IMMUNOHISTOCHEMICAL AND ELECTRON-MICROSCOPIC IDENTIFICATION OF CELLS PRODUCING SEROTONIN AND PROSTAGLANDIN F_2 IN THE RAT THYMUS

S. E. Aleksandrova, I. M. Kvetnoi,
É. S. Kurilets, and V. A. Gulyaev

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The attention of research workers has recently been drawn to the study of the role of hormones in the control of immunologic reactions. Changes in functional activity of endocrine cells have been demonstrated in various immune disturbances. It has been shown that biogenic amines and certain peptide hormones can be secreted by apudocytes, which are components of immunocompetent organs [3]. In addition, a close connection definitely exists between the immune and endocrine control systems in the method of function of the thymus – the key organ of the immunity system – itself [4, 8, 13]. Before the 1960s the thymus was not regarded as an endocrine organ. The function of the thymus which had been investigated was that primarily connected with the formation and differentiation of lymphoid cells. An important role in the study of the immunologic properties of the thymus was played by thymectomy

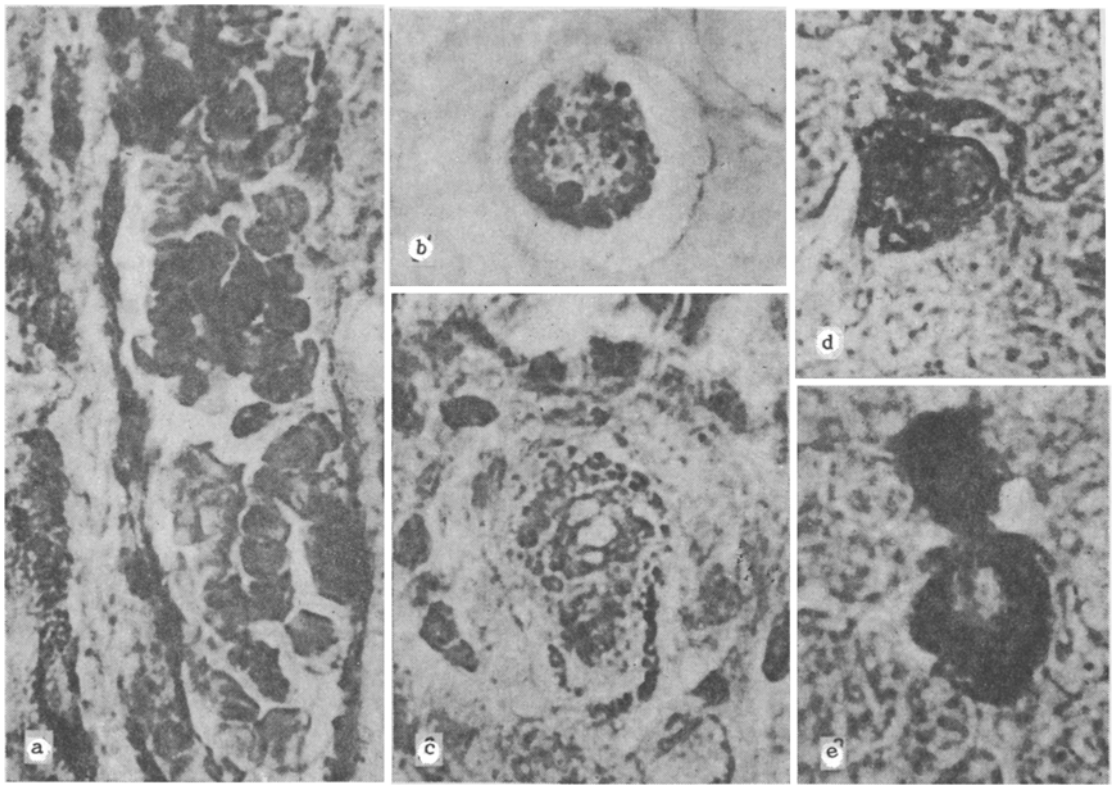

Fig. 1. Endocrine cells in the thymus. a) Grimelius' argyrophilic reaction; group of cells located along a blood vessel in the medulla. 600 ×; b) cells of Hassall's corpuscles, Grimelius' reaction. 900 ×; c) identification of serotonin in medullary cells of the thymus. Immunoperoxidase reaction. 900 ×; d, e) immunoperoxidase reaction. Demonstration of prostaglandin F_2 in cortex of thymus. 720 ×.

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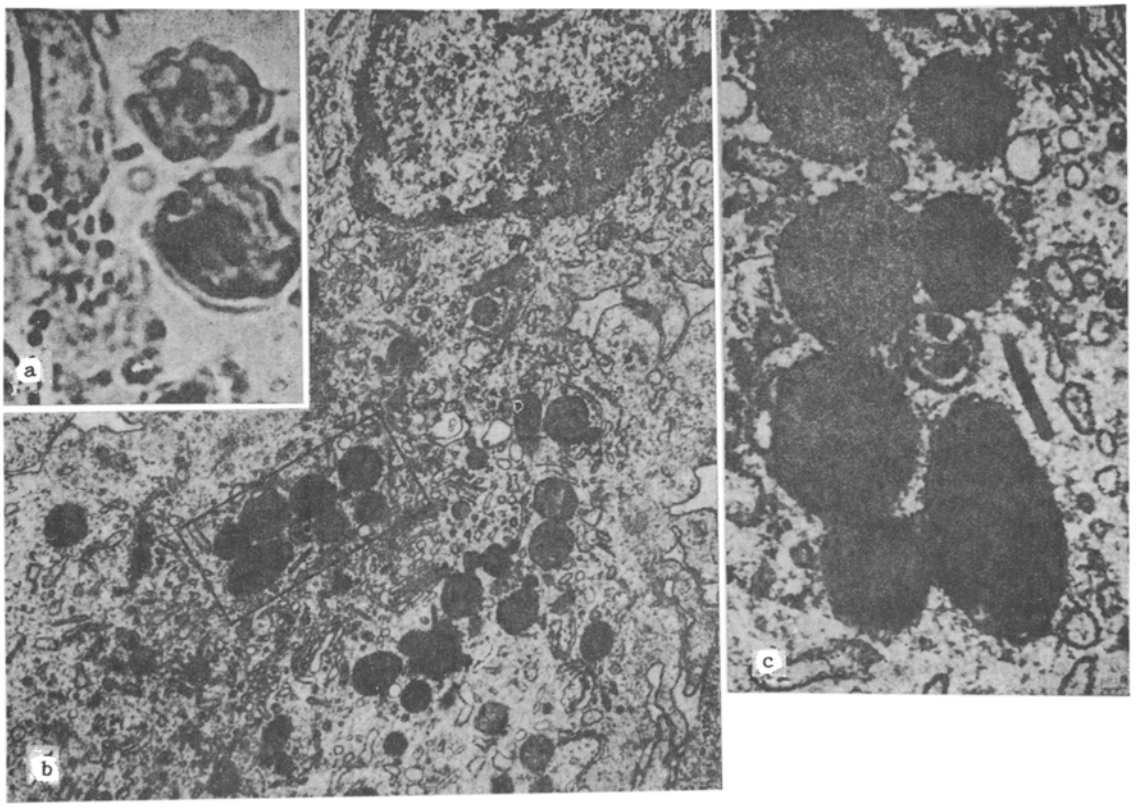

Fig. 2. Electron-microscopic identification of serotonin-containing cells in thymus. a) Immunoperoxidase reaction with antiserum to serotonin in medullary cell of the thymus. 900 ×; b) reticuloepithelial cell of the thymus, containing secretory granules characteristic of serotonin in cytoplasm. Block trimmed after positive immunohistochemical reaction. 13,400 ×; c) secretory granules containing serotonin in reticuloepithelial cell of thymus. Detail of Fig. 2b. 35,000 ×.

in newborn infants, the study of individuals with congential defects or absence of the thymus and correction of these disturbances by administration of its extracts. These circumstances had the result that the endocrine function of the thymus was underestimated for a long time, and its role as an organ of immunity was dominant. Miller [12] opened the "golden age" of thymology. In recent years sufficient evidence has been obtained of the endocrine function of the thymus [3, 4, 9]. The presence of epithelial cells in the thymus is yet another indirect sign of the endocrine function of this organ [4, 9, 10]. More than 20 physiologically active substances, whose secretion is connected with the epithelial cells and Hassall's corpuscles, have been isolated from the thymus [3, 10]. Injection of thymus factors have been shown not only to prevent and correct disturbances in the immunity system, but also to reduce the frequency of development of spontaneous tumors, to increase the duration of survival and increase the resistance of the host to tumor growth, to prevent the development of transplantable leukemia, to exert a protective action against radiation, and to stimulate regeneration when it is inhibited in experimental animals [1, 2, 5, 6, 13]. The presence of endocrine-like cells also has been demonstrated in the medulla of the chick thymus, and they give an argentaffin reaction [11]. All this provides the theoretical basis for a further search for hormonally active substances in the epithelial cells of the thymus.

The aim of this investigation was to study the possibility of synthesis of serotonin and prostaglandin F_2 in the epithelial cells of the thymus by methods of immunohistochemistry and electron microscopy. The identification of these substancs in the thymus is theoretically possible and biologically justified for by virtue of their biological properties these hormones are essential as modulators of the rate and time of formation of immunocompetent cells.

EXPERIMENTAL METHOD

Pieces of the thymus were taken for investigation from Wistar rats. For the light-optical study the material was fixed in neutral Bouin's fluid and embedded in paraffin wax.

For electron-microscopy the thymus was fixed by Karnovsky's method and embedded in Epon. Staining was carried out by Grimelius' method and the immunohistochemical reaction on de-waxed, semithin sections, by the indirect immunoperoxidase method. Specific antisera against serotonin and prostaglandin F_2 were used. After the discovery of a positive reaction to antisera against hormones, the blocks were trimmed for the cutting of ultrathin sections, to be studied on the JEM-100C microscope.

EXPERIMENTAL RESULTS

Histochemical staining of sections of the thymus by Grimelius' method showed a positive argyrophilic reaction in the medulla of the thymus. A positive reaction by Grimelius' method was found in a group of cells along the large blood vessels (Fig. 1a) and in cells of Hassall's corpuscles (Fig. 1b). The immunoperoxidase reaction revealed cells containing serotonin in the medulla of the thymus (Fig. 1c) and prostaglandin F_2 in the cortex of the thymus (Fig. 1d, e). To determine the precise location of serotonin in the thymus, the immunohistochemical reaction was carried out on semithin sections (Fig. 2a). After trimming of the block and examination of serial ultrathin sections in the electron microscope it was found that cells giving a positive immunoperoxidase to anti-serotonin serum are reticuloepithelial cells of type 2 according to Clark's classification [9]. These are cells with a large nucleus, containing many large mitochondria, a well developed lamellar apparatus, and rough and smooth endoplasmic reticulum. Numerous secretory granules, with the characteristic structure or serotonin, were present in the cytoplasm of these cells (Fig. 2b, c).

Identification of serotonin- and prostaglandin-containing cells in the thymus considerably widens our ideas on the regulatory role of this organ in living processes. Serotonin is known to be involved in antitumor resistance [7], and a radioprotective mechanism of action of this biogenic amine also is possible [2]. As an inhibitor of cell division, serotonin can control the times and rate of proliferation of immunocompetent cells in the thymus. Prostaglandin F_2 has a significant effect on carbohydrate and lipid metabolism and also has a modulating influence on the function of endocrine organs, including the thymus. The discovery of serotonin and prostaglandin F_2 in the thymus suggests that secretion of other hormonally active substances is also possible in the thymus. The identification and study of these substances from the oncoradiologic aspect will be a topic for our future research.

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