

The pulmonary endothelial cell

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ABSTRACT The surface of the endothelial cells of the pulmonary trunk of the Wistar albino rat was studied by means of silver preparations and by scanning and transmission electron microscopy. This surface is the site of cytoplasmic projections and the opening of caveolae which together appear to be features associated with the active metabolic rôle of the pulmonary endothelial cell.

When one looks at a transverse section of pulmonary trunk, the cytoplasm of the endothelial cells is spread out so thinly as to be barely discernible. Indeed, the endothelial layer is detected largely through the prominent nuclei that bulge from the inner surface of the vessel (fig 1). The apparently dull nature of the pulmonary endothelial cell has led histologists and pathologists to pay it but scant attention. In recent years, however, it has become clear that the surface of pulmonary endothelial cells has an important metabolic rôle. For this reason we thought that as pathologists we should take a closer look at the structure of the pulmonary endothelial cell as shown by silver preparations and by scanning and transmission electron microscopy.

Silver preparations

Technique

The margins of endothelial cells were stained with silver deposits by a modification of one of the methods described by Poole *et al* (1958) and Florey *et al* (1959). A male Wistar albino rat was killed by inhalation of ether. After removal of attached fat the pulmonary trunk was removed and opened longitudinally. Excess blood was washed from the endothelial surface with 0.85% saline, and the specimen was pinned on to clean cork using steel pins. The specimen was then rinsed in distilled water and exposed to osmium tetroxide vapour for 10 minutes, stained with a fresh solution of 0.25% silver nitrate for 30 seconds, and rinsed

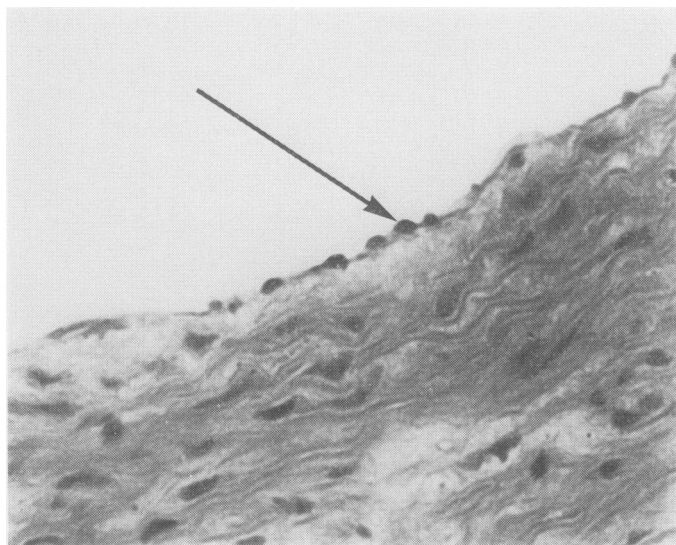


Fig 1 *Transverse section of pulmonary trunk of Wistar albino rat. Bulk of tissue seen comprises smooth muscle cells, nuclei of which are clearly visualised. Elastic fibres form clear undulating strands in media. Surface of pulmonary trunk is lined by endothelial cells that have very thin extensions of cytoplasm but prominent nuclei (arrow). Were it not for latter endothelial layer would be barely visible (Haematoxylin and eosin, $\times 635$).*

again in distilled water and placed in Caulfield's medium (a fixative containing osmic acid at pH 7.4 with added sucrose) for 30 minutes. This was followed by washing in several changes of distilled water for two hours, then dehydrating in ascending grades of ethyl alcohol, clearing in xylol, and mounting in DPX.

Results

When the margins of the pulmonary endothelial cells are stained in this manner by silver deposits, the inner surface of the pulmonary trunk is seen to be lined by a mosaic of cells. The silver preparations show the shape, size, and arrangement of the cells (fig 2) and allow one to determine their dimensions and surface area. Most of the endothelial cells are roughly rectangular, although some have a more ovoid or triangular shape (fig 2a). The cell margins have a wavy outline, and in some cases there are finger-like projections from one cell fitting into a corresponding recess in another (fig 2a). Presumably these aid a tighter locking together of the endothelium. The greater diameter of the cells is usually in the region of 30 to 35 μm and the shorter diameter some 15 to 20 μm . The range of surface area of the individual endothelial cells, as determined by planimetry in 24 cells, is 369 to 627 μm^2 (mean 503 μm^2). Some areas of the inner surface of the pulmonary trunk show more elongated cells in which the diameters

are in the region of 50 and 8 μm (fig 2b). The "waves" may be few and large so that the cell has a dumb-bell shape (fig 2b).

Scanning electron microscopy

Technique

Four female Wistar albino rats were anaesthetised with ether vapour and the thorax and abdomen were opened by a mid-line incision. The carotid arteries and superior venae cavae were ligated. A small incision was made in the inferior vena cava between the origins of the renal veins, and a polythene cannula was passed into the vessel towards the heart. The cannula was tied firmly into place. The abdominal aorta was also similarly cannulated close to its bifurcation. The cannula within the inferior vena cava was connected to a flask containing heparinised saline, which entered the inferior vena cava at a pressure of 20 cm water. This flushed out the inferior vena cava, pulmonary arteries, and pulmonary veins, the blood and surplus saline being collected via the aortic cannula. When a three-way stopcock was turned the saline was replaced by chilled, buffered glutaraldehyde. After this had filled the pulmonary vasculature and had started to flow from the aorta, the aortic cannula was clamped to reduce the flow of fixative to about 1 ml min^{-1} . By this means the full hydrostatic pressure of 20 cm water was applied to the

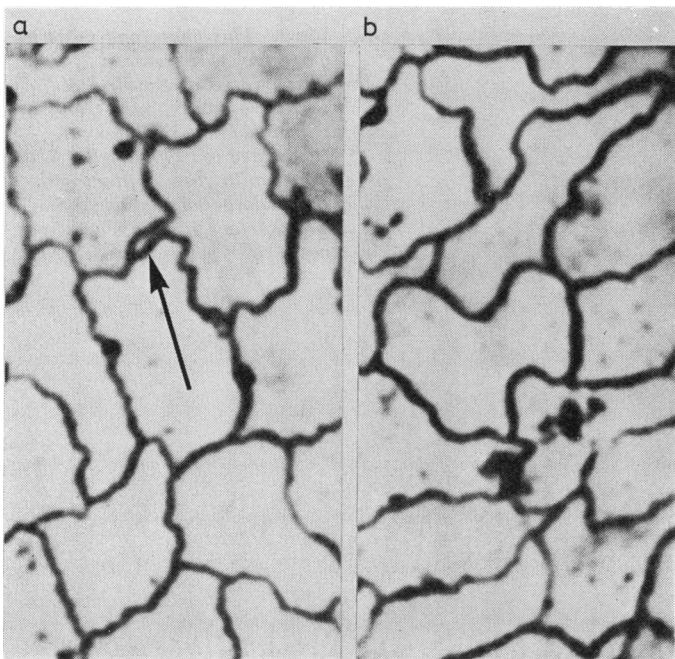


Fig 2a and b. Silver preparations of endothelial surface of pulmonary trunk of Wistar albino rat. Barely discernible endothelial layer of fig 1 is now seen as an extensive mosaic of cells, outlines of which are shown by silver deposits. (a) Roughly rectangular endothelial cells with wavy outlines. One cell shows a finger-like projection (arrow). (b) An area showing more elongated, narrow cells, one of which has a dumb-bell shape (Osmium tetroxide vapour and silver nitrate solution, both $\times 777$).

pulmonary trunk to fix it as close to its dimensions *in vivo* as possible. Fixation was aided by pouring glutaraldehyde on to the outside surface of the pulmonary trunk. It was left to fix for two hours. The thoracic organs were then removed, and the pulmonary trunk was carefully dissected free and stored overnight in glutaraldehyde at 4°C.

An intact cylinder of pulmonary trunk 3 mm long was dehydrated in ascending grades of ethanol and stored in amyl acetate. It was then transferred to a critical-point drying apparatus and the amyl acetate flushed out under pressure with liquid carbon dioxide. After soaking in liquid carbon dioxide for one hour it was dried by the critical-point technique. The dried cylinder was then cut

into two segments by two longitudinal cuts made under a dissecting microscope. The two halves were mounted on stubs, endothelium uppermost, using silver dag as the cement. The mounted vessels were coated with gold-palladium *in vacuo* and examined with a Cambridge Stereoscan scanning electron microscope at a voltage of 7 kV.

Results

Scanning electron micrographs of erythrocytes in close apposition to the surface of the pulmonary endothelial cells (fig 3) serve as a reminder of the intimate approximation of blood cells and plasma on the one hand and the endothelium on the other, and of the potential metabolic significance

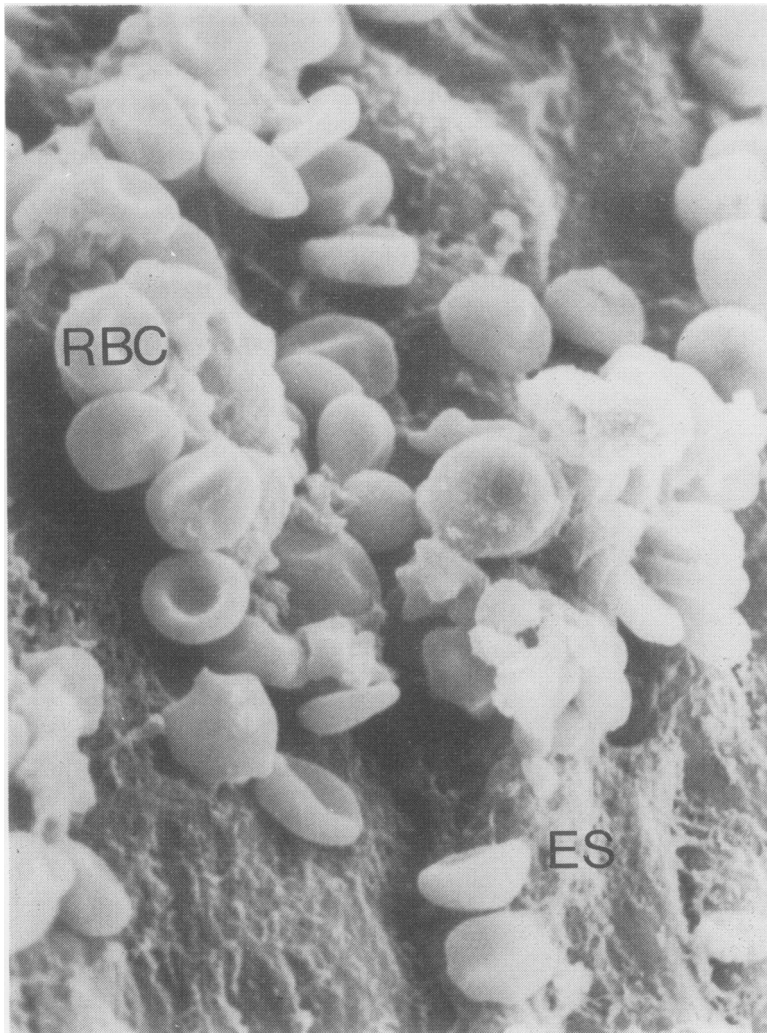


Fig 3 This figure and figs 4 to 6 are scanning electron micrographs of endothelial surface of pulmonary trunk of a female Wistar albino rat. Biconcave red blood cells, RBC, are seen in close contact with the endothelial surface, ES. This surface is roughened by a network of projections ($\times 3200$).

of this. The pulmonary trunk does not have a smooth lining (fig 4). Instead it has a "cobblestone surface" that is produced by the protuberance of the nuclei of the endothelial cells (fig 4). Superimposed on the cobblestone lining produced by the nuclei is a shaggy appearance brought about by large numbers of cytoplasmic flecks and projections (figs 4 to 6). Some are round or oval and others linear and finger-like. They may be single or may be aggregated into complexes in which as many as 15 individual projections can be seen (fig 6). Some of the projections are branched and others show small buds arising from the side of projections. Others assume leaf-like flaps and folds; these comprise the largest projections and they predominate along the margins of cells (fig 6). The endothelial projections range in length up to 2700 nm and in width up to 430 nm. They are seen all over the surface of the pulmonary trunk but are especially numerous over the nuclear prominences. They tend to be large and leaf-like over the cell margins and this facilitates delineation of one cell from another by the observer (fig 6).

The pulmonary endothelial cells tend to be roughly rectangular, oval, or even triangular in

shape, the greater diameter being up to 30 μm in the tissue fixed and processed for scanning electron microscopy, the smaller diameter being 10 to 19 μm (fig 5). The nuclei have a round or oval outline (fig 5). The surface of the pulmonary endothelial cells is studded with small pits (fig 6). These are openings on to the cell surface of the caveolae intracellulares.

Transmission electron microscopy

Technique

Isolation and fixation of the pulmonary trunk were performed according to the method of Smith *et al* (1978) described above. Small arcs of pulmonary trunk were trimmed off the vessel, post-fixed for one hour in 1% osmium tetroxide, stained with uranyl acetate, and embedded in Araldite. Semi-thin sections, 1 μm in thickness, were stained with toluidine blue for the selection of a suitable field. Fine sections were cut with an LKB Ultratome III, stained with lead citrate, and examined with an AEI EM 6B electron microscope.

Results

Transmission electron microscopy of the pul-

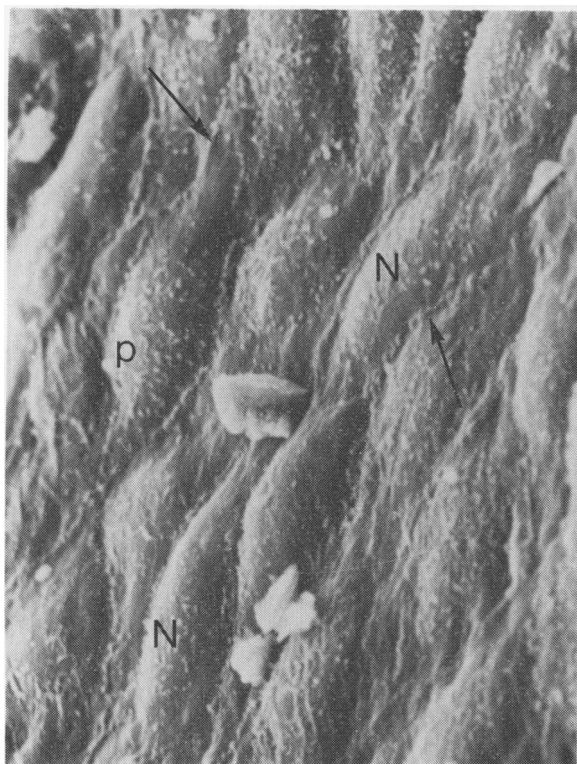


Fig 4 Oblique view of endothelial surface of pulmonary trunk showing a cobblestone appearance due to prominences formed by bulging nuclei, N. Cell margins (arrows) are picked out clearly by endothelial projections at this site. Numerous endothelial projections, p, are also seen over bulging nuclei and over surrounding flatter cytoplasm ($\times 2228$).



Fig 5 *Surface of endothelial cells of pulmonary trunk. Central, or slightly excentric, nuclei show as pale ovoid structures, N. Surrounding flattened areas of endothelial cytoplasm appear dark. Endothelial projections are prominent over nuclei but are also seen over surrounding cytoplasmic sheets. They also occur prominently at cell margins picking out these structures clearly. Dark cracks are artefacts induced by technique of preparation (X2836).*

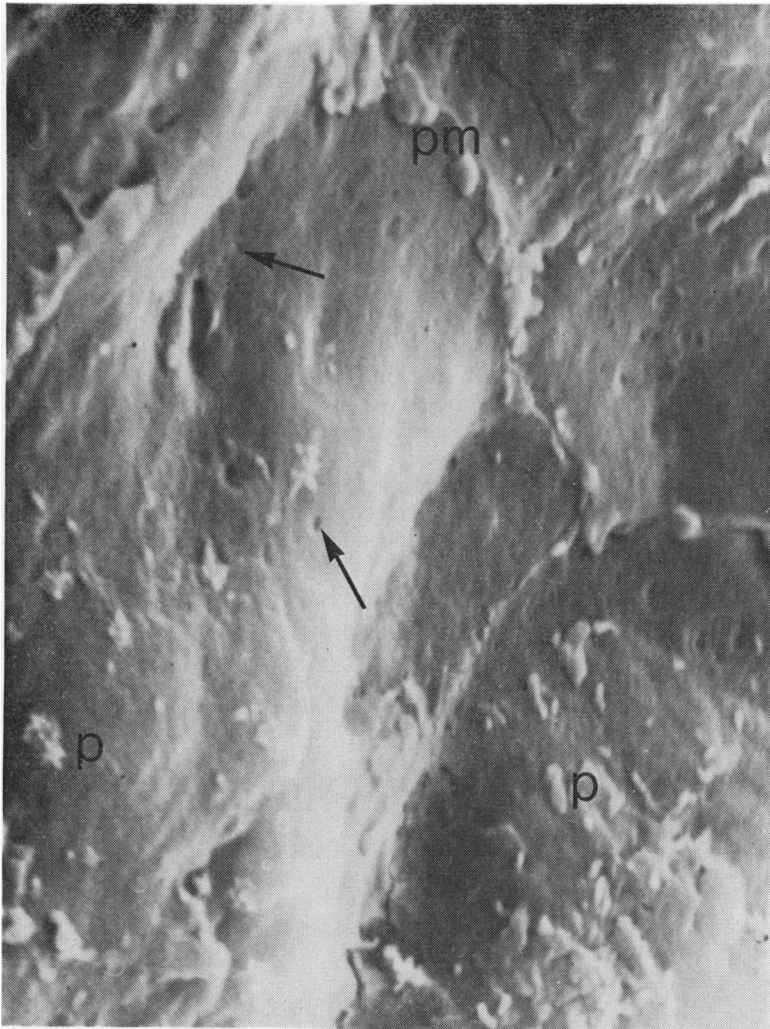


Fig 6 *Cytoplasmic surface has been photographed at a higher magnification to show its distinctive features more clearly. Endothelial projections, p, are seen over cytoplasmic surface and especially at cell margins, pm. Luminal openings of caveolae intracellulares (arrows) appear like small craters on surface of endothelial cell (X7047).*

monary endothelial cells confirms that the "cobblestone appearance" of the lining of the pulmonary trunk is due to the nuclei bulging into the lumen (fig 7). At this point the depth of cytoplasm and nucleoplasm is in the region of $4.7 \mu\text{m}$. The depth of the flat, squamous extremities of the cytoplasm of the endothelial cell falls to a value as low as $0.4 \mu\text{m}$. The cells are held closely and firmly together to produce the mosaic shown in fig 2 by "tight junctions" (fig 8). The endothelial projections seen on scanning electron microscopy are also clearly visible in transmission electron micrographs (fig 9). Some contain caveolae intracellulares and ribosomes.

The surface of the pulmonary endothelial cells is studded with very large numbers of caveolae intracellulares (fig 10). They open on to the luminal surface by a stoma covered by a diaphragm composed of a single lamella. Sections through the stoma show dense osmiophilic bodies at the meeting point of the plasma and caveolar membranes and the caveolar diaphragm (arrow in fig 10). Caveolae are 50 to 90 nm in diameter. The wall around their cavity is of unit membrane type with two lamellae. This wall is not uniform in appearance but is granular with the formation of small nodules. Sometimes several caveolae fuse together to form a complex structure (fig 9) but in doing so the shape and identity of the individual

caveolae are maintained so that the whole comes to resemble a bunch of grapes.

Microtubules are common in the cytoplasm of pulmonary endothelial cells (fig 10) but, it should be noted, there were no bundles of fine filaments akin to the actomyosin filaments of smooth muscle cells.

Discussion

The view that the microscopist customarily has of the endothelium of a transverse section of the pulmonary trunk is that of an immensely thin sheet of cytoplasm all but invisible. Indeed its existence is largely inferred by the protuberances formed by the nuclei of the endothelium (fig 1). This being the case, it is hardly surprising that the pulmonary endothelial cell has received scant attention from histologists and pathologists.

When one looks at the surface of the pulmonary endothelium stained by silver salts rather than at a section of it, however, the appearance is transformed, and one realises that the endothelium of the pulmonary trunk forms an extensive area comprising a mosaic of numerous cells with which the blood comes into intimate contact during its passage through the lung (fig 2).

Scanning electron microscopy reinforces this impression and one appreciates the intimate con-

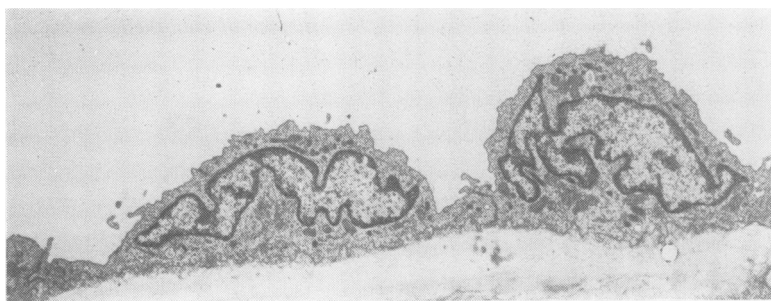


Fig 7 *Transmission electron micrograph of endothelial surface of pulmonary trunk of a Wistar albino rat. Endothelial cells consist of a central protuberant part due to the nucleus with surrounding flattened plates of cytoplasm* ($\times 5217$).

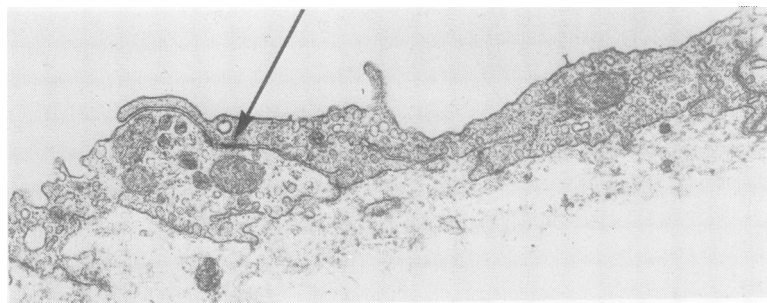


Fig 8 *Transmission electron micrograph of peripheral flattened plates of cytoplasm of two endothelial cells of pulmonary trunk from Wistar albino rats. Cells are kept closely apposed by tight junctions (arrow)* ($\times 16\ 666$).

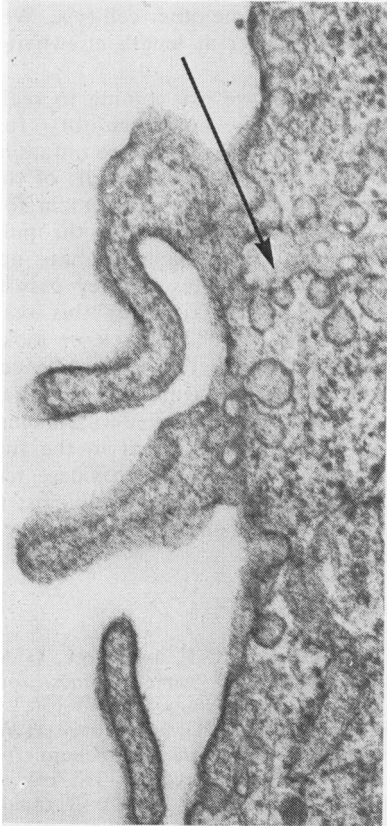


Fig 9 Transmission electron micrograph of superficial portion of an endothelial cell of pulmonary trunk of a Wistar albino rat. Three endothelial projections, whose surface appearance on scanning electron microscopy is shown in figs 4, 5, and 6, are seen. Luminal surface of cell is site of numerous caveolae intracellulares. At one place several caveolae have fused (arrow), and it should be noted that individual caveolae have retained their shape and structure ($\times 60\,000$).

tact between the luminal surface of the pulmonary endothelial cells and the erythrocytes and plasma of the blood (fig 3). This view of the endothelial surface at ultrastructural level brings with it the somewhat surprising realisation that it is not smooth but roughened by a network of finger-like projections and strands (figs 3–6). Some of these projections may be artefact and derived from fibrin in the blood, but there is no doubt that most are true ultrastructural features of the surface of the pulmonary endothelial cell (figs 5 and 6). They can be identified readily in transmission electron micrographs (fig 9). They appear to have been first described by Smith *et al* in 1971 who reported their presence in the pulmonary arteries

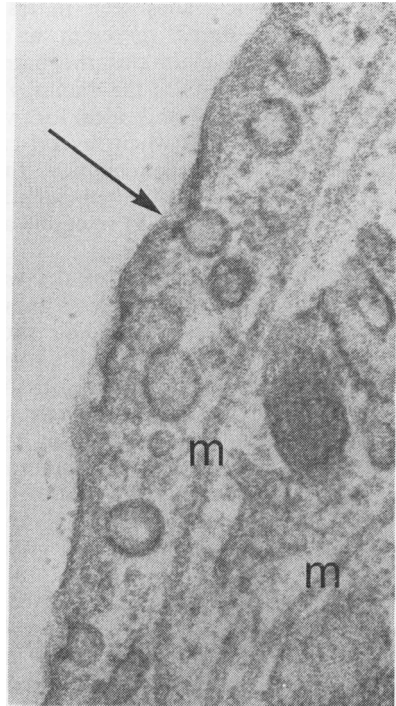


Fig 10 Transmission electron micrograph of superficial portion of an endothelial cell of pulmonary trunk of a Wistar albino rat. Opening on to luminal surface of cell are several caveolae intracellulares. Example indicated by an arrow shows characteristic structure with two osmiophilic knobs either side of stoma, which is covered by a thin diaphragm. Wall of caveola has a granular appearance. Microtubules, m, run through cytoplasm ($\times 82\,758$).

of the dog. These authors ascribe a haemodynamic effect to these endothelial projections. They suggest that the dense, irregular meshwork of finger-like processes could produce an eddying flow of cell-free plasma along the surface of the endothelial cells. This is probably of importance in large pulmonary arteries where nutrient capillaries enter the adventitia and outer media but do not penetrate to the endothelium. A retarded flow of plasma along the surface of the cell could provide favourable conditions for the exchange of metabolites and possibly for the metabolism of circulating hormones. Since the projections are so numerous they must greatly increase the surface area of the cell.

Our study confirms in the pulmonary trunk the same ultrastructure of caveolae intracellulares reported by Smith and Ryan (1972) as occurring in the endothelial cells of the capillaries of the rat lung. Smith and Ryan (1972, 1973) believed that

the dense, osmiophilic knobs seen in transverse section around the stoma represent a circular skeletal structure that maintains the patency of the stoma and the integrity of the diaphragm. Our own observations support this idea for we were impressed by the fact that when several caveolae fused together to form one complex, the individual caveolae still retained their identity and shape so that the whole came to resemble a bunch of grapes (fig 9).

The granular, nodular nature of the wall surrounding the cavity of the vesicle is thought to represent enzyme clusters or binding sites. The distribution of lead phosphate deposits formed on reaction of caveolar 5'-nucleotidase with AMP in the presence of lead nitrate corresponds with the spacing of the globular structures in the wall (Smith and Ryan, 1972). Plasma membrane and caveolae may be harvested in a highly purified state suitable for study by a series of low-speed centrifugations of lung homogenates (Ryan and Smith, 1971).

The recently introduced immunoperoxidase technique allowed Ryan and Ryan (1975) to show an "angiotensin converting enzyme" in globular particles some 6 nm in diameter in the membrane lining caveolae and in undifferentiated plasma membrane of the surface of pulmonary endothelial cells. In this technique, after preliminary incubation of rat lung tissue with normal goat serum to bind non-specific sites, antibodies to this enzyme were coupled to peroxidase and attached to the enzyme sites in the walls of caveolae. Subsequently the peroxidase moiety coupled to the antibodies were allowed to react with hydrogen peroxide and diaminobenzidine, thus labelling precisely the sites of the enzyme at ultrastructural level. Their study showed that the inactive decapeptide angiotensin I is converted into the active octapeptide angiotensin II in the walls of the caveolae of pulmonary endothelial cells, predominantly in the pulmonary capillaries and venules. Thus it would appear that the active metabolic rôle of the lung does not reside in enzymes in the blood but in the enzymes situated in the walls of the caveolae of the pulmonary endothelial cells.

An important negative finding in our study was the absence of bundles of fine filaments in the cytoplasm of pulmonary endothelial cells akin to the actomyosin filaments of smooth muscle cells. Cells possessing such fine cytoplasmic filaments are very characteristic of the various intimal proliferations in hypertensive pulmonary vascular disease. This negative finding in the present study suggests that intimal changes in vascular disease of the lung are not due to proliferation of endo-

thelial cells but to some other cell type. We discuss this important matter at length elsewhere (Smith and Heath, 1979).

More and more we are coming to realise that the lung has many non-respiratory functions. Some of these probably lie in the unfamiliar cells such as the Feyrter and Clara cells of the bronchial tree whose functions still remain to be discovered. Others, however, lie in the pulmonary endothelial cells which have intimate access to hormones and metabolites as they pass through the pulmonary circulation. For this reason we felt it worth while to have a closer look at the ultrastructural features of the surface cells that show so little of their nature and potential in the classical paraffin section stained with haematoxylin and eosin. It seems likely that in the future increasing use of the immunoperoxidase technique will allow the histologist and pathologist to learn more about their behaviour and function in health and disease.

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