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Mercury from Maternal "Silver" Tooth Fillings in Sheep and Human Breast Milk

A Source of Neonatal Exposure

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ABSTRACT

Neonatal uptake of mercury (Hg) from milk was examined in a pregnant sheep model, where radioactive mercury (Hg^{203})/silver tooth fillings (amalgam) were newly placed. A crossover experimental design was used in which lactating ewes nursed foster lambs. In a parallel study, the relationship between dental history and breast milk concentration of Hg was also examined in 33 lactating women. Results from the animal studies showed that, during pregnancy, a primary fetal site of amalgam Hg concentration is the liver, and, after delivery, the neonatal lamb kidney receives additional amalgam Hg from mother's milk. In lactating women with aged amalgam fillings, increased Hg excretion in breast milk and urine correlated with the number of fillings or Hg vapor concentration levels in mouth air. It was concluded that Hg originating from maternal amalgam tooth fillings transfers across the placenta to the fetus, across the mammary gland into milk ingested by the newborn, and ultimately into neonatal body tissues. Comparisons are made to the U. S. minimal risk level recently established for adult Hg exposure. These findings suggest that placement and removal of "silver" tooth fillings in pregnant and lactating humans will subject the fetus and neonate to unnecessary risk of Hg exposure.

Index Entries: Mercury; dental amalgam; lactation; milk.

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Prior to amalgam mixing, 2.6 g of Hg^{203}, which had a specific activity of 19.2 mCi/g (Dupont–New England Nuclear, Boston, MA), was diluted ninefold with 20.8 g of nonradioactive elemental Hg to yield a total Hg mass of 23.4 g. This produced a new specific activity of 2.133 mCi/g. After the placement of 36 fillings (12 in each of three ewes), the remaining unused Hg was 4.5 g. Assuming a 25% loss of mercury during carving and finishing procedures, 14.175 g Hg were actually placed in the three ewes (4.725 g Hg/ewe). Thus, each ewe received a total of approx 10 mCi Hg^{203}. After amalgam placement and trimming of the tooth fillings, the oral cavity was flushed thoroughly with water and aspirated several times to remove amalgam particles. After surgical recovery, the animals were returned to individualized floor pens until normal vaginal deliveries occurred. Normal feeding consisted of fresh hay twice daily, supplemented with 2 kg alfalfa/oat pellet mix once daily, and water ad libitum. Food and water consumption resumed to normal levels within several hours following dental surgery.

The crossover design between lactating ewes and foster nursing lambs is illustrated in Table 1. To facilitate the crossfostering of the lambs born from amalgam-free ewes to the radioactive amalgam ewes—and, conversely, the lambs from radioactive amalgam ewes to the nonradioactive ewes—the ewes' placental afterbirths were saved and smeared on the foster lambs to aid acceptance by the appropriate surrogate lactating ewe. The lambs were then held on the nipple for 10–15 min every 2–3 h to feed. Most ewes accepted the foster lamb within the first 48 h postpartum, but two ewes required a handler in the room to help facilitate lamb feeding for several days.

Ewes and lambs were euthanized with sodium pentobarbital/saturated KCl at 16 d postpartum (approx 30 d after amalgam placement). From the lambs, specimens of kidney, liver, stomach and colon content were taken; from the ewes, kidney, liver, and milk samples were taken. All samples were weighed prior to analysis. All tissue and fluid specimens were analyzed for radioactivity, and total Hg concentrations were calculated as previously described (*2*), with corrections being made for isotopic decay ($t^1/_2$ = 47 d), isotope specific activity, and the dilution factor for nonradioactive Hg added prior to mixing the amalgam. The final calculation value represented the total Hg attributable solely from dental amalgam per g (wet wt) of tissue or fluid.

Human Experiments

Human breast milk samples (1–3 mL collected by a breast pump) and urine specimens were obtained at a dental office from 33 randomly selected lactating women (ages 25–41). In 27 of these patients, the amalgam tooth filling history revealed 2–14 (mean 7.7) occlusal amalgam surfaces and 4–30 (mean 13.8) total amalgam surfaces. The remaining six control patients had no amalgam fillings.

INTRODUCTION

Dental "silver" fillings, technically known as silver amalgam, contain approx 50% mercury (Hg) by weight in combination with other base metals (silver, tin, copper, and zinc), and the Hg is released from these fillings as vapor and microparticles, especially during and immediately following chewing. It is well established that amalgam Hg exposure constitutes the largest nonoccupational exposure source for the general population, and is greater than Hg exposure from all other environmental sources combined, including food. Estimates of amalgam Hg daily dose in humans have varied from 1.2 to 27 μg Hg/d, with a present consensus of approx 10–12 μg absorbed/d for the average person with eight occlusally involved fillings *(1)*.

Previously, we employed an experimental sheep model that received dental amalgam tooth fillings containing a radioactive Hg tracer. That study demonstrated the localization of amalgam Hg in various adult tissues by whole-body image scan and tissue scintillation counting *(2)*. Similar studies employing pregnant sheep indicated that Hg from amalgam fillings appeared in maternal and fetal blood and in amniotic fluid within 2 d after fillings were installed. Both maternal and fetal tissues began to accumulate Hg within several days following amalgam placement, and this accumulation was progressive with advancing gestation. The sheep placenta also accumulated increasing concentrations of amalgam Hg as gestation advanced to term, and milk obtained within 2 d following birth contained levels of Hg from dental amalgam that reached as high as 60 ng Hg/g milk *(3)*.

Results of more recent studies of amalgam Hg detected in human fetal and infant tissues *(4)* are unclear about the lactational transfer contribution of amalgam Hg to infant body burden. Therefore, the first objective of this study was to determine the contribution of newly placed maternal amalgam fillings in sheep upon neonatal uptake of Hg from milk. The second objective was to establish the relationship between dental history in lactating women and their milk concentrations of Hg over various durations of lactation.

METHODS

Animal Experiments

Six adult ewes (Dorset/Suffolk cross) of 3–5 yr of age, with an average body wt of 65 ± 8 kg, were bred by estrus cycle synchronization *(5)*; the day of mating was considered to be d 0 of gestation. At approx 134 d of gestation, three of the ewes were prepared for dental surgery and 12 radioactive occlusal amalgam fillings were placed in the molar teeth of each ewe (three molars in each quadrant of the mouth). Detailed procedures for general anesthesia dentistry in sheep have been described previously *(2)*.

Table 1
Birth and Nursing Record Pairings of Lambs from Three Radioactive Amalgam and Three Nonamalgam Pregnant Ewes

Ewe #	Delivery (days after conception)	Natural lambs (delivered)	Lambs nursed
014[a]	147	V08[a]	V08[a]
		V09[a]	V10
015[a]	148	V13[a]	V14
016[a]	148	V11[a]	V11[a]
		V12[a]	
017	147	V10	V09[a]
018	149	V14	V13[a]
019	Did not deliver; one cycle off		

[a]Denotes radioactivity (Hg^{203}) from maternal dental amalgams.

Intra-oral Hg vapor testing of mouth air was performed in triplicate both before chewing and again 10 min after continuous gum chewing stimulation for each patient by analytical methods previously described *(6)*. Total Hg concentrations in breast milk and urine specimens were determined by cold vapor atomic fluorescence spectrometry *(7)*.

RESULTS

Animal Experiments

Table 1 shows the crossover design for the lactating ewes and the natural or foster lambs. Five of the six ewes delivered within a 48-h period, at 147–149 d gestation; one did not, being one cycle off. Two ewes (#014 and 016) delivered twin lambs, V08–V09 and V11–V12, respectively. The remaining three ewes (#015, 017, and 018) each delivered one lamb. The lambs were numbered by birth order from V08 to V014. Ewe #014, which delivered two lambs, nursed two lambs (one her own and one a foster lamb). Ewe #016, which also delivered two lambs, only nursed one lamb (her own). The other twin (V12) was euthanized on the first day postpartum. The remaining three ewes (#015, 017, and 018) nursed single foster lambs.

Table 2 lists the concentration of total Hg in tissues and fluids from the ewes with radioactive amalgam fillings, 16–30 d after placement. Samples of milk (3–5) were taken from each of the radioactive amalgam bearing ewes at 2–3 d intervals during the first 15 d postpartum. The average amalgam Hg concentrations in milk for this period were 2.71 ng Hg/g, 1.94 ng Hg/g, and 4.33 ng Hg/g (for ewes #014, 015, and 016, respectively). The Hg concentration in kidney and liver attributable solely to dental amalgams for each ewe is also shown. The averages for

Table 2
The Concentration of Total Amalgam Mercury in Tissues and Fluids of Lactating Ewes 16–30 d After Placement of Radioactive Amalgam Tooth Fillings

	ng Amalgam Hg/g tissue (range)		
Ewe #	Kidney (d 30)	Liver (d 30)	Milk (d 16–30)
014	1957	529	2.71 (1.92–5.15)
015	2283	174	1.94 (1.73–2.76)
016	1798	336	4.33 (1.56–6.82)
Mean of 3 ewes	2013	346	2.99 (1.74–4.91)

Table 3
The Tissue Hg Levels in Neonatal Lambs at 16 d Postpartum That Were Exposed to Maternal Amalgam Hg either only *In Utero*,[a] Only from Milk,[b] or Both *In Utero* and from Milk[a,b]

	ng Amalgam Hg/g tissue			
Lamb #	Kidney	Liver	Stomach contents	Colon contents
Twins				
V08[a,b]	18.1	48.7	14.2	40.9
V09[a]	9.6	17.6	4.2	52.3
V11[a,b]	21.8	31.4	8.7	89.1
V12[a] (killed at birth)	7.9	91.0	—	—
Singlets				
V10[b]	7.1	1.4	16.8	459.9
V13[a]	10.5	14.3	14.7	77.8
V14[b]	5.2	2.3	8.2	168.0

[a]Born from radioactive ewe.
[b]Nursed on radioactive ewe.

the three ewes are 2013 ng Hg/g and 346 ng Hg/g for kidney and liver, respectively.

Table 3 displays the tissue Hg levels in neonatal lambs exposed to maternal amalgam Hg only *in utero*, or only from milk, or both from *in utero* exposure and from milk.

Lambs V08 and V09 were twins from a radioactive ewe. V08 remained suckling on its radioactive natural mother for 16 d postpartum, while V09 was crossfostered to a nonradioactive surrogate ewe. The amalgam Hg concentration in kidney of V08 was 18.1 ng Hg/g compared to 9.6 ng Hg/g for V09, while amalgam Hg concentrations in liver were 48.7 and 17.6 ng Hg/g, respectively. This is approximately a

twofold difference between lambs for both tissues. V08's stomach contents contained 14.2 ng Hg/g, compared to V09, which was 4.2 ng Hg/g, a threefold difference. However, the colon contents of V08 and V09 were more similar, being 40.9 and 52.3 ng Hg/g, respectively, which represented a 3–12-fold increase in concentration of amalgam Hg from stomach to colon.

Lambs V11 and V12 were also twins from a radioactive ewe. While V12 was euthanized on the first day postpartum, V11 remained suckling on the radioactive natural mother for an additional 16 d postpartum. The concentration of amalgam Hg in the kidney of V11 was 21.8 ng Hg/g, approx three times higher than the kidney of its twin, 7.9 ng Hg/g. By way of contrast, liver concentration of amalgam Hg in V11 was 31.4 ng Hg/g, only one-third the level of amalgam Hg present in the liver of its twin (91.0 ng Hg/g). The amalgam Hg levels in the stomach and colon contents of V11 were 8.7 ng Hg/g and 89.1 ng Hg/g, respectively, a 10-fold increase in Hg concentration.

Lamb V10, from a nonradioactive ewe, suckled on the same radioactive ewe that nursed lamb V08. V08, exposed to amalgam Hg *in utero* and via milk, had a kidney concentration of amalgam Hg, 18.1 ng Hg/g, that was 2.5-fold higher than the level of 7.1 ng Hg/g found in V10, which was exposed only to radioactive milk. However, the liver concentration of amalgam Hg in V08 was 48.7 ng Hg/g, whereas for V10 it was only 1.4 ng Hg/g (a 35-fold difference). While amalgam Hg levels in the stomach contents of V10 and V08 were comparable (16.8 ng Hg/g and 14.2 ng Hg/g, respectively), the amalgam Hg concentrations in the colon contents were not, being 459.9 ng Hg/g in V10 and 40.9 ng Hg/g in V08. This represented a 3–27-fold increase in concentration of amalgam Hg from stomach to colon.

The singlet lamb V13 was birthed from a radioactive ewe but suckled on a nonradioactive ewe, while singlet V14 was birthed from a nonradioactive ewe but suckled on a radioactive ewe. At 16 d postpartum, the amalgam Hg concentration in kidney of V13 was 10.5 ng Hg/g compared to 5.2 ng Hg/g in V14, a twofold difference. The amalgam Hg concentrations in liver of V13 and V14 were 14.3 ng Hg/g and 2.3 ng Hg/g, respectively, a sixfold difference. Stomach contents had amalgam Hg levels of 14.7 and 8.2 ng Hg/g in V13 and V14, respectively. However, colon contents had amalgam Hg concentrations of 77.8 ng Hg/g for V13 and 168.0 ng Hg/g for V14, a twofold difference for V14 (which only suckled on a radioactive ewe). There was a 5–20-fold increase in concentration of amalgam Hg from stomach to colon.

Human Experiments

Figure 1 shows the mean concentrations (±1 SEM) of Hg in breast milk and urine of lactating women. Those subjects with amalgam fillings had significantly ($p < 0.05$) higher Hg levels in breast milk (0.237 ± 0.034

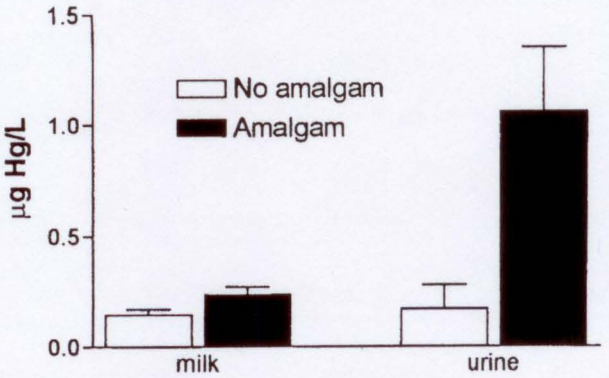

Fig. 1. Mean concentrations of mercury (±1 SEM) in breast milk and urine of lactating women with (n = 27) and without (n = 6) amalgam tooth fillings.

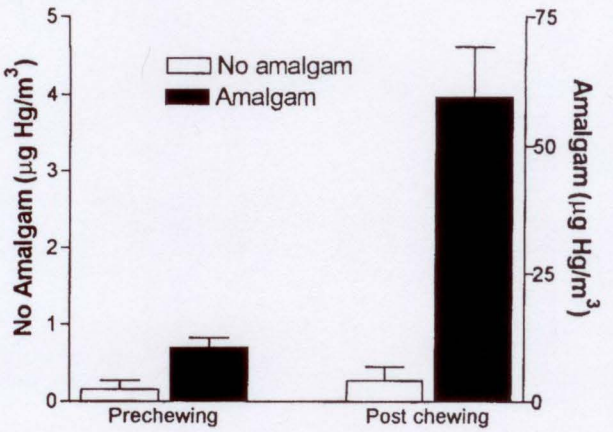

Fig. 2. Mean concentrations of mercury (±1 SEM) in intra-oral air, before and after chewing stimulation, of lactating women with (n = 27) and without (n = 6) amalgam tooth fillings.

μg Hg/L) than did those without amalgams (0.146 ± 0.025 μg Hg/L). Likewise, urine Hg levels in subjects with amalgams were significantly ($p < 0.01$) higher (1.062 ± 0.294 μg Hg/L) compared to subjects with no amalgams (0.170 ± 0.109 μg Hg/L).

Figure 2 shows the mean concentrations (±1 SEM) of Hg in intra-oral air before and after chewing stimulation in control lactating women without amalgam fillings vs lactating women with 2–14 occlusal amalgams. In control subjects, baseline Hg concentrations in mouth air prior to chewing were only 0.160 ± 0.111 μg Hg/m^3, and these concentrations remained similar after chewing at 0.276 ± 0.180 μg Hg/m^3 ($p > 0.50$). By way of contrast, in subjects with amalgams, the baseline Hg concentra-

tions in mouth air before chewing were 10.487 ± 1.959 μg Hg/m^3, which markedly increased to 59.474 ± 9.743 μg Hg/m^3 after chewing ($p < 0.001$). This latter increase was 215 times greater than post chewing levels of Hg in mouth air from lactating amalgam-free women.

A correlation matrix of variables (one-tailed comparison, critical $r = 0.32$, $p < 0.05$) within the experimental group of lactating women with amalgam fillings revealed several significant relationships: milk Hg vs urine Hg ($r = 0.49$); urine Hg vs postchewing intra-oral air Hg ($r = 0.34$); prechewing intra-oral air Hg vs postchewing intra-oral air Hg ($r = 0.75$); postchewing intra-oral air Hg vs number of total amalgam surfaces ($r = 0.50$) or number of occlusal amalgam surfaces ($r = 0.41$); and postchewing intra-oral air Hg vs number of total amalgam surfaces ($r = 0.71$) or number of occlusal amalgam surfaces ($r = 0.60$).

DISCUSSION

The combined findings of the present study clearly demonstrate that Hg, specifically originating from the mother's newly placed dental amalgam tooth fillings, will transfer across the placental barrier into the fetus and will also transfer across the mammary alveolar barrier into milk ingested by the newborn. Some of this Hg will concentrate in neonatal body tissues.

During the first 2 wk of lactation, milk from sheep contained approx 3 ng Hg/mL derived exclusively from newly placed amalgam fillings. Lactating ewes nursing a single lamb during the first 6 wk postpartum produce approx 1500 mL milk/d; when nursing twin lambs, milk production increases to 2500 mL/d *(8)*. Thus, nursing lambs in the present study would have received a total of 3.7–4.5 μg Hg daily from their mother's amalgam fillings.

The results from the animal experiments demonstrate that, during pregnancy, the fetal liver concentrates amalgam Hg at higher levels than does the kidney, a reflection of the hepatic portal circulation in the fetus (lambs V09, V12, and V13 in Table 3). After delivery, the newborn kidney receives additional amalgam Hg from mother's milk (lambs V08 and V11 in Table 3). In those lambs not exposed to amalgam Hg *in utero*, the newborn kidney shows an early ability to concentrate amalgam Hg received from breast milk (lambs V10 and V14 in Table 3). In all cases, there was a significant increase in Hg concentration in the intestinal tract of the newborn as ingested milk moved from the stomach to the colon, in part a reflection of water reabsorption.

Lactating women with aged amalgam fillings excrete significantly more Hg into breast milk and urine than do women without such fillings. This increased Hg excretion is a direct reflection of both the Hg vapor concentration in mouth air of amalgam bearing women and the

number of amalgam surfaces in their teeth. Markedly increased levels of Hg vapor have been reported in mouth air of nonlactating subjects with amalgam fillings *(1)*.

Infant intake of human breast milk averages approx 850 mL/d *(9,10)*. Human breast milk concentrations of Hg have typically ranged from 0.8 to 9.5 ng/mL in Europe *(9)*. A German study of 34 subjects reported that human colostrum Hg levels were 5.5 ng/mL, which declined somewhat to 2.0 ng/mL in mature milk (but no dental histories were obtained on these subjects) *(11)*. In the United States, a maximum allowable daily intake of Hg from human breast milk (based on a 5 kg infant ingesting 700 mL milk/d) is stated to be 2.0 μg *(12)*. This allowable limit *(12)* is exceeded by a factor of two- to fourfold in previous reports of Hg intake for infants *(9,11)*, based on a daily ingestion of 850 mL human milk *(9,10)*.

In the present study, when baseline Hg levels in breast milk of subjects without amalgam are subtracted from Hg levels in milk of amalgam bearers, approx 0.090 μg Hg/L is the concentration attributable exclusively to amalgams. With a daily milk intake of 850 mL, this would mean that maternal amalgam fillings in our patients would have contributed an average of 75 ng Hg/d, an amount well below the stated 2 μg daily limit *(12)*. While there was significantly increased transfer of amalgam Hg to nursing infants via breast milk, the average amount transferred did not approach the present allowable limits. However, two of the patients in our amalgam-bearing group had milk Hg concentrations well in excess of 600 ng/L, a level of Hg that would constitute more than 25% of the daily limit for nursing infants, and an amount that would be additive to the pre-existing amalgam Hg body burden received *in utero* by their infants.

The minimal risk level (MRL) in adults for chronic inhalation of Hg vapor is 0.28 μg Hg/d, a dose substantially lower than average daily Hg vapor exposure levels from dental amalgam. However, MRLs for chronic oral exposure to inorganic or organic Hg have yet to be established that are below the lowest-observed-adverse-effect levels *(13)*. In the present study, when a fetal body wt 1/20 that of an adult is factored in, daily Hg doses *in utero* would likely exceed the adult MRL because Hg vapor readily crosses the placenta. Although inorganic and organic Hg doses in breast milk ingested daily by an infant weighing 1/12 that of an adult cannot be directly compared to known vapor MRLs, chronic oral exposure to Hg in these two forms could still result in neurological effects at very low doses. It is likely that human fetuses and neonates from mothers with more than the average 8 occlusal amalgam surfaces would be at greater risk of Hg exposure. Also, the sheep data strongly suggest that newly placed amalgams would transfer more Hg across the placental and mammary alveolar membranes than would aged amalgams as observed in the human portion of this study.

We conclude that the placement or removal of Hg/silver tooth fillings in pregnant or lactating women produces an unnecessary risk to the fetus and neonate of toxic heavy metal exposure.

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