MECHANISM OF THE HARMFUL ACTION OF PYRIMETHAMINE ON ALBINO RAT EMBRYOS

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The dynamics of the histopathological changes in albino rat embryos following administration of single teratogenic doses of pyrimethamine on the ninth day of pregnancy was investigated. The most sensitive structure to the action of pyrimethamine was found to be the decidual tissue, the first disturbances in which were observed as early as 1 h after treatment. The cells of the various germ layers reacted differently to pyrimethamine. The cytotoxic effect of the compound was particularly marked on the embryonic mesoderm and the extraembryonic ectoderm. It is concluded that hemodynamic disorders connected with disturbance of the decidual tissue play an essential role in the mechanism of the harmful action of some chemical teratogens on mammalian embryos.

KEY WORDS: pyrimethamine; pregnancy; decidual tissue; induced teratogenesis; embryonic death.

Investigation of the pathogenesis of embryonic death and malformations is a useful approach to the understanding of complex morphogenetic processes in both normal and pathological mammalian embryogenesis. The investigation described below is one of a series aimed at studying the mechanisms of induced teratogenesis and embryonic death following exposure to harmful substances during pregnancy.

It was shown previously that the antimalarial pyrimethamine (2,4-diamino-5-para-chlorophenyl-6-ethylpyrimidine) is a powerful teratogen for albino rats [1], it can induce chromosomal aberrations [2, 3], and it causes various biochemical disturbances both in the embryo itself [4, 5] and in its provisional organs and the decidual tissue.

The object of this investigation was to study the dynamics of the histopathological changes in the various embryonic anlagen and the tissues of the uterus after exposure to pyrimethamine during the period of maximal sensitivity of rat embryos to this compound, namely on the ninth day of pregnancy [1].

EXPERIMENTAL METHOD

Noninbred albino rats from the Rappolovo nursery, Leningrad, were used. Pyrimethamine, in a dose of 25 mg/kg, was given as a single injection by gastric tube on the ninth day of pregnancy, in the form of a water–fat emulsion. Tween-20 was used as the emulsifier. With this experimental scheme, as was shown previously [1], pyrimethamine causes death of up to 40% of the implanted embryos and leads to developmental anomalies in all the surviving embryos. The mothers were autopsied at various times after administration of the compound (1, 3, 6, 12, and 24 h). Parts of the uterus containing the implantation sites were fixed with Bouin's or Carnoy's fluids, dehydrated in alcohols of increasing strength, and embedded in paraffin wax; serial histological sections (5-7 μ) were cut and stained with Mayer's hematoxylin and eosin, with Heidenhain's azan, with methyl green and pyronine, or by Feulgen's method. The sections were studied and photographed with the Opton microscope (Photomicroscope II). On average 12 to 15 embryos were analyzed at each time point.

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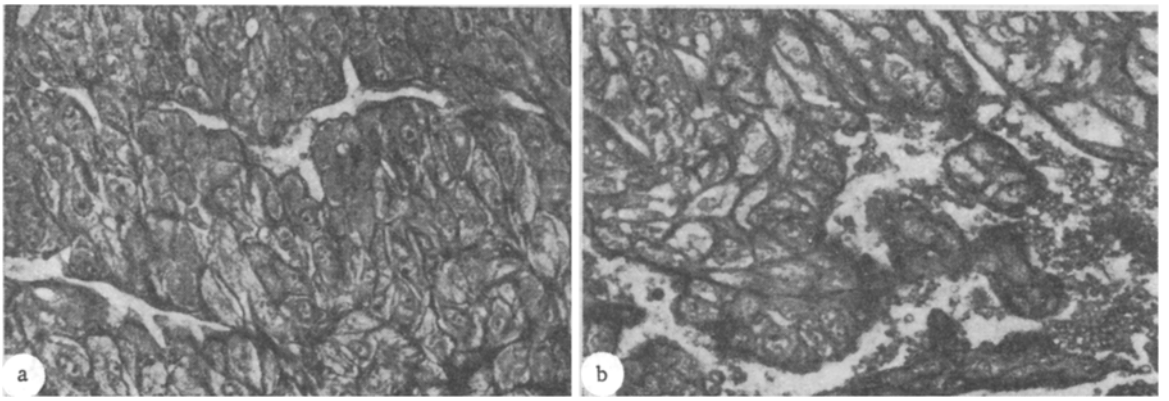

Fig. 1. Decidual tissue of rats on ninth day of pregnancy: a) normal; b) 3 h after injection of 25 mg/kg pyrimethamine. Stained with azan, 280 ×.

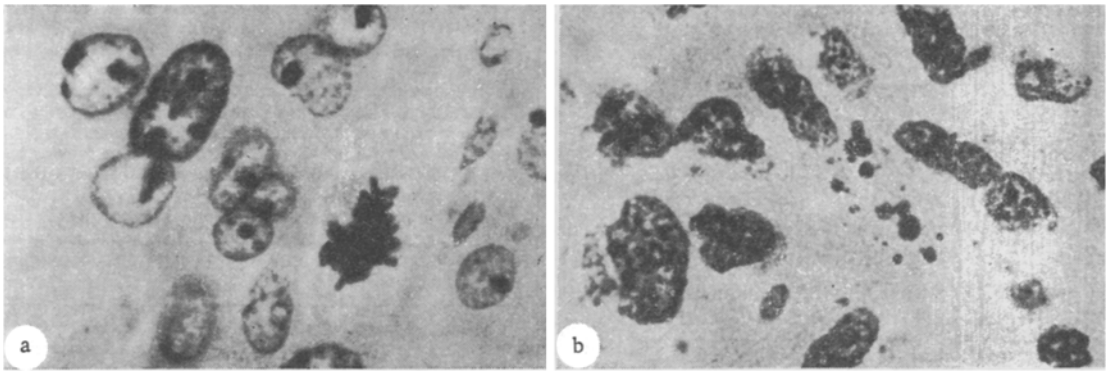

Fig. 2. Cells of antimesometrial part of decidual tissue of rats on ninth day of pregnancy: a) normal decidual cells (stained with methyl green and pyronine, 800 ×); b) karyorrhexis, hyperchromatosis, and deformation of decidual cell nuclei 6 h after injection of 25 mg/kg pyrimethamine (stained by Feulgen's method, 800 ×).

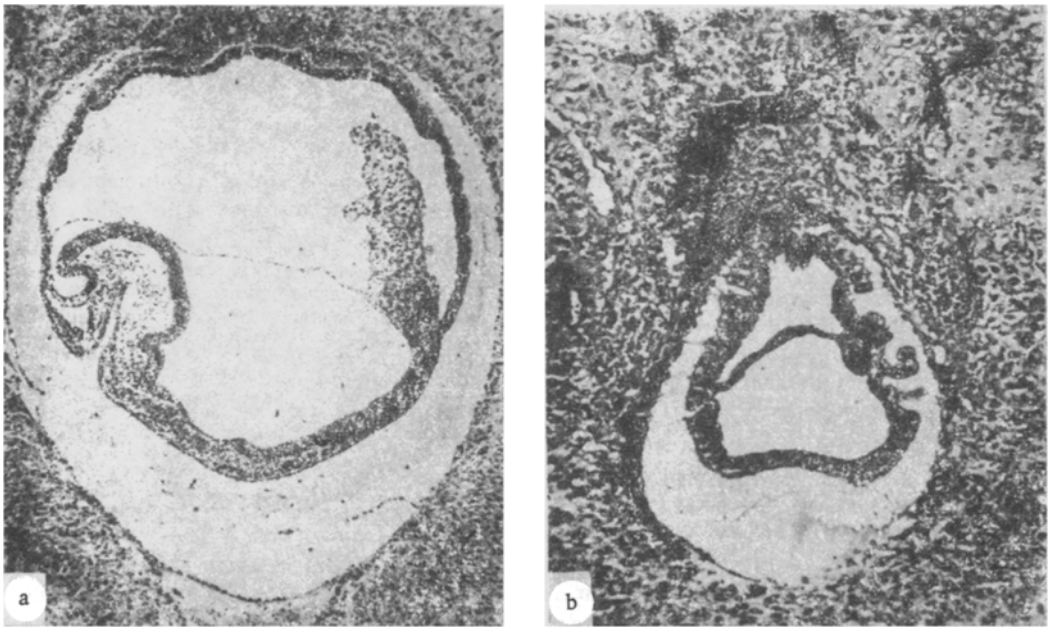

Fig. 3. Embryo on tenth day of development: a) normally developing embryo; b) embryo 24 h after injection of 25 mg/kg pyrimethamine on ninth day of pregnancy (hematoxylin–eosin, 80 ×).

EXPERIMENTAL RESULTS

Morphological changes 1 h after administration of pyrimethamine were found in the decidual tissue only. They consisted of widening of the intercellular spaces filled with exudate, and very slight vacuolation of individual decidual cells. By 3 h the edema was increased in the antimesometrial part of the decidual tissue and the lacunae and sinuses in the intermediate and mesometrial zones, filled with maternal blood, were dilated (Fig. 1). An eosinophilic effusion collected between the syncytium of the giant cells of the trophoblast and Reichert's membrane, in what Merker and Villegas [7] call the periembryonic sinus. In sections stained with azan swelling of some parts of Reichert's membrane itself was observed. On staining with methyl green and pyronine, a reduction in the content of pyroninophilic material was found in the cells of the antimesometrial zone. The nuclei of these cells in sections stained by Feulgen's method appeared swollen, with tiny granules of chromatin concentrated near the nuclear membrane.

The changes described above increased in intensity 6 h after administration of the teratogen and the cell nuclei in the antimesometrial zone became hyperchromatic and shrunken; some of them were completely destroyed (Fig. 2). Death of the cells in the zone immediately adjacent to the implantation chamber took place on a massive scale. In all the embryos studied the typical picture of stasis was observed. Degenerative changes also were found in the ectoplacental cone. Its cambial portion was reduced and the cell nuclei at the base of the cone were hyperchromatic and pycnotic. The cells lost their connections with each other and spaces formed between them. Characteristically it was at this time, i.e., 6 h after administration of the compound, that the first pathological changes were found in the embryo itself. As the histological analysis showed, cells of the different embryonic layers responded differently to pyrimethamine; the cells of the mesoderm were most sensitive. Their cytoplasm was shrunken and their nuclei became elongated and hyperchromatic. Pycnosis was comparatively rare in the embryonic ectoderm, but round basophilic granules were observed in the cytoplasm of the cells. No signs of degeneration were seen in the cells of either the embryonic or the vitelline entoderm. Many cells of the embryonic entoderm had become cylindrical, i.e., had acquired the characteristic shape of cells of the vitelline entoderm.

The most characteristic pathomorphological feature 12 h after administration of pyrimethamine was the marked hemodynamic disturbances in the embryos. Maternal blood from the dilated lacunae and sinuses of the intermediate zone of the deciduoma poured into the region of the ectoplacental cone and into the periembryonic sinus, compressing and deforming the embryo. No secondary giant cells were present. The apical part of the cone was infiltrated with leukocytes. The cambial zone of the ectoplacental cone at this time consisted only of a small group of basophilic cells. Development of the embryonic cylinder also was considerably retarded, its wall was thin and consisted of a layer of grossly hypertrophied cells of the vitelline entoderm, whereas the remaining embryonic anlagen had degenerated.

Maternal blood 24 h after administration of the compound had separated the layers of the ectoplacental cone, accumulated in the periembryonic sinus, and led to complete deformation of the germinal vesicle (Fig. 3). By this time the subsequent fate of the embryos subjected to the action of the teratogen was decided: in some of them severe disturbances of morphogenesis were observed, although they remained viable (these were about 60% of the total number of embryos), whereas the rest had died and were being resorbed.

The results of this investigation indicate that pyrimethamine injures primarily decidual tissue, and only secondarily the embryonic anlagen; the sensitivity of the latter to the compound varies. The harmful action of pyrimethamine was most marked in the mesoderm of the embryo and in the ectoplacental cone, whereas the vitelline entoderm was much less severely affected.

This step-by-step histological analysis thus provides evidence of the decisive role of a disturbance of the decidual tissue in the mechanism of the harmful action of pyrimethamine on albino rat embryos in the postimplantation stages of development. During this period the decidual tissue performs the function of a specialized organ, the hemodynamic matrix, responsible for nutrition of the embryo, for controlling the circulation and affecting the transport of materials from the maternal blood into the embryonic tissues [7, 8]. Under the influence of pyrimethamine not only do the cells of the decidual tissue die, but serious hemodynamic disturbances also arise, leading to the onset of stasis, hemorrhages into the periembryonic sinus, and detachment of the ectoplacental cone; in turn, these disturb the nutrition of the embryo and aggravate the pathological changes in the embryonic anlagen.

The results thus indicate that when the mechanisms of the embryotoxic and teratogenic effects of harmful substances are assessed in experiments on rats, allowance must be made not only for the direct action of the teratogen on the embryonic cells, but also for the vascular reactions of the uterine wall, i.e., disturbances of the function of the decidual tissue as the "hemodynamic matrix."

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