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Fluoride in Bone of Rats Anesthetized during Gestation with Enflurane or Methoxyflurane

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Fluoride concentrations in maternal and fetal bones were measured following exposure of pregnant rats to methoxyflurane or enflurane anesthesia. Fluoride content in fetal bone increased significantly only after exposure to methoxyflurane, and then only when methoxyflurane was administered after 12 days of gestation, when ossification of fetal bone begins. Fluoride concentrations in maternal bone increased following both anesthetics, except in rats exposed to methoxyflurane after 15 days of gestation, when ossification of many fetal skeletal parts is in progress and fluoride is preferentially deposited in the fetal skeleton. Key words: Anesthetics, volatile: methoxyflurane; Anesthetics, volatile: enflurane; Biotransformation (drug), methoxyflurane; Biotransformation (drug), enflurane; Ions; fluoride; Pregnancy, fluoride.)

INCREASED FLUORIDE CONCENTRATIONS in blood,[1–3] urine,[1–7] and bone[8] have been reported to occur following methoxyflurane[1,2,3,6,8] and enflurane[4,5,7,8] anesthesia. Since fluoride concentrations in tissues may reach toxic levels,[1–3] manifested by nephrotoxicity[1,3] or by inhibition of vitally important enzymes,[2,9,10] the use of methoxyflurane for general anesthesia is presently restricted; however, it continues to be used to provide analgesia during labor and delivery. This results in exposing the fetus to methoxyflurane[11,12] and fluoride.[13–15]

The maternal–fetal exchange of fluoride ingested during pregnancy has been studied intensively because of concern over the effect of fluoride on the developing fetus, especially with regard to development of bones and teeth *in utero*.[16,17] Rapid and extensive placental crossing by fluoride was demonstrated when ^{18}F was administered to a woman immediately before therapeutic abortion.[18] Increased fluoride concentrations in fetal blood and skeleton were observed in cattle and experimental animals ingesting increased amounts of fluoride.[19] The capability of fetal bone to retain fluoride is largely due to the large fraction of cancellous bone.[17,20]

In the present study, fluoride concentrations in maternal and fetal bones of rats were measured to determine osseous uptake of fluoride by the fetus following methoxyflurane and enflurane anesthesia administered to gravid rats.

Method

Experimental animals were female Wistar rats approximately 8 months old, fed the same diet, and given drinking water with 0.4 ppm fluoride. Rats mated on the same day and having a positive plug-test were used. The gestation of Wistar rats lasts 21–22 days. Two rats were anesthetized simultaneously in a 20-liter exposure chamber twice for four hours, 20 hours apart. The desired anesthetic concentration was delivered into the chamber from a Pentec II vaporizer using an air flow rate of 8 l/min. Concentrations of anesthetic in the exposure chamber were sampled at 15-minute intervals by gas chromatography.[2,7] Methoxyflurane concentration was maintained at 0.20 per cent (SD 0.02) and enflurane at 1.5 per cent (SD 0.23). The temperatures in the exposure chamber varied between 19 and 22 C.

At the end of gestation (that is, 20 days after mating) mothers were sacrificed by decapitation under ether anesthesia. Fetuses were removed and sacrificed within 2 minutes after the mother's death, unless mentioned otherwise. Maternal and fetal femoral and tibial bones were removed and dried at 105 C and analyzed for fluoride. For analysis, the bones of fetuses from the same litter were pooled. There was an average of 11 fetuses (SD 5) per litter. Bones were decalcified with a sodium EDTA solution, and fluoride was determined in the eluate by a specific fluoride-

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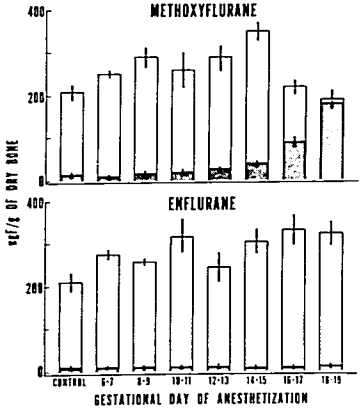

FIG. 1. Fluoride concentrations (means and standard errors) in maternal (*open column*) and fetal (*dotted column*) bones after methoxyflurane or enflurane anesthesia. On the abscissa is the gestational age at which the rats were anesthetized.

ion electrode.[5] The concentrations are expressed as μg fluroide per gram of dry bone.

The first experiments were designed to study the distribution between fetal and maternal skeleton of fluoride released from methoxyflurane or enflurane as a function of the stage of pregnancy when the mother was anesthetized. Since only limited numbers of rats mated on the same day were available at the same time, each drug was studied in two sets of similar experiments, each consisting of 18 rats. Fourteen pregnant rats were anesthetized according to the schedule indicated in figure 1. Four pregnant nonanesthetized rats served as controls.

Additional experiments were designed to study neonatal changes in osseous fluoride concentrations following delivery of fetuses. Eight pregnant rats were anesthetized with methoxyflurane, four animals for four hours each on the fourteenth and on fifteenth days of gestation, and four animals for four hours each on the eighteenth and nineteenth days of gestation. All mothers were sacrificed on the 20th day of gestation, and the fetuses were quickly removed. From each litter, every other fetus was sacrificed immediately, and the rest were allowed to survive 10 hours. Fetal femoral and tibial bones were pooled so that bones of offspring from two litters, exposed simultaneously and sacrificed after the same period of extrauterine life, constituted one sample for fluoride analysis. Similar experiments were carried out with enflurane.

Results

The vapor concentrations used in these experiments produced light anesthesia, from which the animals recovered in less than 10 minutes. No abnormality was observed in the fetuses on gross examination.

The means and standard errors (n = 4) of fluoride concentrations in fetal and maternal bones are presented in figure 1. The increases in fluoride in maternal bones were statistically significant following anesthesia with both drugs, except that in rats anesthetized with methoxyflurane after 15 days of gestation the fluoride concentration returned to normal. The fluoride concentration in fetal bones did not differ from controls following enflurane anesthesia. Following methoxyflurane anesthesia, a significant increase in fluoride content in fetal bones was observed only when methoxyflurane was administered in the last trimester of pregnancy.

Table 1 contains the data on the uptake of fluoride into bone following neonatal metabolism of anesthetic drugs stored in fetal tissues. The fluoride concentrations in the bones of offspring exposed to methoxyflurane on the eighteenth and nineteenth days of gestation were 20 per cent higher among neonatal rats that survived for 10 hours after delivery than they were in animals sacrificed immediately after delivery. The fluoride concentrations in bones of offspring exposed to methoxyflurane on the fourteenth and fifteenth days of gestation were the same whether the animals were sacrificed immediately after delivery or 10 hours after delivery. The concentrations in offspring exposed to enflurane on the eighteenth and nineteenth days of gestation, as well as on the fourteenth and fifteenth days of gestation, were also the same whether the animals were sacrificed immediately after delivery or 10 hours after delivery.

Discussion

Increased fluoride concentrations in maternal and fetal blood have been reported to occur in obstetric patients who received

methoxyflurane analgesia during labor.[13–15] The fluoride concentration in umbilical venous blood was about 1 third of that found in maternal blood at delivery.

In this study we demonstrated a large uptake of fluoride in fetal bones when methoxyflurane anesthesia was administered to rats in the third trimester of pregnancy. The fetus is probably exposed to increased fluoride concentrations regardless of when methoxyflurane anesthesia is administered. But fluoride deposition in fetal bone depends upon the stage of skeletal development and bone mineralization.

The uptake of fluoride into ossification centers of cartilage following injection of radioactive fluoride and calcium has been studied in 15-day-old albino rats and in albino mice in advanced pregnancy.[19,21] The early stages of vertebral condensation and cartilage deposition in the humerus of the rat start on the fourteenth day of gestation, with intensive ossification of the skeleton starting on the seventeenth day of gestation.[22] Fluoride uptake by ionic exchange takes place mainly in cancellous bone. The preferential deposition in fetal bone of fluoride administered to rats in advanced pregnancy has been explained by the different ratios of cancellous bone (exchangeable compartment) to compact bone (non-exchangeable compartment) in mother and fetus.[17] In the adult rat—which grows during most of its life—the ratio between the two compartments is 1:1, but in the fetus the skeleton belongs completely to the "exchangeable compartment."[20] That after the seventeenth day of gestation, the uptake of fluoride released by methoxyflurane metabolism is greater in fetal bone than in maternal bone may also be explained by the different ratio of the two osseous compartments.

In this study no osseous abnormality in fetal development was observed. However, Smith[23] reported that methoxyflurane anesthetization of pregnant mice after the onset of ossification of fetal bone caused a significant increase in the incidence of skeletal defects. Since fluoride affects bone resorption,[24–26] it is possible that the teratogenicity of methoxyflurane observed in some species may be related to fluoride retention in fetal bone.

Clinical reports on the use of enflurane in obstetric anesthesia have been favorable.[27]

TABLE 1. Fluoride in the Bones of 20-day-old Fetuses and 10-hour-old Newborns

	Gestational Exposure (Days)	μg F/g Bone	
		Fetus	Newborn
Methoxyflurane	14–15	37	36
		40	37
	18–19	146	174
		198	251
Enflurane	15–16	4.7	5.6
		6.0	6.7
	20–21	12	15
		10	10

Studies in non-pregnant animals,[2] as well as in man,[4,5,7] indicate that while fluoride concentrations in blood and tissues associated with enflurane anesthesia are increased, the levels to which they rise are significantly less than those observed following methoxyflurane anesthesia.[2] Indeed, in the present study the fluoride content in the bones of fetal rats exposed to enflurane was the same as that in the control group (fig. 1).

Oxidative and reductive biotransformation processes are uniformly deficient in the fetuses of laboratory animals, and the microsomal enzyme systems involved in these processes do not develop until after birth.[28,29] Therefore, in pregnant rats exposed to methoxyflurane or enflurane, degradation of these anesthetics to fluoride takes place only in maternal tissues, and the released fluoride reaches the fetus through the placenta. The increase of the fluoride concentration in bone during the first 10 hours of life (table 1), may result from methoxyflurane stored in fetal tissues and metabolized when microsomal enzymes become functional.

Recent reports[28,29] indicate that in contrast to common laboratory animal fetuses, human fetal liver, adrenal, kidney and gastrointestinal tissue are capable of metabolizing xenobiotics at an early gestational age. Since cytochrome P-450-linked enzymes are developed in human fetal liver, it may be postulated that the human fetus metabolizes inhalation anesthetics. In the human fetus, therefore, two sources of fluoride may be involved following exposure of the mother to methoxyflurane or enflurane: 1) defluorination of anesthetic agent in maternal tissues followed by placen-

tal transfer of fluoride: 2) placental transfer of anesthetic agent followed by fetal metabolism. Exposure from the first source was evaluated by fluoride concentrations in umbilical vein blood,[13–15] but this evaluation did not provide information about the amount of fluoride released by fetal metabolism. Measurement of fluoride concentrations in neonatal blood and urine does not provide this information either, since a large fraction of fluoride is rapidly deposited in the fetal skeleton.

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