Acta Med. Nagasaki 30 : 55−67

Pathological Study of the Pituitary-adrenal System in the Spontaneously Hypertensive Rats (SHR)

—With Special Reference to the Corticotrophs by Immunohistochemical and Morphometrical Technique—

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Received for publication, January 29, 1985

The pituitary gland, especially the corticotrophs, of spontaneously hypertensive rats (SHR) at 4 weeks (prehypertensive stage) and 10 weeks of age (early hypertensive stage) was examined immunohistochemically and morphometrically.

The corticotrophs (ACTH positive stained cells) were found sparsely in the anterior lobe and a few cells were observed in the area adjacent to the intermediate lobe. The corticotrophs appeared small and stellate in shape and extended their cytoplasm to the neighboring sinusoid. The total area and number of the corticotrophs in SHR was significantly greater than that in WKY at 4 weeks of age. Although the total number of corticotrophs in SHR at 10 weeks was also greater, the total area of these cells was not different from that of WKY. The average width of the zona fasciculata and reticularis in SHR was significantly greater than that of WKY at 4 weeks.

These result suggest that there was increased activity of the pituitary-adrenal system in SHR just before their spontaneously developing hypertension.

INTRODUCTION

OKAMOTO and AOKI[1] selectively bred Wistar-derived rats for the development of spontaneous hypertension (the SHR line) as well as a normotensive line (WKY) which maintain normal blood pressure. Since then, spontaneously hypertensive rats (SHR)[1] have been used as a model for human essential hypertension.

It has been reported that the endocrine system, along with the autonomic nervous system and cardiovascular system, plays an important role in the pathogenesis of hypertension in SHR. AOKI[12] has reported on the relationship between endocrine organs and hypertension in SHR. He pointed out that the pituitary-adrenal axis was necessary for the de-

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velopment and maintenance of high blood pressure in SHR. And WEXLER *et al.*[14] reported the Cushingoid pathophysiology of old massive obese SHR, a substain of SHR. On the other hand, BAER *et al.*[3] failed to show that the adrenal gland was involved in pathogenesis of spontaneous hypertension.

Concerning the alternations of the pituitary gland in SHR, some description were reported histologically. However, there has been no report using the immunohistochemical technique on the pituitary gland in SHR.

This paper describes immunohistochemical and morphometrical studies of the pituitary gland and adrenal cortex in male SHR, with special enphasis on the corticotrophs which produce and secrete ACTH.

MATERIAL AND METHODS

Thirty male SHR and 30 male WKY were obtained from the colony of the Japan Rat Co. Ltd. and KYUDO Co. Ltd.

The animals were kept in the Laboratory Animal Center for biomedical research, Nagasaki University School of Medicine where temperature (25°C), fumidity (45-50%) and lighting (12h light, 12h dark cycle) were controlled. The rats were maintained on commercial rats food (FUNABASHI, Chiba) and drank tap water ad libitum.

Their blood pressure was recorded by the indirect tail plethymographic method, which measures the systolic blood pressure only.

Four experimental groups were made as follow:

Half of the rats in both the SHR and WKY groups were sacrificed by decapitation at 4 weeks of age, which the other 15 rats in both groups were sacrificed at 10 weeks. Because the pituitary-adrenal axis is affected by circadian rhythm, all animals were killed at a set time in the morning (0900-1100h).

To determine the plasma level of adrenocorticotropin (ACTH), the trunk blood of 5 rats from each group was collected into polystyrol tubes coated with EDTA, soon after the decapitation. After the blood was centrifuged, the plasma was extracted and stored at −20°C until required for assay. Circulating ACTH was measured by means of radioimmunoassay kits (CIS Radiopharmaceuticals, Gif-Sur-Yvette, France) using human ACTH as the standard.

All pituitary and both adrenal glands were removed rapidly, rinsed with saline and weighed. These specimens were fixed in BOUIN's fluid overnight, washed in three changes of 70 % ethanol for one day each, dehydrated and embedded in paraffin. Horizontal serial sections of the pituitary glands were cut at 5 μm and mounted on albumin-coated glass slide for the immunohistochemical study. Sections of the adrenal glands were cut at 5 μm.

Localization of ACTH in the pituitary glands was carried out immunohistochemically using the unlabeled antibody peroxidase-antiperoxidase (PAP) according to STERUNBERGER *et al.*[10]. Deparaffinized sections, treated with 0.1 % H_2O_2, 100 % methanol and 1

% normal swine serum for 30 min., were mounted with the first antisera, anti $ACTH^{1-24}$ serum by DAKO Co. Ltd., in a moist chamber and allowed to react overnight at 4°C. The reacted sections were washed in three changes of 0.01 mol cold PBS pH 7.2 for 5 min. each. Then the sections were reacted with the second antisera, anti-rabbit swine serum, for 40 min. in a moist chamber at room temperature. The washing after reaction with the second antisera was done as described above. Therefore, reaction with the peroxidase antiperoxidase complex was performed in the same way as the reaction with the second antisera. The sections were then immersed in a solution containing 0.05 mol Tris-HCl buffer, pH 7.6, 0.55 mmol 3.3′-diaminobenzine and 2 mmol H_2O_2 (GRAHAM-KARNOVSKY's solution) for 5 min. The sections were finally counterstained for nuclei with light hematoxylin. These reactions between antigen and antibody were done in constant conditions, that is, humidity, dilution of antisera and reaction time. In order to establish the specificity of the immunohistochemical staining with anti-ACTH antisera, the following control tests were performed. 1) Sections were incubated with anti-ACTH antisera preincubated with human ACTH. 2) The sections were incubated with anti-αFeto protein or anti-human IgG antiserum.

Eight specimens were chosen at random from the previously mentioned 4 experimental groups. Moreover, 6 sections were chosen from each case as long as they had anterior, intermediate and posterior lobes. Four light micrographs randomly sampled from each section were taken at a magnification of x200 and enlarged photographically to x1400. The area and number of the corticotrophs and vascular areas were measured by a modular system for semiautomatic quantitative evaluation images of A.S.M., West Germany.

In order to study the average width of the zona glomerulosa and width of the zona fasciculata and reticularis of the adrenal cortex, 25 sections of each experimental group were chosen at random as long as they had the adrenal medulla in the center and might be the biggest cut surface. These sections were stained with hematoxylin and eosin (H. E.) and WATANABE's method for reticulin fiber. To determine the average width, two parameters of each section were measured randomly at 10 points.

Comparison of these experimental groups for the above parameters was performed by the Student's t-test and F-test.

RESULTS

The systolic blood pressure of SHR did not differ significantly from that of WKY at 4 weeks of age, but at 9 and 10 weeks the systolic blood pressure of SHR was significantly higher than that of WKY (Fig. 1, Table 1). The body weight of SHR did not differ significantly from that of WKY (Table 1). The pituitary weight over body weight (relative pituitary weight) of SHR was greater than that of WKY at 4 weeks, but was not different from that of WKY at 10 weeks (Table 1). The relative adrenal weight of SHR was not different from that of WKY at 4 weeks of age but was significantly lower than that of WKY at 10 weeks (Table 1).

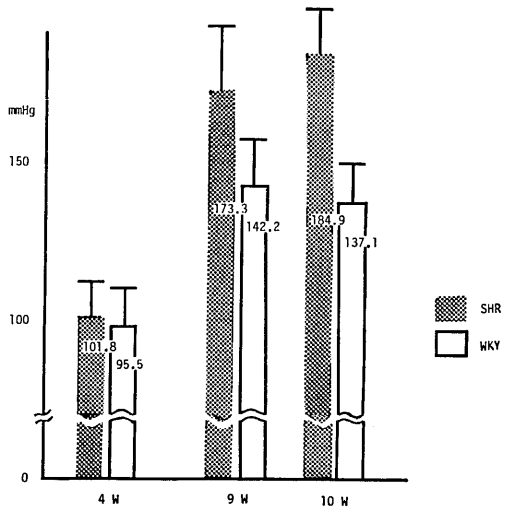


Fig. 1. Blood pressure

Table 1. Comparison of blood pressure, body weight, relative pituitary weight and relative adrenal weight

	4W			10W		
	SHR	WKY	P	SHR	WKY	P
Blood pressure (mmHg)	101.8±11.6	95.5±12.6	N.S.	184.9±14.7	137.1±12.3	0.001
Body weight (g)	62.1± 4.5	83.1±13.4	0.05	240.9±32.3	251.1±17.5	N.S.
Relative pituitary weight (10^{-5})	6.50±2.27	5.29±1.28	N.S.	4.29±0.39	5.12±2.25	N.S.
Relative adrenal weight (10^{-4})	4.75±1.05	4.72±2.01	N.S.	1.86±0.19	2.32±0.32	0.001

Mean ± S. E.
N.S. : not significant

Histological findings. In the anterior lobe of the pituitary gland, corticotrophs were found sparsely and a few cells were observed in the area adjacent to the intermediate lobe. Many ACTH positive stained cells were also found in the intermediate lobe (Fig. 2). The corticotrophs appeared small and stellate or spiderlike in shape and extended their cytoplasm to the neiborghing sinusoid (Fig. 3). The corticotrophs and other cells with a negative reaction for anti-ACTH serum, which might be GH cells, were frequently found in jaxta-position (Fig. 4). In the comparison between the pituitary gland of 4 and 10 weeks of age, the latter had more and larger sinusoid and also large cells with a negative reaction for anti-ACTH serum (Fig. 5).

Morphometrical studies. The ratio of corticotrophs in the examined area, excluding the vascular area, was evaluated. The average ratio of SHR was significantly greater than that of WKY at 4 weeks of age, but was not different at 10 weeks(Fig. 6 and Table 2).

On the other hand, there was a tendency for the ratio to become smaller according to the growth in both SHR and WKY. The total number of corticotrophs was evaluated in the examined area, again excluding the vascular area. The average ratio of the corticotrophs in SHR was significantly greater than that of WKY at 4 and 10 weeks (Fig. 7 and Table 2).

In the adrenal cortex, the average width of the zona glomerulosa of SHR was not different from that of WKY at 4 and 10 weeks of age (Table 2). The average width of the zona fasciculata and reticularis of SHR was significantly greater than that of WKY at 4 weeks (Fig. 8, 9 Table 2).

Radioimmunoassay. Plasma ACTH levels of SHR may have been greater than that of WKY at 4 weeks of age (Table 3), but this difference was not statistically significant because the number of each experimental group was too small to compare.

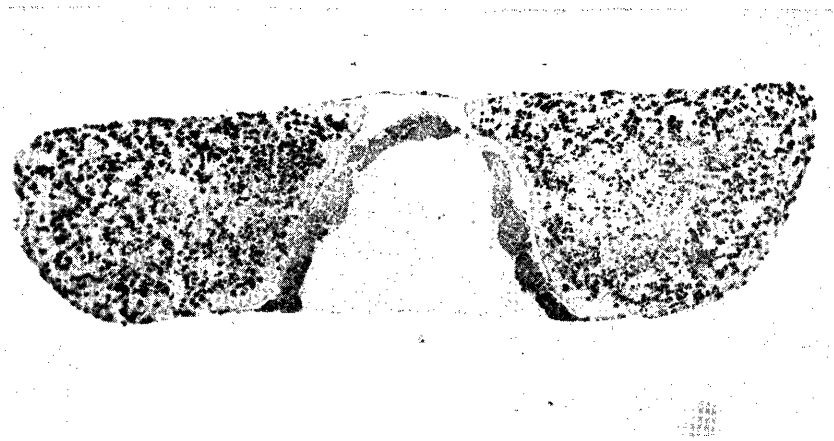


Fig. 2. Complete view of pituitary horizontal section (PAP stain for ACTH, ×20)

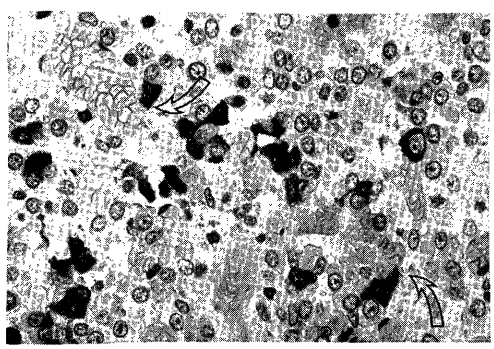


Fig. 3. Anterior lobe of pituitary gland: Corticotrophs extended its cytoplasm to the neiborghing sinusoid (PAP stain for ACTH, ×400)

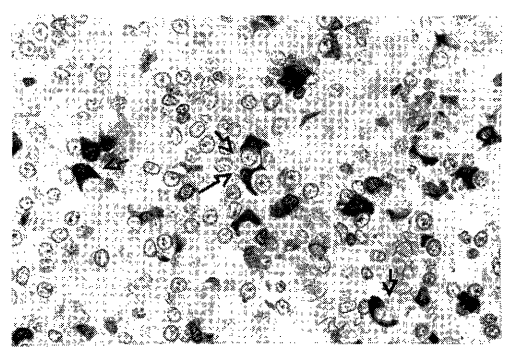


Fig. 4. Anterior lobe of pituitary gland: Corticotrophs and other cells were found in jaxtaposition (PAP stain for ACTH, ×400)

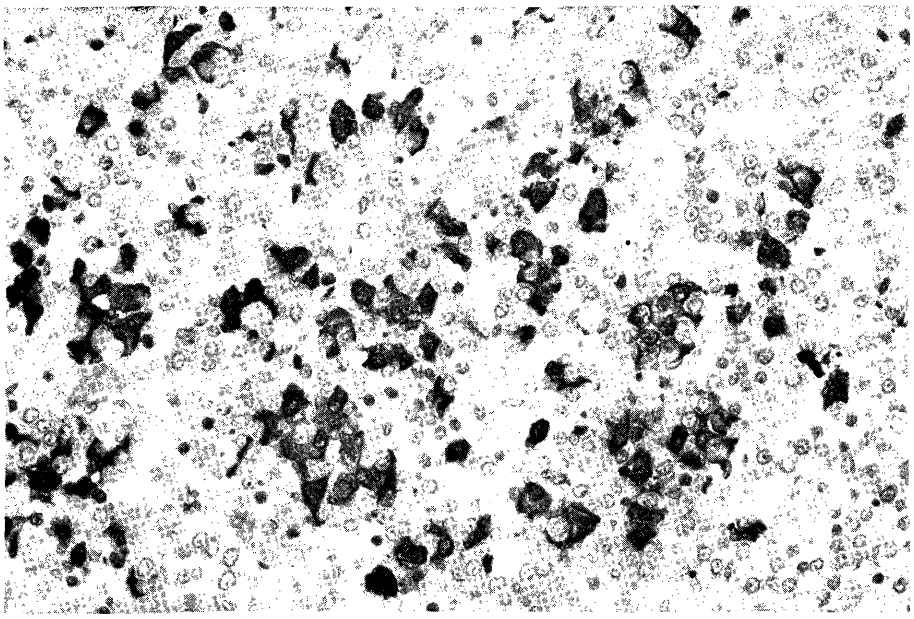


Fig. 5. Pituitary gland (PAP stain for ACTH, ×200)
a: Anterior lobe at 4 weeks of age in SHR

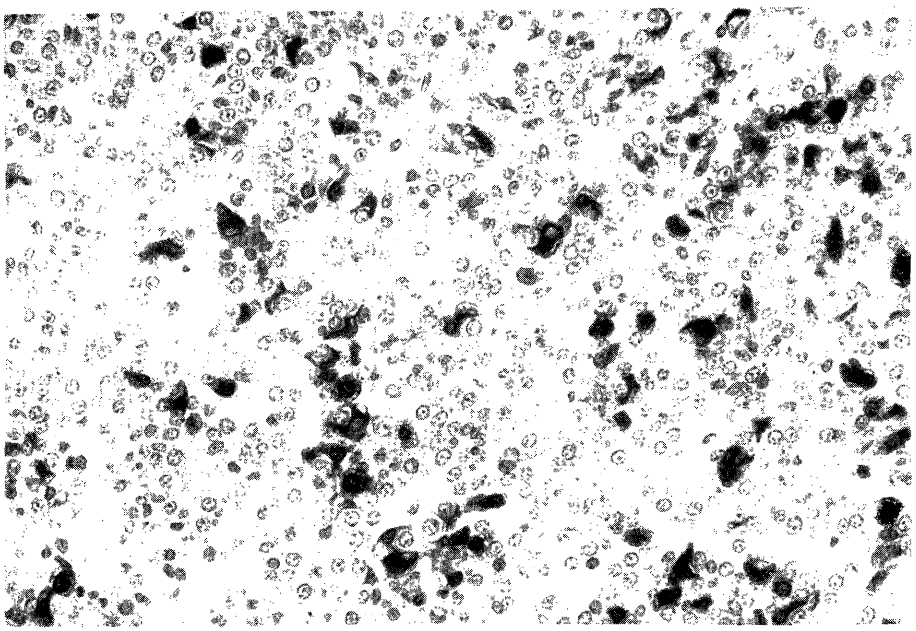


Fig. 5. Pituitary gland (PAP stain for ACTH, ×200)
b: Anterior lobe at 4 weeks of age in WKY

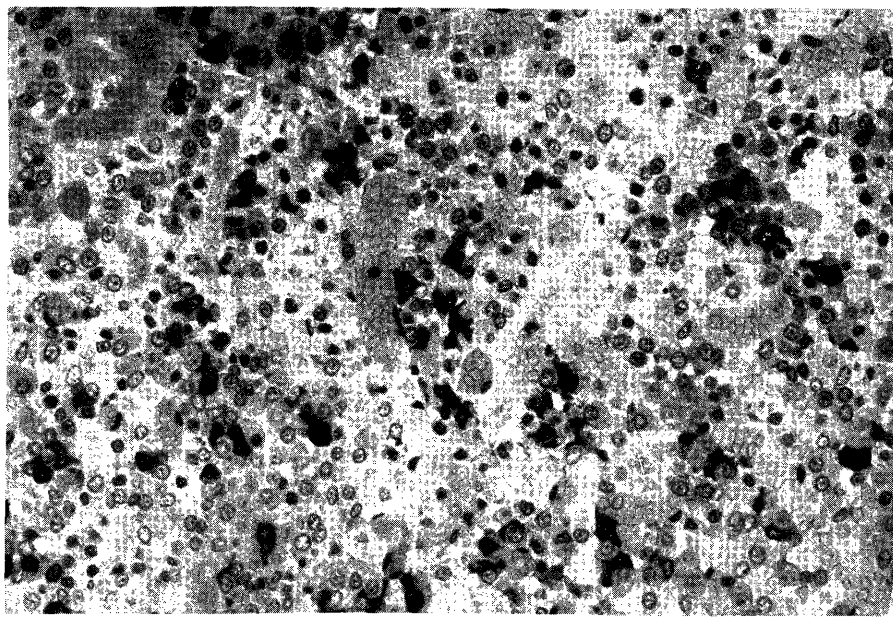


Fig. 5. Pituitary gland (PAP stain for ACTH, ×200)
c: Anterior lobe at 10 weeks of age in SHR

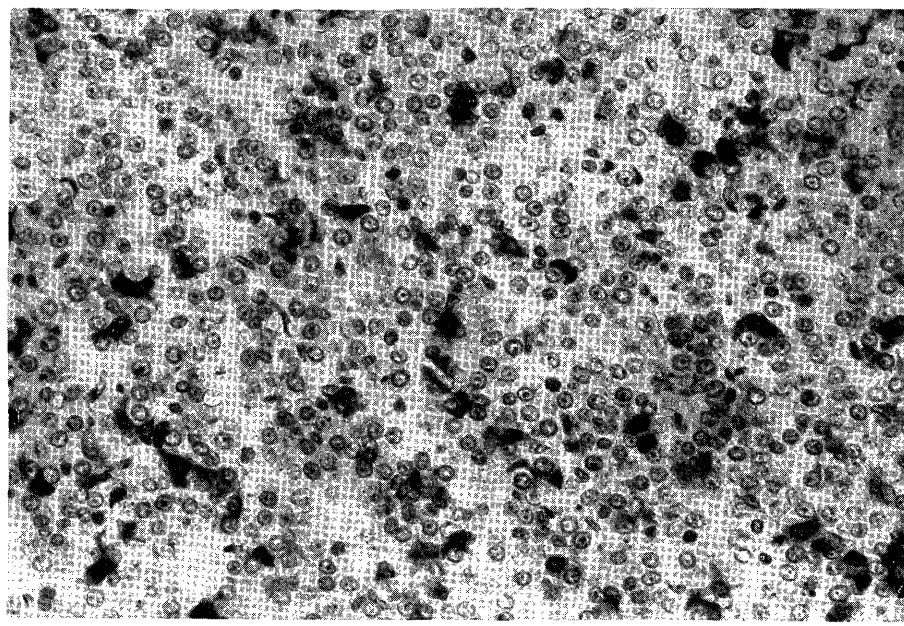


Fig. 5. Pituitary gland (PAP stain for ACTH, ×200)
d: Anterior lobe at 10 weeks of age in WKY

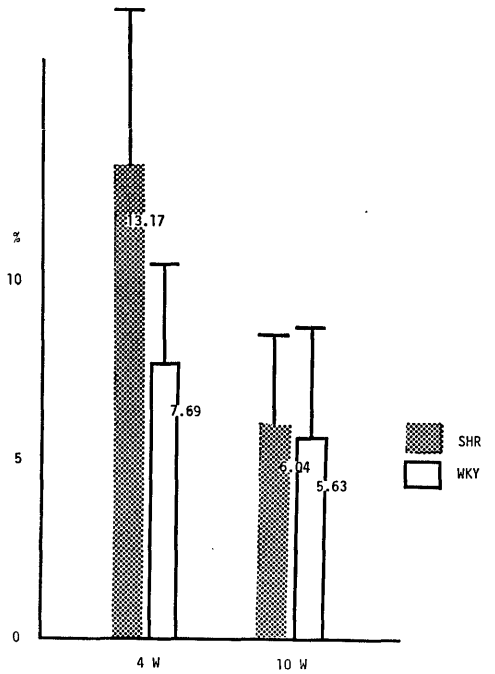


Fig. 6. Ratio of corticotrophs in the examined area (excluding vascular area)

Table 2. Synopsis of morphometrical parameters in the pituitary gland and the adrenal cortex

	4W			10W		
	SHR	WKY	P	SHR	WKY	P
Pituitary gland						
Ratio of corticotrophs in the examined area (%)	13.17±4.47	7.69± 2.79	0.001	6.04±2.35	5.63± 3.11	N.S.
Total number of corticotrophs in the examined area ($/10^4\mu m^2$)	14.61±6.66	8.87± 3.37	0.001	6.99±2.32	6.07± 3.66	0.01
Adrenal cortex						
Width of the zona glomerulosa (10^{-2}mm)	2.10±0.33	2.22± 0.43	N.S.	2.60±0.43	2.82± 0.77	N.S.
Width of the zona fasciculata and reticularis (10^{-2}mm)	63.25±7.88	55.31±11.80	0.01	79.69±5.93	90.27±11.05	0.001

Means ± S. E.
N.S. : not significant

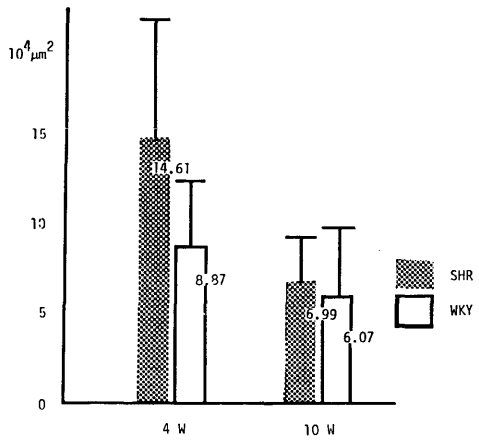


Fig. 7. Total number of corticotrophs in the examined area
(again excluding vascular area)

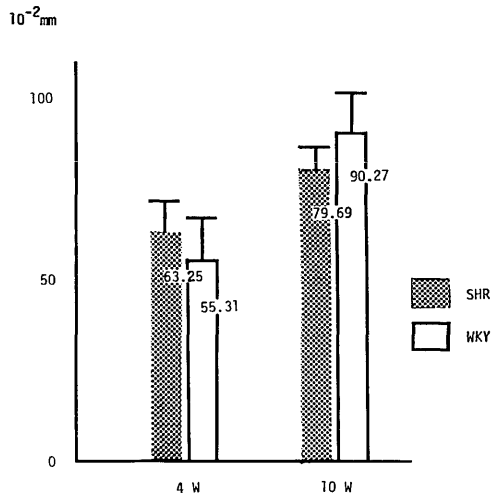
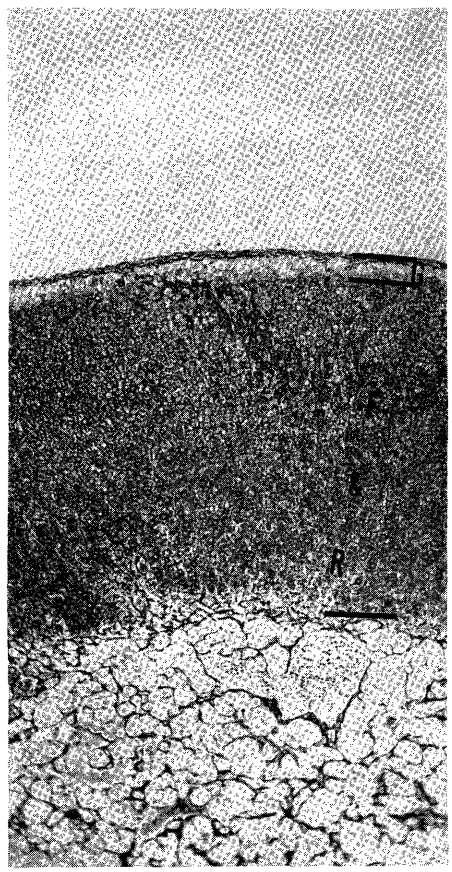


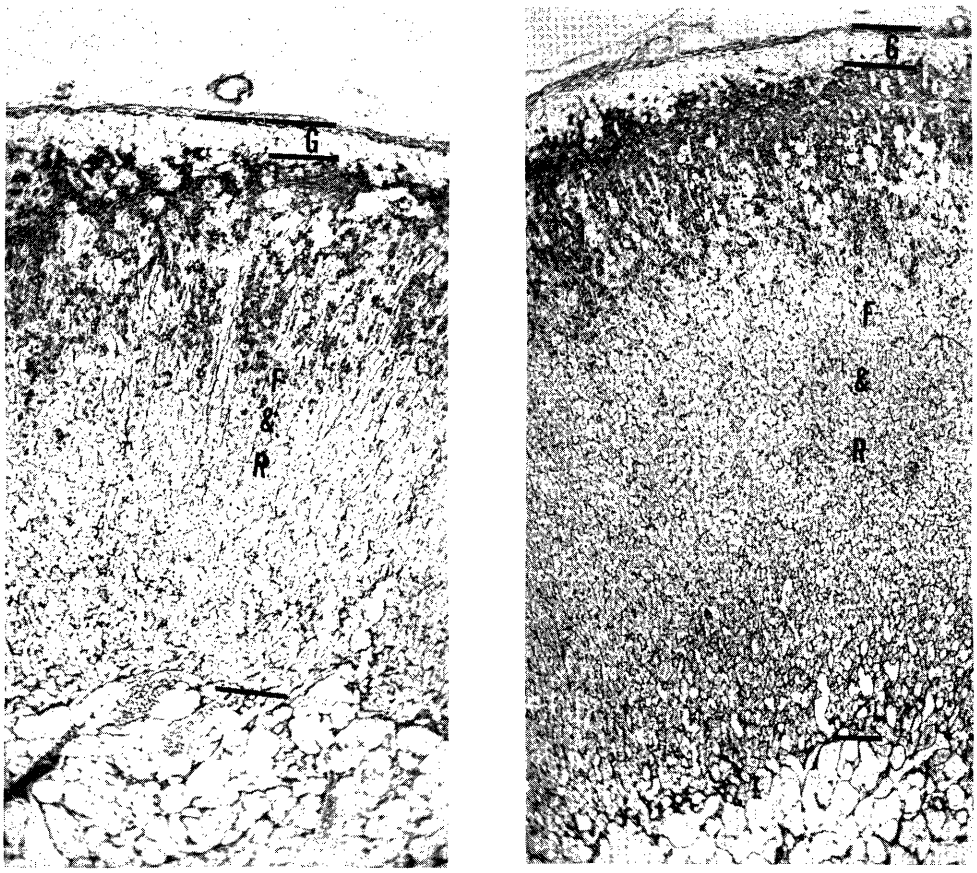
Fig. 8. Average width of the zona fasciculata and reticularis

a: SHR 4 weeks of age



b: WKY 4 weeks of age

Fig. 9. Adrenal cortex G: the zona glomerulosa
F&R: the zona fasciculata and reticularis
(reticulin stain, ×50)



c: SHR 10 weeks of age d: WKY 10 weeks of age

Fig. 9. Adrenal cortex G: the zona glomerulosa
F&R: the zona fasciculata and reticularis
(reticulin stain, ×50)

Table 3. Plasma level of ACTH (pm CIS/ml)

4W			10W		
SHR	WKY	P	SHR	WKY	P
144.3±94.2	44.2±19.4	0.05	212.4±241.2	151.9±100.1	N.S.

Means ± S. E.
N.S. : not significant

DISCUSSION

Previous morphological studies[1)11)12)] indicated a pronounced secretory activity of the pituitary corticotrophs in SHR. AOKI *et al.* described that an increase in the percentage of basophils in the pituitary of SHR started from the prehypertensive stage, and that these changes were exaggerated as hypertension persisted. Moreover, hypertrophy and the "hyaline change" was observed in the basophils[1)]. TABEI *et al.*[12)] described that corticotrophs and thyrotrophs were relatively increased from the prehypertensive stage, compared with the other hormone producing cells identified by their electron microscopic features. In addition, a few of the plasma ACTH data in basal level of the circadian rhythm[5)6)9)] were reported in SHR. In these reports, no difference of the basal ACTH concentrations was found between SHR and WKY.

AOKI *et al.* suggested that the basophils of the pituitary gland secrete TSH and ACTH. But at the present time, some of the corticotrophs are considered to be chromophobes. Furthermore, TABEI *et al.* classified the cells of the anterior pituitary with the electron microscopic feature by KUROSUMI's description (1968)[8)].

STERUNBERGER *et al.*[10)] introduced the unlabeled antibody peroxidase-antiperoxidase (PAP) method in 1979. This method was emphasized with regard to the properties, the specificity and the high sensitivity which compared with enzyme histochemistry, other immunohistochemical and radioimmunoassay techniques. The quantitative study of enzyme immunoassay and radioimmunoassay uses the antigen-antibody reaction as a basis. So if all conditions of each experimental step are constant and there are a number of micrographs to examine for the reproducible data, quantification of immunohistochemistry is possible. Therefore, the immunohistochemical and morphometrical study of the corticotrophs in this paper is considered to more accurate observations than previous reports.

In this study, the average ratio of area and number of corticotrophs in SHR was greater than that of WKY except for the area at 10 weeks. Although the age group of SHR was different, the tendency of this result was similar to that of studies by TABEI and AOKI. However, the percentage of corticotrophs in this study is different from these studies and this fact is probably due to the differences of age group and histochemical procedures in both experiments. The decrease in total area and number of corticotrophs from 4 weeks to 10 weeks of age may result from the increase of the other hormone secreting cells.

The average ratio of the total area of corticotrophs to the examined area in SHR was not different from that of WKY at 10 weeks. This result may suggest that the corticotrophs play a significant role in the development of hypertension.

On the other hand, it is postulated that the adrenal gland plays an important role in regulating the development or maintenance of hypertension in SHR. AOKI *et al.*[1)] reported that hypertrophy and an increase in lipid content of the zona fasciculata in SHR was present from the prehypertensive stage. TSUCHIYAMA *et al.*[13)] and TABEI *et al.*[11)] described the morphological alterations of the adrenal cortex in SHR histochemically and

ultrastructurally and they pointed out that these changes were seen even in the prehypertensive stage and that these finding closely resembled the finding obtained from the adrenal cortex with continuous administration of ACTH for a certain period.

DEVITO *et al.*[4] and HÄSLER *et al.*[7] reported that hypothalamo-pituitary-adrenocortical response to ether stress is markedly enhanced in SHR during early development of hypertension.

Moreover, in this study, the zona fasciculata and reticularis of SHR increased in width compared to that of WKY at 4 weeks.

From these reports and results, it is assumed that a hyperfunctional state in the corticotrophs and adrenal cortex is present in SHR at 4 weeks of age.

However, this study is limited to the pituitary-adrenocortical axis and there are many reports[2)7)] of the relationship among the hypothalamus, adrenal medulla and possibly other organs.

So further morphological studies of these systems in SHR are necessary for an understanding the pathogenesis of hypertension.

ACKNOWLEDGMENT

The author wish to thank greatly Dr. H. TSUCHIYAMA, Professor of Pathology, Nagasaki University for his suggestive and varuable criticism and coments. And the author also express my gratitude to Dr. M. OZAKI, Professor of Pharmacology, Nagasaki university for his valuable coments. The co-operative research students and skillful technical assistants in the Department of Pathology, Nagasaki University are acknowleged.

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