THE CORRELATION OF MEDIAL COMPOSITION OF THE ABDOMINAL AORTA AND RENAL ARTERY WITH BLOOD PRESSURE IN THE DEVELOPING, SPONTANEOUSLY-HYPERTENSIVE RAT(SHR)

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The correlation of medial composition of the abdominal aorta and renal artery with blood pressure in the developing; spontaneously-hypertensive rat (SHR)

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Faculty of Medicine
Memorial University of Newfoundland
May 1980

St. John's Newfoundland

ABSTRACT

The elevation of arterial blood pressure in the spontaneously hypertensive rat (SHR) has been postulated to be the result of increased vascular resistance and that such an augmentation of vascular resistance may be due, at least in part, to changes of the vessel wall. The present study is designed to examine this hypothesis.

Animals from each strain of SHR, WAR and WKY were sacrificed at 2, 4, 5, 6, 7, 8, 12 and 18 weeks of age. Blood pressures from each group were obtained by intra-arterial cannulation. The volume fractions of SMC, collagen and elastin in the media of the abdominal aorta and of the left renal artery of 2, 4, 8, 12 and 18 weeks old animals were estimated by stereological analysis at the EM level. The estimated values of these components in the SHR were compared with those obtained from the WAR and the WKY controls.

The systolic blood pressure in the SHR rose to a significant level from 5 weeks and further increased to a hypertensive level by 18 weeks of age, as compared with the controls. As revealed by stereological analysis of the medial composition of the abdominal aorta and the left renal artery of the SHR, no significant difference was observed when comparing these parameters with those of the controls.

The results of the present study indicate that the medial components of the arteries studied were not significantly different among the three strains of rat, up to 18 weeks of age, while the systolic blood pressure of the SHR was elevated significantly from 5 weeks of age.

The elevation of arterial blood pressure during the development of the SHR therefore appears not to be the result of morphological changes in the media of the abdominal aorta and the renal artery.

ACKNOWLEDGEMENT

To Dr. T. M. Scott goes my heartfelt thanks; without his continuous guidance and encouragement this thesis would neither have been undertaken nor completed. I also wish to thank Dr. C. R. Triggle who read the thesis most carefully and made many constructive suggestions.

The discussion with Dr. D. Bryant on statistical methods used in analysing the data of the present study and the assistance from the staff of the Audiovisual Unit and of the Electron Microscopy Unit of the Faculty of Medicine, MUN, in the preparation of this thesis are greatly appreciated.

Acknowledgement is also due to the Faculty of Medicine, MUN and the Canadian Heart Foundation for their financial support throughout the entire M. Sc. programme.

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INTRODUCTION

I. CLASSIFICATION OF HYPERTENSION

Hypertension and its complications claim many lives every year. It can be defined as a condition with an abnormally elevated arterial blood pressure in a well defined population and has been classified as either "primary" (essential), where there is no single apparent causative factor, or "secondary", where there is a definable pathological abnormality. The former comprises up to 90% of the total "hypertensive" population (Genest, 1977).

II. DEFINITION OF HYPERTENSION

It has always been a controversial issue as to how to distinguish between "hypertensives" and "normotensives", because, except for its high arterial pressures, such a so-called "disease" is virtually, at least in its early stages, symptomless (Pickering, 1972). According to the World Health Organisation, hypertension should be defined as a single, sitting or recumbent, blood pressure higher than 160/95 mmHg (World Health Organisation, 1959). The usefulness of such a definition in clinical applications is, however, questionable, since arterial pressures are known to be highly variable under many influences such as pain, cold, exercise, sleep, anger and fear (Pickering, 1972). Despite these problems, it is necessary, for the purpose of comparison, to define a reference point in hypertension research. Okamoto and Aoki (1963), in developing their spontaneously hypertensive rats (SHR), considered a systolic pressure above 150 mmHg to be hypertensive, below 149 mmHg normotensive, using the tail-cuff as the measuring device. These criteria were in agreement with other workers (Alexander et al, 1954; 1956; Grollman and Grollman, 1962; Phelan and Smirk, 1960; Phelan et al, 1962). In

further discussion of this subject, the term hypertension will be used to describe individuals with systolic pressure over 150 mmHg, unless otherwise stated.

III. MODELS OF HYPERTENSION

Research in chronic diseases, such as hypertension, with human subjects is frustrating, time-consuming and often impossible to control. Animal models, on the other hand, provide well controled and time-compressed experimental conditions. In the past few decades, much of the reasearch in hypertension has been directed to finding an animal model which resembles "essential" hypertension. As a result, many experimental models were produced by intervening in the normal blood pressure control mechanisms using chemical, mechanical and other means.

IV. UNITARY MODELS OF HYPERTENSION

There are at least seven key mechanisms involved in normal blood pressure homeostasis (Guyton et al, 1972). If any one of these control mechanisms is disturbed, it will promptly elicit complex adjustments of virtually all the others, and a hypertensive condition may occur resulting in a unitary model of hypertension. These control mechanisms are:

1. corticohypothalamic-bulbar neurohormonal influences;
2. the baroreceptor reflex mechanism;
3. cardiac and smooth muscle design, function and intrinsic control;
4. renal function;
5. the renin-angiotensin system;
6. aldosterone and other hormonal regulation;
7. the reflex volume control.

By interfering with one or more of these control mechanisms, experimental models bearing some similarities to essential hypertension, have been

produced from genetically normotensive animals. For example, a persistent elevated blood pressure has been produced:

1. in dogs be clamping the renal artery with an adjustable silver clamp (Goldblatt et al, 1934);
2. in rabbits by wrapping the kidney with cellophane or silk (Grollman, 1944; Page, 1939);
3. in rabbits by infusing crude renin intravenously (Blacket et al, 1950);
4. in rats by infusing angiotensin intravenously (Dickinson and Lawrance, 1963; Koletsky et al, 1966; McCubbin et al, 1965);
5. in chicks by using deoxycorticosterone acetate (Selye, 1942);
6. in dogs by disrupting the sinoaortic nerves surgically (Hering, 1927-cited in Nosaka and Wang, 1972);
7. in dogs by placing bilateral lesions in the nucleus tractus solitarii (Carey et al, 1979).

Although these unitary animal models have led to a better understanding of many types of secondary hypertension, e.g. 1-4 are related to renal hypertension, they have proven not to be good models of human essential hypertension.

V. GENETIC MODELS OF HYPERTENSION

The discovery of the genetically linked hypertensive animal models marked the advance in the present knowledge of essential hypertension. In the early 1950s, Alexander et al (1954; 1956) attempted to isolate a colony of spontaneously hypertensive rabbits. Unfortunately, these rabbits did not maintain a sufficiently high steady pressure to be recognised as a model for essential hypertension. Subsequently, during the period from the late 1950s to the early 1970s, four different strains

of hypertensive rats were developed by various groups which are considered to be valuable models of essential hypertension. These are:

1. Sprague Dawley-Brookhaven strain of hypertension-sensitive rats (HSR) developed by Dahl's group (Dahl et al, 1962a; 1962b) in the United States of America.

The HSR are mostly normotensive rats when subjected to a low salt intake, but on a high salt diet, two strains, salt-sensitive and -resistant, are recognised. On an 8% salt intake, for example, the salt-sensitive rats exhibit an elevated systolic pressure well beyond 200 mmHg, while the resistant rats remain relatively normotensive (Dahl et al, 1968). In addition, young HSR appear to be more sensitive, i.e. the severity and rate of rise in pressure, to a given salt load than mature HSR.

2. Wistar-Milan strain of spontaneously hypertensive rats (MHS) developed by Bianchi's group (Bianchi et al, 1973; 1975) in Italy.

The MHS represent a relatively mild form of hypertension as compared with GHS and SHR (see below). The systolic preesure in these rats usually rises from normal to about 160-170 mmHg by the fourth to the seventh week after birth and remains at this level thereafter.

3. Wistar-Otago strain of genetically hypertensive rats (GHS) developed by Smirk's group (Smirk and Hall, 1958) in New Zealand.

The GHS was the first strain of spontaneously hypertensive rats isolated. The systolic pressure of these rats begins to rise in early life and reaches its plateau of 165-175 mmHg at about 6-8 weeks.

4. Wistar-Kyoto strain of spontaneously hypertensive rats (SHR) isolated by Okamoto's group (Okamoto and Aoki, 1963) in Japan.

The SHR represent the most severe form of hypertension among the different strains of spontaneously hypertensive rat; many SHR show a

systolic pressure exceeding 200 mmHg at about 25 weeks of age (Okamoto and Aoki, 1963).

Among the four strains of hypertensive rat, the SHR has been exposed to the most extensive investigations, and is widely accepted as the best animal model so far known for studying essential hypertension (Cutilletta et al, 1977; Imamura, 1978; Kawamura et al, 1976; Lund and Tomanek, 1978; Nosaka and Wang, 1972; Okamoto, 1969; Wexler, 1979; Yamori, 1977). For these reasons, the SHR has been choosen as the experimental model in this study. The SHR will now be considered in more detail.

VI. DEVELOPMENT OF BLOOD PRESSURE IN SHR

The SHR was isolated by Okamoto and Aoki (1963) in Japan. Among the Wistar stock maintained in Kyoto University, they noted that a male rat had developed hypertension spontaneously (by their definition, a systolic pressure of 150 mmHg persisting over a month). By mating this rat with a female which showed a systolic pressure lightly above the average range (130-140 mmHg) and subsequent brother-sister matings of their offsprings, a strain of rats which invariably developed hypertension was isolated. The elevation of blood pressure in the SHR is gradual but at a fairly early age. Usually, at the age of 5-6 weeks, the systolic pressure of the SHR is significantly higher than the control (Lais et al, 1974; Okamoto et al, 1972), but it can be as early as 4 weeks (Lais et al, 1977) or as late as 12 weeks (Moll et al, 1975) of age. The rate of rise in the systolic pressure continues over the next few weeks and reached its plateau of 200-210 mmHg at about 12-16 weeks.

VII. INHERITANCE OF SHR

The inheritance of hypertension in the SHR and its mode appears to be polygenic in nature (Hansen, 1972, Louis et al, 1969a; Okamoto, 1969).

Tanase et al (1972) reported such an inheritance in the SHR involved relatively few major genetic components which seemed to act additively and were transmitted equally well in both males and females, however, the level of hypertension in the latter was somewhat lower throughout their life span (Okamoto, 1969). Although the development of hypertension may well be due to the interaction between genetic and environmental factors (Folkow and Hallback, 1977), it appeared that genetic factors play a dominant role but that the course of hypertension can be modified by environmental factors (Hallback and Folkow, 1974; Lais et al, 1974; Okamoto, 1969). This is substantiated by the fact that since the isolation of the SHR in Japan, many SHR colonies have been established in many parts of the world; they all invariably develop hypertension despite different environmental factors involved, although some of them appear to be milder (Folkow and Hallback, 1977).

VIII. CAUSES OF HYPERTENSION IN SHR

Since the isolation of the SHR, much work has been devoted to trying to find the "primary" cause(s) of the spontaneous hypertension in these animals, hoping that such a discovery may shed some light on the understanding of the aetiology of human hypertension. Up to now, the primary areas considered to be involved are the nervous system, renal function and the cardiovascular system. The following discussion will mainly include information about the SHR. For those areas in which information concerning the SHR is not available, but may be important in initiating and/or maintaining the hypertension in these animals, results from other forms of hypertension such as Goldblatt hypertension will be summarised. No attempt will be made to correlate these data with the type of hypertension.

IX. NERVOUS SYSTEM OF SHR

Investigation of the nervous system, especially the central nervous system (CNS), is often the most confusing and frustrating by virtue of the complexity of its function. From the results obtained by Okamoto's group (Okamoto, 1969; 1972; Yamori et al, 1972) it appears that there are differences between the CNS of the SHR and the normotensive control concerning their efferent nervous and hormonal control of the organ systems and the metabolism. For example, nuclear and cellular size of neurones in supraoptic, paraventricular, arcuate and vagal dorsal motor nucleus were larger in the SHR as compared with the controls. In addition, they also showed that there is an increase in activity in the sympatho-adrenal, the ACTH-corticoid and the TSH-thyroxine systems in the SHR as compared with the control (Okamoto, 1969; 1972). Recently, Lehr et al (1980) showed that the SHR have a larger metencephalon and smalier mesencephalon and diencephalon than the WKY controls.

Young SHR are known to react more to both chronic (Yamori et al, 1969) and acute (Hallback and Folkow, 1974) stress than their normotensive controls; such an activity appears to subside with age (Folkow and Hallback, 1977). This reaction is associated with a higher heart rate, an increase of cardiac output and a moderately raised mean arterial pressure which is characteristic in the pattern of increased sympathetic discharge. Studies on the noradrenaline (NA) turnover in various cardiovascular compartments further support this observation. For example, the NA turnover in the heart of young SHR showed an increase in activity (Yamori, 1972; 1977) and such an activity seemed to decrease in mature animals (Louis et al, 1969b; Nakamura et al, 1972; Vetadzokoska et al, 1972). Similar results were reported in studies on plasma NA levels (Nakamura and Nakamura, 1978).

Chemical sympathectomy by 6-hydroxydopamine at birth prevents hypertension in the SHR, although the blood pressures in these animals still remain higher than the treated control (Folkow et al, 1972). Thus these authors concluded that just the "hyper-reactivity" in the SHR due to the "hyper-activity" of the sympathetic nervous system alone cannot totally account for the elevated blood pressure. Recently, Johnson and Macia (1979) reported that the SHR are resistant to guanethidine-induced chemical sympathectomy which can be overcome by a treatment with antibody to nerve growth factor. Thus, the discrepancies of the 6-hydroxydopamine-induced sympathectomy in the SHR and normotensive controls reported by Folkow et al may well be due to such a unique "resistance" in the SHR.

Baroreceptor function has long been recognised to bear a close relationship to hypertension (Hering, 1927); it is generally accepted that the carotid sinus baroreceptors are reset to maintain a higher blood pressure in chronic renal hypertension (McCubbin et al, 1956) and in hypertension of the SHR (Nosaka and Wang, 1972). In the SHR, the baroreceptor function appeared to exhibit the peak response at a basal mean blood pressure of 160 mmHg, whereas those of the normotensive controls showed the greatest sensitivity at 100 mmHg (Nosaka and Wang, 1972). The mechanism of how the baroreceptors are reset is not known. From the work of Rees et al (1978) on carotid sinuses in experimental hypertensive dogs, structural defects seemed to be confined to the intimal and/or medial areas of the sinus wall. In the SHR, histological studies showed a close correlation between the aortic hypertrophy and baroreceptor resetting (Sapru and Wang, 1976).

Intimately related to the baroreceptor reflexes is the vasomotor regulatory centre which is located in the medulla of the brain. The exact structures and their functions involved in the regulation of blood

pressure are yet to be defined. From the results of recent experiments, the centre appears to include the nucleus tractus solitarius, the nucleus locus coeruleus, the area postrema, the hypothalamus and the reticular formation.

1. THE NUCLEUS TRACTUS SOLITARIUS (NTS)

The structure of the NTS and its connexion to other areas have been described by Palkovits and Zaborsky (1977). Experimental evidence shows that the afferent cardiovascular information is relayed, either directly or indirectly, to the nucleus ambiguus and the dorsal nucleus of the vagus and to a number of reticular nuclei through the NTS (Calaresu et al, 1975- a review; Joy, 1975). The NTS appears to exert an inhibitory effect on the cardiovascular system; stimulation of the NTS produced bradycardia and hypotension (Barnes et al, 1979; Crill and Reis, 1968; De Jong, 1974; Gunn et al, 1968), and lesions of the NTS resulted in hypertension (Carey et al, 1979; Snyder et al, 1978). Furthermore, Struyker-Boudier et al (1975) reported that injection of noradrenaline into the area of the NTS results in a fall in blood pressure and heart rate.

In young SHR, both the dopamine beta-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) activities in the NTS did not differ from the young normotensive controls. Whereas in adult SHR, the DBH activities of the NTS was significantly elevated, while the PNMT activities remained unchanged as compared with the controls (Nakamura and Nakamura, 1978).

2. THE NUCLEUS LOCUS COERULEUS (LC)

The LC and related nuclei are important in influencing cortical (Anden et al, 1967), cerebellar (Anden et al, 1967; Siggins et al, 1971), medullary (Anden et al, 1966), hypothalamic (Dahlstrom and Fuxe, 1964)

and spinal (Sasa et al, 1974) functions, as well as in modulating arterial pressure controls (Ward and Gunn, 1976a; ,976b). Recently, studies showed that there may be abnormalities in the LC of the SHR. Kawamura et al (1978) showed that electrical stimulation of the LC produced frequency-related pressor responses associated with increase heart rate seen in the normotensive controls were not present in the SHR. Nakamura and Nakamura (1978) also reported the DBH activities in the LC of young SHR were significantly elevated, whereas that of the adult, and the PNMT activities in both young and adult did not change as compared with the controls. On the contrary, Renaud et al (1979) found that the basal and the reserpine-induced, elevated tyrosine hydroxylase (TH) activities of the LC and the A-1 neurones (catecholamine nucleus lies ventrolaterally in the medulla) were similar to the controls, in both young and adult SHR. However, reserpine failed to increase the TH activities in the A-2 neurones (catecholamine nucleus lies in the dorsal medullary tegmentum) of mature SHR.

3. THE AREA POSTREMA (AP)

The AP is known to have a close relationship with various nuclei which involved in cardiovascular control mechanisms. Connexions between the AP and the ipsilateral (Brizzee and Neal, 1954) and the contralateral (Morest, 1967) NTS have been observed. Furthermore, Scroop and Lowe (1968) demonstrated that intravertebral artery injection of very low doses of angiotensin II caused an increase in heart rate, blood pressure and cardiac output of the dog; these responses required the integrity of the areas postrema (Joy and Lowe, 1970). Thus Joy (1971) put forward a concept of the angiotensin-area postrema axis as a neurohumoral vasomotor centre afferent pathway. Most recently, Barnes et al (1979) showed that the electrical stimulation of the AP can elicit responses simimlar to the

intravertebral artery injection of angiotensin II.

The function, with respect to cardiovascular control, of the AP and its connexions with other brain structures in the rat, in particular the SHR, deserve more attention.

4. THE HYPOTHALAMUS

The hypothalamus has been suggested to be an important area in the central control of blood pressure (Calaresu et al, 1975-a review; Folkow and Rubinstein, 1966; Nathan and Reis, 1975; Philippu, 1975; Philippu et al, 1970). Electrical stimulation of the posterior hypothalamus elicits a rise in blood pressure, whereas such a manipulation in the anterior hypothalamus results in bradycardia and hypotension (Nathan and Reis, 1975; Philippu, 1975). Similar results were obtained by injection of noradrenaline into the anterior hypothalamus (Struyker-Boudier et al, 1975). These responses seem to be mediated through a massive release of catecholamine either from the adrenal medulla (Nathan and Reis, 1975) or the hypothalamus (Philippu et al, 1970) and the main nucleus involved appears to be the nucleus interstitialis striae terminalis (Lindvall and Stenevi, 1978). The information concerning the function of the hypothalamus in blood pressure control of the SHR is not available at the present time.

5. THE RETICULAR FORMATION

The reticular formation contains many interconnected nuclei and its location extends from the medulla to the mesencephalon. By virtue of its diffuse anatomical arrangement, research in this area is relatively difficult. Miura and Reis (1969) showed that during carotid sinus nerve stimulation polysynaptic responses can be recorded in virtually all the nuclei of the reticular formation. Thus its involvement in cardiovascular control has been proposed. Although projections of the primary cardiovascular fibres to the NTS are well accepted, there is limited amount of

infromation concerning projections from this nucleus (Calaresu et al, 1975-a review). From the electrophysiological studies by Calaresu's group (Calaresu and Henry, 1970; Calaresu and Thomas, 1971; Henry and Calaresu, 1974a; 1974b) and Reis' group (Crill and Reis, 1968; Miura and Reis, 1968; 1971), it appears that the nuclei gigantocellularis, parvocellularis, reticularis ventralis and reticularis lateralis provide an excitatory input to cardiovascular spinal neurones and that the paramedian nucleus, the ventral nucleus of the medullae oblongatae centralis and the raphe nuclei provide an inhibitory input to the same neurones. Relatively little information has been obtained about the structures and functions of the reticular formation in the SHR.

Although the CNS is not the topic of this thesis, it is still of prime importance to recognise the relation of these structures and their function(s) with respect to the cardiovascular control mechanism in order to define the cause(s) of hypertension in the SHR.

X. RENAL FUNCTION OF SHR

Less effort has been concentrated on the renal function of the SHR. With the information on hand, there seems to be no indication of renal disturbance in young SHR. These include the normal blood and plasma volume (Sen et al, 1972a), as well as the sodium content and balance (Baer et al, 1972). The results on the plasma renin activity (PRA), however, are conflicting. Koletsky et al (1972) observed a normal or even subnormal PRA in the SHR, while Sen et al (1972b) reported a modest rise in the PRA in young SHR and a normal to subnormal level in adults. Such a discrepancy may be explained by the difference in age of animals used. Furthermore, the observed rise in the PRA in young SHR of Sen's group may be accounted for by the hyper-activity of the sympathetic nervous

system in young SHR, since renin release is modulated by beta-adrenergic receptor mechanisms (Freeman and David, 1977).

The information of structural changes in the kidney of adult SHR is fragmented. According to Okamoto (1969) and Freis (1972), changes such as fibrous hyperplasia and fibrinoid necrosis of the glomeruli, intimal hyperplasia of arterioles with areas of fibrinoid necrosis and proteinacious material in tubules were commonly found in adult SHR. Furthermore, these changes appeared to occur more rapidly and severely in the SHR which fed with a salt or a fat-cholesterol-salt diet (Hazama et al, 1972). More studies need to be done concerning the morphology of the kidney in the SHR, especially in the pattern of the renal vasculature. From the work of Ljungqvist (1962) on kidneys of patients with essential hypertension, it suggested that there is a reorganisation of the intrarenal vasculature so that the blood supply to the renal cortex is reduced, whereas that of the medulla is improved.

Kidney transplantation has also been performed on the SHR. Using this technique, Kawabe et al (1978) showed that the hypertension was transferred with the kidney graft to the normotensive recipient. This result strongly suggested that the kidney is important in maintaining, if not initiating, the elevated blood pressure in the SHR. The mechanism of how the elevated blood pressure in the SHR is maintained is not known, although some pro-hypertensive factors other than renin release from the hypertensive kidney were proposed (Kawabe et al, 1978).

XI. CARDIOVASCULAR SYSTEM OF SHR

A large body of information is presently available concerning the cardiovascular system of the SHR. Much of this information supports the hypothesis that the cardiovascular system is important in initiating and/

or maintaining the elevation of blood pressure in the SHR. As will be described later, testing this hypothesis is the main objective of this thesis, and for this reason the cardiovascular system of the SHR will now be discussed in detail.

a. HEART

Cardiac hypertrophy is the most common finding associated with many forms of hypertension (Beznak et al, 1969; Goldstein et al, 1974; Grant, 1953; Hall et al, 1953; Malik et al, 1974; Page and McCallister, 1973), including that of the SHR (Imamura, 1978; Kawamura et al, 1976; Lund and Tomanek, 1978; Sen et al, 1974; Takatsu and Kashii, 1972). In the SHR, as in other forms of hypertension, the cardiac hypertrophy is characterised by an increase in muscle fibre diameter, muscle cell volume fraction, and myofibril volume fraction and a decrease in mitochondrial volume fraction and capillary density (Imamura, 1978; Lund and Tomanek, 1978). From the report of Kawamura et al (1976) and Imamura (1978), it appears that the cardiac hypertrophy in the SHR is the result of both the hypertrophy of myocardiocytes and the proliferation of interstitial tissues. The hypertrophy of myocardiocytes appeared to be an addition of sarcomeres, both in series and in parallel, to the existing myofibril (Iaks et al, 1974). This is also evident in cardiac hypertrophy of the SHR (Imamura, 1978; Kawamura et al, 1976). The exact location of sarcomerogenesis in a myocardiocyte is not known. However, it has been postulated that the "new" sarcomeres are laid down at the intercalated disc (Adomian et al, 1974) or at the "Z" bands (Legato, 1970) or both (Imamura, 1978).

The cardiac hypertrophy associated with different forms of hypertension including that of the SHR is thought to be the result of elevated blood pressure. This is substantiated by the fact that there is a

gradient of the location of hypertrophy in the heart, e.g. in the SHR, the changes in the left ventricle are more pronounced than other parts of the heart (Imamura, 1978; Kawamura et al, 1976). Furthermore, significant changes in the SHR, indicative of cardiac hypertrophy, appeared at the age of 11 weeks as compared with the controls. The authors concluded that these changes were the result of the elevated blood pressure, because at the age of 7 weeks the systolic pressure of the SHR was significantly higher than the normotensive controls. On the contrary, Sen et al (1974) showed that cardiac hypertrophy in the SHR appeared at the age of about 3-4 weeks, preceding the elevation of blood pressure. However, their result was based on the ventricular weight which does not distinguish between the connective tissue and the myocardiocytes and it is, therefore, less reliable than the electron microscopic analysis that Imamura (1978) and Kawamura et al (1976) used.

Cardiac hypertrophy in rat (Beznak et al, 1969; Cutilletta et al, 1975; Hall et al, 1953), cat (Carey et al, 1978) and man (Kempner, 1948) can be reversed at various stages after the induction of hypertrophy by relieving the pressure-overload to the heart. The regression is characterised by the reduction of myocardial mass. The connective tissue hypertrophy, on the other hand, does not regress as readily (Cutilletta et al, 1975). However, the development of cardiac hypertrophy persists despite the lowering of blood pressure in the SHR by anti-hypertensive therapy (Sen et al, 1977) and peripheral sympathectomy (Cutilletta et al, 1977; Page and Oparil, 1978). Thus, it follows that cardiac hypertrophy in the SHR may result from more than one mechanisms rather than just the pressure alone.

While the cause of cardiac hypertrophy in the SHR is not known, the

possibility of "cardiogenic" hypertension in these animals has not been ruled out. From the studies of Frohlich's group (Pfeffer and Frohlich, 1973; Pfeffer et al, 1974), the cardiac output appeared to be elevated in young SHR, whereas that of the mature SHR seemed to be at a normal to subnormal level. This transient increase of cardiac output in young SHR may be of great important in defining the process involved in the initiation of hypertension in the SHR.

B. BLOOD VESSEL

Recent haemodynamic studies indicated that in the established stage of hypertension, the SHR exhibit a normal to subnormal cardiac output and an elevated total peripheral vascular resistance (Iriuchijima, 1973; Pfeffer and Frohlich, 1973; Pfeffer et al, 1974; Tobia et al, 1974a). There seems to be a uniform rise in regional resistance in young and mature SHR (Tobia et al, 1974a; 1974b), as judged by evaluation of the distribution of cardiac output. The increased vascular resistance can be the result of various factors which include increased sympathetic activity, vessel wall hypertrophy and altered responsiveness to catecholamines by vascular smooth muscle (Folkow et al, 1969; 1970; Hutchins and Darnell, 1974; Jellinek et al, 1977; Judy et al, 1976). The latter two will now be considered in detail; the increased sympathetic activity has been discussed earlier.

1. VESSEL WALL HYPERTROPHY

It is well known that the vessel wall becomes stiffened and thickened in hypertensive animals (Aabs, 1968; Berry and Greenwald; 1976; Bevan, 1976; Bevan et al, 1976; 1980; Wiener et al, 1977; Wolinsky, 1970; 1971; 1972) and patients (Karsner, 1938; Naeye, 1967; Pickering, 1968; Cook and Yates, 1972). These changes in the blood vessels may account for

the higher peripheral resistance observed in hypertensive patients (Folkow et al, 1958; Sivertsson, 1970) and in the SHR (Folkow et al, 1969; 1970; Iriuchujima, 1973; Pfeffer and Frohlich, 1973; Pfeffer et al, 1974; Tobia et al, 1974a, 1974b). These stiffening and thickening processes appear to be the result of the thickening of the intima, and increase in vascular connective tissue, hypertrophy and/or hyperplasia of the vascular smooth muscle or a combination of all three.

a. TUNICA INTIMA

The thickening of the intima of large arteries is one of the typical changes of hypertensive disease, in general (Robbins and Cotran, 1979). This intimal thickening is characterised by structural changes of the endothelial cells, thickening of the subendothelial layer and an increase of transendothelial permeability.

i). STRUCTURAL CHANGES OF ENDOTHELIAL CELL

Very few studies concerning the morphology of the intima of the SHR have been published. Still (1979) reported that the intimal thickening in the thoracic aorta of the SHR did not seem to be organised in any pattern. Unfortunately, his description on the subject was vague and fragmented. More effort is required to describe the intima of the SHR, with respect to the development of hypertension, in full detail.

Endothelial cells in the early stages of various forms of hypertension other than that of the SHR exhibit an increase of organelles, especially rough-surfaced endoplasmic reticulum, ribosomes, Golgi complex and mitochondria (Gabbiani et al, 1979; Huttner et al, 1970; Suzuki et al, 1975). Furthermore, Gabbiani et al (1979) reported the presence of microfilament bundles in association with the early stages of hypertension. However, these microfilament bundles also present in normal rats when the intima was.

sectioned tangentially (Buck, 1979; Gerrity and Cliff, 1972). It appears that the mode of sectioning for microscopy is important to demonstrate these microfilament bundles. Unfortunately, the former authors did not mention how they sectioned the endothelium for electron microscopic examination. Although the function of these microfilament bundles is not known (Hammersen, 1976), it has been suggested that they may be related to contraction (Becker and Nachman, 1973; Majno et al, 1969; Rostgaard et al, 1972), increased permeability (Gabbiani et al, 1975; Majno et al, 1969), and support or structure (Gerrity and Cliff, 1972; Hammersen, 1976) of the endothelium.

Hypertrophy and hyperplasia of the endothelial cells are said to be associated with many types of experimental hypertension. Gabbiani et al (1979) reported that the thickening of the endothelium, an indication of endothelial cell hypertrophy, was apparent in the early phases following the induction of hypertension and regressed by 40 days. The degree of regression appeared to be varied with different types of hypertension. Their conclusion on the thickening of the endothelium was based on the measurement of the mean thickness of the endothelium. Such a measurement is very much dependent on the perfusing pressure during fixation for microscopy, Unfortunately they did not report the perfusing pressure used during fixation in their study. More comprehensive studies, perhaps using stereologic techniques, are required for such a conclusion. Hyperplasia of the endothelial cells is also known to accompanying hypertension. Schwarts and Benditt (1977) found that there was an 10-fold increase in the rate of replication of endothelium in hypertension produced by coarctation of the renal artery in the rat, using tritiated thymidine labelling techniques. This information concerning hypertrophy and hyperplasia of the endothelial cells has not been acquired for the SHR.

ii). THICKENING OF SUBENDOTHELIAL LAYER

The thickening of the subendothelial layer is one of the prominent findings of many types of experimental hypertension (Gabbiani et al, 1979; Huttner et al, 1970; Suzuki et al, 1975), including that of the SHR (Behrendt and Kuhnel, 1976). The exact composition of this progressive accumulation of connective tissue matrix in the subendothelial layer is not known. It appears to be a basement membrane-like substance (Behrendt and Kuhnel, 1976; Gabbiani et al, 1979; Suzuki et al, 1975) and very rarely elastin (Suzuki et al, 1975). The significance of this accumulation is not clear; it may be important in maintaining the elevated blood pressure in hypertension of both experimental animals and the SHR.

iii). TRANSENDOTHELIAL PERMEABILITY

At the early stages of experimental hypertension, it appears that there is an increase of transendothelial permeability (Gabbiani et al, 1979; Huttner et al, 1970). Using tracers such as horseradish peroxidase (mol. wt. 40,000; diameter about 50 angstroms) and ferritin (mol. wt. 500,000; diameter about 110 angstroms), Huttner et al (1970) found that in normal rats, the peroxidase reaction product appeared in aortic endothelial cell junctions and within plasmalemmal vesicles, whereas the ferritin molecules were found mainly within plasmalemmal vesicles. These provide evidence for the presence of the hypothetical "pore" systems in transendothelial transport namely the endothelial cell junctions correspond to the small pores and the plasmalemmal vesicles the large pores. In hypertension, the mode of transendothelial transport does not seem to be altered although the reaction products are much higher, in particular, the amount of ferritin in the plasmalemmal vesicles, indicative of higher activity (Huttner et al, 1970).

Rippe and Folkow (1977) reported that there appeared to be no increase in capillary permeability in the SHR. However, the less pronounced edema formation in their SHR hindquarter preparation may indicate the overall increase in transendothelial transport in both direction as compared with the normotensive controls.

b. CONNECTIVE TISSUE IN VASCULAR WALL

Both elastin and collagen are said to be increased in the vasculature of renal hypertensive rats (Wolinsky, 1970; 1971; 1972). Recently, much of the work in this area has been focussed on collagen metabolism. From the results of biochemical assay for the enzyme prolyl hydroxylase and tritium-labelled proline incorporation studies, it is well accepted that an increase in vascular collagen synthesis and deposition invariably accompany many forms of hypertension such as DOCA-salt hypertension (Nissen et al, 1978; Ooshima, 1977; Ooshima et al, 1974; 1975) including that of the SHR (Iwatsuki et al, 1977a; Newman and Langner, 1978; Sheridan et al, 1979). In DOCA-salt hypertensive rats, these changes can be reversed by treating the hypertensive animals with anti-hypertensive drugs such as chlorothiazide or reserpine (Ooshima et al, 1975). In addition, there is a decrease in blood pressure and a reduction of vascular collagen turnover in DOCA-salt hypertensive rats treated with beta-aminopropionitrile, a specific inhibitor of the enzyme lysyl oxidase which mediates the first step in the cross-linking of collagen and of elastin in the oxidative deamination of lysine and hydroxylysine (Iwatsuki et al, 1977b). The latter two experiments have not been reported in the SHR.

It appears that the increase of collagen synthesis in the vasculature of hypertensive animals including that of the SHR is important for the maintenance of the elevated blood pressure. This is supported by the

evidence that treatment with a collagen synthesis inhibitor such as beta-aminopropionitrile can prevent the elevation of blood pressure in DOCA-salt hypertensive rats (Ooshima et al, 1974; Iwatsuki et al, 1977b). In the SHR, the increase of collagen synthesis does not appear until 23 weeks of age following the elevation of blood pressure to a significantly level as compared with the normotensive controls (Newman and Langner, 1978). Furthermore, it has also been reported that the increase in collagen biosynthesis in both the DOCA-salt hypertensive rat and the SHR is present in arteries but not in veins (Iwatsuki et al, 1977b). This further supports the notion that collagen synthesis in the vasculature of hypertensive animals is pressure dependent.

Although there is enough evidence to support the belief that high blood pressure can stimulate the collagen biosynthesis and deposition in the vessel wall resulting in a stiffened artery which may be one of the determinants for maintaining the elevated blood pressure, it is still necessary to define the origin and location of the increased collagen. This requires more direct morphological and quantitative studies.

c. VASCULAR SMOOTH MUSCLE (SMC)

Many studies have been reported on the reaction of the SMC to experimental hypertension. These reports seem to suggest that there is an increase in SMC content in the vessel wall of many types of hypertensive animals. Using biochemical methods, Wolinsky (1970; 1971; 1972) observed that the amount of non-collagenous protein, interpreted as the SMC content, was increased with renal hypertension. This increment of SMC content may be the result of hypertrophy and/or hyperplasia. The resulting increased vascular wall thickness in hypertensive patients (Pickering, 1968) and hypertensive rats (Greditzer and Fischer, 1978) has been claimed to be the result of SMC hypertrophy. This is supported by Wiener et al (1977) who reported that there was a 58-60% increase in SMC cross-sectional area

in the thoracic aorta of renal hypertensive rats. Autoradiographic studies, using tritiated thymidine, showed tha SMC mitosis is prominent in experimental hypertension (Bevan, 1976; Bevan et al, 1976; Crane and Dutta, 1963; Fernandez and Crane, 1970), especially in the early stages (Bevan et al, 1980). These results suggest that large arteries such as the aorta may play a role in initiating and/or maintaining the elevated blood pressure in experimental animals.

There has been no study reported on the structure of large arteries during the development of hypertension in the SHR; the information concerning the SMC hypertrophy and hyperplasia has not been published. However, studies on small arteries exhibit conflicting results. Ichijima (1969) reported that the luminal diameter of small arteries of the SHR was generally smaller than that of the controls. Mulvany et al (1978) and Warshaw et al (1979) found that there was an increase in wall thickness in the mesenteric arteries of the SHR. On the contrary, Bohlen (1979), and Bohlen and Lobach (1977) did not detect any changes in the microvasculature of the cremaster muscle in the SHR. This discrepancy may be due to the different vascular bed studied.

There seems to be not enough information concerning the morphology of the SMC in both developing and adult SHR at the present time. It is of prime important to acquire this information, which forms the basis of comparing the cardiovascular system of the SHR with the normotensive controls, in order to define the cause(s) of hypertension in these animals.

2. REACTIVITY OF BLOOD VESSELS

The reactivity of a blood vessel to a given vasoactive agent is defined as the responsiveness, either sensitivity or contractility, of that blood vessel to that given vasoactive agent.

Studies have shown that the basal vascular resistance and the vascular reactivity are increased in hypertensive patients (Conway, 1963) and experimental animals (Folkow, 1971-a review). However, more recent studies on the reactivity of the SHR vasculature present many conflicting results. Generally, reactivity studies are performed on isolated vessels or vascular bed by the perfusing the tissue preparation with, or on vascular strips of various types of artery and vein by incubating the tissue preparation in a physiological solution to which vasoactive agents can be added.

Perfusion studies indicate that there is an overall increase in responsiveness of blood vessels and vascular beds, isolated from various forms of hypertension, to noradrenaline (Hink, 1965; McGregor and Smirk, 1968; McQueen, 1956). In the SHR, this responsiveness appears to be present in young (Bohlen, 1979; Lais and Brody, 1975; Okamoto et al, 1966) but not in mature (Shibayama et al, 1967) animals. However, results on the responsiveness of the vascular system to 5-hydroxytryptamine (5-HT) indicate that there is regional difference. Ahlund et al (1977) and Haeusler and Finch (1972) reported the hindquarter vascular bed of the SHR and of the normotensive controls were insensitive to 5-HT. No significant difference can be detected between the two groups. Furthermore, Ahlund et al (1977) observed that the SMC of the aorta and of the portal vein in the SHR were more sensitive to 5-HT than the controls. Although perfusion studies can be useful in determining the responsiveness of the vasculature of the hypertensive animals, they cannot distinguish whether the increased reactivity is due to structural changes in the vasculature or the hyper-reactivity of the SMC.

In order to test the possibility that the increased resistance in hypertensive animals is due to the hyper-responsiveness of the SMC, isolated vascular rings or strips have been used. From the information available,

it seems that different vessels react differently to a given vasoactive agent. The results from the aorta of the hypertensive animals are confusing. Tissues from different forms of hypertension have been shown to produce less (Mallov, 1959; Redleaf and Tobian, 1958), similar (Clineschmidt et al, 1970; Hallback et al, 1971) or more (Gordon and Nogueira, 1962) contractile force than the normotensive controls when challanged by noradrenaline. In the SHR, aortic strips exposed to noradrenaline appear to produce less (Shibata and Kurahashi, 1972; Spector et al ,1969) or similar (CLineschmidt et al, 1970; Hallback et al, 1971) contractile force than the controls. Recent studies using the femoral artery from renal and DOCA-salt hypertensive rats demonstrated that the reactivity of the SMC to noradrenaline was increased, whereas the contractility was decreased (Bandick and Sparks, 1970; Bohr and Sitrin, 1970; Holloway et al, 1972). Furthermore, the SMC from the SHR appears to react differently to manganese and lanthanum (Bohr, 1974), prostaglandins (McMurtry et al, 1979) and potassium, indicative of sodium-potassium ATPase activity (Webb and Bohr, 1979) when compared with the controls.

In summary, there appears to be an "abnormal" responsiveness in the vasculature of the SHR to vasoactive agents. The cause of this abnormality, whether it is due to structural changes in the vasculature or intrinsic activity in the SMC of the hypertensive animals, is uncertain. It has been postulated that there is a defect in the calcium transport mechanism in the SMC (Goldberg and Triggle, 1978; Noon et al, 1978; Zsoter et al, 1977) and also the cardiac muscle (Limas and Cohn, 1977) from the SHR. Direct evidence supporting this hypothesis has been obtained by Kwan et al (1979) using a plasma membrane fraction isolated from the SHR. They observed that enhanced alkaline phosphatase activity and reduced ATP-dependent calcium accumulation preceded the development of hypertension in the SHR.

Although the elevated peripheral resistance observed in the SHR can be a result of either structural changes of the vessel wall or altered responsiveness of the SMC, the possibility of the alteration of the overall design of the vasculature cannot be ruled out. Hutchins and Darnell (1974) reported that there is a decrease in the number of small arterioles in the cremaster muscle of the SHR.

XII. HYPOTHESES ON THE CAUSATIVE FACTORS IN HYPERTENSION OF THE SHR

Based on the results obtained so far from studying the SHR, the following areas have been implicated in initiating the hypertension in these animals.

1. ABNORMALITIES IN THE CNS (Judy and Farrell, 1979; Judy et al, 1976; 1979; Okamoto, 1969; Yamori, 1977).

Many authors have suggested that neurochemical changes in the CNS structures related to the sympathetic nervous system including those of the vasomotor centre may lead to some adaptive changes in the arterial system resulting a persistent elevated blood pressure. However, substantial evidence supporting this theory is freagmented.

2. CHANGES IN RENAL FUNCTION (Guyton et al, 1974).

In trying to formulate the blood pressure control mechanisms with computor programmes, Guyton et al observed that the volume control function of the kidney can produce an infinite gain. Thus they postulated that changes in this mechanism may result a sustained high blood pressure. Unfortunately, only a limited amount of information is available at present to support this notion.

3. ALTERATION IN VASCULAR DESIGN (Conway, 1963; Folkow, 1978; Folkow and Hallback, 1977; Folkow et al, 1970; 1973; Pickering, 1968; 1977; Sivertsson, 1970).

It is generally accepted that there is an increase in basal vascular

resistance and vascular reactivity in hypertensive patients and in hypertensive animals. The cause of these alteration is not known.

As described by standard pathology textbooks, arterioles in hypertension exhibit medial hypertrophy. Many factors may be attributed to this hypertrophy (see above). Folkow and coworkers speculated that morphological changes such as hypertrophy and/or hyperplasia of the SMC can explain the increased basal vascular resistance and vascular reactivity in hypertension. Firstly, the increased SMC content can encroach the lumen of the blood vessel resulting in a narrowing of the luminal diameter and thus increasing the vascular resistance even when it is maximally dilated. Secondly, the increased vascular reactivity can be explained by the increased SMC mass, without any alteration in the sensitivity or reactivity of the SMC. In response to a given stimulus, the outer most muscle layer of the normal vessel wall, where all the constrictor neuroeffector junctions are located, contracts and pushes the remaining SMC mass towards the lumen. However, in a vessel with medial hypertrophy, an increased bulk of SMC is pushed inwards to produce an exaggerated reaction. This may be interpreted as hyper-reactivity of the vasculature of hypertensive animals and hypertensive patients. Similar theory has been postulated by Conway, Pickering and Sivertsson.

Although many experimental results appeared to support this theory, no well-designed experiment has been published to verify the involvement of medial hypertrophy in initiating and/or maintaining the elevated blood pressure. Furthermore, most of the results obtained for the formulation of this theory were based on either patients or experimental animals with established hypertension. It is impossible to decide from this material whether the changes observed initiated or were the result of the elevated blood pressure; or whether they are responsible for maintenance

of an already elevated blood pressure.

XIII. OBJECTIVE OF THE PRESENT STUDY

The present study is designed to test the hypothesis that the elevation of arterial blood pressure in the SHR is due, at least in part, to morphological changes in the vessel wall.

MATERIALS AND METHODS

The arterial blood pressure was measured during development in WAR, WKY and SHR, and correlated with volume measurements of SMC, collagen and elastin in the media of the aorta and renal artery, and with wall thickness and lumen diameter measurements of these vessels.

I. EXPERIMENTAL ANIMALS

The Wistar-America (WAR), Wistar-Kyoto (WKY) and the Okamoto and Aoki strain of spontaneously hypertensive (SHR) rats used in this study were bred from the colonies maintained in the Animal Unit of memorial University of Newfoundland, Canada. Animals were kept in plastic cages; food (Purina rat chow) and tap water were provided as libitum. Young animals were weaned at the age of four weeks. For each strain, 32 animals were assigned at random into 8 groups, so that each group consisted of 2 males and 2 females. Animals were sacrificed at 2, 4, 5, 6, 7, 8, 12 and 18 weeks of age. A total of 96 animals were studied. The WAR and the WKY served as controls.

II. BLOOD PRESSURE MEASUREMENT

Blood pressure were recorded on a Beckman dynograph recorder (type R-411) via a pressure transducer (Statham P-23AA) through a cannula made from Intramedic polyethylene (PE-90) tubing and a Yale (20G-1") hypodermic needle. Prior to each set of measurements, the machinery was calibrated by a sphygmomanometer (S. Mes blood pressure gauge) so that each millimeter displacement on the recording chart corresponding to 5 mmHg, ranging from 0 to 225 mmHg. After the completion of each set of measurements, the base line was checked to assure the accuracy of the recording.

In each of the 96 animals, blood pressure were measured from either the left femoral (all except 2-week old animals) or the left common carotid

artery (2-week old animals only) under sodium pentobarbital (Somnotol, M.T.C. Pharmaceuticals) anaesthesia (dosage: 30-40 mg/kg). Animals were weighed and the amount of 30 mg/kg of undiluted anaesthetic was injected intraperitoneally. The animals were then tied, in a supine position, to a heated small animal operating unit (#150, Harvard Apparatus Company Inc.) maintaining a core temperature of 37 degrees C. Before operation, the depth of anaesthesia was tested by both corneal and tail reflexes. Supplementary dose of anaesthetic (10% dilution) was given to those which showed signs of "light" anaesthesia. For animals of 2 and 4 weeks of age, all of the anaesthetic was given in a 10% dilution.

An oblique incision was made on the ventral surface of the left thigh along the longitudinal axis of the femoral neurovascular bundle. Blunt dissection was used to separate the femoral fascia until the inguinal ligament was visible. The length of the femoral artery between the inguinal ligament and the origin of the superficial epigastric vessels was isolated from the vein and the nerve. Three pieces of 5-0 silk suture were then passed under the artery. The most distal one was first tied and the most proximal one was used to occlude the vessel while a transverse incision was made closest to the distal end. The cannula, filled with 10 U/ml heparinised (Hepalean, Harris Laboratories) saline, was then passed into the lumen of the artery and was stablised by the middle suture. The proximal suture was then released and the pulsatile blood pressure was allowed to stablise for two minutes. Measurement was then made at the last 2 cm of the tracing; the highest point was recorded as the systolic pressure while the lowest the diastolic pressure.

In 2-week old animals, the left common carotid artery was cannulated. A mid-line incision was made on the ventral surface of the neck region

near the sternum. Blunt dissection was used to separate the two salivary glands. The left sternomastoid muscle was then retracted laterally to expose the common carotid artery which could be seen pulsating under the sternothyroid and the omohyoid muscle. The two muscles were separated and the length of the common carotid artery below the level of the thyroid gland was blunt-dissected free medially from the recurrent laryngeal nerve and laterally from the vago-sympathetic trunk. The rest of the cannulating procedure was similar to that for the femoral artery.

III. TISSUE SAMPLES AND STEREOLOGY

The abdominal aorta and the left renal artery of 2, 4, 8, 12 and 18 weeks old animals were used for stereological analysis at the electron microscopic level.

After the completion of blood pressure measurements, the animal was removed from the operating unit for fixation. A mid-line incision was made in the thoracic region and the fur peeled away so that the pectoralis major muscle was visible. The sternal head of the pectoralis major muscle was then cut to expose the rib cage. An incision was made along the left side of the sternum and care was taken so that the internal thoracic artery remained undisturbed to avoid excessive haemorrhage before perfusion. After opening the pericardium, the apex of the heart was hoisted by a pair of forcepts and the needle of a winged infusion set (Surflo; 19G-3/4") was passed into the left ventricle. Through this infusion set, the animal was perfused at room temperature with a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde (Karnovsky, 1965) in 0.1M sodium cacodylate buffer (pH 7.2) at a pressure equivalent to 80 mmHg for 10 minutes. To ensure the flow of fixative, the inferior vena cava, exposed by laparotomy, and the external jugular vein were cut. After the 10-minute

perfusion period, clear fixative was seen flowing out through the severed veins.

The left renal artery, from its origin to the hilum of the kidney and the abdominal aorta, just below the right renal artery to the bifurcation of the common iliac arteries, were excised for further immersion fixation for 2 hours in fresh fixative. While in the fixative, the arteries were trimmed so that they contained a minimal amount of fat and the vessels were then cut transeversely into 5, approximately equal length, segments.

The tissues were then washed with 0.1M sodium cacodylate buffer containing 5.4% sucrose, postfixed with 1% osmium tetroxide in o.1M cacodylate buffer, stained en bloc with uranyl acetate saturated in 50% ethanol, dehydrated in a series of graded ethanol, cleared in acetone, infiltrated in acetone and Araldite 1:1 mixture for 8 hours and polymerised in pure Araldite fro 24 hours at 60 degrees C.

Stereological analysis was performed on a randomly selected aortic block and the middle segment of the renal artery of each animal. Thin, silver to light gold (600-900 angstroms) interference colour, transverse sections were cut on a Huxley-LKB ultra-microtome from these blocks, collected on 300 mesh copper grids (TAAB) and stained en face with alkaline lead citrate (0.4%) solution. The sections were examined in a Philips EM-300 electron microscope at 60 KV. The microscope was calibrated regularly with a calibration grid (cross grating replica, 54,000 lines/inch). The grid was scanned to locate a square which was completely covered by a section; no structural details could be observed in this mode, thus visual bias was minimised. Seven micrographs, one from each corner and three from the centre field of the choosen square, were photographed at a set

magnification of 6,400 and printed to 19,200 times. Care was taken so that these micrographs did not overlap and were sampled from the tunica media. Volume percent of smooth muscle cells (SMC). collagen and elastin were estimated from the prints by a 100-point sampling grid modified from Weibel et al (1966). The sum of the points from the seven micrographs, expressed in terms of percentage, was considered to be the sample value for that specimen in each animal. The mean of the four sets of estimates in each group was calculated and compared with those obtained from other groups of the same age.

After the stereological sampling had been completed, the sections were examined in more detail with emphasis on the development of the SMC.

IV. WALL THICKNESS AND LUMEN DIAMETER

From the randomly selected segment of the aorta and the middle segment of the left renal artery of each animal, one-half-micron-thick sections were cut in a plane, as close as possible, perpendicular to the vessel wall. The sections were then collected on glass slides, stained with toluidine blue and examined under a light microscope (Heerbrugg, Wild). The lumen and wall of the vessels were photographed at 30 and 400 times magnification, respectively. Measurements were made on contact prints. The smallest diameter of the lumen and the thinnest part of the vessel wall were recorded. For each set of micrographs, the microscope was calibrated with a micrometer slide IAmerican Optical company, Buffalo, N.Y.).

Sections were also cut from the remaining blocks, stained and examined; the number of the SMC layers, the elastic laminae and mitotic figures were noted.

V. STATISTICAL ANALYSIS

Data from this study were summarised either in the form of table or

histogram. Each value was pooled from 4 animals, 2 males and 2 females. In tables, the values are expressed as mean ± standard deviation, whereas in histograms, each block represents the mean value and the error bar, the standard deviation.

Data from each age group were analysed by Student-Newman-Keuls' multiple-range test (Steel and Torrie, 1960) at a significance level of 5%. An example of detailed statistical analysis will be considered in Appendix I.

RESULTS

Among the 96 rats studied, two, a 4-week old male WAR and an 8-week old female SHR, had an auxiliary left renal artery. In those cases, the larger of the two renal arteries was used for analysis. In addition, an 18-week old male WAR was replaced, due to the presence of a "tumour" in the posterior mediastinum just below the apex of the heart, pressing against the descending aorta and related structures; the recorded blood pressure however, did not seem to be different from the rest of the group.

I. BODY WEIGHT

In general, the body weight of the 3 strains of rat doubled from 2 to 4 weeks, tripled from 4 to 8 weeks and by 18 weeks, each rat, on the average, was about 10 times the weight at 2 weeks (Table 1). Both the rate of growth and the maximum body weight, at least up to 18 weeks of age, were higher in males than in females. At 2 weeks, there was no significant difference between males and females, however, by 18 weeks, the body weight of the males was about twice that of the females. This is reflected by the progressively increasing value of the standard deviation with age.

The rate of increase of body weight in the WKY appeared to be quite similar to that in the SHR, although the WKY were somewhat larger than the SHR from 4 weeks onward. The WAR, on the other hand, seemed to grow faster and to a higher weight than the WKY and the SHR. At 2 weeks, the WAR were already bigger than the WKY and the SHR.

II. BLOOD PRESSURE

Blood pressures, obtained through intra-arterial cannulation of the left femoral artery, except in 2-week old animals (through the left common carotid artery), were recorded as shown in Figure 1.

The development of systolic pressure (SBP) in the WAR and the WKY exhibited a similar pattern (Figure 2). In the WAR, the SBP rose from 54 mmHg at 2 weeks to 100 mmHg at 4 weeks, dropped back to 94 mmHg at 5 weeks and gradually increased to 128 mmHg by 18 weeks. The SHR, on the other hand, appeared to follow a different pattern. At 2 weeks, the SBP of the SHR was significantly higher than that of the controls. There was no difference among the 3 strains at 4 weeks. From 5 weeks onward, the SBP of the SHR were significantly higher than the controls and at 18 weeks there was a further increase to a hypertensive level (159 mmHg).

The development of the diastolic pressure in the WAR, the WKY and the SHR appeared to be quite similar to each other (Figure 3). Except at 2 and 18 weeks, in which the mean diastolic pressure for the SHR was significantly higher than that for the WAR and the WKY, there was no significant difference observed among the 3 strains during the first 18 weeks of postnatal development.

III. STEREOLOGY

Stereological analysis was performed on a total of 840 electron micrographs; the estimation of SMC, collagen and elastin components, expressed in terms of volume percent, of the media in the abdominal aorta and the left renal artery of the SHR were compared with those obtained from the WAR and the WKY controls. Typical examples of micrographs obtained from the aorta and renal artery, overlaying by a sampling grid, are shown in Figure 4 and 5, respectively.

The volume percent of SMC (Figure 6), collagen (Figure 7) and elastin (Figure 8) in the media of the abdominal aorta of the SHR varied from 57, 18 and 23, respectively, at 2 weeks to 60, 21 and 19 at 18 weeks. No significant difference could be detected among the 3 strains at any

age group up to 18 weeks in this study. However, there was a tendency that the volume percent of the SMC in the abdominal aorta of the WAR and the WKY decreased and that of the SHR increased during the first 18 weeks of postnatal development. The volume percent of collagen content also tended to increase with age.

The volume percent of SMC (Figure 9), collagen (Figure 10) and elastin (Figure 11) in the media of the left renal artery of the SHR ranged from 67,22 and 11, respectively, at 2 weeks to 61,26 and 13 at 18 weeks. There was no significant difference observed when comparing these parameters with those obtained from the controls during the first 18 weeks of postnatal development.

IV. ULTRASTRUCTURE OF DEVELOPING SMC

The development of the SMC was examined qualitatively under the electron microscope. The process of SMC maturation appeared to be similar among the 3 strains of rat studied; this included changes in myofilaments, intra-cellular organelles and cell contacts.

1. MYOFILAMENTS

Immature SMC were characterised by few filament bundles and rarity of dense bodies (Figure 12 and 13). These bundles were organised along the longitudinal axis of the SMC just below the cell membrane and were in close relation to dense areas. Dense areas were rarely seen along the cell membrane at sites of close cell apposition. In mature SMC, organised filament bundles appeared to fill the entire cell except the areas occupied by the nucleus, the perinuclear organelles and foci of organelles along the cell periphery (Figure 14). Dense bodies were common and seen most frequently in cells of the renal artery. Dense areas were also plentiful along the cell membrane occupying the areas which were not filled with plasmalemmal vesicles. Microtubules were found interposed among the filament

bundles; these were best seen in transverse sections of the SMC.

2. NUCLEUS AND INTRA-CELLULAR ORGANELLES

The elongated nucleus was usually located in the centre of the SMC. In young SMC, it was smooth in contour, whereas in mature SMC a few folds were present.

Most of the volume of the young SMC was occupied by organelles. Rough-surfaced endoplasmic reticulum (RER) and free ribosomes, organised into rosettes, were abundant (Figure 15). Electron opaque materials were often seen within the cisternae of the RER in the SMC of 2-week old animals (Figure 16a). The RER were also seen in close relation to smooth-surfaced endoplasmic reticulum and plasmalemmal vesicles just below the cell membrane (Figure 16b). Mitochondria were plentiful and interposed among the RER. Many Golgi complexes were present usually at the nuclear poles (Figure 17). Occasional lipofuscin granules and a pair of centrioles were also seen gathered at the nuclear poles. Changes were obvious in mature SMC. These included a decreased amount of RER and mitochondria, an increased amount of lipofuscin granules and a small Golgi complex, usually located at poles of the nucleus.

3. CELL CONTACTS

Young SMC were elongated, lying parallel to each other, with very few branching processes. Extensive areas of close membrane apposition were apparent with very few specialised junctional structures (Figure 18). Mature SMC, on the other hand, were highly branched, with the tips of branches of neighbouring cells making close contacts which in some cases were nexuses (Figure 19, 20a and 20b).

Based on the above description, the vessels of the 2-week old animals can be listed according to the order of maturity, from the most mature to

the least mature: renal artery of the SHR, renal artery of the WAR and the WKY, aorta of the SHR, and aorta of the WAR and the WKY. The determination of the maturity of the vessel is only relative because not all the SMC in the same vessel were at the same stage of maturity. There were no detectable differences observed in the aorta and renal artery of 18-week old animals.

V. LIGHT MICROSCOPY

One-half-micron-thick sections, stained with toluidine blue, were examined under a light microscope. The structure of the vessels were observed and the number of SMC layers, elastic laminae (not including the internal elastic lamina) and mitotic cells, the wall thickness and the lumen diameter of the vessel were recorded. Typical examples of the aorta and renal artery studied were illustrated in Figure 21 and 22.

The development of the vessels among the 3 strains studied was similar. Vascular SMC from young animals were arranged almost entirely spiral to the longitudinal axis of the vessel, although it was apparent that not all the SMC layers were arranged in the same plane (Figure 23). This deviation was more pronounced in mature vessels. Longitudinally arranged SMC bundles were also seen outside the external elastic lamina in the mature renal artery. Among the 600 specimens examined, intimal thickening appeared in three occasions: an 18-week old WKY aorta, an 18-week old WAR and an 18-week old SHR renal artery (Figure 24).

There was no difference in the number of SMC layers and elastic laminae observed along the whole length of the abdominal aorta. In young aortae, 5-7 SMC layers and 4-5 elastic laminae were arranged alternately across the vessel wall so that the number of SMC layers between two elastic laminae was ranged from 1-3. The number of SMC layers and elastic laminae

were increased to 7-9 and 4-6, respectively, at 18 weeks. In young renal arteries, the most proximal segment consisted of 5-6 layers of SMC and 2-3 elastic laminae, whereas those of the most distal segment were 4-5 and 1-2, respectively. No change was observed in renal arteries of 18-week old animals.

Mitotic cells, presumably mitotic SMC, were seen quite frequently in the media of vessels from young animals (Figure 25). In aortae of the WAR, the WKY and the SHR, the number of mitotic figures seen were 21, 13 and 10, respectively, at 2 weeks and 4, 3 and 3 at 4 weeks. There were no mitotic figures seen in 8, 12 and 18 weeks old specimens. In renal arteries, the corresponding figures were 4, 1 and 3 at 2 weeks. There was no mitotic cell seen in 4, 8, 12 and 18 weeks old vessels except in one specimen from an 8-week old WAR.

VI. WALL THICKNESS AND LUMEN DIAMETER

During development, the wall thickness and lumen diameter of the WAR abdominal aorta increased from 34 and 486 μm, respectively, at 2 weeks to 57 and 789 μm at 18 weeks. There were no differences observed when these parameters were compared with those od the WKY and the SHR except that the lumen diameter of the abdominal aorta obtained from 12 and 18 weeks old WAR were significantly larger than those of the WKY and the SHR (Table 2).

In the renal artery, the wall thickness and lumen diameter of the WAR increased from 17 and 243 μm, respectively, at 2 weeks to 32 and 447 μm at 18 weeks; there was no significant difference seen among the 3 strains of rat studied(Table 3).

VII. SUMMARY OF RESULTS

1. The WAR grew faster and to a higher weight than the WKY and the SHR,

at least during the first 18 weeks of postnatal life.

2. The systolic pressure of the SHR was significantly higher than that of the WAR and the WKY at the age of 2, 5, 6, 7, 8, 12 and 18 weeks, whereas the diastolic pressure of the SHR was significantly higher than that of the WAR and the WKY only at 2 and 18 weeks.
3. Stereological analysis indicated that there was no significant difference in the medial contents of either the abdominal aorta or the renal artery of the WAR, the WKY and the SHR during the first 18 weeks of postnatal development.
4. Electron microscopic examination revealed that the maturity of the vessels obtained from 2-weeks old animals was in the following order, from the most mature to the least mature: renal artery of the SHR, renal artery of the WAR and the WKY, aorta of the SHR, and aorta of the WAR and the WKY. There was no difference observed in 18-week old vessels.
5. Light microscopic study showed that during the first 18 weeks of development, the wall thickness and lumen diameter of the abdominal aorta and the renal artery obtained from the WAR, the WKY and the SHR were similar except the lumen diameter of the 12- and 18-week old WAR aorta was larger than that of the WKY and the SHR.

VIII. TABLES AND FIGURES

Table 1. Body weight of developing WAR, WKY and SHR.
Each value (mean ± standard deviation) was obtained from 4 animals, 2 males and 2 females. It is apparent that the WAR were bigger than the WKY and the SHR from 2 weeks onward. Furthermore, the high value of standard deviation in more mature rats reflects the body weight of males was greater than the females.

a - mean value of the WAR is significantly higher than the WKY at a significance level of 5%.

b - mean value of the WAR is significantly higher than the SHR at a significance level of 5%.

c - mean value of the WKY is significantly higher than the SHR at a significance level of 5%.

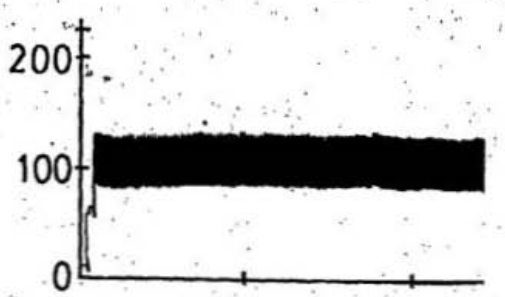
BODY WEIGHT OF DEVELOPING WAR, WKY & SHR.

STRAIN \ AGE (WEEKS)	2	4	5	6	7	8	12	18
WAR	34±1 [a,b]	71±2 [a,b]	77±5 [b]	117±14 [a,b]	178±33 [a,b]	222±28 [a,b]	286±47 [b]	389±137
WKY	21±2	56±5 [c]	70±8 [c]	85±10	105±17	134±28	212±45	254±67
SHR	22±1	42±3	51±6	70±11	94±10	117±16	179±40	247±69

Figure 1. Blood pressures of 12 weeks old WAR, WKY and SHR. Measurements were made on the last 2 centimeters of the tracing; the highest point was recorded as the systolic pressure, the lowest the diastolic pressure.

Strain: WAR
Age: 12 weeks
Sex: male
Body Weight: 301 gm
Blood Pressure: 130/87 mmHg
Amount of Anaesthetic : 10.0 mg

Strain: WKY
Age: 12 weeks
Sex: male
Body Weight: 246 gm
Blood Pressure: 130/80 mmHg
Amount of Anaesthetic : 8.6 mg



Strain: SHR
Age: 12 weeks
Sex: female
Body Weight: 166 gm
Blood Pressure: 145/100 mmHg
Amount of Anaesthetic : 5.8 mg

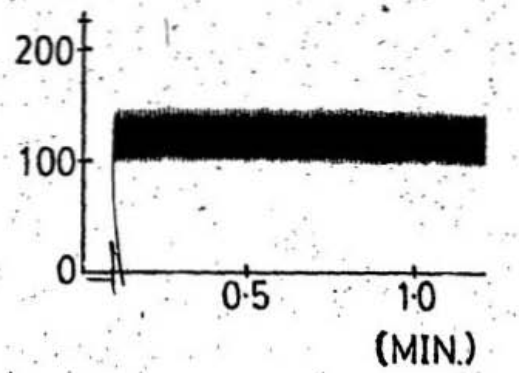
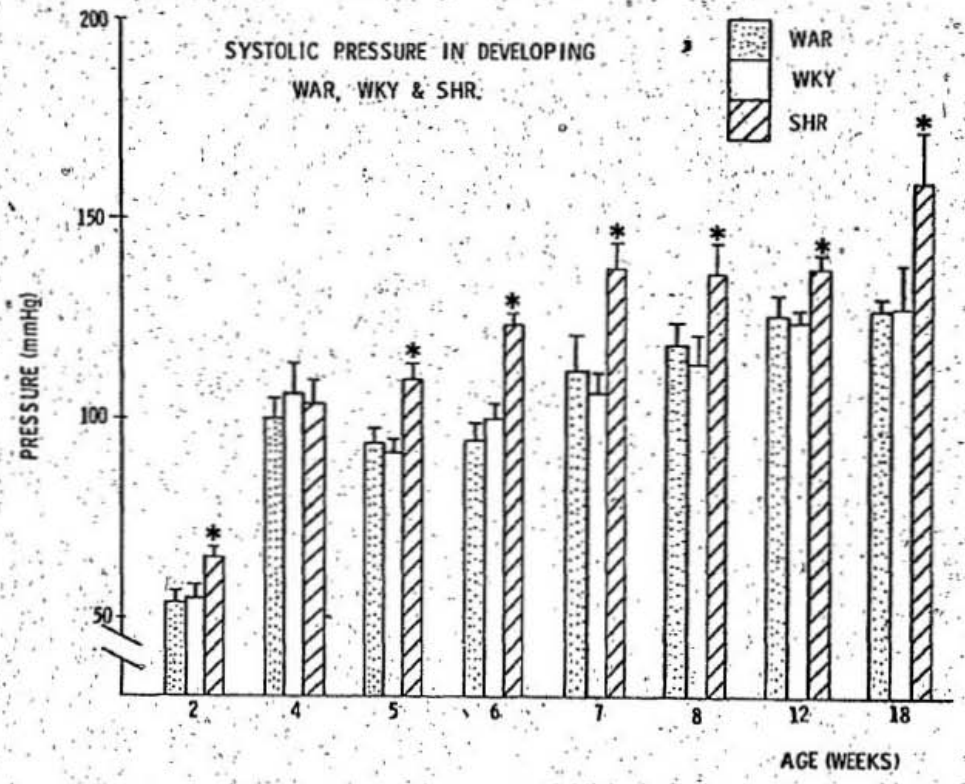


Figure 2. Systolic pressure in developing WAR, WKY and SHR. Except for 2-week old animals in which the left common carotid artery was used, the systolic pressure was recorded through an intra-arterial cannulation of the left femoral artery. The systolic pressure of the SHR was significantly higher than that of the WAR and the WKY at 2, 5, 6, 7, 8, 12 and 18 weeks of age.

Figure 3. Diastolic pressure in developing WAR, WKY and SHR. The pattern of diastolic pressure in the 3 strains of rat studied was similar during the first 18 weeks of postnatal development. The diastolic pressure of the SHR was significantly higher than that of the WAR and the WKY at the age of 2 and 18 weeks.

* - indicated the mean value for the SHR is significantly higher than that of the WAR and the WKY at a significance level of 5%.


SYSTOLIC PRESSURE IN DEVELOPING
WAR, WKY & SHR.
WAR
WKY
SHR
200
150
100
50
PRESSURE (mmHg)
2
4
5
6
7
8
12
18
AGE (WEEKS)

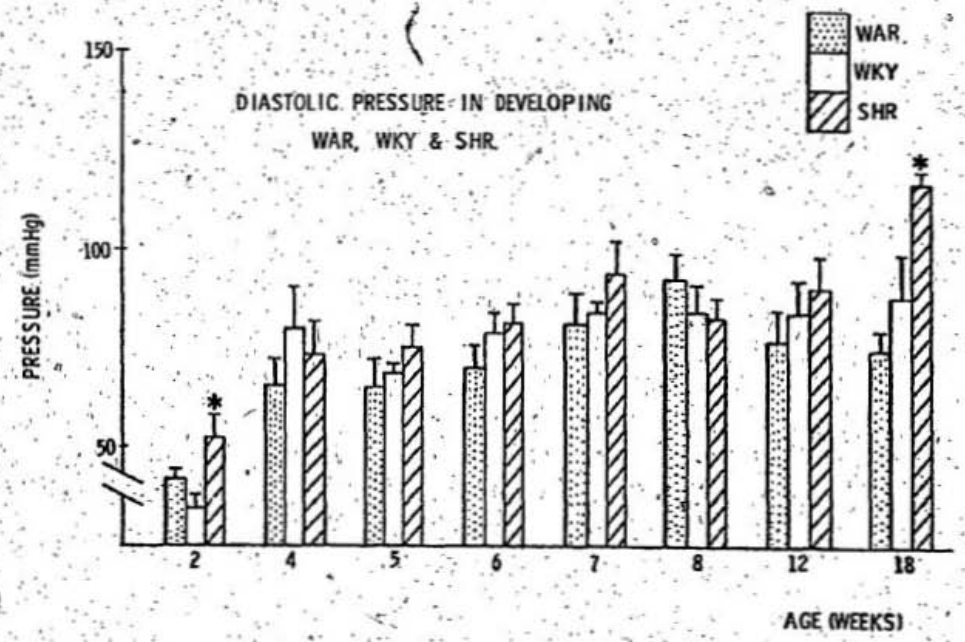

DIASTOLIC PRESSURE IN DEVELOPING
WAR, WKY & SHR.
WAR
WKY
SHR
150
100
50
PRESSURE (mmHg)
2
4
5
6
7
8
12
18
AGE (WEEKS)

Figure 4. An example of electron micrographs, obtained from the abdominal aorta, used for stereological analysis. The volume percent of SMC, collagen and elastin were estimated by laying a 10 X 10 point-counting grid, modified from Weibel et al (1966), onto the micrograph. In this micrograph, the volume percent of the SMC is 66%, because there are 66 points overlying SMC profiles. EM. Aorta, 12-week SHR. Calibration bar = 1 micron.

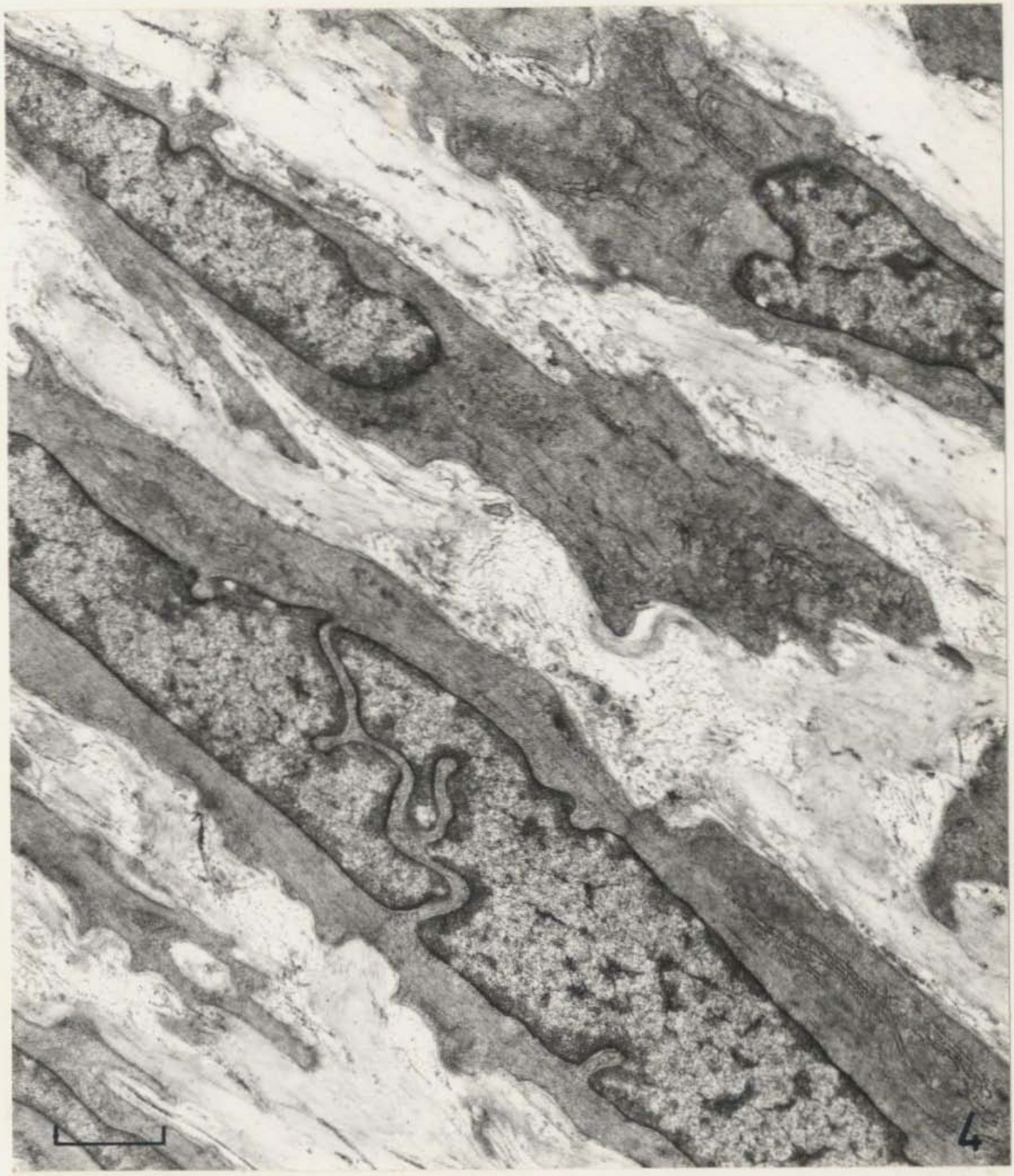

4

Figure 5. An example of electron micrographs, obtained from the left renal artery, used for stereological analysis. The volume percent of SMC, collagen and elastin were estimated by laying a 10 X 10 point-counting grid, modified from Weibel et al (1966), onto the micrograph. In this micrograph, the volume percent of SMC is 69%, because there are 69 points overlying SMC profiles. EM. Renal artery, 12-week SHR. Calibration bar = 1 micron.


5

Figure 6. Volume percent of smooth muscle in the tunica media of the abdominal aorta in developing SHR, WKY and WAR.

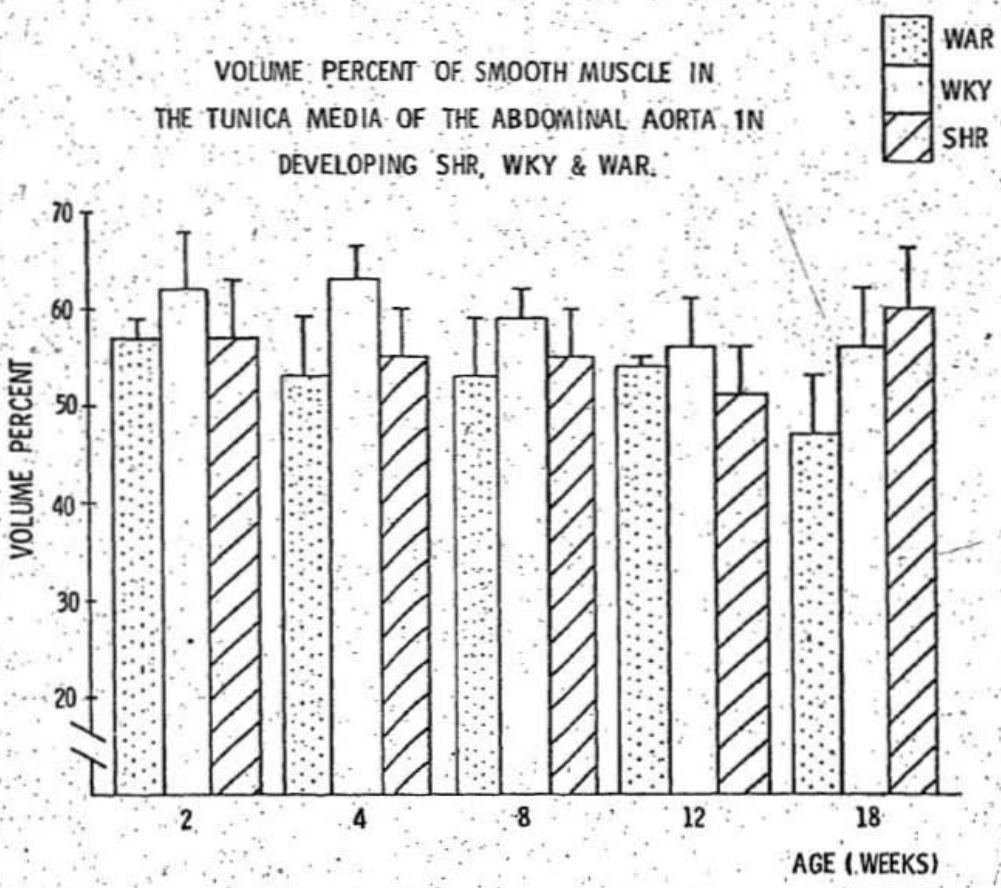
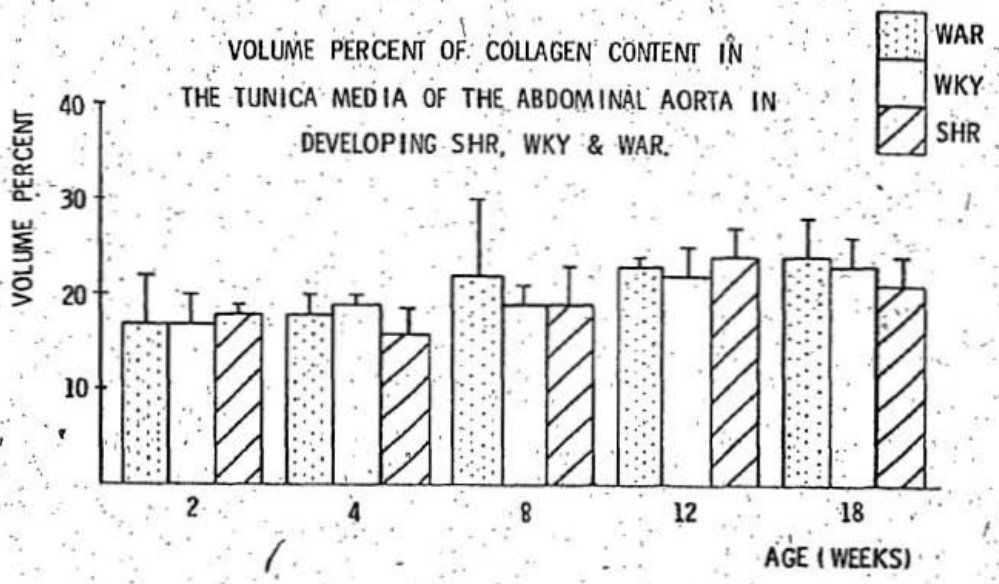

VOLUME PERCENT OF SMOOTH MUSCLE IN
THE TUNICA MEDIA OF THE ABDOMINAL AORTA IN
DEVELOPING SHR, WKY & WAR.
WAR
WKY
SHR
VOLUME PERCENT
70
60
50
40
30
20
2
4
8
12
18
AGE (WEEKS)

Figure 7. Volume percent of collagen content in the tunica media of the abdominal aorta in developing SHR, WKY and WAR.

Figure 8. Volume percent of elastin content in the tunica media of the abdominal aorta in developing SHR, WKY and WAR.


VOLUME PERCENT OF COLLAGEN CONTENT IN
THE TUNICA MEDIA OF THE ABDOMINAL AORTA IN
DEVELOPING SHR, WKY & WAR.
WAR
WKY
SHR
VOLUME PERCENT
40
30
20
10
2
4
8
12
18
AGE (WEEKS)

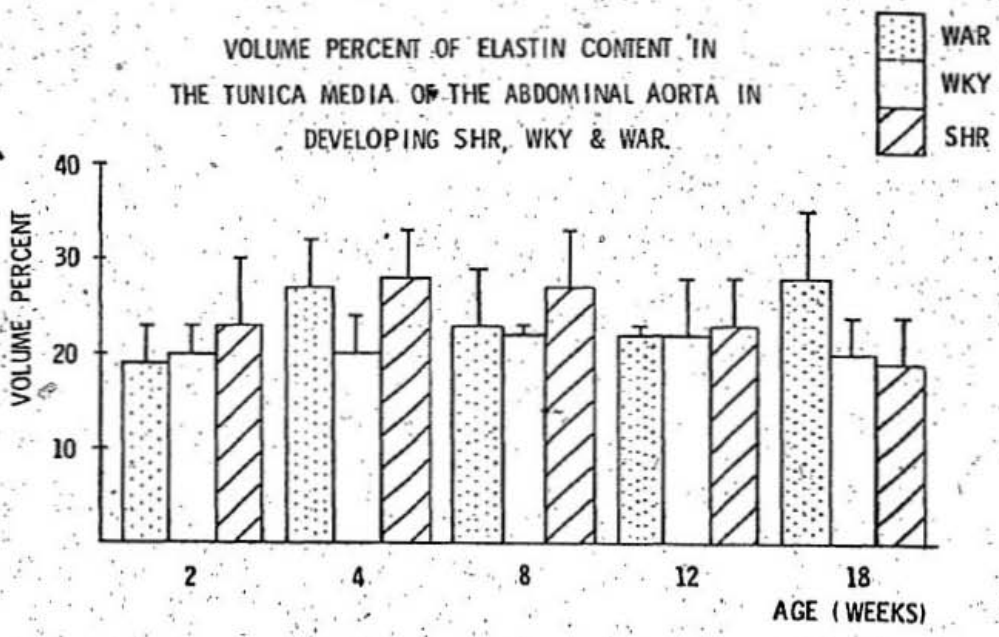

VOLUME PERCENT OF ELASTIN CONTENT IN
THE TUNICA MEDIA OF THE ABDOMINAL AORTA IN
DEVELOPING SHR, WKY & WAR.
WAR
WKY
SHR
VOLUME PERCENT
40
30
20
10
2
4
8
12
18
AGE (WEEKS)

Figure 9. Volume percent of smooth muscle in the tuncia media of the left renal artery in developing SHR, WKY and WAR.

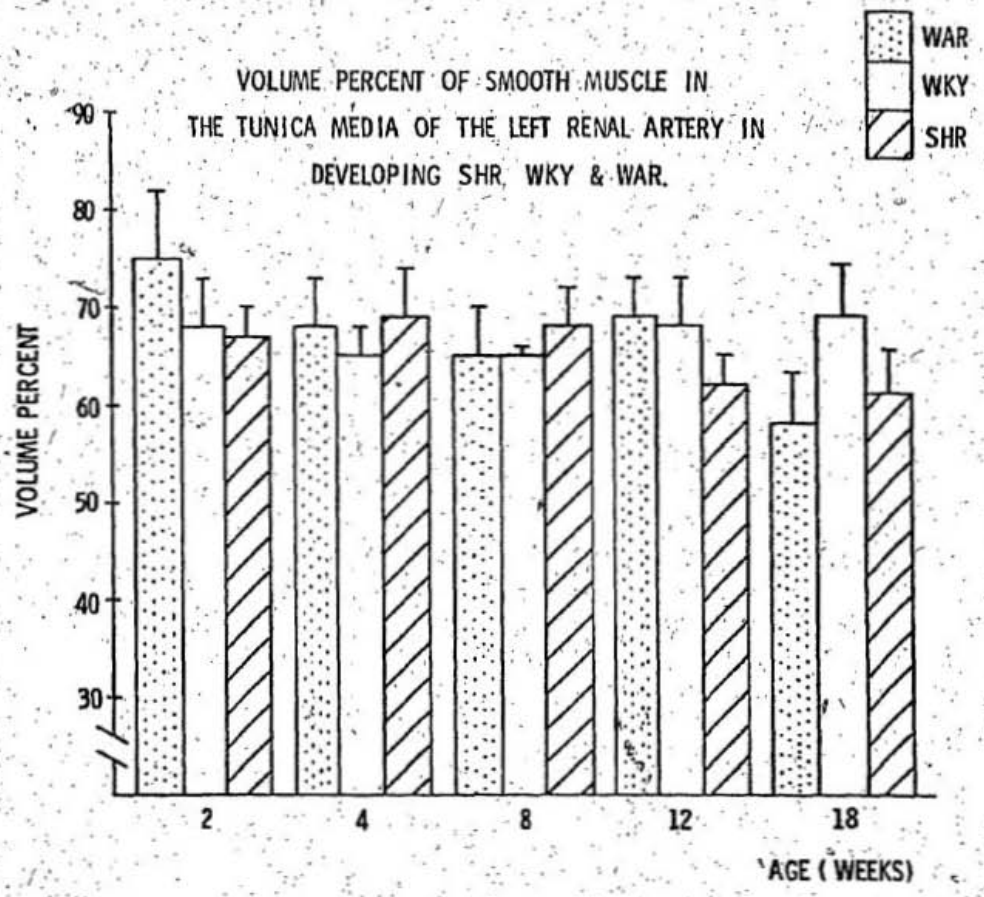
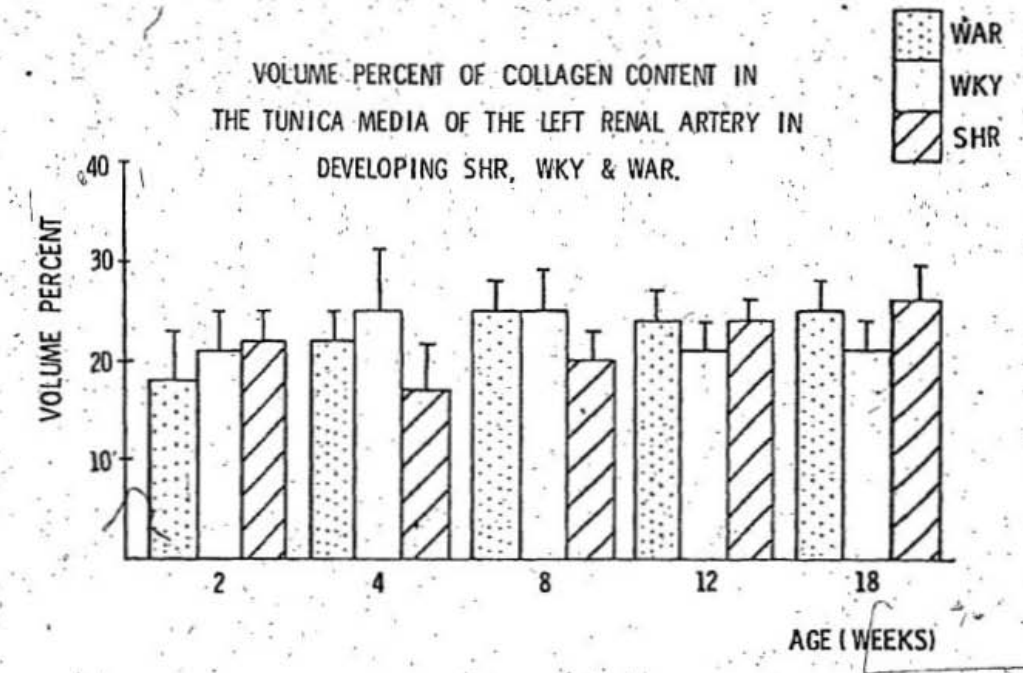

VOLUME PERCENT OF SMOOTH MUSCLE IN
THE TUNICA MEDIA OF THE LEFT RENAL ARTERY IN
DEVELOPING SHR, WKY & WAR.
WAR
WKY
SHR
VOLUME PERCENT
90
80
70
60
50
40
30
2
4
8
12
18
AGE (WEEKS)

Figure 10. Volume percent of collagen content in the tunica media of the left renal artery in developing SHR, WKY and WAR.

Figure 11. Volume percent of elastin content in the tunica media of the left renal artery in developing SHR, WKY and WAR.


VOLUME PERCENT OF COLLAGEN CONTENT IN
THE TUNICA MEDIA OF THE LEFT RENAL ARTERY IN
DEVELOPING SHR, WKY & WAR.
WAR
WKY
SHR
VOLUME PERCENT
40
30
20
10
2
4
8
12
18
AGE (WEEKS)

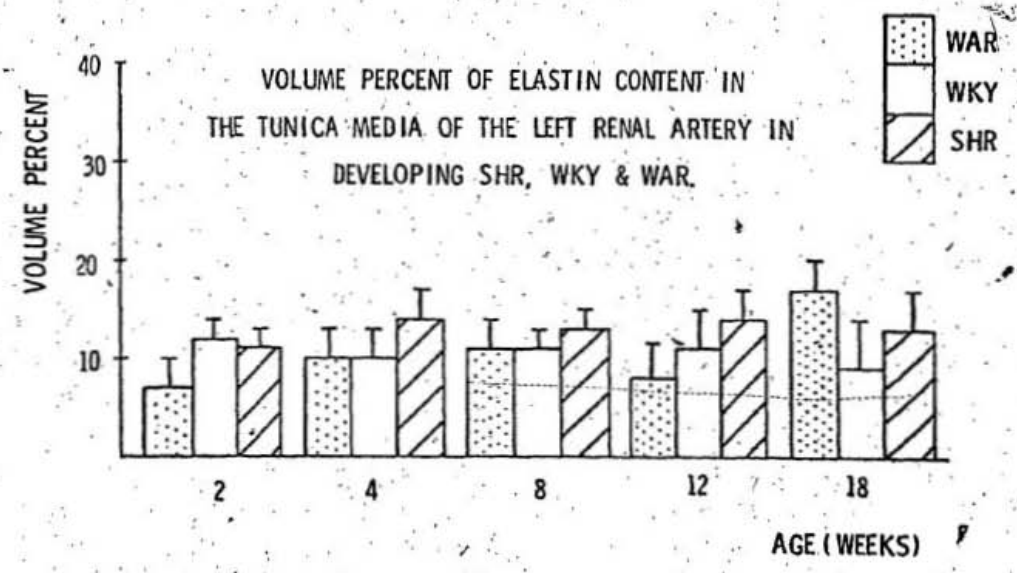
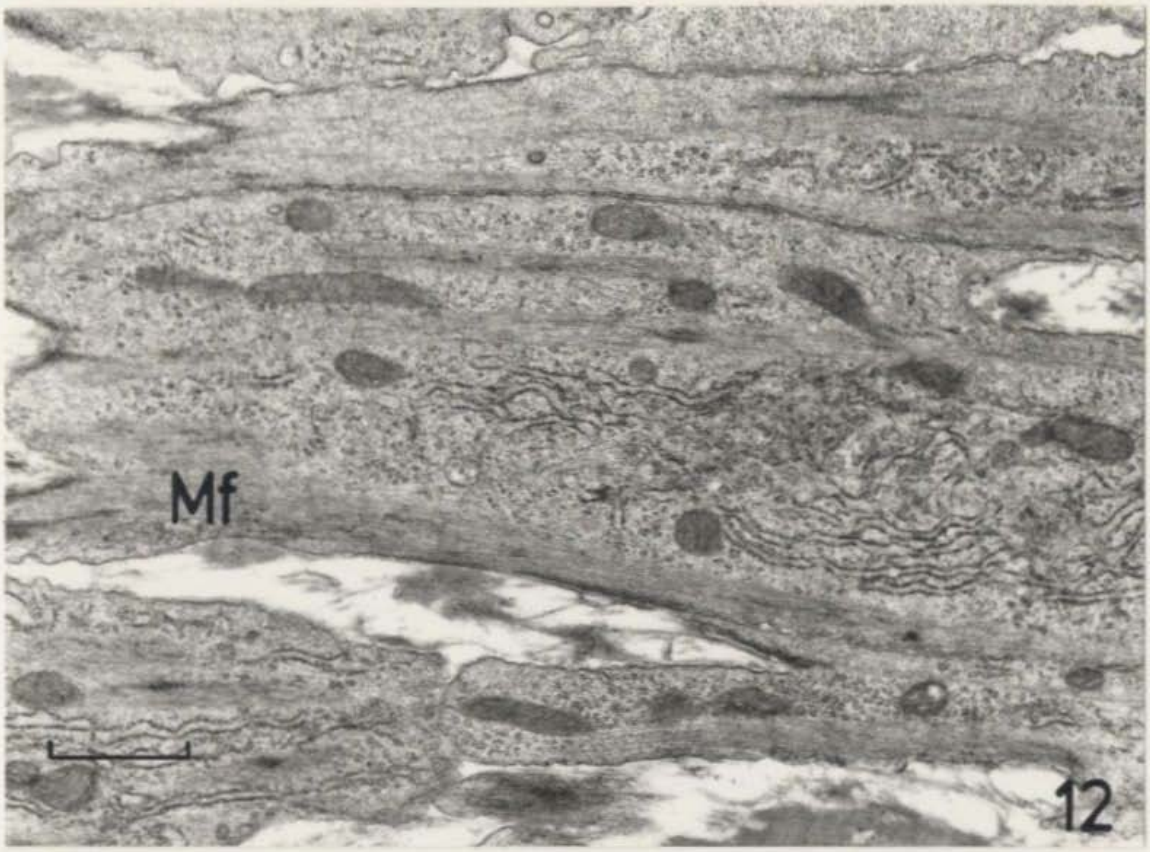

VOLUME PERCENT OF ELASTIN CONTENT IN
THE TUNICA MEDIA OF THE LEFT RENAL ARTERY IN
DEVELOPING SHR, WKY & WAR.
WAR
WKY
SHR
VOLUME PERCENT
40
30
20
10
2
4
8
12
18
AGE (WEEKS)

Figure 12. Young SMC were characterised by relatively fewer myofilament bundles, oriented along the longitudinal axis of the cell, with most of them lying just below the cell membrane. Mitochondria, rough-surfaced endoplasmic reticula and ribosomes, organised into rosettes, were plentiful filling most of the cell volume. EM. Aorta, 2-week WAR. Calibration bar = 1 micron.

Figure 13. The SMC illustrated in this micrograph were more mature than those in Figure 12, judging by the relatively thicker myofilament bundles and lesser area of close apposition. EM. Renal artery, 2-week WAR. Calibration bar = 1 micron.

Abbreviation: Mf = myofilament.


Mf
12

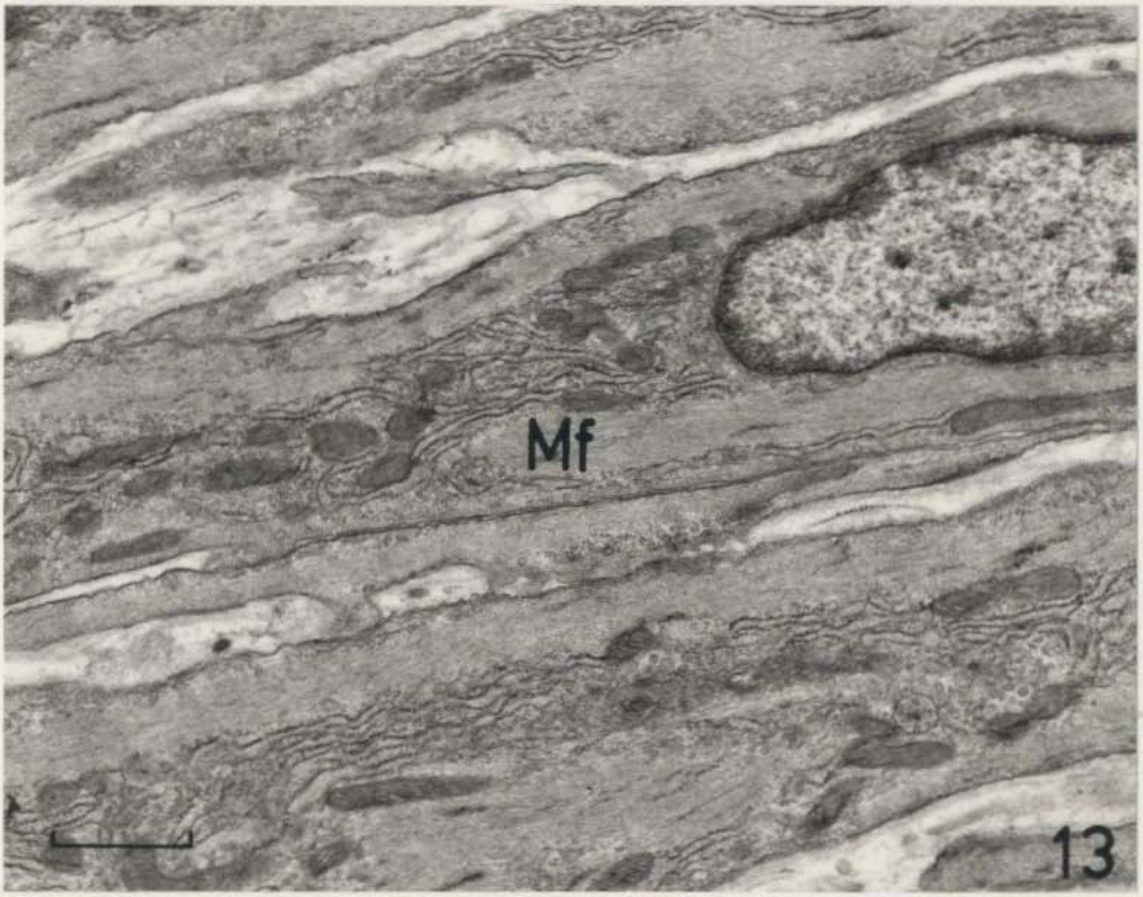

Mf
13

Figure 14. Mature SMC were highly branched, with most of the cytoplasm filled with well organised myofilament bundles along the longitudinal axis of the cell. The nucleus was usually presented with few folds. Intra-cytoplasmic organelles were disposed usually at the nuclear poles, although foci of these organelles were frequently found along the cell periphery. Collagen fibres and some disorganised elastin were plentiful filling most of the remaining extra-cellular space which was not occupied by the elastic laminae. EM. Aorta, 18-week WKY. Calibration bar = 1 micron.

Abbreviation: C = collagen

E = elastin

SMC = smooth muscle cell.

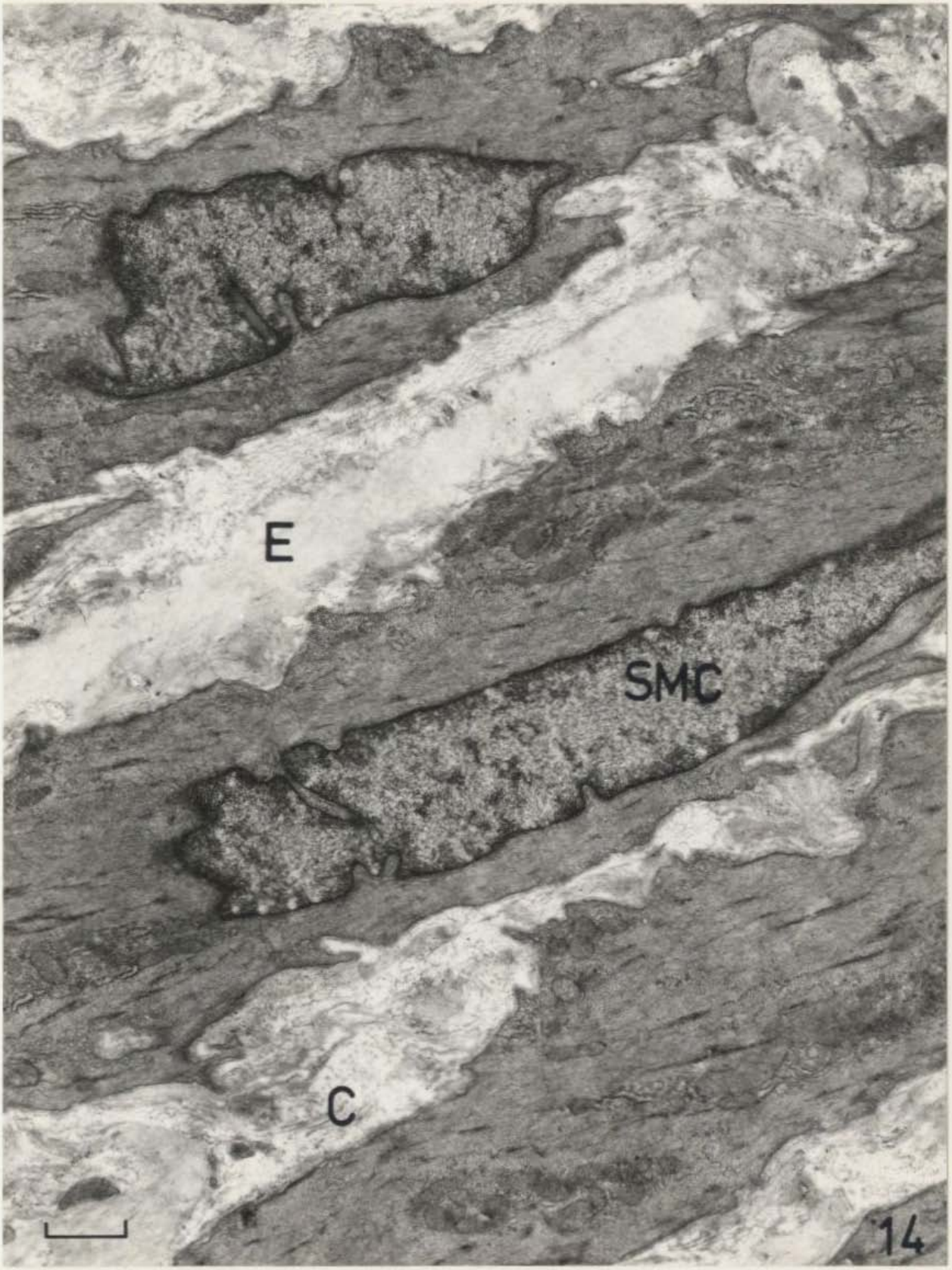

E
SMC
C
14

Figure 15. A large fraction of the cytoplasm in immature SMC was occupied by organelles. The most prominent ones were rough-surfaced endoplasmic reticulum, rosettes of ribosome and mitochondria. Myofilament bundles were organised along the cell periphery. Microtubules (arrow) were also abundant and interposed among organelles and myofilaments EM. Aorta, 2-week WKY. Calibration bar = 1 micron.

Abbreviation: Mf = myofilament
Mit = mitochondria
N = nucleus.
RER = rough-surfaced endoplasmic reticulum.

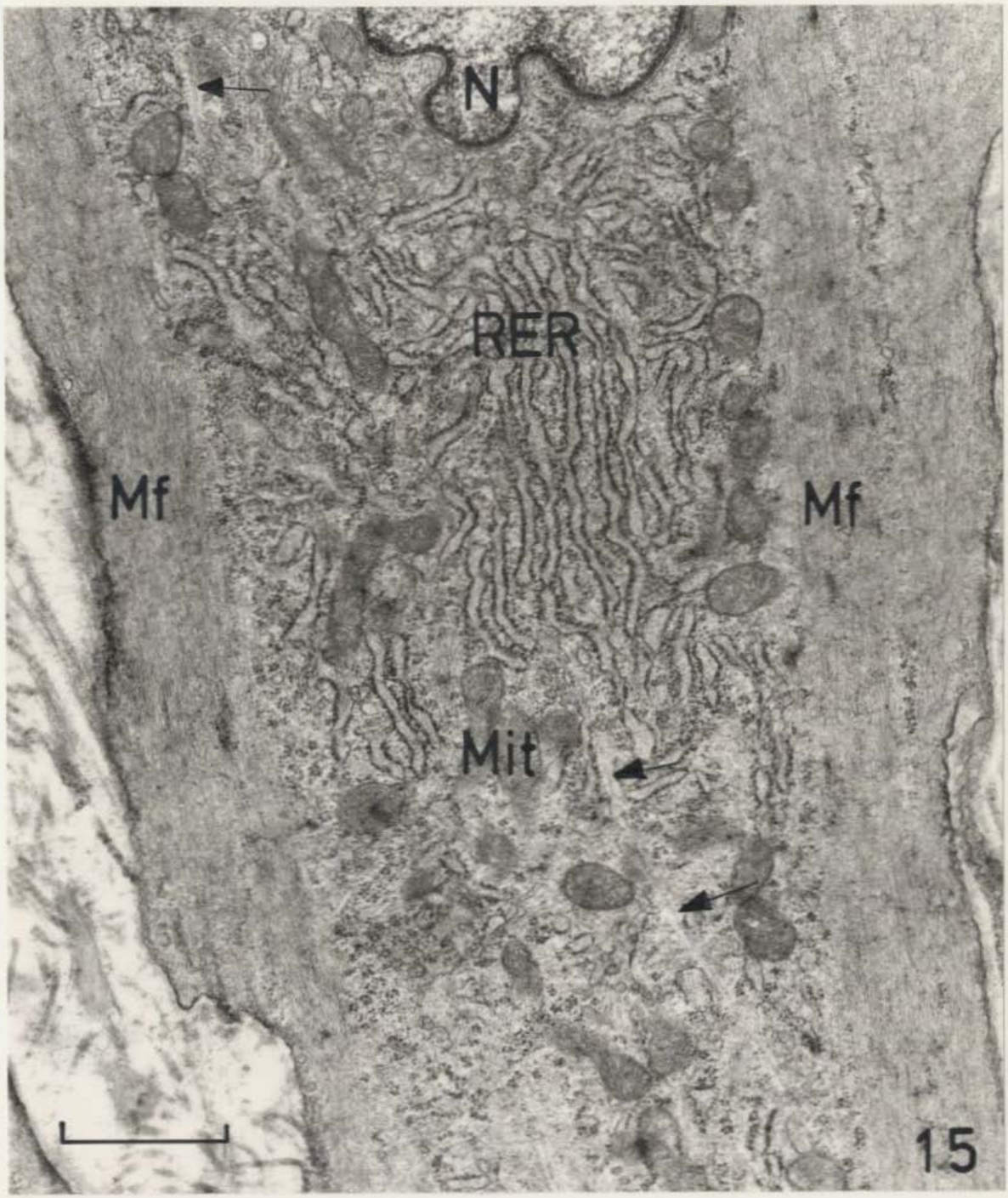

N
RER
Mf
Mf
Mit
15

Figure 16a. Electron opaque materials were often seen within the cisternae of rough-surfaced endoplasmic reticulum (arrow) of immature SMC; this may be indicative of high activity of the SMC in manufacturing contractile proteins and/or connective tissues.
EM. Aorta, 2-week WAR. Calibration bar = 1 micron.

Figure 16b. Rough-surfaced endoplasmic reticulum was frequently found near the periphery of the SMC in close relation with plasmalemmal vesicles and smooth-surfaced endoplasmic reticulum. The latter is known to be continuous with the rough-surfaced endoplasmic reticulum. EM. Renal artery, 2-week WKY. Calibration bar = .1 micron.

Abbreviation: PV = plasmalemmal vesicle
RER = rough-surfaced endoplasmic reticulum
SER = smooth-surfaced endoplasmic reticulum.

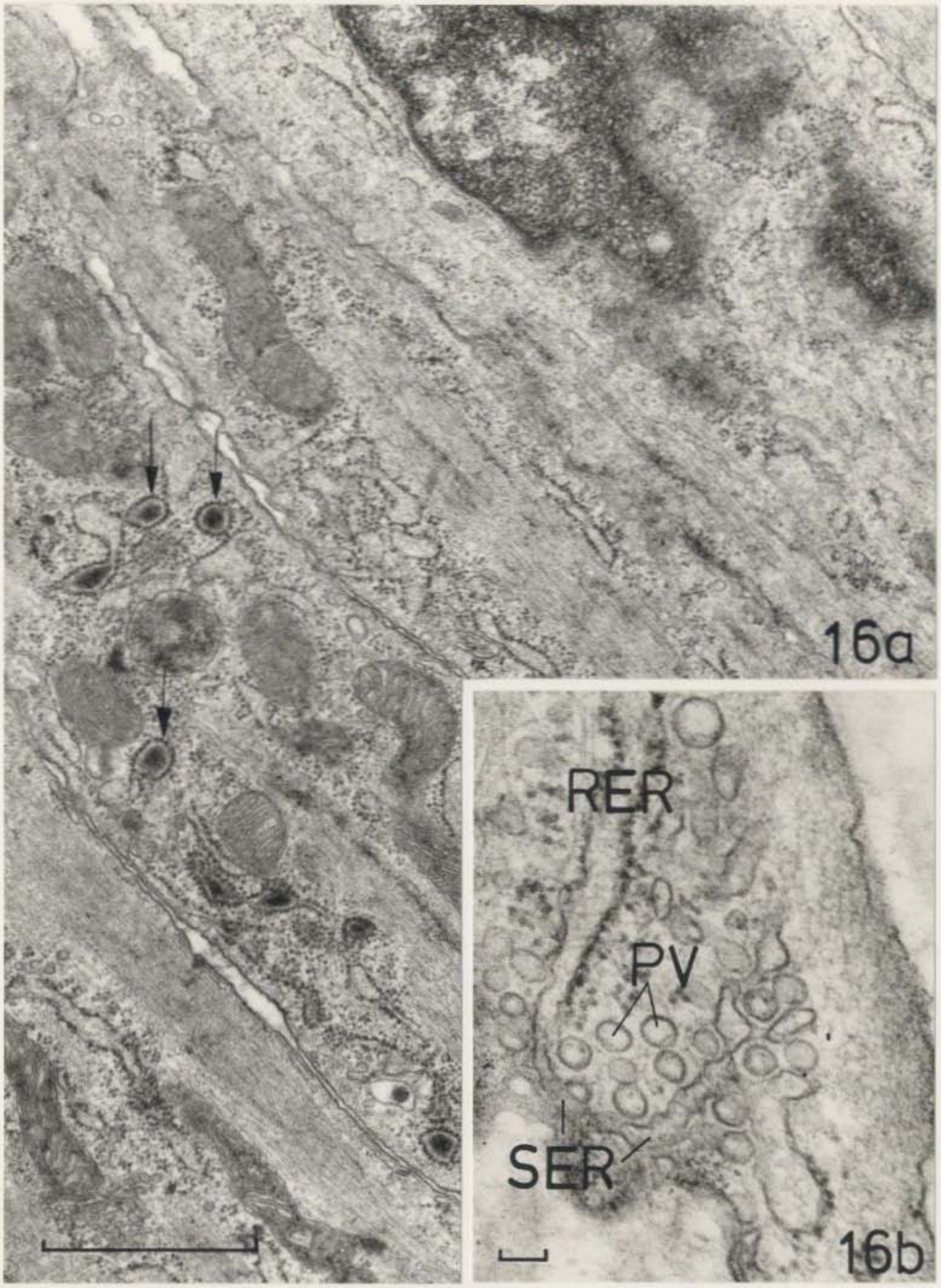

16a
RER
PV
SER
16b

Figure 17. Many Golgi complexes were frequently found near the nucleus and interposed among other organelles of immature SMC. Each Golgi complex consisted of 4-7 saccules and many associated vesicles. High secretory activity is indicated in these young SMC which possess many Golgi complexes. EM. Renal artery, 4-week SHR. Calibration bar = 1 micron.

Abbreviation: G = Golgi complex.

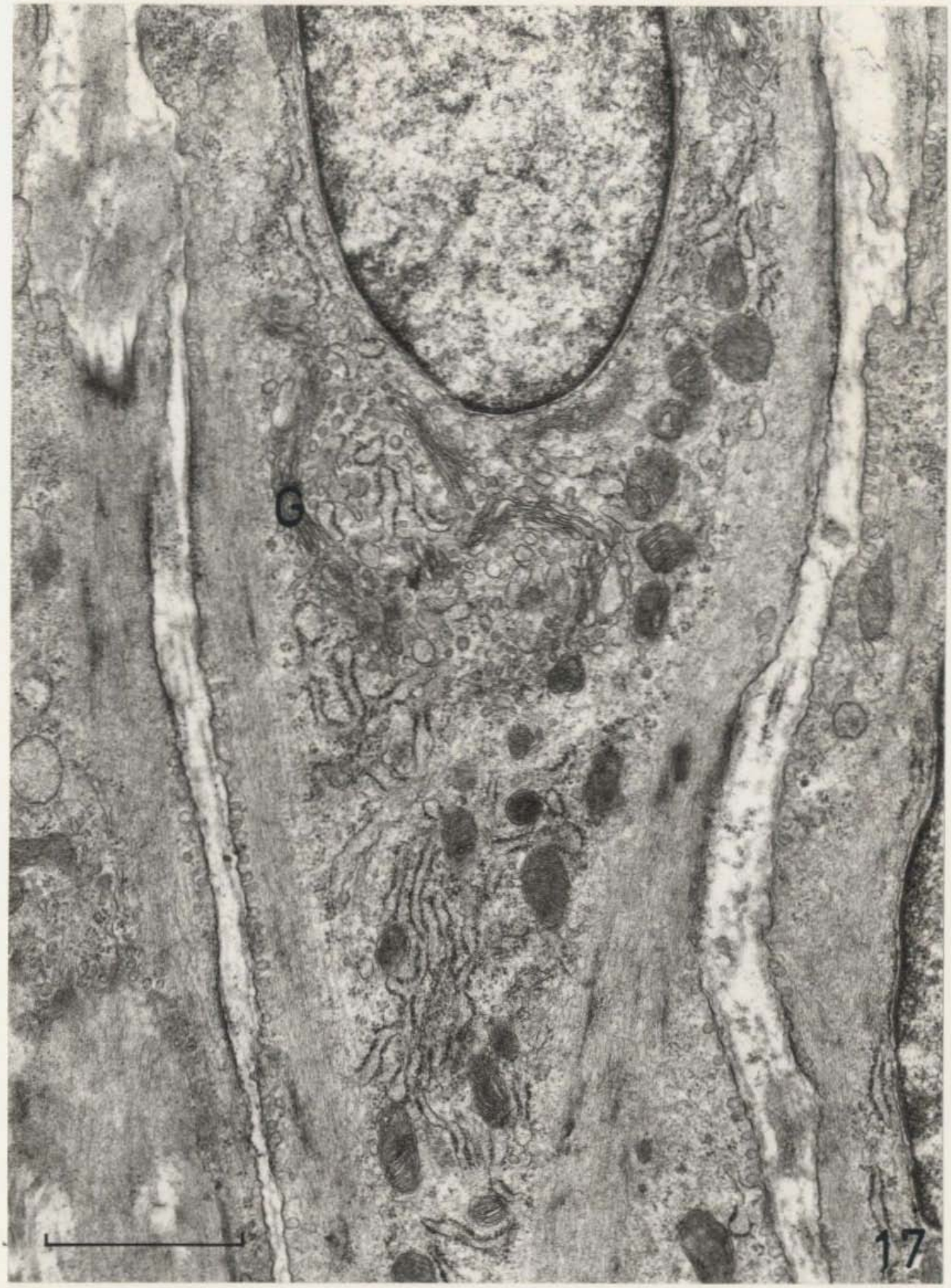

G
17

Figure 18. Extensive area of close membrane apposition (arrow) with no specialised junctional structures were frequently found among young SMC. Dense areas were seldom seen along the cell membrane at the sites of cell apposition. EM. Aorta, 2-week WAR. Calibration bar = 1 micron.

Figure 19. Mature SMC were highly branched with the tips of branches of neighbouring cells making close appositions (arrow). EM. Renal artery, 2-week SHR. Calibration bar = .5 micron.


18

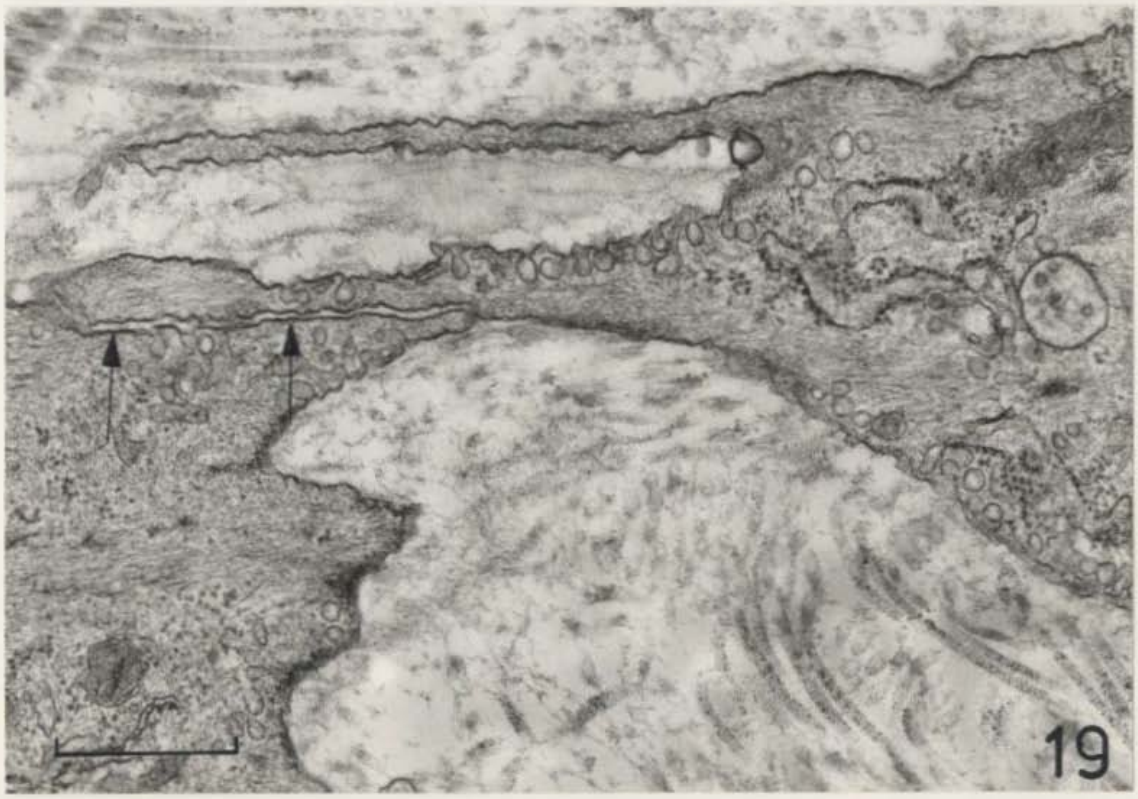
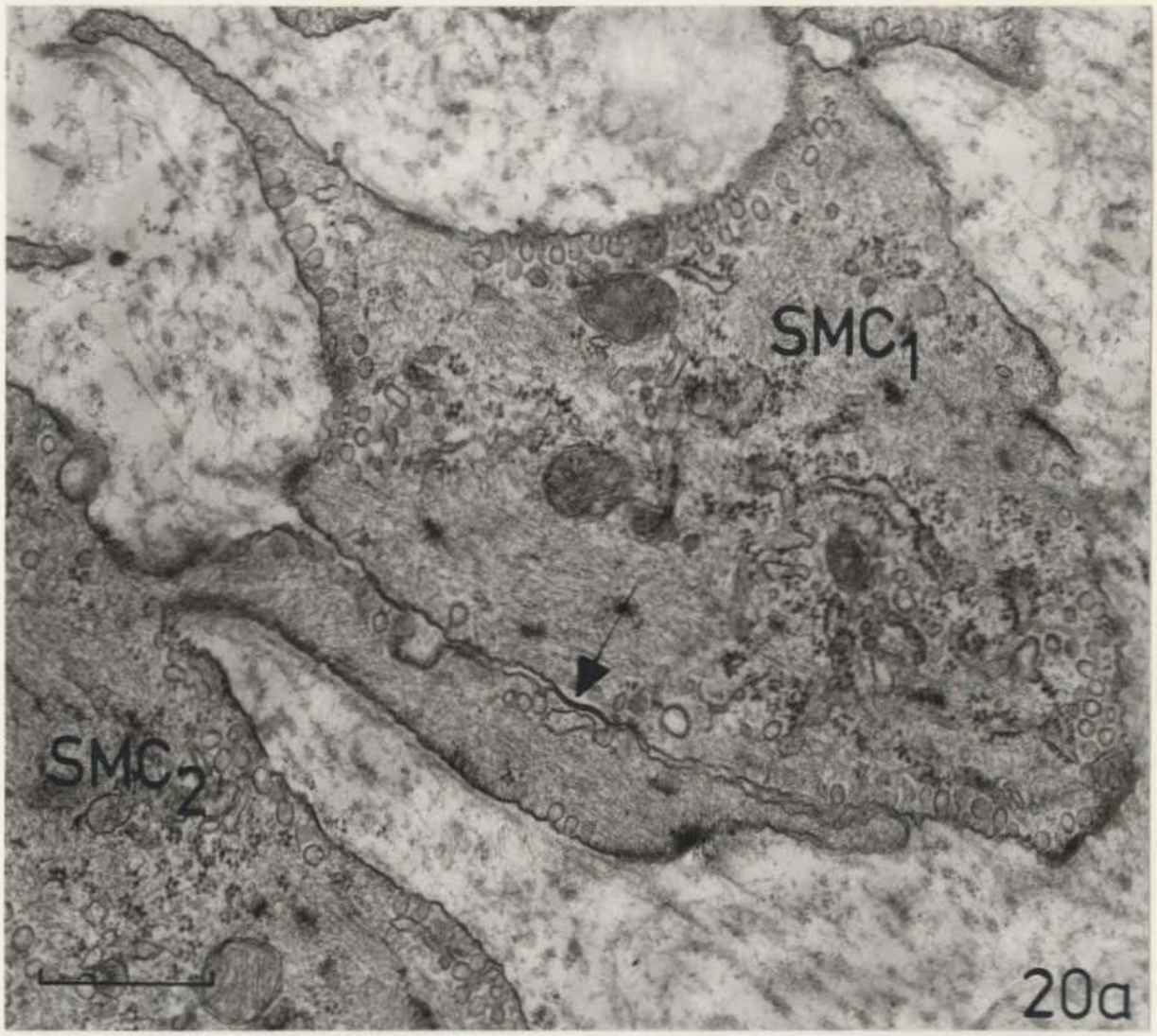
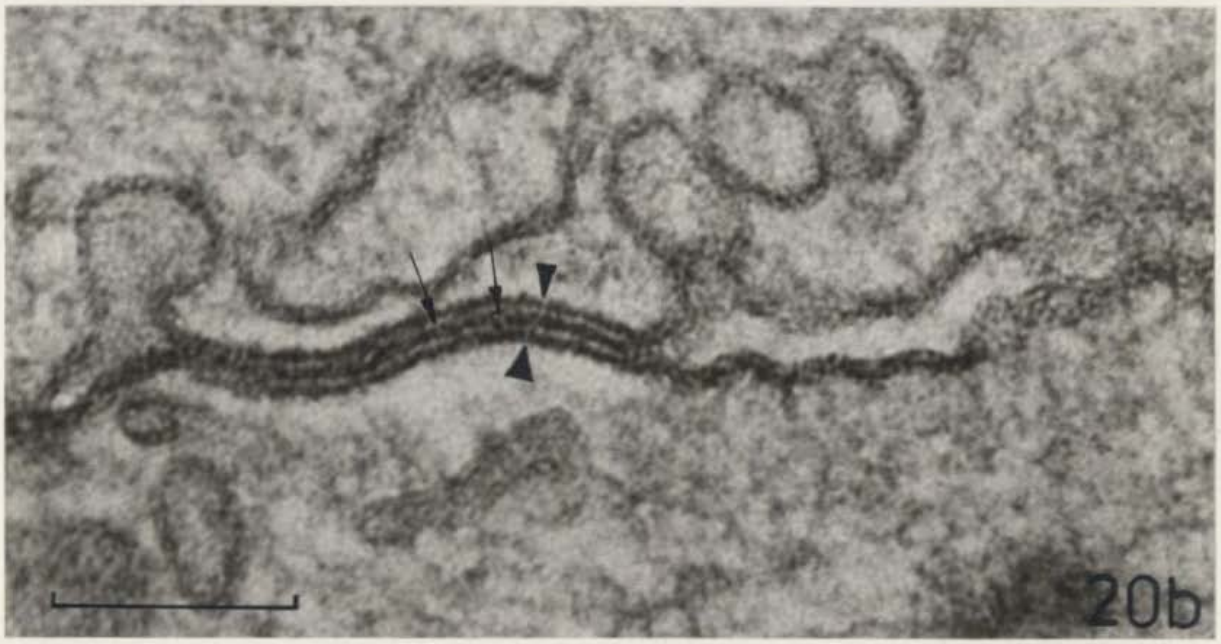

19

Figure 20a. Often the highly branched, mature SMC (SMC_1) extended its cytoplasmic process to make close contacts with the cell proper of another SMC (SMC_2). Sometimes, these junctional structures were specialised into nexus (arrow). EM. Renal artery, 4-week SHR. Calibration bar = .5 micron.

Figure 20b. The nexus illustrated in this micrograph appeared to be a pentalaminal structure with the outer leaflet of the adjacent SMC fused to form the thicker middle lamina. Translucent regions (arrow) were seen along this lamina; these were interpreted as intercellular channels (Campbell et al, 1971; Uehara et al, 1976). Often these "channels" extended through the whole thickness of the nexus (arrow head). EM. Renal artery, 4-week SHR. Calibration bar = .1 micron.



20a



20b

Table 2. Wall thickness (WT, in μm) and lumen diameter (LD, in μm) of the abdominal aorta in developing WAR, WKY and SHR.

* - indicated the mean value for the WAR is significantly higher than that of the WKY and the SHR at a significance level of 5%.

Table 3. Wall thickness (WT, in μm) and lumen diameter (LD, in μm) of the left renal artery in developing WAR, WKY and SHR.

WALL THICKNESS (WT, in μm) AND LUMEN DIAMETER (LD, in μm) OF THE ABDOMINAL AORTA IN DEVELOPING WAR, WKY & SHR.

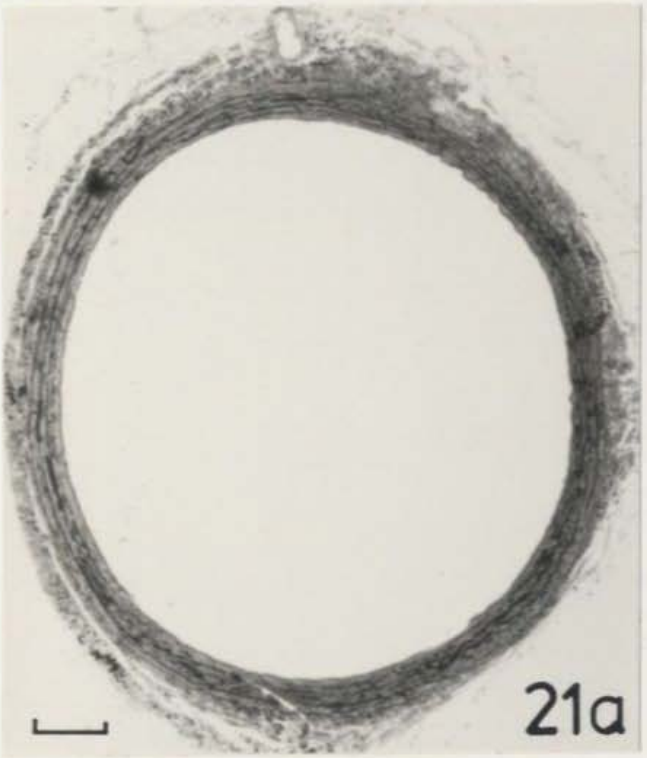
STRAIN \ AGE (WEEKS)		2	4	8	12	18
WAR	WT	34±2	44±3	49±8	53±6	57±5
	LD	486±37	566±71	587±43	745±26 *	789±39 *
WKY	WT	36±5	45±3	49±3	57±3	56±4
	LD	410±44	473±99	536±42	595±41	628±57
SHR	WT	36±3	41±2	50±5	56±3	59±5
	LD	502±64	462±56	563±68	560±65	560±89

WALL THICKNESS (WT, in μm) AND LUMEN DIAMETER (LD, in μm) OF THE LEFT RENAL ARTERY IN DEVELOPING WAR, WKY & SHR.

STRAIN \ AGE (WEEKS)		2	4	8	12	18
WAR	WT	17±1	23±1	30±5	33±2	32±5
	LD	243±49	279±22	326±24	380±50	447±31
WKY	WT	19±2	25±2	29±3	32±5	35±5
	LD	262±42	324±27	339±51	376±74	422±27
SHR	WT	17±2	24±3	27±2	28±4	36±3
	LD	242±28	261±30	314±57	332±49	441±17

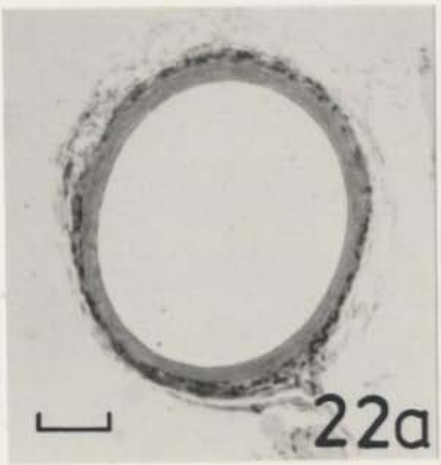
Figure 21. Typical micrographs of the abdominal aorta used for measuring lumen diameter (a) and wall thickness (b).
a. LM. Aorta, 4-week WAR. Calibration bar = 100 microns.
b. LM. Aorta, 2-week SHR. Calibration bar = 10 microns.

Figure 22. Typical micrographs of the left renal artery used for measuring lumen diameter (a) and wall thickness (b).
a. LM. Renal artery, 4-week WKY. Calibration bar = 100 microns.
b. LM. Renal artery, 4-week SHR. Calibration bar = 10 microns.

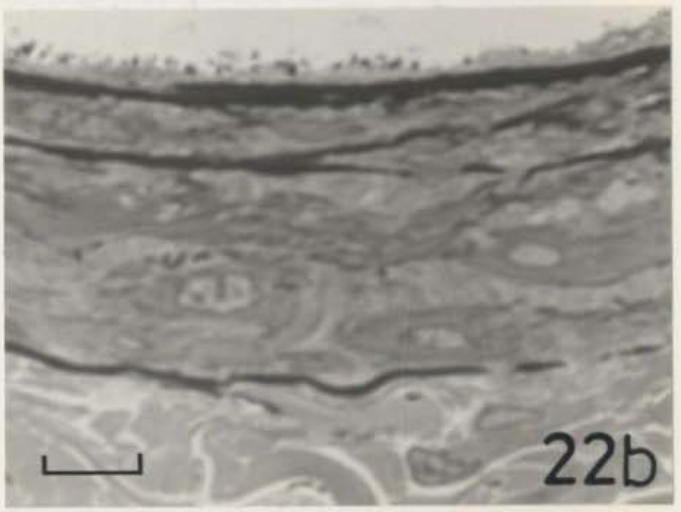


21a

21b



22a



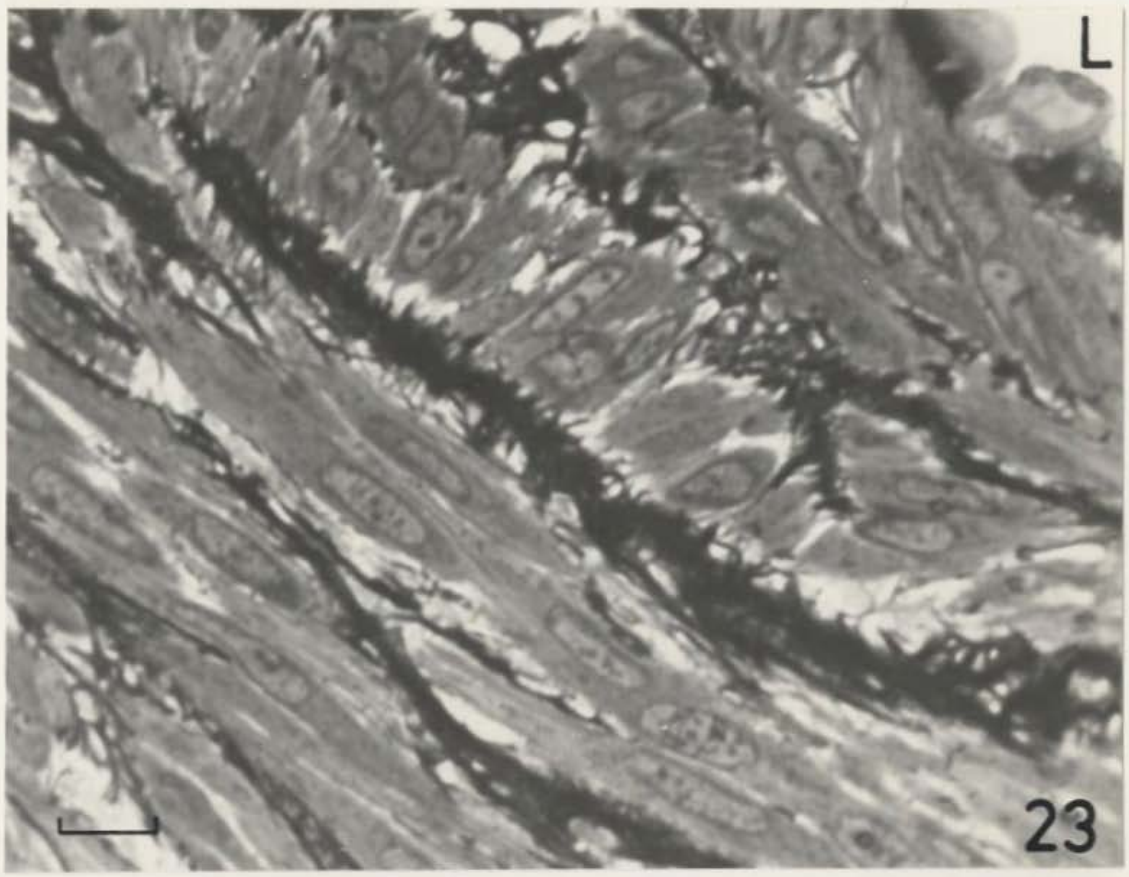
22b

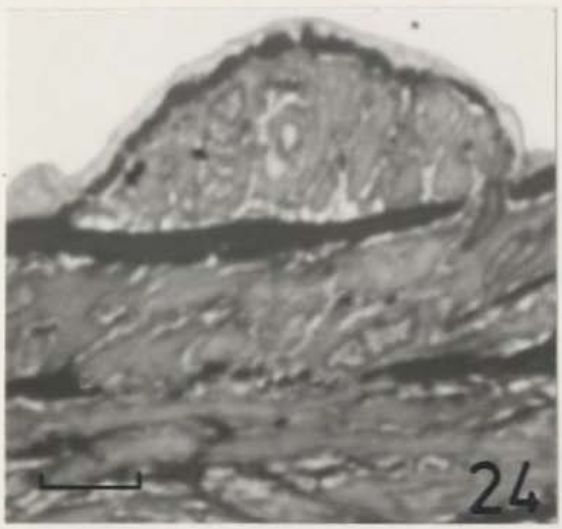
Figure 23. The SMC of the aorta were mostly arranged in a spiral fashion along the longitudinal axis of the vessel and alternated with elastic laminae across the tunica media of the vessel wall, although some of the SMC were disposed in a different plane. LM. Aorta, 2-week SHR. Calibration bar = 10 microns.

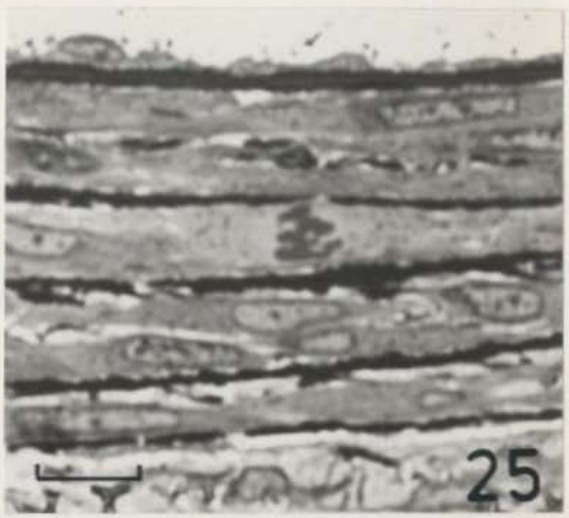
Abbreviation: L = lumen of the vessel.

Figure 24. Intimal thickenings were seen in 18 weeks old animals; the SMC-like cellular component of these thickenings were usually aligned along the longitudinal axis of the vessel. LM. Aorta, 18-week WKY. Calibration bar = 10 microns.

Figure 25. Mitotic SMC were often seen in the vessel wall obtained from young animals. LM. Aorta, 2-week WAR. Calibration bar = 10 microns.


L
23


24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DISCUSSION

Despite a significant elevation of arterial blood pressure in the SHR, as compared with that of the WAR and the WKY, morphological analysis revealed no indication of medial hypertrophy in the abdominal aorta and the renal artery.

I. NORMOTENSIVE CONTROLS

Most of the studies reported in the literature using the SHR as the experimental model utilised either the WAR or the WKY as the control; the latter is the closest normotensive progenitor from which the SHR strain is derived. However, it has also been reported that even the WAR and the WKY do not have the same physical, physiological and biochemical characteristics (Clineschmidt et al, 1970; Frohlich, 1977; Frohlich and Pfeffer, 1975; Pfeffer and Frohlich, 1973; Pfeffer et al, 1974). For example, Clineschmidt et al (1970) reported that both sensitivity and contractility (see INTRODUCTION) of aortic strips isolated from the SHR were similar to those of the WKY, when tested with NA; whereas aortic strips from the WAR tended to be more sensitive to NA and developed higher tension than the SHR. Thus, it has been suggested that both the WAR and the WKY should be used as normotensive controls in research involving the SHR as the experimental model (Frohlich, 1977). Other strains such as the Sprague Dawley rat have also been used by a number of investigators (i.e. Gorog and Kovacs, 1977).

Both the WAR and the WKY were used as normotensive controls in the present study. Since comparable weight is important when comparing cardiovascular parameters, it appeared that the WKY may be a better normotensive control for the SHR than the WAR, judging from the rate of increase in body weight of the 3 strains during the first 18 weeks of postnatal

development. Due to the larger body size of the WAR, compared to age matched SHR, findings from morphological studies of the SHR using the WAR as the control should be interpreted with caution.

II. AGE GROUP

The blood pressure in the SHR has been reported in the literature, to elevate to a significant level than the normotensive controls as early as 4 weeks (Lais et al,1977) or as late as 12 weeks (Moll et al, 1975). The present study was designed to test the hypothesis that if morphological changes in the blood vessels of the SHR had contributed to initiation of the elevation of blood pressure, then such changes should be detectable before or during the period during which the blood pressure of the SHR becomes significantly higher than the controls and manifested to an even higher degree when the blood pressure is established at a hypertensive state. For this reason, animals of 2, 4, 8, 12 and 18 weeks of age were utilised in this study and subjected to stereological analysis.

In comparing the blood pressure measurement with the stereological parameters in this study, it was noted that the blood pressure of the SHR became significantly higher than the controls at the age between 4 and 8 weeks. In order to refine this information about the elevation of blood pressure in the SHR for future reference, the blood pressures of 5, 6, and 7 weeks old animals were also recorded. Thus blood pressures were measured in a total of eight groups of rat from each strain.

III. BLOOD PRESSURE

Many methods have been employed to record blood pressure in rats. These include tail-cuff, acute and chronic intra-arterial cannulation and micropipette. The tail-cuff in the most frequently used method in hypertension studies. Although systolic pressure was the only parameter

reported in these studies, one of the advantages of the tail-cuff is that it does not require any surgery. For a successful measurement in using the tail-cuff, it requires warming and restraining the animals. The former process is used to promote vasodilation of the tail artery of the rat so that the pressure can be detectable. However, both the warming (Wright et al, 1978; Yen et al, 1977) and restraining (Kvetnansky et al, 1979) process are known to affect the blood pressure measurement by the tail-cuff.

Many studies have been performed to evaluate the tail-cuff method. In attempting to measure the "true" blood pressure, a chronic intra-arterial cannula was implanted into the animal and the blood pressure was recorded via this cannula without disturbing or anaesthetising the animal. Pfeffer et al (1971) used chronic carotid artery cannulation to evaluate the tail-cuff method. They reported that there was high degree of correlation between the two methods. However, unilateral cannulation of the carotid artery is known to increase blood pressure in the SHR and the WAR (Hallback, 1975; Pang and Scott, 1980). Furthermore, the error in the blood pressure recording obtained from carotid artery cannulation appeared to be the result of the neck suegery used in the preparation for cannulation (Pang and Scott, 1980). Bunag (1971) reported that the blood pressures recorded by the tail-cuff were higher than those obtained from the chronic aortic cannulation by 50 mmHg in the SHR, 70 mmHg in two-kidney Goldblatt hypertensive Sprague Dawley rats and 18 mmHg in normotensive Sprague Dawley rats. Using a chronic tail arterial cannula, Chiueh and Kopin (1978) reported similar results, although the difference in blood pressure recorded was lower than those obtained from the tail-cuff only by 25 mmHg in the SHR and 10 mmHg in the WKY. They further showed that the difference in blood

pressure was reduced when the blood pressure was recorded from pentobarbital anaesthetised animals by the tail-cuff. Results from these experiments strongly suggested that the tail-cuff method does raise the blood pressure to a much higher level than that obtained from chronic arterial cannula. The degree of increment in blood pressure is dependent on the method employed and sometimes hard to assess.

Because of its accessibility, cannulation of the femoral artery in anaesthetised animals was used in the present study. Although changes in the blood pressure in an anaesthetised animal, due to the anaesthetic, are also hard to determine, a consistent blood pressure recording was obtained by anaesthetising animals to a proximately same level of surgical anaesthesia. Surgical anaesthesia was achieved by intra-peritroneal injection of sodium pentobarbital at a dose of 30-40 mg/kg and was judged by the absence of both corneal and tail reflexes.

As the results from this study show, the SBP of the SHR was significantly higher than the WAR and the WKY at 2, 5, 6, 7, 8, 12 and 18 weeks. With the exception of the 2-week old animals in which the common carotid artery was used, these results were obtained by a femoral artery cannulation. As indicated above, there may be a possible error in the blood pressure measurement obtained through a common carotid arterial cannula and this error may be different among the 3 strains. Therefore the significance of the higher SBP in the SHR at 2 weeks, as compared with the controls, is uncertain and questionable. There was no difference observed in 4-week old rats in the present study, although the level of the SBP was much higher than that of the 5-week old animals, at least in the WAR and the WKY. This was thought to be the result of weaning; this may be the most dramatic change the weanlings had experienced since birth. Furthermore,

the level of SBP of 4-week old animals in the present study (104 mmHg in the SHR, 100 mmHg in the WAR and 106 mmHg in the WKY) was lower than that obtained by Lais et al (130 mmHg in the SHR and 120 mmHg in the WKY; 1977). The higher SBP reported by the latter authors was likely to be the result of restraining and dissecting the animals under local anaesthetics during blood pressure recording, although the possibility that the anaesthetic used in this study may lower the blood pressure, as many general anaesthetics do, cannot be excluded. In the present study, the SBP of the SHR rose gradually to about 137 mmHg by 7 weeks and remained at this level until 12 weeks. By 18 weeks, there was a further increase in the SBP of the SHR to a hypertensive level (159 mmHg), This level is very much lower than 200-210 mmHg at about 12-16 weeks as reported in the literature (Folkow and Hallback, 1977-a review; Okamoto, 1969) when the tail-cuff was employed. This discrepancy is likely to be the result of the different method employed in blood pressure recording. It may also be due to a different substrain of the SHR. Furthermore, the blood pressure measurement obtained by the tail-cuff is likely to be over-estimated and that obtained by arterial cannulation in anaesthetised animals under-estimated, in both the SHR and the normotensive control.

Judging from the results obtained from the present study, it is safe to conclude that the SBP of the SHR colony maintained at this university rises to a significantly higher level than the WAR and the WKY from 5 weeks and increases even further to a hypertensive level (159 mmHg) by 18 weeks of age.

IV. MORPHOLOGY OF THE BLOOD VESSEL AND OF THE SMC DURING DEVELOPMENT

The aorta and the renal artery were choosen as the models in the present study for the following reasons. The former has been used in many pharmacological studies of hypertension in the SHR, although its structure

had not been fully described. The changes in the media of the thoracic aorta of rabbits (Bevan, 1976; Bevan et al, 1976; 1980) and of rats (Wolinsky, 1970; 1971; 1972) in Goldblatt hypertension are well documented. The ultrastructure of the thoracic aorta of developing mice (Karrer and Cox, 1961) and of developing rats (Cliff, 1967; Gerrity and Cliff, 1975) was also studied. Hypertension has been produced in normotensive animals by clamping the renal artery thereby reducing the lumen diameter of this vessel. It would be of great interest to examine the renal artery during the development of hypertension in the SHR to see whether a narrowing of renal artery occurs in this animals. Furthermore, the information concerning the structure of the renal artery is scarce; only one paper was found in the literature which dealt directly with the structural characteristics of the renal artery of the rat (Osborne-Oellegrin, 1978).

Although arterioles are likely to be the candidates involved in the initiation of hypertension, morphological changes of these vessels are difficult to compare due to the lack of anatomical landmarks. Larger arteries, on the other hand, are usually defined with well-established anatomical landmarks which may serve as better models for studying morphological changes of the vessel wall in hypertension. Thus the aorta and renal artery were choosen to be the models in the present study.

The blood vessels used in the present study were obtained from animals with a SBP ranging from 50 to 175 mmHg. In order to compare vessels of different sizes from different ages, it would be beneficial to fix these vessels under a uniform tension within the range of 50-175 mmHg. It has been shown that when rabbit aorta was fixed by perfusion at and above 80 mmHg of pressure, all elastic laminae were straight and there was little

change in the interlamellar distance (Wolinsky and Glagov, 1964). Thus the vessels were fixed in situ by perfusion at a pressure of 80 mmHg.

The ultrastructure of the SMC fixed in this pressure appeared to be satisfactory judging from the preservation of cell membrane and intracellular organelles, although undulation of the internal elastic lamina was seen in some renal arteries obtained from 18-week old animals. Owing to the differences in the systolic pressure among the animals from different age group, the interpretation of the wall thickness and lumen diameter of the vessels was made with caution.

1. CHARACTERISTICS OF DEVELOPING VESSELS

By light microscopy, vascular SMC in both the abdominal aorta and renal artery of young rats appeared to be arranged mostly spiral to the longitudinal axis of the vessel, although it was apparent that not all of the SMC layers were arranged in the same plane. The deviation was more pronounced in mature vessels. This finding is in agreement with that of the thoracic aorta in developing rats (Cliff, 1967).

The present study revealed that in 2-4 weeks old animals, the abdominal aorta consisted of 5-7 SMC layers and 4-5 elastic laminae which were arranged alternately across the vessel wall. The number of layers was increased to 7-9 and 4-6, respectively, at 18 weeks. This is contrary to the findings of Cliff (1967) who reported that the number of SMC layers and elastic laminae did not change in the thoracic aorta of the rat during the first 12 weeks of development.

There was no change observed in the number of SMC layers and elastic laminae in the renal artery during the first 18 weeks of development in rats used in this study. The most proximal segment of the renal artery consisted of 5-6 layers of SMC and 2-3 elastic laminae, whereas those of the most distal segment were 4-5 and 1-2, respectively. There is no

information concerning these measurements in the literature. By applying the technique used in this study to the micrographs published by Osborne-Pellegrin (1978), the number of SMC layers and elastic laminae of the renal artery were comparable to those reported in the present study.

Cliff (1967) reported that there was only one incidence of SMC mitosis observed in all the specimens used in his study. However, mitosis of the SMC in the media was prominent in 2- and 4-week old aorta and 2-week old renal artery in the present study. As pointed out by Cliff (1967), the maturation of the media in blood vessels of the rat is very likely, at least in part, to be the result of hyperplasia of the SMC. The discrepancy between the results of the present study and those of Cliff (1967) is likely due to different state of cell cycle of the SMC during fixation for microscopy.

Longitudinally arranged SMC bundles were seen outside the external elastic lamina of the mature renal artery in the present study. Similar structure was reported in rats by Osborne-Pellegrin (1978), although the author did not specify the strain of rat she used in her study. The function of these SMC bundles is not clear. It has been suggested that they may play a role in supporting the arteries at sites of branching (Osborne-Pellegrin, 1978; Yohro and Burnstock, 1973).

Intimal thickenings were seen in the oldest groups of animals (18 weeks) used in the present study. Although intimal thickening have been found after ligation (Buck, 1961), cholesterol feeding (Buck, 1962; Parker and Odland, 1966) and in repair process (Warren and Brock, 1964) and degenerative process (Simpson and Harnes, 1964) of arteries, the finding in the present study suggested that these changes may be a result of ageing.

2. ULTRASTRUCTURE OF DEVELOPING SMC

The results of electron microscopic examination of the SMC in the

developing rat of the present study were similar to those in the thoracic aorta of the developing mouse (Karrer and Cox, 1961) and rat (Cliff, 1967). The maturation process of the SMC in the blood vessels included changes in myofilaments, intra-cellular organelles and cell contacts. Using these criteria, the order of maturity of the vessels was determined. At 2 weeks, the order of maturity of the vessels, from the most mature to the least mature, was renal artery of the SHR, renal artery of the WAR and the WKY, aorta of the SHR, and aorta of the WAR and the WKY. If it can be assumed that mature SMC can produce a stronger contraction as the result of increased and more organised myofilament bundles, then the unexpected finding is of great important. It follows that the renal artery of the SHR will contract more to a given amount of vasoactive agent and so produce a greater reduction in lumen diameter. Thus the situation is not unlike the 2-kidney Goldblatt hypertension in which the renal artery is narrowed by clamping to produce an elevation of arterial blood pressure. This result needs further studies by more detailed stereological analysis of the myofilaments in the SMC and pharmacological studies of the developing renal artery.

The ultrastructure of the SMC in 18-week old animals in the present study was similar in both the abdominal aorta and renal artery among the 3 strains of rat studied. This is in agreement with that reported by Osborne-Pellegrin (1978).

Although Osborne-Pellegrin (1978) reported that no nexuses were found in either the abdominal aorta or renal artery, the present study confirmed the finding in the thoracic aorta of developing rats (Cliff, 1967) that nexuses were seen quite often between adjacent SMC in mature vessels. Furthermore, nexuses were found more frequently in mature renal artery than in mature abdominal aorta.

3. WALL THICKNESS AND LUMEN DIAMETER

The development of wall thickness and lumen diameter of the abdominal aorta and renal artery in the WAR, the WKY and the SHR were similar as shown by the results of the present study (Table 2 and 3). There were no significant differences observed when these parameters were compared among the 3 strains at any age group used in the present study, except that the lumen diameter of the abdominal aorta of 12- and 18-week old WAR was significantly larger than that of the WKY and the SHR in the corresponding age group. This difference is more likely to be the result of larger body size of the WAR as compared with the WKY and the SHR of the same age rather than the difference in blood pressure between the two groups. This is substantiated by the fact that although the blood pressure of the SHR was significantly higher than the WKY, there were no significant difference in both the body weight and the lumen diameter between the two groups.

Gerrity and Cliff (1975) reported that the wall thickness of the thoracic aorta in the developing rat was 95, 100, 113 and 112 microns at 2, 4, 8 and 12 weeks, respectively, whereas the wall thickness of the abdominal aorta of the WAR in the present study was 34, 44, 49, 53 and 57 microns at 2, 4, 8, 12 and 18 weeks of age, respectively. Gerrity and Cliff also reported in the same study that the lumen diameter of the thoracic aorta was 640, 920, 1240 and 1200 microns at 2, 4, 8 and 12 weeks, respectively, whereas the lumen diameter of the abdominal aorta of the WAR in the present study was 486, 566, 587, 745 and 789 microns at 2, 4, 8, 12 and 18 weeks of age, respectively.

Although Wiener et al (1977) found that both wall thickness and lumen diameter of the thoracic aorta in 2-kidney Goldblatt hypertensive rat were significantly higher than the normotensive control after the induction of hypertension, the results of the present study showed that there were no

significant differences observed in these parameters even when the SBP of the SHR was significantly higher than that of the WAR and the WKY.

The wall thickness of the renal artery in the developing WAR obtained from the present study was 17, 23, 30, 33 and 32 microns at 2, 4, 4, 12 and 18 weeks, respectively, whereas the lumen diameter was 243, 279, 326, 380 and 447 microns at 2, 4, 8, 12 and 18 weeks, respectively. No published information is available for comparison.

In the present study, the results of wall thickness and lumen diameter measurements reveal that there is no sign of vessel wall hypertrophy in either the abdominal aorta or renal artery of the 3 strains of rat studied during the first 18 weeks of postnatal development.

4. STEREOLOGY

Stereological methods fro morphometric cytology, reviewed by Weibel et al (1966), have been proven to be useful in morphometric analysis. Sampling technique similar to those of Weibel et al or their modifications have been applied in morphological studies on numerous occasions, e.g. lung (Weibel, 1963), parotid gland (Cope, 1978), Skeletal muscle (Crowe and Baskin, 1977; Eisenberg et al, 1974), Myocardium (Imamura, 1978; Kawamura et al, 1976; Lund and Tomanek, 1978; Sachs et al, 1977; Tomanek, 1979) and thoracic aorta of developing rats (Gerrity and Cliff, 1975) and 2-kidney Goldblatt hypertensive rats (Wiener et al, 1977).

The development of stereology in cytology is based on the Delesse Principle (Delesse, 1847- cited in Weibel et al, 1966; Appendix II). Based on this principle, the volume fraction of a component in an organ can be estimated from thin section of this organ with a sampling grid. The sampling grid used in the present study consisted of 100 (10 X 10) crosses, arranged as a regular lattice and made to fit the 8 X 10 photographic paper on which the electron micrographs were printed. Estimations of seven micrographs,

with the final magnification of 19,200 times, obtained from each specimen were pooled to give the value of that specimen.

Gerrity and Cliff (1975) also used a 100-point sampling grid in their study on the thoracic aorta of developing rats; the magnification they used was only 6,000. Evaluation of the micrographs that they published, does not allow one to distinguish elastin from collagen when a small amount of elastin was intermingled with the collagen fibres. In order to have a more accurate estimation of these components, a higher magnification was used in the present study. The pooling process of the estimates from the seven micrographs was also used to compensate for the smaller sampling area covered in this study as the result of higher magnification. Furthermore, the method used in the present study is more precise than that of Gerrity and Cliff (1975), judging from the density of sampling points per unit area: 0.64 points per sq. cm. in the present study as compared with 0.48 points per sq. cm. in the study of Gerrity and Cliff (1975).

The number of points of the grid amd electron micrographs used in the present study, and the detailed calculation of point density of sampling grids will be considered in Appendix II.

As indicated in the RESULTS section, although there was a tendency that the volume fraction of the SMC (about 60%) in the abdominal aorta of the WAR and the WKY to decrease and that of the SHR to increase during the first 18 weeks of postnatal development, no significant difference could be detected among the 3 strains at any age group in this study. These results were wuite different from those obtained by Gerrity and Cliff (1975) who showed that the volume fraction of SMC in the thoracic aorta of developing rats decreased from 47% at 2 weeks to 25% at 12 weeks.

Furthermore, they showed that the volume percent of collagen and elastin increased from 5 and 32, respectively, at 2 weeks to 17 and 52 at 12 weeks, whereas, in the present study, the volume percent of both collagen and elastin were about 20. During the first 18 weeks of postnatal development, there was no significant difference observed in the volume percent of collagen and of elastin in the abdominal aorta, although the collagen content tended to increase with age, e.g. the collagen content of the SHR increased from 18% at 2 weeks to 22% at 18 weeks. The difference between the results of the present study and those obtained by Gerrity and Cliff (1975) is likely to be the result of the different segment of the aorta used. Judging from the amount of elastin present, the abdominal aorta appears to be more muscular than the elastic thoracic aorta, although this transition is expected to be gradual. Furthermore, the abdominal aorta and the thoracic aorta seemed to developed along a different pattern. This suggests that the function of the abdominal aorta may be different from the thoracic aorta (Kot and Rose, 1979)

The result of the present study indicated that although the SBP of the SHR was elevated to a significantly higher level than the normotensive controls from 5 weeks and to a hypertensive level by 18 weeks, there was no sign of increased collagen deposition in the media of the vessels examined in the SHR, as compared with the normotensive controls of the same age group. This result is in agreement with that reported by Newman and Langner (1978) who showed that the vascular collagen synthesis of the SHR does not increase until 23 weeks of age, after the SBP of the SHR has risen to a significant level than the normotensive controls.

The volume percent of SMC, collagen and elastin in the renal artery of the SHR in the present study was about 61, 26 and 13, respectively, at

the age of 18 weeks; no significant difference was observed among the 3 strains of rat studied. These results are different from those reported by Osborne-Pellegrin (1978) who estimated that the collagen and elastin component of the distal segment of the renal artery in mature rats occupied about 15-25% of the total medial volume. The difference between the two studies is likely due to the different segment of the renal artery used in stereological analysis; the distal segment of the renal artery is more "muscular" than the middle segment used in the present study.

From the results of the present study, the development of the abdominal aorta and the renal artery appeared to follow a similar pattern of growth. The increase in wall thickness of these vessels was likely to be the result of an addition of SMC, collagen and elastin content so that the medial composition of the vessels remained unaltered. In considering the results of stereological analysis of the medial content together with the wall thickness measurement of the blood vessels examined, there was no indication of medial hypertrophy in either the abdominal aorta or the renal artery during the first 18 weeks of postnatal development. However, the SBP of the SHR was significantly higher than the WAR and the WKY from 5 weeks onward and was increased to a hypertensive level (159 mmHg) by 18 weeks. Thus, it appears that the elevation of blood pressure in the SHR is not the result of medial hypertrophy of these vessels.

It is possible that the large arteries may not be involved in the process of hypertension development. However, the secondary changes of the largest artery, the thoracic aorta, to hypertension, in particular to Goldblatt hypertension has been well documented in the rabbit (Bevan, 1976; Bevan et al, 1976; 1980) and in the rat (Wolinsky, 1970; 1971; 1972). From the results of these studies, it is impossible to determine whether

the change in the blood vessels is a causative factor in hypertension, because hypertension was induced in these animals by artificial means. The development of hypertension in the SHR, on the other hand, is spontaneous. Thus by examining the morphology of these vessels during the development of hypertension, the involvement of these vessels in initiating the hypertension of the SHR can be determined. The results of studying the development of these vessels in the present investigation strongly suggest that the initiation of hypertension in the SHR is not the result of changes in medial composition of the abdominal aorta and the renal artery. However, the hypothesis that medial hypertrophy occurs at later stages of hypertension, indicative of a maintenance role, in the SHR has not been examined.

The hypothesis that medial hypertrophy of small arteries may be an initiating factor in hypertension of the SHR (Folkow, 1978; Folkow and Hallback, 1977; Folkow et al, 1970; 1973; Mulvany et al, 1978; Warshaw et al, 1979) has not been settled. On the one hand, Ichijima (1969) reported that the lumen diameter of small jejunal arteries in the SHR was generally smaller than that of the controls. This is supported by Mulvany's group (Mulvany et al,1978; Warshaw et al, 1979) who found that medial hypertrophy of mesenteric vessels was prominent in the SHR from 6 weeks to 50 weeks of age. On the other hand, Bohlen (1979) and Bohlen and Lobach (1977) did not detect any changes in the microvasculature of the cremaster muscle in the SHR.

CONCLUSION

Based on the results obtained from the present investigation of the SHR, the following conclusions can be drawn:

1. The WKY appeared to be a better normotensive control for the SHR than the WAR in morphological studies.
2. The systolic blood pressure of the SHR colony maintained at the Memorial University of Newfoundland was significantly higher than the WAR and the WKY from 5 weeks and increased to a hypertensive level by 18 weeks of age.
3. Although ultrastructural changes in the abdominal aorta and the renal artery during development were not different among the 3 strains of rat studied, the arteries of the SHR appeared to be more mature than those of the WAR and the WKY normotensive controls at 2 weeks of age.
4. The elevation of arterial blood pressure in the SHR appeared not to be the result of medial hypertrophy of the abdominal aorta and renal artery.

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APPENDICES

APPENDIX I: STATISTICAL ANALYSIS

Data obtained from the present study were analysed by Student-Newman-Keuls' multiple-range test. The procedures used were as the following:

1. Calculate the mean value for each group.

   For example, the systolic blood pressure of 12-week old rats was:

	WAR	WKY	SHR
	130	120	135
	125	125	135
	118	127	140
	130	125	140
mean	125.75	124.25	137.50

2. Rank the means in an order from the highest to the lowest.

SHR	WAR	WKY
137.50	125.75	124.25

3. Calculate $W_p = q_a(p, n_2)\, S_{\bar{x}}$; where a = level of significance (5%)

   p = number of means

   n_2 = error degree of freedom

   $S_{\bar{x}}$ = (error mean square/r)$^{\frac{1}{2}}$

   a. $q_a(p, n_2) = q_{0.05}(3, 9)$, in the present study was determined from the table of "the upper percentage points of the 'Studentised' range (Steel and Torrie, 1960)".

   b. $S_{\bar{x}}$ was calculated from the formula $S_{\bar{x}}$ = (error mean square/r)$^{\frac{1}{2}}$;

   where r = number of animals (4 in the present study) in each group

$$\text{error mean square} = \frac{\text{error sum of squares}}{\text{degree of freedom (9)}}$$

c. Error sum of squares (Error SS) was calculated from the formula

Error SS = Total SS - Treatment SS ;

where Total SS = the sum of the square of all values - C

Treatment SS = (the sum of the square of the column sum divided by 4) - C

C = the square of the sum of all values divided by 12.

In the present study the $S_{\bar{x}}$ = (error mean square/r)$^{\frac{1}{2}}$

= $(16.50/4)^{\frac{1}{2}}$

= 2.03

and $q_{0.05}(3, 9) = 3.95$

therefore $W_p = q_a(p, n_2)\ S_{\bar{x}}$

= 3.95 X 2.03

= 8.02

The difference between two means can be either greater than 8.02, when they are significantly different from each other, or smaller than 8.02, when they are not significantly different from each other, at a significance level of 5%.

From the results of the present study, the following comparisons can be made:

SHR vs WAR = 137.50 - 125.75 = 11.75; significantly different from each other.

SHR vs WKY = 137.50 - 124.25 = 13.25; significantly different from each other.

WAR vs WKY = 125.75 - 124.25 = 1.50; not significantly different from each other.

Thus, it can be concluded that the systolic blood pressure of the SHR is significantly higher than that of the WAR and the WKY; there is no significant difference between the systolic blood pressure of the WAR and the WKY.

APPENDIX II: STEREOLOGICAL METHOD

1. THEORETICAL CONSIDERATION OF STEREOLOGY

The development of stereological methods in cytology was based on the Delesse Principle (Delesse, 1847-cited in Weibel et al, 1966) which states that the planimetric fraction of a section occupied by sections of a given component corresponds to the fraction of the tissue volume occupied by this component. This principle was modified by Glagoleff (1933- cited in Weibel et al, 1966), Chalkley (1943-cited in Weibel et al, 1966)and Attardi (1953-cited in Weibel et al, 1966) as a point-counting volumetry, thus the fraction of test points enclosed in the structure X could be considered as an estimate of the volume fraction occupied by X,

$$\text{i.e. } \frac{V_x}{V_t} = \frac{P_x}{P_t} \quad ;$$

where V_x = the volume fraction of component X

V_t = the total volume of the organ containing X

P_x = the number of points overlying the profiles of X

P_t = the total number of points overlying the section of the organ containing X.

2. SAMPLING GRID

The sampling grid used in the present study was a 10 X 10 regular point lattice which conveniently gave direct percent values for volumetry and was modified from Weibel et al (1966) to fit the 8X10 inches photographic paper on which the electron micrographs were printed. In order to establish the sampling grid used was, at least, as accurate as that proposed by Weibel et al (1966) which also contained 100 points and enclosed in a test area of rectangle with the horizontal side measuring 10 Z and the vertical side 8.66 Z, where Z is the distance between 2 adjacent points. The component of the tunica media were estimated from the

same set of micrographs by the two grids simultaneously. The mean obtained from the modified sampling grid, denoted by $\bar{X}$ and the mean obtained by that of Weibel et al, denoted by $\bar{Y}$ were compared by a paired "t" test at a significance level of 5%. The procedures used were as the following:

a. Set the hypotheses.

Set the null hypothesis $H_0 : \bar{X} = \bar{Y}$;

the alternative hypothesis $H_A : \bar{X} \neq \bar{Y}$.

b. Obtain "t" value from table.

In this case, $t_{28,\ 0.025} = \pm 2.048$

The null hypothesis will be accepted when the calculated "t" values are within the interval from -2.048 to +2.048. If the calculated values are either smaller than -2.048 or larger than +2.048, the null hypothesis will be rejected and the alternative hypothesis accepted.

c. Calculated values.

Collagen: $\bar{X}_C = 23.60$; $\bar{Y}_C = 22.27$; $t_C = 0.84$.

Elastin: $\bar{X}_E = 29.00$; $\bar{Y}_E = 29.27$; $t_E = -0.12$.

SMC: $\bar{X}_{SMC} = 47.07$; $\bar{Y}_{SMC} = 47.93$; $t_{SMC} = -0.32$.

Since all the calculated values are lying within the intervel from -2.048 to +2.048, the null hypothesis is accepted. That is the mean obtained from the modified sampling grid is not differed from that obtained from Weibel et al.

3. NUMBER OF MICROGRAPHS USED IN STEREOLOGICAL ANALYSIS

Since the medial components in the artery are organised, at least in the abdominal aorta, into lamellar units, the random sampling technique used in the present study may create a high variation in the estimates. If the number of micrographs used for estimation is few, this variation can be very large or very small, as compared with the "true" values; the "true"

value can be reached by increasing the number of micrographs used. The minimum number of micrographs at which the variation of the estimates was stablised was determined by plotting the number of micrographs against the standard deviation of the estimates (Figure 16). The standard deviation of the estimate of SMC, collagen and elastin reached its plateau at about 7. Thus 7 micrographs obtained from each specimen were used for stereological analysis in the present study.

4. POINT DENSITY OF SAMPLING GRID

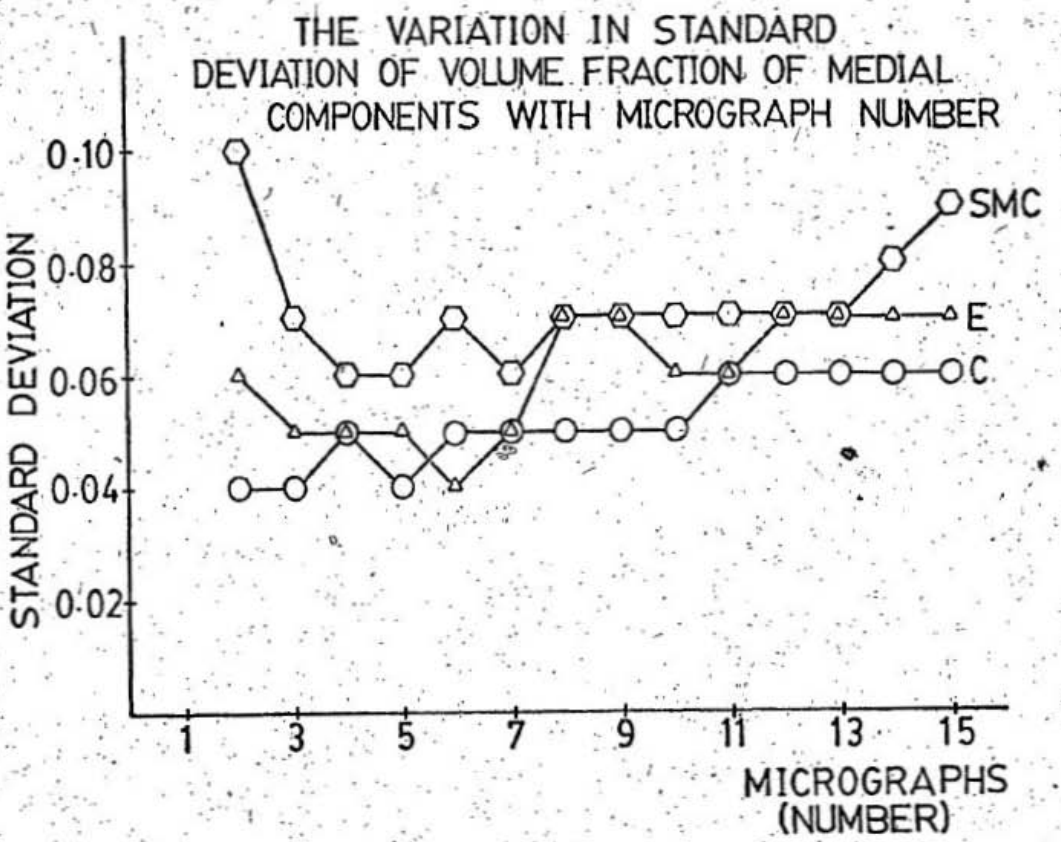
In the present study, the 100-point sampling grid covered an area of 24.8 cm X 19.8 cm = 491.04 sq. cm, thus the point density is 100/491.04 = 0.20 point/sq. cm.

In the study by Gerrity and Cliff (1975), the 100-point sampling grid covered an area of 13.5 cm X 15.3 cm = 206.55 sq. cm, thus the point density is 100/206.55 = 0.48 point/sq. cm.

Since the magnification used in the present study was 19200/6000 = 3.20 times higher than that of Gerrity and Cliff. Therefore the point density of the sampling grid used in the present study is 3.20 X 0.20 = 0.64 point/sq. cm, which is higher than that of Gerrity and Cliff; the estimation of the medial components of the arteries obtained in the present study is more precise than that of Gerrity and Cliff.

Figure 26. The variation of standard deviation of volume fraction of medial components with micrograph number.

The volume fraction of SMC, collagen and elastin was estimated by a 100-point sampling grid and the standard deviation was calculated with the increasing number of micrographs. By plotting the number of micrographs against the standard deviation, the "inherent" variation of the components in the vessel wall can be estimated. As shown on the graph, the standard deviation of the estimates of SMC, collagen and elastin reaches its plateau at about 7.


THE VARIATION IN STANDARD DEVIATION OF VOLUME FRACTION OF MEDIAL COMPONENTS WITH MICROGRAPH NUMBER
STANDARD DEVIATION
0·10
0·08
0·06
0·04
0·02
SMC
E
C
1
3
5
7
9
11
13
15
MICROGRAPHS (NUMBER)

APPENDIX III: FIXATIVE

The half strength Karnovsky fixative (1965) was used for fixation in the present study. The fixative was prepared as the following:

1. Dissolve 2 gm of paraformaldehyde to 20 ml of distilled water by warming the mixture to 60 degrees C.
2. Add 2 drops of 1N sodium hydroxide to clear the solution and cool.
3. Add 10 ml of 25% glutaraldehyde into the cooled solution and bring to 60 ml with distilled water.
4. Add 0.2M sodium cacodylate buffer (pH 7.5) to the solution to obtain a final volume of 100 ml.
5. Adjust pH to 7.2.

Fresh fixative was used in each set of experiments.