Morphological Studies on the Peripheral Small Arteries of Spontaneously Hypertensive Rats*

KUNIO ICHIJIMA†

OKAMOTO and AOKI have developed a colony of spontaneously hypertensive Wistar rats^{1,2} with a 100% incidence of spontaneously occurring hypertension. These rats would seem to be excellent material for experimental research on the essential hypertension of humans. Therefore, extensive studies have been performed in our laboratory on the endocrine system,³⁻⁹ cardiovascular system,¹⁰⁻¹² autonomic nervous system¹³⁻¹⁷ and hypothalamus^{18,19} in an effort to elucidate the pathogenesis of essential hypertension. These studies have shown that there is adenohypophyseal-adrenocortical and adenohypophyseal-thyroid hyperfunction, and overactivity of the autonomic nervous system as well as of the hypothalamus and adrenal medulla. These disturbances may well cause changes in the cardiovascular system. OKAMOTO et al.¹⁰ have reported histopathological studies on the cardiovascular system in the spontaneously hypertensive rats mainly in the late stage of hypertension; further studies of the peripheral vascular system, especially in the early stage, are required to provide a morphological basis for these disturbances.

It is obvious that generalized and persistent constriction or an increased wall/lumen ratio^{20-22,24} of small arteries and arterioles may be responsible for the increased peripheral resistance which is characteristic of hypertension. The nature of vasoconstriction has been the subject of much investigation and speculation. It may be

defined as a decrease in the diameter of the lumen of a blood vessel. This reduction in caliber may be a result of thickening of the arterial wall (structural change) or of shortening of smooth muscle cells without any increase of substance in the arterial wall (functional change). It is well known that chronic hypertension is accompanied by structural changes in the small arteries and arterioles which may be due to proliferation, degeneration or deposition of various substances in the arterial wall with or without inflammation.^{20-22,25-28} Since it is generally accepted that these structural changes are a consequence of angiospasm,^{2,9} it seems important to investigate the nature of angiospasm which may be related to the pathogenesis of hypertension. Angiospasm is generally defined as a reversible change of the vascular wall, and it may be due to contraction of smooth muscle cells in response to a high intraluminal pressure, increased sympathetic activity, elevated circulating substance or irritability of the smooth muscle cells themselves. BYROM^{29,30} has shown angiospasm, which disappeared completely when hypertension was relieved, in both the brain and retina of renal hypertensive rats, and suggested that it was a direct local response to the physical strain of excessive intra-arterial tension.

Satisfactory measurements of the arteries and arterioles cannot be made in ordinary histological preparations because they are collapsed and contracted in various degrees. Quick freezing and

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Department of Pathology, Faculty of Medicine, Kyoto University, Kyoto

(Director: Prof. K. Okamoto)

† Present Address: Department of Clinical Pathology, Kochi City Hospital, Kochi, Japan

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the freeze-substitution method³¹ have the tremendous advantage of reducing the distortion and preserving the functional stage of the blood vessels.³¹⁻³³

The innervation of small arteries and arterioles is considered to be sympathetic in regard to blood pressure homeostasis,³⁴ and it is now generally accepted that noradrenaline is the transmitter of the sympathetic adrenergic system in mammals. The results obtained by the ordinary silver impregnation method^{35,36} or the *intra vitam* method of staining with methylene blue³⁷ for sympathetic nerve fibers around the blood vessels were not always constant probably because of their technical difficulty and specificity. FALCK³⁸ and FALCK and OWMAN⁴⁰ have described a fluorescence method which can demonstrate noradrenaline in the adrenergic fibers. This method has facilitated the morphological study of sympathetic innervation of the blood vessels. Up to the present, however, the morphology of the sympathetic nervous system around the arteries in hypertensive subjects has received little attention except for one study of renal hypertensive rats⁸⁹ and two of spontaneously hypertensive rats.^{12,17}

The enzyme-histochemical method is thought to be one of the most valuable morphological approaches to cell function, and adenosine triphosphatase activity is very probably involved in the contraction of muscle cells.^{41,42} In spontaneously hypertensive rats TABER⁶ showed the adenosine triphosphatase activity was increased in the interlobular arteries of the kidney even in the pre-hypertensive stage.

The present study concerns the morphological changes of the peripheral small arteries and arterioles in spontaneously hypertensive rats as demonstrated by direct observation and by histological, histometric, enzyme-histochemical and fluorescence methods in an attempt to provide additional morphological support for the many reports which have already been published on the abnormalities in spontaneously hypertensive rats. Some preliminary results were reported earlier¹² (in Japanese). The small arteries and arterioles of the jejunum were chosen for this study because evidence^{21,25,26} suggested that these are representative peripheral vascular beds in hypertension. There is also the practical advantage that they are easy to observe and to excise.

MATERIALS AND METHODS

The Wistar strain rats in the control group were supplied by the Animal Center Laboratory, Faculty of Medicine, Kyoto University or were bred from these rats in the Department of Pathology under normal conditions. Only normotensive rats (systolic blood pressure below 149 mmHg) were selected and used as controls. The hypertensive group consisted of F₁₀ to F₁₆ rats of the spontaneously hypertensive rats (OKAMOTO and AOKI) (abbreviated as SHR henceforth). The details of the method of obtaining the SHR and their body weights and blood pressure have already been reported^{1,2} and are summarized briefly as follows: From among the Wistar strain rats, supplied by the Animal Center Laboratory, Faculty of Medicine, Kyoto University, one male rat (P₁) with distinctly high blood pressure (145 to 175 mmHg) and one female rat (P₂) with a blood pressure slightly above the average (130 to 140 mmHg) were selected. After the male rat's blood pressure had remained above 150 mmHg for over a month, he was mated with the female rat to obtain F₁ rats. Of the F₁ rats, males and females with hypertension persisting for over a month were selected and mated to obtain F₂ rats. The same process was repeated successively. These rats showed 100% occurrence of spontaneous hypertension within the first five months of life. These F₉ rats were employed in the present study to obtain F₁₀, F₁₁, F₁₂, F₁₃, F₁₄, F₁₅ and F₁₆ rats by brother-sister breeding or cross breeding. They were divided into three groups according to age and development of hypertension: (1) 40 to 60 days after birth (the first stage), (2) four to six months after birth (the second stage) and (3) over one year (12 to 15 months) after birth (the third stage). In the SHR these stages were considered to correspond to the pre-hypertensive or transitional, initial and advanced stages of hypertension,² respectively. A few three-month-old SHR were also examined. In the present study a total of 181 rats of both sexes were used, including 84 controls.

These animals were housed in an air-conditioned room (22-24°C temperature and 50-60% humidity), fed with stock chow diet (Oriental MF, NMF and CMF, made by Oriental Yeast Co., Tokyo, or CE-2, CA-1 and OA-2, made by Nihon CLEA Co., Tokyo) containing about 0.3% sodium and sometimes green vegetables. All rats were given tap water to drink *ad libitum*.

1. General Observations

The blood pressure and body weight of all rats were measured once a week from four weeks of age until death. The systolic blood pressure was measured indirectly without anesthesia by

Fig. 1. Cross-sectional view of the abdomen of a rat with the abdominal window and vital microscope in position.

the modified tail-water plethysmographic method.¹

2. Direct Observations

The peripheral arterial patterns of the rats were observed in vivo through the very simple abdominal window described by PALMER and WILKINS⁴³ made of a glass or plastic coverslip. Prior to the experiment, rats were fasted for 12 to 24 hours, water being allowed ad libitum. Then they were anesthetized with α -chloralose (40 mg/kg, i.v.). A left femoral artery was exposed through a small incision in the groin and cannulated for recording arterial blood pressure kymographically by means of a mercury manometer, as described in an earlier paper.¹¹ A small dose of heparin was given intravenously to prevent coagulation in the cannula. In some cases, the right femoral vein was also cannulated for the intravenous infusion of drugs. Then the abdomen was opened in the midline and the abdominal window, warmed to body temperature, was inserted as gently as possible, and the muscular edge was fixed firmly by a thread to the ring of the abdominal window, as illustrated in Fig. 1. All these procedures were performed under identical conditions in all the animals examined. The amount of bleeding during these procedures was usually negligible. The vessels of the outer surface of loops of the upper jejunum were examined through a FSC capillary microscope for skin and mucous membrane (magnification of $\times 19$ and $\times 32$, made by Fuji Co., Tokyo), or a microscope (magnification of $\times 5$ to $\times 30$) designed in our laboratory for direct observations. Photographs of the fields were taken with a 35-mm Nikon camera attached to the microscope

with a xenon lamp or electric flash light. By this method it was possible to record the appearance of the jejunal vessels in normotensive control rats and SHR. After this observation, rats were sacrificed and examined to ensure that the jejunal portions observed were about 5 to 10 cm from the pylorus. Some rats were kept for the next histological procedure. The constrictions (segmental narrowing) and tortuosities of the jejunal small arteries and arterioles were estimated in each case and graded (—), (+) and (++) : (—), if the constrictions or tortuosities were very slight or absent, and (++) , if they were extreme.

The numbers of animals used in this study were as follows: In the control group, there were seven males in the first stage (numbers 140, 141, 144, 145, 146, T-1, T-2), 13 males in the second stage (107, 112, 132, 441, 153, 156, Y-7, E-14, D-19, 319, 351, 352, 353), and three females in the third stage (5, 54, 55). In the SHR group, there were six males (numbers 113001, 113003, 123002, 123003, 123004, 123005) and one female (113009) in the first stage, nine males (113003, 113045, 142018, 142019, 155049, 155050, 155051, 155052, 160006M) and three females (153012, 153013, 155048) in the three-month-old group, five males (142013, 153001, 153004, 153005, 153008) and seven females (10158, 113012, 113013, 113015, 113025, 113027, 153011) in the second stage, and two males (10151, 133025) and two females (10118, 123053) in the third stage. The direct femoral blood pressure and body weight of these animals are shown in Table I.

3. Histological and Histometric Examinations

In an attempt to preserve the jejunal small arteries and arterioles in fixed preparations, the jejunal wall was quick-frozen by pouring isopentane cooled to -170°C by liquid nitrogen into the abdominal cavity of rats anesthetized by α -chloralose (40 mm/kg, i.v.) and cannulated as described above for recording arterial blood pressures kymographically. After freezing, portions of the intestine (2 to 3 cm in length) which were the upper part of the jejunum (about 5 to 10 cm from the pylorus) corresponding to the areas studied during direct observation, were quickly excised and placed in a 1.0% acetone solution of osmium tetroxide at -70°C for one week to permit freeze-substitution. They were then washed in acetone at -70°C three times by the technique described by FEDER and SIDMAN.³¹ Subsequently they were permitted to regain room temperature and were opened along the mesenteric border of the jejunum. The outer sides of the jejunal wall, which were directly cooled by isopentane, were excised and cleared by a mixture of methylbenzoate and oil of anise

Fig. 2. Diagram illustrating the First, IInd, IIIrd and IVth branches of the jejunal small artery and arteriole.

Fig. 3. Diagram illustrating the minimal, maximal and mean diameters in each branch shown in Fig. 2.

S: the dimensional area of the branch,
L: the mean length of the branch.

without staining, laid flat on a slide glass and mounted in balsam as described by GIESE⁴⁴. The preparations were studied under light microscope. The vessels could then be compared with their appearance in the photographs of the living jejunum. For histometry, the preparations were magnified by microprojector (Leitz Typ. Xlc Xenon, Kr. 90014, Ernst Leitz GMBH., Wetzlar) and transcribed on section-paper (final magnification of $\times 300$). The branches of the jejunal arteries were named the First, IInd, IIIrd and IVth branches according to the following criteria: After reaching the mesenteric border of the jejunum, the artery (mural trunk) was named the First branch till it divided at its bifurcation into two branches of almost the same caliber. Then from the point of the bifurcation, the next two branches were named IInd branches till they bifurcated with almost the same caliber. The IIIrd and IVth branches were named in the same way (Fig. 2). The narrowest and widest caliber of each branch was measured and named as the minimal and the maximal diameters, respectively. Then the area and length of each

branch was measured. The length of the arterial branch was measured along the central line of the contour of the branch, then the mean diameter (area/length) was calculated (Fig. 3.). Thus the minimal, maximal and mean diameters of the small arteries and arterioles of the jejunal wall were measured and expressed in μ . These average values of 6 to 21 arteries (mural trunks) and their branches were determined for each rat. Then the minimal, maximal and mean diameters of each group were calculated for the Ist, IInd, IIIrd and IVth branches, respectively. Each group contained 10 to 12 rats. Thus, 6294 branches, 3298 from normotensive control rats and 2996 from SHR were examined. The data thus obtained from the controls and SHR groups were analyzed statistically with Student's "t" test. The data were considered to be significant when the "P" values were less than 0.05. In occasional cases, a number of small segments of these arteries were excised from the tissue specimens with a sharp scalpel under a stereomicroscope. They were embedded in paraffin and sectioned serially at 5μ so as to make the cross sections perpendicular to the longitudinal axis of the artery. Alternate sections were stained with hematoxylin and eosin, and a Weigert's elastic procedure counterstained with van Gieson stain.

A total of 31 normotensive control rats and 32 SHR of both sexes were examined. The numbers of animals used in this study were as follows: In the control group, there were 11 males in the first stage (numbers 101, 102, 103, 104, 106, 162, 163, 164, 165, 166, 167), 10 males in the second stage (142, 148, 149, 151, 153, 155, 444, E-10, Y-5, Y-9), and 10 females in the third stage (2, 180, 2-Y, 3-Y, F-1, H-1, O-1, O-3, T-67, Y-20). In the SHR group, there were 12 males in the first stage (numbers 123001, 123008, 123048, 123049, 123050, 133003, 133004, 133005, 133015, 133016, 133017, 133018), 10 males in the second stage (110228, 123006, 123007, 123028, 123039, 123041, 123042, 133001, 133002, 133023), and 10 females in the third stage (10022, 10036, 10070, 10251, 110063, 110070, 110127, 110129, 110131, 113026). The final direct femoral blood pressure and body weight of these animals are shown in Table II.

4. Enzyme-Histochemical Examinations

Blood pressure and body weight were measured indirectly prior to the experiment. After anesthesia induced by α -chloralose and midline laparotomy, the upper portions of the jejunal wall were quickly frozen by cooled isopentane, as described above. Then the tissue specimens were excised and placed in cooled 6.25% tris aminomethane-maleate buffer solution of glutar-

aldehyde at 0°C to 4°C for 30 minutes. The jejunal small arteries and arterioles could be seen under a stereomicroscope in almost the same patterns as in direct observations or in histometry. Proper portions of the jejunum were then selected and excised along the arteries. Next the specimens were soaked in liquid n-hexane and indirectly frozen again by a mixture of crushed solid carbon dioxide and acetone through the medium. These frozen blocks were then stored in a deep freezer temporarily. Forty serial sections (at a distance of 800 μ) were made at intervals of 400 μ by a DITTES-DUSPIVA cryostat (Germany) maintained at -20°C with a thickness of 20 μ . Alternate sections were placed in small cells and stained for two enzyme activities.

Two enzyme activities (adenosine triphosphatase and adenosine monophosphatase) were tested for in these sections from matched controls and SHR at the same time by WACHSTEIN and MEISEL's method⁴⁵ modified slightly in the preparation of the incubation medium as follows: 1 ml of 0.1M magnesium sulfate solution and 4 ml of 0.2M tris aminomethane-maleate buffer at pH 7.2 were mixed and added to distilled water to a total volume of 8.6 ml, followed by the addition of 0.4 ml of 2% lead nitrate solution drop by drop gently to form a coarse turbid product. The substrates for the ATPase and AMPase were 5 mg. The incubation medium was obtained by filtration of the mixture. For staining, the sections were incubated in each medium at 37°C for periods of 30 minutes. The sections were then washed thoroughly in distilled water and immersed in diluted yellow ammonium disulfide solution for about three minutes. After washing in water, the sections were arranged on a slide glass and mounted in Apáthy gummy syrup. Throughout these entire enzyme-histochemical processes, from sacrifice to slide mounting, sections from controls and SHR were examined simultaneously and the sections were mounted in pairs on the same slide glass. Thus, about 120 to 180 sections from controls and SHR were mounted in one slide glass. Findings were compared by placing sections from controls and SHR on the same slide and examining by microscope. In this study, broad zones of reactions were noted, with no attempt at finer cytologic and organelle localization.

A total of 19 control rats and 19 SHR of both sexes were examined. The numbers of animals used in this study were as follows: In the control group, there were six males in the first stage (numbers 239, 241, 266, 267, Y-1,* Y-2*), six males in the second stage (192, 222,* 227,* 242, 243, M-709), and five males (61, 159, 194, 195, S-62) and two females (187, 189) in the third stage. In the SHR group, there were six males in

the first stage (143020, 143022, 150298,* 150301,* 153014, 153015), six males in the second stage (133051, 143019, 143021, 150205,* 150221,* 150226), and five males (123027, 123030, 123060, 123061, 125014) and two females (123034, 123038) in the third stage. Animals with asterisks were also used in the next fluorescent examination. The final indirect blood pressure and body weight of these animals are shown in Table III.

5. Fluorescent Examinations

The upper portions of the jejunum were removed as described above, but usually the tissue specimens examined in this experiment were smaller. Some modifications of the original fluorescence method introduced by FALCK³⁸ and FALCK and OWMAN⁴⁰ were used. After freeze-drying at -35°C to -40°C for six days, the tissue specimens were treated with formaldehyde gas at 80°C for one hour. The specimens were then embedded in paraffin in vacuo. The details of the method were described earlier.¹⁷ Eighty serial sections (at a distance of 800 μ) were made at intervals of 400 μ with a thickness of 10 μ , and alternate sections were either mounted in a mixture of xylene and Entellan (Merck), or stained with hematoxylin and eosin as controls. Care was taken to cut the cross sections at a right angle to the longitudinal axis of the blood vessels. A fluorescence microscope (Carl Zeiss) was used with a dark field condenser for oil immersion. Throughout these procedures, materials from matched controls and SHR were treated and examined simultaneously. All quantitative estimations were made from cross sections. For an objective analysis of the results, every fifth section of the 1st, 11th, 11th and 14th branches (illustrated in Fig. 2) of the jejunal arteries which were almost circular were photographed (every 100 μ) under the microscope with Tri-X film (Eastman Kodak Co., U.S.A.). Exposure time was three minutes. In occasional cases, arterial cross sections were photographed at closer intervals to follow the innervation along the course of a single artery. The fluorescence observed in the external muscular layer of the arteries and arterioles was ascertained by the following procedures and proved to be the neurotransmitter, noradrenaline, in the adrenergic nerve fibers.¹⁷ 1) When the tissue specimens were heated for one hour at 80°C in the desiccator without paraformaldehyde treatment, no fluorescence was seen. 2) Fluorescence disappeared after treatment with sodium borohydride solution, and then reappeared with re-treatment with formaldehyde vapour. 3) The administration of reserpine inhibited fluorescence, and the MAO-inhibitor, nialamide augmented it. Measure-

Fig.4. Arterial pattern of the jejunal wall in control rat (No.319, male, five months of age, direct femoral blood pressure 130 mmHg).

- A. Photograph of the jejunal wall of a living rat observed by vital microscope with abdominal window in position. The artery and vein run parallel to each other, and their calibers are almost uniform. No constrictions or tortuosities are seen. Maximal luminal diameter of the artery is 80μ . $\times 22$.
- B. Jejunal wall prepared by quick freezing and freeze-substitution method of the field shown in A. The arterial pattern is quite similar to that observed by direct observation. 1st and 2nd branches illustrated in Fig.2. are seen. $\times 22$
- C. A higher magnification of B. $\times 40$

ments of the vessels' diameters and of the cross-section areas of fluorescent dots or cloudlets around the vessels were made from the microphotographs (final magnification of $\times 700$), and expressed in μ and in μ^2 . No attempt was made to grade the intensity of the fluorescent dots or cloudlets. The data obtained from the controls and SHR groups were statistically analyzed using Student's "t" test, and the data were considered to be significant when the "P" values were less than 0.05. Thus, a total of 1172 arterial cross sections, consisting of 570 from 15 control rats and 602 from 15 SHR of both sexes were examined.

The numbers of animals used in this study were as follows: In the control group, there were five males in each stage. In the SHR group, there were also consisted of five males in each stage: in the first stage numbers 150298, 150301, 153023, 153024, 153025, in the second stage numbers 148006, 148007, 150205, 150221, 150226, and in the third stage numbers 123029, 123030, 133043, 133044, 133045. The mean final indirect blood pressure and body weight of these animals are summarised in Table IV.

RESULTS

All the findings of the SHR group were compared with those from the matched normotensive

control group of the same sex and with almost the same survival rate.

1. Blood pressure and Body Weight (Tables I, II, III and IV)

The mean systolic blood pressure, measured directly or indirectly, of the control group remained in the range of 75 to 140 mmHg throughout all stages. In the SHR group, it rose steadily with age and was significantly ($P < 0.01$) higher than that of the matched control group throughout all the stages (Tables II and IV). It ranged from 114 to 240 mmHg. In regard to body weight, the mean values of the SHR group seemed to be a little less than in the matched control group in some stages, but there was no statistically significant difference among them (Tables II and IV).

2. Direct Observations (Table I, Figs. 4A, 5A and 6)

1) Segmental Constrictions and Tortuosities of the Jejunal Small Arteries and Arterioles

The absence of unevenness in the surface of the jejunal wall simplifies the vascular picture, and the jejunal small arteries and arterioles can

Fig. 5. Arterial pattern of the jejunal wall in SHR (No. 153001, male, six months of age, initial stage of hypertension, direct femoral blood pressure 190 mmHg).

- A. Photograph of the jejunal wall of a living rat observed through vital microscope with abdominal window in position, showing typical appearance of bead-like structure of the small arteries and arterioles. Note the segmental constrictions alternating with un-constricted segments and marked tortuosities in the course of the artery. No particular changes are seen in the vein. Maximal luminal diameter of the artery is 70μ . $\times 22$
- B. The jejunal wall prepared by quick freezing and freeze-substitution method of the same field in A. The arterial pattern is quite similar to that observed by direct observation, and segmental constrictions and tortuosities are well preserved. 1st, IIrd and IIIrd branches illustrated in Fig. 2 are seen. $\times 22$
- C. A higher magnification of B. $\times 40$

easily be seen through the abdominal window as they run in one plane. The jejunal branches of the superior mesenteric arteries form a series of arcades (Fig. 2) which give rise to numerous straight vessels (vasa recta), measuring from 60μ to 150μ in diameter, supplying the jejunum. After reaching the attached border of the mesenterium, the arteries run between the serous and muscular coats (mural trunks) and divide and subdivide several times to form terminal arteries and arterioles (10μ to 20μ). Anastomoses are numerous at all levels. The veins are wider and flatter than the arteries. The arteries and veins run parallel to each other in the larger portions, but they run increasingly irregularly in the smaller portions.

In the normotensive control group, the calibers of the small arteries and arterioles are almost uniform, and they are slightly narrower than the veins. Constrictions or tortuosities were rare or even absent (Table I and Fig. 4A). In the first stage, no constrictions or tortuosities were observed. In the second and third stages, some rats (107, D-19, 351, 5, 55) showed slight variation in the calibers or tortuosities, but they were limit-

ed to a small percentage of the visible small arteries and arterioles (Table I). The degree of constriction or tortuosity seemed to increase with age.

In the SHR group, they showed almost the same arterial patterns as in the control group except for one in the first stage, rat (123005), with a blood pressure already higher than 150 mmHg (direct method), which showed segmental constrictions of a slight degree but no distinct tortuosities. After three months of age, obvious segmental constrictions and tortuosities of the jejunal small arteries and arterioles were seen and were striking features: i.e. irregular narrowing of one or more small arteries and arterioles, the uneven appearance being accentuated by un-constricted segments of the intervening parts and frequently by tortuosities of the vessels (Fig. 5A and 6A). They were more severe in the second and third stages, and less severe at three months of age. The degree of constriction seemed to be correlated with both the severity and the duration of hypertension. Constriction was present in all jejunal arterial channels less than 150μ as measured by the diameter of the contained blood

TABLE I. INDIVIDUAL FINDINGS OF ARTERIAL CONSTRICTIONS AND TORTUOSITIES IN JEJUNAL SMALL ARTERIES AND ARTERIOLES

Age	Case No. of Normo- tensive Control Rats	Systolic Blood Pressure (mmHg)	Body Weight at time of Experi- ment (gm)	Segmental Constric- tion**	Tortuo- sity**	Case No. of SHR	Systolic Blood Pressure (mmHg)	Body Weight at time of Experi- ment (gm)	Segmental Constric- tion**	Tortuo- sity**
40-60 Days	140♂	120	115	—	—	113001♂	140	146	—	—
	141♂	115-120	130	—	—	113003♂	130	125	—	—
	144♂	125	145	—	—	123002♂	140-145	120	—	—
	145♂	120-125	130	—	—	123003♂	140-145	122	—	—
	146♂	125	106	—	—	123004♂	130-140	106	—	—
	T-1 ♂	90	100	—	—	123005♂	150-170	135	+	—
	T-2 ♂	90	95	—	—	113009♀	140	95	—	—
3 Months						113003♂	140	280	+	+
						113045♂	175-190	340	++	+
						142018♂	170-185	308	++	++
						142019♂	170	270	+	+
						153012♀	168	250	++	+
						153013♀	170	235	++	+
						155048♀	175	235	++	++
						155049♂	180	280	++	++
						155050♂	160-190	295	++	++
						155051♂	160	320	+	+
						155052♂	190	354	++	++
						160006M♂	185	360	+	+
4-6 Months	107♂	120	308	+	+	10158♀	176	202	++	+
	112♂	125-135	352	—	—	113012♀	160-170	210	+	+
	132♂	130	316	—	—	113013♀	174	232	++	+
	441♂	130	262	—	—	113015♀	154	206	+	+
	153♂	120-125	364	—	—	113025♀	150	236	+	+
	156♂	130	370	—	—	113027♀	175	220	++	++
	Y-7 ♂	105-140	382	—	—	142013♂	180	280	++	++
	E-14 ♂	120	412	—	—	153001♂	190	362	++	++
	D-19♂	140	376	+	+	153004♂	190	386	++	++
	319♂	130	305	—	—	153005♂	200	340	++	++
	351♂	135	262	—	+	153008♂	220	350	++	++
	352♂	128	308	—	—	153011♀	230	205	++	++
	353♂	130	326	—	—					
Over 1 Year	5 ♀	130	235	+	+	10151♂	180	380	++	++
	54 ♀	120-125	210	—	—	10118♀	180	265	++	++
	55 ♀	75-95	250	+	+	123053♀	175	230	++	++
						133025♂	195	320	++	++

SHR : Spontaneously Hypertensive Rats

** : Grade of Constrictions and Tortuosities

— : Slight or Absent

+ : Moderate

++ : Marked

column, and it was more prominent in the smaller arteries or arterioles measuring 10μ to 50μ . Irregular segmental constrictions from a few micron to a mm or so in length were seen alternating with un-constricted segments where the caliber was either normal or somewhat dilated. When the segmental constrictions were close together, the un-constricted segments were bulbous and the arteries seemed beaded, but when the segmental constrictions were widely separat-

ed, the un-constricted segments were like a string of tiny sausages. Occasionally, the constricted segments showed such a marked degree of narrowing as to be almost invisible. These changes, though widespread, were usually patchy and some vessels escaped. Uniform dilatations of either larger or smaller arteries were never seen. The constrictions showed no tendency to change in position during more than 12 hours of observation and were only rarely observed to relax in

Fig.6. Disappearance of constrictions and tortuosities by systemic blood pressure fall in SHR (No.155049, male, three months of age).

- A. Photograph of arterial pattern of the jejunal wall observed through vital microscope with abdominal window in position. Segmental arterial constrictions and tortuosities are demonstrated. Systolic blood pressure was 180 mmHg by direct method. $\times 22$
- A'. A higher magnification of the rectangle of A. $\times 80$
- B. The same field twenty minutes after intravenous injection of hexamethonium (20 mg, 0.4 ml/kg). Segmental constrictions and tortuosities are no longer seen, and arterial branches seem close to each other. Systolic blood pressure was 100 mmHg by direct method. $\times 22$
- B'. A higher magnification of the rectangle of B. $\times 80$

smaller arteries or arterioles. No constant changes were observed in the jejunal veins in SHR. The degree of constriction and tortuosity of the small arteries and arterioles in each case is shown in Table I.

2) Variation in the Segmental Arterial Constrictions and Tortuosities with Systemic Blood Pressure Change

While the jejunal vessels were under continuous observation, a hypotensive agent was used in some SHR (155049, 142019, 153011, 133025) to restore the blood pressure to normotensive levels. Hexamethonium (20 mg, 0.4 ml/kg or more) was given intravenously. It caused a sudden fall of blood pressure, and the first discernible arterial and arteriolar dilatations appeared when the blood pressure was lowered sharply. This was accompanied by a shortening of the jejunal wall along the long axis, following the increase of peristaltic activity, and the arteries seemed close to each other. After 20 to 30 minutes, in some cases (155049, 142019), the constrictions disappeared and one could no longer distinguish between constricted and unconstricted segments, and the tortuosities also disappeared (Fig. 6); but in other cases (133025,

153011), the constrictions seemed to persist, though to a lesser degree. These findings were confirmed cinematographically (unpublished data). In one case (113013) blood pressure fell spontaneously to hypotensive levels, but some segmental constrictions were always present.

3) Effect of Local Pressure

The effect of local pressure was studied by applying a blunt sewing-needle to the exposed surface of the jejunal wall. Segmental constriction of the artery appeared at this point, but it disappeared in a few minutes. No difference in responsiveness between controls and SHR was observed. Even when the abdominal window was removed, the arterial patterns did not seem to change. The local pressure caused by the abdominal window, therefore, seemed to have little effect on arterial caliber.

4) Effect of Local Vasodilator

In some SHR (155048, 155050, 155051, 155052, 160006M, 142018), the effect of a vasodilator on arterial segmentation was demonstrated by soaking cotton pledgets in 1.0% papaverine warmed to body temperature and applying them to a small area of the jejunum for 30 to 60 seconds. While the systemic blood pres-

Fig. 7. Histological appearance of the jejunal small artery prepared by quick freezing and freeze-substitution method (paraffin section, Weigert's elastic procedure counterstained with van Gieson stain). $\times 400$

- A. Control rat (No.148, male, five months of age, direct femoral blood pressure 123 mmHg). Internal elastic lamina is smooth and the wall is very thin. Internal diameter is 80μ .
- B. SHR (No.133001, male, five months of age, initial stage of hypertension, direct femoral blood pressure 180 mmHg). Un-constricted segment. The appearance is almost the same as control (A), but the muscular wall and internal elastic lamina seem a little thicker than in the control. No arteriosclerotic changes are observed. Internal diameter is 75μ .
- C. Constricted segment of the artery shown in B. The distance between B and C is 300μ . Internal elastic lamina show undulation, and smooth muscle cells are shortened to increase the thickness of the wall. Internal diameter is 30μ .

sure remained unaltered, marked arterial dilations occurred two to five minutes after local papaverine application, and it was impossible to distinguish between constricted and un-constricted segments in some cases (155051, 155052, 160006M, 142018). About 30 to 60 minutes later, in some cases (160006M, 142018), the segmental constrictions reappeared gradually at the same points, and the arteries regained their previous calibers. In other cases, however, the arteries did not seem to return to their previous calibers, but this was impossible to determine accurately because of edematous changes in the jejunal wall. And in some cases (155048, 155050), although the degree of constriction was less, segmentation was persisted for over one hour.

3) Histological and Histometric Examinations (Table II, Figs. 4B, C, 5B, C and 7)

The specimens prepared by the quick freezing and freeze-substitution method showed vascular patterns similar to those seen in vivo (Figs. 4 and 5).

In the normotensive control group, serial

sections of the arteries showed that the walls of the arteries were quite thin in relation to the size of their lumina. The endothelial cells lining the lumen were flat and quite thin and had elongated nuclei. The internal elastic lamina was not wrinkled, but appeared as a thin circular ring without convolutions. The smooth muscle cells were long and thin, surrounding the intima in a narrow shell of uniform thickness. The lumen was packed with red blood cells (Fig.7A). Occasionally, slight wrinkling of the internal elastic lamina was seen, but marked convolutions were never seen in any of the controls at any stage.

In the SHR group, it was possible to observe the entire sequence of histological changes in a single artery in serial sections extending from the un-constricted area through the region of intense vasoconstriction. In the un-constricted segments, the appearance was almost the same in cross section in controls; the muscular wall and internal elastic lamina seemed a little thicker (Fig. 7B), but no noticeable arteriosclerotic changes could be found in the first and second stages. In the third stage, however, some showed changes like those seen in arteriosclerosis or periarteritis

nodosa. In the constricted segments, endothelial cells were columnar in shape, smooth muscle cells appeared shortened, the wall thickened, the luminal diameter reduced, and the internal elastic lamina markedly convoluted (Fig. 7C). Again no noticeable arteriosclerotic changes could be found in the first and second stages. The lumen was packed with blood cells. It is obvious that the blood column in the vessels, transparent in these preparations, shows the internal diameter of the arteries. The average length of almost all arterial branches was longer in SHR than in matched controls (Table II), probably because of tortuosities, but this difference was not statistically significant. The slightly greater length may be due to the longitudinal constrictions of the arteries, because arterial smooth muscle cells are arranged in a helical fashion.⁴⁶

The mean, minimal and maximal diameters of the Ist, IInd, IIIrd and IVth branches (abbreviated hereafter as I, II, III and IV branches) of each rat and the mean values for each group are summarized in Table II. As might be expected, the greatest difference between controls and SHR was in the minimal diameters, the minimal diameters ranging from 64% to 85% those of the controls. In the first stage, the mean minimal diameters of I, II and IV branches in 12 SHR (38.1 ± 7.4 , 20.2 ± 4.7 , 9.6 ± 3.1 , respectively) were significantly smaller than in 11 matched controls (48.7 ± 5.5 , 24.8 ± 4.9 , 11.4 ± 2.3), but there was no significant difference in III branch, though it was smaller (13.0 ± 4.2) than in matched control (15.3 ± 2.9). In the second stage, all branches were significantly smaller in 10 SHR (36.1 ± 8.6 , 19.7 ± 3.0 , 13.0 ± 2.3 , 8.3 ± 1.2) than in 10 matched controls (48.8 ± 6.6 , 25.2 ± 4.0 , 16.7 ± 2.5 , 12.2 ± 2.0). In the third stage, also all branches were significantly smaller in 10 SHR (30.1 ± 4.7 , 16.6 ± 3.1 , 11.2 ± 3.5 , 7.3 ± 2.8) than in 10 matched controls (41.6 ± 7.1 , 23.5 ± 5.2 , 15.9 ± 3.5 , 11.4 ± 2.5).

The mean diameters of I, II and IV branches were significantly smaller in SHR (53.9 ± 8.1 , 29.1 ± 5.6 , 13.7 ± 3.7) than in matched controls (63.4 ± 5.6 , 34.2 ± 4.7 , 16.2 ± 2.4), but there was no significant difference in III branch in the first stage, though it was smaller (18.5 ± 4.6) than in matched controls (22.0 ± 3.6). In the second stage, I and IV branches were significantly smaller in SHR (58.0 ± 6.4 , 15.2 ± 2.0) than in matched controls (71.2 ± 9.1 , 17.3 ± 2.1), but there was no significant difference in II and III branches, though they were a little smaller (35.2

± 4.7 , 23.6 ± 3.4) than in matched controls (36.3 ± 5.7 , 23.8 ± 3.1). In the third stage, all branches were significantly smaller in SHR (47.6 ± 5.4 , 29.1 ± 3.8 , 20.5 ± 4.5 , 13.6 ± 3.6) than in matched controls (62.4 ± 11.2 , 35.7 ± 6.7 , 24.7 ± 4.6 , 18.6 ± 3.4).

The maximal diameters showed the least difference between controls and SHR. In the first stage, I, II and IV branches were significantly smaller in SHR (74.3 ± 7.2 , 40.7 ± 6.4 , 19.8 ± 5.1) than in matched controls (84.3 ± 7.7 , 46.4 ± 5.3 , 21.5 ± 2.3), but there was no significant difference in III branch, though it was smaller (26.6 ± 6.2) than in matched controls (29.9 ± 4.8). In the second stage, I branch was significantly smaller in SHR (84.7 ± 7.8) than in matched controls (97.4 ± 12.5), but there was no significant difference in II, III and IV branches between SHR (56.7 ± 6.7 , 38.7 ± 7.8 , 25.5 ± 5.0) and matched controls (52.4 ± 8.8 , 34.8 ± 6.5 , 25.5 ± 3.6), the diameters in SHR being the same or even larger than in the controls. In the third stage, I and II branches were significantly smaller in SHR (70.7 ± 6.2 , 47.5 ± 5.3) than in matched controls (83.5 ± 13.6 , 55.1 ± 7.4), but there was no significant difference in III and IV branches, III branch being even a little larger (36.2 ± 7.5) in SHR than in matched controls (34.8 ± 7.5) although IV branch was smaller in SHR (23.3 ± 5.2) than in matched controls (27.1 ± 5.3).

4. Enzyme-Histochemical Examinations (Table III, Fig. 8)

The increase of enzyme activity was scored as follows:

- ↑ : slightly increased
- ↑ : moderately increased
- ↑↑ : markedly increased
- : no difference
- ↓ : slightly decreased

The changes of ATPase and AMPase activities are summarized in Table III.

1) ATPase Activity

ATPase reactions were observed in the arterial walls of the normotensive control group in all stages. Intense reactions were detected in the media (Fig. 8A). However, arteries were not always stained with the same intensity, but showed considerable variation. But the findings were estimated as a whole in this study. The activity tended to rise slightly with age. Among a total of six SHR in the first stage, there was a slight increase in three, a slight decrease in one, and no change in two. In the second and third stages, an

TABLE II. MEAN, MINIMAL AND MAXIMAL LUMINAL DIAMETERS OF THE FIRST, IIND, IIIRD AND IVTH BRANCHES OF JEJUNAL SMALL ARTERIES

Age	Rats	Case No. of Rats	B.P.	B.W.	I				II				III				IV											
					No.		Mean		Min.		Max.		No.		Mean		Min.		Max.		No.		Mean		Min.		Max.	
					Br.	L.	D.	D.	Br.	L.	D.	D.	Br.	L.	D.	D.	Br.	L.	D.	D.	Br.	L.	D.	D.	Br.	L.	D.	D.
Control		101♂	125	92	10	12.6	57.1	40.8	77.2	19	11.5	26.0	16.6	38.3	10	7.1	20.1	13.0	28.6	9	6.1	15.5	9.7	22.7				
		102♂	130	100	11	17.4	64.9	44.0	99.9	18	13.0	33.9	21.3	54.6	13	7.8	18.0	11.0	29.0	2	6.4	16.3	10.8	22.5				
		103♂	125	112	11	13.6	53.6	44.9	80.9	22	12.1	30.9	22.7	43.2	38	6.9	18.1	13.0	24.1	29	5.7	13.3	9.9	18.0				
		104♂	130	140	12	18.1	63.4	47.5	90.0	22	16.2	31.1	20.7	45.6	25	8.5	19.7	13.5	28.6	14	5.6	15.9	11.1	22.1				
		106♂	130	105	6	13.6	58.2	41.7	78.3	12	18.1	31.4	21.0	43.9	15	6.3	19.8	14.6	24.6	11	4.4	15.5	11.4	19.2				
		162♂	125	130	12	19.4	69.4	52.4	88.4	23	15.0	36.7	27.1	50.4	35	8.1	24.6	16.2	36.5	27	4.6	15.3	9.6	22.6				
		163♂	115	110	12	16.4	67.9	53.6	83.8	24	13.5	41.6	30.2	52.8	39	9.9	28.1	18.6	38.6	52	4.7	17.2	11.1	23.5				
		164♂	125	125	13	21.6	69.0	52.9	89.0	21	15.7	39.1	32.4	46.5	34	8.8	28.0	21.4	32.1	34	5.4	22.1	17.3	25.3				
		165♂	120	150	11	20.3	64.9	54.0	80.6	26	10.7	33.6	25.6	43.7	39	6.9	20.1	14.7	26.5	32	4.7	14.3	10.3	18.3				
		166♂	120	95	11	19.5	57.7	47.3	72.0	22	13.9	32.1	24.1	40.0	35	7.6	22.1	16.9	26.4	42	5.3	17.7	12.8	21.4				
		167♂	115	125	11	18.2	71.0	56.6	87.1	22	12.8	40.1	30.8	51.2	39	9.3	23.3	15.2	33.4	47	5.0	15.0	10.3	22.3				
	40-60 Days		124	118	11	17.3	63.4	48.7	84.3	21	13.9	34.2	24.8	46.4	29	7.9	22.0	15.3	29.9	27	5.2	16.2	11.4	21.5				
			126	118	11	13.0	55.6	45.5	77.7	13	12.2	34.7	24.9	45.3	13	11.1	23.6	12.9	44.8	13	10.6	12.4	12.3	22.3				
			135	92	14	24.7	62.0	46.1	76.7	76.7	27	15.3	38.5	28.8	46.7	43	8.5	28.0	21.5	35.6	47	5.8	21.7	16.2	27.0			
		125	85	16	19.2	49.9	34.1	77.6	77.6	31	12.9	25.0	18.2	33.2	50	7.8	15.9	11.4	20.8	46	4.6	12.5	9.0	16.7				
		145	132	12	14.6	47.6	33.5	62.9	62.9	17	15.4	27.2	17.5	39.2	25	5.7	18.0	12.0	27.7	16	4.5	14.9	9.8	22.7				
		123049♂	155	150	12	20.9	60.1	38.2	83.7	83.7	22	14.5	30.0	19.9	42.5	31	7.3	17.9	12.4	25.4	17	5.6	11.8	7.4	17.9			
		123050♂	160	160	12	15.4	56.3	43.9	71.7	71.7	22	16.4	31.6	21.2	44.4	34	8.9	19.7	14.5	29.0	14	7.0	19.1	14.6	27.4			
		133003♂	155	136	11	19.1	55.7	34.5	78.0	78.0	20	16.2	28.1	17.9	39.5	29	9.2	15.4	9.8	22.3	22	5.1	10.5	7.5	14.8			
		133004♂	150	168	11	22.2	43.3	29.2	71.0	71.0	19	10.2	25.9	16.7	42.4	29	7.4	19.4	10.0	35.7	18	6.4	13.3	6.3	26.7			
		133005♂	145	142	12	15.8	36.6	23.4	60.8	60.8	23	11.6	16.8	11.2	24.4	18	6.5	10.1	5.9	14.1	8	4.4	9.1	6.9	12.4			
		133015♂	130	126	12	20.3	58.5	44.3	80.6	80.6	24	14.6	31.6	24.2	46.2	30	6.6	18.2	14.6	25.4	34	5.4	13.4	9.4	19.3			
		133016♂	140	145	14	19.1	63.4	47.5	82.3	82.3	28	15.6	32.8	21.9	44.3	32	7.7	19.7	14.4	26.9	35	4.7	11.4	8.7	16.4			
		133017♂	150	138	13	20.5	58.3	44.4	70.0	70.0	23	14.7	35.5	27.4	45.2	34	10.5	24.8	18.7	32.7	41	5.8	15.3	11.5	20.1			
		133018♂	125	116	13	15.9	55.4	37.8	76.7	76.7	24	14.2	26.7	17.6	39.8	35	6.8	15.4	10.5	23.5	25	4.2	10.9	7.8	16.0			
Control		143**	133	13*	19.0	53.9**	38.1**	74.3**	74.3**	23	14.3	29.1*	20.2*	40.7*	33	7.7	18.5	13.0	26.6	27	5.3	13.7**	9.6**	19.8*				
		142	122	11	13.1	48.1	37.4	77.2	77.2	13	11.9	25.6	14.7	46.4	18	11.3	24.6	14.2	46.2	13	10.9	13.7	12.3	25.1				
		130	280	11	17.1	63.6	38.0	104.8	104.8	22	12.8	25.7	16.7	45.7	35	7.0	17.1	11.0	29.3	31	5.0	13.7	9.1	24.3				
		148♂	123	265	14	19.3	88.8	58.8	110.2	110.2	27	16.1	44.0	29.2	59.9	46	9.1	24.0	18.8	34.5	61	6.7	17.4	12.3	24.3			
		149♂	130	320	13	22.8	69.9	49.1	83.9	83.9	26	16.3	32.4	24.6	46.3	51	9.5	20.6	14.9	27.3	63	5.6	14.8	11.0	20.5			
		151♂	130	270	15	18.3	73.5	50.9	96.7	96.7	30	13.6	42.1	28.2	60.8	48	8.7	27.7	18.0	44.0	52	5.1	20.1	12.8	31.1			
		153♂	120	262	12	22.4	75.0	48.8	112.6	112.6	22	14.8	36.9	25.9	57.8	36	9.1	24.1	16.9	36.9	25	4.6	17.1	12.3	26.6			
		155♂	110	275	15	22.6	81.1	61.8	113.8	113.8	29	14.6	41.5	30.7	58.3	44	9.7	26.7	19.9	37.8	57	5.7	19.0	14.9	27.9			
		444♂	130	286	8	15.0	57.8	40.3	95.0	95.0	15	17.3	34.2	22.2	60.0	22	9.1	24.6	15.1	46.5	19	5.3	16.2	9.2	31.0			
		E-10♂	130	280	13	23.6	72.5	49.0	93.8	93.8	23	15.7	33.0	23.4	44.6	29	7.1	25.2	17.8	30.2	22	5.2	15.8	12.5	21.9			
		Y-5♂	130	325	14	17.2	63.5	48.1	83.6	83.6	26	14.9	33.0	24.9	45.0	43	8.5	22.6	16.7	29.1	32	6.0	18.8	14.5	24.2			
		Y-9♂	140	310	19	18.1	66.5	42.9	79.5	79.5	35	11.8	40.5	26.2	45.3	61	8.7	25.2	17.6	32.2	55	5.3	19.7	13.5	22.8			

4-6																								797
Months	110228♂ 123006♂ 123007♂ 123028♂ 123039♂ 123041♂ 123042♂ 123042♂ 133001♂ 133002♂ 133023♂	175 185 220 240 200 200 175 180 180 200 185	328 300 280 286 306 296 316 282 300 262	12 15 12 9 11 13 13 11 14 15	18.2 16.3 19.9 24.8 22.4 15.3 23.5 19.1 19.9 21.2	49.9 62.2 54.2 49.1 56.7 68.1 66.2 61.3 57.7 54.2	36.1 46.8 32.9 19.5 33.6 45.3 47.7 33.6 34.3 31.1	66.8 83.9 80.6 90.8 80.6 92.2 88.2 90.2 82.1 91.9	23 30 21 17 19 26 25 18 24 32	13.5 16.6 16.7 17.0 13.3 12.9 14.3 21.6 18.1 18.1	28.9 34.8 34.8 39.1 34.3 39.6 33.4 44.5 32.0 30.5	21.0 24.0 19.7 15.4 19.5 22.1 21.5 21.8 16.8 15.6	37.4 52.0 56.1 64.2 55.9 66.1 51.0 76.7 51.3 56.4	21 46 28 28 25 30 24 22 38 33	9.3 8.1 7.1 9.8 10.3 11.3 9.3 11.8 9.4 9.2	23.9 18.5 21.0 29.0 24.8 27.9 23.3 26.2 21.4 20.1	17.2 13.0 15.3 12.5 11.5 13.6 14.3 12.9 9.9 9.9	29.4 27.9 32.2 47.9 41.0 48.9 37.4 48.5 34.8 39.3	18 37 24 31 20 28 28 27 34 23	5.7 4.9 5.1 6.9 6.2 4.2 6.2 6.7 6.9 7.6	13.8 11.4 14.0 18.6 17.3 14.9 16.5 15.8 15.6 14.3	8.6 7.9 9.1 7.3 9.5 9.2 10.1 7.0 7.9 6.5	19.0 17.8 21.1 33.9 29.7 25.3 28.9 27.2 24.9 27.2	
SHR																								
Control																								
Over																								
1 Year																								
SHR

Fig.8. ATPase activity in the jejunal small artery prepared by quick freezing and fixation with cooled glutaraldehyde (stained by metalsalt method). $\times 400$

- A. Control rat (No.192, male, six months of age, final indirect blood pressure 134 mmHg). Activity is detected in the wall of the artery. Internal diameter of the artery is 75μ .
- B. SHR (No.133051, male, six months of age, initial stage of hypertension, final indirect blood pressure 200 mmHg). Un-constricted segment. Marked increase of activity is observed in comparison with the control (A). Internal diameter of the artery is 75μ .
- C. Constricted segment of the artery shown in B. The distance between B and C is 60μ . Marked increase of activity is also observed in comparison with the control (A), but no distinct difference of activity is found between it and the un-constricted segment (B). Internal diameter of the artery is 43μ .

increase of activity occurred in all SHR. Among the six in the second stage, two showed a slight, one a moderate and three a marked increase. In the third stage, seven SHR showed increased activity, four marked, and three moderate.

2) AMPase Activity

In the normotensive control group, rats in the first stage showed a negative or slightly positive reaction. The reaction became stronger in the second and third stages. Thus, in the control group the reaction grew more intense with age. In the SHR group, no increase was observed in the first stage in any of the six rats. In the second stage, among a total of six rats, one showed a slight, two a moderate and one a marked increase, while in two there was no change. In the third stage, among a total of seven SHR, the increase was, moderate in three and slight in three, and in one there was no change.

5. Fluorescent Examinations (Table IV, Figs. 9, 10, 11, 12, 13 and 14)

Typical green fluorescence of nerve fibers was seen around the blood vessels, as described by

other investigators.⁴⁷⁻⁵⁰ Large cells containing strong yellow fluorescence in the cytoplasm (probably containing serotonin-like substance) were seen around the blood vessels here and there. These cells may be of histiocytic origin. In the arteries and arterioles there were relatively abundant fluorescent nerve fibers, but none or only a few in the veins. In cross sections, bundles of green fluorescence typical of adrenergic fibers called the varicosities of the nerve terminals surrounding the external muscular layer of the arteries and arterioles were seen as a ring of fluorescent dots or cloudlets (Figs. 9 and 10), because numerous small bundles of terminals accompany the vessels.⁴⁸ Not infrequently, bundles of nerve fibers, which seemed to reach the arteries or arterioles centrally from a neighboring position, were observed. As the vessel diameters decreased from 140μ down to about 10μ at the precapillary level, the number of bundles of adrenergic terminal also decreased. The small arteries had distinct internal elastic lamina showing greenish autofluorescence as described by FUXE⁴⁸ and NORBERG and HAM-

TABLE III. INDIVIDUAL FINDINGS OF ATPase AND AMPase ACTIVITIES IN JEJUNAL SMALL ARTERIES AND ARTERIOLES

Age	Sex	Case No. of Normotensive Control Rats	Final Systolic Blood Pressure (mmHg)	Body Weight at time of Experiment (gm)	Findings**		Case No. of SHR	Final Systolic Blood Pressure (mmHg)	Body Weight at time of Experiment (gm)
					ATPase	AMPase			
40-60 Days	♂	239	128	220	↓	→	143020	166	180
		241	130	182	→	→	143022	166	184
		Y-2	120	80	↑	→	150298	175	170
		Y-1	130	110	↑	→	150301	152	180
		266	114	180	↑	→	153014	162	250
		267	118	134	→	→	153015	154	150
4-6 Months	♂	192	134	414	↑↑	↑↑	133051	200	380
		242	135	380	↑↑	↑	143019	208	380
		243	134	394	↑↑	↑	143021	234	355
		222	124	384	↑	→	150205	190	305
		227	120	320	↑	↑	150221	174	280
		M709	140	394	↑	↑	150226	190	330
Over 1 Year	♂	159	130	450	↑↑	↑	123027	180	320
		S62	130	390	↑	↑	123030	192	336
		194	124	444	↑	↑↑	123060	206	386
		195	130	428	↑	→	123061	200	427
		61	136	516	↑↑	↑	125014	194	410
	♀	187	130	270	↑↑	↑	123034	200	224
		189	116	265	↑↑	↑	123038	192	232

SHR : Spontaneously Hypertensive Rats

** : Findings in SHR were obtained by comparing them with those of corresponding controls

↑ : Slightly Increased Activity

↑ : Moderately Increased Activity

↑↑ : Markedly Increased Activity

→ : No Difference

↓ : Slightly Decreased Activity

BERGER,⁴⁹ which made it easy to distinguish the arteries from the veins. At the precapillary level, however, the amount of elastic tissue decreased so that it was often difficult to differentiate between precapillary and postcapillary vessels. At this very small vessel size, there were often seen two bundles of terminals of very intense fluorescence. It is possible that some of these vessels lie on postcapillary side. In no cases were fibers seen penetrating down into the muscular layers of the vessels. And probably due to superposition of fluorescence in the 10μ thick sections and to oblique sectioned bundles, two or more bundles seemed to be close to each other, and made a series of bead-like structures, so it was difficult to estimate the number of bundles accurately as indicated by HÖKFELT.⁵¹ However, the fluorescent dots or cloudlets in the SHR group seemed to be larger than in the control group in the first and second stages (Figs. 9 and 10). In the third stage, however, the fluorescent fibers were very weak or scant, indicating degenerative changes in the nerve fibers. It was also

difficult to find any distinct difference in intensity of fluorescence between the control and SHR groups (though it generally seemed more intense in SHR), because the intensity of fluorescence seemed to vary from one artery to another even in the same rat. Therefore, cross-section areas of fluorescent dots or cloudlets were used as indices.

The mean values of the cross-section areas of fluorescent dots or cloudlets of I, II, III and IV branches (illustrated in Fig. 2) of the jejunal arteries from the SHR group were compared with those of the matched control group. The results are summarized in Table IV. In the first stage, the mean cross-section areas of fluorescent dots or cloudlets around all branches in five SHR were significantly larger (3.62 ± 3.16 , 2.41 ± 1.41 , 1.93 ± 1.02 , 1.43 ± 0.86) than in five matched controls (2.02 ± 1.80 , 1.72 ± 1.31 , 1.17 ± 1.14 , 0.66 ± 0.64). In the second stage, also, were significantly larger around all branches in five SHR (8.02 ± 4.76 , 7.69 ± 2.34 , 3.49 ± 1.95 , 1.82 ± 1.35) than in five matched controls (3.13

Fig.9. Fluorescent appearance of the jejunal small artery prepared by quick freezing and freeze-drying method. $\times 400$

- A. Control rat (No.261, male, six months of age, final indirect blood pressure 126 mmHg). Fluorescent dots or cloudlets of adrenergic nerve terminals around the artery are demonstrated. Internal diameter of the artery is 80μ .
- B. SHR (No.148006, male, six months of age, initial stage of hypertension, final indirect blood pressure 194 mmHg). Un-constricted segment. Fluorescent dots or cloudlets are larger than those of the control (A). Internal diameter of the artery is 80μ .
- C. Constricted segment of the artery shown in B. The distance between A and B is 200μ . Fluorescent dots or cloudlets are also larger than in the control (A), but no distinct difference is found between them and those of the un-constricted segment (B). Internal diameter of the artery is 45μ .

Fig.10. Fluorescent appearance of the jejunal small artery prepared by quick freezing and freeze-drying method. $\times 400$

- A. Control rat (No.318, male, 58 days old, final indirect blood pressure 116 mmHg). Fluorescent dots or cloudlets of the artery are seen. Internal diameter of the artery is 45μ .
- B. SHR (No.153023, male, 58 days old, pre-hypertensive or transitional stage of hypertension, final indirect blood pressure 155 mmHg). Fluorescent dots or cloudlets are larger than in the control (A). Internal diameter of the artery is 45μ .

TABLE IV. MEAN CROSS-SECTION AREAS OF FLUORESCENT DOTS OR CLOUDLETS AROUND JEJUNAL SMALL ARTERIES AND ARTERIOLES ($\times 100\mu^2$)

Age	Rats	No. of Rats	Systolic Blood Pressure (mmHg)	Body Weight at time of Experiment (gm)	Branches			
					I	II	III	IV
40-60 Days	Control	5	119.8 $\pm$ 10.0	150 $\pm$ 30	20.2 $\pm$ 1.80 (49)	1.72 $\pm$ 1.31 (56)	1.17 $\pm$ 1.14 (39)	0.66 $\pm$ 0.64 (47)
	SHR	5	145.0** $\pm$ 7.0	165 $\pm$ 10	3.62** $\pm$ 3.16 (63)	2.41** $\pm$ 1.41 (61)	1.93** $\pm$ 1.02 (34)	1.43** $\pm$ 0.86 (28)
4-6 Months	Control	5	129.2 $\pm$ 7.0	354 $\pm$ 34	3.13 $\pm$ 2.26 (57)	2.27 $\pm$ 1.26 (60)	1.28 $\pm$ 1.01 (41)	0.91 $\pm$ 1.07 (35)
	SHR	5	174.2** $\pm$ 12.4	320 $\pm$ 31	8.02** $\pm$ 4.76 (61)	7.69** $\pm$ 2.34 (75)	3.49** $\pm$ 1.95 (50)	1.82** $\pm$ 1.35 (40)
Over 1 Year	Control	5	127.6 $\pm$ 4.7	388 $\pm$ 56	4.12 $\pm$ 2.21 (42)	1.83 $\pm$ 1.18 (61)	1.21 $\pm$ 1.06 (38)	0.88 $\pm$ 0.55 (45)
	SHR	5	187.0** $\pm$ 18.0	366 $\pm$ 43	2.91* $\pm$ 2.53 (63)	2.14 $\pm$ 2.30 (63)	1.57 $\pm$ 1.25 (32)	0.87 $\pm$ 0.97 (32)

SHR : Spontaneously Hypertensive Rats
() : Number of Arterial Cross Sections

$\pm$: Standard Deviation
* : $0.05 > P > 0.01$
** : $0.01 > P$

± 2.26 , 2.27 ± 1.26 , 1.28 ± 1.01 , 0.91 ± 1.07). In the third stage, there were no significant differences in area between five SHR (2.14 ± 2.30 , 1.57 ± 1.25 , 0.87 ± 0.97) and five matched controls (1.83 ± 1.18 , 1.21 ± 1.06 , 0.88 ± 0.55) for II, III and IV branches, respectively, but around I branch the area was significantly smaller in SHR (2.91 ± 2.53) than in matched controls (4.12 ± 2.21).

In order to examine the distribution of innervation along a single artery, the cross-section areas of fluorescent dots or cloudlets at two points (constricted and un-constricted segments) in a single artery were examined: 118 arteries from controls and 130 from SHR. The distance between two segments varied from 20μ to 300μ . The changes in cross-section areas of fluorescent dots or cloudlets were correlated with changes in luminal diameters (Figs. 11, 12, 13 and 14). For I branch (Fig. 11), the coefficients of correlation were $+0.36$ ($0.2 > P > 0.1$), $+0.20$ ($P > 0.2$) and $+0.03$ ($P > 0.2$) in controls, and -0.04 ($P > 0.2$), -0.60 ($0.05 > P > 0.01$) and $+0.20$ ($P > 0.2$) in SHR, in the first, second and third stages, respectively. For II branch (Fig. 12), they were -0.24 ($P > 0.2$), $+0.07$ ($P > 0.2$) and -0.34 ($P > 0.2$) in controls, and $+0.25$ ($P > 0.2$), -0.04 ($P > 0.2$) and $+0.32$ ($P > 0.2$) in SHR. For III branch (Fig. 13), they were $+0.36$ ($P > 0.2$), $+0.44$ ($P > 0.2$)

and -0.49 ($P > 0.2$) in controls, and -0.25 ($P > 0.2$), $+0.29$ ($P > 0.2$) and -0.40 ($P > 0.2$) in SHR. For IV branch (Fig. 14), they were -0.02 ($P > 0.2$), -0.75 ($P > 0.2$) and $+0.47$ ($P > 0.2$) in controls, and $+0.47$ ($P > 0.2$), $+0.08$ ($P > 0.2$) and -0.71 ($P > 0.2$) in SHR. Thus, no significant correlation seemed to exist between the changes in cross-section areas of fluorescent dots or cloudlets and the decrease in luminal diameters in a single artery except in one instance. In I branch of SHR in the second stage, the cross-section areas of fluorescent dots or cloudlets decreased as the luminal diameter decreased.

DISCUSSION

It has been mentioned in previous reports^{1,2} that the average systolic blood pressure in SHR is significantly higher than in normotensive control rats even in the pre-hypertensive first stage (40 to 60 days after birth). In some of them the systolic pressure was already higher than 150 mmHg, especially in the rats used for histochemical studies (Table III). It has been confirmed¹¹ that a minimum anesthetic dose of α -chloralose (40 mg/kg) affects blood pressure less than pentobarbital or ether, and that direct pressures are about 10 to 20 mmHg lower than indirect pressures without anesthesia. In this study, the direct femoral blood pressure was preserved at

Fig. 11. Relationship between the change in cross-section areas of fluorescent dots or cloud-lets and the decrease in luminal diameters along a single artery of normotensive control rats and SHR in the 1st branch.

Fig. 12. Relationship between the change in cross-section areas of fluorescent dots or cloud-lets and the decrease in luminal diameters along a single artery of normotensive control rats and SHR in the 2nd branch.

Fig. 13. Relationship between the change in cross-section areas of fluorescent dots or cloud-lets and the decrease in luminal diameters along a single artery of normotensive control rats and SHR in the 3rd branch.

Fig. 14. Relationship between the change in cross-section areas of fluorescent dots or cloud-lets and the decrease in luminal diameters along a single artery of normotensive control rats and SHR in the 4th branch.

hypertensive levels in almost all SHR during anesthesia. A transient rise or fall of femoral blood pressure occurred following laparotomy, but it usually returned in a few minutes to approximately the initial level.

Though the definition of an arteriole is not definite,^{20,52,53} blood vessels described in this study may be called small arteries and arterioles, because the internal diameters ranged from 150 μ down to 10 μ or smaller, and three layers could be distinguished in the larger ones but not in the smaller ones. GRANT⁵⁴ has shown that local spasm can be provoked in the arteries of the rabbit's ear by moderate local pressure on the vessel wall.

It is known that other mechanical stimuli such as pinching, longitudinal stretching or rubbing may also cause arterial spasm.⁵⁵ In this observation, focal arterial or arteriolar spasm was also noted following local pressure with a sewing-needle on the exposed surface of the jejunal wall. So the influence of factors such as the pressure of the abdominal window as well as peristaltic stretching, anxiety or surgical procedure must be considered. The influence of longitudinal stretching seems to be very slight, except for peristalsis in this study, as was the problem in BYROM's experiment²⁹ with cerebral arteries because of progressive cerebral edema. No definite difference

in peristaltic activity was noted between controls and SHR. Factors such as local pressure or peristalsis have an equal influence in all the experimental groups, and since in the arteries of all normotensive controls the spasm is very slight or absent, it can be assumed that these vascular changes are not due to these factors or that the arteries in SHR are abnormally irritated by these factors. Increased irritability of the arteries in hypertension due to various factors has been demonstrated by many investigators⁵⁶⁻⁵⁸. In the present study, however, the calibers of the small arteries and arterioles did not seem to be changed by the removal of the abdominal window in either controls or SHR, so the present findings, like those of other investigators^{29,59,60,62} suggest that the reversible changes in caliber demonstrated in the jejunal small arteries and arterioles in SHR are hypertensive phenomena and not artefacts, although the influences of anesthesia, anxiety or surgical procedure have not been excluded completely.

Although the cause of angiospasm which underlies hypertension is unknown, the following factors may be responsible: 1) high intraluminal pressure, 2) excessive sympathetic nerve impulse, 3) elevated concentration of circulating pressor substance, 4) increased contractile sensitivity of the muscle cells. Good evidence is available to support each of these possible mechanisms, and no conclusive evidence excludes any of them. BAYLISS⁶³ observed contractions of arteries both *in vivo* and *in vitro* in response to increased intraluminal pressure, and he suggested that the reaction was independent of the central nervous system and was myogenic in nature. BYROM^{29,30} and MEYER, WALTZ and GOTOH⁶² observed angiospasm of the cerebral or retinal small arteries of rats, cats or monkeys following the development of renal hypertension, and suggested that intraluminal pressure plays an important role in the cause of angiospasm. Moreover, RODDA and DENNY-BROWN⁶⁰ showed that intermittent periods of high intraluminal pressure had more effect than a steady but not so high intraluminal pressure in producing a focal response in the cerebral arteriolar muscles of monkeys. In the present study, the degree of constrictions and tortuosities seemed to be correlated with the severity and the duration of hypertension both in direct observations and histometry; that is, the higher the blood pressure and the longer the duration of hypertension, the more marked were the arterial constrictions and tortuosities. The results

support the speculation that high intraluminal pressure is responsible for arterial or arteriolar spasm, and its duration is also related to the degree of angiospasm. Tortuosities of the small arteries and arterioles are also related to the severity and the duration of hypertension as was demonstrated by SHORT²⁴ in the intestinal arteries of patients with hypertension and by MEYER *et al*⁶² in the cerebral arterioles of cats and monkeys with DOCA hypertension. Tortuosities may also produce peripheral resistance as well as constrictions.

In some young adult SHR (three to six months old; one to four months after the onset of hypertension), the segmental constrictions and tortuosities of the jejunal small arteries and arterioles disappeared completely after the blood pressure had been reduced steeply to below normotensive levels, or upon the application of a local vasodilator. In this study, however, the disappearance of irregularities in the caliber does not necessarily mean that the arteries have returned to their original diameters or that there is no hypertrophy in the arterial wall, because the original diameters of the constricted and un-constricted segments are not known. These results are in agreement with those of other investigators^{29,30,60,62} and suggest that no permanent changes had occurred to prevent the relaxation of smooth muscle in these arteries or arterioles. In fact, no sclerotic changes in the arterial walls were observed in paraffin sections (Fig. 7), although accurate measurements of the arterial wall were not taken. HALPERT and GROLLMAN⁶⁴ found no significant histological changes in the large or small arteries of the heart, kidneys, spleen, liver, pancreas, lungs or brain of rats with experimental renal hypertension for periods of one to eight months, although all showed cardiac hypertrophy. However, in some rats in the third stage (over one year old, 10 to 13 months after the onset of hypertension), the irregularities did not disappear completely, so labile angiospasm may here be turning into anatomical narrowing.

Although no exact critical level of blood pressure was found above which angiospasm occurred and below which it was absent, slight constriction of the jejunal arteries and arterioles was noted to occur when the blood pressure exceeded approximately 150 mmHg. This value is slightly lower than that reported by MEYER *et al*⁶² 160/110 mmHg in cerebral arterioles, probably because of the difference of the organs. When a sud-

den reduction in blood pressure from 180 mmHg to 100 mmHg was caused by intravenous injection of hexamethonium (20 mg/kg), progressive arterial dilations appeared, but the segmentation did not disappear as rapidly as the reduction of blood pressure, and it needed a few minutes to disappear after the blood pressure remained at 100 mmHg. So it seems that there is no exact parallelism between the blood pressure and the degree of constriction, at least in the jejunal small arteries and arterioles, and that once contraction of the arterial smooth muscle cells occurs, it cannot be relaxed very quickly.

Most investigations of the architecture of blood vessels walls have not been concerned with the functional aspects. Many such studies have been performed on post-mortem material. But satisfactory measurements cannot be made on arteries or arterioles in regular histological preparations because the vessels are collapsed and contracted, so that an increase in the ratio of wall thickness to luminal diameter, which is commonly employed as an indicator of vascular wall hypertrophy^{20,21} is also seen in normal vessels during constriction; consequently, such arteries may be wrongly identified as being hypertrophied. SHORT^{23,24} tried to distend the arteries and arterioles to such a degree that the internal elastic lamina was rendered quite smooth in the pulmonary and intestinal vessels in human hypertension, and he proposed, on the basis of histometric data, the term "arteriolar contracture" to signify a persistent shortening of the circular elements of the arterial wall, even when no hypertrophy was found in the arterial and arteriolar walls. Recently, the method of quick freezing and freeze-substitution³¹ has been developed, and it is reported that this method has the tremendous advantage of reducing the distortions of regular histological preparations^{32,33,44,65}. Using this method, it was possible in this study to preserve almost the same arterial patterns as those noted in direct observations (Figs. 4 and 5). Measurements of these preparations seem to reflect accurately the luminal diameters of arteries and arterioles in vivo. The results of histometric study, which showed that the luminal diameters (especially minimal luminal diameters) in SHR are narrower than normal, are in accord with many other reports on hypertensive arteries and arterioles. The fact that the diameters are already narrower in SHR than in controls, even in the first stage, suggests that the vascular changes are already present before 40 days of age. This result

is quite consistent with other abnormalities already described in the endocrine system^{4,6} cardiovascular system,¹¹ autonomic nervous system,^{13,16} hypothalamus¹⁸ and adrenal medulla⁷ of SHR. In this study, no data were obtained in SHR before the age of 40 days. This period must be studied in future. The un-constricted segments seemed dilated in contrast to the intervening constricted segments, but histometric data showed that they are slightly constricted, or at least not dilated, especially in the first and third stages (Table II). It is questionable, however, whether the two parts of the arterial branches compared in this study are exactly the same. Indeed, there are great variations in the branchings in the vasa recta, but the number of branches measured in this study seems to be sufficient to reduce this possible error, because the average length of each branch is approximately the same and no significant difference was noted statistically between controls and SHR (Table II). So it can be supposed that the arterial branches compared in this study are almost the same.

In regard to the nervous control of the blood vessels in hypertension, there are many reports suggesting that neural factors are responsible for the development and maintenance of hypertension⁶⁶⁻⁷². In patients with hypertension, DOYLE and SMIRK⁶⁶ measured the magnitude of blood pressure fall following a maximally effective dose of hexamethonium and found the neurogenically maintained fraction of the blood pressure was increased in essential hypertension. BROD⁶⁸ also suggested that increased sympathetic activity was associated with elevated vascular resistance in the early stage of hypertension. In animals, ROTHMAN⁶⁹ noted increased sympathetic activity with increased production of norepinephrine in rabbits with cerebral hypertension given norepinephrine before and after the administration of nembutal. PHELAN, ERYETISHER and SMIRK⁷⁰ and PHELAN⁷¹ also suggested that a neural component is present in rats with inherited hypertension as in human subjects⁶⁶. DICKINSON⁷² proposed that essential hypertension was initiated by a neurogenic mechanism, but that an appreciable component of the hypertension was later sustained by non-neurogenic means.

Several studies conducted in our laboratory^{6,7,11,13,14,16} on the activity of the autonomic nervous system in SHR suggest that the autonomic nervous system is overactive from the hypothalamic center to the peripheral organs. MATSUMOTO¹³ investigated the superior cervical

ganglia histometrically and showed that the ganglia in SHR were both heavier and larger than normal, and that the nerve cells and their nuclei were larger in SHR than in control rats. Moreover he showed histochemically¹⁴ increased activities of acid phosphatase and monoamine oxidase in SHR, but no increase in acetylcholine esterase activity. Tabei⁶ reported that the noradrenaline-storing cell islets in the adrenal medulla, the innervation of which is considered to be sympathetic, were about twice as large as normal in SHR. MORISAWA⁷ confirmed these results and demonstrated that they were already enlarged six days after birth and became twice as large as normal by the 18th day of life. OKAMOTO et al.^{1,11} using infusion of depressor agents and carotid occlusion, suggested that increased sympathetic vasomotor tone was already present in SHR in the earliest stage and concluded that neural factors are responsible for the development of hypertension. Recently, OKAMOTO et al.¹⁶ demonstrated electrophysiologically increased activities of spontaneous discharge from the splanchnic nerve in SHR, especially in the early stage. Moreover, they reported that the fall in blood pressure after *encéphale isolé* was significantly greater in SHR than in controls, and that *cerveau isolé* did not influence the blood pressure in either controls or SHR. They concluded then that the increased sympathetic vasoconstrictor discharge which originated in the pontobulbar portion of the brain stem constituted one important factor in the pathogenesis of hypertension in SHR. The hypothalamus is generally believed to be the most important structure related to cardiovascular control above the medulla oblongata,⁷³⁻⁷⁵ and the overactivity of some hypothalamic nuclei in SHR were also suggested histochemically^{18,19} and histometrically.¹⁹ In summary, all these findings seem to support the concept of increased tonicity of the autonomic nervous system in SHR. And since the innervation of the small arteries and arterioles (resistance vessels) is considered to be mainly sympathetic in regard to blood pressure homeostasis,³⁴ it has been supposed that in SHR the increased activity of the sympathetic nervous system may cause arterial or arteriolar spasm which may be responsible for the development of hypertension.

The morphological demonstration of the autonomic nervous system has been performed mainly by the silver impregnation method^{35,36} or the *intra vitam* method of staining with methylene blue,³⁷ but these results are not always constant,

probably because of their technical difficulty and specificity. Thus, it has been difficult to study quantitatively the difference of sympathetic nerve fibers in various vascular beds or between normal and diseased subjects. A specific and sensitive histochemical fluorescence technique for the demonstration of catecholamines and 5-hydroxytryptamine in tissues has recently been developed,^{38,39} and the technique and its problems have been described in detail.^{38,40,49,76} This method permits the visualization of the intraneuronal distribution of adrenergic transmitter and has been generally accepted as a very valuable tool in morphological, physiological and pharmacological studies of adrenergic structures and mechanisms. Using this method, MALMFORS⁷⁶ suggested that the sympathetic adrenergic nerves could synthesize, take up, store, release and metabolize noradrenaline, which served as their transmitter substance. On the other hand, identification and quantitative studies of catecholamines in various tissues have been carried out by spectrophotofluorometrical methods used for biochemical assay,^{77,79,80} or microscopic spectrofluorometric methods.⁸¹⁻⁸⁴ DE LA LANDE and HEAD,⁷⁷ using the former method, demonstrated more noradrenaline than adrenaline in the central artery of the rabbit. DAHLSTRÖM et al.⁷⁹ estimated fluorometrically the catecholamine content in the area innervated by the cervical sympathetic ganglia. RITZÉN,⁸¹ in a model experiment, showed the relationship between the intensity of fluorescence and the concentration of fluorophore by the latter method. VAN ORDEN et al.⁸³ also employed the latter method in the identification and quantitative estimation of 5-hydroxytryptamine in single neoplastic mast cells grown *in vitro*. With this technique, it has become possible to measure the number and intensity of fluorescent adrenergic fibers in histologic sections. GILLIS et al.⁸⁴ reported that the number and intensity of fluorescent adrenergic fibers of the cat atria stimulated electrically via the sympathetic nerve was much greater than in unstimulated cats, following the intravenous injection of norepinephrine-³H.

The fluorescence method has shown sympathetic noradrenaline-containing nerve terminals around the arteries of the mesentery,^{38,49,51} brain,^{85,86} skeletal muscle,^{47,48} ear^{50,78} and other tissues^{49,79,87} in experimental animals. FALCK³⁸ demonstrated a vascular plexus of adrenergic nerve fibers around the mesenteric small arteries and arterioles in mice, rats and guinea-pigs.

FUXE and SEDVALL⁴⁸ showed the distribution of adrenergic nerve fibers to the blood vessels in skeletal muscle and a fairly rich supply of adrenergic fibers in the intra-muscular arteries, arterioles and metarterioles, but only a sparse supply in the arteries entering the muscles, and they suggested that the extent and type of distribution of sympathetic innervation was related to the functional properties of the vessels. HÖKFELT⁵¹ using araldite as the embedding medium instead of paraffin, made thinner sections (0.5μ to 2μ) of mesenteric arteries of rats in order to determine more correctly the true location and number of varicosities. No data, however, have yet appeared on the quantitative study of the adrenergic nervous system around the vessels in hypertensive subjects except for that of ZUSSMAN⁸⁹ and on SHR in our laboratory.^{12,17} ZUSSMAN reported fluorescent smudging in the adventitia of arterial and arteriolar walls in rats with experimental renal hypertension as early as 30 days after renal artery ligation. In SHR, HAEBARA et al.¹⁷ also reported augmented fluorescence in the adrenergic nerve terminals of cerebral, chorioidal, intestinal and coronary arteries.

There is no doubt that the green fibers observed in this study around the jejunal small arteries and arterioles of rats are sympathetic noradrenaline-containing nerve terminals. The cross-section areas of fluorescent dots or cloudlets in SHR are larger in all branches in the first and second stages than those in controls. Though the nature of this enlargement of fluorescent dots or cloudlets is uncertain, it may be due to enlargement of sympathetic nerve fibers, increased amount of noradrenaline due to overactivity of the sympathetic nerves or decreased secretion of noradrenaline. HOLLAND and SCHÜMANN⁸⁸ reported that noradrenaline synthesis was increased in the adrenal gland by stimulation of the splanchnic nerves. In renal hypertension, ZUSSMAN⁸⁹ using the fluorescence method found that the distribution and quantity of fluorescent material closely paralleled the renal changes and suggested that this might be due to increased adrenergic stimulation. In SHR, histometric studies of the adrenal medulla showed^{6,7} that the ratio of noradrenaline-storing cell islets to the whole medulla tissue was prominently increased even in the pre-hypertensive stage. It was confirmed biochemically⁹ that the noradrenaline content of the adrenals of SHR was about double the normal value. In this study, no attempt was

made to grade the intensity of the fluorescent dots because of the great variation among arteries even in one animal, though it seemed slightly stronger in SHR than in controls. Consequently, no information was obtained on the concentration of noradrenaline in the sympathetic nerve terminals. But it is possible that only a portion of the catecholamine within the adrenergic fibers can be detected by this method, as suggested by ANGELAKOS and KING.^{90,91} The results suggest then that the cross-section areas of sympathetic nerve terminals are larger than normal in SHR. The question still remains, however, as to whether the size of the nerve fibers, i.e. the areas of fluorescent dots or cloudlets (purely morphological indices) accurately reflect neuronal activity. Some investigations have dealt with the relationship between the morphological changes of the cells and cellular activity.^{92,93} MATSUMOTO,¹³ TABER⁶ and FUKUSHIMA¹⁹ in our laboratory, also used the method in combination with histochemical studies to demonstrate cellular activities. The results of histometric examination of the adrenergic nerve terminals and of enzyme-histochemical examination of the walls of arteries innervated by adrenergic nerves seem to support the other findings in SHR.^{9,11,13,16} Though the intensity of fluorescent dots or cloudlets was not determined, it seems that excessive sympathetic nerve impulses exist in SHR and are responsible for the development and maintenance of hypertension. However, it is known⁹⁴ that exercise, anesthesia, surgical operation or disease cause increased secretion of noradrenaline in the adrenal medulla, and MORISAWA⁸ has suggested that both synthesis and secretion of noradrenaline in the adrenal medulla are increased more in SHR than in controls. It is possible that the same changes occurred in this experiment in the sympathetic nerve terminals of the jejunal small arteries and arterioles because of α -chloralose anesthesia, surgical procedure or the supine position of the animals, but this remains to be clarified by future studies.

Failure to demonstrate fluorescent structures in the smooth muscle layer of the jejunal small arteries and arterioles in rats supports the findings of other investigators.^{38,48-50,87} Electron microscopic studies of the innervation of various arteries have also failed to reveal any nerve endings within the muscle layer but only on the outer surface of this layer.⁹⁵⁻⁹⁷ These findings support the finding^{78,87} that in smaller arteries the transmitter substance may be released only in the

medial-adventitial border and reaches the effect-site in the smooth muscle by diffusion. In this study, the cross-section areas of fluorescent dots or cloudlets in the third stage were significantly smaller in the I branch in SHR than in controls, and no difference was found between them in II, III and IV branches, perhaps because of degenerative changes in adrenergic fibers, which were also observed by HASSLER³⁶ in atheromatous lesions stained by silver impregnation. This finding supports the speculation that hypertension is maintained by non-neurogenic means in the late stage,⁷² and that some organic changes in the cardiovascular system secondary to hypertension may be responsible for sustaining high blood pressure.

In this study, special attention was paid in the fact that segmental constrictions tended to remain or reappear at the same points along the affected vessel as were demonstrated by other investigators,^{29,30,60,62} but no difference was found between the constricted and un-constricted segments histologically (Figs. 7B and C), histochemically (Figs. 8B and C) or fluorometrically (Figs. 9B and C, Figs. 11, 12, 13 and 14). The fluorescence method could not demonstrate any correlation in the cross-section areas of adrenergic nerve terminals between constricted and un-constricted segments except in one instance. In this one branch (I branch of SHR of the second stage), the cross-section areas of adrenergic nerve terminals were smaller in the constricted than in the un-constricted segments. FUXE and SEDVALL⁴⁸ observed no definite accumulation of adrenergic fibers in any sections of the vascular bed in skeletal muscle using the fluorescence technique, but the close juxtaposition of the blood vessels suggested that the adrenergic nerves might reach the vessels at various levels; such an arrangement might provide some functional differentiation in the vasoconstrictor control of the various sections of the vascular bed. In this study, bundles of adrenergic fibers which seemed to reach the blood vessels centrally were seen not infrequently. The bundles which were connected with the fluorescent dots or cloudlets of the external muscular layer of the arteries and arterioles were also used in calculations. It may be hazardous, however, to draw any general conclusions concerning vasoconstrictor innervation, because of methodological problems and great variations in the data in this study; this remains to be clarified in the future. BYROM^{29,30} suggested that the segmental constriction of the

arteries might be related to the uneven distribution of vascular muscle fibers. Though this question was not clarified in the present study, this may be one factor. The method used in this study provided no strictly quantitative information on the concentration of noradrenaline in the sympathetic nerve terminals around the arteries, but such information can be readily obtained by the microscopic spectrofluorometrical method described by GILLIS et al.⁸⁴ or others⁸² to determine the number and intensity of fluorescent adrenergic terminals with thinner sections,⁵¹ or by the spectrophotofluorometrical method⁸⁰ used for the biochemical assay of catecholamines.

In regard to pressor substances, the renin-angiotensin system has been proposed by some investigators as the probable mediator of the hypertension,⁹⁸ but the role of this pressor system in hypertensive states is still uncertain.⁹⁹ TOBIAN et al.¹⁰⁰ have suggested that a relationship may exist between granulation of juxtaglomerular cells and extractable renin in rats with renal hypertension. In SHR, OKAMOTO³ showed that juxtaglomerular cell granularity and renal pressor substance were normal in the early stage and decreased in the late stage of hypertension. HAGA et al.¹⁰¹ confirmed the decreased granularity in SHR, and suggested that it was mainly due to the decrease in granularity of each juxtaglomerular apparatus. Moreover, SOKABE¹⁰² reported a decrease in renin content and release in SHR, and concluded that renin seemed to play no part in the pathogenesis of hypertension in SHR. Since the noradrenaline content of the adrenal medulla is known to be increased in SHR,⁹ as mentioned above, it may well be that the increase in catecholamine humoral factors is related to the development and maintenance of hypertension.

The presence of an increased reactivity to pressor substances or various other conditions in subjects with hypertension has been suggested. OGDEN et al.⁵⁶ reported increased sensitivity to pitressin, which was thought to be a direct stimulant of arterial muscle, and abnormal pressor response to noise and fright in rabbits with renal hypertension. Many investigations have shown that an increase in responsiveness to noradrenaline and adrenaline is present in hypertensive animals^{58,69,103,104,106,107} and men.^{67,105} DOYLE and FRASER⁶⁷ reported a significantly greater vascular responsiveness to noradrenaline in young adult normotensive sons of hypertensive parents than in young men of the same age whose par-

ents had normal blood pressure, and they suggested that this might be due to the inheritance of a specific defect. MASUYAMA et al.¹⁰³ suggested that the increased sensitivity of peripheral vessels to noradrenaline in renal hypertension contributed to the pressor mechanism, while the baroreceptors and sympathetic activity were passively adjusted to the higher blood pressure levels. Increased vascular reactivity to infused noradrenaline might be the result of either an impaired inactivation of the amine or an increased sensitivity of the effector cells. DE CHAMPLAIN et al.¹⁰⁷ showed that the accumulation of ³H-norepinephrine one hour after its injection was reduced in the heart, spleen, intestine, skeletal muscle, and kidney of rats with DOCA hypertension, suggesting impaired inactivation of the amine in the sympathetic nervous system (decreased ability to retain norepinephrine in storage granules). By infusing the isolated caudal artery in rats with DOCA hypertension, HINKE⁵⁸ demonstrated muscular hyper-responsiveness and suggested that this might be due to an alteration of the selective permeability to ions in muscle fibers. On the other hand, there have also been some studies reporting a decreased pressor response to noradrenaline^{69,108} or adrenaline^{69,104} or no change of response to noradrenaline.¹⁰⁹ This type of response may be due to a disturbance of cardiovascular metabolism in adrenal-regeneration hypertension¹⁰⁸ or an already existing high concentration of noradrenaline in the effector cells in cerebral hypertension.⁶⁹ In SHR, OKAMOTO et al.¹¹ showed increased sensitivity to noradrenaline and decreased sensitivity to adrenaline. Moreover, they suggested that adeno-hypophyseo-adrenocortical and -thyroid hyperfunction^{4,5} might have some relationship to the changes in the vascular reactivity in SHR, because ACTH, adrenal cortical and thyroid hormones affect the response to noradrenaline.

Biochemical and histochemical studies of ATPase and AMPase activities in arterial walls have been reported. ATPase is believed to be bound to myosin-actomyosin and is thought to be related to the contraction of muscle cells.^{41,42} It is certain that ATPase is responsible for the breakdown of ATP into ADP to supply the energy required for muscular contraction. The presence of ATPase activity in arterial walls has been demonstrated biochemically^{110,111} and histochemically¹¹² in various tissues. KIRK¹¹¹ observed ATPase activity in homogenates of human aorta and pulmonary and coronary

arteries; the coronary artery had higher enzyme activity than the aorta or pulmonary artery. By histochemical methods, STEIN and HARRIS¹¹² showed ATPase activity in human tibial and popliteal arteries and greater activity in arteriosclerotic than in normal subjects.

There have also been some biochemical and histochemical studies of AMPase (5'-nucleotidase) activity in arterial walls. REIS¹¹³ first demonstrated AMPase activity in human arterial tissue and suggested that the high activity of this phosphatase at a physiological pH might be important for the process of tissue calcification. These findings of REIS were confirmed by NEWMAN et al.¹¹⁴ but OKA and ANGRIST¹¹⁵ did not find any relationship between this enzyme and the process of calcification, and they¹¹⁶ observed a positive AMPase reaction in the proliferating cells in the intramyocardial and mesenteric arterial walls of rats with experimental salt hypertension. MCMANUS et al.¹¹⁷ found a positive AMPase reaction in the walls of coronary arteries showing arteritis, but no reaction in the hyaline area of renal arteries. STEWARD and BECKETT¹¹⁸ also described AMPase activity in some vessels located in the regenerating area of traumatic muscle. SANDLER and BOURNE¹¹⁹ reported that AMPase was present in the aortic arch and in the thoracic but not in the abdominal aorta, and they assumed that this might be related to atherosclerotic changes.

MAEKAWA¹²⁰ suggested that a disturbance of the ATP-ATPase system in the blood vessels might be an important factor in the pathogenesis of hypertension. OKA and ANGRIST^{115,116} showed increased activity of ATPase and AMPase in the walls of small myocardial arteries and aorta in rats with salt or renal hypertension. Increased AMPase activity especially was observed in the aorta in an early stage when no other changes were detectable by routine staining methods. They suggested¹¹⁶ that an increase of these reactions might be related to vascular reactivity and this in turn to electrolyte changes, all of which were possibly related to dynamics of vascular function. FUJITA et al.¹²¹ showed increased ATPase activity in the arteries of hypertensive dogs, and decreased activity after reno-portal anastomosis had reduced the blood pressure. In SHR, TABEI⁶ reported increased ATPase activity in the vasa afferens and interlobular arteries of the kidney, especially the latter, even in the pre-hypertensive stage, and he suggested that the interlobular arteries might be more

intimately related than the vasa afferens to the severity and duration of hypertension. In this study, the precise localization and finer changes in the organelles was not determined, but broad zones of reaction were noted. The finding that arterial walls did not stain uniformly, and that individual arteries showed considerable variation in intensity of staining may be explained by direct observations that the constrictions were not uniform and also by the findings of fluorescent examination where great variation was seen in the nerve supply to the blood vessels. ATPase activities in the intestinal arteries with a luminal diameter of 10μ to 150μ were increased throughout all three stages. Since ATPase activity is related to the contraction of smooth muscle cells, the present results suggest that the smooth muscle cells of the intestinal small arteries and arterioles are overactive in SHR, although this may be partly due to sympathetic overactivity, and that it may also be related to the development and maintenance of hypertension. However, it is often difficult to determine whether the observed changes in the arteries are the cause or the result of hypertension. ATPase activity is normally negative or slightly positive in the intestinal arteries of rats as was also demonstrated in other tissues.^{114,115} No distinct difference was found between controls and SHR in the first stage in this study. This finding is contradictory to that of OKA and ANGRIST^{115,116} in renal or salt hypertension, which showed the earliest changes of the AMPase reaction. The reason is not known, but the difference may be related to the difference in pathogenesis between renal or salt hypertension and SHR.

In summary, increased contractile activity of arterial smooth muscle cells and the overactivity of the sympathetic adrenergic nervous system around the artery in SHR were suggested by direct observation, histological, histometric, enzyme-histochemical and fluorescence methods. As mentioned previously, extensive studies have been performed in our laboratory to determine the pathogenetic factors in essential hypertension with the use of excellent material, the spontaneously hypertensive rats. There is no doubt that endocrine organs play a significant role in these animals. On the other hand, the sympathetic nervous system, including the adrenal medulla and hypothalamus is also one of the essential factors in the development and maintenance of hypertension. The morphological demonstration of the changes of the jejunal small arteries and

arterioles as well as the overactivity of the sympathetic nervous system and increased activity of the vascular smooth muscle cells seems to support well the findings mentioned above.

SUMMARY

Morphological studies of the jejunal small arteries and arterioles of spontaneously hypertensive rats (OKAMOTO and AOKI) were performed by direct observation, and by histological, histometric, enzyme-histochemical and fluorescence methods. For making the histological preparations, quick freezing and freeze-substitution or freeze-drying methods were employed to preserve the functional stage of the blood vessels. Rats were divided into three groups: the pre-hypertensive or transitional stage (40 to 60 days after birth), the initial stage (four to six months after birth) and the advanced stage (over one year after birth) of hypertension, and the following results were obtained.

- 1) Segmental constrictions and tortuosities of the jejunal small arteries and arterioles were observed in all cases of spontaneously hypertensive rats after the development of hypertension.

- 2) Minimal luminal diameters of almost all branches of the jejunal small arteries and arterioles in spontaneously hypertensive rats were significantly smaller than in normotensive control rats, in the range of 64% to 85% of the control diameters.

- 3) The mean luminal diameters of the jejunal small arteries and arterioles in spontaneously hypertensive rats were significantly smaller in three-fourths of the branches, while the maximal luminal diameters were significantly smaller in half the branches, than in normotensive control rats.

- 4) Adenosine triphosphatase activity of the jejunal arterial walls of spontaneously hypertensive rats showed an increase in half the cases in the pre-hypertensive or transitional stage and in all cases in the initial and advanced stages.

- 5) Adenosine monophosphatase activity of the jejunal arterial walls of spontaneously hypertensive rats showed no difference in all cases in the pre-hypertensive or transitional stage, an increase in two-thirds of the cases in the initial stage, and in all except one in the advanced stage.

- 6) Adrenergic nerve terminals around the jejunal small arteries and arterioles of spontaneously hypertensive rats were significantly larger than in normotensive control rats in the pre-hypertensive and initial stages, and showed no

change in the advanced stage.

7) From the above-mentioned evidence, it may be concluded that increased activity of the sympathetic nervous system and increased contracting tendency of the vascular smooth muscle cells cause angiospasm, which plays an important role in the development and maintenance of hypertension.

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