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HISTOAUTORADIOGRAPHICAL AND ELECTRON MICROSCOPICAL STUDIES OF THE PRENATAL ADRENAL MEDULLA IN SPONTANEOUSLY HYPERTENSIVE RAT

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The histoautoradiography and fine structure of the adrenal medulla in Spontaneously Hypertensive Rat (SHR, Okamoto and Aoki) was examined in the prenatal stage.

The labeling index of the fetal adrenal medulla in SHR was 10 ± 1.38 on the 20th day of gestation and became lower on the 22nd day of gestation. This tendency was consistent with the decrease of mitotic index. The autoradiographical grain count per labeled cell on the 20th day of gestation didn't differ from that on the 22nd day of gestation. These results indicate that the generation time of the adrenal medullary cell in SHR became more longer on successive day of gestation. However, there was no significant difference of the histology and histoautoradiography between SHR and control.

Ultrastructurally, most of medullary tissue on the 20th day of gestation were composed of the intermediate cells containing many intermediate granules. These granules increased in number during the fetal life. In most of the adrenal medullary cells, the fine structure was similar to that observed in the control group.

These findings suggest that the difference of cell proliferation and cell differentiation in the adrenal medullary cells between SHR and control appears after birth, because the significant difference in the medullary cells of both groups has previously reported in the adult rats.

Spontaneously Hypertensive Rat (10, 11) (abbreviated as SHR) have been considered the most suitable experimental animal as the model of human essential hypertension. Tsuchiyama *et al* reported the ultrastructural changes of the adrenal cortex in SHR even in the prehypertensive stage (17). Moreover, the auther showed that the labeling index of the fetal adrenal cortex in SHR was significantly higher than that of normotensive control rat after the 20th day of gestation (16).

It was also reported that the sympathetic tone was elevated and the content of catecholamine increased in adult SHR (12). Histometrical results of the adrenal medulla demonstrated that the area of noradrenaline storing cell islets of SHR was about twice the size of the control from eighteen day after birth (8, 15).

Recent investigation suggests that the activity of the enzyme phenylethanolamine-N-methyl transferase is regulated by adrenal cortical hormone and the last

Dedicated to Professor Hideo Takamatsu in memory of his retirement.

stage of the differentiation in the adrenal medullary cell is under the control of adrenocortical hormone (3, 11).

In the present investigation, cell proliferation and cell differentiation of the fetal medullary tissue was examined in the comparison between SHR and normotensive Wistar-Kyoto rat using the technique of histoautoradiography and electron microscopy.

MATERIALS AND METHODS

Thirteen pregnant rats from a stock of inbred SHR (F27–29), which revealed a systolic blood pressure of over 150 mmHg, and eleven pregnant rats of normotensive Kyoto-Wistar rats (abbreviated as KWR) were used in this study. All pregnant rats was injected intraperitoneally with 1.0 μci/g body weight of tritiated thymidine (Daiichi Pure Chemicals Co., Ltd., Japan: Thymidine-6-T, 1.0 μci/ml) on the 20th and 22nd day of gestation.

All rats were sacrificed by Nembutal sodium anesthesia one hour after the tritiated thymidine injection. The obtained fetuses of SHR (abbreviated as SHRF) were 120 and the fetuses of KWR were 98 (abbreviated as KWRF).

As the electron-microscopical specimen, the adrenals of one to three fetuses from the each litter were immediately removed and fixed by immersion in 1.4% glutaraldehyde buffered with 0.1 M phosphate to pH 7.4 and followed by 1.3% osmium tetraoxide. After dehydration in acetate the material was embedded in Epon 812.

Observation was carried out with JEM 7A electron microscope.

The remainders were fixed in 10% formalin and prepared for paraffin embedding. Serial sections 5 μ thick passing through the center of adrenals were used. Autoradiographical films, using Sakura autoradiographic emulsion, NR-M2 (Konishiroku Photo. Ind., Co., Ltd., Tokyo, Japan), was made by dipping method (2). The exposure time was 30 days. Those films were developed in Konidol X (Konishiroku Photo. Ind. Co., Ltd., Tokyo, Japan) for 5 minutes at 20°C and treated with Feulgen method or hematoxyline stain. Some sections were treated with hematoxyline-eosin and Snook's silver stain for histological analysis. Five hundred medullary cells were counted without discrimination of many cell type because of difficulty of the compartmentalization.

The percentage of parenchymal cell which were labeled and of mitosis was calculated. Cell limitedly with four or more grains was used for calculation of the labeling index and the mean grain count per labeled cell.

RESULTS

Histological Findings: The adrenal medulla in SHRF was composed of almostly immature chromaffin cells which were in separate clusters surrounded by cortical cells. The future medullary cells were migrating toward the central part of the adrenal gland and gradually coalesced with an increase of volume. On the 22nd day of gestation, congregation of the medullary cells was almostly finished. It was very difficult that the chromaffinoblast is identified from the neuroblast and the spongioblast (13).

TABLE 1. *Mitotic index in per cent of fetal adrenal medulla.*

	Day of Gestation	
	20th	22nd
KWR	0.64±0.16(24)	0.37±0.11*(21) (P_1<0.01)
SHR	0.62±0.12(22)	0.39±0.14*(27) (P_2<0.05)*

Mean±S. D.
*: Significant by F-test.
P_1: KWR (22nd) verusus KWR (20th)
P_2: SHR (22nd) verusus SHR (20th)

The mitotic index of SHRF was 0.62±0.12(%) on the 20th day of gestation and 0.39±0.14(%) on the 22nd day of gestation (Table 1). There was no difference between SHRF and KWRF in the histology and the mitotic index. The mitotic index in each group, however, decreased significantly on successive day of gestation (Table 1).

Histoautoradiographic Findings: Figure 1 reveals the typical autoradiographic distribution of tritiated thymidine administrated on the 20th day of gestation. The labeling index of SHRF was 10.6±1.38(%), and of KWRF was 10.73±2.17 (%). Statistically there was no significant difference between both groups. In the each group, the labeling index on the 22nd day of gestation decreased more significantly than that of the 20th day of gestation (Table 2).

The mean grain count per labeled cell of SHRF was 17.48±4.54 on the 20th

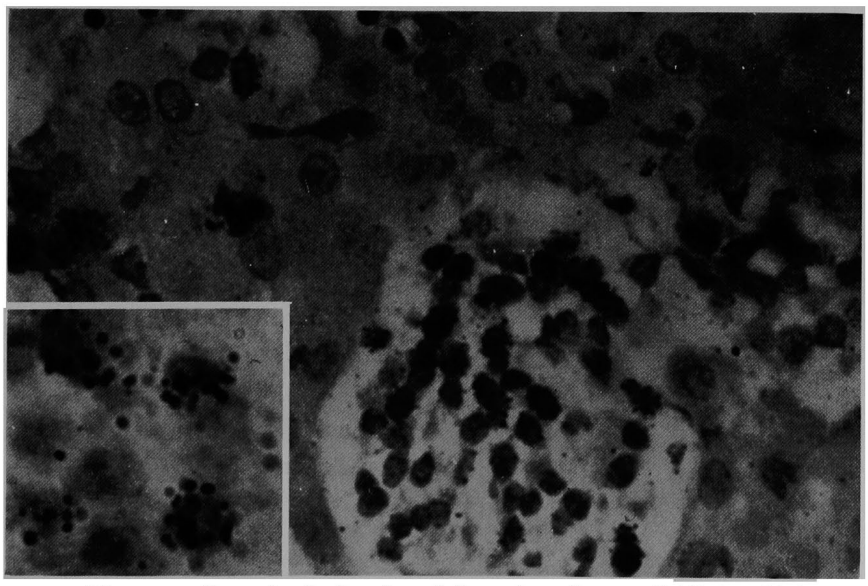

FIG. 1. Histoautoradiograph of adrenal medullary tissue from a 19 1/2-day-old fetus in SHR. The insert shows higher magnification of an area of the medullary cells.

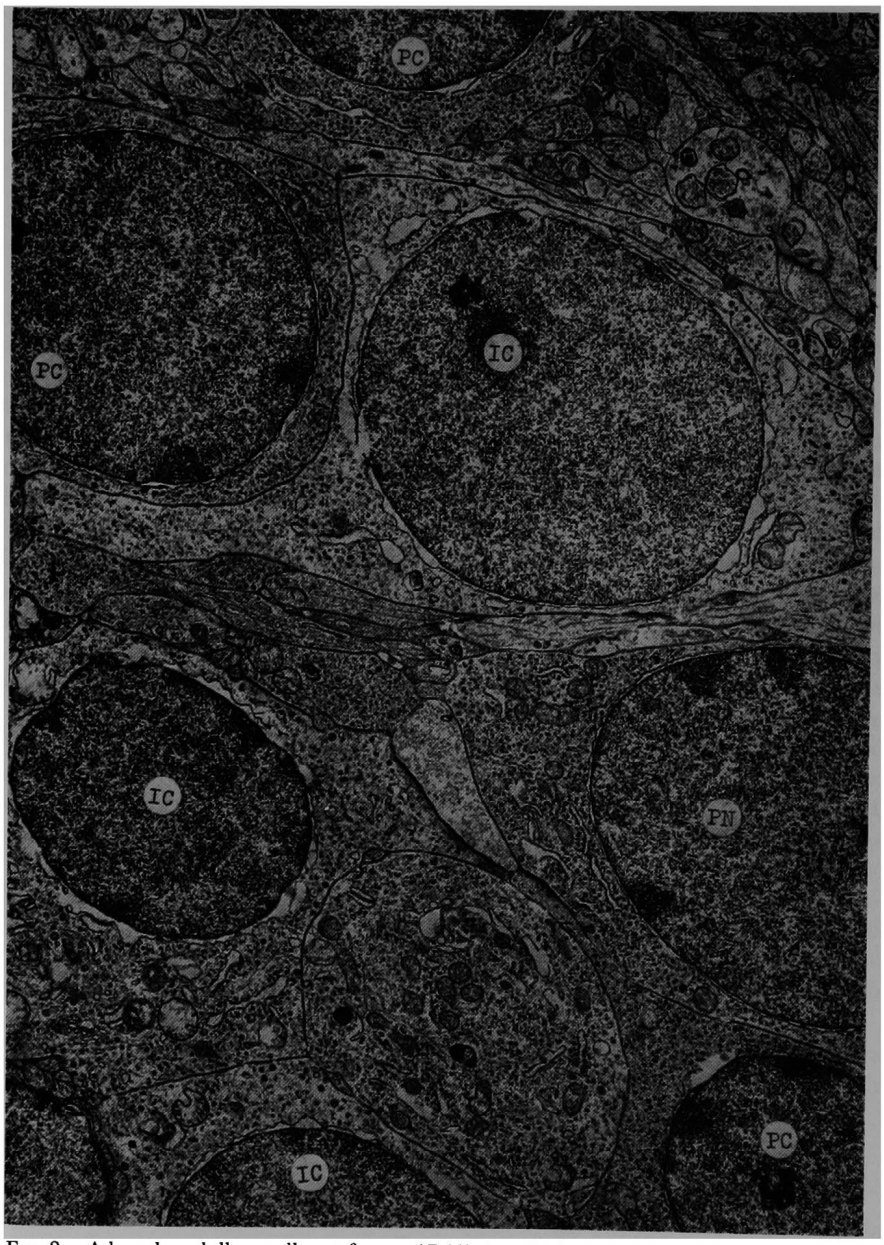

FIG. 2. Adrenal medullary cell nest from a 17 1/2-day-old fetus in control rat. They consist of the intermediate cells (IC), principal neurons (PN) and the primitive sympathetic cells (PC). ×7,500

FIG. 3. Intermediate medullary cells showing clear (ICC) and dark (IDC) cytoplasm from a 19 1/2-day-old fetus in SRH. These cells contain a few intermediate granules and empty- looking organelles. ×11,730

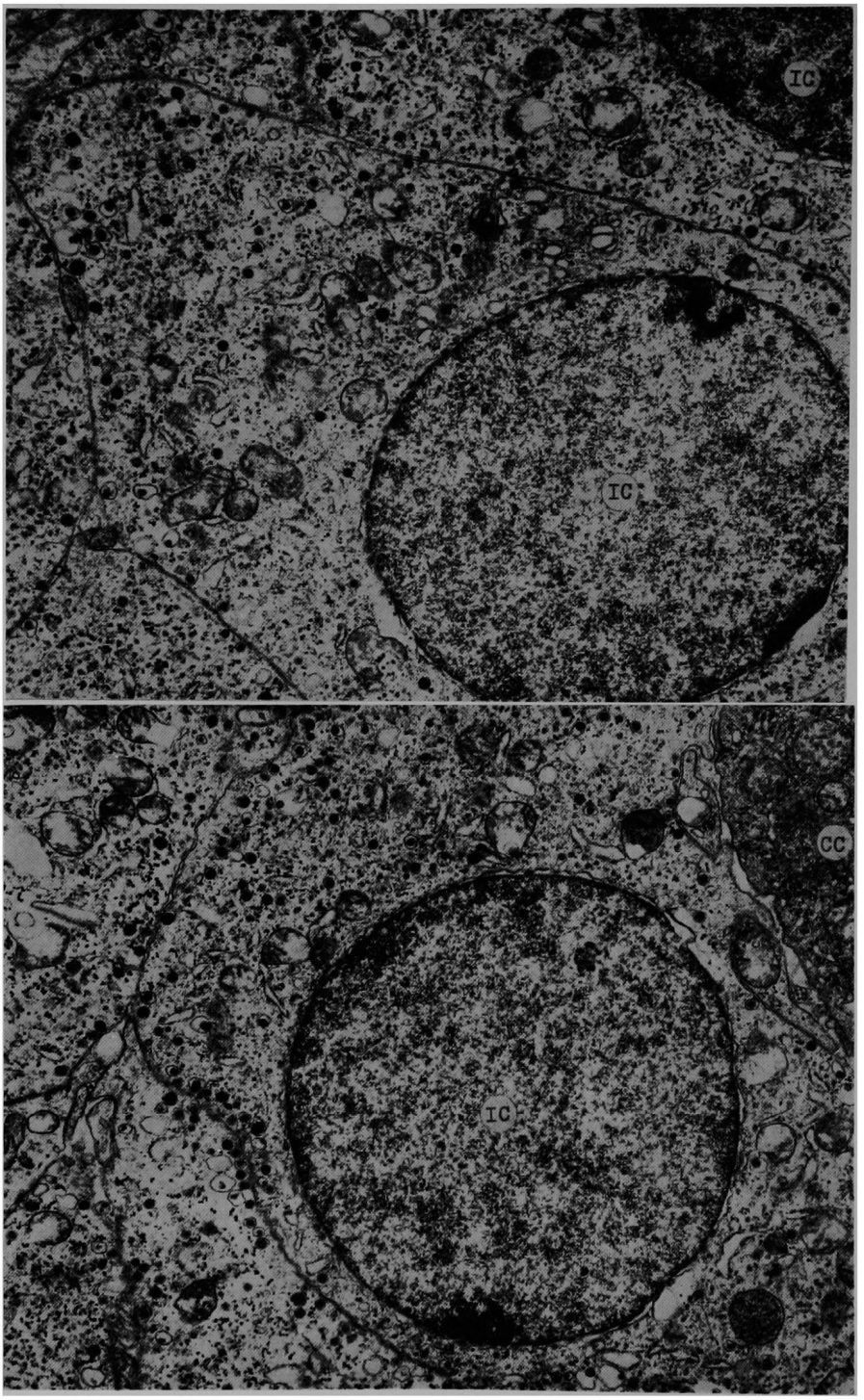
IC
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TABLE 2. *Labeling index in per cent of fetal adrenal medulla.*

	Day. of Gestation	
	20th	22nd
KWR	10.73±2.17(24)	5.07±1.06*(21) ($P_1<0.01$)
SHR	10.60±1.38(22)	4.50±1.86*(27) ($P_2<0.01$)

Mean±S.D.
*: Significant by F-test.
P_1: KWR (22nd) verusus KWR (20th)
P_2: SHR (22nd) verusus SHR (20th)

TABLE 3. *Autoradiographic grain count per labeled cell of fetal adrenal medulla.*

	Day. of Gestation	
	20th	22nd
KWR	16.46±4.04(24)	13.20±6.62(21)
SHR	17.48±4.54(22)	14.88±7.37(27)

Mean±S.D.

day of gestation and 14.88±7.37 on the 22nd day of gestation. No significant difference was statistically observed between SHRF and KWRF.

In each group, there was no difference of the mean grain count on successive day (Table 3).

Ultrastructural Findings: The medullary cell nests of SHRF on the 20th day of gestation consisted of the intermediate cells, principal neurons and the primitive sympathetic cells (Fig. 2). The intermediate cells were composed of two kind of cells with clear and dark cytoplasm. Organelles more developed in the latter than in the former. Among the cell nests the intermediate cells were most predominant. Thesec ells contained the large number of moderately dense cored granules and the small number of highly dense cored granules. Those granules were 700 to 2000 Å in diameter. The mitochondria, Golgi apparatus and the rough-surfaced endoplasmic reticulum were impairly developed. Many materials with high or low density were found in granule and cytoplasm (Fig. 3). Many empty-looking organelles without core were also found in the intermediate cell. In the center of the cell nests, the intermediate cells developed poorly than in the peripheral part surrounded by the adrenocortical tissue. The moderately cored granules (the intermediate granules (1)) and empty-looking organelles increased slightly in number on successive day (Fig. 4).

However, there were no remarkable changes in SHRF as compared with KWRF (Fig. 5).

FIGS. 4 & 5. Part of an intermediate cell (IC) of the adrenal medulla from a 21 1/2-day-old fetus in control rat (Fig. 4) and SHR (Fig. 5). Intermediate granules are increased and many particles with varied density in cytoplasm are found. ×11,500

DISCUSSION

In the previous study, SHR of inbreeding colony in our laboratory revealed a possibility of the active some steroid production of the adrenal cortex in pre-hypertensive stage and the increase of labeling index in intrauterine life (16, 17). On the other hand, Hervonen suggested that glucocorticoids might cause the differentiation of new chromaffin cell from the primitive sympathetic element or from some intermediate form (4).

Therefore, it is important to investigate whether the changes of the adrenal medulla correlated with cell proliferation and methilating property appear or not in intrauterine period.

The histologic findings of the adrenal medulla in SHRF were essentially the same as those reported in normal fetal rats (5, 13). There was no histological difference as compared with the adrenal medulla of KWRF. The labeling index of the adrenal medulla in SHRF was $10.60 \pm 1.38(\%)$ on the 20th day of gestation and decreased significantly on the 22nd day of gestation. The mitotic index indicated the same tendency. These results would be different from the observation by Josimovich (5).

The autoradiographical grain count of SHRF had a marked resemblance in number on the 20th and 22nd day of gestation. No significant difference between SHRF and KWRF was there statistically. This fact indicated that the DNA synthesis and the generation time of adrenal medullary cell of SHRF did not differ from that of KWRF on the 20th and the 22nd day of gestation. No difference of DNA synthesis is seen in each group, but the generation time of adrenal medullary cell of SHRF and KWRF is shorter on the 20th day of gestation than on the 22nd day of gestation, because of $T_G \propto \frac{1}{LI.Gr}$, where T_G is the cell generation time, LI is the labeling index and Gr is the grain count (2).

The large number of immature intermediate cells and principal neurons and the small number of primitive sympathetic cells were seen in the central part of medullary cell nests. The intermetiate cells contained the intermediate granules with various degrees of density. Some granules had very dense materials and other moderately dense materials in the same cell.

These granules increase in number near to the peripheral zone surrounded by adrenal cortical cells. The granules and the empty-looking vesicles increased on successive day. These observations of SHRF were tentatively the same electron microscopic findings as previously reported in normal fetal rats (1). On the each day of gestation, there was no difference of the fine structure between SHRF and KWRF.

O'Brein suggested that the fetal adrenal medullary cell contained vesicles which store mainly ATP and that in course of maturation these vesicles took up cathecholamine and were transformed into chromaffin granules (9).

The decrease of mitotic index and labeling index in both groups and the changes of the ultrastructural appearance would be reflected the cell maturation on successive day of gestation. In the intermediate cell of SHRF and KWRF, a numerous number of particles with various degrees of density appeared in the granules and cytoplasm. Elfvin suggested that the glycogen-like particle close

to the granule in the cytoplasm might be a precursor of cathecholamine (1).

A population of noradrenaline-secreting cell with increased granules and vesicles increased through pre-, initial and advanced hypertensive stage of adult SHR (6, 7, 14, 15). In addition, Ozaki demonstrated the increased contents of noradrenaline and adrenaline of the adrenal medulla in adult SHR (10). Therefore, it may be explained that the significant difference of cell proliferation and cell differentiation in the medullary cell between SHR and control rat appears after birth.

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