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A culture of the trophoblast was obtained from an early human embryonic chorion. Trophoblastic cells of four main types are described: giant, branched, fibroblast-like, and round. All the cells were rich in RNA and glycoproteins and had the complement of enzymes for the Krebs' pentose cycle, but had low succinate dehydrogenase activity. A marked cytotoxic effect of the lymphocytes of the healthy pregnant woman on allogeneic cells of the trophoblast culture and no interaction between neonatal lymphocytes and these trophoblastic cells were found.

KEY WORDS: *trophoblast; cell culture; enzymes; cytopathic action.*

The role of the trophoblast in the regulation of immunological interaction between mother and fetus has been studied intensively [4, 5]. The culture of cells and tissues *in vitro* is a convenient method of studying the mechanisms of intercellular interaction and of determining enzyme activity and antigenic and immunogenic properties. To some extent the data obtained *in vitro* can be used to discuss phenomena observed *in vivo*.

The object of this investigation was to attempt to obtain an *in vitro* culture of the trophoblast and to study the morphology, enzyme activity, and antigenic and immunogenic properties of its cells.

EXPERIMENTAL METHOD

The material used for the investigation was the chorion (seventh to ninth week of pregnancy) obtained during therapeutic abortion on women with a normal physiological course of pregnancy.

Under sterile conditions the chorionic villi were washed with Hanks' solution, cut into small pieces with scissors, and trypsinized in a 0.3% solution of trypsin for 30 min at 37°C, after which they were washed twice in medium No. 199 by centrifugation. The cells were resuspended in medium No. 199 and filtered through a Kapron filter. Suspension containing $6 \cdot 10^6$ – $7 \cdot 10^6$ cells was transferred to 250-ml flasks and cultivated at 37°C. The composition of the nutrient medium was 80% Eagle's medium + 20% bovine serum.

Preparations for morphological investigation were stained with hematoxylin-eosin. The concentration of nucleic acids (Feulgen reaction, gallocyanin and chrome alum, acridine orange), proteins (Amido Black 10B), polysaccharides (PAS reaction), lipids, etc., was studied histochemically. The key enzymes of the pentose cycle and tricarboxylic acid cycle, mitochondrial and lysosomal enzymes, and also the content of monoamine oxidase (MAO), as a characteristic enzyme of the syncytium of the human placenta *in vivo*, were determined. All the reactions were carried out according to Burstone [1].

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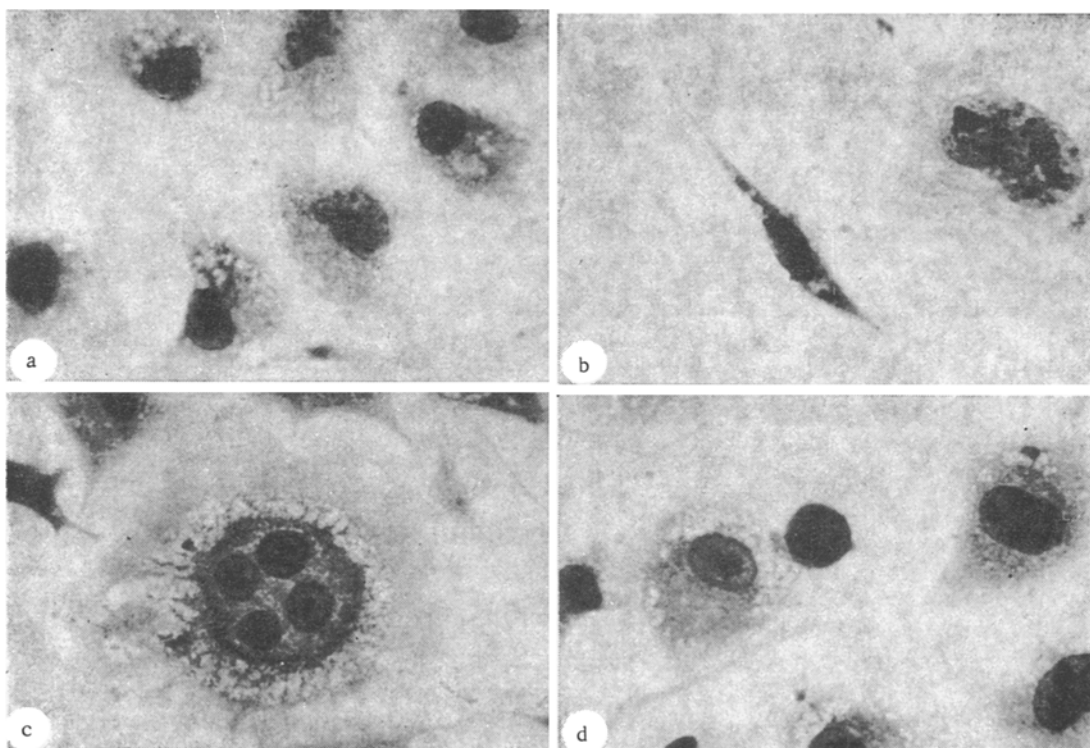


Fig. 1. Types of trophoblastic cells: a) large, branched cells; b) fibroblast-like cells; c) giant multinuclear cell; d) round, unbranched cell. Hematoxylin-eosin, 400 \times .

The cytotoxic action of lymphocytes from a healthy pregnant women on cells of the trophoblast culture was studied by methods described previously [2].

EXPERIMENTAL RESULTS

During the first days of culture examination of unstained and stained preparations in the phase-contrast microscope revealed individual adherent cells of average size with weakly basophilic cytoplasm and with a few branching processes. On the seventh to eighth day colonies of cells began to appear and by the 10th-12th day they were much larger. The cells in the colonies were tightly packed together, with smaller cells in the center and larger trophoblastic cells at the periphery. After subculture, growth took place in the form of a monolayer, with the formation of colonies in some cases.

On the second to third day after subculture cells at various stages of mitosis and phagocytic cells appeared in the culture. During subculture the culture itself became stabilized as regards its cellular composition and it was composed of the following groups of cells (Fig. 1):

1. Large polygonal basophilic cells with a few processes and small vacuoles in the cytoplasm. Basophilic granules were detected on one side of the nucleus, and the nucleus itself contained up to seven or eight large nucleoli, frequently joined by an intervening isthmus. The basophilia of the cytoplasm died away toward the periphery.
2. Giant cells: a) mononuclear, with many long and thin processes and with vacuolated cytoplasm; b) with a few processes, resembling fibroblast-like cells, but much larger. The nuclei often had an irregular outline and sometimes differed in size. The nucleoli were very large and numerous. Chromatin was identified as large and small masses, irregularly distributed in the karyoplasm; c) multinuclear cells with vacuolated cytoplasm.

The cells of these two groups were characterized by basophilic cytoplasm and by perinuclear condensation on one side of the nucleus.

3. Fibroblast-like cells, smaller than those of the previous category, elongated and spindle-shaped, with an oval, clearly outlined nucleus and with basophilic cytoplasm. The chromatin in the nucleus was more densely packed and the nucleoli were few in number.

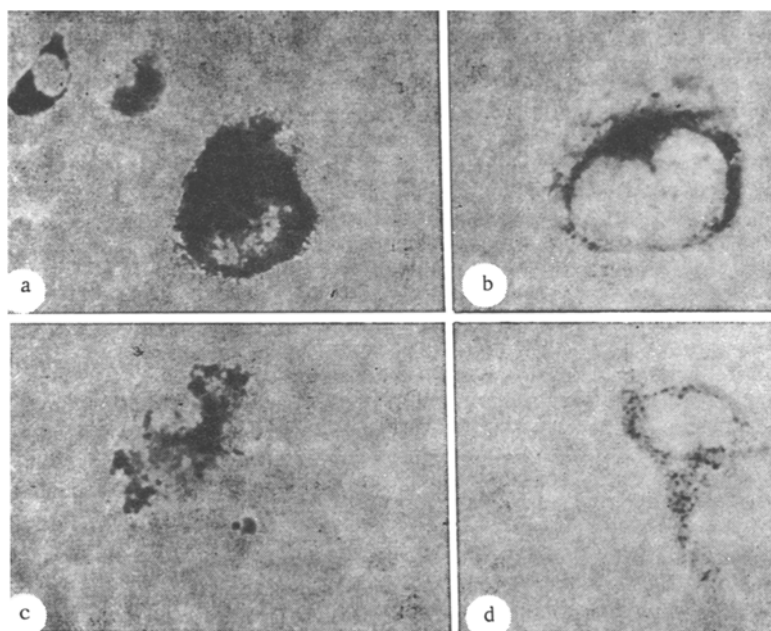


Fig. 2. Histochemically demonstrable enzyme activity in trophoblastic cells: a) α -glycerophosphate dehydrogenase b) glucose-6-phosphate dehydrogenase; c) acid phosphatase; d) monoamine oxidase. 400 \times .

4. Small round cells with a very dense nucleus, a clearly outlined nuclear membrane, and a homogeneous distribution of chromatin. The cells were unbranched, their cytoplasm was uniformly basophilic, and vacuoles were comparatively rare in it. Cells in a state of mitosis and also cells with more than 46 chromosomes were found in the preparations.

Common histochemical features for all cells were a high content of RNA, large nuclei rich in chromatin and with many nucleoli, and a high content of glycoproteins and a low content of glycogen in the cytoplasm.

As regards enzymes (Fig. 2), succinate dehydrogenase activity was rather low and lactate dehydrogenase and α -glycerophosphate dehydrogenase activity high, evidence of the predominance of anaerobic glycolysis over the Krebs's cycle. The activity of enzymes of the pentose cycle was high.

In the course of culture of the trophoblastic cells changes were observed in the basic characteristics and a redistribution of the number of the four cell types described above occurred; cells of type 4 became fewer, the number of multinuclear cells increased, but cells of the first type were predominant.

The cellular structure (including multinuclear structures) was thus preserved in the trophoblast culture, but its histochemical characteristics corresponded to the syncytium (as reflected in high MAO and acid phosphatase activity) or the peripheral cytotrophoblast of the human placenta. The trophoblastic cells had high activity of enzymes responsible for synthesis of RNA, carbohydrates, and proteins, which is not characteristic of Langhans' cells.

In one series of experiments the cytotoxic effect of lymphocytes of a healthy pregnant woman on allogeneic cells of a culture of the trophoblast was studied. A marked cytopathic effect was observed, including an initial stage of adhesion of the lymphocytes to the trophoblastic cells with their subsequent destruction. These observations confirm earlier results [3].

Other facts were obtained to show that neonatal lymphocytes have no cytopathic effect on allogeneic cells of the trophoblast culture (the cytopathic index did not exceed its normal level [2]).

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EFFECT OF CULTIVATION TEMPERATURE ON BLAST TRANSFORMATION OF LYMPHOCYTES FROM PEOPLE OF DIFFERENT AGES

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The effect of the cultivation temperature on blast transformation of lymphocytes from persons aged 20-35 and 90-102 years induced by phytohemagglutinin was studied. Cultivation at 39°C was found to increase, but at 41°C to reduce sharply the index of blast transformation compared with the control (37°C). The effect of cultivation temperature on the blast-transformation process has certain features which depend on the donors' age.

KEY WORDS: *blast transformation; lymphocytes; age; temperature.*

The intensity of blast transformation of lymphocytes under the influence of phytohemagglutinin (PHA) is known to depend to a definite degree on the conditions of cultivation: the PHA concentration, the density of distribution of the cells, the composition of the nutrient medium and of the gaseous phase [6-12, 14]. According to some reports, the mitotic activity of PHA-stimulated lymphocytes depends on the incubation temperature [2, 3].

The object of this investigation was to study the effect of the cultivation temperature on the intensity of blast transformation of the lymphocytes after incubation for different periods. Data in the literature [1, 5, 13, 15] and the writer's own observations [4] are evidence of a change in the response of lymphocytes to PHA during aging, and it was therefore decided to carry out the present investigation from an age aspect.

EXPERIMENTAL METHOD

Altogether 457 cell cultures from 20 people aged 20-35 years and from 12 people aged 90-102 years were investigated.

Leukocytes were isolated from heparinized blood by sedimentation with gelatin (1 ml of 10% gelatin to 10 ml blood) and were cultivated in medium No. 199 containing 25% group IV serum. The final concentration was $1 \cdot 10^6$ cells in 1 ml culture. Difco PHA-P in a dose of 0.002 ml to 1 ml of culture was used as the stimulator. Incubation was carried out at 37 ± 0.3 , 39 ± 0.3 , and $41 \pm 0.3^\circ\text{C}$ for 24, 39, 48, 54, 66, 72, and 78 h. At the end of cultivation the cell suspension was centrifuged and films prepared from the residue and stained by the May-Gruenwald method. At least seven cultures were used at each time. Blast cells and intermediate forms were regarded as transformed lymphocytes. The criteria of transformation were the size of the nucleus and cytoplasm and the structure of the nuclear chromatin. The number of transformed lymphocytes in 1000 cells was counted.

The numerical results were subjected to statistical (dispersion) analysis. The significance of differences was assessed by Fisher's criterion and by the χ^2 criterion in Fisher's modification.

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