Effects of Dietary Sodium Restriction During Pregnancy on the Histochemistry of the Rat Zona Glomerulosa[1,2] (34448)

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Several studies have reported that dietary sodium restriction results in a marked increase of glucose-6-phosphate dehydrogenase (G6PD) activity in the glomerulosa of the rat adrenal cortex (1–4). Sodium restriction also results in lipid accumulation in the zona glomerulosa (2, 3, 5) and in the increased secretion of aldosterone (6). The observed hypertrophy and hyperplasia of the zona glomerulosa in sodium-restricted animals is indicative of the increased aldosterone production in response to inadequate sodium intake (3, 5). Investigations in this laboratory (7, 8) on the effects of varying the levels of dietary sodium during pregnancy in the rat have noted that hypertrophy and hyperplasia of the zona glomerulosa also develop during pregnancy. These responses are intensified when sodium restriction is superimposed upon the stress of pregnancy and the histological picture then becomes one resembling cellular exhaustion. Rinsler and Rigby (9) studied urinary excretions of aldosterone in human pregnancies and noted that a considerable increase in aldosterone secretion occurred during pregnancy, thus suggesting that an increase in sodium requirement occurred. They suggested that this increased aldosterone secretion ". . . is a physiological response by the adrenal cortex to maintain the extracellular fluid in the face of sodium loss and water loss into maternal cells and into the products of conception."

To better understand the physiological adjustments in response to the stress of a restricted sodium intake during pregnancy, the effects of varying the level of sodium intake during pregnancy on the histochemical changes in the rat adrenal cortex were examined.

Materials and Methods. Young adult female albino rats (Sprague-Dawley) were maintained on laboratory chow until they weighed approximately 200 g and regular estrous cycles were established. The animals were then divided into four experimental groups of four animals each. Two groups were mated and two served as their nonpregnant counterparts. The basal ration (10) in which a Na-free modification of the Williams and Briggs (11) salt mix was substituted was supplemented with NaCl at 2 levels: restricted, 0.032% and control, 1.76% (1.1 and 28.7 meq/100 g by analysis). The animals were placed on the control ration approximately 8 days prior to the beginning of the experimental period. On the first day of the experiment (the day mating was confirmed by the presence of sperm in the vaginal smear for the pregnant group) four nonpregnant and four pregnant animals were placed on each of the diets for 22 days. Food and demineralized water were provided *ad libitum* and weekly records of food intake were kept.

On Days 1 and 22, blood samples were taken from the tip of the tail into Strumia-type tubes for hematocrits and heparinized capillary tubes for Na and K concentrations. Five microliters of plasma were analyzed for Na and K content on the IL Flame Photome-

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On Day 22 litters were removed by abdominal section and an adrenal removed from each animal for histological and histochemical examination. The adrenal was cleaned and rapidly frozen with a freon aerosol. Frozen sections were cut at —20° in a cryostat. Histological details were obtained by staining 6-μ unfixed frozen sections by a toluidine blue 0 method for rapid frozen sections (12). The width of the zona glomerulosa and the number of cells comprising the width of the zone were determined at four points and averaged for each of three sections from each animal. Slide labels were masked and coded to obtain objectivity in the readings.

The activity of G6PD in the adrenal cortex was studied histochemically using an incubation medium containing Nitro-BT as electron acceptor. Ten-micron sections were cut, placed on slides, and allowed to dry for 5 min in the cryostat. The slides were then immersed for 2 min in cold acetone (4°) and then washed in two changes of cold 0.1 *M* phosphate buffer (pH 7.4) for periods of 30 sec each. Sections were then covered with freshly prepared substrate and incubated at 37° for 30 min. Sections were usually incubated within 30 min after removal from the animal. The substrate solution was the same used by Cohen (2) with the exception that 0.1 *M* phosphate buffer, pH 7.4, was substituted for 0.1 *M* veronal buffer, pH 7.4. The slides were masked and the width of the enzyme reaction in the zona glomerulosa measured 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.

For evaluation of lipid, 10-μ frozen sections were allowed to dry 5 min in the cryostat, fixed in cold calcium formol overnight, then stained with Sudan black B (13) with nuclear fast red counterstain. Slides were masked and examined for lipid content and distribution.

Results. The data obtained for food intakes, weight gains, hematocrits, and Na and K concentration of the plasma for the small groups in this study confirmed the statistically analyzed data obtained previously in this laboratory (7, 8) and were used, therefore, in the interpretation of the histochemical data.

The average Na and K concentrations of plasma on Days 1 and 22 of the experimental period are presented in Table I. Plasma Na and K in the nonpregnant groups on both levels of dietary Na were unchanged at the end of the experimental period. Pregnancy caused a decrease in plasma Na but the animals in the Na-restricted group had a considerably lower concentration of plasma Na on Day 22 than the pregnant controls. The plasma K concentration was altered only in the Na-restricted pregnant group with an increase of 50% during the experimental period.

The average widths and average number of cells comprising the zona glomerulosa are shown in Table II. The nonpregnant control group had the narrowest zona glomerulosa

TABLE I. Average Plasma Sodium and Potassium Concentrations.

Dietary group (meq Na/100 g)	Nonpregnant				Pregnant			
	Na (meq/liter)		K (meq/liter)		Na (meq/liter)		K (meq/liter)	
	Day 1	Day 22	Day 1	Day 22	Day 1	Day 22	Day 1	Day 22
1.1	137.2 ±0.8[a]	137.6 ±1.7	4.7 ±0.1	4.9 ±0.1	139.9 ±0.7	122.2 ±3.5	5.0 ±0.2	7.5 ±0.5
28.7[b]	135.3 ±1.3	135.6 ±1.0	4.9 ±0.1	4.8 ±0.1	139.0 ±1.2	130.3 ±1.5	4.6 ±0.0	4.6 ±0.1

[a] Standard error of the mean.

[b] Control groups.

TABLE II. Average Widths and Cell Counts of the Adrenal Zona Glomerulosa Stained with Toluidine Blue 0.

Dietary group (meq Na/100 g)	Nonpregnant		Pregnant	
	Width (μ)	Cells (no.)	Width (μ)	Cells (no.)
1.1	93.6 ± 8.0[a]	9.2 ± 0.3	188.2 ± 5.2	12.8 ± 0.3
28.7[b]	70.4 ± 6.0	7.4 ± 0.5	86.0 ± 9.1	8.4 ± 0.5

[a] Standard error of the mean.
[b] Control groups.

measuring 70.4 μ in width. Pregnancy caused a 22% increase in width, and dietary Na restriction caused a 33% increase in width. The combination of dietary Na restriction and pregnancy caused a 167% increase in zona glomerulosa width. There was a direct relationship between the number of cells and the zone width indicating that hyperplasia accompanies the hypertrophy of the zona glomerulosa.

The average widths of the purple formazan stain deposited in the zona glomerulosa was used as a measure of G6PD activity (Table III). The width of enzyme stain deposited in the nonpregnant control group averaged 26 μ. There was a 54% increase in G6PD due to pregnancy and a 68% increase due to dietary Na restriction. The effects due to pregnancy plus Na restriction caused a 656% increase in G6PD. The area of enzyme activity comprised a little over one-third of the zona glomerulosa width in the nonpregnant control group and about one-half the width in both the Na-restricted nonpregnant and pregnant control groups. In the Na-restricted pregnant group enzyme activity filled practially the entire zone. Representative adrenal sections from each group are shown in Fig. 1.

TABLE III. Average Widths of Enzyme Stain and Percentages of the Area of Enzyme Activity in the Adrenal Zona Glomerulosa.

Dietary group (meq Na/100 g)	Nonpregnant width		Pregnant width	
	μ	%	μ	%
1.1	43.7 ± 3.2[a]	50	196.6 ±10.4	98
28.7[b]	26.0 ± 2.0	39	40.0 ± 6.6	50

[a] Standard error of the mean.
[b] Control groups.

Adrenal sections were examined for lipid content and distribution. In the control nonpregnant animals (Fig. 2b) most of the lipid present in the zona glomerulosa was subcapsular. The zona glomerulosa was demarcated from the deeper cortex by a lipid-free transition zone two to three cells wide. Lipid patterns of the Na-restricted nonpregnant group (Fig. 2a) and the control pregnant group (Fig. 2d) were similar. In both of these groups lipid in the zona glomerulosa was slightly greater than in the nonpregnant control group. Lipid was distributed fairly uniformly throughout the zona glomerulosa although in some animals it was mostly subcapsular. The lipid-free transition zone generally appeared well demarcated. In the Na-restricted pregnant animals (Fig. 2c) lipid was absent in one-half of the zona glomerulosa. The lipid-free transition zone was sometimes difficult to distinguish because of the depleted zona glomerulosa and patchy distribution of fascicular lipid.

Discussion. Previous histological studies in this laboratory have demonstrated extreme hypertrophy and hyperplasia of the zona glomerulosa of the adrenal cortex when dietary Na intake is restricted during pregnancy (7). The present study was designed to investigate further our suggestion that dietary Na restriction during pregnancy places a stress on the aldosterone-producing Na-retaining mechanisms in the animal.

The group of pregnant animals on the Na-restricted diet exhibited the deleterious effects of Na deficiency observed in previous studies. The average food intake and average weight gain were less than the control pregnant animals as were the average litter weight, average fetal weight, and average ma-

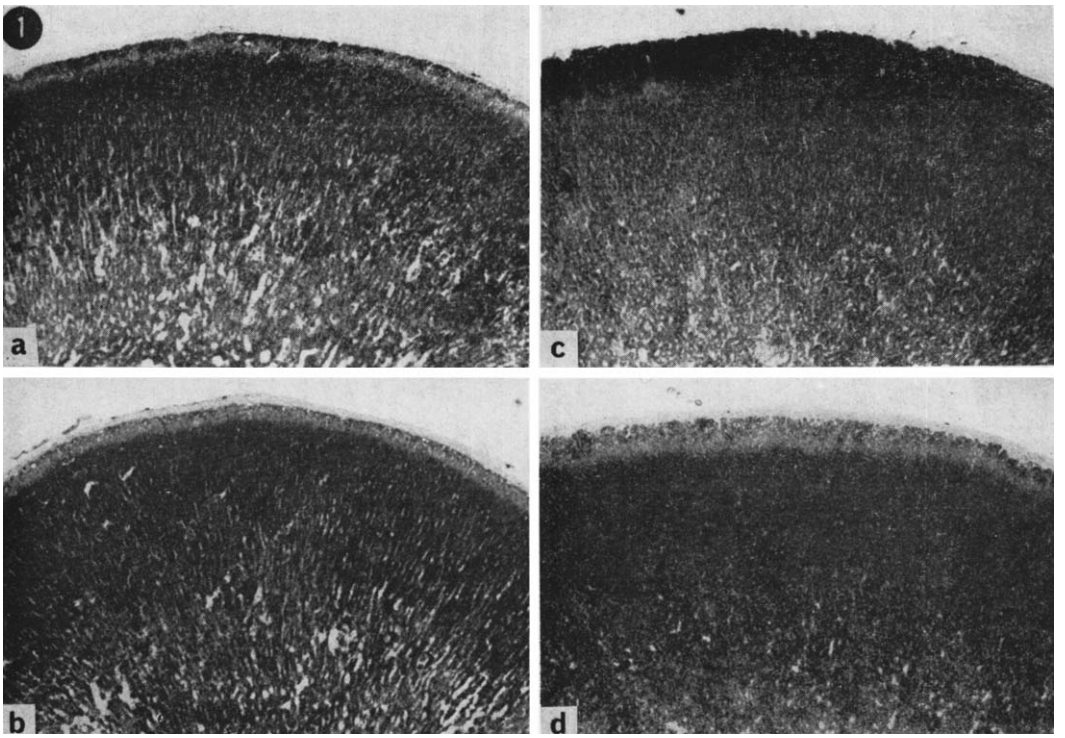

FIG. 1. Glucose-6-phosphate dehydrogenase activity in rat adrenal cortex (×56). a. Nonpregnant rat on Na-restricted diet. b. Nonpregnant rat on control Na diet. c. Pregnant rat on Na-restricted diet. d. Pregnant rat on control Na diet.

ternal tissue gain. These animals also showed the blood changes characteristic of Na deficiency which has been previously observed (7, 8): marked hyperkalemia and hyponatremia. Although a decrease in plasma Na concentration during gestation should be expected because of the normal increase in blood volume (10), the pregnant animals on a restricted intake of dietary Na had a much greater decrease in plasma Na concentration than the pregnant controls. In addition, since the expected decrease in hematocrit value associated with expanded blood volume during pregnancy did not occur, the total circulating Na ions were reduced.

A direct linear correlation has been demonstrated between zona glomerulosa width and the number of cells comprising the zone (7, 8). An increase in the width of the zona glomerulosa has been associated with an increase in aldosterone secretion (5, 6). Results of this study confirm our earlier findings that pregnant rats on a restricted Na intake have the widest zone and the greatest number of cells in the zona glomerulosa and, therefore, the greatest rate of aldosterone secretion. The width measurements of all groups in this investigation tended to be greater than those of previous studies in this laboratory and are attributed to the use of fresh-frozen tissue in this experiment as compared to paraffin sections used in earlier studies.

The histochemical findings of this study lend striking support to our suggestion that dietary Na restriction during pregnancy does stress the aldosterone-producing capacity of the cells of the zona glomerulosa. The zona glomerulosa of the pregnant animals on the Na-restricted diet was highly active in G6PD as compared to the very sparse subcapsular activity in the control nonpregnant group. These findings indicate that the stress of Na restriction during pregnancy stimulated an extremely high rate of aldosterone synthesis. Increased activity of G6PD in the zona glomerulosa has been implicated as an index of

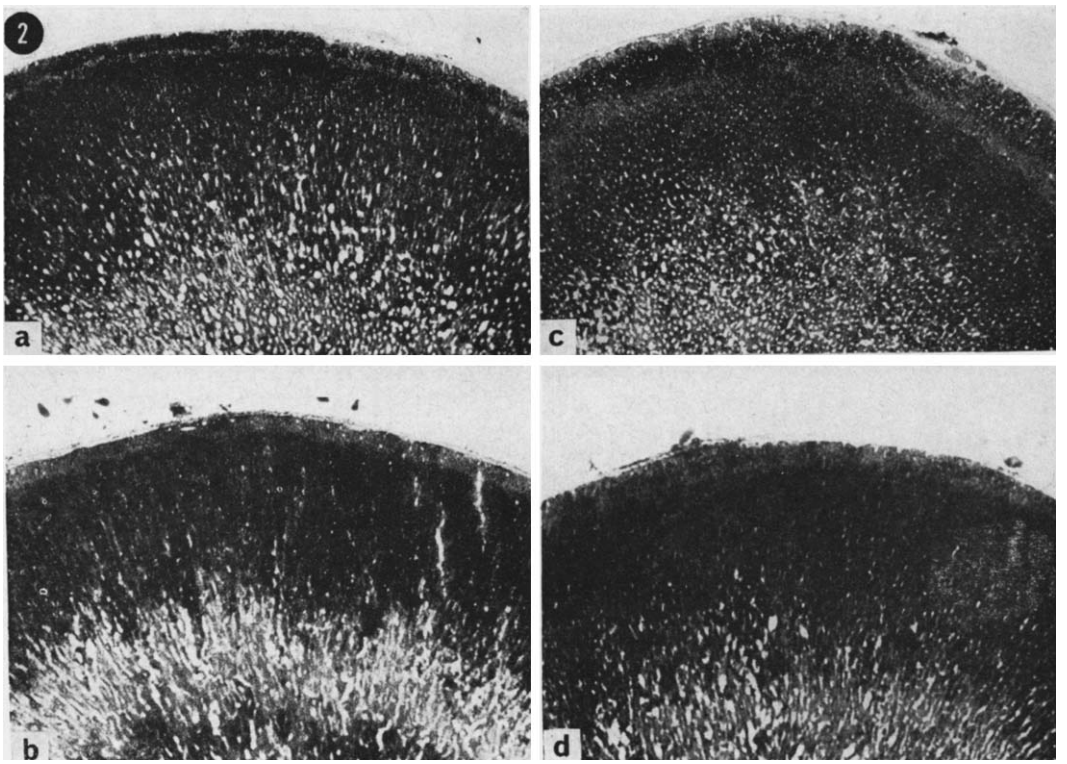

FIG. 2. Sudanophilic lipid in rat adrenal cortex (× 56). Sudan black B counterstained with nuclear fast red. a. Nonpregnant rat on Na-restricted diet. b. Nonpregnant rat on control Na diet. c. Pregnant rat on Na-restricted diet. d. Pregnant rat on control Na diet.

increased aldosterone synthesis (1–4). G6PD specifically catalyzes the bimolecular oxidation of glucose-6-phosphate to 6-phosphogluconolactone and reduction of $NADP^+$ to NADPH in the pentose phosphate pathway. NADPH is required for the reductive processes in the synthesis of fatty acids, cholesterol, and steroids. The highly active G6PD system in the adrenal cortex probably functions as a NADPH generator in corticoid hormone synthesis. The enzyme system may also function in production of ribosyl phosphate for RNA synthesis. It is inferred that the increased demand for aldosterone in the stressed animal increases the requirement for enzymes needed for aldosterone synthesis. Also, the hypertrophy and hyperplasia of the zona glomerulosa of the stressed animal indicate that more glomerulosal cell material is synthesized. Thus, increased RNA is required for synthesis of these enzymes and glomerulosal cell material.

Patterns of sudanophilic lipids were also observed for changes due to dietary Na restriction during pregnancy. Hartroft and Eisenstein (5), and Eisenstein and Hartroft (6) reported that increased amounts of lipid deposited in the zona glomerulosa correlated with increased amounts of aldosterone secreted. In this study, there was a slight increase in zona glomerulosa lipid of animals subjected to either the stress of pregnancy or to the stress of dietary Na restriction. In the adrenals of the animals subjected to both pregnancy and Na restriction there was lipid depletion of the extremely hypertrophied zona glomerulosa, a condition associated with maximal secretion of aldosterone.

Different laboratories have reported slightly different degrees of enzyme activity and lipid content as a result of Na deprivation. The differences reported by other groups as compared to this study can be attributed to the differences of the age of the animal when

placed on experiment, the degree and the duration of Na deprivation.

Previous studies in this laboratory have shown histological exhaustion of the cells of the zona glomerulosa of animals subjected to Na restriction during pregnancy. Prevention or reversal of the exhaustion was observed when Na (8) or aldosterone (14) were administered to pregnant Na-restricted rats for 4 days prior to term, or when rats were continued on the Na-restricted diets and examined 4 days postpartum (15). It is suggested that the increased activity of G6PD in the zona glomerulosa and the changes in the patterns of lipid distribution of animals subjected to sodium restriction and pregnancy are the physiological responses to an increased demand for aldosterone. The high level of G6PD in the lipid-depleted zona glomerulosa of animals subjected to both Na restriction and pregnancy support the previous suggestion from this laboratory that Na restriction during pregnancy exhausts the aldosterone-producing capacity of the cells of the zona glomerulosa.

Summary. Pregnant rats on two levels of dietary Na (1.1, 28.7 meq/100 g) were studied along with their nonpregnant counterparts. At the end of gestation, adrenals were removed and studied histochemically for the demonstration of G6PD and sudanophilic lipids. Plasma Na and K concentrations and hematocrit values were taken as supporting data to aid in the interpretation of the histochemical findings.

Either pregnancy or a restricted intake of dietary Na led to slight hypertrophy and hyperplasia of the zona glomerulosa. The combination of Na restriction and pregnancy resulted in extreme hypertrophy and hyperplasia of the zona glomerulosa.

G6PD in the zona glomerulosa increased by 54% due to pregnancy and by 68% due to dietary Na restriction. However, the combined stress of pregnancy plus Na restriction caused a 656% increase in G6PD.

Alterations in the patterns of sudanophilic lipids were noted. Dietary Na restriction or the stress of pregnancy resulted in a slight increase in zona glomerulosa lipid as compared to the nonpregnant controls. However, the combined stress of Na restriction and pregnancy led to lipid depletion in an hypertrophied zona glomerulosa.

It is suggested that the increased activity of G6PD in the zona glomerulosa and the changes in the patterns of lipid distribution are due to an increased stimulation to aldosterone synthesis. The results support the suggestion that Na restriction during pregnancy exhausts the aldosterone-producing capacity of the cells of the zona glomerulosa.

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