# Teratogenic Effects of Methylazoxymethanol. (31767)

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Cycasin (methylazoxymethanol-β-D-glucoside), occurs in the seeds of *Cycas circinalis* and *Cycas revoluta*. Since plant parts from which cycasin has on occasion been incompletely removed are ingested by humans and animals in tropical parts of the world, it is of interest that cycasin is hepatotoxic and carcinogenic in small laboratory animals(1). Recent studies have shown that the aglycone (methylazoxymethanol, MAM) of cycasin is the proximate carcinogen because MAM causes neoplasms after subcutaneous and intraperitoneal injection as well as feeding while cycasin causes tumors only if it is fed to animals(2). Moreover, cycasin fails to cause tumors if it is fed to germfree animals while MAM can cause tumors under these conditions. This suggests that cycasin must be deglucosylated before it is carcinogenic and that the intestinal flora provide the β-glucosidase activity necessary for this hydrolysis (3).

In addition to its hepatotoxic and carcinogenic effects, MAM has been reported to cause mutations(4), chromosome breakage (5), and methylation of nucleic acids(6). This communication describes the results of experiments in which MAM was tested for teratogenic effects.

*Material and methods.* The method developed by Ferm(7) for screening compounds for possible teratogenic activity was used in this study. Female golden hamsters weighing 90-120 g and obtained from a commercial hamster colony in Newfield, New Jersey were placed with males, and daily vaginal smears were performed. The presence of spermatozoa in the smear marked the first day of gestation. On the eighth day the hamsters were anesthetized with pentobarbital (60 mg/kg of body weight), and MAM was injected into the lingual vein in various amounts as indicated in Table I. The MAM was prepared by the method of Kobayashi and Matsumoto(8) from crystalline cycasin. A solution of 4 mg/ml of MAM in physiological saline was used for injection. Control pregnant hamsters received corresponding amounts of saline.

The hamsters were sacrificed on the 12th day of pregnancy and the fetuses were removed from the uterus and examined under a dissecting microscope in physiological saline for malformations and for viability as evidenced by cardiac contractility. They were then fixed in Bouin's solution for later histological study.

*Results.* The observations are summarized in Table I. The 23 pregnant females which had been treated had 214 living fetuses. No abnormalities were found among the 49 fetuses obtained from females injected with 12 mg of MAM per kg of body weight and among the 39 fetuses from controls. Malformations were noted in all of the living fetuses from animals which had been injected with 20 and 23 mg of MAM per kg of body weight. In addition, several dead and sites of resorbed embryos were found, and nearly all (35/36) of the embryos were resorbed in a group of females which had received 25 mg of MAM per kg.

The following gross malformations were noted: hydrocephalus, microcephalus, cranioschisis, exencephaly, spina bifida, rachischisis, anophthalmia, microophthalmia, oligodactyly, and growth retardation. The severity and type of abnormalities varied within each litter, and a variety of combinations occurred (Fig. 1 and 2). The occurrence of cranioschisis, rachischisis, and exencephaly, often in combination, suggests that the effect of MAM on embryonic development was rapid since the neural tube closes by the ninth day(7). Details of the malformations will be pub-

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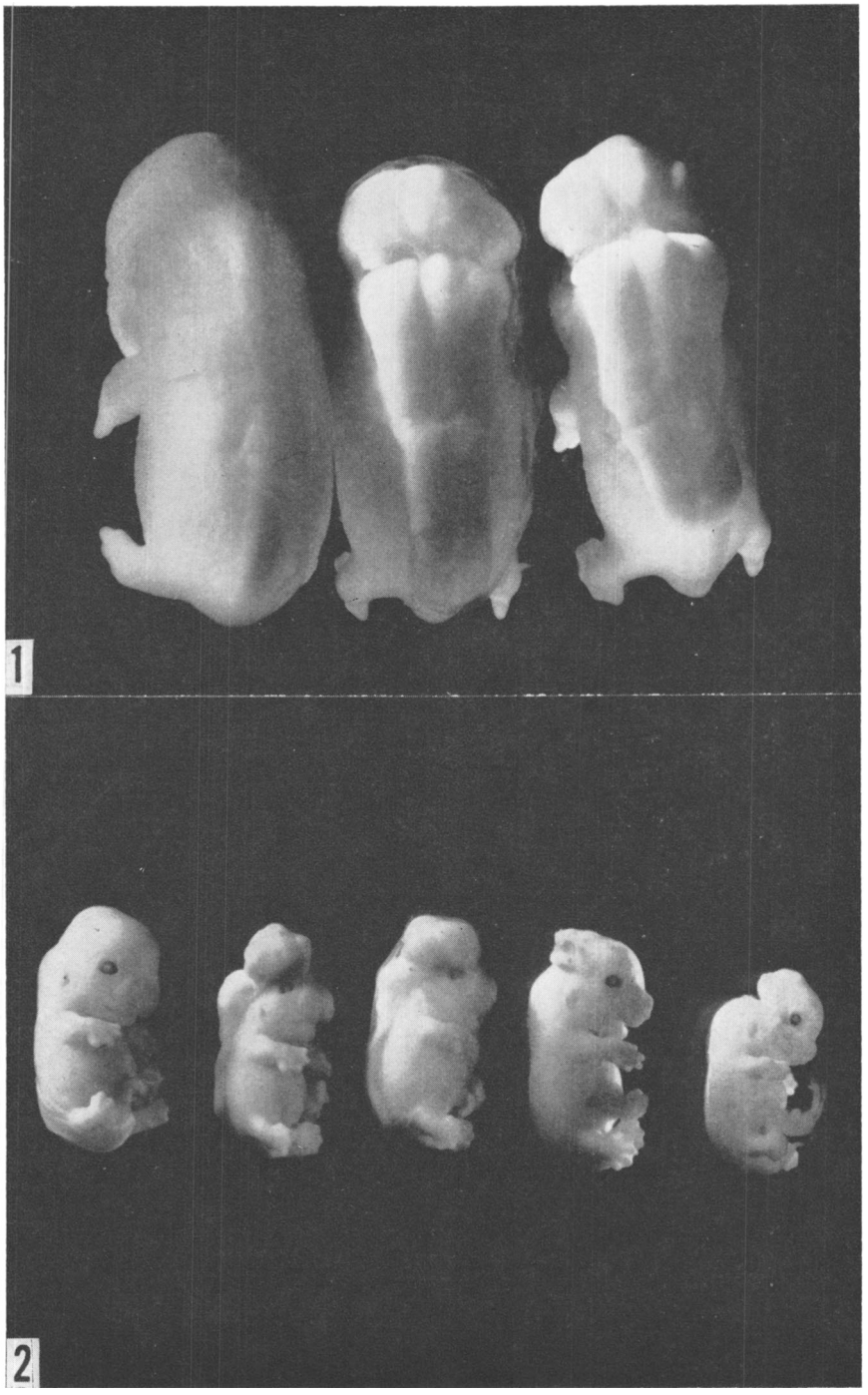


FIG. 1. Examples of fetal malformations observed in MAM treated golden hamsters. 12-day-old fetusees, control fetus, left. × 6.

FIG. 2. 12-day-old fetuses showing different combinations and varying degrees of abnormalities. Control fetus, left. × 3.

TABLE I. Teratogenic Effect of Methylazoxymethanol in Golden Hamsters.

| No. of litters | Treatment Type | Dose, mg/kg | No. of implantations | No. of alive fetuses | No. of malformations | No. of dead fetuses | No. of resorptions |
|---|---|---|---|---|---|---|---|
| 4 | Saline | | 41 | 39 | 0 | 0 | 2 |
| 4 | MAM | 12 | 52 | 49 | " | " | 3 |
| 10 | " | 20 | 123 | 111 | 111 | " | 12 |
| 6 | " | 23 | 80 | 53 | 53 | 17 | 10 |
| 3 | " | 25 | 36 | 1 | 1 | 0 | 35 |

lished after completion of microscopic study of the fetuses.

*Discussion.* Although the mechanisms through which the malformations are induced are presently obscure, observations using thin-layer chromatography have established the presence of MAM in embryos following intragastric administration of cycasin to mothers. This finding is also of considerable significance in view of the recent observations that tumors can be induced with cycad material by the transplacental route(9). A combined teratogenic and carcinogenic effect of a single compound was recently reported in rats by Druckrey, Ivancovic and Preussmann(10) following an intravenous injection of ethylnitrosourea on day 15 of pregnancy. The animals had malformed paws at birth and developed tumors of brain and peripheral nerves by the 160th day.

*Summary.* Methylazoxymethanol (MAM), the aglycone of cycasin, a naturally occurring glucoside of cycad plants, causes retardation of growth and a variety of malformations in embryos of the golden hamster. This same substance was previously shown to be carcinogenic, hepatotoxic and to cause mutations, methylation of nucleic acids and chromosome breakage.

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## Interaction of Progesterone and Aldosterone with Red Blood Cells of The Rat. (31768)

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Interactions between steroid hormones and proteins have been actively investigated(1-3). However, very little is known regarding the binding of steroids to red blood cells (RBC) and the possible role of RBC in the metabolism and transport of steroids. Some investigators(4) have reported that erythrocytes play a minor role in the transport of steroids; others(5,6) suggest that red blood cells might have an important function in the mechanism of transport of steroids. Previous investigations(7,8) on the binding of aldosterone and