EFFECT OF MATERNAL STRESS ON THE HARMFUL ACTION OF 4-METHYLURACIL ON EMBRYOGENESIS OF RATS

É. A. Kosmachevskaya and N. A. Chebotar'

UDC 615.252.441:547.854.7].015.45:618.33-056.716-02:616.45-001.1/3

The action of 4-methyluracil on embryogenesis was studied in experiments on albino rats under conditions of stress (immobilization of the females on the 10th day of pregnancy). Under normal conditions the compound in doses of 600-2000 mg/kg does not give rise to developmental anomalies in rat embryos, and the embryotoxic action of 4-methyluracil is manifested only in a dose of 2000 mg/kg and above. If the pregnant females were immobilized after receiving the compound (2000 mg/kg) its lethal action on the embryos was unchanged, and external examination of the surviving embryos showed no developmental anomalies. However, the examination of microanatomical sections showed that 58.9% of the fetuses had developmental anomalies of the urogenital system.

* * *

4-Methyluracil (4MU) is widely used in various branches of medical practice as a stimulator of physiological and reparative regeneration and also as a regulator of inflammatory processes [5]. The possibility of using 4MU for the treatment of pregnant women makes it necessary to investigate the effect of this compound on development of the embryo under experimental conditions because careful assessment of the action of therapeutic substances on animal embryos is an effective means of safeguarding the antenatal period of development [3].

4MU has a marked embryotoxic action and produces developmental anomalies in chick embryos [4]. In a dose of 50 mg/kg, this compound has no harmful action on rat embryos [6].

The teratogenic and embryotoxic activity of harmful agents can be strengthened or weakened by changes taking place in the maternal organism [1, 7-9]. Immobilization, for instance, potentiates the teratogenic and embryotoxic action of carbutamide and sodium salicylate on rat embryos [2], probably as the result of delay of the excretion of these substances by the pregnant female.

The object of the present investigation was to study the teratogenic embryotoxic activity of 4MU while the pregnant females were in a state of stress produced by immobilization.

EXPERIMENTAL METHOD

Experiments were carried out on 69 pregnant albino rats weighing 150-200 g. The slab recommended by Renaud [10] was used for immobilization (on the 10th day of pregnancy). 4MU was introduced into the stomach as a single dose of 2000 mg/kg body weight body the beginning of immobilization. This dose was chosen on the basis of a series of preliminary experiments in which various doses of the compound, from 600 to 3000 mg/kg, were given by the gastric route. The results of these experiments showed that 4MU in doses of 600-1500 mg/kg has no effect whatever or a weak embryotoxic action on the development of the rat embryo, and doses of 2500-3000 mg/kg caused death of the pregnant animals. A dose of 2000 mg/kg gave a marked embryotoxic effect but not toxic effect on the pregnant animals. The rats were sacrificed on the 19th-20th day of pregnancy. The number of implantation sites and of living and dead embryos in the uterus and the number of corpora lutea in the ovaries were counted. The fetuses were studied under a binocular

Department of Embryology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad (Presented by Active Member of the Academy of Medical Sciences of the USSR D. A. Biryukov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 12, pp. 89-92, December, 1968. Original article submitted October 24, 1967.

TABLE 1. Harmful Action of 4-Methyluracil on Embryogenesis of Albino Rats when Administered on the 10th Day of Pregnancy

Treatment	No. of rats	No. of corpora lutea	No. of embryos			No. of malformed embryos	Living fetuses				
			implanted	dying after implantation	living		hydronephrosis	edema of ureter	absence of one kidney	ectopia of kidneys	malformation of gonads
4MU, 2000 mg/kg	13	112	109	38 (34.8%±4.5)	71	–	–	–	–	–	–
4MU, 2000 mg/kg + immobilization for 2.5 h	15	100	94	39 (41.5%±5.0)	56	33 (58.9±6.5)	18	16	10	12	11
Immobilization for 2.5 h	11	106	106	17 (16.0%±3.5)	83	–	–	–	–	–	–
Control	39	372	330	24 (7.3%±1.4)	306	–	–	–	–	–	–

loupe and fixed in Bouin's fluid. After fixation for 7-10 days, a microanatomical investigation of the embryos was carried out by Wilson's method [11].

The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Administration of 4MU on the 10th day of pregnancy had no toxic action on the pregnant females, but caused death of 34.8% of the fetuses. In the control group the number of dying fetuses did not exceed 7.3%. Macroscopic examination revealed no differences whatever between the surviving embryos and the controls, and no developmental anomalies of the internal organs were present.

In the control group no deaths were observed among the immobilized pregnant animals, the number of dying embryos was 16%, and no developmental anomalies were found among the surviving embryos.

When the pregnant animals were immobilized after receiving 4MU, no developmental anomalies were found in the surviving embryos on external inspection, but microanatomical examination of sections showed that 58.9% of fetuses had various developmental anomalies of the urogenital system. The commonest types of malformations were hydronephrosis and edema of the ureter. Ectopia of the kidneys, unilateral absence of a kidney, curvature of the ureters, cryptochidism and monorchidism, and incomplete development of the Müllerian duct were found in some embryos. No developmental anomalies of other internal organs were found (Table 1). The most typical developmental malformations are shown in Fig. 1.

Under conditions of maternal stress due to immobilization, the teratogenic action of 4MU was thus manifested. No case of congenital developmental anomalies due to the action of 4MU has been described in the literature. However, we consider that it is too early yet to state categorically that this compound is harmless to the human embryo [6], for this investigation carried out by the authors cited involved a small number of albino rats and the internal organs of the embryos were not examined. The dose used in the present experiments was much larger than the therapeutic dose, but the results obtained, indicating the ability of 4MU to give rise to developmental anomalies of the urogenital system in rat embryos during immobilization of the mother not only require detailed study, but also provide evidence of the potential teratogenic properties of this compound. The possibility is not ruled out that if other factors are brought to bear on the mother during pregnancy, the teratogenic properties of 4MU may also be manifested in man.

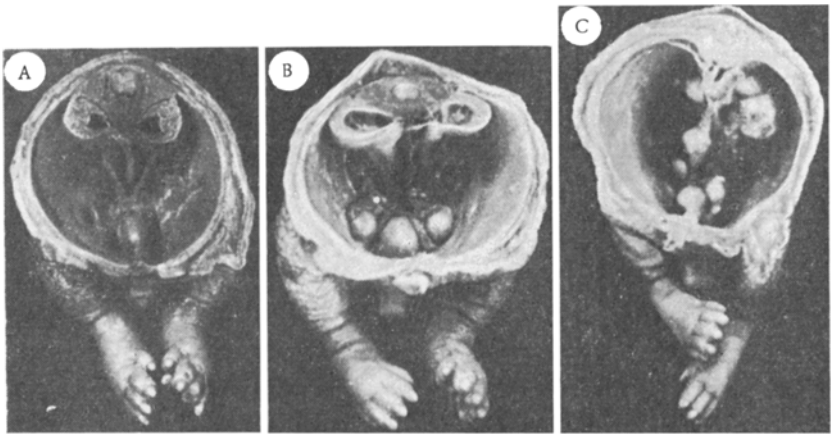

Fig. 1. Section through a 20-day rat embryo. A) Normal; B) hydronephrosis; C) absence of right kidney.

LITERATURE CITED

1. I. R. Barilyak, Farmakol. i Toksikol., No. 5, 631 (1967).
2. I. R. Barilyak and N. A. Chebotar', in: Proceedings of the Tenth Conference of Undergraduate and Postgraduate Students of Morphological Departments and Laboratories of Leningrad Teaching and Research Institute [in Russian], Leningrad (1967), p. 13.
3. A. P. Dyban, Vestn. Akad. Nauk SSSR, No. 1, 18 (1967).
4. É. A. Kosmachevskaya, in: Problems in Pharmacology and Experimental Pharmacotherapy. Transactions of the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR [in Russian], Vol. 9, No. 3, Leningrad (1966), p. 99.
5. N. V. Lazarev, in: Proceedings of a Conference on the Use of Pyrimidine and Purine Derivatives in Oncology and Other Branches of Medicine [in Russian], Leningrad (1966), p. 61.
6. B. V. Monakhov, N. R. Myuller, and K. V. Yaremenko, Vopr. Onkol., No. 2, 111 (1967).
7. N. A. Chebotar', Farmakol. i Toksikol., No. 2, 221 (1967).
8. A. S. Goldman and W. C. Yakovac, J. Pharmacol. Exp. Ther., 142, 351 (1963).
9. A. S. Goldman and W. C. Yakovac, Proc. Soc. Exp. Biol., (New York), 115, 693 (1964).
10. S. Renaud, J. Appl. Physiol., 14, 868 (1959).
11. J. G. Wilson, in: Teratology, Principles and Techniques. Chicago (1965), p. 251.