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Distribution of the Hormonal Thymic Factor Thymalin in Human Fetal Tissues

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It is known that differentiation of T lymphocytes from precursor T cells, which are supplied by the hemopoietic organs, occurs in the thymus. Hormones secreted by thymic epithelial cells are involved in this process [14]. A group of thymic hormones consisting of thymosin α_1 [5], thymulin [2], thymic hormonal factor [19], and thymopoietin [4] has been identified. All of these hormones were obtained from animal thymuses and are currently used as immunomodulators in clinical practice in the west. In the CIS the immunomodulators taktivin [1] and thymalin (timalin) [11] have found a wide application. Thymalin induces the differentiation of T cells [12], normalizes immunological indexes in immunodeficient animals [6], and effectively corrects secondary immune deficiency [18]. It was shown that, upon interacting with the plasma membrane of T lymphocytes, the constituent peptides of thymalin stimulate the expression of specific receptors and enhance their functional activity [11].

Thymalin, as well as thymosin (fraction V) is a mixture of polypeptides with molecular weights varying from 1000 to 5000 D (gel filtration chromatography on Sephadex G-25, G-50). Thymalin is entirely different from thymulin; these peptides have no homologous sequences [12].

The aim of this study was to define the period of embryogenesis when the thymus starts to secrete thymalin and to find out whether thymalin-containing cells are present in the epidermis and in other organs whose epithelium is similar in embryological origin to thymic endothelium.

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MATERIALS AND METHODS

Organs of 40 embryos and fetuses (6-25 weeks of gestation) obtained from essentially healthy women during induced abortions were studied. The epithelium of the organs developing from the anterior head gut - the thymus, epiglotis, larynx, trachea, lung, and esophagus - was examined. The head gut is localized in the anterior part of the embryo [7], and endothelium originating from it possesses certain features of ectoderm and entoderm [8,9]. We also examined skin, intestine, liver and spleen epithelium of the same fetuses.

Physiologically active substances were prepared from bovine thymuses [11,15]. The organs were kept in acetone at -4°C for 48 h and homogenized. Extraction with 3% acetic acid (1:6, v/v) in the presence of $ZnCl_2$ was carried out for 72 h. The precipitate obtained after centrifugation (20 min, 3000 rpm) was treated with acetone and ether until a white powder was formed. The powder was dissolved, sterilized, and lyophilized. Ion-exchange chromatography on carboxyl cationite (Biokarb, Russia), gel-filtration on Sephadex G-25 (Pharmacia, Sweden), and electrophoresis in a thin layer of cellulose (Filtrak, Germany) showed the preparation to consits of several polypeptide fractions (Table 1, [15]).

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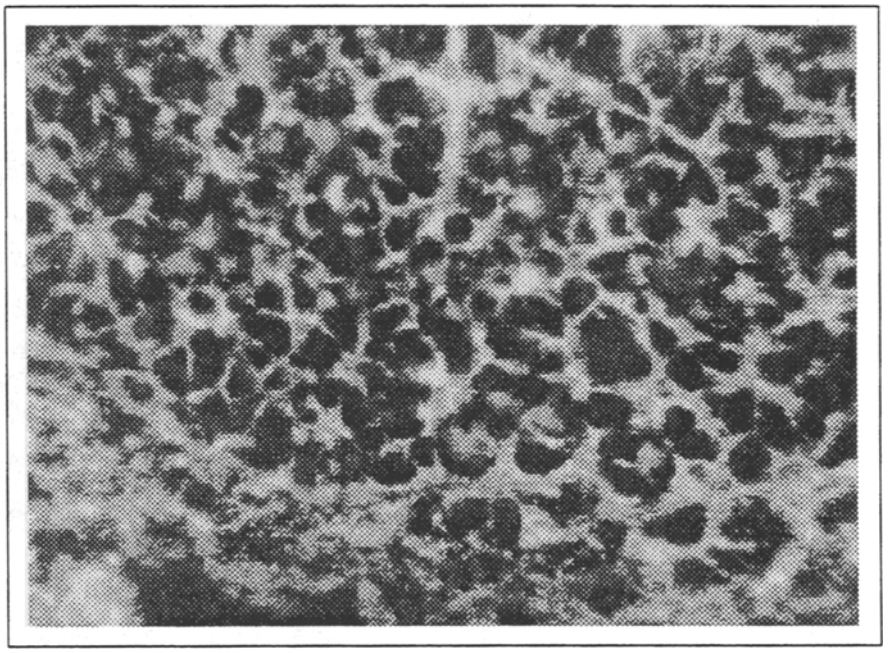

Fig. 1. Localization of thymalin in the thymus of a 6-week-old human embryo. Thymalin-positive cells with processes forming a network. ×8000.

Cryostat sections (4-6 μ thick) were cut from pieces of organs frozen in liquid nitrogen, air-dried, fixed in cold (5°C) acetone, and washed with cold phosphate-buffered saline (PBS, pH 7.4) for 15 min. After incubation with rabbit antithymalin antiserum (Institute of Vaccines and Sera, Moscow) for 40 min, the sections were washed 3 times with cold PBS and incubated for 20 min with FITC-conjugated donkey antirabbit antiserum (Institute of Epidemiology and Microbiology, Moscow). The preparation and evaluation of antithymalin antiserum were described by Moskvicheva *et al.* (1985). The serum was obtained by immunization of rabbits with thymalin conjugated to bovine serum albumin or polyvinylpyrrolidone (40 kD). Its specificity was evaluated by radial immunodiffusion and immunoelectrophoresis in agar gel.

Antithymalin antiserum was used in the indirect agglutination titer 1:3200 and donkey antirabbit antiserum was used in the titer 1:64. For the purpose of reducing nonspecific protein adsorption, prior to incubation the conjugates were treated with human liver powder.

Control sections were incubated with nonimmune rabbit serum or PBS. All sections were incubated in a humidified chamber and examined under a LYuMAM-3 fluorescent microscope (LOMO, St. Petersburg).

RESULTS

At the early stages of embryogenesis (6 weeks), when the thymus is not populated by lymphoid cells, thymalin-positive cells are diffusely distributed over the entire epithelial stroma of the thymus. The processes of these cells interconnect to form a network (Fig. 1). The emergence of thymalin-positive cells in the thymic reticuloendothelium precedes colonization of this organ by lymphoid cells, which is generally observed by the 7.5-8th week of embryogenesis [16].

In 20-25-week-old fetuses, thymalin-positive cells form a thin layer in the subcapsular zone (Fig. 2, *a*) and are concentrated predominantly in the medulla (Fig. 2, *b*).

At the same stage of embryogenesis, thymalin-positive cells are found in the epithelium of the epiglottis, larynx, trachea (Fig. 2, *c*), lung, esophagus, and skin of various localization (Fig. 2, *d*). Fluorescence is confined to immature epitheliocytes. In the airways, these cells are represented by basal and intermediate cells of the stratified epithelium; in the esophagus and skin they comprise

TABLE 1. Physicochemical Characteristics of Components Extracted from the Thymus

Preparation	Component	Content in wholemount, %	Specific weight	Isoelectric point, pH units
Thymic factor (TF)	Fraction 1	80	660	2.5
	Fraction 2	15	5000	4.0
	Fraction 3	5	5000	9.5

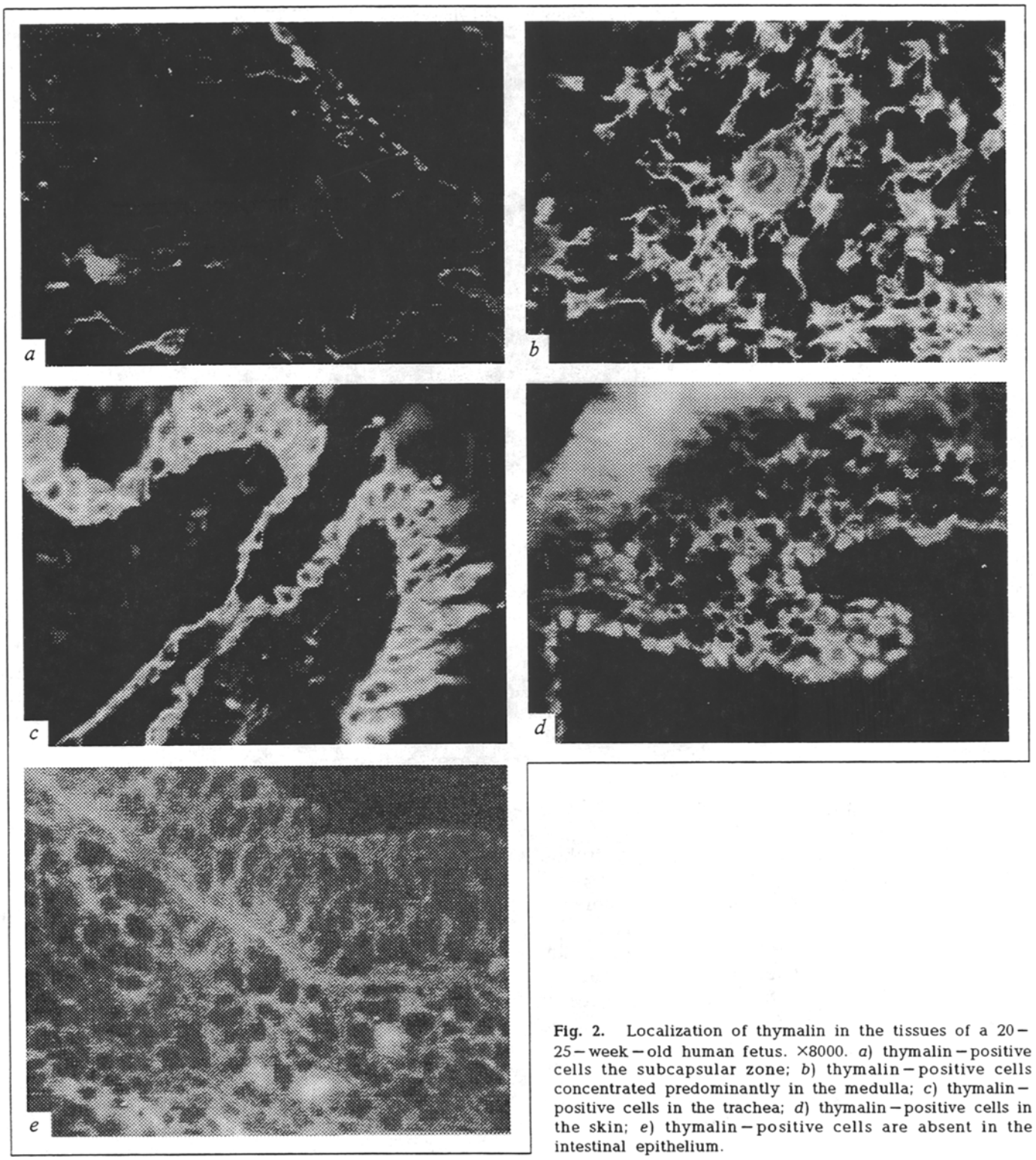

Fig. 2. Localization of thymalin in the tissues of a 20–25-week-old human fetus. ×8000. *a*) thymalin-positive cells the subcapsular zone; *b*) thymalin-positive cells concentrated predominantly in the medulla; *c*) thymalin-positive cells in the trachea; *d*) thymalin-positive cells in the skin; *e*) thymalin-positive cells are absent in the intestinal epithelium.

1 or 2 basal layers. The fluorescence is more intense in the cutaneous and tracheal epithelium and less intense in the esophageal epithelium. No fluorescence is observed in the control sections. Thymalin-positive cells are not found in the small and large intestine epithelium (Fig. 2, *e*) or in the liver, i.e., in the organs that develop from the endoderm.

Our findings indicate that the human thymus starts to produce thymalin in the early stages of gestation, prior to its colonization by lymphoid cells. Thymalin-positive cells are present in the

thymus during the whole period of embryogenesis. Similarly to cells that secrete thymosin α_1, thymulin, and thymopoietin [17], thymalin-secreting cells are located in the subcapsular and medullary zones.

The observation that thymalin is present in the basal layer of the skin, epiglottis, larynx, trachea, and esophagus are in line with published data indicating the presence of thymopoietin in skin keratinocytes of adult humans [3] and in some epithelial tissues of adult mice [13].

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The Protective Effect of Sodium Hypochlorite on Morphology and Transcription of Rat Central Neurons in Acute Nembutal Poisoning

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Hypoxia plays a key role in the pathogenesis of acute barbiturate poisoning [1,3]. Accordingly, in brain tissues, which naturally have a high level of energy metabolism, barbiturates cause damage accompanied by a decrease of creatine phosphate and of the total amount of ATP, ADP, and AMP [1,2]. In addition, in CNS neurons, in particular in the pyramidal cells of the cortex, barbiturates

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