

# Lead in the Placenta, Membranes, and Umbilical Cord in Relation to Pregnancy Outcome in a Lead-Smelter Community

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As part of a cohort study of the effects of chronic exposure to lead on pregnancy outcome and child development, lead concentrations in the umbilical cord and placental tissues (body and membranes) from 9 late fetal deaths, 23 preterm births, and 18 births associated with premature rupture of the amniotic membranes were compared with the lead concentrations in the tissues obtained at 22 normal births. Modest elevations in lead concentration were found in the placental body of late fetal deaths (stillbirths) and preterm births as well as in the cord tissue associated with preterm births and premature rupture of membranes. The geometric mean lead concentration in the membranes from late fetal deaths was 2.73  $\mu\text{g/g}$  of dry tissue (95% confidence limits 0.69–10.8), which was 3.5 times higher than the mean found in normal births (0.78  $\mu\text{g/g}$ , 95% confidence limits 0.61–1.00). The concentration in the membranes of preterm births was also significantly high, being 1.24  $\mu\text{g/g}$  (0.91–1.67). Low correlations of membrane and antenatal blood lead concentrations suggest that other factors in addition to exposure to environmental lead may influence the amount of lead accumulated in the placental membranes.

## Introduction

Lead in large amounts has been shown to be fetotoxic in both animals and humans (1). The question of whether chronic antenatal exposure to lower levels of lead contributes to the incidence of adverse outcomes of pregnancy such as spontaneous abortion, premature rupture of membranes, preterm birth, and late fetal death in humans is still being debated (2,3). Even greater controversy surrounds the debate over whether such exposure, both antenatal and postnatal, has a deleterious effect on the subsequent neuropsychological development of the child (4).

One difficulty that stands in the way of resolving these issues is the need for a reliable biological indicator of long-term human exposure to lead. The ease with which it may be determined has made blood-lead concentration the most popular index of exposure, but since it may only reflect the burden received over the preceding 2 to 3 months (5), serial determinations are necessary for a reliable assessment of chronic exposure. Tooth-lead may

give an indication of longer term exposures, but proof of this is not yet available. Other tissues that accumulate lead (such as bone, kidney, and brain) are not generally accessible.

The placenta is a large accessible organ, across which lead can pass from mother to fetus from as early as 12 weeks gestation (6) and has been suggested as an alternative to repeated maternal blood sampling for assessing lead exposure *in utero* (7). Early work was not encouraging. Engelhart et al. (8) found only small differences in the lead content of placentas taken at births from industrial and rural areas of Germany and concluded that the placenta was not a useful organ for assessing lead burden. It is important to note, however, that their analyses were confined to tissue taken from the main body of the placenta.

Very few studies have looked at the lead content of umbilical cord and placental tissues associated with abnormal births. In addition to the study by Fahim and colleagues showing a relationship between elevated lead concentration in placental membranes and premature rupture of those membranes (2), Wibberley and co-workers (9,10) reported higher placental lead concentrations associated with late fetal deaths and malformations but pointed out that this association may be a consequence of lead accumulation by the placenta in times of fetal stress. Engelhardt et al. (8) were unable to demonstrate that the placental content of lead was any higher in preterm births than in term births.

The outcomes of 831 pregnancies studied in relation to maternal blood lead concentration in the lead-smelter town of Port Pirie, South Australia, and its surrounding rural areas have already been reported (11,12). More recently, evidence of adverse

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effects of cumulative lead exposure upon the early mental development of children within this cohort has been reported (13,14). As part of the first phase of this cohort study, placentas were routinely collected and frozen. This paper compares the lead concentrations in placental tissues taken from three groups of adverse outcomes of pregnancy (premature rupture of membranes, preterm birth, and late fetal deaths) with the concentrations measured in placentas collected from a random selection of normal births.

## Materials and Methods

### Source of Specimens

Of the 749 pregnancies followed beyond week 20 of gestation, 12 terminated in late fetal death (stillbirth), 25 deliveries were preterm, and 21 births were preceded by premature rupture of membranes. A birth was classified as preterm if the gestational age of the infant was assessed as 36 weeks or less using the Dubowitz procedure (15) and premature rupture if the amniotic membranes ruptured spontaneously 24 hr or more before the onset of labor. An attempt was made to obtain the placenta and cord from all pregnancies, but in some instances delivery occurred outside the study area, and the placenta was not collected. Placental material was obtained in 9 of the 12 instances of late fetal death but because of the macerated condition of some specimens, the number of analyzable cords and membranes was reduced to 6. A total of 23 placentas were collected from preterm births, 2 of these being from women normally resident outside Port Pirie; 18 of the placentas associated with premature rupture of membranes were obtained, 7 of these coming from outside Port Pirie. Of the 22 normal births randomly sampled from the remaining deliveries, 5 were in women from outside Port Pirie.

### Sample Collection and Preparation

Whole placentas together with umbilical cord were placed in a plastic bag at delivery with strict precautions against contamination. They were stored frozen at  $-20^{\circ}\text{C}$ . Prior to analysis, thawed specimens were drained and divided into the following three components: cord, detached at point of entry to the placenta; membrane, the frill was separated, but no attempt was made to recover that part of the membrane adherent to the body of the placenta; placenta body, all the remaining tissue. The protocol for blood collection has been described elsewhere (11).

### Lead Determinations

Each entire tissue component was individually cut into small pieces, minced and homogenized, and four aliquots were transferred to preweighed borosilicate tubes with screw caps. After a standard addition of  $0.25\ \mu\text{g}$  of aqueous lead nitrate to two tubes (for checking recovery), all samples were dried to constant weight so that results could be expressed as micrograms per gram dry weight of tissue. Samples were then subjected to a wet-digestion technique using high purity acids. To prevent frothing, 1.0 mL of concentrated nitric acid was added to each tissue sample and allowed to stand for 2 to 3 days prior to heating. A further 3 mL of nitric acid was required for each tissue sample, and with the aid of an anti-bumping granule, most of the nitric acid

fumes were driven off by increasing the temperature gradually to around  $200^{\circ}\text{C}$ , leaving a clean, pale yellow solution. After cooling, 0.5 mL perchloric acid was added to each tube, which was then reheated until dense white perchloric acid fumes appeared (about  $250^{\circ}\text{C}$ ). The tubes were then allowed to cool, and following the addition of 4.0 mL of deionized water, they were reheated to around  $100^{\circ}\text{C}$  and capped.

To overcome the problems brought about by the large quantity of iron present in the placenta (16), 0.2 mL of ascorbic acid (250 g/L) was added to each tube to reduce any ferric ions to the ferrous form. The iron was then complexed with ferrozine [in preference to potassium cyanide as used in other reported methods (16)] by the addition of 4.0 mL of a solution containing 12.5 g/L ferrozine dissolved in a triammonium citrate buffer (500 g/L), from which lead contamination had been removed by complexing with ammonium pyrrolidine dithiocarbamate and extraction with methyl-pentan-2-one. The pH following this addition was about 5. The sample lead was then complexed with 1.0 mL of 10 g/L ammonium pyrrolidine dithiocarbamate and extracted into 1 mL of 4-methyl-pentan-2-one (the purple iron complex remaining in the aqueous phase).

Following centrifugation of the organic phase,  $1\text{-}\mu\text{L}$  aliquots were read by electrothermal atomic absorption spectrometry (17) using a Varian Model 775 atomic absorption spectrophotometer with a CRA-90 carbon rod atomizer. Peak height measurements were made at a wavelength of 217.1 nm, and injections were repeated until three values were obtained within 10 absorbance units, the mean of the three values being used for subsequent calculations. With iron removed from the sample, no other interfering substances could be demonstrated. There were neither matrix effects in the furnace nor any detectable background signal, so that no correction was necessary. A set of duplicate calibration standards containing 0, 0.10, 0.25, 0.50, and  $0.75\ \mu\text{g}$  of lead was taken through the entire procedure described above with the exception that only 1 mL of nitric acid was used in the digestion. The amount of lead in each tube was calculated and expressed in units of microgram per gram dry weight of tissue.

Blood lead determinations (which do not require prior acid digestion and removal of iron) were carried out using the same steps of complex formation with ammonium pyrrolidine dithiocarbamate and extraction into 4-methyl-pentan-2-one, followed by atomic absorption spectrometry (11).

### Quality Control Considerations

All glassware was cleaned by soaking in nitric acid and rinsing several times in deionized water. Other equipment was cleaned with a nonionic detergent/EDTA mixture and rinsed. Each batch of disposable plastics used was checked for lead contamination prior to use. The method described above permits weighing, digesting, and extracting to be carried out in a single tube, thus reducing potential sources of error in transferring materials.

Lead determinations were performed on quadruplicate subsamples with a standard addition of lead nitrate to two tubes. The acceptance criteria for a single tissue sample were: *a*) each duplicate subsample had to be within 7.5% of the mean of the two measurements; and *b*) recovery of added lead to subsamples had to be within 85 to 115%.

Batch quality control was monitored by including, within each

**Table 1. Estimated geometric mean lead concentrations and 95% confidence intervals in maternal blood, cord, and placental tissues of different pregnancy outcomes.**

| Pregnancy outcome                       | n   | Antenatal maternal blood, $\mu\text{g}/\text{dL}$ |           | Cord tissue, $\mu\text{g}/\text{g}^a$ |            | Placental body, $\mu\text{g}/\text{g}^a$ |           | Placental membrane, $\mu\text{g}/\text{g}^a$ |            |
|---|-----|---|-----------|---------------------------------------|------------|--|-----------|--|------------|
|   |     | Mean  | 95% CI    | Mean                                  | 95% CI     | Mean                                     | 95% CI    | Mean   | 95% CI     |
| Normals                                 | 22  | 8.7   | 7.7, 9.9  | 1.42                                  | 0.87, 2.32 | 0.48                                     | 0.38,0.61 | 0.78   | 0.61, 1.00 |
| Premature rupture of membranes          | 18  | 8.5   | 7.4, 9.8  | 1.91                                  | 0.94, 3.88 | 0.41                                     | 0.32,0.53 | 0.72   | 0.55, 0.95 |
| Stillbirths                             | 6-9 | 8.2   | 6.0,11.1  | 1.18(6)                               | 0.24, 5.77 | 0.76(9)                                  | 0.44,1.30 | 2.73(6)                                      | 0.69,10.8  |
| Preterm births                          | 23  | 11.9  | 10.2,13.9 | 2.47                                  | 1.51, 4.05 | 0.66                                     | 0.49,0.90 | 1.24   | 0.91, 1.67 |
| High maternal blood lead at 14-20 weeks | 7   | 16.2  |           | 3.31                                  | 0.96,11.4  | 0.64                                     | 0.39,1.03 | 1.16   | 0.42, 3.25 |
| High cord blood lead                    | 7   | 14.0  |           | 1.26                                  | 0.70, 2.24 | 0.96                                     | 0.66,1.39 | 1.35   | 0.90, 2.03 |

<sup>a</sup>Dry tissue.

batch, two samples (approximately 0.5 g) from a dried stock material prepared by pooling the homogenates from a number of placentas. Over 10 batches, the estimated mean for this stock material was 0.251  $\mu\text{g}/\text{g}$  dry weight with a coefficient of variation of 7.5%. A batch was considered acceptable if the mean value of the two stock samples lay within two standard deviations of this mean, i.e., if it was between 0.21 and 0.29  $\mu\text{g}/\text{g}$ .

The Adelaide Children's Hospital laboratory, where all lead determinations were performed, takes part in three major external quality-control programs: the Wolfson Research Laboratories' National Quality Control Scheme, the Commonwealth of Pennsylvania's Department of Health's Lead Proficiency Testing Survey, and the program that is conducted by the CHG Committee of the Standards Association of Australia.

## Statistical Analysis

Since tissue lead concentrations are typically skewed, all reported means are geometric rather than arithmetic, unless otherwise stated. Differences between the four main comparison groups (normal, preterm, premature rupture, and late fetal death) were assessed by a one-way analysis of variance of the logarithm of the measured lead concentration.

## Results

Estimated geometric mean lead concentrations are reported in Table 1. Although estimates for cord lead are slightly higher among specimens taken at births that were either preterm or at which premature rupture of membranes was observed, these differences were not statistically significant ( $F_{3,68} = 1.13$ ,  $p = 0.34$ ). Placental lead was also elevated relative to controls for late fetal deaths and preterm births, and this finding was of marginal ( $p = 0.10$ ) significance. Greatest differences were observed in the lead concentrations of the placental membranes. Samples taken from stillbirths contained on average 3.5 times higher lead concentrations than those from normal births; samples from preterm births were 60% higher.

Average maternal blood lead concentrations, which are also presented in Table 1, are remarkably similar (between 8 and 9  $\mu\text{g}/\text{dL}$ ) for controls, premature rupture of membranes, and late fetal deaths, but significantly higher (11.9  $\mu\text{g}/\text{dL}$ ) for preterm births, as reported previously (11). (The values reported here are not identical with those reported previously due to the use in this paper of geometric rather than arithmetic means.)

Tissue lead content of 14 placentas chosen for analysis solely

on the basis of a high blood lead concentration either in mid-pregnancy or in the cord blood and which came from otherwise obstetrically normal births are shown in the last two lines of Table 1. These observations were included in the investigation of co-relationships between the various measures of lead exposure, but excluded from the comparisons of the various birth outcome groups because of the nonrandom way in which they were selected for analysis.

Estimates of Pearson correlation coefficients between tissue lead concentration and blood lead concentrations measured both antenatally and at delivery for all birth outcomes combined are shown in Table 2. Cord lead concentration was not significantly associated with any blood lead measurement, and the only statistically significant correlate of membrane lead was the lead concentration in cord blood. Placental lead concentration was positively associated with all the blood lead determinations, the correlation being weakest for maternal blood at delivery ( $r = 0.08$ ,  $p = 0.47$ ) and strongest for the average of all antenatal measurements ( $r = 0.32$ ,  $p = 0.003$ ).

## Discussion

Since Port Pirie has a large lead smelting plant and a history of past contamination with lead-bearing dusts (18), it is informative to compare the observed lead contents in the cord and placenta collected at normal births with values reported in other studies. Table 3 presents summary data for 16 previous studies. Extracted results have all been converted, where necessary, to units of micrograms per gram dry tissue weight. For results published per unit of wet tissue weight, a conversion factor of 6.0 was used (8). Since in most cases only arithmetic means were reported, data reported elsewhere in this paper are re-expressed as arithmetic means in the last line of Table 3 to facilitate comparison.

**Table 2. Estimated Pearson correlation coefficients ( $r$ ) of log lead concentrations of cord and placental tissues with those of antenatal and delivery blood samples.**

| Blood lead measure          | $r^a$ |      |           |
|-----------------------------|-------|------|-----------|
|                             | Cord  | Body | Membranes |
| Maternal, average antenatal | -0.02 | 0.32 | -0.04     |
| 14-20 weeks gestation       | 0.02  | 0.27 | 0.02      |
| Around 32 weeks gestation   | -0.06 | 0.38 | 0.01      |
| Delivery                    | 0.03  | 0.08 | -0.05     |
| Cord                        | 0.04  | 0.31 | 0.22      |

<sup>a</sup>A maximum of 86 pairs of observations may contribute to each estimate but actual numbers vary slightly due to missing values.

Table 3. Previously reported lead concentrations in placental and cord tissues.

| Reference             | Country   | Year | Wet (w) <sup>a</sup><br>or<br>dry (d) | Lead, $\mu\text{g/g}$ dried tissue |           |           |
|-----------------------|-----------|------|---------------------------------------|------------------------------------|-----------|-----------|
|                       |           |      |                                       | Cord                               | Body      | Membrane  |
| Horiuchi et al. (19)  | Japan     | 1959 | w                                     | —                                  | 3.42      | —         |
| Collucci et al. (20)  | U.S.      | 1973 | w?                                    | —                                  | 5.76      | —         |
| Einbrodt et al. (21)  | Germany   | 1973 | d                                     | —                                  | 3.39      | —         |
| Baglan et al. (7)     | U.S.      | 1974 | d                                     | —                                  | 1.83      | —         |
| Thiene et al. (22)    | Germany   | 1974 | d                                     | —                                  | 0.16–0.20 | —         |
| Fahim et al. (2)      | U.S.      | 1976 | w                                     | 0.60                               | 0.42–0.48 | 0.30–0.60 |
| Engelhardt et al. (8) | Germany   | 1976 | w,d                                   | —                                  | 1.94–2.30 | —         |
| Wibberly et al. (9)   | England   | 1977 | w                                     | —                                  | 5.58      | —         |
| Hubermont et al. (23) | Belgium   | 1978 | w                                     | —                                  | 0.67      | —         |
| Roels et al. (24)     | Belgium   | 1978 | d                                     | —                                  | 0.50      | —         |
| Jacyszyn et al. (25)  | Germany   | 1982 | d                                     | —                                  | 0.33      | —         |
| Takacs et al. (26)    | Hungary   | 1984 | d                                     | —                                  | 1.78      | —         |
| Takacs et al. (27)    | Hungary   | 1984 | d                                     | —                                  | 0.56–0.65 | —         |
| Tsuchiya et al. (28)  | Japan     | 1984 | w                                     | 0.34                               | 0.27      | —         |
| Korpela et al. (29)   | Finland   | 1986 | w                                     | —                                  | 0.14      | 0.52      |
| Ward et al. (30)      | England   | 1987 | d                                     | —                                  | 2.35      | —         |
| This report           | Australia | 1988 | d                                     | 2.38                               | 0.56      | 0.92      |

<sup>a</sup>When results were reported per unit of wet tissue weight, a conversion factor of 6.0 was applied.

There is a wide variation in reported concentrations of lead in the placenta. The subjects studied were not especially chosen as being at high-risk of exposure to lead, although they were frequently classified according to the degree of industrialization at the place of residence, and yet there is a 14-fold variation in the figures for the United States; a 21-fold difference for the values reported from Germany; and the values reported by Wibberley (9) are three times higher than those reported by the same group in occupationally exposed women 3 years later (10) (data not shown). It seems likely that most of this variation is due to methodological difficulties associated with the determination of such small concentrations of lead and does not reflect genuine differences in the study populations.

The methods of analysis used in the studies summarized in Table 3 are not always described in sufficient detail to enable a meaningful comparison, but certain points are still worth noting. Several authors who used a solvent extraction technique omit any mention of the problem of interference by iron—a mandatory consideration with this method. In the method used in this study, no furnace matrix effects were observed, no background signal was detected, and no correction to the final reading was necessary. Injection of a clean extract rather than solubilized tissue permitted calibration with aqueous standards, thereby avoiding the difficulties of interpretation associated with calibration by addition of analyte to samples. In almost all published reports, only a small sample of each tissue was analyzed, under the tacit assumption that the lead was uniformly distributed throughout the entire specimen. However, the data from this present study suggest that calculations based on results from a small sample of placental body containing membrane tissue might overestimate the lead concentration of the entire placental body. The common practice of expressing lead concentrations per unit of wet weight is also vulnerable to changes in moisture content, particularly if specimens are frozen and thawed prior to analysis.

In the final comparison of results, the placental lead concentrations found in this study are closely comparable to those of Takacs et al. (27), and only four studies report lower values, so it would seem reasonable to suggest that the levels found in the

placenta from normal births at Port Pirie are not excessive. This result is consistent with previous findings that the nature of the lead contamination problem in Port Pirie is such that only the children, and not their mothers, have high blood lead concentrations relative to other urban populations (31,32). There are very few published results for cord and membrane, but from Table 3 it is evident that the values recorded in this study are higher than all previously reported data.

Table 2 exhibits, with the exception of maternal blood at delivery, a fairly consistent association of all blood lead concentrations with placental body lead concentration and reflects, presumably, the fact that the placenta is permeable to lead. The estimated correlation coefficient for log placental lead and cord blood lead of 0.31 agrees very closely with the value of 0.28 reported by Roels et al. (24), but their estimate of 0.22 ( $n = 474$ ) for the correlation between maternal blood lead and placental lead is much higher than the 0.08 observed in this study.

The apparent lack of a correlation of membrane and cord lead with any maternal blood lead measurement may indicate that these tissues reflect aspects of maternal lead burden that are quite different from those indicated by maternal blood lead concentrations either antenatally or at delivery. This observation becomes of further interest in view of the concurrent finding that the membrane lead concentrations were quite significantly elevated in placentas from late fetal deaths and preterm births.

Studies in which the lead content of the cord and the amniotic membranes were separately analyzed have revealed differences in the abilities of the various tissues to accumulate lead (2,28,29). Fahim et al. (2) in a study of 249 births in Missouri, reported lead concentrations in the placental membranes that were 4 to 6 times higher than the values for placenta and cord, but, most interestingly, the latter findings were restricted to births that were preterm or in which there was early membrane rupture (defined as spontaneous rupture more than 12 hr before the onset of labor). The lead content of membranes from normal-term births were, if anything, lower than those of placenta and cord. But Korpela et al. (29) found the lead content of amniotic membranes was also four times higher than that of the placenta body in a small study of six normal births.

If it is accepted that the blood lead measurements are indeed a reliable index of maternal exposure, then the absence of correlations between membrane lead concentration and maternal blood lead concentration would suggest that the accumulation in membranes is determined by factors additional to the woman's exposure to environmental lead. These findings are therefore compatible with the cautious interpretation advanced by Khera et al. (10) that lead is more readily absorbed by the placenta in times of fetal stress and that the elevated levels found in lead-accumulating tissues are a consequence rather than a cause of the adverse outcomes. An assumption underlying this argument is that late fetal deaths and preterm births are preceded by a prolonged period of fetal stress. This may be a reasonable assumption for most stillbirths, but is less likely to be valid for many preterm births.

An alternative explanation as to why, first, the correlations between lead concentrations in placental tissues and maternal blood are generally low ( $< 0.4$ ) and, second, the placental tissue lead concentrations in stillbirths and preterm births are relatively high, is that there is considerable interindividual variation in the efficiency of transfer of lead from maternal blood to placental tissue. It is possible that in women in whom this transfer is efficient, risk of adverse pregnancy outcome is raised. By analogy, there is some evidence of significant interindividual variation in maternal-to-fetal transfer of zinc, such that, paradoxically, an inverse relationship between maternal and fetal blood zinc concentrations occurs (33).

As a corollary to both these arguments, however, it might be supposed that other heavy metals would be accumulated simultaneously, but recent work by Ward et al. (30), in which 37 elements were determined for each placental sample, found that only lead and cadmium were negatively associated with gestational age at birth. The mechanisms by which lead and cadmium are accumulated in placental tissues [and possibly also in bone (34)] may therefore be reasonably specific.

The authors are also extremely grateful to a referee who drew attention to a review by Simons (35) of effects of calcium that are either mimicked or antagonized by lead and to the fact that fetal stress is often accompanied by hypoxia, often manifested at the cellular level by loss of cellular calcium homeostasis. In this connection, it is noteworthy that a relationship between dietary calcium and blood lead concentrations in Port Pirie women has been reported previously (31), while Marche et al. (36) have found a calcium binding protein in placenta that is identical to that present in the adult intestine. It may therefore be desirable, in future work, to determine both lead and calcium in the same tissue samples to see if the lead accumulation is secondary to a calcification process.

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