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THE EPITHELIAL TUBULES OF THE THYMUS ARE THYMOSIN-DEPENDENT STRUCTURES

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KEY WORDS: thymus, epithelial tubules, thymosin.

Considerable progress in the study of the thymus has led to the unanimous conclusion that it is an organ with dual function: lymphopoietic and hormonal [5, 6, 7]. More than 14 different substances with biological activity have been isolated from the thymus, and some of them have been synthesized artificially [1, 9, 12, 15]. Using monoclonal antibodies to thymosin and thymulin, the sources of synthesis of the thymus hormones have been localized, namely the epithelial cells of the cortex and medulla [13, 15]. Methods of determination of hormones in the blood have been developed [9]. However, the question of how thymus hormones enter the circulation has not yet been settled, and the role of the corpuscles and epithelial tubules of the thymus in this process has not been identified.

The possibility that the epithelial tubules may be involved in the secretory process is indicated by histochemical and histoautoradiographic investigations [2, 10], which have demonstrated the ability of the epithelial cells lining the walls of the tubules to synthesize proteins and glycoproteins has been confirmed. Epithelial tubules are detectable constantly in the early postnatal period of development [2, 10, 11]. The character of the morphological changes arising in the epithelial tubules of the thymus following injection of exogenous thymus hormones has not been established, and the investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

The test object was the thymus of Wistar rats aged 1 month. The experimental animals were given an injection of thymosin-5, obtained by the usual method [12] on the 1st, 2nd, and 3rd days after birth in the interscapular region, in a single dose of 50 μg in 0.05 ml of solution. The control animals received 0.05 ml of physiological saline at the same times. The intact, control, and experimental animals were decapitated on the 1st, 3rd, 7th, 14th, and 30th days after birth. The thymus was quickly removed and fixed in Bouin's or Carnoy's fluid. Sections 5-7 μ thick were stained with hematoxylin and eosin, alcian blue, and Schiff's reagent. Between 30 and 50 serial longitudinal and transverse sections were cut from each block, and each was examined visually for the presence of epithelial tubules.

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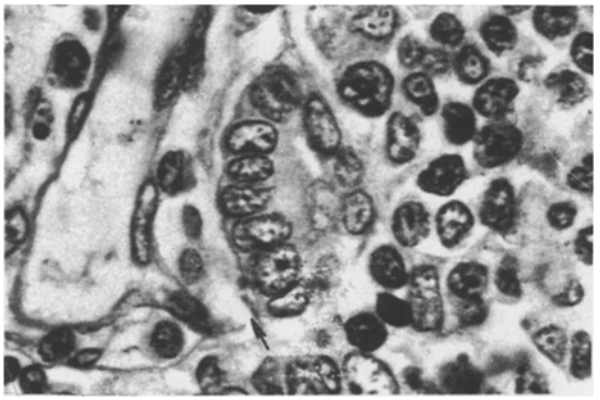

Fig. 1. Small epithelial tubule filled with alcianophilic contents, in close contact with blood vessel (arrow), in cortex of thymus. Here and in Fig. 2: alcian blue. Nuclei counterstained with Carazzi's hematoxylin. Objective 100, ocular 6.3.

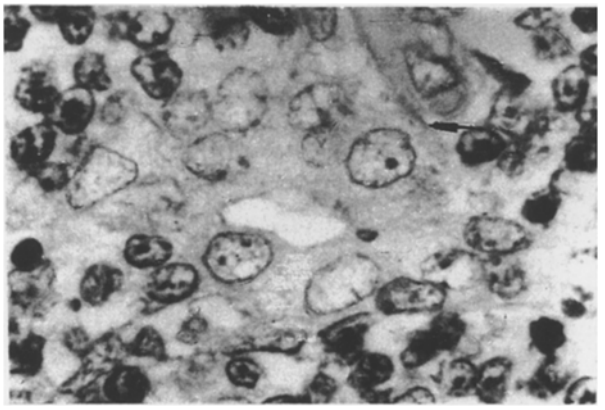

Fig. 2. Resorption of contents of epithelial tubule in contact with blood vessel, in thymus of 3-day-old animal.

EXPERIMENTAL RESULTS

Epithelial tubules were discovered in the thymus of all the intact and control newborn rats (Table 1). They were tiny, circular in shape in transverse section, whereas in longitudinal section they appeared as tubules, which could be traced through 10-20 serial sections. Their diameter varied from 20 to 30 μ. The walls of the tubules were lined with prismatic epithelial cells, which actively secreted PAS-positive and alcianophilic substances into the lumen of the tubules (Fig. 1). The tubules had close contact with vessels of the microcirculatory bed. Virtually no lymphocytes were seen between the epithelial cells or in the lumen of the tubules. After the injection of thymosin-5, epithelial tubules also were found in the thymus of all the experimental newborn rats after the first injection of thymosin-5, but they were smaller than in the controls.

Epithelial tubules were constantly identified 3 days after birth in the thymus of the intact and control animals, but their morphology was changed. They were larger and in transverse sections they most frequently were oval-shaped. The longitudinal dimensions were unchanged. Cubical and flattened cells appeared among the epithelial cells. Areas of double-layered epithelium could be seen. The secretory activity of the epithelial cells remained high, but empty epithelial tubules appeared (Fig. 2). Near the walls of the tubules vessels were constantly seen. By contrast, in the experimental rats epithelial tubules were not seen after the third injection of thymosin (Table 1).

TABLE 1. Frequency of Discovery of Epithelial Tubules in Thymus of Rats Receiving Thymosin-5 (number of animals studied given in parentheses)

Group of animals	Age of animals, days				
	1	3	7	14	30
Intact	6(6)	12(12)	13(13)	0(14)	1(7)
Control (injection of physiological saline)	3(3)	3(3)	3(3)	0(3)	1(3)
Exptl. (injection of thymosin)	6(6)	0(10)*	0(14)*	5(13)*	6(10)*

Legend. *$p < 0.05$ — statistically significant results.

The most epithelial tubules were detected in intact and control animals 7 days after birth. In a few animals several tubules were encountered in a single slice of the organ. On transverse sections the shape of the epithelial tubules may vary from round to polygonal on account of invaginations in the tubule wall. In the majority of tubules the epithelium becomes multilayered. Between the epithelial cells and the lumen of the tubules small- and medium-sized lumphocytes are constantly found (Fig. 3). The synthesis of glucosaminoglycans and proteoglycans in epithelial cells of tubules drops noticeably and the majority of epithelial tubules are empty. In the experimental animals epithelial tubules are not detected in the thymus gland.

No epithelial tubules could be identified in the thymus of the intact and control animals 2 weeks after birth (Table 1), unlike in animals receiving thymosin. Large concentrations of epithelial cells and fully formed thymic corpuscles were seen in the thymus of all three groups of rats against the background of a well developed cortex and medulla.

The morphology of the epithelial tubules in the thymus of the experimental animals resembled patterns observed in the control rats on the 1st and 3rd days after birth.

Epithelial tubules were seen in month-old animals of the experimental group three times more often than in the control and intact groups. The morphology of the epithelial tubules was highly variable.

The main function of the thymus is to supply the body with immunologically mature T lymphocytes [5, 6]. The maximal intensity of thymus gland function is observed 1 week after birth [5], when the peripheral lymphoid organs are rapidly colonized by T lymphocytes. Against this background epithelial tubules of the most varied shapes appear continually [2, 10, 11]. Epithelial cells lining the walls of the epithelial tubules actively secrete proteoglycans and glycosaminoglycans into the lumen of the tubules. The tubules have close contacts with blood vessels of the microcirculatory bed. Electron-microscopic investigations demonstrated the presence of trans-cellular mechanisms of transport of pinocytotic vesicles from the lumen of the tubules into the vessels, and vice versa, in the epithelium [14]. All these morphological features indicate involvement of the epithelial tubules in secretion.

Besides the early postnatal period of development, epithelial tubules also were constantly discovered during the first hours and days after exposure of the animal to various conditions: starvation, irradiation, skin grafting, injection of hormones, etc. [3, 11]. Any of these conditions induced more intensive migration of T lymphocytes from the thymus. Both mature T-lymphocytes, and earlier immature forms, which were able to mature under the influence of thymic factors in the peripheral lymphoid organs, migrated under these circumstances [4, 7, 8].

The question of which structures of the thymus participated, and how, in the secretion of thymic factors into the circulation remains a matter for debate. On the basis of data showing continuous detection of epithelial tubules with signs of active secretion in newborn rats against the background of massive migration of leukocytes from the gland, an attempt has been made to link the discovery of tubules in the thymus with its hormonal factors. In the first 3 days after birth of the animals exogenous thymosin-5, which acts both on bone-marrow precursors of T-lymphocytes and on immature lymphocytes, migrating from the thymus, was injected into the animals [4, 7, 8].

Epithelial tubules were discovered 6 h after a single injection of thymosin into the newborn animals in the thymus of all the rats, but they were smaller than in the intact and control animals. After the third injection of thymosin in the course of the first 3 days after birth, no epithelial tubules could be seen in the thymus of the experimental animals of the 3rd and 7th days after birth. Meanwhile, in the intact and control animals at these times epithelial tubules were seen in all individuals, and the time course of the morphology of the tubules was closely linked with the development of the gland as a whole. Active secretion

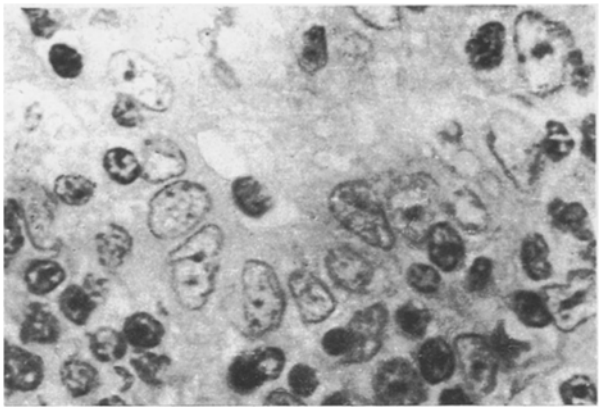

Fig. 3. Depopulated epithelial tubule with lymphocyte in lumen, located on cortico-medullary boundary in thymus of 7-day-old rat. Hematoxylin and eosin. Objective 100, ocular 6.3.

of proteoglycans and glycosaminoglycans by the epithelium of the tubules, contacts of the tubules with vessels of the microcirculatory bed, and changes in the morphology of the tubules in the course of a change in functional activity of the thymus in the intact and control animals, are evidence of involvement of the epithelial tubules in realization of the function of the gland. Injection of thymosin inhibited the formation of epithelial tubules in the thymus of the experimental animals, proof of the thymosin-dependent character of the tubules. The causes of inability to find epithelial tubules in the thymus of the experimental animals may be connected with the replacement action of thymosin on the epithelial cells of the tubules by a feedback mechanism. The possibility of a transient decrease in hormone synthesis by the epithelial cells of the thymus after injection of the exogenous hormone was mentioned in [15].

Considering data showing that the formation of the thymus, with its lobes, lobules, cortex, and medulla, is undisturbed in the experimental animals in the absence of epithelial tubules, in our view the injected thymosin had a replacement action on the epithelium, causing T-precursor cells to be supplied to the thymus, and these were responsible for the formation of the gland. The function of the epithelial tubules is therefore to secrete thymosins, which may perhaps regulate the flow of T-precursor lymphocytes from the bone marrow into the thymus during a period of intensive functional activity of the gland. As the T-precursor lymphocytes are supplied to the thymus, and undergo differentiation and proliferation, the secretory activity of the epithelial tubules is depressed. Due to the increase in the number of lymphocytes the tubules become difficult to distinguish, and this may explain why in adult animals developing in the usual way epithelial tubules are found in 20-30% of cases [11].

It can be concluded from these results that the epithelial tubules of the thymus are thymosin-dependent structures, which can be included among the reactive formations of the gland.

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THE USE OF VENOUS ALLOGRAFTS IN VASCULAR SURGERY

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The availability of adequate vascular prostheses remains an urgent problem in emergency reconstructive vascular surgery. The main reason is that the severity of the patient's state in an emergency situation calls for the shortest possible duration of surgical intervention. Synthetic prostheses can be used successfully to repair the aorta or arteries of large diameter [2, 10], whereas reconstruction of arteries of average and small caliber can best be achieved by replacement with an autologous vein [4, 8]. However, in 15-30% of cases it has a dispersed type of structure, a small diameter, or changes resulting from previous thrombophlebitis, and it is therefore unsuitable for use as a prosthesis [5, 9, 11].

The use of venous allografts is therefore interesting for such purposes. By now, the use of "fresh" allografts has achieved popularity, when used within 1-2 h after preparation [1, 7].

The aim of this investigation was to develop a simpler method of conserving venous allografts based on a study of changes in their biomechanical properties, morphology, and tissue metabolism.

EXPERIMENTAL METHOD

A 2% solution of neutral formalin and an isotonic buffered salt solution (Hanks' solution) were used for conservation.

Venous grafts were taken from 54 patients undergoing operations for varicose veins of the lower limbs without any trophic disturbances. The patients' ages were from 23 to 60 years and the duration of the disease from 1 to 20 years. After removal, the samples of the grafts were kept in the above-mentioned solutions at 4°C and their biomechanical, biochemical, and morphological properties were studied on the 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th days of conservation.

Changes in the mechanical properties of the grafts were studied by the method of uniaxial stretching of flat specimens. Specimens measuring 20 × 5 mm were prepared in the longitudinal direction and their properties tested on the "Instron-1122" universal testing machine. Tests were carried out on 187 specimens.

The following characteristics of the material were determined:

a) the tensile strength σ = p/f mPa, where p denotes the load required for complete rupture of the test specimen, and f the area of cross section of the specimen;

b) the deformation $\varepsilon = (l - l_0)/l_0$, where l and l_0 denote initial and deformed length of the specimen;

c) the tangential modulus of elasticity (E, in mPa) was determined as the tangent of the angle of slope to the abscissa, the tangent and the deformation curve, and calculated for several values of stretching tensions.

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