whereas *gag*-coded proteins were expressed only in one. Moreover, if the *pro-pol* sequences were detected in other cell culture, the amount of 30 and 14 kD proteins reacting with serum against *gag*-coded proteins of MPMV varied from culture to culture. An important result of this study is a convenient cell culture model for investigation of genetic information of type D retroviruses from the lymphocytes of patients with B-cell lymphosarcomas.

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The Role of Genetic Differences in the Mother—Fetus System in Biotransformation of Proteratogens

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Culturing of postimplantation embryos of C57Bl/6 and DBA mice differing in the ability to biotransform 3,4-benz(a)pyrene, reciprocal crossing of these animals, and transfer of preimplantation embryos into sham-pregnant females is used to model different genetic states by the Ah locus in the mother—fetus system. The role of metabolic factors at the early stages of embryogenesis in the realization of embryo- and genotoxic effects of 3,4-benz(a)pyrene is evaluated.

Key Words: *embryonal metabolism of xenobiotics; teratogenesis; sister chromatid exchanges*

A variety of synthetic compounds are biotransformed in human and animal body with formation of teratogenic, mutagenic, and/or blastomogenic metabolites. Deleterious effects of such a transformation on rat embryos at the initial stages of organogenesis have been demonstrated for cyclophosphamide, ethanol, adriamycin, and other compounds [2,3,5]. Recent studies show that metabolic activation of some substances, specifically, polycyclic aromatic hydrocarbons (PAH), aromatic amines, and dioxines occurs not only in mother's body, but also in embryos at the early stages of prenatal life [6,7]. The ability to transform PAH is controlled by the Ah locus and is inherited by the autosomal monogenic pattern as a dominant trait [8]. Some animals, for example, C57Bl/6, CBA, and C3H mice, carry the dominant allele Ah^b and are homozygous for this allele (Ah^b/Ah^b), indicating a high ability to biotransform PAC. Some other mouse strains (DBA, AKR, and SWV) are homozygous for the recessive allele (Ah^d/Ah^d) and exhibit a weak response to PAH [8].

The discovery of genetically determined ability to bioactivate xenobiotics in adult animals and early embryos reshapes traditional concepts concerning the teratogenesis of chemicals in mammals. The contribution of metabolic factors to this process at the early stages of embryogenesis is unclear, particularly in cases when mother but not fetus carries the recessive alleles Ah^d. This problem is particularly im-

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portant because metabolization of xenobiotics by embryo is possible at the initial stages of organogenesis, i.e., when the embryo is particularly sensitive to the damaging effect of xenobiotics.

The mother—fetus system should be regarded as a system consisting of two compartments with independent abilities to biotransform xenobiotics. As a result of various crossings (reciprocal, inverse, etc.), these compartments may acquire different genetic states characterized by different saturation of their loci by dominant and recessive alleles and, consequently, different ability to activate proteratogens.

Using C57Bl/6 and DBA mice which differ in the ability to transform PAH, we created various genetic states in the mother—fetus system by reciprocal crossings and transfer of preimplantation embryos into sham-pregnant females. The role of metabolism at the early stages of embryogenesis and the contribution of each compartment to embryotoxic (embryolethal, teratogenic, and growth-inhibiting) and genotoxic effects (by the sister chromatid exchange test) of 3,4-benz(a)pyrene (BP) were assessed.

MATERIALS AND METHODS

C57Bl/6 and DBA mice from the Rappolovo breeding center were used. Pregnancy was dated starting from the night when females were put in cages with males. The day when vaginal plug was detected was recorded as day 1 of gestation. Two series of experiments were performed. The first series was designed to assess the ability of C57Bl/6 and DBA mouse embryos to bioactivate BP. For this purpose 8-day-old embryos were put in a culture medium (50% Waymouth MB752/1 medium with 50% inactivated rat serum) containing BP (Serva) dissolved in dimethyl sulfoxide to a final concentrations of 1-100 μM (0.25 to 25.0 μg/ml). After a 48-h incubation, embryonal status, embryolethal, teratogenic, and growth-inhibiting effects, and the frequency of sister chromatid exchanges (SCE) in endodermal cells of the visceral wall of the yolk sack were assessed using the criteria and methods described previously [2,4]. At least 20-25 embryos were used to evaluate the effects of each BP concentration.

In order to obtain genetically uniform embryos in the second series of experiments, both reciprocal crossing of C57Bl/6 and DBA mice and transfer of preimplantation embryos (the morula—early blastocyst stage) of a given genotype into sham-pregnant females of the same or another genotype were performed. This enabled us to create different genetic states for the dominant Ah^b allele in the mother—fetus system (Table 1). On day 8 of gestation, all mice received a single intraperitoneal injection of BP (200 mg/kg). The embryos were studied on day 10, i.e., simultaneously with the embryos in the first series of experiments. The same criteria were used to assess the development of embryos; the frequency of SCE in the bone marrow cells of females was determined using the standard scheme [1]. Statistical analysis of results was performed using Student's *t* test.

RESULTS

The results of the first series of experiments (Fig. 1) show that DBA and C57Bl/6 mouse embryos differ in the ability to metabolize BP to embryo- and genotoxins. DBA embryos (Ah^d/Ah^d) virtually did not react to BP, while the frequency of SCE and the intensity of embryotoxic (embryolethal+teratogenic) effect on C57Bl/6 embryos (Ah^b/Ah^b) increased considerably. At 10 and 100 μM BP caused death of 20 and 54% of embryos, respectively, and induced hypoplasia of the mandibular processes, anophthalmia, and neural tube abnormalities in the surviving 45 and 58% of these embryos. At 100 μM BP produced a growth-inhibiting effect, which manifested itself as a decrease in the craniocaudal size of embryos and in the total protein content

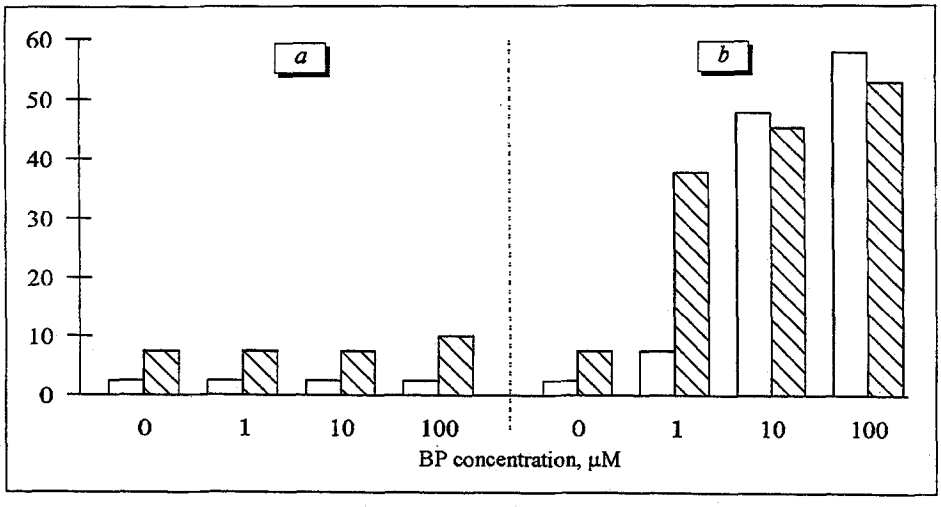

Fig. 1. Embryo- and genotoxic effects of 3,4-benz(a)pyrene (BP) in cultured DBA (*a*) and C57Bl/6 (*b*) mouse embryos. Ordinate: total embryotoxic effect, % (white bars) or frequency of sister chromatid exchanges, exchange per cell (shaded bars).

TABLE 1. Effects of Allelic Differences (by Ah Locus) in the Mother—Fetus System on Embryo- and Genotoxic Effects of 3,4-Benz(a)pyrene (*M±m*)

Distribution of Ah alleles, mother: fetus	Allelic characteristics of			Number of females	Number of embryos	Effects, %				
	males	females	embryos			embryo-lethal	terato-genic	total embryo-toxic	SCE in embryos	SCE in maternal bone marrow
0:0	Ah^d/Ah^d	Ah^d/Ah^d	Ah^d/Ah^d	8	61	9.8±4.2	4.3±3.0	13.7±4.8	11.2±0.6	10.3±0.6
1:0	Vasectomy	Ah^b/Ah^d	Ah^d/Ah^d	5	28	21.4±7.8	3.8±3.8	25.0±8.2	18.6±0.5	39.8±1.7
		transfer								
0:1	Ah^b/Ah^b	Ah^d/Ah^d	Ah^b/Ah^d	9	69	11.6±3.9	19.7±5.1	29.0±5.5	40.7±2.0	10.5±0.5
1:1	Vasectomy	Ah^b/Ah^d	Ah^b/Ah^d	5	35	22.9±7.1	22.2±8.0	40.0±8.3	38.4±1.4	39.7±1.7
		transfer								
2:0	Vasectomy	Ah^b/Ah^b	Ah^d/Ah^d	6	35	37.1±8.2	13.6±7.3	45.7±8.4	26.3±1.2	42.2±1.9
		transfer								
0:2	Vasectomy	Ah^d/Ah^d	Ah^b/Ah^b	6	31	13.8±6.4	28.0±8.9	37.9±9.0	39.9±1.6	11.6±0.5
		transfer								
2:1	Ah^d/Ah^d	Ah^b/Ah^b	Ah^b/Ah^d	8	52	30.8±6.4	41.7±8.2	59.6±6.8	41.4±2.0	43.3±2.1
1:2	Vasectomy	Ah^b/Ah^d	Ah^b/Ah^b	6	32	21.9±7.2	42.3±9.7	56.3±8.8	42.2±1.3	44.7±2.0
		transfer								
2:2	Ah^b/Ah^b	Ah^b/Ah^b	Ah^b/Ah^b	9	56	39.3±6.5	55.9±8.5	73.2±5.9	44.1±2.1	41.6±2.0

Note. Allelic characteristics: DBA) Ah^d/Ah^d; C57Bl/6) Ah^b/Ah^b; (C57Bl/6×DBA) F_1) Ah^b/Ah^d.

to 82 and 68% of the control, respectively. The frequency of SCE was 7.8 times as high as in the control.

The results of *in vitro* experiments indicate that BP metabolites with embryolethal, teratogenic, and embryotoxic activities are formed at the early stages of embryogenesis.

In the second series of experiments, the role of BP metabolites formed in early embryogenesis and the relationship between BP metabolism and genetic status of the mother—fetus system were studied. Genetic patterns of the mother and embryo compartments created by crossings and transfer of embryos made it possible to assess the contribution of allelic differences in the mother—fetus system (by the dominant Ah^b allele) to the teratogenic effect of BP (Table 1). When both mother and embryo were homozygous for a dominant or recessive allele (Ah^b distribution in each compartment is 0:0 or 2:2), the embryolethal, teratogenic, and total embryotoxic effects of BP were contralateral as well as the SCE frequencies (Table 1). An increase in Ah^b in the mother compartment (0:0, 1:0, or 2:0) coincided with a tendency toward a higher embryolethal and teratogenic activity of BP. In contrast, an increase in Ah^b allele in the embryo compartment (0:0, 0:1, or 0:2) coincided with a stronger tendency toward a higher teratogenic activity. The same tendencies were observed when Ah^b increased in both compartments. It is impossible to obtain the 2:0 and 0:2 Ah^b patterns by natural crosses. In an attempt to detect allelic dependence of the effect by changing "gene dose," we created these patterns by transfer of preimplantation embryos. In contrast to embryotoxic effect, the SCE levels in mother's bone marrow cells and in embryonal tissues depended not on the number of alleles, but on their presence in a given compartment.

This study shows an important role of the metabolism of proteratogenic xenobiotics at the early stages of embryogenesis in the realization of the teratogenic effect. Our findings indicate that extrapolation on humans of the embryotoxic activity data obtained on animals homozygous for a recessive allele may yield an incorrect result, since fetus, in contrast to mother, may be capable of bioactivating a proteratogen.

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