blood plasma: a tendency for the plasma 17β-estradiol concentration to fall was observed at 10 a.m., and at 5 p.m. the decrease in its level was significant ($P < 0.05$). Other workers also have observed a similar decrease in the 17β-estradiol level [12].

The results of these experiments suggest that normal development of the preimplantation embryo is closely connected with the blood 17β-estradiol concentration on the day before implantation has also been observed in hamsters [9], rats [14], and rabbits [6].

Consequently, besides the possible direct effect of prostaglandin $F_{2\alpha}$ on the embryos, an important role in the mechanism of action of prostaglandins may perhaps also be played by their effect on synthesis of estrogens, changes in whose level may, in turn, affect somehow or other the preimplantation development of the embryo and implantation and subsequent development of the fetus in utero.

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ULTRASTRUCTURE AND DIFFERENTIAL ANTIGENS OF HUMAN T-LYMPHOCYTES DURING EMBRYOGENESIS

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Thymocytes of human embryos at the 7-8-week stage have irregularly shaped nuclei with one to three distinct nucleoli, characterized by absence of compact chromatin or heterochromatin. The electron-dense cytoplasm of these cells contains polysomes and a few mitochondria. Receptors for sheep's red blood cells and T-antigen are absent on the surface of the cells. In 11-12-week human embryos, the T-lymphocytes are reduced in size, clumps of heterochromatin appear in their nuclei and receptors for sheep's red blood cells (79%) and T-antigen (60%) appear on the surface of the cells. Later, the quantity of compact chromatin in the nuclei of the thermocytes increases and the cells acquire their definitive properties and structure.

KEY WORDS: human embryogenesis; antigens of T-lymphocytes; ultrastructure of T-lymphocytes.

The properties and kinetics of the T-lymphocyte population of human embryos and fetuses have recently been described [1, 3, 6, 11, 12]. Meanwhile the early stages of development of human thymocytes, their fine

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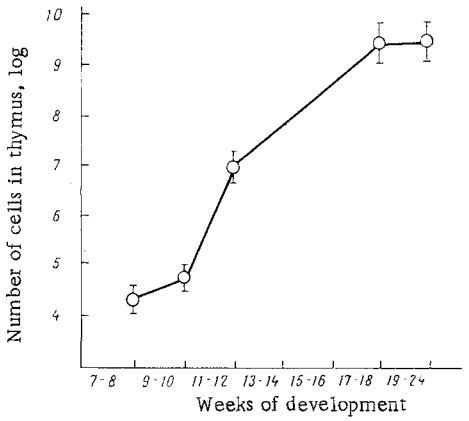

Fig. 1. Number of lymphocytes in thymus of human embryos and fetuses at different stages of development. Each point represents mean of three experiments.

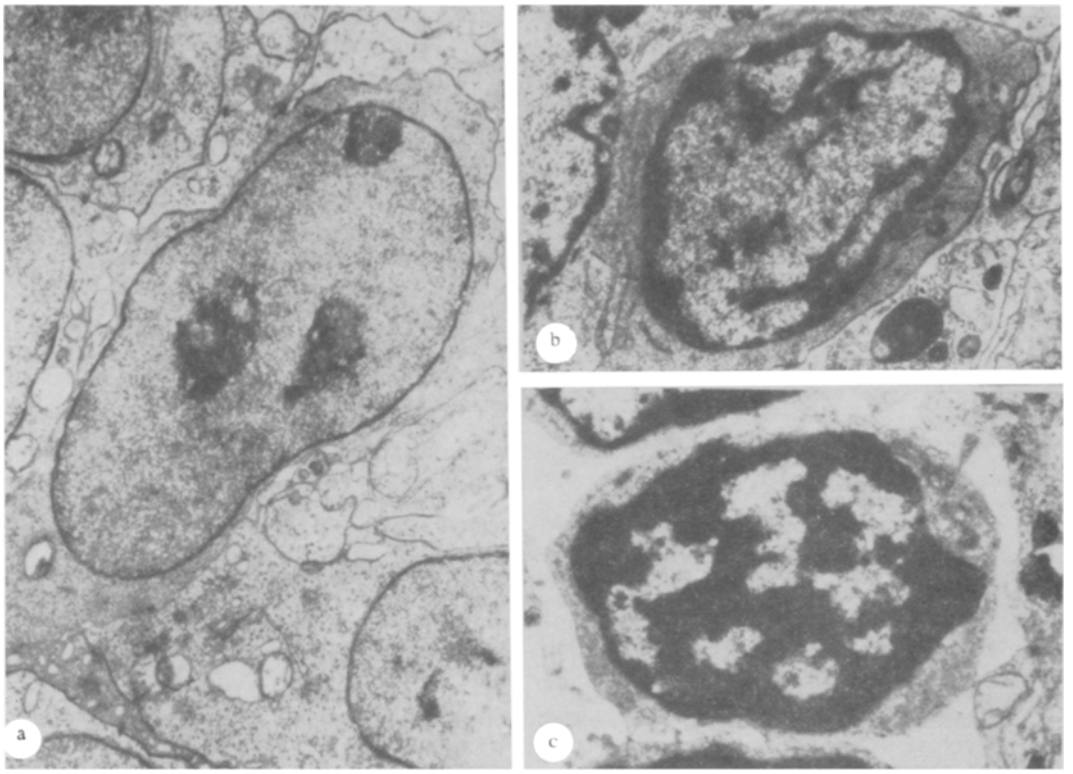

Fig. 2. Ultrastructure of lymphocytes from human embryonic and fetal thymus: a) 7-week embryo. Compact chromatin absent from nucleus of lymphocyte, three nucleoli visible, 12,000×; b) 11-week human embryo. Compact chromatin in nucleus of lymphocyte located near nuclear membrane, with separate granules of chromatin in nucleoplasm, 12,000×; c) 23-week human fetus. Compact chromatin occupies much of the area of the lymphocyte nucleus, 11,00×.

structural features, and the time of appearance of specific receptors for T-lymphocytes on the cell surface have not been adequately studied [6].

This paper describes the results of an electron-microscopic investigation of thymocytes of human embryos and fetuses from 7.5 to 24 weeks of development and detection of differential antigens—receptors against sheep's red blood cells (SRBC) and T-antigen, on the surface of the thymocytes.

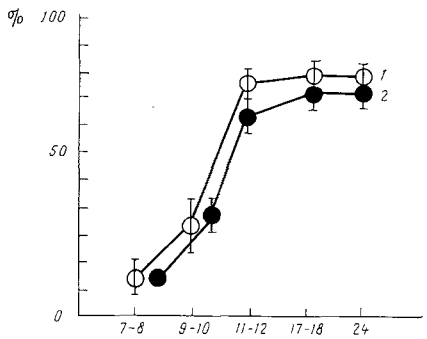

Fig. 3. Number of RFC and lymphocytes lysed by antiserum against T-lymphocytes in thymus of human embryos and fetuses: 1) RFC; 2) lysed cells.

EXPERIMENTAL METHOD

Human embryos and fetuses (56) from 7.5 to 24 weeks of development were obtained at medical abortions on healthy women and at premature labor. The age of the embryos and fetuses was determined from the crown-rump length, the length of the foot, and the mother's menstrual cycle. The thymus was removed under a magnifying glass, weighed, and cut in half. One half of the thymus, or sometimes the whole thymus or a smaller part of it was fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2), containing 4.5% sucrose. The other part of the thymus or the whole gland was used for preparation of a cell suspension. Pieces of thymus tissue, fixed in glutaraldehyde for 1 h at 0°C, were postfixed in 1% OsO_4, dehydrated, and embedded in Epon-Araldite mixture. Sections were cut on an LKB Ultrotome, stained with uranyl acetate and lead citrate, and examined in the TEM 100B electron microscope. Semithin sections were stained with toluidine blue and examined under the light microscope.

Cell suspensions were prepared by crushing the thymus tissue gently in a glass homogenizer and in medium No. 199, cooled to 4°C, and passing the product through two layers of Kapron gauze. Most of the free cells in the suspensions were lymphocytes, 94-98% of which were living, according to the result of the trypan blue test. The total number of lymphocytes in the thymus was counted.

Lymphocytes with surface receptors for SRBC were detected by the rosette-formation test, performed by the method of Jondal et al. [8].

The presence of T-antigen on the surface of the lymphocytes was determined by the cytotoxic test of Komuro and Boyse [9]. Serum against human T-lymphocytes used for the test was provided by N. A. Kraskina, to whom the writers are grateful. The test was carried out as follows: 0.2 ml of lymphocyte suspension containing 10^4-10^7 cells/ml was mixed with 0.2 ml serum taken in various dilutions (from 1:5 to 1:1000), after which 0.1 ml guinea pig serum adsorbed by human fetal thymocytes was added as complement. The mixture was incubated at 37°C for 45 min. Lysed cells were identified by staining with trypan blue. In the control, cell suspensions were incubated with buffered physiological saline instead of antiserum. The number of lysed cells was expressed in percent by the formula $(a - b)/a$, where a is the number of living cells in suspensions without antiserum, and b the number of living cells in suspensions with antiserum, among 100 cells counted.

EXPERIMENTAL RESULTS

The total number of lymphocytes in the thymus was determined at all times of investigation. It increased logarithmically from the 7.5-week embryo to the 24-week fetus (Fig. 1). A small change in the direction of the logarithmic curve was observed at the 12th week of human embryonic development, possibly indicating critical changes in lymphocyte structure at that time.

Investigation of thymocytes of 7.5-8-week human embryos by light and electron microscopy showed that the cells contained irregularly shaped or round nuclei with one, two, or even three nucleoli and that heterochromatin or compact chromatin was absent from the nuclei of 97-97.4% of cells. The electron-dense cytoplasm of these cells contained a few mitochondria and many polysomes (Fig. 2). Most cells had cytoplasmic

outgrowths. Cytoplasmic outgrowths of human fetal thymocytes of this kind were first described by Haar. Thymocytes of 7.5-8-week human fetuses had virtually no receptors for SRBC and the number of rosette-forming cells (RFC) did not exceed 4% and averaged 1.3%. In all dilutions tested the antiserum had no cytotoxic action on these lymphocytes (Fig. 3).

Thymocytes from 9-10-week human embryos were almost indistinguishable in structure from thymocytes from 7.5-8-week human embryos. However, in a few cells clumps of chromatin could be seen in the nuclei near the nuclear membrane (Fig. 2). The number of RFC in the thymus of the 9-10-week human embryo averaged 23%, and 24% of the cells were lysed by antiserum in a dilution of 1:10 (Fig. 3).

Thymocytes of 11-12-week human embryos were reduced in size, they were rounder in shape, and regions of compact chromatin, the granules of which could be seen near the nuclear membrane of lying freely in the nucleoplasm, appeared in the nuclei of 90-97% of cells. Compact chromatin was distributed in some cells as a narrow band near the nuclear membrane. The nucleoli were reduced in size, the outgrowths disappeared, and the number of polysomes was reduced (Fig. 2). Rosettes with SRBC were formed by 79% of thymocytes from 11-12-week human embryos and 60% of the cells were lysed by antiserum in a dilution of 1:10 (Fig. 3).

The thymocytes of 17-24-week human fetuses were small, with a narrow rim of electron-dense cytoplasm, containing a few mitochondria and polysomes. Compact chromatin occupied a large part of the cell nuclei, in which it formed a curious and complex pattern (Fig. 2). Receptors for SRBC were found on 80% of thymocytes, and 70% of the cells were lysed by antiserum in a dilution of 1:10 (Fig. 3).

The results of this investigation are evidence of a clear and regular order of development and differentiation of thymocytes during human embryogenesis. Stem cells which enter the thymus of 7.5-8-week human embryos from the liver are characterized by absence of compact chromatin in the nuclei, and of T-antigen and receptors for SRBC on the cell surface. The 11th-12th week of development of human embryos is the critical period during which compact chromatin is formed in the nuclei of the thymocytes and receptors for SRBC and T-antigen appear on their surface. These observations agree on the whole closely with those of Sugimoto et al. [13], who showed that many lymphocytes of the developing thymus in chick embryos between the 12th and 13th days of incubation differentiate into lymphocytes with T-antigen on their surface and typical heterochromatin in their nuclei.

It can be concluded from analysis of these results that the hematopoietic stem cell not only of the embryo, but also of the adult, is characterized by absence of compact chromatin from its nucleus. Evidence in support of this conclusion is given by the fact that in the 13-day mouse fetal thymus, containing lymphocytes in which only diffuse chromatin, uniformly distributed throughout the nucleus [7] is present, polypotent hematopoietic stem cells giving rise to different types of colonies in the spleen of irradiated recipients [4] can be found. Moreover, in the series of differentiation of adult cells which has been most completely studied, namely differentiation of the spermatogonium, compact chromatin is absent in the nucleus of the stem cell – the type A_0 spermatogonium – and the appearance of even small regions of heterochromatin is associated with repression of certain genes and loss of its stem-cell properties [5]. Similar observations have recently been made also on stem cells of the adult intestinal epithelium [10]. This conclusion is in harmony with modern views on the mechanism of regulation of gene activity in eukaryotes and it calls for further discussion and investigation [2].

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