RELATIONSHIP BETWEEN LEVEL OF CHROMOSOMAL ABERRATIONS IN PREGNANT MICE AND EMBRYOS AND INCIDENCE OF SPONTANEOUS INTRAUTERINE FETAL DEATH

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UDC 618.439-07:576.312.36]-092.9

The level of chromosomal abberations was determined in bone marrow cells of pregnant mice and in the cells of embryos obtained from these same animals, and correlated with the incidence of spontaneous intrauterine fetal death. No difference in the frequency of aneuploid cells was found. There was likewise no significant difference between the number of cells with chromosomal aberrations in the bone marrow of pregnant mice with different numbers of dead fetuses or with none. At the same time, the frequency of chromosomal aberrations in living embryos increased with an increase in the antinatal death rate. It is concluded that the possible mutagenic action of endogenous factors must be taken into account when the genetic risk of substances in the period of embryogenesis is assessed.

Cytogenic studies in the last decade have shown a correlation between chromosomal disturbance in embryos and developmental anomalies. An increase in the frequency of chromosomal and genome disturbances is observed both in spontaneously aborted embryos and stillborn fetuses, as well as in children dying during the first months after birth [1, 2, 4-9, 11-14].

TABLE 1. Number of Living and Dead Embryos of Mice Used in Experiments

Group of mice	Animal No.	No. of corpora lutea	No. of living embryos	No. of embryos dying		
				total	before implantation	after implantation
1	1	7	7	0	0	0
	2	8	8	0	0	0
	3	8	8	0	0	0
	4	8	8	0	0	0
	5	8	8	0	0	0
2	6	8	6	2	0	2
	7	9	7	2	1	1
	8	5	4	1	0	0
3	9	8	3	5	4	1
	10	9	5	4	4	0
	11	5	1	4	4	0
	12	6	3	3	2	1
	13	6	3	3	3	0

The increasing scale of human contact with substances used in industry and agriculture could lead to an increase in the general level of chromosomal mutations in man, including during his embryonic development. At the same time, in each individual case the conclusion regarding the etiology of chromosomal aberrations in embryos is difficult to draw because of the lack of study of the role of endogenous factors in the development of chromosomal aberrations. It is accordingly interesting to study the chromosomal apparatus of animal embryos during disorders of pregnancy not induced by external causes.

The object of the present investigation was to determine the level of chromosomal aberrations in pregnant mice and in their embryos and to correlate this with the frequency of intrauterine fetal death.

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TABLE 2. Level of Chromosomal Aberrations and Aneuploidy in Living Embryos and in Bone Marrow of Pregnant Mice in Relation to Frequency of Spontaneous Intrauterine Fetal Death (M ± m)

Group of animals	No. of dying embryos	No. of metaphases examined	No. of metaphases with aberrations		No. of aberrations				No. of aneuploid cells (in %)		
			abs.	%	total	single fragments	paired fragments	translocations	total	hypoploid	hyperploid
1-	0	a 700	6	0,85±0,34	6	6	0	0	3,57±0,70	3,00	0,57
		b 300	2	0,66±0,46	2	2	0	0	4,33±1,17	4,33	0,00
2-	1—2	a 450	5	1,11±0,49	5	5	0	0	4,22±0,94	4,00	0,22
		b 400	4	1,00±0,50	4	4	0	0	3,50±0,91	3,50	0,00
3-	3—5	a 650	44	6,76±0,97	56	44	8	4	2,92±0,66	2,92	0,00
		b 300	2	0,76±0,46	4	4	0	0	4,00±1,13	4,00	0,00
Males	—	b 250	2	0,80±0,17	2	0	0	0	4,80±1,34	4,80	0,00

Legend: a) embryos; b) bone marrow.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice 3 months old. Pregnant females were killed by decapitation on the 12th-13th day after fertilization. Colchicine solution (5 μg/g body weight) was injected intraperitoneally into the animals 2 h before sacrifice. Preparations of chromosomes were made from bone marrow cells by Ford's method [10] with slight modification of the hypotonic treatment. The number of corpora lutea and of living and dead embryos was counted. The liver of the embryos was used for obtaining chromosomal preparations. The method of obtaining these preparations, as described previosuly [3], was simplified by omitting the trypsinization stage. After mechanical disaggregation of the liver in medium No. 199 the suspension was pressed through two layers of gauze, centrifuged, the residue suspended in a fresh portion of medium No. 199, and poured into penicillin flasks. Colchicine was added at the rate of 0.5 μg/ml medium. The flasks were incubated for 2.5 h at 37°C. A 0.075 M solution of KCl was used for the hypotonic treatment. Not less than 50 metaphases of bone marrow and 100 metaphases of embryos were examined from each animal. The preliminarily numbered specimens were analyzed concurrently by two workers.

EXPERIMENTAL RESULTS

The bone marrow and embryos of pregnant mice with different numbers of dead embryos or with none were subjected to cytological analysis. The animals were divided into 3 groups (Table 1). Group 1 consisted of mice in which no resorption had taken place, group 2 consisted of animals with 1-2 dead embryos, and group 3 of mice with 3-5 dead embryos. The results of chromosomal analysis are given in Table 2.

For the analysis of aneuploidy, cells containing not less than 37 chromosomes and with moderate scatter were studied. No difference was found between the 3 groups as regards the number either of hypoploid or of hyperploid cells in the bone marrow. Differences between the numbers of aneuploid cells in the embryos of the different groups of mice likewise were not significant.

The number of metaphases with aberrations in the bone marrow of the pregnant mice remained at a low level despite differences in the number of dying embryos (0.66-1.00%). The bone marrow of the males used for fertilization contained 0.5% of cells with chromosomal aberrations.

The frequency of structural chromosomal aberrations in the embryos of the group 1 mice (without resorption) was 0.85%. The chromosomal aberrations consisted of chromatid ruptures, one for each aberrant cell. The incidence of gaps was 1.57%, and of fusions 0.42%.

In group 2, with a low incidence of intrauterine fetal death, the level of aberrations in the living embryos showed no significant change (metaphases with chromosomal aberrations 1.11%, with gaps 3.55%, with fusions 0.44%).

A significant increase in the number of aberrant metaphases (6.76%; $P < 0.01$) was found in living embryos obtained from females with 3 and more dead fetuses. Many of the aberrations consisted of chromosomal breaks and translocations. At the same time, the number of breaks per aberrant cell and the total number of gaps (4.00%) were increased. The number of fusions (0.61%) was close to that observed in the embryos from the group 1 mice.

With an increase in the antinatal mortality, the incidence of chromosomal aberrations in the living embryos thus increased also.

This fact must be taken into consideration when the level of spontaneous chromosomal aberrations in embryonic tissues of animals is determined and also when the mutagenic action of various substances is studied experimentally.

It is important to note that in the case described the chromosomal aberrations were the result and not the cause of the pathological state. An analogous situation can evidently arise in any abnormality of intrauterine fetal development in man, and for this reason disturbances of the chromosomal apparatus of the cells in spontaneously aborted embryos must not be attributed entirely to the action of mutagenic external environmental factors.

The authors are grateful to Professor N. N. Kolesnik for his interest in this research and for his valuable comments.

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