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EFFECT OF THE PERIOD OF PREGNANCY ON GROWTH-REGULATING PROPERTIES OF THE MATERNAL BLOOD SERUM *in vitro*

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The use of blood sera from various animals and man as additional components in media for organ and tissue culture *in vitro* is widely familar [6, 7]. More recently many investigations have been published in which homologous sera were used for this purpose: rat [5, 8-10], rabbit [3], etc. By means of allogenic rat serum, New [8] cultured whole embryos and thereby proved that the sex of the animal from which the serum was obtained is of no importance.

In investigations to study the nature and mechanisms of action of growth-regulating factors in embryogenesis of the lung, conducted by the method of primary monolayer culture of embryonic rat lung [2], we have used different homologous blood sera from females and, in particular, blood serum from pregnant animals. With the onset of pregnancy, various specific biological substances, whose composition and concentration vary depending on the stage of pregnancy, enter the blood stream and may perhaps be reflected in the character of growth of the monolayer. The investigation described below was devoted to the study of this problem.

EXPERIMENTAL METHOD

A cell suspension for primary monolayer culture was obtained from the lungs of 19-day Wistar rat fetuses. The technique of preparation of the material and of culture was described previously [2]. In the present investigation 10% homologous serum from rats at the stages of 9, 9.5, 10, 12, and 18 days of pregnancy was added to the medium No. 199. According to the classification suggested by Professor A. P. Dyban's laboratory [1], this corresponded to stages 10, 11, 12, 14-15, and 20-21 of embryonic development of rats. At each time five series of experiments were performed. The numerical data were analyzed on the Nairi-K computer. The significance of differences was evaluated by Student's t-test.

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TABLE 1. Changes in Number of Cells and Mitoses (in °/oo) Depending on Addition of Blood Sera from Rats at Different Stages of Pregnancy to the Culture Medium (66 h of growth *in vitro*) (M ± m)

Test object	Stage of pregnancy, days				
	9	9	10	12	18
Fibroblast-like cells	709,40±13,07	712,33±23,92	775,00±52,10	728,00±25,49	669,00±62,00
Epithelial-like cells	235,60±14,22	244,83±19,33	173,33±27,10	159,20±25,86	251,25±42,96
Mitoses	4,23±0,52	4,91±0,53	5,32±0,42	3,02±0,36	3,94±0,72

EXPERIMENTAL RESULTS

A well-formed monolayer was observed in the primary culture of embryonic rat lung on the 3rd day of growth (66 h), in which two main types of cells could be distinguished: epithelial-like and fibroblast-like; the morphological differences between them were described previously [2]. Addition of blood serum from rats at different stages of pregnancy to the culture medium changed the character of growth of the culture. Quantitative data for fibroblast-like and epithelial-like cells are given in Table 1. The number of fibroblast-like cells increased ($P > 0.01$) after the addition of blood serum from a rat on the 10th day of pregnancy (P-10) to the medium compared with the action of P-9 serum (Fig. 1). P-12 serum (12th day of pregnancy) reduced the number of cells of this type in the monolayer. A further decrease in the number of fibroblast-like cells took place ($P < 0.01$) on the addition of P-18 serum (18th day of pregnancy) to the growth medium. Blood serum from a rat on the 10th day of pregnancy thus has some stimulating effect on growth of fibroblast-like cells in primary monolayer culture of rat embryonic lung (compared with the action of sera obtained at different stages of pregnancy).

The increase in the number of epithelial-like cells under the influence of blood sera from rats at different stages of pregnancy also followed a course of its own. The number of cells of this type in the culture as a whole was less than the number of fibroblast-like cells, but it varied depending on which serum was used. If the course of the curves reflecting these processes are compared (Fig. 1), it will be seen that one is a mirror image of the other. For instance, the addition of P-10 serum to the medium led to a decrease in the number of epithelial-like cells in the culture ($P > 0.01$), whereas the number of fibroblast-like cells increased. Substances entering the blood stream of the rat in the later stages of pregnancy (18 days) caused an increase in the number of epithelial-like cells ($P \geq 0.01$), but the number of fibroblast-like cells decreased under these circumstances. Comparison of the curves shows that during pregnancy substances capable of acting selectively on growth of different types of cells in culture *in vitro* enter the maternal blood stream.

The growth-regulating properties of the blood serum of the pregnant rats were revealed by analysis of total mitotic activity (Table 1, Fig. 1, 3), which includes not only the main types, but several other types of embryonic lung cells constantly present in a primary culture. On the 10th day of pregnancy the blood contained substances causing (compared with blood on the 9th day of pregnancy) an increase in mitotic activity ($P > 0.01$), but this was reduced by the action of P-12 serum ($P < 0.01$). Serum from rats in the later stages of pregnancy (P-18) caused a small increase once again in the number of dividing cells ($P > 0.01$), although not so great as P-10 serum.

The development of mitotic activity also was studied under the influence of blood serum from rats at different stages of pregnancy, for which purpose cultures were fixed after growth *in vitro* for 20, 44, and 66 h. The results of this series are given in Fig. 2. In the period of adaptation (20 h) and during growth of the culture (44 h), sera P-9, P-10, and P-18 had almost identical stimulating action. P-12 serum differed sharply from the rest, and caused a decrease in the number of dividing cells. During growth of the culture for 66 h all sera caused an increase in mitotic activity except P-9 and P-18, in the presence of which the number of mitoses fell somewhat by 66 h of growth *in vitro*.

Differences in the action of the blood sera on cell growth in a primary monolayer culture of embryonic rat lung thus appear in the early stages of culture. The curve of mitoses under the influence of P-12 serum merits attention. It differs sharply from the others both in character and in level. On the 12th day of pregnancy some changes evidently take place in the maternal organism which lead to changes in either the quality or the quantity of substances

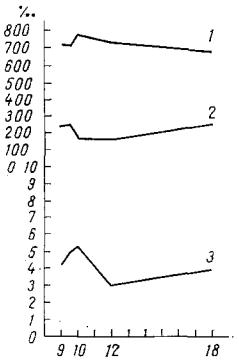

Fig. 1

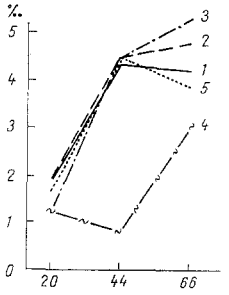

Fig. 2

Fig. 1. Dynamics of action of blood sera from rats at different stages of pregnancy on mitosis (3) and on fibroblast-like (1) and epithelial-like (2) cells in culture after 66 h of growth *in vitro*. Abscissa, stage of pregnancy in days; ordinate, number of cells and mitoses (in °/₀₀).

Fig. 2. Formation of mitotic activity in primary monolayer culture of embryonic lung under the influence of blood serum from rats at different stages of pregnancy. 1) 9 days, 2) 9.5 days, 3) 10 days, 4) 12 days, 5) 18 days of pregnancy. Abscissa, time of growth of culture *in vitro* (in h); ordinate, mitotic activity (in °/₀₀).

capable of influencing proliferative processes in culture. Very likely these are certain specific growth-stimulating or inhibiting substances (at least this is a possibility which cannot be ruled out), as a result of a change in the hormonal balance of the pregnant female.

The property of rat blood serum, revealed by the investigation described above, of regulating the character of growth of a primary monolayer culture of embryonic lung during pregnancy thus must be taken into account when experiments are planned in which blood serum from pregnant animals is to be used.

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