EXPERIMENTAL INVESTIGATION OF THE ACTION OF CHRONIC HYPOXIA ON EMBRYONIC DEVELOPMENT

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Wistar rats were exposed in a pressure chamber to chronic hypoxic hypoxia from the 1st to the 6th, 1st to the 10th, 1st to the 20th, or 7th to the 10th days of pregancy. The results were analyzed on the 20th day of pregancy when the number of embryos dying at the preimplantation and postimplantation stages of development was determined and the embryos were studied for the presence of developmental anomalies of the viscera and skeletal system. Hypoxia was found to have a marked embryotoxic but only a weak teratogenic effect. The harmful action of hypoxia was exhibited about equally at the pre- and postimplantation stages of intrauterine development. Developmental anomalies, predominantly of the urogenital system, occurred in 9.4% of the rat embryos.

Hypoxia is an unavoidable accompaniment of many diseases of pregnancy and it often becomes the dominant factor in the pathogenesis of disturbances affecting the fetus and newborn infant [1, 2, 5–7, 13].

Oxygen lack influences the embryonic development of animals [1, 2, 8–10, 14, 15]. Numerous investigations have shown that hypoxia may lead to the termination of pregancy or give rise to developmental anomalies, and that the harmful effect of hypoxia depends on the stage of pregnancy.

The object of this investigation was to study the effect of moderate chronic hypoxia on the embryonic development of rats depending on the period of pregnancy at which exposure took place.

EXPERIMENTAL METHOD

Wistar rats obtained from the Rappolovo nursery, Academy of Medical Sciences of the USSR, were exposed in a pressure chamber to chronic hypoxic hypoxia. The animals were kept at an "altitude" of 3000–3200 m above sea level, corresponding to a barometric pressure of 500 mm Hg (partial pressure of oxygen 105 mm Hg), from the 1st to the 20th, the 1st to the 10th, the 1st to the 6th, or the 7th to the 10th days of pregnancy inclusive. The experimental conditions in the pressure chamber were kept constant throughout the 24-h period except for an interruption of 1 h to allow the animals to be fed and the cages cleaned. Control rats were kept under normal animal-house conditions.

The animals of the experimental group tolerated the hypoxic conditions well and were clinically indistinguishable from the controls.

The animals were sacrificed on the 20th day of pregnancy and the number of corpora lutea in the ovaries, the number of implantation sites in the uterine cornua, and the number of living (normal and abnormal) and dying fetuses were counted. The crown-rump length of the living embryos was measured.

Some of the embryos were fixed in Bouin's fluid, after which the state of their viscera was investigated by Wilson's microanatomical method. In some embryos, fixed in 96° ethanol, the state of the skeletal system was studied in total sections stained with alizarin red.

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TABLE 1. Effect of Chronic Hypoxic Hypoxia on Development of Rat Embryos

Period of exposure in days	Number of rats in group	Number of corpora lutea in ovaries	Number of embryos dying before implantation		Number of implantation sites	Number of embryos dying after implantation		Number of feruses	Total number of embryos dying	
			abs.	%		abs.	%		abs.	%
1—10	10	96	35	36,4±4,9	61	35	57,4±6,4	26	70	72,9±4,6
1—6	10	93	30	32,2±4,9	63	17	27,0±5,6	46	47	50,5±5,2
1—20	31	283	83	29,3±3,0	200	136	68,0±3,3	64	219	77,4±2,5
7—10	10	92	5	5,4±2,3	87	22	25,3±4,7	65	27	29,3±4,8
Control	39	375	23	6,1±1,2	352	20	5,7±1,2	332	43	11,5±1,6

The criteria of the harmful action of hypoxia on embryonic development were preimplantation and postimplantation death of the fetuses and the teratogenic effect. When the postimplantation mortality of the embryos was assessed, death of the fetuses was noted before the formation of the placenta (early death), as shown by the presence of a decidual reaction and of implantation sites showing necrotic changes in the uterus, and death of the fetuses in the period of placental formation (late death), when a well-formed placenta and a macerated fetus at various stages of development were present in the uterus.

Altogether 100 pregnant rats (including 39 control) were used in the experiments; 533 living embryos (332 in the control) were obtained from them.

The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results are given in Table 1.

Moderate chronic hypoxic hypoxia caused death of the rat embryos at both preimplantation and postimplantation stages of development. Comparison of the results obtained in all the groups of rats exposed to hypoxia at various periods of pregnancy showed a high mortality among the embryos, much higher than in the control. The most marked embryotoxic effect of hypoxia occurred from the 7th to the 10th days.

Hypoxia led to preimplantation death of about 30% of the embryos. In the case of exposure to hypoxia from the 7th to the 10th days, i.e., after implantation, the number of dying embryos in the early stages of development was indistinguishable from that in the control rats.

Some of the embryos exposed to hypoxia in the preimplantation period evidently still remained capable of implantation in the uterine wall but died soon after. Analysis of the postimplantation mortality (Fig. 1) shows that 27% of embryos died through exposure to hypoxia in the early stage of development after implantation, and of this number 19% died before placentation and 8%, corresponding to the control figure, at later stages.

Hypoxia from the 7th to the 10th day of pregnancy caused postimplantation death of 25.3% of the fetuses, and all of them died in the period of placentation. The mortality among embryos exposed to hypoxia throughout pregnancy was indistinguishable from that among those exposed during the first 10 days of pregnancy, namely about 60%. Presumably in these rats the postimplantation mortality was made up of 30% due to the action of hypoxia in the initial periods of embryogenesis and 30% at the postimplantation stages of development.

Chronic hypoxia thus has a harmful action on both the preimplantation and postimplantation stages of intrauterine development.

After exposure to hypoxia the living embryos were considerably retarded in development, for their crown-rump length was significantly less than in the control embryos (27.2 ± 0.3 and 32.5 ± 0.4 mm, respectively). These results are in agreement with those obtained by other workers [2, 3, 8] who showed that development is retarded and the weight of the organs reduced in embryos exposed to hypoxia.

On a detailed examination of the living embryos under the MBS-1 binocular loupe no developmental anomalies could be discovered. No anomalies likewise could be found in a study of total preparations of

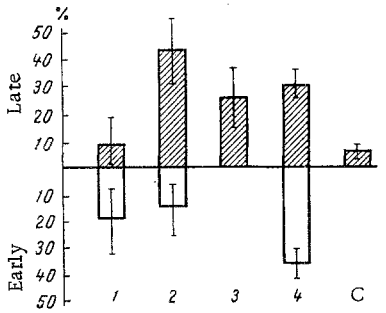

Fig. 1. Postimplantation mortality of rat embryos exposed to chronic hypoxia: 1) action of hypoxia from 1st to 6th days of pregnancy; 2) from 1st to 10th days; 3) from 7th to 10th days; 4) from 1st to 20th days of pregnancy; C) control.

the skeletal system of 63 fetuses. However, on investigation of 138 embryos, 15 different developmental anomalies of the viscera were discovered in 13 embryos (9.4%). Their frequency was about equal in all the groups of rats exposed to hypoxia at different periods of pregnancy. Developmental defects of the urogenital system were commonest: absence or maldevelopment of the kidney in two embryos (1.4%), hydronephrosis in four (2.9%), hypertrophy of the urinary bladder in one embryo (0.7%), and monochidism in one embryo (1.4%). In addition, hydrocephalus was found in five embryos (3.6%) and stenosis of the upper third of the esophagus in two embryos (1.4%).

Consequently, moderate hypoxia has a teratogenic effect. This effect is probably exerted primarily on the blood vessels [1, 2], and the disturbance of their development subsequently leads to various anomalies of organogenesis.

The mechanism of the embryotoxic action of hypoxia is still unexplained. Death at postimplantation stages of embryogenesis possibly takes place through a disturbance of the development of the allantois, which fails to grow to make contact with the ectoplacenta [2]. An examination of the amniotic sacs on the 11th day of pregnancy in these experiments revealed inadequate development of the allantois in 32% of fetuses, and 25% of the embryos died before placentation. In the early stages of embryogenesis the direct action of hypoxia on the embryonic cells, i.e., its cytostatic effect [12], evidently plays the leading role, and this conclusion is supported by results obtained by other investigators [4, 9, 11]. Very possibly it is this cytostatic action of hypoxia which leads to arrested or delayed cleavage of the mammalian ovum.

On the other hand, hypoxia may also exert a cytogenetic effect. According to the writers' preliminary data, chronic hypoxic hypoxia causes an increase in the number of aneuploid and polyploid sets of chromosomes in the bone marrow cells of rats. Possibly hypoxia has a similar effect on embryonic cells. These hypotheses require further experimental confirmation.

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