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LUMINAL CONSTRICTIONS ON CORROSION CASTS OF CAPILLARIES AND POSTCAPILLARY VENULES IN RAT EXOCRINE PANCREAS CORRESPOND TO PERICYTE PROCESSES+

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Abstract

The rat exocrine pancreas was studied as a model to demonstrate morphological features of different types of capillaries, using scanning electron microscopy of vascular corrosion casts and transmission electron microscopy of tissue sections. Two types of capillaries were discerned. The first type represents less undulated, slender, straight capillaries with numerous, shallow, circular or semilunar furrows on its cast's surface. In tissue sections, this type probably corresponds to non-fenestrated capillaries. The numerous grooves on its cast correspond to pericyte processes beneath the endothelial lining. The second type comprises capillaries of an undulated course and variable diameter with less numerous furrows. In addition, these casts showed circumscribed, smooth surfaced bulging areas defined by the grooves described. In tissue sections, this type probably corresponds to fenestrated capillaries, the bulging areas on its cast correspond to fenestrated regions of the endothelium. Fenestrated areas of capillary endothelium are less reinforced; pericyte processes are not present beneath these regions in tissue sections. The hypothesis that pericyte processes are responsible for surface indentations on capillary casts was supported by observations on postcapillary venules. Casts of these vascular segments showed also numerous circularly running furrows. Accordingly, the wall of postcapillary venules is provided with pericytes while smooth muscle cells are missing.

+In commemoration of Professor Dr. Joseph Hyrtl, 1810-1894.

Key words: Pericytes, corrosion casting, capillaries, veins, scanning electron microscopy, transmission electron microscopy, rat.

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Introduction

The morphological background of venous sphincters, occurring as drastic constrictions on cast preparations caused by bundles of circularly running smooth muscle cells, is now well established (Aharinejad *et al.*, 1990, 1991, 1992; Schraufnagel and Schmid 1988a, b; Schraufnagel and Patel, 1990). These sphincter constrictions are pronounced, wide, and drastic furrows which may reduce the cast's diameter down to 50% the original width (Aharinejad *et al.*, 1992). In this manner, they differ from the multiple shallow and narrow groove reliefs, circular or semilunar, described on capillary and venous casts surface in a series of recent papers (Aharinejad *et al.*, 1990; Castenholz, 1991; Christofferson and Nilsson, 1988; Konerding *et al.*, 1988; Nakai *et al.*, 1989; Ohtani and Gannon, 1982; Ohtani *et al*, 1986; Schraufnagel and Schmid, 1988a, b; Schraufnagel and Patel, 1990; Syed Ali, 1984). These surface details have been tentatively interpreted as being caused by pericytes (Castenholz, 1983; Ohtani *et al.*, 1986; Syed Ali, 1984). When studying the microvascular bed of the exocrine pancreas in rats, we observed circular smooth muscle cells in the regions of venous sphincters (Aharinejad *et al.*, 1990) but smooth muscle cells were absent on capillaries. Hence, most probably pericytes are spatially related to the shallow grooves observed on capillary casts (Aharinejad and Böck, 1991). As further structural details, many capillary casts show circumscribed areas bulging into the tissue, areas which are defined by similar surface indentations as mentioned above. In the reticular zone of rats adrenal gland, such capillary protrusions in corrosion casts were discussed as fenestrated areas of the endothelial lining (Nakamura and Matsuda, 1981). However, both these interpretations still require critical examination.

In the present paper the rat exocrine pancreas served as a model for demonstrating protrusions and grooves on capillary corrosion casts, as well as similar grooves and constrictions on initial and collecting veins. Microvascular corrosion casts were studied by scanning electron microscopy (SEM), while the wall composition of

corresponding vascular segments in tissue sections was analyzed by means of transmission electron microscopy (TEM).

Material and Methods

Scanning electron microscopy

Five Sprague Dawley rats of both sexes (weighing 200-220 g) were anesthetized with sodium pentobarbital (intraperitoneally, 40 mg/kg body weight) and the abdomen and thorax were opened by a median cut. We cannulated the thoracic aorta with a plastic catheter (Argyle 0.8 x 19 mm, Sherwood Medical, St. Louis, MO, USA), connected the cannula to a two-way connector (LS2, Braun-Melsungen, Germany), and rinsed the circulatory system with 37 °C heparinized Tyrode solution (5,000 IU/l) until the efflux of the incised caudal vena cava was clear. Then, 16 ml Mercox CL-2B (Dainippon Ink & Chemicals, Tokyo, Japan) diluted with 4 ml monomeric methylmethacrylate (Hodde, 1981), was injected through the aorta. Animals bodies were left two hours at room temperature, thereafter put in a 60 °C water bath overnight, and then macerated in 15% KOH solution at 40 °C for 2 days or longer. The specimens were cleaned in 2% formic acid and several passages of distilled water and freezed in a small volume of the latter. Most of these frozen cast preparations were cut at -20 °C with a specially adapted circular saw into 1-2 mm thick slices (Aharinejad *et al.*, 1991). Thereafter, all the frozen specimens were freeze-dried and mounted onto copper foils with silver paste using the conductive bridge method of Lametschwandtner *et al.* (1980, 1990). Mounted cast preparations were evaporated with carbon for 3 seconds and then sputter-coated with gold for 600 seconds (Aharinejad *et al.*, 1989). The thickness of the resulting layers was about 10 nm. Observation was performed with a Cambridge 90 M SEM operating at an accelerating voltage of 15 kV.

Islets of Langerhans were identified on account of their particular vascular bed. Endocrine capillaries are tortuous in shape and wider, mean diameter 12 μm, than exocrine capillaries which show a mean diameter of 7 μm (Ohtani *et al.*, 1986). In addition, the capillary bed in the islets of Langerhans is often clearly separated from the microvasculature of the exocrine pancreas by a boundary region (peri-insular exocrine portion) which is nearly free of capillaries (Böck, 1989). In this peculiar zone the insulo-acinar connections are found.

Transmission electron microscopy

Five rats of the same weight category were used. Perfusion fixation was performed with 2.5% glutaraldehyde in 0.2 M phosphate buffer, small tissue blocks were removed and immersion fixed in the same fixative for additional 2 hours. After washing in phosphate buffer, postfixation was carried out with 1% osmium tetroxide for 2 hours, followed by dehydration in ethanol and embedding in Epon 812. Thin sections were cut on a Reichert OmU2 ultramicrotome, double stained with uranyl acetate and lead citrate, and studied in a Zeiss EM 9-S2 TEM.

For statistical evaluation of pericytes and different types of capillaries, four additional rats of both sexes (same weight category as described above) were used. After perfusion fixation, eight tissue blocks were prepared from the pancreatic tail and body. From each block ten ultrathin sections were cut and analyzed in TEM. The student t-test was used for the statistical analysis.

Fig. 1. Low magnification scanning electron micrograph showing the cast capillary network of the rat exocrine pancreas. Two types of capillaries can be discerned. The first type is more delicate and takes a rather straight course (arrowheads), the second type is undulated with circumscribed smooth surfaced dilatations (arrows). Calibration bar = 50 μm.

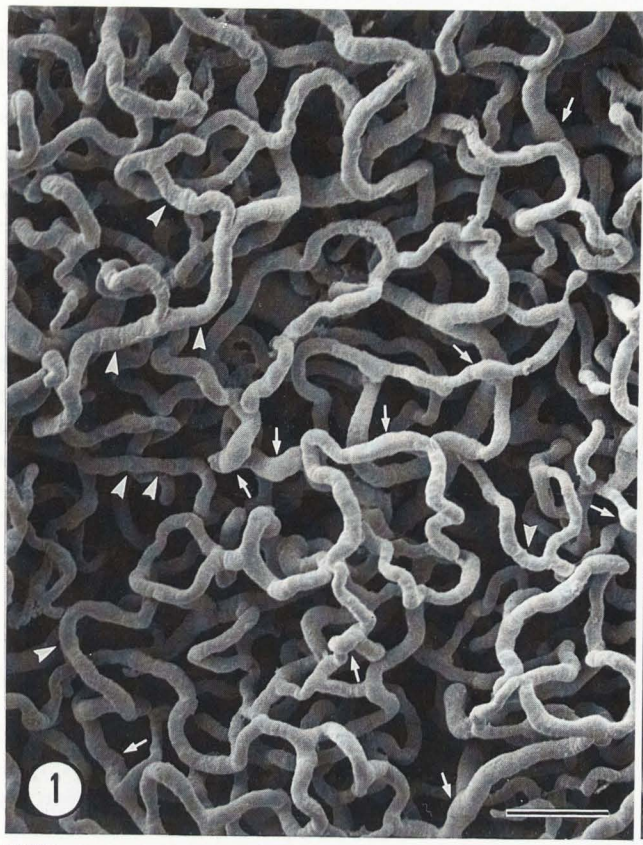
Fig. 2. Higher magnification micrograph shows the circumscribed bulging segments in the capillary network (arrows). Accentuated circular grooves or furrows are seen on the surface of nearly all capillary casts. Calibration bar = 15 μm.

Fig. 3. Transmission electron micrograph showing a non-fenestrated capillary in the rat exocrine pancreas. This type of capillary is well provided with pericyte processes (p). Note the absence of any connective tissue fibers in spatial relationship to the capillary wall. Calibration bar = 1 μm.

Fig. 4. Transmission electron micrograph showing a fenestrated capillary in the rat exocrine pancreas. Fenestrated areas (f) of the endothelium are devoid of pericyte processes. In these regions, the capillary wall merely consists of a thin endothelial layer and its basal lamina. Connective tissue fibers are missing. Insert shows that pericyte processes are located beneath non-fenestrated endothelial segments (nf) of this type of capillary. Calibration bar = 1 μm.

Results

The acinar capillary network of the rat exocrine pancreas reveals two types of capillaries (Fig. 1). First, there are less undulating capillaries which show series of shallow surface constrictions. These capillaries measure 7-10 μm in diameter. Second, there are more undulating capillaries which reveal similar but less frequent constrictions on the cast's surface. This latter type of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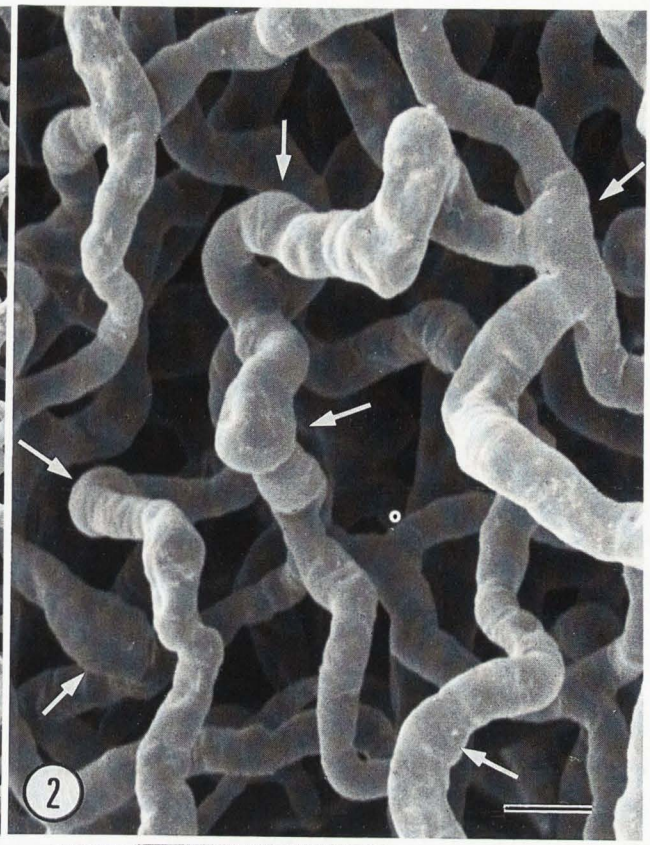
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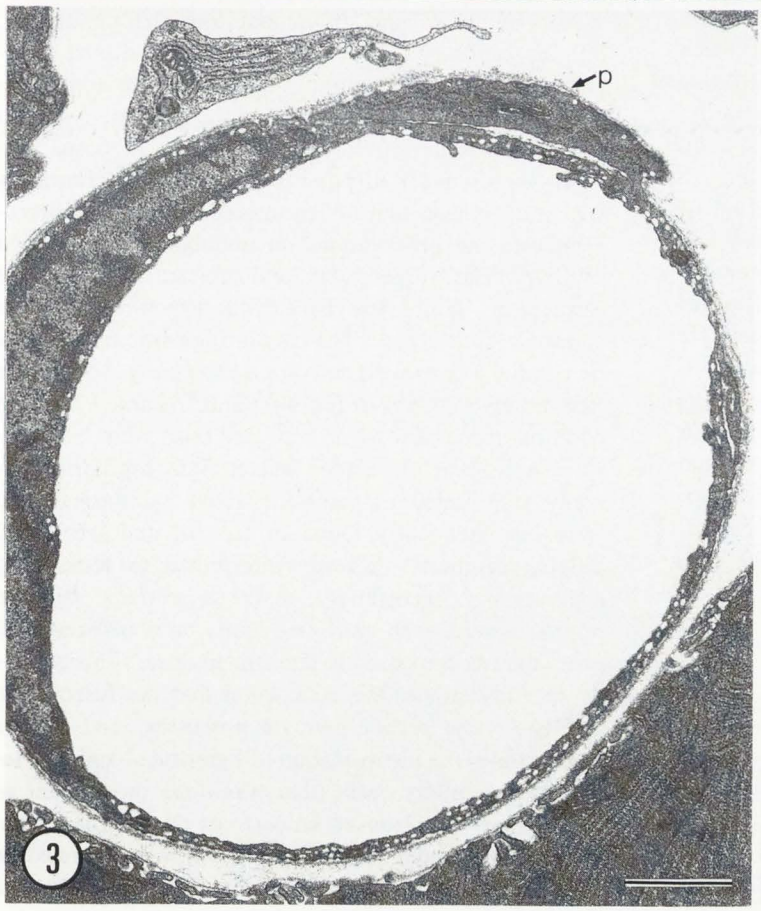
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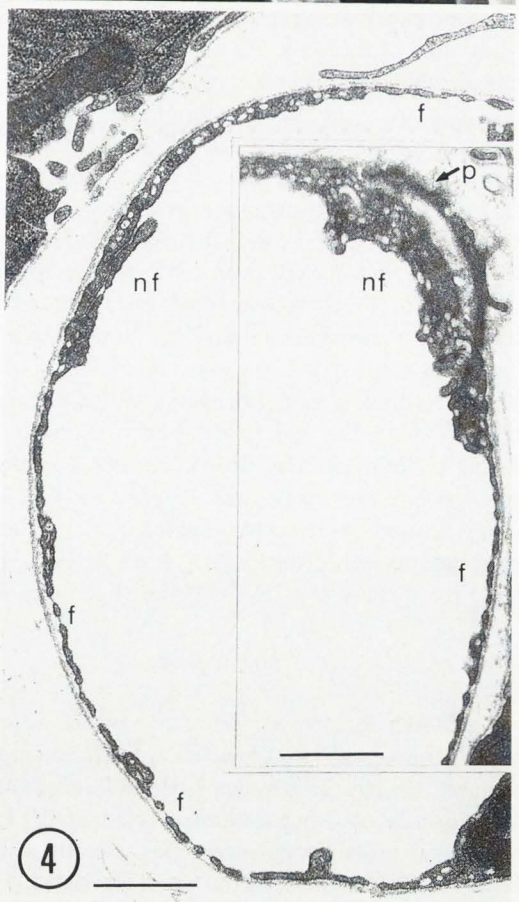
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capillary offers smoothly surfaced circumscribed flat protrusions or dilatations, defined by the grooves mentioned above. These bulging areas are the reason for the varying diameter of this capillary type, measuring between 7 and 12 μm (Fig. 2).

In corresponding tissue sections of the rat exocrine pancreas, two types of capillaries can also be discerned. The first type comprises non-fenestrated capillaries, well provided with pericyte processes (Fig. 3). The second type represents capillary profiles with fenestrated and non-fenestrated endothelial segments, alternatively forming the wall circumference (Fig. 4). Non-fenestrated capillaries were observed less frequently, i.e., 27.5%. In addition, pericytes were more frequently noted in the wall of non-fenestrated capillaries (2.5 $\pm$ 1.3%) than in fenestrated ones (1.3 $\pm$ 0.7%). In fenestrated capillaries (72.5%) the rare pericyte processes were regularly positioned external to non-fenestrated regions of the endothelial lining (Fig. 4) but not beneath the fenestrated areas.

In the rat pancreas, microvascular casts of postcapillary venules and small collecting veins also revealed multiple surface indentations similar to the reliefs described on capillary casts (Fig. 5). A further attempt was undertaken to compare the microcirculatory pathways of the periductular vascular plexus with that of the exocrine pancreas. The periductular vasculature differs from that of the exocrine pancreas in showing much more anastomosing venous vessels. These veins are also provided with circular and semilunar surface constrictions, some of which appeared deeper than those on capillaries (Fig. 6).

In addition to comparison of surface morphology of capillary casts in SEM with distribution of pericytes and fenestrated endothelial areas in corresponding vascular segments in TEM, we searched for structural features indicative of pericyte contractility. Some of the pericyte processes were found to show remarkably folded cytoplasmic outlining, as did the subjacent endothelial lining together with the interposed basal lamina. In such cases, endothelial cells protruded into the lumen and vascular diameter was drastically reduced (Fig. 7). Capillary profiles with such wrinkled pericyte processes, suspected as being contracted, were noted in the exocrine lobuli as well as in periductular capillaries.

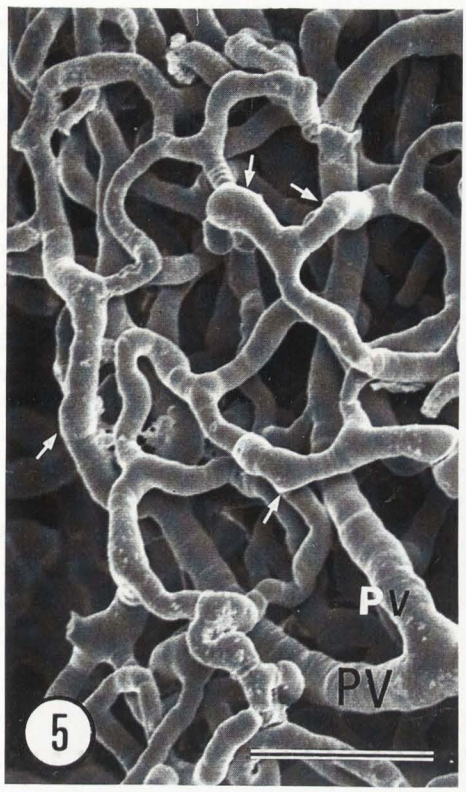
Discussion

In order to correlate different vascular segments in corrosion casts and tissue sections, the following criteria are used. First, the frequency of the subject under consideration in cast preparations as compared to the frequency of probably corresponding structures in tissue sections. By far most of the capillary casts of the exocrine pancreas were of the undulated type with circumscribed shallow dilatations, showing few circular or semi-lunar furrows on the casts surface. Accordingly, most capillaries in tissue sections were of the fenestrated type. The reverse was true for non-fenestrated capillaries in tissue sections and the slender, straight capillary casts which showed multiple surface constrictions. Therefore, it is fair to assume that the undulating, bulging capillary casts correspond to fenestrated capillaries, the slender, straight capillary casts to non-fenestrated ones. Second, the size of the particular subject serves as a criterion for identification. In tissue sections, the diameter of fenestrated capillaries constantly was smaller than that of fenestrated ones. This corresponds to the observations on undulating and straight capillary casts, respectively, and substantiates the above assumption. Third, the shape of the structure under discussion is considered. The circularly positioned grooves on capillary casts well correspond to pericyte processes (see the micrographs of Fujiwara and Uehara, 1984). In addition, these furrows on capillary casts were frequently seen in case of slender and straight capillaries (already interpreted as non-fenestrated capillaries), but were less frequently found in case of undulating and bulging capillaries (already interpreted as fenestrated capillaries). Accordingly, in tissue sections, pericyte processes were seen more frequently on non-fenestrated capillaries as compared to fenestrated ones. This coincidence substantiates the conclusion that the furrows on capillary casts reflect pericyte processes, and further substantiates the identification of fenestrated and non-fenestrated capillary casts (that was done on account of other criteria). Grooves on casts of postcapillary venules and small collecting veins are also interpreted as being caused by pericytes, since these vascular segments

Fig. 5. Capillary network in the exocrine rat pancreas. Arrows indicate smooth-surfaced bulging areas of capillary casts. Two postcapillary venules (PV) are seen. These small draining venules show numerous furrows on the casts surface. Calibration bar = 50 μm.

Fig. 6. Periductal plexus of capillaries and postcapillary venules. All vessels show numerous surface indentations (arrowheads) along their course. Calibration bar = 50 μm.

Fig. 7. Tissue section showing two capillaries of a periductal plexus. Pericyte processes (p) embracing the capillary marked "A" show folded outlining as does the endothelium beneath. Together with reduced luminal width of the capillary, this situation strongly suggests a contracted state of the pericyte. By contrast, pericyte processes related to the second capillary marked "B" show smooth outlining. Calibration bar = 4 μm.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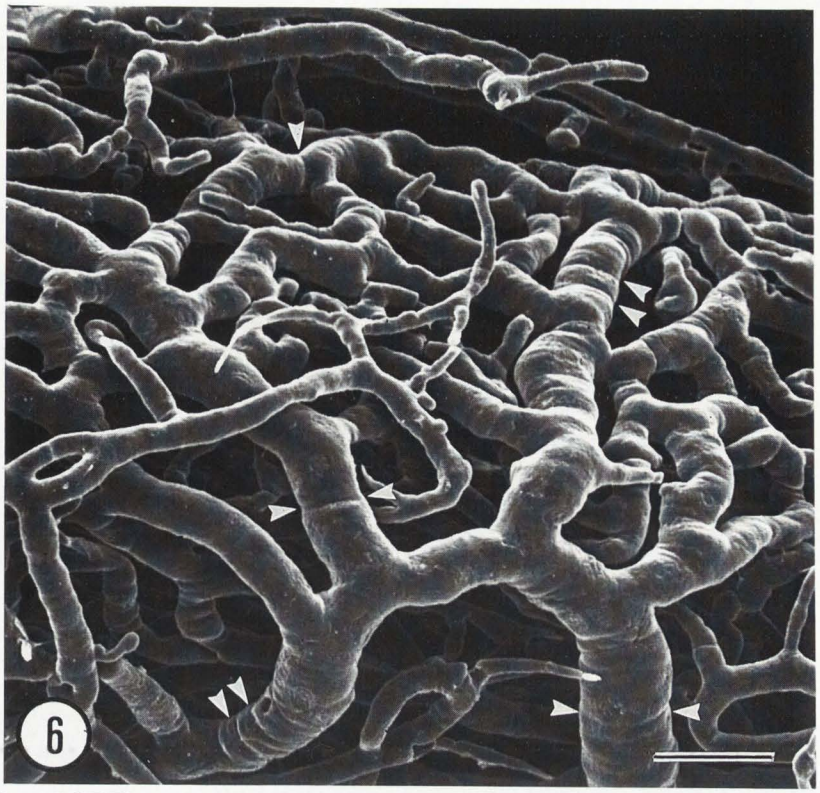
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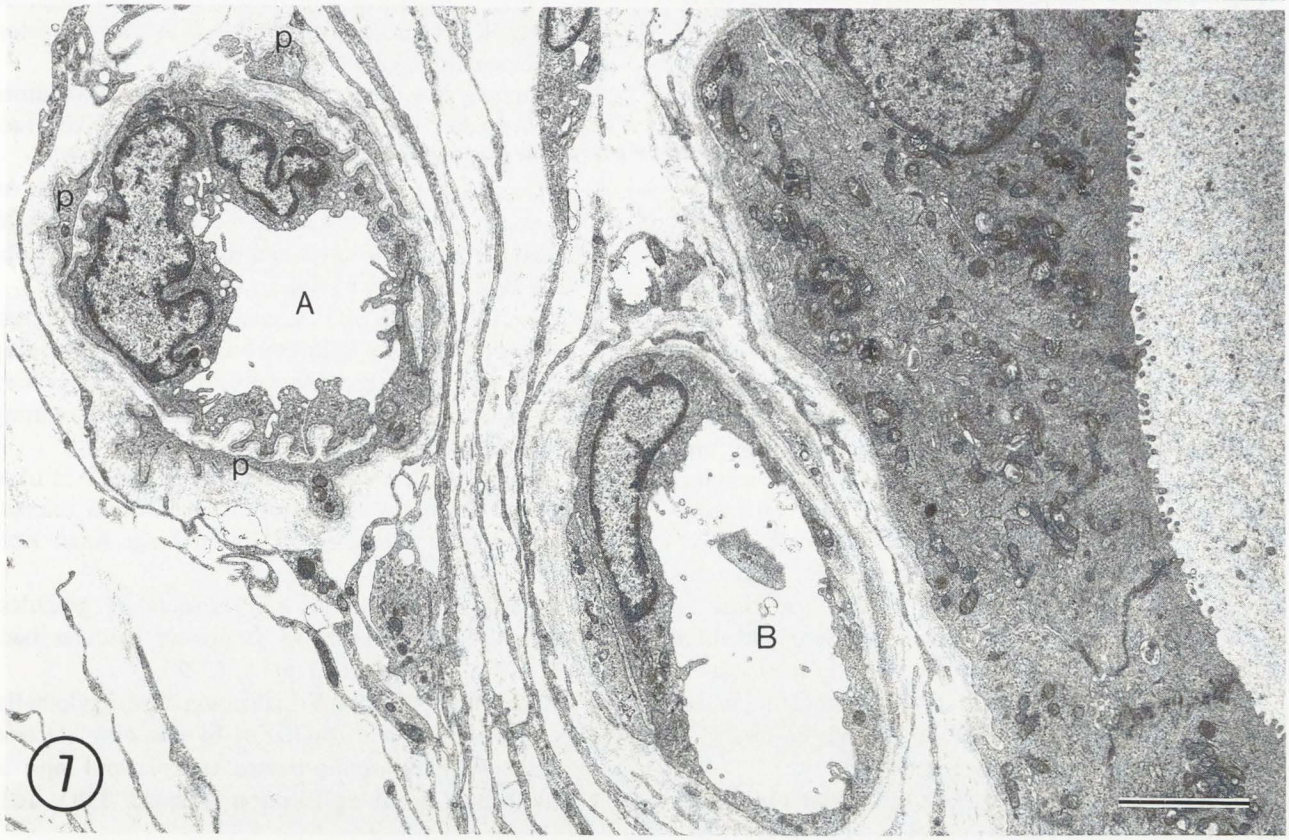
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are evidently well provided with pericytes (Rhodin, 1968).

The bulging smooth-surfaced areas on casts of the second type of capillaries most obviously correspond to areas of fenestrations in the endothelial outlining. This interpretation is also achieved by applying the above criteria. Moreover, tissue sections showed that pericyte processes in these capillaries are restricted to the non-fenestrated endothelial areas, so they assist defining of bulging fenestrated region by causing the nearby grooves.

It should be stressed, however, that measurements of furrows on capillary casts or venous casts cannot be simply compared to measurements of pericyte processes in tissue sections. The endothelial layer and its basal lamina are interposed between pericyte processes and vascular lumen, so that the pericyte impressions on luminal casts are blurred by these structures. In addition, it should be considered that only the bundles of cytoplasmic filaments in pericyte processes are candidates for active contraction or resistance against increasing luminal pressure. Therefore, it should be stated more precisely that pericyte filament bundles are causing surface indentation in cast perpetration but not pericyte processes. Pericyte processes are generally assumed to be ramified, and they only rarely embrace the entire circumference of the related capillary or venule (Zimmermann, 1923; Fujiwara and Uehara, 1984). Hence, circular surface constrictions on capillary casts deserve further explanation. We suggest that the common basal lamina of endothelium and pericytes is responsible for this phenomenon. This basal lamina acts as a circularly running belt that transduces the contractile force of a segmental pericyte process over the entire circumference.

Pericyte contractility has been suggested as a consequence of indirect morphological evidence (Fig. 7). Meanwhile, histochemical data and observations on cultured pericytes established this assumption (Diaz-Flores *et al.*, 1991). These findings well justify the interpretation that pericyte's contractile forces are responsible for the delicate surface reliefs observed in cast preparations of capillaries and postcapillary venules. When this conclusion is accepted, the relation of bulged areas on capillary casts to fenestrated areas of the endothelium becomes plausible. Pericytes mechanically reinforce the capillary wall. Fenestrated areas of endothelium, where pericyte processes are mostly absent, can be easily dilated during casting medium perfusion.

Besides of few observations on axonal varicosities in close spatial relationship to pericytes (Forbes *et al.*, 1977), which favor the neural control of these cells, plasma components, endothelium-derived substances, and metabolites from surrounding tissues may also govern pericyte contraction. Moreover, it is reasonable to assume that pericytes contraction is also initiated by other stimuli, such as thermal ones, or by unphysiologic agents, injected during cast preparation (Aharinejad *et al.*, 1992). Consequently, all these stimuli have to be considered as relevant for pericytes contraction. However, not all pericytes within a definite specimen show the same state of contraction or relaxation. As shown in Fig. 7, strongly contracted pericytes are seen near to less contracted or even relaxed ones. Therefore, the number of pericyte processes would be underestimated when simply counting surface constrictions in cast preparations.

Acknowledgments

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Discussion with Reviewers

K. Nakai: Basically I agree with the authors' conclusions that the furrows in the capillary cast are of pericytic origin. To reach the conclusions, however, authors may need a further confirmation that the capillary lumen under SEM is really correspondent with that under TEM, because the materials for each technique were prepared by different methods (Mercox was injected into un-fixed animals, whereas the animals observed under TEM were perfusion-fixed by glutaraldehyde). From my experience and published observations, although showing mild shrinkage of the tissue, corrosion casts from the perfusion-fixed animals showed more constant morphology, at least in brain blood vessels. Anyhow, the authors should observe phenomena prepared under similar conditions for both SEM and TEM as far as possible, in order to correlate findings from different observation method and strengthen the statement to be accepted by the wide range of audience, otherwise the authors should discuss about the technical aspect concerning the reasons why they prepared the corrosion casts from un-fixed animals.

Authors: Casting of unfixed animals is the standard technique in our laboratory. When applying this technique, we observed the grooves and furrows described on casts of capillaries and postcapillary venules. TEM studies were done later on to identify the structural components which might be responsible for this surface relief but not to find capillary profiles of similar shape. When performing casting with prefixed tissue, two possibilities can be considered: (i) The surface of capillary casts is smoothly outlined, or (ii) The surface of capillary casts shows similar grooves as seen without prefixation. In the first case, the result would not provide further evidence to explain the grooves on casts from unfixed tissue. In the second case, the question would remain: which structures are responsible? We did a few

pilot experiments with prefixed pancreatic tissue and observed grooves on capillary casts also under this condition. Hence, we did not continue this work.

D.E. Schraufnagel: We found that male rats seem to contract their pulmonary venous sphincters more than female rats (Schraufnagel DE, Thakkar MB: Alpha-adrenergic antagonism blocks pulmonary venous sphincters in neurogenic pulmonary edema. Submitted for publication). Did you notice a difference between your male and female rats?
Authors: We did not note such a difference in the exocrine pancreas.

D.E. Schraufnagel: How do the fenestrated capillaries that you describe differ from the pancreatic endocrine capillaries?
Authors: In corrosion casts, the endocrine capillaries appear as smooth-surfaced, swollen, sinusoidal capillaries (main diameter 12 μm) which run a strikingly tortuous course and often take U-shaped turns (Ohtani *et al.*, 1986, text reference). In tissue sections, endocrine capillaries show extended fenestrated areas which often surround the entire cross-section profile. Pericytes are rarely seen. Fenestrated capillaries in the exocrine pancreas are provided with more pericytes. Cross-section profiles show only segments of fenestrated endothelium, the remainder of the circumference consists of non-fenestrated endothelium. Pericyte processes are mostly beneath the non-fenestrated regions, the fenestrated segments are facing glandular acini. In cast preparations, fenestrated capillaries of the exocrine pancreas show varying width because of interposed smooth surfaced areas which bulge into the surrounding tissue. These bulges are interpreted as corresponding to fenestrated areas of the endothelium.

A. Castenholz: As a main result of your paper, a differentiation has been made between two types of capillary vessels. Are you able to correlate on type of these vessels, on the base of tissue-cast-analysis, to the secretory terminal parts of the gland and the other to the fine ductular system?
Authors: Such a strict correlation, as suggested by the question, is not given. Fenestrated capillaries of the exocrine pancreas are clearly related to the glandular acini (see the preceding question) but non-fenestrated capillaries are not exclusively related to intercalated ducts. We rather feel that acinar arterioles primarily give rise to non-fenestrated capillaries which continue as undulating and branching fenestrated capillaries. The latter provide the main mass of capillaries which are in common for glandular acini and intercalated ducts. The excretory ducts, by contrast, are provided with a distinct periductular and periductal capillary plexus that is separately fed by arterioles (Ohtani *et al.*, 1986, text reference).

A. Castenholz: From which topographic area of the pancreas were the pictures shown in Figures 6 and 7 (which are designated as casts of periductular vascular structures), taken. Are these structures located outside the parenchyma of the gland?
Authors: These figures are taken from larger ducts which are embedded into interlobular connective tissue. Hence, these ducts are essentially outside the parenchyma.

O. Ohtani: In Discussion you write that to identify the furrows as being caused by pericyte processes you have applied two criteria "frequency" and "shape and size" of the subject under consideration in SEM as compared to those of probably corresponding structures in tissue sections. Do you have sufficient data that show how the morphology of the casts corresponds to the shape and distribution of pericytes?
Authors: We are well aware that there is only indirect evidence in favor of our interpretation that the furrows on capillary casts are caused by pericyte processes. However, we feel that our conclusion is logical and this hypothesis is, as yet, the only one to interpret the furrows seen on capillary casts. Employment of new methods, different to ours, is required to provide additional and probably more sufficient data. In this direction, pharmacological relaxation and stimulation of pericytes prior to casting may be promising.

O. Ohtani: In the legends to Figures 3 and 4, you have described that connective tissue fibers associated with capillary wall are missing. As you may know, connective tissue fibers cannot be well stained by routinely used double staining with uranyl acetate and lead citrate. If tissue sections are immersed in tannic acid prior to the double staining, collagen fibers (and elastic fibers) associated with capillaries will be well demonstrated.
Authors: We only stated that significant amounts of collagen fibrils "in spatial relationship to the capillary wall" are missing. Tannic acid impregnation to increase electron density of elastin and collagen is also used in our laboratory; moreover, a switch to dark field illumination allows rapidly to identify collagen fibrils which otherwise show minimum electron contrast under bright field illumination [Böck P, Stockinger L (1984) Light and electron microscopic identification of elastic, elaunin and oxytalan fibers in human tracheal and bronchial mucosa. Anat. Embryol. **170**, 145-153]. All together, we feel that collagen bundles on capillaries of the rat exocrine pancreas are too scarce and too delicate as to

account for the furrows found on capillary casts.

O. Ohtani: In general, there are abundant collagen fibers (or fibrils) surrounding blood vessels including capillaries, even if collagen fibrils are only sparsely seen in ultrathin sections under a TEM. Recent work by Ohtani *et al.* may help you understand how abundant collagen fibers exist in various tissues including pancreas [Ohtani O (1987) Three-dimensional organization of the connective tissue fibers of the human pancreas: A scanning electron microscopy study of NaOH treated-tissues. Arch. Histol. Jpn. **50**, 557-566; Ohtani O, Ushiki T, Taguchi T, Kikuta A (1988) Collagen fibrillar networks as skeletal frameworks: A demonstration by cell-maceration/scanning electron microscope method. Arch. Histol. Cytol. **51**, 249-261).
Authors: Your excellent work on human pancreas (Ohtani, 1987) clearly shows a continuous collagen sheath around insular capillaries (your Fig. 5), while this envelop appears extremely fine on exocrine capillaries (your Fig. 2). However, casts of exocrine capillaries show the circular furrows under discussion, while casts of endocrine capillaries appear smooth surfaced (see text reference Ohtani *et al.*, 1986; in particular, Fig. 3 in this paper shows circular grooves and furrows on exocrine capillaries and Fig. 5 shows smoothly outlined endocrine capillaries). Therefore, we conclude that the fine collagen envelop of exocrine capillaries is not responsible for the furrows seen on casts of these vessels.

R. Christofferson: Your physiological interpretations: active contraction of pericyte filaments and passive distension of fenestrated endothelium, respectively, of furrows and bulging areas on the surface of capillary casts, are attractive. They cannot represent the whole truth, however, since I have observed bulging areas in capillaries not known to be fenestrated, as well as the absence of furrows in capillaries known to possess pericytes (while other capillaries in the same specimen exhibit furrows). Do you have any explanation for this?
Authors: As to the latter point, we assume that only contracted pericytes cause furrows on capillary casts. Relaxed pericytes do not cause surface indentations and therefore are not identified on capillary casts. It is fair to assume that the physiological contraction state of pericytes varies in different microcirculatory domains, even within one and the same specimen. Consequently, also variations of the casts surface relief have to be considered. In conclusion: in case the described furrows on capillary casts are found, they indicate the presence of pericytes; in case the furrows are missing, this is no proof for the absence of pericytes. As to the observation of bulging areas on casts of non-fenestrated capillaries, we have to qualify our hypothesis in similar manner: where fenestrated areas are present, these areas preferentially correspond to bulging regions of capillary casts (at least in the rat exocrine pancreas), but there is no proof that fenestrations are absent in other segments of the cast, as there may be bulges in non-fenestrated capillaries as well. Therefore, the observation of bulges on capillary casts is no proof for fenestrated areas but it should stimulate to search for fenestrated regions in tissue sections (as in your observation).

R. Christofferson: Can't the furrows and bulging areas be artifacts caused by, e.g., heterogenicity of the resin, interactions between hydrophobic resin and the wash-out medium, or uneven perfusion pressure?
Authors: High perfusion pressure certainly will accentuate the relief on capillary casts (bulges become wider and furrows appear relatively deeper). However, the structural components responsible for this relief remain the same and therefore we would not classify the relief as artifact. It seems unlikely that furrows and bulging areas on capillary casts are merely caused by inhomogeneous polymerization of the casting resin or by other physical parameters which influence the preparation process, since these phenomena should cause comparable surface reliefs on casts of small arteries and arterioles, which, by contrast, appear smooth surfaced.

R. Christofferson: Do you agree that some furrows, especially towards the venous side of the capillary tree, can be caused by inelastic connective tissue elements?
Authors: We agree that this possibility cannot be excluded in general. In the exocrine pancreas, we looked for collagen fiber bundles in spatial relationship to capillaries. Fibers of appropriate dimension were too scarce to account for all the furrows seen on capillary casts (see also discussion with Prof. Ohtani above). This result indicates that the majority of furrows on capillary casts are caused by pericyte processes but does not exclude the possibility that some of the furrows may be caused by collagen fibers.

R. Christofferson: Both furrows and bulging areas would theoretically impede the resin flow; were furrows more frequent in incompletely cast specimens, and hence bulging areas more frequent in specimens when a higher resin infusion pressure was used?
Authors: We did not quantify the frequency of furrows and bulges with respect to varying infusion pressure. We also expect that the surface relief on capillary casts will become more pronounced as perfusion pressure is increased. Incompletely cast specimens were selected out and not further studied. Such specimens, however, may provide additional evidence.

R. Christofferson: While reviewing your paper, I was (unsuccessfully) thinking of how to design an experiment to validate your hypothesis. Do you think casting during intravital microscopy of a vascular segment (in e.g., the rat mesentery), or TEM of cast and immersion-fixed specimens, would do that?

Authors: We already studied TEM sections of non-macerated specimens, fixed after casting. Because of the heat generated during resin polymerization, tissue preservation was poor, but still sufficient to identify all structural components of interest. However, analysis of their three-dimensional arrangement, readily done in TEM, requires laborious reconstruction work and so we stopped this study. Vital microscopy during casting seems more promising. This approach would provide essential information on effects of casting media on contractile cells of the vascular wall.