On Histochemical Studies of the Various Organs of Spontaneously Hypertensive Rats*

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IT IS GENERALLY accepted at present that hypertension is associated with many endocrine disorders in man and many experimental observations indicate that various endocrine glands are intimately related to blood pressure homeostasis.

In 1963, selective production of a strain of spontaneously hypertensive rats was reported by OKAMOTO and AOKI¹⁾. These rats are absolutely without parallel from the points of showing 100 per cent incidence of spontaneous occurrence of hypertension and a more severe hypertension than "inherited hypertension in rats" by SMIRK et al.²⁻⁴⁾, and "spontaneously hypertensive rabbits" by ALEXANDER et al.⁵⁾⁶⁾. Spontaneously hypertensive rats may be considered to be analogous to essential hypertensive humans, because of the absence of severe organ changes, especially in the cardiovascular-renal system before the development of hypertension⁷⁾.

OKAMOTO⁸⁾, AOKI et al.⁹⁾ and AOKI¹⁰⁻¹²⁾ have studied primarily the endocrine organs of spontaneously hypertensive rats and have detected some slight but regular changes in them even from the pre-hypertensive stage and observed that these changes become more severe and intense according to age. Moreover, it has been demonstrated that removal of some of the endocrine organs in the pre-hypertensive stage prevents the development of hypertension¹⁶⁾.

In the present study, the author histochemically examined various organs of spontaneously hypertensive rats, aiming at getting

nearer to the functional aspects of these organs, and detected a few clear findings compared with normotensive control rats, some of which were presented from the pre-hypertensive stage. These will be considered from the standpoint of playing some significant roles in the development and maintenance of hypertension in this animal and further will be judged as a clue to elucidate the possible mechanism of human essential hypertension.

MATERIALS

The Wistar strain rats of the control group consisted of rats supplied by the Animal Center Laboratory, Faculty of Medicine, Kyoto University and of the offsprings bred from these rats in the Department of Pathology under normal conditions. From among them only normotensive (below 149 mm Hg) rats were selected and used for the control (abbreviated as N hereafter). The hypertensive group consisted of F₇ to F₁₁ rats of spontaneously hypertensive rats (OKAMOTO and AOKI)¹¹⁾¹³⁾ (abbreviated as SH henceforward). Although the method of selective production of spontaneously hypertensive rats, their body weight, blood pressure and incidence of hypertension have been already reported¹¹⁾¹³⁾ the method is briefly mentioned as follows :

Many weanling rats of the Wistar strain supplied by the Animal Center Laboratory, Faculty of Medicine, Kyoto University were kept in the Department of Pathology under normal conditions. From among these one male rat showing distinctly high blood pressure (145-175 mm Hg) and a female rat with a blood pressure slightly above the average (130-140 mm Hg) were selected. After this male rat had persisted in high blood pressure of above 150 mm Hg for over a month, the male rat was mated with the female rat to obtain F₁ rats. Many

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of these F_1 rats showed spontaneous hypertension. The process of mating these rats was repeated successively. In these rats, 100 per cent occurrence of spontaneous hypertension in F_2 rats onward was observed within the first 5 months of life. By repeating this process, the generations have reached F_{12} at present.

A total of 90 rats was used in this study. All rats were sacrificed by decapitation after being divided into 3 groups according to survival duration: 40–60 days old (Stage I), 5–7 months (Stage II) and 13–17 months of age (Stage III). In the N group; rats in Stage I consisted of 16 male rats (case numbers, 252, 41, 48, 47, 42, 44, 43, 319, 321, 320, 45, 46, 381, 369, 383, 382), in Stage II, of 17 males (66, 68, 69, 242, 246, 227, 245, 316, 261, 14, 21, 313, 311, 312, 323, 338, 17), and in Stage III, of 7 males (103, 133, 57, 64, 54, 58, 60) and 5 females (121, 107, 109, 117, 59). In the SH group¹³⁾; rats in Stage I consisted of 17 male rats (case numbers, 9078, T9027, T9054, T9053, 10095, 10096, 10013, 10053, 10059, 10055, 10056, 10057, T9123, T9124, 10241, 10242, 116001), in Stage II, of 16 males (T8022, T8011, 9057, 9058, 9055, 9064, 9065, T9011, 9074, 9075, 9077, T9032, 10030, 10063, T9086, 9099), and in Stage III, of 7 males (7046, 8001, 8100, 8102, 8107, 8109, N9001) and 5 females (7084, 7056, 7053, 7082, N9004).

All animals were kept in an air-conditioned animal room with $25^\circ\text{C} \pm 0.5^\circ\text{C}$ of temperature and 50–60 per cent of humidity. These rats were fed on Oriental's stock chow diet (NMF and MF) (Oriental Yeast Co., Japan) or boiled Manitoba wheat mixed with cooked dried sardine, and seasoned with a little salt (0.3 per cent Na) and sometimes green vegetables. Tap water was given ad libitum.

METHODS

GENERAL OBSERVATIONS:— The blood pressure and body weight of all rats were measured once a week all through life beginning at 4 weeks of age. The systolic blood pressure was measured indirectly without anesthesia by the modified tail-water plethysmographic method (OKAMOTO and AOKI¹⁾.

ORGAN WEIGHT:— Immediately after decapitation, the adrenals, thyroid, kidneys, heart, liver and pancreas were taken out and removed of coagulated blood, adipose and connective tissues adhering to them. The adrenals and thyroid were weighed on a torsion balance. The kidneys, heart, liver and pancreas were weighed on a lever balance. The organ weight was indicated as the organ weight to body weight ratio:

$$\begin{array}{ll} \frac{\text{organ weight (gm)}}{\text{body weight (gm)}} \times 10^5 & \text{for the adrenals and thyroid} \\ \frac{\text{organ weight (gm)}}{\text{body weight (gm)}} \times 10^3 & \text{for the kidneys, heart, liver and pancreas} \end{array}$$

ENZYME HISTOCHEMICAL EXAMINATION:— Six organs, i.e. adrenals, thyroid, kidneys, heart, liver and pancreas were examined. The left adrenal, one lobe of the thyroid, a slice from the mid portion of the kidney, a transverse section of the heart, a sagittal section of the liver and the tail part of the pancreas were mounted on blocks with a small quantity of water, soaked in liquid n-hexan and indirectly frozen by a mixture of crushed solid carbon dioxide and acetone through the medium. These frozen blocks were then stored in a deep freezer temporarily. Frozen sections were made in cryostats partly by a HARRIS cryostat (Model No. M 40, U.S.A.) and partly by a DITTES-DUSPIVA cryostat (Germany) maintained at -20°C with a thickness of 15μ and were attached to cover slip glass by momentary thawing by the heat of fingers. These sections were stored again in the deep freezer.

Then twenty-one kinds of enzyme activity were examined on these six kinds of section from the SH group and corresponding control group at the same time. The kinds of enzymes examined histochemically and the staining method applied are given in Table I.

Each incubation period applied in the present study is illustrated in Table I. In these methods, the following 5 improvements were added:—

First, for the demonstration of ACPase activity, GOMORI's metal-salt method¹⁷⁾¹⁸⁾ was modified to a slight extent, especially in preparation of the incubation medium, with due regard to the advice of Prof. HIDEO TAKAMATSU³³⁾ (Dept. of Pathology, Tuberculosis Research Institute, Kyoto University, Kyoto) as follows: 13 ml of 3 per cent sodium- β -glycerophosphate solution and 10 ml of 0.25 M acetate buffer at pH 4.6 were mixed and added to distilled water to a total volume of 90 ml, followed by the addition of 10 ml of 2 per cent lead nitrate solution drop by drop gently to form a coarse turbid product. The incubation medium was obtained by filtration of the mixture. Unfixed cryostat sections were incubated in an oven at 37°C for periods of 60 minutes. The slides were then washed thoroughly in distilled water and immersed in diluted yellow ammonium disulfide solution for about 3 minutes. After washing, post-fixation and washing again, the slides were mounted.

Second, adenosine triphosphate, the substrate for the ATPase was reduced by one-fourth the quantity used in the original method¹⁹⁾ and glucose-6-phosphate disodium salt, the substrate of the G-6-PD was reduced similarly by one-third²⁹⁾, owing to the occurrence of too intense reactions.

Third, in the cases of ATPase¹⁹⁾, glucose-6-phosphatase²⁰⁾, 5'-nucleotidase²¹⁾ and steroid- 3β -ol dehydrogenase³⁰⁾, each substrate solution was filtered before incubation.

TABLE I HISTOCHEMICAL EXAMINATION METHODS OF VARIOUS ENZYMES

		Incubation Period (minutes)
<i>Phosphatases</i>		
1. Alkaline Phosphatase (Naphthol AS Phosphate Method)	Burstone ¹⁴⁻¹⁶⁾	60
2. Acid Phosphatase (ACPase) (Metal-Salt (Pb) Method)*	Gomori ¹⁷⁾¹⁸⁾	60
3. Adenosine Triphosphatase (ATPase) (Metal-Salt (Pb) Method)*	Wachstein and Meisel ¹⁹⁾	50
4. Glucose-6-Phosphatase (Metal-Salt (Pb) Method)*	Wachstein and Meisel ²⁰⁾	50
5. 5'-Nucleotidase (Metal-Salt (Pb) Method)*	Wachstein and Meisel ²¹⁾	20
<i>Esterases</i>		
6. Esterase (Naphthol AS-D Acetate Method)	Burstone ²²⁾²³⁾	180
7. Lipase (Tween 80 Method)*	Gomori ²⁴⁾	240
8. Cholinesterase (Acetylthiocholine Method)	Gomori ²⁴⁾	80
<i>Oxidases</i>		
9. Monoamine Oxidase (MAO) (Tetrazolium Method)	Glennner, Burtner and Brown ²⁵⁾	80
10. Cytochrome Oxidase (N-Phenyl-p-Phenylenediamine Method)	Burstone ²⁶⁾	60
<i>Dehydrogenases (All by Tetrazolium Method)</i>		
11. Diphosphopyridine Nucleotide Diaphorase	Burstone ²⁷⁾	15
12. Triphosphopyridine Nucleotide Diaphorase (TPND)	Burstone ²⁷⁾	20
13. Succinic Dehydrogenase	Nachlas, Tsou, Souza, Chang and Seligman ²⁸⁾	40
14. Lactic Dehydrogenase*	Hess, Scarpelli and Pearse ²⁹⁾	20
15. Glucose-6-Phosphate Dehydrogenase (G-6-PD)*	Hess, Scarpelli and Pearse ²⁹⁾	30
16. Steroid-3 β -ol-Dehydrogenase*	Levy, Deane and Rubin ³⁰⁾	60
17. Glutamic Dehydrogenase	Hess, Scarpelli and Pearse ²⁹⁾	60
18. Ethanol Dehydrogenase	Hess, Scarpelli and Pearse ²⁹⁾	90
19. Malic Dehydrogenase	Hess, Scarpelli and Pearse ²⁹⁾	120
<i>Glycosidase</i>		
20. β -Glucuronidase (Post-Coupling Azo-Dye Method)	Seligman, Tsou, Rutenburg and Cohen ³¹⁾	180
<i>Proteolytic Enzyme</i>		
21. Leucine Aminopeptidase (Azo-Dye Method)	Nachlas, Crawford and Seligman ³²⁾	120

*: Slightly modified in this study as explained below.

Fourth, in determination of lipase activity²⁴⁾, sodium cholate, as an activator, was added to the incubation medium by 0.005 per cent in final concentration³⁴⁾³⁵⁾.

Fifth, the substrate of lactic dehydrogenase²⁹⁾, stock 0.5 M sodium lactate itself is fairly high in pH value, so that it was lowered to approximately pH 7.4 by the addition of 1 N hydrochloride solution in advance.

Before incubation the slides were brought to room temperature and dried by air. For staining, each kind of unfixed cryostat section was incubated in each medium. After staining, these sections were post-fixed in 8 per cent neutral formalin for over 30 minutes, after mounting in Apáthy gummy syrup. Throughout this enzyme histochemical process, from sacrifice to slide mounting, materials from the hypertensives and those controls were examined simultaneously and the slides were mounted in pairs on the same slide glass. On the other hand, each technical control section was employed, omitting substrate from the media.

Findings were obtained by comparing the section of hypertensive to that of control, mounted on the same slide, with a light microscope. On that occasion, the reaction observed in the technical control section was not regarded as a nonspecific product.

HISTOCHEMICAL AND HISTOMETRICAL EXAMINATION FOR THE ADRENAL MEDULLA: —

a) Noradrenalin Reaction using the Glutaraldehyde-Osmium Tetroxide Technique³⁶⁾. The right adrenals were removed both from the normotensives and the hypertensives and sliced sagittally including the mid-plane of the medulla about 3 mm thick. After being fixed by immersion in cold (0°–4°C) 6 per cent glutaraldehyde buffered to pH 7.3 with 0.1 M phosphate for a period of more than 2 hours, these adrenal slices were cut on the thermo-electro freezing microtome (Komatsu Electronics Inc., Japan) at 15 μ and the sections afterwards transferred to 1 per cent cold veronal acetate-buffered osmium tetroxide (pH 7.3) for a period of about 70 minutes. They were then washed in distilled water and afterwards mounted in Apáthy gummy syrup.

b) **Histometrical Examination.** The areas of the medulla in cross-sections stained with this technique were projected with an ERNST LEITZ microprojector (Type VX501,-3b with xenon lamp, Germany) at about 310 magnification on sheets of section paper to measure the whole cross-sectional area and the area of noradrenalin-storing cell islets with a planimeter.

Three sections containing the medulla were chosen at random from every adrenal and similarly measured. For each section the dimensional ratio of noradrenalin-storing cell islets to the whole adrenal medulla was calculated as follows :

$$\frac{\text{area of noradrenalin-storing cell islets}}{\text{area of whole adrenal medulla}} \times 100(\%)$$

These three values were averaged for each rat. The mean values were measured for each animal group in Stages I, II and III of both N and SH groups, each of which consisted of five cases. Afterwards, the relationship of the dimensional ratio of the SH group to that of the N group was calculated for each stage :

$$\frac{\text{dimensional ratio of the SH group}}{\text{dimensional ratio of the N group}}$$

STATISTICAL ANALYSIS:— The data thus obtained from the normotensive and hypertensive groups were statistically analyzed using “t” test, and the data were considered significant when the “p” values were less than 0.01.

RESULTS

All the finding of the SH group were compared with those from the N group of the same sex and with almost the same survival duration.

BODY WEIGHT:— There was no significant difference between the N and SH groups (Tables II, IV, VI, VIII, X, XII) within each stage and within each histochemical group.

BLOOD PRESSURE:— The mean systolic blood pressure of the N group remained in the range of from 110 to 126 mm Hg. In the SH group, it rose steadily with age and was significantly higher than that of the N group. There exist significant differences between the N and SH groups in mean systolic blood pressure as follows : G-6-PD group ; in Stages I and II and males in Stage III, TPND group ; in all stages, noradrenalin reaction group ; in all stages, ACPase group ; in Stages II and III, ATPase group ; Stages I and II and males in Stage III, MAO group ; in all stages.

ORGAN WEIGHT:— The ratios of organ

weight to body weight of rats in both the N and SH groups used in this study are shown in Tables II, IV, VI, VIII, X and XII.

Adrenals:— There was no significant difference among histochemical groups using adrenals in all stages.

Thyroid:— The thyroids of males of the SH group in Stage III were significantly heavier in weight than those of the N group but there was no significant difference in Stages I and II.

Kidneys:— The weight of kidneys of the SH group for the investigation of ATPase activity was significantly higher in Stage II but there was no significant difference in Stage I or in either sex of Stage III.

Heart:— In the SH group, the weight of the heart was significantly greater in Stage II and in males in Stage III than in the N group, but there was no significant difference in Stage I or in females in Stage III.

As to the age of the animals, the organ-body weight ratio of the adrenals (in G-6-PD, TPND, and noradrenalin reaction groups) was largest in Stage I, and was larger in Stage II than in males in Stage III both in the N and SH groups. In Stage III, females of the N group showed a ratio larger than males of the N group but smaller than in Stage I. Though the females in Stage III consisted of only a few cases, they seemed to show the same tendency as the males in Stage III concerning the adrenals. As to the thyroid, no difference was found between any stages of the N and SH groups. The kidneys, in both N and SH group rats in Stage I showed greater value relative to body weight than those in Stages II and III but no difference between Stages II and III respectively. The heart-body weight ratio was slightly greater in rats of the N group in Stage I than in Stages II or III but there was no difference between Stages II and III. In Stages II and III the SH group showed significantly greater heart-body weight ratios than the N group, and the SH group revealed an almost unchanging ratio value through Stages I, II and III.

Besides the organs mentioned above, the liver and pancreas showed no remarkable change in the organ-body weight ratio.

TABLE II INDIVIDUAL FINDINGS OF G-6-PD ACTIVITY IN THE ADRENAL CORTEX

Experi- mental Stage	Sex	Case No. of SHR	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mmHg)	Duration of Hyper- tension (day)	Mean Body Weight at Sacrifice (gm)	Adrenal Weight/ Body Weight $\times 10^5$	Findings		Case No. of Normo- tensive Control	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mmHg)	Mean Body Weight at Sacrifice (gm)	Adrenal Weight/ Body Weight $\times 10^5$
								Z. fasci- culata and reticu- laris	Z. glome- rulosa					
I	♂	T9027	42D	140	0	130	19.2	↑	→	41	42D	106	150	18.7
		T9054	45	133	0	120	27.5	↑	→	48	46	117	120	35.0
		T9053	50	149	0	140	23.6	↑	→	47	52	115	130	33.9
		T0095	43	143	0	80	40.0	↑	→	42	43	125	100	20.0
		T0096	43	148	0	85	43.5	↑	→	44	59	116	80	41.3
		T0013	54	129	0	120	29.2	↑	→	43	54	126	160	20.6
		T0059	52	139	0	115	27.8	↑	→	321	48	108	108	25.0
		T0055	56	128	0	118	22.0	↑	→	320	52	114	134	22.4
		T0057	56	133	0	142	20.4	↑	→	382	51	116	140	27.2
		T9123	59	139	0	164	20.7	↑	→	45	57	116	166	27.7
		T0242	47	138	0	124	24.2	↑	→	381	59	109	166	21.1
II	♂	9057	6M	214	99	248	12.5	↑↑	↑↑	242	6M	128	325	13.2
		9058	6	229	124	226	26.1	↑↑	↑↑	246	6	126	320	11.2
		9055	6	209	98	238	28.2	↑	↑	227	7	135	365	8.5
		9065	6	225	105	265	14.7	↑	↑	245	6	114	368	13.0
		T9011	5	223	61	170	30.6	↑	→	316	5	125	250	12.4
		9075	6	199	104	278	12.9	↑	↑	261	6	120	264	10.6
		9077	6	220	104	280	13.6	↑	↑	14	5	127	280	20.0
		T0063	5	196	68	282	11.3	↑↑	↑↑	311	5	113	300	13.0
III	♂	7046	17M	208	453	372	11.8	↑	→	103	14M	127	335	9.6
		8001	14	200	341	322	9.9	↑	↑	133	12	123	275	11.3
		8100	13	196	330	332	11.4	↑	↑	57	14	121	360	10.0
		8102	13	206	330	360	10.8	↑	↑	64	14	124	360	12.8
		8107	13	200	342	374	10.2	↑	↑	54	15	119	350	12.0
		8109	13	199	328	350	16.3	↑	↑	58	14	111	320	16.2
		N9001	16	185	388	320	12.0	↑	↑	60	16	118	410	9.2
	♀	7056	16	164	412	260	21.2	↑↑	↑	107	14	125	255	20.0
		7053	17	232	461	233	34.8	↑	↑	109	14	124	262	15.3
		7082	17	192	417	255	24.3	↑↑	↑	117	14	130	325	14.8

Symbols and Abbreviations (Used in all tables)

↑ : Increase of the enzyme activity

↑ : Slightly increased

→ : No difference

D : Days

M : Months

SHR : Spontaneously hypertensive rats

Findings in rats of the SH group were obtained by comparing with those of the N group in all tables.

HISTOCHEMICAL FINDINGS: —The most positive findings were found in rats of the SH group compared with each of the same sex rats of the N group with almost the same survival duration. The increase of the enzyme activity was scored as follows:

- ↑ : slightly increased
- ↑↑ : moderately increased
- ↑↑↑ : markedly increased
- ↑↑↑↑ : most markedly increased
- ↓ : slightly decreased
- ↓↓ : moderately decreased

1) G-6-PD Activity in the Adrenal Cortex. (Table II, Figs. 1-4). The G-6-PD reaction was observed in the adrenal cortex of the N group in all stages. Extensive reaction was detected in cells of the zona fasciculata and reticularis with the greatest concentration present in the middle and outer part of the fasciculata. The glomerulosa showed considerably less activity.

Zona fasciculata and reticularis: Moderate

increase of G-6-PD activity in the adrenal cortex, especially in the middle and outer part of its zona fasciculata, of the SH rats was detected from Stage I and a more prominent increasing tendency of this activity was observed after Stage II, most remarkably in Stage III (Figs. 1-4). In Stage II, among a total of 8 cases which showed increase of the activity, 3 cases increased markedly. In Stage III, both males and females showed elevation and in a total of 10, 7 showed a marked increase of the activity.

Zona glomerulosa: No increase was observed in Stage I (Figs. 1, 2). In Stage II, increase was observed in all cases except one, and among them 2 cases increased markedly (Figs. 3, 4). SH rats in Stage III, all but one showed a moderate increase. Although the activity increased in the glomerulosa, it was not as extensive as in the fasciculata and reticularis in both the N and SH groups.

Fig. 1. G-6-PD activity in the adrenal cortex of a normotensive rat (No. 42, male, 43 days old). (By tetrazolium method). ($\times 100$). The reaction is mainly observed in the zona fasciculata and reticularis. The glomerulosa shows considerable less activity.

Fig. 2. G-6-PD activity in the adrenal cortex of a SH rat (No. 10095, male, 43 days old, pre-hypertensive stage). (By tetrazolium method). ($\times 100$). The activity shows moderate increase, especially in the middle and outer part of its fascicular zone compared to the control (Fig. 1). No increase is observed in the zona glomerulosa.

Fig. 3. G-6-PD activity in the adrenal cortex of a normotensive rat (No. 242, male, 6 months of age). (By tetrazolium method). ($\times 100$). The reaction is observed intensely in the zona fasciculata and reticularis. The glomerulosa also shows moderate activity.

Fig. 4. G-6-PD activity in the adrenal cortex of a SH rat (No. 9057, male, 6 months of age, initial stage of hypertension). (By tetrazolium method). ($\times 100$). More prominent increase of the activity is observed in the zona fasciculata and reticularis. The activity is enhanced also in the zona glomerulosa.

TABLE III TOTAL AVERAGE VALUES OF G-6-PD ACTIVITY IN THE ADRENAL CORTEX

Experi- mental Stage		Sex		No. of Animals	Mean Systolic Blood Pressure at Sacrifice (mm Hg)		Duration of Hyper- tension (day)	Mean Body Weight at Sacrifice (gm)		Adrenals Weight/ Body Weight $\frac{\text{gm}}{\text{gm}} \times 10^5$	Findings	
					mean	S.D.		mean	(range)		Z. fasci- culata and reticula- ris	Z. glome- rulosa
I	42-59 Days Old	♂	Normotensive Control	11	115 $\pm$ 6.3		0	132	(80-166)	26.6 $\pm$ 7.3	↑	→
			SHR	11	138 $\pm$ 7.0**		0	122	(80-164)	27.1 $\pm$ 8.0		
II	5-7 Months of Age	♂	Normotensive Control	8	124 $\pm$ 7.4		0	309	(250-368)	12.8 $\pm$ 4.0	↑	↑
			SHR	8	214 $\pm$ 12.2**		61-124	248	(170-282)	18.7 $\pm$ 8.0	↑	↑
III	13-17 Months of Age	♂	Normotensive Control	7	120 $\pm$ 5.2		0	344	(275-410)	11.6 $\pm$ 2.3	↑	↑
			SHR	7	199 $\pm$ 7.5**		328-453	347	(320-374)	11.8 $\pm$ 2.2	↑	↑
		♀	Normotensive Control	3	126 $\pm$ 3.2		0	281	(255-325)	16.7 $\pm$ 2.9	↑	↑
			SHR	3	196 $\pm$ 34.2 ⁺		412-461	249	(233-260)	26.7 $\pm$ 7.1	↑	↑

S.D.: Standard deviation of the mean

⁺ $0.01 < p < 0.05$

* $0.001 < p < 0.01$

** $p < 0.001$

↑: About half the strength shown by ↑

TABLE IV INDIVIDUAL FINDINGS OF TPND ACTIVITY IN THE ADRENAL CORTEX

Experi- mental Stage	Sex	Case No. of SHR	Days or Months after Birth	Mean Systolic Blood- Pressure at Sacrifice (mm Hg)	Duration of Hyper- tension (day)	Mean Body Weight at Sacrifice (gm)	Adrenal Weight/ Body Weight $\times 10^5$	Findings		Case No. of Normo- tensive Control	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Mean Body Weight at Sacrifice (gm)	Adrenal Weight/ Body Weight $\times 10^5$
								Z. fasci- culata and reticu- laris	Z. glome- rulosa					
I	♂	1 T9027	42D	140	0	130	19.2	↑	→	41	42D	106	150	18.7
		2 10095	43	143	0	80	40.0	↑	→	42	43	125	100	20.0
		3 10096	43	148	0	85	43.5	↑	→	44	59	116	80	41.3
		4 10013	54	129	0	120	29.2	↑	→	43	54	126	160	20.6
		5 10055	56	128	0	118	22.0	→	→	320	52	114	134	22.4
		6 10056	56	125	0	131	25.2	↑	→	321	48	108	108	25.0
		7 10057	56	133	0	142	20.4	↑	→	382	51	116	140	27.2
		8 T9123	59	139	0	164	20.7	↑↑	→	45	57	116	166	27.7
		9 T9124	59	135	0	158	27.8	↑	→	46	57	119	172	20.9
		10 10241	47	136	0	116	23.3	→	→	381	59	109	166	21.1
II	♂	1 T8011	7M	222	93	235	23.8	↑	↑	69	6M	124	285	12.6
		2 9057	6	214	99	248	12.5	↑	↑	242	6	128	325	13.2
		3 9058	6	229	124	226	26.1	↑	↑	246	6	126	320	11.2
		4 9055	6	209	98	238	28.2	↑	↑	227	7	135	365	8.5
		5 9065	6	225	105	265	14.7	↑	↑	245	6	114	368	13.0
		6 T9011	5	223	61	170	30.6	↑	→	316	5	125	250	12.4
		7 9075	6	199	104	278	12.9	↑	→	261	6	120	264	10.6
		8 9077	6	220	104	280	13.6	↑	→	14	5	127	280	20.0
		9 10063	5	196	68	282	11.3	↑	→	311	5	113	300	13.0
III	♂	1 7046	17M	208	453	372	11.8	↑	→	103	14M	127	335	9.6
		2 8001	14	200	341	322	9.9	↑	↑	133	12	123	275	11.3
		3 8100	13	196	330	332	11.4	↑	→	57	14	121	360	10.0
		4 8102	13	206	330	360	10.8	↑	→	64	14	124	360	12.8
		5 8107	13	200	342	374	10.2	↑	↑	54	15	119	350	12.0
		6 8109	13	199	328	350	16.3	↑	→	58	14	111	320	16.2
		7 N9001	16	185	388	320	12.0	↑	↑	60	16	118	410	9.2

Fig. 5. TPND activity in the adrenal cortex of a normotensive rat (No. 246, male, 6 months of age). (By tetrazolium method). ($\times 100$). The reaction is observed in the fasciculata and reticularis, and slightly less in the glomerulosa.

Fig. 6. TPND activity in the adrenal cortex of a SH rat (No. 9058, male, 6 months of age, initial stage of hypertension). (By tetrazolium method). ($\times 100$). Moderate enhancement of the activity is observed in the zona fasciculata and reticularis compared to the control (Fig. 5). An increasing tendency of the activity is also observed in the zona glomerulosa.

The findings of the G-6-PD activity detected in the adrenal cortex are summarized in Table III.

2) TPND Activity in the Adrenal Cortex. (Table IV, Figs. 5, 6). In the adrenal cortex of the N group, intense TPND activity was observed in the fasciculata and reticularis in all stages, but in sharp contrast to the case of the G-6-PD, these sections also showed comparatively intense enzyme activity in the glomerulosa. The increase of the activity in the glomerulosa, however, did not exceed that observed in the fasciculata and reticularis in any of the N group.

Zona fasciculata and reticularis: Increasing tendency of the activity in the SH group was observed from Stage I. Out of 10 rats, the increase was detected in 8 cases, one of which showed markedly, 4 of which showed slightly. In Stage II and in males in Stage III, moderate enhancement was observed in all of 9 and 7 cases, respectively (Figs. 5, 6). Although the TPND activity in the fasciculata and reticularis showed a tendency to rise ac-

cording to age, the gradient of the enhancement was not so remarkable as that of G-6-PD.

Zona glomerulosa: There was no change in Stage I in any of 10 cases. In Stage II among 9 cases, 5 showed increasing tendency of the activity with 3 of these showing slightly positive (Figs. 5, 6). The others showed no change. In Stage III, among 7 males, 3 showed a moderate increase while 4 did not change. As far as TPND activity in the glomerulosa is concerned, about half of the cases showed an increase both in Stages II and III. There was no definite tendency as in the case of G-6-PD activity. The increasing degree in TPND activity in the glomerulosa was less than in the fasciculata and reticularis and the intensity of the increase was not different between Stages II and III.

The findings of the TPND activity are summarized in Table V.

3) Noradrenalin Reaction for the Adrenal Medulla. (Table VI, Figs. 7-10).

a) Histochemical findings: Noradrenalin reaction positive cell islets was identified scat-

TABLE V TOTAL AVERAGE VALUES OF TPND ACTIVITY IN THE ADRENAL CORTEX

Experi- mental Stage	Sex		No. of Animals	Mean Systolic Blood Pressure at Sacrifice (mm Hg)		Duration of Hyper- tension (day)	Mean Body Weight at Sacrifice (gm)		Adrenals Weight/ Body Weight $\frac{\text{gm}}{\text{gm}} \times 10^5$	Findings	
				mean	S.D.		mean	(range)		Z. fasci- culata and reticula- ris	Z. glome- rulosa
I	42-59 Days Old	♂	Normotensive Control	10	116 ± 6.8	0	138	(80-172)	24.5 ± 6.6	↑	→
			SHR	10	136 ± 7.2**	0	124	(80-164)	26.0 ± 8.5		
II	5-7 Months of Age	♂	Normotensive Control	9	124 ± 7.0	0	306	(250-368)	12.7 ± 3.1	↑	↑
			SHR	9	215 ± 16.7**	61-124	247	(170-282)	19.3 ± 7.7 ⁺		
III	13-17 Months of Age	♂	Normotensive Control	7	120 ± 5.2	0	344	(275-410)	11.6 ± 2.4	↑	↑
			SHR	7	199 ± 7.5**	328-453	347	(320-374)	11.8 ± 2.1

Fig. 7. Noradrenalin reaction for the adrenal medulla of a normotensive rat (No. 382, male, 51 days old). (By glutaraldehyde-osmium tetroxide technique). ($\times 40$). Noradrenalin-storing cell islets are identified scattered in the adrenal medulla.

Fig. 8. Noradrenalin reaction for the adrenal medulla of a SH rat (No. 10056, male, 56 days old, pre-hypertensive stage). (By glutaraldehyde-osmium tetroxide technique). ($\times 40$). The noradrenalin-storing cell islets appear larger and more numerous.

tered in the adrenal medulla of both the N and SH groups (Figs. 7-10). The intensity of the reaction was similar in the N group through all stages, but the number and size of reaction positive cell islets showed slightly an increasing tendency according to age. Usually in the hypertensives, from the pre-hypertensive stage, the reaction-positive cell islets in the adrenal medulla appeared larger, more numer-

ous and more densely stained. These tendencies became most intense in Stage III.

b) Histometrical findings: As shown in Table VII, the dimensional ratio of these reaction positive cell islets to the whole adrenal medulla of the N group was 11.0 per cent in Stage I, 13.3 per cent in Stage II and 14.8 per cent in Stage III. Thus the dimensional ratio in the N group histometrically

TABLE VI INDIVIDUAL FINDINGS OF NORADRENALIN REACTION FOR THE ADRENAL MEDULLA, THE DIMENSIONAL RATIO OF NORADRENALIN-STORING CELL ISLETS TO THE WHOLE ADRENAL MEDULLA

Experi- mental Stage	Sex	Case No. of SHR	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Duration of Hyper- tension (day)	Mean Body Weight at Sacrifice (gm)	Adrenal Weight/ Body Weight $\times 10^5$	Dimen- sional Ratio of SHR	Case No. of Normo- tensive Control	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Mean Body Weight at Sacrifice	Adrenal Weight/ Body Weight $\times 10^5$	Dimen- sional Ratio of Control
I	♂	1 10053	49D	132	0	102	20.6	18.9%	319	45D	108	103	33.0	8.9%
		2 10059	52	139	0	115	27.8	19.9	320	52	114	134	22.4	12.0
		3 10056	56	125	0	131	25.2	24.4	382	51	116	140	27.2	7.6
		4 10057	56	133	0	142	20.4	20.2	321	48	108	108	25.0	12.9
		5 116001	49	140	0	120	31.7	21.2	383	46	104	140	20.7	13.7
II	♂	1 9074	6M	208	91	276	15.6	20.1	21	6M	128	320	15.9	9.5
		2 9075	6	199	104	278	12.9	36.6	313	5	130	282	12.4	11.9
		3 9077	6	220	104	280	13.6	31.0	323	7	132	330	10.9	18.5
		4 T9032	6	204	100	320	13.7	23.1	14	5	127	280	20.0	14.2
		5 9099	7	187	145	314	15.0	37.7	338	5	115	320	17.8	12.2
III	♂	1 8100	13M	196	330	332	11.4	28.9	57	14	121	360	10.0	11.9
		2 8102	13	206	330	360	10.8	32.3	64	14	124	360	12.8	17.5
		3 8107	13	200	342	374	10.2	25.4	54	15	119	350	12.0	17.4
		4 8109	13	199	328	350	16.3	28.9	58	14	111	320	16.2	14.0
		5 N9001	16	185	388	320	12.0	27.5	60	16	118	410	9.2	13.3

TABLE VII TOTAL AVERAGE VALUES OF NORADRENALIN REACTION FOR THE ADRENAL MEDULLA, THE MEAN DIMENSIONAL RATIO OF NORADRENALIN-STORING CELL ISLETS TO THE WHOLE ADRENAL MEDULLA

Experi- mental Stage	Sex	No. of Animals	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Duration of Hypertension (day)	Mean Body Weight at Sacrifice (gm)	Adrenals Weight/ Body Weight $\frac{\text{gm}}{\text{gm}} \times 10^5$	Dimensional Ratio	#
I	♂	5	110 $\pm$ 4.9	0	125 (103-140)	25.7 $\pm$ 4.8	11.0 $\pm$ 2.6	$\times 1.9$
		5	134 $\pm$ 6.1**	0	122 (102-142)	25.1 $\pm$ 4.8	20.9 $\pm$ 2.6**	
II	♂	5	126 $\pm$ 6.7	0	306 (280-330)	15.4 $\pm$ 3.8	13.3 $\pm$ 3.4	$\times 2.2$
		5	204 $\pm$ 12.1**	91-145	294 (276-320)	14.2 $\pm$ 1.1	29.7 $\pm$ 7.9*	
III	♂	5	119 $\pm$ 4.8	0	360 (320-410)	12.0 $\pm$ 2.8	14.8 $\pm$ 2.5	$\times 1.9$
		5	197 $\pm$ 7.7**	328-388	347 (320-374)	12.2 $\pm$ 2.4	28.6 $\pm$ 2.5**	

#: The relationship of the dimensional ratio of the SHR group to that of the normotensive control group in each stage.

Fig. 9. Noradrenalin reaction for the adrenal medulla of a normotensive rat (No. 323, male, 7 months of age). (By glutaraldehyde-osmium tetroxide technique). ($\times 100$). Noradrenalin-storing cell islets are scattered in the medulla. On the right side a part of the cortex can be seen.

Fig. 10. Noradrenalin reaction for the adrenal medulla of a SH rat (No. 9077, male, 6 months of age, initial stage of hypertension). (By glutaraldehyde-osmium tetroxide technique). ($\times 100$). Noradrenalin-storing cell islets in the medulla indicate a stronger reaction with about twice the dimensional ratio to the whole adrenal medulla histometrically.

also increased slightly according to age. On the other hand, that of the SH group was found to be 20.9 per cent in Stage I corresponding to about 1.9 times that of the mean of the N group in the same stage. In Stage II, the mean was shown to be 29.7 per cent corresponding to about 2.2 times compared to that of the N group of the same age. In Stage III, the SH group mean was 28.6 per cent, about 1.9 times compared to that of the N group. Thus the mean dimensional ratio of noradrenalin-storing cell islets to the whole adrenal medulla of the SH group showed about twice that of each corresponding N group through all the stages from I to III. There was no plain interrelationship between the severity of hypertension and the intensity of reaction in the adrenal medulla of the SH group within each stage.

4) ACPase Activity in the Follicular Cells of the Thyroid. (Table VIII, Figs. 11, 12). The activity was recognized by the presence of reaction product in the cytoplasm of follicular epithelial cells of the thyroid both in the N and SH groups. Thyroid sections of the N group showed moderately positive reaction in the follicular cells through all stages and had follicles, showing rather strongly stained epithel, scattered among moderately positive ones. On the other hand, in the SH group, almost all the follicular epithel demon-

strated stronger average activity. In Stage I, all 7 cases displayed a clear increase, 3 markedly and the others moderately. In Stages II and III, all cases had a distinct increasing tendency with 6 in Stage II and 4 in Stage III respectively marked (Figs. 11, 12).

The findings of ACPase activity in the thyroid are summarized in Table IX. The increase was observed in the SH group through all stages and became more definite in Stages II and III.

5) ATPase Activity in the Vasa Afferens and Interlobular Arteries of the Kidney. (Table X, Figs. 13-16). In the kidney of the N group, ATPase activity was remarkably detected in the vascular walls such as the arcuate and interlobular arteries, vasa afferens and glomerular capillaries through all stages. The activity was almost the same in all vascular walls.

Vasa afferens: In the SH group, the activity was no different in any case in Stage I, but all of 10 cases in Stage II and all of 9 in Stage III showed an increase (Figs. 13, 14).

Interlobular arteries: In the SH group, among 9 cases in Stage I, 4 showed no change and 5 increased moderately. All SH rats in Stages II and III showed increase, 3 out of 10 in Stage II, 5 out of 9 in Stage III displaying marked increase (Figs. 15, 16). In Stage II, there was no distinct interrelationship between the severity and duration of hypertension and

TABLE VIII INDIVIDUAL FINDINGS OF ACPase ACTIVITY IN THE THYROID

Experi- mental Stage	Sex	Case No. of SHR	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Duration of Hyper- tension (day)	Mean Body Weight at Sacrifice (gm)	Thyroid Weight/ Body Weight $\times 10^5$	Findings	Case No. of Normo- tensive Control	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Mean Body Weight at Sacrifice (gm)	Thyroid Weight/ Body Weight $\times 10^5$
I	♂	1 10095	43D	143	0	80	17.5	↑↑	42	43D	125	100	13.0
		2 10096	43	148	0	85	20.0	↑	44	59	116	80	13.8
		3 10013	54	129	0	120	13.3	↑	43	54	126	160	10.6
		4 T9123	59	139	0	164	9.1	↑↑	45	57	116	166	9.6
		5 T9124	59	135	0	158	7.6	↑↑	46	57	119	172	8.1
		6 10241	47	136	0	116	8.6	↑	381	59	109	166	12.0
		7 10242	47	138	0	124	8.9	↑	369	59	124	160	8.8
II	♂	1 T8011	7M	222	93	235	15.7	↑↑	69	6M	124	285	14.0
		2 9057	6	214	99	248	12.5	↑↑	242	6	128	325	6.8
		3 9058	6	229	124	226	10.2	↑↑	246	6	126	320	10.9
		4 9065	6	225	105	265	9.1	↑↑	245	6	114	368	9.2
		5 T9011	5	223	61	170	11.2	↑↑	316	5	125	250	10.4
		6 10063	5	196	68	282	12.1	↑↑	311	5	113	300	9.0
		7 T9086	5	192	68	260	11.9	↑	312	5	124	300	6.7
III	♂	1 7046	17M	208	453	372	13.7	↑	103	14M	127	335	7.5
		2 8001	14	200	341	322	12.1	↑↑	133	12	123	275	9.8
		3 8100	13	196	330	332	10.8	↑	57	14	121	360	7.8
		4 8102	13	206	330	360	11.4	↑↑	64	14	124	360	7.5
		5 8107	13	200	342	374	15.0	↑	54	15	119	350	8.0
		6 8109	13	199	328	350	11.7	↑↑	58	14	111	320	7.5
		7 N9001	16	185	388	320	9.4	↑↑	60	16	118	410	6.4

Fig. 11. ACPase activity in the follicular cells of the thyroid of a normotensive rat (No. 242, male, 6 months of age). (By metal-salt method). ($\times 400$). The follicular epithelial cells show moderately positive reaction.

Fig. 12. ACPase activity in the follicular cells of the thyroid of a SH rat (No. 9057, male, 6 months of age, initial stage of hypertension). (By metal-salt method). ($\times 400$). The follicular epithelial cells display a marked increase of the activity compared to the control (Fig. 11).

TABLE IX TOTAL AVERAGE VALUES OF ACPase ACTIVITY IN THE THYROID

Experimental Stage	Sex		No. of Animals	Mean Systolic Blood Pressure at Sacrifice (mm Hg)		Duration of Hypertension (day)	Mean Body Weight at Sacrifice (gm)		Thyroid Weight/Body Weight $\frac{\text{gm}}{\text{gm}} \times 10^5$	Findings
				mean	S.D.		mean	(range)		
I	43-59 Days Old	♂	Normotensive Control	7	119 $\pm$ 6.2	0	143	(80-172)	10.9 $\pm$ 2.2	↑ ↑
			SHR	7	138 $\pm$ 6.1	0	121	(80-164)	12.2 $\pm$ 4.9	
II	5-7 Months of Age	♂	Normotensive Control	7	119 $\pm$ 6.2	0	307	(250-368)	10.9 $\pm$ 2.2	↑ ↑
			SHR	7	216 $\pm$ 14.8**	61-124	241	(170-282)	11.8 $\pm$ 2.1	
III	13-17 Months of Age	♂	Normotensive Control	7	120 $\pm$ 5.2	0	344	(275-410)	7.8 $\pm$ 1.0	↑ ↑
			SHR	7	199 $\pm$ 7.5**	328-453	347	(320-374)	12.0 $\pm$ 1.8**

Fig. 13. ATPase activity in the vasa afferens of the kidney of a normotensive rat (No. 69, male, 6 months of age). (By metal-salt method). ($\times 400$). The activity is shown in the walls of a vas afferens.

Fig. 14. ATPase activity in the vasa afferens of the kidney of a SH rat (No. T8011, male, 7 months of age, initial stage of hypertension). (By metal-salt method). ($\times 400$). A stronger activity is observed compared to the control (Fig. 13).

TABLE X INDIVIDUAL FINDINGS OF ATPase ACTIVITY IN THE RENAL VESSELS

Experi- mental Stage	Sex	Case No. of SHR	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Duration of Hyper- tension (day)	Mean Body Weight at Sacrifice (gm)	Kidney Weight/ Body Weight $\times 10^3$	Findings		Case No. of Normo- tensive Control	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Mean Body Weight at Sacrifice (gm)	Kidney Weight/ Body Weight $\times 10^3$
								Vasa Afferens	Inter- lobular Arteries					
I	♂	1 9078	42D	140	0	93	10.2	→	↑	252	46D	118	85	9.7
		2 T9054	45	133	0	120	10.3	→	↑	48	46	117	120	10.0
		3 10095	43	143	0	80	12.5	→	↑	42	43	125	100	10.5
		4 10096	43	148	0	85	11.8	→	↑	44	59	116	80	10.9
		5 10013	54	129	0	120	11.1	→	→	43	54	126	160	11.0
		6 T9123	59	139	0	164	8.5	→	→	45	57	116	166	10.6
		7 T9124	59	135	0	158	8.9	→	→	46	57	119	172	8.2
		8 10241	47	136	0	116	10.3	→	→	381	59	109	166	9.8
		9 10242	47	138	0	124	10.8	→	↑	369	59	124	160	9.1
II	♂	1 T8022	5M	170	67	225	7.2	↑	↑	68	6M	127	260	6.7
		2 T8011	7	222	93	235	9.7	↑	↑	69	6	124	285	7.5
		3 9057	6	214	99	248	8.5	↑	↑	242	6	128	325	7.1
		4 9058	6	229	124	226	8.5	↑	↑	246	6	126	320	7.1
		5 9055	6	209	98	238	8.9	↑	↑	21	6	128	320	7.2
		6 9064	6	210	105	292	7.5	↑	↑	227	7	135	365	6.9
		7 9065	6	225	105	265	7.9	↑	↑	245	6	114	368	6.8
		8 T9011	5	223	61	170	8.8	↑	↑	316	5	125	250	7.4
		9 10063	5	196	68	282	7.8	↑	↑	311	5	113	300	8.2
		10 T9086	5	192	68	260	7.6	↑	↑	312	5	124	300	7.6
III	♂	1 7046	17M	208	453	372	7.6	↑	↑	103	14M	127	335	6.9
		2 8001	14	200	341	322	9.1	↑	↑	133	13	123	275	7.6
		3 8100	13	196	330	332	8.7	↑	↑	57	14	121	360	6.6
		4 8102	13	206	330	360	7.5	↑	↑	64	14	124	360	6.4
		5 8107	13	200	342	374	7.8	↑	↑	54	15	119	350	12.6
		6 8109	13	199	328	350	7.5	↑	↑	58	14	111	320	7.4
	♀	7 7084	14	176	323	240	7.8	↑	↑	121	13	125	220	7.1
		8 7056	16	164	412	260	7.9	↑	↑	107	14	125	255	7.5
		9 7053	17	232	461	233	8.7	↑	↑	109	14	124	262	6.4

Fig. 15. ATPase activity in the renal interlobular arteries of a normotensive rat (No. 21, male, 6 months of age). (By metal-salt method). ($\times 400$). The activity is detected in the wall of the vessel.

Fig. 16. ATPase activity in the renal interlobular arteries of a SH rat (No. 9055, male, 6 months of age, initial stage of hypertension). (By metal-salt method). ($\times 400$). Marked increase of the activity is observed compared to the control (Fig. 15).

TABLE XI TOTAL AVERAGE VALUES OF ATPase ACTIVITY IN THE RENAL VESSELS

Experimental Stage	Sex		No. of Animals	Mean Systolic Blood Pressure at Sacrifice (mm Hg)		Duration of Hypertension (day)	Mean Body Weight at Sacrifice (gm)		Kidneys Weight/Body Weight $\frac{\text{gm}}{\text{gm}} \times 10^3$	Findings	
				mean	S.D.		mean	(range)		Vasa Afferens	Interlobular Arteries
I	42-59 Days Old	♂	Normotensive Control	9	119 $\pm$ 5.4	0	134	(80-172)	10.0 $\pm$ 0.9	→	↑
			SHR	9	138 $\pm$ 5.6**	0	118	(80-164)	10.5 $\pm$ 1.3 ⁺		
II	5-7 Months of Age	♂	Normotensive Control	10	124 $\pm$ 6.5	0	309	(250-368)	7.3 $\pm$ 0.4	↑	↑
			SHR	10	209 $\pm$ 18.4**	61-124	244	(170-292)	8.3 $\pm$ 0.8**		
III	13-17 Months of Age	♂	Normotensive Control	6	121 $\pm$ 5.5	0	333	(275-360)	7.9 $\pm$ 2.3	↑	↑↑
			SHR	6	202 $\pm$ 4.6**	328-453	352	(322-374)	8.1 $\pm$ 0.7		
		♀	Normotensive Control	3	125 $\pm$ 0.2	0	246	(220-262)	7.0 $\pm$ 0.5	↑	↑
			SHR	3	191 $\pm$ 36.3 ⁺	323-461	244	(233-260)	8.1 $\pm$ 0.5

Fig. 17. MAO activity in the heart muscle of a normotensive rat (No. 109, female, 14 months of age). (By tetrazolium method). ($\times 100$). The reaction is observed rather diffusely in the heart muscle.

Fig. 18. MAO activity in the heart muscle of a SH rat (NO. 7053, female, 17 months of age). (By tetrazolium method). ($\times 100$). Prominent elevation of the activity is observed.

TABLE XII INDIVIDUAL FINDINGS OF MAO ACTIVITY IN THE HEART MUSCLE

Experi- mental Stage	Sex	Case No. of SHR	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Duration of Hyper- tension (day)	Mean Body Weight at Sacrifice (gm)	Heart Weight/ Body Weight $\times 10^3$	Findings	Case No. of Normo- tensive Control	Days or Months after Birth	Mean Systolic Blood Pressure at Sacrifice (mm Hg)	Mean Body Weight at Sacrifice (gm)	Heart Weight/ Body Weight $\times 10^3$
I	♂	1 9078	42D	140	0	93	4.7	↑	252	46D	118	85	4.5
		2 T9027	42	140	0	130	5.4	→	41	42	106	150	4.1
		3 T9054	45	133	0	120	4.7	→	48	46	117	120	5.4
		4 10095	43	143	0	80	6.3	↑	42	43	125	100	5.5
		5 10096	43	148	0	85	5.7	→	44	59	116	80	5.3
		6 10013	54	129	0	120	5.0	→	43	54	126	160	4.4
		7 T9123	59	139	0	164	4.5	→	45	57	116	166	4.1
		8 T9124	59	135	0	158	4.1	→	46	57	119	172	4.0
		9 10241	47	136	0	116	4.5	→	381	59	109	166	4.3
		10 10242	47	138	0	124	4.6	→	369	59	124	160	3.6
II	♂	1 T8022	5M	170	67	225	4.1	↑↑	66	5M	126	285	3.2
		2 T8011	7	222	93	235	7.5	↑	69	6	124	285	4.1
		3 9057	6	214	99	248	5.5	↑	242	6	128	325	3.8
		4 9058	6	229	124	226	6.0	↑	246	6	126	320	3.8
		5 9055	6	209	98	238	6.4	↑	227	6	128	320	3.1
		6 9064	6	210	105	292	4.0	↑	245	7	135	365	3.2
		7 9065	6	225	105	265	4.3	↑	316	6	114	368	3.0
		8 T9011	5	223	61	170	6.5	↑	313	5	125	250	3.7
		9 10030	5	223	71	302	4.7	↑	311	5	130	282	3.4
		10 10063	5	196	68	282	4.0	↑	311	5	113	300	3.3
III	♂	1 7046	17M	208	453	372	5.1	↑↑↑	103	14M	127	335	3.7
		2 8100	13	196	330	332	4.6	↑↑↑	57	14	121	360	3.6
		3 8102	13	206	330	360	4.3	↑↑↑	64	14	124	360	3.3
		4 8107	13	200	342	374	4.4	↑↑↑	54	15	119	350	3.4
		5 8109	13	199	328	350	5.2	↑↑↑	58	14	111	320	3.7
		6 N9001	16	185	388	320	4.4	↑	60	16	118	410	3.8
	♀	7 7084	14	176	323	240	4.5	↑	121	13	125	220	3.9
		8 7056	16	164	412	260	4.2	↑↑↑	107	14	125	255	3.7
		9 7053	17	232	461	233	6.1	↑↑↑	109	14	124	262	3.5
		10 N9004	16	197	388	255	4.9	↑	59	16	113	250	4.0

↑: Slightly decreased
 ↓: Moderately decreased

TABLE XIII TOTAL AVERAGE VALUES OF MAO ACTIVITY IN THE HEART MUSCLE

Experi- mental Stage		Sex		No. of Animals	Mean Systolic Blood Pressure at Sacrifice (mm Hg)		Duration of Hyper- tension (day)	Mean Body Weight at Sacrifice (gm)		Heart Weight/ Body Weight $\frac{\text{gm}}{\text{gm}} \times 10^3$	Findings
					mean	S.D.		mean (range)			
I	42-59 Days Old	♂	Normotensive Control	10	118± 6.5	0	136 (80-172)	4.5±0.7	Indefinite		
			SHR	10	138± 5.3**	0	119 (80-164)	4.9±0.7			
II	5-7 Months of Age	♂	Normotensive Control	10	125± 6.8	0	310 (250-368)	3.5±0.4	↑		
			SHR	10	212± 17.8**	61-124	248 (170-302)	5.3± 1.3**			
III	13-17 Months of Age	♂	Normotensive Control	6	120± 5.5	0	356 (320-410)	3.6±0.2	↑↑		
			SHR	6	199± 8.2**	328-453	351 (320-374)	4.7± 0.4**			
		♀	Normotensive Control	4	122± 5.9	0	247 (220-262)	3.8±0.2	↑↑		
			SHR	4	192± 29.0*	323-461	247 (233-260)	4.9±0.8 ⁺			

intensity of reaction either in vasa afferens or interlobular arteries. In Stage III, there seemed to exist a parallel relationship between the severity of hypertension and the intensity of the activity increase in the renal interlobular arteries in both sexes.

Arcuate arteries and glomerular capillaries: The activity was unaltered through all stages.

The findings on the ATPase activity are summarized in Table XI.

6) MAO Activity in the Heart Muscle. (Table XII, Figs. 17, 18). In the N group, rats in Stage I showed a slightly positive reaction and had a tendency to stain slightly unevenly. The staining was somewhat stronger in the papillary muscles of the left ventricle. The reaction became more even and more diffuse in Stage II. The reaction became stronger and displayed quite diffusely in a section in Stage III (Figs. 17, 18). Thus the reaction grew more intense in the N group according to age. In the SH group, among 10 cases in Stage I, 4 showed no change, one moderately and another one slightly increased and 2 showed slightly and another 2 were moderately decrease in the activity when compared to the N group in Stage I. Thus SH group in Stage I showed no definite tendency. In Stages II and III, all cases displayed an increase of the activity. Among 10 cases

in Stage II, 4 showed slight, another 4 moderate and 2 marked increase. Of a total of 10 cases in Stage III, 4 showed moderate, 2 marked and 4 most marked increase. The longer the duration of survival, the stronger the observed reaction in the heart of both the N and SH groups. The SH group had a greater proportion in increase of the activity than the N group, and displayed more dominant increase in Stage III than in Stage II.

The findings on MAO activity are summarized in Table XIII. No characteristic findings were observed in the position of the heart conducting system in any stage.

Some other additional findings are hereunder listed. In the N group, in all experimental histochemical groups, strength of the histochemical reaction had little fluctuation through all stages, except MAO activity. Variation according to sex in Stage III was not demonstrated in any kind of histochemical reaction.

Among SH rats, within each stage, interrelationship between the severity and duration of hypertension and the increasing degree of histochemical activity was not demonstrated except in renal interlobular arteries in Stage III.

Finally, in the adrenals, thyroid, kidneys and heart, no particular results were found in other kind of enzyme histochemistry save for

those described above. Comparative histochemical tests of the liver and pancreas of rats in both groups produced no significant findings whatsoever.

DISCUSSION

It has been in accord with reports concerning the SH rats that there is no significant difference in body weight between the animals of the N group and the SH group¹⁾⁹⁾¹⁸⁾. Blood pressure rose in almost all the SH rats from the pre-hypertensive stage and became significantly high after the development of hypertension. As shown in the present materials and as reported earlier¹⁾¹³⁾, the course of blood pressure of SH rats in Stage I (40–60 days old), in Stage II (5–7 months of age) and in Stage III (13–17 months of age) correspond to those in the pre-hypertensive stage, initial stage and advanced stage of hypertension respectively.

The above-mentioned comparative histochemical findings were obtained by the examination of twenty-one kinds of enzyme activity on six organs in each of the three stages. Among these findings, conspicuous and relevant ones will be considered.

The findings of the G-6-PD and TPND activity in the adrenal cortex of the N group are in accord with those of COHEN³⁷⁾. Unusually high levels of G-6-PD activity were found biochemically in adrenal cortical tissue by KELLY et al³⁸⁾. The enzyme, G-6-PD in the adrenal cortex, as COHEN said, is considered to act as a reduced triphosphopyridine nucleotide (TPNH) generator. This TPNH is required as a cofactor for the hydroxylases which is important for steroid synthesis. Consequently, the elevation of the G-6-PD activity, especially in the middle and outer part of the zona fasciculata and reticularis from the pre-hypertensive stage, and in the glomerulosa after the incidence of hypertension seems to suggest the increase of steroid synthesis in each zone of the adrenal cortex of the SH rat from each corresponding stage. It is inferred, therefore, that the synthesis in the zona fasciculata and reticularis is promoted after the incidence of hypertension and becomes manifest in the advanced stage of hypertension. On the other hand, the augmented TPND

activity in the adrenal cortex of the SH group from the pre-hypertensive stage in the zona fasciculata and reticularis, and after the incidence of hypertension in the glomerulosa, means also the rise of the oxidation of much of the TPNH *via* this enzyme in these zones in each stage. But the increasing degree of TPND activity is less than that of G-6-PD according to age. This finding should be thought of as an incidental change to the enhancement of the G-6-PD activity in each zone respectively. Glucocorticoids³⁹⁾ are secreted from the fasciculata and hypertension can be induced experimentally by the administration of them^{40–44)}. The hyperfunction of the zona fasciculata of the adrenal cortex in SH rats drawn from the histochemical findings concurs with the histologically determined hypertrophy of the zone⁹⁾. This hyperfunction can be considered as a cause of the development and maintenance of hypertension in this rat. The hyperfunction of the zona fasciculata might go with the histological findings in the anterior pituitary of SH rats that increase in the percentage ratio of basophils existed from the pre-hypertensive stage⁹⁾.

On the other hand, it is generally believed that the zona glomerulosa is the site of aldosterone production³⁹⁾⁴⁵⁾. In the glomerulosa of SH rats, rise of G-6-PD and TPND activity was observed histochemically after the development of hypertension, hypertrophy detected histologically and increase in its lipid content was detected also from the pre-hypertensive stage. From these findings, it should be inferred that hypersecretion of aldosterone in the SH group is likely to be present at least after the incidence of hypertension. Meanwhile, the zona glomerulosa appears to react demonstrably to alteration in electrolyte concentration^{46–48)}. It is not clear, however, whether or not this is regulated by the increase in renin release or of angiotensin formation^{39)47)49–54)}. At any rate, hyperfunction of the glomerulosa is considered to occur in the SH group at least after the incidence of hypertension and it is probable that hyperfunction of this zone plays a certain role in hypertensive condition.

SOBEL⁵⁵⁾⁵⁶⁾ verified histologically, histochemically and biochemically that the ACPase activi-

ty in the follicular cells of the rat thyroid showed rise when they received exposure to cold, and reduced when they got thyroxin treatment. This fact means the alteration in ACPase activity in the thyroid indicates the rate of thyroid hormone synthesis. Increase of ACPase activity in the follicular cells of the thyroid of SH rats through all stages is consistent with that of the cases exposed to cold by SOBEL⁵⁵⁾⁵⁶⁾. The histological and histochemical findings on the thyroid of SH rats also suggest the increase of the function. Consequently, it seems to suggest that there exists an increase of secretory activity of the thyroid in SH rats from the pre-hypertensive stage becoming more marked after the incidence of hypertension as determined by both histological and histochemical studies. Moreover, the thyroid of SH rats gained manifestly in weight in the advanced stage of hypertension. Thyroxin increases cardiac output in cooperation with the effect of catecholamine so that pulse pressure and heart rate are increased⁵⁷⁾. Patients with hyperthyroidism frequently display hypertension⁵⁸⁾. When thyroid powder was administered to SH rats, the blood pressure rose much higher than that of normotensives¹¹⁾. In addition, it has been confirmed in rats that thyroxin enhances hypertension due to corticosteroids⁵⁹⁾. Consequently the hyperfunction of the thyroid in SH rats is also a factor for the occurrence and maintenance of hypertension attending on the adrenal cortical hyperactivity in this rat. The hyperfunction of the thyroid may correspond also to the increase of the percentage ratio of basophils in the anterior pituitary from the pre-hypertensive stage⁹⁾.

It is suggested that the presence of ATP-ATPase system in the vascular walls is an indispensable prerequisite to the contraction of vascular smooth muscle and that a disturbance of this ATP-ATPase system in the blood vessels is important concerning the pathogenesis of hypertension⁶⁰⁾. NAKAJIMA⁶¹⁾ did studies on ATPase in dog kidneys and revealed that all the fractions which possessed the ATPase activity caused a remarkable rise of blood pressure when injected into another dog intravenously. MOTOMURA⁶²⁾ revealed

that "acid ATPase" activity in the serum is higher in the hypertensives than in the normotensives. In the meantime, NAKAMOTO⁶³⁾ planned a histochemical method for the demonstration of acid ATPase with the incubation medium at pH 5.5 and reported that the activity was demonstrated moderately to markedly in such renal vascular walls as arteries of medium caliber, vasa afferens and glomerular capillaries using this technique. Consequently, histochemical findings on ATPase activity in the renal vascular system in rats of both the N and SH groups might be considered to be analogous to that of acid ATPase activity, at least from the histochemically detected localization point of view. Based on this, it can be assumed that the renal interlobular artery in some cases of SH rats showed a tendency to gain in contraction even from the pre-hypertensive stage, and both the interlobular artery and vasa afferens contract more intensely in all cases than those of normotensives after the development of hypertension. This must be also a factor concerning the hypertensive state. From the histochemical findings on ATPase activity of the renal vessels of SH rats, the interlobular arteries might be more intimately related with the severity and duration of hypertension. It cannot be settled at present, however, whether these changes in renal vessels play some role as a cause of hypertension in SH rats or occur rather as an effect of it.

The pattern of distribution of noradrenalin-storing cell islets in the adrenal medulla of a rat, using the glutaraldehyde-osmium tetroxide technique is reported to correspond to that observed after the application of the iodate or fluorescent methods³⁶⁾⁶⁴⁾. Two possible causes can be considered from the finding, observed in the adrenal medulla of SH rats with this technique, that the noradrenalin-storing cell islets of SH rats indicate a stronger reaction with about twice the mean dimensional ratio to the whole adrenal medulla than is found in normotensives through all stages. One is the increase of synthesis of noradrenalin in these reaction positive cells, the other is the increase of retention of noradrenalin because of the reduction of release. This latter is

unaffected by the production rate of noradrenalin in these cells which is normal or below average. On the other hand, the noradrenalin content of the adrenals of SH rats was reported to show a slight increasing tendency in the hypertensive state (This was announced in only a brief excerpt⁶⁵⁾ and in personal communication by OZAKI.). Further, according to the unpublished data of the Department of Pathology, Faculty of Medicine, Kyoto University, (OKAMOTO, YAMORI and SUZUKI), the noradrenalin content of adrenals of SH rats significantly indicated about double that of normotensives in the initial stage of hypertension. This evidence coincides with the above histochemical finding.

The adrenal medulla is mainly innervated by the sympathetic nervous system without effective counteraction of the parasympathetic system. If this histochemical finding depends on the former, the enhancement of the noradrenalin synthesis, it may be induced that overactivity of the sympathetic nervous system exists in SH rats from the pre-hypertensive stage and that the release of hormone also increases. Anyway, from this finding concerning the adrenal medulla of SH rats alone, it cannot be decided which interpretation is more probable.

Increase of MAO activity in the heart muscle of SH rats was detected after the development of hypertension with the histochemical technique after GLENNER²⁵⁾, using tryptamine hydrochloride as substrate. Though this finding may be partly due to the specific reaction to tryptamine as substrate, it seems to be mostly due to the presence of a real increase of MAO activity itself. NOVICK⁶⁶⁾ obtained a significant increase of MAO activity in the heart after administration of thyroid hormone. Consequently there is good evidence to assume that increase of cardiac MAO activity is partly caused by the thyroid hyperfunction in SH rats. CROUT et al.⁶⁷⁾ called MAO to account as the most relevant enzyme concerning the metabolism of endogenous noradrenalin in the heart of the rat. There is information to the effect that cardiac noradrenalin stores of SH rats tend to rise slightly after the development of hypertension (OZAKI

in a brief excerpt⁶⁵⁾ and personal communication). Moreover, in SH rats, dominant cardiac hypertrophy was observed after the incidence of hypertension, so that the cardiac catecholamine stores and MAO activity also seem to be playing a greater part in the mechanism of hypertension. On the basis of these changes found in the adrenal medulla and heart muscle, it should be conceived that the catecholamine synthesis and metabolism, such as noradrenalin synthesis in the adrenal medulla, cardiac catecholamine stores and its metabolism, primarily the oxidative deamination, in the heart muscle of SH rats increase generally in the hypertensive condition and as a result of these, the mechanism terminating the biological response to catecholamine as an important role in the heart is obviously promoted after the development of hypertension in SH rats.

It has been observed that there are no significant changes in heart and adrenal catecholamine content in dogs with renal or neurogenic hypertension, both of which were examined 2-3 weeks after operation⁶⁸⁾, or in rats with renal hypertension which were sacrificed 8-12 weeks after operation⁶⁹⁾. Augmented cardiac adrenalin content has been reported in some cases of chronic condition of essential hypertension⁷⁰⁾. There have been no reports, however, on a regular change in the cardiac MAO activity in hypertension. Considering this, the presence of marked elevation of MAO activity in the heart muscle of SH rats after the development of hypertension may be caused by superimposition of the factors that these animals survive with the higher extent and the longer duration of hypertension, and that they may show some characteristic reaction in the heart.

The urinary excretion of catecholamine was not significantly changed in chronic renal hypertensive dogs, 3-8 weeks after operation⁷¹⁾. Although patients with essential hypertension have been reported to excrete slightly elevated⁷²⁻⁷⁷⁾, normal^{76), 78-85)}, and reduced⁸⁴⁾ amounts of noradrenalin and its metabolites in their urine, there is little doubt that the majority of them excreted no more than normal quantities. As generally noted, few positive results have been reported concerning the role of

catecholamine in experimental and essential hypertension from such points of view as, for instance, the content in organs or volume of excretion. On the other hand, in spite of reports that significant increase of catecholamine has seldom been recognized in hypertension, especially in the essential type, most kinds of antihypertensive drugs are substances which act to tranquilize the autonomic nervous system, to inhibit the secretion or the synthesis of catecholamine or to promote the release of it.

Considering this, changes detected in noradrenalin reaction of the adrenal medulla and in MAO activity of the heart muscle of SH rats seem possibly to perform some significant role concerning the development and maintenance of hypertension in these animals. Furthermore, from this standpoint, increase of the mean dimensional ratio of noradrenalin-storing cell islets in the adrenal medulla of SH rats might have another important meaning as an indicator of the existence of the sympathetic overactivity in these animals.

Enhancement of vascular reactivity to noradrenalin has become known in experimental and essential hypertension⁸⁵⁾⁸⁶⁾. Moreover, thyroxin and glucocorticoids potentiate the effects of catecholamine, primarily noradrenalin to the vascular wall⁸⁷⁾⁸⁸⁾. RAAB⁸⁹⁾ suggested that the reactivity of the vascular wall was augmented by the increase of the sodium amount in it and such vessels became more sensitive to aldosterone or angiotensin. These elements may be also responsible for the mechanism of hypertension in SH rats.

From the evidence that the histochemical changes in the adrenal cortex and medulla, and thyroid and histological findings in the anterior pituitary⁹⁾ were observed in SH rats from the pre-hypertensive stage and that these findings showed an increasing tendency according to age, the following presumption seems to be applicable: Namely, in SH rats, from the pre-hypertensive stage, hyperfunction of the adeno-hypophyseo-adrenocortical axis, the adeno-hypophyseo-thyroid system and, in addition, possible overactivity of the system of sympathetic nerve-adrenal medulla exist coincidentally. These organized changes may

play the principal part in producing hypertension in SH rats.

SUMMARY

Spontaneously hypertensive rats (OKAMOTO and AOKI) were sacrificed in the pre-hypertensive stage (40 to 60 days after birth), initial stage (5 to 7 months after birth) or advanced stage of hypertension (13 to 17 months after birth). Twenty-one kinds of enzyme activity were examined histochemically in the adrenals, thyroid, kidneys, heart, liver and pancreas, and noradrenalin reaction was also applied to the adrenal medulla. Each positive finding in spontaneously hypertensive rats was obtained by comparing it with the reaction of the same sex normotensive Wistar strain rats with almost the same survival duration.

1) Glucose-6-phosphate dehydrogenase and triphosphopyridine nucleotide diaphorase activity increased in the zona fasciculata and reticularis of the adrenal cortex of spontaneously hypertensive rats with dominant elevation of the former activity in the middle and outer part of the fascicular zone from the pre-hypertensive stage. More prominent increase was observed after the incidence of hypertension.

2) The noradrenalin-storing cell islets in the adrenal medulla of spontaneously hypertensive rats indicated a stronger reaction with about twice the mean dimensional ratio to the whole adrenal medulla in all stages than was found in normotensives.

3) Acid phosphatase activity in the follicular cells of the thyroid in spontaneously hypertensive rats was elevated from the pre-hypertensive stage.

4) Adenosine triphosphatase activity of the renal interlobular arteries of spontaneously hypertensive rats showed an increase in about half the cases in the pre-hypertensive stage and in all cases after the incidence of hypertension.

5) Glucose-6-phosphate dehydrogenase and triphosphopyridine nucleotide diaphorase activity in the zona glomerulosa of the adrenal cortex of spontaneously hypertensive rats rose after the incidence of hypertension, though

the latter activity showed only a slightly increasing tendency.

6) Increase of adenosine triphosphatase activity in the walls of renal interlobular arteries and vasa afferens of spontaneously hypertensive rats was detected in all cases after the incidence of hypertension.

7) Gradual elevation of monoamine oxidase activity in the heart muscle of spontaneously hypertensive rats occurred after the incidence of hypertension.

8) No constant difference was found in the results of other kinds of enzyme histochemical comparisons of adrenals, thyroid, kidneys or heart, or in the enzyme histochemistry of the liver or pancreas, either.

9) From the above-mentioned evidence, it may be assumed that there exists from the pre-hypertensive stage in spontaneously hypertensive rats, an increase of (1) glucocorticoids synthesis in the adrenal cortex; (2) either synthesis of noradrenalin, or retention of it in the adrenal medulla; (3) secretory activity of the thyroid and (4) the contracting tendency of the renal vessels of medium caliber. In addition, after the incidence of hypertension, elevation of (5) aldosterone synthesis in the zona glomerulosa of the adrenal cortex; (6) the contraction of the renal vessels and (7) the catecholamine metabolism, primarily the oxidative deamination in the heart muscle is also indicated.

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