diol and [^{3}H]-E_2 with UEBP (7.6×10^6 and 5.5×10^7 M^{-1}) is in satisfactory agreement with the values of RCA. Together with data on the competitive character of inhibition of [^{3}H]-E_2 binding with unlabeled ligands this is evidence of very close coupling between the binding sites of estrogens and androgens in the UEBP molecule. The results of the investigation correspond most closely to two types of interconnection between these sites: 1) The subcenters of the binding sites for estrogens and androgens are different, but the binding sites themselves partly overlap; 2) the subcenters for the A ring of estrogens and androgens coincide whereas the subcenters for the D ring of these steroids are different.

Comparison of the values of RCA for interaction of estrogens and androgens and their natural metabolites with UEBP and with the receptors of classical target organs for these hormones [1, 2] shows that there is no direct correlation between the intensity of estrogenic or androgenic activity of the steroids and their affinity for UEBP. Meanwhile evidence is constantly accumulating that several of the sex steroids also possess other forms of biological activity (anabolic, lipotrophic, and so on), probably mediated through other types of receptors [1, 2]. It is quite possible that UEBP serves to distribute the flows of steroids with qualitatively different biological activity. Another possibility is that the function of UEBP is linked with regulation of metabolic conversions of sex steroids in cells of the liver — the central organ of steroid metabolism.

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MYELOPOIESIS IN THE EMBRYONIC HUMAN LIVER

A. A. Rakov, T. S. Blinova,
B. V. Afanas'ev, V. D. Kargin,
and V. N. Shabalin

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Interest in the *in vitro* study of hematopoiesis in human embryonic organs at different stages of their development has recently shown a marked increase, mainly as a result of attempts to use these organs for transplantation into patients with combined immunodeficient states and with hypoplastic and aplastic anemias [8, 9, 14].

However, there have been a few such investigations so far and the conclusions drawn are based on an insufficient quantity of factual data and are often contradictory [3, 4, 10, 11].

It was accordingly decided to study myelopoiesis in the most active human organ of embryonic hematopoiesis, namely the liver (from the 6th to the 28th week of fetal development), using a method of cloning hematopoietic cells in semisolid nutrient media.

EXPERIMENTAL METHOD

A suspension of embryonic liver cells from 113 human fetuses obtained after abortions and by minor caesarian section was used.

Leningrad Research Institute of Hematology and Blood Transfusion. Academician I. P. Pavlov First Leningrad Medical Institute.

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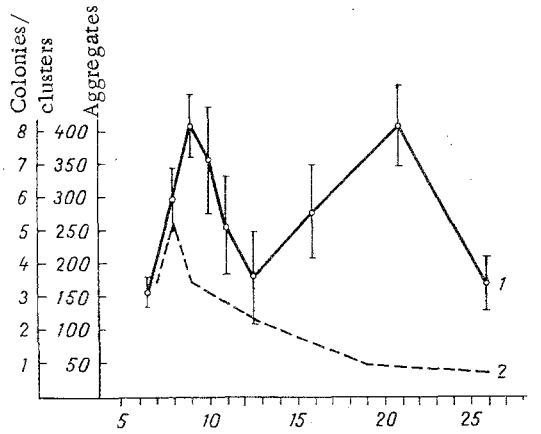

Fig. 1. Time course of myelopoiesis *in vitro* in embryonic human liver depending on age of fetus. Abscissa, age of fetus (in weeks). 1) Number of cell aggregates; 2) ratio of colonies to clusters.

Under aseptic conditions the liver was removed from the fetus and pressed through a fine sieve under a jet of cold 0.9% NaCl solution. The resulting cell mass was passed through a fine injection needle to obtain a homogeneous suspension. Hematopoietic cells were cultured by the classical method in semisolid media, as developed in experiments on animals [6, 13], and subsequently used for cloning human hematopoietic cells [12]. Modified culture media of Howell [5, 7] and McCoy's 5A medium were used. Instead of embryonic calf serum, these media contained human placental blood serum [1]. For each sample of test cells five to 10 petri dishes (diameter 40 mm) were prepared with a two-layered agar culture. The bottom layer of the culture was a nutrient support (feeder) with blood donors' leukocytes (10^6 in 1 ml). The top layer contained nucleated liver cells ($2 \cdot 10^5$ in 1 ml). The petri dishes with the cell culture were incubated for 8-9 days in glass exsiccators or special plastic chambers, in which the necessary conditions for active cell proliferation were created (37°C, 100% relative humidity, CO_2 concentration in the gaseous layer of the chamber 10 vol. %).

Cell aggregates formed in the semisolid nutrient media were extracted and films prepared from them for morphocytochemical study of nucleated cells by techniques developed and modified by the writers [2, 5]. These included staining with aceto-orcein and by the Romanovsky–Giemsa method, cytochemical reactions for peroxidase by Sato's method in Quaglino's modification, staining by Burstone's method for naphthol-AS-D-chloroacetate esterase, by Hayhoe's method for α-naphthyl acetate esterase, and by Lepehne's method for hemoglobin.

The results of cloning were estimated from the number and size of the cell aggregates formed (under 20 cells — clusters; over 20 cells — colonies), the morphocytochemical characteristics of the nucleated cells composing them, and the ratio between the number of different types of colonies. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

During cloning of hematopoietic cells from human embryonic liver in a semisolid nutrient medium, the minimal number of cell aggregates was formed at the 6th-7th week of intrauterine embryonic development — on average 154.2 per 200,000 nucleated cells (Fig. 1). The number of aggregates formed then rose sharply and by the 9th week of embryonic development it reached its maximum, namely 412.9 ($P < 0.001$). In the period from 10 to 14 weeks the cloning ability of the nucleated liver cells fell rapidly almost to the 7-week level (181.5). The number of aggregates formed then increased once again, but this time the increase was smaller, so that by the 21st week it had reached almost the same level as at the 9th week (402.3; $P < 0.001$). After 22 weeks of fetal development the cloning ability of the nucleated cells in semisolid medium fell again.

In the period up to the 14th week of fetal development the number of colonies in the culture was several times greater than the number of clusters. The ratio of colonies to

clusters (Fig. 1) reached a maximum (5.2) at the 8th week of embryonic development. Starting with the 19th week clusters became more numerous than colonies, and after 22 weeks the number of clusters was almost twice the number of colonies.

The morphocytochemical study showed that until the 12th week of fetal development most of the hematopoietic cells in the embryonic liver were of the monocyte-macrophage series. The predominant colonies, of diffuse type during this period, consisted of large mononuclear cells, giving a negative reaction for chloroacetate esterase and a strongly positive reaction for nonspecific esterase, completely inhibited by sodium fluoride.

The cytochemical reaction for hemoglobin also revealed a few small clusters of cells of the erythroid series.

Analysis of aggregates formed in cultures produced by seeding liver cells from fetuses aged 18 weeks or more showed that cells in such aggregates (chiefly clusters) were young cells of the myeloblast and promyelocyte type, although a few mature granulocytes also were found. The reactions of such cells for peroxidase and chloroacetate esterase were positive. The reaction for non-specific esterase also was positive but activity of this enzyme was not inhibited by sodium fluoride, a characteristic feature of cells of this type.

The formation of erythroid clusters could not be detected in cultures of hematopoietic cells from the liver at this period of fetal development.

The experiments *in vitro* revealed the following particular features of myelopoiesis in the human embryonic liver: two statistically significant peaks (at 9 and 21 weeks) were discovered for the number of myeloid precursor cells in the liver (several times higher than in adult human bone marrow); during the period of the first rise myelopoiesis is monocytic-macrophagal in character with absolute predominance of colonies over clusters; some activity of precursor cells of erythropoiesis also is observed; the second period of rise is distinguished by the granulocytic character of myelopoiesis, the predominance of clusters, and absence of spontaneous erythropoiesis.

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