SENSITIVITY OF EARLY MOUSE EMBRYOS TO THE ANTIFOLIC DRUG PYRIMETHAMINE

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The effect of the antifolic drug pyrimethamine (2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine) on cleavage of CBA mouse embryos was studied. Pyrimethamine does not affect the development of early mouse embryos in utero but considerably inhibits cleavage of embryos explanted into medium containing the drug. The sensitivity of mouse and rat embryos to pyrimethamine is compared and it is concluded that there are no interspecific differences in their response to the teratogen at the level of the embryonic cells themselves.

KEY WORDS: early embryos; explantation; embryogenesis; teratogen; antifolic drug.

Folic acid inhibitors have been successfully used to study induced teratogenesis and also to shed light on the mechanisms controlling normal embryogenesis [3]. It has been shown, in particular, that the antifolic drug pyrimethamine (2,4-diamino-5-chlorophenyl-6-ethylpyrimidine), used for the treatment of malaria and other protozoal infections, exhibits a powerful teratogenic action in experiments on pregnant rats [4]; embryos at the first to second days of development were most sensitive to this substance [1].

The sensitivity of embryos of other mammals, including mice, to pyrimethamine has not been studied. Nevertheless, the answer to this question would be of great interest not only with respect to the choice of the most adequate test system for investigating the teratogeneic activity of this group of pharmacological agents. Investigation of species differences in the response of mammalian embryos to antifolic agents would help to clarify the relative importance of this vitamin at the different stages of normal embryogenesis.

This paper describes a study of the action of pyrimethamine on cleavage of mouse embryos in utero and in cultures in vitro.

EXPERIMENTAL METHOD

Mice of strain CBA from the "Rappolovo" nursery were used. To study the effect of pyrimethamine on embryos developing in utero one group of animals was given this compound by the intragastric route (as a suspension in water with Tween) on the first day of pregnancy in a dose of 50 mg/kg body weight. The mice were killed on the fourth day of pregnancy, the number of corpora lutea in the ovaries was counted, and under the MBS-1 binocular loupe the embryos were flushed out of the oviduct and uterus and their number was counted. The embryos were then examined under the phase-contrast microscope and fixed; cytological specimens were prepared [11] and the number of nuclei in them and the number of cleavage divisions counted [9]. Pyrimethamine is insoluble in water and, for that reason, to study its action on embryos developing in culture, the blood serum of rats to which pyrimethamine had been administered was used. Pyrimethamine was given by the intragastric route to donor rats as a suspension in water with Tween-20 in different doses (50, 25, 12, 6, and 3 mg/kg body weight). Serum for preparation of the culture medium was taken 6 h after administration of pyrimethamine to the animals, for at that time the concentration of the drug in the rats' blood reaches its maximum [2]. Mouse embryos from the stage of two and four blastomeres were cultivated at 37°C in microdrops under a layer of mineral oil [7] with constant aeration with a mixture of 7% CO_2 and 93% air in modified Biggers' medium [5] to which 20% rat blood serum was added instead of bovine albumin. After the end of cultivation the

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TABLE 1. Effect of Pyrimethamine on Development of Cleaving Mouse Embryos (M ± m)

Development in utero					Development in culture									
		number of embryos					number of developing embryos			including blastocysts				
Dose of pyrimethamine, mg/kg body weight	number of animals	absolute	% of number of corpora lutea	number of blastomeres in embryos	dose of pyrimethamine, mg/kg body weight of donor animal	number of explanted embryos	absolute	%	P	absolute	%	P	number of blastomeres in embryos	P
50	15	79	76,2±2,2	38,8±3,4	3	18	17	94±5,5	>0,05	14	82±3,0	>0,05	43,7±5,1	>0,05
					6	20	13	68±10,7	<0,01	5	38±9,1	<0,01	21,3±2,1	<0,01
					12	56	34	61±7,3	<0,01	15	44±6,6	<0,01	17,9±1,1	<0,01
					25	81	47	58±5,4	<0,01	11	23±4,6	<0,01	16,0±1,1	<0,01
					50	23	12	56±10,3	<0,01	2	15±7,4	<0,01	13,4±2,8	<0,01
Total	15	79				198								
Control	10	51	78,4±1,9	39,0±2,8		62	62	100	—	57	92±3,4	—	43,6±3,1	—
Grand total	25	130				260								

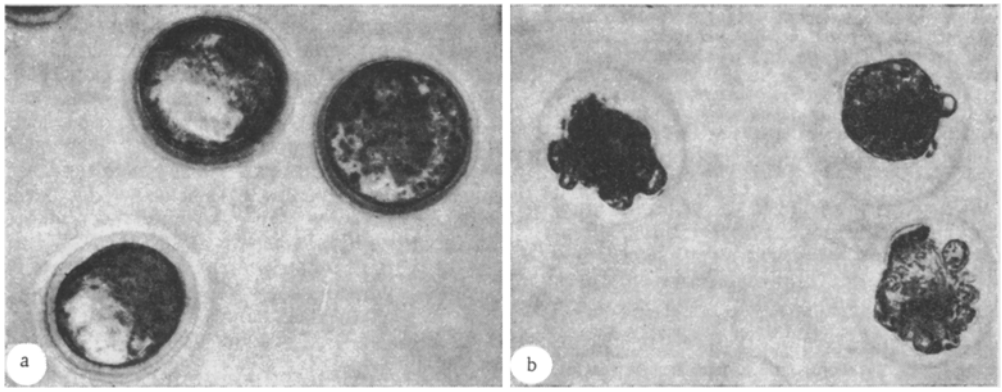

Fig. 1. Development of mouse embryos in vitro. Phase contrast, 70 ×: a) embryos after cultivation for 48 h in medium containing blood serum of intact animals. Normal embryos at blastocyst stage; b) embryos after cultivation for 48 h in medium containing blood serum of experimental animals treated with pyrimethamine in a dose of 6 mg/kg. Inhibition of cleavage and cavitation, disintegration of blastomeres.

embryos were investigated intravitally and later in fixed cytological preparations by the same method as the embryos developing in utero. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that after administration of pyrimethamine to the mice on the first day of pregnancy cleavage of the embryos was not disturbed and on the fourth day these embryos were indistinguishable from normal as regards both the number of blastomeres and the degree of development. It must be emphasized that a very large dose of pyrimethamine, which completely arrests cleavage and causes death of 100% of rat embryos [4], was given to the pregnant mice.

The mouse embryos developed well in culture in a medium containing blood serum of intact rats. At the time of fixation these embryos were normal blastocysts consisting of a fairly large number of blastomeres (Table 1; Fig. 1a).

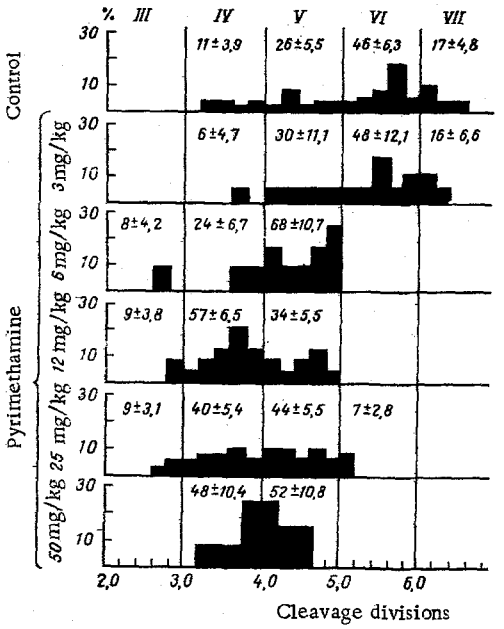

Fig. 2. Histogram. Cleavage of mouse embryos explanted at stage of four blastomeres into medium containing blood serum of intact animals (control) or serum from animals treated with pyrimethamine in doses of 3, 6, 12, 25, and 50 mg/kg body weight. Roman numerals show cleavage division; Arabic numerals total number of embryos (in %) at the corresponding stage of cleavage. Ordinate, number of embryos (in %) having passed through corresponding number of cleavage divisions; abscissa, number of cleavage divisions.

A different picture was observed after the addition of blood serum from rats receiving pyrimethamine to the culture medium. Embryos developing in this medium had considerably fewer blastomeres and many of them still remained at the stage without conversion into blastocysts (Table 1; Fig. 1b). Furthermore, whereas the control embryos at the time of fixation had completed cleavage division VI-VII, in the experimental group most embryos were still at division IV-V (see the histogram in Fig. 2).

An inhibitory effect was observed after all doses of pyrimethamine from 6 mg/kg or higher; with an increase in the dose of pyrimethamine the harmful action of the blood serum on development of the embryos was intensified: There were fewer blastomeres, they were deformed and some of them were fragmented, and cell debris was observed in the widened perivitelline space (Table 1; Fig. 1b). Pycnotic nuclei and micronuclei were observed in fixed preparations. Characteristically, even in large doses pyrimethamine did not stop cleavage immediately: the blastomeres managed to divide once or twice before embryonic development came to a halt (see histogram in Fig. 2).

Unlike rat embryos, early mouse embryos developing in utero thus did not react to the pyrimethamine administered to their mothers, whereas during cultivation in vitro they were severely damaged by this compound. Consequently, species differences exist between mouse and rat embryos in their relationship toward pyrimethamine, although the differences appear only when the substance is actually administered to the pregnant animals. The absence of a harmful action of pyrimethamine on embryos in utero can evidently be explained by certain special features of the pharmacodynamics of this substance in mice compared with rats. In mice pyrimethamine is perhaps absorbed more slowly or excreted more rapidly and its concentration thus does not reach the threshold level at which embryonic development is disturbed. In mice the wall of the oviduct has been shown to be a biological barrier [8] and the possibility cannot be ruled out that pyrimethamine penetrates badly or not at all into the lumen of the oviduct, so that it cannot act on the cleaving mouse embryos.

Since mouse embryos developed well in the medium with heterologous rat serum, the blood of intact rats evidently does not contain factors inhibiting cleavage of mouse embryos. Meanwhile, after the addition of blood serum of donor rats treated with pyrimethamine to the medium, development of the mouse embryos was disturbed; the intensity of the effect depended on the dose of pyrimethamine given to the donors. Consequently,

the blood of rats receiving pyrimethamine contains a factor inhibiting development of mouse embryos, and there is every reason to suppose that this factor is pyrimethamine itself or its metabolites. Characteristically mouse embryos reacted by inhibition of cleavage to blood serum from donor rats receiving 6 mg/kg pyrimethamine. This is the threshold dose which disturbs the development of rat embryos in utero [1]. The sensitivity of of the embryonic cells themselves to this antifolic compound was thus just as high in the mouse embryos as in rat embryos.

Since the mechanism of action of pyrimethamine consists of inhibition of dihydrofolate reductase [6, 10], there is reason to suppose that the very earliest stages of development of rat and mouse embryos are equally dependent on the normal function of this key enzyme of the folate cycle in the blastomeres; moreover, the zygotes of these animals evidently have no reserves of folates which could maintain the processes taking place during cleavage.

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EFFECT OF ANTENATAL ADMINISTRATION OF DIETHYLSTILBESTROL AND PROGESTERONE ON THE BLOOD SYSTEM OF THE NEWBORN PROGENY

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The effect of sex hormones injected into pregnant animals on the blood system of their progeny was determined. Changes were observed in various sections of the system: peripheral blood, bone marrow, spleen, and liver. Sex hormones stimulate hematopoiesis in the bone marrow and spleen of the fetus but inhibit it in the liver. The changes observed are interpreted as a process of acceleration of functional maturation of the fetal blood system.

Sex hormones have a considerable effect on the hematopoietic system for they cause changes in erythro-, leuko-, and thrombopoiesis [2, 3, 5-8]. Inhibition of hematopoiesis as a result of the action of sex hormones is manifested as a decrease in the number of cells of the erythroid and myeloid series and of karyocytes, delayed maturation of cells, and an increase in the number of reticular and endothelial cells in the bone marrow, spleen, and lymph nodes.

This paper describes a study of the effect of a combination of sex hormones (diethylstilbestrol and progesterone), injected into female rats during pregnancy, on the state of the blood system of their progeny.

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