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Neurobehavioral Deficits After Low Level Lead Exposure in Neonates: The Mexico City Pilot Study

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Lead Umbilical cord Maternal Newborns Neurobehavioral development Stress

THE site of the study reported here, Mexico City and its environs, is a densely populated, heavily industrialized urban center. Over 20,000,000 people live in 10,000 square kilometers along with nearly 50% of the industrial and manufacturing capacity of the country. Nearly 4,000,000 motor vehicles contribute strongly to environmental contamination. The city is in a high mountain valley with a floor of over 2200 m and is almost completely surrounded by mountains as high as 5400 m. Frequent temperature inversions, especially during the 7 to 8 month dry season, compound the contamination problem. Data collected from a sample of Mexico City school teachers at the turn of the present decade revealed mean blood lead levels over 22 μg/dl (4), and a sample of pregnant women at delivery had blood lead levels of 20 μg/dl (6). Umbilical cord lead levels of the babies born to women of the latter study were 13 μg/dl. Thus, the Mexico City metropolitan area is a good site to study the effects of a wide range of lead exposures in a nonoccupationally exposed population.

Several recent studies have demonstrated the damaging effects of low-level in-utero lead exposure on subsequent infant development. Umbilical cord blood lead is associated with abnormal reflexes and soft neurological signs in 48 hour infants (3). Cord lead as low as 10 μg/dl is associated with slowed mental development in the first two years of life (1).

Since maternal lead readily passes to the fetus through the placenta [see (7) for recent review], information about maternal lead levels before birth may allow us to estimate fetal exposures prior to umbilical cord measurements. Although many factors may operate to alter maternal-fetal correlations of lead, measures of maternal lead at various stages of pregnancy provide us with the most easily obtained means to assess the effects of the history of fetal lead exposure upon later development.

The results reported here are from a pilot study of a larger prospective study currently underway. In addition to providing training for staff and resolution of operational problems for the prospective study, the pilot study allows us to exam-

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ine the effects of prebirth maternal lead levels upon outcome, to reveal factors responsible not only for absolute lead levels of mother and child but for changes in maternal lead levels during the last stages of pregnancy and differences in lead between the mother and the child, and to extend the period of detailed measurement of neurobehavioral outcome of the child from 2 to 30 days after birth. Our results show that change in lead of the mother from 36 weeks to birth is related to gestation age, the ability of the baby to regulate behavioral state at 15 and 30 days after birth and the ability of the baby to regulate autonomic state at 30 days after birth. As the mother's lead at the child's birth (hereafter referred to as mother's lead at birth, for brevity) tends to increase over mother's lead at 36 weeks, gestation age and behavioral regulation tend to decrease, while autonomic regulation tends to increase. These findings appear independent of average lead levels of mother and child. We discuss how these findings, if replicated, may alter our view of the relationship between perinatal lead levels and later development.

TABLE 1

CHARACTERISTICS OF SAMPLE (n=50)

	Mean	SD	Range
Age of mother	25.1 years	5.7	15–38
Weight of child	3.14 kg	0.41	2.00–4.00
Gestation age	39.5 weeks	1.2	36–42
Blood lead of mothers at 36 weeks (M36)	15.0 μg/dl	6.4	5.5–42.0
Blood lead of mothers at birth (MB)	15.5 μg/dl	5.7	6.0–33.5
Blood lead of babies at birth (UC)	13.1 μg/dl	6.0	3.0–33.5
Pearson r (M36 × MB)	0.70		
Pearson r (M36 × UC)	0.78		
Pearson r (MB × UC)	0.83		

METHOD

Pregnant women visiting the outpatient clinic at the National Institute of Perinatology and the General Hospital in Mexico City on or before their 36th week of pregnancy, as determined from the date of last menses, were contacted for inclusion in the pilot study. Those who met one or more of the following exclusion criteria were not included:

1. Consumption of one or more alcoholic drinks per day.
2. Drug addiction or habitual use of prescribed, over-the-counter or folk medicines.
3. Under 15, over 40 years of age.
4. Kidney disease.
5. Psychosis.

Babies born to mothers accepted into the study were excluded for the following reasons:

1. Birth weight under 2000 g.
2. Apgar at 5 minutes 6 or under.
3. Gestation age under 36 weeks.
4. Serious birth defects.

The project director at each site interviewed each patient to explain the nature of the experiment and to obtain informed consent. A project social worker gathered SES data, nutritional habits, drinking, smoking and drug habits, history of past and present pregnancies, etc. Additional information was obtained from hospital records.

Data from 42 to 50 mother-baby pairs, depending on the outcome measure used, are presented here.

Blood Samples

A sample of blood up to 7 ml was obtained by venapuncture during the 36th week of pregnancy. Samples were stored in a refrigerator at 4 degrees C in Becton-Dickonson purple top Vacutainers with EDTA until analysis. Two sterile prepackaged alcohol wipes were used, each with single wipes in the same direction, to clean the skin immediately before venapuncture.

At the moment of birth an umbilical cord blood sample was drawn. A maternal sample was drawn within 30 minutes of delivery. These samples were stored as those above.

Birth Record

A project physician attending the birth noted birth conditions, complications, physical measurements, 1 and 5 minute Apgar and determined gestational age.

Brazelton Neonatal Behavioral Assessment Scale (NBAS)

At 48 hours after birth psychologists certified in the use of the NBAS by the Child Development Unit of Children's Hospital, Boston, MA, administered the NBAS to the infant in the hospital. The test was administered again in the subject's home at 15 and 30 days after birth.

The seven NBAS cluster scales were calculated from each NBAS protocol sheet. Examiners were not aware of the lead status of the babies they tested.

Blood Lead Analysis

Refrigerated Vacutainers were packed in foam and delivered by air courier for analysis by ESA Laboratories, Inc., Bedford, MA within 48 hours of packing. All analyses were done in duplicate by anodic stripping voltammetry using an ESA 3010A Trace Metals Analyzer. Mean values of the duplicate analysis were used as data. For each duplicate analysis the 95% confidence range for the difference in each analysis of each sample was 1.2, 1.6 and 1.8 μg/dl for maternal blood samples at 36 weeks, at birth of child and the umbilical cord, respectively. Empty Vacutainers from the same manufacturing lots were also sent as blanks. Blood lead values reported below were not corrected for hematocrit. ESA Laboratories has a rigorous internal quality control program and participates in several quality assurance programs, among them the American Association for Clinical Chemistry/College of American Pathologists Blood Lead Surveys as a participant, and the Blood Lead Proficiency Testing Program of the US Public Health Service, Centers for Disease Control as a reference laboratory.

Statistical Analyses

The data were analyzed in several stages. Descriptive statistics and bivariate correlations were calculated for independent, dependent and control variables. In the first set of analyses lead measurements were used as dependent varia-

TABLE 2

VARIABLES PREDICTING LEAD

	LogM36	
Smoking (y/n)† Frequency Eat Bread† Frequency Eat Beans†	Home Remedies last 3 months (y/n)† Years in City† Drink Alcohol last months (y/n)†	No. Cigarettes† Age† Single Mother (y/n)*
	LogMB	
Bleeding (y/n)†	Signs of Fetal Suffering (y/n)†	
	LogUC	
Years in City†	No. Cigarettes*	
	M36-MB	
Smoking (y/n)† Single Mother (y/n)† Income* Age*	Bleeding (y/n)† Home Remedies last 3 months (y/n)* No. Cigarettes† Drink Alcohol last months (y/n)*	Problems in Pregnancy (y/n)* Delivery (with/without spinal block)† Gravidity†
	M36-UC	
Nervous Disorder (y/n)*	Frequency Eating Tortillas*	
	MB-UC	
Income† Meds during Delivery (y/n)† Frequency Eating Vegetables* Frequency Eating Tortillas* Age*	Bleeding (y/n)† Delivery (with/without spinal block)† Used Opiates (y/n)* Smoking (y/n)*	Single Mother (y/n)† Unmarried Mother (y/n)*

*$p<0.10$.
†$p<0.05$.
Log lead values are natural logs.
Composite lead variables are differences in lead between the two variables.

bles to determine factors responsible for lead levels in the subjects. Significant bivariate correlations between control variables and lead levels were used in forward multiple regression analyses to determine the best joint predictors of lead in the subjects. In the second set of analyses lead measurements were used as independent variables to determine the contribution of lead levels to the outcome of the babies. Significant bivariate correlations between control variables and outcome as measured by the NBAS scales were used in forward multiple regression analyses to determine the best joint predictors of the NBAS scales over the first 30 days of life. After the best multiple regression model was constructed from the nonlead variables, each lead variable was separately forced into the model to determine the relationship between the lead variable and the adjusted NBAS scale. The overall plan of analysis follows Bellinger *et al.* (op. cit.). Analyses were performed with SAS and with Statgraphics. All probability values reported are 2-tailed.

While not the most conservative analytic scheme, this plan seemed the best compromise for handling the small number of subjects available for analysis and the large number of potential control and confounding variables. In all analyses reported below, where lead significantly predicted outcome of the child, we repeated the analyses with a stepwise multiple regression model. In these analyses, control variables were entered if they were significantly associated with outcome on entry at, $F=2.9$ (approximately $p=0.10$) or better, as above, but were removed if subsequent entries of variables reduced their significance in the model. This procedure allowed the entry of different control variables, not originally associated with outcome through their partial correlations. In each case, the lead variable in the stepwise model was more significant, and accounted for more variance in outcome, than in the forward regression models presented here.

RESULTS

Table 1 summarizes the characteristics of the subjects used in the reported analysis. The mothers are mostly in their 20's, have lived for most of their lives in the Valley of Mexico, have small families, and are in the lower half of the socioeconomic scale. Mean lead levels of the subjects reported here did not differ statistically from the lead levels of subjects lost to the study for various reasons.

TABLE 3

SIGNIFICANT JOINT PREDICTORS OF LEAD FORWARD MULTIPLE REGRESSION

Variable	Coefficients	Partial r^2	Model r^2	*t*	Probability
	LogM36 (n=50)				
Years in City	+.010	.110	.110	1.98	0.054
Smoking (y/n)	−.405	.058	.168	−2.91	0.006
Beans	+.115	.075	.243	2.34	0.024
Bread	−.110	.059	.302	−2.21	0.032
	LogMB (n=50)				
Fetal Suf. (y/n)	−.187	.096	.096	−2.44	0.018
Bleeding (y/n)	−.285	.072	.158	−2.27	0.028
	LogUC (n=50)				
Years in City	+.014	.065	.065	2.10	0.041
	M36-MB (n=50)				
Smoking (y/n)	−2.544	.125	.125	−1.77	0.085
Single Mom (y/n)	+3.171	.084	.209	2.22	0.032
Problems in Pregnancy (y/n)	+2.087	.066	.275	2.10	0.042
Alc. Prv. Mnt. (y/n)	−4.589	.064	.339	−3.07	0.004
Use block (y/n)	−4.272	.050	.389	−3.60	<0.001
Grava	+.986	.094	.483	2.96	0.005
Income	+.683	.023	.506	1.71	0.094
	M36-UC (n=50)				
Nervous Disorder (y/n)	−4.470	.090	.090	−2.41	0.020
	MB-UC (n=48)				
Del. Meds. (y/n)	−2.041	.107	.107	−2.70	0.010
Income	−1.273	.119	.226	−4.27	<0.001
Vegetables	+1.617	.144	.370	4.03	<0.001
Use Block (y/n)	+2.339	.086	.456	2.82	0.007

See Table 2 for description of variables. All variables shown in order of entry (F>2.9 to enter). All statistics shown are for final model.

Table 2 shows the control variables that had significant bivariate correlations (Pearson r or Spearman r for interval and ordinal measures respectively) with lead measurements, or significant F tests (for nominal variables) with lead measurements. All variables significantly associated with each lead variable at $p<0.10$ were entered into a forward multiple regression against the lead variable to determine the best joint predictors of lead level.

Table 3 shows the results of those analyses. Although we will discuss these results more fully later, please note that the variables significantly predicting maternal log lead at birth, lead change in mother and lead difference between mother and baby include signs of fetal suffering, vaginal bleeding and other reported problems during pregnancy, use of medications and spinal block during delivery. Other factors contributing to the various lead levels include time of residence in the Valley of Mexico, smoking, drinking and dietary habits.

A similar analytical scheme was used to determine the best joint predictors of infant outcome. Table 4 reports all significant bivariate correlations of lead measures with

TABLE 4

SIGNIFICANT BIVARIATE CORRELATIONS OF NBAS SCALES AND LEAD

Lead Variable	NBAS Scale	r
M36	—	—
MB	—	—
UC	—	—
M36-MB	Regulation of State 15 days	+.398†
	Regulation of State 30 days	+.411†
	Autonomic Regulation 30 days	−.389*
M36-UC	Autonomic Regulation 30 days	−.332*
MB-UC	Regulation of State 30 days	−.404†

*$p<0.05$.
†$p<0.01$.

TABLE 5

EFFECT OF LEAD ON NBAS SCALES IN MULTIPLE REGRESSION MODEL

NBAS Scale*	Lead	N	*t*	Partial r^2	Probability	Coefficient
Regulation of State 15 days	M36-MB	42	2.04	.068	0.049	+.101
Regulation of State	M36-MB	42	1.98	.061	0.055	+.091
30 days	MB-UC	42	−2.10	.071	0.042	−.136
Autonomic Regulation	M36-MB	42	−1.85	.048	0.073	−.022
30 days	M36-UC	42	−1.84	.047	0.074	−.024

*NBAS habituation scale not tested because of low number of subjects with complete data.

TABLE 6

EFFECT OF PRIOR ENTRY OF AVERAGE LEAD VARIABLES UPON COMPOSITE LEAD VARIABLES IN MULTIPLE REGRESSION MODEL

Outcome	Lead	N	*t*	Probability	Coefficient
Regulation of State 15 days	M36-MB	42	2.09	0.045	+.110
Regulation of State	M36-MB	42	2.04	0.048	+.097
30 days	MB-UC	42	−2.20	0.034	−.145
Autonomic Regulation	M36-MB	42	−2.33	0.026	−.027
30 days	M36-UC	42	−1.98	0.056	−.026
Gestation Age	M36-MB	50	2.17	0.035	+.072

NBAS scales with a probability less than or equal to 0.05. Change in lead of the mother between 36 weeks and birth is significantly associated with NBAS regulation of state scale at 15 and 30 days after birth, and with autonomic regulation scale at 30 days after birth. Note that higher maternal birth lead than 36 week lead predicts improvement in autonomic regulation and poorer outcome in regulation of states. The different signs of the two outcome measures associated with this composite lead measure will be discussed below. Lead difference between mother and baby at birth is significantly associated with regulation of state scale at 30 days, and lead difference between mother at 36 weeks and the baby is significantly associated with autonomic regulation at 30 days.

All control variables significantly associated with outcome on each NBAS scale were entered into a forward multiple regression against outcome. All variables with F greater than or equal to 2.9 (significance level approximately equal to 0.10, two-tailed) at the moment of entry were retained in the regression equation regardless of their significance in the model when considered jointly. Each lead measurement was then separately entered last into the model to determine if the addition of the lead measurement significantly improved the ability of the model to predict outcome. Table 5 lists all NBAS scales significantly associated with lead in the presence of the control variables.

All lead variables significantly associated with NBAS outcome in bivariate tests remained significant in the multiple regression models, though with reduced levels of significance. The change in lead in the mother between 36 weeks and birth predicted regulation of state at 15 days ($t=2.04$, $p=0.049$), regulation of state at 30 days ($t=1.98$, $p=0.055$) and autonomic regulation at 30 days ($t=1.85$, $p=0.073$). Lead difference between mother and baby predicted regulation of state at 30 days ($t=2.10$, $p=0.042$) and lead difference between the mother at 36 weeks and the baby predicted autonomic regulation at 30 days ($t=1.84$, $p=0.074$).

The associations between the composite lead variables and NBAS outcome variables remained significant when the average of the two lead values were forced into the multiple regression ahead of the composite lead measures. Table 6 shows that significance levels of all the associations improved slightly in the presence of the average lead levels over significance levels obtained without average levels, even though entry of average lead levels by themselves did not improve the models' predictive ability. (Due to small sample size, a small improvement in the prediction ability of the model could go undetected when the association of outcome with average maternal or maternal-child lead is tested. However, a very large number of births (558) would be required to detect with 90% power that a 2% increase in r^2 exists in the population. A 2% increase in r^2 has been considered a small effect in the behavioral sciences. Possibly a larger percent increase would be considered small in Mexico City relative to typical U.S. populations because of the larger variability of lead in persons in Mexico City and the subsequent reduced population size required to find a significant association of lead with outcome.)

Several physical measurements were taken on the babies

TABLE 7

SIGNIFICANT BIVARIATE CORRELATIONS BETWEEN PHYSICAL OUTCOME MEASURES AND LEAD

Lead Variable	Outcome Measure	r
M36	—	—
MB	—	—
UC	—	—
M36-MB	Gestation Age	+.285*
M36-UC	Weight	+.286*
MB-UC	—	—

*$p<0.05$.

at birth and were submitted to the same analyses. Tables 7 and 8 show significant bivariate correlations of the lead measures with physical outcome measures and the results of the multivariate analyses with lead measures entered last in a forward multiple regression with all significant control variables. Change in lead of the mother was significantly associated with gestation age and difference between lead in the mother at 36 weeks and the baby was significantly associated with birth weight in the bivariate correlations. In the presence of control variables, maternal lead change still significantly predicted gestation age ($t=2.20, p=0.033$) in the multiple regression. However, lead difference between mother and baby no longer significantly predicted weight when the confounding variable of gestation age was jointly considered with the lead measure ($t=1.60, p=0.116$).

Forced entry of average maternal lead level ahead of maternal lead change did not materially affect the association between maternal lead change and gestation age ($t=2.17$, $p=0.035$) in the multiple regression model.

DISCUSSION

Limitations of Study

The principal shortcoming of this study is the small sample size, ranging from 42 to 50 mother-baby pairs, depending on the outcome measure used. Small sample size reduced the power of statistical tests to detect real lead effects, for any given significance level of the test.

Although we ran scores of statistical tests, with 6 NBAS scales, 7 physical outcome measures and 6 lead measures, we have taken no account of study-wise error. We tested 144 bivariate associations between the lead measures and the outcome measures to select the seven significant associations to use in the multivariate analyses. The bivariate associations were significant at probabilities between 0.007 and 0.045. We might expect about 3 of these significant relationships to occur by chance.

Small sample size in multivariate studies also increases the risk that outliers in the data will unduly affect the results. Two subjects not excluded by the exclusion criteria mentioned above were eliminated from the analysis because their mother-baby lead differences were extreme outliers. Their results, along with two other excluded subjects, will be discussed more fully below.

Using whole blood lead determinations as the measure of lead exposure has some uncertainties. Blood lead measurements reflect recent lead exposures more accurately than earlier exposures, although the average 4 week interval between the two maternal measurements minimizes this problem in the present study. More important, however, is the lack of clear evidence relating blood lead levels to structural, physiological and biochemical changes in the organs of humans, especially in brains. Thus, blood lead measurements at best are imperfectly correlated with alterations to target organs responsible for changes in behavior noted in most lead studies. This problem is increased by the usual method of obtaining umbilical cord lead used in most studies, including this one, which collects placental, not fetal blood. If low-level lead exposure is causally associated with behavioral abnormalities in children, the rather low percentage of variance in behavior accounted for by blood lead level in many studies (between 2 and 8%) may, in part, be a function of the nature of our assessment of lead.

In this study, NBAS measurements 48 hours after birth were taken in a hospital examining room, under more or less constant conditions. However, the 15 and 30 day measurements were taken in the homes of the subjects, under widely varying conditions. In addition, no control measures of quality of home environment or nutritional state were made at these later times. In an unplanned follow-up at 6 months after birth maternal WAIS IQ and HOME scale measurements were made on 25 subjects. There were no significant bivariate correlations between either of these two scales and any of the NBAS outcome measures significantly associated with lead for these subjects. Nevertheless, all of these factors could have influenced the measured pattern of development of the child.

The lead measures of greatest interest in this report are the composite measures, lead change in the mother and lead difference between mother and child. While lead change in the mother can be rationally described by using the measurement at an earlier time as a reference against which direction and amount of change at later times are compared, there is no similar rational for describing lead difference between mother and child. Thus, the statements, as lead level of the child approaches and exceeds the lead level of the mother, and, as lead level of the mother approaches and falls below the lead level of the child, are equivalent for the pres-

TABLE 8

EFFECT OF LEAD ON PHYSICAL OUTCOME IN MULTIPLE REGRESSION MODEL

Physical Outcome	Lead	N	*t*	Partial r^2	Probability	Coefficient
Gestation Age	M36-MB	50	2.20	.061	0.033	+.071
Weight	M36-UC	50	1.60	.024	0.116	+19.71

TABLE 9

EFFECT OF PRIOR ENTRY OF LEAD VARIABLES ON RELATIONSHIP OF LEAD TO OUTCOME IN MULTIPLE REGRESSION MODEL

Outcome	Lead	N	*t*	Probability	Coefficient
Regulation of State 15 days*	MB	42	−1.71	0.098	−.105
Regulation of State 30 days†	MB	42	−2.39	0.022	−.174
Autonomic Regulation 30 days‡	M36	42	−1.64	0.110	−.022
Gestation Age*	MB	50	−1.95	0.057	−.074

*M36 entered first.
†M36 and UC entered first.
‡MB and UC entered first.

ent data and we have no a priori rule for deciding which lead variable moves relative to the other in describing the effect of the composite lead variable upon outcome. For convenience we have adopted the convention of fixing the lead of the mother as the reference and the lead of the child as the variable for determining direction of the mother-child difference. Serial blood lead measurements in the mother in the weeks immediately preceding delivery may show if the maternal lead level remains stable or shows sharp changes at birth and help validate our use of the mother's lead as the reference value.

Comparisons of Results with Other Studies

In design, this study was most like the Cleveland study of Ernhart *et al.* [see, (3)]. Whereas the Cleveland study used a single NBAS application, we extended our child assessments over the first 30 days. The Cleveland study, reporting on all babies for whom both an umbilical cord lead level and a NBAS examination was obtained, showed a significant effect of umbilical cord lead on reflexes at 48 hours. When the sample was restricted to those babies for whom maternal lead levels at birth were also obtained, there was no significant effect of umbilical cord lead on abnormal reflexes, though a related measure, the Graham-Rosenblith neurological soft-signs scale, remained significant. Noting the high correlation usually present between maternal and umbilical cord lead at birth ($r=.70$), and the failure of maternal lead at birth to predict outcome, the authors of the Cleveland study examined the role of the part of umbilical cord blood lead uncorrelated with maternal lead in predicting outcome. In the presence of maternal lead, as a covariate, the significance levels and proportion of variance in outcome accounted for by cord lead improved. The researchers, noting an earlier suggestion of accumulation of lead in fetal, but not maternal, tissues after fetal distress (8), raised the possibility that stress-related neurological soft-signs may have to be accounted for in trying to associate umbilical cord lead and outcome.

With our sample size less than $^1/_3$ of that of the Cleveland group, we failed to find a significant association of lead and reflexes. However, gestation age, regulation of state at 15 and 30 days of age and autonomic regulation at 30 days are all associated with lead in this study. Regulation of state measures factors such as cuddliness, consolability and self-quieting, while autonomic regulation measures factors such as tremors, startles and skin color.

The curious finding that the same change in lead of the mother predicts different directions of outcome using regulation of states and autonomic regulation scales deserves some comment. A Pearson correlation between the two scales yielded a value of −.031, whereas a partial correlation between the two scales, taking out the effect of the composite lead variable was +.153. The partial correlations between the composite lead measure and autonomic regulation, holding regulation of state constant, and between the composite lead measure and regulation of state, holding autonomic regulation constant, were −.412 and +.433, respectively, little different than the Pearson correlations between the lead measure and the two outcome scales (see Table 4). These results indicate that a different subgroup of subjects improved in autonomic regulation than got worse in regulation of states as maternal birth lead increased over maternal 36 week lead. Mean maternal lead level did not play a role in determining which baby would improve in autonomic regulation or get worse in regulation of state as the partial correlation between the two outcome measures, holding mean maternal lead constant, was −.039. Small sample size precludes a more extensive analysis to identify possible variables distinguishing the two subgroups.

Since change of lead in the mother and difference of lead between mother and baby were variously associated with outcome, we performed additional analyses, similar to those of the Cleveland group. Table 9 shows the results of forcing maternal lead at 36 weeks ahead of maternal lead at birth into the multiple regression model for gestation age and 15 day regulation of state scale. Since two composite lead measures, containing both of the maternal lead levels and the umbilical cord lead, were significant for 30 day regulation of state and 30 day autonomic regulation, all three lead measures were forced at the end of the multiple regression for these outcome measures.

With 36 week maternal lead entered into the model maternal lead at birth still predicted gestation age ($t=1.95$, $p=0.057$), the higher the maternal lead the lower the gestation age. The same results holds for entry of maternal lead at birth after 36 week lead for 15 day regulation of state, though the association was only marginally significant ($t=1.71$, $p=0.098$).

With both maternal lead at 36 weeks and umbilical cord lead in the model, maternal lead significantly predicted 30 day regulation of state (t=2.39, p=0.022), the higher the maternal lead, the poorer the outcome. Prior entry of maternal lead at birth and umbilical cord lead resulted in maternal 36 week lead failing to reach significance for 30 day autonomic regulation (t=1.64, p=0.11).

These additional analyses suggest, at least for gestation age and 30 day regulation of state, that some proportion of variance associated with increase of maternal lead at birth independently of maternal lead at 36 weeks and of umbilical cord lead may be responsible for poor outcome.

The factor associated with higher absolute maternal lead (Table 3) is reported vaginal bleeding. Reported problems during pregnancy and not using spinal block during delivery are associated with higher maternal lead at birth relative to maternal 36 week lead. For instance, the average lead levels at birth for mothers reporting vaginal bleeding during pregnancy was 19.0 μg/dl, and for mothers not reporting bleeding 14.7 μg/dl, F(1,48)=4.47, p=0.040. Similarly the average maternal lead change between 36 weeks and birth in mothers receiving spinal block was −0.3 μg/dl and for mothers not receiving spinal block +2.6 μg/dl, F(1,48)=4.16, p=0.047. If unblocked mothers experience more stress during delivery than blocked mothers, increased stress related to the pain of later stages of labor and of delivery may play a role in elevating maternal lead at birth independently of earlier maternal lead levels. This is not to deny that the conditions leading to the use of spinal block, such as unbearable pain during earlier labor, unusually prolonged labor and preparation for surgical intervention in the delivery are not stressful. However, spinal block, as used in the participating hospitals, does reduce the pain and associated stress normally accompanying later stages of labor and delivery. The role of stress in the relationship between maternal lead and outcome of the baby needs further examination.

None of the measures just discussed were considered threats to the pregnancy or delivery serious enough to eliminate the subject from the study. The exclusion criteria for mother and child, as stated in the Method section, have been widely used in prospective lead studies. Complications during pregnancy ranged from subjective complaints to transient foot swelling controlled without drugs. Subjects with severe and persistant hypertension or edema were not recruited into the study. And, none of these variables were significantly associated with the outcome measures. The inclusion of subjects with positive responses on these variables allows an expansion of the search for factors controlling lead relationships during pregnancy.

The results of four subjects, not included in the above analyses, provide additional data related to the issue of stress and both maternal-umbilical cord lead relationships and infant outcome. The first case was excluded from the study because of hypoxia and low Apgar score (5-minute Apgar=4). The mother of this child had a lead level of 6.0 μg/dl at birth, the umbilical cord lead 13.5 μg/dl. The second case was excluded from the study because of failure to obtain NBAS scores. The mother of this child had a lead level of 10.0 μg/dl, the umbilical cord lead 16.0 μg/dl. The presence of meconium was noted at birth. The third and fourth cases were eliminated from the study because the maternal-umbilical cord lead differences were outliers in the data set. One case had maternal lead levels of 13.0 μg/dl, and umbilical cord lead of 34.0 μg/dl. The other case had respective values of 9.0 μg/dl and 20.5 μg/dl. Both of these babies showed no obvious signs of fetal distress during labor and delivery (5 minute Apgar=8), but both showed a strongly increasing frequency of abnormal reflexes on the NBAS during the first 30 days of life.

Our results and those of the Cleveland group suggest that pre- and perinatal stress can alter lead relationships over time in the mother and between the mother and baby at birth. Two major questions require further data. If stress influences maternal and maternal-umbilical cord lead relationships, what are the types of stress that alter the relationship and what are the mechanisms through which this occurs? Stress may alter placental transfer of lead to the fetus. Stress may mobilize lead in the mother or fetus in compartments not normally contributing to maternal and fetal blood lead levels. Or, stress may reduce the elimination of lead by the mother or fetus. And, if the hypothesized stress variables influence child development, do they act independently of lead level or in conjunction or interaction with lead level?

Public Health Implications for Mexico

Nearly 70% of our sample of newborns had umbilical cord lead levels at or greater than 10 μg/dl, an exposure which some authors associate with subsequent developmental abnormalities. Our sample was carefully selected for low risk and we do not know the extent to which it represents the population of the Valley of Mexico. Since the mean umbilical cord lead level of our sample corresponds well with a much larger, unselected sample taken at the beginning of the decade (op. cit.), the data suggests that a substantial proportion of the population of newborns in the Valley is at risk due to lead exposure. Time of residence in the Valley is a significant predictor of 36 week maternal lead and umbilical cord lead, not age of mother. Although some clues to the sources of lead contamination in pregnant women are indicated here (the association between frequency of bean consumption and maternal lead may be related to traditional methods of preparation in low-temperature pottery), both the extent of lead contamination and identification of sources requires a larger sample.

SUMMARY AND CONCLUSIONS

Taking due note of the limitations of the present study, the previous finding that part of the lead burden of the mother-child pair independent of the absolute level of lead significantly predicts infant outcome has been extended here. As maternal lead at birth of the child tends to increase over earlier maternal lead levels, the consolability and self-regulating behavior of the infant tends to decrease out to 30 days of life. Maternal lead elevations at birth are also associated with decreased gestation age. Since elevations of maternal lead at birth are associated with stressful pre- and perinatal events, there is some possibility that these stressful events might act with, or independently of lead to affect outcome. In four cases not included in the statistical analyses large elevations in umbilical cord lead over maternal lead at birth are associated with either stressful birth, poor outcome or both. Although there is a large and growing body of work establishing a relationship between lead exposure and developmental problems in humans, further research is necessary to establish if the relationship is causal, and if so, by what route the connection is based in the range of exposures studied.

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