LITERATURE CITED

1. I. A. Alov, "Experience of the study of the cerebrospinal fluid: the meninges and vascular plexuses of the brain," Doctoral Dissertation (place not given) (1954).
2. A. A. Arkhipovich, "The microvascular system of the dura mater of the human brain. A macro-microscopic investigation," Doctoral Dissertation, Kiev (1973).
3. M. A. Baron, F. M. Lyass, and N. A. Maiorova, Med. Radiol., No. 8, 60 (1964).
4. E. V. Busharov, Arkh. Anat., No. 2, 51 (1970).
5. N. Ya. Vasin, "Structure of the vascular system of the dura mater and general principles of its collateral circulation," Candidate's Dissertation, Moscow (1959).
6. G. F. Dobrovol'skii, Vopr. Neirokhir., No. 6, 5 (1969).
7. G. F. Dobrovol'skii, Vopr. Neirokhir., No. 2, 29 (1970).
8. G. F. Dobrovol'skii, in: Abstracts of Proceedings of the Second All-Union Congress of Neurosurgeons [in Russian], Moscow (1976), pp. 96-98.
9. V. V. Kupriyanov, Ya. L. Karaganov, and V. I. Kozlov, The Microcirculatory System [in Russian], Moscow (1975).
10. N. A. Maiorova, "Experimental investigation of the resorption of red blood cells from the intermeningeal spaces of the brain," Candidate's Dissertation, Moscow (1953).
11. V. A. Shakhlamov, Capillaries [in Russian], Moscow (1971).
12. K. H. Andres, Z. Zellforsch., 79, 272 (1967).
13. J. D. Waggener and J. Beggs, J. Neuropath. Exp. Neurol., 26, 412 (1967).

STRUCTURAL AND IMMUNOMORPHOLOGICAL CHARACTERISTICS OF THE HUMAN THYMUS DURING EMBRYONIC DEVELOPMENT

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The thymus of 100 human fetuses was studied between the 4th and 34th weeks of intrauterine development by means of histological, histochemical, immunomorphological, and electron-microscopic methods. Development of the organ from the standpoint of development of the functional system is described. The anlage of the thymus can be detected at the 5th week of fetal development; it reflects the properties of the epithelium of a foregut organ. By the 7th-8th week differentiation of the reticuloendothelium and population of the organ with lymphocytes are beginning to take place and antigenic specificity is found on the surface of the lymphocytes. The zone of growth of the reticuloendothelium of the thymus, the significance of Hassall's corpuscles, the appearance of two subpopulations of T lymphocytes, and their quantitative changes are described. In the period from the 11th until the 34th week of fetal development the number of T lymphocytes forming rosettes with sheep's red blood cells virtually does not change (70-90%), whereas the number of T lymphocytes forming rosettes with autogenous red cells increases during this period from 23 to 70%.

KEY WORDS: human fetal thymus; lymphocyte; rosette-forming cells.

After the discovery of cellular immunity and identification of the T system of cells among lymphocytes [14] and determination of the role of the thymus, the central organ of lymphopoiesis, in immunologic reactions [2, 7, 12], the morphology and character of changes in the epithelial basis of the thymus during prenatal human development still remain inadequately studied. Since the work of Galustyan [1], the basic question of the nature of this

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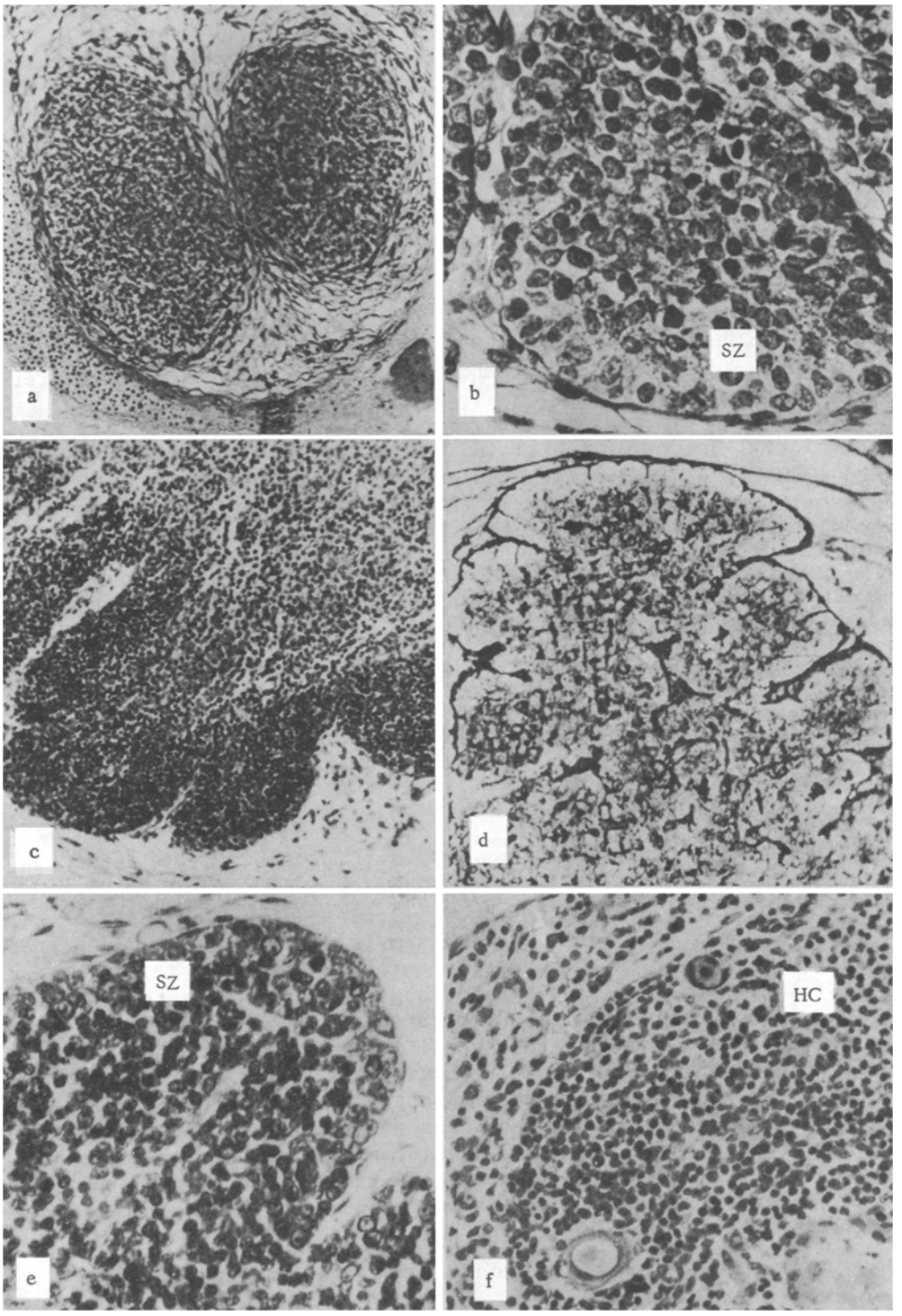

Fig. 1. Structure of the thymus in 7-34-week human fetuses: a) thymus of 7-week embryo (PAS method, 120×); b) thymus of 10-week fetus (PAS, 500×); c) thymus of 12-week fetus (PAS, 120×); d) reticulin fibers of thymus of 10-week fetus (Gordon's stain, 250×); e) thymus of 22-24-week fetus (Brachet's stain, 500×); f) thymus of 34-week fetus (hematoxylin-eosin, 250×). HC) Hassall's corpuscles, SZ) subcapsular zone.

epithelium has still not been settled. Clark [9] states that it is ectodermal in origin and describes the principal features of the epithelial cells of the mouse thymus, which differ from other structures of the organ in that desmosomes are present between the cells and tonofilaments in their cytoplasm. Donarin [10] considers that the epithelium of the thymus is

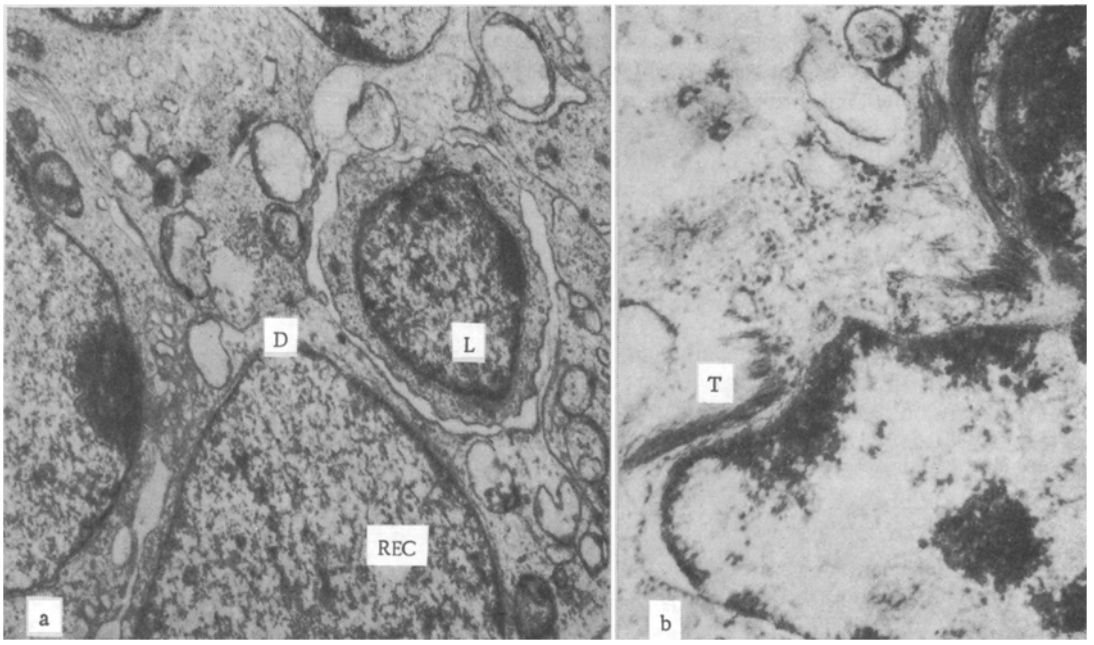

Fig. 2. Electron micrographs of hyman fetal thymus: a) thymus of 8-week embryo (12,000×); b) thymus of 12-week fetus (34,000×); D) desmosome; L) lymphocyte; REC) reticuloendothelial cell; T) tonofilaments.

entodermal in nature. The opinion has been expressed that it is derived from a special anlage — the precaudal plate — in the same way as the epithelium of organs developing from the foregut [3]. In systems of immunogenesis investigated in single fetuses [8, 13, 15] the presence of immunocompetent cells of the T-system has been demonstrated in the thymus of the human fetus. It is not yet known whether one or several subpopulations of T cells appear in the embryonic thymus, what is the character of their differentiation, or where the cambial cells of the epithelium of the thymus providing for development of the organ are concentrated.

This paper describes an attempt to study the development of the human fetal thymus as part of a functional system and to answer some of the questions listed above.

EXPERIMENTAL METHOD

The thymus of 100 human embryos and fetuses between the 4th and 34th weeks of development, obtained at termination of pregnancy for medical reasons, was studied.

The age of the fetus was determined from the assumed date of ovulation of the mother and from the body length of the fetus and the absolute and relative weight of the thymus. Material was fixed in Carnoy's fluid and 10% neutral formalin solution and embedded in paraffin wax. Sections were stained with hematoxylin-eosin, by Brachet's method, the PAS method, for reticulin fibers by Gordon's method, and for keratin by the methods of Romeis' and Shubich [5]. Pieces of thymus for electron microscopy were treated by Palade's method and studied in the JEM-100B electron microscope. The percentage of T lymphocytes forming rosettes with sheep's red blood cells (RFC) and with autogenous red blood cells (autoRFC) was counted in a suspension of the fetal thymus [11]. In a control series lymphocytes were incubated with serum against human T lymphocytes, as a result of which rosette formation was inhibited.

EXPERIMENTAL RESULTS

The period of embryonic development of the thymus was divided into five stages, each with its own particular developmental features.

Embyros Aged 4-6 Weeks. The analage of the thymus can be detected at the 5th week of development in the form of two bands of stratified epithelium, one on each side at the level of the developing larynx. No blood vessels or reticulin fibers are present in the anlage. As regards the content of neutral mucoproteins (NMP) and RNP, its epithelium has common features with the epithelium lining the developing larynx and it is evidently derived from the precaudal plate. Mitoses are seen in the cells of all parts of the bands, but they do not contain lymphocytes.

Embryos Aged 7-8 Weeks. Epithelial bands descend into the retrosternal region and move close together (Fig. 1a). Single narrow blood vessels and thin reticulin fibrils grow into them. A reticuloendothelium appears in the middle of the bands as a feature of special cell differentiation characteristic of the thymus. The cells become branched in shape, NMP disappear from their cytoplasm, and their RNP content is reduced. Desmosomes are clearly visible between the cells. The organ becomes populated with lymphocytes, which can be seen among the reticuloendothelial cells (Fig. 2a). RFC can be detected in the suspension of the organ, evidence that differentiation of T lymphocytes has begun.

Fetuses Aged 10-12 Weeks. The absolute weight of the thymus is 41±1.25 mg and its relative weight 0.089%. Invasion of the reticuloendothelium by mesenchyme with blood vessels is beginning and the band is divided into primary lobules, in which the cortex and medulla can be distinguished by the 11th-12th week (Fig. 1c), and Hassall's corpuscles are beginning to appear. The network of blood vessels and reticulin fibers becomes more concentrated, especially in the medulla (Fig. 1d). The subcortical zone of epithelial cells is clearly distinguishable in the thymus (Fig. 1b). It borders on the mesenchyme, consists of three or four layers of pyroninophilic epithelial cells, and is not populated with lymphocytes. Tonofilaments begin to appear in the cytoplasm of the epithelial cells (Fig. 2b). The suspension of the organ contains 71% RFC and 23% autoRFC.

Fetuses Aged 15-16 Weeks. Active invasion of the primary lobules of the thymus by mesenchyme, reticulin fibers, and blood vessels continues and they separate into new (secondary) fragments. Together with mesenchyme, young epithelial cells of the subcortical zone move from the periphery into the medulla of the lobule, and arrange themselves in it around the blood vessels. The same relations are observed here between the blood vessels, epithelium, and lymphocytes as at the periphery of the organ. This may account for the presence of an identical blood–thymus barrier everywhere in the thymus. Because of widening of the region of cells of the subcortical zone the cortex and medulla of the thymus begin to grow more rapidly and there is a marked increase in the absolute and relative weights of the organ (156.3±2.9 mg and 0.1% respectively). The suspension was found to contain 90.5% RFC and 27% autoRFC.

Fetuses Aged 17-34 Weeks. The absolute (from 284±1.8 mg at 17 weeks to 6711±14.1 mg at 34 weeks) and relative weight (0.11% at 17 weeks to 0.41% at 34 weeks) of the thymus increases. This is accompanied by histological changes in the gland reflecting predominantly the quantitative aspect of development. The area of the lobules increases and toward the end of the embryonic period their breaking up into smaller subdivisions takes place more slowly. This can be judged from the great reduction in the density of the blood vessels and reticulin fibers in the medulla as a result of their ingrowth on a reduced scale and the marked proliferation of the medullary substance. An increase in the weight of the medulla is a characteristic feature of this period. By the 26th week of development it occupies an extensive total area in the center of each of the two lobes of the gland. At the periphery of the lobes it is continuous with the medulla of each small lobule, so that the latter are separated from the general mass of the lobe only at the tip and at the sides, where the cortex is located. At the base of the lobule, its medulla is continuous with the general mass of the medulla of the gland itself, in which the number of Hassall's corpuscles is increased. Some workers consider that the subcortical zone disappears in the embryonic period, and different times are stipulated for its disappearance, but there is no general agreement regarding its functional importance. According to observations made on the largest number of fetuses, the subcortical zone does not disappear until the 34th week of development, but undergoes the following changes. Parallel with the ingrowth of some of its cells into the medullary substance of the lobules and proliferation of the lobules it becomes discontinuous. In 20-23-week fetuses (Fig. 1e) this zone can be identified as two or three layers of epithelial cells at the apex of the lobules. At the sides it is reduced in thickness to one or two layers, and at the base of the lobules in between them it becomes filled with lymphocytes and essentially disappears. With growth of the organ the regions which have lost their subcortical zone increase in size, so that the impression is obtained that the zone has disappeared completely. By the 30th week of development the subcortical zone can be identified in the form of one or two layers of cells only at the apex of the lobules, where the lobules still retain their capacity for breaking up into smaller subdivisions. The presence of three parallel processes is suggested: a decrease in the area and thickness of the subcortical zone, an increase in the weight of the medulla, and enlargement of the Hassall's corpuscles in the thymus during the period from the 17th to the 34th week of intrauterine development. Sometimes Hassall's corpuscles can be seen lying

close to the basement membrane in regions where the subcortical zone has disappeared (Fig. 1f), and they contain keratin. This account shows that the epithelium of the subcortical zone constitutes the growth zone of the reticuloendothelium of the thymus, and the Hassall's corpuscles are aggregations of differentiated and keratinizing epithelial cells. In the period described the number of RFC is unchange at 81.4-90%, but the number of autoRFC increases from 40% at 17 weeks to 60-70% by the 34th week of development.

A study of the thymus in 100 fetuses thus showed that differentiation of the reticuloendothelium of the gland at the 7th-8th week of embryogenesis coincides with the beginning of colonization and differentiation of lymphocytes, a manifestation of the general biological rule of development of functional systems of organs. By the end of embryonic development the subcortical zone is broken up into separate fragments and can be clearly identified only at the apex of the lobules, where the capacity for further subdivision is still preserved. The spectrum of antigenic specificities on the surface of the T lymphocytes in the thymus is found early in the course of embryonic development, namely at the 7th-8th week. In the period from the 11th-12th week until the end of intrauterine development the number of T lymphocytes forming rosettes with sheep's red blood cells remains virtually unchanged. The present writers describe for the first time the appearance of cells forming rosettes with autogenous red cells in the thymus of the human fetus, and their dynamics. The number of cells forming rosettes with autogenous red cells is smaller than the number forming rosettes with sheep's red cells and it shows a tendency to increase with age of the fetus.

LITERATURE CITED

1. Sh. D. Galustyan, Structure of the Thymus in the Light of Experimental Analysis [in Russian], Moscow (1949).
2. R. V. Petrov, Immunology and Immunogenetics [in Russian], Moscow (1976).
3. V. P. Soustin, in: The Morphology of the Epithelium of the Anterior Division of the Digestive and Respiratory Systems [in Russian], Moscow (1971), p. 35.
4. Z. S. Khlystova, N. A. Chuich, S. P. Shmeleva, et al., in: Systems Properties of Tissue Organizations [in Russian], Moscow (1977), p. 231.
5. M. G. Shubich, Arkh. Anat., No. 6, 114 (1963).
6. G. E. Asma, W. Pichler, S. Henrica, et al., J. Clin. Exp. Immunol., 29, 278 (1977).
7. F. M. Burnet, Cellular Immunology [Russian translation], Moscow (1971).
8. M. D. Cooper and A. R. Lawton, in: Molecules and Cells, No. 6 [Russian translation], Moscow (1977).
9. S. L. Clark, Am. J. Anat., 112, 1 (1963).
10. N. M. Donarin, J. Exp. Med., 142, 17 (1975).
11. M. Jondal, G. Holm, and H. Wigzell, J. Exp. Med., 136, 207 (1972).
12. J. F. A. P. Miller and P. Dukor, Biology of the Thymus [Russian translation], Moscow (1967).
13. M. Papiernik, Blood, 36, 470 (1970).
14. J. Roitt, Essential Immunology, Oxford (1974).
15. D. Stites, J. Coldwell, M. C. Carr, et al., in: Transactions of the Seventh Karolinska Symposium on Research Methods in Reproductive Endocrinology, Copenhagen (1974), p. 306.