Virchows Arch [Pathol Anat] (1981) 393:133–144

Virchows Archiv A

Ultrastructure of Fetal Stem Arteries of Human Placenta in Normal Pregnancy*, **

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Summary. Fetal stem arteries of the 3rd order of 50 normal placentas obtained at term of unevenful pregnancies were examined by transmission electron microscopy with the aim at providing a base-line information for the assessment of similar arteries in hypertensive disorders of pregnancy.

The results showed that the endothelial cells often protruded with a considerable portion of their cellular body into the lumen, and that the intercellular junctions between the neighbouring cells were confined largely to their short basal segments. Numerous myoepithelial junctions were formed between the processes of the endothelial cells and those extending from the smooth muscle cells (SMC) of the adjacent medial layer. At times the "participating" processes of the SMC were entirely enclosed within the endothelial body.

Collagen fibrils increased in number between the SMC and the interstitium broadened progressively from the inner to the outer arterial layers. Small cellular processes devoided of a basement membrane and of most cytoplasmic organelles were numerous in the interstitium; these were traced to the main bodies of the medial SMC.

It is postulated that the "naked" SMC-processes are susceptible to injury of a nature much more subtle than that affecting the main body of the SMC, since these processes were often swollen and appeared edematous in an otherwise innocuous mural environment.

Key words: Term, normal human placenta – Fetal arteries – Ultrastructure – Specialized processes of smooth muscle cells

* Supported by grants-in-aid of research No. PR 499 from the Ministry of Health, Province of Ontario, and T.3–11 from the Ontario Heart Foundation, Toronto, Ontario, Canada

** Presented in part at the Annual Meeting of the USA/Canadian Division of the International Academy of Pathology, New Orleans, Louisiana, February 23–29, 1980

*** Recipient of a Medical Research Council of Canada Fellowship, 1975–1978

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0340-1227/81/0393/0133/$02.40

Whereas there are detailed reports in the literature on the subject of topography (Arts 1961; Bøe 1953) of placental vasculature, few and contradictory contributions (Bøe 1953; Burstein et al. 1956; Paine 1957; Becker 1963; Nikolov and Schiebler 1973) have been made since the classical histological study of Eden (1897) concerning the actual structure of the fetal vessels and the variations to be expected to exist at the various segments of that vascular tree. Perhaps it is not astonishing therefore to find in the literature also contraditions in the findings and statements relating to the morphological status of the placental vasculature under pathological conditions (Riviere 1930; Hunt et al. 1940; Paine 1957; Burstein et al. 1957; Fox 1967; Bender et al. 1976).

Recently, a study concerned with the morphology of fetal stem arteries in normal full-term placentas and those in hypertensive disorders of pregnancy was carried out by light microscopy (Las Heras et al. 1979, 1980). To amplify the above, an assessment of the ultrastructural features of fetal stem arteries in normal full-term placentas was undertaken to provide the base-line information necessary for the assessment of the ultrastructure of similar arteries in hypertensive disorders of pregnancy. The purpose of this communication is to report the results of the study undertaken with the above aim.

Materials and Methods

The material for this study consisted of tissues removed from placentas of 50 normal full-term pregnancies. The placentas were collected at the hospital immediately after delivery, assessed macroscopically, and cut perpendicularly to the basal plate into slices measuring approximately 2.5 cm in width. Upon examination of all slices, tissue blocks were removed from the central area of placentas which appeared normal and disected. One portion was processed routinely for light microscopic examination (Las Heras et al. 1979) and the other was fixed for 90 min at 4° C in 3% buffered glutaraldehyde for electron microscopy. Following fixation in glutaraldehyde and washing in 0.1 M phosphate buffer (pH 7.4), small pieces of tissues (1 to 2 mm) were postfixed for 90 min at 4° C in a solution of 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) and for additional 30 min at room temperature. Following dehydration the tissues were embedded in Epon-812 according to the method of Luft (1961).

One-micron-thick sections were cut with glass knives on either Sorvall MT-1/Porter-Blum or Reichert ultramicrotomes; they were stained with alkaline toluidine blue for light microscopy and selection of fetal stem arteries. Only fetal stem arteries of the 3rd order (Arts 1961), i.e., measuring 100–300 μ in diameter were the subject of the present study (Figs. 1 and 2). Thin sections were cut with diamond knife, mounted on unsupported copper grids, stained doubly with uranyl acetate and lead citrate, and examined with a Philips EM-300 electron microscope.

Results

The innermost lining of the fetal stem arteries (Figs. 1 and 2) was composed of endothelial cells whose large portions projected into the lumen "free", i.e., without contacts or structural junctions with the neighbouring cells (Figs. 3 and 4). Only over a short distance at the basal portion was there an organized apposition between adjacent cells in the form of a simple junction. Whereas it was not within the scope of the present investigation to study these junctions in detail, it appeared on the basis of the medium power micrographs that their overall structure was similar to that reported to occur in large arteries (Hüttner et al. 1973). The nucleus was large and often simply infolded or bean-shaped (Fig. 4), its nucleolus, when

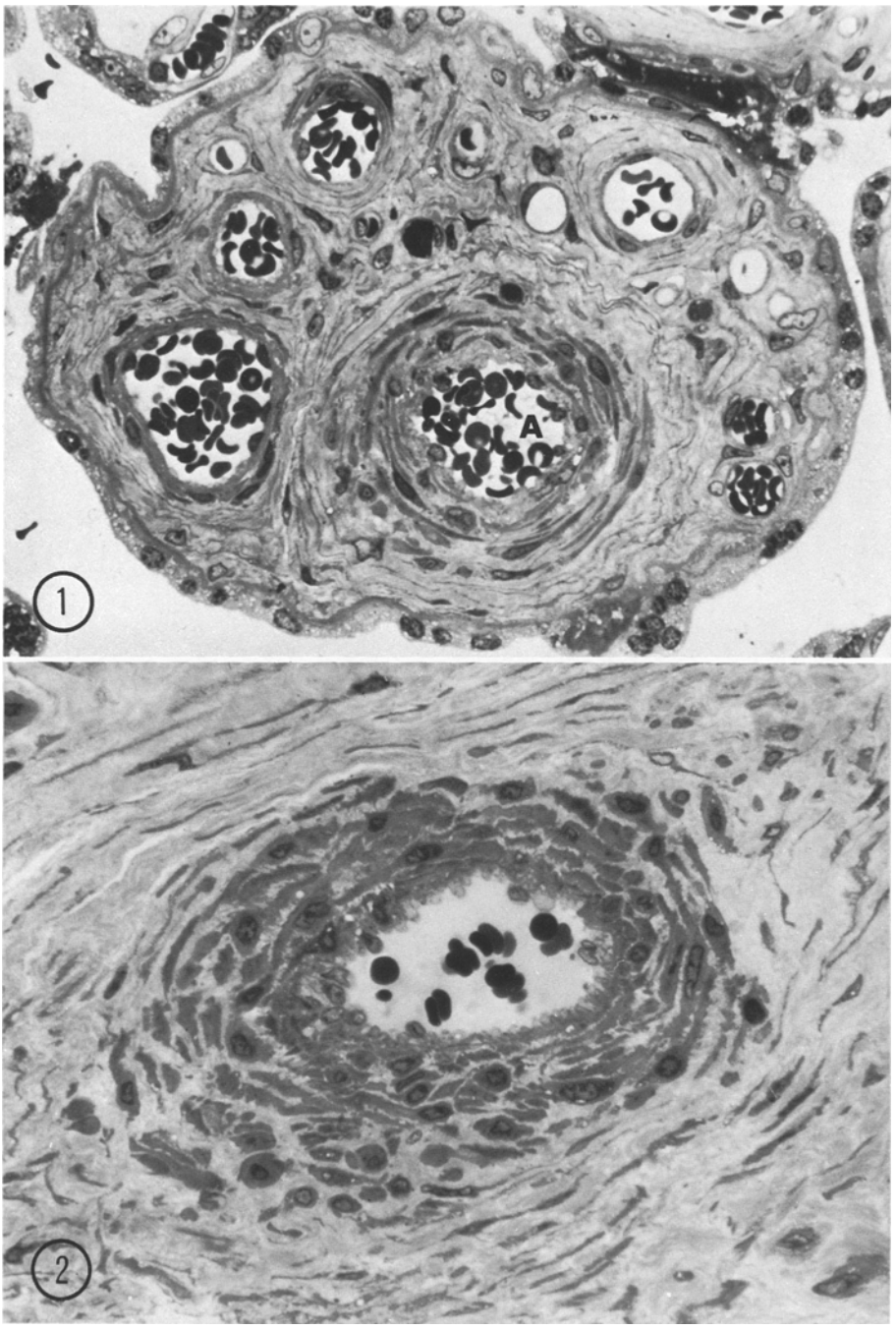
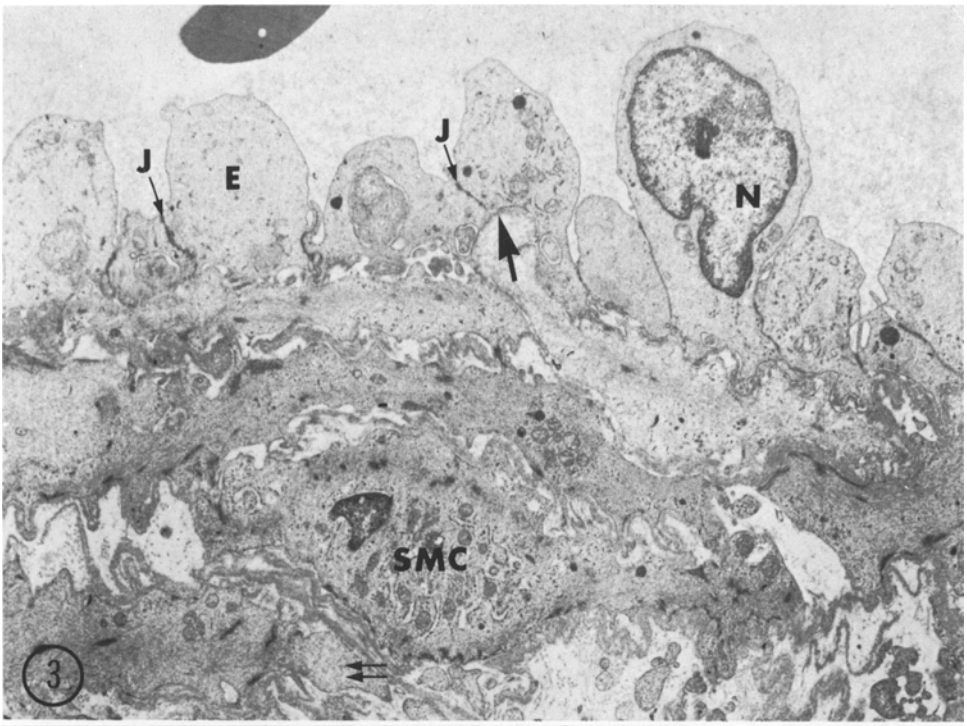


Fig. 1. Light microscopic appearance of a normal fetal stem artery of 3rd order in term placenta. The artery (A) is seen with the accompanying vein, arterioles and smallest vascular channels. Glutaraldehyde-osmic acid fixation; plastic-embedded tissue; toluidine blue staining. Magnification = ×400

Fig. 2. Tissue similar to that illustrated in Fig. 1. The coat of this (slightly tangentially cut) fetal stem artery of 3rd order consists of 5–7 layers of smooth muscle cells. Magnification = ×680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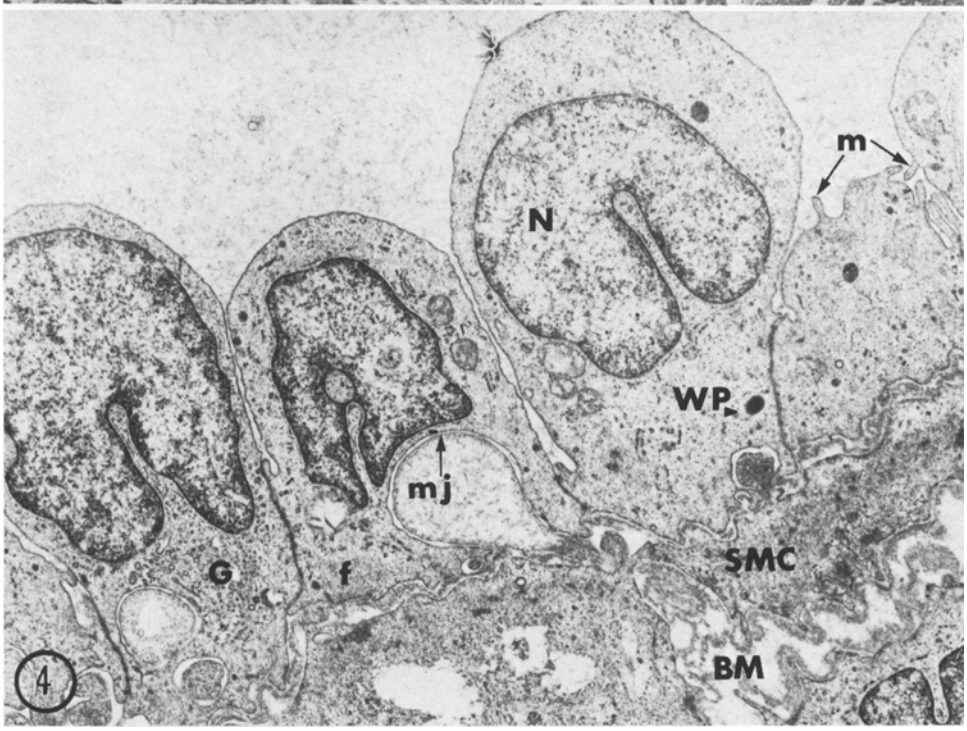
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present, prominent (Fig. 3), and the chromatin had a distribution characteristic of nuclei of the endothelial cells. The cytoplasm of the cell that projected into the lumen appeared to have a simpler structure than that of its basal portion. The latter often contained cytoplasmic fibrils and a prominent Golgi zone (Fig. 4), whereas in the luminal portion, there were only a few cytoplasmic organelles. In keeping with other endothelial cells there were also a few small profiles of rough endoplasmic reticulum, a few round mitochondria and many caveolae cellulares. The caveolae were particularly numerous at both the basal and luminal aspects of the cells. Microvillous projections into the lumen were observed occasionally (Fig. 4), but were not a prominent feature. Many cells contained cytoplasmic osmiophilic structures suggestive of the Weibel-Palade bodies, i.e., the supposed hallmarks of the endothelial cells (Weibel and Palade 1964). However, the internal structure of these organelles was not resolved with ease in the cells studied. Mitotic figures were observed on occasion in the endothelial cells (Fig. 5). The junctions of such cells with the adjacent cells were usually of a considerable length (Fig. 5). On the basal aspect, the endothelial cells exhibited infoldings and processes (Figs. 3–9), and rested on a prominent basement membrane. This basement membrane (BM) varied little in thickness, but showed small areas of reduplication or aggregation (Fig. 5).

Adjacent to the medial aspect of the BM were the smooth muscle cells (SMC) of the medial coat. A few collagen fibrils intervened between them on occasion (Figs. 6 and 8). The medial SMC were often of a complex branching and interdigitating variety, and were surrounded by their own BM (Figs. 3 and 6). The innermost "row" of the SMC was circumferential, whereas in the deeper layers of the media the cells had a spiral and longitudinal arrangement (Figs. 3 and 6). In many areas the medial SMC immediately adjacent to the endothelium were in close contact with the latter by means of cellular processes forming the so-called "myoendothelial junctions". Basement membrane did not intervene between the two cell types at such "junctions" (Figs. 3–5 and 8), however, these areas showed no special organization. The cellular processes of the SMC in contact with the endothelial cells appeared slightly but distinctly "swollen" (Figs. 4 and 7). Occasionally, some of these SMC processes were entirely surrounded by or apparently enclosed within the cytoplasm

Fig. 3. Detail of the intima and inner media of the normal fetal stem artery of 3rd order. The endothelial cells (*E*) project into the lumen and at their basal portion form with the adjacent cells a simple junction (*J*). The nucleus (*N*) is infolded and the nucleolus prominent. The smooth muscle cells (*SMC*) have a circular, spiral and longitudinal arrangement, depending on the layer of the media. Note the myoendothelial contacts (*arrow*) without the intervening basement membrane. Some cellular processes extend from the SMC (*double arrow*), and are devoided of basement membrane. Magnification = ×5,500

Fig. 4. Electron micrograph of an area of the intima and innermost media from a normal fetal stem artery of 3rd order. The nuclei (*N*) of the endothelial cells are large and simply folded. That portion of the cytoplasm of the cells that projects into the lumen contains few cytoplasmic organelles; myofibrils (*f*) and a prominent Golgi zone (*G*) are present at the basal portion of the cytoplasm. A few microvilli (*m*) extend into the lumen. The smooth muscle cells (*SMC*) of the innermost media are in contact with the endothelial cells by means of myoendothelial "junctions" (*mj*). Osmiophilic bodies (*WP*) suggestive of those described by Weibel and Palade are seen in some endothelial cells. The basement membrane (*BM*) of SMC shows different thickness and sometimes areas of reduplication or aggregation. Magnification = ×10,600

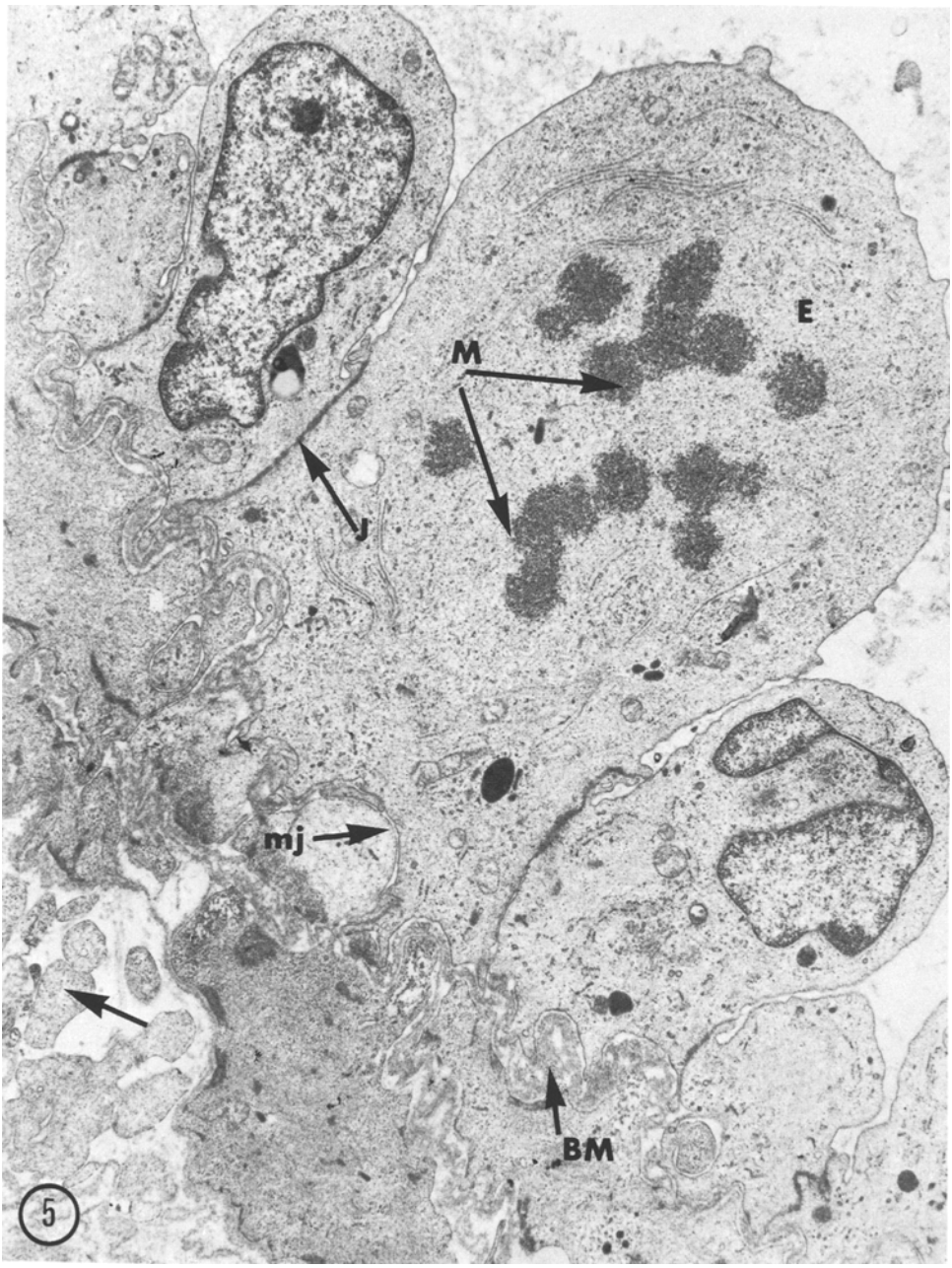


Fig. 5. Detail of the intima and inner media of the normal fetal stem artery of 3rd order. One of the endothelial cells (*E*) is seen in mitosis (*M*) and the junctions (*J*) of this cell with te adjacent cells are of considerable length. The basement membrane (*BM*) of the endothelial cells shows different thickness and sometimes areas of reduplication or aggregation. Myoendothelial junctions (*mj*) are formed between the endothelial and smooth muscle cells. Small cellular processes devoided of basement membrane are seen in the interstitium (*arrow*). Magnification = ×12,300

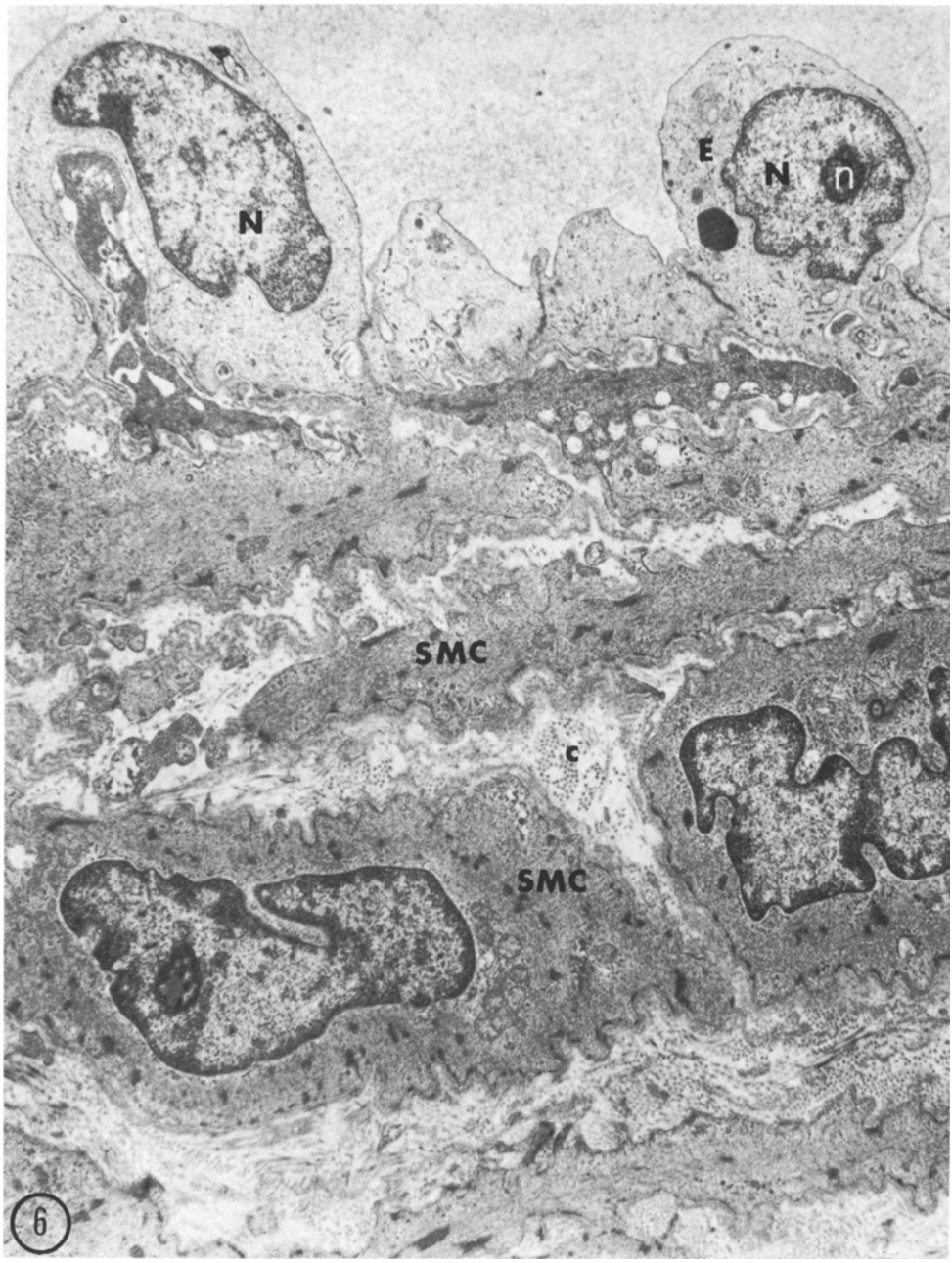
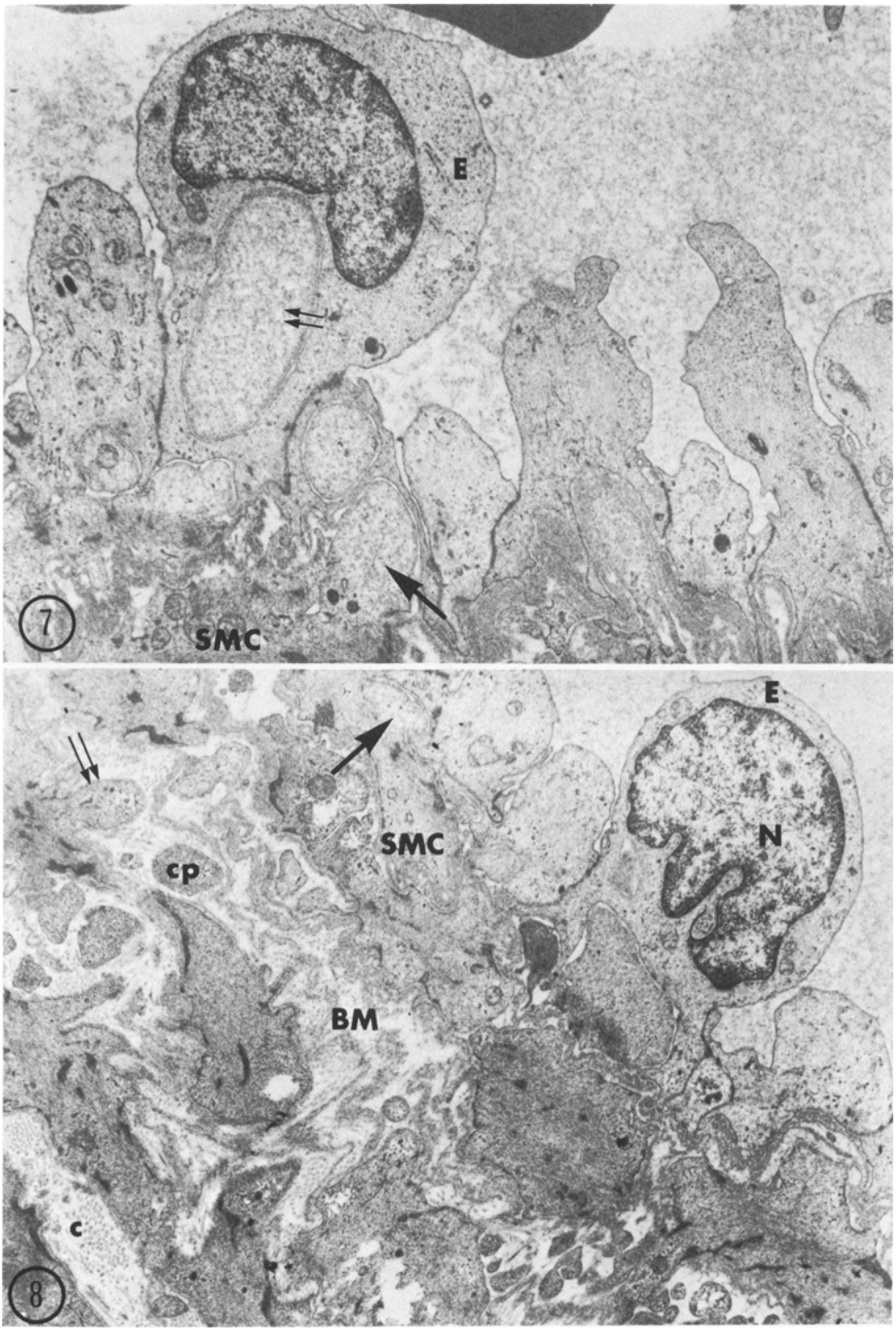


Fig. 6. Normal fetal stem artery of3rd order. The intima shows prominent endothelial cells (*E*) with large nuclei (*N*) and prominent nucleoli (*n*). The chromatin has a distribution characteristic of the endothelial cells. In the media, the smooth muscle cells (*SMC*) are of the complex branching variety and arranged in circumferential and longitudinal fashion. The interstitial spaces are occupied by bundles of collagen fibrils (*c*). Magnification = ×6,400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of the endothelial cells. In these instances, the resulting image conveyed the impression of a distinct intracytoplasmatic structure limited by a double unit membrane (Fig. 7).

All medial SMC contained a nucleus with the characteristic fence-like contour and chromatin distribution, cytoplasmic myofilaments, oblong and triangular densities, and numerous caveolae cellulares; only a few mitochondria and a few profiles of rough surface endoplasmic reticulum were observed in the majority of these cells.

In most instances the innermost medial SMC formed a continuous layer, the cells being in close contact with each other (Figs. 3 and 6). The medial cells of the outer layers were almost always separated from each other by an interstitial space which was broadened towards the outer media. This intervening interstitium contained collagen fibrils which increased in number towards the adventitia (Fig. 6), and a BM-like substance which was either accumulated focally or duplicated over a considerable distance (Figs. 5 and 8).

Numerous small cellular processes, whose nature was not immediately apparent, were observed often in the broadened interstitium (Figs. 3, 5, and 8–10). Since the small cellular processes seldom contained cytoplasmic organelles and were not surrounded by a basement membrane, it appeared at first that they represented slender processes of connective tissue cells, notably fibroblasts. However, in none of the electron micrographs studied could fibroblasts be identified within the inner arterial wall. In fortuitous sections, however, it was possible to establish that cellular processes, morphologically identical with those observed "free" in the interstitium (see above), were extending from the main body of the SMC (Figs. 3 and 10). In such instances the processes "penetrated" the surrounding BM of the SMC and extended "naked" beyond it into the interstitium. These still attached processes often appeared slightly swollen (Fig. 3) and resembled similar processes originating from the endothelial aspect of the innermost layer of the SMC, i.e., those that were involved in the formation of the myoendothelial "junctions" (see above).

Thorough search failed to disclose the presence of any, even the smallest, elements of elastic tissue in the media. The transition zone between the media and the surrounding connective tissue was not explored in detail since in the present study the interest was directed towards the innermost mural components of the fetal stem arteries of the 3rd order.

Fig. 7. Tissue as in Figs. 3–6. The intima shows endothelial cells (*E*) whose main bodies project into the lumen. The cellular processes of the smooth muscle cells (*SMC*) in contact with the endothelial cells appear swollen (*arrow*). Some of these processes are apparently enclosed within the cytoplasm of the endothelial cells (*double arrow*). Several endothelial cells contain small osmiophilic bodies reminiscent of Weibel-Palade bodies. Magnification = ×8,300

Fig. 8. Tissue as in Figs. 3–7. One of the endothelial cells (*E*) contains a large folded nucleus (*N*). The process of a smooth muscle cell (*SMC*) at one of the myoendothelial junctions is slightly swollen (*arrow*). The interstitial spaces between the smooth muscle cells are occupied by collagen fibrils (*C*), basement membrane substance (*MB*) and small cellular processes (*cp*). A similar process is seen in continuity with a smooth muscle cell (*double arrow*). Magnification = ×8,500

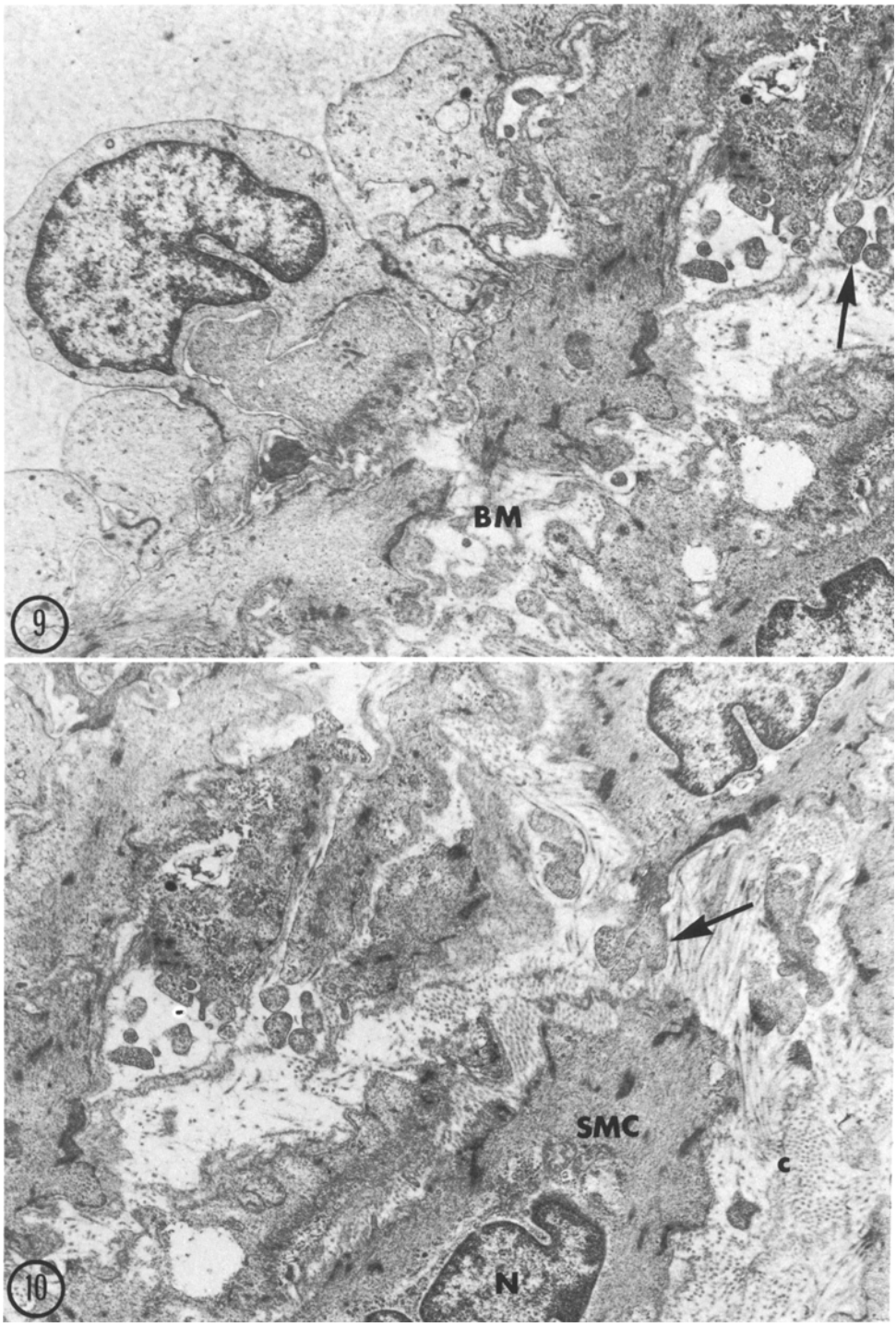

Fig. 9. Tissue as in Figs. 3–8. The interstitial spaces are occupied by collagen fibrils, basement membrane (*BM*) and cellular processes (*arrow*). These processes are not surrounded by a basement membrane. Magnification = ×7,800

Fig. 10. Detail of the inner media of a normal fetal stem artery of 3rd order. One of the medial smooth muscle cells (*SMC*) shows a portion of a nucleus (*N*) with a fence-like contour. The cytoplasm shows a few organelles and contains myofilaments, and triangular and oblong densities. Some small cellular processes (*arrow*) extend from the SMC and are devoided of basement membrane. There are numerous collagen fibrils (*C*) in the broadened interstitial space. Magnification = ×7,100

Discussion

Present studies indicate that the 3rd order, fetal stem arteries (Arts 1961) of normal, full-term human placentas exhibit several structural peculiarities; some of these may represent functional adaptation to the changing requirements of fetal circulation. Thus, the abundance of endothelial cytoplasm with protrusion into the lumen; absent elastic tissue elements (whose presence would promote recoiling); the loose arrangment of the smooth muscle cells (SMC) in the media with the exception of those in the innermost layer; and the broadened intercellular spaces with only a moderate number of collagen fibrils but abundant basement membrane-like substance, all are features that may be interpreted as mural arrangements facilitating the distensibility without the consequent recoiling and promoting a bi-directional transmural transport.

It would appear that the cohesiveness and synchronization of function between the endothelial layer and the medial coat (in the absence of internal elastic lamina or its less continuous equivalent), may be ensured by the myoepithelial junctions, and between the medial SMC by the basement membrane (MB). This BM rarely enveloped two or a group of SMC, a feature commonly observed in other small and also large arteries. Rather, the abundant BM-like, often folded substance appeared to "connect" the SMC which were separated by the broad interstitial spaces. It is interesting to speculate that the folded rows of the BM could serve as the distensibility-limiting devises.

Mitotic figures in the endothelial cells were observed on several occasions, but not in the SMC, not even upon repeated search. One must conclude that the propensity to proliferate is greater in the former than in the latter. Since degenerating or necrotic endothelial cells were not observed in the areas adjacent to the mitotic figures – containing cells is would be difficult to interpret the phenomenon of cellular proliferation as a physiological regeneration. However, it is possible that this process may provide a reservoir of endothelial cells available, should further distensibility of fetal vessels be required in the adaptation to increasing demands upon fetal circulation.

The seemingly intraendothelial vacuoles bounded by double limiting membranes (Fig. 7) represent processes of the SMC that herniated through the BM and invaginated into the endothelial cells. Subsequently, these may be pinched off from the parent cytoplasmic body, and ultimately herniate through the thinned-out enclosing rim of the endothelial cytoplasm into the lumen. This feature was observed in various segments of the arterial tree by several investigators (Dingemans and Wagenvoort 1977; Stetz et al. 1979) and a similar phenomenon (of herniation) was reported to involve the medial SMC (Joris and Majno 1977). The presence of the herniating processes was interpreted as a morphological marker of vascular SMC-contraction (Joris and Majno 1977).

In the present study the small cellular processes devoided of a basement membrane and of most cytoplasmic organelles were numerous in the interstitium. They often appeared edematous, and when still attached to their parent cellular bodies, they were often the only segments of the cytoplasm affected by edema. This phenomenon was reminiscent of that observed in the gelatinous early lesions of human atherosclerosis; here, focal intracytoplasmic edema was present often in the

SMC of the affected intima when the segment of the adjacent BM was discontinuous or permeated by edema fluid in the course of the pathological process (Haust 1977). It is postulated that the "naked" SMC-processes are susceptible to injury of a nature more subtle than that affecting the main body of the SMC. Moreover, the cellular BM may not only play a supportive but also a protective role.

Acknowledgements. The authors wish to acknowledge gratefully the continuous support provided by Dr. Paul Harding, Professor of Obstetrics and Gynaecology at the University of Western Ontario, who has been collaborating in most phases of the ongoing programme on the role of placenta in maternal and perinatal disease processes, and thank Ms. Irena Wojewodzka, Mr. Roger Dewar and Mrs. Wendy Thomas for their invaluable technical assistance, and Mrs. Lya Motesharei for the efficient typing of the manuscript.

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Accepted June 26, 1981