Morphological Study of the Adrenal Cortex in Stroke-prone Spontaneously Hypertensive Rats (SHRSP)

—Special Reference to the Zona Glomerulosa by Electron Microscopy and Morphometry—

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The adrenal zona glomerulosa of stroke-prone spontaneously hypertensive rats (SH RSP) at 18 week old was examined morphologically and morphometerically. An increase in width of the zona glomerulosa was found including hypertrophy and hyperplasia of the cells. The area of cells in this zone of SHRSP was significantly greater than that of Wistar-Kyoto strain rats (WKY). The mitochondria of this zone were smaller in size, pleomorphic in shape and increased in number, and the percent area of the mitochondrial compartment of SHRSP was significantly lesser than that of WKY. The lipid droplets in zona glomerulosa cells of SHRSP were numerous and the percent area of lipid droplers compartment of SHRSP was significantly greater than that of WKY. Therefore, the zona glomerulosa cells were appeared to be hypertrophic. The smooth-surfaced endoplasmic reticulum and the Golgi apparatus were well developed. The relationship between these pathological changes and functional state in the zona glomerulosa of SHRSP was discussed.

INTRODUCTION

The spontaneously hypertensive rat (SHR)[26] has been used as a model of human essential hypertension. Recently, OKAMOTO and YAMORI et al.[27] established a substrain (stroke-prone SHR, SHRSP) from SHR; this strain is characterized by severe hypertension and a high incidence of cerebrovascular lesion.

The association of hypertension with endocrine disorders in human and in experimental animals has been described in previous studies. AOKI[1] has reported on the relationship between endocrine organs and hypertension in SHR. And he pointed out[2]

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that the adrenal gland was necessary for the development and maintenance of high blood pressure in SHR. However, BAER et al.[3] failed to show that the adrenal was involved in pathogenesis of spontaneous hypertension.

Several authors have reported the studies on the plasma renin activity (PRA)[15)18)29)] and the plasma aldosterone concentration (PAC)[19)25)] in SHRSP. OGINO et al.[25] revealed that PAC of 5 month old SHRSP was nearly equal to PAC of WKY.

Concerning the alterations of the adrenal cortex in SHR, some descriptions[1)4)9)12)17)22)23)31)] were reported histologically, histochemically and morphometerically. On the other hand, there is no report on the adrenal cortex in SHRSP. This paper describes on morphologic and morphometric studies of the adrenal cortex in male SHRSP, with special emphasis on the zona glomerulosa, which is the major site of aldosterone production.

MATERIALS and METHODS

Nine male SHRSP and nine male WKY which were obtained from the colony of the Department of Pharmacology in Nagasaki University were used. All animals were given a standard food stock diet (FUNABASHI, Chiba) and maintained in a regular day and night rhythm. The blood pressure was recorded by tail plethysmographic method.

All rats were sacrified by decapitation at 18 week old. Both adrenal glands were removed rapidly and trimmed of adherent fat and the right adrenal gland was weighed. The left adrenal gland was divided into two equal parts with sharp blade. A half was fixed in 10% aqueous formaldehyde solution, embedded in paraffin and cut in serial sections. These sections were stained with hematoxylin and eosin, Van Gieson's method for elastic fiber, Watanabe's method for reticulin fiber, PAS and Mallory-Azan's methods.

The other half was cut into smaller fragments and fixed in 0.1M phosphate-buffered 4% glutaraldehyde followed by 1.3% osmium tetroxide. After dehyderation in acetone, the tissue was embedded in Epon 812. Semithin sections were cut and stained with toluidine blue to identify three zones of the adrenal cortex for ultrathin sections. Ultrathin sections were stained with uranil acetate and lead citrate. Specimens were observed with a JEOL 100B electron microscopy.

As morphometric procedure, the average width of the zona glomerulosa and the area of cells, nucleus, mitochondria and lipid droplets in the zona glomerulosa were recorded. The percentage of the cytoplasm area (percent area) occupied by mitochondria or lipid droplets was also examined.

In order to study the average width of the zona glomerulosa 10 light micrographs of toluidine blue semithin sections from each animal were taken at a magnification of ×200 and enlarged photographically to ×1,400. Five to eight electron micrographs of the zona glomerulosa were taken from each specimen at a magnification of ×2,000 or ×5,000 and enlarged to ×8,000 or ×20,000 for the measurement of the area of cells,

nucleus, mitochondria and lipid droplets. On these randomly sampled micrographs each parameter was determined by a modular system for semiautomatic quantitative evaluation images of A. S. M., LEITZ, West Germany.

Comparison of group means for these parameters was performed by Student's t-test.

RESULTS

The systolic blood pressure of SHRSP was significantly higher than that of WKY. The body weight of SHRSP was lesser than that of WKY. The adrenal weight per 100 g body weight (relative adrenal weight) of SHRSP did not differ significantly from that of WKY. These data are shown in Table 1.

At autopsy, the incidence of cerebral lesions was 6/9 of SHRSP, and none of WKY had the cerebral lesion.

Histological findings. The zona glomerulosa was increased in width and consisted of six to seven cell layers in SHRSP(Fig. 1-a, b), whereas the zone was consisted of three to four cell layers in WKY(Fig. 2-a, b).

The zona glomerulosa cells in WKY were round, polygonal or elongated in shape and had clear cytoplasm with abundant lipid droplets (Fig. 2-b). The cells of this zona in SHRSP were the same shape and had enlarged clear cytoplasm containing numerous lipid droplets (Fig. 1-a, b). Therefore, the zona glomerulosa cells of SHRSP represented hypertrophic character.

In both groups of WKY and SHRSP, the "transitional zone" between zona glomerulosa and zona fasciculata was composed of two or more cell layers (Fig. 1-b, Fig. 2-b). The transitional zone cells were smaller in size than the zona glomerulosa cells, elongated in shape and contained few lipid droplets in the cytoplasm. But this zone in SHRSP (Fig. 1-b) was increased in width compared with WKY (Fig. 2-b).

Intracortical nodules constituted from zona fasciculata cells, which were large and round cells with lipid-rich cytoplasm, were found in SHRSP (Fig. 3) more frequently than in WKY.

Capsular arteries of SHRSP showed moderate medial thickening (Fig. 4) but few

Table 1 Comparison of Blood pressure, Body weight and Relative adrenal weight of SHRSP with WKY

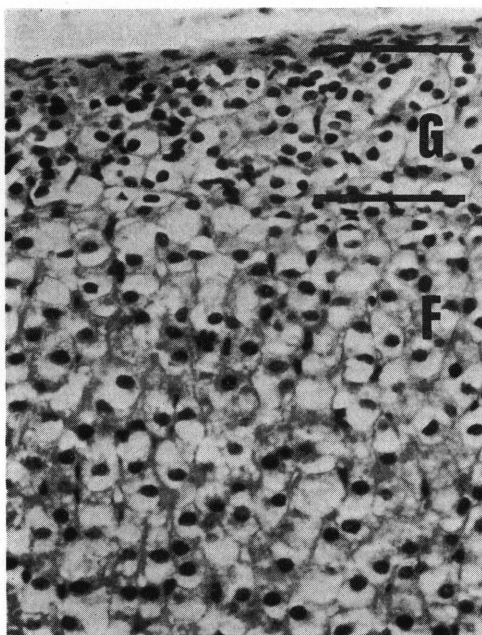|	SHRSP	WKY
Blood Pressure (mmHg)	263 ± 3.9	125 ± 2.5
Body Weight (g)	286 ± 15.4	331 ± 9.2
Relative Adrenal Weight (mg/100gBW)	7.4 ± 0.36	7.8 ± 0.50

Each value represents the group mean ± SE

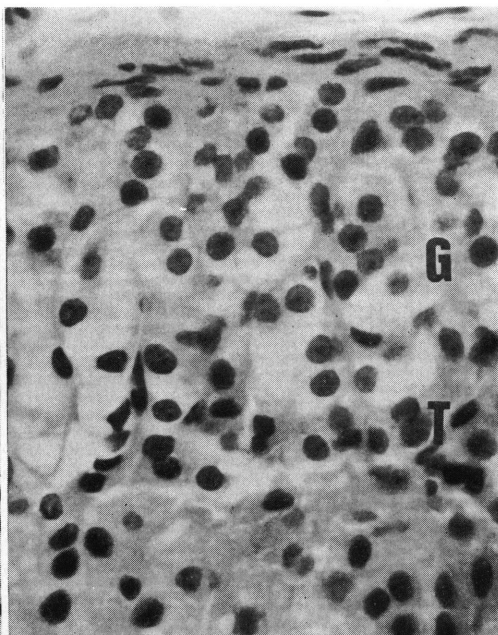Fig. 1-a. Outer adrenal cortex of SHRSP. G= zona glomerulosa, F=zona fasciculata. (×200).

Fig. 1-b. Zona gromerulosa(G)of SHRSP shows an increase in width with cellular hypertrophy and hyperplasia. Transitional zone (T) is prominent. (×400).
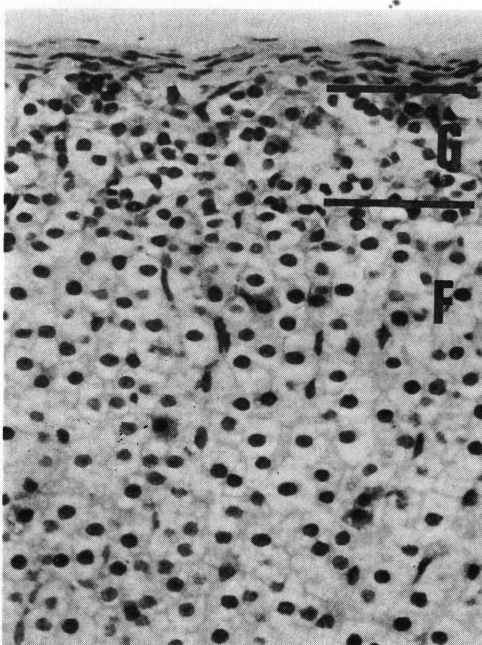

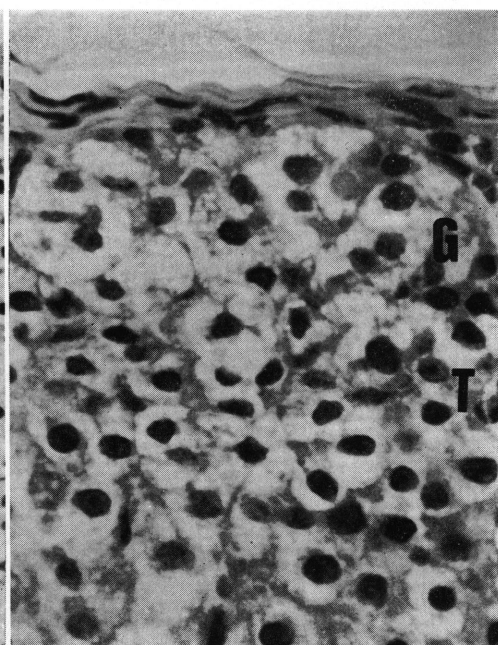Fig. 2-a. Outer adrenal cortex of WKY. G= zona glomerulosa, F=zona fasciculata. (×200).

Fig. 2-b. Zona glomerulosa (G) and transitional zone (T) of WKY. (×400).
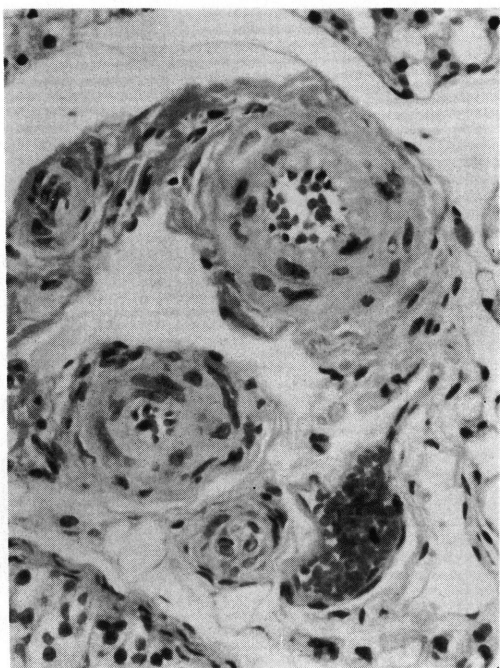
Fig. 3. Cortical nodule (arrow) of zona fasciculata in SHRSP. (×40).

Fig. 4. Medial thickening of cortical arteries in SHRSP. (×200).
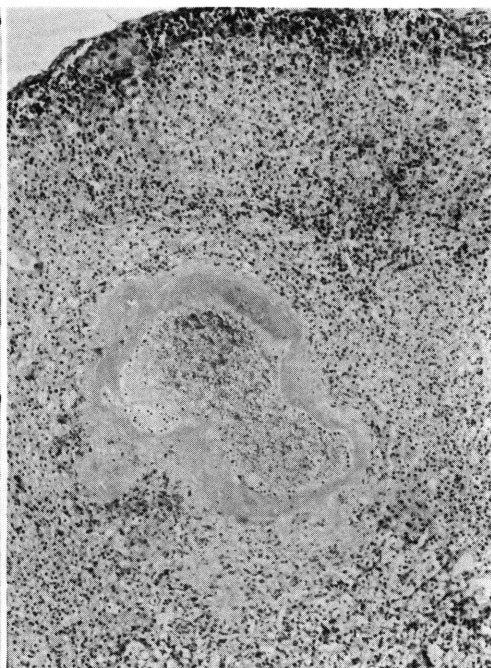
Fig. 5. Fibrinoid necrosis of cortical artery in SHRSP. (×40).

intimal fibrous thickening or thromboembolism was appeared. Fibrinoid necrosis of the
cortical artery was found in only one rat of SHRSP(Fig. 5).

   Ultrastructural findings.   In WKY, the zona glomerulosa cells were arranged in
cords and here and there arranged in stratified or perivascular rosette–like structure (Fig.
6).   The cells were round, polygonal or elongated in shape with round or ovoid shaped
nucleus. The cells were separated by narrow space sometimes interrupted by desmosome–
like junction structure (Fig. 7).   The mitochondria were various in shape and in size.
The mitochondrial matrix was slightly electron–dense and two mitochondrial membranes
were clearly observed (Fig.7). The mitochondria generally had short tubular cristae, sit-
uated near the periphery of these organelles. Lipid droplets with various size were found
in the cytoplasm. Sometimes lipid droplets were surrounded by smooth–surfaced endoplas-
mic reticulum in tubular appearance but had no remarkable membranous material. The
smooth–surfaced endoplasmic reticulum was seen in the cytoplasmic matrix of the zona
glomerulosa cells among other organelles.   This organelle seemed to be well developed
and vesicular in shape (Fig. 7). A few profiles of rough–surfaced endoplasmic reticulum,
free or clustered ribosomes and glycogen particles were scattered throughout the cytoplasm.
The Golgi apparatus were generally well developed near the nuclei associated by amor-
phous dense–bodies and mitochondria and were formed by many lamellar raws of cisternea
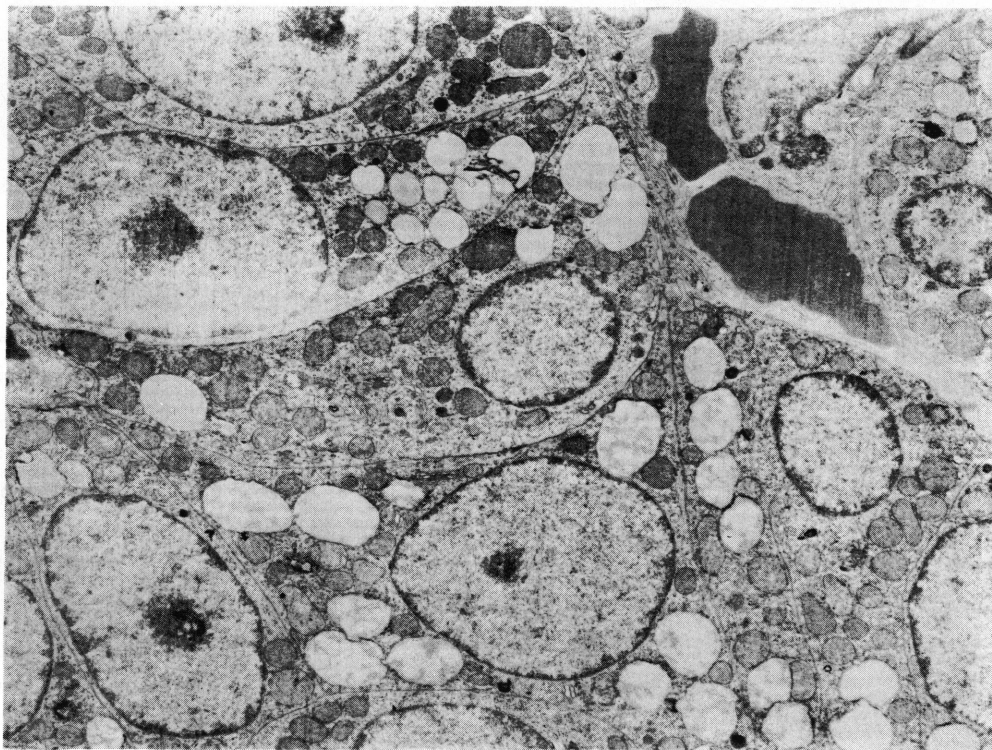(Fig. 7).

Fig. 6.   Zona glomerulosa cells of WKY. Note the arrangement in peri–vascular
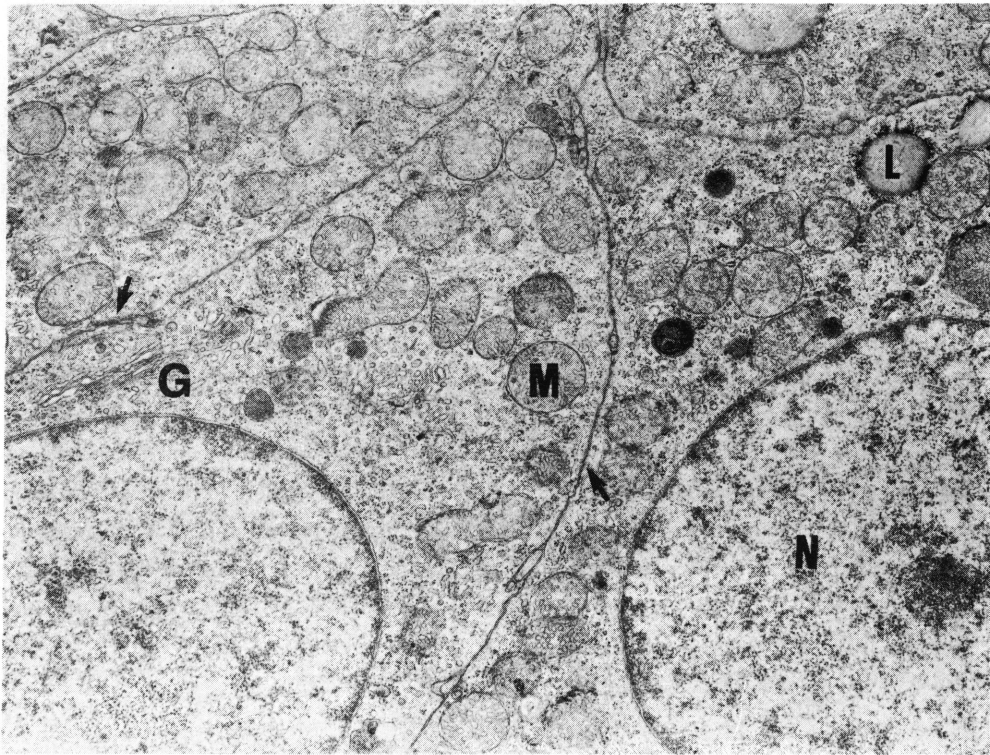rosette–like structure. (×6,700).

Fig. 7. Zona glomerulosa cells of WKY. N=nucleus, M=mitochondria, L=lipid
droplets, G=Golgi apparatus. Desmosome-like structure (arrow). (×12,000).

In SHRSP, the zona glomerulosa cells (Fig. 8-11) had no remarkable difference
in the arrangement or structure and in shape from WKY. The nucleus was similar to
that of WKY. The mitochondria of the zona glomerulosa cells in SHRSP showed more
various in shape (Fig. 9, 10) than in WKY; round, polygonal, elongated, dumb-bell
shaped, kidney shaped, etc. These organelles represented smaller in size but increased
in number. The mitochondrial matrix was moderately electron-dense. The mitochondrial
cristae was tubular as WKY but was prominent compared with WKY (Fig. 10). Some
elongated mitochondria exhibited regular arrays of lamellar cristae which usually run
parallel to long axis of the mitochondria (Fig. 10, 11-a). Amorphous electron-dense
intramitochondrial droplets appeared more frequently in SHRSP (Fig. 11-b) than in
WKY. The zona glomerulosa cells in SHRSP had a great deal of lipid droplets in the
cytoplasm (Fig. 8-a). Among these droplets very large lipid droplets measuring up to
12-14 μm^2 in area, which were never seen in WKY, appeared sometimes (Fig. 8-b).
The smooth-surfaced endoplasmic reticulum in SHRSP was well developed and showed
more vesicular or dilated appearance (Fig. 10). The Golgi apparatus, ribosomes and
glycogen particles were also observed and prominent in SHRSP (Fig. 9, 10).

Morphometrical findings. Data of various zona glomerulosa cell components are
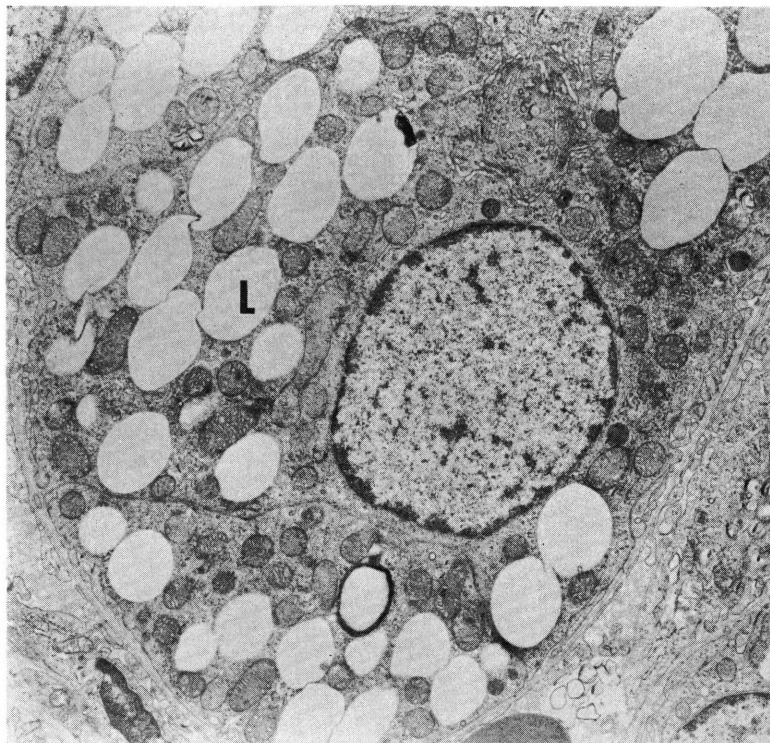
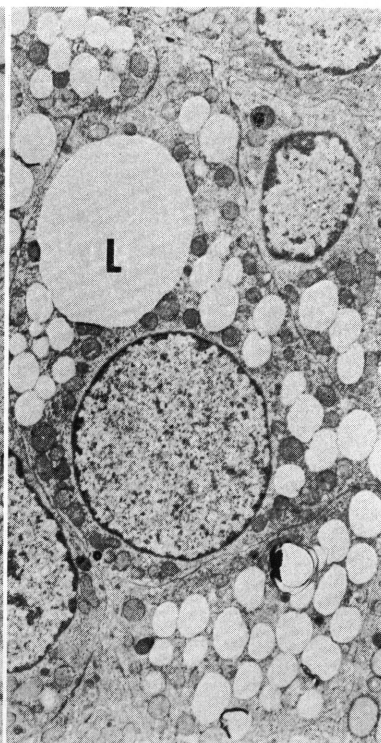Fig. 8-a. Zona glomerulosa cells of SHRSP. Numerous lipid droplets (L) are seen. (×7,900).
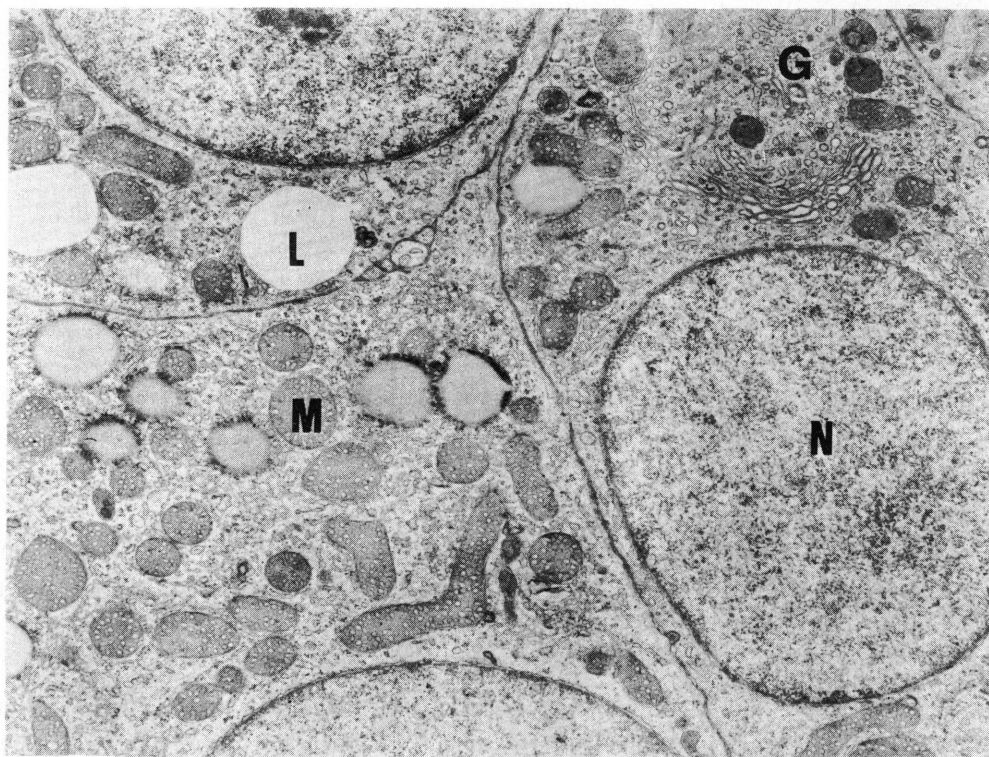
Fig. 8-b. Note large lipid droplets (L). (×5,000).

Fig. 9. Zona glomerulosa cells of SHRSP. N=nucleus, M=mitochondria, L=lipid droplets, G=Golgi apparatus. Note pleomorphism in mitochondria. (×12,000).
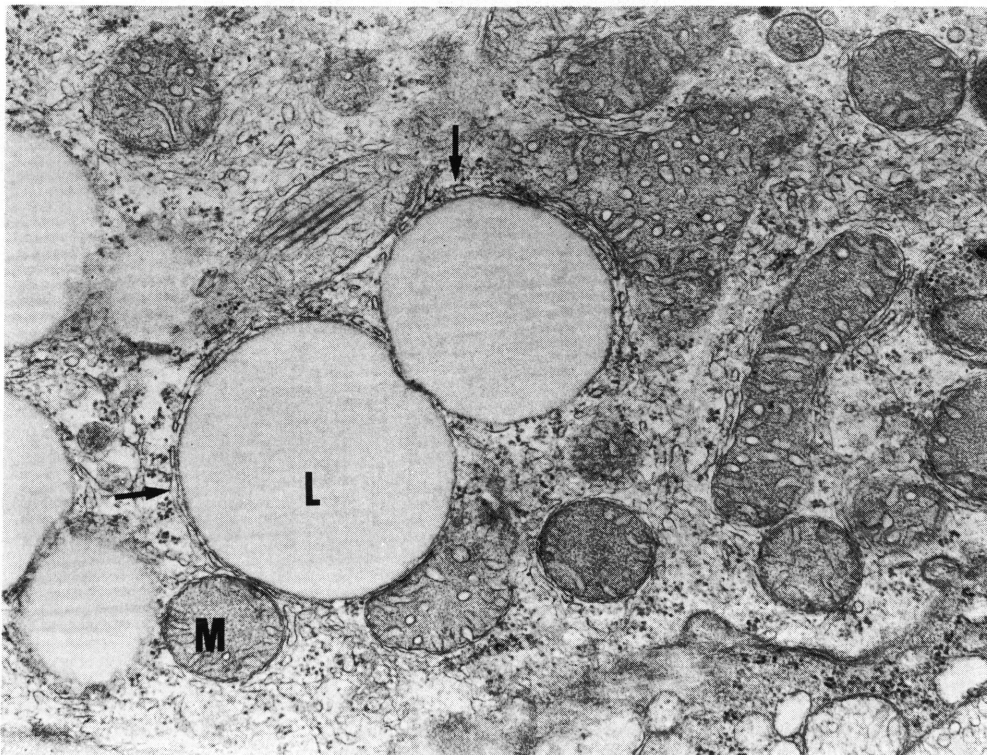
Fig. 10. Zona glomerulosa cell of SHRSP. Mitochondria (M) have electron–dense matrix and tubular cristae. Lipid droplets (L) are surrounded by smooth–surfaced endoplasmic reticulum (arrow). (×34,000).

given in Table 2.   The average width of the zona glomerulosa of SHRSP (60.7 ± 1.60 μm) was significantly ($p < 0.001$) greater than that of WKY (49.0 ± 1.03 μm).   The width of the zona glomerulosa in SHRSP with cerebral lesions (67.0 ± 8.03 μm) was greater than that in SHRSP without cerebral lesions (54.2 ± 12.58 μm).   However, this difference was not statistically significant because the number of each group was too small to compare.

The area of the zona glomerulosa cells of SHRSP (75.4 ± 2.15 μm^2) was significantly ($p < 0.001$) larger than that of WKY (62.7 ± 2.28 μm^2), whereas the area of nucleus did not differ significantly from each other (SHRSP: 23.4 ± 0.56 μm^2 vs WKY: 22.1 ± 0.70 μm^2).

The area of mitochondria of SHRSP (0.28 ± 0.006 μm^2) was significantly ($p < 0.001$) lesser that that of WKY (0.39 ± 0.010 μm^2).   And the percent area of the mitochondrial compartment of SHRSP (15.6 ± 0.38 %) was significantly ($p < 0.001$) lesser than that of WKY (21.6 ± 0.60 %).

Lipid droplets were observed on 90% of the zona glomerulosa cells in SHRSP and on 69% of that in WKY.   Although the area of lipid droplets did not differ significantly from each other (SHRSP: 0.80 ± 0.031 μm^2 vs WKY: 0.88 ± 0.052 μm^2), the percent area

Fig. 11-a. Zona glomerulosa cell of SHRSP. Note regular arrays of lamellar cristae in elongeted mitochondria. (×48,000).

Fig. 11-b. Zona glomerulosa cell of SHRSP. Note electron-dense intramitochondrial droplets (arrow). (×48,000)

Table 2 Synopsis of morphometric parameters of the zona glomerulosa

		SHRSP	WKY	p
Width of the zona glomerulosa	(μm)	60.7±1.60	49.0±1.03	<0.001
Area of cells	(μm²)	75.4±2.15	62.7±2.28	<0.001
Area of nucleus	(μm²)	23.4±0.56	22.1±0.70	NS
Area of mitochondria	(μm²)	0.28±0.006	0.39±0.010	<0.001
Percent area of mitochondria	(%)	15.6±0.38	21.6±0.60	<0.001
Area of lipid droplets	(μm²)	0.80±0.031	0.88±0.052	NS
Percent area of lipid droplets	(%)	21.6±1.09	16.9±1.40	0.001<<0.01

Each value represents the group mean ± SE
p, level of significance of the differece between SHRSP and WKY
NS, not significant

of lipid droplets compartment of SHRSP (21.6±1.09 %) was significantly (0.001<p<
0.01) greater than that of WKY (16.9±1.40 %).

DISCUSSION

It is postulated that the adrenal gland plays an important role to regulate the
development or maintenance of hypertension in SHR. TSUCHIYAMA et al.[31] described
the morphological alterations of the adrenal cortex in SHR histochemically and ultra-
structurally, and pointed out that these changes were seen even in prehypertensive stage.
NICKERSON showed by quantitative morphological techniques that the zona glomerulosa
and the zona fasciculata of 21 week old male SHR[22] and the zona reticularis of aging
female SHR[23] differed from those of WKY. Another morphological studies on the zona
glomerulosa with relation to its functional alterations have been reported in SHR. The
present study is the first description of the zona glomerulosa in SHRSP.

In this paper, the zona glomerulosa of SHRSP was characteristically increased in
width. Morphometrically the average width of the zona glomerulosa of SHRSP was sig-
nificantly greater than that of WKY. This widened zone of SHRSP consisted of hyper-
trophy and hyperplasia of the zona glomerulosa cells. The width of the zona glomerulosa
in SHR was also greater than that of controls[22]. The increased zona glomerulosa was
observed in a response to ACTH administration[24], renovascular hypertension[28], renal
encapsulation-induced hypertension[13] and sodium-depletion[11]. Biochemically, the renin-
angiotensin system of SHRSP was activated[15,18,19,29], but PAC was not elevated[25], and
participated in maintaining high blood pressure. MATSUNAGA et al.[18] and KAWA-
SHIMA et al.[15] pointed out the close relationship between PRA and cerebral lesions in
SHRSP, that is, when the higher PRA was found in SHRSP the higher incidence of
cerebral lesions was observed. It is of interest that the width of the zona glomerulosa
in SHRSP with cerebral lesions was greater than that in rats without cerebral lesions,
though it was not statistically significant. Considering these facts, the activated renin-
angiotensin system may play an important role[28] to induce hypertrophy and hyperplasia
of the zona glomerulosa in SHRSP.

The area of the zona glomerulosa cells of SHRSP was significantly greater than
that of WKY. The significant increase of the area of cells in SHRSP may probably
due to increased lipid droplets compartment because the area of nucleus and the percent
area of mitochondria were not increased in these cells. The smooth-surfaced endoplasmic
reticulum and the Golgi apparatus, though they were not measured, seemed to be not
effective enough to increase the area of cells.

Under the condition of sodium or magnesium depletion and ACTH administration
the motochondria are increased in number and in size, and the smooth-surfaced endo-
plasmic reticulum and the Golgi apparatus appear to be well developed. On the other
hand, lipid droplets tend to be decreased in number and in size[6,8,11,14]. Opposite alter-

ations of these organelles have been observed under a hypofunctional state by hypophysectomy[24], sodium supplement[12] or aldosterone administration[8].

There are at least two steps in the biochemical process of aldosterone formation which involve mitochondrial enzymes[7]. One is the transformation of cholesterol to pregnenolone and the other, from 18-hydroxycorticosterone to aldosterone[5].

In this study on the zona glomerulosa cells of SHRSP, the mitochondria were small in area and pleomorphic in shape but increased in number. NICKERSON[22] observed a decreased volume of mitochondria also in SHR. YAMORI et al.[33] and MOLL et al.[20] reported impairments of mitochondrial enzymes in SHR. Therefore, above-mentioned changes of mitochondria may be induced by the impairment of mitochondrial enzymes or the impairment of maturation of mitochondria.

The presence of straight tubular cristae in some mitochondria has been reported in the case of functional solicitation; sodium depleted animals[11], aldactone administration[8], hypophysectomy in animals[10] and primary aldosteronism in human[30]. This type of cristae was also described in the mitochondria of a few cells of the zona glomerulosa in normal rats[10]. At the present study this alteration was found not only in SHRSP but also in WKY.

The percent area of lipid droplets compartment of the zona glomerulosa cells in SHRSP was significantly increased, but the area of lipid droplets in SHRSP did not differ from in WKY. AOKI[1], TSUCHIYAMA et al.[31] and FRINK et al.[9] reported that lipid droplets of the zona glomerulosa cells in SHR were increased. On the other hand, a decreased volume of lipid droplets in SHR was shown quantitatively by NICKERSON[22]. The cause of this difference is not known but it will be related with diet, strain or developmental environment.

The smooth-surfaced endoplasmic reticulum tended to be well developed in SHRSP. 3β-hydroxysteroid dehydrogenase system and 21-hydroxylase system are located within the smooth-surfaced endoplasmic reticulum[32]. Therefore, these organelles may reflect the activity of the zona glomerulosa cells.

The Golgi apparatus also appeared to be hypertrophic in SHRSP. This organelle takes a part in conjugation of steroid hormone[16]. The changes of the smooth-surfaced endoplasmic reticulum and the Golgi apparatus in SHRSP may be the response to an activated state of the zona glomerulosa cells.

These fine structural alterations may indicate the following possibilities. The zona glomerulosa cells in SHRSP are stimulated in the steroidogenesis by elevated demands for aldosterone. It may be supported by the fact that PRA in SHRSP was elevated[15][18]. And so a lot of cholesterol are taken into the zona glomerulosa cells as a precursor of aldosterone. Then, if the mitochondria of SHRSP have some kinds of dysfunction, it may lead to the increase in number of the mitochondria to compensate for unfavorable function. But it may be limited to spend cholesterol because the impairment of mitochondrial function causes an insufficient conversion to steroid hormone. Therefore, lipid droplets where cholesterol is stored[21] are increased in number in cytoplasm. The significant

increase of cell area may be induced by increased lipid droplets. And the zona glomerulosa cells may have undergo hypertrophy to hyperplasia.

Biochemically, though PRA of SHRSP was elevated[15)18)] OGINO et al.[25)] reported that PAC of SHRSP did not differ from that of WKY at 5 month old. These results will support the speculation in the present study.

However, further studies on the renin-angiotensin aldosterone system in SHRSP are necessary to understand the pathogenesis of hypertension.

ACKNOWLEGEMENT

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