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The Morphogenesis Of The Experimental Poststenotic Dilatation Of The Canine Carotid And Femoral Arteries

Alberto Trillo

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THE MORPHOGENESIS OF THE EXPERIMENTAL POSTSTENOTIC
DILATATION OF THE CANINE CAROTID AND FEMORAL ARTERIES

by

Alberto Trillo, M.D.

Department of Pathology

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Canada
1972

© Alberto Trillo, M.D.
Con Amor a Mi Madre y a Mi Esposa
It is to experiment on the living animal that final reference must always be made to reach an understanding of organic properties. ..... 

Claude Bernard
Memoir on the pancreas
ABSTRACT

The present study was undertaken to test whether structural changes do take place in experimentally produced poststenotic dilatations in dogs. Poststenotic dilatation of the common carotid and femoral arteries was induced by incomplete ligature in six dogs. The contralateral arteries served as controls. Of the six animals, two were kept for one month, two for three months and the remaining two for six months.

In the control carotid arteries, two populations of smooth muscle cells were observed: deep-dark staining and pale cells. The cellular junctions between these cells were of the desmosome type, however, areas of apparent cytoplasmic continuity were observed along the apposing plasma membranes.

In addition, the extracellular spaces in the walls of carotid and femoral arteries contained small, membrane-bound bodies refered to as granulo-vesicular bodies. The possibility that granulo-vesicular bodies are produced by the smooth muscle cells and "secreted" into the extracellular spaces where they may play a role in connection with the process of remodeling of elastic fibres was suggested.
Changes in arterial architecture were observed by both, light and electron microscopy as early as one month following the arterial ligature. The morphological changes appeared to represent two different tissue responses: a proliferative or reparative and a degenerative.

The earliest alterations consisted of diffuse intimal thickening with proliferation of smooth muscle cells and connective tissues. Rupture of the internal elastic lamina and irregularities of its surface were features consistently present in all dilated segments.

The most conspicuous changes however, were observed in the media, where the elastic lamellae appeared fragmented, the interstitial spaces widened and the collagen fibrils increased. The fragments of elastic tissue seemingly underwent "dissolution" and the collagen fibrils aggregated in bundles that gradually lost their fibrillar structure and periodicity becoming amorphous electron dense masses.

On the basis of the present observations, it may be concluded that the main morphological substratum of the poststenotic dilatation consists of degenerative changes of the elastic elements with a concomitant increase of the interstitial fibrous tissue.
ACKNOWLEDGEMENTS

It is difficult to convey adequately in words the deep appreciation and gratitude I feel towards the persons and institutions which in different ways contributed to make the conclusion of this work possible.

During the last three years I have had the privilege of working under the direction of Dr. M. Daria Haust, Professor of Pathology. My association with Professor Haust has signified the most rewarding and gratifying journey that I have so far endeavoured, and I now fully understand the feeling that I sensed in the words of my early mentor, Professor Isaac Costero, when he talked about his good fortune to have studied under Ramon y Cajal and Rio Hortega.

I was fortunate to have been able to count on the advice and opinions of Dr. Margot R. Roach, Professor and Chairman of the Department of Biophysics, and a leading researcher in the field of poststenotic dilatation.
Special gratitude is due to Dr. A. C. Wallace, Professor and Chairman of the Department of Pathology, for allocating the necessary space and making the facilities of his department available to me.

The ever present willingness and technical skill of Miss Irena Wodjewodzka and Mr. Roger Dewar were also of invaluable help.

The staff of the Health Sciences Library, especially Mrs. Agnes Kutas, deserve many thanks for their untiring efforts in helping me to collect the reference works.

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I. INTRODUCTION AND PURPOSE OF THESIS

Poststenotic dilatation refers to a hydrodynamic phenomenon that occurs when in a closed circulating system a segment of the conduit is constricted circumferentially and a dilatation distal to the constriction develops. The poststenotic dilatation has been considered as a paradox because its development does not conform with the general laws of hydrodynamics. According to these laws, in a closed circulating system constricted circumferentially the pressure is expected to rise proximally to the stenosis and decrease beyond it; thus, the dilatation might be expected to occur at the high pressure pre-stenotic segment. Surprisingly enough an opposite effect takes place in post-stenotic dilatation, as it occurs at the low pressure area, i.e. immediately beyond the constriction.

To explain this phenomenon many hypotheses have been suggested, and although to date none has been conclusively proven nor accepted, there is a general agreement
that turbulence seems to be prerequisite for the development of poststenotic dilatation.

In patients, poststenotic dilatation has not been reported often, however, it may occur beyond almost every type of arterial stenosis both of intrinsic and extrinsic origin (Roach, 1962; 1963 b; Roach and MacDonald, 1970). It has been described commonly in association with a cervical rib compressing the subclavian artery (Halsted, 1918; Holman, 1954 b; Steinberg, 1957); stenosis of the pulmonary artery (Brock, 1952; De Vries and Van den Berg, 1958; Keith et al., 1958); aortic valve (Jarchow and Kincaid, 1962); renal artery (McCormack, 1961; Keith et al., 1958; Roach and MacDonald, 1970); and compression of the popliteal artery by a displaced ligament or tendon (Gedge et al., 1961).

In animals poststenotic dilatation has been reproduced by various means in the thoracic aorta of calf (De Vries and Van den Berg, 1958); newborn pups (Gerbode and Hultgren, 1951); abdominal aorta of adult dogs (Halsted, 1916; Reid, 1916; 1924); aortic arch of sheep (De Vries and Van den Berg, 1958) and chicks (Rodbard et al., 1963; 1967), and in carotid and femoral arteries of adult dogs (Roach, 1962; 1963 b).

In vitro production of poststenotic dilatation has
been achieved in isolated segments of human and canine iliac arteries (Roach, 1962; 1963 a) and in segments of rubber tubes (Holman, 1955; De Vries and Van den berg, 1958; Bruns et al., 1959).

The hemodynamics of poststenotic dilatation have been studied extensively; however, there is paucity of morphological studies, and the few reported are somewhat contradictory and were carried out only at the light microscopic level. It has been proposed on the basis of roentgenographic measurements and analysis of tension-length curves, that poststenotic segments dilated experimentally for as long as 10 months (Roach, 1970), return to normal diameter and function upon removal of the constrictive devices. These functional studies were not accompanied by morphological evaluation of the arterial wall.

Return to normal diameter and function of the arterial wall does not preclude the possibility, however, that the long period of unphysiological state does not cause subtle changes of architecture. Indeed, it is difficult to conceive that such changes do not take place and would not be reflected ultimately in altered function, were the vascular wall left in situ for a long period of time following the relief of the constrictive device.
The present study was undertaken to test whether under the conditions of experimentally produced poststenotic dilatation in dogs structural changes do take place. Since these changes could be of subtle nature and difficult to detect on light microscopic examination, electron microscopy was employed as well. The purpose of this thesis is to report the results of morphological studies undertaken with the above aim and to interpret these results in terms of morphogenesis of the lesions observed.

It is not within the scope of this thesis to discuss biophysical or other implications of these results.
II. REVIEW OF THE LITERATURE

A. Nomenclature and Early Descriptions

The term poststenotic dilatation was introduced to the medical literature relatively recently; it was coined by Halsted in 1916 to describe and emphasize the peculiar hemodynamic characteristics of this entity. In the literature the terms poststenotic dilatation and poststenotic aneurysm have been used as synonyms (Halsted, 1916; Holman, 1954 a; 1955; Frederiksen and Poulsen, 1961; Wellington and Martin, 1965). From the historical point of view there has been disagreement in defining the correct meaning of the word aneurysm, as well as of its etymology; thus Lancisi (1728) in his monumental work *De Aneurysmatibus Opus Posthumum* dedicated one chapter to the explanation of the origin and significance of an aneurysm. He wrote: "The word aneurisma, which the Arabians corrupted into emborysma, aporisma, and hyporisma, but which the Latin writers confined strictly to the dilatation of an artery, has been derived by some of the moderns from three Greek terms."
Hieronimus Montanus derives the word *aneurisma* from a priva-
tive and *νευρον*, that is to say, a *nervus*. This deriva-
tion is not much approved by Marcus Aurelius Severinus who
cannot understand how it can hold good, there being a
specific difference between a nerve which is not the seat
of the disease and an artery which is, yet, he attempted an
explanation if we understand by *enervatio* a debility of the
artery. Johan Baptista Silvaticus, one of the most diligent
writers amongst the followers of Galen on this disease, has
derived the word *aneurisma* from the Greek verb *ευρονω*
dilato; for in ordinary aneurisms the artery is always
dilated. Lastly, Severinus thinks that *aneurisma* is derived
from *ευρενεξιλίρε* or *aflluerere*.

This rather confusing delineation of the word
aneurysm has unfortunately persisted to date, as evidenced
by the definition offered in Stedman's Medical Dictionary:
"A circumscribed dilatation of an artery, or a blood con-
taining tumor connecting directly with the lumen of an
artery". This definition is not easily applicable to our
present knowledge on the subject of aneurysms, as it contains
the Galenic concepts of this disease. A more concise and
generally accepted definition of an aneurysm is that of
Gore (1968), who wrote: "Aneurysm is any abnormal localized
dilatation of a vascular channel". If we accept Gore's definition of an aneurysm as valid, then a poststenotic dilatation can be rightfully considered as an aneurysm. Halsted (1916) provided perhaps the best definition of a poststenotic dilatation: "A circumscribed dilatation of an artery distal to the site of a constriction". Accordingly, a poststenotic dilatation may be considered as a poststenotic aneurysm.

In this communication however, the term poststenotic dilatation will be used to conform with the terminology of the majority of the workers in this field; exceptions will be made when quoting authors who refer specifically to poststenotic aneurysms (Warren, 1849; Holman, 1954a; 1955; Frederiksen and Poulsen, 1961; Wellington and Martin, 1965).

B. Observations on Poststenotic Dilatation in Man

The first published observation of the occurrence of a circumscribed arterial dilatation associated with arterial constriction was reported in the literature in the nineteenth century. In 1831 Mayo reported a case of pulsating tumor above the right clavicle in a patient
suffering from a large exostosis of the first rib. Adams (1836) discussed a similar case, later confirmed by post-mortem examination. In 1842 Cheevers described a localized dilatation of the ascending aorta beyond an aortic valve stenosis. Seven years later Warren (1849) published a case of a patient with a cervical rib and an "aneurysmal tumor" in the left subclavian artery. In 1861 Coote reported on a large pulsating aneurysm distal to the site of compression of the subclavian artery by a cervical rib. Poland in 1869 observed a pulsating, circumscribed dilatation of the right subclavian artery which he claimed was treated successfully by manual compression. In 1884 Gould described a tense, pulsating dilatation of the right subclavian artery in a patient with a cervical rib. Ehrich (1895) cited an instance of a dilatation of the left subclavian artery secondary to a cervical rib; following the removal of the rib, the dilatation subsided. At the turn of the present century, Murphy presented one case of right and another of left subclavian aneurysms; a cervical rib on the corresponding side was present in both patients. In 1918 Halsted published a detailed and well documented case of a patient with a large localized dilatation of the right subclavian artery. The dilatation was surgically treated by the
application of an aluminum band to the innominate artery until the pulse of the dilated segment was almost obliterated. To his surprise a year later a systolic bruit over the carotid artery distal to the site of the band was heard, and after a lapse of four years a large cylindrical dilatation of the common carotid artery developed. In the largest series of cases with cervical rib collected up to 1924, Halsted found that a fusiform, cylindrical localized enlargement of the subclavian artery was present in 27 instances out of a total of 716 cases.

In what is perhaps the largest series of patients with coarctation of the aorta, Clagett et al. (1954) reported the presence of aneurysmal dilatations distal to the site of coarctation in 10 of the 124 cases collected. Generally, the aneurysms found were at the origin of the first or the second intercostal artery distal to the constriction. In a curious case of coarctation between the origins of the left carotid and subclavian arteries Clagett et al. (1954) observed two aneurysms in the area distal to the narrowed segment.

In a detailed study of incidence, distribution and functional aspects of poststenotic dilatation, Holman (1955) pointed out that although this entity is more often diagnosed
in the subclavian arteries, there was increasing radiological evidence that it occurs in other arteries as well. To illustrate this point, Holman (1955) presented one case of congenital subaortic stenosis with poststenotic dilatation of the ascending aorta, two cases of dilatation beyond stenosis of the pulmonic valve, and one of poststenotic dilatation of the descending aorta associated with coarctation. In 101 cases of aortic coarctation collected by Frederiksen and Poulsen (1961) poststenotic dilatation was present in four. Dramatic complications may result from poststenotic dilatation as exemplified by a patient reported by Robicsek and co-workers (1961), in whom a poststenotic dilatation associated with aortic coarctation ruptured into the esophagus. Poststenotic dilatation has also been observed in the so-called pseudo-coarctation of the aorta. Pseudo-coarctation of the aorta was defined by Souders et al. (1951) as a subclinical form of coarctation caused by kinking and angulation of the aortic arch when pull is exerted upon the latter by the ligamentum arteriosum. Souders et al. (1951) described the first case, and Steinberg (1956) presented a series of 16 cases of pseudo-coarctation of the aortic arch, all of them associated with poststenotic dilatation. One year later Steinberg (1957) reported an
additional case of pseudo-coarctation, this time with aneurysm of the aortic sinuses.

In cases of a rare vascular disease diagnosed as fibromuscular dysplasia McCombs and Crocker (1967) reported three cases of poststenotic dilatation of the renal artery recognized angiographically and later confirmed at surgical exploration. Intrinsic stenosis of the vascular lumen caused by severe atherosclerosis may be complicated by poststenotic dilatation. Roach and MacDonald (1970) presented a series of 16 cases of atherosclerotic stenosis accompanied by poststenotic dilatation.

C. Observations on Experimental Poststenotic Dilatation

1. Experiments in animals

There is general agreement among the workers in this field that William Halsted should be credited as the pioneer in the experimental study of poststenotic dilatation. It is of interest to note that in one of his communications Halsted (1918) cited the work of Porta on pathological alterations of canine arteries caused by ligatures and torsions published in 1854. In his report Porta includes two remarkable drawings of aortic dilatation distal to the site of ligation; nevertheless, he did not elaborate
on the significance of the dilatation. Perhaps the most lucid description of poststenotic dilatation and the first attempt at explaining its physical basis is contained in a series of publications by Halsted (1916; 1918; 1924). In these, Halsted reported the results of experiments on 30 dogs with surgically induced aortic constriction by means of application of an aluminum or a silver band. Seven animals developed a marked dilatation in the abdominal aorta a short distance beyond the site of constriction. Halsted concluded that the reduction in diameter of the arterial lumen should be almost total in order to produce a poststenotic dilatation, and that several months must elapse after the application of the band before a dilatation will occur.

Elaborating on the possible cause of arterial dilatations Halsted wrote: "The abnormal whirlpool-like play of the blood in the relatively dead pocket below the site of the constriction and the lowered pressure, may be the chief factor concerned in the production of the dilatation".

Gerbode and Hultgren (1951) devised a surgical procedure to produce aortic coarctation in puppies. It consisted of an application of a ligature around the aorta without diminishing its lumen. This, however, limited the
vessel to its original diameter and as the puppies grew to maturity it became an area of coarctation. When the animals were killed one and two years later, dilatation beyond the stenosis was invariably present. Another interesting surgical technique to induce arterial stenosis in dogs was devised by Holman (1954 a). The technique consisted of anastomosing the severed proximal end of the left subclavian artery to the distal end of the severed descending aorta using the "V-plasty technique". At necropsy twenty-four days later, a dilatation of the subclavian artery beyond the anastomosis was observed.

In a large series of experiments in adult dogs, Roach (1962; 1963 b) induced poststenotic dilatation by ligating the femoral and carotid arteries. Three degrees of stenosis were produced by the ligatures; a minimal stenosis which did not result in dilatation, a moderate stenosis with presence of distal thrill and bruit resulting in the formation of poststenotic dilatation, and severe stenosis which caused only a temporary proximal dilatation.

2. Experiments in artificial circulating systems

Holman (1954 a) was perhaps the first to reproduce poststenotic dilatations in artificial circulating systems
in an attempt to correlate the results between the *in vivo* and *in vitro* experiments. These experimental devices consisted of a closed circulating system of rigid conduits with an interposed segment of thin transparent elastic tubing. The circulation of the fluid was provided by a pump calibrated to produce 120 strokes per minute. An incomplete stenosis was produced in the middle portion of the elastic tubing. After 21 hours of continuous pumping a dilatation developed beyond the constriction. On the basis of this and the experiments in dogs (Holman, 1954 a), the author suggested that the play of hydraulic forces against the arterial or rubber tubing wall just beyond a stenosis is the main factor responsible for the production of poststenotic dilatation. To explain this, he postulated that the change of laminar to turbulent flow produces eddies of alternating high and low pressure. These eddies would bombard the wall producing high potential energy and lateral pressure. The repeated impacts against an elastic wall over prolonged periods of time eventually yield to "structural fatigue" and secondary dilatation.

Robicsek (1955) and Robicsek et al. (1958) observed turbulence and cavitation in the pathway of the flow of a coloured fluid circulating through a closed system with an
interposed glass model reproduction of a poststenotic
dilatation. The author suggested that in a living individual
a turbulent flow and cavitation also could develop, injuring
the elastic elements of the arterial wall and resulting in
dilatation.

De Vries and Van den Berg (1958) studied the
pressures of constant and pulsating flows in the pre-, and
poststenotic areas produced in rigid and rubber tubes and
in isolated segments of animal aorta. In these experiments
an increase in the lateral pressure could not be detected;
rather, the pressure decreased during the systole giving
rise to a paradoxical behavior of the poststenotic pressures.
To explain the formation of poststenotic dilatation in the
area of low pressure they suggested that the alterations
of arterial walls or elastic tubes may be the result of high
frequency vibrations of the wall as a consequence of the
formation of eddies developing beyond the stenosis.

Bruns et al. (1959) designed an experiment in which
static pressures were produced in a circulating model of
latex rubber tubing. A vibrating blade was inserted through
one of the stoppers at the end of a tube. The vibrating
rate was maintained at 96 cycles per second, and the
pressures between 130 and 140 mm Hg. A dilatation was noted
in the tubing wall in the area subjected to vibration after seven to 120 hours. Bruns et al. (1959) suggested that this observation could be extrapolated to the living systems where the turbulence of the blood may fluctuate pressure-wise in a nearly periodic fashion owing to the presence of an obstacle to the blood flow. The passage of the blood through the obstacle, i.e. narrowed lumen, is likely to produce a thrill with vibration of the wall which may induce "structural fatigue" and dilatation.

D. Biophysical Concepts in Poststenotic Dilatation

Perhaps the most comprehensive attempt to study the hydrodynamics and pathogenesis of poststenotic dilatation has been that of Roach and her associates (1962; 1963 a; 1963 b; Roach and Harvey, 1964; Roach and MacDonald, 1970). Analysis of murmurs and pressure-volume curves in canine arterial segments dilated experimentally in vitro showed that the murmurs had a wide frequency spectrum with no resonant points and no components over 1500 cycles per second. Roach (1962; 1963 a; 1963 b) concluded that this type of sound would be produced likely by turbulence rather than by cavitation or vortex formation. The analysis of pressure-volume diagrams suggested that in the poststenotic
segment the dilatation was caused by a weakness of the arterial wall. Roach (1963 a) also observed that arterial segments subjected to turbulence had increased distensibility. To test whether this increased distensibility was caused by changes in the elastic properties of collagen or elastic fibers, or both, she measured the "elastance" (Young's modulus or coefficient of elasticity X arterial wall thickness) of human iliac arterial segments treated with trypsin or formic acid, as well as that of segments of untreated arteries (Roach and Burton, 1957). The statistical analysis of the results suggested that the modifications in distensibility, both circumferential and longitudinal, were caused by changes in the elastic fibers. To prove that low frequency vibrations produced by turbulence were the cause of the changes observed in the distensibility curves, segments of human iliac arteries were exposed to vibrations 80 to 300 cycles per second for two to nine hours. The results showed that after two to seven hours of vibration the arterial segments became more distensible than those of controls.

Rodbard and co-workers (1967) proposed the action of drag forces as being responsible for the development of poststenotic dilatation. To test this assumption they devised a circulating system with an interposed portion made of
plaster of Paris with a narrowed area simulating a constric-
tion of the lumen. An aqueous solution of hydrochloric
acid was maintained at a constant flow and a few hours
later an erosion of the wall was observed in the area beyond
the narrowed lumen. This they interpreted as being caused
by the drag resulting from a change of laminar to turbulent
flow.

E. Theories of Pathogenesis

Of the many theories that have been proposed to
explain the pathogenesis of poststenotic dilatation, only
the most popular will be reviewed.

1. Congenital weakness of the arterial wall

Laubry (1943) and later Van Buchem (1956) proposed
that in cases of poststenotic dilatation associated with
congenital malformations, an accompanying weakness of the
vascular wall might lead to dilatation. This theory,
however, has been dismissed by several authors (Holman,
1954 b; Rodbard et al., 1967) on the basis that poststenotic
dilatation develops often in patients without congenital
defects, and it may be produced experimentally in vivo as
well as in vitro.
2. Traumatic injury to the arterial wall

Damage to the vasa vasorum and nerves caused by pressure on the arterial wall of either a cervical rib or a displaced ligament in man and by experimental ligation in animals has been proposed as the cause of arterial dilatation (Cavina, 1915; Halsted, 1916; Tuci and Cinti, 1956). This assumption, however, cannot explain why the dilatation occurs distal to the site of ligature. Moreover, it has been shown that stripping of adventitia has not resulted in dilatation of arteries (Holman, 1954 a). Further evidence against this theory has been presented by Taylor et al. (1950) when they reported that destruction of aortic vasa vasorum and nerves by local freezing was not followed by arterial dilatation.

3. Cavitation

Robicsek et al. (1958) and Bruns et al. (1959) indicated that cavitation was perhaps responsible for poststenotic dilatations, since under certain conditions the sudden change in velocity and pressure such as those produced when the blood flows through a narrowed arterial lumen may result in an alternate formation and collapse of gas bubbles. This creates innumerable micro-explosions
which, if sustained, could injure the arterial wall. Roach (1962) suggested that although cavitation cannot be definitely excluded as a pathogenetic mechanism, conditions required for its production (i.e. frequencies over 3000 cycles per second) are seldom found under physiological or even pathological conditions with the possible exception of decompression sickness.

4. Vortex formation

Bruns et al. (1959) suggested that vortices can be formed when the blood flows through a narrowed lumen and that as a result the laminar flow changes into turbulent. This turbulence produces vibration creating a zone of unevenly distributed shocks of increased pressure that may injure the arterial wall resulting in dilatation. Roach (1962) objected in part to this suggestion stating that along with the vibration of the circulating fluid the arterial wall also vibrates, therefore being involved in the production of murmurs.

Foreman and Hutchison (1970) have also expressed the view that blood flowing through arterial segments with a stenosis induces arterial wall vibration over a wide range of frequencies.
Boughner (1971) showed that arteries dilate when vibrated at frequencies capable of producing murmurs, and that the age of a given artery determines the frequency range necessary for production of dilatation.

5. Turbulence

Flasher (1951) was perhaps the first to suggest that turbulence plays an important role in the production of poststenotic dilatation. Holman (1954 b) also advocated turbulence as a decisive factor. This view has been endorsed by Roach (1962; 1963 a; 1963 b) and Roach and Harvey (1964) who stated that turbulence is responsible for both murmurs (vibration) and damage to the vascular wall which ultimately leads to poststenotic dilatation.

F. Morphology of Mammalian Elastic and Muscular Arteries with Special Reference to Canine Carotid and Femoral Arteries

Classically the arteries have been divided into three types namely elastic or conducting, muscular or distributing, and arterioles. The aorta and its major branches, e.g., the innominate, subclavian and common carotid arteries belong to the elastic type. Most of the remaining, including the femoral arteries are of the muscular type (Ham, 1965; Maximow and Bloom, 1957; Michels, 1962).
Macroscopically, the elastic arteries have a relatively thin wall for the size of the vessel and abundant elastic tissue which imparts a yellow color upon the freshly cut wall. Microscopically, the endothelial cells are polygonal in shape; the internal elastic lamina is not always distinct and therefore, there is no sharp demarcation between intima and media. The latter consists largely of smooth muscle cells, collagen fibers and fenestrated elastic lamellae arranged in a concentric repeating pattern. The number of elastic lamellae in mammalian arteries including man is directly proportional to the radius of the vessel (Wolinsky and Glagov, 1967).

The muscular arteries have a relatively thick wall owing to the large number of smooth muscle cells present in the media. The internal elastic lamina is well defined and sharply demarcates the intima from the media. The latter consists almost exclusively of helicoidally arranged smooth muscle cells, scanty elastic lamellae, and other connective tissue elements between the muscular layers. The differences between elastic and muscular arteries are not always clearly recognized; furthermore, there are transitions from one type to another. Laing (1964) and French (1966) have reported that in the "middle age" the porcine thoracic aorta changes
gradually from elastic to muscular type as it descends into the abdomen. Similar observations have been made in the aorta of baboons (Katzberg, 1966) and man (Haust, 1971).

A more detailed account of the specific microscopic characteristics of elastic and muscular arteries is as follows:

1. Elastic arteries

In adult life the tunica intima of elastic arteries constitutes approximately one sixth of the total thickness of the arterial wall (Ham, 1965). The intima is lined by a continuous layer of endothelial cells the cytoplasm of which cannot be observed always by light microscopy. In paraffin sections the cellular details of these cells is not demonstrated with ease with exception of the usual prominence of nuclei. The existence of a subendothelial layer or space has been debated by several authors. Buck (1958) reported that in the aorta of rats, the endothelial cells rest directly upon the internal elastic lamina. Laing (1964) stated that in the aorta of newborn pigs, the endothelium lies almost directly on a prominent internal elastic lamina with the exception of the most proximal part of the aortic arch where an amorphous, thin layer of ground substance can be seen
intervening between the endothelium and the internal elastic lamina. Pease and Paule (1960) observed only traces of basement lamina separating the endothelium and internal elastic lamina in the thoracic aorta of rats. Karrer (1961) reported that in aortae of young mice, the endothelial cells rest immediately upon the inner-most elastic lamina without interposition of any other structures. Karrer however, found that in the aorta of aging mice a 300 to 700 μ wide space separates the endothelium from the internal elastic lamina. Similar observations were reported by Paule (1963) in the aorta of rats. Wolkoff (1924) and French (1966) pointed out that in small mammals, such as mouse and rat, the aortic endothelium is almost directly resting on the internal elastic lamina so that when observed in transverse sections by light microscopy these two components may appear to be in contact with each other.

In the excellent reviews on the structure of the aorta of pigs Seifert (1962), French (1965; 1966), and Laing (1964) concluded that the intima in the thoracic region is relatively broad and poorly demarcated from the media since no well defined internal elastic lamina is present. The subendothelial space contains a meshwork of collagen and elastic fibers loosely arranged toward the
endothelial surface. Similar observations have been recorded in carotid arteries of rabbits (Trillo and Chavez, 1969).

The presence of cellular elements in the subendothelial space has been noted by several authors in different arteries and animal species. According to Seifert (1962) the cells in the subendothelial space as seen in electron micrographs are of two types: round cells without cytoplasmic differentiation, and long, drawn-out narrow cells with branching cytoplasmic processes. The former he considered to be mesenchymal stem cells which can develop into macrophages and possibly other forms, and the latter as a special form of a fibrocyte concerned in the metabolism of intimal connective tissues. In the aorta of swine Lee and co-workers (1970) have described what they termed "poorly differentiated cells" having the morphological features of both smooth muscle cells and monocytes.

Musculo-elastic intimal cushions or pads occur widely in the arteries of mature mammals including the human aorta. Pfister (1927), Fox (1933) and French (1966) have described these formations in the transition zone between the thoracic and abdominal aorta. In this transitional region a relatively thin internal elastic lamina
separates the subendothelial layer from the medial smooth muscle cells. The subendothelial space at this point contains elastic fragments and the thin internal elastic lamina has numerous gaps.

Haust et al. (1965) have described the relation of cells and fibers in the aortic intima of human fetus and newborn. In the fetus a continuous endothelium is separated from the internal elastic lamina by a narrow gap containing fine filamentous material and a few collagen fibers apparently arranged in a helicoidal manner. Occasional subendothelial smooth muscle cells were observed in older fetuses. In the abdominal aorta of the newborn, one or two layers of smooth muscle cells and increased connective tissue elements are sometimes found in the subendothelial space.

The endothelium in elastic arteries in several species has fairly similar structural features. Light microscopy has not contributed much to the knowledge of the structure of endothelial cells because of the limitation in optical resolution. However, when examined en face, or by the so-called "Häutchen" technique, it yields significant information. Efskind (1941), Poole et al. (1958) and Florey et al. (1959) using this method described the
endothelium as consisting of a continuous layer of flat cells bound to each other by a "substantial zone" of cement material. Cohnheim (1867) observed in silver nitrate impregnated preparations small loops where the lines did not enclose a nucleus, and dark spots or deposits in the lines themselves. He termed the loops and spots stomata and stigmata respectively and these were interpreted as openings between cells. Auerbach (1865) reported the occurrence of pinched-off cytoplasmic fragments which were later considered to be artifacts. With the electron microscope structures corresponding to naturally occurring gaps (stomata or stigmata) have not been observed (Buck, 1958; Florey, 1966; French, 1966). Recently Shimamoto and co-workers (1971) using scanning and transmission electron microscopy described intercellular bridges in the aortic endothelium of rat, rabbit, monkey and dog.

The endothelial cells are attached to each other by interdigitating junctions which have been described by French (1966) as resembling the external compound membrane observed by Robertson (1959) in Schwann cells, and as tight junctions or zonula occludens by Farquhar and Palade (1963) in several types of epithelium. Fenestrae or intercellular gaps similar to those occurring in the endothelium of
capillaries (Bennett et al., 1959; Fawcett, 1959) have not been observed in the endothelium of either elastic or muscular arteries (French, 1966).

The endothelial cells have the usual cytoplasmic organelles observed in most cell types. Hackensellner and David (1965) reported that the endothelial cells of carotid arteries of the rabbit have few mitochondria usually clustered around the nucleus. Rhodin (1962) observed a marked paucity of endoplasmic reticulum in the endothelial cells of femoral artery of mice and this he interpreted as being an indication of low synthetic and metabolic activity of these cells. Geer et al. (1961) described in the aorta of man and dog abundant profiles of endoplasmic reticulum. Similar observations were reported in endothelial cells from the aorta of chick embryo (Karrer, 1961). A common feature of arterial endothelial cells in all species reported is the presence of pinocytotic vesicles (Palade, 1953; Keech, 1960; Rhodin, 1962; Florey, 1966; French, 1966). These structures have been related to the active transport of substances across the endothelium (Palade, 1961; Florey, 1966). Another structure common to endothelial cells is a basement lamina; it separates the endothelium from the subendothelial space (Palade, 1961; Bennett, 1963; Haust et
al., 1965; French, 1966).

A polysaccharide-rich coat has been described to line the apical surface of endothelial cells in capillaries (Luft, 1966) and in the rabbit aorta (Trillo and Chavez, 1969). This layer, however, cannot be visualized with the commonly used stains for electron microscopy; it may be demonstrated when the tissues have been treated with ruthenium red.

The tunica media represents from one third to one half of the total wall thickness. It consists of concentri-cal layers of smooth muscle cells separated by fenestrated elastic lamellae, collagen bundles and elastic fragments (Keech, 1960; Geer et al., 1961; Rhodin, 1962; Bierring and Kobayashi, 1963; Laing, 1964; Haust et al., 1965). In addition to collagen fibers and ground substance, a new connective tissue component was described in elastic arteries (Haust, 1965; Haust et al., 1965; Haust and More, 1967).

The elastic lamellae vary in number depending upon the caliber of the vessel, animal species, age and body weight (Wolinsky and Glagov, 1967). Keech (1960) reported that in the aorta of rats there were from seven to 11 elastic lamellae; in human aorta Ham (1965) found that in the newborn infant there are about 40 elastic lamellae,
while in the adult the number may reach up to 70. Wolinsky and Glagov (1967) in a comparative study of mammalian aortae found that in the adult mouse, i.e., the smallest animal, there were five elastic lamellae and in the swine, the largest mammal examined, there were 72 lamellae. The authors also described in aortae of rabbits fixed under pressure, fine interlamellar elastic fibrils connecting adjacent elastic lamellae. These observations were made, however, on tangential sections, and should be interpreted with caution.

The cellular composition of normal tunica media has been a matter of debate for several decades. For many years, the classical histologists have described by light microscopy the existence of smooth muscle cells and fibroblasts or fibrocytes in the media (Altschul, 1944; Maximow and Bloom, 1957; Ham, 1965). These observations however, have not been substantiated by electron microscopic examinations, and at present there is a general agreement that the only normal cellular element in the medial compartment is the smooth muscle cell (Berrian, 1953; Pease and Paule, 1960; Pease and Molinari, 1960; Keech, 1960; Karrer, 1961; Geer et al., 1961; Rhodin, 1962; Bierring and Kobayashi, 1963; Paule, 1963; Cliff, 1967; Wolinsky and Glagov, 1967).
The medial smooth muscle cells are described by most authors as being fusiform or elongated in shape (Keech, 1960; Pease and Paule, 1960; Karrer, 1961; Pease, 1962; Bierring and Kobayashi, 1963; Paule, 1963). This, however, seems to depend on whether or not the vessel was fixed under pressure (Bunce, 1965). Geer et al. (1961) have stated that in arteries fixed under pressure in situ, the smooth muscle cells appear fusiform, while if fixed after removal from the body, they appear irregular in shape, shorter and broader. With respect to the orientation of smooth muscle cells in the arterial media, most authors agree that they are oriented obliquely to both, the radial and longitudinal axis of the vessel (Keech, 1960; Pease and Paule, 1960; Bierring and Kobayashi, 1963; Paule, 1963).

The cellular junctions between medial smooth muscle cells have been described as being of two types, one which resembles the desmosomes of epithelial cells and intercalated discs of myocardial fibers (Keech, 1960), and the other consisting of fusion of the adjacent plasma membranes or nexus (Barr et al., 1965; Johnson and Sommer, 1967; Moss and Benditt, 1970).

An important anatomic and functional feature has been reviewed by Wolinsky and Glagov (1967) regarding the
existence of vasa vasorum in the arterial media. In a comparative study, these authors established that in any artery vasa vasorum are present in the media only if the number of medial elastic lamellae exceeds 29.

Elastic arteries do not have a well-defined external elastic lamina; however, Ham (1965) suggested that the outermost elastic lamella of the tunica media could be considered as the external elastic lamina, this being the border between the media and adventitia. The latter in most elastic arteries consists of incomplete, concentrically and longitudinally arranged elastic fibers alternating with less organized collagen bundles and fibroblasts (Keech, 1960; Pease and Paule, 1960; Pease, 1962; Paule, 1963; Ham, 1965). Occasionally, islets of longitudinally oriented smooth muscle cells can be observed in the innermost adventitia (Keech, 1960; Laing, 1964). Vasa vasorum and nerves are scattered among the connective tissue elements.

2. Muscular arteries

The smooth muscle cells are the major component of the tunica media. In the human gastroepiploic artery Westman and Nylander (1965) found that the tunica media measures up to 200 μm and that it contains about 20 layers
of circularly arranged smooth muscle cells. Mattews and Gardner (1966) stated that the number of layers of smooth muscle cells increases with the size of the vessel. In agreement with most authors (Smirnov, 1955; Pease and Molinari, 1960; Westman and Nylander, 1965), Mattews and Gardner (1966) found that in muscular arteries, e.g., the mesenteric artery, the medial smooth muscle cells are arranged in a helicoidal fashion with respect to the long axis of the vessel and that the cells are interlocked in such a manner that the nuclear region of one cell lies against the thin tapering ends of adjacent cells.

The ultrastructural details of the medial smooth muscle cells in muscular arteries are similar to those described for elastic arteries and arterioles (Parker, 1958; Geer et al. 1961; Rhodin, 1962; Westman and Nylander, 1965).

In general, the adventitia is thicker than the media. In the femoral artery of the dog (Smirnov, 1955), it represents two thirds of the total thickness of the wall. In the human gastroepiploic artery (Westman and Nylander, 1965) and in mesenteric artery of the rat (Mattews and Gardner, 1966) it accounts for one half of the mural thickness. The adventitia consists of thick incomplete elastic
fibers oriented in both longitudinal and helicoidal manners. The wide spaces between elastic fibers are occupied by fibroblasts, fibrocytes, vasa vasorum and nerves.

G. Histology of Poststenotic Aneurysms in Man and Experimental Animals

Arterial aneurysms may be classified morphologically as saccular, which communicate with the lumen through a relatively narrow neck; fusiform or cylindrical, involving the entire circumference of the arterial wall, and dissecting, characterized by abnormal passage of blood along and within the arterial wall (Gore, 1968).

Poststenotic dilatation fits into the category of the fusiform or cylindrical aneurysms, therefore attention will be paid to this particular type. However, saccular intracranial aneurysms also have been considered to be of the poststenotic type on pathogenetic grounds (Sahs, 1966; 1969).

There is little detailed information in the literature regarding histological changes of poststenotic aneurysms. Reviews of large autopsy series such as those of Abbott (1928 a, 1928 b) and Reifenstein and co-workers (1947) include details of clinical and gross pathological aspects, but no microscopical data. Reifenstein and
co-workers (1947) reported that in the segment distal to aortic coarctation the wall appeared thin and hypoplastic, and had poorly differentiated coats. Dunnill (1959) in a review of nine cases with aortic coarctation and poststenotic aneurysm found histological changes in two of these. The changes consisted of diffuse intimal thickening and formation of cysts-like spaces in the media. There was a decreased number of elastic lamellae in the dilated segments when compared with those in the normal areas.

Clagett et al. (1954) found poststenotic aneurysms in ten cases in their series of 124 patients with coarctation of the aorta. In these, histological changes were not found often and when present they consisted of intimal thickenings and occasional "fibrous ridges".

Frederiksen and Poulsen (1961) reported that in 101 patients with aortic coarctation four had poststenotic aneurysms and in one of these, histological examination of the resected segment revealed subacute endarteritis which probably was a complication of an endocarditis later found at post-mortem examination.

For many years the origin of non-mycotic intracranial aneurysms has been the subject of dispute. Stehbens (1963 a; 1963 b) considered that the intracranial aneurysms,
especially the fusiform-shaped, often seen at the base of the skull in the vertebral arteries, should be considered similar in nature to the poststenotic dilatations. This proposal has been endorsed by Sahs (1969).

Detailed morphological studies of the distended wall of both saccular and fusiform intracranial aneurysms have been carried out by several authors (Nystrom, 1963; 1965; Stehbens, 1963 a; Sahs, 1966; 1969). In a well documented study, Stehbens (1963 a) distinguished what he called "early aneurysmal changes" consisting of funnel-shaped dilatations of the vessel with minimal intimal proliferation and focal disappearance of the internal elastic lamina. These defects in the internal elastic lamina were more conspicuous in the areas where the media was thin and fibrotic. Sahs (1966) observed thinning of the wall, loss and poor stainability of elastic fibers and minimal to moderate thickening of the intima. In large aneurysms the intimal thickenings contained foam cells and occasionally foci of calcification, and there was marked fibrosis of the media and fragmentation of the elastic fibers.

Sahs (1966; 1969) believed that small outpouchings in which the fragmentation of the elastic lamellae was the
main feature, could represent potential or early stages of aneurysms. In addition, this author described larger out-pouchings in which the arterial wall consisted largely of collagenous tissue containing elastic fragments. These lesions corresponded to the funnel-shaped dilatations of Stehbens (1963a).

Nystrom (1963; 1965) studied with the electron microscope intracranial aneurysms resected from seven patients. The most striking changes were hypertrophy of the endothelium with deposition of intracytoplasmic lipid droplets and vacuolization. Splitting of the internal elastic lamina was present in all the cases. The typical pattern of bundle arrangement of collagen and reticular fibrils was lacking and the collagen fibrils were distributed randomly in the ground substance. In the thinned media the smooth muscle cells were often "hyalinized".

In experimental poststenotic dilatation little has been said about histological changes. In poststenotic dilatation produced experimentally in canine aortae Reid (1916) reported elastic tissue disruption, but little change in other connective tissues. In poststenotic dilatation of canine carotid and femoral arteries Roach (1962; 1963b) described subendothelial hyperplasia.
Jensen and Svane (1967) described intimal proliferation and medial fibrosis with reduced number of elastic lamellae in canine aortae dilated experimentally. Rodbard and co-workers (1967) reported that in poststenotic dilatation of the aorta of chicks, the wall was atrophic at the expense of medial elastic and muscular elements. These authors pointed out that the degree of changes appeared to increase with the duration of experiments, and that in short term experiments no changes were present. In similar experiments Habib and Nanson (1969) observed an increase in thickness of the arterial wall owing to cellular hypertrophy.
III. ORIGINAL OBSERVATIONS

A. Material and Methods

Six mongrel dogs supplied by the vivarium of the Health Sciences Center of the University of Western Ontario were used in the present study. Upon arrival the dogs were placed in individual metal cages for two weeks for adaptation and observation. During this period and throughout the experiments the animals were fed Purina dog chow and tap water ad libitum.

Sex, age and weight of animals, and the duration of experiments are shown in Table I. In addition to the normal diet, the dog number two from group I was fed sucrose (4gm/kg/day) dissolved in drinking water and 100 mg per day of propylthiouracil (Propyl Thyrocil obtained from Frosst Laboratories). This animal was included in the series since no specific arterial changes were observed following the short term thiouracil-sucrose feeding.
1. Experimental production of poststenotic dilatation

In all animals poststenotic dilatation of the left common carotid and right femoral arteries was induced by incomplete ligature. The contralateral arteries served as controls in every case.

The dogs were anesthetized with pentobarbital (Carbinal obtained from Parke-Davis Laboratories) at a dosage of 30 mg/kg. The anterior region of the neck and the right leg were shaved and cleaned with soap and water, rinsed with a 1:500 aqueous solution of Zephiran and painted with a 3% alcoholic solution of iodine.

Under sterile conditions the left common carotid and right femoral arteries were dissected and exposed. The arteries were then ligated with a 0.5 mm wide nylon taffeta band tightening the ligature until a thrill was felt in the portion distal to the site of ligation. The wound was then closed by surgical planes and sutured with 00 silk thread. To prevent a possible infection, procaine penicillin at a dosage of 400,000 U.I./day was administered intramuscularly for a week. Of the six animals, two were kept for one month, two for three months, and the remaining two for six months (Table I).
2. Collection and processing of specimens

Under pentobarbital anesthesia a set of arteriograms of the ligated arteries were carried out by intra-arterial injection of 30 ml of sodium diatrizoate (Hypaque Sodium obtained from Winthrop Laboratories) in each pair of dogs at the time just prior to sacrifice.

To achieve good structural preservation and consistent architectural relations between the various mural elements (Wolinsky and Glagov, 1964; Bunce, 1965), most of the arteries were fixed in situ by perfusing the entire circulatory system. The perfusion apparatus (Fig. 1) consisted of two bottles with a capacity of one gallon each, connected to two intravenous infusion sets and through a portion of these (afferent tubing) with a pressure chamber and a mercury manometer. Pressure was supplied to the chamber by a manual blower. The efferent plastic tubing of the infusion sets were connected to a three-way stopcock having a tubing outlet with a 16 gauge blunt needle inserted into the right common carotid artery. A cannule was inserted into the left femoral artery for the purpose of draining the blood and infusion fluid.

One of the bottles contained 2,000 ml of Palay's rinsing solution (Palay et al., 1962) while the other
contained an equivalent amount of 3% phosphate-buffered glutaraldehyde solution (Sabatini et al., 1963). Following the draining the blood was washed out by the rinsing solution until the outflowing fluid became clear; subsequently, the glutaraldehyde fixative was infused under pressure maintained at 140-150 mm Hg.

Following perfusion, the common carotid and femoral arteries of both sides were removed and the dilated segments and their contralateral controls were divided into segments as indicated in Fig. 2. From the carotid and femoral arteries two ring-shaped cross sections, each approximately one mm in width, were removed from the opposite ends of segments 2 and 6 respectively on the experimental sides and from segments 4c and 8c on the control sides (Fig. 2). Thus 4 ring-shaped arterial cross sections from each dog totalling 24 sections in 6 dogs were removed from the experimental sides (maximally dilated segment). Equal number of tissue sections was obtained from the control sides for examination. Therefore, 48 tissue sections were obtained for processing and selection for electron microscopic examination.

From the intervening areas of the same segment one, ring-shaped cross section measuring approximately 2 mm in
width was removed and processed for light microscopy. Thus, 24 segments from experimental (12) and control (12) sides were removed and processed in toto for light microscopic examination.

For electron microscopy, the ring-shaped arterial slices were placed immediately in cold 3% buffered glutaraldehyde (pH 7.4) (Sabatini et al., 1963), and prefixed in the same but fresh solution for 90 minutes at 4°C. The tissues were subsequently washed several times in 0.1 M phosphate buffer (pH 7.4) with 0.0685 gm of sucrose added per milliliter, and postfixied in cold 1% phosphate-buffered osmium tetroxide (Millonig, 1961). Following fixation the tissues were washed in fresh phosphate buffer solution and dehydrated in ascending concentrations of ethanol. The arterial slices were embedded flattly in Epon-812 according to the method of Luft (1961).

One micron thick sections were cut with glass knives on either Porter-Blum MT-1 or Reichert ultramicrotomes and stained with alkaline toluidine blue for light microscopy. From each ring-shaped cross arterial section embedded in toto in Epon-812, approximately three to four segments were selected for electron microscopy on the basis of examination of the thick sections. The selected segments of the rings
were removed from the original large blocks and re-embedded. Thus, 252 blocks selected from the experimental and control sides were ultimately examined by electron microscopy. Thin sections were cut with a diamond knife, mounted on either unsupported or formvar-coated copper grids and stained doubly with uranyl acetate (Stempack and Ward, 1964) and lead citrate (Reynolds, 1963). Thin sections were examined with a Philips EM-300 electron microscope.

For light microscopy the arterial segments were fixed in 10% phosphate-buffered formalin solution for 48 hours. After fixation the tissues were dehydrated and cleared with tetrahydrofuran (Hausa, 1959) and sectioned at three to five micra. The sections were stained with hematoxylin-phloxine-saffron (HPS) and Weigert-Hart resorcin fuchsin-metanil yellow-nuclear red for elastic fibers.
B. Results

1. General considerations

Poststenotic dilatation was successfully produced in both carotid and femoral arteries in all dogs. Twelve operations were performed and there were no infections. A granulomatous foreign body reaction was present at the sites of arterial ligature in most cases; the vessels were, however, dissected with a relative ease.

Prior to ligation the diameters ranged from three to four mm in the carotid and from two to two and a half mm in the femoral artery. The poststenotic dilatation was always fusiform in shape (Figs. 3 and 4) and therefore the diameters were measured in the central area of maximal dilatation; they ranged from five and a half to eight mm in the carotid, and from four to six mm in the femoral artery (Table II).

2. The normal carotid artery

a). Gross appearance and topography. In the dog both common carotid arteries arise from the brachiocephalic artery approximately one cm apart from each other. The left common carotid artery emerges at the level of the
dorsal end of the second rib and anterior to the trachea. It is loosely bound to the esophagus dorsomedially by the deep cervical fascia. Its branches and termination are similar to those of the right vessel (Fig. 5).

Upon emerging from the brachiocephalic artery the right common carotid diverges from the left and obliquely crosses the ventrolateral surface of the trachea as it courses towards the head. In the neck it lies in the angle formed dorsally by the longus capitis muscle and ventromedially by the trachea (Fig. 5). At the thoracic inlet the vagosympathetic nerve trunk becomes associated with the dorsal surface of the artery and accompanies it along its course through the neck. The internal jugular vein is also associated with the right common carotid artery in the mid-half of the neck. The fascia that binds these structures together attaches them rather loosely and is called the carotid sheath. The right common carotid artery ends at or near the body of the hyoid bone giving origin to internal and external carotid arteries. The gross caliber of the common carotid artery is approximately twice that of the femoral artery (Fig. 6).
b). **Light microscopic observations.** The wall of the common carotid artery is composed of three compartments or tunicae as described for elastic and muscular arteries above. The tunica intima consists of a thin endothelium and a narrow subendothelial space barely discernible in sections of paraffin-embedded tissues (Fig. 7). It is clearly seen in one-micron-thick sections in Epon-embedded tissues (Fig. 8).

The endothelium consists of a single layer of flattened cells with slender cytoplasmic processes and prominent nuclei which protrude into the lumen. Often appearing normal otherwise, the intima shows small areas of a widened subendothelial space containing smooth muscle cells, delicate elastic strands and other connective tissue elements (Fig. 8).

The intima and the tunica media are separated from each other by the internal elastic lamina which appears as a broad and undulating band when fixation takes place without distending pressure (Fig. 7) or as a straight band when the artery is fixed under pressure (Fig. 8). The internal elastic lamina often shows interruptions or gaps up to 200 micra wide; when the gaps reach these proportions, the intima and the media communicate and the smooth muscle
cells located close by in the innermost medial layers change their polarity and align themselves perpendicularly to the long axis of the vessel. Small areas of splitting and fraying are present at intervals in the internal elastic lamina. The intima over these areas is often broadened by the presence of elastic fibrils, other connective tissue elements, and smooth muscle cells which are often surrounded by delicate elastic strands (Fig. 8).

The tunica media consists of alternating layers of elastic lamellae and interlamellar spaces containing smooth muscle cells, collagen fibrils and small strands of elastic substance (Figs. 7 and 8). Within the media the number of elastic lamellae is fairly constant, ranging from 11 to 13. They are oriented in a helicoidal fashion and in cross sections may appear either as merging with each other, or have areas of discontinuities (Figs. 7 and 8). Thin elastic filaments that criss-cross the interlamellar spaces and apparently connect the elastic lamellae are observed in the outer interlamellar spaces.

The cellular elements of the media are identified as smooth muscle cells and only in a few instances cells resembling connective tissue cells are observed in the interlamellar spaces of the outer media.
The smooth muscle cells occupy most of the interlamellar space and are disposed, singly or in small bundles, in a helicoidal manner. The inner interlamellar spaces contain few smooth muscle cells, but these increase in number towards the outer layers (Fig. 8). In the outermost interlamellar spaces, bundles of smooth muscle cells are often disposed longitudinally (Figs. 7 and 9). The smooth muscle cells are either fusiform or ribbon-like in shape with centrally located elongated nuclei that, depending upon the state of contraction during fixation, may show smooth or indented contours (Fig. 10). The cytoplasm of smooth muscle cells is packed with myofilaments which appear refractile in sections stained with HPS.

Two populations of smooth muscle cells are identified in the media of the common carotid arteries: deep-dark staining and pale cells. The differences in staining properties are noted more readily in sections of Epon embedded tissues stained with toluidine blue. The dark cells are distributed singly or in groups of three to four. They are usually surrounded by pale staining cells which make up the bulk of the medial cell population (Figs. 9 and 10). The intercellular spaces between the dark and pale cells appear to be narrower than the spaces between adjacent
pale cells, and at times appear to be obliterated (Figs. 9 and 10). In the dark cells the myofilaments are more tightly packed than in the pale cell (Fig. 10). Vasa vasorum are not present in the tunica media of the common carotid artery. The outermost elastic lamella is the equivalent of the external elastic lamina of the muscular arteries, and separates the tunica media from the adventitia. The latter consists of tightly bundled collagen fibers oriented in both circular and longitudinal fashions, and traversed by thick elastic fibers. Occasionally, small islets of longitudinally arranged smooth muscle cells are observed in the innermost portion of the adventitia (Fig. 7). Vasa vasorum and nerves are present usually in close proximity to the border between the media and adventitia.

c). *Electron microscopic observations.* The intima consists of a single layer of endothelial cells resting on a basement lamina which in turn is separated from the internal elastic lamina by an irregularly wide subendothelial space. The latter contains single collagen fibrils arranged into a loose meshwork, clumps of basement lamina-like material and, in many instances, smooth muscle cells (Figs. 11, 12).
The cytoplasm of the endothelial cells is of low electron density and contains relatively few organelles; of these, the more conspicuous are small mitochondria, discrete profiles of rough endoplasmic reticulum, free ribosomes and, occasionally, small dense bodies such as those described by Weibel and Palade (1964). Pinocytotic vesicles are numerous in both luminal and basal cellular regions (Figs. 11 and 13).

Contact between adjacent endothelial cells is of two types: one is by simple apposition of plasma membranes in which the line of contact may be straight, and the other by junctional folds or interdigitations (Figs. 12 and 13). In the latter the overlapping part of an endothelial cell may project into the lumen producing the so-called endothelial flaps or marginal folds (Fig. 13). Along apposing plasma membranes there are zones of increased electron density in which the adjacent plasma membranes appear more firmly attached to each other than elsewhere (Fig. 13). The intercellular spaces at the cell junctions appear fairly uniform, ranging in width from 150 to 250 Å; there are, however, areas in which adjacent cells may be separated by wider spaces.

The intima is separated from the media by the
internal elastic lamina. The latter appears as a ribbon-shaped, 1500 to 2000 µm wide structure. The texture of the internal elastic lamina varies; it may appear homogeneous, almost amorphous, or it may be traversed by electron dense filaments oriented transversally and longitudinally (Figs. 11, 12 and 13). The internal elastic lamina often is interrupted by pores or gaps of variable width ranging from just a few, up to several micra. These gaps are usually occupied by cytoplasmic processes of smooth muscle cells (Fig. 12).

The media represents approximately two thirds of the total thickness of the arterial wall. It consists of layers of smooth muscle cells alternating with elastic lamellae. The interstitial spaces are largely occupied by bundles or individual collagen fibrils, microfibrils, ground substance and elastic strands (Fig. 11). The smooth muscle cells are arranged either in a circular or helicoidal fashion. Their shape is fusiform or ribbon-like with tapering cytoplasmic processes which at times appear to be in continuity with those of adjacent cells (Fig. 11). The nucleus of smooth muscle cells is centrally located and has an elongated cigar-shaped outline with numerous indentations (Figs. 11 and 12). Young active smooth muscle cells have prominent
cytoplasmic organelles, while less active older elements have their cytoplasm largely occupied by contractile elements. In the former a well developed Golgi complex, profiles of rough endoplasmic reticulum and mitochondria are often seen in the perinuclear region or at the cell periphery (Figs. 14, 15 and 16). The mitochondria are small, have a dense matrix and short slender cristae (Figs. 14 and 16). The cytoplasm of smooth muscle cells is in general uniformly dense owing to the presence of closely packed myofilaments; occasionally the perinuclear area is less dense. The myofilaments are arranged in a parallel fashion, the orientation coinciding with the long axis of the cell. Individually, the myofilaments have a diameter of approximately 80 Å. Electron-dense, irregular patchy areas are seen dispersed randomly throughout the cytoplasm of smooth muscle cells. Occasionally these dense areas are present at the cell periphery beneath the plasma membrane (Figs. 11, 12, 14, 17 and 19). The myofilaments seem to attach themselves to dense areas at the cell periphery, but appear to traverse those in central locations (Figs. 12 and 14). The plasma membrane of smooth muscle cells is studded with small inpocketings, or pinocytotic vesicles, having an average diameter of 600 Å. In most instances these vesicles
contain a dense amorphous material (Figs. 14, 15 and 16).

The smooth muscle cells are surrounded by a 600 Å thick basement lamina (Figs. 17 and 18). It has a fibrillo-granular texture with occasional dominance of either the granular or fibrillar component. In the latter case, it appears to be composed of an intricate meshwork of 50 Å thick filaments (Figs. 15 and 16). The basement lamina is intimately associated with condensations of microfibrils and newly formed elastic tissue (Fig. 16).

The cellular junctions between smooth muscle cells are of two types. One of these consists of close apposition along the sides of two adjacent cells; often there is fusion of the plasma membranes with resulting obliteration of the intercellular space (Fig. 15). This type of cell junction corresponds to the so-called nexus (Dewey and Barr, 1962). The second type of junction occurs when the ends of two adjacent cells are in apposition but the plasma membranes do not fuse; thus a narrow intercellular space remains. Beneath the plasma membrane of apposing cells, there are corresponding areas of increased electron density resembling the desmosomal junctions of epithelial cells (Figs. 17, 18, 19 and 20).

Dark and pale staining cells corresponding to those
observed with the light microscope are identified with ease by electron microscopy. The two types of cells are often in contact with each other, forming an "end to end" cellular junction of the desmosome-like type. However, in some areas the intercellular space is not visible and seemingly the cytoplasm of one cell type appears to be in continuity with the adjacent cell of the other variety (Fig. 19). The dark cells appear to contain more tightly packed myofilaments than do the pale cells; the pinocytotic vesicles also seem to be more numerous in the former.

The interstitial spaces between individual or groups of smooth muscle cells are occupied by bundles of collagen fibrils, microfibrils, elastic strands and partially or entirely membrane-bound granulo-vesicular bodies to be described in detail below. The orientation of the collagen bundles is rather haphazardous since they are found arranged in circular, helicoidal and longitudinal fashions (Figs. 11, 19 and 20).

In addition to the connective tissue elements mentioned above, the interstitial spaces contain small, membrane-bound bodies subsequently referred to as granulo-vesicular bodies. These structures have a spherical or elliptical shape and measure from 0.2 to 0.5 μ in diameter.
They are limited by a trilaminar membrane and their core has a sieve-like appearance (Fig. 21). In search of the possible origin of these structures, the medial smooth muscle cells were explored; the granulo-vesicular bodies are present within the cytoplasm of these cells and observed in various stages of development and at different cellular locations.

Structures presumably representing immature forms of the granulo-vesicular bodies are identified among the vesicles and sacs of the Golgi complex, or in close topographic relation to rough surfaced endoplasmic reticulum, often devoid of a complete limiting membrane (Fig. 22). In what is considered to be a more advanced stage of development, the granulo-vesicular bodies are observed in the perinuclear area either partially or completely surrounded by a membrane (Figs. 23 and 24). In a further stage of development and migration towards the cell periphery the granulo-vesicular bodies appear in close proximity to the cell border, and both, their limiting and the plasma membrane are seemingly fused (Fig. 25).

When observed in the extracellular spaces, the granulo-vesicular bodies show a striking relation to basement laminae, microfibrils and elastic elements (Figs. 21,
26 and 27). They seem to have a tendency to attach themselves to elastic fibers and fuse their limiting membrane with the homogeneous component of the elastic elements (Fig. 27).

The elastic lamellae, although possessing fenestrations, could be viewed as a nearly continuous sheet of elastic substance (Fig. 11). The appearance of the elastic tissue is variable, owing to the inconsistent uptake of the heavy metals used as stains for electron microscopy (uranium and lead). Tissue sections treated with the same staining solutions may disclose areas in which the elastic tissue appears markedly electron dense, while in others it is almost translucent. The internal structure of the elastic lamellae is formed by 80 Å thick filaments embedded in an amorphous matrix (Figs. 11 and 16). While the borders of mature elastic tissue are in general smooth, in newly formed elastic tissue they are studded with microfibrils (Fig. 16).

Elastic fibers connecting perpendicularly to the elastic lamellae such as those described in the aorta of rabbits (Wolinsky and Glagov, 1964), were not observed in our material.

Nerve axons are rarely observed in the arterial
media. When present, they are usually in the outermost interlamellar spaces and surrounded by Schwann cells (Fig. 28). Nerve endings in or around smooth muscle cells were not observed.

A well defined single external elastic lamina is seldom present; it separates the media from the adventitia (Fig. 29). The latter consists of thick, randomly oriented elastic fibers and bundles of collagen fibrils (Fig. 29). Nerve axons wrapped by Schwann cells are commonly found among the collagenous elements (Fig. 29). In the outer portion of the adventitia, the elastic fibers are less conspicuous and the connective tissue skeleton is looser. Schwann cells enveloping several nerve axons are more numerous here (Fig. 30). Small vasa vasorum surrounded by collagen bundles and slender fibroblasts are also common in the outer adventitia (Fig. 31).

3. The normal femoral artery

a). Gross appearance and topography. The femoral artery is the actual continuation of the external iliac artery from the vascular lacuna to the thigh. In the proximal half of the thigh it runs parallel to its accompanying vein and either caudal or medial to the saphenous
nerve; it has a superficial position in the femoral triangle (Fig. 6). The femoral vessels are covered only by the thin skin of the leg and the deep medial fascia. Upon leaving the femoral triangle at the middle of the thigh, the femoral artery and vein incline laterally along the medial border of insertion of the abductor muscle, where they are covered by the caudal belly of the semi-membranosus muscle. When the femoral vessels reach the popliteal surface of the femur, they continue between the lateral and medial heads of the gastrocnemius muscle as the popliteal vessels.

b). **Light microscopic observations.** The femoral artery, like all the large and medium sized arteries, is composed of three compartments namely the tunica intima, media and adventitia. In general the structure of the femoral artery is similar to that of the carotid described in the preceding chapter, therefore only the differences will be presented to avoid repetition.

The intima consists of a single layer of endothelial cells separated from the internal elastic lamina by a subendothelial space (Fig. 32) focal and diffuse intimal thickening are more common and thus the intima is wider than in the carotid artery (Figs. 32 and 33). The
intimal thickenings consist of slender smooth muscle elements, collagen, and delicate elastic strands sometimes surrounding the smooth muscle cells (Fig. 33).

The internal elastic lamina shows numerous fenestrae or gaps, reduplication and irregularities in thickness (Figs. 32 and 33). The tunica media is considerably thinner than that of the carotid artery. In cross sections the tunica media appears divided into an inner and an outer portion. The former contains smooth muscle cells and interrupted, circularly arranged, elastic lamellae, while the latter has almost exclusively smooth muscle cells longitudinally oriented.

In the inner portion the smooth muscle cells have a fusiform shape and contain abundant myofibrils which stain strongly with basic dyes (Figs. 32, 33 and 34). In contrast the smooth muscle cells of the outer portion have a pale cytoplasm and a centrally located, vesicular nucleus imparting an "owl eye" appearance (Figs. 32 and 35).

The adventitia represents two thirds of the total thickness of the arterial wall. It consists of coarse elastic fibers often interrupted and oriented in a nearly circular fashion (Figs. 34 and 35). The wide spaces between the elastic fibers are occupied by collagenous
connective tissue and vasa vasorum.

c). Electron microscopic observations. The endothelial cells have prominent nuclei and slender cytoplasmic processes. The cytoplasm contains relatively few organelles and the cellular junctions are identical to those described in the endothelium of carotid artery. The subendothelial space is in many areas occupied by smooth muscle cells, elastic elements and collagen (Fig. 36). The internal elastic lamina shows numerous fenestrations or gaps which, if small, are partially filled by cytoplasmic processes of endothelial cells (Fig. 37). The border of the internal elastic lamina, especially that facing the subendothelial space, has a scalloped appearance owing to the protrusion of "buds" of elastic substance and attached microfibrils (Figs. 36 and 37).

The inner half of the media consists of helicoidally oriented smooth muscle cells alternating with bundles of collagen fibrils and elastic lamellae (Figs. 36 and 37). The elastic lamellae are often interrupted or split (Figs. 36 and 37). These interruptions or gaps may reach considerable width, therefore some areas may appear to be devoid of elastic lamellae (Fig. 36). The ultrastructural
characteristics of the smooth muscle cells in the inner half
of the media are similar to those of carotid artery de-
scribed in the preceding chapter.

The outer half of the media is composed of bundles
of smooth muscle cells running parallel to the long axis of
the vessel. These cells are characterized by having a
centrally located nucleus which has evenly distributed
chromatin and a discrete nucleolus. The perinuclear area
appears usually devoid of myofibrils. This "clear" appear-
ing cytoplasmic zone contains a well developed Golgi
complex, sparse profiles of rough surfaced endoplasmic
reticulum and a few small mitochondria (Fig. 38). The
peripheral portion of the cells is occupied by myofilaments
oriented in both tangential and longitudinal fashions with
respect to the long axis of the cell (Fig. 38). Con-
densations are numerous among the myofilaments and beneath
the plasma membrane. The latter shows a moderate number of
pinocytotic vesicles (Fig. 38).

The interstitial spaces are occupied by loose bundles
of collagen fibrils oriented largely longitudinally, and
scanty elastic elements. The smooth muscle cells in the
outer media are oriented longitudinally; they have centrally
placed nuclei surrounded by a clear halo of cytoplasm
containing just a few organelles. The periphery of the cells is occupied by helicoidally oriented myofilaments (Fig. 38). A thick and well defined external elastic lamina separates the media from adventitia (Fig. 39). The adventitia consists of rather poorly organized, helicoidal, thick elastic fibers with a filamentous texture (Fig. 39). The spaces between the elastic fibers are occupied by compact bundles of collagen fibrils running parallel to the long axis of the artery.

4. Poststenotic dilatation of the carotid artery

The findings to be presented here are those observed in the areas of maximal dilatation (marked as number two in Fig. 2). Changes in arterial architecture were observed by both, light and electron microscopy as early as one month following the arterial ligature; these progressed quantitatively in the dilated segments of arteries examined three and six months following ligation.

a). Light microscopic observations. In the segments obtained one month after arterial ligation, the intima appears diffusely thickened. The intimal thickening consists of one to three layers of smooth muscle cells and connective tissue elements present in narrow intercellular
spaces (Fig. 40). The intimal smooth muscle cells are oriented preferentially in a longitudinal fashion.

The internal elastic lamina displays large areas of attenuation, splitting and ruptures (Fig. 40). In the media, the innermost elastic lamellae are thinned and interrupted. The smooth muscle cells in the inner interlamellar spaces have an altered polarity as evidenced by a longitudinal orientation of cellular groups. The intercellular spaces are occupied by scanty connective tissue and small strands of elastic substance closely applied to the boundaries of cells (Fig. 40).

In arterial segments examined three months following ligation, the internal elastic lamina is interrupted in large areas of circumference, thus a virtual communication between intima and media is established (Fig. 41). The innermost elastic lamellae appear fragmented or absent with only small remnants present in the interstitial spaces (Fig. 41). A large number of medial smooth muscle cells now appear to be oriented longitudinally and the intercellular spaces are widened and largely occupied by connective tissue elements (Fig. 41).

In arterial segments dilated for six months, the gaps in the internal elastic lamina are even larger. The
b). **Electron microscopic observations.** In arterial segments dilated for one month the endothelium appears relatively normal. The endothelial cells form a continuous layer, but occasionally there is a partial separation of the plasma membrane of two adjacent cells (Figs. 44 and 45) while other cytoplasmic membranes remain closely apposed (Fig. 46). The cytoplasm of the endothelial cells is thinned out occasionally and separated from the basement
lamina creating a space between the latter and the endothelial plasma membrane (Fig. 44). The cytoplasm of some endothelial cells contains patches of filaments arranged in a nearly parallel fashion. The filaments are present usually near the cellular borders and seem to attach to the plasma membrane. The filaments measure approximately 80 Å in thickness and resemble the myofilaments of smooth muscle cells (Fig. 46).

The subendothelial space is widened and occupied largely by smooth muscle cells, proliferating basement lamina of endothelial and smooth muscle cells, microfibrils, collagen fibrils and newly formed elastic elements (Figs. 44, 45 and 46).

The internal elastic lamina is often interrupted by gaps. The latter contain collagen and small masses of elastic substance (Fig. 44). The limiting border of the internal elastic lamina is rather smooth along the medial side, but its intimal surface has a characteristic scalloped appearance; the electron dense margins are studded with numerous microfibrils (Fig. 44). The central portion of the internal elastic lamina is less electron dense and almost translucent; it is traversed by filaments which in some areas converge to form small pools (Fig. 44).
The elastic lamellae vary in thickness and in many areas contain collagen fibrils; the latter appear to split the lamellae with the consequent formation of pools or lacunae partially occupied by microfibrils in addition to collagen fibrils (Fig. 47). The images of "pervaded" elastic lamellae with the formation of lacunar spaces, suggest that the lamellae break by a process of "erosion" (Fig. 48). Ruptured elastic lamellae occur more often in arteries dilated for three months than in those dilated for one month (Figs. 48 and 49). Collagen fibrils accumulate around the edges of interrupted elastic lamellae in a seeming attempt at filling the gaps. In many instances, the collagen fibrils arrange themselves parallel to the elastic lamellae and anchor at the edges of the lamellae on each side in a manner suggestive of bridging (Figs. 49 and 50). This mechanism of repair seems, however, ineffective as the fragmentation of elastic tissue progresses and the collagen fibrils are no longer capable of bridging the ever enlarging gaps (Fig. 51). Bundles of tightly arranged collagen fibrils oriented at random together with fragments of elastic lamellae occupy conspicuously the interstitial spaces (Fig. 51) already at this stage of poststenotic dilatation.
The changes in arterial segments dilated for six months are even more advanced. The elastic elements have altered staining qualities; areas of increased electron density alternating with less dense elastic tissue may be observed at times in the same field (Figs. 52 and 53). The borders of both internal elastic lamina and elastic lamellae are markedly irregular as exemplified in Figures 52 and 53. The intimal surface of the internal elastic lamina is studded with masses of homogeneous, electron dense elastic material which protrude into the subendothelial space (Fig. 52).

In the media, the elastic lamellae have lost their fibrillar texture and now appear homogeneous. Their edges have a frayed appearance suggesting a process of rarification (Figs. 53 and 54). The collagen fibrils aggregate into compact bundles which seem to lose gradually their fibrillar structure and periodicity becoming electron dense, amorphous masses (Figs. 54, 55 and 56). At this stage, it is difficult to assess the nature of some of the amorphous electron dense accumulations since they may represent degenerating elastic substance or alternatively, aggregated structureless collagen fibrils. This general pattern of the alterations described above, can be appreciated in
Figure 57 which is a photomontage of the inner two thirds of the arterial wall dilated for six months.

The cellular junctions between medial smooth muscle cells are also altered in the dilated arterial segments. In segments dilated for one month, the nexuses are still present (Fig. 44), but they seem to disappear after three and six months of dilatation as the cells separate from each other when the intercellular spaces become widened. The intercellular spaces at the desmosome-like junctions that in the control arteries are nearly obliterated, now appear broadened; they are occupied by collagen fibrils arranged in a parallel or a criss-crossing array and seemingly anchor to the opposite cellular borders (Figs. 58 and 59).

Degenerative changes are observed occasionally in the medial smooth muscle cells in the dilated segments. These changes consist of intracytoplasmic lipid droplets (Fig. 57) and myelin figures which in some instances occupy large areas of the cytoplasm (Fig. 60).

The adventitia shows relatively few changes, the most conspicuous consisting of hyperplasia of the walls of vasa vasorum. The endothelial cells in the vasa vasorum acquire a somewhat spherical shape, and their cytoplasm has an increased density owing to the presence of numerous
polyribosomes, cisternae of endoplasmic reticulum, and patches of filaments similar in appearance to myofilaments of smooth muscle cells. The subendothelial space is largely occupied by proliferating basement lamina-like material of both endothelial and underlying smooth muscle elements, and newly formed elastic substance which in some areas show an attempt at formation of an internal elastic lamina (Fig. 61).

5. Poststenotic dilatation of the femoral artery

The changes occurring in poststenotic dilatation of the femoral artery are qualitatively comparable to those of the dilated carotid artery. Quantitative differences are to be expected owing to the structural variations between these two types of arteries.

a). Light microscopic observations. Arterial segments examined following one month of dilatation reveal a diffuse intimal thickening, characterized by the presence of one to two layers of cells, delicate elastic strands and few collagen bundles. The internal elastic lamina shows many areas of splitting and reduplication (Fig. 62). In the media the elastic lamellae are conspicuously interrupted and large areas, at times involving the entire wall thickness, appear devoid of continuous elastic lamellae and only
short strands of elastic substance remain in the interstitial spaces (Fig. 62).

In arterial segments dilated for three months, the intimal thickenings are quite prominent particularly above large gaps in the internal elastic lamina (Fig. 63). In the media the fragmentation of elastic lamellae is conspicuous (Fig. 63). Arterial segments examined six months following ligation reveal still more advanced alterations. The internal elastic lamina is absent in large segments of the arterial circumference and in its place, a delicate elastic membrane appears beneath the endothelial layer (Fig. 64). In the media, the interstitial spaces are considerably broadened and contain connective tissue elements embedded in an amorphous material which is metachromatic in sections stained with alkaline toluidine blue (Fig. 64). The elastic lamellae, particularly those located in the inner portion, are almost absent and only remnants can be identified in the interstitial spaces.

b). **Electron microscopic observations.** Examination of arterial segments dilated for one month reveals that in some areas, the intima is thickened by the presence of smooth muscle cells, leukocytes, proliferating basement lamina,
elastic substance and bundles of collagen fibrils (Fig. 65). The endothelial cells do not appear altered although occasionally, they contain lipid droplets (Fig. 66). The internal elastic lamina shows numerous gaps and irregularities of its borders, especially on the intimal side (Fig. 66). In the media, the interstitial spaces are widened and contain an increased number of collagen bundles. The elastic lamellae are rarefied, in many areas virtually absent, and only short strands and small fragments are scattered in the interstitial spaces (Fig. 66).

In tissues removed from arterial segments dilated for three months, the changes appear to be increased in magnitude and are especially conspicuous in the media: here the interstitial spaces are broad and contain an increased number of collagen fibrils organized into compact bundles and remnants of rarefying elastic lamellae. In many areas the collagen fibrils form electron dense aggregates which are virtually indistinguishable from the degenerating elastic fragments, especially when both types of tissue are adjacent to each other (Figs. 67 and 68). At higher magnifications it is possible to recognize at times the collagenous nature of some of the dense aggregates owing to the persistence of a faint outline of collagen
fibrils (Figs. 68, 69 and 70). The picture remains practically unchanged in sections from arterial segments dilated for six months, except that the elastic tissue is more rarefied, and the dense collagenous aggregates and remnants of elastic lamellae are in some areas closely applied to the cellular borders (Fig. 71).
IV. DISCUSSION

A. General Considerations

The results of present studies confirm previous observations that poststenotic dilatation may be produced experimentally in canine carotid and femoral arteries almost at will providing the arterial ligature is applied in a manner that results in creating a thrill distal to the site of ligation. Of the 12 ligated arteries all developed a poststenotic dilatation. These results are comparable to those of Roach (1962), who, too, produced one hundred percent poststenotic dilatation in her experimental series.

The morphological changes observed in poststenotic dilatation of the carotid and femoral arteries were comparable qualitatively, therefore, these will be discussed together.

The normal structure of elastic and muscular arteries in a wide variety of animal species has been studied extensively (Benninghoff, 1930; Maximow and Bloom, 1957; Parker,
1958; Keech, 1960; Pease and Molinari, 1960; Pease and Paule, 1960; Seifert, 1962; Bierring and Kobayashi, 1963; Paule, 1963; Laing, 1964; Ham, 1965; Matthews and Gardner, 1966). However, only a few studies on the structure of carotid and femoral arteries, specifically those of dog, have been reported (Smirnov, 1955). It was for this reason that a detailed morphological study of these arteries was undertaken in order to allow an evaluation of changes in arterial segments of poststenotic dilatation.

B. The Normal Structure of the Carotid and Femoral Arteries

The canine carotid and femoral arteries have intimal structural features that in other arteries and animal species have been the subject of extensive studies and diverse interpretation. These intimal features include focal thickenings, proliferation of subendothelial smooth muscle cells and connective tissues, and fragmentation and reduplication of the internal elastic lamina.

In general, focal intimal thickenings or cushions have been regarded as an early step in the development of atherosclerotic lesions. There is however, strong evidence that similar changes of more diffuse nature occur normally during growth in the young adult vertebrate including man.
Intimal thickenings occur in the aorta of the adult cat and dog (Krause, 1922), cow and horse (Wolkoff, 1924) and pig (Laing, 1964). In newborn animals intimal cushions have been reported in the aortae of puppies just a few days old (Morehead and Little, 1945). In an electron microscopic study Geer et al. (1961) described the replacement of the internal elastic lamina of normal dogs by small elastic fibers and presence of smooth muscle cells in the subendothelial space. In the aortae of human fetuses and newborn babies, Haust and co-workers (1965) reported the presence of intimal cushions composed of smooth muscle cells, elastic and collagen fibers along with discontinuities and irregularities of the internal elastic lamina.

It is clear that the assessment of the normal arterial structure is difficult. Departures from what some authors have considered normal structural pattern in some vessels are quite common in a variety of arteries of several animal species. On the other hand, the morphological changes brought about by the process of aging should be taken into consideration when evaluating the "normality" of arterial structure.

The interpretation of structural changes in experimental material has to be approached with caution, since
what might be considered as a pathological alteration may
indeed represent a normal variation of the arterial structural
organization.

1. The granulo-vesicular bodies

Granulo-vesicular bodies were observed with regularity
in all sections studied (Figs. 14, 20-27, 49, 55, 68 and 69).
It is surprising that in the extensive literature on arterial
ultrastructure in both normal and pathological conditions,
no reference has been made to structures corresponding to
those referred in the present studies as "granulo-vesicular
bodies". In a structural survey of normal chicken aorta,
Moss and Benditt (1970) included an illustration of what
they interpreted as "rounded, membrane-bound aggregates of
closely packed dense granules". These granules conceivably
may represent some stages of development of granulo-vesicular
bodies. Recently, Trillo and Haust (1972) reported the
presence of granulo-vesicular bodies in the aorta of normal
rats and pigs, and observed that the number of these
structures was increased in the aortae of hypertensive rats.

In the present study, the granulo-vesicular bodies
were observed in control arteries within smooth muscle cells
where they were present in various stages of development and
at different cellular locations. This suggests that they are produced by the smooth muscle elements and "secreted" into the extracellular spaces. In the latter, they were topographically related to elastic tissues and connective tissue microfibrils suggesting that the granulo-vesicular bodies may play a functional role in connection with the process of remodeling of elastic fibers. Their increased number in processes such as hypertension in which remodeling of mural elements is a prominent feature would support this theory. However, the true significance of these structures cannot be established on the basis of present information. It is hoped that further electron microscopic and cytochemical studies will shed some light on the nature and function of these arterial granulo-vesicular bodies.

C. Poststenotic Dilatation of the Carotid and Femoral Arteries

Diffuse intimal thickening with proliferation of smooth muscle cells and connective tissues was a feature consistently found in the poststenotically dilated segments.

The most conspicuous changes, however, were observed in the media where structural alterations appear to represent two different tissue responses: a proliferative or reparative, and a degenerative. In most instances prolifera-
tive and degenerative changes were not observed in a "pure" form since they often overlapped so that in the same section the two processes could be seen taking place simultaneously.

For the convenience of the exposition, the intimal changes will be discussed first; these will be followed by a discussion of medial alterations. Exception will be made only when dealing with changes of structures common to both, intima and media.

1. Mural components

The arterial wall, especially the intima, seems to be a sensitive organ which reacts readily to a variety of noxious stimuli in a manner that appears to be conditioned by the nature of the injury. The latter is usually categorized as physical, chemical and biological. In the present discussion, and without disregarding the possibility of a mixture or combinations of pathological stimuli, the physical factors will be considered largely since it seems likely that in poststenotic dilatation these are of prime importance as the originating injurious elements.

The role of physical factors in vascular pathology was recognized by Duguid (1926; 1952) a few decades ago and further stressed by several authors (Burton, 1954; Rodbard,
1956; 1959; Texon et al., 1962; Esterly and Glagov, 1963; Fry, 1968; 1969 a; 1969 b; Pflieger and Goerttler, 1970; Aars, 1971). Duguid (1957) suggested that impairment of the elasticity or flexibility of the intima might lead to structural disorganization, and that this in turn would cause shearing and tearing of the endothelium. According to Rodbard (1959) high and low distending forces may cause intimal alterations with peculiar characteristics in each instance. High distending forces tend to compress and flatten the endothelium, thus inhibiting its growing capacity. Low distending forces (e.g., poststenotic dilatation) may cause the endothelial cells to become rounded and stimulate local proliferation resulting in formation of intimal cushions.

Shearing stress secondary to a turbulent flow has been claimed to cause intimal damage. Rodbard and co-workers (1967) and Fry (1968) suggested that a turbulent flow usually produces endothelial erosion. Fry (1968) proposed that the normal endothelium can withstand shearing stresses up to a critical point, and that, if this limit is not surpassed, the endothelium behaves as an elastic body remaining tightly adherent to its basement lamina in an orderly fashion, but beyond the limit of resistance the endothelial
cells change configuration, and may detach and desquamate.

An example of the effect of physical forces on the arterial intima is found in areas of arterial curvature where, according to Texon et al. (1962), the laminar flow changes to turbulent, exerting a lifting or pulling effect on the endothelium. In support of this suggestion one should mention the work of Duguid (1926), Laing (1964), and Pflieger and Goerttler (1970). All these authors reported a higher incidence of spontaneous and experimental intimal lesions in arterial curvatures and branching of vessels. Meyer (1972) has found similar results in curved areas of the tortuous splenic artery in man.

In the present study, gaps in the junctions between endothelial cells and blebs or lacunae between the plasma membrane of endothelial cells and its basement lamina (Figs. 44 and 45) have been observed consistently. In arterial segments dilated for three and six months this was accompanied by a conspicuous diffuse intimal thickening and extensive rupture of the internal elastic lamina. These alterations may be attributed conceivably to the action of the turbulent flow in the poststenotic area. Subsequent arterial distension and secondary dilatation may enhance the intimal changes while the arterial wall acquires a curved
shape.

The presence of white blood cells identified as leukocytes and monocytes in the subendothelial space is an interesting phenomenon observed in the intima of dilated arteries. These types of cells have been observed occasionally in the intima of normal arteries (French, 1966). The presence of circulating cells in the intima has been related by several authors to alterations in endothelial permeability or to severe endothelial damage (Buck, 1958; Esterly and Glagov, 1963; Esterly, 1965; Still, 1967; 1968; 1970).

It has been well documented that local and systemic hypertension results in an increase in the tension of the arterial wall causing influx of blood constituents into the arterial wall. Influx of plasma proteins and intimal deposition of mononuclear cells are common findings in large arteries and arterioles of experimental animals with local or systemic hypertension (Buck, 1958; Esterly and Glagov, 1963; Esterly, 1965; Ooneda et al., 1965; Wiener et al., 1965, Still, 1968; 1970; Bondar, 1970; Bondar et al., 1971; Trillo et al., 1970; 1971). The presence of white blood cells in the intima of the dilated arteries would at first appear paradoxical since the poststenotically dilated segment has
a decreased intravascular pressure (Holman, 1955). However, one should consider that in this area the vascular surface is overstretched and the endothelial cells pulled apart, thus resulting in a relative increase of permeability and loss of focal mural resistance in the presence of low luminal pressure.

Perhaps the most conspicuous and functionally significant change in poststenotic dilatation is represented by the alterations of the elastic elements. As stated earlier, there are two types of response of mural elements to the stretching force: an early response characterized by proliferation and repair, and a late phase of degenerative events.

The early proliferative phase was observed mainly in arterial segments dilated for one month. It is characterized by the presence of numerous microfibrils, proliferating basement lamina of smooth muscle cells, and newly formed elastic elements (Fig. 44).

The mode of formation and site of origin of elastic elements in arteries and other elastic-containing organs has been a controversial issue.

For many decades the classical histologists speculated on the origin of elastic fibers and were divided into
two groups: one favouring the cellular origin, the other proposing that elastic fibers were formed from the intercellular matrix. Another area of discussion centered around the type of cell involved in the direct or indirect production of elastic fibers. Authors of the stature of Schwann (1839), Jores (1907) and Orsos (1926) believed that the elastic fibers were produced by either fibroblasts or undifferentiated connective tissue cells. Loisel (1897) and Krompecher (1930) suggested that formation of elastic fibers was restricted to a specific type of mesenchymal cell which was not capable of forming other types of fibers. Loisel (1897) coined the term "elastoblast" to designate this cell.

Positive identification and morphological characterization of the elastoblast were never achieved. In spite of this, this suggestion was supported by many authors until recently when the problem was clarified with the use of the electron microscope. Berrian (1953), Pease and Molinari (1960), Pease and Paule (1960) and Keech (1960) among others, have stated that the smooth muscle cell is the only cell normally present in the arterial media of both elastic and muscular arteries.

The development of elastic tissue has been studied
in detail by Haust and co-workers (1965; 1967) in aortae of human fetuses, skin of infants and aortae of newborn pigs, by Greenlee et al. (1966) in the ligamentum nuchae from fetal calves and digital tendons from newborn rats, and by Jones and Barson (1971) in the lung of chicks.

According to Haust and co-workers (1965; 1967) there is a continuous growth of elastic elements resulting from the accretion of microfibrils to form elastic units; these in turn coalesce giving origin to larger elastic elements. Haust and co-workers (1965; 1967), and Greenlee et al. (1966) have observed that the newly formed elastic elements are characterized by the presence of filaments within the elastic matrix, while in mature elastic fibers no definite filaments can be identified. Another distinctive feature of the young elastic elements is that their surfaces are studded with microfibrils and small elastic units which tend to disappear as the fibers become mature.

In arterial segments dilated for one and three months, structures suggestive of a process of elasto-genesis were consistently observed in the intima as well as in the media (Figs. 44 and 46). In the arterial segments dilated for three months, formation of new elastic elements was less conspicuous, and degenerative process predominated. The
latter was still more marked in the arteries dilated for six months.

The degenerative changes of the elastic and collagenous tissue consisted of rupture of the internal elastic lamina and the medial elastic lamellae, and coalescence or aggregation of collagen fibrils with loss of their periodicity. Fusion of degenerating fragments of elastic "substance" and amorphous collagen aggregates were also common.

The degenerative changes in the poststenotically dilated arterial segments are likely responsible for the alterations in the elastic properties of the dilated segments; Roach (1962; 1963 a) studied these properties in a series of measurements of arterial distensibility.

In intact arteries, distension usually is well tolerated without causing permanent dilatation. It is the property of elasticity of the wall that enables the vessels to maintain a tone which balances the stretching force and retains a margin of extensibility before the limit is reached. When the arterial wall is stretched until it is rendered tense, it can still be lengthened to a certain extent while retaining the power of recoiling to its original dimensions; only when the distending pressure is such as to
stretch the wall beyond these limits the power of recoiling is lost and the distension becomes permanent.

In poststenotic dilatation the normal arterial distensibility and the ability to recoil are expected to be greatly altered as a result of the destructive process and paucity of elastic fibers. The initial attempt at regenerating elastic elements is perhaps made ineffective by the repetitive stretching forces until the arterial wall "gives up" and becomes permanently distended. It is surprising, however, that the arterial distension does not seem to progress with time of ligature. It remains within certain limits (expressed by measurement in diameters) regardless of the duration of the experiments (Table II). Another factor which undoubtedly influences the extent of arterial dilatation is the distensibility of collagen and smooth muscle cells. Collagen is extremely resistant to stretching and in the dilated segments this resistance is further increased by the increase in collagen in the interstitial spaces. The aggregation of collagen fibrils with loss of their periodicity and apparent fusion with the remaining elastic elements may be viewed perhaps as an attempt at reinforcing the vanishing elastic capability of the arterial wall. It has been postulated that collagen fibrils under special
circumstances may transform themselves into elastic substance (Hall, 1955; Burton et al., 1955; Rodgers et al., 1967).

Hall (1955) and Burton et al. (1955) have shown that collagen fibers treated with an alkaline buffer and sodium metaperiodate become remarkably similar to elastic substances. To substantiate this, they showed that collagen fibers become stainable with Hart's modification of Weigert's stain for elastic fibers and that electron microscopically structureless, branching fibrils similar to elastic tissue appear as a continuation of collagen fibrils with periodicity.

Chemical similarities between collagen and elastic fibers have been a matter of debate for some time. Banga (1953) found that during hydrothermal contraction of collagen fibers in 40 per cent potassium iodide solution, a mucopolysaccharide and a soluble protein similar to protocollagen are dissolved or "liberated" from the normal typical collagen fiber, leaving behind metacollagen. Metacollagen was described as a rubber-like material with elastic properties similar to those of elastin. This substance differs from elastin by a distinct mucoprotein content. Both the elastic and the collagen fibers contain two
mucopolysaccharides. One of these is called sheath mucoid. Collagen and elastic fibers contain a different kind of sheath mucoid.

Ramachandran (1963) treated collagen with calcium chloride and obtained a substance with an X-ray diffraction pattern similar to that of elastin. He believed that elastin is analogous to collagen at the molecular level and that it is built on a triple chain arrangement of amino acid residues.

Ultrastructural studies (Haust, 1965; Haust and More, 1967) suggest that the fine fibrils (microfibrils) in the extracellular spaces and the filaments observed in elastic tissues are morphologically identical. On the basis of her observations, Haust (1965) proposed that there is one organized morphologic precursor, the microfibril, which participates in the formation of both collagen and elastic tissue.

Destructive and degenerative changes of elastic fibers in arteries have been studied extensively in a wide variety of pathological conditions in man and in the experimental animal. According to Balo' (1963) the elastic fibers are destroyed in three ways: by elastolysis, by granular degeneration and by calcification.
Elastolysis can be best studied in vitro, incubating elastic tissues in elastase solution. In situ, this phenomenon is observed as an autolytic process in the cadaver. In both instances the process is similar and consists of swelling and subsequent decreased staining intensity of the elastic fibers. At a later stage erosion, indentations and droplet-like defects occur in the fiber.

Granular desintegration was first described by Dmitrijeff (1897) and consists of irregularities in staining properties and breakdown of elastic fibers into irregular granules which arrange themselves along the course of the original fibers. Granular disintegration is more often seen in the internal elastic lamina and inner elastic lamellae. It is also quite conspicuous in and around atheromatous plaques (Balcó, 1963).

Of the three types of elastic degeneration calcification has been studied perhaps most extensively. Blumenthal and co-workers (1944) stated that calcification is very common and that invariably it precedes the formation of atheromatous plaques in the intima. Lansing et al., (1948) suggested that calcification is a process inherent in aging and that it can occur in both elastic and muscular arteries. Calcification of elastic fibers is often present
in spontaneous arterial lesions, especially in the rabbit (Haust and More, 1965; Schenk et al., 1966). Recently, Haust and Geer (1970) have analyzed the mechanism of calcification in the spontaneous aortic lesions of the rabbit. They suggested that the process of calcification follows a sequential pattern; the first event consists of an accentuation of the filamentous components of the elastic fibers and increased intensity of their staining properties. Secondly, the filaments arrange themselves radially to a central, structureless electron-lucent core of an elastic fiber, and subsequently calcium salts are deposited in the central core resulting in the formation of masses of calcium which distend the elastic fiber. Haust and Geer (1970) postulated that the calcium salts gain access into the elastic matrix via the helicoidal central spaces of the component microfibrils.

2. Theories of pathogenesis

As pointed out in the Review of Literature, many theories have been proposed to explain the development of poststenotic dilatation. Of these, the theory implicating the turbulence and vibration of the arterial wall seems to have gained widest acceptance (Robicsek, 1955; Robicsek et

Hinze (1959) defined turbulence as follows: "turbulent fluid motion is an irregular condition of flow in which the various quantities show a random variation, resulting in a superimposition of eddies of various sizes and vorticities". The author further elaborated that turbulence can be generated by friction forces at fixed walls or by the flow of layers of fluids with different velocities passing over one another. The turbulence that is continuously affected by fixed walls is designated as wall or shear-flow turbulence.

With the above considerations in mind, the following question may be formulated: how does turbulence affect the mural elements in the arteries, specifically those of elastic tissues, to such an extent as to cause their destruction? Definite answer has not been provided to date and in any event can be only speculative. Of the several suggestions that have been made to explain the pathogenesis of poststenotic dilatation, perhaps that offered by Roach (1962; 1963 a), Roach and Harvey (1964), and Boughner (1971) deserves some consideration. These
authors have proposed that stenosis in an artery or a rubber conduit produces a turbulent flow which in turn causes the wall to vibrate. The repetitive vibration over a period of time would eventually lead to "structural fatigue" of the wall components (mainly of elastic elements) causing a breakdown of elastic fibers in the case of an artery, or a molecular disarray in the case of rubber tubes.

The concept of structural fatigue has been known and applied for a long time in metallurgy, however, its association with biological materials was not proposed until recent times by Holman (1955). In general, the conditions which produce structural fatigue "demand" frequent and recurrent stresses and strains. In biological systems the response to an applied stress consists of a response characterized by repair and proliferation. However, it has been proposed (Holman, 1955) that if the stimulus (stress) is acting constantly and over a prolonged period of time, the reparative and regenerative processes will eventually fail and structural fatigue, especially of the non-cellular elements (e.g., fibrous proteins) will result.

Consideration should be given to other factors known to affect the integrity and function of elastic tissue.
Among these, the most relevant to human and experimental pathology are anoxic states causing shifts in the acid-base balance in the direction of acidity (Frazeekas, 1935; Buchner and Luft, 1936; Hueper, 1956; Balo, 1963); (Buchner and Luft (1936), and Hueper (1956) have described severe degenerative changes of elastic fibers in the aortae of rabbits kept in an atmosphere low in oxygen, or oversaturated with carbon dioxide.) and intoxication by a variety of substances such as thyroxin (Balo, 1939), vasopressin (Byrom, 1937), and hydrochloric acid (Haldane, 1921).

Deficiency of trace elements, particularly copper, produces experimentally alterations similar to those observed in poststenotic dilatation such as destruction of internal elastic lamina and elastic lamellae (Simpson and Harms, 1964; Carnes et al., 1965; Waisman and Carnes, 1967; Waisman et al., 1969).
The role and functional significance of the granulovesicular bodies are yet to be determined with certainty. Nevertheless, there is circumstantial evidence that these bodies may play a role in the process of remodelling of elastic tissue; this is suggested by the increased number of these structures in the aortae of hypertensive rats (Trillo and Haust, 1972) and in poststenotic dilatation (Figs. 68 and 69).

Morphological observations suggest that the granulovesicular bodies, once extruded into the interstitial spaces, become associated with the elastic elements. Their limiting membranes fuse with the elastic elements and eventually disappear. Provided these bodies are of secretory nature, it is possible that they release their contents when fusing with elastic structures, thus intervening either in the crosslinking of the proelastin molecules, the accretion of microfibrils, or in the elastin-elastase system. The latter action may be affected perhaps by blocking the elastoproteinase inhibitor normally present in the lipoprotein fraction of the serum (Loeven, 1963).

Medial smooth muscle cells undoubtedly play an important role in the preservation of the normal tonicity and distensibility of the arterial wall. The muscular tone
of the wall has to be uniform and thus both, the contraction and relaxation of the smooth muscle cells must be synchronized at all times. This is achieved by an effective propagation of the electrical impulse from cell to cell.

Evidence that the smooth muscle cells in the arterial wall behave as an electrical syncytium has been provided by Barr et al. (1968) by electrophysiological experiments. The transmission of the electrical impulse in smooth muscle cells, as in the myocardial cells (Barr et al., 1965; Johnson and Sommer, 1967; Bencosme et al., 1969) is established via the areas of lesser electrical resistance. It has been shown (Dewey and Barr, 1962; 1964; Barr et al., 1965; Tomita, 1967; Abe and Tomita, 1968; Barr et al., 1968) that the nexus or tight junctions are areas of low electrical resistance and it is likely that they represent the points of transmission of the stimuli. In the presently examined tissues there was a marked paucity of nexuses between medial smooth muscle cells, especially in arterial segments dilated for six months. This is being interpreted as a result of overdistension of the arterial wall, and an increase of fibrous tissue in the interstitial spaces. The disruption of the nexuses is likely to result in a relative blockade of the electrical impulses with possible uncoordinated
contraction and relaxation of the smooth muscle cells and thus, lack of the normal tonicity. The distending force acting upon an arterial wall that has lost its tonicity would render this wall more prone to permanent dilatation.

Further evidence of the effects of overstretching upon the junctions of medial smooth muscle cells is represented by the changes observed in the desmosome-like junctions; these changes consist of separation of the apposing cellular borders and proliferation of connective tissue in the widened intercellular spaces.

On the basis of present observations one may conclude that the complex pathogenetic mechanisms involved in poststenotic dilatation induce an equally diverse and complex reaction of the mural elements. It seems however, that the main morphologic substratum of the poststenotic dilatation consists of degenerative changes of the elastic elements with a concomitant increase of interstitial fibrous tissue.

In future investigations special consideration should be afforded to cellular changes that no doubt occur in the dilated wall. The elastic and collagenous components depend upon cellular activity and any change in their configuration and quantity would undoubtedly reflect an altered cellular metabolic state.
Whereas models of artificial circulatory systems proved helpful in the study of altered physiological conditions, their usefulness is limited because such systems do not provide an insight into the underlying biomolecular processes of the wall. The complex multifactorial nature of the arterial wall and the variability of the biological responses make the use of living models more meaningful with respect to unravelling of the basic changes underlying the phenomenon of poststenotic dilatation.
V. SUMMARY AND CONCLUSIONS

Poststenotic dilatation is a hydrodynamic paradox that occurs when in a closed circulating system an area of the conduit is constricted and a dilatation develops beyond the constriction.

In patients, poststenotic dilatation may occur beyond almost every type of arterial stenosis both of intrinsic and extrinsic nature. It has been proposed that poststenotic segments dilated experimentally for as long as 10 months return to normal diameter and function upon removal of constrictive devices. However, morphological evaluation of possible changes in the dilated arterial wall are not well documented.

The present study was undertaken to test whether structural changes do take place in experimentally produced poststenotic dilatation in dogs.

Poststenotic dilatation of the common carotid and femoral arteries was induced by incomplete ligature. A
total of twelve operations were performed in six adult dogs. The contralateral arteries served as controls. Of the six animals, two were kept for one month, two for three months and the remaining two for six months. The arteries were fixed in situ under pressure, and representative areas of the dilated and control arteries were processed for light and electron microscopy.

In the control carotid arteries, two populations of smooth muscle cells were observed: deep-dark staining and pale cells. The cellular junctions between these cells were of the desmosome type, however, areas of apparent cytoplasmic continuity were observed along the apposing plasma membranes. In addition, the extracellular spaces in the walls of carotid and femoral arteries contained small, membrane-bound bodies referred to as granulo-vesicular bodies. Structures presumably representing immature forms of granulo-vesicular bodies were identified within smooth muscle cells. The possibility that granulo-vesicular bodies are produced by the smooth muscle cells and "secreted" into the extracellular spaces where they may play a role in connection with the process of remodelling of elastic fibers was suggested.

Changes in arterial architecture were observed by
both light and electron microscopy as early as one month following the arterial ligature; these alterations progressed quantitatively in the dilated segments of arteries examined three and six months after ligation. The morphological changes appeared to represent two different tissue responses: a proliferative or reparative and a degenerative. The earliest alterations consisted of diffuse intimal thickening with proliferation of smooth muscle cells and connective tissues. Rupture of the internal elastic lamina and irregularities of its surface were features consistently found in all dilated segments.

The most conspicuous changes, however, were observed in the media, where the elastic lamellae appeared fragmented, the interstitial spaces widened and the collagen fibrils greatly increased. Collagen fibrils tended to bridge gaps between fragments of elastic lamellae and between separated cellular junctions of smooth muscle cells. Subsequently, fragments of elastic tissue seemingly underwent "dissolution" and collagen fibrils aggregated in bundles that gradually lost their fibrillar structure and periodicity becoming amorphous electron dense masses.

On the basis of present observations the following conclusions may be drawn:
1. The reactions of the mural elements in poststenotic dilatation observed in this study were of complex and diverse nature.

2. The main morphological substratum of the poststenotic dilatation consists of regeneration and degenerative changes of the elastic elements with a concomitant increase of interstitial fibrous tissue.

3. Granulo-vesicular bodies observed also in normal arterial walls were increased in number in the extracellular space in the dilated segments. Similar structures were present in and originate from the mural smooth muscle cells. It is possible that these bodies are associated with the re-modelling of elastic fibers.
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Figure 1: Perfusing apparatus. It consists of two bottles connected to a pair of intravenous infusion sets as indicated, as well as a pressure chamber and a mercury manometer. Pressure is exercised through a manual rubber blower. The efferent tubings of the infusion sets are connected to a three-way stopcock with a tubing outlet ending in a 16 gauge blunt needle. The latter is inserted into the right common carotid artery for infusion of the rinsing solution (Palay's) and the fixative. A cannule is inserted into the left femoral artery to drain the blood and infused fluids.
Figure 2: The dilated portions of the left common carotid and right femoral arteries were divided into three segments: the beginning of the dilatation (1 and 5), the area of maximal dilation (2 and 6), and the end of the dilatation (3 and 7). Corresponding areas from the contra-lateral sides (4c and 8c) were used as controls.
<table>
<thead>
<tr>
<th>Groups</th>
<th>Dog Number</th>
<th>Sex</th>
<th>Weight</th>
<th>Age</th>
<th>Duration of Ligation</th>
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<tr>
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<td>M</td>
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<td></td>
<td>6</td>
<td>F</td>
<td>10</td>
<td>6</td>
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</tbody>
</table>

+ diet supplemented with sucrose-Propylthiouracil feeding

* in lbs. at beginning of experiment

** approximate in months at beginning of experiment

*** in months
### TABLE II

Diameters* of Control and Dilated Arterial Segments*

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Carotid Artery</th>
<th>Femoral Artery</th>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Maximum Dilatation</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>8</td>
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<td>2</td>
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<tr>
<td>6</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

* External diameters

* In mm.
Figure 3: Arteriogram of the carotid artery taken six months after ligation. A fusiform dilatation developed above the site of ligature (arrow).

Figure 4: Arteriogram of the femoral artery taken six months after ligation. A fusiform dilatation, approximately twice the normal diameter, is present below the site of ligature (arrow).
Figure 5: Schematic representation of topographic anatomy of the left and right common carotid arteries. They arise from the brachiocephalic artery and at origin the left common carotid artery is anterior to the trachea, while the right diverges from the left and crosses the ventrolateral surface of the trachea to right. (Adopted from: Miller, M. E., Anatomy of the dog. Philadelphia: Saunders, 1964).

Figure 6: Schematic representation of topographic anatomy of the femoral artery. The femoral artery may be regarded as an extension of the external iliac artery; it runs parallel to its accompanying vein. (Adopted from: Miller, M. E., Anatomy of the dog. Philadelphia: Saunders, 1964).
Figure 7:
General view of a cross section of control carotid artery fixed without pressure. The thin and flattened endothelium (en) lies almost directly on the internal elastic lamina. The inner half of the media is composed of bundles of circularly oriented smooth muscle cells (arrow), whereas the outer half contains bundles of longitudinally oriented smooth muscle elements (arrow-heads). Wavy elastic lamellae (el) separate the bundles of smooth muscle cells. HPS; X=250

Figure 8:
Detail of the intima and adjacent media of control carotid artery fixed under pressure. Note the prominent internal elastic lamina (iel) showing minute gaps (arrows). The elastic lamellae (el) appear as straight bands. Tissues removed under pressure. Epon embedded tissue; Toluidine Blue; X=400

Figure 9:
Control carotid artery. Islet of longitudinally oriented medial smooth muscle cells, showing two different cell populations: clear (cc) and dark staining (dc) cells. Epon embedded tissue; Toluidine Blue; X=1000

Figure 10:
Control carotid artery. Higher magnification of the dark (dc) and clear staining cells (cc). The dark staining cells have vesicular nuclei and their cytoplasm is largely occupied by tightly packed myofilaments. Epon embedded tissue; Toluidine Blue; X=1000
Figure 11: Low power survey of a cross sectioned control carotid artery. The intima is lined by a single layer of endothelial cells. The endothelial cells have slender cytoplasmic processes containing few organelles. The more conspicuous of these are small dense bodies (DB). The subendothelial space contains in some areas smooth muscle cells (SM) and basement lamina-like material (BL). The internal elastic lamina (IEL) appears homogeneous in some areas, while in other it has a fibrillar texture. The media consists of layers of smooth muscle cells (SM) alternating with elastic lamellae (EL). The interstitial spaces contain longitudinally and helicoidally arranged bundles of collagen fibrils (COL0). X=7,000
Figure 12: Electron micrograph of an area of intimal thickening in a control carotid artery. The endothelial cells (EN) establish two types of cellular junctions: interdigitating processes (CJb) and plain apposition of adjacent plasma membranes (CJa). The subendothelial space is occupied by smooth muscle cells (SM), basement lamina-like material (BL), newly formed elastic elements (NEL) and loose collagen fibrils (COL). The internal elastic lamina (IEL) has numerous gaps. Through these gaps, cytoplasmic processes of intimal and medial smooth muscle cells protrude (arrow). X=6,400

Figure 13: Details of the two types of endothelial junctions (CJa and CJb) shown in Fig. 12. Cytoplasmic processes of marginal folds (arrow) protrude into the lumen. A subendothelial smooth muscle cell (SM) appears partially surrounded by newly formed elastic strands (NEL). Note the filamentous nature of the internal elastic lamina (IEL). X=16,200
Figure 14:
A young medial smooth muscle cell. The paranuclear zone is largely occupied by a well developed Golgi Complex (G) and mitochondria (M). A granulo-vesicular body (GVB) is among the Golgi vesicles. Free ribosomes and polyribosomes (R) are abundant. Numerous pinocytotic vesicles (PV) are present at plasma membrane. X=15,400

Figure 15:
Lateral cellular junction of the nexus type (NJ) between two adjacent smooth muscle cells. The intercellular space is obliterated. The basement lamina (BL) is fibrillar. X=25,000

Figure 16:
Portion of two active young medial smooth muscle cells. The cells are partially surrounded by a thick, fibrillar basement lamina (BL) which in some areas appears proliferating (arrow-head). Newly formed elastic elements (EL) surrounded by microfibrils (MF), are at some points in contact with the plasma membrane (arrow). Numerous pinocytotic vesicles (PV) are present along the plasma membrane. The cytoplasm contains profiles of rough surfaced endoplasmic reticulum (RER) and mitochondria (M). X=37,000
Figure 17: Electron micrograph of two medial smooth muscle cells (SM) from a control carotid artery. Almost the entire surfaces of both cells appear to be involved in the desmosome-like cellular junction. The plasma membranes in some areas are closely apposed to each other (circle) leaving an almost negligible intercellular space. At some points (arrow-heads) along the cellular borders, electron dense "patches" face each other. X=7,400

Figure 18: Detail of a desmosome-like cell junction between two smooth muscle cells. Note the electron dense areas (arrow-heads) of both cytoplasms "facing" each other. The myofilaments seem to attach themselves to these dense areas. X=20,500
Figure 19: Clear smooth muscle cell (CSM) in contact with a dark cell (DSM) in the media of a control carotid artery. The cellular junction is of the desmosome type, however, at some points the cytoplasms seem to be in continuity with each other (arrows). X=38,000
Figure 20:

Low power survey of the media of a control carotid artery. Fenestrated elastic lamellae (EL) are seen at the upper left and lower right corners. The intercellular spaces contain randomly oriented collagen bundles (COL). At this magnification, it is somewhat difficult to visualize the small, intra-, and extracellular granulo-vesicular bodies (arrows). X=5,450

Figure 21:

Granulo-vesicular bodies (GVB) when seen in the intercellular spaces, are associated with the elastic lamellae (EL). The granulo-vesicular bodies are limited by a trilaminar membrane and have an electron dense core with a sieve-like appearance. X=36,100

Figure 22:

When present within smooth muscle cells, granulo-vesicular bodies (GVB) are seen at various stages of development. In this cell one granulo-vesicular body is seen among the Golgi vesicles (G) and another closely applied to a cisterna of rough endoplasmic reticulum. X=41,000
Figure 23:
Detail of the perinuclear area of a smooth muscle cell, showing a granulo-vesicular body (GVB) partially limited by a membrane. X=28,000

Figure 24:
Perinuclear area of a smooth muscle cell with a granulo-vesicular body (GVB) completely surrounded by a membrane. Note the "clear" rim between the core and the limiting membrane. X=30,000

Figure 25:
A smooth muscle cell (SM) with a granulo-vesicular body located at the cell border. The limiting membrane of the granulo-vesicular body (GVB) is closely apposed to the plasma membrane (arrow-head). X=21,000

Figure 26:
Two granulo-vesicular bodies (GVB), here devoid of limiting membranes, are located in the extracellular space in close proximity to the basement lamina (BL) and to a loose network of microfibrils (Mf). Observe the apparent continuity of the basement lamina with the elastic elements at the upper left quadrant of the photograph. X=38,200

Figure 27:
Two granulo-vesicular bodies show a striking spatial relation to elastic elements. The limiting membrane of the granulo-vesicular bodies (GVB) appears to be in contact with the periphery of the homogeneous component of the elastic elements (EL). X=25,000
Figure 28: Outer portion of the media of a control carotid artery. Two Schwann cells (SC) enclose several nerve axons (AX). X=8,300

Figure 29: Transverse section of a control carotid artery. The media and the adventitia are separated by a narrow but well defined external elastic lamina (EEL). The adventitia consists of compact bundles of collagen (COL), elastic fibers (EL), nerve axons (AX), and fibroblasts (F). X=8,950
Figure 30: Transverse section of adventitia of a control carotid artery. Several Schwann cells (SC) are seen embedded in a collagenous matrix. The Schwann cells are enwrapping numerous nerve axons (AX). X=12,900

Figure 31: Vasa vasorum of the adventitia often have prominent endothelium (EN) and pericytes (P). Dense collagen bundles (COL) and fibroblasts (F) with tortuous and slender cytoplasmic processes surround the vasa vasorum. X=6,000
Figure 32: Transverse section of a control femoral artery. The intima appears thickened by the presence of smooth muscle cells beneath the endothelium (en). In the media, bundles of smooth muscle cells (sm) oriented in a helicoidal fashion alternate with interrupted elastic lamellae (el). The smooth muscle cells in the outer media are longitudinally oriented. Epon embedded tissue; Toluidine Blue; X=250

Figure 33: Transverse section of a control femoral artery. The intimal thickening is enhanced by the presence of delicate elastic strands at the periphery of the subendothelial smooth muscle cells (arrows). Note the helicoidal arrangement of the medial smooth muscle cells and the numerous fenestrae in the elastic lamellae (el). Epon embedded tissue; Toluidine Blue; X=250
Figure 34:
Transverse section of a control femoral artery showing the division of the media into two portions. In the inner, the smooth muscle cells (sm) are helicoidally oriented, and fusiform in shape. In the outer portion the smooth muscle cells are longitudinally oriented with a centrally located nucleus and clear cytoplasm. The adventitia consists of coarse elastic fibers (el) arranged in a nearly circular fashion, thick bundles of collagen fibers (col) and vasa vasorum (arrow). HPS; X=250

Figure 35:
Photomicrograph of the outer portion of the media of a control femoral artery. The smooth muscle cells (sm) are oriented longitudinally (here seen in cross section). They have a central round nucleus and pale cytoplasm. Epon embedded tissue; Toluidine Blue; X=250
Figure 36: Low power survey electron micrograph of a control femoral artery. The subendothelial space is occupied by smooth muscle cells (SM) and small fragments of elastin (arrow-head). In the media, groups of slender smooth muscle cells (SM), arranged helicoidally, alternate with elastic lamellae. The elastic lamellae (EL) appear fragmented and are rather scarce. The interstitial spaces are occupied by compact bundles of collagen fibrils (COL) oriented circularly and longitudinally. X=8,000
Figure 37: Detail of the intima and inner media of a control femoral artery. The endothelial cells are seen at the top of the electron micrograph. The subendothelial space is here virtually non-existent. The internal elastic lamina (IEL) shows a gap which is occupied by an endothelial cytoplasmic process (arrow). The elastic lamellae (EL) are slender and show splitting and interruptions (arrow-heads). X=9,600
Figure 38: Electron micrograph of a smooth muscle cell located in the outer media of a control femoral artery. The smooth muscle cells in this area are longitudinally oriented and have centrally placed nuclei (N) with evenly dispersed chromatin. The nucleus is surrounded by a halo of cytoplasm containing few organelles other than a well developed Golgi complex (G). The myofilaments occupy the cell periphery and are arranged in a helicoidal fashion. X=35,000

Figure 39: Portion of the outer media and adventitia of a control femoral artery. A thick, well developed external elastic lamina (EEL) separates these two coats. The adventitia consists of thick elastic fibers (EL) separated by compact bundles of collagen (COL). X=9,200
**Figure 40:** Transverse section of a carotid artery dilated for one month. The intima shows diffuse intimal thickening caused by the presence of one to three layers of smooth muscle cells in the subendothelial space. The internal elastic lamina (iel) shows areas of attenuation and fragmentation. In the media, the innermost elastic lamellae appear fragmented (arrow-heads). Epon embedded tissue; Toluidine Blue; X=400

**Figure 41:** Transverse section of the area of maximal dilatation of a carotid artery three months following ligation. The internal elastic lamina (iel) is absent over large areas, and there is no distinct delineation between intima and media. There is fragmentation and paucity of the inner elastic lamellae (el). Epon embedded tissue; Toluidine Blue; X=400
**Figure 42:** Section of a carotid artery diluted for six months. The internal elastic lamina (iel) shows wide gaps. Delicate elastic strands have developed in the intima (arrow). The smooth muscle cells have altered polarity and the interstitial spaces are widened. The elastic lamellae are fragmented (el). Epon embedded tissue; Toluidine Blue; X=400

**Figure 43:** Section of a carotid artery diluted for six months. The internal elastic lamina is absent in large areas of the arterial circumference. The smooth muscle cells are randomly oriented. There is marked paucity of the elastic lamellae (el) and only small strands of elastic tissue remain scattered in the interstitial spaces (arrow-heads). Epon embedded tissue; Toluidine Blue; X=400
Figure 44: Electron micrograph of an area of the intima and innermost media from a carotid artery dilated for one month. The apposing plasma membranes of endothelial cells appear separated with the exception of the apical and basal points where the cells are still attached (arrow). Blebs (B) are created by detachment of the basement lamina (BL) of endothelial cells. The subendothelial space is occupied by smooth muscle cells (SM), basement lamina-like material and collagen fibrils (COL). The internal elastic lamina (IEL) has a fibrillar texture and scalloped borders. Gaps (arrow-heads) are often observed. In the media, the smooth muscle cells are separated by widened interstitial spaces containing elastic elements (EL) and collagen fibrils. Nexuses between smooth muscle cells are still present (circle). X=7,000
Figure 47: Detail of the media of a carotid artery one month after ligature. The elastic lamella appears split (EL) by penetration of collagen fibrils (COL) with resulting formation of lacunae containing microfibrils (arrows). X=15,000

Figure 48: Ruptured elastic lamella (EL) has a spongy appearance owing to the penetration of the elastic matrix by collagen fibrils (COL). X=11,000

Figure 49: Detail of a fragmented elastic lamella (EL). The gap between the borders is occupied by collagen fibrils and granulo-vesicular bodies (GVB) which are devoid of a limiting membrane and are closely apposed to the edges of fragmented elastica. X=25,000
Figure 50: Media of a carotid artery dilated for three months. Collagen fibrils (COL) align themselves parallel to the elastic fiber (EL); some of them fill the gap and seemingly fuse with the elastic matrix. X=34,000

Figure 51: Media as in fig. 50, showing a more advanced disruption of an elastic lamella (EL). The collagen (COL) fibrils are increased and randomly oriented. X=10,000
Figure 52: Detail of the intima in a carotid artery dilated for six months. Irregular masses of elastic substance are attached to the internal elastic lamina (IEL) and protrude into the subendothelial space. Note the proliferation of basement lamina (BL) from endothelial as well as from subendothelial smooth muscle cells (SM). In the inner media, collagen fibrils (COL) coalesce, resulting in the formation of electron dense masses (DM). X=13,000

Figure 53: Tissue as in Fig. 52. This electron micrograph depicts an irregularly outlined elastic lamella (EL). The staining properties of the elastic substance vary. Collagen fibers (arrow) pervade the elastic matrix. X=38,000
Figure 54: Media of a carotid artery six months after ligation. The fragmented elastic lamellae (EL) show uneven staining properties and frayed edges. The interstitial spaces are occupied by compact bundles of collagen fibers (COL) which lose their periodicity and aggregate to amorphous dense masses (DM). X=16,000
Figure 55: Portion of media of a carotid artery after six months of ligation. Dense masses of collagen aggregates (COL) devoid of their typical periodicity, partially occupy the interstitial spaces. Observe a granulovesicular body (GVB) in the smooth muscle cell (SM). X=31,800

Figure 56: Tissue as in Fig. 55. Sometimes the nature of the dense masses (DM) is not very apparent as they may represent elastic fragments fusing with collagen fibrils or aggregated structureless collagen fibrils. X=25,600
Figure 57: Photomontage of a transverse section of the carotid artery six months after ligation. The subendothelial space is occupied by smooth muscle cells (SM), fragments and strands of elastic substance (arrow), and collagen fibrils. The internal elastic lamina (IEL) has an irregular inner outline caused by fusion of irregular masses of elastic substance. Large gaps partially occupied by smooth muscle cells (SM) communicate with the intima and the media. The interstitial spaces in the media appear widened and contain randomly oriented bundles of collagen fibrils (COL) and fragmented elastic lamellae (EL). Occasionally, lipid droplets (L) may be seen in the smooth muscle cells. X=5,900
**Figure 58:** Portion of two medial smooth muscle cells (SM) of a carotid artery after three months of ligation, showing collagen fibrils (COL) aligned in a parallel array occupying the widened intercellular space. The collagen fibrils seem attached to the cell borders (arrow-heads). X=15,000

**Figure 59:** Detail of a desmosome-like cellular junction between two smooth muscle cells (SM) of a carotid artery after three months of ligation. There is widening of the intercellular space which is largely occupied by collagen fibrils (COL) which are seemingly anchored at the cellular borders. X=50,500
**Figure 60:** Detail of a medial smooth muscle cell in a carotid artery six months after ligation. Part of the cytoplasm is occupied by myelin figures (MF) and lipid droplets (L). X=6,800

**Figure 61:** Portion of an adventitial vasa vasorum in a carotid artery six months after ligation. The endothelial cells have abundant cytoplasm containing patches of filaments (arrows) similar to those of smooth muscle cells (SM). The subendothelial space is occupied by proliferating basement lamina (BL) and newly formed elastic elements (NEL). X=7,000
Figure 62:
Transverse section of the area of maximal dilatation of a femoral artery one month after ligation. There is reduplication of the internal elastic lamina and fragmentation of the medial elastic lamellae (el). Epon embedded tissue; Toluidine Blue; X=250

Figure 63:
Transverse section of a femoral artery three months after ligation. There is a cushion-like area of intimal proliferation above a large gap of the internal elastic lamina (arrow-heads). Note the paucity of elastic lamellae (el). Epon embedded tissue; Toluidine Blue; X=250

Figure 64:
Transverse section of a femoral artery six months after ligation. The internal elastic lamina is absent in large areas and is being replaced by delicate and interrupted elastic strands (arrows). In the media, the interstitial spaces appear widened and the smooth muscle cells have altered polarity. The elastic lamellae (el), especially in the inner third, are largely absent and only fragments remain in the interstices. Epon embedded tissue; Toluidine Blue; X=400
Figure 65: Intima of a femoral artery one month after ligation. The subendothelial space is occupied by leukocytes (LK) and smooth muscle cells (SM). The basement lamina (BL) of the endothelial cells (EN) appears to be proliferating. X=6,500

Figure 66: Femoral artery, one month after ligation. The intima is thickened by the presence of smooth muscle cells (SM), bundles of collagen fibrils (COL) and fragments of elastic material (arrows). The internal elastic lamina (IEL) is fragmented and shows variable staining properties. In the media, the elastic lamellae are fragmented with only small strands remaining (EL). The interstitial spaces are largely occupied by collagen bundles (COL). X=5,000
**Figure 67:** Low power survey electron micrograph of a transverse section of a femoral artery three months after ligation. The internal elastic lamina (IEL) has irregular outlines with masses of elastic substance protruding into the subendothelial space (arrow). In the media, the interstitial spaces appear widened and contain an increased amount of collagen (COL) and electron dense amorphous masses, representing fragments of elastic lamellae (EL) and aggregates of collagen fibrils (DM). X=5,800

**Figure 68:** Femoral artery three months after ligation. Intercellular space between two smooth muscle cells showing fragments of elastic lamellae (EL) and aggregates of collagen fibrils, without their normal periodicity (DM). Note a granulo-vesicular body (GVB) closely apposed to a fragment of elastic substance. X=27,500
Figure 69:
Femoral artery three months after ligation. A dense mass (DM) probably representing aggregated structureless collagen fibrils. Note two granulo-vesicular bodies (GVB) among the collagen fibrils. X=31,800

Figure 70:
Femoral artery three months after ligation. Interstitial space between two smooth muscle cells showing the appearance of a fragment of an elastic lamella (EL) and a dense mass probably collagenous in nature (DM). X=15,000

Figure 71:
Portion of the media of a femoral artery six months after ligation. The paucity of elastic lamellae is noticeable and only dense masses (DM) of aggregates of collagen and fragmented elastic elements remain in the interstitial spaces. X=4,900
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