcine ACTH at 12 hr intervals, starting at 8 days of incubation.

Embryos of groups 1 and 2 were necropsied at Hamburger-Hamilton stages 21 through 30 (3.5–7 days) and at 24 hr intervals from day 8 through hatching. Some chicks between the ages of 1 and 14 days were also necropsied. Embryos of groups 3 and 4 were necropsied at 24 hr intervals. The younger embryos were frozen *in toto* while in older embryos and chicks, only a section of the sacrum with intact adrenals was frozen.

Dehydroepiandrosterone (DHA) and pregnenolone (P) were used as substrates for the histochemical visualization of 3β-HSD activity by a modification of the method of Levy, Deane and Rubin (5). The enzyme activity observed with the substrate DHA was designated DHA-3β-HSD and that with pregnenolone P-3β-HSD. The activities, based on density of formazan deposition, were rated according to a 1 to 6 scale.

Results. In the avian adrenal the interrenal (cortical) cells are distributed throughout the gland in the form of irregularly arranged cords, interspersed with groups of chromaffin cells, without cortical or medullary zones as seen in the mammalian adrenal.

Normal embryos. Traces of DHA- and P-3β-HSD activity were seen in the migrating adrenocortical cells of normal stage 22 (3.5–4 days) embryos. Both of these activities revealed a steady increase to the eighth day of incubation, however, the P-3β-HSD was always greater than the DHA-3β-HSD activity (Fig. 1). These activities appeared to be evenly distributed throughout the cortical

[1] Supported by NIH Grants AM-03895 and AM-09926 and a NIH General Research Support Grant.

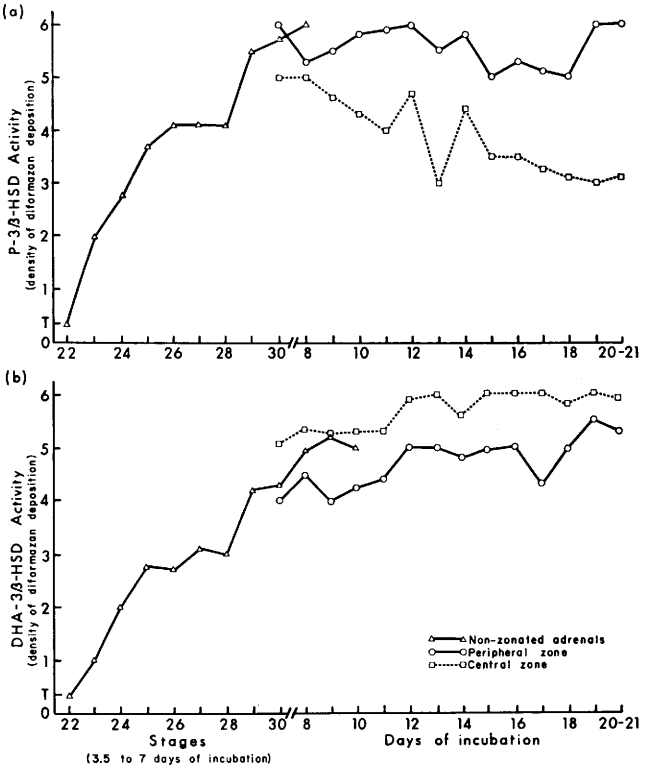

FIG. 1. The adrenal 3β-HSD activity in normal embryos (a) using the substrate pregnenolone (P-3β-HSD) and (b) the substrate dehydroepiandrosterone (DHA-3β-HSD).

cell groups and cords through stage 29.

At stage 30 (6.5–7 days) the first indications of a zonation were observed in the adrenals of some embryos. The P-3β-HSD activity was higher in some of the cortical cords at the periphery of the gland than in those more centrally located. From 9 days, and continuing through the remainder of the incubation period and the first 14 days posthatch, the last stage studied, all adrenals revealed a peripheral zone of high and a central zone of lower activity. At 9 and 10 days, the diameter of the central zone was about equal to the width of the peripheral zone. After 10 days, there was a pronounced increase in the size of the central zone so that in the last third of the incubation period, and the first 14 posthatch days, the adrenals consisted of a narrow peripheral cortical zone of high and a large central one of lower P-3β-HSD activity.

Peripheral zone P-3β-HSD activity reached a maximum between 10 and 12 days and again at the time of hatching (20–21 days) (Fig. 1). Central zone P-3β-HSD activity was at a maximum when this zone first appeared between 6.5 and 8 days but after 8 days there was a steady decrease in activity reaching a low level at the time of hatching. During the first 14 posthatch days the peripheral and central zone activities remained at the level observed at time of hatching.

The adrenals of some stage 30 embryos showed indications of a zonation when examined for DHA-3β-HSD activity. However, the distribution of this activity was just the opposite of that of P-3β-HSD, that is, the DHA-3β-HSD activity was greater in the central than in the peripheral zone. This zonation was more difficult to determine than that with P-3β-HSD but it occurred in most of the older embryos and in chicks. All adrenals showing DHA-3β-HSD zones also

showed a P-3β-HSD zonation but not all of the latter showed DHA-3β-HSD zones. Central zone DHA-3β-HSD activity reached a maximum at 12 days and was maintained at this level through hatching (Fig. 1). Peripheral zone DHA-3β-HSD activity increased gradually throughout the incubation period and reached a maximum at time of hatching. The DHA-3β-HSD activity levels at hatching were maintained in posthatch chicks. The DHA-3β-HSD activity, in adrenals which did not appear to be zonated, was evenly distributed throughout the cortical cords and was equal to that of central zone DHA-3β-HSD of zonated adrenals.

Hypophysectomized embryos. No differences were detected, in the morphological development of the adrenals from stage 22 to 9 days, between normal, sham-operated or hypophysectomized embryos. Beginning at about 10 and continuing to 19 days, the last stage studied, the cortical cords of hypophysectomized embryos revealed hypertrophy, a reduction in number and larger than normal groups of chromaffin (medullary) cells.

No modification in the development of DHA- or P-3β-HSD activity between sham-operated and hypophysectomized embryos was observed from stage 22 through 8 days (Fig. 2). From 9 through 19 days only about three fourths of the adrenals of hypophysectomized embryos showed P-3β-HSD zones while all the adrenals of sham-operated embryos revealed these zones. Beginning at 10, and continuing through 19 days, the peripheral cortical cords failed to form a continuous peripheral zone of high P-3β-HSD activity owing to the large amount of intervening chromaffin tissue. During this period the central zone was smaller and the peripheral larger than those of sham-operated

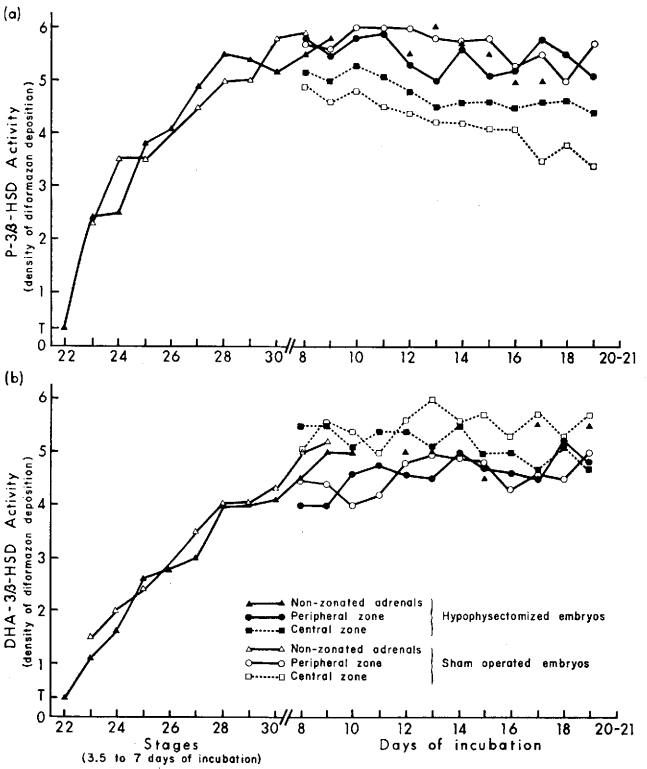

FIG. 2. The adrenal 3β-HSD activity in hypophysectomized and sham-operated embryos (a) using the substrate pregnenolone (P-3β-HSD), and (b) the substrate dehydroepiandrosterone (DHA-3β-HSD).

embryos of corresponding ages.

The average peripheral zone P-3β-HSD activity of hypophysectomized embryos was about equal to that of sham-operated embryos through 11 days and lower from 12 through 19 days except for an increase at days 17 and 18. The average central zone P-3β-HSD activity of hypophysectomized embryos was higher than that of sham-operated embryos from 8 through 19 days. About one fourth of the adrenals of hypophysectomized embryos showed no P-3β-HSD zonation. The average activity of these nonzonated adrenals was about equal to the peripheral zone P-3β-HSD activity of hypophysectomized embryos.

The number of adrenals of hypophysectomized embryos showing a DHA-3β-HSD zonation was lower than that of sham-operated embryos. The peripheral zone DHA-3β-HSD activity in the adrenals of hypophysectomized embryos was about the same as that of controls. The central zone DHA-3β-HSD activity of hypophysectomized embryos was about equal to that of sham-operated embryos from 8 through 12 days but lower through the remainder of the incubation period. The DHA-3β-HSD activity level of the nonzonated adrenals of hypophysectomized embryos was close to that of the peripheral zone of zonated adrenals of hypophysectomized embryos except at 17 and 19 days when it approximated the central zone level.

Treated hypophysectomized embryos. The peripheral and central cortical zones of adrenals from 9 through 19 day hypophysectomized embryos with well developed adenohypophyseal transplants and ACTH-treated hypophysectomized embryos of the same age revealed about the same level of DHA- and P-3β-HSD activity as these zones in the adrenals of sham-operated embryos at the corresponding age. Histologically, the adrenals of these treated hypophysectomized embryos were indistinguishable from those of sham-operated and normal embryos of the same age.

Discussion. The 3β-HSD histochemical technique facilitates the identification of steroidogenic cells. We observed 3β-HSD activity in the adrenocortical cells of stage 22 (3.5–4 days) embryos. Straznicky, Hajos and Bohus (7) assayed adrenal homogenates for 3β-HSD and first observed this enzyme in 14–15 day embryos. Using histochemical methods, Sivaram (8) first observed the enzyme in 10 day embryos, Boucek, Gyori and Alvarez (9) at 5 days, and Chieffi *et al.* (10) at 4.5 days. These discrepancies in observations on the time of appearance of this enzyme in the embryonic chick adrenal may, in part, be attributed to variations in the early development of the chick embryo. Since we observed considerable variation in the degree of development of embryos of comparable incubation ages, morphological characteristics (Hamburger-Hamilton) were employed in determining the age of embryos prior to 8 days of incubation.

Our observations on the overall level of P-3β-HSD activity, from 4 to 8 days and that of central zone activity from 8 days through hatching, are similar to those of Boucek, Gyori and Alvarez (9) who reported that the P-3β-HSD activity, observed in the adrenals of 5 day embryos, increased to a maximum between 11 and 12 days and thereafter showed a gradual decrease until posthatch day 14. These investigators made no mention of the presence of cortical zones. Chieffi *et al.* (10) observed DHA-3β-HSD activity in chick adrenals from 4.5 days incubation to 4 days posthatch but did not mention the presence of cortical zones.

Although the cortical and medullary tissues are intermingled in the avian adrenal, it has been reported that the cortical tissue at the periphery of the gland differs from that more centrally located in embryos (8, 11, 12) and adults (13). Sivaram (11) reported the presence of zones beginning at 15 days. In our study zonation was first observed at stage 30 (6.5–7 days) and was seen in all adrenals by 9 days.

Hypophysectomy had no apparent effect on either DHA- or P-3β-HSD activity before 8 days. The first indications of a zonation occurred at this time in both normal and hypophysectomized embryos. However, from 9 through 20 days all normal embryos and approximately three fourths of the hy-

pophysectomized ones revealed zonated adrenals. In the latter the peripheral zone was larger and the central zone smaller than in normal embryos. Peripheral zone P-3β-HSD activity after 10 days was somewhat lower in hypophysectomized embryos while that of the central zone was higher after 13 days. The adrenals of the remaining one fourth of the hypophysectomized embryos gave no indications of a zonation and the P-3β-HSD activity of these was about equal to the peripheral zone activity of hypophysectomized embryos. These results are not in agreement with those of Manelli (14) who observed no effect of hypophysectomy on adrenal 3β-HSD (substrate not given) in embryos necropsied after 12 to 15 days of incubation.

The absence of zones in the adrenals of some hypophysectomized embryos could be attributed to a failure of central zone development or to an increase in central zone P-3β-HSD level of activity to that of peripheral zone. The presence of a smaller than normal central zone, with a higher than normal P-3β-HSD activity, could be the result of a retarded development of central zone cortical tissue with a consequent infiltration of peripheral zone tissue, or to an increase in central zone P-3β-HSD activity, thus reducing the size of the lower activity central zone. Miller (15) reported atrophy of the cortical tissue in the central zone of hypophysectomized pigeons while that of the peripheral zone remained unchanged for as long as 128 days. From this one may conclude that development of a central zone of low P-3β-HSD and high DHA-3β-HSD activity is under pituitary control. Our results on pituitary transplants and ACTH administration in hypophysectomized embryos support this view. In the absence of the pituitary the central zone showed little or no development.

The results of these experiments show that transplanted adenohypophyses from 17 day donor embryos synthesize and release ACTH in the absence of hypothalamic releasing factors.

Our observations on the intensity and distribution of adrenal DHA- and P-3β-HSD activity in normal embryos suggest the presence of two substrate-specific 3β-hydroxysteroid dehydrogenases. In the early stages of embryonic development, P-3β-HSD activity was always higher than DHA-3β-HSD while in the final stages central zone DHA-3β-HSD activity was noticeably higher than the central zone P-3β-HSD and about equal to peripheral zone P-3β-HSD activity. The existence of different substrate-specific 3β-hydroxysteroid dehydrogenases was previously reported (16) as a result of observations on the Leydig cells of the fetal mouse and (9) in work on the embryonic gonads of the chick. Additional studies are required to fully delineate the hormonal control of 3β-HSD activity in the tissues of the adrenal and to clarify the biological significance of this enzyme in adrenal steroidogenesis.

Summary. The adrenals of white Leghorn embryos were examined for histochemically demonstrable 3β-hydroxysteroid dehydrogenase (3β-HSD) using pregnenolone (P) and dehydroepiandrosterone (DHA) as substrates. Traces of P- and DHA-3β-HSD activity were seen as early as stage 22 (3.5–4 days) in adrenocortical cells of normal embryos. These activities gradually increased through 8 days of incubation and were evenly distributed throughout the adrenal, however, the P-3β-HSD was always greater than the DHA-3β-HSD activity. From 9 days through hatching the adrenals showed a narrow peripheral cortical zone of high P-3β-HSD and low DHA-3β-HSD and a large central zone of low P-3β-HSD and high DHA-3β-HSD activity. Many of the adrenals of hypophysectomized embryos lacked cortical zones, however, when they did occur the peripheral was always wider and the central smaller than those of normal or sham-operated controls. From 12 through 19 days, peripheral zone P-3β-HSD activity in hypophysectomized embryos was lower than in controls, except for an increase at 17 and 18 days, and central zone activity was higher than in controls. The peripheral zone DHA-3β-HSD activity of hypophysectomized embryos during this period was equal to that of controls while that of central zone was lower. The DHA- and P-3β-HSD activity of non-zonated adrenals approximated the peripheral

zone levels of zonated adrenals. The DHA- and P-3β-HSD activity levels of adrenals from hypophysectomized embryos which received adenohypophyseal transplants or exogenous ACTH were at levels of adrenals from controls. Histologically, the adrenals of treated hypophysectomized embryos were similar to those of controls.

The authors express their appreciation to Dr. Russell L. Kutz, Armour Pharmaceutical Company, Kankakee, IL, for the generous supply of ACTH. We also thank Mrs. Lucia Smelte and Mrs. Shanta George for their excellent technical assistance.

1. Case, J. F., Anat. Rec. **109**, 277 (1951).

2. Case, J. F., Ann. N.Y. Acad. Sci. **55**, 147 (1952).

3. Betz, T. W., Gen. Comp. Endocrinol. **9**, 172 (1967).

4. Adjovi, Y., Arch. Anat. Microsc. Morphol. Exp. **59**, 185 (1970).

5. Levy, H., Deane, H. W., and Rubin, B. L., Endocrinology **65**, 932 (1959).

6. Fugo, N. W., J. Exp. Zool. **85**, 271 (1940).

7. Straznicky, K., Hajos, F., and Bohus, B., Acta Biol. Acad. Sci. Hung. **16**, 261 (1966).

8. Sivaram, S., Can. J. Zool. **43**, 1021 (1965).

9. Boucek, R. J., Gyori, E., and Alvarez, R., Gen. Comp. Endocrinol. **7**, 292 (1966).

10. Chieffi, G., Manelli, H., Botte, V., and Mastrolia, L., Atti Accad. Naz. Lincei [8] **37**, 82 (1964).

11. Sivaram, S., Anat. Rec. **148**, 366 (1964).

12. Sivaram, S., Histochemie **12**, 316 (1968).

13. Kondics, L., and Kjaerheim, A., Z. Zellforsch. Mikrosk. Anat. **70**, 81 (1966).

14. Manelli, H., Boll. Zool. Agr. Bachicolt. **31**, 555 (1964).

15. Miller, R. A., Acta Endocrinol. **55**, 108 (1967).

16. Baillie, A. H., and Griffiths, K., J. Endocrinol. **31**, 63 (1964).

Received Mar. 2, 1972. P.S.E.B.M., 1972, Vol. 140.