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Scanning electron microscopic investigations of the human umbilical artery intima

A new conception on postnatal arterial closure mechanism

G. Röckelein[1] **and A. Scharl**[2]

[1] Institute of Pathology, University of Erlangen-Nürnberg, Krankenhausstraße 8–10, D-8520 Erlangen Federal Republic of Germany
[2] Department of Obstetrics and Gynaecology, University of Cologne, Kerpener Straße 34, D-5000 Köln 41 Federal Republic of Germany

Summary. Umbilical cord arteries were investigated using a scanning electron microscope using different methods of preparation: Perfusion of one artery under pressure from a 100 cm water column caused artefacts and the preparatory work took at least 10 min after delivery. To shorten this time fully patent umbilical cords were double clamped and fixed immediately after birth. However, the removal of blood after fixation caused the endothelial layer to be lost. Therefore umbilical cords were double clamped, snap frozen and stored in liquid nitrogen until preparation. The endothelial lining of the fully patent umbilical artery at birth is composed of longitudinally arranged, spindle-shaped cells, connected by cellular junctions. The basement membrane contains numerous gaps. Because of these gaps postnatal vasoconstriction causes herniation of the subendothelial myofibroblasts forming subendothelial vacuoles. The vacuoles produce displacement of the endothelial cell cytoplasm towards the vascular lumina resulting in protuberances and blebs on the endothelial cells. Rupture of vacuoles leads to crater-like injuries.

Beneath the basement membrane a thin layer of myofibroblasts is arranged longitudinally. Oblique or transversely ordered bundles of myofibroblasts are interposed at wide and irregular intervals. These transverse bundles are able to trigger localized contraction rings called "folds of Hoboken", the initial stage of postnatal arterial closure.

Key words: Endothelium – Umbilical cord – Postnatal closure – Scanning electron microscopy

Offprints requests to: G. Röckelein

Introduction

After delivery the blood flow in the umbilical cord is rapidly interrupted by an active closure of the umbilical artery. The mode of this vasoconstriction is unique. Due to localized contractions of the muscularis numerous, oblique folds of the whole arterial wall ("folds of Hoboken") appear within the first minute after delivery. Spread of this contraction process leads to complete occlusion of arterial sections of varying length, separated by arterial sections full of arrested blood (Scharl 1986).

Our knowledge of the mechanisms responsible for the regulation of umbilical and placental perfusion and especially for closure of vessels at birth is still limited. The lack of nerves in the umbilical cord (Becker 1981) and the velocity of the contraction suggest humoral control. Vascular tone is regulated essentially by the endothelium (Furchgott and Zawadzki 1980; Furchgott 1983; Busse 1984; Busse and Bassenge 1984a, b; Förstermann 1986, 1987) and the structure of the arterial intima may thus determine the mode of vasoconstriction: Local changes may occur as contraction begins.

Since the conventional histological methods such as light and transmission electron microscopy are unsuitable for the investigation of extensive areas of endothelium, scanning electron microscopy was used in this study.

Materials and methods

Umbilical cords were obtained from infants born after normal pregnancies by vaginal delivery or by cesarean section for positional anomalies. Ergometrine was not given until after the cord had been clamped. A 10-cm length of the median third of the umbilical cord underwent the following procedures:

Fully patent cord specimens ($n=9$) were clamped at both ends simultaneously, cut off and fixed in formalin 5% at room temperature immediately after delivery. In the fixed vessels, however, blood could not be removed without injuring the endothelium.

One artery of umbilical cord specimens ($n=9$), double clamped and cut off immediately after delivery, was cannulated and perfused with buffered glutaraldehyde under pressure from a 100 cm water column. Perfusion started with the distal end open. After complete removal of blood the distal end was closed. The distended specimen was then fixed in buffered glutaraldehyde for 24 h. Because of the preparatory work fixation was delayed until at least 10 min after delivery, when postnatal arterial closure had already started. Perfusion caused focal damage of the endothelium.

In order to avoid these artefacts, preserve the fully patent arterial structure and arrest the postnatal vasoconstriction at different stages, umbilical cord specimens ($n=6$) were double clamped and cut off at varying time intervals ranging from 10 s to 2 min after delivery and snap frozen in liquid nitrogen. The specimens were cut into 1–2 cm length segments. By thawing in heparin containing physiological NaCl solution and dissecting the arteries longitudinally blood could be carefully washed away from the endothelium. The specimens were fixed in buffered glutaraldehyde.

In fixed samples at least one vessel was halved longitudinally (Fig. 1). Arteries with long-distance contractions were cross-sectioned. Both fully patent arteries and samples containing folds and long-distance contractions were dehydrated in alcohol, dried and covered with gold as described by Brunk et al. (1981). From 5 to 10 segments of each specimen were examined using a CamScan scanning electron microscope.

Results

Transverse, predominantly semilunar folds ("folds of Hoboken") were found at irregular intervals along the constricted umbilical cord arteries apparently caused by local contraction of the entire muscular coat (Fig. 1).

The endothelial lining of non-constricted umbilical arteries was composed of uniform spindle-shaped endothelial cells lying longitudinal to the vascular axis. Wide clefts often observed between them were bridged by regularly arranged cytoplasmic processes (Fig. 2b).

In contracted arterial segments many endothelial cells exhibited numerous finger-like blebs of their luminal cytomembrane. These blebs were absent or only rarely seen in non-contracted arteries indicating that they result from vasoconstriction (Fig. 2a, b, c). So-called subendothelial vacuoles were found in constricted arterial segments (Fig. 2c). Larger vacuoles, swelling up through the basement membrane directly underneath an endothelial cell, displaced the cytoplasm of the cell towards the vascular lumina thus giving it a cap-like appearance (Fig. 2b). However, when a vacuole protuded between two endothelial cells it tended to rupture forming a crater-like structure with edges consisting of burst cytomembranes (Fig. 3b). These endothelial "windows" are not real gaps or channels but are formed only after rupturing of the endothelial cells and their myofibroblasts. They effect a leak between blood and the arterial media. These changes were found in large numbers in all contracted arterial segments, but were most abundant in "folds of Hoboken".

Numerous gaps in the basement membrane (Fig. 3a) are essential for herniation of vacuoles towards the endothelium. Scanning electron mi-

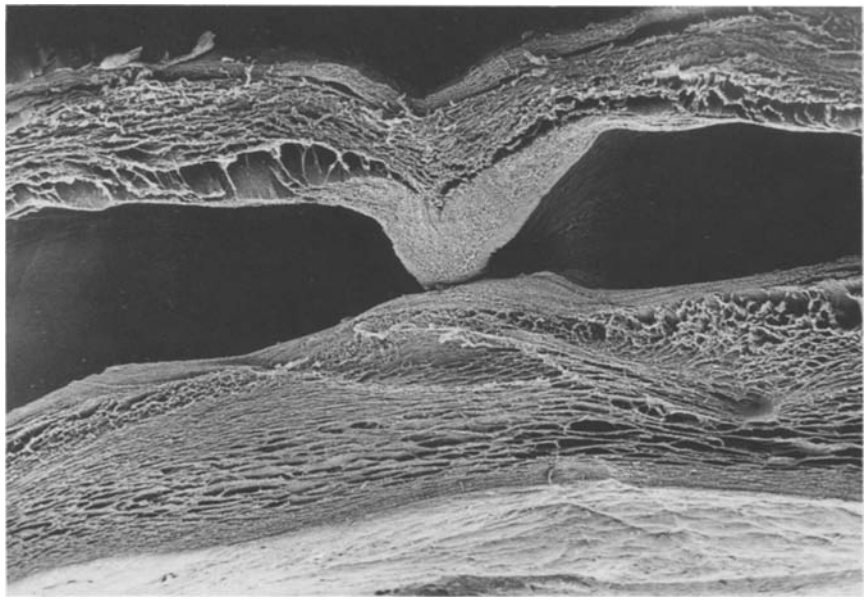

Fig. 1. "Fold of Hoboken": The localized one-side waist of the arterial wall is caused by contraction of the entire muscular coat (×6)

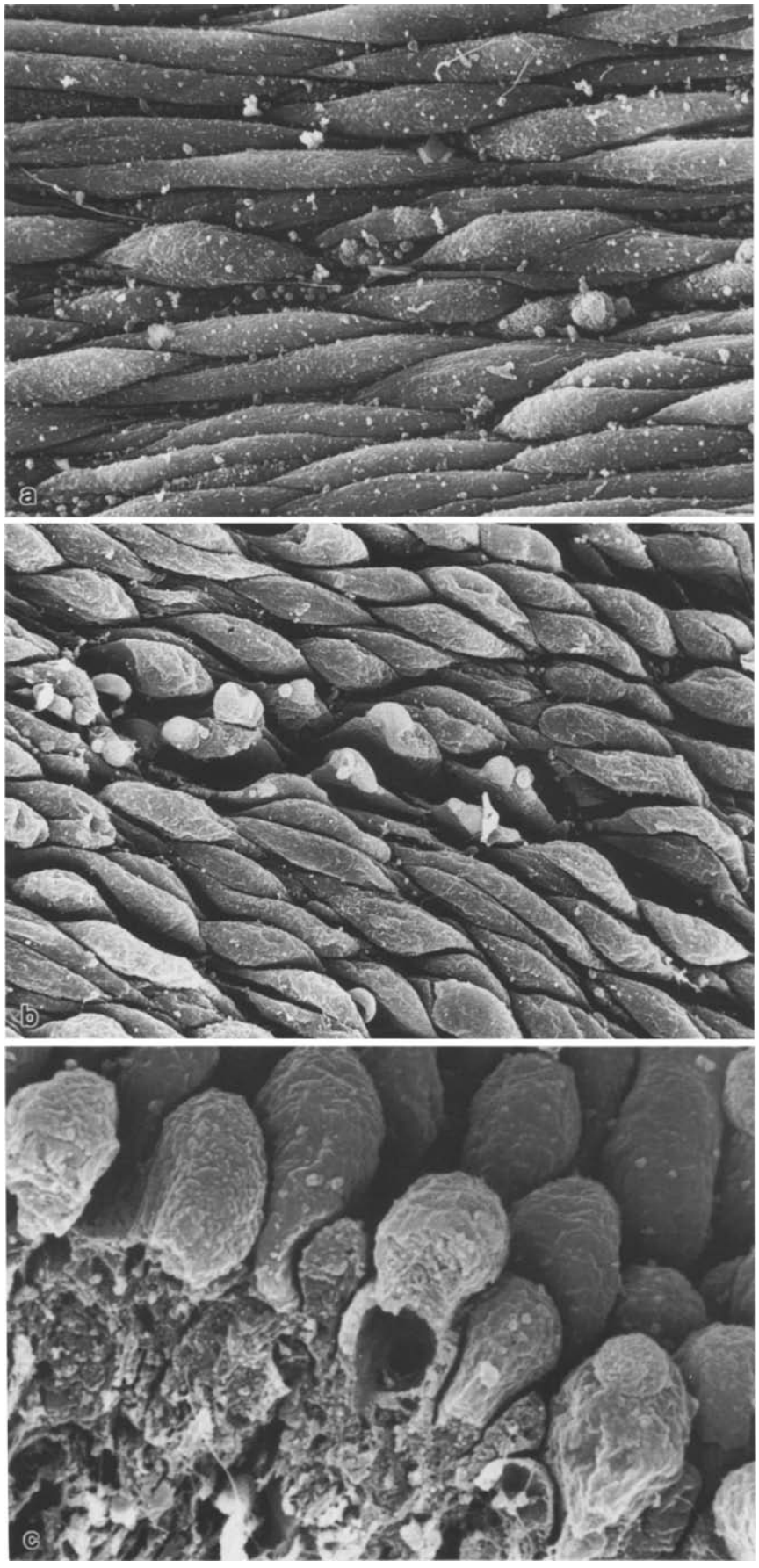

Fig. 2. Endothelium of umbilical cord artery: In the fully patent umbilical artery spindle-shaped endothelial cells are densely packed and arranged longitudinal to the vascular axis. (**a** ×760). In partly-contracted vessels endothelial cells are aggregated to clusters, their cytoplasms are pushed up towards the lumen and by that they assume a cap-like appearance (**b** ×570). In "folds of Hoboken" endothelial cells are displaced and rounded out towards the lumen by basal vacuoles. At the foot of the central vacuole its hernial opening through the basement membrane is visible (**c** ×1425)

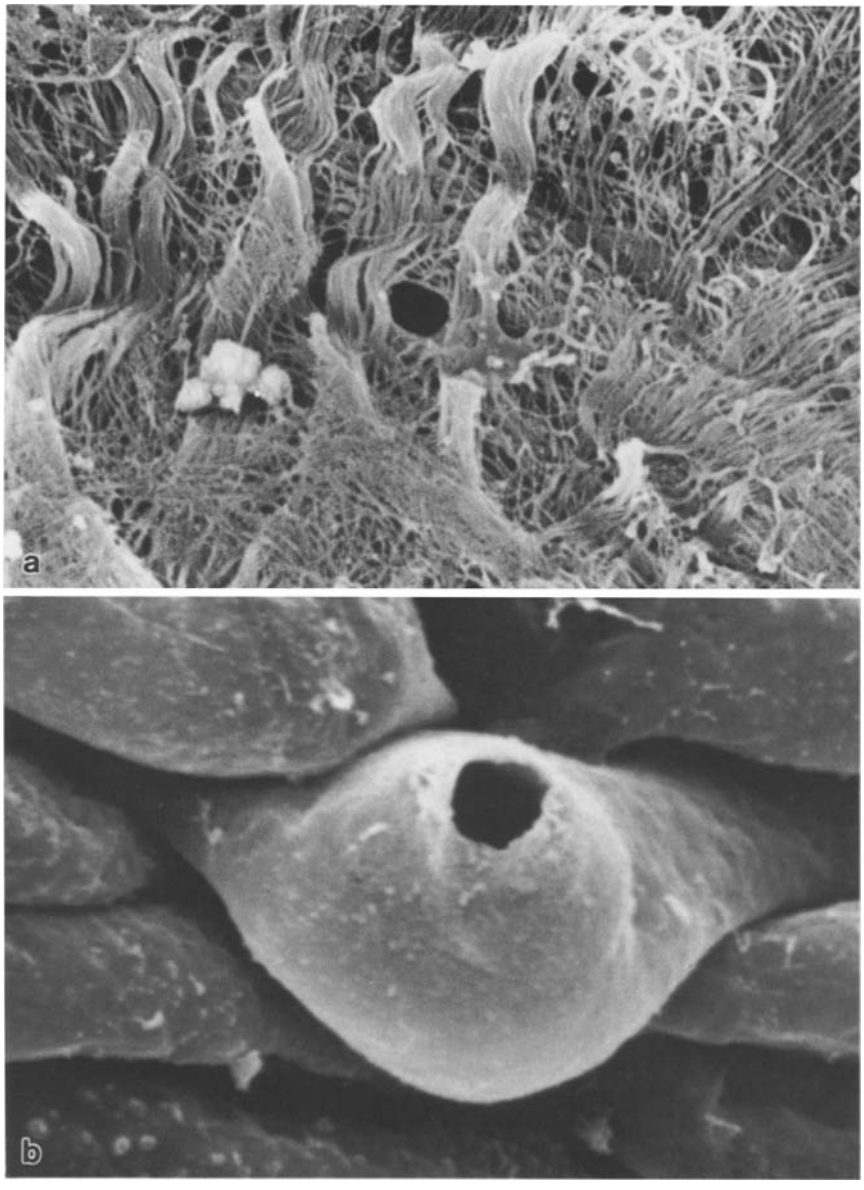

Fig. 3. Basement membrane of umbilical cord artery: The basement membrane is composed of a few fascicules of connective tissue fibers and contains numerous gaps with maximal diameters of 1 µm. Underneath subendothelial fibromyoblasts are visible (**a** ×1425). Vacuoles prolapse through the gaps of the basement membrane towards the endothelium and sometimes cause perforation of endothelial cells (**b** ×2850)

croscopy gave a view of a whole area of the basement membrane and revealed slit-like, oval and round gaps with maximal diameters of 1 µm (Fig. 3a).

A thin layer of spindle-shaped myofibroblasts was found immediately underneath the basement membrane. These lay almost exclusively longitudinal to the vascular axis. At wide and irregular intervals oblique or transverse bundles of myofibroblasts were interposed, often extening to cover a large part of the vessel circumference (Fig. 4).

In vessels, snap frozen immediately after delivery, deposits composed of fibrin and thrombozytes were observed on individual endothelial cells. These deposits were not extensive areas of thromboss, but were limited to individual cells. The appearance of these thromboses must be due to loss of the anti-thrombotic endothelial surface, which may be caused by cell degeneration (Fig. 5).

Discussion

Investigation of endothelial structures using scanning electron microscopy have been performed in almost every part of the human circulation (Motta et al. 1977; Johannessen 1980), but not in the vessels of the umbilical cord. This fact is not surprising since due to the rapid postnatal contraction

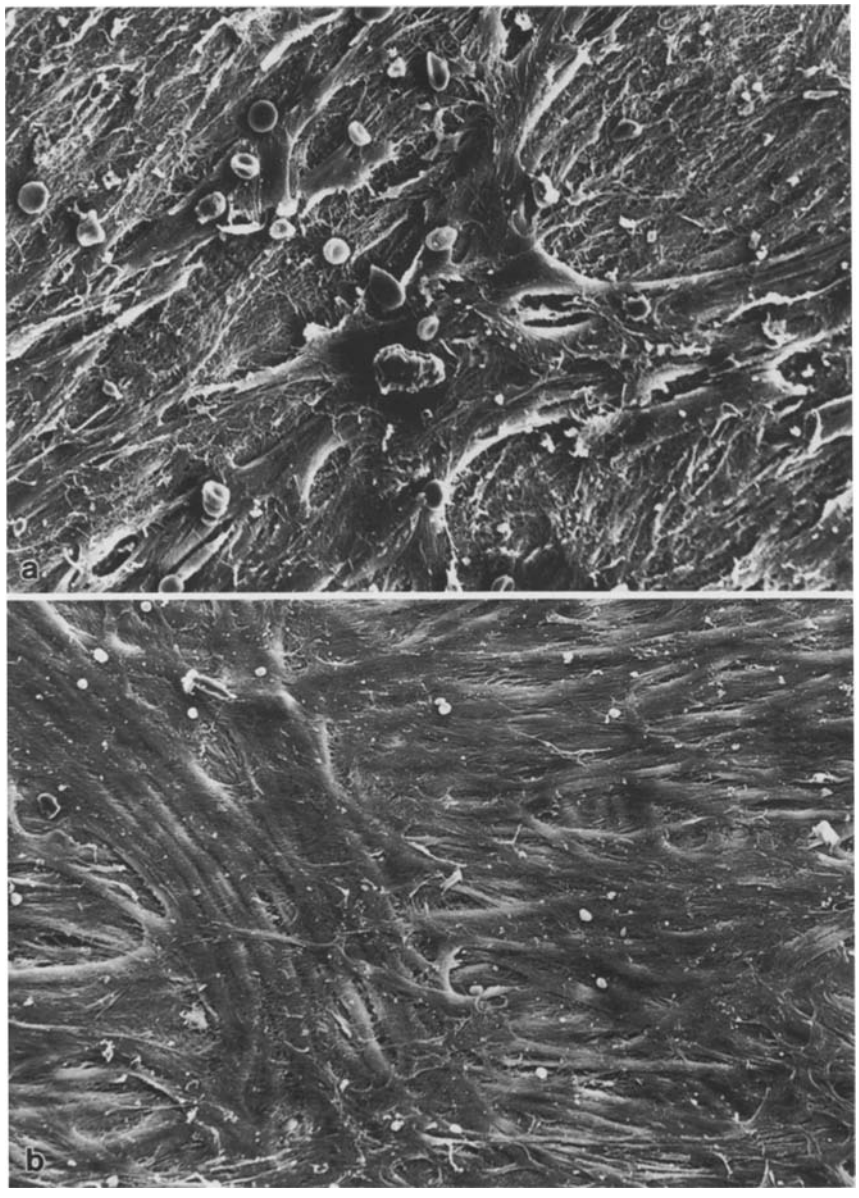

Fig. 4. Myofibroblasts of umbilical cord artery: Immediately underneath the basement membrane a thin layer of myofibroblasts is arranged almost parallel to the vascular axis. Oblique or transverse myofibroblast bundles of varying size are interposed at irregular intervals (× 57)

(Scharl 1986) it is difficult to obtain umbilical artery specimens free of artefacts. To investigate the structure of the fully patent umbilical artery endothelium the cord specimen must be obtained and fixed at once after delivery to capture the true state of the tissue before the beginning of the postnatal contraction. Immersion fixation is not useful since in vessels containing blood the endothelium cannot be preserved and in vessels free of blood vasoconstriction alters conditions. Perfusion fixation using physiological pressure is able to preserve vessels with normal diameter and free of blood. Even so, artefacts caused by mechanical damage or excessive dilatation must be expected (Röckelein and Hey 1985). Owing to postnatal arterial contraction, which is not fully reversible (Scharl 1986), fixation must occur rapidly post natum. Perfusion fixation, however, takes at least 10 min (Staubesand et al. 1984).

Snap freezing fixes the cord within a few seconds and thereby preserves the state of the tissue at the time of clamping. After thawing, muscle cells are no longer contractile. Thus blood can be removed without causing severe alteration of arterial structure or producing artefacts. This method allows sampling of both fully patent arteries and vessels at different stages of contraction determined by the time of clamping and freezing. By

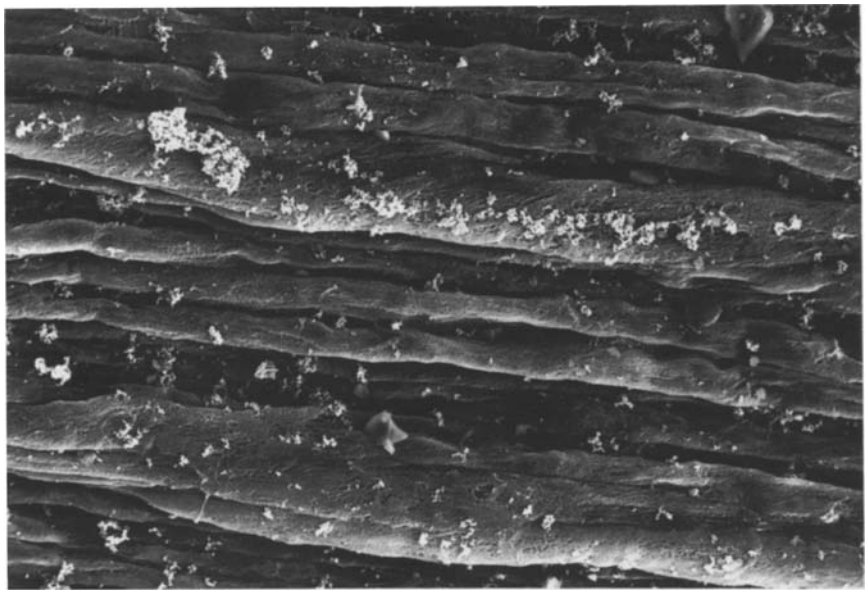

Fig. 5. Deposits of fibrin on endothelial cells: In snap frozen arteries delicate deposits composed of fibrin and a few thrombocytes are observed on individual endothelial cells (× 760)

that means we obtained specimens with no signs of initial contraction.

The endothelial lesions described by Asmussen (1979, 1980) and regarded as morphological evidence of maternal nicotine abuse, represent mainly myoendothelial hernia. This assessment is confirmed by the later observation that Asmussen's endothelial degeneration occurs just as frequently in umbilical cords from non-smoker pregnancies (Staubesand et al. 1984). Similar herniations, though fewer and less marked (Joris and Majno 1977, 1981), have also been observed in other human vessels, e.g. pulmonal vessels (Hall and Haworth 1986), especially in cases of pulmonary hypertonia with hyperplasia of the muscularis. Here also, their occurence is faciliated by the vascular architecture. Myoendothelial herniations are not in themselves pathological phenomena but result from the contraction of subendothelial muscle cells depending on special conditions of the vascular architecture (Las Heras and Haust 1981; Röckelein and Hey 1985).

"Folds of Hoboken" result from contractions of the whole double layered arterial media (Chacko and Reynolds 1954; Gebrane-Younes et al. 1986; Scharl 1986). Directly beneath the arterial intima a thin layer of myofibroblasts (Röckelein and Hey 1985) is arranged almost parallel to the vascular axis. This layer is interrupted at wide and irregular intervals by oblique or nearly transverse ordered bundles of myofibroblasts. Owing to their arrangement these transverse bundles are able to trigger the formation of "folds of Hoboken": Comparatively weak contraction results in a small fold which by conduction of the muscular spasm to the adjacent muscle cells of the media leads onto the typical waist.

The purport of this local constriction is to establish vessel barriers for rapid postnatal interruption of umbilical perfusion (Hughes 1966; Scharl 1986).

Our knowledge on the initial trigger of these local postnatal vasoconstrictions is very limited. With the exception of a few millimeters at the fetal end the umbilical cord is devoid of neural structures (Becker 1981). Regulation of the muscular tone of the umbilical arteries must therefore be based on non-neural, local or humoral mediator systems. Delivery changes the local environment of the umbilical cord. Regulation by the umbilical amniotic epithelium is therefore one possibility. However, the wide gap containing Wharton's jelly that lies between the amniotic epithelium and the muscular coat of the vessels makes this unlikely. Furthermore, if released by local reactions of the amniotic coat, contraction would be expected to occur in all umbilical vessels in the same manner and at the same location. This is not the case. On the contrary the "folds of Hoboken" are irregularly distributed (Scharl 1986).

There is a substantial body of data suggesting that release of vasoconstriction agents is localized in the endothelium, especially since vasa vasorum are lacking there (Roach 1973). Very active metabolism has been demonstrated in endothelial cells of the umbilical vessels (Gebrane-Younes et al.

1986). Endothelia are capable of producing enormous amounts of prostaglandins (Kawano and Mori 1983) and related substances, e.g. "endothelial derived relaxing factor" (ERDF). ERDF is released by intact endothelia onto the myocytes of the media and effects relaxation (Furchgott and Zawatzki 1980; Furchgott 1983; Busse 1984; Busse and Bassenge 1984a, b; Förstermann 1986, 1987). An impressive demonstration of maintainance of vasotonus by the endothelium was given by Furchgott and Zawadzki (1980) and Busse and Bassenge (1984). Perfusion of isolated vessel specimens with solution containing vasoconstrictive substances causes contraction when endothelial cells have been mechanically damaged and dilatation when endothelial cells are intact. Uninjured endothelium is able to convert the muscular reaction to a stimulus. Junctions between endothelial cells and myofibroblasts of the umbilical artery intima (Nikolov and Schiebler 1973) respresent the anatomical substrate of this functional myoendothelial unity.

Owing to their short half times neither prostaglandins nor related vasoactive substances can conceivably act systemically. Instead they act as local tissue hormones. The primary stimulus to the endothelium remains unknown.

Our investigation has revealed changes of the endothelia caused by vasoconstriction. In non-contracted vessel segments, however, no alteration of the endothelium was evident. The location of the "folds of Hoboken" must therefore be determined in other tissue layers. The subendothelial layer of myofibroblasts with its transverse fascicules may determine the position of the "folds". However, the irregular distances between these transverse fascicules are much shorter than between the final "folds of Hoboken" indicating that not each fascicule results in a "fold". It is more probable that only the stronger bundles are able to shorten vessel diameter and prime a "fold of Hoboken".

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