A STUDY OF THE TROPHIC INFLUENCE OF THE SYMPATHETIC INNERVATION ON VASCULAR SMOOTH MUSCLE IN NORMAL AND DISEASE STATES

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A study of the trophic influence of the
sympathetic innervation on vascular smooth
muscle in normal and disease states

by

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ABSTRACT

While there are probably many factors which influence the structure of blood vessels, recent evidence has suggested that one of the main influences is exerted by the innervation.

It has been suggested that the innervation of vessels plays a large part not only in vasoconstriction, but also in the growth and maintenance of vessel walls. This trophic influence of nerve on muscle is well documented in skeletal muscle, but less so in the case of vascular smooth muscle. The present study was designed to examine this trophic relationship between the catecholaminergic innervation and the structure of the media of jejunal arteries during normal development and in disease states in which the arterial wall structure is known to change, and in an experimental model in which the innervation has been altered.

The wall thickness, lumen diameter (and subsequently medial smooth muscle cell mass) and number and disposition of fluorescent sympathetic fibers were determined in varied sets of rats sampled at twelve weeks of age. These sets of rats included normal developing Sprague Dawley rats, spontaneously hypertensive rats (SHR), SHRs treated with capsaicin to lower blood pressure to normotensive levels, Wistar-Kyoto rats (WKY) treated with capsaicin, normal WKY rats, Sprague Dawley rats treated with streptozotocin to induce

diabetes, Sprague Dawley rats chemically sympathectomized
with 6-hydroxydopamine (6-OHDA), and Sprague Dawley rats
with renal artery constrictions to induce hypertension.

The results of the study indicate that an increase
in the medial smooth muscle cell mass observed in the SHR
strain of rat is the result of a sympathetic hyper-
innervation separate from the influence of an elevated
arterial pressure. Furthermore this trophic effect did not
extend to the normal state nor to experimentally induced
disease states.

iv.

ACKNOWLEDGEMENT

TABLE OF CONTENTS

This thesis is dedicated to my parents and

to my daughter Kiera

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

I. Overview of the cardiovascular system

The overall function of the vascular component of the cardiovascular system is to transport nutrient and other materials to and from various organs in order to fulfill metabolic requirements. The heart provides the energy for this circulation and the blood vessels are the channels through which it takes place. The force of cardiac contraction drives the blood to the tissues through thick walled vessels, the arteries, and back to the heart by a system of thinner walled vessels, the veins. In the tissues, the blood is driven through a fine meshwork of vessels, the capillaries, the walls of which consist of a single layer of cells which allows interchange of materials between blood and the tissue fluid.

The structure of the peripheral vasculature is adapted in various regions to local functions. The large diameter elastic arteries are able to adjust to a wide range of intraluminal pressures while the thick-walled muscular arteries distribute blood at a high pressure. Further "downstream" are the arterioles. Their lumens typically measure from 50-100 microns (Rhodin, 1962; Short, 1966); however, they possess a specially adapted ability to vary this diameter to altering peripheral resistance and thus contribute greatly to the overall control of blood pressure.

In the hypertensive human as well as the spontaneously hypertensive rat (SHR) there are both structural and functional changes in blood vessel walls which have been suggested to be contributing factors to the elevated blood pressure seen in these two species (Folkow, Hallback, Lundgren and Weiss, 1969; Folkow et al, 1970; Folkow, Hallback, Lundgren, Silvertsson and Weiss, 1973; Folkow and Hallback, 1977; Pfeffer, Frolich, Pfeffer and Weiss, 1974; Tobia, Lee and Walsh, 1974; Ichiyima, K. 1967).

The normal histological structure of the vascular component of the cardiovascular system has been studied by a number of investigators (Pease and Molinari, 1960a, 1960b; Rhodin, 1962). This structure follows a general plan although the relative thickness and proportions of the various tissue elements will vary in relation to both the type of vessel (eg. whether muscular or elastic) and its calibre.

Three distinct layers or tunics can be identified in normal tissue. The inner layer, tunica intima, is comprised of a continuous sheet of endothelial cells separating the blood vessel wall from the plasma and an internal elastic lamina separating the intima from the middle layer (the tunica media). An additional subendothelial layer situated between the internal elastic lamina and the endothelial cells contains a dense connective tissue matrix with occasional smooth muscle cells (Morse, 1979).

The diagonally oriented smooth muscle cells of the medial layer are surrounded by collagen, some elastin and a

ground substance comprised mainly of mucopolysaccharides.
The smooth muscle cells are arranged in several circular or
diagonal layers. Their spatial relationship with each other
and with the nearby endothelial cells of the intima will
vary according to the type of vessel. The outermost border
of the media is bounded by external elastic lamina. A sleeve-
like adrenergic neural plexus surrounds and sometimes pene-
trates this muscle layer. The relationship of the noradrener-
gic innervation to the structure of the blood vessel will be
considered in greater detail in a later section.

The outer layer (tunica adventitia) consists of
fibroblasts and a few smooth muscle cells intermingled bet-
ween bundles of longitudinally arranged collagen fibrils.
The size of the lumen is dependent on a number of factors,
including vessel size and degree of vasoconstriction in
normal humans and control animals. Medial hypertrophy and
or hyperplasia will reduce lumen diameter in vessels from
hypertensive humans and SHRs (Nordborg and Johansson,
1978; Bevan and Tsuru, 1981).

This is the basic model that can be employed in
descriptions of the structure of the various vessel types.
Again, differences in the various components within tunics
is one variable which gives each vessel type its identity.
Each will now be considered in a general structural overview.

Elastic arteries

Elastic arteries are largest in calibre and conduct
blood from the heart to the muscular arteries and therefore
must be able to cope with a wide range of intraluminal
pressures. Typically these arteries are thin walled with large
lumens and possess a high content of fenestrated elastic
fibers with virtually no smooth muscle (Morse, 1979). In
humans the intima is relatively thin. Cells are joined by
an organized arrangement of tight and gap junctions. The
internal elastic lamina in these arteries has been observed
to fuse with the tunica media which is the widest tunic
comprised of as many as 70 elastic sheets interspersed with
ground substance and a few elongated smooth muscle cells
(Wolinsky and Glasgov, 1969). Total thickness of the
media in elastic arteries may measure up to 1 mm in the
adult human. The adventitia is typically comprised of
collagen bundles, elastin, fibroblasts, vasa vasorum and
rare longitudinal smooth muscle cells.

Muscular arteries

The principal difference in muscular as compared to
elastic arteries involves the rapid change to a well-developed
muscular component of the arteries with a corresponding
extensive loss of the elastic component. In descriptions
of human muscular arteries Rhodin (1970) and Ham (1974)
reported a large variation in diameters of muscular arteries.

Although no recognized anatomical boundaries exist muscular arteries are generally subdivided into large, medium-sized and small arteries. No appreciable differences have been described in the intima of elastic vs. muscular arteries. As in the case of the elastic arteries the basal lamina is thin and the subendothelium is markedly and continuously reduced as a function of decreasing diameter. As many as 40 helical layers of diagonally arranged smooth muscle cells interspersed between collagen and elastic fibers have been described as comprising the media of the larger muscular arteries (Schatman and Becker, 1977) although the number of layers is again a function of vessel size. The adventitia of muscular arteries is thick in comparison to the larger arteries containing vasa vasorum. Lymphatics and nerve fibers of the adventitia generally terminate at the medial boundary (Burnstock, 1975; Morse, 1974).

Microvascular components

As previously mentioned the arterioles are specially adapted in their ability to alter lumen diameter as a function of sympathetic drive.

In view of the relevance to the present study the arterioles will be considered in some detail. In an exhaustive study devoted to elaborate descriptions of the microvasculature of the rabbit medial thigh muscles Rhodin (1967) divided the microvasculature into three groups in order of decreasing diameter; arterioles (50-100 microns),

terminal arterioles (<50 microns) and precapillary sphincters (approximately 10 microns).

Arterioles tended to run parallel with venules while terminal arterioles bifurcated at right angles to the arterioles, following courses independent of smaller venules.

The smooth muscle cells of the media of the arterioles were spindle shaped, all characteristically arranged in a circular fashion between the internal and external elastic laminae. Layers of smooth muscle cells gradually decreased as a function of decreasing arteriolar diameter although exceptions to this observation were present. Cells averaged 30-40 microns in length in arterioles with typical widths of 5 microns. Within the media interspersed between the smooth muscle cells, collagen fibril bundles and isolated fibrils were observed. Cells were surrounded by a thin basement membrane although neighbouring cells sometimes penetrated basement membranes of adjacent cells.

The endothelial cells of the arterioles occur as flat cells, about 2 microns, with lengths averaging approximately 50 microns, that formed a single fenestrated sheet with overlapping end-to-end contacts. As a rule the intima exhibited a thin basal lamina and at least 2 thin basement membranes separating the intima from the media. A continuous layer of elastic components were also described between the endothelial cells and the media. Elastic laminae are, however, absent in some species (Fernando and Morat, 1964).

The adventitial layer of these arterioles usually contain loosely arranged collagen fibril bundles and an occasional spindle-shaped fibroblast. Some of the fibroblasts, termed veil cells, were flattened and elongated in Rhodin's (1968) study. Occasionally also, mast cells and macrophages were described.

Terminal arterioles

The intima of terminal arterioles is arranged in a manner which is basically similar to that seen in the larger arterioles. No fenestrations are present in the endothelial sheet; however, there is a prevalence of pinocytotic vesicles occurring both at the luminal and basal surfaces of the intima. Filaments approximately 50 Å in diameter are common and occur in 0.1 micron bundles oriented longitudinal to the vessel (Albert and Nayak, 1976).

Elastic components separating the intima from the media in larger arterioles are absent in terminal arterioles. A basement membrane separates the endothelial cells from the medial smooth muscle cells although occasionally smooth muscle cells break through this basement membrane and form myo-endothelial junctions with the endothelial cells of the intima (Rhodin, 1967). The cell to cell contacts of junctions typically range from 0.5 micron to 1 micron in length, part of which will form a "tight" contact. Near these contact points bundles of filaments are always present.

Even though the smooth muscle cell is recognized to be by far the most important contributor to the control of arteriolar lumen diameter it is generally accepted that the endothelial cells of the intima are capable of performing the same function albeit to a lesser degree (Pascual and Bevan, 1980). It is probable that the filaments described by Rhodin (1976) may be similar in function to those described by Somlyo and Somlyo (1968).

Within the adventitia of terminal arterioles occasional fibroblasts and collagen bundles are generally present. The adventitia is much thinner than in the larger arterioles.

Precapillary sphincters

Precapillary sphincters have also been described by a number of early investigators (Sandison, 1931; Chambers and Zweifach, 1944; Wells, 1955). They are identified by their characteristic branching pattern at a right angle to the terminal arterioles and a circular arrangement of the smooth muscle cells now reduced to 1-2 layers, as distinct from the spirally arranged cells of the terminal arterioles. The endothelial cells of the intima are shorter, thicker and extend towards the luminal surface reducing lumen diameter. Both endothelial cells of the intima and smooth muscle cells of the media are surrounded by a basement membrane.

Myoendothelial junctions are present connecting endothelial cells with medial smooth muscle cells but are

more elaborate in precapillary junctions with increased
tight intracellular connections (Rhodin, 1967).

The adventitia is reduced in these vessels, but
richer in fibroblasts than larger arterioles.

Capillaries, venules and veins

Reviews similar to those cited describing arteries
and arterioles are present in the literature for capillaries, venules
and veins, and therefore it will not be reviewed here.

Fawcett (1959), Rhodin (1968) and Simionescu,
Simionescu and Palade (1974) provide excellent accounts of
the fine composition of these structures.

Innervation of blood vessels

As a background to the present study the role of the
sympathetic nervous system in the innervation of blood ves-
sels will be considered. It is anatomically well established
that the sympathetic system has many preganglionic fibers
which synapse both in the sympathetic chain as well as the
abdominal plexuses to subsequently innervate blood vessels
supplying the abdominal viscera (Gaskill, 1886). Because
of the small diameter and vast number of arterioles in this
region a large proportion of the total vascular resistance
can be attributed to these vessels. This total peripheral
resistance to blood flow is an important mechanism in the
overall maintenance of systemic arterial blood pressure.

Furthermore, the control of lumen diameter of these arteries and arterioles, and therefore peripheral resistance at any given time is controlled largely by the release of catecholamines (noradrenaline, adrenaline, dopamine) at the neuroeffector junction between the sympathetic postganglionic fibers and the contractile smooth muscle cells of the arterioles (Vanhoutte and Leusen, 1978). With the exception of umbilical and placental circulation, in most species, all blood vessels are innervated by adrenergic nerve endings (Bevan, 1979; Vanhoutte, 1980, 1983).

The axons of noradrenergic fibers, instead of ending in another synapse or a motor endplate terminate in an arrangement of bulblike varicosities usually at the medial/adventitial border and inner adventitia. This network of varicose fibers is devoid of Schwann cells (Bevan, 1979) and contain two types of spheroid to oblong, dense-cored vesicles, one approximately 500 Å and the other approximately 1000 Å in diameter (Vanhoutte, 1983).

It is these dense-cored vesicles which serve as the storage sites for noradrenaline and the final enzyme in its synthesis and release. Vanhoutte (1980, 1983) has discussed these two types of vesicles in some detail. The larger vesicles are described as the newly synthesized ones which have been transported by axoplasmic flow from the cell body. The smaller vesicles (approximately 500 Å in size) are those which have been recycled as the result of local reuptake. Differences in electron density among vesicles

have also been noted. Such differences have been directly related to amount of stored noradrenaline (Burnstock, 1975; Vanhoutte, 1983).

This arrangement of varicose terminals innervating the blood vessels rarely forms true synapses with the vascular smooth muscle effector cells of the tunica media (Bevan, 1979; Bevan, Bevan, Purdy, Robinson and Waterson, 1972; Burnstock, 1975 a, b and Burnstock, Gannon and Iwayama, 1970). Depending on the size of the vessel, the neuromuscular relationship will vary. Generally this relationship is more intimate in smaller than in larger blood vessels (Rhodin, 1968; Bevan, 1979).

Noradrenaline is released by a process of exocytosis into the extracellular space at varying distances of neuromuscular separation. The literature in this area is equivocal. Burnstock (1975b) reported the closest apposition between noradrenergic varicosity and effector cell, at 50 nm for arterioles, small arteries and veins, to 200-500 for medium to large arteries.

Vanhoutte (1983) puts this figure at 80 nm and Bevan (1979) has reported the amount of separation to vary functionally from a few hundred Angstroms in arterioles and small arteries to several thousand in medium and larger vessels.

In general the larger elastic arteries typically are more sparsely innervated with substantially large neuromuscular distances (100-200 nm)(Bevan, Bevan, Purdy, Robinson

and Waterson, 1972).

The density of the innervation in the various anatomical elements of the vascular system have been reviewed by a host of investigators (Burnstock et al, 1970, 1975b; Burnstock and Bell, 1974; Bevan et al, 1972; Furness and Burnstock, 1975). There is considerable variation in the pattern and distribution of the innervation as related to the physiological role. In large elastic arteries the density of innervation is at a low level with neuromuscular separations of approximately 2000 nm although considerable species differences do exist (Burnstock, 1975b). Gap junctions have been identified although they appear to be less frequent than in smaller arteries (Somlyo and Somlyo, 1968; Bevan and Su, 1973), indicating that electrotonic transmission may be of primary importance in these vessels. As one moves "downstream" an increase in the innervation is noted in muscular arteries (De la Lande, Jellett, Lazner, Parker and Waterson, 1974) with considerably reduced neuromuscular distances. Arterioles and precapillary sphincters are the most heavily innervated vascular structures, although striking differences in the innervation have been noted with a relatively sparse distribution in the cerebral and coronary as compared with a heavy innervation of the mesenteric circulation (Burnstock, 1979).

The evidence for capillary adrenergic innervation is incomplete. No studies are available on the functional transmission of noradrenaline on capillary beds although a

few nerves have been observed in capillaries situated proximal to the heart (Hudlicka, 1973). It has been argued however that these are probably traversing fibers which do not actually innervate the capillaries (Burnstock, 1975b). Several general statements can be made with regard to the adrenergic innervation of veins.

Veins, as a rule, tend to have a less dense network of adrenergic varicosities than do arteries (Bevan, Hosmer, Ljung, Degram and Su, 1974). Their distribution and density are more variable across different vessels and the minimum neuromuscular separations tend to be small; under 100-150 nm, for example, in the rat portal vein (Holman, Kasby, Suthers and Wilson, 1968).

Finally, large muscular veins are more heavily innervated than conducting veins (Burnstock, 1979).

Vanhoutte (1980) reported that the density of the innervation of vascular smooth muscle will depend largely on the extent to which individual vascular beds contribute to centrally induced changes in vascular diameter. As has been described, innervation density is not the same in all vessels of the same bed, and further, the innervation changes dramatically from the origin of a blood vessel to its capillary network (Bevan, 1979).

Burnstock's (1975a, b) observations suggest that the more densely innervated a particular vascular segment the closer the apposition between nerve varicosity and effector cell. Further, more densely innervated vascular segments tend

to be under more localized noradrenergic control. This
idea was later considered by Bevan (1979). Variations in
innervation density and neuroeffector separation were
regarded as responsible not only for final concentrations
of noradrenaline at the postsynaptic membrane but also for
the particular distribution of released noradrenaline within
the different tunics of the blood vessel and in and around the
varicosity. Narrower neuroeffector junctions increase peak
concentrations of noradrenaline and also increase the
likelihood that noradrenergic modulation of the postsynaptic
membrane remains localized to the immediate area.

Bevan's et al (1972) study had shown that innervation
density and magnitude of neurogenic response were positively
correlated suggesting innervation density was one important
factor influencing the reactivity of vascular smooth muscle.
A later study (Bevan, 1977) illustrated that neuronal uptake
was a function of innervation density.

Thus in the vascular components largely responsible
for maintaining total peripheral vascular resistance (ie.
small arteries and arterioles and in precapillary sphincters)
density of innervation has been shown to be at a peak.

Overview of the autonomic nervous system

The autonomic nervous system includes those nervous
elements whose function in the body is in the regulation of
the activity of smooth or involuntary muscle. An early
Greek physician, Galen, was the first to suggest that there

was 'sympathy' or 'consent' between the different parts of the body, but it was not until the eighteenth century that the term 'sympathetic' was used to characterize a particular autonomic nerve (Day, 1979). On the basis of work by Gaskill (1916) in anatomy and Langley (1921) in physiology, the autonomic nervous system has since been divided into a parasympathetic subdivision and a sympathetic subdivision with essentially antagonistic actions.

Unlike the neurons of the voluntary or striated muscle the sympathetic and parasympathetic neurons synapse before reaching their effector organs. The sympathetic preganglionic neurons with cell bodies primarily in the intermediolateral cell column exit from the spinal cord via the motor roots of all thoracic and the upper two lumbar spinal nerves and most form synapses in a paired chain of sympathetic ganglia before innervating their effector organs as postganglionic neurons (Day, 1979).

At the level of the preganglionic synapse acetylcholine is the neurotransmitter while at the site of the neuroeffector junction it is almost exclusively noradrenaline. Postsynaptic sympathetic fibers innervating the heart, smooth muscle, certain glands and blood vessels all have in common noradrenaline as their neurotransmitter.

Synthesis and uptake of noradrenaline

Noradrenaline is present in adrenergic fibers and in certain pathways within the central nervous system. Adrenaline

constitutes most of the catecholamine present in the adrenal medulla although small concentrations do appear in various organs and in the central nervous system (Goth, 1976). The synthesis of noradrenaline involves the acquisition of tyrosine by the adrenergic neuron from the extracellular fluid. Tyrosine is then transformed in the neuroplasm to L-DOPA by the cytoplasmic enzyme, tyrosine hydroxylase. The amount of catecholamine produced will depend on the availability of this enzyme. As L-DOPA, the substance is decarboxylated by L-aromatic amino acid decarboxylase and stored in the synaptic vesicles as dopamine. The final step in the synthesis of noradrenaline is the transformation of dopamine to noradrenaline by the enzyme dopamine β-oxidase which is found bound to the membranes of the storage vesicles (Vanhoutte, 1980).

Noradrenaline acts on both alpha and beta receptors on the postsynaptic effector membrane, however a considerable proportion of the released noradrenaline is taken up by the surrounding adrenergic nerves.

The neuronal uptake mechanism for noradrenaline involves a neuronal amine pump system. The active carrier is coupled to Na, K and ATPase. A portion of the released noradrenaline however, on its route to the storage vesicle is degraded mainly to 3-4 dihydroxyphenylglycol by neuronal mitochondrial monoamine oxidase (Vanhoutte, 1983).

Structural and functional changes in the SHR

As already discussed the peripheral resistance of vascular beds is an important mechanism in the maintenance of systemic arterial blood pressure. There is general agreement that there is an increase in the vascular resistance and reactivity both in human patients (Folkow, Grimby and Thulesius, 1958; Conway, 1963; Silvertsson, 1970) and in the spontaneously hypertensive rat (SHR) (Folkow et al, 1969, 1970, 1973, 1977; Pfeffer and Frolich, 1973; Pfeffer et al, 1974; Tobia et al, 1974), a strain of Wistar rats selected for systemic hypertension (Okamoto and Aoki, 1963), however the cause of these alterations has not fully been explained. Vascular hyperresponsiveness to neurogenic (ie. noradrenaline) or circulating humoral substances is one suggestion which has been forwarded to explain maintenance of increased vascular resistance (Lais and Brody, 1978; Webb, Vanhoutte and Bohr, 1981).

On the other hand Vanhoutte (1980) has reported that in the spontaneously hypertensive rat, the early stages in the development of hypertension are characterized by a hyperfunction of the peripheral adrenergic nerve varicosities which promotes an increased release of noradrenaline and which may therefore be the important initiating factor for increased vascular resistance.

One approach undertaken by investigators has concentrated on the altered membrane properties observed in

the SHR and in hypertension (Hermsmeyer, 1976; Campbell,

Chamley-Campbell, Short, Robinson and Hermsmeyer, 1981).

Such alterations raise the possibility that the sympathetic

innervation may be active in altering the membrane

permeability of arterial smooth muscle in the genetically

hypertensive SHR model. It was shown that suppression of

the Na, K pump by cold temperatures reduced membrane

potentials in these animals (Hermsmeyer, 1976) and further

that when arteries from control WKY animals were trans-

planted to SHRs the control arteries acquired the altered

membrane properties of the SHR arteries (Abel and Hermsmeyer,

1981; Campbell et al, 1981). Further surgical denervation

induced by superior cervical ganglionectomy prevented

any such acquired properties in transplanted arteries.

There appears therefore to be a sympathetic influence

genetically inherent to the SHR which leads to membrane

hyperactivity, increased permeability, decreased potential

and to exaggerated vasoconstriction and hypertension (Abbond,

1982).

Many investigations have been devoted to the reaction

of the smooth muscle cell to experimental hypertension.

Scott and Pang (1981) have reported that an increase in the

medial wall thickness in the SHR resulted in an increase

in both muscular and connective tissue components. This

medial hypertrophy of small arteries and arterioles in

various organs of hypertensive humans (Short and Thomson,

1959; Short, 1966; Cook and Yates, 1972; Naeye, 1976) and in

SHRs (Ichijima, 1969; Mulvany and Halpern, 1977; Henrich,
Hertel and Assman, 1978; Warshaw, Mulvany and Halpern,
1979; Nordberg and Johansson, 1979; Hart, Heistad and
Brody, 1980) is well known. Cerebral, renal and mesenteric
vascular beds all undergo structural changes during the
development of hypertension (Naeye, 1976).

Ichijima (1969) reported significant reductions in
lumen diameters in small arteries and arterioles of the
SHR as compared with the WKY and suggested that this
difference was due to an increased thickening of the media
in the SHR.

Of concern to recent investigators has been the
actual contributing morphological factor to medial
thickening. Are the medial changes observed due to an
increased cell size (hypertrophy) as had been assumed or
due to an increased cell number (hyperplasia)? Bevan,
Burnstock, Johansson, Maxwell and Nedergaard (1976) have
reported the increase in vessel wall dimensions in
experimentally induced hypertension in rabbits is due
partially to a hyperplasia of the cells. Later however,
Owens, Rabinovitch and Schwartz (1981) reported that in
SHRs smooth muscle cell hypertrophy was the single con-
tributing factor to increased arterial wall mass. It is
possible that the etiology of the increased medial
thickening in the hypertensive state may be different for
experimental vs. genetic models of hypertension.

There have been varying reports as to the relation-
ship between the initiation and/or maintenance of elevated
blood pressure and the development of medial hypertrophy.
Are the morphological changes observed in the media adaptive
changes to elevated blood pressure or is some other factor
responsible for the medial changes observed; and if so, is
the increase in blood pressure a reaction to the hyper-
trophy? The data is equivocal.

Cox (1982) examined the effects of Goldblatt
hypertension on the mechanical properties and medial
composition of carotid and tail arteries of Wistar rats.
He reported an increase in the relative cell volume of the
arterial wall of the carotid artery and thereby concluded
that such changes were a direct result of the elevation of
arterial pressure. Using 14-week old captopril (which
lowers blood pressure to normotensive levels) treated
SHRs, Cox compared cross-sectional medial areas of blood
vessels from these animals with control SHR and WKY rats.
Only the untreated SHR developed medial hypertrophy. The
author therefore concluded that medial hypertrophy is an
adaptive change to hypertension rather than a genetically
inherent trait.

Scott and Pang (1983), however, using the anti-
hypertensive agent capsaicin rendered SHR normotensive
and, using a similar procedure found that despite the con-
trol of blood pressure at normotensive levels wall
thickening similar to that observed in uncontrolled

hypertensive rats occurred thereby illustrating pressure independent hypertrophy. They further observed that a hyperinnervation is present before the blood pressures of the animals became significantly higher than those of the normotensive WKY controls and thus may be responsible at least for the initiation of the medial hypertrophy.

In the literature there is a wealth of other such conflicting data on this issue (Greenberg, Palmer and Wilborn, 1978; Greenberg, Gaines and Sweatt, 1981; Henrichs, Unger, Birecek and Ganten, 1980; Mulvany, Korsgaard, Nyborg and Nilsson, 1981).

What must be kept in mind when considering this line of experiments is that the development of hypertension and arterial medial hypertrophy is probably initiated and maintained by a number of separated yet related factors. One of these factors certainly involves the vascular segment under study. Cox (1982) was using the carotid artery as his sample while Scott and Pang (1983) took their observations from the mesenteric arterial bed. These studies also used two separate models of hypertension. The hemodynamics as well as the innervation of a bed of resistance vessels cannot be compared to a high pressure conductance artery such as the carotid. Similarly Goldblatt renal hypertension is clearly a different pathological model than genetically determined hypertension.

Clearly then, there are many factors at play in this area. It is possible that the sympathetic innervation may

initiate cell replication or may modulate metabolic processes in the vascular smooth muscle and that the corresponding increase in peripheral resistance and subsequent elevated blood pressure may maintain a hypertrophied media.

Ichijima (1969) examined the development of the innervation in the SHR by several histological techniques. His investigations carried out on prehypertensive SHR , advanced hypertensive SHR and normotensive WKY controls indicated that noradrenergic nerve varicosities were significantly larger in prehypertensive SHR, as well as in advanced hypertensive SHR than in control WKY. It was concluded that in the SHR there is the potential for an increased role of the sympathetic system in the initiation and maintenance of hypertension.

Since it is now generally accepted that a trophic influence is exerted on the blood vessel wall of arteries, arterioles and veins (Aprigliano and Hermsmeyer, 1977; Abel, Aprigliano and Hermsmeyer, 1980; Bevan and Tsuru, 1981) and that chemical or surgical denervation can cause profound structural alterations, it is interesting to speculate that medial hypertrophy in hypertension may be related to exaggerated sympathetic tone.

The relationship of the catecholaminergic innervation and medial arterial structure therefore would appear to be of primary interest to the previously discussed line of research. The present study attempts to examine this relationship in different models of hypertension and in

control animals.

Trophic influence of the sympathetic nervous system

While there are probably many factors which influence the structure of blood vessels, recent evidence has suggested one of the most important of these is exerted by the innervation (Ramirez and Luco, 1973). It has further been suggested that the sympathetic innervation of vessels is not only important in vasoconstriction but also in the growth and maintenance of blood vessel walls (Bevan, 1976).

A trophic influence of nerve on muscle has been well documented for somatic nerve on striated muscle (Guth, 1968; Hofmann and Thesliff, 1972), however, the evidence for a trophic relationship between the autonomic nervous system and vascular smooth muscle is only beginning to come to light.

In the literature the necessity of nerves for the development of voluntary muscles, their importance in cell regulatory mechanisms and their role in protein and enzyme metabolism has received a good deal of attention (Thesliff, 1960; Guth, 1968). Research by Luco and Luco (1971) and later Ramirez and Luco (1973) has shown that sympathetic preganglionic axons, if allowed to reinnervate voluntary muscle can exert trophic effects similar to those exerted by somatic nerves.

Bevan (1976) has suggested that the sympathetic innervation influences the proliferation of vascular

smooth muscle in growing rabbits. In examining the effect of surgical denervation on the uptake of tritiated thymidine into the rabbit ear arteries, she reported a significant reduction in uptake of DNA precursor with fewer labelled vascular smooth muscle cell nuclei in the tunica media of denervated rabbits as compared with control animals.

Using the same model, later investigators (Bevan and Tsuru, 1981; Rowan, Bevan and Bevan, 1981) emphasized the necessity of an intact sympathetic nervous system for normal development and maintenance of the arterial wall. It was reported that long term sympathetic denervation restricted cell division during development resulting in both structural and functional changes; structural in that medial wall thickness of the arteries was decreased and functional in that a supersensitivity to noradrenaline was observed.

In studies by Hart et al, (1980) unilateral superior cervical ganglionectomy was performed on stroke-prone spontaneously hypertensive rats. Despite the fact that vessels on both sides of the brain were exposed to equally high pressures, the ipsilateral vessels showed significant reductions in wall to lumen ratios when compared with those vessels on the contralateral side providing further evidence for the supporting function of the sympathetic innervation.

Aprigliano and Hermsmeyer (1976) have defined the
trophic influence of the sympathetic nervous system on
blood vessels as "long term interactions affecting or
regulating the muscles in addition to the more immediate
action of junctional transmission". The results of their
studies on portal veins of rats chemically sympathectomized,
using 6-hydroxydopamine, (a compound which produces a
marked destruction of the peripheral adrenergic innervation)
support the hypothesis of a trophic influence of the
sympathetic system on vascular muscle and confirm Bevan's
earlier report of supersensitivity to noradrenaline; however,
they do not allude to the exact nature of this trophic
influence. Conclusions from these studies suggest that
while noradrenaline is certainly involved, it is not the
only trophic factor. Abel and Hermsmeyer's work demon-
strated that membrane excitability changes were likely due
to a removal of the trophic effect of the sympathetic
nervous system. This and later research from the same
group strongly indicated that the neurotransmitter nor-
adrenaline was responsible for the development of altered
membrane properties of vascular muscle, however, some other
factor in addition to noradrenaline was also operating
(Abel et al, 1980; Abel and Hermsmeyer, 1981).

In the past several years there have been attempts
to elucidate other possible factors responsible for the
observed trophic effects. Local application of colchicine,
which restricts axoplasmic flow to the hypogastric plexus,

has recently been shown to elicit denervation-like super-
sensitivity to noradrenaline in the rat vas deferens (Goto,
Masaki, Saito and Kasuya, 1979). The results of this and
later studies (Saito, Kasuya and Goto, 1982) support the con-
jecture of Abel and Hermsmeyer that some neurotrophic
factor in addition to the noradrenaline is involved in the
regulation of smooth muscle responsiveness. The presence
therefore of a trophic factor(s) separate from and/or indepen-
dent of the neurotransmitter itself in the adrenergic neuro-
effector system appears to be fairly well established.

Acetylcholine has already been shown to underly the
trophic effects of nerve on muscle in the somatic-voluntary
muscle system (Guth, 1968), however, the identification of
a non-noradrenergic trophic factor in the noradrenergic-vascular
smooth muscle neuroeffector system as yet remains an important
research area.

Vascular changes in diabetes

Diabetes has been defined as a heterogeneous group
of disorders that shares glucose intolerance as its common
feature (Gepts and LeCompte, 1981). Diabetes mellitus, the
most common form of this group is a disease characterized by
diminished rates of insulin secretion by islet cells, and
is generally classified in two principal forms; juvenile
onset and maturity onset diabetes, the latter occurring in
later life and generally observed to afflict obese

individuals (Crepaldi, 1978).

In man the initial changes which occur in the early stages are primarily of biochemical origin involving carbohydrate, lipid and protein metabolism (Reinila, 1981). Later in the course of the disease, however, these become manifest as structural abnormalities mainly of a vascular nature and it is this class of changes which is of principal concern to this research. The primary diseases of the blood vessels in the diabetic human may be con- sidered in two principal divisions: (1) disturbances to the large blood vessels (macrovascular or atherosclerotic lesions) seen in larger muscular arteries (Warren, LeCompte and Legg, 1966; Ganda, 1980) and (2) disturbances of the small blood vessels (microangiopathies) described in capillaries in both the human diabetic (Kilo, Vogler and Williamson, 1972; Pardo, Perez-Stable, Alzamora and Cleveland, 1972; Williamson and Kilo, 1977) and in the experimentally induced diabetic animal (Fox, Darby, Ireland and Sonksen, 1977; Joyner, Mayhan, Johnson and Phares, 1981).

Those lesions of the large blood vessels fall into three principal categories of variable significance to the diabetic. These are the formation of fatty intimal plaques, medial calcification and diffuse intimal fibrosis (Reinila, 1981). Further these vascular lesions are not restricted to any one form of diabetes. Rather, such atherosclerotic lesions are more often unrelated to the severity or duration of the disease (Bradley, 1971) and although

these vascular disturbances are also present in "nondiabetes"
it has been shown that in the diabetic such lesions are more
extensive (Robertson and Strong, 1968) and involve smaller
diameter arteries (Strandness, Priest and Gibbons, 1964).
The reasons for such vascular complications have still not
been identified (Reinila, 1981). What is clear, however,
is that such macrovascular complications account for 75% of
all diabetic deaths.

Microangiopathies have received considerable attention
in the literature (Ditzel, 1954, 1968; Ditzel and Sagild,
1954; Ditzel and Moinat, 1957; Ditzel et al, 1960; Kohner
1971, 1972; Kohner, Hamilton, Saunders, Sutcliffe and
Bulpitt, 1975; McMillan, 1975). It has been clearly shown
on the basis of these studies that the majority of
microvascular disturbances have occurred either in the
retina or in the renal glomeruli, however, in recent studies
of animals rendered diabetic using alloxan or streptozotocin,
it has been realized that these changes are much more
widely distributed than had earlier been thought. Within
the microvasculature of a host of other anatomical regions,
including the bulbar conjunctiva, inner ear, stomach mucosa,
heart, skin and muscles, and placenta (Ditzel, 1968) such
microangiopathies have been described.

It appears that the development of these micro-
angiopathies requires simultaneous functional and
anatomical changes in the microvessels (Joyner et al, 1981).
Manifestations of these lesions may be derived from the

various metabolic and endocrine alterations in diabetes.
Elevation in glucose, glucagon and growth hormone levels
(Lundback, Christensen, Jensen, Johansen, Olsen, Hansen,
Orskov and Osterby, 1971; Unger, 1974; Unger and Orci, 1975;
Chang, Noble and Wyse, 1977) and depression of circulating
insulin levels combined with increased levels of renin-
angiotensin and circulating catecholamines (Christensen,
1974) may contribute, although the complexity of these hormonal
interactions render any links to morphological changes in
these vessels purely speculative (Joyner et al, 1981).

The types of degenerative changes which have been
described in experimentally induced diabetic animals have
largely been consistent with the types of microvascular
changes reported in the human diabetic. Bohlen and Niggl
(1979a) have demonstrated that the arterioles of the
cremaster muscle in streptozotocin diabetic mice are
characterized by a decreased number, a loss of vascular
tone and a reduced cross-sectional area of the vessel walls.
Fenton, Zweitach and Worthen (1979) have shown a similar
decreased number of small blood vessels in diabetes. It
was further shown that this latter morphological change
normalized in later life (Bohlen and Niggl, 1979b). However,
this decrease in the number of perfusing vessels resulted
in a 45% reduction in blood flow per milligram of tissue
in adult life, indicating that this curtailment of
perfusing arterioles remains a serious complication of the

adult stage of juvenile-onset diabetes. Bohlen and Niggl's work with diabetic mice demonstrates therefore that the normal arteriolar development with respect to numbers of arterioles is retarded in juvenile-onset diabetes.

Wolinsky, Goldfisher, Capron, Coltoff-Schiller and Kasak (1978) have reported depressed levels of hydrolase enzymes in aortic vascular muscle in juvenile-onset diabetes. In juvenile rats after four weeks of diabetes they observed a marked reduction in the thickness of the aorta as compared to control animals. It is therefore plausible that such metabolic disturbances of the vascular smooth muscle cells may be related to the earlier described reports of reductions in numbers of perfusing arterioles. Such intracellular disturbances as described by Wolinsky may prevent full development of the media and restrict branching of small calibre blood vessels.

Additional medial changes which have been observed include increased basement membrane-like material between the smooth muscle cells in renal arterioles of diabetic children (Urizar, Schuartz, Top, Jr. and Vernier 1969). The media is also the site of calcification generally seen in elderly or long-term diabetics (McMillan, 1975) and this medial calcification has been seen to be related to duration of diabetes as well as decreased blood flow (Christensen, 1973). Reinila (1981) has also reported that in his streptozotocin diabetic rats some inner medial smooth muscle cells were necrotic while the basement membrane of

certain of these smooth muscle cells from the same rats were patchily thickened.

Certain other changes of concern have been reported in the literature associated with microvascular deterioration. These include venular dilation as well as basement membrane thickening and increased tortuosity. Venular dilation has been documented in all forms of diabetes (Ditzel, Beaven and Renold, 1960). Joyner et al (1981) have suggested that this dilation could be due to an increased intravascular pressure and result in extravasation of fluid, largely in the form of plasma and large molecules such as immuno-globulin.

Biochemical analysis and anatomical studies of basement membrane conducted in the 60's and 70's (Lazarow and Speidal, 1963; Beisswenger and Spiro, 1970; Westburg and Michael, 1973; Beisswenger, 1976) have concluded that the capillary basement membrane in both human and experimental diabetes is both thickened and altered in composition and such thickening is not confined to the basement membrane of blood vessels (Warren et al, 1966). Although there is a wealth of conflicting evidence regarding the extent of thickening as a function of duration of diabetes, what is at present unclear is the actual role of the basement membrane thickening in the pathology of diabetic microangio-pathy (Macmillan, 1975). As regards changes in chemical composition Beiswenger and Spiro (1970) have postulated, on the basis of work in alloxan diabetic rats, that the

unusually high carbohydrate content of basement membrane was
due to an abnormal change in the protein synthesis pattern
whereby precursor segments with a high carbohydrate content
are used to manufacture basement membrane in the diabetic
state. It is therefore conceivable that the product of
such basement membrane precursor exists in a more permeable
form, aiding plaque formation.

The primary classes of cardiovascular changes which
occur in both human and experimental diabetes have been dis-
cussed. Atrophy of the media, disturbances in basement
membrane and reduction in the numbers of perfusing vessels
are of considerable concern to this research. Clear
hypotheses for these types of morphological changes in
diabetes although well studied have not been tendered. It
is the intent of this research to direct attention towards
the innervation of the microvasculature in diabetes as a
possible agent in the deterioration. The innervation of
small arteries have not been studied during the development
of diabetes although a reduction in the innervation may be
responsible for the medial changes observed.

Animal models

The animals employed in the present study are either
genetic, in the case of the SHR, or experimentally induced
models of the disease states previously reviewed and their
corresponding controls.

The spontaneously hypertensive rat

The SHR is a strain of Wistar rats developed by the
Japanese workers Okamoto and Aoki in 1963. With successive
generations this strain has evolved to development of
essential hypertension in 100% of offspring. SHR have
been used extensively in hypertension research as a close
approximation of essential hypertension in man. Features
common to both include greater severity in males, complications
such as brain hemorrhage and myocardial necrosis and in-
creasing severity with advancing age (Bishop, Kawamura and
Detweiler (1979). It has been shown that the development of
hypertension in the SHR has a clear genetic basis since F2
backcrosses of the normotensive WKY from which the SHR were
developed show intermediate levels of hypertension. The
structural alterations in the vascular system of the SHR
have already been reviewed.

Goldblatt renal hypertension

By partially restricting blood flow through one of
the renal arteries (2 kidney - 1 clip) in developing normo-
tensive animals, a marked and persistent elevation in
systolic blood pressure can be produced (Goldblatt, 1944).
This technique has been used extensively as a non-genetic
animal model of human hypertension by an intervention in
the renin-angiotensin system. Carretero, Oza, Scicli and
Schork (1974) have shown that in the 2 kidney - 1 clip model
plasma renin activity levels are significantly increased as

early as one month after renal artery constriction with renin activity in the kidney increased fourfold.

The vascular alterations associated with Goldblatt hypertension include a general thickening of the arterial wall in both large and small arteries of renal artery constricted animals (Mulvany et al, 1981). This method therefore appears to be a well-documented procedure for inducing non-genetic hypertension and will be the model employed in this study.

Streptozotocin induced diabetes

Several classes of agents have been employed in inducing diabetes in experimental animals (Mordes and Rossini, 1981). The two most common are the broad spectrum antibiotics Alloxan and Streptozotocin. The cytotoxic effects of this class of agents are specific to islet cells causing a marked primary insulin deficient state. Streptozotocin (2-deoxy-2-(3 methyl-3 nitrosurea-D glucopyranose) or STZ was chosen for use in this study because it has been shown by recent investigation to render a stable, non-insulin dependent diabetic state in rats with a single intravenous injection of 50 mg/kg (Penpargul, Schaible, Yipintsoi and Scherer, 1980).

Cell membrane binding is the first step in the destructive process with the toxic effects being mediated through a specific recognition by some receptor on the cell surface. Fischer and Rickert (1975) have suggested that the glucose

component of streptozotocin enhances the uptake into the cell concentrating the cytotoxic effects of the nitrosurea element. The current body of literature in the area of diabetes research has shown STZ to be the method of choice for general induction of diabetes in experimental animals. Atheromas and microangiopathies in STZ treated animals have been reviewed earlier.

6-hydroxydopamine chemical sympathectomy

In 1967 it was discovered that 6-hydroxydopamine (6-OHDA) produced a selective destruction of the terminal ground plexus of peripheral noradrenergic neurons (Tranzer and Thoenen, 1968). The identification of this compound has yielded a technique for the study of the neural input to various tissues, organs and organ systems, superior in many instances to the conventional methods of lesioning or removal of peripheral ganglia.

This so-called 'chemical sympathectomy' is the result of a chain of destructive events within the catecholamine containing neurons which are well described in the literature, including depletion of stored noradrenaline in post ganglionic fibers (Kostrzewa and Jacobowitz, 1972), destruction of cytoplasmic enzymes and actions which suppress the axon's conduction capabilities (Thoenen and Tranzer, 1968; Wagner, 1971; Iverson and Uretsky, 1970).

Studies of the effect of chemical sympathectomy on blood vessels are abundant in the literature; however

general the detrimental effect of 6-OHDA seen in other
tissues is much less extensive in the case of blood
vessels (Goldman and Jacobowitz, 1971). Finch, Haeusler,
Kuhn and Thoenen (1972) observed that noradrenergic in-
nervation of the treated animals (68 mg/kg iv. twice in a
6 hour interval) was present one week after treatment and
had returned to control levels after 2 weeks.

Nevertheless depletion of noradrenaline from
noradrenergic varicosities innervating blood vessels of
experimental animals has been induced in a number of in-
vestigations with good success.

The method employed in the present study involved an
extensive treatment schedule at a high dose commencing 1 day
after birth. This procedure is considered in detail in the
section entitled Methods.

Capsaicin treatment

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is an
acrid and irritating derivative of hot peppers from plants
of the genus Capsaicum. Its principal action, when adminis-
tered to adult animals, is in the depletion of substance P
from primary sensory motor neurons (Jansco, Kirally and
Jansco-Gabor, 1977) involving destruction of a particular
population of primary sensory neurons. In the present
study capsaicin will be used for rendering genetically
hypertensive animals (SHR) normotensive. A control for
the possibility of some peripheral action of capsaicin

(other than the lowering of blood pressure) on the structure
of blood vessels, studied in the present investigation will
however be included.   This procedure is outlined in the
Methods section.

Objectives of the present study

While there are probably many factors which influence
the structure of vessels, recent evidence has suggested that
one of the main influences is exerted by the innervation.

It has been suggested that the innervation of vessels
plays a large part not only in vasoconstriction, but also in
the growth and maintenance of vessel walls. This trophic
influence of nerve on muscle is well documented in skeletal
muscle, but less so in the case of vascular smooth muscle.
The general objective of this research is to examine the
trophic relationship between the catecholaminergic inner-
vation and the structure of the media of small diameter
arteries during normal development and in disease states in
which the arterial wall structure is known to change, and in
an experimental model in which the innervation has been
altered.

The basic vascular structure to be studied in the
proposed experimental models is the jejunal artery. This
vessel is easily accessible, its wall thickness and lumen
diameter can be measured with a minimum of error, and the
number of nerve fibers at the adventitial/medial border can
be easily determined by fluorescence microscopy. The wall

thickness, lumen diameter and number and disposition of
fluorescent sympathetic fibers will be determined in varied
sets of rats sampled at twelve weeks of age. These sets of
rats will include normal developing Sprague Dawley rats,
spontaneously hypertensive rats (SHR), SHR treated with
capsaicin to lower blood pressure to normotensive levels,
Wistar-Kyoto rats (WKY) treated with capsaicin, normal WKY
rats, Sprague Dawley rats treated with streptozotocin to
induce diabetes, Sprague Dawley rats chemically sympathectomized
with 6-hydroxydopamine (6-OHDA), and Sprague Dawley rats with
renal artery constrictions to induce hypertension.

It is hoped that from the results it will be possible
to determine the limits of the trophic influence of the
sympathetic innervation on the arterial wall during normal
development, in hypertension and in diabetes. An under-
standing of the trophic role of innervation on vascular
structures will allow further investigations of how the
trophic capacity of the nervous system can be used to
prevent or reverse the changes which take place in the vas-
cular system in diseases such as hypertension or diabetes.

MATERIALS AND METHODS

I.   Experimental and control animals

1.   Streptozotocin induced diabetes

Twelve 4 week old male Sprague Dawley rats (SD) (obtained from Charles River of Canada) weighing between 90g and 110g were anaesthetised with ether and their femoral veins exposed. Diabetes was induced by a single intravenous injection of streptozotocin (STZ) (Upjohn), 50 mg/kg, dissolved in 0.05M citrate buffer, pH 4.5 (Penpargkul, Schaible, Yipintsoi and Scherer, 1980). The incisions were closed and animals allowed to recover from anaesthesia. Diabetic rats were housed 2 rats per cage with free access to normal Purina rat chow and water.

Rats were weighed and urine tested for glucose and ketone bodies using indicator reagent sticks (Labstix®) weekly until they were sacrificed 8 weeks later at 12 weeks of age.

Tables of weights and glucose/ketone body readings are presented in Table 1.

2.   Capsaicin treated SHR and WKY

SHR and normotensive WKY (origin Charles River of Canada stock) were treated at 3 and 4 days postnatally with a subcutaneous injection of capsaicin (Caps) (Sigma) 25 mg/kg (10% soln,

dissolved in 10% ethanol, 10% tween 80, in saline).
Following weaning at 4 weeks 12 male SHRs and 12 male
WKYs were randomly selected from the litters, housed 4
animals per cage and allowed free access to Purina rat chow
and water until they were sacrificed at 12 weeks of age.

3. 6-Hydroxydopamine chemical sympathectomy

Pregnant female Sprague Dawley rats were obtained
from Charles River of Canada. The newborn litters of these
animals were treated at 1 day of age with intraperitoneal
injection of 6-hydroxydopamine hydrobromide (6OHDA), 100 μg/g
body weight dissolved in 1% ascorbic acid in physiological
saline. At 4 and 7 days postnatally and weekly through until
4 weeks of age this dose was repeated. Animals were then
weaned and 12 males randomly selected and continued on this
weekly dose until 6 weeks of age. The dose was then reduced
to half (50 μg/g) and administered every 2 weeks until 12
weeks of age (Sinaiko, Cooper and Mirkin, 1980).

Animals were housed 4 per cage and allowed Purina
rat chow and water ad libitum.

Degree of denervation was determined at 12 weeks in
animals fixed and processed for viewing of fluorescent
profiles at the medial/adventitial border.

3. Goldblatt hypertension

According to the procedure described by Goldblatt,
the two kidney, one clip procedure (Goldblatt, 1934), at 4

weeks of age these animals were anaesthetized with ether
and a midline incision made, as for laparotomy, extending
from the level of the bladder cranially to the level of the
xyphoid process, the intestinal viscera expelled from the
abdomen and retracted to the right, and the left kidney
exposed (see Figure 1).

Using fine forceps a portion of the left renal artery
occurring between the origin of the left suprarenal artery
and kidney hilus was isolated from the left renal vein and
surrounding fascia. Using a 5-0 curved needle a 5-0 silk
suture was looped around the isolated segment and a 6-0 curved
needle introduced between the vessel segment and the suture
loop. The loop was tightened occluding blood flow. The
kidney appeared pale. When the 6-0 needle was removed the
kidney regained its colour indicating that blood flow had
been restored.

Following this surgery the surgical field was
sponged and rinsed with physiological saline. The rectus
abdominus muscle was next closed using a continuous suture
of 5-0 chromic catgut. Finally the skin and subcutaneous
tissue were closed using a vertical mattress suture with
5-0 silk. Animals were then removed from the operating
area and allowed to recover from anaesthesia under a heating
lamp. When the animals had recovered from the anaesthetic
they were housed 4 per cage and allowed free access
to normal Purina rat chow and water. The success of
the operations in inducing experimental hypertension was

confirmed by blood pressure measurements (described later) at 12 weeks of age.

The criterion for experimental hypertension used was a systolic pressure of greater than 150 mmHg, which is the universal reference point between normotensive and hyper- tensive states in experimental research (Okamoto and Aoki, 1963). Twelve of the operated animals which fulfilled this criterion were randomly selected from this group and sacrificed.

5. Control series

Four sets of controls have been employed in this study and are outlined as follows:

(a) normal untreated SD rats as controls for SD treated with streptozotocin, or 6OHDA, or surgically ren- dered hypertensive using the Goldblatt technique;

(b) normal untreated SHRs as controls for SHR treated with capsaicin;

(c) WKY , the normotensive strain of rats from which the SHR were developed, treated with capsaicin as a control for any effects of capsaicin unrelated to the lowering of blood pressure in treated SHR;

(d) normal untreated WKY rats as controls for the capsaicin treated WKY rats.

II. Assignment of animals to experimental and control groups

The procedure for measurements of arterial blood pressure was the same for each experimental and control group. At 12 weeks of age animals in each experimental and control group were randomly divided into 2 subgroups of 6 animals per subgroup.

For each experimental and control group one of these subgroups was perfused according to a method for Araldite resin embedding for wall thickness/lumen diameter measurements while the other subgroup was perfused according to a modified Falck (1962) protocol for wax embedding and subsequent visualization of catecholamines (Loren, Bjorklund, Falck and Lindvall, 1980). Both methods are described in detail below.

III. Procedure for blood pressure measurements

Blood pressures were taken from all animals assigned to the resin embedding subgroups for all experimental and control groups and in both subgroups for the renal artery constricted group. All arterial blood pressures were measured from the left femoral artery. The procedure for blood pressure measurements was as follows.

Animals were weighed and anaesthetised with Somnotol (Sodium Pentobarbitol 35 mg/kg, 32.5% solution in tyrodes buffer). The animals were placed in the supine position on a draped operating board and a 3 cm oblique incision made along the ventral surface of the femoral field of the

animals, perpendicular to the longitudinal axis of the left hindleg. The inguinal ligament was visualized by blunt dissection of the superficial femoral fascia. A 1.5 cm segment of the femoral artery was located between the inguinal ligament and the origin of the superficial epigastric artery and isolated from the neurovascular bundle, by blunt dissection. Using very fine curved forceps, three 5-0 silk sutures were next looped around the segment and the most distal suture tied off. The most proximal suture was used to occlude the blood flow while a transverse incision was made in the artery close to the distalmost suture providing access to the lumen of the vessel.

A cannula (PE-90 Intramedial tubing) filled with heparinized saline (Hepalean) and drawn to a narrow diameter was introduced into the lumen of the vessel and secured by tying the middle suture. Pressure on the proximal suture was released and the pulsing blood allowed access to the fluid filled cannula for recording of blood pressure. In no instance did the time of the operation and blood pressure measurement exceed 20 minutes.

Recording of arterial blood pressure was carried out on a R411 Beckman Dynograph recorder via a Statham pressure transducer (P-23AA) which was attached to the cannula by a Yale 20G-1" hypodermic needle for each animal. Calibration of the recording apparatus using a sphygmomanometer (W.A. Baum and Co.) was carried out prior to blood pressure

measurement. This procedure involved calibration such that each mm of pen displacement in the recording chart corresponded to 5 mmHg with a range from 0-200 mmHg. In addition the trace was allowed to stabilize for 2 minutes or until a steady blood pressure was established. Following each measurement the chart was checked to ensure the trace returned to the baseline level.

IV. Perfusion of mesenteric arteries and tissue processing

1. Perfusion of mesenteric arteries

Following blood pressure measurements, animals, still under sodium pentobarbital anaesthesia, were transferred from the operating table to the perfusion room where they were placed in the supine position on a metal rack over a sink for drainage. A longitudinal midline incision was made through the fur and skin of the abdominal lumbar areas and following separation of this tissue from the underlying musculature and linea alba, a further midline incision was made along the linea alba extending from the level of the xyphoid process caudally to the level of bladder exposing the visceral organs. The abdominal content was next carefully removed from the abdominal cavity and the mesenteric vascular bed of jejunal arteries arranged such that they fanned out from the mesenteric artery to the jejunum.

The abdominal aorta was next cleared and cannulated with a 18G-2" catheter (intramedic) at a point approximately 2 cm below the origin of the right renal artery. The inferior

'vena cava was then cut at the thoracic segment at the point where it emerges from the diaphragm. Following exsanguination with 100 ml of cold tyrodes buffer the animals were perfused transaortally with a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.2 (Karnovsky, 1965) at 120 mmHg until 100 ml had been delivered.

2. Resin embedding

The jejunal arteries attached to the mesenteric artery were dissected from the perivascular fat under a dissection microscope (Olympus) and placed in the same fixative on a rocker table for an additional 2 hours fixation. The tissue was then processed as follows: rinsed in 0.1M sodium cacodylate buffer in 5.4% sucrose; postfixed with 1% osmium tetroxide, in the same buffer; stained en bloc with 50% ethanol saturated uranyl acetate; dehydrated in a series of graded ethanols; cleared using acetone; infiltrated with a 1:1 mixture of acetone and Araldite for 16 hours; and polymerized in unadulterated Araldite for 24 hours at 70°C. Prior to polymerization the jejunal arteries were separated from the mesenteric artery and arranged in order in the block.

3. Wall thickness and lumen diameter measurements

Measurements of the tunica media of the arterial wall and internal lumen diameter were taken from the third

jejunal artery in all animals studied using light micro-
graphs.

Cross-sections were cut at one half micron from these
samples on a Cambridge-Huxley ultramicrotome at a point
approximately 1 mm from their origin at the superior
mesenteric artery. Sections were collected on a glass slide,
stained using Rapid Polychrome Stain (Sato and Shamoto, 1973)
and examined under a light microscope (Wild).

Photographs of the lumen and tunica media were then
taken at 30 and 400 magnification respectively and
measurements made from negatives. The thinnest segment of
media yielded wall thickness measurement while lumen diameter
was calculated by taking a measurement at the widest and
narrowest points on each vessel, added and divided by 2. Each
set of micrographs was calibrated using a slide micrometer
(American Optical Co.).

4. Procedure for Visualization of Catecholamines

Animals perfused and processed for visualization of
catecholamines were anaesthetised using Somnotol (sodium
pentobarbitol 35 mg/kg i.p. in tyrodes buffer) and following
laparotomy, cannulation of the abdominal aorta and severing
of the inferior vena cava as described earlier, perfused as
follows (modified from Loren, Bjorklund, Falck and Lindvall,
1980): Exsanguination with 100 ml ice cold tyrodes buffer
followed by perfusion with 150 ml ice cold aluminum sul-
phate (10% solution in tyrodes buffer). Under a dissection

microscope (Olympus) the mesenteric arcade of jejunal vessels was dissected and the jejunal arteries rapidly separated from the corresponding veins and perivascular tissue on ice cooled glass. The vessels were next blotted and mounted on a 2 x 3 cm piece of Whatman filter paper, vessel samples frozen in liquid hexane, and subsequently freeze dried for 6 hours at -40°C. The dry tissues were next exposed to paraformaldehyde vapour at 80°C and 80% humidity for one hour.

Following vapour exposure vessel samples were embedded in wax under vacuum and vessel cross sections cut on a rotary microtome (American Optical) at 7 μm. Sections were mounted in a 5:1 oil/xylene mixture, heated on a slide warmer (Fisher) and examined under ultraviolet light (HBO mercury vapour lamp) from a Wild microscope at BG38/12 filter settings at 40x magnification.

5. Examination of fluorescent profiles

Profiles of fluorescent fibers were examined and counted at the medial/adventitial border of 6 sections from each artery examined for each animal. Animal means were then combined to derive group means for each experimental and control group.

RESULTS

I. General observations

Of the various treatments administered to the ex-
perimental groups in this study, STZ-induced diabetes
clearly produced the most pronounced overt detrimental
effects. Diabetic rats appeared to groom less frequently
than other groups, had distended abdomens and fur with a
typical yellow tinge. Three of these animals developed
retinopathies after less than eight weeks of diabetes.
After eight weeks of diabetes none of the animals had died
but all had maintained a level of glucosuria exceeding 2
g/dl for eight weeks. Three of these animals had developed
ketosuria from moderate to strongly positive prior to
sacrifice at 12 weeks as measured by Labstix (Table 1).

The 6-OHDA-treated animals were observed to groom
extensively in the genital area. These animals were much
less excitable and, in general, appeared sluggish in com-
parison to (SD) controls.

Two animals from the Goldblatt hypertensive group
were discarded as their systolic blood pressure did not
exceed 150 mmHg. The 12 animals comprising this group
were therefore randomly selected from the 14 remaining
animals which fulfilled the criterion for elevated blood
pressure.

II. Body weight

The body weights of developing STZ treated rats are

presented in Table 1 and Figure 6. The most rapid growth
appeared between the ages of 5 and 10 weeks, a one week
lag behind that of SD control animals, a likely result of
injection of STZ at 4 weeks.

The mean body weights of the animals in experimental
and control groups at 12 weeks are presented in Table 2 and
Figures 7 and 8. The STZ treated animals had significantly
lower body weights ($p < .05$) than their SD controls.
Caps treated SHR and WKY also exhibited significantly
lower body weights ($p < .05$) than their SHR or WKY counter-
parts. SHR (Caps) and SHR rats did not however differ from
WKY (Caps) and WKY respectively. Neither 6-OHDA treated nor
Goldblatt hypertensive animals differed in body weight from
their SD controls.

Variance in the weights of animal groups employed in
this study was low with the exception of the three groups
which received drug treatments: STZ, 6-OHDA and SHR.

III. Blood pressure

Blood pressures were taken at 12 weeks from the
femoral artery of 6 animals from each experimental and control
group. These data are presented in Table 3 and in Figure
8. The Goldblatt renal artery constricted animals had
significantly higher ($p < .05$) blood pressures than con-
trols while STZ treated and 6-OHDA treated animals exhibited
a significantly lower ($p < .05$) blood pressure than controls.

SHR (Caps) animals had blood pressures significantly higher ($p < .05$) than their WKY counterparts but significantly lower ($p < .05$) than untreated SHR and not different from WKY untreated. The SHRs had significantly elevated pressures ($p < .05$) when compared with WKY and the WKY (Caps) animals showed significantly lower ($p < .05$) pressures than WKY controls. The SD and the WKY had comparable pressures.

IV. Fluorescence

Noradrenergic fibers could be seen as amorphous fluorescent granules of various size disposed at the medial/ adventitial border of vessel cross-sections, although fibers could occasionally be seen within the media and/or adventitia. Figure 5 shows typical cross-sections of jejunal arteries at 12 weeks for each experimental and control group. Table 4 and Figure 8 present the numbers of fluorescent profiles observed in treatment and control groups.

The prolonged 6-OHDA treatment was confirmed to have produced a complete ablation of visible noradrenergic fibers at 12 weeks. This absence of noradrenergic fibers had earlier been the case when 4 animals were sacrificed and examined for fluorescence at 6 weeks in a confirmation experiment.

There was a hyperinnervation observed in the STZ treated group when compared with controls ($p < .05$). No differences were seen in the Goldblatt hypertensive animals

as compared with controls. Both the SHR (Caps) and the SHR
untreated animals exhibited a hyperinnervation (p < .05) when
compared with SKY (Caps) or WKY untreated controls. The
two different strains of control animals, SD and WKY, did not
differ from each other with respect to numbers of fluorescent
profiles. Similarly WKY (Caps) and WKY did not significantly
differ.

V. Morphometric data

Three measures on the 0.5 micron sections taken from
the aldehyde fixed vessels of treatment and control animals
were made from photographic negatives; medial thickness, lumen
diameter and number of smooth muscle cell (SMC) layers within
the media. Typical cross-sections of jejunal arteries from
treatment and control animals are presented in Figure 4.
Data for medial thickness/lumen diameter and of numbers of
SMC within the media are presented in Tables 5 and 7 res-
pectively and in Figure 8.

The STZ treated group had a significantly thinner media
(p < .05) than did controls, however lumen diameter measure-
ments remained unchanged while the Goldblatt hypertensive
animals had both a significantly thicker media (p < .05) and
a significantly reduced lumen (p < .05). 6-OHDA treated
animals did not differ significantly from controls.

SHR (Caps) and SHR untreated groups had significantly
thicker media (p < .05) but did not differ in lumen diameter
from WKY (Caps) treated or WKY untreated controls.

WKY treated did not differ from WKY untreated with

respect to either medial thickness or lumen diameter.

To simplify this data a single measurement termed medial SMC mass, which represents the amount of smooth muscle cell present in cross sectional area, takes into account both medial thickness and lumen diameter and is estimated by the formula given in the legend, Table 6. Medial SMC mass estimations are given in Table 6 and Figure 8.

STZ treated animals had significantly (p < .05) smaller medial masses than did controls. 6-OHDA treated animals, although showing significant reductions in medial thickness did not differ from controls in terms of SMC mass.

Goldblatt hypertensive animals had significantly higher SMC masses (p < .05) when compared with controls as did both SHR (Caps) and SHR untreated (p < .05) when compared with their controls. The WKY (Caps) did not differ from the WKY untreated.

A graph of the relationship between blood pressure and the medial SMC mass is presented in Figure 9. As a function of blood pressure in the Goldblatt hypertensive animals the medial SMC mass increased significantly as compared with controls (p < .05). The STZ treated animals also differed significantly (p < .05) from controls with respect to medial SMC mass, as a function of blood pressure but in the opposite direction from the Goldblatt hypertensive group. The SHR (Caps) group did not differ from the WKY untreated while the SHR showed significant difference from its WKY control.

A second graph depicting the medial SMC cell mass as a function of number of fluorescent profiles is presented in

Figure 10,

As a function of number of fluorescent profiles the STZ treated animals show a significantly reduced ($p < .05$) medial SMC mass. Both SHR (Caps) and SHR untreated also showed a significant ($p < .05$) increase in medial SMC mass as a function of number of fluorescent profiles.

Figure 11 illustrates a series of key comparisons with respect to medial SMC mass, mean arterial pressure and number of fluorescent profiles in graphical form. Significant differences between groups are indicated with an asterisk.

The number of smooth muscle cell layers (Table 7) was significantly increased in Goldblatt hypertensive, SHR (Caps) treated and SHR untreated animals as compared with their controls while in the STZ treated animals a significant decrease ($p < .05$) was noted.

VI. Statistical Analysis

Data from this study are summarized either in the form of a table, graph or histogram. In all cases, the values are expressed as the means ± standard deviations of the means.

Data from each independent measure across treatment and control groups were tested for significance by the Student-Newman Keuls t-test. Relationships between medial smooth muscle cell mass and numbers of fluorescent profiles were obtained by plotting means and standard deviations for these comparisons.

Table 1. Table of weights, urine glucose readings and
          development of ketone bodies in STZ treated
          rats.   (Weights are expressed as the mean $\pm$
          the standard deviation.   n = 12).

AGE (wks)	WEIGHT (gms)	GLUCOSE (g/dl)	# ANIMALS DEVELOPING KETONE BODIES
4	INDUCTION OF DIABETES		
5	122 ± 9.1	>2	0
6	173 ± 15.8	>2	0
7	192 ± 22.6	>2	0
8	215 ± 28.38	>2	2
9	231 ± 39.6	>2	2
10	255 ± 45.9	>2	3
11	277 ± 52.2	>2	3
12	270 ± 64.7	>2	4

Table 2. Table of weights of experimental and control
animals at time of sacrifice. (Weights are
expressed as the mean ± the standard deviation.
Significant differences from controls are
marked with an *. n = 12)

ANIMAL GROUP	WEIGHT AT 12 WEEKS
STZ treated	270 ± 64.7*
6-OHDA treated	377.2 ± 62.5
Goldblatt hypertension	424.7 ± 14.4
SHR (Caps)	282.2 ± 58.3*
WKY (Caps)	274.8 ± 29.4*
SHR (untreated)	409.3 ± 25.2
WKY (untreated)	373.5 ± 29.5
SD	413.7 ± 11.1

Table 3. Mean arterial blood pressure (M.A.P.) for treatment and control groups where 1 mmHg = 0.133 kpa. (Values are expressed as the mean + the standard deviation. Significant differences from controls are marked with an *. n = 6)

GROUP	MEAN ARTERIAL PRESSURE
STZ treated	78.9 ± 18.9
6-OHDA treated	81.0 ± 12.7*
Goldblatt hypertension	138.0 ± 11.6*
SHR (caps) treated	101.1 ± 5.2
WKY (caps) treated	62.1 ± 6.8*
SHR (untreated)	136.0 ± 3.4*
WKY (untreated)	91.2 ± 4.7
SD control	94.5 ± 9.5

Table 4. Numbers of fluorescent profiles visible around cross-sections of jejunal arteries from treatment and control animals. (Values are expressed as the mean ± the standard deviation of the mean. Significant differences from controls are marked with an *. n = 6)

TREATMENT GROUP	NUMBER OF FLUORESCENT PROFILES
STZ treated	72.6 ± 11.63*
6-OHDA treated	none visible
Goldblatt hypertension	57.6 ± 5.23
SHR (caps)	81.2 ± 7.76*
WKY (caps)	61.8 ± 6.14
SHR (untreated)	77.4 ± 8.10*
WKY (untreated)	55.9 ± 7.40
SD control	52.9 ± 2.45

Table 5. Morphometric characteristics of treatment
and control animals. (Medial thicknesses
and lumen diameters are expressed as the
mean ± the standard deviation. Significant
differences from controls are marked with
an *. n = 6)

GROUP	MEDIAL THICKNESS (μm)	LUMEN DIAMETER (μm)
STZ treated	8.18 ± 1.57*	341.95 ± 32.02
6-OHDA treated	8.73 ± 0.75	357.26 ± 35.88
Goldblatt hyptn.	14.15 ± 1.76*	291.44 ± 32.26*
SHR (caps) treated	13.20 ± 1.40*	281.10 ± 20.10
WKY (caps) treated	10.90 ± 1.90	260.90 ± 19.80
SHR (untreated)	12.50 ± 1.16*	283.68 ± 42.82
WKY (untreated)	10.11 ± 1.48	298.68 ± 17.29
SD control	9.91 ± 0.93	334.94 ± 16.06

Table 6. Cross-sectional medial SMC mass as measured
from the external elastic lamina to the
lumen. Values for SMC mass were estimated
by application of the formula shown below
for calculation of total medial SMC mass:

$$\frac{3.14(\text{lumen diam.}+\text{medial thickness})^2}{2} - \frac{3.14(\text{lumen diam.})^2}{2}$$

ANIMAL GROUP	MEDIAL SMC MASS
	$(\mu m^2 \times 10^4)$
STZ treated	8.99 ± 1.65*
6-OHDA treated	10.03 ± 0.86
Goldblatt hypertension	13.58 ± 1.88*
SHR (caps)	12.20 ± 0.94*
WKY (caps)	9.30 ± 1.29
SHR (untreated)	11.61 ± 1.60*
WKY (untreated)	9.80 ± 0.87
SD	10.73 ± 0.49

Table 7. Number of SMC layers within the media of
cross-sections from jejunal arteries of
animals from treatment and control groups.
(Values are expressed as the mean $\pm$ the
standard deviation.   n = 6)

ANIMAL GROUP	# SMC LAYERS
STZ treated	5.17 ± 0.43*
6-OHDA	4.08 ± 0.58
Goldblatt hypertension	6.36 ± 1.02*
SHR (caps)	5.70 ± 0.46*
WKY (caps)	3.57 ± 0.53
SHR (untreated)	6.44 ± 0.66*
WKY (untreated)	4.44 ± 0.93
SD control	4.57 ± 0.43

Figure 1. Schematic illustration of a neuromuscular
junction of a blood vessel. Noradrenaline
(NA) is stored in dense cored vesicles
within the noradrenergic nerve varicosity.
Upon release by exocytosis the neurotransmitter
occupies receptor sites on the effector cells.
Some of released noradrenaline, however, is
taken up by the same or by neighbouring varicosi-
ties   (redrawn from Shepherd, 1983)

FIGURE1: Schematic diagram illustrating the typical arrangement of a neuromuscular junction.
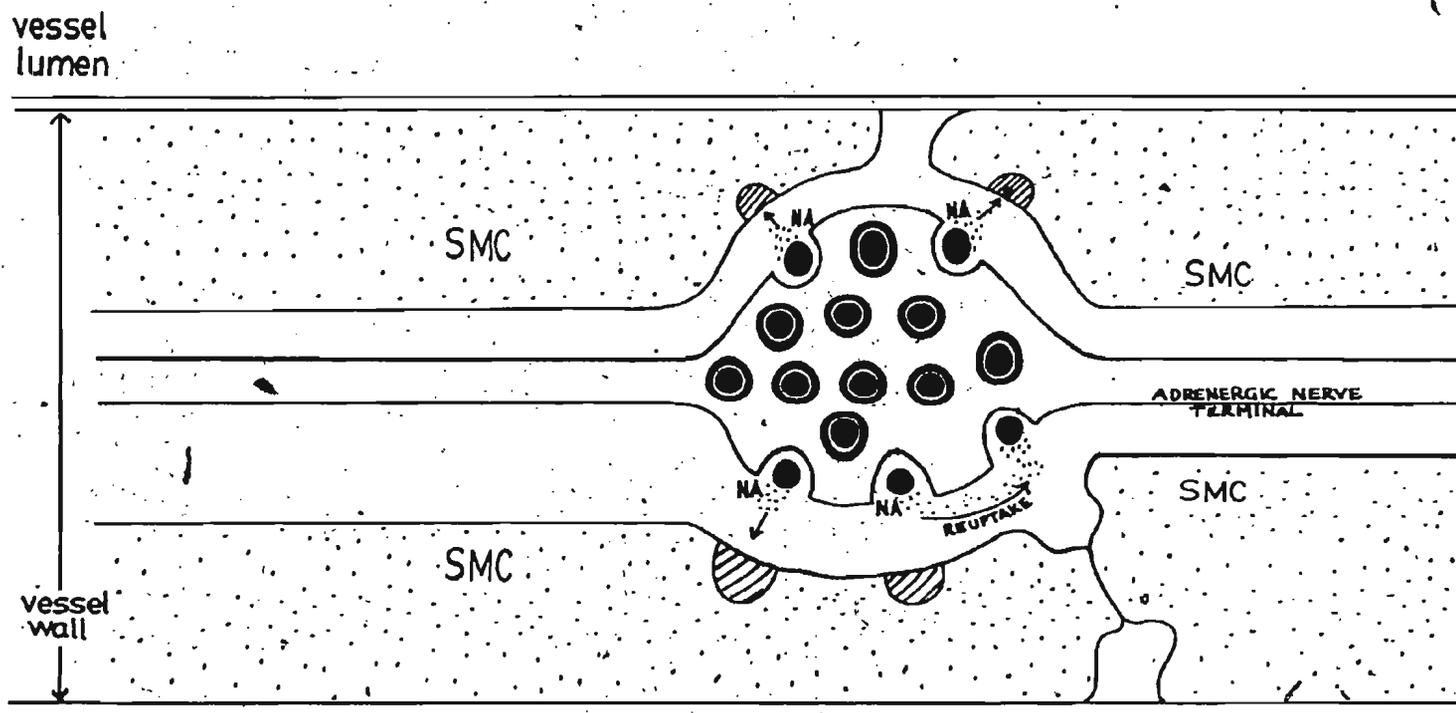
Figure 2. Photographs showing the surgical field (A) in
the procedure for the induction of Goldblatt
renal hypertension at 4 weeks, and in post-mortem
examination (B,C), eight weeks later. The
arrow in A shows the segment where the renal
artery was constricted and in B shows the suture
in place after 8 weeks. C shows the relative
size of the kidney in which the renal artery has
been constricted (C) in relation to the normal
(N).

Figure 3. Representative chart recordings showing blood
pressure measurement traces from each treatment
and control group. (Measurements in mmHg where
1 mmHg = 0.133 kpa).

FIGURE 3.

mmHg

A.   STZ treated
290 gms
M.A.P. = 55 mmHg

B.   60HDA treated
392 gms
M.A.P. = 87 mmHg

C.   G. REN. HYPT'N.
410 gms
M.A.P. = 139 mmHg

D.   S.D.
370 gms
M.A.P. = 98 mmHg

FIGURE 3.

mmHg

E. SHR (caps)
    279 gms
    M.A.P. = 98 mmHg

200

0

F. WKY (caps)
    271 gms
    MAP = 80 mmHg

200

0

G. SHR
    407 gms
    MAP = 148 mmHg

200

0

H. WKY
    369 gms
    MAP = 100 mmHg

200

0

Figure 4. Photographs showing representative
cross-sections of jejunal arteries in treat-
ment and control animals from which lumen
diameter and wall thickness measurements
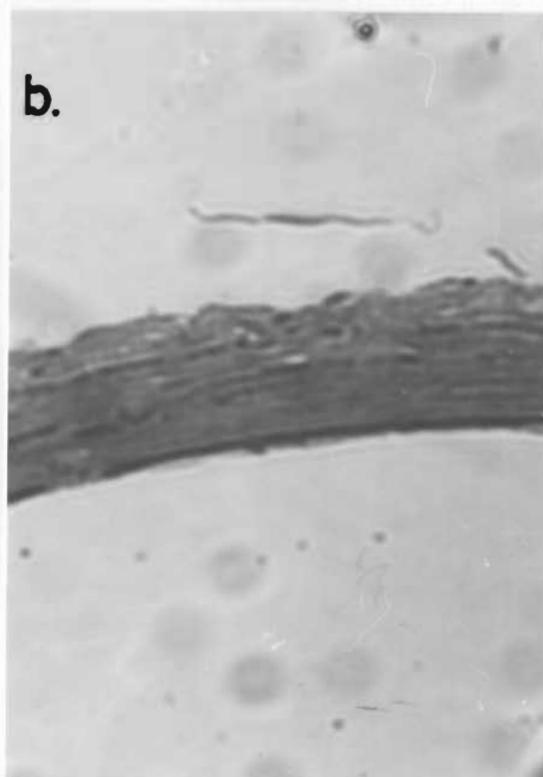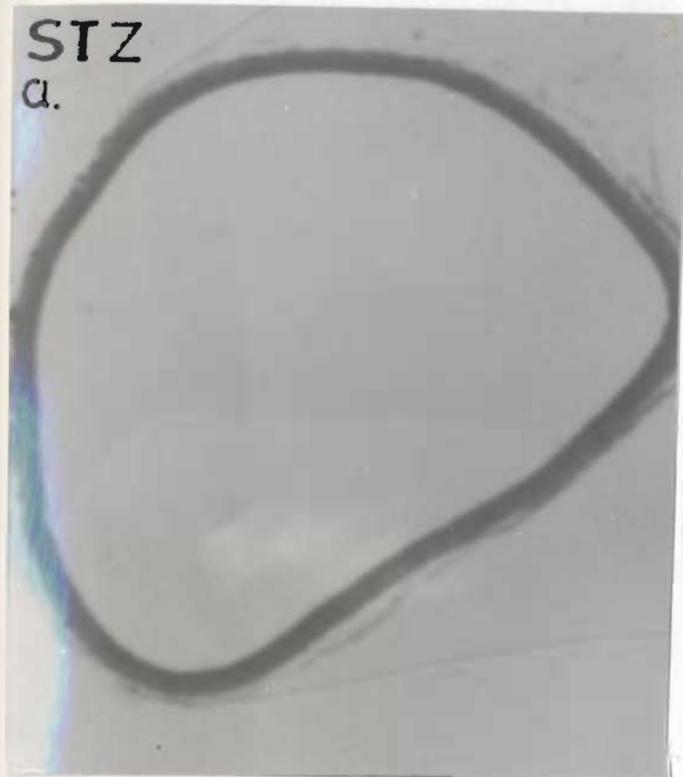were made. Final magnifications 150X and
840X respectively.

STZ

6 OHDA
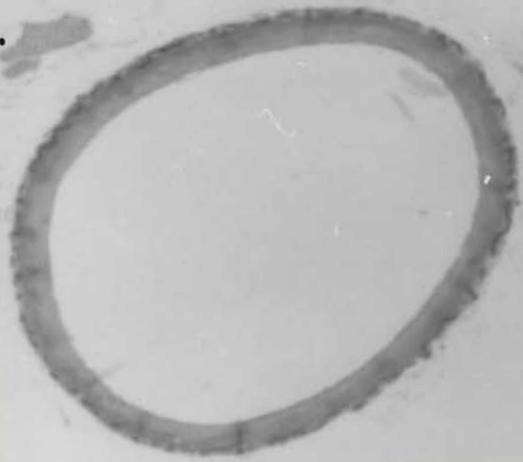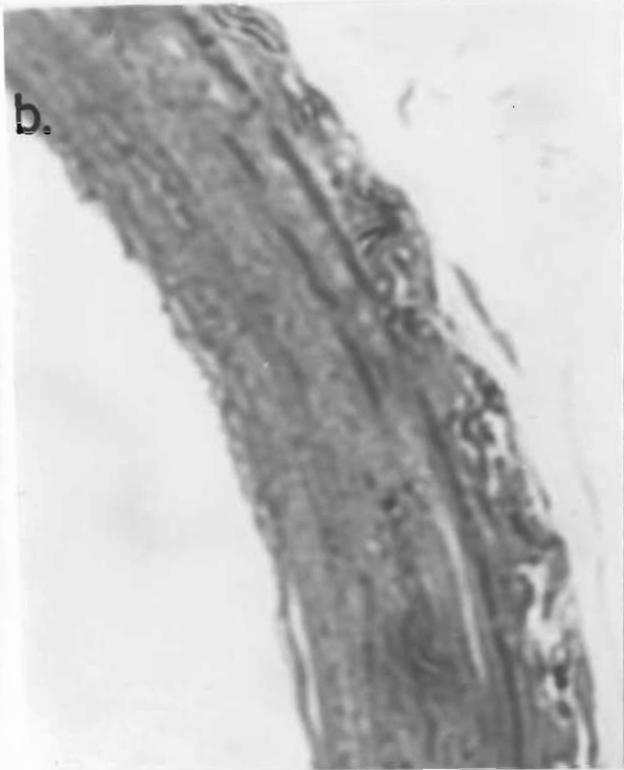
FIG. 4

78.
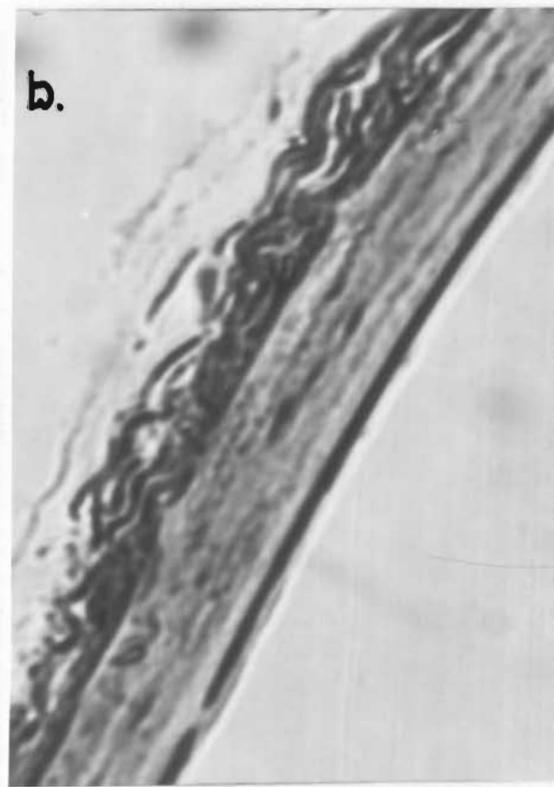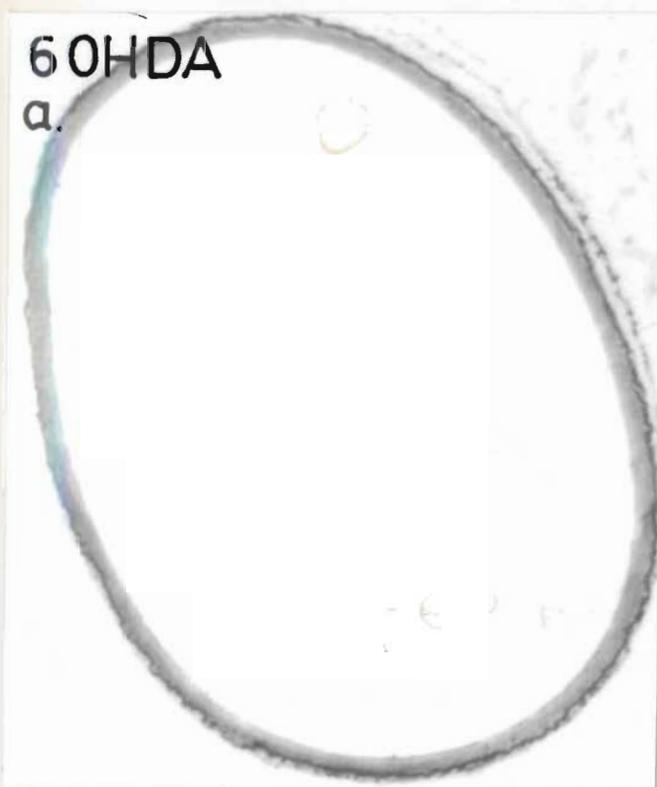
GOLD.
a.
b.

SHR (caps)
a.
b.

FIG. 4 (cont.)
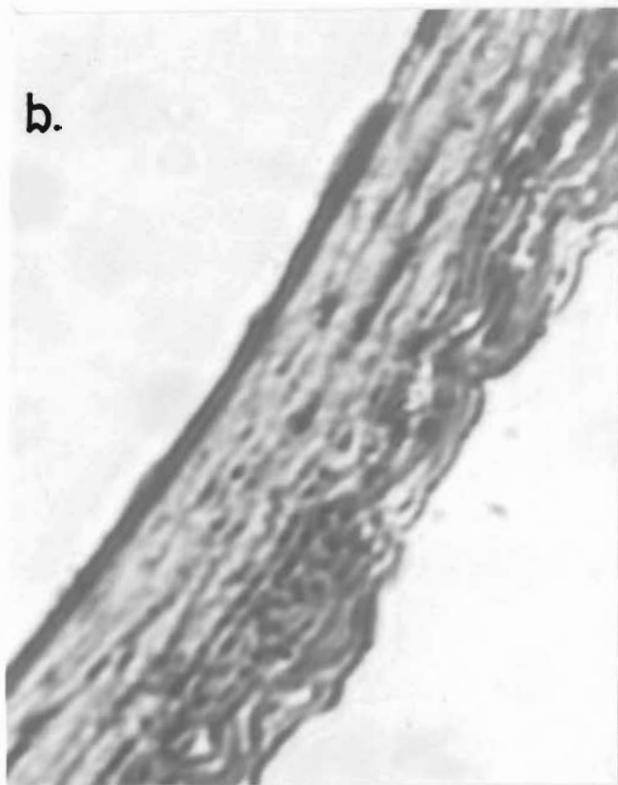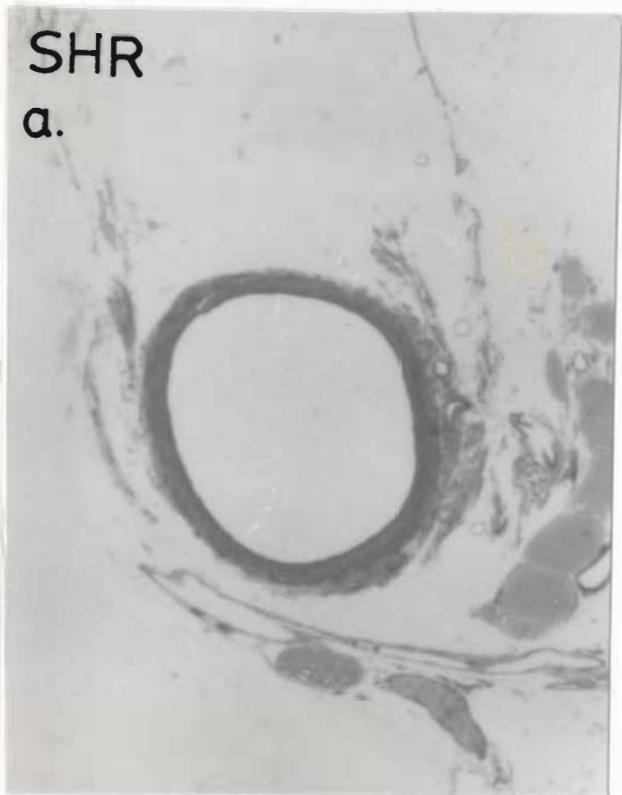
WKY (caps)

a.

b.

SHR

a.

b.
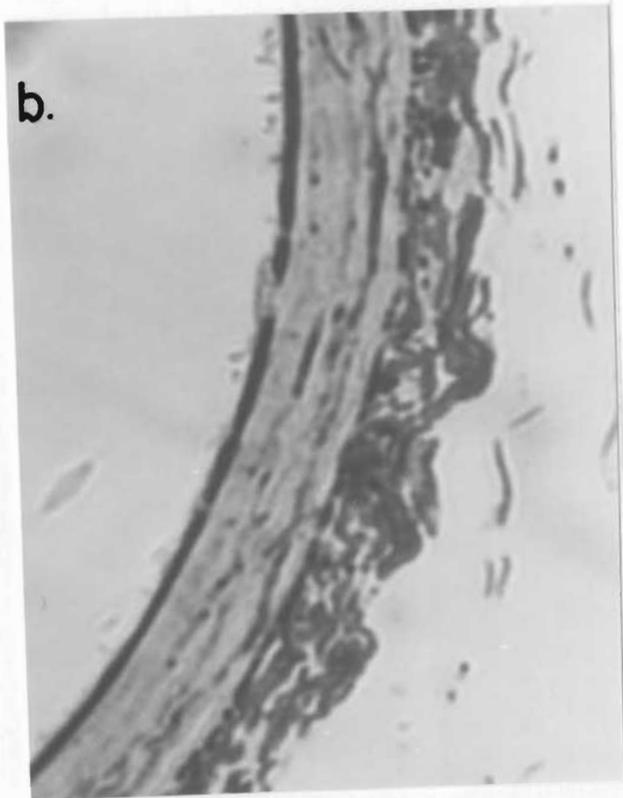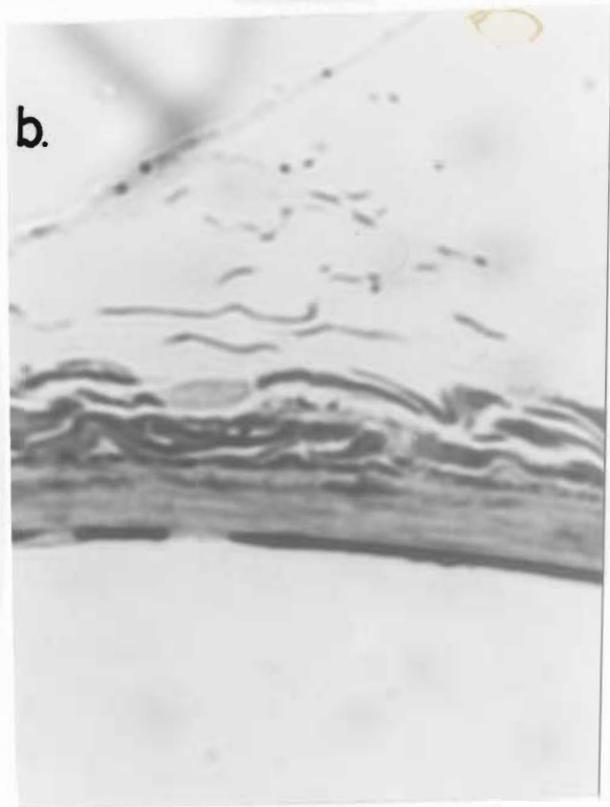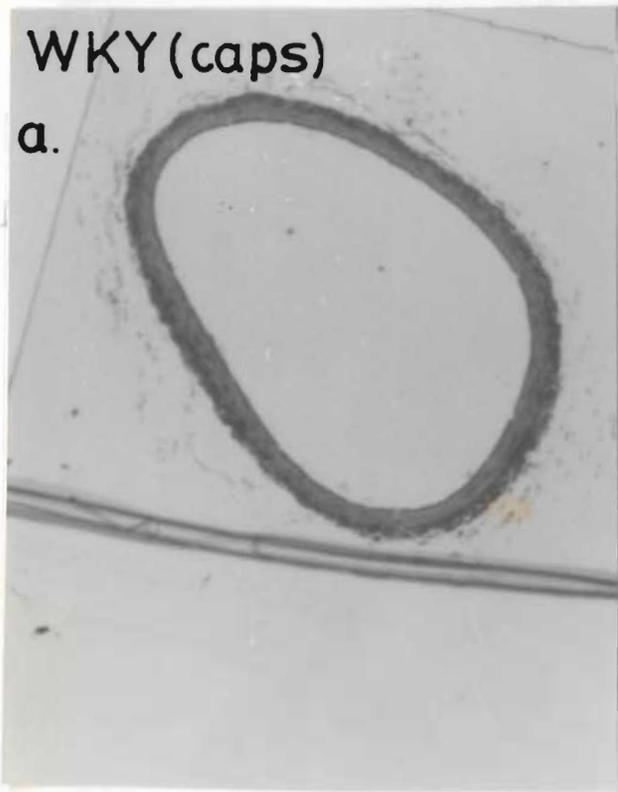
FIG. 4 (cont.)

WKY
a.
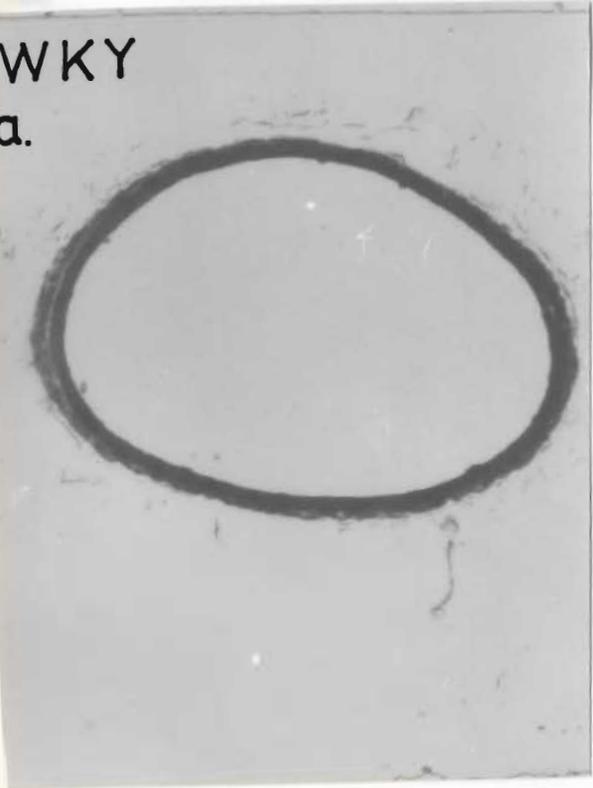
b.
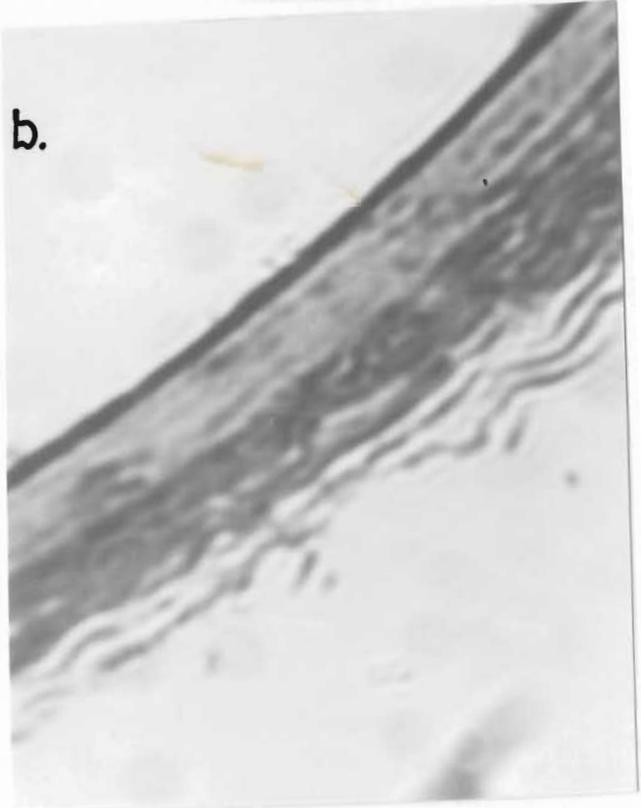
SD
a.
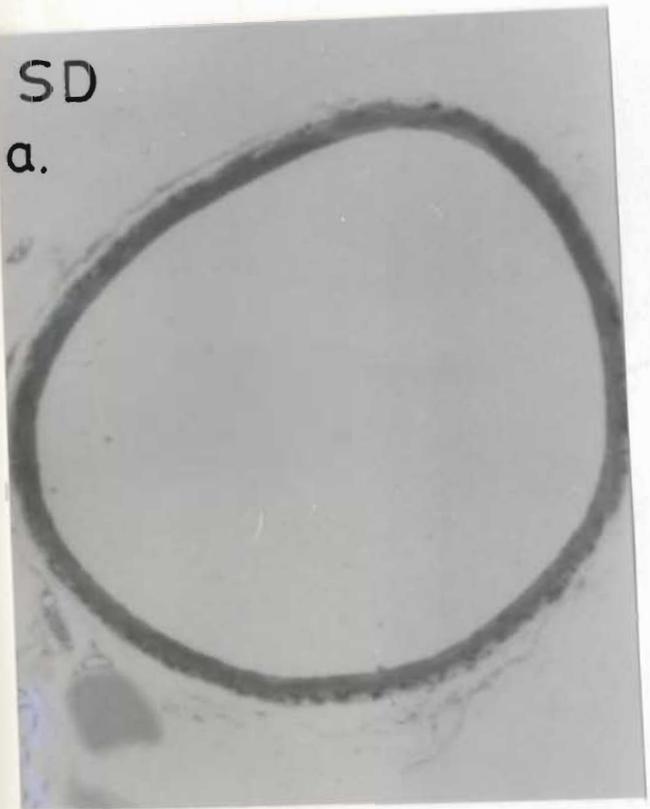
b.
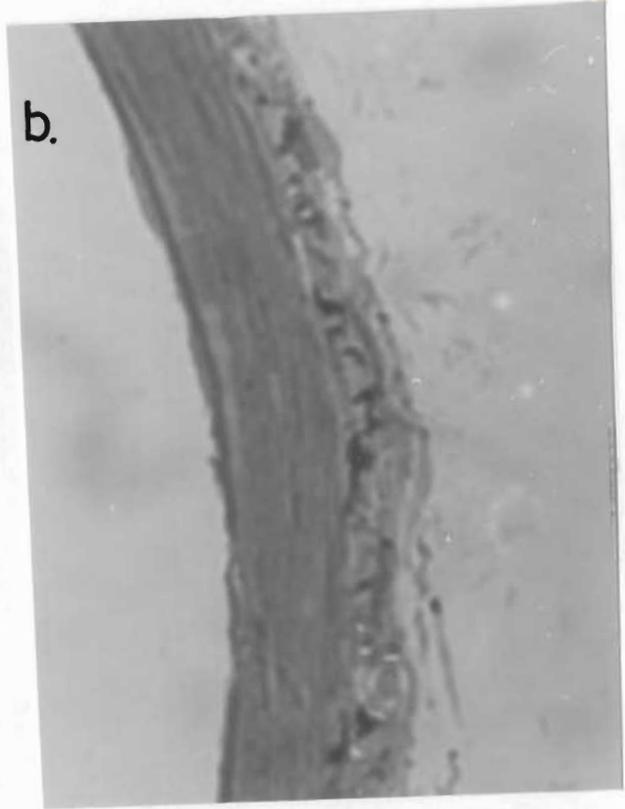
FIG. 4 (cont.)

Figure 5. Representative fluorescent cross-sectional
           micrographs of jejunal arteries from treat-
           ment and control animals showing the typical
           arrangement of fluorescent profiles at the
           medial/adventitial border (X 480)

STZ
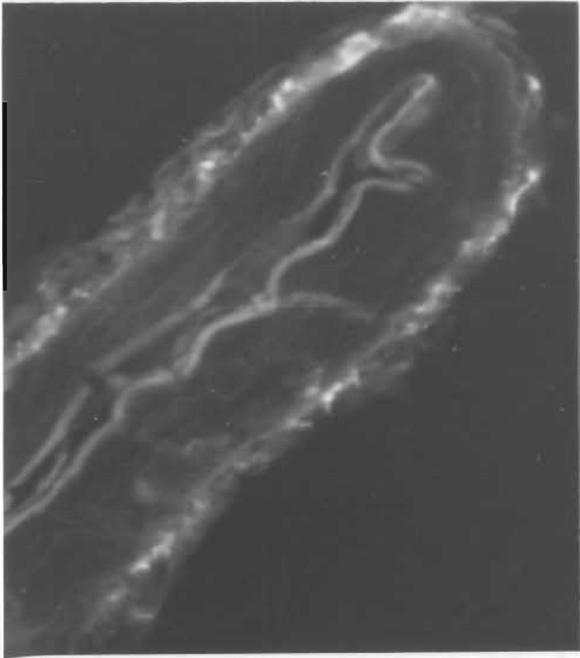
60HDA

GOLD.
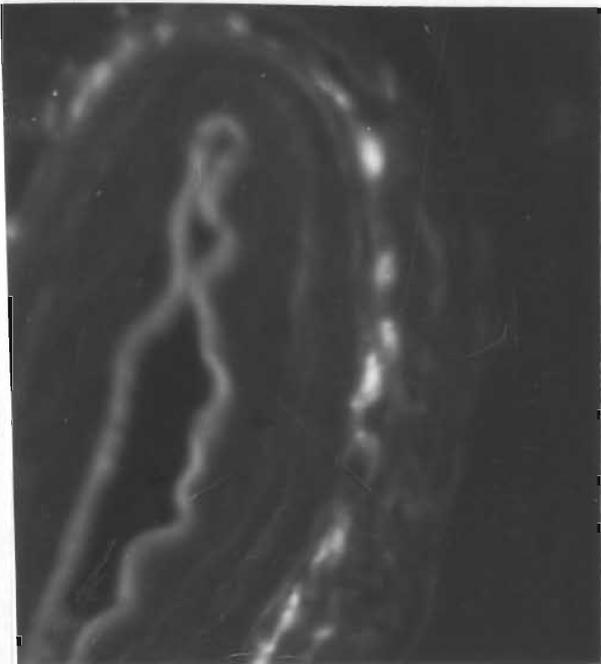
SHR(caps)
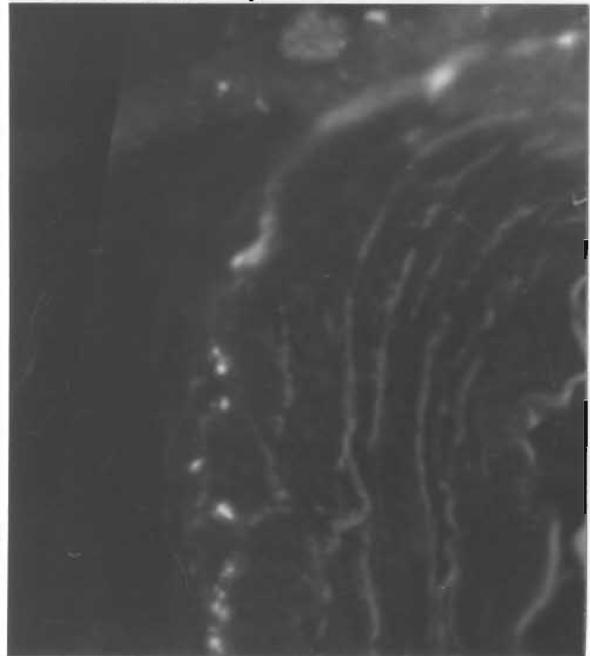
FIG. 5

WKY(caps)
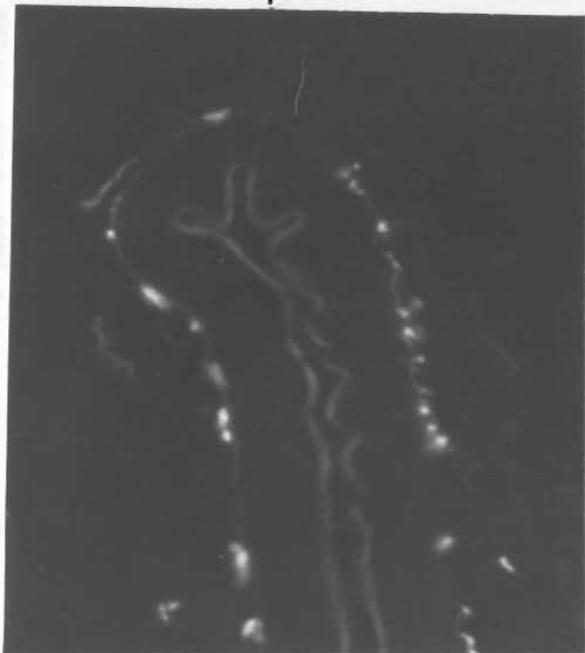
SHR
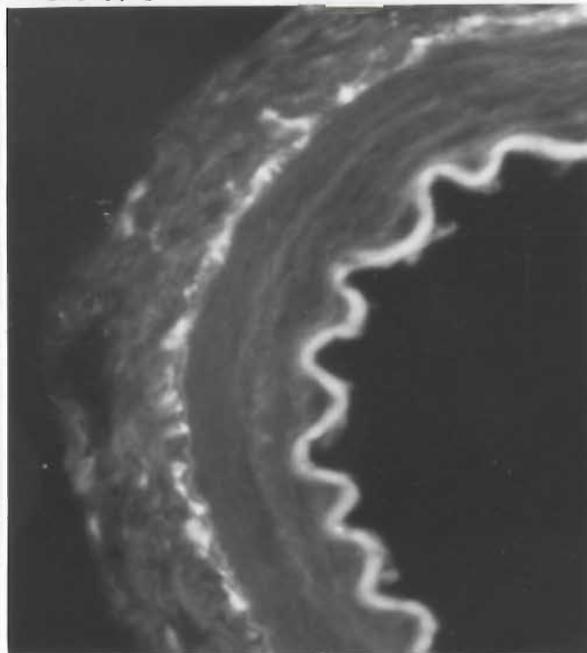
WKY
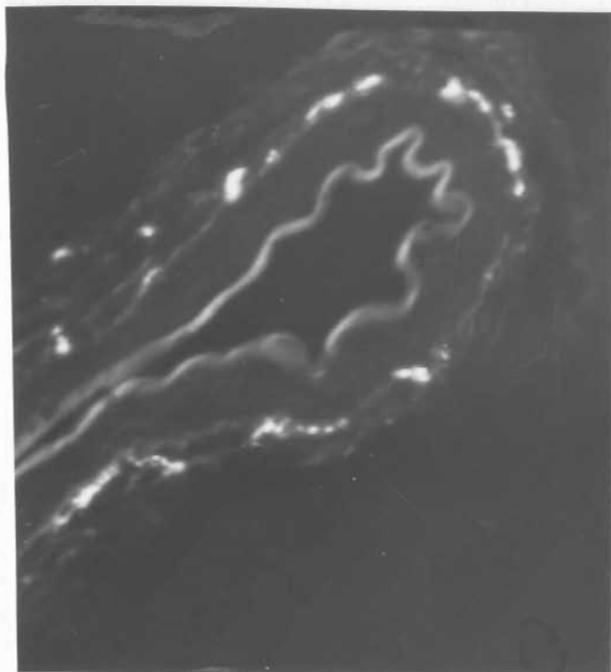
SD
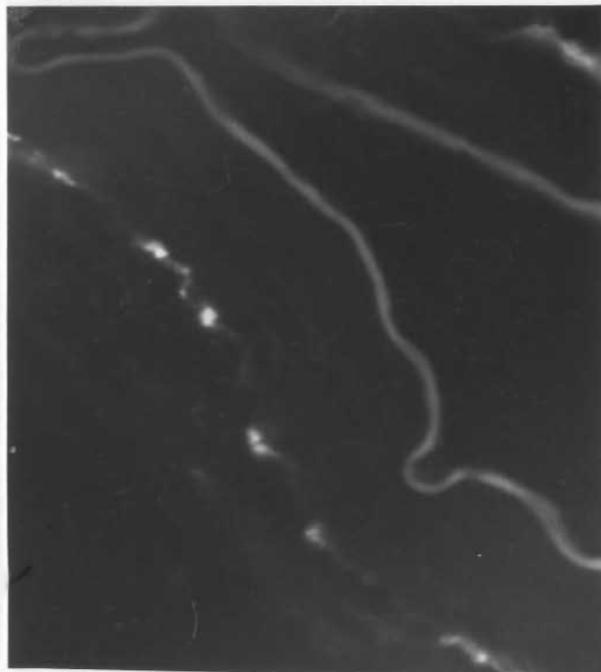
FIG. 5 (cont.)

Figure 6. Mean body weights of developing STZ treated and
normal Sprague Dawley rats. (Bars represent one
standard deviation of the mean. n = 12)

FIGURE 6: Weights of developing STZ treated and normal S.D. rats (n = 12)
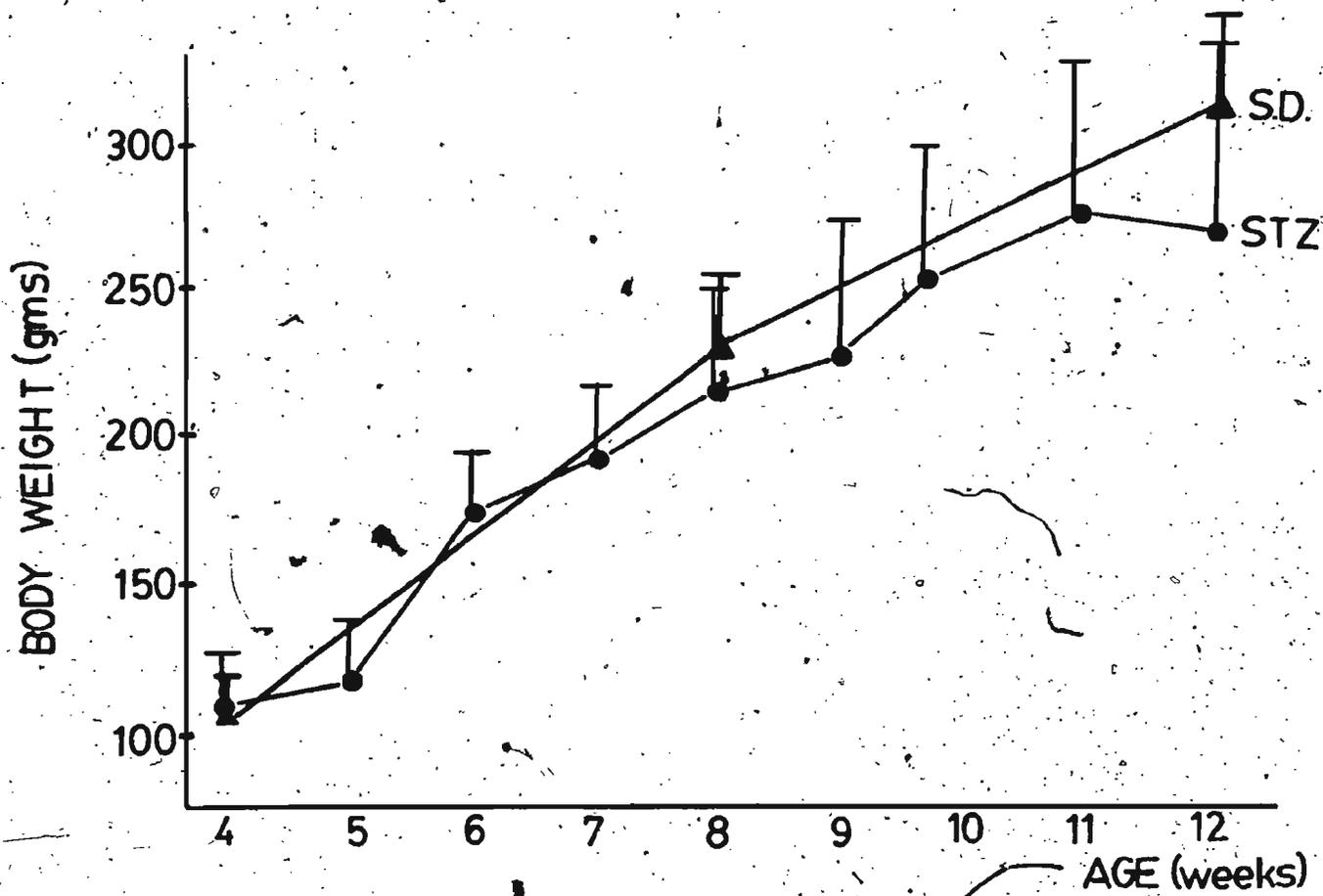
Figure 7.   Histogram illustrating the mean body weight
            of animals in treatment and control groups.
            (Bars indicate the mean $\pm$ the standard
            deviation. n = 12)

FIG. 7: Mean body weight for treatment and control groups.
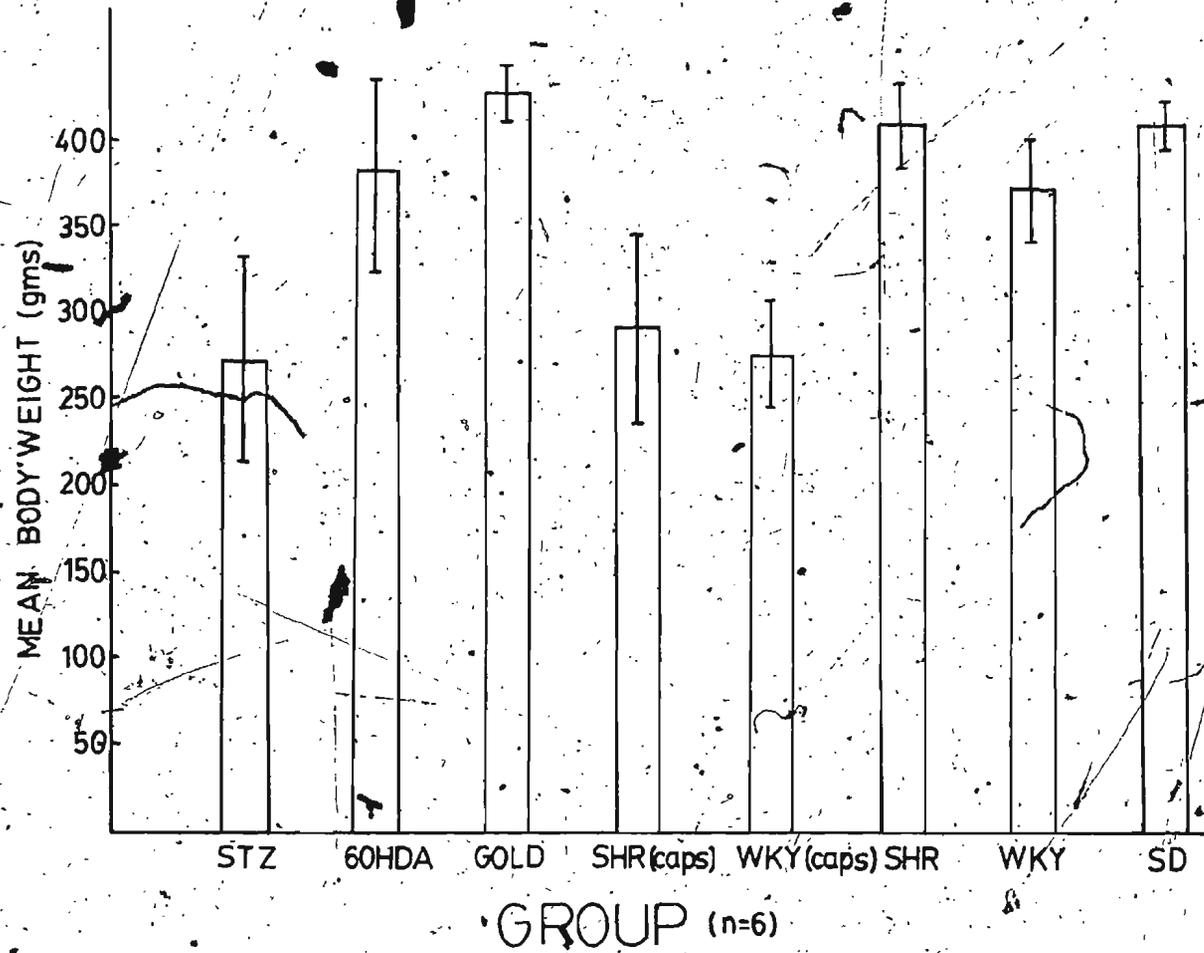
Figure 8.   Compilation of data based on numbers of
fluorescent profiles, mean arterial blood
pressure and medial smooth muscle cell (SMC)
mass across treatment and control groups.
(Bars indicate the mean + the standard
deviation. $n = 6$.)

FIG.8: Data based on fluorescent profiles, blood pressure and medial S.M.C. mass in treatment and control groups.

GROUP (n=6)
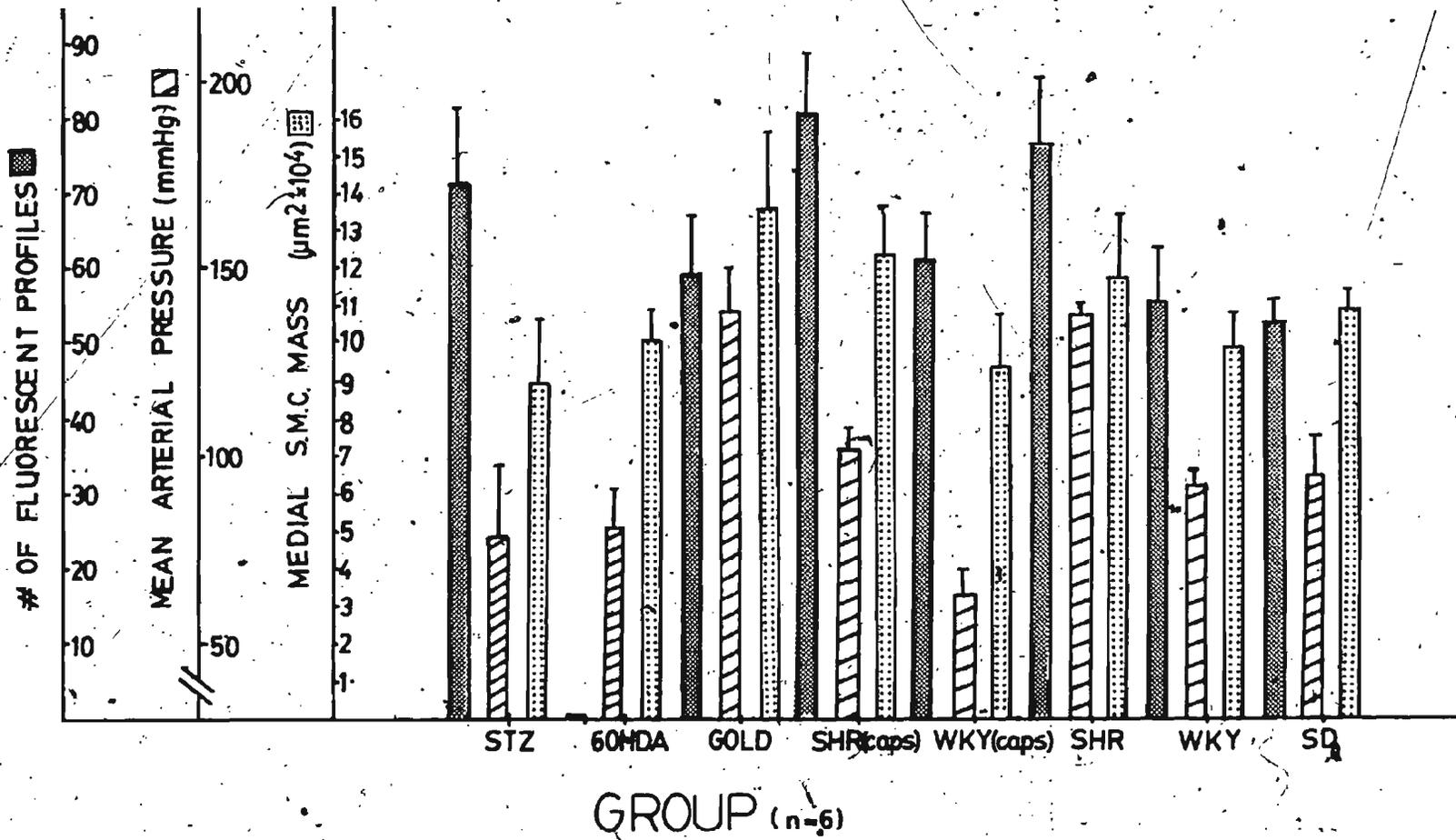
Figure 9.   Medial smooth muscle cell (SMC) mass plotted
against mean arterial pressure.   (Points on
the graph represent the mean ± 95% confidence
limits for the respective variables.   An *
indicates treatment groups where the relation-
ship between mean arterial pressure and medial
SMC mass is significantly different from their
controls.   n = 6)

FIG.9: Medial S.M.C. mass plotted against mean arterial pressure. (n=6)
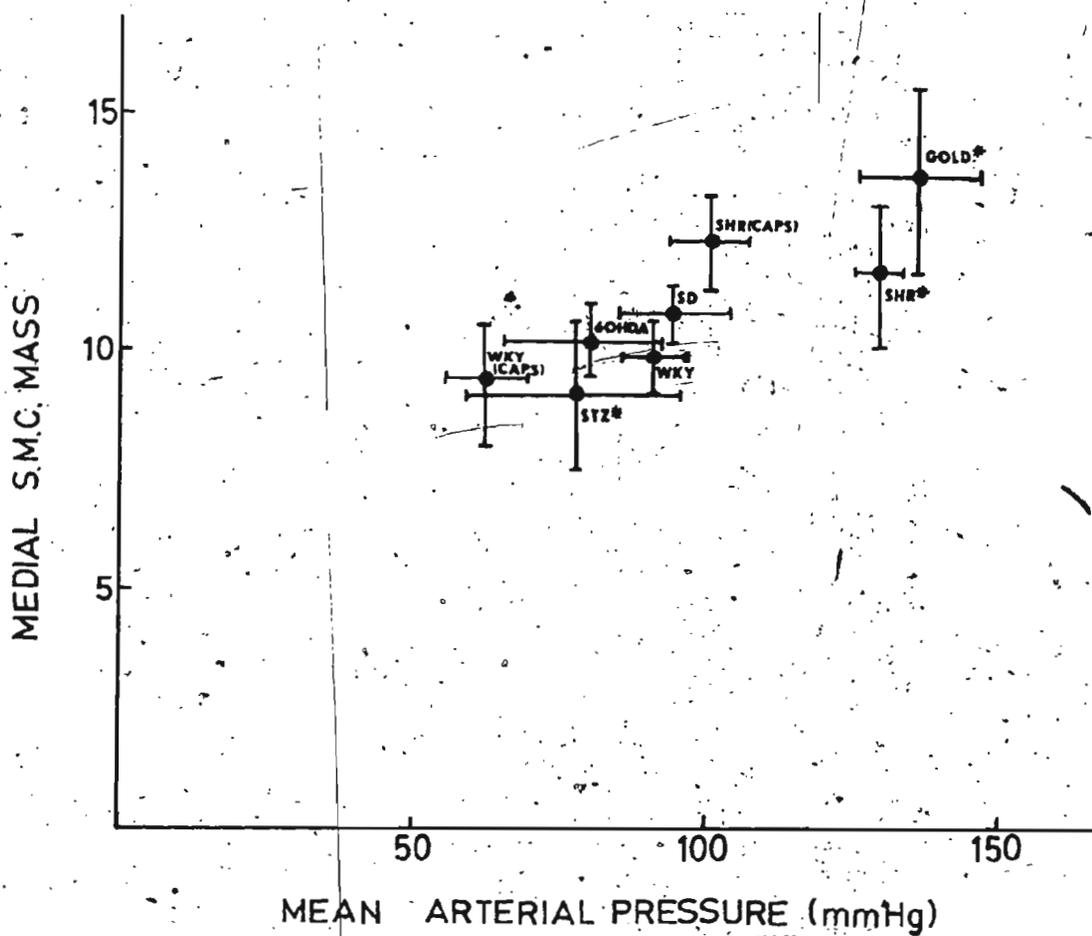
Figure 10.   Medial smooth muscle cell (SMC) mass plotted
             against number of fluorescent profiles.
             (Points on the graph represent the mean + 95%
             confidence limits for the respective variables.
             An *.is indicated treatment groups where the
             relationship between number of fluorescent
             profiles and medial SMC mass is significantly
             different from their controls. n = 6)

FIG.10: Medial S.M.C. mass plotted against # of fluorescent profiles. (n=6)

Y-axis: MEDIAL S.M.C. MASS

X-axis: # OF FLUORESCENT PROFILES
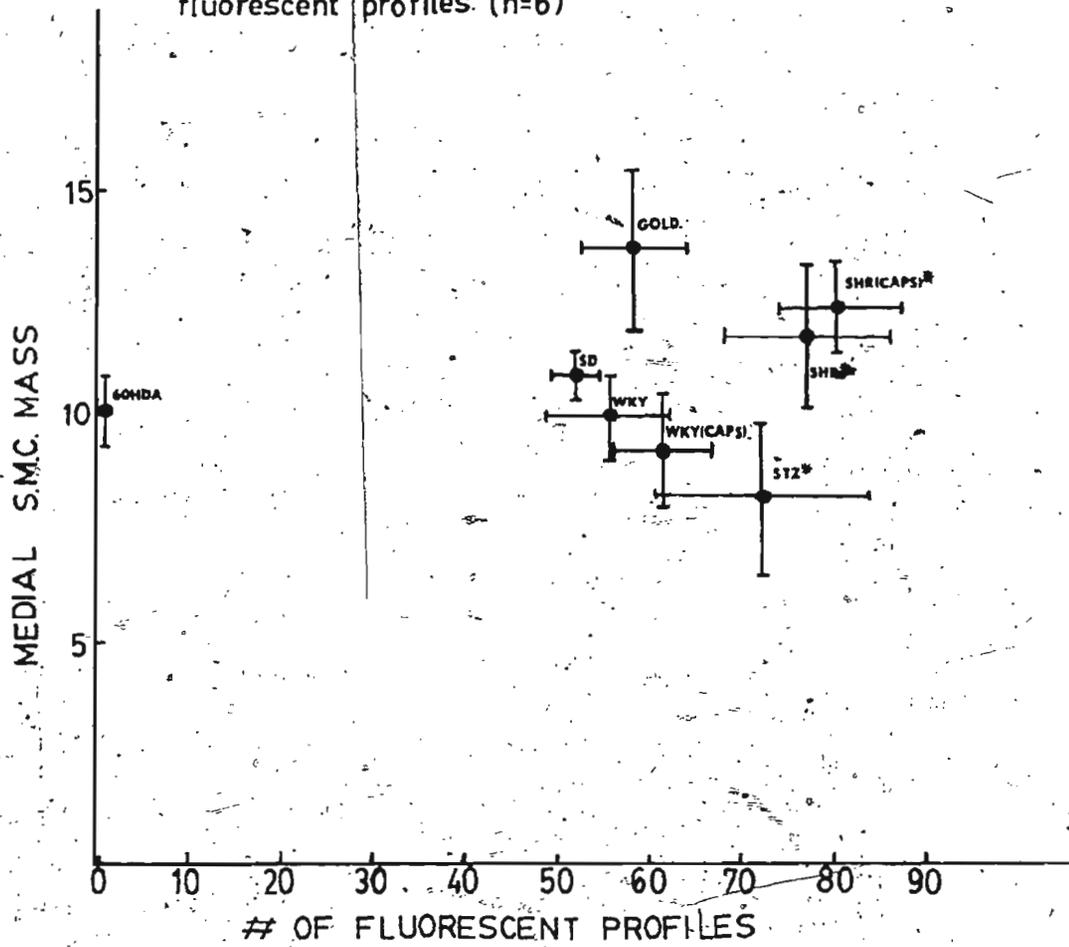
Figure 11.   Series of individual graphs showing key
comparisons between sets of treatment and
control groups with respect to medial SMC
mass, mean arterial pressure (M.A.P.) and
number of fluorescent profiles. A.: STZ
vs. Sprague Dawley (SD); B.: Goldblatt renal
hypertension (GOLD) vs. SD; C.: SHR (caps) vs.
WKY (caps); D.: SHR vs. WKY; E.: 6-OHDA vs.
SD; F.: SHR (caps) vs. SHR; G.: WKY (caps)
vs. WKY. (n = 6)

FIG.11:

A. medial s.m.c. mass ($\mu m^2 \times 10^4$) — •

m.a.p. (mmHg) — - - □

# of fluorescent profiles — ▲

stz GROUP ... sd

B. medial s.m.c. mass ($\mu m^2 \times 10^4$) — •

m.a.p. (mmHg) — - - □

# of fluorescent profiles — ▲

gold GROUP ... sd

C. medial s.m.c. mass ($\mu m^2 \times 10^4$) — •

m.a.p. (mmHg) — - - □

# of fluorescent profiles — ▲

shr(caps) GROUP ... wky(caps)

D. medial s.m.c. mass ($\mu m^2 \times 10^4$) — •

m.a.p. (mmHg) — - - □

# of fluorescent profiles — ▲

shr GROUP ... wky

E

medial s.m.c.mass (μm² × 10⁴)

m.a.p. (mmHg) - - - ▶ - □

# of fluorescent profiles ⟶

6ohda GROUP ──────── sd

G

medial s.m.c.mass (μm² × 10⁴)

m.a.p. (mmHg) ───── □

# of fluorescent profiles ⟶

wky(caps) GROUP ──────── wky

F

medial s.m.c.mass (μm² × 10⁴)

m.a.p. (mmHg) - - - - □

# of fluorescent profiles - - - ⟶

shr(caps) GROUP ──────── shr

DISCUSSION

The major finding of this study, in agreement with the reports from other laboratories (Ichijima 1969, Aprigliano & Hermsmeyer 1977, Abel et al 1980, Bevan & Tsuru 1981), indicates a trophic influence of the sympathetic nervous system on certain morphological characteristics of the blood vessels of the SHR. The data, however, also suggest that these trophic effects may be unique to this particular strain of experimental rat.

I. Body Weight

Table 2 shows that the mean body weight of STZ treated rats was significantly lower than that of controls, with a similar significant difference existing for capsaicin treated animals. This introduces the confounding variable of differing body weights between experimental and control groups and raises the question of whether or not these groups can be considered to belong to a homogeneous population and indeed whether valid comparisons can be made. To try to equilibrate weights between diabetic and control rats would, however, invalidate the homogeneity of the age group. It may be argued that animal weight is a more valid classifying variable than age, however, in the case of the diabetic rat, allowing the animals to survive to attain weights comparable to control values would either result in animals developing long-term diabetic complications (Reinila,1981) further confounding any comparisons, or in

animals which never reach control weight levels. From the graphical representation of developing STZ treated rat weights (Figure 6), it will be seen that at 10 weeks this group's weight began to plateau and from 10 to 12 weeks this group had gained only 15 gm (Table 1).

The difference in weights for capsaicin treated SHR and WKY as compared with untreated SHR and WKY again raises the question whether valid comparisons can be made between animal groups with such differing body weights. The use of capsaicin as an experimental antihypertensive drug is a relatively new tool in preventing the development of hypertension in the SHR. The antihypertensive effect of capsaicin treatment in SHR neonates was reported by Scott and Pang (1982) however their study did not report the relative weights of treated animals.

The author has allowed both capsaicin treated SHR as well as WKY to survive to 20 weeks, however, these animals even at 20 weeks do not grow to within the control weight range. Furthermore it was noted that these capsaicin treated animals at 20 weeks have weights comparable to those of 8 week control animals. Since Scott and Pang (1983) showed no significant increase in medial thickness of jejunal arteries from 8 to 12 weeks in normal developing SHR and WKY it seems therefore acceptable to make comparisons between capsaicin treated animals and their untreated controls. Again it is noted that one must decide on some classification variable on which to compare experimental and control animals in any

experiment. Since using body weight as that variable introduces a host of extraneous influences as a function of differing ages the age has been selected as the classification variable in this study.

II. Streptozotocin induced diabetes

The significant decrease in blood pressure and in medial SMC mass (Table 3, Figure 8 and 11a) is in accordance with previous reports (Bohlen and Niggl, 1979a; Bohlen and Hawkins, 1982). The reduction in the number of perfusing arterioles accompanied by lumen dilation observed in mice by Bohlen and Niggl (1979), is one factor purported to contribute to this reduction in blood pressure in the diabetic state and may be a contributing factor towards the reduced blood pressure observed in STZ treated rats in the present study although no measurements on number of perfusing vessels were actually made. A more likely explanation of the lowered blood pressure however is probably related to the increased lumen diameter:wall thickness ratio seen in these animals as compared with their controls.

The reduction in medial SMC mass may be considered in the light of three possible contributing factors: the sympathetic innervation, the reduction in blood pressure and the nature of vascular deterioration inherent in diabetes.

If one is to assume that a trophic influence of the sympathetic nervous system also operates outside the SHR strain of rat and extends to other vascular disease states,

then the hyperinnervation of noradrenergic fibers seen in
the diabetic animals of this study cannot be used to explain
why a decrease in medial SMC mass was observed. Since the
number of SMC cell layers significantly exceeded control levels
(5.17 as compared with 4.57) one must concede that the medial
SMC mass is a more reliable measure than number of SMC layers;
further, similar values for mass may be obtained from specimens with
differing numbers of layers. In any event the hyperinnervation
observed in the diabetic rats has not been described previously.

The significant reduction in blood pressure seen in the
diabetic group must be considered as a possible contributing
factor to the reduced medial SMC mass observed. A reduction in
blood pressure could serve to reduce medial mass in a purely
pressure-dependent manner. The reduced dilation of vessel lumens
in this group could serve to decrease blood pressure and lessen
any medial SMC growth to subnormal levels.

The third possibility, a microvascular breakdown
inherent in diabetes, has been reported by Bohlen and Niggl (1979a).
Bohlen and Niggl (1979b) reported that the normal development of
arterioles in mice is depressed in juvenile onset diabetes. The
report of Wolinsky et al (1978) shows a depression of hydrolase
enzymes in aortic vascular muscle from animals with juvenile
onset diabetes and suggests that the low enzyme activity may
explain the depression of arteriole development. The reduction in
medial SMC mass observed in diabetic rats in the present

study may, as Wolinsky (1978) suggested, be due to arteriolar abnormalities characteristic of diabetes.

III. 6-hydroxydopamine chemical sympathectomy

The prolonged schedule of administration of 6-OHDA employed in this study produced a complete ablation of visible catecholaminergic fluorescence in sections from jejunal arteries. 6-OHDA has been reported to be similarly effective in inducing sympathectomy by a host of other investigators (for a review see Kostrzewa and Jacobowitz, 1974). The mean arterial pressure in this group was also significantly reduced (Table 3, Figure 8 and 11e), a finding earlier reported by Sinaiko et al (1980). This reduction in blood pressure poses another confounding factor to proving the hypothesis that a reduced or absent innervation is operative in any observed reduction in the media of chemically sympathectomized rats.

In 6-OHDA treated animals, while medial thickness was significantly reduced ($p \leq .05$), the estimated medial SMC mass was not significantly different from control animals. Such a total ablation of the sympathetic innervation of the jejunal arteries studied should be expected to produce a more pronounced effect on the smooth muscle cell content of the media if a trophic effect of the sympathetic nervous system was operating in this model. Indeed the trend towards the reduction of the medial SMC mass (10.73 mm to 10.03 mm) could probably be accounted for on the basis of a reduced blood pressure alone.

The literature concerning the trophic role of the sympathetic nervous system on vascular smooth muscle clearly points to structural changes in the arterial wall of denervated arteries in normal (Bevan and Tsuru, 1981, Rowan et al, 1981) as well as the SHR (Hart et al, 1980) over similar time periods as those employed in this study. The results of this study, however, show that for the 6-OHDA treated SD rats, no pronounced regression of medial SMC mass due to sympathectomy could be extrapolated from the data. It is possible however that had animals remained sympathectomized for a longer period, the trend towards a reduction in medial SMC mass would have continued to a level of statistical significance.

IV. Goldblatt renal hypertension

It will be noted from a summary of the results (Figures 8 and 11b) that while numbers of fluorescent profiles (Table 4) visible around jejunal arteries from renal artery constricted animals remained at control levels, the mean blood pressure of this group was significantly elevated $(p \leq .05)$ as was the volume of the medial SMC mass $(p \leq .05)$. Clearly the Goldblatt method employed for induction of hypertension in these animals was effective in elevating the pressure of the operated animals with only 2 of the operated animals failing to reach the critical level of a systolic pressure greater than 150 mmHg.

Goldblatt renal hypertension does not appear to

exert any increase or reduction in sympathetic nerve activity
as measured by profile counts. For the purposes therefore
of studies of the role of the sympathetic system in
hypertension this method provides a reliable experimentally
hypertensive animal with an unaltered sympathetic nervous
system (at least from a structural point of view). The
maintenance of control levels of catecholaminergic innervation
with a significantly higher medial SMC cell mass in this
group (Figure 11b) clearly is indicative of a pressure-dependent
increase in medial SMC mass operating in this particular model.

As previously discussed (see Introduction) Owens et
al (1981) reported that in the SHR, medial SMC hypertrophy was
the single contributing factor to the increase in arterial
wall mass. Bevan et al (1976) reported a hyperplasia of
arterial SMCs in experimentally induced hypertension in
rabbits. Table 7 indicates for the present study, no increase
in number of layers of SMCs in the media of Goldblatt
experimentally induced hypertensive SD rats, suggesting that
experimental hypertension in the animals used in this study
induced a hypertrophy and not a hyperplasia of medial SMCs.

V.   SHR Capsaicin treated and SHR

Table 3 shows the mean arterial pressures of the SHR
(Caps) and the SHR untreated to be significantly different
($p \leq .05$) at 101.1 ± 5.2 and 136 ± 3.4 mmHg respectively.

The blood pressure of capsaicin treated SHR thus remained
at normotensive levels. The number of fluorescent profiles
however was significantly increased (p ≤ .05) in capsaicin
treated as well as untreated SHR (Table 4, Figure 8 and
Figure 11c and 11f) as was the medial thickness and medial
SMC mass (p ≤ .05) when compared with SKY (Caps) and WKY
untreated controls. This hyperinnervation has been previously
reported in the SHR (Scott and Pang, 1983 and Ichijima, 1969),
however, prior to this study no such data were available
for a genetically hypertensive rat with a reduced blood
pressure as in the case of the capsaicin treated SHR.

In a consideration of the trophic effect of a
hyperinnervation of the sympathetic nervous system in pro-
ducing a medial hypertrophy such as that seen in the SHR it
has been difficult to try and separate the effects of
elevated blood pressure (pressure-dependent hypertrophy)
with those of the observed hyperinnervation (pressure independent
hypertrophy) in the observed medial hypertrophy (Cox, 1982;
Mulvany et al, 1981; Greenberg et al, 1978 and 1981). To
what factor then does one attribute the hypertrophy observed
in the SHR? Clearly in the capsaicin treated SHR where blood
pressure was lowered to normotensive levels, a pressure-dependent
hypertrophy may be ruled out. Thus the significant
hyperinnervation (p ≤ .05) of the capsaicin treated animals
(81.26) as compared with WKY capsaicin treated (62.1) or WKY
untreated controls (55.9) would appear to be a contributing

factor to the observed hypertrophy, a pressure independent hypertrophy.

There appears therefore to be a trophic influence of the sympathetic nervous system operating in the genetically hypertensive SHR strain which has been demonstrated to be separate from the effects of elevated blood pressure.

VI.   Control series

The numbers of fluorescent profiles between capsaicin treated and untreated WKY did not differ. Thus the effect of administration of capsaicin did not appear to affect development of the sympathetic nervous system.

Volume of medial SMC mass was also similar in these two control groups of animals.

Capsaicin treatment lowered blood pressure in WKY animals as well as in SHR with capsaicin treated WKY having significantly lower ($p \leq .05$) blood pressures as compared with untreated WKY.

The blood-pressure lowering effect of capsaicin is therefore not unique to the hypertensive state in the SHR but extends to normotensive animals.

VII. General Discussion

Graphs plotting medial SMC mass against mean blood pressure, and against number of fluorescent profiles are presented as Figures 9 and 10 respectively.

It will be noted that in Fig. 9 the experimental groups marked with an asterisk show a significantly different relationship between mean arterial pressure and medial SMC mass than do their controls. Similarly for Fig. 10 those groups marked with an asterisk show a significantly different relationship between number of fluorescent profiles around jejunal arteries and medial SMC mass.

The data show that in the Goldblatt renal hypertensive group, while there existed significantly higher blood pressures ($p < .05$) and medial SMC mass, relationship between these two variables was irrespective of number of fluorescent profiles.

In the SHR untreated group, Fig. 9 illustrates the significant relationship ($p < .05$) between mean blood pressure and medial SMC mass ($p < .05$), however, Fig. 10 shows a similar significant relationship ($p < .05$) between numbers of fluorescent profiles and medial SMC mass. The blood pressure lowering effect of capsaicin serves to separate the effects of pressure as opposed to sympathetic innervation on the development of a hypertrophied media (Fig. 11f). Only the

increase in numbers of fluorescent profiles was found to be
significantly related to the mass of cells of the media in
these animals (p $\leq$ .05). Therefore, separating the effect
of blood pressure on the medial hypertrophy observed in the
SHR suggests a more singular role of the sympathetic nervous
system operating in this genetic strain.

The question of whether the sympathetic nervous
system is active in non-genetically hypertensive states and
in diabetes is a primary concern of the present research.
Fig. 9 illustrates a significant difference (p .05) from control,
between mean arterial pressure and medial SMC cell mass for
animals experimentally rendered hypertensive by the Goldblatt
method. No such relationship, however, was seen to exist
between numbers of fluorescent profiles and medial SMC mass
when compared with their SD controls. Similarly, ablation
of the sympathetic nervous system by 6-OHDA sympathectomy
showed no significant reduction in the volume of cells
within the media nor in the numbers of smooth muscle
cell layers for that of controls. Thus in the non-genetically
experimentally induced hypertensive SD animals there is
apparently only a pressure dependent effect on the medial
SMC mass. Furthermore the fact that no relationship was
shown to exist between the absence of a sympathetic
innervation of jejunal arteries and the volume of SMCs in
the media of 6-OHDA treated SD rats lends support to the

conjecture that the trophic effect of the sympathetic
nervous system seen in the SHR strain does not extend to
other strains of rats. .It is possible, however, that a
longer period of sympathectomy may have increased, the trend
toward reduction in these parameters. A study by Todd
(1969) suggesting that the innervation of femoral, superficial
epigastric and tail arteries of Wistar rats reached a plateau
at 30 days of age, however, casts doubt on this possibility.
Scott and Pang (1983) however, later reported that at least
in the SHR and WKY the sympathetic innervation of jejunal
arteries increased to at least 12 weeks. This, therefore,
may be an important difference in the trophic effect of the
sympathetic nervous system which may be operating in the
SHR/WKY strains but not in the SD strain of rat observed in
this study. More complete comparisons between various
genetic strains of experimental animals would appear to be
an important research area for further investigations.

A second concern of the present research was the
question of whether the trophic influence of the sympathetic
nervous system extended to the disease-state of diabetes.
While the significant increase in the innervation cannot be
accounted for it certainly does not exert a growth promoting
effect on the cells of the media in diabetic animals. Indeed
a negative relationship exists. It would seem therefore that
this trophic influence neither extends to the models of the

normal nor to the disease state studied in the present investigation.

Finally, it must be conceded that although the number of fluorescent profiles offers a measurable index of noradrenergic activity at the neuromuscular junction in the jejunal arteries examined in this study, it is possible that another index of noradrenergic activity may be the more appropriate measure. For example, perhaps the total amount of noradrenaline released per stimulus or tonic activity of the sympathetic nervous system would be more appropriate measureable variables. Number of fluorescent profiles have, however, been generally accepted as a valid measure of sympathetic activity (Todd, 1969, Scott and Pang, 1983).

CONCLUSIONS

The general objectives of the present study were:

1) To examine the trophic relationship between the catecholaminergic innervation and the structure of the media of small diameter arteries during normal development and in both the genetically and experimentally induced disease state.

2) To determine the limits of this trophic influence on arterial wall structure.

The general conclusions to be drawn from the investigation are:

1) An increase in the medial smooth muscle cell mass observed in the SHR strain of rat appears to be the result of a sympathetic hyperinnervation and not due to the influence of an elevated arterial pressure.

2) This trophic effect of the catecholaminergic innervation did not extend to the normal state nor to experimentally induced disease states.

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