ANTENATAL EFFECT OF PYRIMETHAMINE AND VITAMIN A ON BEHAVIOR OF THE RAT PROGENY

V. G. Malakhovskii

UDC 616.831.31-009.81-039:616.85/-02:/615.283+615.356:577.161.11/.015.4:612.64

The behavior of month-old rats was studied following administration of pyrimethamine (1 mg/kg) and vitamin A (150,000 i.u.) to their mothers during pregnancy in doses much less than those causing malformations of the fetal brain. Although in their outward appearance the rats were indistinguishable from the controls, their motor activity was lower and their aggressive response weaker than with normal rats of the same age. They differed sharply from intact animals also in their rate of defensive conditioning and in the rate of their response of choosing the correct path of avoidance.

Evidence has been obtained that the intrauterine action of radiation or of chemical agents may not only lead to malformations, but may also disturb the behavioral responses of outwardly normal animals after birth. The W.H.O. experts have recommended the term "functional" to describe defects of this type. After exposure to pyrimethamine in utero, malformations of the fetus have been found [1, 2]. Comparatively little is known of the teratogenic action of pyrimethamine on the human fetus, but from the few reports which have been published, such an effect seems likely [8, 12]. The teratogenic action of hypervitaminosis A is well known [5, 9, 11].

The object of this investigation was to look for disturbances of behavior in rats exposed antenatally to pyrimethamine and vitamin A in doses too small to produce externally visible developmental disturbances.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 160-220 g were used. Pyrimethamine, dissolved in peach oil, was injected intraperitoneally in a single dose on the 9th day of pregnancy, the time of maximum teratogenic activity of the compound [3], and the time of first appearance of the primitive central nervous system [10]. A concentrated oily solution of vitamin A was given by mouth at the same time of pregnancy.

The motor activity of the rats was studied in a chamber by recording the number of short-circuits between contacts in the floor during running. Records of movements of the animal in the horizontal plane were taken every 10 min for 1 h [7]. The aggressive response was recorded by the method of Kudrin and Polevoi [4]. The number of sites and the presence of a squeak in response to electrical stimulation were recorded.

Conditioned defensive reflexes were studied in a chamber divided into two compartments. The rats were stimulated by an electric current through the floor. The number of combinations necessary for the first spontaneous conditioned-reflex response (going into the other compartment) and the number of combinations necessary for 3 correct consecutive responses, considered to indicate a stabilized conditioned reflex, were determined. The response of choosing the path of avoidance during electrical stimulation was assessed [6].

Central Research Laboratory, Leningrad Pediatric Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 3, pp. 34-36, March, 1971. Original article submitted June 10, 1970.

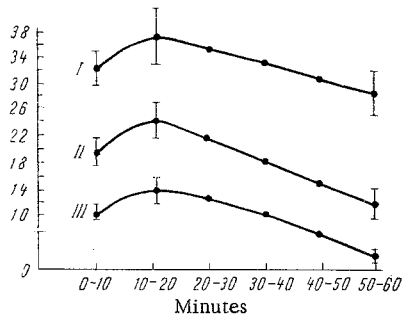

Fig. 1. Motor activity (number of short circuits of floor contacts as rats moved about in chamber; ordinate) of control month-old rats (I) and of rats exposed antenatally to pyrimethamine(II) and vitamin A (III).

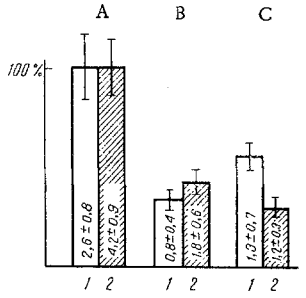

Fig. 2. Aggressive response (relative to control, taken as 100%) of month-old control rats (A) and of rats exposed antenatally to pyrimethamine (B) and vitamin A (C): 1) frequency of squeak response; 2) number of sites.

EXPERIMENTAL RESULTS AND DISCUSSION

In the experiments of series I, pyrimethamine was injected into pregnant females in a dose of 8 mg/kg. At autopsy on the 18th day of pregnancy, 210 fetuses were found, of which 117 had developmental defects, notably meningocele in 32% and anencephaly in 14% of cases. Following administration of vitamin A in a dose of 300,000 i.u. per rat, meningoceles were found in 30% of cases.

In the experiments of series II, after administration of pyrimethamine in a dose of 1 mg/kg and of vitamin A in a dose of 150,000 i.u. per rat, the progeny was indistinguishable from the control in weight, in external appearance, and in the mean number of young rats in the litter. When the rats attained the age of 1 month (428 animals) their behavior was compared with the behavior of control rats (96) whose mothers had received peach oil by intraperitoneal injection during pregnancy.

The motor activity of the young rats whose mothers had received pyrimethamine and vitamin A during pregnancy was much less marked than that of the controls (Fig. 1). The aggressive response of the experimental young rats was much weaker (Fig. 2).

The rate of defensive conditioning was determined in experiments on 269 animals as the quotient obtained by dividing 100 by the number of combinations needed to produce the conditioned-reflex response. In rats exposed antenatally to pyrimethamine, the rate of conditioning was 2.1 ± 1.4 (control 6.2 ± 1.9), and the rate of stabilization 3.2 ± 1.6 (control 8.8 ± 2.4). The corresponding figures for rats exposed to vitamin A were 2.8 ± 1.2 and 4.2 ± 1.4. The differential stimulus was applied after the reinforced conditioned-reflex response. The number of correct responses was counted for each group of rats. The result of this investigation was 68% for the control animals, 11% for rats exposed to pyrimethamine, and 24% for rats exposed to vitamin A.

In experiments on 217 rats the ability of the animals to find the correct choice of path to avoid electrical stimulation was determined. The time taken by the rats to leave the bottom floor of the chamber, in seconds (t), was converted into a rate (100/t). The rate of leaving the bottom floor of the chamber was 3.4 ± 0.17 for rats exposed antenatally to pyrimethamine, 7.8 ± 0.1 for rats exposed to vitamin A, and 12.2 ± 0.2 for intact rats. The "teratogenic" rats made 4 and 2.6 times respectively more mistakes when going into the side compartment of the chamber than the control rats.

Neither macroscopic examination nor histological study of all parts of the brain of these animals, with sections stained with hematoxylin-eosin and by Nissl's method, revealed any abnormality, so that the brain defect in these rats was evidently latent in character.

Another important fact is that during the investigation of pyrimethamine and vitamin A the general level of abnormalities in the behavioral responses of the rats was about equal, although the dose of pyrimethamine given in the experiments of series I was 8 times smaller, and the dose of vitamin A was 2 times smaller than doses capable of producing externally visible malformations of the fetal head and brain.

LITERATURE CITED

1. A. P. Dyban and I. M. Akimova, Akush. i Gin., No. 5, 21 (1965).
2. A. P. Dyban, L. D. Udalova, and I. M. Akimova, Dokl. Akad. Nauk SSSR, 167, No. 1, 228 (1966).
3. A. P. Dyban and I. M. Akimova, Arkh. Anat., No. 8, 228 (1966).
4. A. N. Kudrin and L. G. Polevoi, Farmakol. i Toksikol., No. 1, 95 (1964).
5. E. A. Lotosh, Dokl. Akad. Nauk SSSR, 169, No. 1, 238 (1966).
6. V. G. Malakhovskii, Farmakol. i Toksikol., No. 3, 372 (1968).
7. V. G. Malakhovskii, A. D. Berkovich, and V. B. Prozorovskii, Farmakol. i Toksikol., No. 3, 336 (1969).
8. A. G. Pap, in: Antenatal and Neonatal Care [in Russian], Kiev (1964), p. 227.
9. S. O. Cohlan, Pediatrics, 13, 556 (1954).
10. V. Hamburger, Quart. Rev. Biol., 38, 342 (1963).
11. H. Kalter and J. Warkany, Am. J. Path., 38, 1 (1961).
12. G. Piekarski, Münch. Med. Wschr., 102, 842 (1960).