junctions (communicating junction, nexus)[8,20-29]. Tight junctions between arterial SMC have been demonstrated with a freeze-etching study only by Tani et al.[16] in arteries of Willis' circle in adult dogs. Gap junctions are thought to be composed of intercellular channels that mediate electrotonic coupling and the movement of ions and small molecules between cells (for review, see Bennett and Goodenough[30]). Details of the number and arrangement of gap junctions in smooth muscle effector bundles in different organs and their relation to density of innervation have not yet been determined. In conclusion, at the moment when muscular hyperplasia begins, the venous patch wall shows SMC ultrastructurally similar to the SMC of the wall of the host common carotid artery. The origin of the SMC observed in the venous patch wall has to be explored further using thymidine-labeling experiments.

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Gap junctions in myo-endothelial bridges of rabbit carotid arteries[1]

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Summary. Myo-endothelial contacts have been studied in rabbit carotid arteries. Structures, featuring as gap junctions, have been depicted by means of lanthanum hydroxide.

Cytoplasmic bridges passing through fenestrae of internal elastic lamina and apparently connecting endothelium to smooth muscle cells were first detected in coronary arteries by Thoma[2]. Similar structures have been revealed by light and electron microscopy in arteries, arterioles, precapillary sphincters and capillaries[3-9]. Nevertheless, their exact nature and functional significance have not yet been established. It is not clear whether these myo-endothelial bridges form specific contacts between the 2 cytotypes[9] or represent an anchorage of endothelium to the underlying vascular wall. Since different types of myo-endothelial bridges have been detected in large elastic arteries[10,11] we have chosen to investigate the ultrastructure of the intercellular contacts they establish between smooth muscle and endothelial cells in rabbit carotids by means of lanthanum hydroxide. With this technique we have detected pentalaminar structures featuring as gap junctions at sites where opposing membranes, belonging to a smooth muscle and to an endothelial cell, are joined very closely. The morphological and functional significance of these structures will be briefly discussed.

Materials and methods. New Zealand rabbits of both sexes weighing 2-3 kg were anesthetized with pentobarbital. A longitudinal laparatomy was made and a polyethylene catheter inserted into the abdominal aorta up to its thoracic tract. Then a perfusion with oxygenated Krebs-Ringer bicarbonate was started and blood washed out through the severed jugular veins that had been previously exposed. After the blood had been completely eluted, carotids were fixed with 2% gluteraldehyde solution buffered at pH 7.2 with 0.1 cacodylate for 20 min. All solutions were perfused at controlled physiological pressure and temperature. The samples were washed overnight, then immersed for 2 h at room temperature in a solution containing 1% osmium tetroxide and 1% lanthanum hydroxide. Then the tissue was dehydrated in an ascending series of alcohols and lanthanum hydroxide was added to all fluids up to the absolute alcohol. Once embedded in Epon 812, specimens were cut with the aid of an LKB ultramicrotom. Series of 10-15 thin sections with or without lead citrate counterstaining were collected and observed under a Philips 301 electron microscope.

Results. By light micoroscopy, cytoplasmic bridges, passing through the fenestrae of internal elastic lamina, were seen frequently, connecting the endothelium to the first row of smooth muscle in the underlying media. In random sections

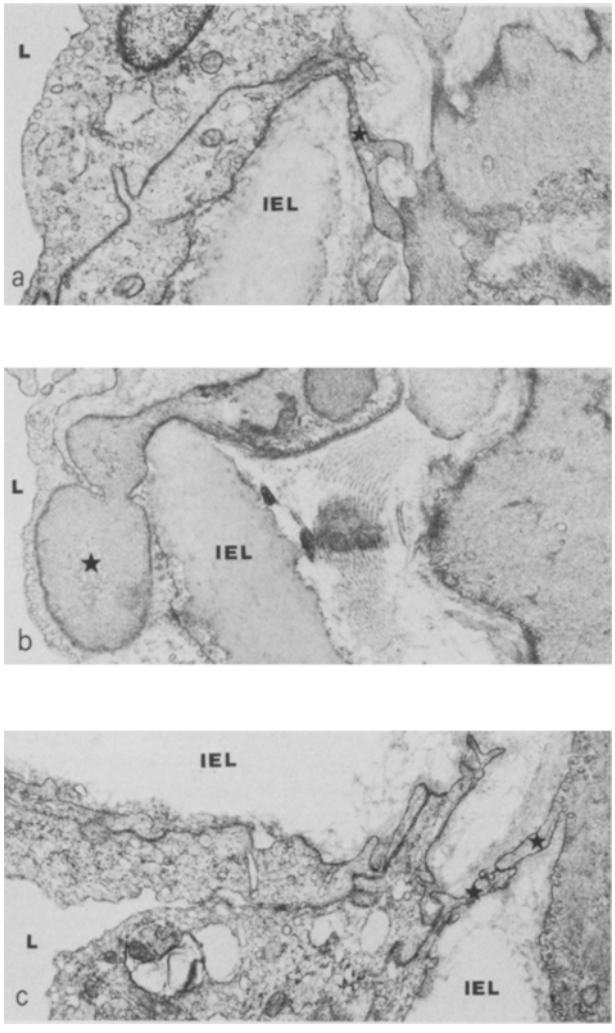

Figure 1. 3 patterns of myo-endothelial bridges can be depicted depending on their origin either from an endothelial cell (a, × 15,230) or a smooth muscle cell (b, × 16,400) or both (c, × 11,715). IEL, Internal elastic lamina; L, vascular lumen.

about 1 out of 4 fenestrae was occupied by an intercellular bridge. In electron microscopy the organization of these myo-endothelial bridges showed 3 patterns depending on their origin either from an endothelial cell or a smooth muscle cell or both (fig. 1a, b, c). It is worth noticing that even the myo-endothelial bridges originating from endothelial cells showed abundant filaments. At the site were the 2 cells joined the cytomembranes were separated by a distance of 120–200 Å without the interposition of the basement membrane. Lanthanum hydroxide permeated some of these junctional areas giving rise to pentalaminar patterns, characterized by a central electron dense line 50–70 Å thick separated by 2 thin electron lucent spaces from the internal leaflets of the opposing cytomembranes (fig. 2). Their length was between 700 and 1600 Å.

Discussion. This work has demonstrated that in large elastic arteries myo-endothelial bridges, like those in other vascular segments[3-9] establish close contacts between endothelium and medial smooth muscle cells. With the use of lanthanum hydroxide, as an electron dense tracer, the intercellular contact sites of some myo-endothelial bridges displayed a pentalaminar feature consistent with the gap junction structure reported by Revel and Karnovsky[12]. We do not have any morphometric data on the frequency of the gap junctions, but it is our impression that they are in a 1 : 1 ratio with myo-endothelial bridges. Given their modest dimensions, gap junctions could only be studied using serial sections.

In other tissues and in vitro cultures the gap junctions have been shown to be involved in intercellular transport of ions (electronic coupling) and of low molecular-weight substances (biochemical coupling) in which they act as low-resistance pathways[13,14]. Recently Sheridan[15] observed in microvessels a transfer of Lucifer yellow from endothelial cells to cells resembling SMC, thus showing that the 2 cells are biochemically coupled. Accordingly, our findings in large arteries seem to provide morphological support for the previous hypothesis that endothelium and smooth muscle cells act as a coupled system for the transmission of signals from receptors localized on the endothelial surface to the arterial wall and vice versa, from smooth muscle cells to the endothelium[9,16,17].

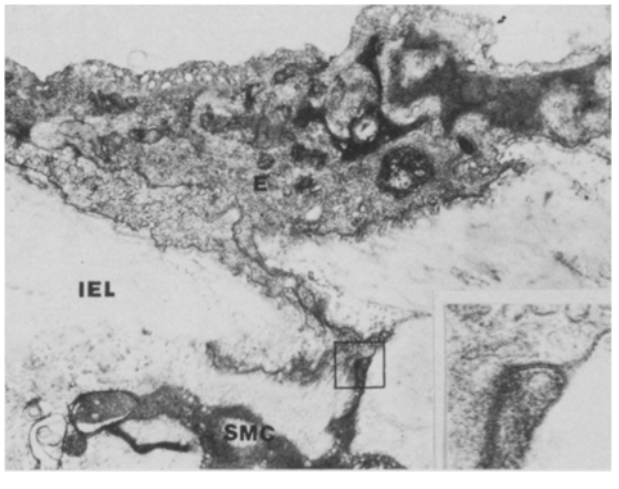

Figure 2. A gap junction revealed by lanthanum hydroxide (inset) at the site of contact between 2 cell processes arising respectively from an endothelial (E) and a smooth muscle cell (SMC) of the tunica media. IEL: internal elastic lamina. × 10,330, inset × 51,650.

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