features of this cell, i.e. the high number of free ribosomes and the relative scarcity of cytoplasmic organelles, suggest an early stage of differentiation. Successively, a certain differentiation takes place although advanced evolutional stages have not yet been observed. As far as the membrane-rich smooth endoplasmic reticulum is concerned, it is possible for it to be a transient device for producing a remarkable amount of membranes in a relatively early stage of cell differentiation: in this regard it has to be noted the abundance of membranes both in the I and in the II type mature cells; in the former, in fact, are typical and abundant the elongated curvilinear rough cisternae, in the latter, scattered vesicle- and tubule-like structures permeate the whole cytoplasmic area.

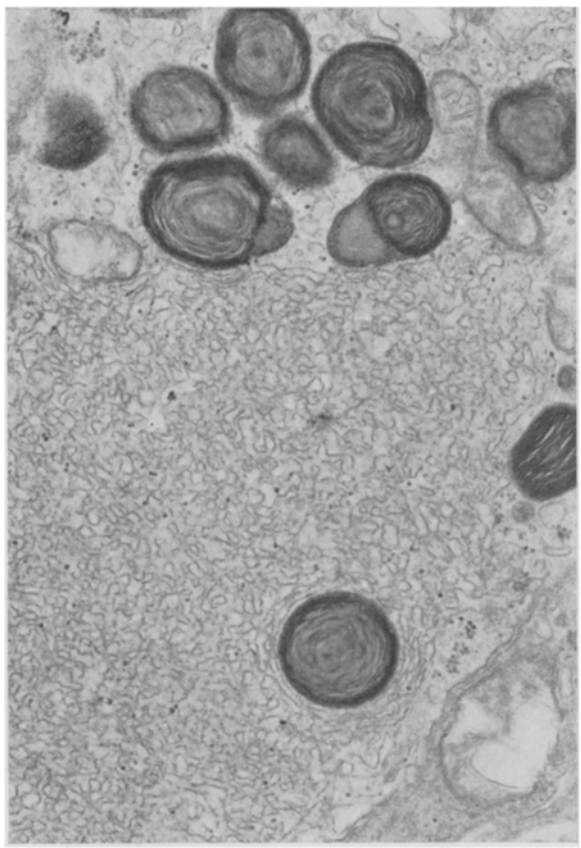

Fig. 3. Many dense bodies mainly formed by closely apposed membranes are clumped in the high part of the figure. Note the one immersed in the smooth endoplasmic reticulum and surrounded by smooth cisternae circularly arranged. Glycogen-like particles are interspersed among the smooth network. ×22,000.

The occurrence of pleomorphic and often concentric dense bodies in close topological relationship with smooth endoplasmic reticulum membranes suggests the possibility of their derivation from the smooth endoplasmic reticulum itself – as often occurs in Leydig cells in normal and experimental conditions[5]; notwithstanding, morphological aspects of gradual changes from whorls of smooth endoplasmic reticulum to dense bodies has not yet been observed. Furthermore, it must be remarked that concentric dense bodies quite similar to that described in the present report are described in the type I cells of the taste bud[3]. Beyond this fact, also the frequent occurrence of a reciprocal proximity of these 2 cell types within the taste bud suggests the hypothesis that the cells described may be a transitional cell type precursor of and evolving toward the mature I type cell of the taste bud.

Riassunto. Nella presente nota è brevemente analizzato l'aspetto ultrastrutturale di una cellula talora osservabile nel calice gustativo e caratterizzata da una notevole abbondanza di reticolo endoplasmatico liscio.

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Significance of Fixation Procedure for Preservation of Arteries

It has been shown that platelets and subsequently leucocytes adhere to subendothelial structures after removal of endothelium[1,2] or severe vascular injury[3]. In order to study particular phases of this dynamic process by electron microscopy, the fixation procedure should rapidly interrupt this process. During these experiments and experiments dealing with attachment and detachment of endothelium[4], we realized that the ultrastructure of arteries was highly dependent on how the initial fixative was applied.

Burgunder rabbits of either sex weighing 2.5–3.5 kg were anesthetized with Numal®. 2.5% glutaraldehyde in 0.1 *M* phosphate buffer, pH 7.4, 560 mOsm, at 20–22°C was used as the initial fixative and applied as follows: 1. Immersion: for 1–3 h. 2. In situ: fixative was dropped on the ouside of the clamped aorta in situ for 20 min. 3. Perfusion without pressure: Approx. 1 cm of the abdominal aorta near the renal arteries was dissected and ligated proximally. A silastic catheter was introduced to perfuse 10 ml of Krebs-Ringer's solution followed by the fixative for 20 min. Simultaneously the iliac arteries were opened. The containers with the Krebs-Ringer's solution and the fixative were placed 100 cm above the aorta. 4. Perfusion with pressure: Same as 3, but without open-

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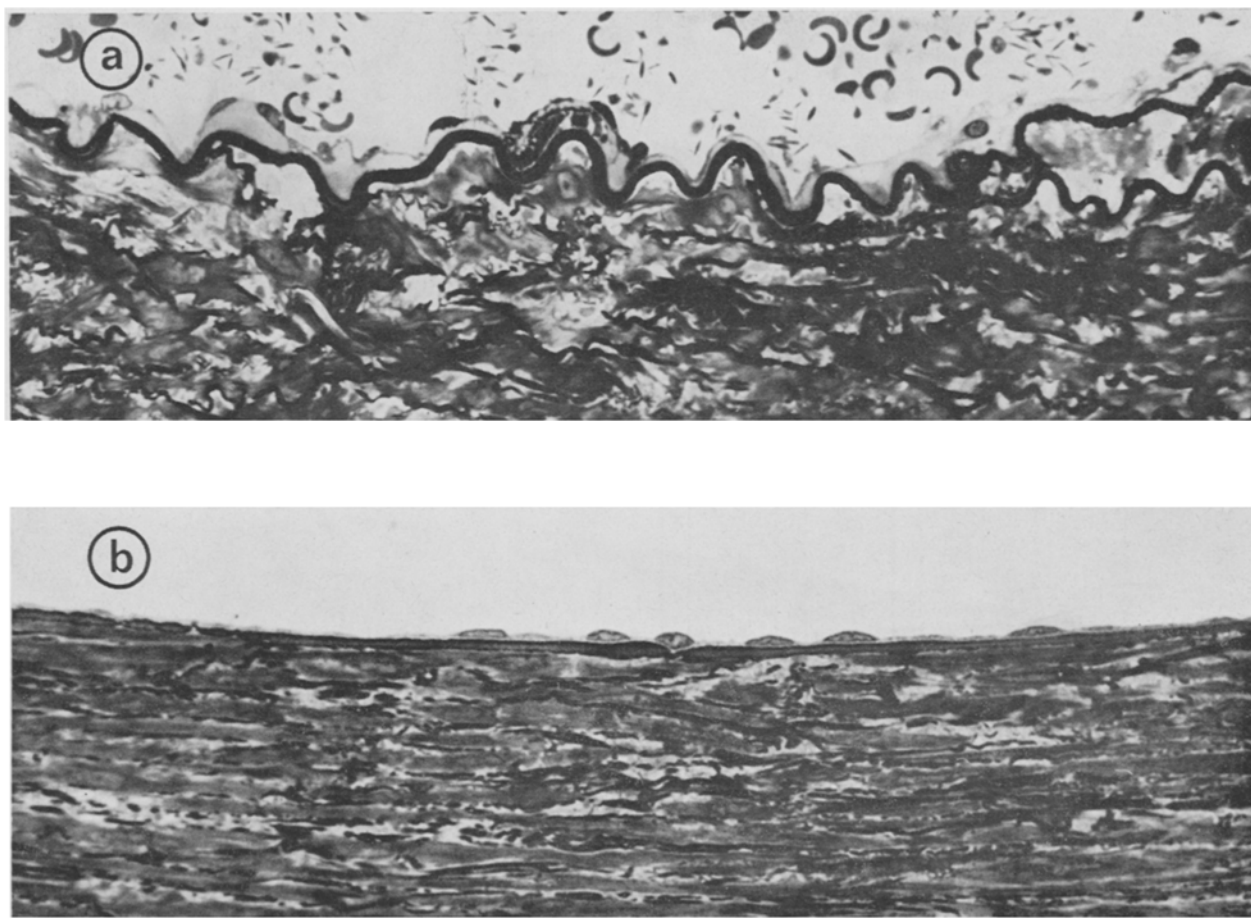

Fig. 1. Light micrographs of rabbit aorta. a) Fixation in situ from outside. Wave-like elastic laminae, partial detachment of endothelium, numerous platelets and erythrocytes in the lumen but not adhering to the wall. b) Fixation by perfusion with defined pressure. Straight elastic laminae, flat endothelium which tightly adheres, empty lumen. 0.8 µm thick Epon sections. Basic fuchsin and toluidine blue[3]. ×900.

ing the iliac arteries. 5. Perfusion with defined pressure: 1 cm of the abdominal aorta near the renal arteries and 1 cm at the bifurcation were dissected, leaving about 7 cm of the vessel untouched. A silastic catheter was introduced proximally and the vessel perfused with Krebs-Ringer's solution. During this rinsing, a second silastic catheter was introduced distally. The outflow of this catheter was placed 60 cm above the aorta (corresponding to approx. 82 mm Hg pressure). The vessel was subsequently perfused with the fixative for 20 min. The containers with the Krebs-Ringer's solution and the fixative were placed 100 cm above the aorta (corresponding to approx. 136 mm Hg). The dissected vessel segments after procedures 1–5 were subsequently immersed in the same fixative for 1 h, stored overnight at 4°C in 0.1 *M* phosphate buffer containing 7% sucrose, postfixed for 1 h in 2% osmium tetroxide in 0.1 *M* phosphate buffer at room temperature, dehydrated with graded ethanols and embedded in Epon 812.

Fixation procedures 1, 2 and 3 yielded wave-like elastic laminae (Figure 1a), whereas procedures 4 and 5 resulted in straight elastic laminae (Figure 1b). This confirms recent histometrical investigations of Guski et al.[5] on formaline-perfused rabbit aortas. They found straight elastic laminae and a decrease of the thickness of the media by 46% with a mean perfusion pressure of 100 mm Hg. In our hands 82 mm Hg was sufficient to obtain straight elastic laminae. This is near the average diastolic pressure of 80 mm Hg measured in our anesthetized rabbits. These data indicate that the elastic laminae of the abdominal aorta are stretched in vivo. Hence the wave-like appearance of elastic laminae of arteries commonly seen in anatomy and pathology textbooks is considered to be a fixation artefact.

Another parameter for studying the effect of the initial fixation procedure was the inner circumference of semithin sections cut perpendicularly to the vessel axis. It was measured with a calibrated ocular in steps of 100 µm. Figure 2 shows that initial perfusion with defined pressure yielded significantly wider inner circumferences than perfusion without pressure. The shortest inner circumference occurred with fixation procedure 2. Contraction of the aorta which was observed during dissection may account for this. Hence traumatization, as well as collapse due to low perfusion pressure, should be avoided in order to get optimally preserved arteries.

The ultrastructure of endothelium was also highly dependent upon the initial fixation. Especially immersion,

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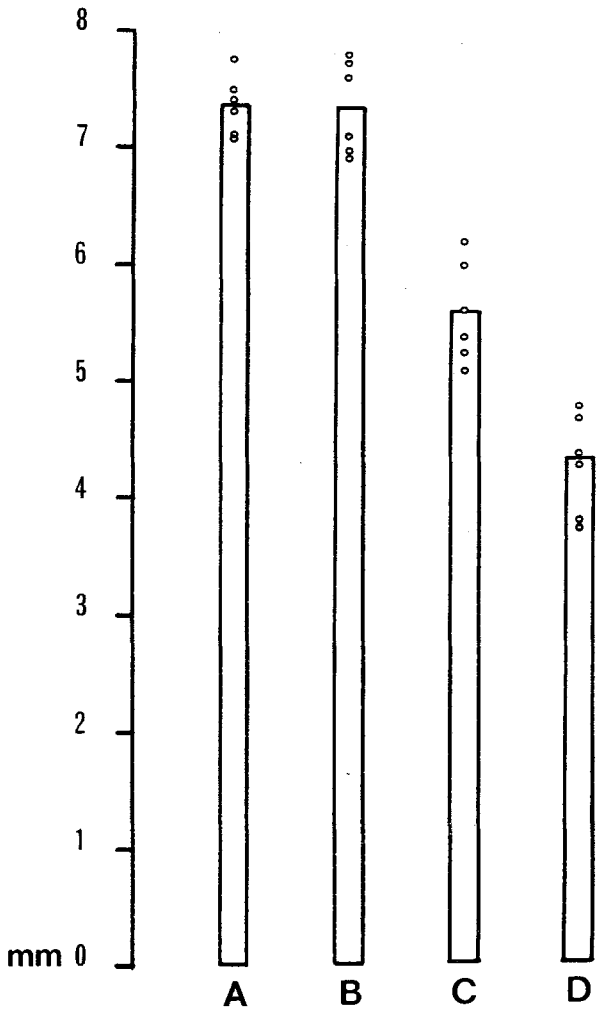
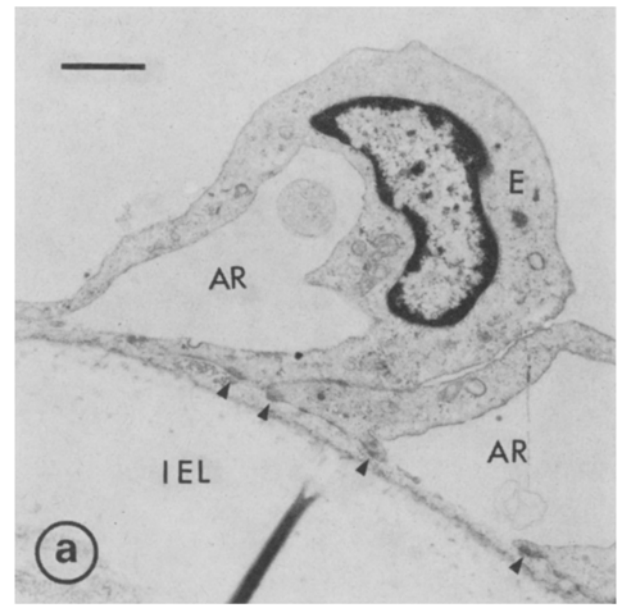
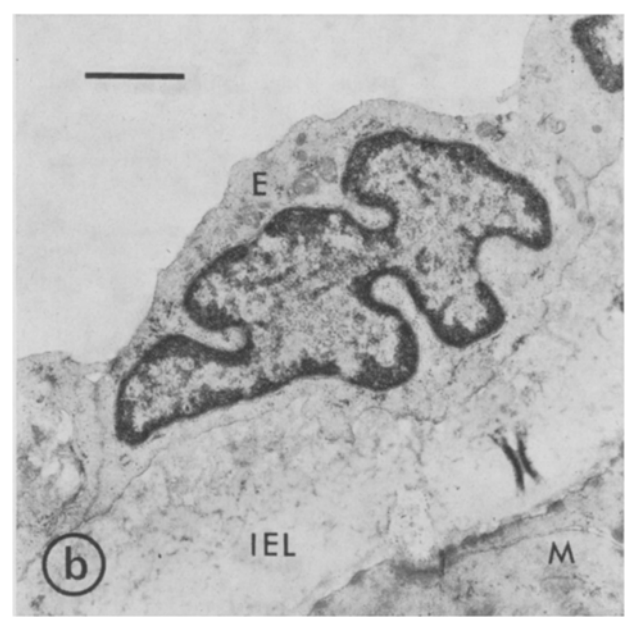


Fig. 2. Inner circumference of rabbit aortas. 3 rabbits were used for each experiment. a) Fixation by perfusion with pressure of 82 mm Hg. b) Fixation by perfusion with pressure resulting from peripheral resistance. c) Fixation by perfusion without pressure. d) Fixation in situ from outside.





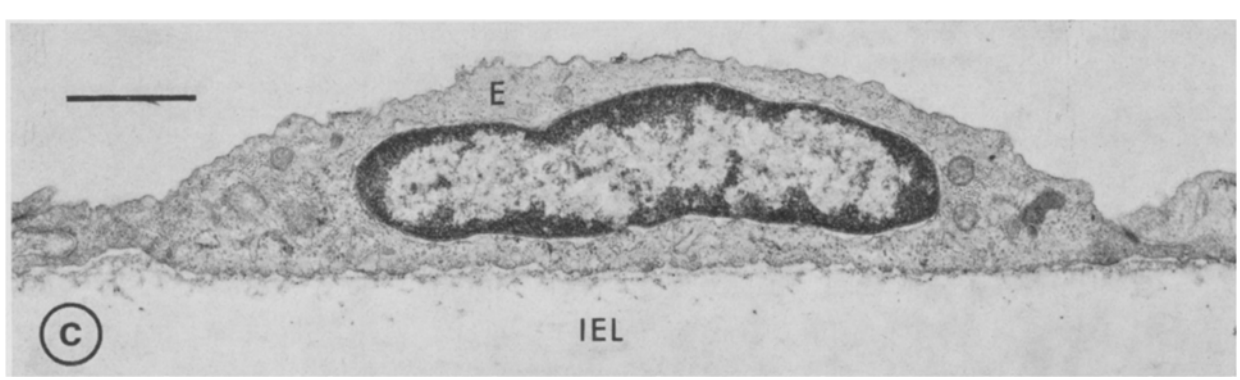


Fig. 3. Electron micrographs of endothelial cells (E) of rabbit aortas. a) Fixation in situ from outside. Endothelial cells (E) bulge into the lumen (on top) and form 'arcades' (AR). Small electron dense zones (▶) of cytoplasm are seen at adhering sites, resembling those found by Ts'AO and GLAGOV[6] after partial ligation of rabbit aortas. Internal elastic lamina (IEL). b) Fixation by perfusion without pressure. Contracted endothelial cell (E) with infoldings of nucleus. Smooth muscle cell (M). c) Fixation by perfusion with defined pressure. Flat endothelial cell (E) with elongated nucleus is tightly attached at the internal elastic lamina (IEL) and other subendothelial structures. Uranyl acetate and lead citrate. Bar = 1 μm.

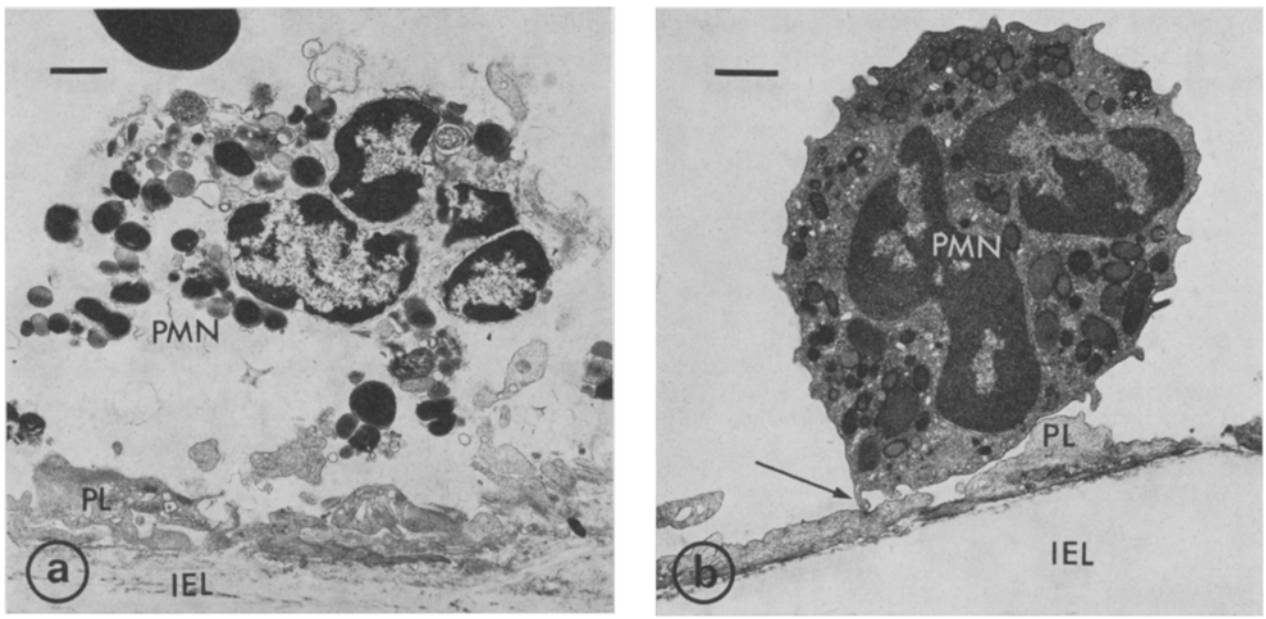


Fig. 4. Electron micrographs of rabbit polymorphonuclear leucocytes (PMN) adhering to the wall of the aorta after remova lof endothelium. a) Fixation in situ from outside. The leucocyte (PMN) is disintegrated, its outer membrane has disappeared and its granules are dispersed. Relationship of the PMN to the platelets (PL) is poorly defined. b) Fixation by perfusion. The leucocyte (PMN) is intact and adheres to the platelets (PL) by a small pseudopod (arrow). Internal elastic lamina (IEL). Uranyl acetate and lead citrate. Bar = 1 μm.

but also fixation from outside, resulted in endothelial cells which bulged into the lumen and were often partially detached from the subendothelial structures, forming 'arcades' (Figure 3a). In contrast, endothelium fixed by perfusion (procedures 3, 4 and 5) always adhered tightly to the subendothelial structures. Without pressure (procedure 3) endothelial cells were thicker and had infolded nuclei (Figure 3b). These are considered signs of contraction of endothelium[7]. We presume that this contraction most probably occurred coincidently with a contraction or a collapse of the whole aorta. The most regular aspect of endothelium was obtained with procedure 5: Endothelium was mainly flat (Figure 1b) and revealed numerous structural details (Figure 3c). These data demonstrate that fixation by perfusion is optimal for the ultrastructural preservation of endothelium and that the degree of contraction in which endothelial cells of untreated vessels are preserved depends on the applied perfusion pressure.

Preservation of particular phases of pathophysiological processes at the inner surface of vessels such as adhesion of blood cells at the damaged vessel wall[8] might especially depend on how fast the initial fixative comes into contact with these cells. To test this assumption, endothelium was selectively removed from aortas[1] in order to induce adhesion of platelets and subsequently leucocytes at the damaged vessel wall. 3 h later the aortas were fixed. Leucocytes in aortas fixed from outside were often in a state of disintegration (Figure 4a), whereas perfusion-fixed leucocytes were intact and had small pseudopods by which they remained attached despite the perfusion of the fixative (Figure 4b). Corresponding experiments in iliac and carotid arteries yielded similar results.

Our data indicate that, in arteries fixed by immersion or in situ from outside, the following morphologic findings may represent fixation artefacts: 1. Narrowing of the lumen reflected by a decrease of the inner circumference and by wave-like elastic laminae, 2. partial detachment, forming of 'arcades', contraction and ill-defined ultrastructure of endothelial cells, 3. disintegration of leucocytes adhering after removal of endothelium. The endothelium of arteries as well as pathophysiologic processes such as leucocyte adhesion to platelets and subendothelial structures were best preserved by initial perfusion fixation at adequate pressure.

Zusammenfassung. Die Morphologie der Aortenwand, die Ultrastruktur des Endothels und der nach Endothelentfernung haftenden Leucocyten wurden am besten erhalten durch Perfusionsfixation mit einem Druck von ca. 80 mm Hg. Demgegenüber führten Immersionsfixation und Fixation in situ durch Auftropfen von aussen zu Einengung des Lumens, wellenförmigen elastischen Lamellen, teilweiser arkadenförmiger Ablösung von Endothelzellen, Kontraktion von Endothelzellen sowie zu Lyse von Leukocyten, die nach Endothelentfernung an der Gefässwand haften.

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