CYTOLOGICAL STUDY OF EARLY EMBRYONIC MORTALITY IN LABORATORY MICE

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Early embryogenesis was studied cytologically in laboratory mice. Preimplantation embryonic mortality among DBA/2JY, C57BL/6JY, and 101/HY mice and among CBWA females crossed with B6 males did not exceed 4-6%. An exception was the AKR/JY mice, with a preimplantation mortality of 29.6%. Investigation of the nature of chemically induced dominant lethals showed that the arrest of cleavage and death of the embryos were caused by gross structural changes in the chromosomes.

KEY WORDS: embryonic mortality; chromosomal aberrations; implantation.

The obtaining of exact data on natural preimplantation embryonic mortality in laboratory mice of different genotypes is essential in order to study problems in general biology and in experimental genetics when this parameter is used as an indicator of the genetic effect of physical and chemical mutagens. Values of the preimplantation mortality obtained by different workers vary considerably and may reach 45.6% [3] or even 48.5% [7]. However, some of this preimplantation mortality is due to unfertilized ova, and this fraction may be increased after exposure of the males to external factors through a decrease in the fertilizing power or number of spermatozoa [8].

The object of this investigation was to study preimplantation embryonic mortality in inbred and hybrid mice of different genotypes, using a chemical mutagen inducing dominant lethal mutations that cause preimplantation death of the embryos.

EXPERIMENTAL METHOD

Experiments were carried out on inbred AKR/JY, DEA/2JY, C57BL/6JY (B6) and 101/HY mice and tetrahybrid CBWA mice crossed with B6 males, aged 2-4 months. Fertilized females were selected depending on the presence of a vaginal plug. The embryos were examined on the 4th day of development. At this time the embryo was in the blastocyst stage and lay freely in the uterine cavity. To obtain the embryos the uterine cornua were irrigated with warm medium No. 199. The embryos were examined under the MBR-1 microscope. Dominant lethals causing death of the embryos before implantation were induced in spermatids of C57BL/6 males by injection of thio-TEPA solution in a dose of 5 mg/kg body weight intraperitoneally. Chromosome preparations were made for cytogenetic analysis by Tarkowski's method [9] in Dyban's modification [2].

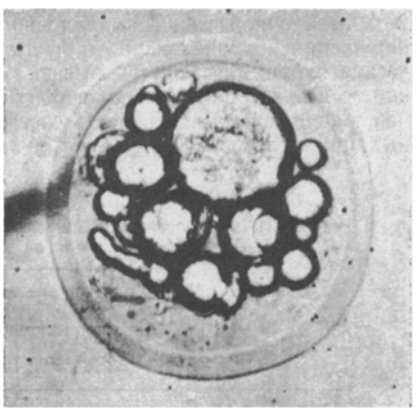

Fig. 1. Degenerating ovum (600 ×).

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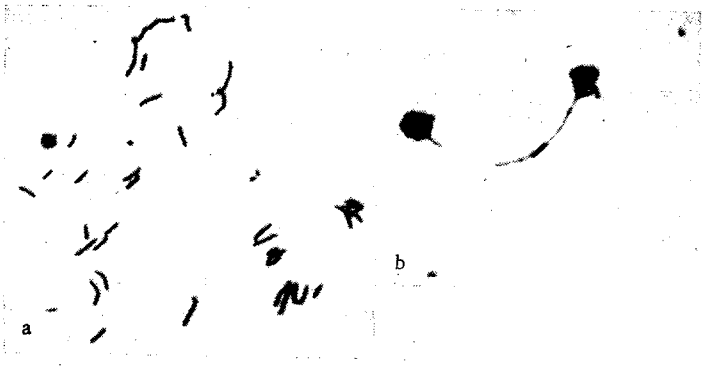

Fig. 2. Chromosomal aberrations in metaphase (a) and anaphase (b) of cleaving ovum fertilized 1 week after injection of thio-TEPA into males in a dose of 5 mg/kg (900 ×).

TABLE 1. Results of Investigation of 3.5-Day Mouse Embryos after Treatment of Late Spermatids of B6 Males with Thio-TEPA in a Dose of 5 mg/kg

	No. of females	No. of ova and embryos at different stages of development studied				
		total	1 cell	2–8 cells	9–16 cells	blasto-cysts
Experiment	37	317	40	203	74	—
Control	30	261	35	9	4	213

EXPERIMENTAL RESULTS

In 44 tetrahybrid CBWA females crossed with B6 males and killed on the 4th day of pregnancy, 343 ova were obtained. Cytological investigation revealed 32 (9.3%) noncleaving ova among the developing embryos (Fig. 1). A similar percentage of noncleaving ova was found after removal of the embryos at the end of the first day of development, at a time when they were in the oviducts. Among 526 ova the percentage that failed to develop was 10.27. The presence of degenerating ova, at the unicellular stage, was thus accounted for not by embryonic mortality, but by the existence of ova that were unfertilized or incapable of fertilization. Allowing for the high normal rate of fertilization (97%) in mice, which remains high even after treatment of spermatozoa and spermatids with chemical mutagens [6], it seems more likely that the absence of cleavage of some of the ova was due to the inability of the latter to undergo fertilization or development.

In order to determine moment of death of the embryos from genetic causes and also to obtain material for investigation in sufficient amount, CBWA females were crossed with B6 males 1 week after the latter had received thio-TEPA in a dose of 5 mg/kg body weight. Similar treatment in the same crossing led to infertility of the females caused by preimplantation death of the embryos as a result of dominant lethal mutations induced in the spermatids by the mutagen [5]. The results of this experiment are given in Table 1. The embryos died at the stage of 2-8 blastomeres (in most cases) or 9-16 blastomeres. Equal numbers of undeveloped ova were observed in the experimental and control groups, indicating no decrease in the normal level of fertilization. Cytogenetic analysis of embryos dying at the 2- and 4-cell stages revealed many structural changes in the chromosomes — translocations and fragments (Fig. 2). Genetic disturbances can thus cause the arrest of cleavage and death of the embryos before implantation. In tetrahybrid CBWA control mice the true embryonic mortality was 5%. On the basis of these results, during the investigation of the different strains of mice, embryos containing 2-22 blastomeres were considered to be dead. The results of cytological analysis of 3.5-day embryos are given in Table 2. They show that preimplantation embryonic mortality among the mice of the various strains varied only very slightly (from 4 to 6%), except in the case of AKR mice, in which the mortality was 29.6%. The proportion of undeveloped

TABLE 2. Cytological Investigation of Preimplantation Mortality in Mice of Different Strains

Strain of mice	No. of females	Number of ova ovulated						
		total	dying embryos		undeveloped ova		total loss	
			abs.	%	abs.	%	abs.	%
C57BL/6JY	25	210	9,0	4,3 (1,9—7,6)	30	14,3 (9,8—19,5)	39	18,6 (11,8—22,0)
AKR/JY	21	260	77	29,6 (24,0—35,4)	48	15,7 (11,4—20,5)	125	45,3 (39,1—51,6)
101/HY	29	230	9,0	3,9 (1,7—6,8)	16	7,0 (4,0—10,8)	25	10,9 (7,1—15,4)
DBA/2JY	24	256	16	6,25 (3,5—9,5)	10	4,0 (1,9—6,8)	26	10,25 (6,8—14,3)
CBWA×C57BL/6JY	29	252	13	5,2 (2,8—8,3)	39	15,5 (11,3—20,3)	52	20,7 (15,8—26,0)

Legend. Confidence limits calculated for P = 0.95 given in parentheses.

ova also varied between 4-7% (DBA/2JY and 101/HY) and 14-15% in the mice of the remaining genotypes. A similar percentage of degenerating ova (13-17%) has been reported by other workers for C57BL/6 and CBA/T6 mice [1]. The age of the females (and, probably, their hormonal balance) has been observed to have a marked effect on the total preimplantation mortality. For instance, in females fertilized at the age of 1.5-2 months at the first ovulation a very low mortality level was observed: only 1.8% for CBWA tetrahybrids crossed with males of the same genotype [4].

The high preimplantation embryonic mortality in laboratory mice, as usually described in the literature, thus depends on the presence of undeveloped ova and the actual preimplantation mortality in the mice of the strains investigated there is around 5%. The exception to this rule is mice of strain AKR, with a high incidence of leukemia. The causes of the high mortality in this strain are of undoubted interest and should be investigated.

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