# AN EXAMINATION OF THE HYPOTHESIS THAT STRUCTURAL CHANGES WHICH OCCUR IN HYPERTENSION, ARE A RESULT OF THE INCREASE IN ARTERIAL PRESSURE



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# An examination of the hypothesis that structural changes which occur in hypertension, are a result of the increase in arterial pressure.

by

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ABSTRACT.

The hypothesis that the medial hypertrophy which occurs in jejunal arteries of the SHR during development is a result of elevation of arterial pressure, was examined. The morphological changes occurring in the jejunal arteries of SHR and normotensive WKY during development were compared with those occurring in jejunal arteries transplanted between the two strains, in WKY in which the blood pressure had been elevated by renal artery constriction, and in SHR which had been rendered normotensive by capsaicin treatment. Although it is still premature to draw the final conclusion on the causes of medial hypertrophy in jejunal arteries of the SHR, the results of the present studies suggest that the medial hypertrophy is neither the result of inherent genetic factors in the arterial wall, nor the increase in arterial pressure, but due to some other factors such as innervation of the arteries or circulating hormones in the blood.

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# INTRODUCTION.

## I. Classification and definition of hypertension.

A common symptom of many cardiovascular diseases is an elevation of arterial blood pressure. This elevation of arterial blood pressure, or more commonly hypertension, 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 majority (up to 90%) of human hypertension is of the former type (1).

Hypertension, as recommended by the World Health Organisation (2) should be defined as a single, sitting or recumbent, blood pressure of the patient higher than 160/95 mmHg (110/70 in normal person). However, it is well known that arterial blood pressure fluctuates during normal daily activities (3). Thus, this definition may be erroneous and misleading. Despite these problems, it is necessary, for the purpose of comparison, to define a reference point in hypertension research. In experimental hypertension, animals with systolic blood pressure of 150 mmHg or higher were considered to be hypertensive (4, 5, 6, 7, 8, 9). In further discussion of this subject, the term "hypertensive" will be used to describe individuals with systolic pressure over 150 mmHg, unless otherwise stated.

## II. Changes in the arterial system in hypertension.

In the established phase, hypertensive patients as well as experimental hypertensive animals exhibit an elevated total peripheral resistance and a normal to subnormal cardiac output. Thus, in the past few decades, many studies have focussed on finding the cause of this increase in total peripheral resistance. Up to now, the cause of the increase of peripheral resistance in hypertension still remains the subject of much discussion.

Arterial hypertrophy has been demonstrated in the vascular system of hypertensive humans and experimental animals. The cause of this hypertrophy is unknown. Because of the close relationship between hypertension and arterial hypertrophy, it has been postulated that the alteration in the arterial wall structure is a result of the increase of arterial blood pressure. The present investigation was designed to examine the hypothesis that structural changes which occur in arteries in hypertension, are a result of the increase of arterial blood pressure.

In order to promote better understanding and further discussion on the arterial system which will be subjected to hypertension, the normal structure and function of the arterial system will be reviewed in this section. Futhermore, since the spontaneously hypertensive rat (SHR) will be used as a model, its characteristics, in particular that of the vascular system, will be described in the

latter part of this section. Other information relevant to different approaches used in the present investigation will be reviewed in the objective and rationale section.

Normal structure and function of the arterial system.

One of the most remarkable characteristics of the mammalian cardiovascular system is its ability to modify blood flow to various vascular beds according to the local metabolic requirements. This can be achieved by constricting or dilating the main "feeding" artery to that vascular bed. These control mechanisms are believed to be located at the arteriolar level. Since arterioles are the simplest form of arteries and are important in controlling blood flow, the present discussion includes some of these control mechanisms whereby the lumen diameter of arterioles of a particular region can be modified which in turn alters the microcirculation to that region. Although regional variations and species differences of these control mechanisms are apparent, the prime concern of this review will focus only on the general principles rather than distinguish these variations.

I. Definitions.

The vascular portion of the cardiovascular system is a series of tubular components through which the

"oxygenated" and "deoxygenated" blood are carried to and from various organs, respectively to fulfill the local metabolic needs. By definition, arteries are vessels which carry the blood away from the heart, whereas veins are those that do the opposite. The space of these vessels through which the blood is being transported, is termed the lumen.

Arterioles are the smallest arteries of the arterial system. According to the classical definition, these are vessels with lumen diameter ranging from 50-100 microns (10, 11). Depending on its state of contraction and dilatation, as well as the mode of fixation, the vessel diameter varies greatly in fixed specimens. It has been suggested that arterioles should be classified according to the vessel wall components rather than the lumen diameter (12). In this context, arterioles are referred to as small arteries which consist of only 1-2 layers of smooth muscle cells in the tunica media. Thus, arterioles (10), terminal arterioles (10), and precapillary sphincters (10, 14, 15) described in the literature are all included in this category.

## II. Arteriolar wall components.

Encircling the lumen of arterioles is the arteriolar wall which is composed of both cellular and fibro-elastic components. These components are arranged concentrically

and can be identified microscopically as three distinct tunicae. The tunica intima consists of a single layer of endothelial cells and a thin basal lamina, and generally lacks elastic laminae (14), although some arterioles in certain species do contain a delicate internal elastic lamina, i.e. glomerular afferent arterioles in the kidney of the rat (15). The tunica media contains 1-2 layers of circularly arranged smooth muscle cells with collagen fibrils occupying the intercellular space. Close contacts between the endothelial cells and the smooth muscle cells are found more frequently in arterioles than other vessels (10, 15); these are called myoendothelial junctions. Most of the tunica adventitia is filled with bundles of longitudinally arranged collagen fibrils and scattered fibroblasts. Nerve terminals of the non-myelinated type are rich and present near the adventitio-medial junction (10, 12, 15).

From a functional point of view, both the endothelial cells and smooth muscle cells are the active components in the arteriolar wall which are capable of altering the lumen diameter of arterioles. By virtue of its structural characteristics and contractile ability, the smooth muscle cell is considered to be, by far, the most important candidate in controlling the lumen diameter of arterioles. Thus the core of the present discussion will concentrate mainly on the smooth muscle cell structure and functions. The possibility that the endothelium may be involved in

these control mechanisms will also be considered.

## The vascular smooth muscle cell (SMC).

### I. Structural characteristics.

Like other muscle cells, the vascular SMC is characterised by the presence of filamentous structures which occupy a large fraction of the cell volume. A set of organelles and inclusions, common to most cell types is usually present and gathered at the two poles of a flattened and generally centrally located nucleus. Only those structures which are directly related to the present discussion will be considered. These are myofilament, dense bodies, sarcoplasmic reticulum and surface vesicle, and cell junction.

#### A. Myofilament.

Three types of filament can be seen in transverse sections of properly fixed vascular SMC. These are thick, thin and intermediate filaments.

Thick or myosin filaments of vascular SMC are approximately 15-18 nm in diameter and about 2.2 microns in length (16). Although myosin filaments of vascular SMC are longer than those of the striated muscles (1.5-1.6 microns), they are indistinguishable microscopically from

those of the skeletal or cardiac muscle (17, 18). Each myosin molecule consists of a light and heavy meromyosin subunit; the latter is composed of 2 globular S-2 and filamentous S-2 subfragments. The exact packing of myosin molecules to form the thick filament is not clear, nor is the precise arrangement of thick filaments in the vascular SMC cytoplasm. However, lateral projections suggestive of cross-bridges and the absence of the central bare zone, corresponding to the "M" band in striated muscles have been noted (19, 20).

Thin or actin filaments are abundant in vascular SMC. The ratio of thin : thick filaments is about 15 : 1 (19). These filaments are of 5-8 nm in diameter and unknown length; the latter has not been successfully measured. The G-actin mononers and F-actin filaments are similar to those of the skeletal muscle (21). In the skeletal muscle the regulatory proteins, tropomyosin and troponin, are located on the actin filaments. Although immunochemically distinct tropomyosin has been isolated, the components corresponding to the skeletal muscle troponin were absent in vascular SMC actin filaments (22). Without the presence of troponin, the function of tropomyosin in the vascular SMC actin filament has to be re-defined.

Intermediate filaments, sometimes referred to as 10 nm filaments are readily found in vascular SMC, surrounding or making contact with the dense bodies or areas (16).

Filaments similar to intermediate filaments have also been found in many other cell types. However, intermediate filaments from SMC are immunochemically distinguishable from those of other cell types (23). For example, antibodies to gizzard intermediate filament failed to stain those of the fibroblasts or nerve cells. This demonstrates polymorphism exists in these filaments. The function of these filaments is not known, although they appeared not to relate to contraction (16). Its function as a cytoskeleton has been suggested (24).

B. Dense bodies.

Amorphous, electron-opaque structures of 0.2 micron wide and about 5 microns long, are often seen in the vascular SMC. These patches can be classified into two forms according to their location. Dense bodies are the free-floating type and are found lying among the myofilaments along the longitudinal axis of the vascular SMC; whereas dense areas are those attached to the plasma membrane and are usually found alternating with rows of surface vesicles along the cell periphery. Dense bodies are commonly surrounded by intermediate filaments, whereas insertion of thin filaments into both dense bodies and areas can readily be demonstrated (16). The function of dense bodies and areas is not known, although the analogy between these structures of the vascular SMC and the "Z" lines of the striated muscle has been drawn (25).

C. Sarcoplasmic reticulum and surface vesicle.

The sarcoplasmic reticulum (SR), usually referred to as the smooth type in striated muscles, of the vascular SMC is not as well-developed as in the striated muscle and is usually present in close relation with the surface vesicles along the sarcolemma. That the SR is a true intra-cellular system of tubules has been demonstrated with extra-cellular markers such as ferritin, colloidal lanthanum and horseradish peroxidase (26, 27, 28); these markers do not enter the SR.

The SR of the vascular SMC is thought to function as a calcium storage, release and uptake site, as in the striated muscle. Using strontium as a substitute for calcium, the divalent cation-accumulating ability of the SR has been demonstrated (29). Although calcium binding proteins such as calsequestrin, which are present in the terminal cisternae of the skeletal muscle (30), have not been identified in the vascular SMC, particles corresponding to the calcium-ATPase, have been demonstrated in the lumen of the SR by freeze-fracture and biochemical techniques (31) as well as by electron microscopy (32). The result of the latter study further suggested that the rough SR and mitochondria are also contained calcium-ATPase.

No "T" tubules or their equivalent have been demonstrated in the vascular SMC, although the close relation between the SR and surface vesicles in these cells

has been suggested to be an analogue of the triad in skeletal muscle or diad in cardiac muscle (26).

As in endothelial cells and fibroblasts, flask-shaped surface vesicles are present in the vascular SMC. These vesicles are 50-80 nm in diameter and appear to be invaginations of the sarcolemma. The presence of these vesicles increases the surface membrane area per cell volume by 25-70 % in SMC (34, 35). In vascular SMC, surface vesicles are usually aligned in rows along the sarcolemma; many of them open to the extracellular space (33). Surface vesicles of the vascular SMC are also known to be associated with the SR and mitochondria (29, 32).

The function of surface vesicles is not clear. It has been suggested that these vesicles are engaged in ion transport (36). However direct evidence has not been obtained (37). That the surface vesicles of the vascular SMC are as the analogue to the transverse tubular system of the striated muscle has also been suggested (32). Although there were structural resemblance of the fused surface vesicles to the "T" tubules of embryonic skeletal (38) and cardiac (39) muscle, functional demonstration has not been attempted.

D. Cell junction.

In the vascular SMC, a spectrum of cell junctions, ranging from simple cell-to-cell apposition to

desmosome-like attachments, can be identified (40).

Simple appositions are found in the vascular SMC (40, 41), particularly in developing SMC. Apart from the closeness (about 10 nm) of cell membranes, there is no specialisation noticeable in this junctional area. No function has been assigned to these junctions.

Desmosome-like attachments are frequently observed in the vascular SMC (40). The inter-cellular space between two adjacent membranes is usually 50 nm wide and there is an increased cytoplasmic density in the region of attachment. Although the function of this junction in the vascular SMC is not clear, it is possible that the desmosome-like attachments may serve to hold the SMC together, similar to the desmosomes present among the epithelial cells in the stratum spinosum of the skin.

Gap junctions or nexuses, are often found in the vascular SMC (40), and are often mistaken as tight junctions in osmicated materials. With both electron microscopy and freeze-fracture technique, it was demonstrated that there is a 2-4 nm gap between the two adjacent membranes of this junction (42, 43). Nexuses are implicated to play an important role in ionic cell-to-cell communication. It was shown that nexus can provide low-resistance pathways for rapid spread of electrical signals (44, 45) between the adjoining cells. Thus the appearance of large amount of gap junctions in the

myometrium during parturition reported by Daniel's group (46) may illustrate the importance of nexuses in the coordination of muscle contraction during labour. This study also demonstrated the dynamic nature of the gap junctions.

Although proteins, nucleic acids and other macromolecules have not been demonstrated to cross the nexus, small molecules such as amino acids, sugars, and nucleotides "penetrate" through the junction quite readily (47). Gap junctions may also function as a means of metabolic coupling between adjacent cells.

## II. Functional characteristics.

Functionally vascular SMCs can be classified into i). spontaneously active SMC which exhibits pacemaker activity, conduction of excitation, and membrane potential spikes (e.g the portal vein of rats) and ii). quiet SMC which does not exhibit membrane potential spikes ( e.g. rat tail artery and rabbit aorta) (52).

### A. Resting membrane potential and action potential.

The resting membrane potential in the SMC from various sites of the vascular tree of different species has been measured with intra-cellular microelectrodes and these results were summarised in a review by Somlyo and Somlyo (48). These values ranged from -25 to -65 mv. The cause of

the diversity in this transmembrane potential of the vascular SMC is not known. Jones (49) reported that the concentration gradient of ions across the membrane of the vascular SMC varied from various sites, as well as from different species. This may form the basis for the differences observed in the resting membrane potential in the vascular SMC.

The action potential in the vascular SMC also varies from spike-like configuration (50), either singly or in bursts, to that resembling the cardiac action potential (51). Thus spontaneous periodic activity in the vascular SMC is not uncommon (e.g. rat portal vein). The mechanism by which the action potential varies among the SMC from different blood vessels is not clear. It has been suggested that the difference in the relative contribution of calcium and sodium ion currents in the vascular SMC may result in the difference of action potential (52).

B. Excitation-contraction coupling.

It was pointed out by Johansson and Somlyo (52) that in spike-generating vascular SMC, the action potentials are normally involved in triggering contraction. However, action potentials are certainly not the only means by which contraction can be initiated or maintained. For example, Uvalis and Johansson (53) showed that a sustained active tension can be evoked in the portal vein even after the blockade of action potentials. Furthermore, there is

evidence suggesting that the action potential itself, as an electrical phenomenon, does not trigger contraction directly but through a chemical mediator; this mediator is most likely to be calcium ion (54).

C. Contractile mechanism.

Although the exact mechanism of the vascular SMC contraction is not known, it is generally agreed that the mechanism of SMC contraction is similar to the sliding filament model proposed for the striated muscle (55, 56). However, there are subtle differences in the contractile process of the two systems. Some of these differences will now be considered.

The energy requirement for contraction in both striated and smooth muscle appears to derived mainly from magnesium-ATP (54). Like the skeletal muscle, the liberation of energy from this complex molecule in the SMC involved the hydrolysis of ATP by an enzyme, ATPase. Unlike the skeletal muscle, the ATPase activity of the vascular SMC is extremely slow.

In both striated and smooth muscle, an increase in the intra-cellular calcium concentration causes contraction, and the reverse causes relaxation (52). In skeletal muscles, the site for storage, release and uptake of calcium seems to reside in the SR. As pointed out earlier, the SR system in the vascular SMC is not as well-developed

as in the striated muscle. However, the SR system, both the smooth and rough type, as well as the mitochondria of the vascular SMC are capable of accumulating calcium ions.

Both actin (57) and myosin (58) of the vascular SMC are immunochemically distinct from the striated muscle; variation in amino acid sequence of both actin (59) and myosin (60) among the SMC from various sites has also been reported.

Associated with the striated muscle actin are the regulatory proteins, tropomyosin and troponin. No troponin or its equivalent has been been identifed. It appears that the regulatory mechanism in actomyosin interaction during contraction of the vascular SMC is different from that of the striated muscle; it may not require tropomyosin and troponin. Furthermore, it has been suggested that the regulatory system of actomyosin interaction in the SMC resided on one of the myosin light chains (61, 62).

As indicated earlier, myosin of the vascular SMC is longer than that of the skeletal muscle. The lack of the central bare zone may suggest that the packing of myosin molecules into myosin filaments is different. In striated muscle, the myosin molecules are oriented in both directions along the thick filament (63); whereas those of the vascular SMC may be aligned in a unidirectional fashion (64). This may increase the maximum distance of filamentous sliding, i.e. the degree of shorting is greater, during the

contraction of vascular SMC.

The endothelial cell.

Lining the luminal surface of the entire cardiovascular system is a single layer of endothelial cells. These cells are important in normal vascular physiology as well as in vascular pathology such as atherosclerosis.

I. Structural characteristics.

In the arterioles, endothelial cells are flattened and elongated, and contain a small number of organelles and inclusions. Inclusions such as the Weibel-Palade bodies are unique in endothelial cells and can be used to identify these cells in culture (65). Organelles such as pinocytotic vesicles and microfilament which are directly related to the present discussion will be considered in detail. The endothelial cells are connected by simple apposition and by different types of junction (10, 66, 67). In the arterioles, a complex of occluding and communicating (nexus) junctions are often seen similar to those of the larger arteries (67). In addition, tight junctions are also present, both between the endothelial cells and between the endothelial and smooth muscle cells. The latter occurs more frequently in arterioles than in larger arteries (12). Due to their location, morphological characteristics and

relation to the smooth muscle of the arteriolar wall, the endothelial cells may play a role in altering the lumen diameter of the arterioles. Directly related to these control mechanisms are the transendothelial transport and contractile function of the endothelium.

As described in the Vascular Smooth Muscle Cell section that innervation to the blood vessels is usually restricted to the adventitio-medial area. However, recent electron microscopic evidence showed that nerve terminals are present in close proximity to the endothelium and sometimes abut directly onto the endothelial cells of brain capillaries (68).

II. Functional characteristics.

The endothelium can be considered as a blood-tissue barrier which limits the interaction between the circulating substances such as amines and polypeptides, and the underlying tissues. This is well documented in the cerebral vasculature and referred to as the blood-brain barrier. This barrier, even in the cerebral vasculature, is by no means complete; substances are known to pass through this membraneous barrier by special transport systems. Chambers and Zweifach (13) proposed the "two-pore" system of transendothelial transport.

Electron microscopic studies provide evidence for the

existence of the "two-pore" system. Using tracers such as horseradish peroxidase (mol. wt. 40,000; diameter about 5 nm) and ferritin (mol. wt. 500,000; diameter about 11 nm), Huttner et al (69) found that the peroxidase reaction product appeared in the endothelial cell junctions and within pinocytotic vesicles, whereas the ferritin molecules were found mainly within pinocytotic vesicles. These provide evidence for the presence of the hypothetical "pore" systems in transendothelial transport, viz the endothelial cell junctions correspond to the small pores and the pinocytotic vesicles the large pores. The study by Simionescu et al (70) confirmed these results.

Microfilaments of 4-7 nm in diameter are often found at the cell periphery. These filaments often form bundles and attach to dense patches along the abluminal surface of the endothelium; cross-banding along the filamentous bundles is also observed (71). Although the function of these microfilament bundles is not known, it has been suggested that they may be related to contraction (72, 73, 74), or increased permeability (75, 73) of the endothelium. Recent immunochemical study by Becker (76) demonstrated the presence of actomyosin and tropomyosin in the endothelium. The presence of these contractile elements implies that the endothelial cells are capable of contraction.

III. Possible role in vascular control mechanism.

As pointed out earlier the endothelial cell is one of the two active components in the arteriolar wall which is capable of altering the arteriolar lumen diameter. The presence of the contractile apparatus in the endothelium suggested that these cells can contract upon stimulation. The contraction of the endothelium can be conducted through the myoendothelial junctions to activate the adjoining vascular SMC. Direct evidence that the endothelium may play a role in altering vascular tone is demonstrated by Pascual and Bevan (77). Furthermore, the contraction of the endothelium may be related to an increase of permeability of monoamines (78). The penetration of monoamines may have an excitatory effect on the underlying vascular SMC; this may lead to a vasoconstriction which in turn reduces the blood flow to the associated area.

Mechanisms involved in the control of arteriolar lumen diameter.

Vascular SMCs are mostly confined to the tunica media and arranged in a spiral or helical fashion with the dominant orientation being circular (79, 80, 81). In general, the angle between the helical turn and longitudinal axis increases with the decrease of artery size, e.g. 30 degrees in mouse femoral arteries (82) and 72 degrees in arterioles (83). Thus, the angle increases until in small arterioles, the SMCs are arranged in a truly circular fashion.

As described earlier, vascular SMCs are interconnected with each other in the media by a wide range of types of cell junction. Stimuli, such as those originating from the nerve endings which are mainly located at the adventitio-medial junction, can initiate changes in the activities to both "innervated" or "non-innervated" SMCs. The latter response is most likely brought about through the nexuses. This results in a highly synchronised contraction or relaxation of the related SMCs to produce a constriction or dilatation of the artery, respectively.

I. Basal vascular tone.

The basal vascular tone is referred to as a residual tonic activity in the blood vessel after the removal of all

the extrinsic influences such as vasoactive neurohumoral or hormonal substances. This concept was promoted and proven conclusively by Folkow (84, 85). Further experiments from the same laboratory demonstrated that the basal vascular tone is myogenic and is confined mostly to the precapillary resistance vessels (86). The mechanism whereby the basal vascular tone is produced is not known. It has been suggested by Johansson and Somlyo (51) that the basal vascular tone is probably a consequence of the rhythmic, spontaneous variations of membrane potential in the vascular SMC. When these variations are pronounced enough they may result in a complete membrane depolarisation and contraction.

II. Vascular control mechanism.

During normal daily activities of an individual, the cardiovascular system is challenged with the problem of satisfying the highly variable circulatory demands of the different tissues without exceeding the limits of the cardiac function. By careful regulation of the vascular tone and cardiac activity, the economy of the cardiovascular system is maintained and its purpose properly fulfilled. Thus organised adjustments of the vascular tone to various regions can maximise the existing cardiac output and assure regional blood supplies in relation to the functional priorities and metabolic states

of different organs.

Many factors can alter the vascular tone resulting in either vaso-constriction or -dilatation. Extrinsic factors which originated from sites outside the vascular tissue, whether they are vasoactive, neurohumoral or hormonal substances, are classified as the remote control mechanism, whereas intrinsic factors are those originating from the vascular tissue itself; the latter is referred to as local control mechanism.

A. Neurohumoral component of remote control mechanism.

Although many substances such as substance P, GABA, 5-HT, histamine, somatostatin, endorphines and bradykinins have been suggested to be neurotransmittor and/or neuronal modulator substances, the importance of these putative substances involves in monitoring vascular control of blood flow has not been implicated. Thus, only the sympathetic adrenergic fibre system, the autonomic cholinergic vasodilator fibre system and the autonomic purinergic vasodilator fibre system will be considered in this section.

1. Sympathetic adrenergic fibre system.

The mammalian vascular system is innervated predominantly by postganglionic sympathetic adrenergic fibres (87). The terminals of these fibres usually form plexuses in the adventitial and/or adventitio-medial region of the vessel wall. Species diversities and regional

variations of these fibres are well known (88-a review). Upon stimulation, noradrenaline (NA), together with ATP, dopamine beta-hydroxylase and chromogranin A, are released from the axon terminal. The mechanism of how the NA is released is not known. However, the presence of calcium ions is a prerequisite. The modulation of the release of NA is also present through many types of presynaptic receptors. For example, NA will, after release, act on the presynaptic alpha-adrenergic (alpha 2-adrenergic) receptor to reduce, or even inhibit, transmitter release (89).

The released NA then diffuses across the synaptic cleft and interacts with the receptor sites on the vascular SMC membrane. There are at least 2 types of adrenergic receptor on the SMC membrane, viz alpha 1- and beta 2-adrenergic receptors. The activation of these receptors produces different effects depending on which type is dominating the receptor population of the vascular SMC membrane.

The activation of alpha 1-adrenergic receptors on the SMC membrane produces a depolarisation or increases the frequency of action potential, when present, resulting in a contraction of the vascular SMC. The mechanism whereby the electromechanical coupling is linked is not clear. However, NA is capable of increasing membrane permeability to sodium, potassium, and chloride ion simultaneously in rabbit blood vessels (90). Excitation of vascular SMC by NA

is also known to associate with an increase of calcium ion permeability; this introduces the concept of activator calcium ion as the link for electromechanical coupling (90).

The increased activity, either electrical or mechanical, of the vascular SMC via alpha 1-adrenergic receptor is coupled with other SMCs presumably through nexuses to produce an synchronised contraction around the circumference of the arteriolar wall resulting in a vasoconstriction which in turn reduces the blood flow to the associated vascular bed.

The activation of beta 2-adrenergic receptor on the SMC membrane results in a hyperpolarisation or inhibits the spike discharge, where such is present, of the vascular SMC. The end effect of this hyperpolarisation is a relaxation of the SMC. Again, the mechanism of how the hyperpolarisation has come about is not known. An increase of membrane permeability of specific monovalent ions (chiefly chloride and potassium) and/or divalent ions such as calcium has been proposed (90). The synchronised relaxation of arteriolar SMC results in vasodilatation of the arteriole which will lead to subsequent increase of blood flow to the related vascular bed.

At high enough concentration, the NA is known to act on both alph- and beta-adrenergic receptor. However, the ratio of alpha- and beta-adrenoceptor present on the SMC

membrane seems to dictate the activity of the SMC and vary from region to region. For example, small coronary vessels show a marked beta-adrenergic receptor mediated response (91, 92). Other vessels contain both alpha- and beta-adrenoceptor in more or less similar concentration (93). In most cases, alpha-adrenergic receptor mediated response is the greatest (94, 95, 96).

The termination of NA activities can be achieved by enzymatic actions of both MAO and COMT; uptake, both uptake 1 and uptake 2, mechanism; as well as binding to connective tissues viz the collagen and elastin. The interaction between NA and receptors can also be considered as a means of inactivating the released transmitter.

2. Autonomic cholinergic vasodilator fibre system.

Cholinergic fibres are those fibres which utilise acetylcholine (Ach) as the neurotransmitter and can be identified in blood vessels of the skeletal muscle (97), brain (98), and probably heart (99, 100), lung (101) and kidney (102). Those fibres which innervate the blood vessels of the skeletal muscle are of the sympathetic origin, whereas the rest originates from the parasympathetic nervous system (103). The activation of cholinergic fibres from both origins produces vasodilatation of the innervated blood vessels. This can be blocked by atropine, a cholinergic muscarinic receptor antagonist (97).

Upon stimulation of the cholinergic fibres, Ach is released from the nerve endings. The release of transmitter is likely via an exocytotic process. The released Ach is then diffused through the synaptic cleft and activates the effector SMC. The interaction of the Ach and the cholinergic muscarinic receptor causes a hyperpolarisation in the effector cell. The mechanism whereby this hyperpolarisation is produced is not known. In arteries which have been exposed to depolarisation (high potassium ion) solution, Ach can still cause vasodilation (104). Thus it seems that Ach does not alter membrane potentials. A more direct effect of Ach on calcium ion permeability, which prevent the initiation and propagation of SMC activity, has been proposed (104). The hyperpolarisation of the effector cell membrane by the released Ach results in relaxation of the vascular SMC, which in turn produces a vasodilatory response.

The inactivation of Ach is believed to be the result of the enzymatic action of acetylcholinesterase which has been localised at the neuromuscular junction of the vascular SMC (105).

3. Autonomic purinergic vasodilator fibre system.

In addition to the classical cholinergic and adrenergic fibres in the autonomic nervous system, there exists a third component which appears to be

*non-cholinergic and non-adrenergic* (106, 107). Upon stimulation of these fibres, a purine nucleotide, probably ATP, is released, thus these nerves are classified as purinergic fibres (108, 109). Although the exact distribution of the purinergic fibres in the body is not known, evidence indicated that these fibres are present in the coronary (110), skeletal (111) and pulmonary (110) circulation. The origin of these fibres is also not clear.

Purine nucleotides are potent vasodilators (112) and have been proposed to be physiological regulators of blood flow (108, 109). When the purinergic fibre is activated, a purine nucleotide, probably ATP, is released. The mechanism of how ATP is released is not clear, although release through exocytosis has been proposed (108).

The released ATP is then diffused across the synaptic cleft and activates the postsynaptic SMC through P-1 and P-2 purinergic receptors (113). The activation of purinergic receptors results in a hyperpolarisation (114) and subsequent relaxation of the SMC. The overall relaxation of the SMCs in the vessel wall causes a vasodilatory response.

The mechanism of how the hyperpolarisation of the SMC is produced is not known. However, adenosine is known to have a direct effect on the calcium ion permeability of the vascular SMC (115). It has also been suggested that adenosine may act on adenylate cyclase receptors which

leads to a production of cyclic-AMP. The cyclic-AMP may then modulate the availability of intracellular calcium ion, thereby resulting a change in contractile activity (116).

Although the mechanism whereby the released purine nucleotides are inactivated is not clear, adenosine uptake by the presynaptic neurone and the enzymatic deamination of adenosine to inosine have been proposed (108).

B. Hormonal component of remote control mechanism.

Vasoactive substances can reach the site of action through the circulation. In order for these blood-borne vasoactive agents to react with the vascular SMC to produce an effect, they must pass through the blood-tissue barrier of the endothelium; the "permeability" of the endothelium and the interaction between the endothelium and the vascular SMC has been considered in the Endothelial Cell section.

Although other vasoactive substances such as 5-HT may exert an important vasoconstrictor effect on the vessel at the site of vascular injury, there is no indication that this substance is involved in controlling vascular resistance under normal conditions. Thus only the influence of the adrenomedullary system, the renin-angiotensin system and the neurohypophyseal system on the vascular system will be discussed.

1. The adrenomedullary system.

During resting condition, both adrenaline (A) and noradrenaline (NA) are released from the adrenal medulla directly into the blood stream, thus they are, by definition, considered as hormones. Ach apparently modulates this release as in the ganglion. The amount of A released is usually much higher than that of the NA. During the fight and flight reaction, the amount of catecholamines released can increase to 20-30 times the resting value (117). Although this amount is relatively small in comparison with those released from the nerve endings during vasocontrictor nerve stimulation, the enhancement of vasoconstriction due to adrenomedullary hormones cannot be overlooked.

Indeed, an increase of A or NA in the blood can produce vasoconstriction in many vascular beds (118, 119). However, infusion of A to the vascular bed of skeletal muscle usually results in a vasodilatory response (120-a review). This result suggested that beta-adrenoceptors are localised predominantly in the vascular beds of the skeletal muscle.

The mechanisms by which catecholamines exert their effect on the vasculature has already been discussed (see sympathetic adrenergic fibre system).

2. The renin-angiotensin system.

The formation of angiotensin is initiated by an elevation of sodium ion concentration and/or osmolarity of the fluid in the distal tubules of the kidney. These changes are recognised by the specialised epithelial cells of the macula densa which in turn initiate the release of renin from the juxtaglomerular cells. The renin converts an alpha 2-globulin, angiotensinogen, present in the blood plasma into angiotensin I. The conversion of angiotensin II usually takes place in the pulmonary tissue by a dipeptidase.

Angiotensin, in particular angiotensin II, is an extremely potent direct contractile stimulant to vascular SMC (120). In physiological concentration, angiotensin II also induces constrictor responses in intact arterioles (121). However these responses are relative small when compared with the alpha-adrenoceptor mediated vasoconstriction (122).

The mechanism whereby vasoconstriction is produced by the angiotensin II is not known. The stimulatory effect of electrical activity of the SMC by the angiotensin II in the vessels has been proposed (123) although these changes cannot relate to alteration of the ion fluxes across the SMC membrane (124, 125).

Perhaps the most important vasoactive effect of angiotensins is their indirect vascular actions. Angiotensins are capable of releasing endogenous stores of

NA from the sympathetic postganglionic nerve endings (126), enhancing the response of some vascular SMC to sympathetic nerve stimulation (127), and interfering with the inactivation of postganglionic nerve ending-released NA by inhibition of the neuronal uptake process for this amine (127).

Up to now, there is no definitive function assigned to the renin-angiotensin system with respect to the general vasomotor control system. However, its production is dependent on the release of renin from the kidney indicating that its primary function may reside in the kidney.

3. The neurohypophyseal hormonal system.

The vasopressin, or more appropriate antidiuretic hormone (ADH), is one of the hormones secreted in the neurohypophysis. The most important role of ADH in the body is the regulation of water reabsorption in the renal tubules. Although some arterioles can constrict in the presence of ADH, in particular 8-arginine ADH (122), it is generally believed that most arterioles are relatively insensitive to this hormone (128).

C. Local control mechanism.

Mention has been made earlier that the presence of basal vascular tone is very likely due to a local myogenic response. In many in vivo studies, autoregulation of blood flow has been observed; autoregulation implies that despite the alteration in arterial pressures, the flow remains more or less the same. Indeed it is true in the vascular bed of the brain, myocardium and kidney. Of the many mechanisms proposed for autoregulation, the local myogenic response and the alteration local ion concentration related to metabolism offer a more comprehensible explanation for this phenomenon. The following discussion encompasses some evidence which supports these theories.

1. Local myogenic response.

As mentioned earlier, Folkow and coworkers demonstrated fairly conclusively the presence of the basal vascular tone and this tone is likely to be the result of spontaneous activity present in most arterioles. Furthermore, in vitro and in situ experiments by Sparks (129), Johansson and Bohr (130), and Johansson and Mellander (131) indicated that the application of either short or sustained force to vascular SMC may produce an active response. This response appears to be myogenic because pretreatment with catecholamine-depleting agents and alpha-adrenergic inhibitor (130) or prefusion with an oxygen-rich mixture (132) does not abolish the response.

There is also an increase in both mechanical and electrical activity during the response (131).

The mechanism by which the myogenic response is produced is not understood. However, the alteration of sodium ion permeability, either through direct stretch (133) or through indirect mechanoreceptors of the vascular SMC (134), is a possibility. An increase of sodium ion permeability may initiate the activity of activator calcium ion (88).

2. Alteration of local ion concentration related to metabolism.

An increase in potassium ion concentration and in osmolarity, and a decrease of oxygen tension are produced during exercise in skeletal muscle. These alterations can cause a vasodilation in the skeletal muscle vasculature. Each of these factors will be considered individually.

During exercise, there is an increase of osmolarity in the skeletal muscle; this increase is at least partly due to the liberation of inorganic phosphate. The elevation of osmolarity can lower the local vascular resistance, thus the blood flow to the region is increased. This response is apparent in skeletal muscle, coronary, pulmonary and renal circulation.

The increase of osmolarity can influence directly on the SMC or indirectly through interruption of

neurotransmission to produce the vasodilatory response. For example, an increase of osmolarity can decrease the NA release from the nerve terminals (135), and reduce the myosin-ATPase activity in the vascular SMC (136); each of these can lead to relaxation of the SMC.

The requirement of oxygen for the generation of ATP through oxidative phosphorylation pathway is well known. It is logical to assume that the maintenance of tension in the SMC via the contractile mechanism requires oxygen. Thus if there is a deficiency of oxygen, the SMC will relax because the production of ATP is interrupted. Indeed, vasodilator response due to oxygen deficiency can be demonstrated in many vascular beds (137, 138).

Although the direct effect of oxygen on the contractile process can explain the vasodilator response, the release of a vasoactive substance within the vascular wall in response to the change of oxygen tension cannot be overlooked.

Potassium is also one of the many substances released by the skeletal muscle during exercise. In vitro, intra-arterial infusion of high potassium ion solution can lead to vasodilation in the coronary and skeletal muscle vasculature.

The mechanism by which the increase of potassium ion produces a vasodilator response is similar to those

proposed in the increase of osmolarity.

D. Interaction of control mechanisms.

In this section, many of the important vascular control mechanisms have been considered. These are the remote and local control mechanisms. An activation of these control systems produces a vasodilator or vasoconstrictor response through a direct or an indirect influence on the contractile process of the vascular SMC. Depending on the local conditions, vascular SMC may be exposed to influence from several control systems. Thus synergistic or antagonistic interactions are expected, which can lead to modified effector responses different from those mentioned in this section.

Summary and conclusion of vascular control mechansims.

The present discussion highlights some of the control mechanisms by which the lumen diameter of arterioles can be altered. The complexity of these control systems is also evident.

The neurohumoral remote control mechanisms offer the most powerful control dominating over many vascular beds. The hormonal remote control mechanisms do not seem to exert an important alteration to the vascular tone under the resting condition. However, their enhancement of the effect resulting from the neurohumoral remote control mechanisms

during the fight and flight reaction cannot be overlooked.

The local control mechanisms serve as the fine tuning mechanism for the massive reaction result from the activation of the remote control mechanisms. These mechanisms take into consideration the local metabolic requirements of the affected vascular bed.

The importance of the endothelial function has also been reviewed. The contractile ability and transendothelial transport mechanisms are capable of altering the lumen diameter of arterioles.

From the results reviewed in the present discussion, it is obvious that the mechanism of action in most of the vascular control mechanisms mentioned is not known. Thus it is premature to try and compile an overall scheme for a complete vascular control mechanism.

## Characteristics of the spontaneously hypertensive rat.

Research in chronic diseases, such as hypertension, with human subjects is time-consuming and often impossible to control. Animal models, on the other hand, provide time-compressed and well controlled experimental conditions. Thus, in the past few decades, much of the effort in hypertension research has been directed to finding an animal model which resembles human essential

hypertension. As a result, many experimental models were produced in genetically normotensive animals by intervening in the normal arterial blood pressure control mechanisms using chemical, mechanical and other means. These models are generally referred to as the unitary models of hypertension. Although these unitary models have led to a better understanding of many types of secondary hypertension, they have proven not to be good models of human essential hypertension.

The discovery of genetically linked hypertensive animal models marked the advance in the present knowledge of essential hypertension. In the early 1950s, Alexander et al (4, 5) attempted to isolate a colony of spontaneously hypertensive rabbits. Unfortunately, these rabbits did not maintain a sufficiently high and steady blood pressure to be recognised as a model for studying essential hypertension. Subsequently, during the period from the late 1950s to the mid 1970s, 6 different strains of spontaneously hypertensive rats were developed by various groups which are considered to be valuable models of essential hypertension. These are:

1. Wistar-Otago strain of genetically hypertensive rats (GHS) developed by Smirk's group (139) in New Zealand;

2. Sprague Dawley-Brookhaven strain of hypertension-sensitive rats (HSR) developed by Dahl's group (140, 141) in the United States of America;

3. Wistar-Kyoto strain of spontaneously hypertensive rats (SHR) isolated by Okamoto's group (7) in Japan;

4. Wistar-Milan strain of spontaneously hypertensive rats (MHS) developed by Bianchi's group (142, 143) in Italy;

5. Wistar-Lyon strain of spontaneously hypertensive rats (LH) developed by Dupont's group (144) in France;

6. Sprague Dawley-Sabra strain of hypertensive sensitive rats (SBH) developed by Ben-Ishay's group (145) in Israel.

Among the six strains of spontaneously hypertensive rats, the SHRs represent the most severe form of hypertension; many SHRs show a systolic blood pressure exceeding 200 mmHg at about 25 weeks of age (7). Furthermore, the SHR has been exposed to the most extensive investigation and is widely accepted as the best animal model so far known for studying human essential hypertension (146, 147, 148, 149, 150, 151, 152, 153). Although the main objective of the present investigation is not concerned with the determination of the cause of hypertension in the SHR, because of the severity and the spontaneous development of hypertension, the SHR has been chosen as the experimental model in this study. Characteristics of the SHR will now be considered in detail.

I. Development of arterial blood pressure in SHR.

In 1963, Okamoto and Aoki reported the isolation of a strain of rats which develop hypertension spontaneously; this strain of rats is now known as the spontaneously hypertensive rat of Okamoto and Aoki strain or in short, the SHR.

Among the Wistar stock maintained in Kyoto University, Okamoto and Aoki noted that a male rat developed hypertension spontaneously (a systolic blood pressure of 150 mmHg or higher persisting over a month, by their definition). By mating this rat with a female which showed a systolic blood pressure slightly higher than average range (130-140 mmHg) and subsequent brother-sister matings of their offsprings, a colony of rats which invariably developed hypertension was isolated.

The development of hypertension in the SHR is gradual but at early age. The systolic blood pressure of the SHR is usually significantly higher than the control rat at the age of 5-6 weeks (154, 155), but it has been reported as early as 4 weeks (156) or as late as 12 weeks (157) of age. The rate of rise in systolic blood pressure continues over the next few weeks and reaches its plateau of 200-210 mmHg at about 12-16 weeks.

II. Inheritance of SHR.

The inheritance of hypertension in the SHR is well documented and its mode of transmission appears to be polygenic in nature (158, 159, 151). Tanase et al (160) reported that such an inheritance of hypertension in the SHR involved relatively few major genetic components which seems to act additively, and were transmitted equally well in both males and females. However, the level of hypertension in the males was somewhat higher than the females throughout their life span (151). Although the development of hypertension in the SHR may be due to the interaction between genetic and environmental factors (161), it appeared that genetic factors play a dominant role but that the course of hypertension can be modified by environmental factors (151, 154, 162). This is evident 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 developed a somewhat lower maxium arterial blood pressure than the original strain (161).

III. Normotensive control for SHR.

Since the development of hypertension in the SHR is spontaneous, it is necessary to find a strain of normotensive rats to serve as the controls. Most of the

studies reported in the literature using the SHR as the experimental model utilised either the Wistar-America (WAR) or the Wistar-Kyoto (WKY) rats as the controls. 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 WKY do not have the same physical, physiological and biochemical characteristics (163, 164, 165, 166, 167). For example, Clineschmidt et al (163) reported that both sensitivity and contractility of aortic strips isolated from the SHR were similar to those of the WKY, when tested with noradrenaline (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 (164). The results of Pang and Scott (168) indicated that the body weight of WAR is much heavier than those of the WKY and SHR. This suggests that the WKY is a better control for the SHR in morphometric analysis of the vascular system, especially when the genesis of hypertension in the SHR is not the main concern in a particular study. Other strains such as the Sprague Dawley rat have also been used by a number of investigators (e.g. 169).

## IV. Pathogenesis of hypertension of 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, hormonal 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 types of hypertension.

## V. Nervous system of SHR.

Research into 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 (151, 170, 171) it appears that there are differences between the CNS of the SHR and the normotensive control concerning their efferent nervous system and the metabolism. For example, nuclear and cellular size of

neurones in the supraoptic, paraventricular, arcuate and vagal dorsal motor nucleus were larger in the SHR as compared with the controls. Recently, Lehr et al (172) showed that the SHR have a larger metencephalon, and smaller mesencephalon and diencephalon than the WKY controls.

Young SHR are known to react more to both chronic (173) and acute (162) stress than their normotensive controls; such an activity appears to subside with age (161). 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 (171, 153) and such an activity seemed to decrease in mature animals (174, 175, 176). Similar results were reported in studies on plasma NA levels (177). Chemical sympathectomy by 6-hydroxydopamine at birth prevents hypertension in the SHR, although the blood pressure in these animals still remains higher than the treated control (178). 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 (179) 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 (180); it is generally accepted that the carotid sinus baroreceptors are reset to maintain a higher blood pressure in chronic renal hypertension (181) and in hypertension of the SHR (150). 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 (150). The mechanism of how the baroreceptors are reset is not known. From the work of Rees et al (182) 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 (183).

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.

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 have full understanding of the function of the vascular system.

V. Renal function of SHR.

Less effort has been concentrated on the renal function of the SHR. With the information on hand, there appears to be no indication of renal disturbance in young SHR. These include normal blood and plasma volume (184), as well as the sodium content and balance (185). The results on the plasma renin activity (PRA), however, are conflicting. Koletsky et al (186) observed a normal or even subnormal PRA in the SHR, while Sen et al (184) 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 the 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

(187).

Enhanced vascular reactivity was demonstrated in isolated perfused kidneys from the SHR (188, 189). However, the information of structural changes in the kidney of adult SHR is fragmented. According to Okamoto (151) and Freis (190), 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, 191). Limas et al (192) showed that structural changes such as medial thickening in the intra-renal vasculature appeared at 10 weeks of age. However, judging from the micrographs published, it is obvious that the intra-renal vasculature was not fixed by perfusion. Thus, the results they presented were doubtful. 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 (193) on kidneys of patients with essential hypertension, it suggested that a reorganisation of the intra-renal vasculature occurs 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 (194) 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 was proposed (194).

VI. Hormonal function of SHR.

Studies by Okamoto and co-workers (151, 170, 171) indicated that there may be differences between the central nervous system of the SHR and normotensive control concerning their hormonal control of organ systems. For example, it was shown 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 (151, 170). No detailed study was reported to determine whether these changes are involved in initiating and/or maintaining the hypertension in the SHR. Because these results were obtained from adult SHR, one can only speculate that these are mechanisms which are concerned with the maintenance of hypertension in the SHR.

Recently, a report by Greenberg et at (195) demonstrated that parabiosis of WKY with SHR results in

hypertrophy and decreased extensibility of the portal veins obtained from the WKY. This suggested that venous hypertrophy in the SHR may have resulted from the action of circulating factors but not the elevated arterial blood pressure on the venous smooth muscle since the blood pressure in the portal vein of the SHR and WKY used in their study was similar. A study by McMurtry et al (196) further indicated that circulating factors may induce the elevation of arterial blood pressure in the SHR. They observed that genetically normotensive Sprague-Dawley rats nursed by SHR foster mothers developed sustained high blood pressure. The transmission of these factors is likely through the milk. Morphometric analysis of the vasculature of the "hypertensive" cross-suckled rats has not been performed, thus it is not known whether medial hypertrophy of the vasculature is present in these hypertensive Sprague-Dawley rats.

## VIII. 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 the elevation of blood pressure in the SHR. As has been described earlier, the main objective of this thesis is to use the vascular system of the SHR to examine the hypothesis that structural changes in the artery in hypertension are a result of the elevated blood pressure. The cardiovascular system of the SHR will now be considered in detail.

### A. Heart.

Cardiac hypertrophy is the most common finding associated with many forms of hypertension (197, 198, 199, 200, 201, 202), including that of the SHR (147, 148, 149, 203, 204). 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 (147, 149). From the report of Kawamura et al (148) and Imamura (147), 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 (205). This is also evident in cardiac hypertrophy of the SHR (147, 148). The exact location of sarcomerogenesis in a myocardiocyte is not known. However, it has been postulated thal the "new" sarcomeres are laid down at the intercalated disc (206) or at the "Z" bands (207) or both (147).

-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 in other parts of the heart (147, 148). Furthermore, significant changes, indicative of cardiac hypertrophy, in the SHR appeared at the age of 11 weeks as compared to the controls. The authors concluded that these changes were the results 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 (203) 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 (147) and Kawamura et al (148) used.

Cardiac hypertrophy in rat (197, 200, 208), cat (209) and man (210) 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 (208). However, the development of cardiac hypertrophy persists despite the lowering of blood pressure in the SHR by anti-hypertensive therapy (211) and peripheral sympathectomy (146, 212). Thus, it follows that cardiac hypertrophy in the SHR may result from more than one mechanism rather than just 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 (166, 167), 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 importance in defining the process involved in the initiation of hypertension in the SHR.

B. Blood vessels.

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 resistance (166, 167, 213, 214). There seems to

be a uniform rise in regional resistance in young and mature SHR (215), as judged by evaluation of the distribution of cardiac output. The increased vascular resistance can be the result of various attributive factors which include increased sympathetic activity, vessel wall hypertrophy and altered responsiveness to catecholamines by vascular smooth muscle (216, 217, 218, 219, 220). The latter two will now be considered in detail; the increased sympathetic activity has been discussed above.

1. Vessel wall hypertrophy.

It is well known that the vessel wall becomes stiffened and thickened in hypertensive animals (221, 222, 223, 224, 225, 226, 227, 228, 229) and patients (230, 231, 232, 233). These changes in the blood vessels may account for the higher peripheral resistance observed in hypertensive patients (234, 234) and in the SHR (166, 167, 213, 214, 215, 216, 217). These stiffening and thickening processes appear to be the result of the thickening of the intima, an increase in vascular connective tissue, hypertrophy and/or hyperplasia of the vascular smooth muscle or a combination of all three.

a. Tunica intima.

Thickening of the intima of large arteries is one of the typical changes of hypertensive disease, in general (236). 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 cells.

Very few studies concerning the morphology of the intima of the SHR have been published. Still (237) reported that the intimal thickening in the thoracic aorta of the SHR did not seen 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 (238, 239, 240). Furthermore, Gabbiani et al (238) reported the presence of microfilament bundles in association with the early stages of hypertension. However, these microfilament bundles are also present in normal rats when the intima was sectioned tangentially (241, 242). 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 (243), it has been suggested that they may be related to contraction (244, 245, 246), increased permeability (245, 247), and support or structure (242, 243) of the endothelium.

Hypertrophy and hyperplasia of the endothelial cells are said to be associated with many types of experimental hypertension. Gabbiani et al (238) 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 vary 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 perfusion pressure during fixation for microscopy. Unfortunately, they did not report the perfusion 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 accompany hypertension. Schwartz and Benditt (248) found that there was an 10-fold increase in the rate of replication of endothelium in hypertension produced by narrowing 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 (238, 240, 249), including that of the SHR (250). 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 (238, 240, 250) and very rarely elastin (240). 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.

As pointed out earlier, results obtained by Gabbiani et al (238) and Huttner et al (239) provided evidence for the presence of hypothetical "pore" system in transendothelial transport. In hypertension, the mode of transendothelial transport does not seem to alter although the reaction products are much higher, in particular, the amount of ferritin in the surface vesicles, indicative of higher activity (239).

Rippe and Folkow (251) 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 directions as compared with the normotensive controls.

b. Connective tissue in the vessel wall.

Both elastin and collagen are said to be increased in the vasculature of renal hypertensive rats (227, 228, 229). Recently, much of the work in this area has been focussed on collagen metabolism. Based on 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 (252, 253, 254, 255) including that of the SHR (256, 257, 258). In DOCA-salt hypertensive rats, these changes can be reversed by treating the hypertensive animals with anti-hypertensive drugs such as chlorothiazide or reserpine (255). 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 (259). 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 (254, 259). In the SHR, the increase of collagen synthesis does not appear until 23 weeks of age following the elevation of blood pressure to a significant level as compared with the normotensive control (257). This is substantiated by the results reported by Pang and Scott (168) which showed by stereological analysis that there is no increase of collagen deposition in the media of the abdominal aorta and renal artery of the SHR, at least up to the age of 18 weeks. 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 (259). This further supports the notion that collagen synthesis in the vasculature of hypertensive animals is pressure dependent.

Although there is enough evidence to believe that high blood pressure can stimulate the collagen biosynthesis and deposition in the vessel wall resulting in a stiffening of the 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.

c. Vascular smooth muscle (SMC).

Many studies have been reported on the reaction of the SMC to experimental hypertension. These reports seemed to suggest that there is an increase in SMC content in the vessel wall of many types of hypertensive animals. Using biochemical methods, Wolinsky (227, 228, 229) 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 (232) and hypertensive rats (260) has been claimed to be the result of SMC hypertrophy. This is supported by Wiener et al (226) 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 that SMC mitosis is prominent in experimental hypertension (223, 224, 261, 262), especially in the early stages (225).

Limas et al (192) reported that medial thickening was present in the thoracic aorta at about 10 weeks of age; whereas, Pang and Scott (168) demonstrated that there was no significant difference in the medial thickness of the abdominal aorta and renal artery between SHR and WKY, at least up to the age of 18 weeks. The discrepancy between these two reports is likely due to the difference in the

segment of artery used in the analysis. Studies on small arteries exhibit conflicting results. Ichijima (263) reported that the luminal diameter of small arteries in the SHR was generally smaller than that of the controls. Mulvany et al (264) and Warshaw et al (265) found that there was an increase in wall thickness in the mesenteric arteries of the SHR. On the contrary, Bohlen (266) and Bohlen and Lobach (267) 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.

Although medial hypertrophy in large and small arteries of the SHR at the early stage of hypertension is still in dispute, this alteration in the tunica media of large and small arteries of the SHR in the established stage of hypertension is well documented. Results reported by Owens et al (268) recently, suggested that smooth muscle hypertrophy alone accounts for the increased smooth muscle cell mass in aortae of the SHR.

From the information presented thus far, it seems to suggest that medial hypertrophy occurs in some segments of the vascular system in the SHR. When it has occurred, medial hypertrophy seems to develop after the elevation of arterial blood pressure. Thus, it is possible even in the SHR that medial hypertrophy of arteries is a result of the increased arterial blood pressure. However, from the

information obtained so far, it is impossible to rule out the possibility that such change in the arterial wall is not related to the genetic of the arterial wall structure, i.e. medial hypertrophy in the arteries of the SHR is genetically predetermined.

2. Reactivity of blood vessels.

The reactivity of a blood vessel to a given vasoactive agent is defined as the response, either due to sensitivity or contractibility, 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 (269) and experimental animals (270-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 beds by perfusion with a physiological solution, or on strips of various types of artery and vein by incubating the vessels in a tissue bath 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 (271, 272, 273). In the SHR, this responsiveness appears to be present in young (266, 274,

275) but not in mature (276) animals. However, results on the responsiveness of the vascular system to 5-hydroxytryptamine (5-HT) indicate that there is regional difference. Ahlund et al (277), and Haeusler and Finch (278) 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 (277) 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 (279, 280), similar (163, 281) or more (282) contractile force than the normotensive controls when challanged by noradrenaline (NA). In the SHR, aortic strips exposed to NA appear to produce less (283, 284) or similar (163, 281)

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 NA was increased, whereas the contractility was decreased (285, 286, 287). Furthermore, the SMC from the SHR appears to react differently to manganese and lanthanum (288), prostaglandins (289) and potassium, indicative of sodium-potassium ATPase activity (290) 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 of 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 (291, 292, 293) and also the cardiac muscle (294) from the SHR. Direct evidence supporting this hypothesis has been obtained by Kwan et al (295) 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.

Summary and conclusions of the characteristics of the SHR.

The SHR is considered to be the best animal model thus far known for studying human essential hypertension. Due to the severity and spontaneous development of hypertension in these rats, the SHR may also be a good model for studying the relationship between the development of arterial blood pressure and arterial change, such as medial hypertrophy, in hypertension.

The cause of hypertension in the SHR is unknown. The areas which may be involved in the initiation and/or maintenance of hypertension in the SHR are the nervous system, renal function, hormonal function and cardiovascular system. A large body of information was presented and suggested the important role played by the vascular system in the genesis and maintenance of hypertension in the SHR. Although there is a close relation between the development of the arterial blood pressure and arterial hypertrophy in the SHR, direct evidence has not been presented to demonstrate the involvement of arterial pressure and genetic factors of the arterial wall on the development of medial hypertrophy in hypertensive arteries.

Objective and rationale of the present investigation.

As pointed out earlier, medial hypertrophy has been demonstrated in the vascular system of hypertensive humans and experimental animals, including that of the SHR. This alteration in the arterial wall is closely related to the development of hypertension. Thus, it has been suggested medial hypertrophy in hypertensive arteries is the result of hypertension. In experimental models other than the SHR, this possibility is very likely. However, in the case of the SHR, this possibility is further complicated by the fact that hypertension is hereditary. Then, it is possible that the changes of the arterial wall in the SHR is not related to hypertension, but rather a genetically predetermined structural variation. Thus, the following series of experiments was designed to determine whether the medial hypertrophy in the arterial system of the SHR is pressure dependent or genetically predisposed. The rationale of these experiments namely confirmation study, transplant study, renal hypertension study and capsaicin study will now be considered individually.

I. Confirmation study.

Medial hypertrophy has been demonstrated in various vascular beds of adult SHR (192, 263, 296). This alteration in the vascular wall is thought to be one of the factors responsible for the elevated peripheral resistance in these

animals (297, 298).

Ichijima (263) reported that the branching pattern of arteries from the mesenteric arcade in the rat is quite consistent; he, and subsequently Mulvany and coworkers (264, 265) demonstrated that medial hypertrophy of mesenteric resistance vessels is present in both young and adult SHR as compared with the normotensive control. However, the youngest group (6 weeks) these authors used had already shown signs of increased blood pressure (263, 265). In order to determine whether or not the alteration of vascular wall structure is pressure related, it is necessary to study animals younger than 6 weeks of age. There is no detailed study of these vessels in the SHR during the early postnatal development, especially when the vessels were fixed in situ. Furthermore, it is well known that substrains of SHR do exist in different research centres (161). Valid comparison of results on studies of the SHR from various centres can only be made when full studies have been reported.

The present study is designed to correlate the development of jejunal arteries with the elevation of arterial blood pressure in the SHR.

II. Transplant study.

In order to determine whether medial hypertrophy in

hypertensive vessels is genetically "predetermined" or is the result of increased arterial pressure, it was proposed that if a segment of genetically hypertensive artery is isolated from a SHR and transplanted into a WKY, then by examining changes in the transplanted artery of the SHR which is subjected to a normotensive environment, the cause of medial hypertrophy in hypertensive vessels may be determined.

III. Renal hypertension study.

Hypertension can be induced by partially constricting one of the renal arteries in genetically normotensive animals (299). This has been referred to as Goldblatt renal hypertension and used as one of the models for studying human hypertension.

The alterations in arteries of Goldblatt renal hypertensive rats are well documented. It is generally agreed that there is a thickening of the arterial wall in both large and small arteries of hypertensive animals. Wolinsky (227) reported that there was an increase in non-collagenous protein after the induction of hypertension; this was interpreted as an increase of smooth muscle component of the arterial wall. The latter was confirmed by morphometric analysis (226). Similar results were reported in small mesenteric arteries of renal hypertensive rats (300, 301).

The objective of the present study is to confirm that medial hypertrophy which occurs in hypertensive arteries is present in jejunal arteries of renal hypertensive WKYs.

IV. Capsaicin study.

The main effect of capsaicin (8-methyl-N-vanillyl-6-nonenamide), the pungent and irritating ingredient of hot peppers of the genus Capsicum which includes Mexican chile pepper and Hungarian red pepper (paprika), whether by intravenous or subcutaneous administration, is the depletion of substance P from primary sensory neurones (302). In adults, administration of capsaicin produces functional impairment of the primary sensory neurones without causing their degeneration (303) and lowers blood pressure of the animal (304); whereas administration of capsaicin in neonates induces selective degeneration of a distinct population of primary sensory neurones which are involved in mediation of chemogenic pain (303) and prevents the development of hypertension in the SHR (305).

Because of its primarily central effect, its antihypertensive property, and its simplicity in administration, capsaicin is used as a tool to study the effect of lowering of pressure on jejunal arteries of the SHR.

## MATERIALS AND METHODS

The four different studies in the present investigation utilised the jejunal artery as a model to correlate morphometric data of this vessel with the development of hypertension. Except for the transplant study, all the experiments required preparation of jejunal arteries for microscopic examination and measurement. These procedures are described in detail in the confirmation study. Measurement of arterial blood pressure was made through the femoral artery in each experiment; these procedures are considered in the same study. EM examination and stereological analysis of the jejunal arteries were employed only in the confirmation study.

### I. Confirmation study.

The development of the medial thickness and luminal diameter, as well as the medial composition of jejunal arteries were correlated with the elevation of arterial blood pressure during the first 20 weeks of postnatal maturation in both the SHR and WKY.

#### A. Experimental animals.

Male, age-matched Okamoto-Aoki (7) strain of spontaneously-hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats, bred from the stocks maintained at the Memorial University animal care facility, were

sacrificed at the age of 4, 8, 12, 16 and 20 weeks of age for light and electron microscopic analysis. Original breeding pairs of SHR and WKY were purchased from the Charles River Breeding Laboratories. Animals were kept in plastic cages; food (Purina rat chow) and tap water were provided ad libitum. Adult rats were bred at random. Weaning was carried out at the age of 4 weeks. At 4-week intervals between 4 and 20 weeks of age, animals were sacrificed and the jejunal arteries were processed for light and electron microscopical examination and analysis. A total of 40 animals were studied. The WKYs served as controls.

B. Blood pressure measurement.

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

In each of the 40 rats, the arterial blood pressure was measured from the left femoral artery under sodium pentobarbital (Somnotol, M.T.C. Pharmaceuticals)

anaesthesia (dosage: 35-42 mg/kg). The animal was first weighed and the amount of 35 mg/kg of undiluted anaesthetic was injected intraperitoneally. The animal was then placed, in a supine position, onto a heated small animal operating unit (no.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; surgical anaesthesia was judged by the absence of both corneal and tail reflexes. Supplementary dose of anaesthetic in 10% dilution was given to those which showed signs of "light" anaesthesia. For animals of 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 neuovascular 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 nerves. 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 artery 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 stabilised by the middle suture. The proximal suture was released and the pulsatile blood pressure was allowed

to stabilise for 2 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.

C. Tissue preparation and sampling.

After the completion of blood pressure measurement, the animal was removed from the operation unit for perfusion fixation. A mid-line incision was made and the fur peeled away so that the musculature of the anterior thoracic and abdominal region were visible. The abdomen was first opened by an incision along the linea alba to expose the abdominal viscera. After the jejunum was reflected to the left, the inferior vena cava was severed just below the renal veins. An incision was made along the left side of the sternum to expose the heart. After opening the pericardium, the left ventricle of the heart was punctured with a cannula. The cannula was joined to 2 or 3 syringes which were connected in parallel. Through this infusion set, the animal was perfused at room temperature with a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde (306) in 0.1M sodium cacodylate buffer (pH=7.2) at a pressure of 120 mmHg (Appendix B). The perfusion was achieved by a syringe pump (Compact Syringe Pump, Model 975, Harvard Apparatus), and the perfusion pressure was measured through the femoral arterial cannula. A total of 60 ml of fixative was used during the perfusion.

Clear fixative was seen flowing out the inferior vena cava after 50 ml of fixative had gone through.

Besides giving rise to a pancreatico-duodenal, a colic and an ileo-caeco-colic branch, there are 14-16 jejunal arteries which branch from the superior mesenteric artery (Appendix A); these arteries anastomose with each other on the mesenteric side of the small intestine to form arterial arcades. The first 5 mm of the third, fourth and fifth jejunal arteries were excised for further immersion fixation for 2 hours in fresh fixative. While in the fixative, the perivascular fat was trimmed, the jejunal arteries and the superior mesenteric artery were processed in toto.

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

D. Medial thickness and lumen diameter measurement.

The medial thickness and internal lumen diameter were measured in the third, fourth and fifth branch of the

jejunal arteries which were obtained from 4, 8, 12, 16 and 20 week old SHR and WKY by light microscopy.

One-half-micron sections were cut perpendicular to the longitudinal axis of the arteries. The sections were then collected on a glass slide, stained with toluidine blue and examined under a light microscope (Wild). The lumen and tunica media of the arteries were photographed at 30 and 400 times magnification, respectively. Measurements were made on the negatives. The diameter of the lumen was calculated from the internal circumference, and the width of the thinnest part of the media was recorded. For each set of micrographs, the microscope was calibrated with a micrometer slide (American Optical Company, Buffalo, N.Y.).

E. Stereology and electron microscopy.

The third and fifth branch of jejunal arteries of 4, 8, 12 and 20 weeks old SHR and WKY were used for EM stereological analysis.

Thin, silver to light gold (60-90 nm) interference colour, sections were cut on a Huxley-LKB ultramicrotome 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 under 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 occupied by the arterial wall; no structural details could be observed in this mode, thus visual bias was minimised. Ten micrographs were photographed within the tunica media at a magnification of 11,660 and printed to 35,000 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 (307). The sum of the points obtained from the ten micrographs, expressed in terms of percentage, was considered to be the sample value for that specimen in each animal. The mean of the eight sets of estimates in each group was calculated and compared with those obtained from the other group of the same age. Theoretical consideration of stereology and number of micrographs used for estimation will be outlined in Appendix C.

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

II. Transplant study.

Microvascular surgery was employed in this study in the transplantation of jejunal arteries isolated from either SHR or WKY donors into the femoral vessels of either SHR or WKY as an arterio-venous shunt. Arterial transplant

was performed at the age of 4 weeks. At the age of 4 weeks, the arterial blood pressure of the SHR and WKY were similar.

A. Animal groups.

The rats used in this study were male SHR and WKY. Arterial tranplantation was performed in the following combinations:

WKY to WKY = WKY artery transplanted into WKY host;
SHR to WKY = SHR artery transplanted into WKY host;
SHR to SHR = SHR artery transplanted into SHR host;
WKY to SHR = WKY artery transplanted into SHR host.

Here, the transplant between rats of the same strain served as the control.

B. Preliminary results.

Preliminary studies showed that the transplanted artery "survived" after the surgery and the inflammatory response associated with the surgery seemed to subside by 4 weeks following the operation (see Appendix D). Thus, morphometric analysis was performed on transplanted arteries at 4 weeks after the surgery.

C. Operative procedure.

The operative procedures are summarised in Figure 1. Animals were anaesthetised in pairs with a combination of ether and sodium pentobarbital: anaesthesia was induced by

ether, followed by an intraperitoneal injection of sodium pentobarbital at 20 mg/kg and an additional inhalation of ether for another 5-10 minutes maintained the animal at a surgical anaesthesia for about 1-2 hours. Arterial transplantation began with the isolation of 2 jejunal arteries from a donor rat which would be transplanted into 2 recipient rats one after the other. In the donor rat, the mesentery was exposed by laparotomy and the third and fifth branches of the jejunal artery were dissected free from the periarterial fat and vein. An incision was made in the femoral region of the recipient rat to expose the segment of femoral vessels between the inguinal ligament and the origin of the superficial epigrastric artery. A segment of 8-10 mm of the fifth jejunal branch of the superior mesenteric artery was then isolated from the donor and perfused with warm heparinised saline. The femoral artery of the recipient was occluded; a transverse incision was made on the recipient femoral artery nearest to the origin of the superficial epigastric artery and an end-to-side anastomosis was performed to join the jejunal artery to the femoral artery with 10-0 sutures under an operating microscope. 4-6 interrupted sutures were required in this anastomosis. After the completion of the arterio-arterial anastomosis the occlusion of the artery was released to check the patency of the anastomosis. The arterio-venous anastomosis was begun by occluding the femoral vein; a transverse incision was made 6-8 mm proximal to that of the

arterio-arterial anastomosis and the distal end of the jejunal artery was tucked into the lumen of the femoral vein. The incision on the femoral vein was then closed by 1-2 interrupted sutures. A few drops of 3% magnesium sulphate solution were applied onto the transplanted artery to promote vasodilatation which is essential for the success of the transplant. The transplanted artery was then positioned in the groove between the femoral vessels; this allowed easy location of the transplanted artery during sampling. After the skin incision was closed, the animal was injected with heparin (500 U/kg) intraperitoneally. The third branch of jejunal arteries was transplanted into another recipient rat using the procedures identical to those described above. The time required to complete a set of two arterial transplants was on the average 105 minutes. The anastomoses of each transplant usually took 30-40 minutes.

The animals were then sacrificed at 4 weeks after the surgery and processed for light microscopic examination.

D. Blood pressure measurement.

At the age of 8 weeks, 4 weeks after the surgery, the arterial blood pressure was measured in the operated rats under sodium pentobarbital anaesthesia before sacrifice. The procedures used for blood pressure measurement through a femoral arterial cannula were identical to those described before. Care was taken so that the tip of the

cannula was nearest and distal to the arterio-arterial anastomosis of the shunt.

E. Tissue preparation and sampling.

At the time of sacrifice, the transplanted arteries were perfusion-fixed and processed for light microscopic examination. The fixative and the procedures used were identical to those described earlier. Before embedding in Araldite, the transplanted artery with the femoral vessels was cut transversely into two halves. The cut was made mid-way between the 2 anastomoses.

F. Medial thickness and lumen diameter measurement.

Half-micron-thick, transverse sections were taken from the end nearest and distal to the middle of the transplanted artery, collected onto a glass slide, stained with toluidine blue and examined under a light microscope. Only medial thickness and lumen diameter of the transplanted arteries were measured and compared with other transplanted groups. The methods of these measurements were same as those described before.

G. Criteria for acceptance.

Preliminary results indicated that the transplanted artery showed considerable variations thus the following criteria were set up to eliminate some of these undesirable variations.

The transplanted artery was accepted for morphometric analysis if and only if:

a. pulsation of the transplanted artery was clearly visible;

b. absence of inflammatory reactions, such as intimal thickness and granuloma in the area surrounding the transplanted artery;

c. absence of clot in the transplanted artery;

d. absence of undulation of the internal elastic lamina in perfused specimen.

III. Renal hypertension study.

Hypertension was induced in normotensive WKYs and the changes of the jejunal arteries were correlated with the development of hypertension in these rats.

A. Experimental animals and operation procedures.

Male WKYs, bred from our stock were used in this study. Hypertension was induced in these animals by 2-kidney-1-clip Goldblatt procedures.

At the age of 12 weeks, rats were operated under ether anaesthesia. After the abdominal content was exposed by laparotomy and retracted to the right, the segment of the left renal artery between the origin of the inferior suprarenal artery and the hilus of the kidney was dissected free from the renal vein and nerve, and other periarterial

tissue. The renal artery was then tied onto a curved needle of 0.3 mm in diameter with two 5-0 silk sutures. After the sutures were secured, the kidney appeared to be pale; this indicated that the renal artery was completely constricted. The redness of the kidney was restored when the needle was withdrawn. After the completion of surgery, the abdominal musculature was closed by 5-0 chromic catgut sutures and the skin by 3-0 silk sutures.

A total of 20, 16 renal hypertensive and 4 sham-operated, rats were examined in this study.

B. Selection of animals and blood pressure measurement.

Four weeks after the surgery, arterial blood pressure of the operated animals was checked by femoral arterial cannulation under ether anaesthesia. In half of those animals which showed signs of hypertension, the incision in the femoral artery through which the arterial blood pressure was measured was repaired by 10-0 monofilament sutures and the animals were allowed to survive for another 4 weeks. At the time of sacrifice (i.e. 8 weeks after the surgery), the arterial blood pressure was measured again through a femoral arterial cannula under sodium pentobarbital anaesthesia (dosage 35-42 mg/kg); techniques used were identical to those described earlier. The other half of "hypertensive" animals were allowed to recover from ether for one day and arterial blood pressure was measured

through a femoral arterial cannula under sodium pentobarbital anaesthesia (dosage 35-42 mg/kg); techniques used were identical to those described earlier. The other half of "hypertensive" animals were allowed to recover from ether for one day and arterial blood pressure was measured through a femoral arterial cannula under sodium pentobarbital anaesthesia. The animals were then perfusion-fixed and the jejunal arteries of these rats were processed for microscopic analysis.

Sham-operated animals were treated similarly.

C. Tissue sampling and microscopy

At the end of the 4- and 8-week period after the surgery, the operated animals were killed and the jejunal arteries were processed for microscopic analysis. The medial thickness and lumen diameter of the 3rd, 4th and 5th branch of jejunal arteries obtained from renal hypertensive rats were compared with those obtained from the corresponding sham-operated rats. The sampling techniques and preparatory procedures were identical to those described earlier.

IV. Capsaicin study.

Capsaicin was used as an antihypertensive agent in this study. The medial thickness and lumen diameter of jejunal arteries of the SHR and WKY, 12 weeks following

capsaicin treatment were measured and correlated with the development of arterial blood pressure.

A. Experimental animals and drug administration.

Only male rats were used in this study. Capsaicin, (Sigma Chemical Co., U. S. A.), dissoved in a solution containing 1 part of Tween 80, 1 part of ethanol and 8 parts of 0.9% saline, was injected subcutaneously on the back of 2-3 day old WKYs or SHRs with a single injection at a dosage of 50 mg/kg. After the injection, the rats were wiped clean and returned to the mother. Rats were then sacrificed at the age of 12 weeks.

B. Blood pressure measurement and tissue sampling.

At the age of 12 weeks, the arterial blood pressure of the capsaicin treated animals was measured through a femoral arterial cannula under sodium pentobarbital anaesthesia, and the jejunal arteries were excised and processed for light microscopic examination and measurements. The methods used in blood pressure measurement, and tissue preparation and sampling were identical to those described in the confirmation study. To ensure that arterial blood pressure in the capsaicin treated SHR had not risen to a hypertensive level, arterial blood pressure was also measured at the age of 4 weeks.

V. Statistical analysis.

Results from the confirmation study, the renal hypertension study and the capsaicin study were analysed by Student's "t" test at a significance level of 5%; whereas those from the transplant study were tested by Student-Newman-Keuls' multiple-range test at the same significance level (308). Details of these statistical analyses will be presented in Appendix E.

## RESULTS

I. Confirmation study.

The development of the thickness of wall and diameter of lumen was measured and correlated with that of the arterial blood pressure in the SHR and WKY during the first 20 weeks of postnatal maturation. EM stereological analysis was also employed to estimate the volume percent of smooth muscle, collagen and elastin in the arterial wall of these animals during this developmental period.

A. Body weight.

The body weight of the SHR was comparable to that of the WKY up to the age of 20 weeks (see Table 1). Among the ages studied, the most rapid growth was between 4 and 8 weeks, thus at 8 weeks the weight of each animal was three times that at 4 weeks. The animals then continued to grow for the next 8 weeks at a somewhat slower rate and reached their mature weight by 16 weeks of age.

B. Blood pressure.

At 4 weeks, the mean arterial blood pressure (MAP) was similar to that of the WKY (see Table 1). The MAP of the SHR was significantly higher than that of the control by 8 weeks and was maintained at a hypertensive level (i.e, systolic blood pressure higher than 150 mmHg) from 12 weeks onward. The systolic blood pressure of the WKY was never

never higher than 125 mmHg in this study.

C. Medial thickness and lumen diameter.

Medial thickness and internal lumen diameter of the jejunal arteries of SHR and WKY were measured at 4, 8, 12, 16 and 20 weeks of age (see Table 2). Examples of micrographs used in this study are presented in Figure 2 and 3.

Light microscopic analysis of the jejunal arteries showed that there were no differences in the medial thickness and lumen diameter of the SHR arteries at 4 weeks, as compared with the WKY controls. At 8 weeks the medial thickness of hypertensive arteries was significantly thicker than that of the normotensive ones; whereas the lumen diameter of these arteries was similar. From 12 weeks onward, jejunal arteries of the SHR had a thicker medial and smaller lumen than those of the WKY. The tunica media of jejunal arteries in the SHR at 8, 12, 16 and 20 weeks was thicker by 17, 33, 38 and 42% , respectively, than that of the WKY; whereas the lumen diameter of the WKY vessels at 12, 16 and 20 weeks was 39, 28 and 37%, respectively, larger than that of the SHR.

D. EM Stereology.

Jejunal arteries obtained from 4, 8, 12 and 20 week old rats of each strain were subjected to stereological analysis (see Table 3). An example of micrographs used is

shown in Figure 4. The results of electron microscopic stereological analysis revealed that there were no significant differences between the hypertensive and normotensive vessels at the ages studied, although there was a trend that the muscular component of the vessel wall decreased and the connective tissue components increased with age. Thus at 4 weeks the mean volume percent of the smooth muscle, collagen and elastin of the normotensive arteries were 80.1, 17.7 and 2.3, respectively, and by 20 weeks these estimates were 73.9, 22.9 and 3.1, respectively.

E. Ultrastructure of developing SMC.

Smooth muscle cells, which were mostly arranged in layers and perpendicular to the longitudinal axis of the arteries, were confined in the media of jejunal arteries. Although the number of SMC layers remained the same during development, there was difference between the SHR and WKY jejunal arteries. There were 4-6 and 5-7 layers of SMC in the media of jejunal arteries of the WKY and SHR, respectively.

Age-related ultrastructural changes, interpreted as the process of maturation in the SMC appeared to be similar between the jejunal arteries of the SHR and WKY; these included changes in myofilaments, intra-cellular organelles and cell-to-cell contacts.

1. "Immature" SMC.

"Immature" SMCs were characterised by few myofilament bundles and rare dense bodies (see Figure 5). These bundles were usually 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 of SMC at sites of close cell apposition. Most of the volume of "young" SMC was occupied by organelles. These included rough-surfaced endoplasmic reticulum (RER), free ribosomes which were usually organised into rosettes, mitochondria, Golgi complexes, occasional lipofuscin granules and a pair of centrioles. Extensive areas of close membrane apposition with very few specialised junctional structures were frequently encountered.

2. "Mature" SMC.

In "mature" SMC, organised myofilament bundles filled most of the cell except the areas which were occupied by the nucleus, the perinuclear organelles and foci of organelles along the cell periphery (see Figure 6). Dense areas were also plentiful along the cell membrane occupying the areas which were not filled with plasmalemmal vesicles. The changes in the amount of organelles were obvious in mature SMC. A decreased amount of RER and mitochondria and an increased amount of lipofuscin granules together with a small Golgi complex were usually located at the poles of the nucleus. Many short cytoplasmic processes were extended

from mature SMC to make close contacts with neighbouring cells. Occasionally these junctions were specialised into desmosome-like structures. Nexuses of the SMC in jejunal arteries were rarely seen.

II. Transplant study.

Out of the 50 transplanted arteries, 27 had patent lumen, but only 16, 4 in each group, were accepted for morphometric analysis using the criteria set up based on the results of the preliminary study. As expected, the number of animals required in order to obtain a group of 4 successfully transplanted arteries in the cross-transplants was much higher than that required for the same strain. The number of transplants required in WKY to WKY, SHR to SHR, WKY to SHR and SHR to WKY was 8, 11, 16 and 15, respectively. The appearance of successfully transplanted arteries in either strain was similar to that described in the preliminary study (Appendix D).

A. Body weight.

At the age of 8 weeks, 4 weeks after the arterial transplantation, the body weight of rats from either strain was about 170 g; there was no significant difference among all four groups studied (see Table 4).

B. Blood pressure.

The arterial blood pressure was measured from all animals through a femoral arterial cannula. The mean arterial blood pressure of 8-week old WKY, 4 weeks after the surgery, whether the transplanted artery was from the SHR or WKY, was about 80 mmHg; whereas that of the SHR was about 105 mmHg (see Table 4). There were no significant differences between the two groups of the same strain. However, the mean arterial blood pressures of the 2 SHR groups were significantly higher than those of the 2 WKY groups.

C. Medial thickness and lumen diameter.

The medial thickness and lumen diameter of the transplanted arteries were measured in half-micron-thick, toluidine blue-stained sections by light microscopy. Examples of micrographs used in this study are illustrated in Figure 7 and 8.

At the age of 8 weeks, 4 weeks post-operation, the medial thickness and lumen diameter of the transplanted arteries in the 2 WKY recipient groups were similar (see Table 5). These parameters were not different between the 2 SHR host groups. However, when these figures were compared between the recipient groups from different strains, it was evident that the transplanted arteries in the SHR hosts had thicker media and smaller lumen than those in the WKY recipients. Thus, at the age of 8 weeks, 4 weeks after arterial transplantation the mean medial thickness and

lumen diameter of transplanted arteries in the WKY hosts were about 10 and 370 microns, respectively; whereas those of transplanted arteries in the SHR recipients were about 13 and 310 microns, respectively.

III. Renal hypertension study.

Two-kidney-1-clip Goldblatt procedures were employed to produce renal hypertension in 12 weeks old genetically normotensive WKYs. The operated rats survived quite well after the surgery. Out of the 16 renal artery-tied rats, 8 showed signs of hypertension at the age of 16 weeks, 4 weeks after the surgery.

Of the renal hypertensive rats studied in the present experiment, all had a well perfused kidney on the operated side. The size of the kidney on the operated side was smaller than the contralateral one in most cases (6 out of 8); there was no significant difference in the size of the kidney on both sides in the other 2 renal hypertensive rats.

A. Body weight.

There was no significant difference between the body weight of renal hypertensive and the corresponding sham-operated rats at 4 weeks (16 weeks of age) and 8 weeks (20 weeks of age) after the surgery, although that of the

20-week old renal hypertensive rats was a little smaller (see Table 6).

B. Blood pressure.

At the age of 16 and 20 weeks, 4 and 8 weeks after the surgery, respectively, the mean arterial pressure of the renal hypertensive rats was significantly higher than that of the corresponding sham-operated WKY (see Table 6). Thus at the age of 16 weeks, the mean arterial pressure of renal hypertensive WKYs was higher than that of the controls by 13%; whereas that of the 20-week old renal rats was 30% higher than that of the controls.

C. Medial thickness and lumen diameter.

The medial thickness and lumen diameter were estimated in the jejunal arteries of renal hypertensive and sham-operated control WKYs under the light microscope. Examples of micrographs used in this study are shown in Figure 9 and 10.

The medial thickness and lumen diameter of the jejunal arteries obtained from 16-week old renal hypertensive rats, 4 weeks after the surgery were similar to those of the corresponding sham-operated controls (see Table 7). However, at 8 weeks after the surgery, the 20 week old renal hypertensive WKYs exhibited a 35% increase in the medial thickness of jejunal arteries when compared with those of the sham-operated WKYs; whereas the lumen diameter

of the experimental and control groups at the age of 20 weeks, 8 weeks after the operation, was similar.

IV. Capsaicin study.

Capsaicin was injected subcutaneously on the back of WKY and SHR neonates, 2-3 days after birth.

Almost immediately after the injection, the rats became cyanotic; quite often artificial respiration was required to restore respiration and redness of the capsaicin injected rats. The latter manoeuvre was required more frequently in the SHR than WKY neonates.

There were differences in gross appearance between SHR and WKY after capsaicin treatment. The treated WKYs usually lost whiteness of the fur colour by 12 weeks of age. However, the fur colour of the treated SHRs was comparable to that of the untreated WKYs. Very often, ulcerated areas were seen in the capsaicin treated WKYs but never in the treated SHRs. These areas were usually confined to the head region nearest to the base of the ears or the forehead.

About 60-70% of the capsaicin treated SHR and WKY showed a reduction in arterial blood pressure. Only those animals which showed a reduction of arterial blood pressure were included in this study and their jejunal arteries processed for microscopical analysis.

A. Body weight.

After the capsaicin treatment, the body weight of rats used in this study was about 50 g at 4 weeks and 210 g at 12 weeks of age (see Table 8). There were no significant differences detected between the body weight of the treated WKY and SHR when compared with the corresponding age groups.

B. Blood pressure.

Arterial blood pressure was measured in 4 and 12 week old WKY and SHR after capsaicin treatment by femoral arterial cannulation. These results were summarised in Table 8.

At 4 weeks, the mean arterial pressures of both treated SHR (79.2 ± 3.5) and WKY (62.3 ± 6.3) were lower than those of the untreated rats (SHR:82.3 ± 3.0; WKY:81.6 ± 4.8; see Table I). Thus, the reduction in mean blood pressure in the treated SHR and WKY was 4 and 24%, respectively as compared with the untreated groups. At 12 weeks, the reduction of mean arterial pressure in the treated SHR and WKY was 26 and 32%, respectively when compared with the untreated groups at the corresponding age. At both ages, the mean arterial pressure of the treated SHR was significantly higher than that of the treated WKY.

C. Medial thickness and lumen diameter.

Only the jejunal arteries from 12 week old capsaicin treated animals were processed for microscopic examination and measurement (see Table 9, and Figure 11 and 12). After capsaicin treatment, the jejunal arteries in the SHR were 23% thicker than those of the WKY. There was no significant difference in the diameter of lumen between the jejunal arteries of the treated SHR and WKY at this age. The jejunal arteries of the capsaicin treated SHR had larger lumen and thicker media than those of the untreated SHR. There were no significant differences in the wall thickness of the jejunal arteries between the treated and untreated WKY, although the jejunal arteries of the treated WKY had smaller lumen than those of the untreated.

# V. Tables and figures.

Table 1. Development of body weight and mean arterial pressure in SHR and WKY.

| Parameter | | 4 weeks | 8 weeks | 12 weeks | 16 weeks | 20 weeks |
|---|---|---|---|---|---|---|
| Body weight (g) | SHR | 52.9±2.9 | 172.8±3.2 | 270.6±11.7 | 313.6±20.3 | 322.9±22.9 |
| | WKY | 54.6±1.1 | 169.0±6.9 | 273.5±15.3 | 314.4±12.0 | 346.2±8.2 |
| Mean arterial pressure (mmHg) | SHR | 82.3±3.0 | 120.0±5.1* | 136.0±3.4* | 143.6±5.1* | 141.0±13.6* |
| | WKY | 81.6±4.8 | 84.8±1.5 | 91.2±4.7 | 93.3±2.4 | 83.8±5.7 |

Each value (mean ± S.D.) was obtained from four male rats.

* indicates the mean value of the SHR is significantly higher than that of the WKY at a significance level of 5%.

Table 2. Morphometric data of jejunal arteries in developing SHR and WKY.

| Parameter | | 4 weeks | 8 weeks | 12 weeks | 16 weeks | 20 weeks |
|---|---|---|---|---|---|---|
| Medial thickness | SHR | 10.2±0.6 | 10.9±1.0* | 11.3±0.9* | 14.8±1.5* | 15.0±1.6* |
| (micron) | WKY | 9.6±0.8 | 9.4±0.6 | 8.5±0.8 | 10.7±0.8 | 10.6±1.2 |
| Lumen diameter | SHR | 194.2±26.6 | 238.7±21.3 | 223.0±29.0* | 257.2±36.5* | 273.1±27.9* |
| (micron) | WKY | 194.4±28.0 | 238.2±20.7 | 309.7±26.3 | 329.8±29.7 | 373.3±48.7 |

Each value (mean ± S.D.) was obtained from 12 measurements (3 per animal).

* indicates the mean value of the SHR is significantly higher than that of the WKY at a significance level of 5%.

Table 3. Stereological data of jejunal arteries in developing SHR and WKY.

| Parameter | | 4 weeks | 8 weeks | 12 weeks | 20 weeks |
|---|---|---|---|---|---|
| Smooth muscle cell | SHR | 79.5±1.5 | 78.1±1.1 | 80.1±1.4 | 76.6±2.7 |
| | WKY | 80.1±1.7 | 78.4±0.7 | 78.0±2.0 | 73.9±2.9 |
| Collagen | SHR | 18.0±1.8 | 18.6±1.1 | 16.6±0.9 | 20.6±1.3 |
| | WKY | 17.7±1.4 | 18.5±0.3 | 17.4±1.7 | 22.9±2.3 |
| Elastin | SHR | 2.5±1.0 | 3.3±0.4 | 3.5±0.5 | 3.5±1.1 |
| | WKY | 2.3±1.0 | 3.0±0.6 | 2.9±0.7 | 3.1±1.4 |

Each value (mean ± S.E. in volume percent) was obtained from 8 measurements (2 per animal).

Table 4. Characteristics of 8-week old SHR and WKY, 4 weeks after arterial transplantation.

| Animal | Body weight (g) | Mean arterial pressure (mmHg) |
|---|---|---|
| WKY to WKY | 181.7±11.3 | 81.7±3.1 |
| SHR to WKY | 170.0±8.1 | 82.9±0.8 |
| SHR to SHR | 166.2±7.9 | 107.3±6.3* |
| WKY to SHR | 163.6±8.5 | 105.0±2.4* |

Each value (mean ± S.D.) was obtained from four male rats.

* indicates mean value of the SHR blood pressure is significantly higher than the blood pressure of the WKY host at a significance level of 5%.

Table 5. Morphometric data of transplanted jejunal arteries obtained from 8-week old SHR and WKY hosts, 4 weeks after the arterial transplantation.

| Animal | Medial thickness | Lumen diameter |
|---|---|---|
| WKY to WKY | 10.3±1.3 | 372.0±25.4 |
| SHR to WKY | 10.5±1.5 | 369.0±35.6 |
| SHR to SHR | 13.5±1.6* | 313.4±69.1* |
| WKY to SHR | 12.8±1.3* | 309.8±71.5* |

Each value (mean ± S.D. in micron) was obtained from 4 measurements (1 per animal).

* indicates mean value of the transplanted artery in the SHR host is significantly different from that of the transplanted artery in the WKY host at a significance level of 5%.

Table 6. Characteristics of renal hypertensive WKY.

| | Animal | Body weight (g) | Mean arterial pressure (mmHg) |
|---|---|---|---|
| 16-week old, | 2K1C | 294.3±6.0 | 106.0±6.0* |
| 4 weeks postoperation | Sham | 295.7±31.7 | 92.5±1.2 |
| 20-week old, | 2K1C | 253.6±37.5 | 131.7±11.1* |
| 8 weeks postoperation | Sham | 289.1±41.0 | 92.5±5.9 |

Each value (mean ± S.D.) was obtained from 4 male WKYs.

* indicates the mean value of the 2K1C rat is significantly higher than that of the sham rat.

2K1C = 2-kidney-1-clip Goldblatt renal hypertensive rat; Sham = sham-operated rat.

Table 7. Morphometric data of jejunal arteries in renal hypertensive WKY.

| | Animal | Medial thickness | Lumen diameter |
|---|---|---|---|
| 16-week old, | 2K1C | 11.6±1.5 | 290.3±30.4 |
| 4 weeks postoperation | Sham | 10.2±0.7 | 311.1±50.1 |
| 20-week old, | 2K1C | 17.6±3.6* | 348.3±39.2 |
| 8 weeks postoperation | Sham | 11.5±0.8 | 322.7±26.2 |

Each value (mean ± S.D. in micron) was obtained from 12 measurements (3 per animal).

* indicates the mean value of the 2K1C rat is significantly higher than that of the sham rat.

2K1C = 2-kidney-1-clip Goldblatt renal hypertensive rat; Sham = sham-operated rat.

Table 8. Characteristics of SHR and WKY after capsaicin treatment.

| | | Body weight (g) | Mean arterial pressure (mmHg) |
|---|---|---|---|
| 4 weeks | SHR | 52.7±4.8 | 79.2±3.5* |
| | WKY | 54.0±2.5 | 62.3±6.3 |
| 12 weeks | SHR | 202.0±32.9 | 101.0±5.2* |
| | WKY | 217.5±20.0 | 62.1±6.8 |

Each value (mean ± S.D.) was obtained from 4 male rats.

* indicates the mean value of the SHR is significantly higher than that of the WKY at a significance level of 5%.

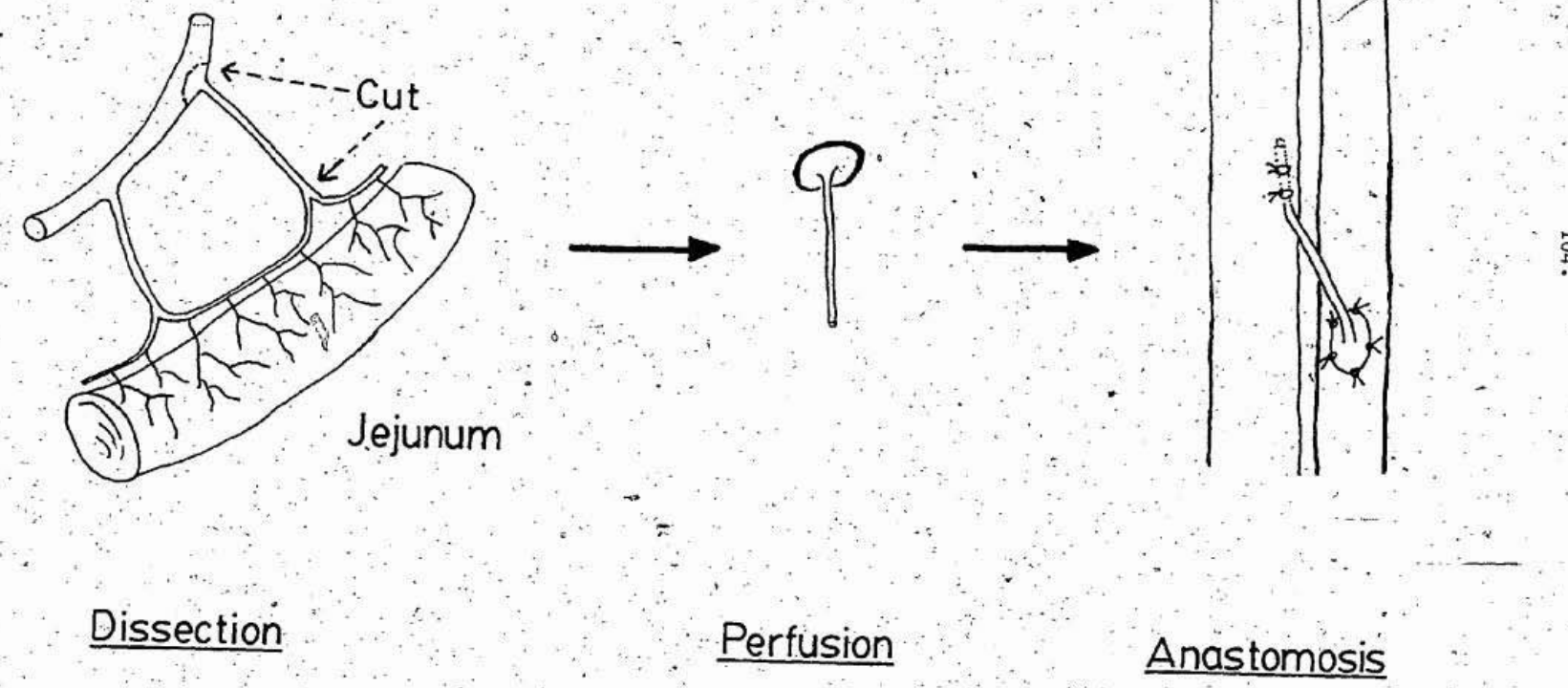
Table 9. Morphometric data of jejunal arteries in 12-week old capsaicin treated SHR and WKY.

| | Medial thickness | Lumen diameter |
|---|---|---|
| SHR | 13.2±1.4* | 281.1±20.1 |
| WKY | 10.1±2.0 | 261.0±19.9 |

Each value (mean ± S.D. in micron) was obtained from 12 measurements (3 per animal).

* indicates the mean value of the SHR is significantly higher than that of the WKY at a significance level of 5%.

Figure 1. Surgical technique of arterial transplantation. Arterial transplantation was begun by isolating jejunal arteries from a donor rat. A branch of jejunal arteries was excised and dissected free of periarterial tissues. It was then perfused with warm heparinised saline to wash out the blood and anastomosed into the femoral vessels of a recipient rat as a shunt.




Surgical technique used in arterial transplantation.

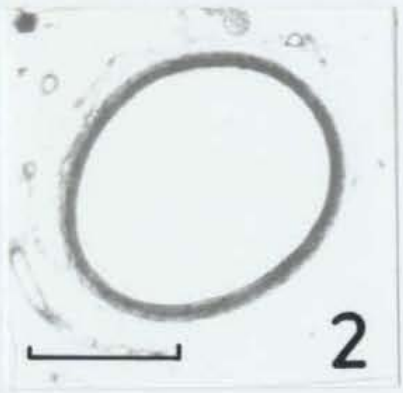



Figure 2. An example of micrographs of jejunal arteries used for measuring lumen diameter in the confirmation study. LM. 20-week SHR. Calibration bar = 200 microns.

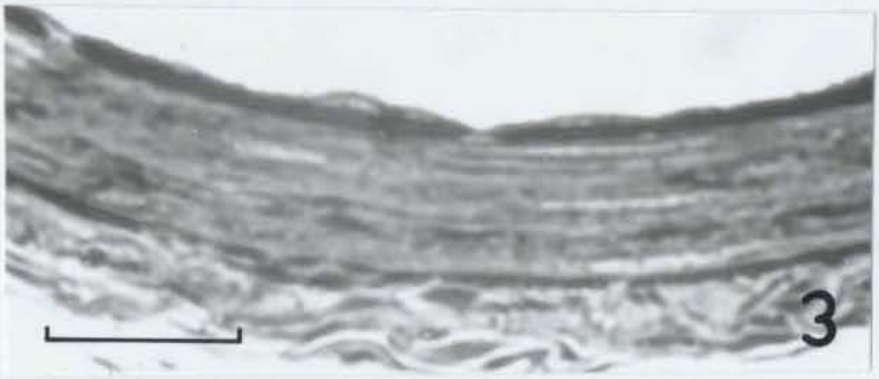



Figure 3. An example of micrographs of jejunal arteries used for measuring wall thickness in the confirmation study. LM. 20-week SHR. Calibration bar = 20 microns.

Figure 4. An example of electron micrographs of jejunal arteries used for stereological analysis in the confirmation study.
The volume percent of SMC, collagen, and elastin were estimated by laying a 10 x 10 point-counting grid onto the micrograph. EM. 12-week SHR. Calibration bar = 1 micron.

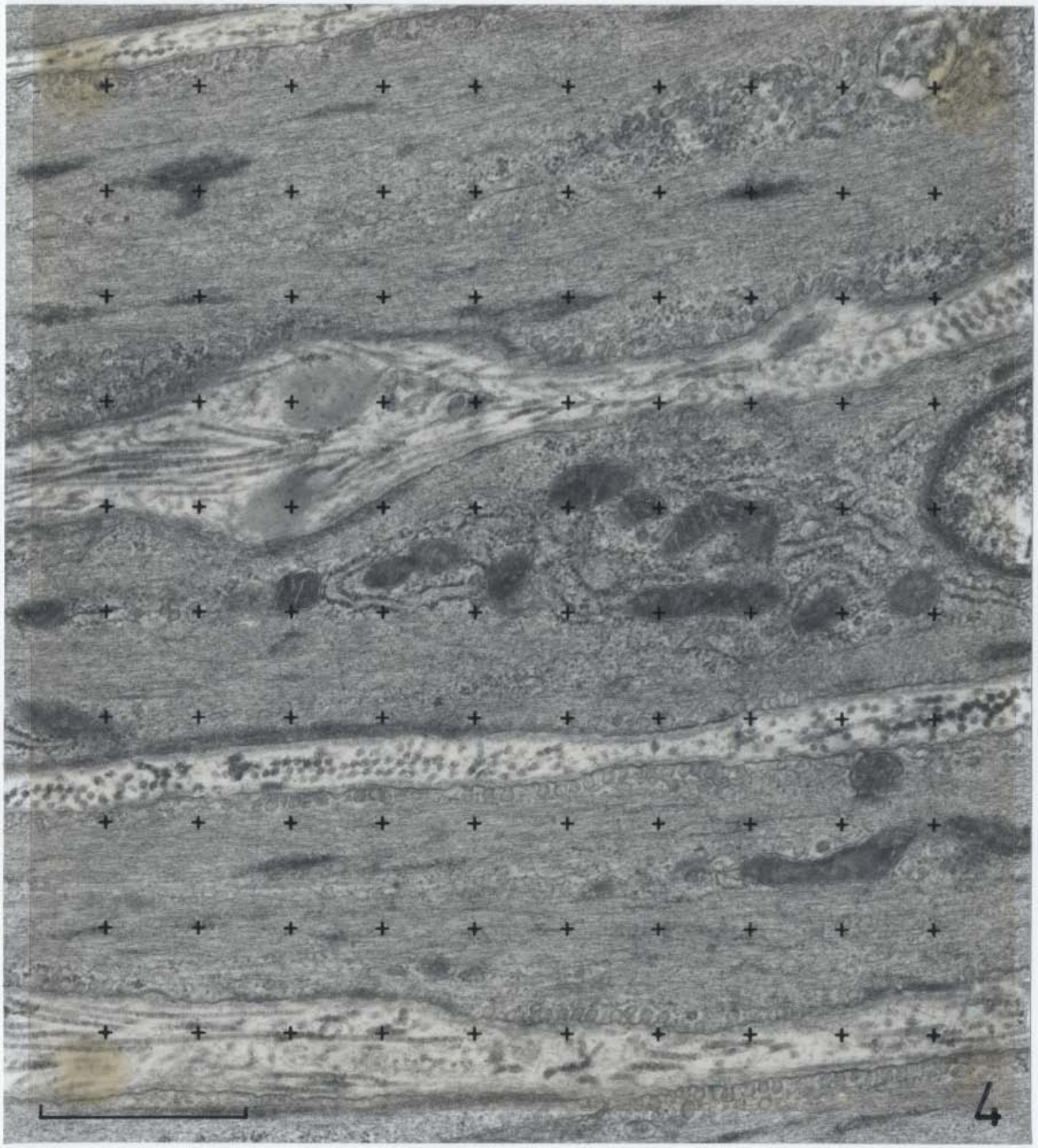

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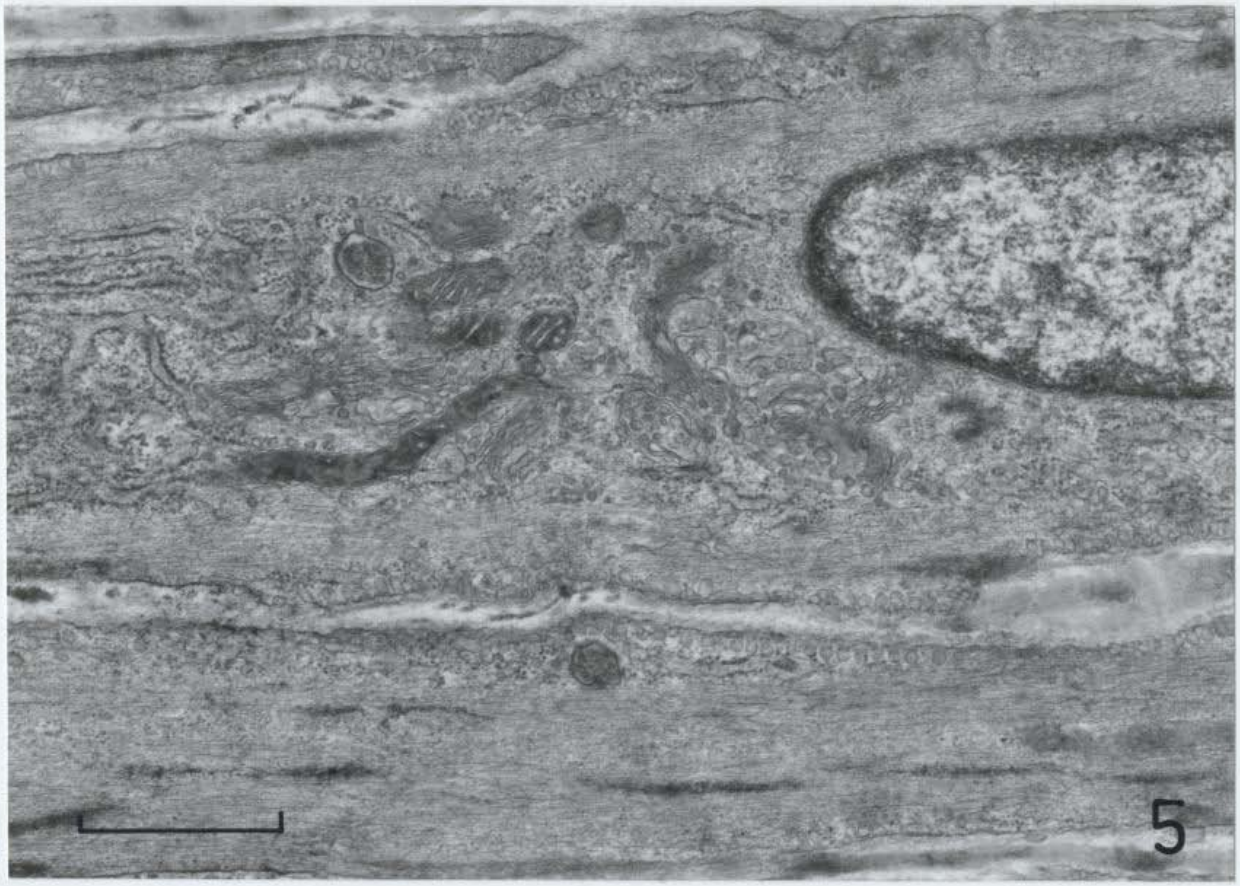
Figure 5. Immature smooth muscle cell.
Immature SMCs were characterised by few myofilament bundles and rare dense bodies. Most of the volume of young SMC was occupied by organelles. These included rough-surfaced endoplasmic reticulum, free ribosomes which were usually organised into rosettes, mitochondria, Golgi complexes and a pair of centrioles. EM. 4-week WKY.
Calibration bar = 1 micron.

Figure 6. Mature smooth muscle cell.
In mature SMC, organised myofilament bundles were filled most of the cell except the areas which were occupied by the nucleus, and the perinuclear organelles. Dense areas were plentiful along the cell membrane occupying the areas which were not filled with plasmalemmal vesicles. EM. 20-week SHR. Calibration bar = 2 microns.



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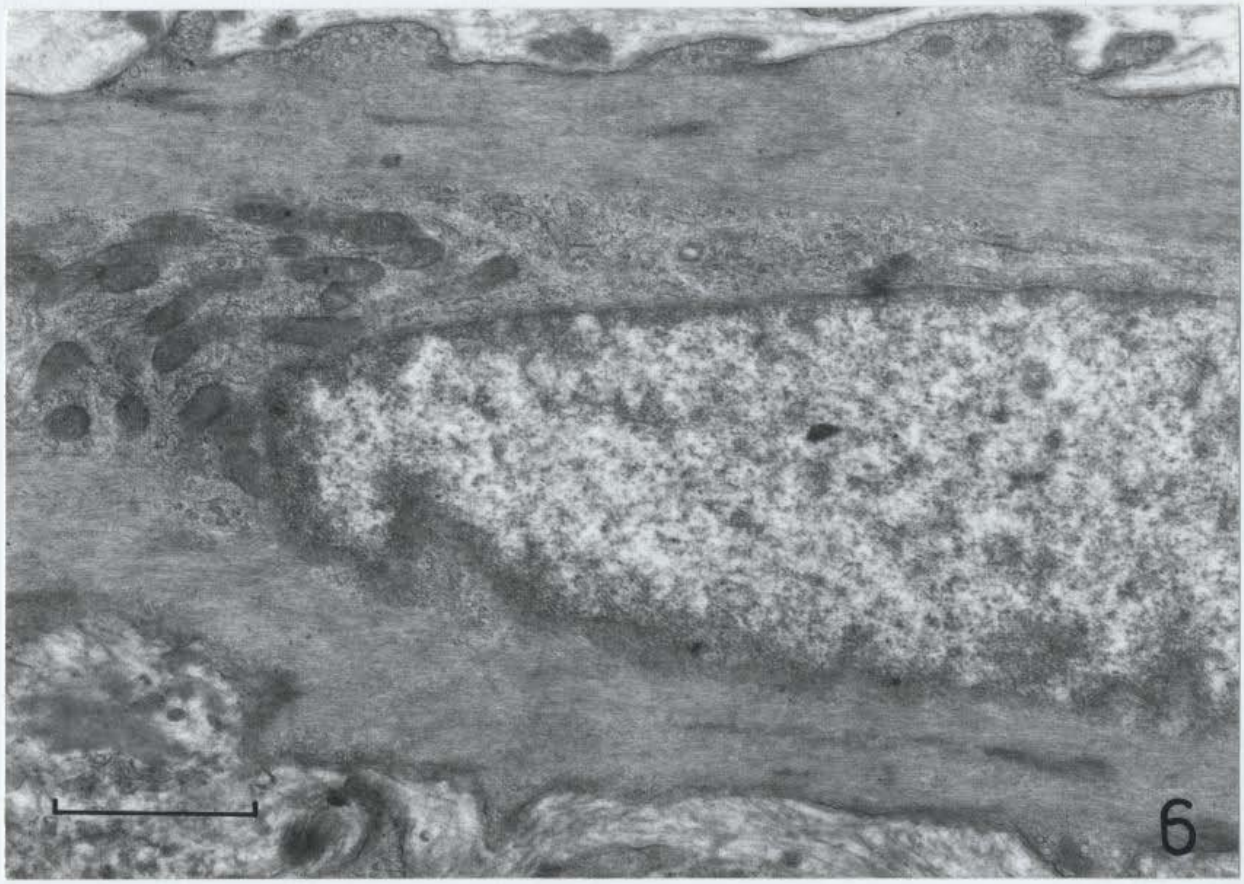

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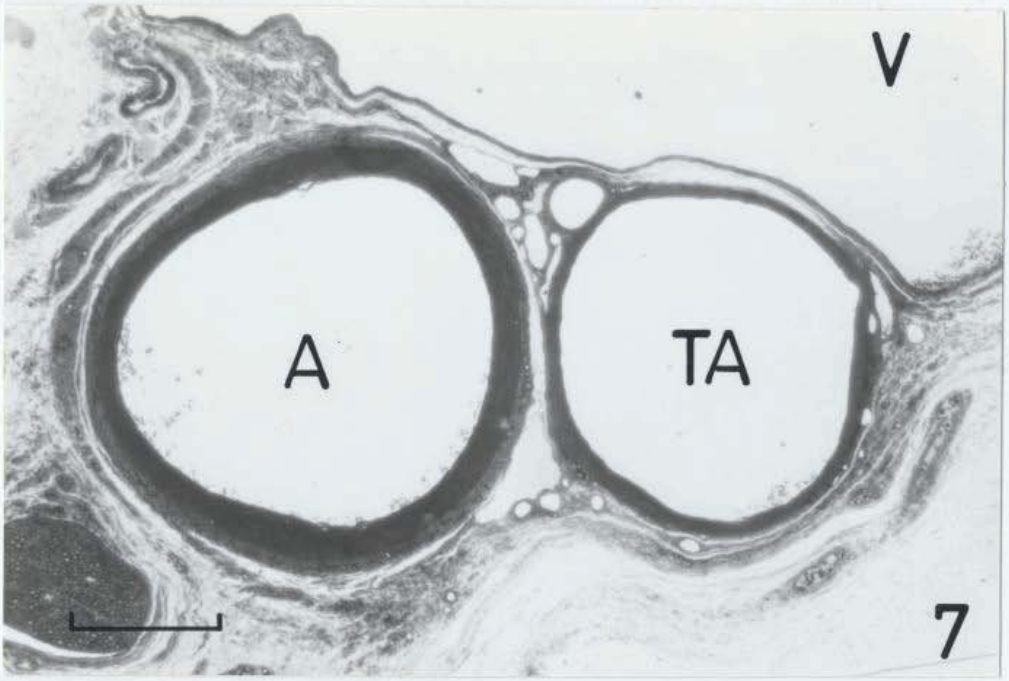


Figure 7. An example of micrographs of transplanted jejunal arteries used for measuring lumen diameter. LM. 8-week WKY. WKY to WKY. Calibration bar = 200 microns.

TA = transplanted artery; femoral artery (A) & vein (V).

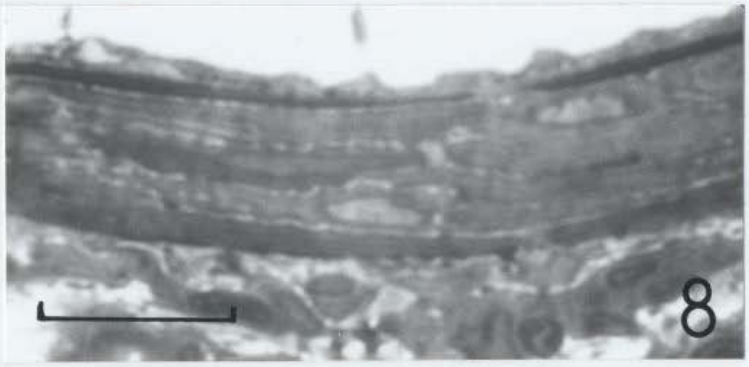


Figure 8. An example of micrographs of transplanted jejunal arteries used for measuring wall thickness. LM. 8-week SHR. WKY to SHR. Calibration bar = 20 microns.

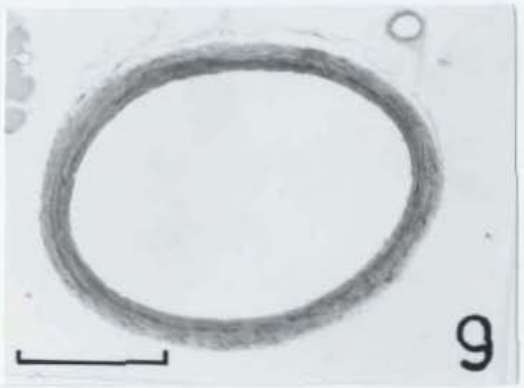


Figure 9. An example of micrographs of jejunal arteries in renal hypertensive WKY used for measuring lumen diameter. LM. 20-week WKY. Calibration bar = 200 microns.

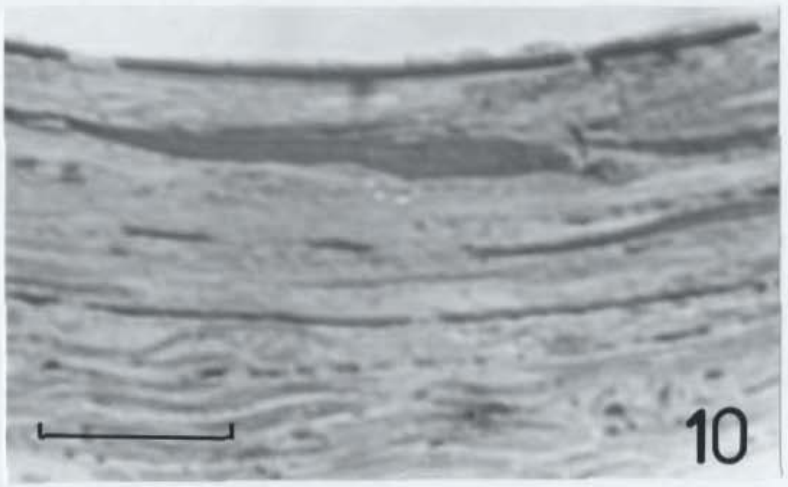


Figure 10. An example of micrographs of jejunal arteries in renal hypertensive WKY used for measuring wall thickness. LM. 20-week WKY. Calibration bar = 20 microns.

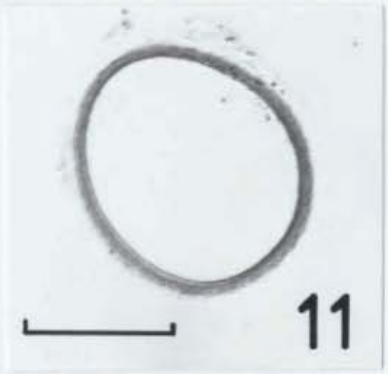


Figure 11. An example of micrographs of jejunal arteries in capsaicin treated rats used for measuring lumen diameter. LM. 12-week SHR. Calibration bar = 200 microns.


Figure 12. An example of micrographs of jejunal arteries in capsaicin treated rats used for measuring wall thickness. LM. 12-week SHR. Calibration bar = 20 microns.

## DISCUSSION

The results of the present investigation demonstrate that medial hypertrophy in jejunal arteries of the SHR is neither the result of elevated arterial pressure nor of genetic predisposed factors intrinsic to the arterial wall.

### I. Confirmation study.

The results of the present study showed that the elevation of arterial blood pressure of the SHR occurred between 4 and 8 weeks. An increase in medial thickness of jejunal arteries in the SHR also took place during this period. The lumen diameter of SHR arteries was smaller than that of the WKY from 12 weeks onward.

#### A. Body weight.

The results of the present study confirmed the finding that the development of body weight in the SHR was similar to that of the WKY during the earlier maturation in these rats (168).

#### B. Blood pressure.

The elevation of mean arterial pressure in the SHR occurred between 4 and 8 weeks of age. This is in agreement with an earlier report from this laboratory which showed that the mean arterial pressure of the SHR was significantly higher than that of the WKY by 5 weeks of age

(168). The mean arterial pressure of the SHR was significantly higher than that of the WKY at 8 weeks and was maintained at a hypertensive level from 12 weeks onward. These data were comparable to those of the parent strain of SHR in Japan (7, 151, 263).

C. Medial thickness and lumen diameter.

By observing small mesenteric arteries in vivo, Ichijima (263) noted that there is an increase in tortuosity in the arteries of the SHR as compared with those of the WKY. Recently, electron microscopic analysis indicated that medial hypertrophy is present in 6- and 50-week old SHR mesenteric arteries (265). These results were obtained after the vessels were studied pharmacologically in vitro. Furthermore, it is not possible from the data presented to determine whether medial hypertrophy occurred before or after the increase in arterial blood pressure, since the blood pressure of the SHR was significantly higher than that of the WKY at 6 weeks in that study.

The first appearance of medial hypertrophy of jejunal arteries in hypertensive animals was present at 8 weeks of age in this study; this finding confirmed that medial hypertrophy does occur in jejunal arteries of the SHR, and that the degree of severity of medial hypertrophy in hypertensive arteries increases with age, at least up to the age of 20 weeks (see Table 2).

D. Electron microscopy and stereological analysis.

Electron microscopy showed that the development of SMC in the media of jejunal arteries from both strains was characterised by changes in the amount of myofilament and intra-cellular organelles, and in cell-to-cell contacts. These were similar to those in the development of SMC in the media of the abdominal aorta and renal artery (306).

EM stereological analysis indicated that medial hypertrophy of the arteries studied was the result of increase in both muscular and connective tissue components. The increase of the muscular component may be due to hyperplasia and/or hypertrophy of vascular smooth muscle cells. A recent report by Owens et al (268) showed that medial hypertrophy in SHR arteries, at least in the aorta, is due to vascular smooth muscle cell hypertrophy, although there was no mention about the age of animals used. Hypertrophy of vascular smooth muscle cell in the SHR is most likely due to the increase in the amount of myofilaments present in the smooth muscle such as that described in the cardiocyte hypertrophy (147, 148, 149).

E. Significance of confirmation study.

The results of this study showed that medial hypertrophy is present in jejunal arteries of the SHR. This increased SMC mass may explain the increased basal vascular resistance and vascular reactivity in hypertension (297,

298).

The cause of medial hypertrophy in the SHR is at present unknown. The results of the present study suggest that the alteration in SHR arteries may be a result of the increase of arterial blood pressure, since the arterial pressure was elevated before the medial hypertrophy appeared. These results fit with those reported by Mulvany et al (307) who showed that there is no sign of medial hypertrophy in the SHR mesenteric resistance vessels at 6 weeks. That elevation of arterial blood pressure can induce changes in vessels is well accepted; vascular changes were reported after the artificial induction of hypertension in genetically normotensive rats (227, 228, 229). However, other possibilities such as vascular smooth muscle defects (295, 301), hormonal influences (195, 196) and sympathetic innervation (308, 309) may play a role in altering medial structures during the course of hypertension. These require further investigation.

II. Transplant study.

The most important finding in this study is that irrespective of their origin, jejunal arteries transplanted into the SHR recipients developed medial hypertrophy; whereas SHR jejunal arteries did not show medial hypertrophy in the WKY hosts.

A. Body weight.

Four weeks after arterial transplantation, the body weight of 8-week old rats from either strain was about 170 g which was similar to that of the unoperated rats. This suggests that arterial transplantation did not produce any ill effect on the operated animals resulting in a reduction in body weight.

B. Blood pressure.

The mean arterial blood pressure of 8 week old WKY, 4 weeks after the surgery, whether the transplanted artery was from the SHR or WKY, was about 80 mmHg; whereas that of the SHR was about 105 mmHg. These data were comparable to those of the unoperated animals reported in the confirmation study (see Table 1), although the mean arterial pressure of the operated SHR was slightly lower.

C. Medial thickness and lumen diameter.

The results of the present study showed that irrespective of their origin, the transplanted jejunal arteries in the SHR hosts exhibited a thicker media and smaller lumen than those in the WKY recipients. The wall thickness of jejunal arteries transplanted between the same strain was similar to that of the unoperated jejunal arteries of the corresponding groups reported in the confirmation study, although the lumen diameter of the transplanted arteries was much larger. This is most likely

due to the change in function and location of the jejunal arteries to a higher pressure segment, such as the femoral artery, of the vascular system.

As pointed out earlier in the INTRODUCTION section, the SHR was isolated from the WKY, an inbred Wistar strain in Kyoto University, Japan. It is logical then to assume that the "genetics" of the 2 strains are similar. Thus, it is likely that cross-transplantation of tissues (in this case jejunal arteries) between the two strains will be compatible. The results of Kawabe et al (194) who studied the effect of kidney transplantation between the SHR and WKY, and of Hermsmeyer and co-workers (319, 320) who studied the effect of cross-transplantation of tail arteries into the anterior chamber of the eye between these 2 strains suggest that there is no sign of tissue rejection. Indeed, results of the preliminary study (see Appendix D) in the present investigation seem to support this contention. Although no detailed analysis was performed to quantify the rejection process in the cross-transplantation of jejunal arteries between the SHR and WKY, the fact that there were over 50% of the transplants were successful in the present study suggested tissue rejection was not a major problem. Therefore, the use of immuno-suppressant drugs was not necessary.

It is possible that surgical intervention may induce changes in the transplanted arteries and that these changes may be different in the SHR than in the WKY arteries. The fact that SHR and WKY arteries transplanted into the SHR recipients developed

medial hypertrophy and that those transplanted into the WKY hosts did not indicate that the suspected alteration did not occur in the present study.

Although direct arterial blood pressure had not been measured in the lumen of the transplanted artery, it is assumed that the blood pressure in the transplanted artery was similar to that of the femoral artery since that femoral blood pressure obtained in this study was recorded very near to the opening of the transplanted artery. Clotting which may have obstructed the lumen of the transplanted arteries was not evident. This is also supported by the results of Zweifach's group who showed that blood pressure of the SHR remains elevated even in the arterioles of 40 microns in diameter (310).

D. Significance of transplant study.

The present study was designed to determine whether the medial hypertrophy which occurs in the jejunal arteries of the SHR is due to the result of increase in arterial blood pressure or to genetically "predisposed" factors in the arterial wall. The fact that SHR jejunal arterties did not develop medial hypertrophy in WKY hosts, and WKY jejunal arteries became thicker in SHR recipients strongly suggested that the determinants governing the development of medial hypertrophy in arteries of the SHR are resident in the hosts. Since the mean arterial pressure of the SHR hosts was significantly higher than that of the WKY recipients during the development of medial hypertrophy

in the transplanted jejunal arteries, it is logical to deduce that this change may have been the result of the elevated arterial blood pressure. The influence of other factors such as innervation and circulating hormones on the development of medial hypertrophy in the transplanted jejunal arteries has not been ruled out and will be discussed later.

III. Renal hypertension study.

The results of this study indicate that hypertension can be induced in genetically normotensive WKY by 2-kidney-1-clip Goldblatt procedures. Associated with the elevation of arterial blood pressure in the renal hypertensive rats was the thickening in the media of jejunal arteries.

A. Body weight.

The body weights of the operated WKYs, both renal hypertensive and sham-operated controls at 16 weeks of age, 4 weeks after the induction of hypertension were similar; these figures were equivalent to those of the normal WKY presented in the confirmation study (see Table 1). Thus, both the operation and the slightly elevated blood pressure in the renal hypertensive rats did not affect the body weight of the operated WKYs. However, the body weight of the 20 weeks old WKYs, in particular the renal hypertensive rats was lighter than that of the normal unoperated WKYs. Although the difference in body weight in the sham-operated and normal WKY was not known, it was most likely due to the long term body reaction to the operation. The lighter body weight of the 20-week old renal hypertensive WKYs was most likely the result of both the surgery and the ill effect of hypertension.

B. Blood pressure.

The development of hypertension resulting from artificial renal arterial stenosis appeared to be gradual. At 4 weeks after the operation, the increase in mean arterial blood pressure of the renal hypertensive rats was slight, although statistically significant as compared with that of the sham-operated WKYs. This increase of arterial pressure in the renal hypertensive WKYs continued to rise in the next few weeks and became hypertensive by 20 weeks of age, 8 weeks post-operation. These results were similar to those of Mulvany et al (301) and Ten Berg and De Jong (311), although the time required for the development of increased blood pressure in those studies was much shorter.

C. Medial thickness and lumen diameter.

The thickening of media in the jejunal arteries of renal hypertensive rats was apparent by 8 weeks after the operation, although there was no detectable difference in the medial thickness of the 16 week old renal hypertensive and sham-operated WKYs, 4 weeks after the operation. Thus, it is possible to induce medial hypertrophy in the jejunal arteries of genetically normotensive WKYs by 2-kidney-1-clip Goldblatt procedures. These results were comparable to those reported by Mulvany et al (301).

D. Significance of renal hypertension study.

Although medial hypertrophy of the jejunal arteries in the renal hypertensive rats is likely to be the result of

the increase in arterial blood pressure as suggested by others (311, 312), the possibility that this alteration in the arteries of hypertensive animals is due to some other factors such as sympathetic innervation (310, 313) and hormonal function (195, 311) has not been ruled out.

IV. Capsaicin study.

The most important finding of the present study is that the anti-hypertensive action of capsaicin treatment did not prevent the development of medial thickening in jejunal arteries of the SHR.

A. Body weight.

As pointed out earlier, the main effect of capsaicin is the depletion of substance P from primary sensory neurones which are involved in the mediation of chemogenic pain, e.g. dorsal root ganlion cells. In addition, administration of capsaicin in neonates induces selective degeneration of a distinct population of these neurones. Although it was not well documented in the literature, the effect of capsaicin treatment in the neonates on the development of body weight was an interesting one. After capsaicin treatment, the body weights of 4 week old SHR and WKY were similar to those of the untreated ones (see Table 1). However, at 12 weeks the treated animals were lighter than the untreated. Since the intestinal wall contains a

large amount of substance P (314-a review), it is possible that the capsaicin pre-treatment in the neonates may produce a permanent impairment in the gastrointestinal tract of the treated animals. This impairment was not apparent before weaning (at 4 weeks of age) because the neonates were still dependent mainly on the milk from the mother rat.

B. Blood pressure.

The anti-hypertensive effect of capsaicin treatment on the mean arterial pressure of adult SHR and WKY was reported by Virus et al (304) and Scott and Pang (305). Scott and Pang (305) in the same report further demonstrated that capsaicin treatment in the neonates can prevent the development of hypertension in the SHR. At the age of 12 weeks, the mean arterial pressure of the SHR was significantly higher than that of the WKY after capsaicin treatment in the neonatal stage, although the mean arterial pressure of the SHR was by no means hypertensive. The mean arterial pressure of the 12-week old WKY after capsaicin treatment was hypotensive. In fact the mean blood pressure of capsaicin treated SHR (101.0 $\pm$ 5.2 mmHg) was comparable to that of the untreated WKY (91.2 $\pm$ 4.7 mmHg) at the age of 12 weeks (see Table 1).

C. Medial thickness and lumen diameter.

The results of morphometrical analysis of jejunal

arteries obtained from 12-week old SHR and WKY after capsaicin treatment showed that these arteries still developed medial hypertrophy. At the age of 12 weeks, jejunal arteries of SHR were 23% thicker than those of the WKY after treatment. Similarly, in the untreated animals, jejunal arteries of the SHR were 25% thicker than those of the WKY at 12 weeks of age (see Table 2). Although the mean arterial pressure of the capsaicin treated WKY was hypotensive, the medial thickness of the jejunal arteries from these animals was comparable to that of the untreated WKY. The jejunal arteries of the capsaicin treated SHR had larger lumen and thicker media than those of the untreated SHR.

D. Significance of capsaicin study.

The finding that the anti-hypertensive effect of capsaicin treatment in the SHR did not prevent the development of medial hypertrophy obtained from the present study is different from those of Mulvany et al (301) and Warshaw et al (315) who showed that anti-hypertensive agents such as Felodipine (a pyridine derivative), reserpine, hydrochlorothiazide and hydralazine can lower arterial blood pressure as well as reduce medial hypertrophy in mesenteric arteries of the SHR. In the latter cases, in order to have an effective treatment, the therapy has to be administered repeatedly so that the concentration of the drug is maintained in the animal for

the whole treatment. Such a lengthy exposure of the anti-hypertensive agent to the artery of interest is very likely to produce a direct effect on the structure of arterial wall.

It has been shown by light and electron microscopy that capsaicin treatment on neonates results in degeneration of a distinct population of primary sensory neurones in the central nervous system of the rat (303, 316). However, there were no signs of degeneration of either myelinated or unmyelinated fibres in sciatic nerve of adult rats after local application of capsaicin (317). These results strongly suggested the effect of capsaicin is mainly central.

Although the direct effect of capsaicin on the structure of jejunal arteries has not been reported, it is unlikely that this "damage", if any, will have a long term effect on the arteries, especially when the analysis was performed 12 weeks after the capsaicin treatment. Saria et al (318) reported that 17 hours after subcutaneous injection of capsaicin, there was only a trace amount of the drug detectable in the blood.

Reports by others (303, 304, 316, 317) have demonstrated that there was no undesirable effect produced by injection of vehicle alone. Thus, it is unlikely that the injection of vehicle may have an effect on the wall structure of the jejunal arteries. As pointed out earlier,

the present study was not designed to investigate the action of capsaicin on jejunal arteries, but rather to use capsaicin as a tool to study the effect of lowering of blood pressure on the structure of jejunal arteries of the SHR. Thus, even if the anti-hypertensive effect of capsaicin treatment was due to the vehicle, the conclusion made from this study would not be altered.

V. Overview and overall conclusion of the present investigation.

The close relationship between elevated arterial pressure and medial hypertrophy in experimental hypertension has long been recognised. Although the exact mechanisms whereby the hypertension in 2-kidney-1-clip Goldblatt hypertensive rats is developed is not clear (311), it is generally agreed that alterations such as medial hypertrophy in arteries of these rats are a result of the increase in arterial pressure (312). The discovery of the SHR in the early 1960's provided an interesting model for studying the cause and effect relationship between elevated arterial pressure and arterial alterations in a naturally occurring situation. Studies of this strain may provide insights into the mechanisms involved in the pathogenesis of hypertension in the SHR.

From the present study (Table 1 & 2), and from other reports (263, 264), it is clearly demonstrated that medial

hypertrophy of mesenteric arteries does occur in the SHR, and that thhe development of hypertension and arterial hypertrophy in these arteries are closely related. These studies however do not distinguish between the influence of arterial pressure and genetic factors on the arterial wall in the development of medial hypertrophy in SHR arteries. It has been suggested that the latter may be one of the factors involved in the initiation and/or maintenance of hypertension in the SHR (161, 217).

When the morphometric data of unoperated and transplanted arteries are compared it appears that a general increase in medial thickness and lumen diamter occurred in transplanted arteries. Despite these increases, which may have been a direct result of transplantation, the SHR arteries transplanted into WKY hosts did not thicken, when compared to similarly transplanted WKY arteries into WKY hosts suggesting that intrinsic genetic factors in the arterial wall cannot dictate the development of medial hypertrophy in these arteries.

Although the influence of other factors such as circulating substances (195, 196), and innervation (319, 320), have not been considered, the fact that transplanted WKY arteries developed medial hypertrophy in SHR hosts suggested that this alteration in WKY arteries may be related to the higher arterial pressure of the SHR. Indeed, the renal hypertension study provided evidence that medial

hypertrophy can be induced in jejunal arteries of genetically normotensive WKY. However the antihypertensive effect of capsaicin treatment did not prevent the development of medial hypertrophy in jejunal arteries of the SHR, indicating that this change is not related to the increase in arterial pressure.

Due to various problems associated with both the transplant and the capsaicin study, as outlined earlier, it is still premature to draw the final conclusion on the causes of medial hypertrophy in jejunal arteries of the SHR. Overall, the results of the present investigation do suggest that the development of medial hypertrophy is not related to intrinsic genetic factors in the walls of SHR arteries, and that the increase in arterial pressure is not a prerequisite for the development of medial hypertrophy in SHR arteries. This is in accordance with the conclusions of Hermsmeyer and collaborators (319, 320). By transplanting segments of tail artery into the anterior chamber of eyes between SHR and WKY, they demonstrated that alteration in vascular smooth muscle membrane properties of the SHR is dependent on the reinnervation from the hosts rather than from the original arteries. The second conclusion from the present study further supplements the results of the latter studies which did not take arterial pressure into consideration, in that elevated arterial is not one of the determinants for the development of medial hypertrophy in SHR arteries.

The results of the present investigation support the hypothesis that the alteration in SHR arteries is a result of some factors which exert trophic influences on arterial structure (319). The finding that denervation can influence both the functional and structural properties of arteries in experimental hypertensive rabbits (223) also suggests that these trophic factros probably originate from the adrenergic innervation of SHR arteries.

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# APPENDICES.

## Appendix A : Jejunal arteries of the rat.

### I. Latex injection study.

Latex injection was used to establish the branching pattern of jejunal arteries in the rat. Animals from each strain of SHR and WKY, were anaesthetised with sodium pentobarbital. Latex (Vultex, General Latex Ltd.) was injected into the animal through the heart; prior to the latex injection, the animal was perfused with warm saline to wash out the blood.

After the latex was set, the mesenteric vascular bed including the part of the abdominal aorta from which the superior mesenteric artery originated, was excised for further dissection under an operating microscope. The small arteries which branched from the superior mesenteric artery were dissected free of all periarterial tissues; the small intestine was then removed by cutting the small arteries at the position nearest to the mesenteric side of the gut.

The results reported in this study were based on 10 dissections, 6 from WKYs and 4 from SHRs. Besides giving rise to a pancreatico-duodenal, a colic (a. colica dextra) and an ileo-caeco-colic branch, there were 14-16 small arteries which branched ffom the superior mesenteric artery (Figure 13). Often another colic branch (a. colica media) was found branching off from the superior mesenteric artery before giving rise to the caudal pancreatico-duodenal

artery. Although there is no unity of definition, according to Hebel and Stromberg (321) the jejunum in the rat is the longest segment of the small intestine. Thus, the 14-16 small arteries which branched from the superior mesenteric artery were termed jejunal arteries. This definition is different from that of Furness (322) and of Wei et al (323).

There were no differences observed between the branching pattern of jejunal arteries of the SHR and WKY.

The first jejunal branch was defined as the artery which supplied the segment just distal to the emergence of the small intestine from a retroperitoneal position. It was discovered that the bifurcation pattern of the first 3-6 branches of jejunal arteries was relatively consistent. Because of their location, it was decided that the third, fourth and fifth jejunal branches could easily be accessible and would be used for the present investigation.

II. Thickness of wall and diameter of lumen of jejunal arteries.

In order to determine the variation of wall thickness and lumen diameter of the jejunal artery along its length, jejunal arteries were processed for microscopical examination. Seven half-micron-thick sections were taken from the first 6 mm of the jejunal artery (one per mm) starting from its origin at the superior mesenteric artery.

The thickness of wall and diameter of lumen of jejunal artery in each section were then measured and compared consecutively. For example, the wall thickness of the first 6 mm of a jejunal artery from proximal to distal were 8.6, 7.9, 7.8, 7.8, 7.6, 7.7, 7.4 microns. Similarly, the diameter of lumen of jejunal arteries was quite constant at least up to 5 mm from its branching point at the superior mesenteric arteries. For example, the lumen diameter of the first 6 mm of a jejunal artery from proximal to distal were 342.2, 317.1, 315.2, 317.1, 316.5, 304.4 and 289.2 microns. Thus, it was decided that jejunal arteries would be sampled at about 5 mm from their origin.

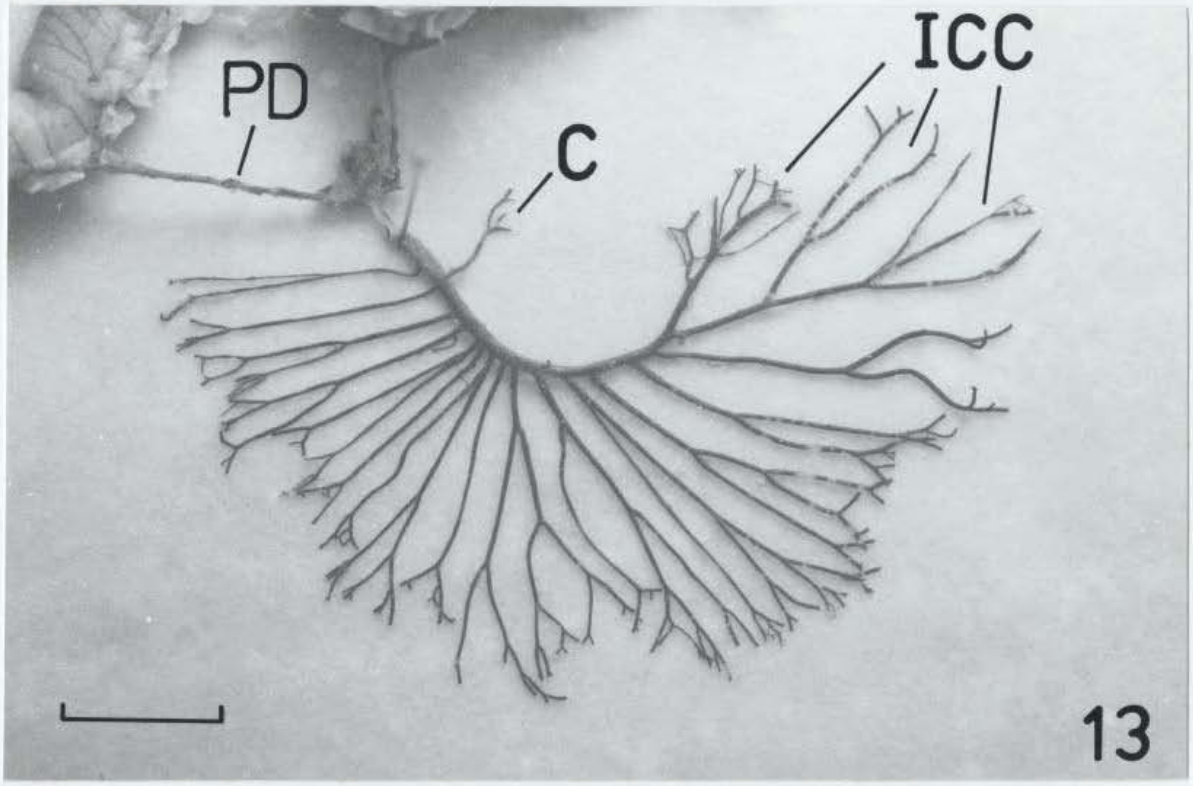



Figure 13. Branching pattern of the superior mesenteric artery in the rat.

Besides giving rise to a pancreatico-duodenal (PD), a colic (C) and an ileo-caeco-colic (ICC) branch, there are 14-16 jejunal arteries which branch from the superior mesenteric artery (321). Latex injected. Adult WKY. Calibration bar = 1 cm.

## Appendix B : Fixation technique.

Perfusion fixation has been used in studying large arteries such as the abdominal aorta and renal artery of the rat (168). In order to determine the suitability of the established perfusion technique on the fixation of the jejunal arteries, the following studies were performed.

I. Perfusion pressure.

From previous study (168), it was demonstrated that the fixation on vascular tissues was satisfactory with half strength Karnovsky fixative delivered by a syringe pump to the animals through the heart at 80 mmHg. However, blood clots were frequently found in the mesenteric bed when these vessels were prefusion-fixed at this pressure. Thus, various perfusion pressures were tested and it was discovered that no blood clot was found in the mesenteric vascular bed when the perfusion pressure was higher than 120 mmHg; the latter was measured through a femoral arterial cannula. Preservation of arterial tissues was also satisfactory under this pressure. It was decided then to fix jejunal arteries at 120 mmHg perfusion pressure in the present investigation.

II. Perfusion fixation at different age.

The present investigation involved studies of animals at various age groups. It is well known that peripheral resistance changes with age. In order to standardise the

perfusion pressure used, WKYs of 4, 8, 12 and 20 weeks of age were studied. Animals were first cannulated through the femoral artery for continuous pressure recording, and perfusion fixation was employed as before. The fixative was delivered by a syringe pump at various flow rate. By adjusting the speed of the syringe pump, the flow rate can be determined for animals of different age so that the pressure through the femoral arterial cannula remained at 120 mmHg. The results were summarised in Figure 14 and applied in the present investigation accordingly.

III. Perfusion fixation with vasodilator.

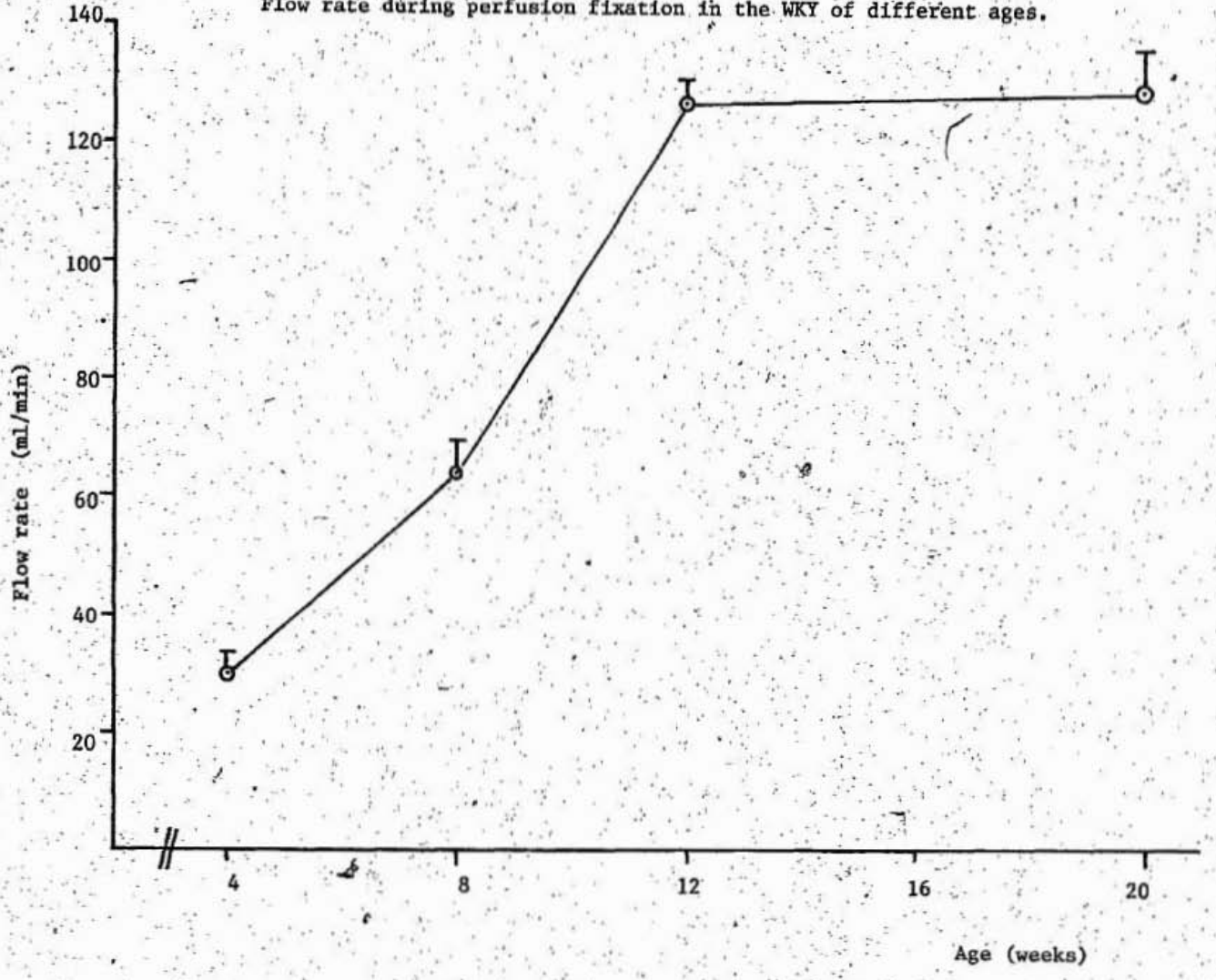
To determine whether or not a vasodilator may improve the perfusion fixation, papaverine was used according to the method reported by Folkow et al (324). The morphometric data of jejunal arteries perfusion-fixed at 120 mmHg pressure with or without papaverine treatment appeared to be similar. The wall thickness and lumen diameter of jejunal arteries in adult WKY (2 animals per group) were 13.5 and 406.7 microns respectively with papaverine treatment, and 14.0 and 389.6 microns respectively without papaverine treatment; these figures were not significantly different from each other. Thus, it was determined that vasodilator treatment does not improve the perfusion-fixation technique and is not necessary in the present investigation.

Figure 14. Flow rate during perfusion fixation in the WKY of different ages.

Animals were first cannulated through the femoral artery for continuous pressure recording, and perfusion fixation was achieved by half strength Karnovsky fixative. The fixative was delivered by a syringe pump at various flow rate. By adjusting the speed of the pump, the flow rate was determined for animals of different ages, so that the pressure through the femoral arterial cannula remained at 120 mmHg.

Each value (mean ± S.D.) was obtained from 3 rats.

Flow rate during perfusion fixation in the WKY of different ages.



## Appendix C : Stereological technique.

### I. Theoretical consideration.

The development of stereological methods in cytology was based on the Delesse Principle (325-cited in 307) 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 (326-cited in 307), Chalkley (327-cited in 307) and Attardi (328-cited in 307) 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,

i.e. $V_x/V_t = P_x/P_t$;

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.

### II. Sampling grid.

The sampling grid used in the present study was a 10 by 10 regular point lattice which conveniently gave direct percent values for volumetry and was modified from Weibel et al (307) to fit the 8 X 10 inches photographic paper on which the electron micrographs were printed.

III. Number of micrographs used in stereological analysis.

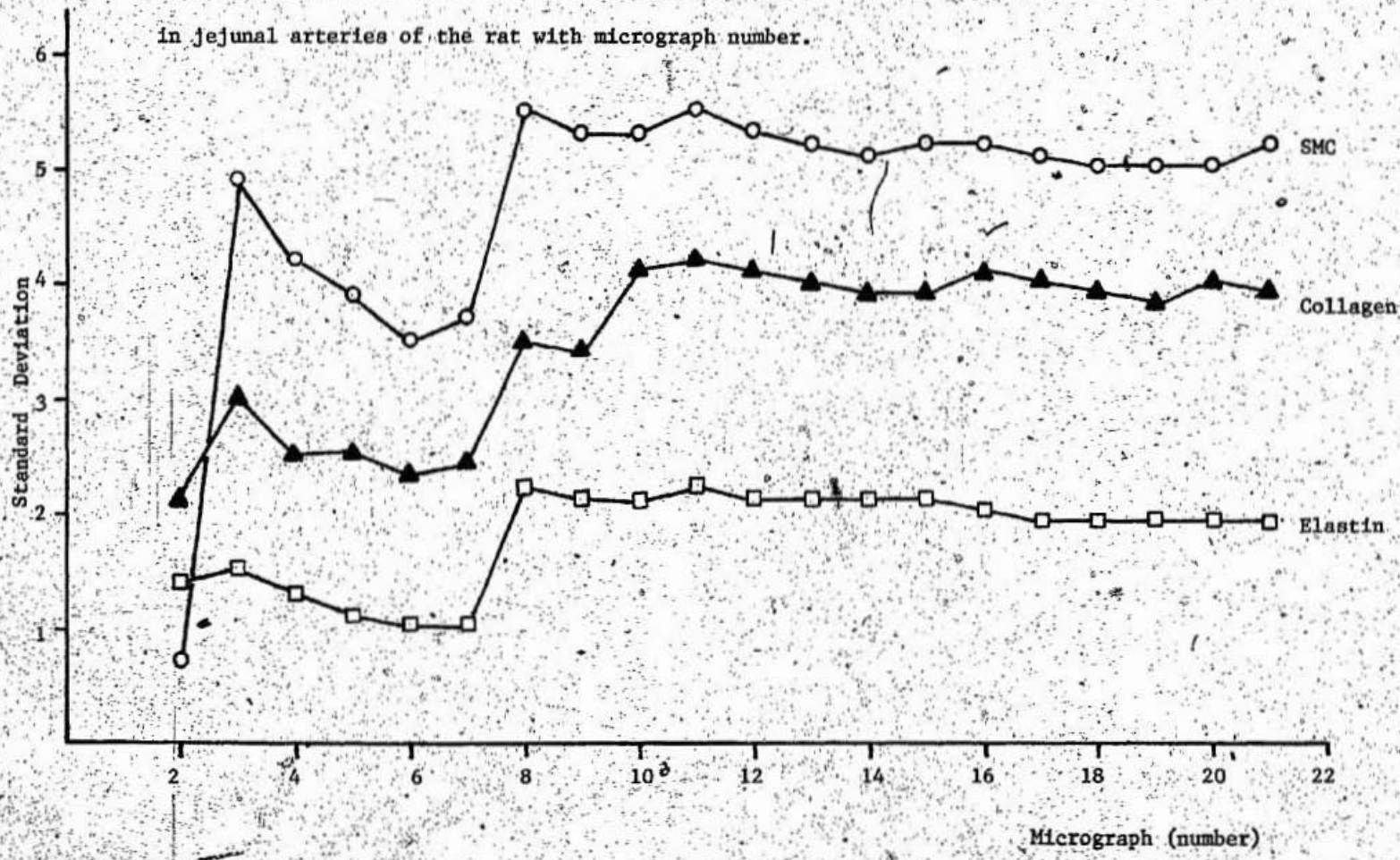
Since the medial components in the artery are organised into lamellar units, random sampling technique used in the present study may create high variation in the estimates. If the number of micrographs used for estimation is few, this variation can be very large or very small, as compared with the "true" value; 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 stabilized was determined by plotting the number of micrographs against the standard deviation of the estimates (Figure 15). The standard deviation of the estimate of SMC, collagen and elastin reached their plateau at about 10. Thus, 10 micrographs obtained from each specimen were used for stereological analysis in the present study.

IV. 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 at the magnification of 35,000 times.

Figure 15. The variation of standard deviation of volume percent of medial components in jejunal arteries of the rat with micrograph number.
The volume percent of SMC, collagen, and elastin was estimated by a 100-point sampling grid and the standard deviation was calculated with 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 in the graph, the standard deviation of the estimates of SMC, collagen, and elastin reaches its plateau at about 10.

The variation of standard deviation of volume percent of medial components in jejunal arteries of the rat with micrograph number.



## Appendix D : Preliminary study on arterial transplantation.

### I. Arterial transplantation.

In order to determine whether or not arterial transplant is a feasible model for studying morphological alterations of the vascular system in the SHR, jejunal arteries were transplanted between the SHR and WKY. Operative procedures were identical to those described in the transplant study.

Animals were killed 1-4 weeks after surgery and the transplanted arteries were processed for light and electron microscopy.

### II. Morphology of transplanted arteries.

Out of the 21 transplanted arteries, 14 were patent. These transplants showed a hugh variation in morphology, ranging from small to large luminal diameter as well as various degrees of vascularisation in the tunica adventitia. Thus, a list of criteria, as described earlier, was set up to eliminate some of these variations.

One week after the surgery, the intima of the transplanted artery contained many layers of cells, some of which were smooth muscle-like (Figure 16). The inter-cellular space also increased, thus the intima appeared to be thickened. By the third week, the intima contained a single layer of endothelial cells; these cells appeared to

be immature. Monocytes were sometimes seen penetrating through the intima (Figure 17).

The smooth muscle cells of the media first became disorganised with an increase of inter-cellular space. Occasionally, "dark" cells were seen in light microscopy; with the electron microscope, these cells appeared to be immature smooth muscle which contained fewer myofilament bundle (Figure 16). By the third week, the media resumed its normal appearance; the medial smooth muscle cells were mature and aligned in a classical helical or circular fashion (Figure 17).

Fibrin strands and other debris were prominent in the adventitia of the transplanted artery one week after the surgery; macrophages were also plentiful (Figure 18). By the third week after the surgery, a mature granuloma was formed surrounding the transplanted artery, thus the adventitia was filled with mononuclear phagocytes (Figure 17). The granulomatous inflammatory response then seemed to subside and vascularisation around the transplanted artery was prominent (Figure 19).

From the results of this preliminary study, the transplanted arteries seemed to survive well in the recipient rats, at least up to 4 weeks postoperatively. Thus, it was concluded that this preparation is a feasible model for studying morphological alterations of the vascular system in the SHR.

Figure 16. Jejunal artery, one week after transplantation.

a. Vessel wall of the transplanted artery. LM. Calibration bar = 20 microns.

b. Smooth muscle-like cells in the intima of the transplanted artery. EM. Calibration bar = 5 microns.

c. Immature smooth muscle cell in the media of the transplanted artery. EM. Calibration bar = 5 microns.

Abbreviation: I = intima; M = media; A = adventitia; L = lumen; E = endothelium; SMC = smooth muscle cell; ↑ = immature smooth muscle cell.

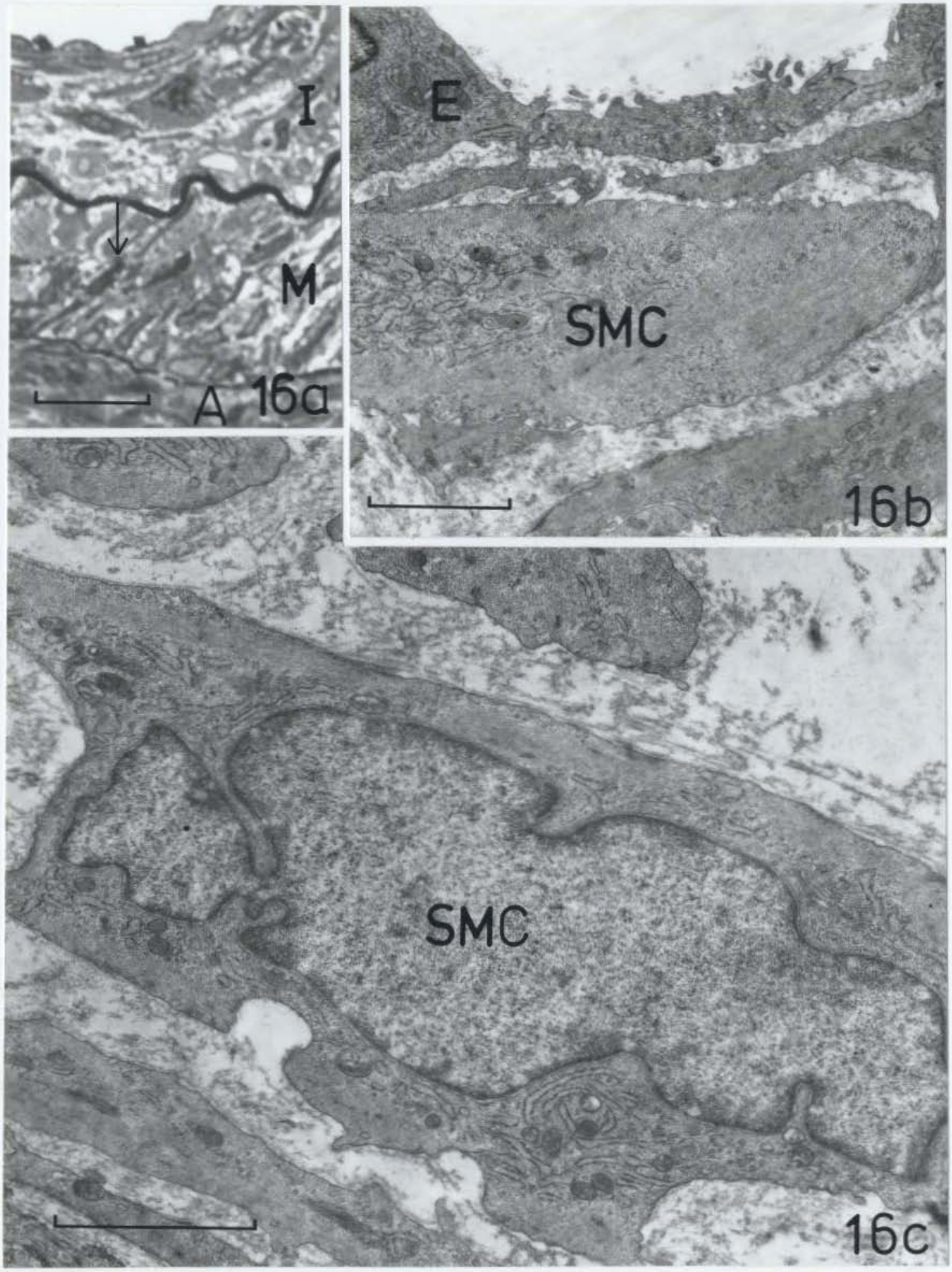

I
M
A
16a
E
SMC
16b
SMC
16c

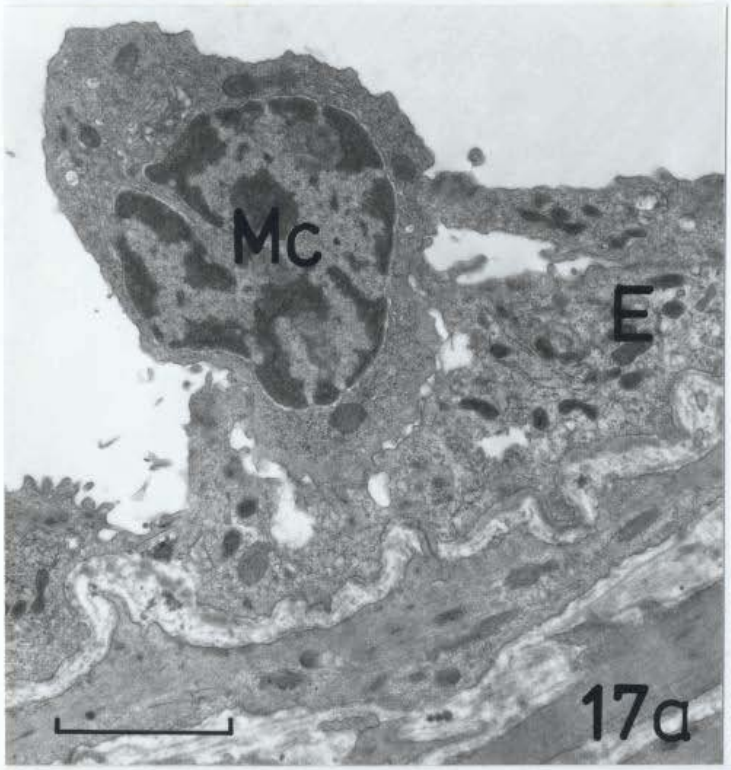
Figure 17. Jejunal artery, 3 weeks after transplantation.

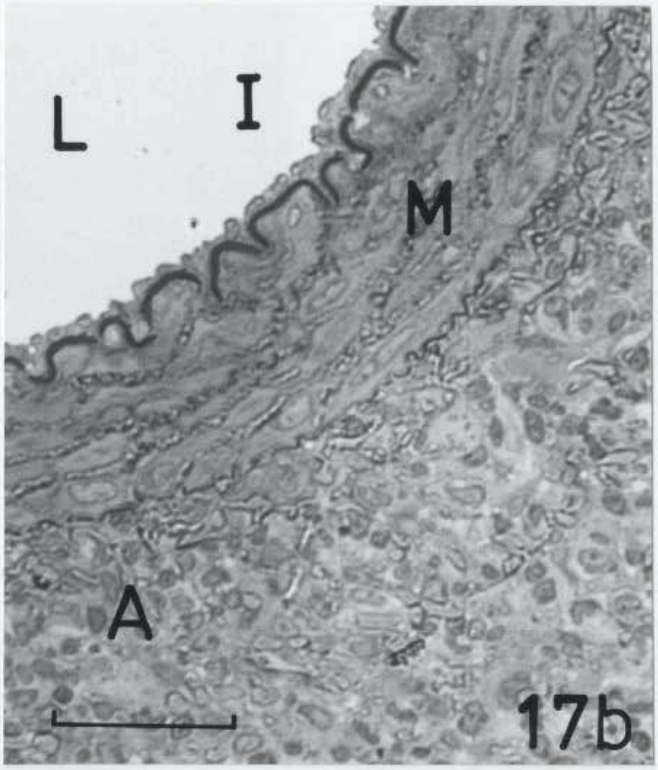
a. Monocyte infiltration through the intima of the transplanted artery. EM. Calibration bar = 4 microns.

b. Vessel wall of the transplanted artery. LM. Calibration bar = 20 microns.

c. Mature smooth muscle cell in the media of the transplanted artery. EM. Calibration bar = 2 microns.

Abbreviation: I = intima; M = media; A = adventitia; L = lumen; E = endothelium; SMC = smooth muscle cell; Mc = monocyte.


Mc
E
17a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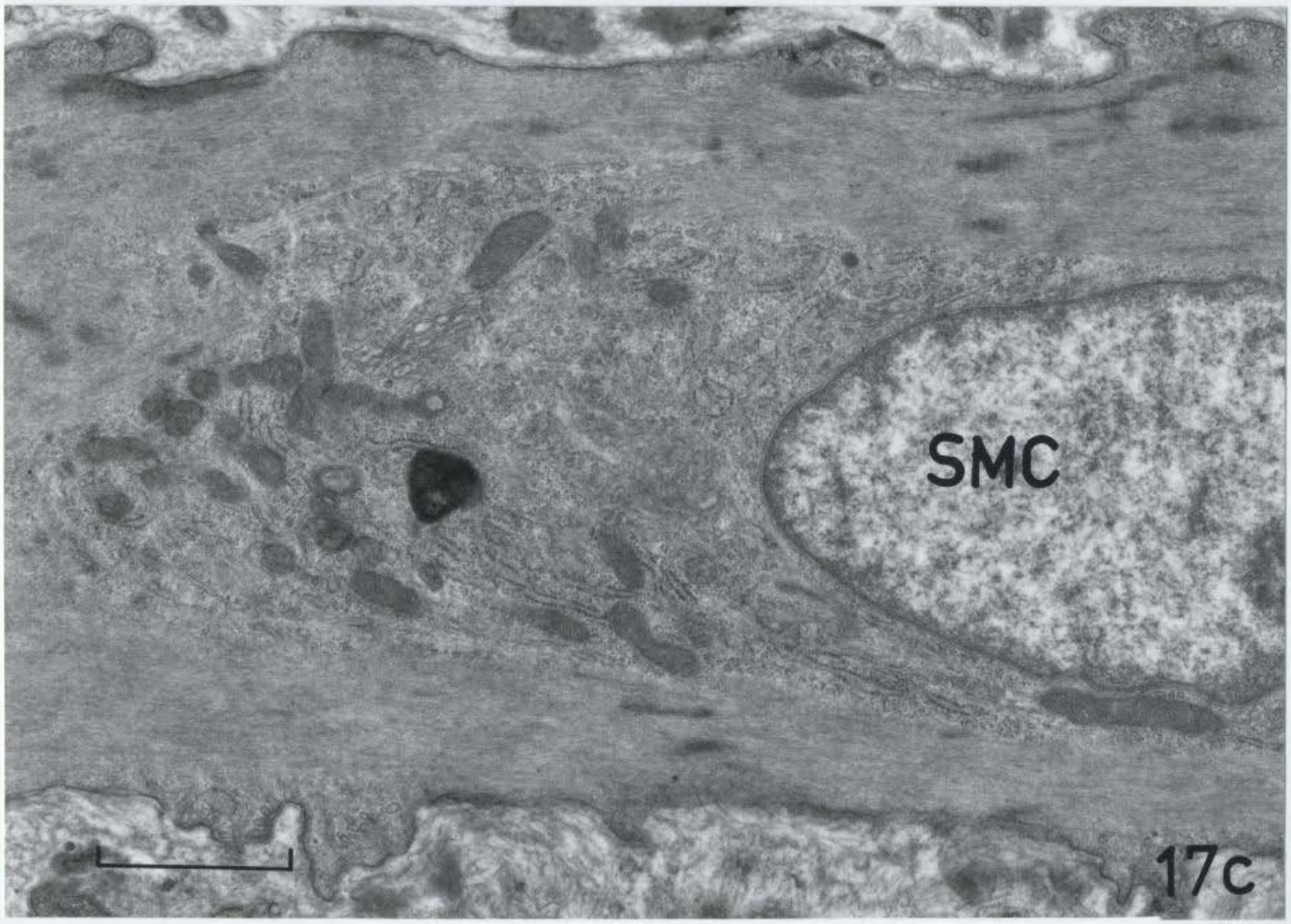
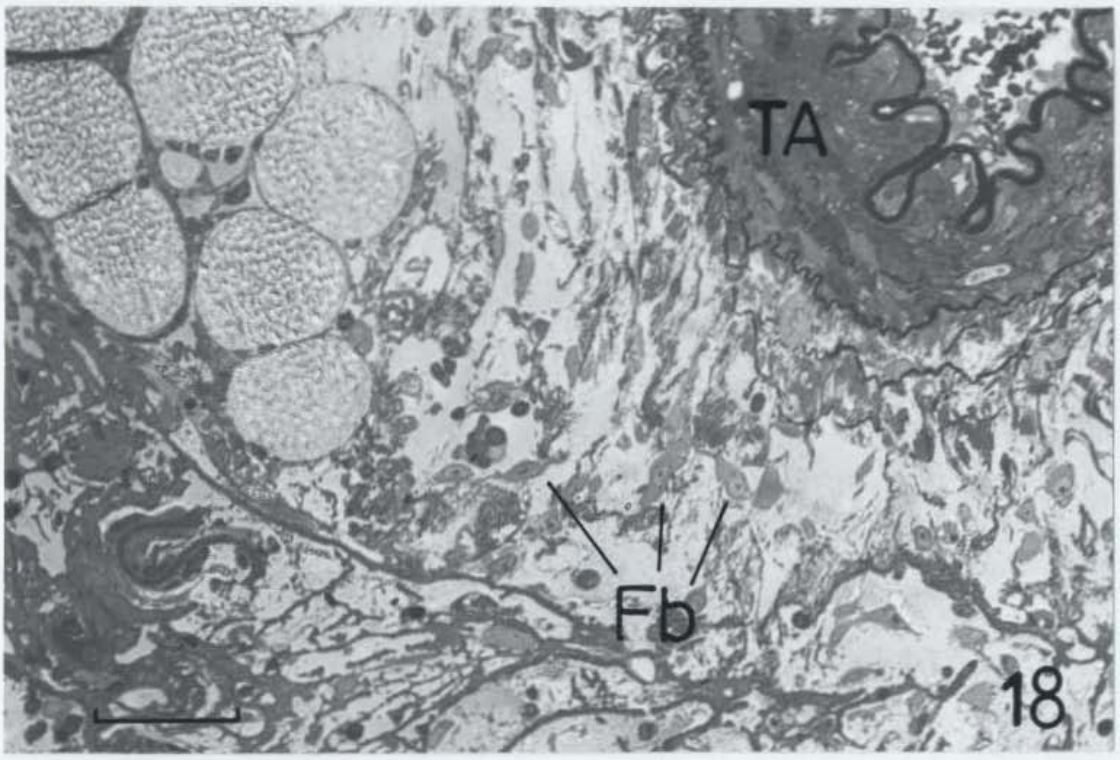
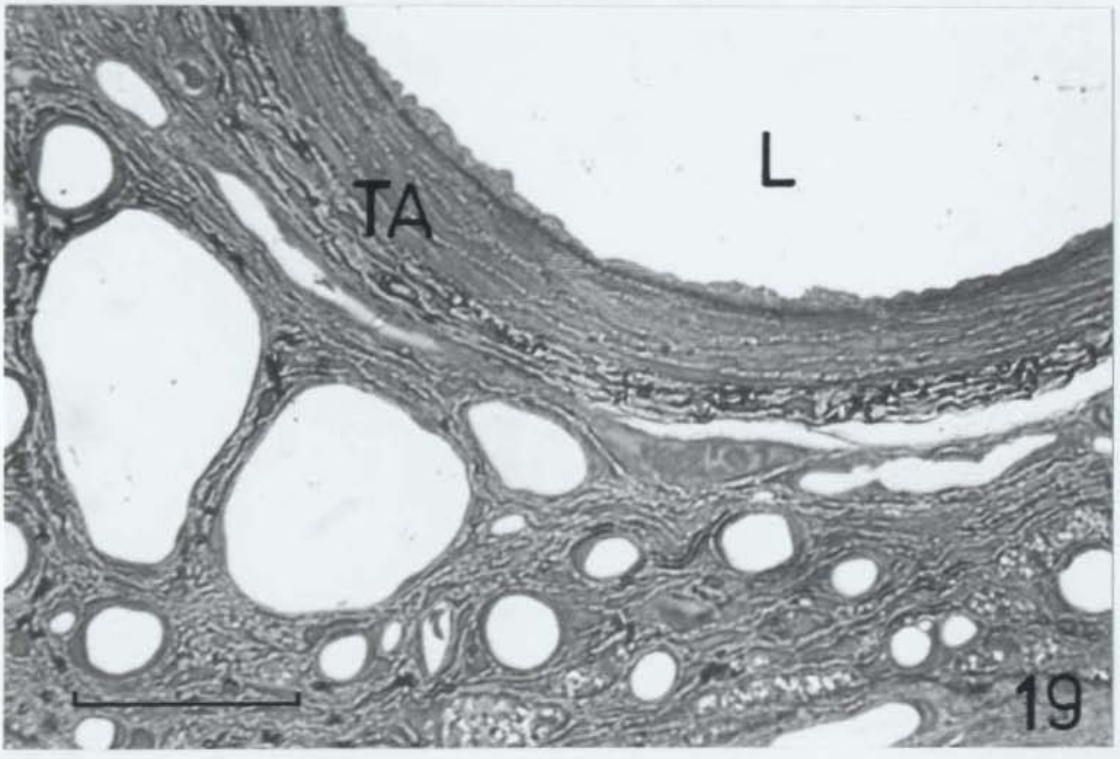

SMC
17c

Figure 18. Jejunal artery, 3 days after transplantation. Many fibroblasts were present in the adventitia of the transplanted artery. LM. Calibration bar = 50 microns.

Figure 19. Jejunal artery, 4 weeks after transplantation. Vascularisation was prominent in the adventitia of the transplanted artery. LM. Calibration bar = 40 microns.

Abbreviation: Fb = fibroblast; TA = transplanted artery; L = lumen.


TA
Fb
18


TA
L
19

## Appendix E : Statistical analysis.

### I. Student's "t" test.

Results from the confirmation study, the renal hypertension study and the capsaicin study were analysed by a 2-tailed Student's "t" test at a significance level of 5%. The procedures were demonstrated in the following example.

The mean arterial pressure of 8-week old rats were 119.67, 116.33, 116.67 and 127.33 mmHg for the SHRs (denoted by X) and 85.00, 84.67, 86.67 and 83.00 mmHg for the WKYs (denoted by Y).

To test whether or not the mean arterial pressure of the SHR was significantly different from that of the WKY, the following procedures were used:

a. Set the hypotheses.

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

and the alternative hypothesis $H_A : \overline{X} = \overline{Y}$.

b. Obtain "t" value from table.

In this case, $t_{0.025, 6} = \pm 2.447$.

That is, the null hypothesis will be accepted when the calculated "t" values are within the interval from -2.447 to +2.447. Thus, if the calculated values are either smaller than -2.447 or larger than +2.447, the null

hypothesis will be rejected and the alternative hypothesis accepted.

c. Calculated values.

The mean of X, or $\bar{X} = 120.00$;
and the mean of Y, or $\bar{Y} = 84.84$.

The value of "t" was calculated from the formula:

$$t = \frac{\bar{X} - \bar{Y}}{\sqrt{\frac{1}{n_1} + \frac{1}{n_2}} \sqrt{\frac{\Sigma X_i^2 - n_1 \bar{X}^2 + \Sigma Y_i^2 - n_2 \bar{Y}^2}{n_1 + n_2 - 2}}}$$

and in this case $t = +13.20$.

Since the calculated "t" value lies beyond +2.447, the null hypothesis is rejected and the alternative hypothesis accepted. That is, the mean arterial pressure of the SHR is significantly higher than that of the WKY.

II. Student-Newman-Keuls' multiple-range test.

To compare the results of 4 groups of operated animal in the transplant study, Student-Newman-Keuls' multiple-range test was used. The procedures used were as follows:

a. Calculate the mean value for each group.

For example, the mean arterial pressure of 8-week old rats, 4 weeks after the surgery was:

| | WKY to WKY | SHR to WKY | SHR to SHR | WKY to SHR |
|---|---|---|---|---|
| | 81.67 | 83.33 | 110.00 | 106.67 |
| | 86.67 | 81.67 | 106.33 | 106.67 |
| | 80.00 | 83.33 | 101.67 | 101.67 |
| | 81.67 | 88.33 | 101.67 | 105.00 |
| mean | 82.50 | 84.17 | 104.92 | 105.00 |

b. Rank the means in a descending order.

| WKY to SHR | SHR to SHR | SHR to WKY | WKY to WKY |
|---|---|---|---|
| 105.00 | 104.92 | 84.17 | 82.50 |

c. 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) $^{1/2}$.

1. $q_a\,(p, n_2) = q_{0.05}\,(4, 12)$ , in the present study was determined from the table of "the upper percentage points of the 'Studentised' range" (308).

2. $S_{\bar{x}}$ was calculated from the formula

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

where r = number of animals in each group ( =4);

error mean square = error sum of squares / 12.

3. Error sum 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 16.

In the present example the $S_{\bar{x}} = 4.82$;

and $q_{0.05}(4, 12) = 4.20$;

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

$= 4.20 \times 4.82$

$= 20.25$

The difference between two means can be either greater than 20.25, when they are significantly different from each other, or smaller than 20.25, when they are not significantly different from each other, at a significance level of 5%.

From the data presented in this example, the following comparisons can be made:

WKY to SHR vs SHR to SHR = 0.08;

WKY to SHR vs SHR to WKY = 20.83;

WKY to SHR vs WKY to WKY = 22.50;

SHR to SHR vs SHR to WKY = 20.75;

SHR to SHR vs WKY to WKY = 22.42;

SHR to WKY vs WKY to WKY = 1.67.

Thus, it can be concluded that the mean arterial blood pressure of the SHR hosts is significantly higher than that of the WKY hosts; there is no significant difference between the mean arterial pressure of the hosts of the same strain.