

## EFFECTS OF POTASSIUM LOADING AND OTHER STIMULATING CONDITIONS ON THE GLOMERULAR ZONE OF FETAL ADRENAL CORTEX OF RATS

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**Effects of several conditions known to stimulate the zona glomerulosa of the adrenal cortex in the adult were examined on the fetal adrenal cortex of rats histochemically and electron microscopically. The stimulating conditions were the following; administration of Na restricted diet to the mother, administration of KCl or angiotensin II to the fetus, spironolactone or insulin to the mother. In these, only an intraperitoneal injection of KCl solution to the fetus affected the fetal zona glomerulosa, in which increased activity of glucose-6-phosphate dehydrogenase and hyperfunctional alteration of the mitochondria and the smooth endoplasmic reticulum were observed. This fact shows the fetal glomerulosa cells can react to one of the specific stimuli on the adult glomerulosa cells.**

The zona glomerulosa of adrenal cortex in the adult produces mineralcorticoids such as aldosterone. Aldosterone has already been detected in the fetuses of human, chicken, mice and guinea pigs (1, 2, 3, 4, 6, 8), and the zona glomerulosa of the fetus is thought to be functional in the late stage of gestation (1, 2, 5, 14). According to our previous study, (12, 13, 14), the zona glomerulosa of fetal rats is almost well differentiated in the latest stage of gestation. However, whether the zona glomerulosa of fetuses can react to any stimuli has barely been studied. The reactivity of the endocrine organ to its own stimulus is essential in order to discuss its differentiation. In this study, the effect of several stimuli which are known to act on the zona glomerulosa in the adult, were examined in the adrenal cortex of fetal rats.

### MATERIALS AND METHODS

Wistar female rats aged 4 to 6 months weighing 250–300 g, were bred, and upon confirmation of the date of insemination by the smear test, the 18 1/2 to 21 1/2 day old male fetuses were used for this study. The following experimental conditions were given to the fetuses directly or indirectly. Those evoke the stimulating conditions in the adult zona glomerulosa.

- (a) Sodium restricted condition: The mother was given a sodium restricted diet (Na 2.53 mEq/100 g, Oriental Yeast Company, Tokyo) throughout her pregnancy.

- (b) Direct administration of potassium chloride to the fetus: Under ether anesthesia the mother was laparotomized and through the wall of her uterus, 0.1 ml of 1% KCl solution per a fetus was directly injected into the abdominal cavity of the fetus. The slaughtering date of the fetus was settled at 21 1/2 days old, and it was arranged to have KCl solution dosed at 1/6, 1/2, 1, 2 and 3 days respectively prior to their death.
- (c) Direct administration of angiotensin II (Ciba Geigy Japan Ltd.) to the fetus: Angiotensin II 1, 2 or 4  $\mu\text{g}$ , was administered directly to the fetus by the same method as (b). The slaughtering date was 21 1/2 days old and angiotensin II was injected 1/2, 1, 2 and 24 hr prior to the death respectively.
- (d) Insulin administration to the mother: The mother was given insulin 1 i.u./100 g body weight daily throughout her pregnancy, because hypoglycemia is thought to stimulate the zona glomerulosa in the adult.
- (e) Spironolactone (Aldacton-A, Dainippon Seiyaku Co. Ltd.) administration to the mother: Spironolactone, 200 mg/kg body weight, was given to the mother throughout her pregnancy.
- (f) The effect of ACTH (Cortrosyn-Z, Organon-Daiichi, Seiyaku Co. Ltd.) for the fetal zona glomerulosa was studied previously by the authors (9).

The day of observation was set at 21 1/2 days of the gestational age. After the above-mentioned treatments the adrenal glands of the fetus were taken out under decapitation. One side of the adrenal gland was sectioned at 10  $\mu\text{m}$  thickness by a cryostat and was prepared for lipid histochemical examination by oil red O staining, and for the enzyme histochemical examinations by glucose-6-phosphate dehydrogenase by Rudolph's method (11), steroid 3 -OH dehydrogenase by Levy's method (9), secondary alcohol dehydrogenase by Hardonk's method (5), alkaline phosphatase by Mayahara's method (10) and acid phosphatase by Gomori's method. Another side of the adrenal gland was fixed in 2% glutaraldehyde and 1% osmic acid, dehydrated with acetone and embedded in Epon 812. A thin section was stained by uranyl acetate and lead citrate, and observed under electron microscope, JEM-100B, 60 KV. A semithin section stained with toluidine blue was also observed.

## RESULTS

Neither spironolactone given to the mother, insulin given to the mother nor angiotensin II directly given to the fetus affected the zona glomerulosa of adrenal cortex of fetal rats in the histochemical and electron microscopical observations.

In the experiment of sodium restricted diet given to the mother, the lipid droplets in the zona glomerulosa of the fetus were finely granular in comparison with the control. No detectable change in the fine structure was observed.

In the experiment of KCl solution directly given to the fetal abdominal cavity, distinct effects were observed. By its administration for 3 days from 18 1/2 to 21 1/2 days of gestational age, the histochemical activity of glucose-6-phosphate dehydrogenase in the zona glomerulosa increased and became moderately positive in comparison with its negative activity in the control (Fig. 1). The lipid droplets of the zona glomerulosa showed a slight depletion with a finely granular appearance. Other layers such as the zona fasciculata and zona reticularis had no change. By

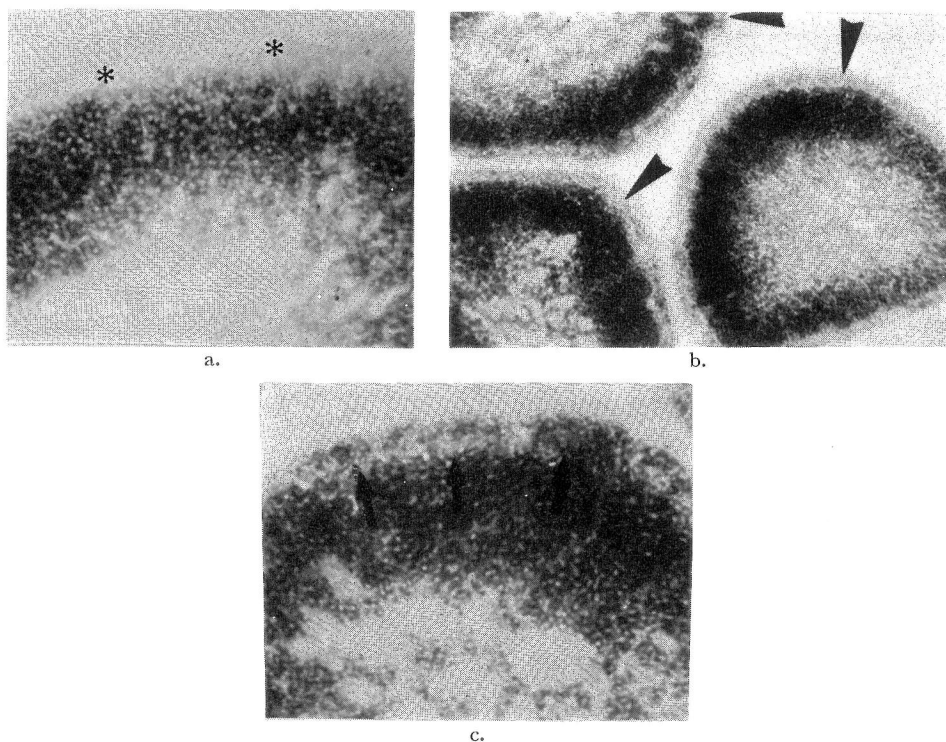


FIG. 1. Histochemical activity of glucose-6-phosphate dehydrogenase in the adrenal cortex of fetal rats.

- a. 21 1/2-day-old, untreated fetus. The activity in zona glomerulosa (\*) is negative.  $\times 100$
- b. 21 1/2-day-old fetus directly administered with KCl solution daily for 3 days. Three adrenal glands are shown here. Note positive activity in the zone of glomerulosa (arrow).  $\times 40$
- c. High magnification of b. Zona glomerulosa shows the moderate activity (arrow).  $\times 100$

the electron microscopical observation, several findings in the zona glomerulosa cells were noted. Round or oval mitochondria increased in number and its cristae showed short tubular or tubulovesicular (Fig. 3.). The mitochondria of the zona glomerulosa cells in the control are long- or oval-shaped, with tubular cristae (Fig. 2). In addition to the mitochondrial change, alteration of smooth endoplasmic reticulum (SER) was characteristic. SER developed diffusely in the tubulovesicular form within the cytoplasm and also became string-like connecting the mitochondria (Fig. 4.). The lipid droplets were seen with a tight connection to the mitochondria and SER. These findings were observed in the group of 3 days, and no obvious change was noted in other groups such as 1/6, 1/2, 1 and 2 days.

#### DISCUSSION

The reactivity to several stimulating conditions of the zona glomerulosa of the adrenal cortex in the fetal rat was examined. The direct intraperitoneal administration of KCl solution affected only the adrenal cortex histochemically and electron microscopically. The following facts were clarified in this study. (1) The zona

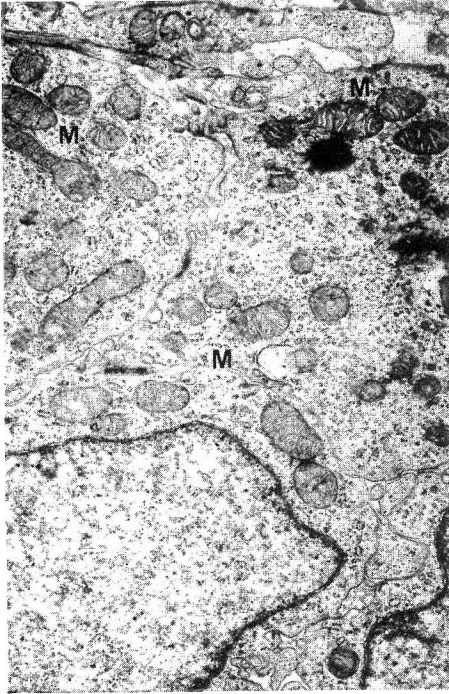


FIG. 2.

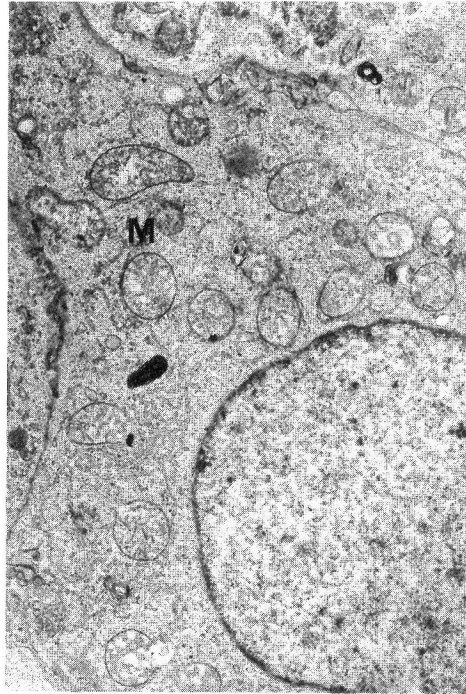


FIG. 3.

FIG. 2. Electron micrograph of untreated glomerulosa cells of adrenal cortex of fetal rat aged 21 1/2-day-old. Long mitochondria (M) have tubular cristae.  $\times 9,000$

FIG. 3. Glomerulosa cells directly administered KCl solution for 3 days, aged 21 1/2-day-old. Increased mitochondria with tubulo-vesicular cristae (M) and diffusely developed smooth endoplasmic reticulum are shown.  $\times 7,000$

glomerulosa of the fetal adrenal cortex was able to react to the stimulus in the late gestational stage. (2) Its stimulus was, for example, the direct administration of KCl solution to the fetus. A sodium restricted diet given to the mother did not distinctly affect the fetal adrenal cortex morphologically. Neither angiotensin II, spironolactone nor insulin had any effects. (3) In the positive group of KCl administration, fine structural changes were seen in mitochondria, SER and lipid droplets. These findings described in "the Result" are thought to be features of hyperfunction of glomerular cells. Accompanying these findings, histochemical changes such as an increased activity of glucose-6-phosphate dehydrogenase means that the glomerular cells of the fetus can react functionally to specific stimulus (7). The condition of sodium restriction of the mother alters the aldosterone secreting rate of the human fetus (1, 2, 6). Although the degree of sodium restriction given to the mother in this experiment is standard in the rat, the sodium restriction experiment in the rat must be repeated not only morphologically but also quantitatively, changing the degree of sodium restriction.

ACTH is a popular stimulus to the zona fasciculata of adrenal cortex in the adult and in the fetus. ACTH could also alter the fetal glomerular cells in our previous



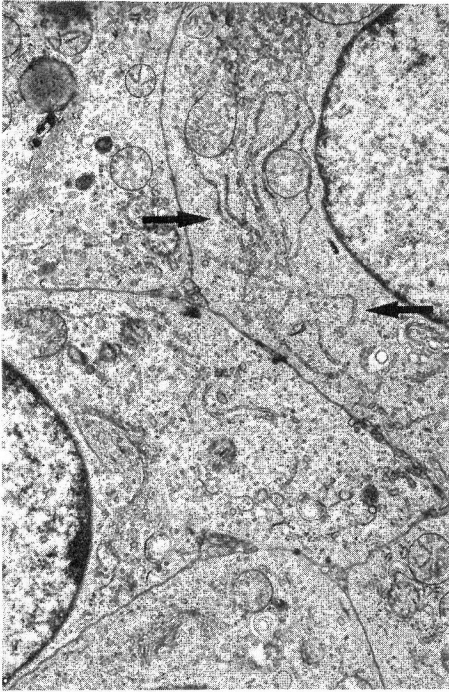


FIG. 4.



FIG. 5.

FIG. 4. Glomerulosa cells in same condition as Fig. 3. Smooth endoplasmic reticulum develop like strings and appear to connect mitochondria (arrow).  $\times 7,000$

FIG. 5. Glomerulosa cells 24 hr after ACTH-treatment. Note string-like smooth endoplasmic reticulum (arrow) and tubulo-vesicular cristae in mitochondria (From author's previous study. This figure is same as *Acta Path. Jap.* 27; 482, 1977.)  $\times 8,000$

study (9). Its alteration in the fine structure is similar to the change of potassium loading in this study (Fig. 5). Through these two experiments of ACTH and potassium loading, it is thought that the reaction of fetal glomerulosa cells to potassium loading is physiological to the specific stimulus and the change by ACTH is also normal as the fetal glomerular cells. Because such a morphological change of the fetal glomerular cells by ACTH is not observed in the adult, it seemed to be related to the prematurity of glomerular cells in the fetus.

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