PHYSIOLOGY

Effect of Neonatal Blood Serum on the Early Stages of Mouse Embryogenesis *in Vitro*

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The effect of blood sera from newborns delivered after complicated pregnancy and abnormal labor (CPAL) on 4-cell mouse embryos is studied. The embryos develop normally in the control sera, 78-100% of them reach the blastocyst stage, the mean number of blastomeres per embryo varies from 68 to 88 cells, and the mortality rate is 3-7%. The CPAL sera have an almost 100% embryolethal effect. The development of mouse embryos is markedly delayed. The most potent pathological effect is elicited by the sera of newborns whose mothers suffered from hypoxia.

Key Words: *embryo; embryotoxic effect; newborn*

Most pathological states are accompanied by the synthesis of biologically active peptides and proteins [2,3,5]. It has been demonstrated that the amniotic fluid of women with complicated pregnancy and labor contains transfer factors that elicit an embryotoxic effect when injected in the amnion of laboratory animals and induce various disturbances in the postnatal development [1,4]. Sera of women whose babies died during delivery or shortly thereafter induce high mortality of chick embryos and abnormalities of development of brain structures [8]. Sera of newborns with marked defects of the axial rudiment and other abnormalities induce similar pathologies in chick embryos [6]. A similar pathogenic effect was demonstrated in cultured rat embryos *in vitro* for the sera of monkeys with a high rate of spontaneous abortions, stillbirths, and neonatal deaths [7]. Culturing of mouse blastocysts *in vitro* in the presence of sera from women with different disorders of reproductive function slowed down development. The synthesis of DNA, cytokeratin, fibropectin, and alkaline phosphatase was suppressed [9]. It is logical to assume that sera of women with complicated pregnancy contain biologically active factors capable of impairing the normal development of embryos in the preimplantation stages of embryogenesis. In order to confirm this possibility we attempted to study the effects of sera from healthy and sick newborns on dividing mouse embryos.

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MATERIALS AND METHODS

Blood was obtained from the umbilical vein of 16 newborns (4 were delivered by healthy women and 12 by women with complicated pregnancy and labor). Sera were then prepared and used for *in vitro* culturing of 4-blastomere mouse (CBA/C57B1) embryos. Culturing was carried out at 37°C in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Sera of nonpregnant women, rat sera, and medium M_{16} were used as controls. The num-

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ber of living and dead embryos was counted after 72 h of culturing. Surviving embryos were fixed on cover slips and stained with Giemsa stain, and the number of nuclei (blastomeres) was counted in each embryo. A total of 703 embryos were analyzed. The effect of the sera was evaluated by the number of dead embryos, the number of cells in each embryo, and the percentage of embryos reaching the blastocyst stage. The results were analyzed using Student's *t* test.

RESULTS

It can be seen from Table 1 that in the sera obtained from nonpregnant women (group 1), from rats (group 2), in M_{16} medium (group 3), and in the sera of women who delivered normally (assessment of the embryos according to the Apgar scale was 8-8-8-9 points and all of them were discharged healthy after 5-6 days) newborns developed normally, 78-100% of them reached the blastocyst stage, the mean number of blastomeres per embryo being 68-88, and only 3-7% of the embryos died.

Sera of newborns (groups 8-19) whose mothers had pathologically complicated pregnancies elicited an embryotoxic effect which varied considerably (7-100%). The number of blastomeres was markedly decreased (35-62 cells), and the frequency of reaching the blastocyst stage varied from 13 to 97%. During pregnancy the women of these groups had the following complications: chills in the second half of pregnancy (groups 11, 12, and 15-18), aggravation of chronic pyelonephritis (groups 9 and 16), trichomonad and yeast colpitis with candidiasis of the vagina (groups 8-11), chronic adnexitis (group 19), rhesus incompatibility (groups 18 and 19), or preeclampsia (group 9). These women gave birth to 8 boys and 4 girls. Eleven newborns were full-term and one was premature (35-36th week). According to the Apgar scale, 2 newborns scored 5-6 and 6-7 points (groups 14 and 19), 3 newborns 7-8 points (groups 9, 12, and 13), and the others 8-8-8-9 points. Nine newborns fell ill in the maternity home (groups 10 and 12-19), four of them were admitted to the hospital (groups 12, 14, 16, and 17), and only 3 (groups 8, 9, and 11) were clinically healthy when discharged from the maternity home. Thus, embryotoxic activity was displayed by sera of newborns born of mothers after a complicated pregnancy and labor, and most of these newborns

TABLE 1. Effect of Sera from Newborns of the Development of Preimplantation Mouse Embryos *in Vitro* ($M \pm m$)

Group of sera	Number of embryos					Mean number of cells per embryo
	total	dead		blastocysts		
		number	%	number	%	
1	72	5	6.9±3.0	61	84.7±4.2	67.8±4.7
2	33	1	3.0±2.9	28	84.9±6.2	67.6±5.5
3	63	4	6.3±3.1	49	77.8±4.7	75.8±5.8
4	19	–	–	17	89.5±7.0	73.4±9.6
5	27	–	–	27	100	87.5±6.4
7	47	2	4.4±3.1	45	95.6±3.1	72.3±6.4
8	28	17	60.7±9.2*	11	39.3±4.2*	47.2±10.0*
9	30	16	53.3±9.1*	6	20.0±7.3*	43.9±4.7*
10	30	12	40.0±8.9*	4	13.3±6.2*	34.6±2.2*
11	51	5	9.8±4.2*	44	86.3±4.8	51.2±3.4*
12	30	8	26.7±8.1*	22	73.3±8.1	47.4±4.3*
13	31	2	6.5±4.4*	9	29.0±8.2*	42.6±2.6*
14	28	28	100*			
15	30	30	100*			
16	30	22	73.3±8.1*	5	16.7±6.8*	35.3±5.6*
17	34	8	23.5±7.3*	26	76.5±7.3	39.1±2.8*
18	30	30	100*			
19	27	5	18.5±7.5*	22	81.4±7.5	61.5±5.7

Note. Asterisk indicates values statistically different from the control ($p<0.05$).

were sick in the early neonatal period (groups 10 and 12-19). It is impossible to characterize the embryotoxic effect of sera of newborns depending on the mother's disease because of the relatively small number of observations and the difficulties encountered in differentiating the diagnostics of a vast array of pathologies. However, it should be noted that the embryotoxic effect is considerably potentiated by hypoxia. The sera of 4 newborns after prenatal hypoxia (groups 14-17, as evidenced by the presence of meconium in the amniotic fluid) induced death of mouse embryos in 100, 100, 73, and 23% of cases, respectively. A 100% embryolethal effect was seen in the serum of a newborn after hemolytic (ABO) disease (group 18). This effect may be due to the similarity of the ABO system of humans and mice.

Thus, our results indicate that *in vitro* culture of preimplantation mouse embryos can be used for the evaluation of embryotoxicity of sera from newborns.

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Effect of Stress on the Reticular Zone of the Adrenal Cortex

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The reticular zone (RZ) of the adrenal cortex is shown to be involved in the formation of the organism's response to stress. A new scheme of physiological regulation of RZ is presented.

Key Words: *stress exposure; adrenal reticular zone; cholesterol esterification*

The adrenocortical reticular zone (RZ) secretes androgens (mainly dehydroepiandrosterone and dehydroepiandrosterone sulfate [2,12]) in amounts surpassing the total sum of all the other adrenal hormones, including glucocorticoids. However, the physiological function of the RZ in humans and animals is still unknown [1,8,12], as is its contribution to the development of the organism's response to stress. The system of physiological regulation of RZ is not clear either [14].

The aim of our research was to study changes in the activity and physiological regulation of the adrenocortical RZ under the effect of stress.

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MATERIALS AND METHODS

Experiments were carried out with male Wistar rats weighing about 200 g. The animals were castrated

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