EFFECT OF UTERO-PLACENTAL ISCHEMIA ON TISSUE PROTEIN SYNTHESIS IN THE RABBIT EMBRYO

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Correlation between growth of the body and metabolism during embryogenesis is a subject which has long attracted the attention of investigators, and a very important aspect of the problem is to determine relationships between biochemical and physiological indices of growth of the embryo and the formation of its functional system. Exchange of materials between mother and fetus is dependent on the functional capacity of the placenta. Its disturbances lie at the basis of most forms of fetal and neonatal pathology; they can lead not only to disturbances of development of the fetus, but may also threaten its life.

In the investigation described below the resistance of various tissues of the developing organism to harmful factors was studied from the comparative aspect. The interference consisted in impairment of the circulation in the uterus in the region of attachment of the placenta, by an operative method described by the writers previously [4]. On the 4th day after the operation disturbance of protein synthesis in the heart and in the soma (the embryo without its head and the anlagen of the internal organs) was studied separately. From these data it was possible to shed some light on the vulnerability of the embryo during the period of intensive development of the fetal and placental circulation. Vascularization of the placenta and the volume of blood in its maternal part also were studied.

EXPERIMENTAL METHOD

Experimental ischemia was created in the experimental rabbits on the 8th day of pregnancy, under aseptic conditions, by ligating about half of the branches of the preplacental vessels next to the implantation sites of each second embryo. Embryos of experimental and control animals were investigated on the 12th day of pregnancy. To determine the degree of retardation of development of the experimental embryos more precisely, 11-day embryos also were studied from intact animals. The fetuses were removed without loss of blood, by the method described in detail by Konstantinova et al. [4]. The wet weight and, after drying to constant weight, the dry weight of some embryos was determined, and in others the total hemoglobin content by Zhakhova's method [2] followed by Crosby and Furth's color reaction for hemoglobin [7]. Cytoplasmic proteins were extracted from the heart and soma with phosphate buffer (pH 7.4), as described by Zhakhova et al. [3]. Their total content in the extracts was then determined by Lowry's method. High concentrations of the reagents were not used, because they distort the results of analysis [1]. For quantitative determination of proteins the method of electrophoresis in polyacrylamide gel was used, in conjunction with a model 69 instrument and reagents from Reanal, Hungary. The conditions of electrophoresis were described by Zhakhova et al. [3]. To determine the blood volume of the placentas, the method of counting the area occupied on histological sections by maternal lacunae, fetal blood vessels (with their accompanying mesenchyme), and the trophoblast, was used. Details of the method of counting were given by a paper by Lebedeva and Tripol'skaya [5].

Altogether 615 embryos from 100 rabbits were used in the experiments, during which 470 hearts, 486 somas, 78 vitelline membranes, and 28 placentas were investigated.

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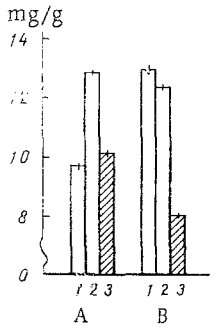

Fig. 1. Changes in content of cytoplasmic proteins in heart (A) and soma (B) of intact rabbit embryos on 11th and 12th days of development (1 and 2, respectively) and in 12-day embryos developing under conditions of uterine ischemia (3). Ordinate, content of cytoplasmic proteins (in mg/g).

TABLE 1. Effect of Uterine Ischemia on Growth and Development of Rabbit Embryo and Its Placenta

Embryos	Wet weight, mg, of			Dry weight of embryo, mg	Hemoglobin content in body of embryo, μg	Fetal vessels, % of area	Maternal blood, % of area
	embryo	heart	membranes				
Control:							
11-day	23,0±0,68 n=74	0,52±0,03 n=171	—	1,60±0,05 n=31	14,5±1,65 n=22	—	—
12-day	75,0±1,67 n=79	1,08±0,07 n=217	17,7±1,62 n=15	4,77±0,19 n=21	103±8,48 n=27	27,32±1,20 n=14	15,98±0,61 n=14
Experimental 12-day	47,0±3,28 n=19	0,79±0,04 n=82	12,1±2,46 n=19	2,70±0,30* n=29	59,6±11,0 n=19	21,34±1,78 n=14	30,60±3,97 n=14
P	<0,001	<0,01		<0,001	<0,001	<0,02	<0,001

*Taken from an investigation by Ovchinnikova [6], conducted under the same conditions.

EXPERIMENTAL RESULTS

From the results it was possible to judge the normal rate of growth of rabbit embryos during the 24 h from the 11th to the 12th days of development. It will be clear from Table 1 that in this series of the investigation the wet and dry weight of the embryo increased on average threefold from the 11th to the 12th days of intrauterine development, whereas the total hemoglobin content in the body increased sevenfold. It will be clear from Fig. 1 that under normal conditions during 24 h of development, the content of cytoplasmic proteins per gram wet weight increased significantly in the heart of the embryos by almost one-third. The number of separated protein fractions also increased significantly under these circumstances. On the 11th day it varied between 13 and 17, and on the 12th day between 16 and 25. During this period, i.e., from the 11th to the 12th days, the content of cytoplasmic proteins per gram weight in the soma of the embryos remained practically unchanged.

Embryos which developed for 3 days under conditions of a reduced blood supply to the uterus were retarded in their development. This was shown by a decrease in the wet and dry weight of the embryo and a decrease in weight of the vitelline membrane and of the heart (Table 1). The rate of growth of the experimental embryos was so retarded that by the 12th day of development (4 days after the operation) they weighed only 60% as much as the controls, but nevertheless, they weighed more than control 11-day embryos, i.e., the delay in growth was less than by one day. However, delay in growth of the heart compared with that of the embryo as a whole in this case was less marked; the wet weight of the heart in the experimental 12-day embryos was 23% less, and the body weight 37% less than in control embryos at the same stage of development. Moreover, inhibition of synthesis and differentiation of the cytoplasmic proteins of the heart was less marked than in the soma (Fig. 1). The content of proteins and the number of their fractions in the heart of the experi-

mental 12-day embryos reached the characteristic level for 11-day control embryos. In the soma of these embryos, however, the content of cytoplasmic proteins was 71% less than in the soma of the control 11-day embryos. The number of protein fractions in the soma of the experimental embryos varied within wide limits — between 10 and 20. In some cases only 10-14 protein fractions were found, whereas in the control on both the 11th and the 12th days of development, fewer than 14 fractions were distinguished. The disturbance of hemoglobin synthesis was less severe than the delay of protein synthesis in the soma. The hemoglobin content in the experimental embryos was only 41% less than in the control 12-day embryos.

On histological study of the placentas hyperemia of the maternal part, which varied in degree, was found. Collections of maternal blood were found most frequently in the decidual basement membrane, bordering directly on the labyrinth, and in the deep parts of the labyrinth. The mean area occupied by maternal blood on the sections was almost twice that in the control (Table 1). Meanwhile, vascularization of the placentas was delayed. The area occupied by fetal vessels with their accompanying mesenchyme was 22% less than in the control. The development of congestive hyperemia in the maternal part of the placenta was evidently caused by disturbance of the venous outflow, for the veins were ligated together with the arteries. Delayed vascularization of the fetal part of the placenta could be connected with its formation under conditions of chronic uterine hypoxia, with the congestive hyperemia subsequently developing in the maternal part of the placenta, and also with delayed growth of the embryo and of the vitelline membrane under hypoxic conditions.

The results of this investigation showed that so far as processes of protein synthesis in the various tissues of the embryo are concerned, at the stage of embryogenesis when subsequent development is determined by the rapid rate of formation of the hemodynamic system, hemoglobin synthesis was not so drastically disturbed, and synthesis of cytoplasmic proteins in the tissues of the embryonic heart was more "protected," more resistant to the effects of harmful factors than in the somatic tissues.

LITERATURE CITED

1. E. M. Beier, Lab. Delo, No. 10, 590 (1976).
2. N. L. Garmasheva, Z. N. Zhakhova, and A. A. Cheremnykh, Akush. Ginekol., No. 1, 8 (1967).
3. Z. N. Zhakhova, N. A. Tripol'skaya, and N. N. Konstantinova, Akush. Ginekol., No. 10, 65 (1977).
4. N. N. Konstantinova, N. A. Tripol'skaya, and Z. N. Shakhova, Akush. Ginekol., No. 6, 61 (1977).
5. I. M. Lebedeva and N. A. Tripol'skaya, Arkh. Anat., No. 12, 50 (1973).
6. G. A. Ovchinnikova, Patol. Fiziol., No. 6, 54 (1974).
7. W. H. Crosby and F. Furth, in: Clinical Laboratory Investigations in Pediatrics (I. Todorov, ed.) [in Russian], Sofia (1968), p. 293.