

## COMPARATIVE STUDY OF THE VACUOLE APPARATUS IN EPITHELIAL CELLS OF A MOUSE THYMUS GLAND

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The epithelial cells of the thymus (T) are a source of humoral factors of great importance. It is suggested that the vacuole apparatus plays a certain role in the process of their synthesis and elimination (14, 16). Besides, the epithelial cells of the core, cortico-medullar zone and medulla itself, possess definite peculiar features (10, 12, 16, 18), which are not thoroughly investigated concerning their apparatus. This facts make us run the present study.

### Material and methods

Thymus materials of 14 four-months-old conventional mice (Swiss) were investigated by the standard method of election-microscope: prefixation in 5% glutaraldehyde (0.1 mol. phosph. buf. with pH-7.4 for 2 hours at 4° C), postfixation in 1% osmious tetraoxyde (0.1 mol. phosph. buf. with pH-7.4 for 1½ at 4° C), dehydration in ascendant alcohols and binding to Durcupan ACM. Semithin cuts (sections) were stained and ultra-thin cuts were double-contrasted after Reynolds E. (1963). The electron-microscope JEM 7A was used in our study.

### Results and discussion

The epithelial cells of various regions of the thymus lobules have characteristic features and at least two varieties can be detected in either region — dark and pale epithelial cells. The vacuole apparatus in these cells possesses definite ultrastructural peculiarities. It is presented by 3 types membrane-bounded structure in the dark cortical epithelial cells: I<sup>st</sup> type — vacuoles bounded with a single, more rarely double membrane; most often to be found. Their content varies a lot — from electron-denseless and homogeneous to more complex: consisted of smaller or bigger electron-dense granules and conglomerates or membraneous fragments with various organization, combined and manifested in a different ratio (fig. 1 — a, b). Sometimes there are particles of  $\alpha$ -cytomembranes or ribosomes in their lumen. The membranes are organized in myelin-like figures in certain vacuoles, while in others — there is a tendency of forming multivesicular bodies (masses) of the grouped-together vesicules. The neighbouring vacuoles often communicate between themselves. II<sup>nd</sup> type — vacuoles found in definite single epithelial cells. They are bounded with a thin membrane and consist of various amount of nuclear material in their lumen (fig. 2). Usually they are located round the thymocyte with a nuclear pycnosis phagocytied in the cytoplasm. III<sup>rd</sup> type — membrane-bounded bodies with a dense

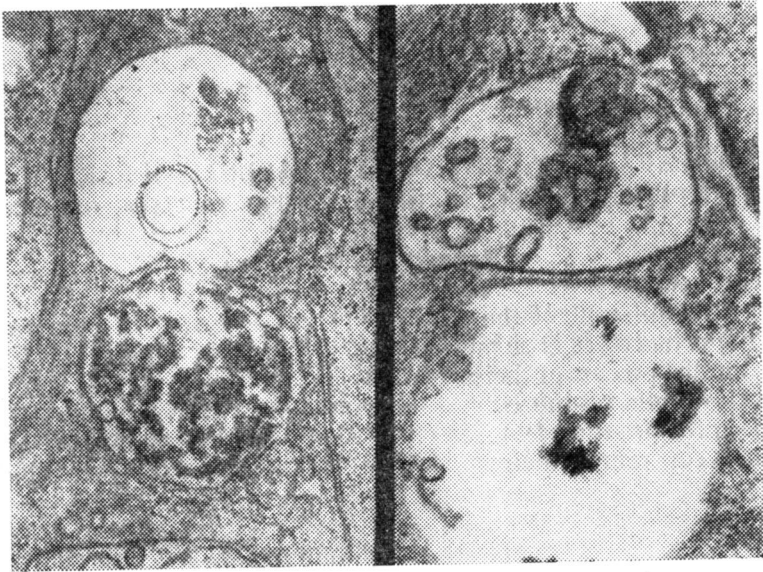


Fig. 1: Vacuoles of the first type in cytoplasm of cortical epithelial cells

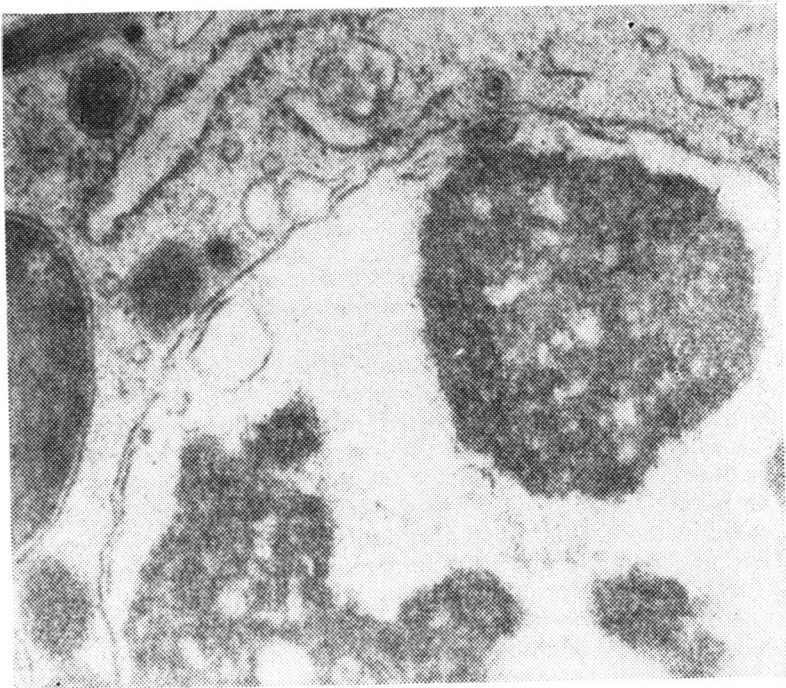


Fig. 2: Cortical epithelial cells: part of vacuole of the second type]

matrix and included homogeneous membranous components or lipid drops (fig. 3 — a, b). Dense particles in a process of confluencing can be found in some of the cells. Rarely, small corpuscles with electron-dense homogeneous matrix bounded with membranes are detected. The big variety of the established structure is usually combined with a loose perinuclear area in the dark epithelial cells and concentrated organells in the thin cytoplasmatic sprouts; a well developed granulative endoplasmatic reticulum, several Goldji-fields, numerous free ribosomes and polysomes. The membrane-bounded corpuscles in the pale cortical epithelial cells, though considerably less in number, preserve the same characteristics.

Single, isolated vacuoles of the first type, with a membranous or fine granulative material in the lumen are most often found in epithelial cells of pale vacuoles with microvilli, and large intranuclear ciliary cysts are established simultaneously. They maintain different features and genesis; therefore, we consider them as a component of the cell vacuole apparatus.

The dense membrane-bounded corpuscles of the third type, presenting various size and form in the sections, predominate in medullar epithelial cells (fig. 4). Their grouping in the cytoplasma (characteristic complexes) is often found. They are rare in the pale and numerous in the dark epithelial cells, where they are combined with greater variety of organells in the cytoplasmatic sprouts (projections). These cells possess vacuoles of the first type as an exception, while vacuoles of the second type are not found in our study at all.

Our results show that the epithelial cells in T of mature animals possess a well developed vacuole apparatus. Though the great variety, 3 main types of membrane-bounded structures can be detected. Similar to our findings are established in epithelial cells in T of other animals but their characteristics is incomplete (8, 9, 11) and the opinions of their origin — quite contradictive (7, 12, 17). We refer them to the group of autophagosomes concerning the vacuole morphological feature (cytoplasmatic components in the lumens) and complying with the bibliographical data of an expressed acid-phosphatase and arylsulphatase activity of their content and bounding membranes (14, 18). Structures of the second and third type, established in our study, have not been an object of a profound analysis until now. The vacuoles of the second type, their structural features and location, can be referred to the heterophagosomes. The ultrastructural characteristics of membrane-bounded corpuscles of the third type makes us consider them to be thelolysosomes including mainly lipofuscine granules and multilamellar corpuscles.

Concerning the established differences of the structure of the vacuole apparatus in epithelial cells of various regions (zones) of T, it can be concluded that the corticle epithelial cells are considerably rich of autophagosomes. Thelolysosomes, heterophagosomes and single primary lysosomes can be found together with them. Thelolysosomes predominate in medullar epithelial cells. Lysosomes are scanty in epithelial cells of the cortico-medullar zone. Usually the vacuole apparatus is more developed (richer) in the dark epithelial cells than in the pale ones, and this is in combination with all cytological signs of an increased functional activity in each zone of T. The so established features of the lysosomal corpuscles contribute to

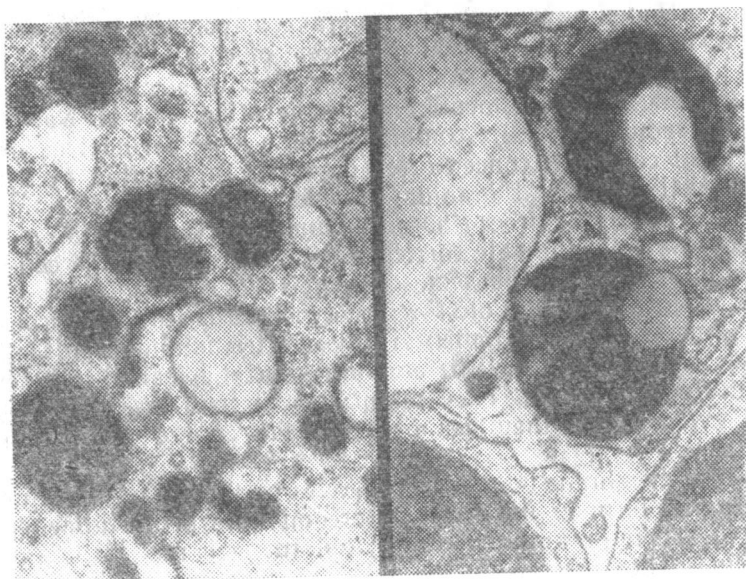


Fig. 3: Cortical epithelial cells: variety of the lysosomes

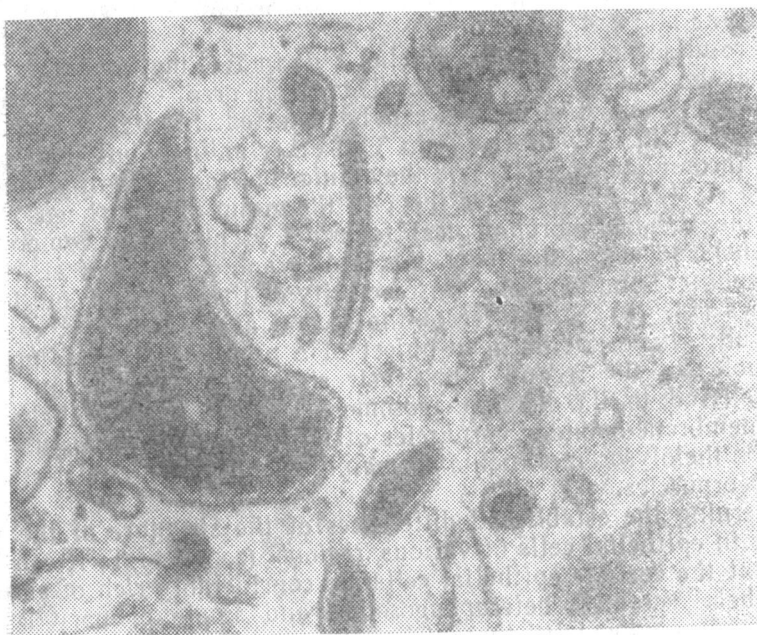


Fig. 4: Dense corpuscles in cytoplasm of medullar epithelial cells

a relatively precise determination of the regional belonging of the corresponding epithelial cell.

Scarce and contradictory suggestions concern the functional activity of the vacuole apparatus in epithelial cells of T. Presuming our data of an expressed morphological heterogeneity of lysosomal corpuscles, it can be concluded that there are certain functional differences between the lysosomes which are connected with the metabolic (functional respectively) features of the cell (1). Our results suggest that the lysosomes of thymus epithelial cells participate in 3 (at least) main biological activities of the cell: autophagia, heterophagia and secretion. The autophagosomes are directly engaged in the process of autophagia. Considering their increase in number and size with the age of rats, Pfoch. M. (1971) relates their function to the early involution of T. It is known that the cortical epithelial cells of T are able to phagocyte (swallow) thymocytes (3, 6, 7). Duning H, C. (1973) accepts that the swallowed material is phagocytosed in some vacuoles and this influences the elimination of the thymocytes. The established in our study heterophagosomes confirm the aforementioned opinion. It is suggested that the vacuoles of the first type participate in the secretory activity of the corresponding cell (6, 12, 14, 16). According to Sagimoto M. (1976) the vacuole content is an activator of the predecessor of the thymus humoral factor located in the dense corpuscles of the epithelial cells. This activator is able to transform the predecessor (precursor) in its active form which is facilitated by the confluence of the bounding membranes of both lysosomes. The participation of the phagosomes and autophagial vacuoles in epithelial cells of some endocrine glands in the regulation of the synthetic process is already known (2, 4). Our data support the possibility of a similar functional activity of autophagosomes in epithelial cells of T. However, the lysosomes of these cells participate in the cell metabolism in a different degree and the process is facilitated by their various relations.

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**СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ВАКУОЛЯРНОГО АППАРАТА ЭПИТЕЛЬНЫХ КЛЕТОК ВИЛОЧКОВОЙ ЖЕЛЕЗЫ У МЫШЕЙ***Ц. Маринова, Е. Бошнакова***Р Е З Ю М Е**

Объекты вилочковой железы четырехмесячных конвенциональных мышей (Swiss), забитых посредством декапитации, обработаны стандартным способом электронно-микроскопического исследования.

В эпителиальных клетках коры, кортико-медулярной зоны и медуллы наблюдаются обилие и разнообразие лизосомальных тел с преобладанием аптофагозом в различном соотношении, а также и с наличием гетерофагозом и телолизом. Устанавливаются характерные региональные особенности в их тонкой структуре и взаимоотношениях, дополняющих цитологическую характеристику различных видов эпителиальных клеток.

Обсуждается морфологическая гетерогенность лизосомальных тел в связи с функциональной активностью вилочковой железы.