

The present investigation showed that during menstruation the destruction and desquamation of the endometrium are focal in character, and under these circumstances fragments of the functional layer lined by preserved epithelium always remain. Most cells of the residual glands are diploid and, for that reason, cannot divide by mitosis; consequently, they cannot take part in the re-epithelization of the raw surface of the uterine mucous membrane during menstruation.

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#### ACTION OF ALLOGENEIC SERUM FROM A PREGNANT PNEUMONECTOMIZED RAT ON EMBRYONIC LUNG TISSUE

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UDC 591.3.591.39

Allogeneic blood serum from a rat undergoing unilateral pneumonectomy during pregnancy was shown to stimulate growth of a primary monolayer culture of embryonic Wistar rat lung, directed chiefly toward fibroblasts. This effect was manifested most clearly on the 3rd day of culture.

KEY WORDS: allogeneic serum; primary monolayer culture; pneumonectomy.

Much clinical and experimental evidence has now been obtained to show that injury to an organ of a pregnant animal affects the development of the homonymous organ of its fetus [1, 3, 5-9, 11, 13]. Murashova [10] showed that unilateral pneumonectomy on a pregnant rat leads to the more rapid development of the lungs in its fetuses. Under these circumstances a specific growth-stimulating factor appears in the blood and reaches the homonymous fetal organ by the humoral route. During postnatal development the progeny of such an animal develops a state of inborn predisposition to diseases of the corresponding organs and systems and they constitute a group of increased risk [4, 14].

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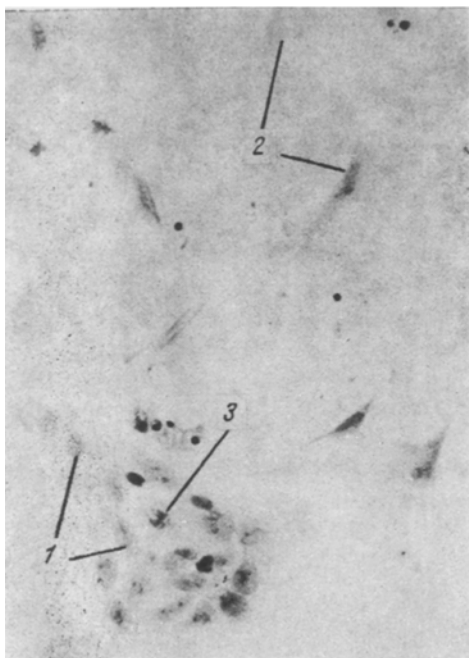


Fig. 1

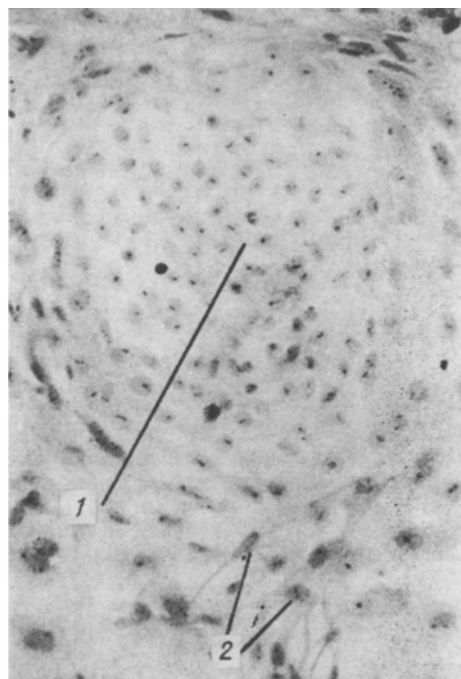


Fig. 2

Fig. 1. Primary monolayer culture of rat embryonic lung with addition of PE serum (20 h of growth). 1) Epithelioid cells, 2) fibroblast-like cells, 3) mitosis. Stained with Harris' hematoxylin, 70 $\times$ .

Fig. 2. Primary monolayer culture of rat embryonic lung with addition of PE serum (44 h of growth). 1) Epithelial islet, 2) fibroblast-like cells. Stained with Harris' hematoxylin, 70 $\times$ .

TABLE 1. Changes in Mitotic Activity (in %) of Cells of Culture Following Addition of Blood Serum from Pregnant Control (PC) and Pregnant Pneumonectomized (PE) Rat ( $M \pm m$ )

Time of culture, h	PC (control)	PE (experimental)	P
20	$1,94 \pm 0,52$	$1,45 \pm 0,28$	$>0,1$
44	$4,99 \pm 0,42$	$11,76 \pm 0,60$	$<0,001$
66	$5,16 \pm 0,62$	$9,78 \pm 0,86$	$<0,01$

The object of this investigation was to study changes in the tissues of the embryonic rat lung in vitro under the influence of blood serum containing such a growth-regulating factor.

#### EXPERIMENTAL METHOD

Female Wistar rats were killed on the 19th day of pregnancy; the lungs were taken from their fetuses, cut into small pieces measuring about 1 mm, and incubated in a 0.25% solution of trypsin for 20 min at 37°C. The material was then trypsinized on a magnetic mixer, and every 20 min the solution with the cells was decanted into centrifuge tubes and centrifuged for 5 min at 1000 rpm. The residue was washed with Hanks' solution and resuspended in medium 199 to which 10% serum was added. A monolayer culture was grown on coverslips in penicillin flasks seeded with a dose of  $10^6$  cells/ml. After fixation in formalin fixative (100 ml 40% formalin, 850 ml distilled water, 50 ml 18% sodium chloride) the cultures were stained with Harris' hematoxylin.

As a rule to study monolayer cultures of mammalian and human lung cells artificial media with the addition of heterologous sera, chiefly calf or bovine serum, have been used [12, 15]. To study the action of the growth-regulating humoral factor appearing in the blood of the pneumonectomized pregnant rat, it was decided

TABLE 2. Changes in Number of Fibroblast-like Cells (in ‰) in Culture Following Addition of Blood Serum from Pregnant Control (PC) and Pregnant Pneumonectomized (PE) Rat ( $M \pm m$ )

Time of culture, h	PC (control)	PE (experimental)	P
20	762 $\pm$ 51	778 $\pm$ 67	>0,1
44	727 $\pm$ 10	731 $\pm$ 15	>0,1
66	668 $\pm$ 18	848 $\pm$ 19	<0,001

TABLE 3. Changes in Number of Epithelioid Cells (in ‰) in Culture Following Addition of Blood Serum of Pregnant Control (PC) and Pregnant Pneumonectomized (PE) Rat ( $M \pm m$ )

Time of culture, h	PC (control)	PE (experimental)	P
20	145,8 $\pm$ 45,9	185,5 $\pm$ 62,4	>0,1
44	212,5 $\pm$ 14,9	293,2 $\pm$ 20,5	>0,1
66	272,7 $\pm$ 16,8	103,8 $\pm$ 18,3	<0,01

to use allogeneic sera exclusively: 1) from a pregnant (9 days) pneumonectomized rat (experimental serum, serum PE) 6 h after removal of the left lung; 2) from an intact pregnant rat (control serum, serum PC). The cultures were fixed 20, 44, and 66 h after seeding. Evaluation of the monolayer included analysis of the cell morphology, the rate of formation of the continuous layer, differentiation, and the mitotic activity of the cells at different periods of culture. The numerical results were subjected to statistical analysis by the Fisher-Student method.

## EXPERIMENTAL RESULTS

After growth for 20 h (Fig. 1), i.e., in the stage of adaptation, two main types of cells were found in both cultures: fibroblast-like and epithelioid. Other cells found included macrophages, blood cells, and cells whose tissue affiliation was difficult to determine. As a rule the epithelioid cells were clustered in small groups, whereas the fibroblast-like cells were either arranged singly or connected by their processes to form a network. No significant difference was found in mitotic activity between cultures with PC or PE serum at this period of investigation (Table 1). When the number of fibroblast-like cells (Table 2) and epithelioid cells (Table 3) was counted no significant differences were found between the action of sera PC and PE.

On the 2nd day of culture (Fig. 2) mitotic activity was increased in both cultures (Table 1); in the culture with addition of serum PE it was increased by 8.1 times and in that with the addition of serum PC by 2.6 times (compared with the previous period of observation). The beginning of differentiation of the cell monolayer also was observed, for the fibroblast-like cells surrounded islets of epithelium, to form structures resembling alveoli. The number of fibroblast-like cells in the experimental and control groups (Table 2) remained almost equal as before (the differences were not statistically significant). Epithelioid cells (Table 3) were somewhat more numerous in the experimental series, but this difference likewise was not significant.

On the 3rd day of growth (Fig. 3) the formation of a continuous monolayer of cells and the formation of well-marked structures resembling alveoli were observed. The walls of these "alveoli" consisted of fibroblast-like cells joined together to form a meshwork, within which the epithelioid cells were arranged. In the control, in the overwhelming majority of cultures no continuous layer was formed, and only the beginning of formation of the structures resembling alveoli was observed. The difference in mitotic activity between the experimental and control series (Table 1) was significant. The number of fibroblast-like cells on the 3rd day of growth of the culture (Table 2) under the influence of serum PE was 27% greater than their number in the culture growing under the influence of serum PC (the differences were statistically significant). The number of epithelioid cells (Table 3) also differed significantly in the experimental and control cultures: In cultures growing under the influence of serum PE it was only 38% of the control value.

This investigation showed that the humoral growth-regulating factor in the blood of the pneumonectomized pregnant rat exhibits activity as early as 6 h after the operation. Its action in a monolayer culture of intact

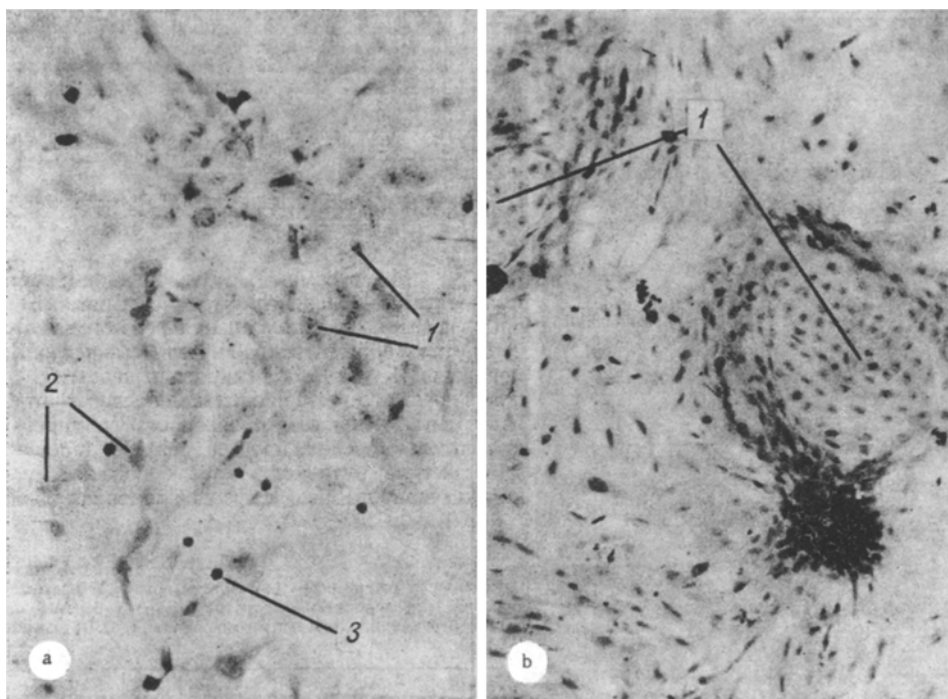


Fig. 3. Primary monolayer culture of rat embryonic lung after addition of serum PC (a) and PE (b). a: 1) Epithelioid cells, 2) fibroblast-like cells, 3) macrophage. Stained with Harris' hematoxylin, 70 $\times$ . b: 1) Structures resembling alveoli. Stained with Harris' hematoxylin, 42 $\times$ .

rat embryonic lung is manifested on the 2nd day of growth, when the mitotic index in a culture to which serum PE was added was twice that in the control, with the result that the density of the cell monolayer was high and it began to differentiate. It is also important to note that the factor contained in serum PE had a stronger growth-stimulating action on the fibroblast-like cells, a feature clearly distinguishable on the 3rd-4th day of development of the culture. This could lead to the more intensive development of stromal structures at the expense of parenchymatous. It is perhaps this specific action of the factor in vivo which lies at the basis of the lowered resistance to unfavorable external environmental influences found in such animals in the postnatal period [4], and this in turn could lay the foundations for the development of an inborn predisposition to diseases of the homonymous organs and systems in the progeny [14].

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## EFFECT OF ACUTE HYPOXIA ON MORTALITY AND SEX RATIO OF THE EMBRYO

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UDC 618.33-008.922.1.04-036.88

The action of acute hypoxia on embryogenesis and its effect on the sex ratio in the progeny was studied in mice. Mice on the 7th-10th days of pregnancy were kept for 3 h daily under hypoxic conditions at an atmospheric pressure of 230 mm Hg. The mice were killed on the 18th day of pregnancy, when the gonads of the embryos were examined. The sex ratio in the progeny was determined as the ratio of the number of males to the number of females. Investigation showed that acute hypoxia on the 7th-10th day of pregnancy, while leading to high mortality among the adult animals, did not significantly affect embryonic development. No selective death of mouse embryos of either sex was found in the experimental and control groups of animals.

KEY WORDS: hypoxia; embryonic mortality; sex ratio.

Several investigations into the effect of hypoxia on embryogenesis have been published [2, 3, 7, 8]. Attention has been concentrated in particular on the study of the action of chronic hypoxia on fetal development [1, 6]. It has been shown, for instance, that chronic maternal hypoxia disturbs the development and causes death of the embryos. However, the effect of acute hypoxia on embryogenesis has received much less study.

The object of this investigation was to study the action of acute hypoxia on embryogenesis in mice. The opportunity was also taken to analyze the effect of acute hypoxia on the sex ratio in the progeny, in view of earlier reports that spontaneous embryonic mortality is unrelated to the sex of the embryo [4, 5].

### EXPERIMENTAL METHOD

Experiments were carried out on C3H inbred mice from the Rappolovo nursery. After mating the day of discovery of a vaginal plug was taken as the first day of pregnancy. On the 7th-10th days of pregnancy the mice were kept for 3 h under hypoxic conditions at an atmospheric pressure of 230 mm Hg. The mice were killed on the 18th day of pregnancy. The embryos were removed from the uterus, laparotomy was performed, and the gonads were examined with the MBS-1 microscope. The sex ratio in the progeny was determined as the ratio of the number of males to the number of females.

Altogether 166 pregnant animals (86 experimental and 80 control) were used. The sex of 789 embryos (309 experimental and 480 control) was determined.

### EXPERIMENTAL RESULTS

Keeping the pregnant mice for 3 h under hypoxic conditions at 230 mm Hg led to high mortality among the adult animals. Of the 86 experimental mice 35 (40.6%) died.

However, as Table 1 shows, acute hypoxia did not significantly increase the embryonic mortality compared with the control (19.7% and 14.4% of embryos died in the experimental and control series respectively). Meanwhile, analysis of mortality among the embryos on individual days of pregnancy showed that in the case of exposure to hypoxia on the 9th day the embryonic mortality was higher than in the control (27.1% in the

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Department of Embryology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 86, No. 7, pp. 88-89, July, 1978. Original article submitted November 10, 1977.