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Endothelium in Contracted Arteries*

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With 4 Figures in the Text

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In 1957, one of us (R. A.) called attention to a peculiar reaction of endothelial cells.

The central artery of the rabbit's ear had been pierced to find out whether or not such an injury might elicit cell division. No signs of mitosis were found, but apparently under the stimulus of the lesion a very intense contraction of the artery had occurred, with a maximum after 24 hours. The endothelial cells or at least their nuclei were "squeezed out" from the grooves between the folds onto the crests where they became more numerous. After only 12 hours and again after 36 hours, the reaction was much less evident. Similar reactions but of lesser intensity could be produced by injections of epinephrine. Piercing of the femoral artery in the rabbit produced no contraction of similar intensity. However, "riding" and "piling up" of endothelial nuclei could readily be found under the most varied conditions in human and animal tissues, although not as characteristic as in the central artery of the rabbit's ear. It is likely that in most of these instances the displacement of endothelial nuclei — the cytoplasma proper is not clearly visible in routine preparations — is due to an agonal or post-mortal contraction. This passive gliding of endothelial cells has been confirmed later by STAUBESAND and by VAN CITTERS et al. The latter authors mention also a columnar shape of endothelial cells in contracted arteries. This, however, is most difficult to see in the routine preparation studied by light microscopy and is not illustrated in their publication. A protrusion of endothelial cells into the lumen of the rabbit's contracted femoral artery is mentioned by BUCK.

We have followed up the experiments on the central artery of the rabbit's ear by complementing the light-microscopic investigation with electron-microscopy. Thus we obtained results which confirmed the passive movement of endothelial nuclei in contracted arteries, but showed, in addition, considerable changes in the configuration of the endothelial cytoplasm, which surpassed by far the reaction of endothelial cells described by BUCK for the femoral artery.

Material and methods

The central artery of the ear of 22 rabbits was pierced by a clean needle. After 24 hours the animals were anesthetized with nembutal and a segment of the artery in the vicinity of the perforation removed, fixed in PALADE'S fixative with or without sucrose, embedded in methyl metacrylate and cut on a Porter-Blum microtome. One μ thick sections were lightly stained with iron-hematoxylin and studied by means of the light-microscope; ultra-thin sections were studied by means of Philips electron microscopes EM 75 and EM 100 B.

Control segments from non-injured arteries (7 specimens) were also studied, and so were three specimens rinsed with acetylcholine to counteract contractions and from three animals injected with acetylcholine prior to biopsy.

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Results

Fig. 1 and 2a show light-microscopic pictures of the inner wall of contracted arteries prepared by formalin fixation and paraffin embedding. Fig. 1 shows a sector from a normal pulmonary artery of a rabbit, killed by injection of nembutal and originally studied for another purpose. One can readily distinguish the

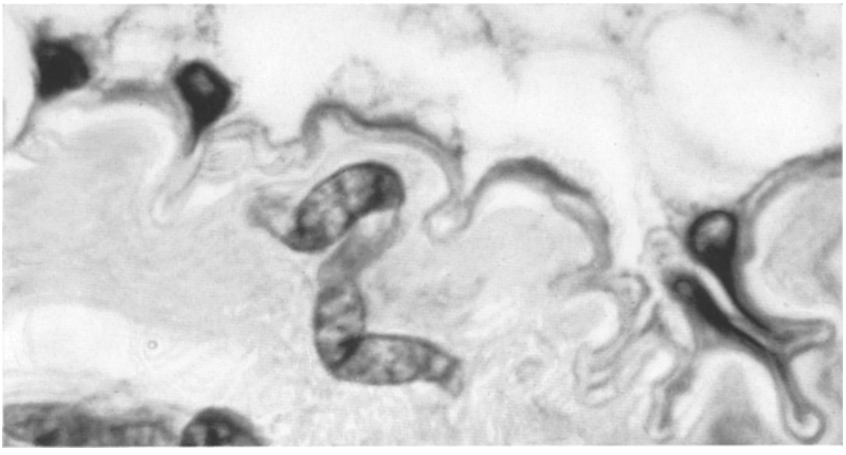

Fig. 1. Rabbit. Contracted pulmonary artery with four endothelial nuclei being squeezed from the grooves between the folds of the corrugated wall. Hematoxylin-eosin. ×2135. Note in Fig. 1—4 the corrugated elastica interna

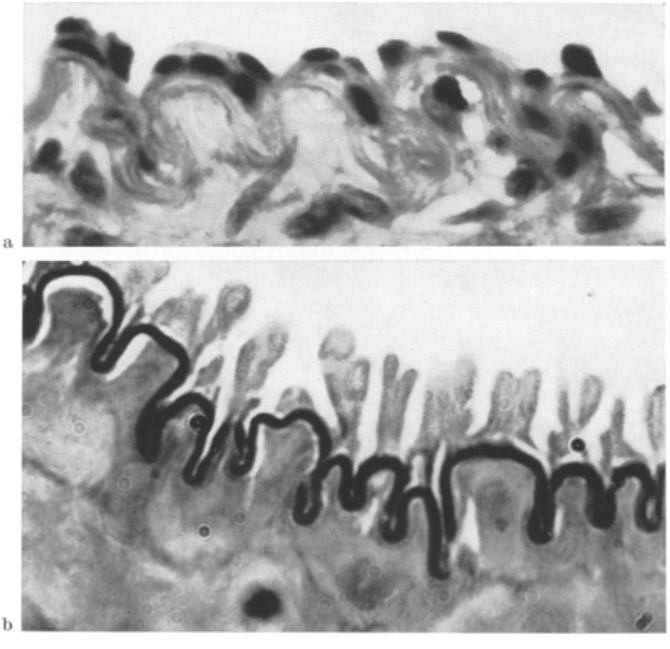

Fig. 2. a Rabbit. Contracted central auricular artery, 24 hours after injury. Note the nuclei "riding" on the crests of the folds and the "empty" appearing grooves. Iron hematoxylin-eosin. ×910. b Identical experimental conditions as in a, but fixation and embedding as for electron-microscopy. Thickness of section 1 μ. Faintly stained with iron hematoxylin. Photographed by light-microscopy. ×910

contracted elastica interna and four nuclei of endothelial cells being squeezed from the grooves formed in the contracting wall. In Fig. 2a, from apparently a later stage of contraction, in this case following injury to the central auricular artery, the "riding" of nuclei on the crest of the folds and the lack of nuclei inside the groove is quite distinct. In Fig. 2b, also from a pierced central auricular artery of a rabbit, the picture shows the cytoplasm of the endothelium protruding into the lumen, a feature practically missing in the first two figures. The reason for this must be sought, we believe, in the different fixation and embedding

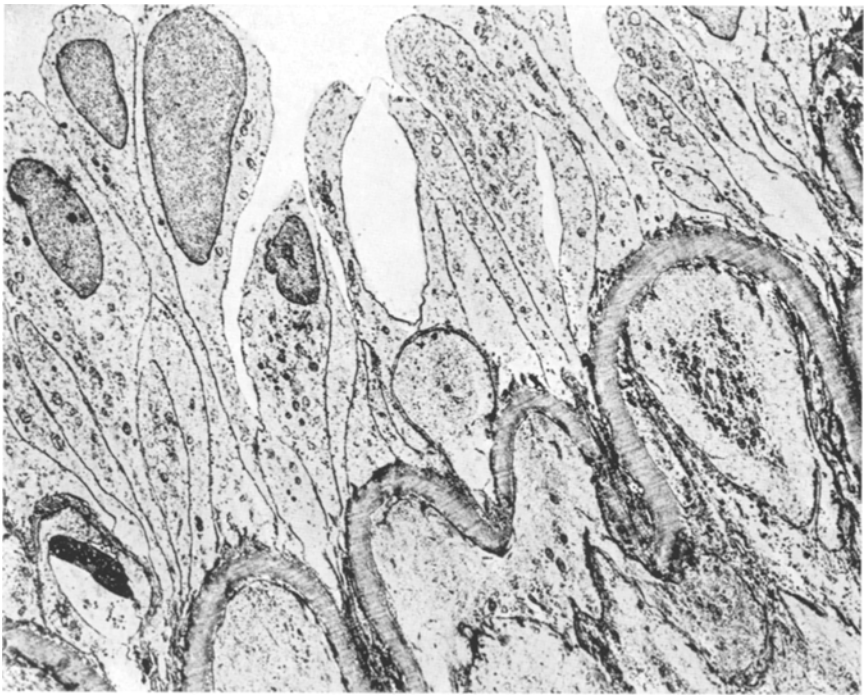

Fig. 3. Rabbit. Contracted central auricular artery, 24 hours after injury. Fixed and embedded as in Fig. 2b, but ultra-thin section. Electron micrograph. Magnification by electron microscope: ×2500

technic. Although Fig. 2b allows no fine study of the endothelium as one expects it today from electron microscopy, it demonstrates clearly the disadvantage and unreliability of formalin fixation and paraffin embedding.

By using the electron microscope, one is able to establish quite readily that the endothelial cytoplasma is protruding into the lumen to a considerable height, it being sometimes separated in its upper aspects from the neighboring cells, sometimes not only not losing contact with them but coming into a more intimate and extensive contact with neighboring endothelial cells (Fig. 3). It is noteworthy that the minute vesicles pithing the surface of the cytoplasma towards the lumen and towards the base do not disappear during this "stretching process" and remain preserved where the cytoplasma is bordering neighboring cells (Fig. 4). The part of the cytoplasm which remains in the grooves appears darker, i.e., it has a higher electron density and represents apparently a condensation of the cytoplasma due to compression.

In several instances other cells appear in the endothelial layer, with their nuclei closer to the elastic membrane than the nuclei of endothelial cells. Sometimes it is possible to find a nucleus in the gap ("fenestra") of the elastica showing an hour glass configuration and belonging likely to a cell wandering into the lining. As to the nature of the cells which migrate apparently from the media into the endothelial layer, some of them are smooth muscle cells, some seem to be histiocytes and a few are white blood cells. Whether the last have immigrated from the media or from the lumen could, so far, not be established.

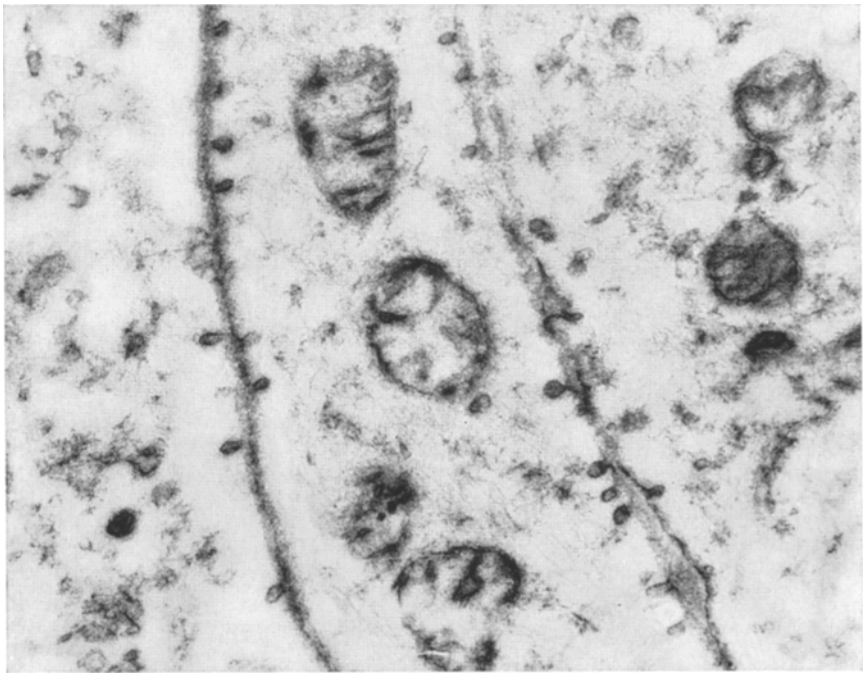

Fig. 4. Same conditions as in Fig. 3. Note mitochondria, and vesicles on the cell membranes which face neighboring cells. Electron microscopic magnification: ×35000 (reduced to $^{19}/_{20}$)

We have also studied under the electron microscope the appearance of endothelial cells in controls from uninjured central arteries of the rabbit's ear. However with the technic required for electron microscopy, the trauma of biopsy is sufficient to cause contraction of the artery with corrugation of the elastica. Thus the endothelial cells showed also in these specimens a certain increase in height, although it did not reach the degree seen in the vicinity of the injury. Injections of acetylcholine as used by VAN CITTERS et al. did not inhibit this traumatic contraction but rinsing the artery with acetylcholine lessened it and the endothelium appeared flat. Unfortunately, this chemical treatment prior to fixation damages the cytoplasm of the endothelium and interferes with a meticulous reproduction by electron microscopy.

Discussion

A columnar type of endothelium has been described as early as 1881 by RENAUT in the fibrous tissue of the corium, in interfascicular connective tissue of nerves and in lymphatic tissue. Under pathological conditions the size of endothelial cells

in renal vessels is said to increase (BELL) and protrude into the lumen (KANTROWITZ and KLEMPERER). Also in small vessels of the human uterine mucosa and in the uterus of bats a columnar endothelium has been described (see ALTSCHUL 1954). More recently, HIBBS et al. have found by electron microscopy a high endothelium in the capillaries of the skin, thus confirming RENAUT's statements from 1881. However the height of the cells in their preparations is much less than that of endothelial cells in the contracted central artery of the rabbit's ear and apparently not due to contraction.

What we wish to point out is the following: in our case of contracted arteries of the rabbit's ear, it is not correct to use the term columnar for the endothelial cells, because it appears that the endothelium is extended parallel to the axis of the vessel and therefore rather leaf-like. Moreover the height differs considerably before, during and after the contraction and therefore such a classification of the endothelium appears to be futile. In view of the mechanical impact on the endothelial cells caused by contraction, and their subsequent movement and change of shape, one is led to ponder about possible consequences of such events. On one hand, one should assume that endothelial cells in contracted arteries after becoming very tall and protruding into the lumen, may become desquamated. As a matter of fact, desquamation of endothelial cells has been reported repeatedly but its occurrence has also quite often been denied (for the extensive literature, see ALTSCHUL 1954). If such desquamation occurs, the replacement remains to be explained because there are no signs of mitosis. The possibilities of amitotic division, of replacement by underlying cells or by monocytes from the blood have been envisaged but not proven. It is indeed hard to understand that endothelial cells subjected to the impacts of blood pressure, contraction and compression by external forces should last throughout life without replacement. However if one considers the mechanical forces acting on and in smooth muscle cells—as clearly illustrated by the contorted nucleus of a smooth muscle cell in Fig. 1—one hesitates to assess the seriousness of the just mentioned impacts.

Summary

In contracted arteries the position and form of endothelial cells undergo considerable changes. The cells are "squeezed" from the grooves onto the crests of the folds formed by contraction of the elastica interna and of the media. The cytoplasm becomes very high and, in relation to the width of the artery, very narrow. During this process much of the luminal and of the basal surface of the raised cell comes into contact with the luminal and basal surface of the neighboring endothelial cell thus losing for the duration of the contraction contact with blood. However, the vesicles which are found on the luminal and on the basal surface of the flat endothelium do not disappear during these changes. By preparation of the tissues for light-microscopy, the cytoplasm becomes so shrunken and faint that its changes during contraction are only indicated or not detectable at all.

Das Endothelverhalten in kontrahierten Arterien

Zusammenfassung

In kontrahierten Arterien ändern sich Anordnung und Form der Endothelzellen beträchtlich als Folge der Faltung der Elastica und Media. Die Zellen werden sehr hoch und ein großer Teil der in den nicht kontrahierten Arterien das

Lumen auskleidenden Zellmembran grenzt während der Kontraktion an benachbarte Endothelzellen und verliert dadurch Kontakt mit dem Blut. Trotz der mechanischen Kräfte, die auf die Zellmembran einwirken, verschwinden die im elektronenmikroskopischen Bild feststellbaren Grübchen und Bläschen nicht. Die althergebrachte Behandlung der Gewebe für lichtmiksoskopische Untersuchungen schädigt die Zellen derart, daß die cytoplasmatischen Formveränderungen entweder nur angedeutet sind oder sich nicht feststellen lassen.

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