Studies on Methylazoxymethanol: Methylation of Nucleic Acids in the Fetal Rat Brain (34220)

YUKIO NAGATA[1] AND HIROMU MATSUMOTO
(Introduced by G. L. Laqueur)
Department of Agricultural Biochemistry, University of Hawaii, Honolulu, Hawaii 96822

Recently, Spatz and Laqueur (1) reported that they were able to produce microencephaly uniformly in the offspring of rats injected with the aglycone of cycasin, methylazoxymethanol (MAM). The compound was effective when injected on Day 14 or 15 of pregnancy and the effect appeared to be limited to the cortical hemispheres of the brain. This observation raised the question of whether or not MAM, a powerful alkylating agent (2, 3) entered and interacted with compounds in the fetal brain and initiated metabolic changes which subsequently resulted in the observed anatomical changes. This paper presents evidence for the reaction of MAM with the nucleic acids and proteins of the fetal brain. It also reports on the distribution of MAM in the tissues and fluids of pregnant rats injected with the compound.

Materials and Methods. MAM-acetate. Tritum-labeled MAM-acetate (230 μCi/m mole) was prepared as previously reported (4).

Animals. The Osborne-Mendel strain of female rats, in Day 14 of pregnancy, were used in all experiments. ^{3}H-MAM-acetate in 0.9% NaCl solution was injected intraperitoneally at a dosage of 20 mg MAM–acetate/kg body weight.

Preparation of tissues. Eighteen hours after the injection of ^{3}H-MAM–acetate the mother rats were decapitated and the organs were removed immediately and kept cold on ice. The fetus was separated in two parts, head and the rest of the body, and homogenized in buffer.

Proteins. One part of homogenate was mixed with two parts of cold 5% trichloracetic acid (TCA) and the precipitated proteins were collected by centrifugation and washed with 5% TCA, followed by ethanol, ethanol–ether (3:1 v/v), twice with ether, then dried in a desiccator over calcium chloride. The quantity of protein was determined by the method of Lowry *et al.* (5).

Nucleic acids. RNA was prepared according to Hiatt (6) and DNA by the method of Colter *et al.* (7). The quantity of RNA was assayed with orcinol (8) and DNA by the indole method (9).

Radioactivity determination. RNA and proteins were dissolved in hyamine hydroxide (10) and DNA was dissolved in a mixture of dioxane and water (11), then added to a toluene scintillation mixture and counted in a Beckman SP-100 scintillation spectrometer. Radioactivity in the tissues, serum, and urine of the mother rat was measured by the method of Meade and Stiaglitz (12).

Identification of derivatives of bases. A portion of the isolated RNA was hydrolyzed with *N* HCl at 100° for 1 hr (13) while DNA was treated with 6 *N* HCl for 3 hr. The hydrolyzates were spotted on Whatman No. 1 paper and chromatographed in a solvent previously used in this laboratory (2). The presence of bases was detected with ultraviolet radiation and the area where 7-methylguanine was found was cut out, eluted with hyamine hydroxide, and the quantity of radioactivity determined (11).

Results and Discussion. The incorporation of radioactivity into the nucleic acids and proteins of the fetus is shown in Table I. There was definite evidence of incorporation of radioactivity into the DNA of the fetal rat brain. This reaction of MAM with DNA was

[1] Present address: Faculty of Agriculture, Gifu University, Kakamigahara, Gifu, Japan.

not unexpected as earlier observations had indicated that MAM methylated nucleic acids readily *in vitro* (2) and *in vivo* (3).

The presence of tritium-labeled methyl group as 7-methylguanine in the hydrolyzates of RNA and DNA was detected by paper chromatography. The recovery of 61% of the radioactivity present in DNA as 7-methylguanine strongly suggests that the methylation of nucleic acids by MAM occurs almost exclusively on the guanine molecule. There were indications that a small amount of another base was also methylated but the results were inconclusive.

There was also some radioactivity incorporated into the proteins of the fetus but no labeled amino acid could be detected on hydrolysis. It is believed the formyl group was incorporated into the proteins, since one of the products of MAM decomposition is formaldehyde (14).

Table II shows the distribution of the injected MAM in tissues and fluids of the mother rat. A surprisingly significant amount, 4.1% of the injected radioactivity was found in the fetus and another 2.2% in the placenta. A large portion of the radioactivity was found in the liver of the mother, as expected. The kidney retained a relatively low amount of MAM since it is apparent that at least another 10% of the injected compound passed through it, as indicated by the quantity of radioactivity found in the urine collected during the 18-hr period after injection, plus that recovered from the bladder at the time the animal was sacrificed.

TABLE I. Incorporation of Radioactivity from ^{3}H-MAM-Acetate into Proteins and Nucleic Acids of Fetal Rat.

Substance	Radioactivity (dpm/mg)	% Radioactivity recovered	R_F[a]
Head protein	819		
Body protein	1246		
Brain RNA	980		
Brain DNA	2340		
RNA hydrolyzate	992		
Derivative in RNA hydrolyzate	473	48	0.37
DNA hydrolyzate	1735		
Derivative in DNA hydrolyzate	1065	61	0.37
7-Methylguanine	—		0.37

[a] Solvent: propan-2-ol (680 ml), 11.6 *N*–HCl (176 ml), water to 1 liter.

TABLE II. Radioactivity Incorporated in the Various Organs and Fluids of Rat Injected with ^{3}H-MAM-Acetate.

Organ	Radioactivity (dpm)	Recovered (%)
Liver	5,209,500	33.5
Kidney	292,000	1.8
Fetus	731,800	4.1
Placenta	336,600	2.2
Serum[a]	684,700	4.4
Urine	1,604,400	10.0
Unaccounted[b]	6,680,000	44.0
^{3}H-MAM-acetate injected[c]	15,540,000	100.0

[a] Total radioactivity calculated as serum volume equals 6.7% of body weight of animal (16).

[b] By difference.

[c] 20 mg/kg body weight; specific activity 230 μCi/mmole.

The results provide evidence that MAM interacts with compounds in the fetal brain. They also confirm the chromatographic observations of Spatz and Laqueur (15) that MAM passes through the placenta.

Summary. Methylazoxymethanol, the aglycone of cycasin, reacted with nucleic acids and proteins in the fetus of rats injected with the compound on Day 14 of pregnancy. Guanine methylated in the seven position was found in both DNA and RNA. The protein reaction products were not identified. The distribution of the injected compound in the tissues and fluids of the mother rat was determined.

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