Puerperal Acute Toxicity Indirectly Induced by 4-Amino-N^{10}-Methyl-Pteroylglutamic Acid (Methotrexate) in Mice[1] (35607)

MARIO RUSTIA AND PHILIPPE SHUBIK

The Eppley Institute for Research in Cancer, University of Nebraska College of Medicine, Omaha, Nebraska 68105

As a part of an investigation into the carcinogenicity of 4-amino-N^{10}-methyl-pteroylglutamic acid (methotrexate, MTX),[2] a group of newborn mice was tested. It was found that a single sc injection of 100 μg given to each newborn resulted in the death of 50% of the untreated mothers. Of the progeny, about 50% survived their mothers. It is assumed that MTX entered the mothers' circulatory systems, apparently via the excreted urine of the newborn mice (1).

In the acute toxicity test in adult mice, the LD_{50} was approximately 300 mg/kg per os, with variations from prior reports, such as 94 $\pm$ mg/kg ip (2) and an LD_{65} of 500 mg/kg sc (3). However, 50% of the mothers in our test group were fatally intoxicated by an amount which could not have exceeded 800 μg each (or 24 μg/g). Tolerance postpartum thus appears to be manyfold lower than that at other stages of life.

Since some newborns survived the death of their mothers, the tests were extended to observe whether newborns surviving similar treatment could be raised by foster mothers. Two new groups, consisting of 20 litters each, were used. Nursing by the foster mothers was begun 24 and 48 hr, respectively, after treatment of the newborns. We were surprised to observe that some of the foster mothers also died.

Materials and Methods. Swiss albino mice from our colony were used. This strain of mice was originally obtained from the Roscoe B. Jackson Memorial Laboratory (Bar Harbor, Maine) and has been randomly bred in our laboratory since 1951. The mothers were housed with their progeny in clear plastic cages on sterilized granular cellulose bedding at room temperature (72–76°F), were fed Rockland diet in pellets (A. E. Stanley Mfg., Co., Decatur, Illinois), and were given tap water *ad libitum*.

Prior to treatment each animal was weighed separately. The newborns averaged 1.6 g, the mothers, 32.7 g. Newborns, less than 24 hr old, were treated with a single sc injection of MTX (0.05 ml = 100 μg of 0.2% solution alkalized with sodium bicarbonate) into the interscapular region.

The experimental groups were distributed as follows:

Group 1 (the original group). Twenty treated litters consisting of 5 to 8 siblings/litter. Each sibling received 100 μg of MTX.

Group 2. Ten untreated and 10 treated litters of 6 to 8 siblings each. Treated newborns received the same dosage as those in Group 1. Foster nursing began 24 hr after treatment.

Group 3. Ten untreated and 10 treated litters consisting of 7 to 8 siblings. Treated newborns received the same dosage as those in Group 1. Foster nursing began 48 hr after treatment.

Foster nursing involved transferring the treated newborns from odd numbered cages to untreated, even numbered cages and vice versa.

Results and Discussion. Results by groups are shown below:

Group 1. From 20 treated litters, 10 of 20 mothers died in from 3 to 7 days after treatment of their newborn. About 50% of the siblings survived after their mothers' deaths and were then killed. The mothers surviving longer than 7 days manifested symptoms of

[1] Supported by Contract PH-43-68-959.

[2] Methotrexate, generously supplied by Dr. H. Kiltie, Lederle Laboratories, Pearl River, New York.

intoxication but to a lesser degree and eventually recovered.

Group 2. When foster nursing was begun 24 hr after treatment, 4 foster mothers out of 10 died, compared with 8 of 10 natural mothers. In two cases, both the natural mothers and the foster mothers died. In another instance the natural mother outlived the foster mother, while in a third case the natural mother survived the intoxication but the foster mother died.

Group 3. When foster nursing was begun 48 hr after the treatment, 6 out of 10 natural mothers died within 4 to 7 days. The foster mothers did not manifest any observable sign of intoxication and none of them died.

In none of the experiments did the animals alter their behavior or manifest any symptom of the intoxication during the first 2 days. The newborns apparently suckled well, as they were well fed. The first clinical signs appeared on the third day when some of the mothers suddenly died.

Symptoms included ruffled hair, labored breathing, anorexia, weakness, and diarrhea, followed by dehydration, weight loss, and increasing lethargy. At the autopsy, congestion of the lung and distention of the stomach and of the intestines, which contained a yellowish fluid, were the conspicuous features. Microscopically, marked lesions were observed, primarily in the small intestine and in the bone marrow. The intestinal mucosa revealed severe desquamation, ulceration, and edema. Also observed were vast destruction of the bone marrow and depletion and dilation of the sinusoids, which were filled with large amounts of blood, giving them a lake-like appearance.

It is difficult to estimate the amount of the antimetabolite supplied to the mothers (via the urine of the treated newborns), but the amount, however minute, was sufficient to kill them. Tests on adult mice show that MTX is excreted, mostly in the urine and probably unchanged (4, 5). When parenterally administered, the elimination is largely completed within 24 hr. A small, but significant, amount is excreted up to 48 hr (4).

It seems probable that the mothers in this investigation were folic acid deficient and when exposed to a very small amount of antimetabolite their extreme intolerance became apparent.

During pregnancy the rate of cell growth and metabolism is rapidly increased, producing a higher folate utilization which continues throughout the gestation period and lactation (6). Mice and rats maintained on a basal, folic acid-deficient diet failed to rear their offspring and lactation was not adequate (7, 8). In humans at parturition the folate level measured in mothers' serum is significantly reduced. In contrast the folate level may increase fourfold in newborns (9). A low level of serum folate is believed to reflect a subclinical deficiency state which may predispose the subject to excessive toxicity when treated with methotrexate (10). Mice maintained on folic acid-deficient diets, even for a short time, become extremely sensitive to small doses of methotrexate (11).

Summary. The administration of 100 μg of MTX to newborn mice resulted in fatal intoxication of 50% of untreated mothers. The mortality rate of foster mothers when foster nursing was begun 24 hr after treatment of the newborns was 40%. None of the foster mothers died, however, when the foster nursing began 48 hr after the treatment. MTX is rapidly excreted, largely in the urine, within from 24 to 48 hr after administration. It is assumed that the antifolate entered the mothers' circulatory systems via the excreted urine of the newborns. Both natural and foster mothers died after a maximum of 24 μg/g, which is a manyfold lower dose than that tolerated in adults (LD_{50} = 300 μg/g per os). It is thought that this striking deviation in tolerance reflects the folic acid deficient state in the immediate postpartum period in mice.

We acknowledge the editorial assistance of Mrs. R. K. Van deBogart.

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Received Feb. 9, 1971. P.S.E.B.M., 1971, Vol. 137.