

## ELECTRON MICROSCOPE INVESTIGATION OF THE INTERMEDIATE FILAMENTS IN THYMUS EPITHELIAL CELLS DURING ONTOGENESIS AND AFTER HORMONAL TREATMENT

Tz. Marinova

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The presence of cytofilaments most of which are organized in the form of bundles is one of the characteristic common and constant morphologic features of thymic epithelial cells of vertebrates and man (7, 9). Recently, it is established that there are several kinds of epithelial cell filaments: actinic (microfilaments), intermediate (of keratin nature) commonly designed by the term tonofilaments, and myosin ones (3, 13). Proceeding from the possibilities of the electron microscope method of investigation and from certain controversies and obscurities in literature data available, we started an investigation of the intermediate filaments in epithelial cells of conventional mice thymus.

### Material and methods

Thymus material of 80 conventional mice at different age (between 13—14 day old fetuses and 24 month old animals) was processed according to the routine method for electron microscopic study (1). An experiment was carried out on 11 animals as 9 mice were intraperitoneally injected L-3, 3', 5-Triiodothyronin (Berlin-Chemie) diluted in saline after J. Scheiff's et al. (1977) method while 2 animals were controls and injected saline only.

### Results and discussion

Intermediate filaments were observed in all epithelial cells of our animals studied. They were commonly organized in the shape of bundles often ending in the area of desmosome contacts between neighbouring epithelial cells. On cross-section filaments looked like tubules (several subunits connected with each other surrounded an optically pale inside) and had an outer diameter of 9—10 nm (fig. 1-a, b). That was a striking fact that the relative share of intermediate filaments varied in different morphological epithelial cell types as distinct from the rest cytoplasmic organellae. They were most numerous in certain cortical epithelial cells designed by us (1) as epithelial cells, type 1<sup>st</sup> («supporting») where filament bundles were orientated towards the longitudinal cell axis and occupied a great part of the cytoplasm. Simultaneously, only single intermediate filament bundles with characteristic intracellular localization could be observed in «pale» epithelial cells, type II<sup>nd</sup>, of the cortex and medulla (1). These filaments were grouped together in cytoplasmic regions where Golgi-vesicles and condensing Golgi-vacuoles were in the close proximity or contact with the cell membrane. These differences in the relative share of the intermediate filaments in various epithelial cell types help the interpretation of their functional

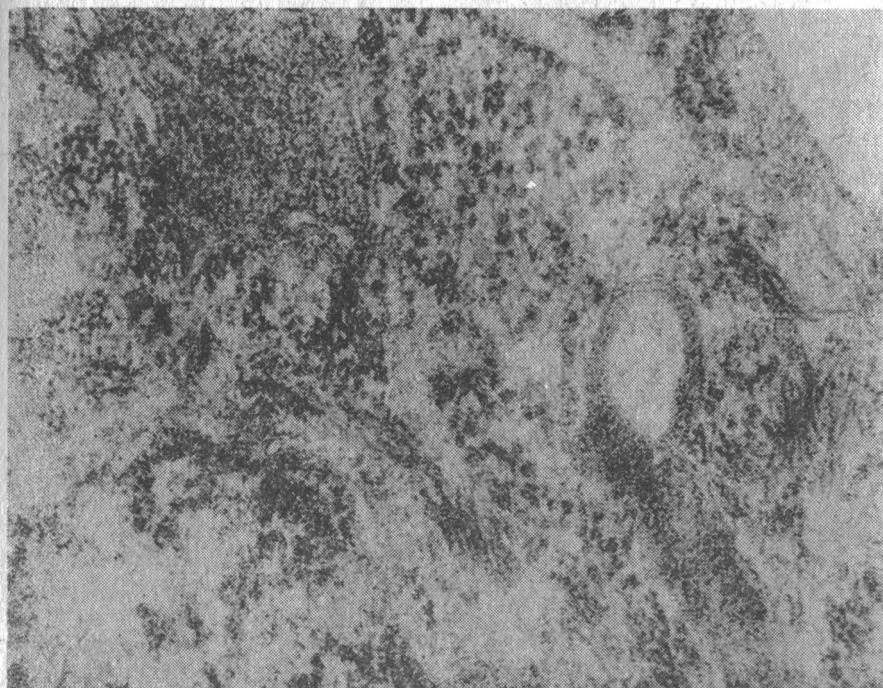
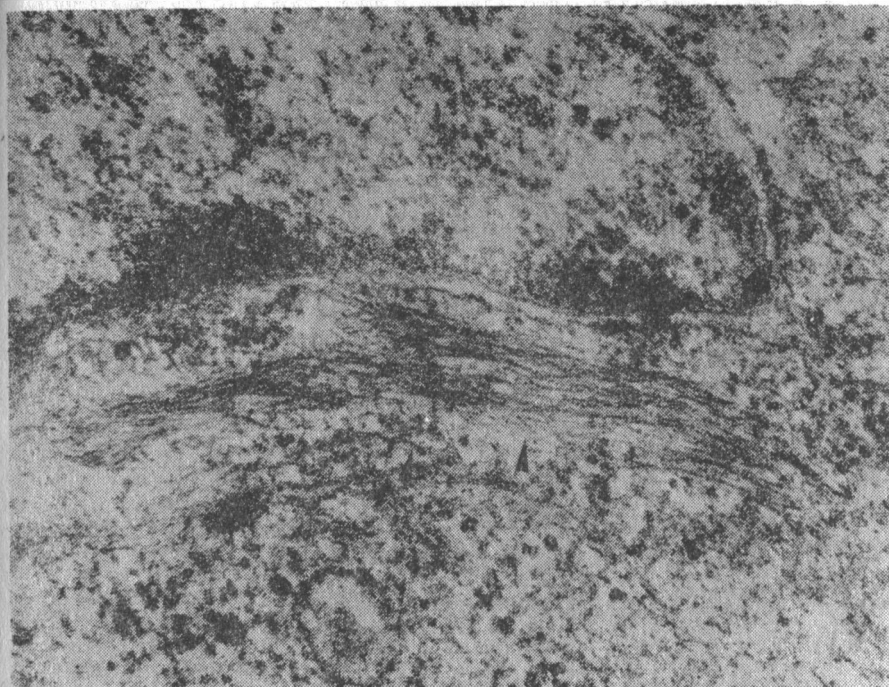


Fig. 1 — a, b. Intermediate filaments oriented by a different manner on the cellular section in the cytoplasm of two cortical epithelial cells. Magn.x 50 000  
a — 3-month old mouse; b — 18-month old mouse

peculiarities. It can be assumed that cortical epithelial cells, type 1<sup>st</sup>, play a role of a supporting skeleton for free cellular elements of the thymus cortex. The close spatial interrelations between intermediate filament bundles and

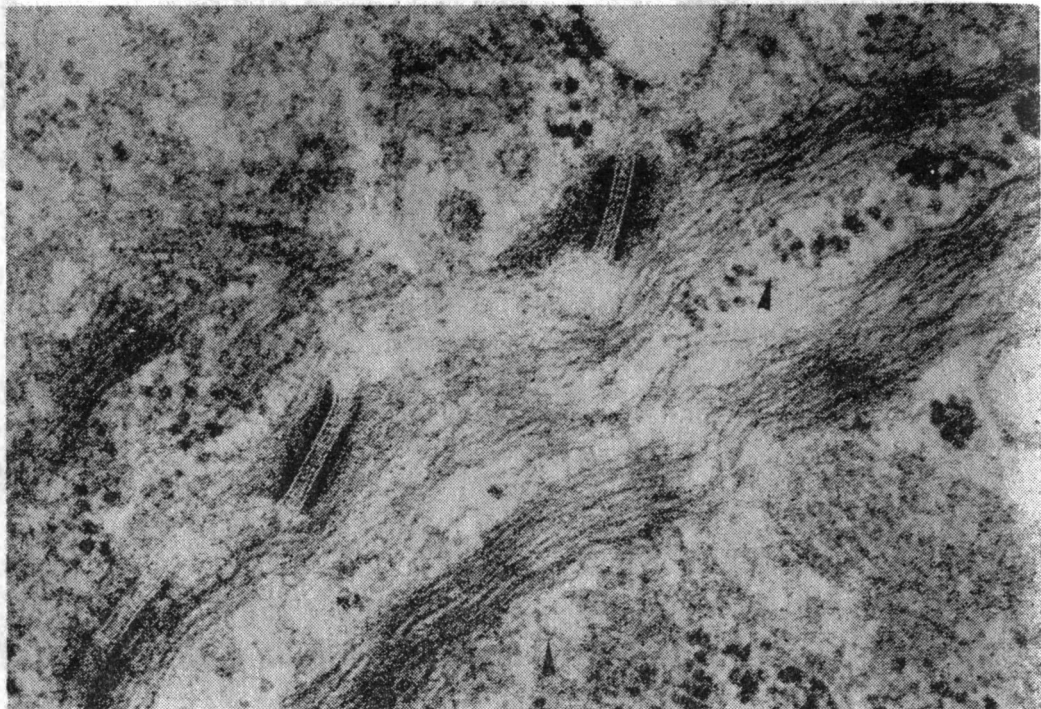


Fig. 2. Intermediate filaments organized in bundles in thymus epithelial cells of 24-month old conventional mouse. Magn.x30 000

condensing Golgi-vacuoles in the epithelial cells of «productive» type allow us to accept that cytofilaments are engaged in the process of liberation of thymus humoral factors by the epithelial cells as it has already been established in cells of some endocrine organs (5, 14).

There were interesting changes of the presentation of intermediate filaments of the epithelial cells during ontogenesis. They could be seen in the cytoplasm of the epithelial cells in thymus of even as early 13—14 day old fetuses and were organized in the shape of very fine bundles. There were no considerable alterations in their amount up to the age of 18 months. It was a notable fact that the amount of intermediate filaments increased to a great extent especially in cortical epithelial cells after this age — bundles with an expressed width occupied the major part of the cytoplasm of the latter. These peculiarities became intrinsic of epithelial cells from both thymus regions in 24-month old mice thymus (fig. 2). Taking into consideration the cytoskeletal function of the intermediate filaments (6) we could suppose that with age they formed a more stable cytoskeletal network in thymic epithelial cells performing a supporting-mechanical function. The fact that the relative part of cytofilaments increased significantly in thymus epithelial cells of lethargic mice (characterized with a very early spon-

taneous thymus involution) as well as in old cultures of thymus epithelial cells (4, 12) allowed us to assume that this was a characteristic ultrastructural peculiarity of «aging» thymic epithelial cells.

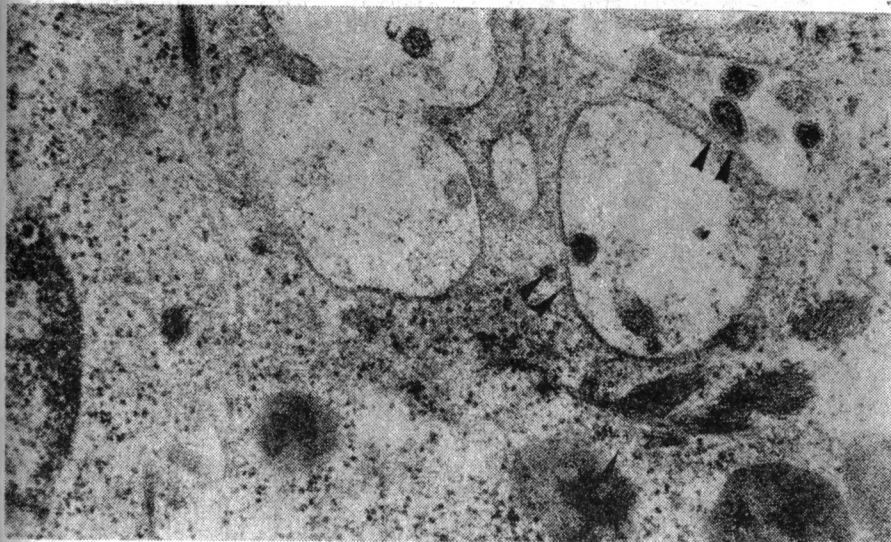


Fig. 3. Intermediate filaments in thymus epithelial cell of 3-month old mouse. Most of them are organized in bundles and located near to oncornaviruses, type C (double arrow). Magn. x20000

C-type oncornaviruses were established in thymus epithelial cells of four animals studied (1). Intermediate filaments in the same cells showed interesting peculiarities — they were organized in bundles concentrated around the viral group (fig. 3). Besides bundles were orientated towards different directions thus forming very whimsical figures. This concentration of intermediate filaments around virus particles (together with certain peculiarities of microtubule organization) located near the nucleus was analogous to the cytoskeletal reorganization into virus-transformed (with iridoviruses, with rheoviruses, with polioviruses) or neoplastically transformed cells under the influence of various factors already described by other authors (8, 10). The reasons for similar alterations were not clarified yet. However, it was assumed that cytoskeletal elements helped the intracellular transport of viral particles and maintained the virus group near the nucleus. It seemed that they facilitated by this way the process of integration of viral and cellular genomes (10).

Interesting changes were observed in intermediate filament organization when epithelial cells were treated with triiodothyronine. These changes were more demonstrative in cortical epithelial cells where intermediate filaments were organized in the shape of bundles forming various figures and occupying relatively large cytoplasmic fields in which other cytoplasmic organelles were hard to be seen (fig. 4). It was possible that there was a more active participation of these cytoplasmic organelles in the process of liberation of substances synthesized more intensively by epithelial cells of «productive type» or the rising number of intermediate filaments resulted (together with increase of desmosome number) from the morphogenetic action of thyroid hormones (2).

Our investigation shows that intermediate filaments (together with vesicular and vacuolar structures and cell nucleus — 1) are one of the most dynamic thymus epithelial cell organellae which change typically with age, in latent virus infection conditions and after some hormonal influences.

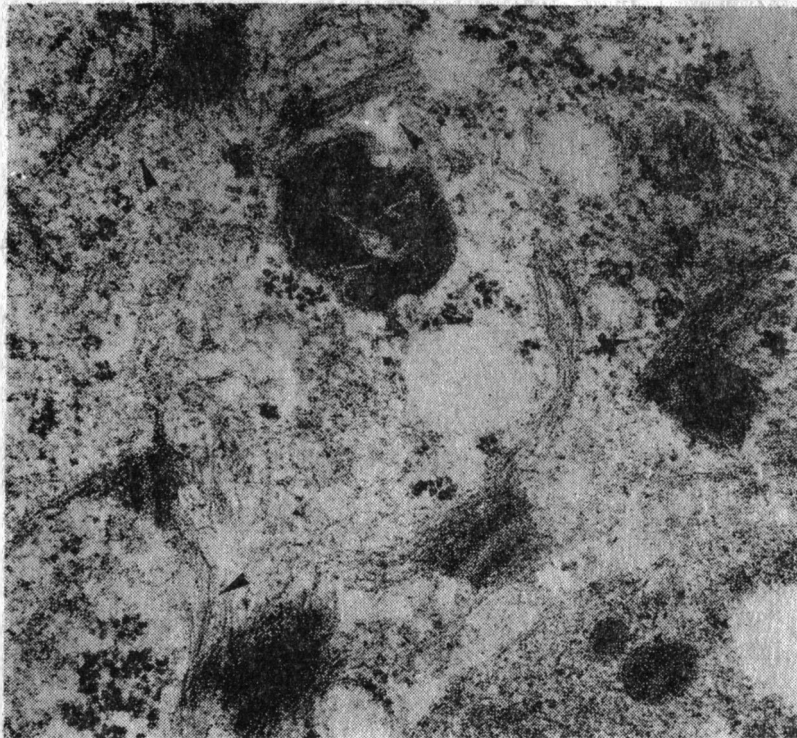


Fig. 4. Numerous intermediate filaments in cortical epithelial cell, type 2, of triiodothyronine-treated animal (3-month-old conventional mouse). Magn. x 20 000 Note: Intermediate filaments are indicated by single arrow

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**ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ ИНТЕРМЕДИЕРНЫХ ФИЛАМЕНТОВ В ЭПИТЕЛИАЛЬНЫХ КЛЕТКАХ ТИМУСА В ХОДЕ ОНТОГЕНЕЗА И ПОСЛЕ ГОРМОНАЛЬНОГО ВОЗДЕЙСТВИЯ***Ц. Маринова***Р Е З Ю М Е**

С помощью стандартного метода электронной микроскопии исследованы интермедерные филаменты эпителиальных клеток тимуса конвенциональных мышей в возрасте от 13—14 дней до 24 месяцев. Часть этих животных были инъецированы интраперитонеально L-3, 3,5-трийодтиронином. Установлены различия в удельном весе, внутриклеточной организации и взаимоотношениях интермедерных филаментов с другими клеточными органеллами, находящимися в зависимости от региональной принадлежности соответствующих эпителиальных клеток и наличия в них С-типа онкорнавирусов, от возраста животных и от наличия гормонального воздействия определенного характера. Приведенные факторы характеризуют филаменты как исключительно динамические органеллы эпителиальных клеток тимуса.