ULTRASTRUCTURAL ASPECTS OF ENDOTHELIAL CELLS' SECRETORY FUNCTION IN RABBIT MAJOR BLOOD VESSELS

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Introduction

Most electron microscope studies on the endothelium of the major blood vessels deal with its permeability function (Hüttner et al — 1973, O. Stein and Y. Stein — 1972), contractility (Yohro, Burnstock — 1973) and role in atherogenesis (Veress et al — 1972). Lately, data have been made available pointing to a synthesis capacity of endothelial cells (EC) (Jaffe, Nachman — 1975, Young — 1973, Thomsen — 1976). However, the issue of fine morphology of the secretory process remains unclarified, i. e. of the morphological substrate of synthesis and excretion of the material produced by the cells toward the lumen and/or intimal matrix.

We undertook the task to study the ultrastructure and dynamics of EC synthetic-secretory apparatus in the ontogenesis of rabbit major blood vessels.

Material and method

Ten- and twenty-day old fetuses, newborns, 12-, 20- and 60-day old, as well as grown up rabbits were used in the experiment. Material for electron microscopic study was taken from the thoracic aorta, pulmonary trunk and venae cavae (intrathoracic segments). Consecutive immersion fixation was made in 3 per cent glutaraldehyde and 1 per cent osmium tetroxide in 0.1 M phosphate buffer with pH=7.4, at 4° C. Dehydration with alcohol and acetone; flat embedding in ACM durcupan. The ultrathin sections were stained with uranyl acetate and lead citrate, and the preparations were examined in electron microscope JEM 7A.

Colchicine experiment. Twenty-day-old rabbits underwent treatment with 0.125 mg per 100 g body weight colchicine intraperitoneally; within four hours, the animals were killed, and material from the aorta and pulmonary trunk was immediately obtained and processed after the method described; untreated rabbits of comparable age were used as controls.

Results

EC of the fetal arterial and venous vessels under study do not show ultracytological differences. They have a well developed granular endoplasmic reticulum (ER), clearly outlined Golgi complex, numerous polysomes and microtubules (MT) (Fig. 1). The ER cisterns occupy a substantial area of the cytoplasm, and their interior is filled with finely granulated material with density exceeding that of the cellular matrix. A considerable amount of vacuo-

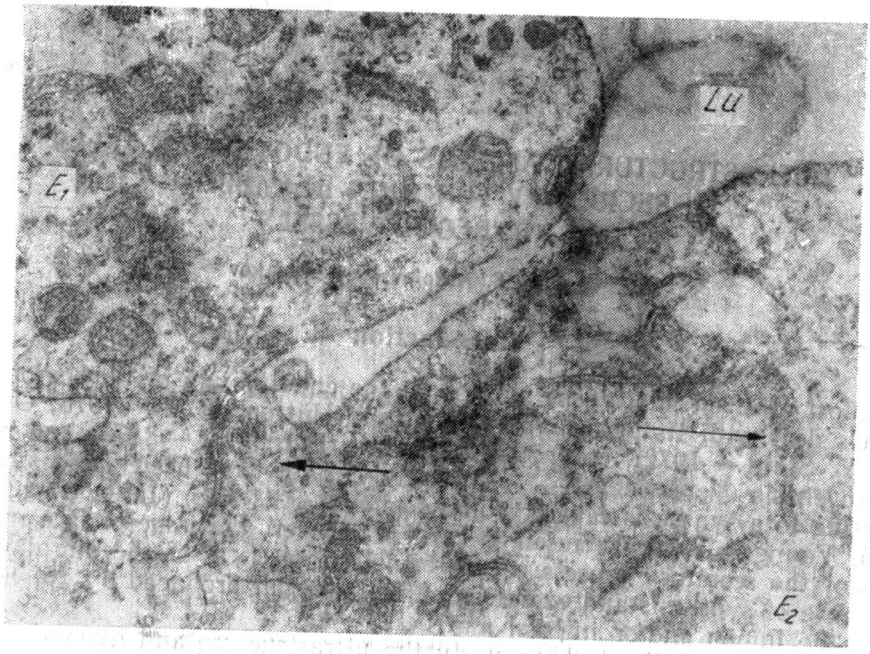

Fig. 1: Endothelial cells (E_1, E_2) from the anterior vena cava of 20-day fetus. Lu — lumen. Dilated ER cistern (arrow), microtubules (double arrow). Magnif. x 20 000

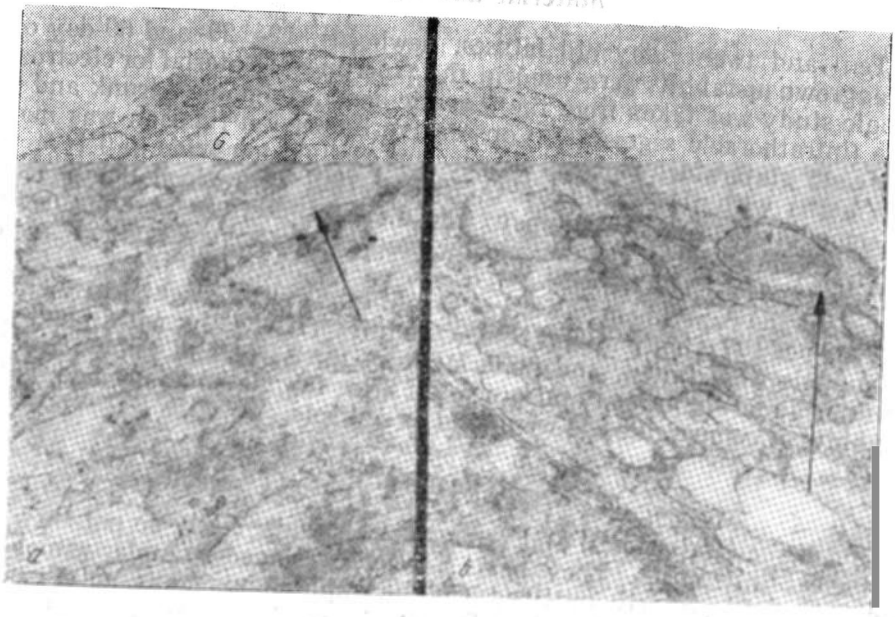

Fig. 2: Endothelial cells from posterior vena cava of newborn rabbit;
2-a) type one vacuole (arrow). G — Golgi complex. Magnif. ×20 000
2-b) type two vacuole (arrow pointing to the transition between type one and two vacuoles). Magnif. ×30 000

les make part of the Golgi complex composition. Some of them are located near by the cytolemma (both luminal and basal one). MT display mainly subcytolemmal arrangement (Vankov et al — 1977). At this particular age EC establish contacts with young elastic fibers, elastic microfibrils and flocculated material with medium electron density.

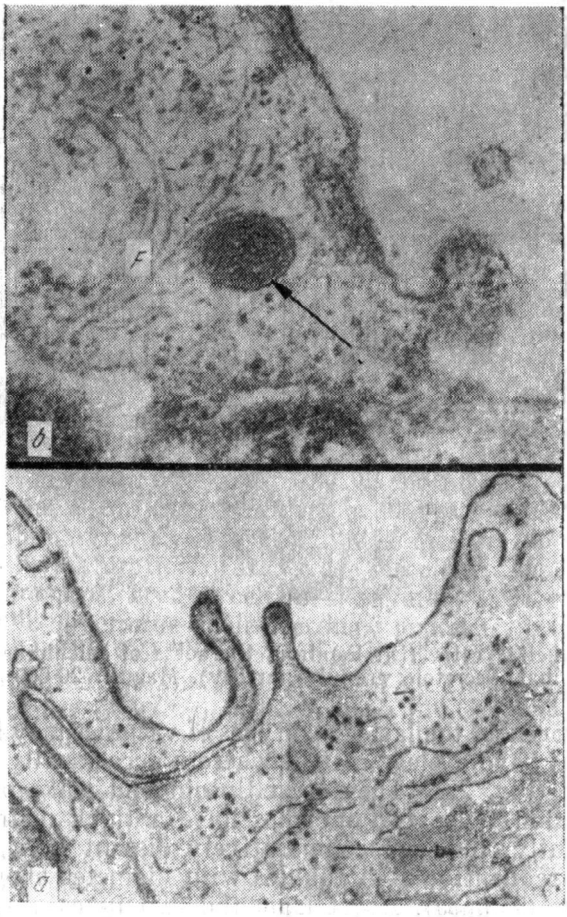

Fig. 3: Venous endothelial cells.
3-a) type two vacuole, containing tubules (arrow). Posterior vena cava from newborn animal. Magnif. 35 000
3-b) Weibel-Pallade corpuscle (arrow) close to intermediary filaments (F). Anterior vena cava from 12-day-old rabbit. Magnif. 50 000

After birth, the amount of the organelles described shows a considerable reduction at the expense of micropinocytotic vesicles occurrence. The latter process runs a quicker course in the veins, whereas up to the eleventh day of the postnatal life, arterial EC preserve a comparatively well developed ER, Golgi complex and ribosomes with micropinocytotic vesiculation being likewise rather strongly pronounced. Among poor in organelles venous EC, even in grown ups, cells with typically developed Golgi complex, presenting

several fields containing numerous cisterns with dilated lateral zones and abundant vesicular and vacuolar population are also established. The size of the vacuoles varies from 300 to 1000 nm, while their form is oval to ellipsoid. They appear enveloped in a single membrane. The vacuoles, regardless of their intracellular location display differences in the morphology of the proper

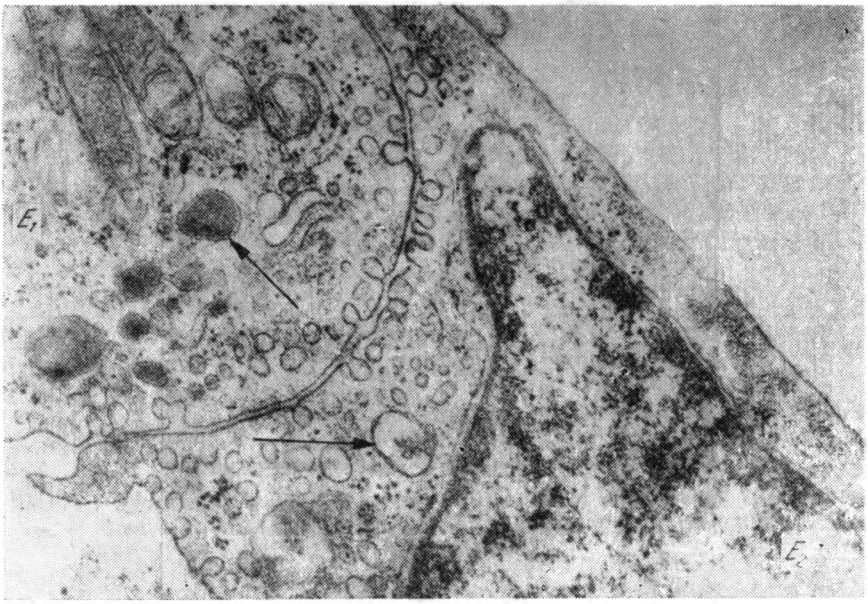

Fig. 4: Endothelial cells (E_1, E_2) from the aorta of colchicine treated animal. Secretory granules (arrows). Magnif. 20 000

matrix. On the ground of the latter two types of vacuoles may be distinguished. **Type one** vacuoles have a clear matrix wherein occasionally filamentous material is observed (Fig. 2a). They are ellipsoid and make part of the Golgi complex composition. **Type two** vacuoles have a thick matrix, greater sizes and, most frequently, they are comparatively distant from the Golgi fields (Fig. 2b). Not infrequently, 3—4 tubules with diameter about 12 nm are established in them (Fig. 3a). Apart from the two types of vacuoles, typical Weibel-Pallade corpuscles are also established (see Vankov, Nikolov, Chaldakov — 1967). Some of them are situated in the immediate vicinity of the luminal cytolemma. Often, intermediary filaments measuring 9—10 nm diameter are noted around them (Fig. 3b).

In EC from colchicine treated animals, numerous secretory granules are observed while MT are not detectable in these cells (Fig. 4).

Discussion

The electron microscope study shows that cells secreting the vascular wall extracellular elements possess a strongly developed organelle apparatus (Kadar et al — 1971 — smooth muscle cells; Thomsen — 1976 — EC). Our

results warrant the assumption that synthesis and secretion of substances, needed for the intimal matrix build up, are among the basic functions of the EC in the prenatal and early postnatal age, particularly with a reference to the aorta and pulmonary trunk. The strongly developed ER and Golgi complex, abundance of polyribosomes and MT, in our opinion, constitute the morphological substrate of the synthesis of proteins, glucosaminoglycans and glycoproteins, of their ammassment into vacuoles originating from the Golgi complex, and release in the subendothelium with ensuing intimal matrix formation. The adequately developed MT system may be engaged in the intracellular transport of the secretory products (Ehrlich et al — 1974; Poisner, Cook — 1975). An analogical interpretation could be made of the results of colchicine experiments where a loss of MT and accumulation of secretory granules is observed in the arterial EC of treated animals.

During the postnatal ontogenesis, EC of the vessels under study undergo differentiation in terms of permeability (Vankov et al — 1975). The latter process involves rather slightly the aorta and pulmonary trunk, and because of that the prenatal cytological peculiarities of the secretory EC described are preserved for a longer period of time. Against the background of prompt venous endothelium specialization in terms of permeability, isolated cells stand out, even in adults, characterized by strongly developed and differentiated Golgi complex, including the already described types of vacuoles and Weibel—Pallade corpuscles as well. ER in these endothelial cells is rather weakly developed. The latter finding points to a functional specialization of some cells within the framework of the venous endothelium, whilst the dynamics of their organelles in the ontogenesis presupposes a period of time required for the maturation of a definite secretory apparatus type. Hence the described EC are considered as cells of a secretory type. Naturally, we are not in a position to determine their secretory products, but the presence of an adequately developed Golgi system suggests that, in all likelihood, it is a matter of glucosamine glycans (Young — 1973). The prevailingly supranuclear localization of the Golgi complex in these EC, and the vicinity, respectively, merging of vacuoles with the luminal cytolemma doubtlessly suggest that the secretory products are excreted into the lumen of vessel. The morphology outline in these cells marks the existence of a direct Golgi complex — lumen secretory route.

In our opinion, of particular interest are also the types of vacuoles described. Beyond any doubt they have a Golgi origin. It might be assumed with a high degree probability that the well developed Weibel—Pallade corpuscles exactly in these secretory EC similarly originate from the Golgi complex (see Sengel, Stoebner — 1970 — small blood vessels), developing from other vacuoles. Therefore, the Weibel—Pallade corpuscles seem related to the secretory process in EC, and obviously promote its function (specialized type of secretory granules). Insofar as their orientation towards the lumen is concerned (Vankov, Nikolov, Chaldakov — 1976), it may be presumed that the intermediary filaments situated near by play a definite role in this respect.

In conclusion, it may be pointed out that in the prenatal age, all EC show morphological evidence of synthesis and secretion of substances needed for the build up of their own wall. After birth, and especially within the first ten days of life, the secretory function loses its morphological substrate in most

of the cells. Only some venous endothelium cells develop morphological qualities of secretory specialized cells whose production is effected within the vascular lumen.

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УЛЬТРАСТРУКТУРНЫЕ АСПЕКТЫ СЕКРЕТОРНОЙ ФУНКЦИИ ЭНДОТЕЛИАЛЬНЫХ КЛЕТОК КРУПНЫХ КРОВЕНОСНЫХ СОСУДОВ У КРОЛИКА

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РЕЗЮМЕ

Описывается морфология и динамика синтетическо-секреторного аппарата эндотелиальных клеток в пре- и постнатальном онтогенезе аорты, легочного ствола и полых вен у кролика. Зародышевые эндотелиальные клетки имеют хорошо развитые эндоплазматический ретикулум, полисомы, комплекс Гольджи и микротубулы; обсуждается вопрос о возможности их участия в построении матрикса интимы. Роль микроканальцев в секреционном процессе исследована на третированных колхицином животных. В ранном онтогенезе артериальные эндотелиальные клетки сохраняют эту морфологию. Только одна популяция венозных эндотелиальных клеток показывает дальнейшую дифференцировку системы Гольджи, что может быть обнаружено на всех этапах постнатального онтогенеза. Описываются два типа вакуолей, которые происходят из комплекса Гольджи. Обсуждается их роль предшественников телец Вейбел—Паладе. Эти эндотелиальные клетки реализуют непосредственно свою секрецию по пути от аппарата Гольджи к просвету сосуда.