TOXIC ACTION OF ETHANOL AND ITS BIOTRANSFORMATION PRODUCTS ON POSTIMPLANTATION RAT EMBRYOS IN CULTURE

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Recent experience has shown that the embryotoxic properties both of original chemical compounds and of their biotransformation products can be assessed differentially and with a high degree of sensitivity directly in cultures of postimplantation rat embryos, with the aid of enzymes from the microsomal fraction of rodent and human liver [2, 4]. At the same time it will be evident that metabolic conversions of chemical substances can take place also with the aid of nonmicrosomal enzymes, such as enzymes of the mitochondria, the soluble fraction (SF) of the cells (cytosol), and so on. The modeling of the processes *in vitro* undoubtedly widens the technical possibilities for testing chemical preparations for embryotoxicity and must contribute to a better understanding of the mechanisms of their action. One such substance whose initial stage of biotransformation takes place chiefly in SF of liver cells is ethanol [10], which is known to act as a teratogen, inducing the fetal alcohol syndrome [7]. However, the mechanism of development of this syndrome is not yet clear. Evidence has recently been obtained that an important role in the etiology of alcohol damage to the fetus is played by the nearest metabolite of ethanol, namely acetaldehyde [9, 11], formed by the action of alcohol dehydrogenase, an enzyme present in liver SF.

In the investigation described below an attempt was made to carry out biotransformation of ethanol in cultures of postimplantation rat embryos by using SF from rat liver and to assess the embryotoxic properties of the biotransformation products and to compare them with the direct action of ethanol and acetaldehyde on embryos in culture.

EXPERIMENTAL METHOD

The investigation was carried out on 65 noninbred albino rats. Dated pregnancy was obtained in the usual way [1]. At 9.5 days of pregnancy the female rats were killed by cervical dislocation and, using the technique in [8], the embryos, in the stage of a developed head process and first pairs of somites, were explanted into nutrient medium (homologous blood serum). Culture was carried out in rotating tubes into which 7.5-8.0 ml of medium was poured and not more than eight embryos added. The conditions of culture were described by the writers previously [2]. In the experiments of series I the embryotoxic properties of ethanol were studied after its direct addition to the culture medium. Concentrations of 6.5-108 mM (0.3-5.0 mg/ml) were tested.

In the experiments of series II biotransformation of ethanol to acetaldehyde was carried out. For this purpose ethanol was added to the medium in a concentration nontoxic for embryos in culture (based on the results of the experiments of series I), and NAD (0.25 mM) and SF of rat liver (0.2-0.55 mg protein/ml) also were added. To convert the NADH formed in the course of the reaction back into NAD, sodium pyruvate (2.3 mM) also was added to the medium. The lactate dehydrogenase present in SF catalyzed the conversion of pyruvate into lactate, accompanied by oxidation of NADH. The toxicity of lactic acid was tested in additional experiments in which a concentration of 2.2 mM lactate was created in the medium. In other additional experiments it was shown that further metabolic conversions of acetaldehyde could not take place under the conditions created for biotransformation of ethanol into acetaldehyde.

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TABLE 1. Development of Rat Embryos after Addition of Various Components Essential for Ethanol Biotransformation to the Culture Medium

Experimental conditions	Ethanol, mM	NAD, mM	Protein of SF, mg/ml	Pyruvate, mM	Lactate, mM	Number of embryos	Number of embryos which died		Number of living embryos	Somites	Cranio-caudal length, mm	Protein, μg per embryo	Number of abnormal embryos	
							absolute	%					absolute	%
Control experiments	—	—	—	—	—	48	1	2,1±2,1	47	27,2±0,1	3,6±0,1	371±7	2	4,3±2,9
	17	—	—	—	—	38	2	5,3±3,6	36	27,3±0,1	3,5±0,1	358±11	—	
	—	0,25	—	—	—	16	—		16	27,3±0,1	3,6±0,1	368±12	—	
	—	—	0,55	—	—	16	—		16	27,2±0,1	3,6±0,1	360±12	—	
	—	—	—	2,3	—	15	—		15	27,2±0,2	3,5±0,1	372±16	1	6,7±6,5
	—	—	—	—	2,2	11	—		11	27,1±0,1	3,5±0,1	366±12	—	
	17	—	0,55	—	—	15	—		15	26,8±0,2	3,5±0,1	345±25	1	6,7±6,4
	17	0,25	—	2,3	—	23	—		23	27,1±0,1	3,6±0,1	344±26	1	4,3±4,2
	—	0,25	0,55	2,3	—	29	—		29	27,0±0,1	3,5±0,1	361±12	—	
Biotransformation	17	0,25	0,55	2,3	—	32	32	100	—	(5—7)				
	17	0,25	0,55	—	—	24	24	100	—	(10—18)				
	17	0,25	0,2—0,35	—	—	22	3	13,6±7,0	19	24,7±0,3	3,1±0,1	259±36	17	89,5±7,0

Legend. Number of pairs of somites in embryos which died given in parentheses.

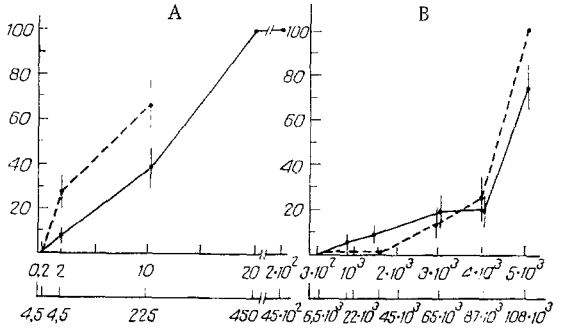

Fig. 1. Embryolethal and teratogenic action of acetyaldehyde (A) and ethanol (B) on rat embryos in culture. Abscissa: top axis — concentration of compounds in medium (in μg/ml), bottom axis — the same (in μM); ordinate, number of dying or abnormal embryos (in %). Continuous line — embryolethal effect; broken line — teratogenic effect. Control values given in Table 1.

To obtain SF the livers of male rats were homogenized in cold phosphate-salt buffer, at the rate of 2 ml solution to 1 g tissue. The homogenate was centrifuged at 105,000g for 1 h. The protein concentration in the supernatant (SF), determined by Lowry's method [6], was 28-30 mg/ml; alcohol dehydrogenase activity was between 12 and 15 nmoles/mg protein/min. The resulting SF as a rule was used on the same day in the experiments.

The embryotoxic properties of acetaldehyde were studied in the experiments of series III, for which purpose a concentration of between 4.5 μM and 45 mM of it was created in the medium (0.2-2000 μg/ml). The pH of the medium did not change appreciably in the course of all the experiments.

At the end of culture the number of embryos which died and which developed anomalies and the number of formed pairs of somites were counted, the craniocaudal length of the embryos was measured, and the total protein content per embryo was determined by Lowry's method [6].

EXPERIMENTAL RESULTS

In the experiments of series I addition of ethanol to the culture fluid caused a statistically significant increase in the embryolethal effect ($P < 0.05$), starting with a

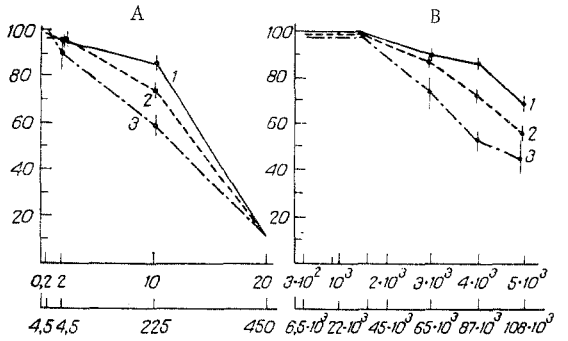

Fig. 2. Action of different concentrations of acetaldehyde (A) and ethanol (B) on craniocaudal length, protein content, and number of formed somites in embryos in culture. Abscissa: top axis — concentration of compounds in medium (in μg/ml), bottom axis — the same (in μM); ordinate, values of parameters (in % of control). 1) Number of formed somites; 2) craniocaudal length; 3) protein content.

concentration of 65 mM (Fig. 1B). A substantial decrease in the number of formed somites, in the craniocaudal length, and in the total protein content (Fig. 2B) and the appearance of abnormal embryos were characteristic of the embryos which continued to develop. The most marked anomalies were hypoplasia of the first three cerebral vesicles and an overall decrease in size of the cranial end of the neural tube, which can be described as an initial form of microcephaly. Even under the influence of a concentration of 108 mM, regarded as lethal for man, some embryos nevertheless continued to survive although their development was abnormal. A concentration of 33 mM was inactive. However, in subsequent experiments with biotransformation of ethanol it was used in an even lower concentration (17 mM) for in the investigation of Brown et al. [3] a concentration of 33 mM induced a small decrease (not statistically significant) in the growth parameters.

In the experiments of series II, besides ethanol (17 mM), NAD, SF, and sodium pyruvate also were added to the culture medium in concentrations nontoxic for embryos in culture (Table 1). All the explanted embryos were dead after 12 h in culture, evidence that toxic products of ethanol biotransformation were formed in the medium. In the absence of any of the components (except pyruvate) essential for ethanol biotransformation from the medium, incidentally, the embryos developed normally (Table 1). If no pyruvate was added the embryos were able to develop for 20-24 h, after which they all died.

A reduction in the SF protein concentration in the medium to 0.2-0.35 mg/ml led to weakening of the embryolethal effect and to an increase in the number of abnormal embryos (Table 1). The use of SF after keeping for 1 week in the frozen state, as a result of which alcohol dehydrogenase activity was lowered by 33-50%, had the same effect. The character of the anomalies differed from that of those induced by ethanol. Mainly inhibition of neurulation was observed, as a result of which the neural tube at the cranial end of the embryo remained open (exencephaly); deformation of the parts of the brain, microcephaly, incomplete axial rotation of the embryos, inhibition of growth of the allantois, and a sharp fall in the growth parameters also were observed.

Differences in the character of induced anomalies obtained by biotransformation of ethanol and its direct addition to the medium could be due to the specific action of acetaldehyde — the immediate metabolite of ethanol. Accordingly, in the next experiments the embryolethal and teratogenic action of acetaldehyde was studied (experiments of series III). Concentrations of acetaldehyde from 45 mM to 450 M caused death of all the explanted embryos practically immediately. The embryos had only to be put into medium with acetaldehyde in a concentration of 450 μM for 1 h or a concentration of 225 μM for 3 h for most of them to die during subsequent culture in medium without acetaldehyde. If the concentration was lowered to 225 and 45 μM, many abnormal embryos were observed (Fig. 1A) and growth was inhibited (Fig. 2A). The character of the anomalies corresponded exactly to that observed in the biotransformation experiments.

It can be concluded from the results that in the stages of initial organogenesis, when the placenta, liver, and other structures capable of metabolizing acetaldehyde were not present [12], this product of ethanol metabolism may represent a greater risk for embryonic development than ethanol itself, and moreover, in concentrations which may actually arise in human blood after taking alcohol [5, 12]. Incidentally, the spectrum of anomalies induced by acetaldehyde is wider than that induced by ethanol; the embryotoxic effect of acetaldehyde, moreover, is realized within a short time (a few hours). However, despite the much greater embryotoxicity of acetaldehyde than of ethanol, which the experiments in vitro revealed, it is probably more correct when speaking of embryos developing *in utero* of their combined action of the fetus, for each of these substances possesses embryotoxic properties to some degree or other. The degree of the harmful action of each compound (ethanol or acetaldehyde) on the fetus probably depends on the character (quantity and frequency) of alcohol consumption and on individual features of the metabolism of these substances in the mother.

LITERATURE CITED

1. A. P. Dyban, V. F. Puchkov, V. S. Baranov, et al., in: Objects in Developmental Biology [in Russian], Moscow (1975), p. 510.
2. V. B. Popov, B. L. Vaisman, and V. F. Puchkov, Byull. Éksp. Biol. Med., No. 5, 613 (1981).
3. N. A. Brown, E. H. Goulding, and S. Fabro, Science, 206, 573 (1979).
4. A. G. Fantel, J. C. Greenway, et al., Life Sci., 25, 67 (1979).
5. M. A. Karsten, S. Matsuzaki, L. Feinmen, et al., New Engl. J. Med., 292, 386 (1975).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
7. A. P. Streissguth, S. Langesman-Dwyer, J. C. Martin, et al., Science, 209, 353 (1980).
8. D. A. T. New, in: Methods in Mammalian Embryology, San Francisco (1971), p. 305.
9. K. S. O'Shea and M. H. Kaufman, J. Anat., 132, 107 (1981).
10. R. Rognstad and N. Grunnet, in: Biochemistry and Pharmacology of Ethanol, Vol. 1, New York (1979), p. 65.
11. P. V. Veghelyi and M. Osztovics, Experientia, 34, 195 (1978).
12. H. Weiner, in: Biochemistry and Pharmacology of Ethanol, Vol. 1, New York (1979), p. 125.

STRUCTURE OF THE HUMAN SPLEEN AND IMMUNOMORPHOLOGICAL PARAMETERS OF ITS LYMPHOCYTES DURING EMBRYOGENESIS

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The spleen is a complex multifunctional organ. It produces more immunoglobulins (IgM and IgG) than other organs [9, 11]. After splenectomy their content in the body falls and the risk of development of septicemia and of the patient's death is increased [13]. Recent investigations have shown that most of the T suppressors [4] affecting the immunologic reactivity of the body are concentrated in the spleen. Finally, the spleen performs the function of blood depot, and many metabolic processes take place in it. The spleen thus plays an active part in various reactions aimed at maintaining normal homeostasis. However, there have been few studies on the structure and function of this organ in man, especially in the embryonic period of development, when the main components of the spleen are formed. The authors of several publications [1-3, 6, 8, 10, 12] have emphasized the need for the study of the spleen during this period of development.

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