Cyclamate and Cyclohexylamine: Transfer Across the Hemochorial Placenta[1] (34352)

R. M. PITKIN, W. ANN REYNOLDS, AND L. J. FILER
(Introduced by J. T. Bradbury)

Departments of Obstetrics and Gynecology and Pediatrics, University of Iowa Hospitals, Iowa City, Iowa 52240; and Department of Anatomy, University of Illinois at the Medical Center, Chicago, Illinois 60680

Widespread use of cyclamates as non-nutritive sweetening agents, estimated at 8000 tons per year in the United States, has led to concern regarding their safety. Two recent reports of cytogenetic effects raise serious questions about the advisability of cyclamate ingestion in human pregnancy. Stone *et al.* (1) described cyclamate-induced chromosome breaks in human leukocytes while Legator *et al.* (2), reported an increased incidence of chromosome disruption with cyclohexylamine in both *in vitro* preparations of a rat-kangaroo cell line and *in vivo* studies of rats. Cyclohexylamine is the compound from which cyclamate is synthesized and to which it is metabolized by a proportion of humans estimated at approximately 20% (3).

The questions of placental transmission of cyclamate and its effect on the fetus have received scant attention. Taylor *et al.* (4) reported observations on a single rat fetus whose dam was given ^{35}S-sodium cyclamate. The Interim Report on Non-nutritive Sweeteners (3) referred to an unpublished study on three pregnant rats given ^{14}C-cyclamate at varying intervals before delivery. Thus, information about placental transfer and fetal tissue levels of radioactive cyclamate is limited to one species, the rat, and involves a total of four fetuses. No data regarding placental transfer of cyclohexylamine or other biotransformation products are available.

Methods. Four rhesus monkeys (*Macaca mulatta*) were studied in the last trimester of pregnancy. The duration of gestation, estimated retrospectively from standard tables of fetal weight and crown-heel length for this species (5), varied from 140 to 160 days. After an overnight fast, the animals were anesthetized with phencyclidine hydrochloride (Sernylan, Parke-Davis and Co., Detroit, Michigan) 2 mg/kg and atropine 0.1 mg intramuscularly. An ether–air mixture was administered by inhalation for approximately 10 min prior to surgery. Polyethylene catheters were inserted into a maternal antecubital vein and into the interior vena cava through a femoral vein. Through midline celiotomy, the uterus was exposed and the anterior and posterior placentas and interplacental vessels were identified by uterine transillumination (6). An interplacental vein was isolated and catheterized with polyethylene tubing in the direction of the primary placenta. Another polyethylene catheter was inserted into the amniotic sac at a different site in the uterus. A retention catheter was placed in the maternal urinary bladder.

^{14}C-sodium cyclamate dissolved in normal saline was infused into each of two animals in amounts of 60 μCi (15.8 μCi/mg) over a period of 110 min. Fifty μCi of ^{14}C-cyclohexylamine hydrochloride (5 μCi/mg) dissolved in normal saline were infused into each of the other two over a period of 180 min. In each experiment the isotope was administered by a constant infusion pump (Harvard Apparatus C., Dover, Massachusetts) into the maternal antecubital vein. Samples were collected in heparinized calibrated capillary tubes at 15–30-min intervals from: (i) maternal inferior vena cava, (ii) interplacental vein, (iii) amniotic sac, and

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(iv) maternal urinary bladder. In two experiments (one cyclamate and one cyclohexylamine), the mother was killed by intravenous pentobarbital at the conclusion of the infusion and a variety of maternal and fetal tissues were obtained for tissue counting and autoradiography. In the other two, the sampling process was continued for 1 hr after conclusion of the infusion, at which time cesarean section was done and various fetal tissues were obtained. Tissue levels and autoradiographic localization will be reported elsewhere.

Blood samples were treated with Nuclear Chicago Solubilizer and decolorized with saturated benzoyl peroxide in toluene. Instagel (Packard Instrument Co.) was added to amniotic fluid and urine samples. Radioactivity was measured in a liquid scintillation counter.

Results and Comment. Figure 1 is a composite representation of mean values from two experiments with ^{14}C-cyclamate infusion. Comparison of maternal and fetal blood levels reveals that, while cyclamate traversed the hemochorial placenta, the degree of transmission was somewhat limited, at least during the 2–3-hr period of observation. The area under the curve of fetal radioactivity levels is 28.6% that of the maternal value. Radioactivity appeared in the maternal urine 60 min after the infusion was begun and increased rapidly in amount, reaching a maximum of 68×10^5 dpm/ml.

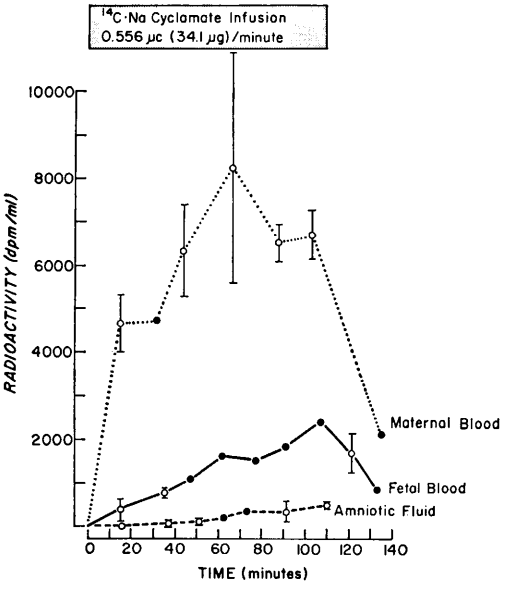

FIG. 1. Radioactivity levels in maternal and fetal blood and amniotic fluid with ^{14}C-cyclamate infusion into mother: (○), means of determinations from each of two experiments; and (●), single observations.

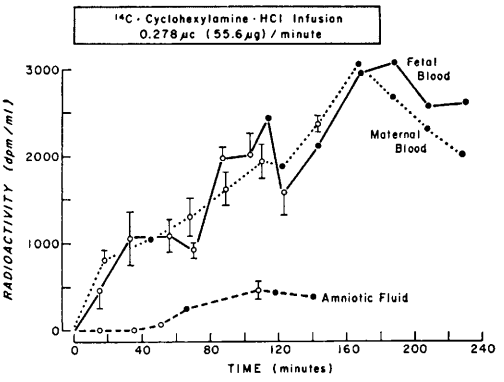

FIG. 2. Radioactivity levels in maternal and fetal blood and amniotic fluid wtih ^{14}C-cyclohexylamine infusion into mother: (○), means of determinations from each of two experiments; and (●), single observations.

In the case of cyclohexylamine (Fig. 2), maternal and fetal levels of radioactivity were virtually identical, indicating that this substance freely diffuses the primate placenta. The area under the curve of fetal radioactivity levels is 96.5% that of the maternal value. Cyclohexylamine appeared in maternal urine 60 min after the infusion was begun and increased to a maximum of 9.9×10^5 dpm/ml. In comparison with ^{14}C-cyclamate, the lower quantitative levels of ^{14}C-cyclohexylamine in all fluid samples are presumably due to the greater tendency of cyclohexylamine to penetrate cells (7).

Summary. Maternal–fetal transfer of ^{14}C-cyclamate and ^{14}C-cyclohexylamine was studied with the fetus *in utero* and the amniotic sac intact in rhesus monkeys. Both substances crossed the placenta. With cyclamate, maternal–fetal blood levels of radioactivity were in the approximate ratio of 4:1, suggesting a somewhat limited degree of transmission. By contrast, cyclohexylamine was found to diffuse freely across the primate placenta.

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