Acute hypertension was produced in rats by the infusion of angiotensin amide for 2 to 4 hours. These animals were injected intravenously prior to sacrifice with either colloidal carbon or iron dextran particles. The mesenteric vessels from hypertensive and control animals were processed for electron microscopy. Ultrastructural alterations are found in dilated segments of small arteries. Initially there is severe contraction of medial smooth muscle cells and the formation of processes of smooth muscle cytoplasm. This is followed by lysis of cell processes and bodies, and passage of plasma and colloidal iron into the media. Subsequently, carbon, platelets, fibrin and cellular debris are seen within these foci of medial necrosis. These changes appear as a sequence whose severity reflects the duration of the angiotensin infusion and degree of elevation of the systolic pressure. The morphologic alterations are discussed in relation to the generalized increase in vascular permeability that is associated with the hypertensive state (Am J Pathol 72:221–240, 1973).

CONSIDERABLE LITERATURE is now available concerning the ultrastructure of the vascular lesions in both human and experimental hypertension. Additional studies have characterized the increase in vascular permeability that is a continuous and progressive development of the hypertensive state. The increase in the permeability of arterial vessels differs in magnitude in various regions and occurs in arteries that exhibit both endothelial and medial alterations of variable extent. Most of these studies have tended to describe advanced rather than early lesions.

Definition of the early vascular alterations in most models of experimental hypertension is difficult to establish, since the onset of the hypertensive state and height of blood pressure rise are variable and the timing of vascular changes is unpredictable. The acute hypertension that follows the infusion of angiotensin in the rat offers several advantages in this regard. The elevation in blood pressure is rapid in on-
set and occurs with a minimum of operative manipulation. Both morphologic and permeability alterations appear to occur in a more uniform sequence.

The ultrastructure and permeability of mesenteric vessels from rats with acute hypertension due to angiotensin administration have been examined in the present study. Morphologic changes in the walls of these vessels are described. Vascular labeling technics utilizing colloidal particles disclose an increase in the permeability of the mesenteric vasculature that is a consequence of necrosis of medial smooth muscle cells.

Materials and Methods

Fifteen male Holtzman rats weighing 200 to 300 g were used in these experiments. All animals were anesthetized with intramuscular injections of Nembutal® (sodium pentobarbital, Abbott Laboratories, North Chicago, Ill), 4 mg/100 g body weight.

Hypertensive Animals

Acute hypertension was produced in 9 Holtzman rats by the constant infusion of synthetic angiotensin amide (Hypertensin®, Ciba Pharmaceutical Co, Summit, Nj) into the femoral vein for 2 to 4 hours. The angiotensin was dissolved in normal saline (33 μg/ml) and infused at a rate of 1.7 μg/min (ie, 0.31 ml/100 g/hr). One hour prior to sacrifice, 6 animals received an intravenous injection of a carbon suspension (Pelikan C11/1431a, Gunther Wagner Co, Hanover, Germany) containing approximately 100 mg of carbon/ml in a dose of 0.1 ml/100 g body weight, as a vascular label. 22 Three animals received Imferon® (iron dextran, Lakeside Laboratories Inc, Milwaukee, Wisc) 15 minutes before sacrifice, in the amount of 1 ml/100 g body weight, as another vascular label.

Upon termination of the infusions and injection of colloidal particles, 30 ml of a formaldehyde-glutaraldehyde mixture 23 was injected into the peritoneal cavity of each animal. Twenty minutes later the peritoneal cavity was opened, the mesentery excised and loops of small intestine with attached mesentery pinned flat to dental wax and immersed in fresh formaldehyde-glutaraldehyde for 3 to 5 hours at room temperature.

Under a dissecting microscope, mesenteric vessels on the surface of the intestine were cut into strips parallel to their longitudinal axis; care was taken to include vessels labeled with carbon. The strips of tissue were then washed several times in 0.1 M cacodylate buffer (pH 7.6) and stored overnight at 4 C. The specimens were subsequently fixed in cacodylate-buffered (pH 7.6) 1% osmium tetroxide, dehydrated in acetone and embedded in Araldite.

Sections (2 μ thick) were cut from the plastic-embedded specimens and examined under the phase contrast microscope to locate mesenteric vessels, as well as to assess the presence of vascular labeling by carbon. Thin sections of selected areas were then cut with diamond knives, stained with uranyl acetate and lead citrate, and examined with a Philips EM 200 electron microscope. To detect iron particles more easily, some sections from animals receiving Imferon were also examined in the electron microscope without staining.
Controls

Six animals served as controls. Two rats were infused with saline for 3 and 4 hours, respectively, at the same rate as in rats given angiotensin and then were injected intravenously with colloidal carbon 1 hour prior to sacrifice. Two animals received the constant infusion of saline for 2% and 3 hours, respectively. They were injected intravenously with Imferon 15 minutes prior to sacrifice. Another animal was injected intravenously with colloidal carbon 1 hour before sacrifice. Blocks of mesenteric vessels from these animals were processed as described previously.

The last animal did not receive any injections and was used to define the ultrastructure of the endothelial cell junctions of mesenteric arteries. Blocks of mesenteric arteries from this animal were rapidly excised, fixed and processed for uranyl acetate staining en bloc and lanthanum staining.

Uranyl Acetate Staining En Bloc

The specimens were fixed in a formaldehyde-glutaraldehyde mixture buffered with 0.1 M cacodylate (pH 7.4) for 3 hours, washed with buffer and then postfixed in 1% OsO₄ buffered with s-collidine (pH 7.4) for 2 hours. The blocks were subsequently washed in cold 0.05 M sodium hydrogen maleate-NaOH buffer (pH 5.2). They were then placed in the dark in 0.5% uranyl acetate dissolved in sodium hydrogen maleate-NaOH buffer (pH 6.0) at 4°C. After 2 hours, the specimens were washed in three changes of maleate buffer, dehydrated in acetone and embedded in Araldite.

Lanthanum Staining

The specimens were fixed in a formaldehyde-glutaraldehyde mixture containing a 1% solution of neutralized lanthanum nitrate for 3 hours, washed in cacodylate buffer containing lanthanum and postfixed in 1% OsO₄ buffered with s-collidine containing lanthanum for 2 hours. After fixation the tissues were rapidly dehydrated in acetone and embedded in Araldite. Section from uranyl acetate- and lanthanum-treated tissues were stained solely with lead citrate prior to electron microscopic viewing.

Systolic blood pressures were monitored both prior to and at intervals during the course of the infusions by an indirect method described previously. The systolic pressure in control animals was usually in the range of 75 to 115 mmHg and never exceeded 120 mmHg. Maximal systolic pressure in the rats infused with angiotensin was 175 mmHg and generally ranged between 135 to 160 mmHg.

Results

Light Microscopic Observations

There was no evidence of vascular leakage in control animals injected with carbon. An occasional mesenteric artery from these animals exhibited an area of constriction under the dissecting microscope. Animals infused with angiotensin exhibited alternating zones of constriction and dilatation in the arteries adjacent to the mesenteric border of the small intestine. Some of these dilated segments were labeled with
carbon. In addition, smaller vessels labeled with carbon were visible through the serosa. Phase contrast examination of thick sections revealed that the labeled arteries (about 40 to 100 μ in diameter) were located in the submucosa of the small intestine. In longitudinal section, the smooth muscle cells of small arteries from animals receiving angiotensin exhibited scalloping of their adventitial surface.

**Electron Microscopic Observations**

The ultrastructure of small mesenteric arteries generally conformed with previous descriptions of other small arteries. The media of arteries near the mesenteric border of the intestine consisted of two layers of smooth muscle cells, while the media of more distal arteries contained only a single layer of smooth muscle (Figure 1). The luminal portions of endothelial cells frequently exhibited sarcomere-like arrays of filaments that tended to align parallel to the long axis of the vessels. The individual filaments measured about 60 to 70 Å in diameter. Electron-dense bands of material measuring about 1000 Å in width and varying from two to eighteen in number were distributed along the filament bundles at a regular spacing of approximately 0.5 μ. The endothelial cell junctions generally exhibited a convoluted course from luminal to abluminal aspects of the cells. The width of the intercellular clefts measured 100 to 150 Å, except at specific loci near the lumen, where adjacent cell membranes were in closer apposition and delimited a gap of about 30 to 40 Å (Figure 2). Lanthanum filled the luminal portions of endothelial clefts, permeated the gap junctions and extended through the abluminal segments of the clefts to the adjacent basal lamina (Figure 3).

Particles of colloidal carbon were not found within the walls of small arteries, capillaries or venules of control animals. However, particles of Imferon, with electron-dense cores measuring about 25 Å in diameter, were seen within pinocytotic vesicles of the endothelium as well as in the subendothelial space of mesenteric arteries (Figure 4). On the other hand, iron particles were not found within endothelial intercellular junctions. A rare arterial smooth muscle or endothelial cell in animals receiving saline contained a large vacuole with granular contents measuring up to 3 μ in diameter; some of these had indented adjacent cell nuclei.

**Hypertensive Animals**

Ultrastructural alterations were most prominent in small arteries. These changes appeared as a sequence whose severity generally reflected
the duration of the angiotensin infusion and the degree of elevation of the systolic pressure. Initially there was severe contraction of medial smooth muscle cells and the formation of processes of smooth muscle cytoplasm. This was followed by lysis of the cell processes and bodies, and passage of plasma and colloidal iron into the media. Subsequently, carbon, platelets, fibrin and cellular debris were seen within these foci of medial necrosis.

Contraction of medial smooth muscle cells was manifested by a decrease in the size of the cells relative to the longitudinal axis of the vessels (Figure 5). In addition, there was a change in the orientation of smooth muscle cell nuclei, numerous nuclear indentations (Figure 5), striking prominence of attachment devices and irregularity of the adventitial contours of these cells (Figures 5 and 6). Subsequently, smooth muscle cells detached from their limiting basement membranes; the adjacent cells remained in contact only in the region of the nexi (Figure 7). Slender elongated processes of smooth muscle cytoplasm then projected from the main cell bodies (Figures 6 and 7). The resultant spaces within the media exhibited layers of basement membrane-like material that were irregularly applied to the surfaces of the cell bodies (Figure 7).

The next stage was characterized by lysis of the cell bodies and their processes (Figures 7–10). Irregularly shaped smooth muscle fragments of variable size and lacking the usual complement of cytoplasmic organelles and filaments occupied the space delimited by the original smooth muscle cell basement membrane. These foci of cytolysis contained granular material resembling plasma (Figure 10). Aggregates of iron particles, but not carbon, were also present within these lesions as well as in the adjacent adventitia. The lytic process was limited to single cells (Figures 8–10) initially but then extended to involve adjacent medial smooth muscle cells (Figure 11).

The most severe lesions exhibited separation of endothelial cell junctions (Figure 12) and accumulation of platelets, fibrillar material occasionally exhibiting the 240 Å periodicity of fibrin, particles of iron and carbon (about 250 Å in diameter) and plasma within the media (Figures 13 and 14). Cytoplasmic fragments containing concentric whorls of membranes and a variety of cellular debris were also present. The accumulation of these elements within the media resulted in a bulging outwards of involved portions of the vessels.

The medial foci containing plasma constituents and colloidal particles were found in dilated segments of mesenteric arteries. Colloidal particles
of carbon were also found in gaps between the endothelial cells of venules as well as between the endothelial and perivascular cells of venules and continuous capillaries. Carbon particles were also seen, rarely, in the lumina of adjacent lymphatic capillaries.

Discussion

The renin-angiotensin system plays a central role in the regulation of blood pressure. Giese originally showed that infusion of angiotensin in rats results in both an acute elevation of blood pressure and the development of alternating segments of constriction and dilatation in mesenteric arteries.²⁷ He further demonstrated that the dilated segments of the mesenteric arterial tree exhibit increased permeability, as manifested by the intramural accumulation of intravenously injected carbon particles.²⁸ Chronic hypertension and widespread thickening of arterioles can result from repeated infusions of angiotensin.²⁹ More recently the degree of carbon deposition within the mesenteric arteries following angiotensin infusion has been shown to be a function of the mean arterial pressure.³⁰ and a consequence of necrosis of endothelial cells.²¹ The subsequent passage of plasma, fibrinogen and platelets into the media purportedly destroys the smooth muscle cells and leads to the accumulation of intramural fibrin.

The severe lesions noted in the present study in the mesenteric vasculature are essentially comparable to alterations recently described by Goldby and Beilin following angiotensin infusion.²¹ However, in the present investigation the primary lesion following the infusion of angiotensin seems to be necrosis of smooth muscle cells rather than endothelial cell damage. The passage of plasma, fibrinogen, platelets and colloidal particles into the media appears to be a secondary event. Furthermore, the increase in vascular permeability appears to be due to separation of endothelial cell junctions rather than to necrosis of endothelium.

Foci of medial degeneration have been observed previously by electron microscopy in different sized arteries during the hypertensive state.³,6,8,9,11–14,20,3¹,3² The suggestion that areas of smooth muscle cell necrosis represent loci minoris resistentiae,²⁰ where impaired contractility together with increased intraluminal pressure can result in a breach of blood-vascular barriers, is confirmed by the present investigation. The accumulation of plasma, colloidal particles, platelets and fibrin follows the focal degeneration of smooth muscle cells. The end result is segmental dilatation of the vasculature, the final diameter of
the vessel being determined by the limiting effects of its connective tissue constituents and surrounding adventitia.

The mechanisms responsible for the medial necrosis are not known. Angiotensin acts directly on the vascular wall, binding to the nuclei of endothelial and smooth muscle cells or endothelial surface membranes. Specific angiotensin receptors have also been demonstrated in a microsomal fraction of smooth muscle, and displacement of calcium ions from microsomal particles follows angiotensin-receptor interaction. The resultant increase in calcium concentration may account for the contraction of myofilaments. Morphologic evidence in the present study showed that the smooth muscle cells are strongly contracted. The smooth muscle necrosis may be due to sustained contraction beyond the metabolic capability of these cells. Treatment with vasodilator and/or vasoconstrictor agents can also produce necrosis of medial smooth muscle cells in arterial vessels.

Separation of endothelial cell junctions of femoral and coronary arteries as well as an increase in vascular permeability of coronary arteries and aorta have been described previously following the injection of angiotensin. The cross-striated arrays of filaments found in the endothelial cells of mesenteric arteries are identical to filament arrays observed in the endothelium lining cerebral cortical and retinal arterial vessels. It has been suggested that such filament arrays could represent the structural templates for endothelial contraction that in conjunction with increased hydrostatic pressure may lead to separation of endothelial cell junctions.

The magnitude of the change in the permeability of vessels in different organs in hypertension may be due to differences in the ultrastructure of their endothelial cell junctions. As shown by the present study, the endothelium lining mesenteric arteries has gap junctions, while the endothelium of cerebral cortical and retinal arteries has tight junctions. Thus, the similar structure of endothelial cell junctions that is shared by cerebral and retinal arteries and capillaries also holds true for visceral vessels, the endothelium of visceral arteries, in addition to that of continuous capillaries, possessing gap junctions. It seems possible that this type of junction is more readily separated by a comparable force than tight junctions, thereby explaining the greater permeability of visceral arteries over those in brain and retina in hypertension.

Iron dextran particles are observed in pinocytotic vesicles of the endothelium, extracellularly in the subendothelial space, but not within
the endothelial cell junctions of arteries of control animals. The absence of intercellular passage of iron particles measuring about 25 Å in diameter (exclusive of the dextran shell) is in keeping with the observed width (30 to 40 Å) of the gap junctions between endothelial cells. Iron particles are found not only in the subendothelial space but also in the media and adventitia of angiotensin-treated animals. These deeper intramural aggregates may be due to an increase in vesicular transport across the endothelium in acute hypertension. However, the presence of interendothelial separations precludes definitive evaluation of the role of vesicular transport, since the particles may also have gained entrance to the vessel walls through open junctions. Increased vesicular transport across endothelium has been described in aorta, cerebral and mesenteric arteries in experimental hypertension, as well as following the injection of angiotensin. In any case, systematic investigation of the role of vesicular transport in the generalized increase in vascular permeability that accompanies the hypertensive state remains to be accomplished.

There is general agreement that carbon particles do not ordinarily traverse vascular endothelium. In the present study, carbon particles have been found in vessels of hypertensive animals with advanced lesions, while intramural aggregates of iron are seen in earlier lesions. In this regard, the accumulation of colloidal iron within the walls of visceral vessels of rats with malignant hypertension has been shown to occur before the accumulation of carbon particles and to be associated with less severe alterations of the vasculature.

The increase in permeability of mesenteric arterial vessels due to angiotensin treatment is accompanied by an increase in the permeability of mesenteric venules and capillaries. This is manifested by the presence of aggregates of colloidal carbon within open endothelial cell junctions as well as between endothelial and perivascular cells. The increased permeability of arteries, capillaries and venules can explain the increased flow of thoracic duct lymph produced in rats by the administration of angiotensin. Impaired contractility of the arteries due to necrosis of medial smooth muscle cells and increased intraluminal pressure could expose capillaries and venules to abnormally high pressures and result in the altered permeability. The endothelium of venules from angiotensin-treated rats is also permeable to circulating fluorescent proteins. Intradermal injection of angiotensin II in rabbits is associated with an increase in the permeability of skin capillaries that is not inhibited by antihistaminics.
The large vacuoles seen in the endothelium and smooth muscle cells of both control and angiotensin-treated rats have previously been described in normal vascular smooth muscle, as well as in a variety of experimental situations. These vacuoles are thought to represent swollen cell processes at junctional areas between smooth muscle cells as well as between endothelial and smooth muscle cells. The swelling of these contacts has been ascribed to anoxia or to a sudden increase in the diffusion of electrolytes and water through vessel walls as in angiotensin-induced hypertension. In the present study such vacuoles could be the result of relative anoxia from overcontraction and/or increased permeability to fluid and solutes.

References


Acknowledgments

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Legends for Figures

Fig 1—Electron micrograph of longitudinal section of mesenteric artery from normal animal injected with carbon. The endothelium contains sarcomere-like arrays of filaments. Electron-dense bands of material are distributed along the filament bundles at a spacing of about 0.5 \( \mu \). The long axis of the nucleus of the smooth muscle cell is oriented along the length of the vessel. Compare with Figure 5. \( L = \) lumen (\( \times 16,000 \)).

Fig 2—Endothelial cell junction of mesenteric artery from normal animal stained en bloc with uranyl acetate. The intercellular cleft measures 100 to 150 \( \AA \) in width, except for a constricted area (*) where the apposed membranes are separated by a gap of about 40 \( \AA \). \( L = \) lumen (\( \times 80,000 \)). Inset—The constricted area in the intercellular cleft shown at higher magnification (\( \times 250,000 \)).

Fig 3—Mesenteric artery from a normal rat. Lanthanum permeates the entire cleft between endothelial cells, including the constricted area (arrows) comprising the gap junction. There is also filling of the adjacent subendothelial space. \( L = \) lumen (\( \times 61,000 \)).
Fig 4—Electron micrograph of mesenteric artery from animal infused with saline for 3 hours and injected with Imferon. Iron dextran particles are seen within pinocytotic vesicles of the endothelium (arrows). Many colloidal particles of iron are also present in the subendothelial space and lumen of this blood vessel. L=lumen (x 70,000).

Figures 5–14 are electron micrographs of mesenteric arteries from animals infused with angiotensin for 2 to 4 hours.

Fig 5—Longitudinal section of artery. Severe contraction of the medial smooth muscle cells is manifested by a decrease in the size of the cells relative to the long axis of the vessel, indentation and change in the orientation of the nuclei (lower left corner), prominent attachment devices and scalloping of the adventitial surface (x 5000).
Fig 6—The adventitial surface of this smooth muscle cell is irregular. The body of the cell is contracted, exhibits several attachment devices and is delimited by reduplicated layers of basement membrane-like material. Numerous smooth muscle cell processes (double arrows) are seen at the periphery of this cell. \( L = \) lumen \((x\ 16,000)\).

Fig 7—Similar to Figure 6. Note the region of contact between adjacent cell processes (arrows). Some of the cell processes (\( P \)) exhibit lytic changes. \( L = \) lumen \((x\ 16,000)\).
Fig 8—A portion of a smooth muscle cell (SMC) lacking the usual complement of cytoplasmic filaments. A few mitochondria and remnants of the sarcoplasmic reticulum, as well as a lipid droplet (D), are contained within the process. IEL=internal elastic lamina (× 13,000).

Fig 9—Swollen mitochondria and glycogen granules are seen within a SMC undergoing lysis (× 22,000).

Fig 10—Single cell necrosis in the media of small artery. Granular material resembling plasma and iron particles (circles) are enclosed by the original SMC basement membrane. Colloidal particles are also present in the adventitia and lumen (L). The endothelium contains cross-striated arrays of filaments (× 13,000).
Fig 11—More extensive necrosis of media of small artery. L = lumen (× 13,000). Fig 12—Separation of endothelial cell junction (arrows) in an area with severe medial lesions (× 17,000).
Fig 13—Survey micrograph of vessel with foci of necrosis of varying extent. The lesion in the lower right corner involves an area previously occupied by several smooth muscle cells, protrudes into the adventitia and is shown at higher magnification in Figure 14 (× 2000).

Fig 14—The media contains granular material resembling plasma, fibrin (F) and a variety of cellular debris. (× 9000). Inset—The fibrillar material displays the usual periodicity of fibrin (230 to 240 Å) (× 43,000).