

Δ^5 - 3β -Hydroxysteroid Dehydrogenase Activity in the Adrenal Cortex of the Sodium-Restricted Pregnant Rat¹ (37183)

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In previous investigations we had studied the response of the rat adrenal to the stress of dietary sodium (Na) restriction during pregnancy. We had noted hypertrophy and hyperplasia of the zona glomerulosa (1) and changes in the fine structure which indicated cellular exhaustion (2). We had also reported histochemical studies which showed increased glucose-6-phosphate dehydrogenase (G6PD) activity and changes in lipid distribution (3).

In the present work we investigated the histochemistry of adrenal Δ^5 - 3β -hydroxysteroid dehydrogenase (3β HSD) activity since it is a major enzyme in the pathway of steroid biogenesis. 3β HSD catalyzes the conversion of pregnenolone to progesterone in the early stages of steroid synthesis and has been demonstrated biochemically and histochemically in the rat adrenal (4-8).

We had expected the pattern of 3β HSD distribution in the adrenal stimulated by Na restriction during pregnancy to be somewhat similar to G6PD, indicating an increase of activity in the zona glomerulosa in response to the increased need for aldosterone. Examination of adrenal sections, however, revealed a different pattern. This report presents measurements of the active areas of 3β HSD activity in the region of the glomerulosa, the transitional zone and the zona fasciculata. These measurements are compared with those we had reported previously for G6PD and lipid patterns. The transitional zone of the adrenal cortex is discussed in relation to the observations of 3β HSD distribution.

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Materials and Methods. Female Sprague-Dawley rats weighing approximately 200 g each were divided into three groups of four animals each. All animals were fed a Na-free basal diet (1) supplemented with a control Na level (28.5 mEq/100 g diet by analysis) for approximately 8 days prior to the experimental period. Two groups of animals were mated and one group served as the nonpregnant counterpart. The nonpregnant and one pregnant group were continued on the control Na diet; the second pregnant group was fed the basal diet supplemented with a restricted Na level (1.4 mEq/100 g diet) for the duration of the experiment. On Day 22 of the experiment one adrenal was removed from each animal for histochemical examination. The methods for lipid and G6PD histochemistry have been reported (3). Zona glomerulosa and transitional zone widths were measured on adrenal sections stained with Sudan black B since these show the zone glomerulosa delineated from the sudanophobe transitional zone, permitting each zone to be measured separately.² These measurements were used as a guide for locating zone limits in sections stained for 3β HSD and G6PD.

For the study of 3β HSD, 10 μ m fresh frozen sections were cut and allowed to dry for 5 min in the cryostat. The slides were

² In the previous histochemical studies (3), zona glomerulosa widths were measured on adrenal sections stained with toluidine blue O. We now believe that these earlier measurements which we had called zona glomerulosa widths, included part of the transitional zone. Measurements of sections stained with Sudan black B does not change the significance of difference in zona glomerulosa widths due to Na restriction and pregnancies of the earlier reported studies.

then immersed for 7 min in cold acetone (4°), washed in 2 changes of cold phosphate buffer (0.1 M, pH 7.4) for 1 min each, covered with substrate and incubated at 37° for 30 min. Sections were then postfixed in calcium formol and mounted in glycerol jelly. The substrate per 1 ml consisted of 0.28 mg dehydroisoandrosterone in acetone, 0.5 mg NAD, 0.5 mg Nitro-BT in 0.05 M phosphate buffer, pH 7.4. For objectivity in the measurements, the slides were masked and measurements were made at four points in each of three adrenal sections from each animal. The four readings from each section were averaged as were the results for each animal to obtain the mean for the group. The *t* test was used to determine significance of

the differences between means.

The second adrenal was removed and prepared for study with the electron microscope by methods reported elsewhere (2). One micrometer thick sections were stained with toluidine blue and examined with a light microscope for orientation purposes. The RCA electron microscope (EMU 3G) was used to study the fine structure of the transitional zone.

Results and Discussion. 3β HSD activity in the zona glomerulosa was light in the nonpregnant and pregnant animals fed the control level of Na (Fig. 1). The area of greatest activity was an intense band which appeared to be in the region of the transitional zone and included part of the outer fasciculata.

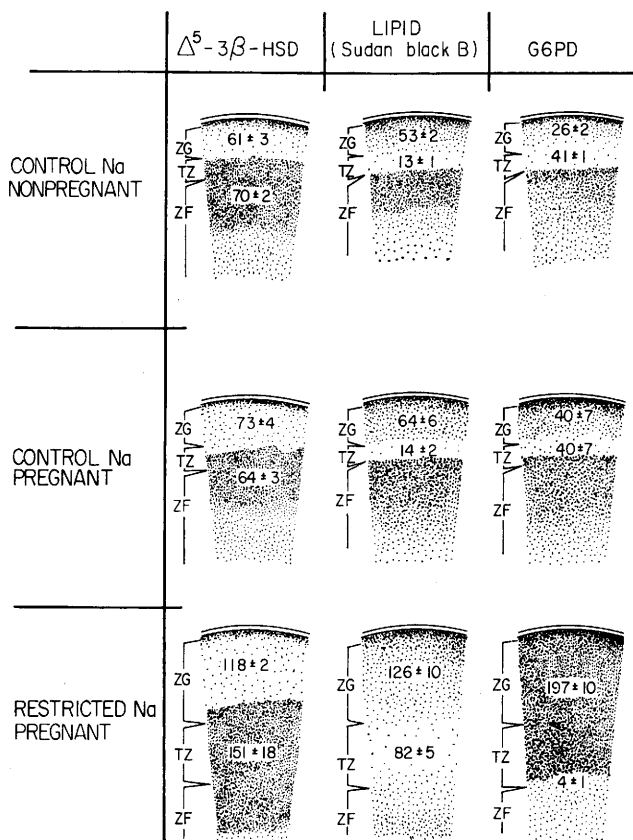


FIG. 1. A diagrammatic presentation of $\Delta^5-3\beta$ -hydroxysteroid dehydrogenase, sudanophilic lipid and glucose-6-phosphate dehydrogenase activity in rat adrenal cortex. Sections are shown for nonpregnant rats fed control Na, pregnant rats fed control Na and pregnant rats fed restricted Na. Parallel markers are used to indicate the limits of the zona glomerulosa (ZG), transitional zone (TZ) and zona fasciculata (ZF). The zone limits are based on measurements made in sudanophilic lipid sections. Width measurements are in micrometers.

Activity in the remainder of the fasciculata was moderate. Activity in the Na-restricted pregnant animals remained light in the glomerulosa region, but in these animals also there was an intense zone of activity in the region of the transitional zone and the outer fasciculata. The width of this intense band of activity increased significantly ($p < 0.001$) in the Na-restricted pregnant animals as compared to either the nonpregnant or pregnant controls.

Others workers who have studied 3β HSD histochemistry in the rat have shown photomicrographs of adrenal sections of normal rats similar to our control rats (5, 6). However, the adrenal response of Na-deprived weanling male rats (48 g) reported by Marx and Deane (5) differed somewhat from ours. They reported an increase of 3β HSD activity starting at 2 wk of Na deprivation in the part of the zona glomerulosa bordering on the fasciculata. Maximum levels were reached at 6 wk at which time activity filled the entire glomerulosa. In the present study, adult female rats were used and may have been better able than the weanlings to adapt to the stress of Na deprivation.

The distribution of 3β HSD activity in the adrenal cortex was different from the pattern of lipid distribution or of G6PD activity (Fig. 1). The adrenals of control Na rats showed a moderately lipid-filled zona glomerulosa clearly demarcated from a lipid-filled zona fasciculata by a sudanophobe transitional zone. The adrenals of Na-restricted pregnant animals, however, showed lipid depletion in the hypertrophied glomerulosa as well as in the fasciculata, and an increase in the width of the sudanophobe zone ($p < 0.001$).

The G6PD activity in the animals receiving the control Na diet was moderate in the zona glomerulosa and very slight in the transitional zone. In the Na-restricted pregnant animals, however, intense G6PD activity extended from the transitional zone throughout the entire glomerulosa, and the width of this area of activity was significantly wider than in the other animals ($p < 0.001$). This distribution differed from that of 3β HSD in which increased activity of Na-restricted pregnant animals was seen in the

outer fasciculata and transitional zones but not in the zona glomerulosa.

The pattern of 3β HSD activity extends our earlier observations on the effects of Na restriction and pregnancy. The patterns of lipid and G6PD activity as well as histological (1) and ultrastructural studies (2) confirmed the involvement of the zona glomerulosa in response to dietary sodium limitation during pregnancy. However, the pattern of 3β HSD activity indicates that the transitional zone as well as the zona glomerulosa is stimulated. In pregnant animals on Na-restricted diets the transitional zone was markedly wider than that of control animals and showed intense 3β HSD activity.

The transitional zone is not yet well understood and its function is the subject of considerable controversy. This zone has also been referred to as zona intermedia, lipid-free zone, sudanophobe zone, subglomerulosa zone and zone of compression. Cater and Lever (9) described the cells of this zone as compressed and basophilic in nature. They reported it to be a region of low mitotic activity and demonstrated a zone of capillary compression which corresponded to the transitional zone plus a variable width of outer fasciculata.

Although the transitional zone is often referred to as being sudanophobe or lipid-free, as we also reported (3), this would not necessarily mean that it is free of lipid. Sudanophobe refers to the nonstaining of tissue with Sudan dyes and histochemical tests for the detection of lipid are not sensitive enough to detect small quantities or specific types of lipid. The results of this study suggest that the cells of the transitional zone are enzymatically active and thus necessarily involved in the synthesis and transfer of steroid molecules.

3β HSD is associated with the provision of precursors needed for corticosteroid synthesis. The reaction catalyzed by 3β HSD, the conversion of pregnenolone to progesterone, is an early step in steroid biogenesis. 3β HSD is present in all the cortical zones but this study indicated its activity predominantly in the region of the transitional zone and part of the outer fasciculata. It is suggested that the region of greater 3β HSD activity func-

tions as the major source of progesterone, the precursor in the production of mineralocorticoids in the zona glomerulosa.

To help determine the extent of transitional zone activity electron micrographs of the tissue were studied. The fine structure of the transitional zone of nonpregnant animals fed control Na (Fig. 2) was similar to that described by previous workers (10). Mitochondrial structure of these cells helps to distinguish them from those of the zona glomerulosa and zona fasciculata. Mitochondria of the transitional zone contain both tubules and vesicles, whereas mitochondria in the zona glomerulosa are predominantly tubular and those of the zona fasciculata are primarily vesicular.

Cells of the pregnant animals fed restricted Na differed from control animals primarily in internal mitochondrial structure. There was a decrease in intramitochondrial elements so that few tubules and vesicles remained (Fig. 3).

The ultrastructural changes observed in the transitional zone were not striking when compared to those seen in zona glomerulosa cells (2). There was no evidence of cellular degradation or pathology. There was, there-

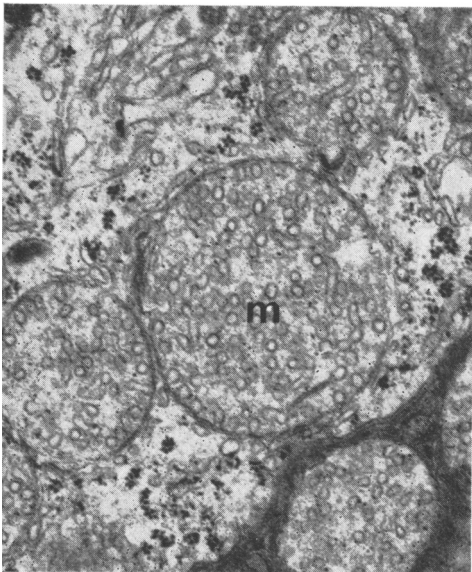


FIG. 2. Mitochondria (m) in part of a cell of the transitional zone of a nonpregnant rat fed the control Na diet. The mitochondria contain tubulo-vesicular elements ($\times 30,000$).

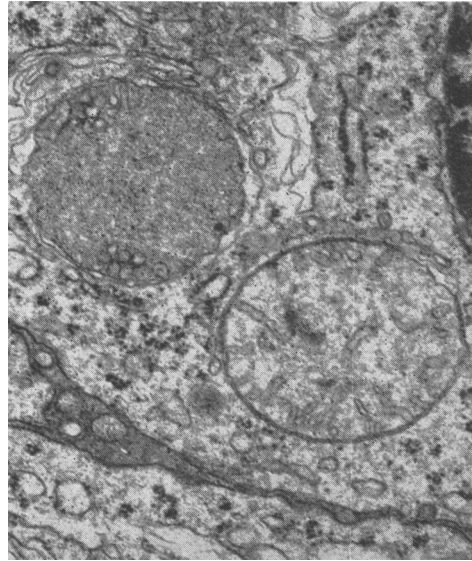


FIG. 3. Mitochondria in part of a cell of the transitional zone of the pregnant rat fed restricted Na. There are fewer internal elements in these mitochondria than in those of control animals ($\times 28,000$).

fore, no indication that the cells of the transitional zone were exhausted in Na-restricted pregnant rats as were those of the zona glomerulosa. It appears that the transitional zone was actively involved in synthesizing precursors for aldosterone production but, unlike the zona glomerulosa, its capacity for synthesis was not exhausted even under the combined stimuli of sodium restriction and pregnancy.

Summary. 3β HSD activity was studied in the adrenal cortex of rats under conditions of sodium restriction, pregnancy or both. The enzyme was shown to be active primarily in the region of the transitional zone and outer fasciculata, indicating that these areas of the adrenal cortex, as well as the zona glomerulosa, are involved when aldosterone production is stimulated. Ultrastructural studies confirmed the histochemical evidence of the involvement of the transitional zone but also provided additional information suggesting a difference in the functional capacities of the zona glomerulosa and the transitional zone. The combined stimuli of sodium restriction and pregnancy led to cellular degradation of the zona glomerulosa (2) but

caused no pathological changes in the transitional zone. A suggested role for this zone is that of providing transitional or precursor cells for both the zona glomerulosa and the zona fasciculata. This might account for the fact that its physiological capacity appears to be greater than that of the zona fasciculata.

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