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A study of the ultrastructure of developing human umbilical vessels

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ABSTRACT

Electron microscopic techniques were used to examine the ultrastructure of developing human umbilical arteries and vein (8–12, 13–17 and 37–40 wk gestational age). These showed that with increasing age there is (1) an increase in the size of the lumen and the thickness of the media; (2) an increase in the ratio of contractile smooth muscle phenotypic cells; (3) an increase in the myofilament content of the smooth muscle cells and the number of Weibel–Palade bodies; (4) a decrease in the glycogen content; (5) an appearance of microvilli on the luminal surface of the endothelium. Lipid vesicles, nerves and vasa vasorum were not observed in any region of the umbilical vein or arteries.

Key words: Umbilical vessels; smooth muscle; endothelium.

INTRODUCTION

Recognition of the importance of blood vessel endothelium in the control of vascular tone has greatly increased since the demonstration by Furchgott & Zawadzki (1980) that acetylcholine only relaxes precontracted vascular tissue in the presence of an intact endothelium. It is now known that the action of a variety of vasodilators and vasoconstrictors is dependent upon an intact endothelium (Angus & Cocks, 1989).

The umbilical vessels have been shown to be devoid of nerves (Spivak, 1943; Reilly & Russell, 1977; Fox & Khong, 1990), suggesting that a nonneuronal control of the fetoplacental circulation may be particularly important in these vessels. Thus the endothelium may play a paracrine role in the regulation of umbilical blood flow. Though there are many publications to date describing the ultrastructure of human umbilical vessels at term (Roach, 1973; Asmussen & Kjeldsen, 1975; Takagi et al. 1985), there are few published studies comparing the ultrastructure of these vessels during gestation. Parry & Abramovich (1972) studied the endothelium of human umbilical vessels through pregnancy and found marked differences in the appearance of the rough endoplasmic reticulum (RER) between 10 wk and full term, but did not study other parts of the vessel wall.

It is of great importance to investigate the fetoplacental circulation early in gestation, since during the first 3 months the fetoplacental circulation is separate from the maternal circulation (Hustin & Schaaps, 1987) and hence develops in a hypoxic environment (Rodesh et al. 1992). Following the establishment of the intervillous circulation at the beginning of the second trimester (Jauniaux et al. 1992), a continuous decrease in fetoplacental vascular resistance with advancing pregnancy is seen (Macara et al. 1993).

The purpose of this study was to determine the ultrastructure of the vasculature within the umbilical cord during pregnancy, to see if this can aid in the understanding of the mechanisms regulating fetoplacental circulation.

MATERIALS AND METHODS

Umbilical cords from legal terminations of pregnancy at two gestational age ranges, 8–12 wk (n = 7), and 13–17 wk (n = 9), and from normal vaginal deliveries (gestational age 37–40 wk, n = 4), were fixed as soon

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as practically possible. Cords from term deliveries were clamped immediately after delivery. Sections of cord, 2 cm in length were taken from the placental to midregion of the cord and were fixed for 2 h in 4% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer. The vessels were then carefully dissected out from the cord, cut into 0.5 cm segments and fixed overnight at 4 °C. The vessels were subsequently postfixed in a 1% osmium tetroxide solution with 0.1 M cacodylate buffer for 1 h; some of the samples were stained for 45 min in aqueous 2% uranyl acetate solution at 4 °C. All samples were then dehydrated using graded ethanol. The samples were finally treated with propylene oxide and embedded in Araldite.

Semithin sections (1 μm) were cut using a diamond knife and stained with toluidine blue for observation under a light microscope. Ultrathin sections of the vessels in cross-section were then counterstained with uranyl acetate and lead citrate prior to examination under a JEOL 1010 electron microscope.

RESULTS

Light microscopy

Throughout gestation, the intima of the umbilical arteries and vein had a single layer of endothelial cells (EC) (Fig. 1). Initially, from 8–15 wk, the media appeared to consist mainly of circular bands of smooth muscle cells (SMC), but by 16 wk (Fig. 1*d*, *i*) other orientations of SMC could be observed. There was no clear adventitial layer and no nerves were present.

Electron microscopy

8–12 weeks

Endothelial cells. The umbilical arteries and vein consisted of a single layer of small, rounded, nucleated EC (intima). The number of organelles varied in section but, in general, the nucleus appeared to be dominant (Fig. 2*a*), irregular in shape with a very prominent nucleolus. The nucleoplasm was finely granular with a few condensations of chromatin attached to the nuclear envelope. The number of organelles—rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), multivesicular bodies (MVB) and mitochondria—varied in sections but seemed to have increased by 12 wk. Weibel-Palade bodies (Weibel & Palade, 1964) were more numerous in the vein at 12 wk but were also present in the arteries. Golgi apparatus was well developed, indicating the synthetic activity of the cell. The EC of the arteries contained more organelles, but the RER appeared more prominent in the vein because of its more dilated appearance (Figs 2*b*, 4*a*). Large accumulations of glycogen were observed only in the endothelium of the arteries (Fig. 4*b*) and were more prominent on the luminal side. Cell contact between EC consisted of tight and gap junctions, with gap junctions appearing every 2–3 cells in the arteries and less frequently in the vein. The contact between the endothelium and SMC was initially extensive in both vessels, arising from long thin processes, generally originating from both the endothelium and SMC (Fig. 2*c*, *d*). However, towards 12 wk of gestation, the processes became fewer, shorter and fatter, and appeared to originate predominantly from the SMC (Fig. 4*b*). Whole body contact was observed between a few EC and SMC. The EC appeared to sit on a patchy basal lamina, with some areas becoming continuous with the extracellular matrix of the SMC.

Smooth muscle cells. The wall of the arteries appeared as an ordered structure, but with an uneven thickness. At 9.5 wk the arteries consisted of approximately 8–9 and the vein 5–6 circular layers of SMC, around the endothelium (Fig. 3*a*). The muscle cells appeared as individual circular bands becoming less distinct towards 12 wk, with groupings of smaller cells in the outer regions. There was much contact between the bands via small processes. SMC appeared very active (Fig. 3*b*), in that it was abundant in synthetic organelles, such as RER and SER, mitochondria, plasmalemmal vesicles, free ribosomes, Golgi apparatus and glycogen. Mitotic figures were observed throughout the muscle layer and were not restricted to any one region. Apart from the SMC of the outer region of the muscle layer, SMC were uniform in appearance. Glycogen was rich in the SMC of both arteries and vein, granules were scattered around the organelles and gathered in large accumulations throughout the cytoplasm (Fig. 4*b*, *c*). Dense bodies (electron dense structures scattered in the cytoplasm; see Gabella, 1981) and caveolae were occasionally seen. An intact basement membrane was observed around most SMC.

The SMC furthest from the lumen appeared as a mixed population, with some cells lacking the distinct characteristics of SMC and were more fibroblast-like in appearance (Fig. 3*c*). The cytoplasm contained an increased number of mitochondria, Golgi apparatus, SER and RER, but lesser amounts of glycogen were observed. The filament content was reduced, resulting in patchy condensations. Whilst not common, dense bodies, dense bands (reinforcements on the cyto-

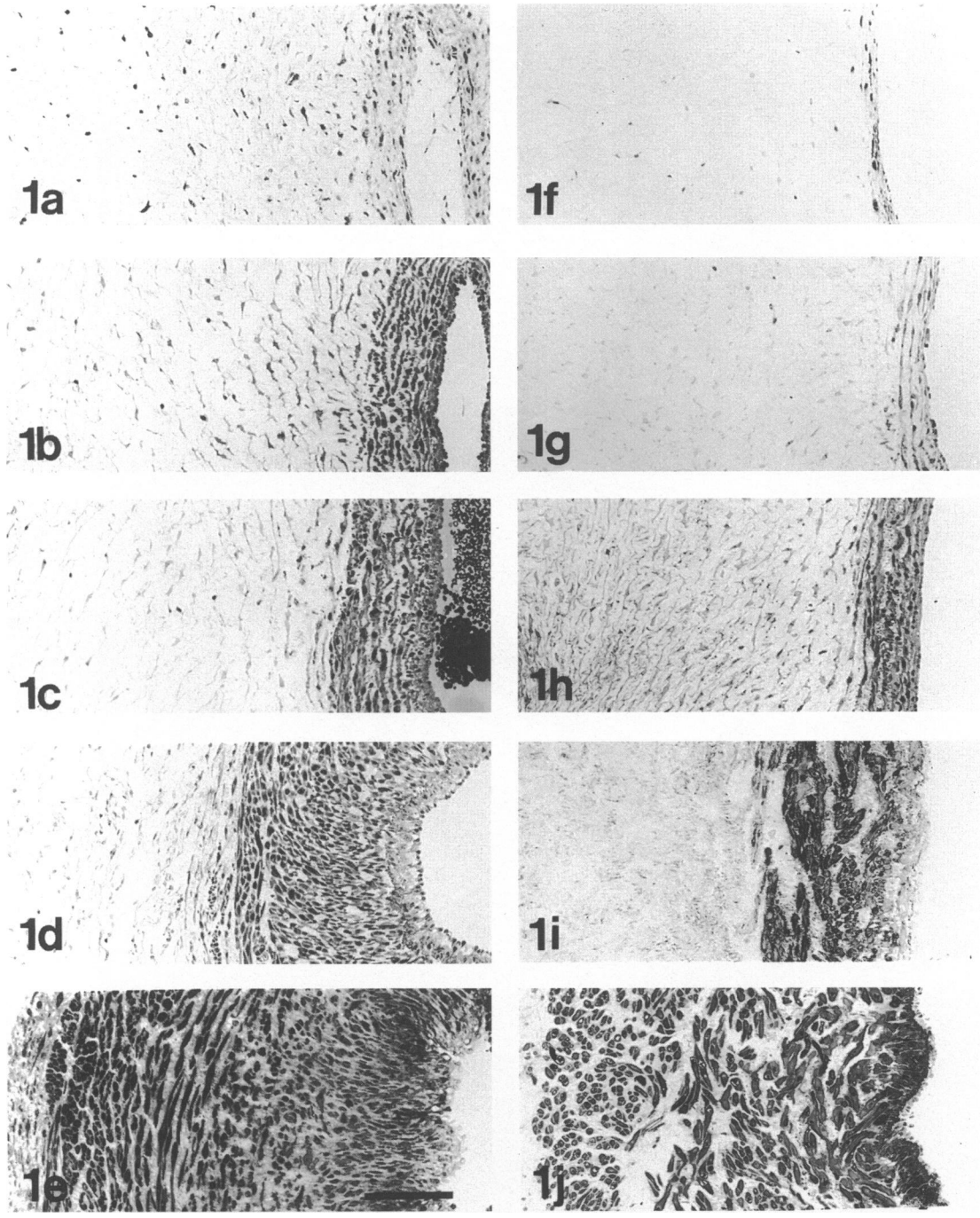

Fig. 1. Light micrograph showing the patterns of increase in the size of the wall of the umbilical artery (*a–e*) and umbilical vein (*f–j*) at 8 wk (*a, f*), 9.5 wk (*b, g*), 14.8 wk (*c, h*), 16 wk (*d, i*) and 39 wk (*e, j*). Bar, 100 μm.

plasmic side of the membrane, found between caveolae; see Gabella, 1981), and caveolae were present. The basement membrane was incomplete. The extracellular matrix consisted of collagen, evenly distributed as small bundles, which became denser further from the lumen. Elastic fibres, which appeared to have an amorphous core surrounded by fibrils with a diameter of 10–11 nm, were distributed throughout the muscle wall as small fibres, and closely associated with collagen and the basement membrane.

13–17 weeks

Endothelial cells. EC were similar in appearance to those obtained at 8–12 wk. However, there were some differences: (1) the number of organelles and amount

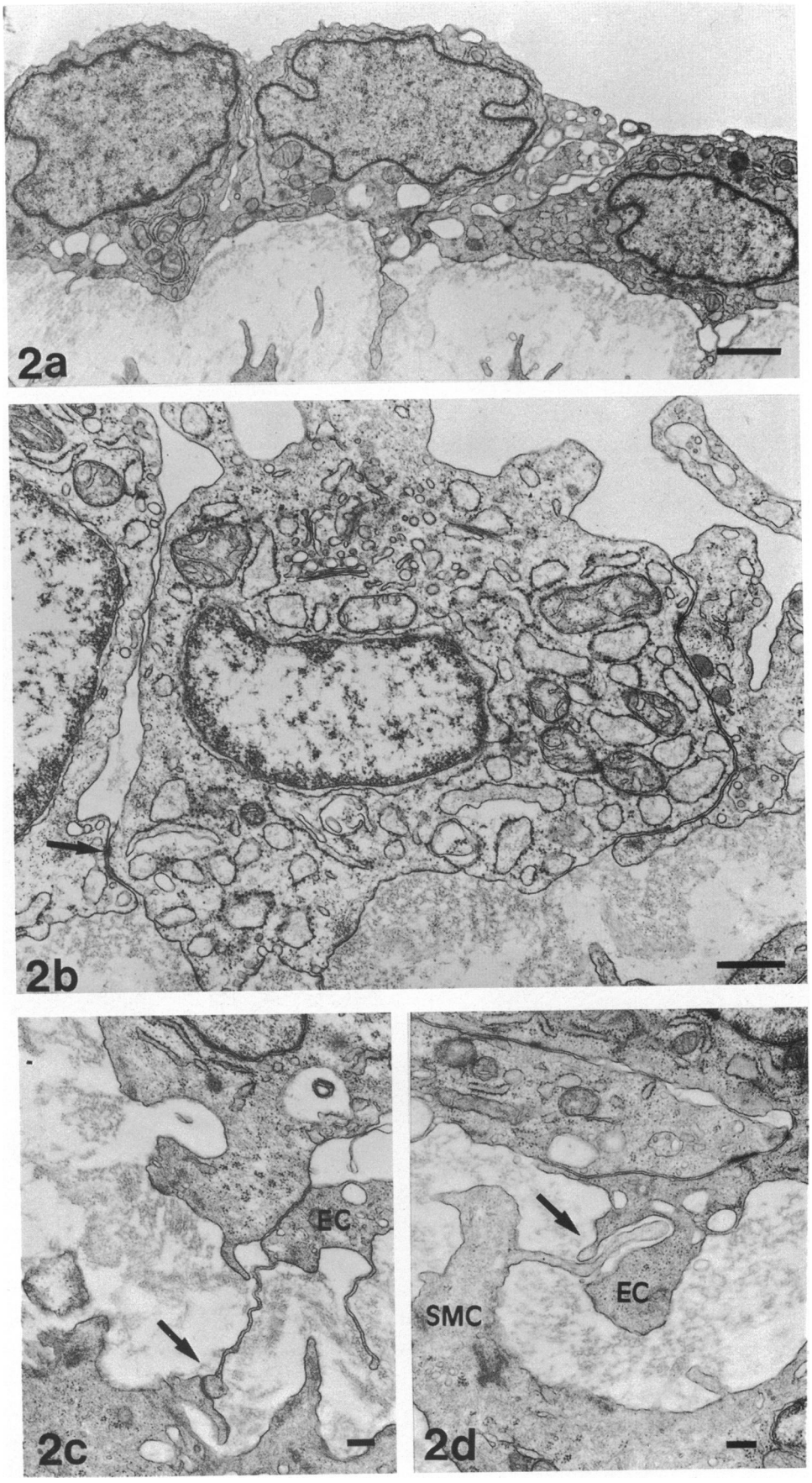

Fig. 2. For legend see opposite.

of glycogen had increased; (2) Weibel–Palade bodies appeared to have decreased in the arteries; (3) the contact between the EC and SMC had decreased but was greater in the arteries than the vein; (4) invaginations of the SMC by processes from EC were reduced and (5) the basement membrane was more obvious and the internal elastic lamina (IEL) was now noticeable.

Smooth muscle cells. The arteries and vein had greatly increased in size (Fig. 1), such that by 13 wk the wall of the vein was approximately 12–14 cells thick and increasing to an average of 24–28 cells by 16 wk. The arteries had increased from 19–21 cells thick at 13 wk to 27–45 cells thick by 16 wk. The IEL of the vein was now clearly distinguishable, though more distinct in the vein and was approximately 200–300 nm thick. The amount of endothelial-muscle cell contact was reduced. The SMC appeared to have a less organised structure in size and orientation. Compared with the samples from 8–12 wk, there was a greater variability in size, with the cells in the middle of the media being the largest. Several SMC throughout the vessel wall appeared slightly more active but with a decrease in the number of contractile filaments (Fig. 5*a*, *b*). Fibroblast-like cells were observed in the outer media and seen in the outer region of the vessel wall.

37–40 weeks (term)

Endothelial cells. The intima of the arteries and vein in the term samples consisted of a single layer of EC as in the 8–12 and 13–17 wk vessels. These, however, were larger in size and more elongated in shape. Many EC, mainly in the vein, contained large microvilli-like structures that projected into the lumen (Fig. 6*a*), which were rich in RER, mitochondria and ribosomes. Lysosomes and Wiebel–Palade bodies were not uniformly present. Although fine filaments were present in all endothelial cells they were more numerous in some. Venous endothelial cells appeared to contain more organelles than arterial EC. Although contact existed between endothelial cells and the underlying muscle cells in the arteries, contact was greatly reduced in the vein (Fig. 6*a*, *b*). The IEL of the vein was large and fibrous with a few gaps allowing contact between the EC and the underlying SMC. The presence of elastic fibres in the arteries was observed. Occasionally, in the vein, a second IEL was observed further from the lumen which was also fibrous and sporadically broken (Fig. 6*c*).

Smooth muscle cells. The intimal/medial border of the arteries appeared to contain a second cell type which was difficult to identify. These cells resembled SMC but were very rich in RER and other organelles. They also showed prominent dense bands, a lower filament content and a few caveolae were found, predominantly under the intima. Contact existed between these cells and with normal muscle cells (Fig. 6*b*).

The SMC of the media now appeared to be randomly distributed and not in distinct bands or bundles. Their general structure appeared very irregular with branching of the cytoplasmic membrane. When the arteries or vein was in a state of contraction, the branches of many SMC lacked all cell content, also glycogen was not observed within any region of the branches (Fig. 6*d*). Although contact between individual SMC was high, no gap junctions were observed. Further into the media, the SMC became less dense with an increase in the branching of the cytoplasmic membranes. An increase in the space around the nucleus was also observed. An external elastic lamina was seen but this was not as distinct as the IEL, being more distinct in the vein but not always continuous.

The border between the media and adventitia was unclear. This region consisted mainly of individual collagen fibres, with the occasional fibroblast-like cell, fibroblast and single SMC scattered within the connective tissue. The presence of membrane whirls on the medial border was apparent. Nerves and vasa vasorum were not observed in this or any other region of either the arteries or vein.

DISCUSSION

Structurally, the vessel walls of the umbilical arteries and vein were similar at 8–12 wk of gestation; both consisted of a single layer of active EC, which progressed from small rounded cells in early gestation, to a more stellate appearance in later gestation. The organelle content, particularly the dilated RER and Weibel–Palade bodies, was more prominent in vessels from 8 to 16 wk. This implies that endothelial cell function may vary throughout pregnancy. Dilated

Fig. 2. Electron micrographs. (*a*) Small rounded closely opposed EC of the umbilical vein (9.5 wk) showing variation in activity and in the proportion of dilated cisternae; bar, 1 μm. (*b*) An EC of the umbilical vein (12 wk) with dilated RER, Golgi apparatus, pinocytotic vesicles and mitochondria, and a tight junction (arrow); bar, 500 nm. (*c*) The umbilical vein at 9.5 wk showing the contact between EC and SMC with a long thin process arising from an endothelial cell (EC) making contact (arrow) with the underlying smooth muscle cell (SMC); bar, 200 nm. (*d*) A process from an SMC invaginating an EC (arrow); bar, 200 nm.

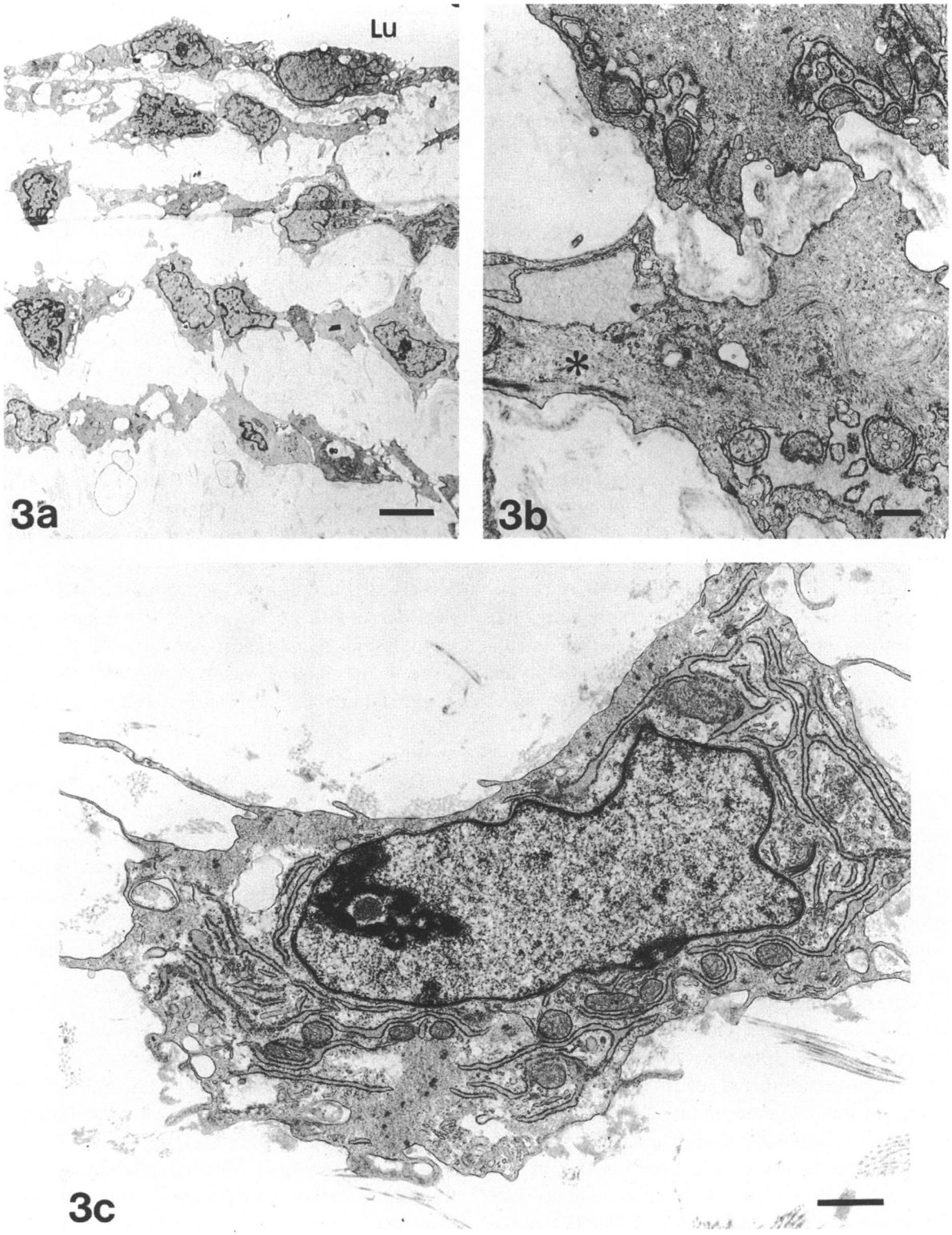

Fig. 3. (*a*) Low power micrograph showing the arrangement of SMC in orderly bands around the lumen (Lu) in the umbilical vein (9.5 wk). The large areas of extracellular space contain small groupings of collagen fibres; bar, 2 μm. (*b*) Dilated peripheral region (asterisk) of one of two SMC from the umbilical arteries (10.7 wk) in a transition state between the two phenotypes, synthetic and contractile; bar, 1 μm. (*c*) Fibroblast-like cell in the umbilical vein (10.7 wk) from the outer region of the media. Dense bodies and caveolae are seen, but a continuous basement membrane is lacking, making the distinction between SMC and fibroblast cell type difficult. Clear regions within the cytoplasm are artifactual, due to the extraction of glycogen during sample preparation. Bar, 1 μm.

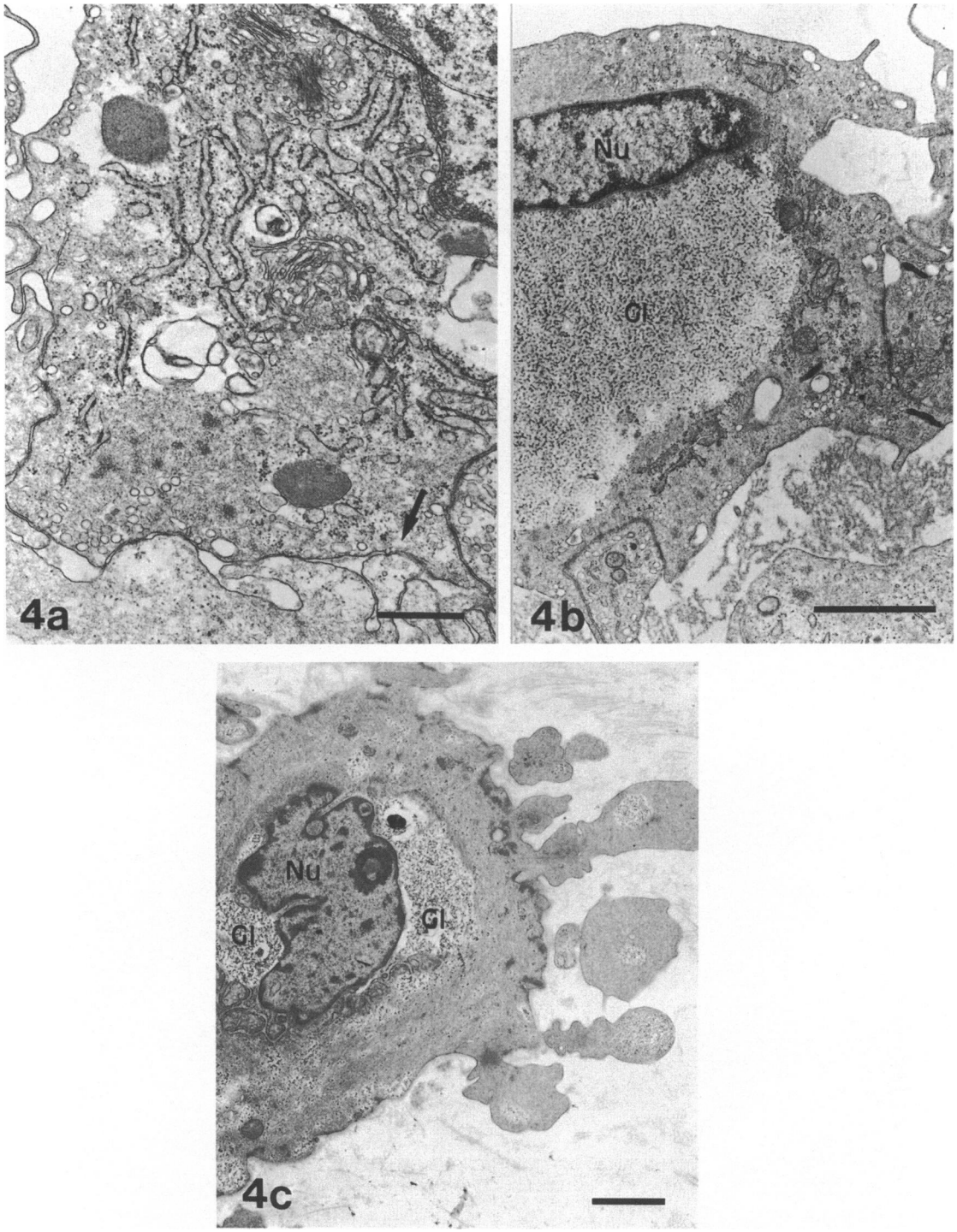

Fig. 4. (*a*) EC from an umbilical artery (12 wk) with dilated RER and a high content of organelles. Contact with the underlying SMC mainly originates from the muscle (arrow) and the processes are short and fat, as compared with those at 9.5 wk (see Fig. 3*c*, *d*). The clear patches represent glycogen, extracted during the postfixation procedure. Bar, 1 μm. (*b*) EC from an umbilical artery (12 wk) with preserved glycogen (Gl), achieved by omitting the uranyl acetate step of the postfixation procedure. Large accumulations of glycogen can be seen around the nucleus (Nu). Bar, 1 μm. (*c*) SMC of umbilical vein (15.4 wk) found in the outer region of the media. There are large accumulations of glycogen (Gl) around the nucleus (Nu) and a few smaller accumulations in the peripheral cytoplasm. Bar, 1 μm.

RER has also been described by Parry & Abramovich (1972) in endothelial cells from early gestation, with maximum dilation at 15 wk. Asmussen & Kjeldsen (1975) observed dilated RER in EC from term arteries of smoking mothers, as have Dadak et al. (1984) in the umbilical artery of babies born to pre-eclamptic mothers.

The EC of the arteries and vein at term remain very active suggesting a contribution to the regulation of the fetoplacental circulation by the possible synthesis

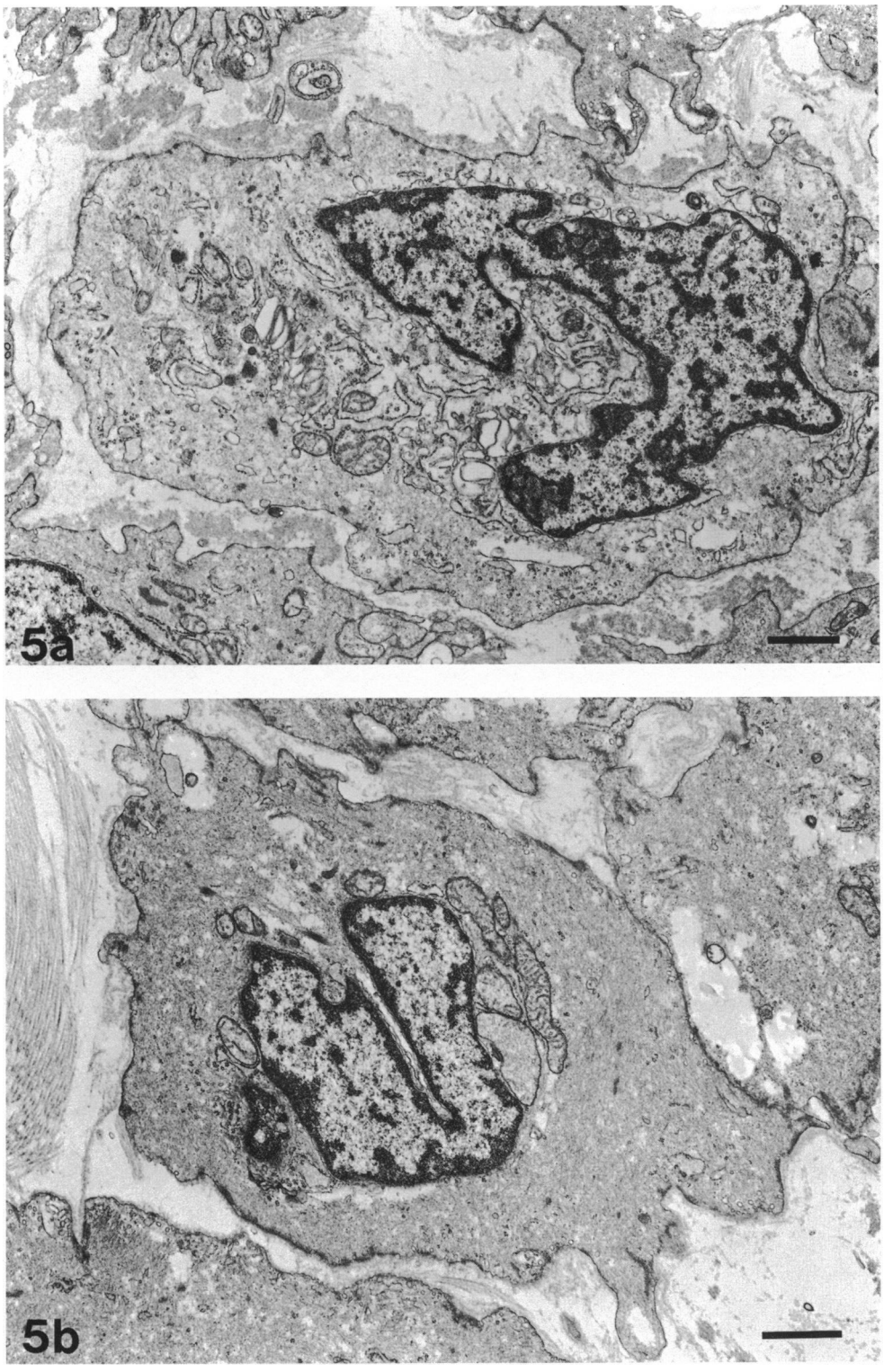

Fig. 5. Electron micrograph of umbilical artery (15 wk) illustrating the heterogeneity of the SMC. (*a*) SMC of the synthetic phenotype located 2–3 cells into the media. It has a low filament content, dilated RER, mitochondria, free ribosomes. (*b*) SMC of the contractile phenotype located in the outer region of the media. Note that it has a greater filament content and fewer organelles. A basement membrane is present and collagen can be seen in bundles, but elastic fibres are not observed. Bar, 1 μm.

and release of vasoactive substances. It has been shown that human term umbilical arteries and vein endothelial cells contain many vasoactive substances, such as 5-hydroxytryptamine, histamine, endothelin (Sexton et al. 1994), neuropeptide Y, atrial natriuretic peptide (Cai et al. 1993*a*), vasoactive intestinal peptide, calcitonin gene-related peptide, substance P (SP) and arginine vasopressin (Cai et al. 1993*b*).

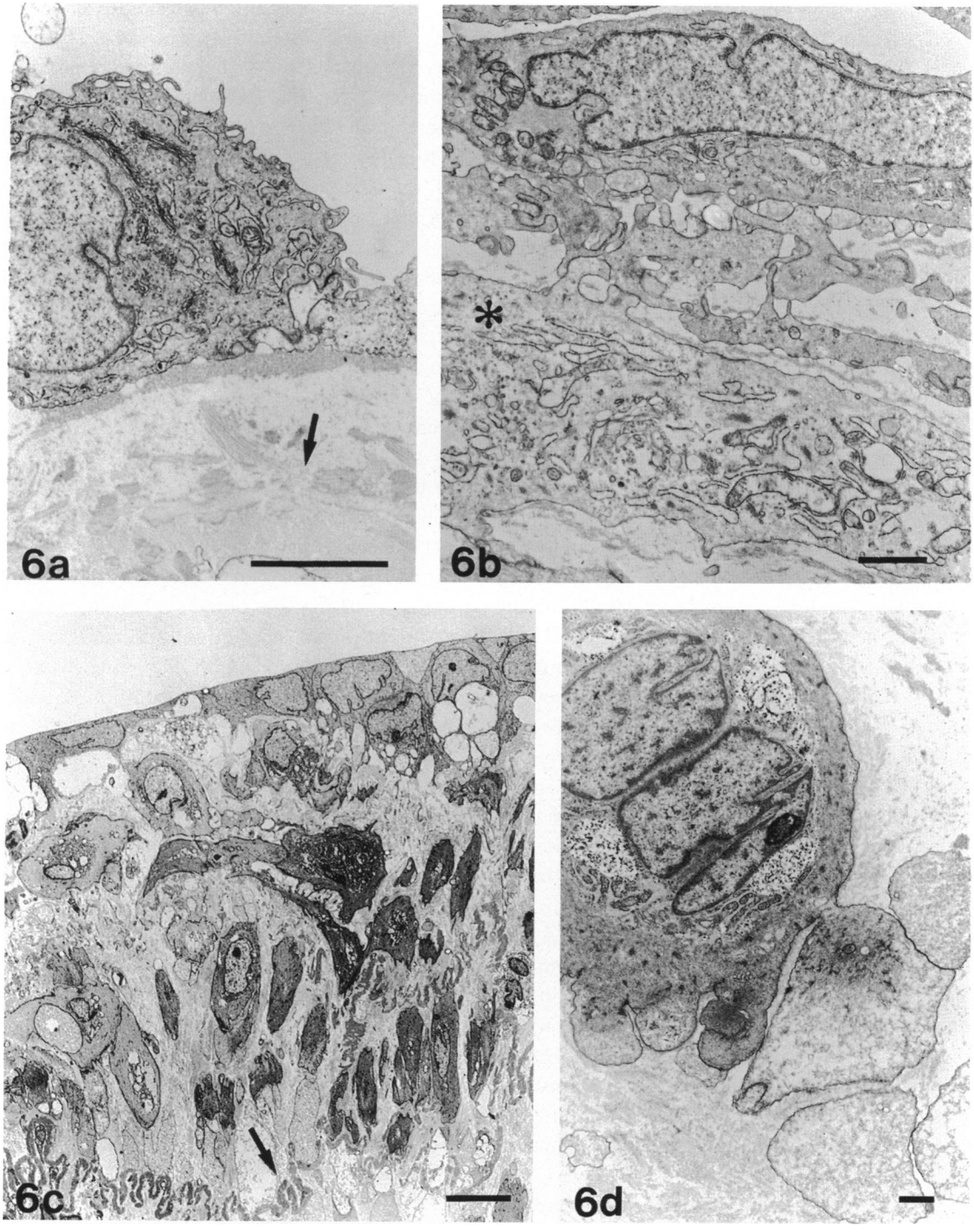

Fig. 6. (*a*) Umbilical vein EC with luminal projections (term), showing a well developed Golgi apparatus and RER, but not all dilated. A distinct basement membrane is present. The elastic lamina is not always in continuous contact with the basement membrane (arrow). Collagen is present in the interstitial space. Bar, 2 μm. (*b*) Electron micrograph of an EC of the umbilical artery showing a fragmented basement membrane and contact with the cytoplasmic processes of the smooth muscle. A cell type (asterisk) restricted to the endothelial/medial border of the artery appears rich in synthetic organelles with a low filament content. Bar, 1 μm. (*c*) Low power electron micrograph of the smooth muscle layer of the umbilical vein (term) showing the elastic lamina as a fibrous fragmented band; the extracellular space is full of cellular processes. There is variable activity between cells. A second elastic lamina (arrow) can be seen deeper in the media. Bar, 2 μm. (*d*) SMC from the outer region of the media; glycogen can be observed around the nucleus, but is reduced in the peripheral cytoplasm. 'Blebbing' of the cytoplasmic membrane of the SMC contains a matrix but lacks filaments. Bar, 1 μm.

Under shear stress they are also capable of releasing vasoactive substances such as adenosine triphosphate, SP and acetylcholine (Milner et al. 1990). The presence of the microvilli-like projections on the luminal side of the term EC observed in the present study seems to increase the surface area thereby aiding any secretory

role. In samples of umbilical vein EC before 17 wk, the basal accumulations of vesicles may possibly contain and secrete the components of the internal elastic lamina.

Arterial EC were full of glycogen, unlike the vein, throughout gestation and in very reduced amounts in the vein at term. Glycogen was identified as occupying the space around the nucleus in both the EC and SMC. Glycogen has been shown to dissolve during the uranyl acetate block staining step of the fixation procedure and in transmission electron microscopy is identified by the existence of vacant areas within the cell cytoplasm (Zhou & Komuro, 1992).

There have been various suggestions as to the orientation of the SMC. Spivak (1943) suggested that two distinct layers existed: an internal longitudinal layer surrounded by an external circular layer. Boyd & Hamilton (1970) suggested that the arrangement is mainly longitudinal with intermittent circular layers, whereas Gebrane-Younes et al. (1986) proposed a predominant helicoidal layer in term vessels. In the present study, it has been shown that in the 8–12 wk arteries and vein the circular layer was dominant; however, in the term vessels there are clearly several orientations of the SMC layers although the precise arrangement is difficult to define, because of regional variations. This may be achieved using 3-dimensional reconstructions of the vessel from serial sections of confocal optical slices.

The SMC showed a change from the 'synthetic/secretory/proliferative' cell phenotype to the 'contractile' cell phenotype during gestation. A few synthetic SMC were observed at term in the muscle layer just below the EC. These cells may be undifferentiated cells remaining from early development, or a result of phenotypic modulation (a reversal of the contractile state) which occurs prior to proliferation, or as a result of arterial repair, hormonal stimulus, or because of alterations in the cell's environment (Campbell et al. 1981).

Moving away from the lumen to the outer region of the vessel wall, fibroblast-like cells were observed in all vessels. These cells have been described in detail by Takechi et al. (1993) as 'myofibroblasts', cells of Wharton's jelly, which act as an adventitial layer around all 3 vessels of the umbilical cord. Myofibroblasts have also been identified in the large vessels of the chorionic plate and stem villi of the human placenta by the presence of the same intermediate filaments; desmin, vimentin and keratin (Khong et al. 1986; Beham et al. 1988; Bradbury & Ockleford, 1990; Takechi et al. 1993). These intermediate filaments have also been found in the mesenchymal cells of the cores of the chorionic villi of the human placenta. It has been suggested that myofibroblasts may play a role in contractility or motility, affecting both maternal and fetoplacental circulation (Ockleford et al. 1993). Kohen et al. (1993) have suggested that 'myofibroblasts' are components of the extravascular contractile system as first described by Spanner in 1936.

Numerous membrane whirls were seen in the outer medial region in the term vessels, compared to a few in earlier vessels. These are probably caused by the retraction of the cytoplasmic membranes after the muscle cell had been stretched during relaxation; these were also observed in samples before 17 wk but were very much reduced in number. They are unlikely to arise due to degeneration since they were observed in this particular region only.

Throughout the whole of this investigation, neither nerves nor vasa vasorum were observed. If the umbilical vessels lack both of these components, common in all other blood vessels, the question arises as to how the smooth muscle cells are activated. Gebrane-Younes et al. (1986) suggested that the umbilical vessels structure is compatible with a liquid movement, therefore enabling substances to permeate easily through the vessel wall.

In summary, the vessel walls of the umbilical arteries and vein are similar in structure, both containing a large and active endothelial layer, which seems likely to be synthesising substances that are used in the local regulation of fetoplacental circulation and possibly even play a role in fetal physiology.

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