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Light and Electron Microscopic Studies on Rat Arterial Lesions Induced by Experimental Arterial Contraction

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Summary. Experimental contraction was produced in the rat mesenteric arteries and the arterial segments were studied morphologically. When the rat mesenteric artery was exposed in physiological saline solution at 37° C and 2–3 mg of methoxamine hydrochloride (10 mg/ml) was dripped onto it, intense contraction was observed for about 30 min but elevation in blood pressure was slight. During the contraction, numerous vacuoles were seen in the medial smooth muscle cells of the arterial segments, and these vacuoles were shown electron microscopically to have double unit membranes, indicating that they were formed by herniation of a part of the adjacent smooth muscle cell body. In the arteries 1–6 h after the end of the contraction, cellular, nuclear and vacuolar membranes and myofilaments of the medial muscle cells were partially lost. 12–24 h after the contraction the arteries exhibited necrosis and desquamation of endothelial cells and platelet adhesion. In the media, smooth muscle cells were completely deprived of cell membranes, myofilaments, nuclei, intracytoplasmic organelles other than mitochondria, and vacuolar membranes. The cytoplasm was filled with fine granular and granulo-vesicular material, and fibrin insudation was observed in these severely damaged cells. Arterial contraction may be an important factor in the induction of arterial lesions.

Key words: Arterial contraction – Herniation – Methoxamine hydrochloride – Smooth muscle cell necrosis – Vacuole.

Introduction

It has been reported that medial necrosis of artery is produced by vasoconstrictor such as methoxamine (Herbertson and Kellaway, 1960; Giese, 1964), noradrena-

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rin (Giese, 1964), angiotensin (Byrom, 1964; Wiener and Giacomelli, 1973). In some of these studies, arterial contraction was also observed besides hypertension and it was suggested that not only hypertension, but also arterial contraction may be a cause of medial necrosis (Byrom, 1964; Wiener and Giacomelli, 1973). In these experiments, however, vasoconstrictor was usually given not topically but systemically and as a result hypertension would have been inevitably produced. The present study aimed to observe arterial lesions induced by arterial contraction. For this purpose, arterial contraction was produced topically in rat without inducing hypertension and the contracted arterial segments were investigated by light and electron microscopy.

Materials and Methods

Sixty one normotensive male Wistar rats, weighing 150–250 g, were laparotomized under anesthesia induced by intramuscular injection of 1 ml/100 g of a 10% urethane. The mesenteric arteries were exposed in physiological saline solution at 37° C in a watch-glass. In 51 rats, 2–3 mg of methoxamine hydrochloride (10 mg/ml) was dripped on one of the exposed arteries in order that its contraction could be observed by a stereomicroscope. In five of these rats, the contracted artery was rapidly frozen with isopentane which was refrigerated with liquefied nitrogen in order to observe the size of the artery and in ten of them, blood pressure was messured by a direct method. During the contraction and at 1–6 h and 12–24 h after the end of the contraction, thirty six rats were sacrificed, and the contracted arterial segments were studied by light and electron microscopy. The remaining ten rats were controls which were treated in the same way as the experimental group without the application of methoxamine hydrochloride. In three of these rats, the artery was rapidly frozen with isopentane refrigerated with liquified nitrogen, and in seven of them, the artery was studied by light and electron microscopy.

Preparation of Frozen Samples. Isopentane refrigerated to −170° C with liquefied nitrogen was poured on the mesenteric artery which was thus frozen rapidly. The frozen artery was removed, minced, and fixed in a 1% osmium tetroxide-acetone solution at −80° C for one week. After washing 3 times with acetone at −80° C, the arterial segment was restored to room temperature and deacetonated and embedded in Epon 812. Sections 3-μ-thick were prepared and stained with toluidine blue for light microscopy.

Blood Pressure Measuring. Blood pressure was measured continuously by direct method. A polyethylene tube filled with a heparin-saline solution was inserted into the carotid artery, and the other end was connected with a glass tube filled with mercury. The blood pressure was recorded at 5 min intervals for 30 min before and 1 h after methoxamine hydrochloride administration. The mean blood pressure of ten animals was calculated at each time for statistical study.

Light Microscopy. The mesenteric arteries were fixed by perfusion with 10% buffered neutral formalin solution, injected from the abdominal aorta at pressure of 100 to 120 mm Hg. The arteries were then removed and further fixed in the same solution. After dehydration, serial paraffin sections were prepared from the arteries, and stained with haematoxylin and eosin and Weigert's elastic stains for light microscopy.

Electron Microscopy. Caulfield's fixative (Caulfield, 1957) was perfused into the abdominal aorta at pressure of 100 to 120 mm Hg in order to fix the mesenteric arteries. These were then removed and cut into small pieces. After 1.5 h refixation in the same fixative, they were dehydrated and embedded in Epon 812. Ultrathin sections were prepared and doubly stained with uranyl acetate and lead citrate for electron microscopy.

Results

Stereomicroscopy. Mesenteric arteries exposed in physiological saline showed a uniform thickness and active pulsation (Fig. 1a). When methoxamine hydrochloride was dripped onto the arteries, intense contraction took place (Fig. 1b). Most arteries regained their former dimensions after about 30 min of contraction.

Findings in Frozen Samples. In the mesenteric artery of control rats, the lumen was well distended and the internal elastic lamina was extended without showing any corrugation (Fig. 2a). During the contraction, however, the arterial lumen was extremely constricted to about $^1/_4$ in diameter compared with that of the control rat (Fig. 2b). Internal elastic lamina was corrugated and endothelial cells were protruded into the luminal side. The arterial wall was thickened to about 3 times that of the control (Fig. 2b).

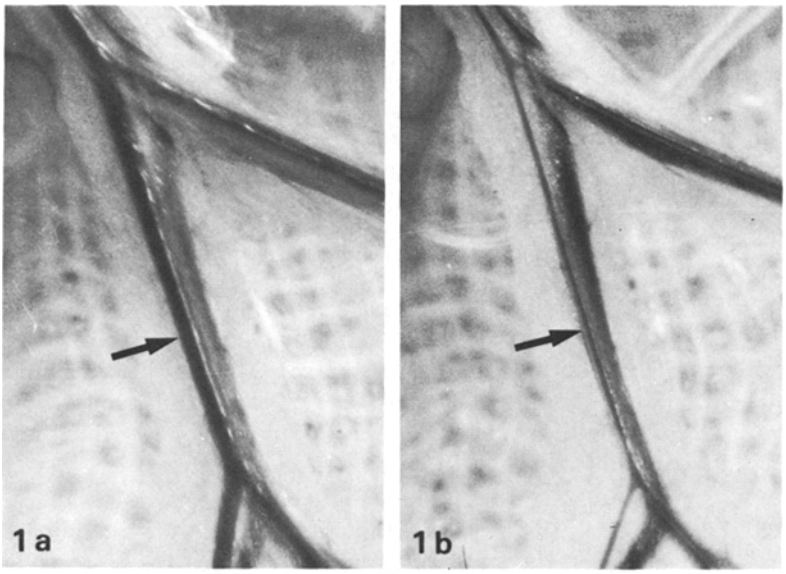
Blood Pressure. During 30 min before methoxamine hydrochloride administration, means of blood pressure at 5 min intervals were stable ranging 94.5–95.6 mm Hg (Fig. 3). During the first 30 min after the administration, the means of blood pressure at 5 min intervals ranged 96.3–99.6 mm Hg (Fig. 3). These were higher than the initial ones in statistical significance ($P < 0.05$) and the maximal rise was 5.1 mm Hg. During a period of 30–60 min after the administration, the means at 5 min intervals ranged 92.1–95.1 mm Hg (Fig. 3), and these were significantly lower than during the first 30 min after the administration ($P < 0.05$), but not significantly different from the pre-administration levels ($P > 0.1$).

Light Microscopy. In control rats the mesenteric arteries were distended and a one-layered endothelium was observed, closely attached to the internal elastic lamina. In the media, smooth muscle cells were arranged in 5–6 layers and their cytoplasm was uniformly stained with eosin but did not contain vacuoles.

During contraction, endothelial cells arranged in a single layer protruded into the narrowed arterial lumen and the internal elastic lamina was corrugated (Fig. 4). In the media, smooth muscle cells were contracted and often contained elliptic or egg-shaped vacuoles (Fig. 4).

1–6 h after the end of the contraction, endothelial cells were flattened and found to be closely attached to the gently undulating internal elastic lamina. Medial smooth muscle cells were fusiform and their cytoplasm and nuclei were well preserved, with intracytoplasmic vacuoles observed as are seen during contraction.

In arterial segments showing mild damage, after 12–24 h contraction the endothelium was well preserved, being closely attached to the internal elastic lamina. In the media, faintly eosinophlic smooth muscle cells were seen and vacuoles were not evident in them. The nuclei of smooth muscle cells were decreased in number, and those remaining showed low affinity for stains. In the segments with severe damage the endothelium exhibited desquamation with



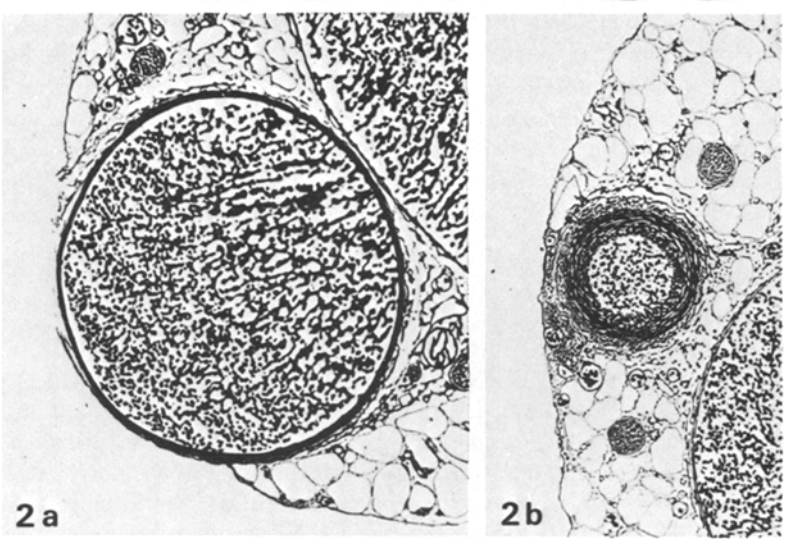


Fig. 1a and b. Mesenteric artery before and after the application of methoxamine hydrochloride. **a** Uniform vascular thickness (*arrow*) before the application. **b** Intense contraction through the whole length of artery (*arrow*) at 11 min after the application

Fig. 2a and b. Frozen sample of mesenteric artery. **a** Control artery. **b** Artery during contraction. Its caliber is $^1/_4$ that of control and its wall is about 3 times thick. ×100

platelets adherence (Fig. 5). The arterial lumen was distended and the internal elastic lamina extended, without fragmentation or lytic change. The media was uniformly and intensely stained with eosin, and nuclei of the smooth muscle cells were completely lost (Fig. 5). The external elastic lamina was elongated and often fragmented. The adventitia was oedematous and infiltrated by neutrophils, plasma cells and lymphocytes.

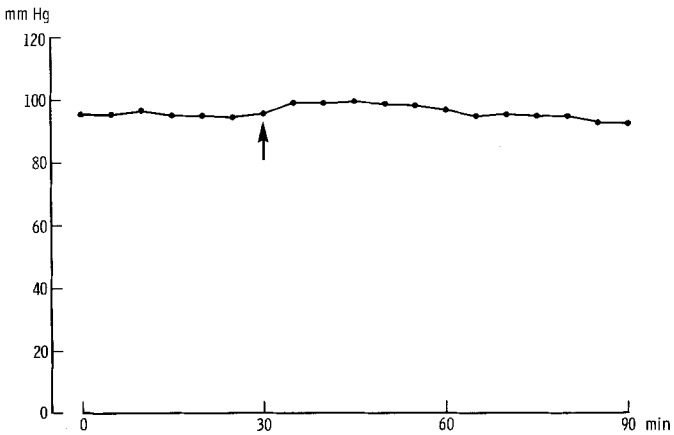


Fig. 3. Mean blood pressures of 10 rats before and after methoxamine hydrochloride application at 5 min intervals. An arrow indicates the time of the application

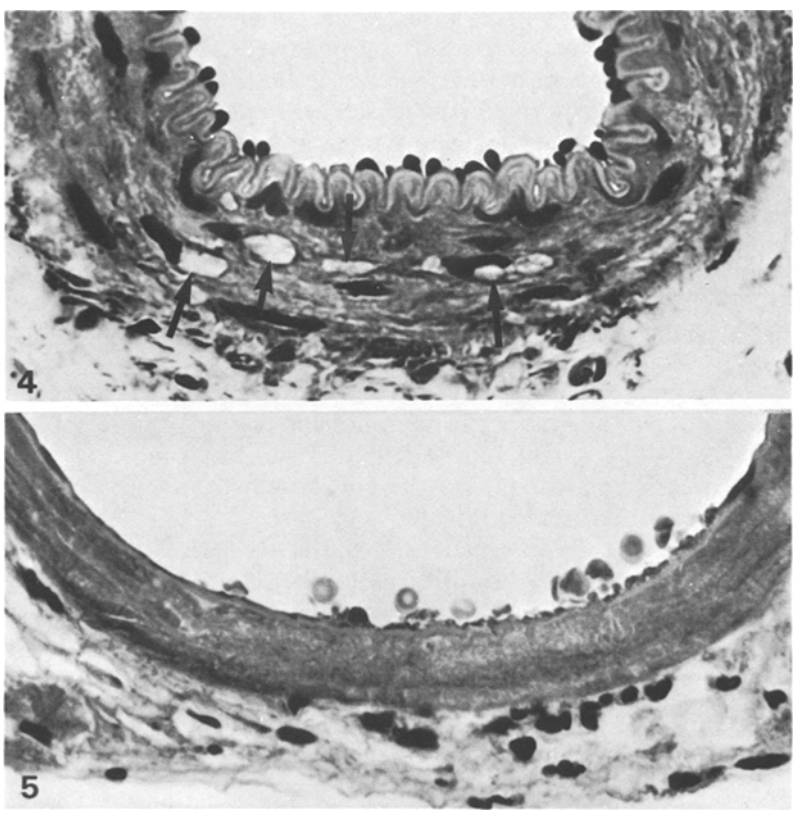


Fig. 4. Mesenteric artery during contraction. Internal elastic lamina is markedly undulated, and many vacuoles (*arrows*) are seen in medial smooth muscle cells. Hematoxylin and eosin stain. ×400

Fig. 5. Mesenteric artery at 24 h after the end of contraction. Endothelium shows partial desquamation, where platelets are adherent. Media has undergone necrosis, being uniformly stained with eosin, and the nuclei of the muscle cells are lost. Hematoxylin and eosin stain. ×400

Electron Microscopy. In control rats the endothelial cells contained mitochondria, Golgi apparatus, rough-surfaced endoplasmic reticulum and numerous pinocytotic vesicles and the nuclei were slightly indented. Neither opening of endothelial cell junctions nor enlargement of the subendothelial space was found. Medial smooth muscle cells contained numerous myofilaments, dense bodies, dense attachments and many pinocytotic vesicles and the nuclei were slightly indented. The medial smooth muscle cells were surrounded with basement membrane which was often partly defective. Where the basement membrane was not evident adjacent smooth muscle cell membranes were close to each other, forming double unit membranes (Fig. 6). Vacuole formation was rarely seen in smooth muscle cells.

In contracted arteries, the nuclei of the endothelial cells were markedly indented and cell organelles were well preserved (Fig. 7). The subendothelial space was often enlarged and fine granular or amorphous debris and vacuoles were seen there (Fig. 7). Junctions between endothelial cells did not open and the boundaries of medial smooth muscle cells were serrated with marked indentation and protrusion but with well preserved cell membranes. In the cytoplasm numerous straight myofilaments were seen, well preserved cell organelles were also present. Mitochondria were mostly found near both poles of the nuclei (Fig. 8) which were markedly indented. The muscle cells often contained double unit membrane-bounded vacuoles in their cytoplasm, which were filled with fine granular or filamentous material associated with ribosomes and vesicles (Fig. 8). These vacuoles were often connected with the adjacent smooth muscle cells by a narrow cytoplasmic stalk.

1–6 h after the end of contraction, the nuclei of the endothelial cells had lost the indentation of their boundaries, cell organelles were well preserved and neither opening of endothelial cell junctions nor enlargement of the subendothelial space was found. In the media, some of myofilaments of the smooth muscle cells disappeared and fine granular material were seen there. Parts of the cytoplasmic and nuclear membranes and most of the pinocytotic vesicles were lost (Fig. 9). Myofilaments were also visible in vacuoles similar to those observed during arterial contraction, but the double unit membranes coating each vacuole had partly disappeared (Fig. 9).

In arteries 12–24 h after the termination of contraction necrotic endothelial cells were encountered frequently, their cytoplasm was electron dense containing swollen mitochondria and vacuoles. The internal elastic lamina was stretched but neither fragmentation nor lysis was seen. In the media, the degenerate smooth muscle cells had lost cell membranes, myofilaments, nuclei and all cell organelles other than mitochondria. Their cytoplasm was filled with fine granular or vesicular material (Fig. 10). Mitochondria were swollen with their cristae becoming indistinct and the double unit membranes of vacuoles observed during the contraction were either single-layered owing to partial dissolution, or had completely disappeared (Fig. 10). Severely necrotic medial smooth muscle cells frequently exhibited insudation of fibrin with cross striation with a periodicity of 150–200 Å evident on section (Fig. 11) and platelets in their cytoplasm. Because of this infiltration the damaged muscle cells became highly electron dense (Fig. 11). Endothelial cells were desquamated where the media was damaged with platelets adhering to the denuded areas (Fig. 11).

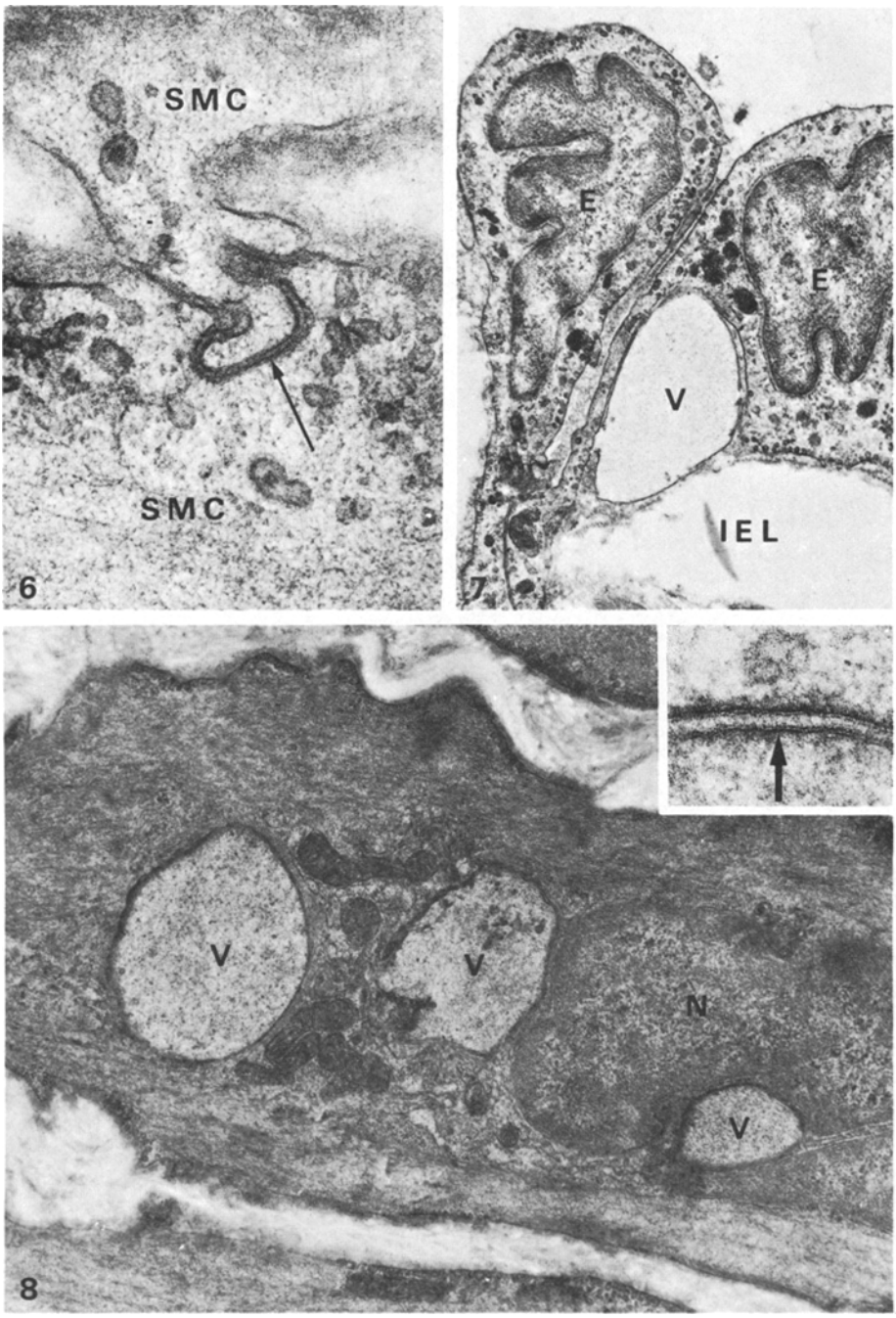


Fig. 6. Media of normal mesenteric artery. In basement membrane-deficient part between smooth muscle cells (*SMC*), double unit membrane is formed as the result of closer approach of the neighbouring cell membranes (*arrow*). ×46,200

Fig. 7. Mesenteric artery during contraction. Nuclei of the endothelial cells (E) are indented and vacuole (*V*) is seen in subendothelial space. IEL, internal elastic lamina. ×10,200

Fig. 8. Medial smooth muscle cell during arterial contraction. Mitochondria are gathered near the pole of a nucleus (*N*), and myofilaments and pinocytotic vesicles are well preserved. In the cytoplasm are seen double unit membrane-bounded vacuoles (*V*), which are filled with fine granular and vesicular material. ×12,100 *Inset:* Double unit membranes of a vacuole (*arrow*). ×99,000

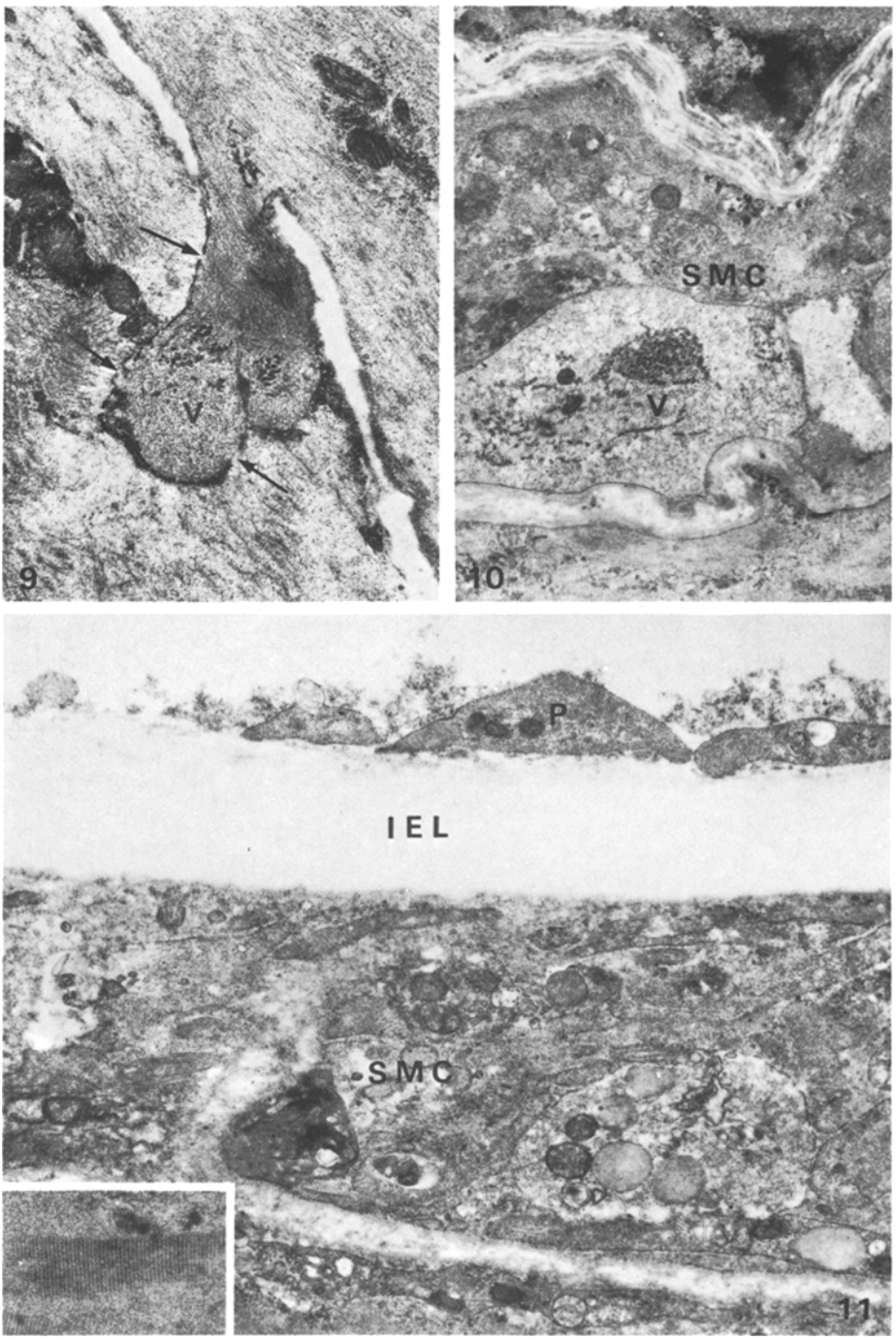


Fig. 9. Arterial media at 1 h and 20 min after the end of contraction. Intrusion (*V*) of a cytoplasmic portion of a smooth muscle cell into an adjacent muscle cell is visible, where the membranes are partly lost (*arrows*). There is, in the cytoplasm, a transitional picture of myofilaments turning into fine granular material through degeneration. × 13,100

Discussion

Arterial Contraction and Medial Smooth Muscle Cell Necrosis

The administration of vasoconstrictor such as methoxamine (Herbertson and Kellaway, 1960; Giese, 1964; Wexler, 1972), angiotensin (Byrom, 1964; Wiener and Giacomelli, 1973) and noradrenalin (Giese, 1964) induces necrosis of the arterial media. Herbertson and Kellaway (1960) observed a medial necrosis of artery after parenteral injection of methoxamine in rat, and they considered that hypertension or arterial contraction might be a cause of the necrosis. After infusion of methoxamine into nephrectomized rat, Giese (1964) observed the same medial necrosis as our experiment. He recorded a high blood pressure during the infusion and he considered the hypertension to be a cause of the necrosis. Wiener and Giacomelli (1973) infused angiotensin into rat, and observed lysis and necrosis of medial smooth muscle cell in the mesenteric artery. They attributed these findings to arterial contraction. In these studies, it was considered that the medial necrosis might be due to arterial contraction, or hypertension induced by systemic administration of the vasoconstrictor. In the present study we have produced contraction confined to the artery into which a vasoconstrictor (α-stimulant) – methoxamine hydrochloride – was applied (Figs. 1b, 2b). Necrosis of medial smooth muscle cells was observed in the artery (Figs. 5, 10, 11). After the administration of methoxamine hydrochloride, blood pressure in carotid artery was elevated about 5 mm Hg, but level of the blood pressure is still within the normal range (Fig. 3). It is thus considered that the elevated blood pressure is not a cause of the medial smooth muscle cell necrosis but the arterial contraction may be the cause of the necrosis in this study.

Contraction of smooth muscle cells has been considered to be responsible for indentation of cellular and nuclear boundaries and for the gathering of cell organelles around the nuclei (Lane, 1965). In the present study smooth muscle cells exhibited indentation of cellular and nuclear boundaries during contraction and straight-running myofilaments, mitochondria gathered around the nuclei were also seen (Fig. 8). These are characteristic findings during arterial contraction. Wiener and Giacomelli (1973) observed the contraction of medial smooth muscle cells by electron microscope, and further investigated the disappearance of cytoplasmic organelles and myofilaments in smooth muscle cell, and subsequent smooth muscle cell necrosis. In the present study, 1–6 h after the termination of contraction the myofilaments in medial smooth muscle cells were dissolved forming fine granular debris. The cell membranes and pinocytotic

Fig. 10. Media of the mesenteric artery at 24 h after the end of contraction. Medial smooth muscle cell (*SMC*) is severely damaged, and its cytoplasm is filled with fine granular or vesicular material. Degenerated mitochondria and vacuole (*V*) are seen. ×12,500

Fig. 11. Mesenteric artery at 24 h after the end of contraction. Endothelial cells are lost, where the platelets (*P*) are adherent. Necrotic medial smooth muscle cells (*SMC*) are filled with fine granular or granular material, degenerated mitochondria and vacuoles, with a high electron density. *IEL*, internal elastic lamina. ×17,500 *Inset:* Deposition of fibrin with periodic cross striation (periodicity: 150–200 Å) in the necrotic part of a medial muscle cell. ×28,600

vesicles had partly disappeared (Fig. 9). 12–24 h after the contraction, myofilaments, cell organelles other than mitochondria and nuclei were not seen in medial smooth muscle cells, which had thus undergone severe necrosis (Figs. 10, 11). It was seldom that we saw focal cytoplasmic necrosis of medial muscle cells, as in hypertensive rats (Takebayashi, 1970; Kojimahara and Ooneda, 1970; Suzuki and Ooneda, 1972). These are considered to be characteristic findings of smooth muscle cell necrosis induced by arterial contraction.

Arterial Contraction and Vacuoles in Medial Muscle Cells

In the media of normal arteries the basement membrane around smooth muscle cells shows fenestrations bringing about membrane-to-membrane contact of adjacent smooth muscle cells (Pease and Molinari, 1960; Rhodin, 1962). However, in these studies vacuoles have not been observed in the medial smooth muscle cell. In the present study similar contact was observed in the normal arterial media with the formation of double unit membranes (Fig. 6), and vacuole was rarely seen in a medial smooth muscle cell.

In contracted arteries the medial smooth muscle cells contained numerous vacuoles bounded by double unit membranes (Figs. 4, 8). The vacuoles were found to contain cell organelles or myofilaments and to be continuous with the adjoining cell body by a narrow cytoplasmic stalk (Fig. 9). It is assumed that these vacuoles may have been formed as the result of herniation of a portion of a muscle cell body into an adjacent muscle cell (Merrillees et al., 1963; Tapp, 1969; Joris and Majno, 1977). Joris and Majno (1977) observed in the rat, especially in tis uterine artery, that a part of cytoplasm of the medial smooth muscle cell was herniated into the neighbouring smooth muscle cell forming double-membraned vacuoles, in which intracellular organelles and microfilaments were contained. They considered that such herniation between the smooth muscle cells might be produced by arterial contraction, where smooth muscle cells made a close contact. Their findings of vacuoles in the medial smooth muscle cell resemble those of the contracted artery in the present study (Figs. 4, 8). However, they failed to compare the contracted artery with the non-contracted and it is not certain whether the vacuoles may be formed only in the contracted artery. In the present study, vacuoles were rarely seen in non-contracted arteries but numerous vacuoles were observed in contracted vessels (Figs. 4, 8, 9, 10). It is assumed that strong contraction of arteries would have compressed the smooth muscle cells so forcibly that a portion of the cell body was intruded into an adjacent cell through a deficiency in the basement membrane and intercellular ground substance, thus forming the vacuole.

In arteries 1–6 h after the termination of contraction the double membranes of the vacuoles were partly lost (Fig. 9), 12–24 h the vacuoles themselves were incorporated in the cytoplasm of necrotic smooth muscle cells (Fig. 10). The vacuoles may have reversibly disappeared together with the intrusion or herniation, or may have been dissolved and incorporated into necrotic muscle cells.

The double-membraned vacuoles in endothelial cells or in subendothelial spaces are seen in pulmonaly arteries and veins of hypoxic rats (Smith and Heath, 1977; Dingemans and Wagenvoort, 1978). In this study, such vacuoles were also observed in subendothelial space or in endothelial cells (Fig. 7). Dingemans and Wagenvoort (1978) observed these vacuoles to be continuous with medial smooth muscle cells, and they considered that the vacuoles might be produced by the cytoplasmic protrusion of the medial smooth muscle cell into the endothelial cell owing to arterial contraction. It is assumed that the double-membraned vacuoles in endothelial cells observed in the present study may also have the same derivation as the vacuoles in medial smooth muscle cells and that arterial contraction may be the cause.

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