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Prenatal and Perinatal Lead Exposures Alter Acoustic Cry Parameters of Neonate

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ROTHENBERG, S. J., S. CANSINO, C. SEPKOSKI, L. M. TORRES, S. MEDINA, L. SCHNAAS, A. POBLANO AND S. KARCHMER. *Prenatal and perinatal lead exposures alter acoustic cry parameters of neonate.* NEUROTOXICOL TERATOL **17**(2) 151–160, 1995.—We performed acoustic analyses on cries elicited from a subset of healthy babies born to the Mexico City Prospective Lead Study at 2 days (n = 75), 15 days (n = 176), and 30 days (n = 166). Lead was measured in maternal blood every 8 weeks during pregnancy from week 12 to delivery and in umbilical cord (1–38 μg/dL, 0.05–1.84 μmol/L). Percent nasalization and number of cries decreased in babies born to mothers with higher lead levels in the last two trimesters while median fundamental frequency increased in babies born to mothers with higher lead at 12 weeks of pregnancy, and with higher cord lead in multiple regression analysis. Decreased percent nasalization was related to increased brainstem auditory evoked response latencies and interpeak intervals in a subset of the sample. The results suggest an effect of gestational exposure to lead on apparatus innervated by cranial nerves and/or lead effect on cry mediated by lead-altered auditory function. Altered baby cry and auditory function associated with lead might contribute to developmental delays by affecting early communication between caretaker and baby.

Acoustic analysis   Neonatal cries   Prenatal and perinatal lead (Pb)   Brainstem   Auditory evoked response   Development

PRENATAL and perinatal low level lead exposure has been described as a developmental neurotoxicant (4). Subtle deficits in psychometric test performance have been associated with prenatal or perinatal lead exposure down to 10 μg/dL (0.48 μmol/L), or lower (2,6). However, psychometric tests used in infants and young children to 2 years of age have low reliability and predictive validity, are relatively nonspecific in identifying deficits, even though many have subscales, and are also subject to cultural bias (16,20,21,30). Notwithstanding that psychometric tests have been successfully used by some studies to detect early deficits associated with lead exposure, the shortcomings limit the application of psychometric tests to cultural areas with established test norms. Their lack of specificity may underestimate the damage produced by lead if lead selectively damages limited parts of the CNS. Furthermore, the tests are relatively weak in identifying neurological alterations that may underlie the behavioral deficits produced by lead.

Measuring the cry of an infant overcomes many of the problems of existing psychometric tests for infant assessment. The cry has no cultural bias, can be elicited at the earliest postnatal age and various features of the cry, although complicated, may be uniquely related to anatomy and the nervous system (10). On the other hand, its validation as a predictive measure for subsequent infant development is still in preliminary stages (17). The acoustic properties of baby cries can be

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used to distinguish infant states such as hunger and pain (35) and are differentially associated with brain disorders (24,34), pre- and perinatal complications (36) and hyperbilirubinemia (33). Recently, spectral features of the baby cry have been related to in utero exposure to substances such as cocaine (3,19), marijuana, (18) and alcohol (22), some of which are suspected or proved teratogens.

We are interested in the effects of lead on acoustic features of infant cries as part of our effort to develop culturally unbiased assessments of lead effect on CNS functioning and child development usable in all populations and for its potential utility as an early biomarker of the CNS impact of toxicants. The specificity of the technique makes it particularly attractive when there is prior evidence that the toxicant under study alters the functions of the organs producing or modulating the cry.

The literature provides a few clues that lead may affect the mechanisms involved in voiced sound production. Pharyngeal paralysis was suspected in several lead poisoned cattle (12) who had difficulty drinking water. Researchers investigating a cluster of illness and death among cows and horses grazing downwind of a smelter discovered unusual sensitivity to lead intoxication, occurring at blood lead levels as low as 30 μg/dL (1.45 μmol/L) (13). They reported that sick horses had difficulty breathing, with roaring noises suggesting pharyngeal involvement. The presence of gangrenous pneumonia among the cases also implied problems in closing the glottis during swallowing.

Of the many parameters of the cry available with acoustic analysis, two were selected a priori to reflect laryngeal and pharyngeal activity. The fundamental frequency of the sound is controlled in part by the tension on the vocal folds of the larynx, the greater the tension the higher the fundamental frequency. Nasality of the cry is produced by raising the velum of the velopharynx. A third parameter, number of cry utterances, was selected as it was found to be depressed with in utero cocaine exposure (3). New born rats exposed to lead in utero produced a lower rate of ultrasonic calls to cold stress on the first day of life and were delayed in reaching peak call rate during early development compared to nonexposed rats, but these effects were not dose dependent (5).

As part of our prospective lead study, we recorded cries during the first month of life. We collected data on medical history, diet and drug use during the current pregnancy and a medical history of any previous pregnancies along with delivery room data. This procedure gave us the opportunity to study the effects of prenatal and perinatal lead exposure upon the selected baby cry parameters adjusting the lead effects for covariates.

We hypothesized that prenatal or perinatal lead exposure of the fetus would result in moderate CNS damage and be reflected in altered fundamental frequency due to damage to laryngeal function and decreased percentage of nasal cry due to damage to pharyngeal function. In accord with the literature we expected to see decreased number of utterances per trial as an indication of higher risk associated with lead exposure.

METHOD

Subjects

Women arriving at the outpatient obstetrical clinic of the National Institute of Perinatology in Mexico City around their 12th week of pregnancy were screened for inclusion in the study. Exclusion criteria were:

1. Younger than 15 years of age, older than 42 years
2. Active diabetes, german measles, hepatitis, or toxoplasmosis, as determined by laboratory test
3. Habitual use of alcohol or drugs
4. Taking prescription medications
5. High blood pressure controlled with medication
6. Active psychosis

Women not excluded were interviewed by the project physician. He explained the nature of the project and read the informed consent approved by the institutional review board. Women agreeing to participate signed the informed consent.

The women underwent a standard obstetrical exam and venous blood was drawn for a clinical blood screen and PbB analysis. This procedure was repeated every 8 weeks during the pregnancy.

We administered a questionnaire between 12 and 20 weeks of pregnancy to elicit socioeconomic information, dietary habits, drug, alcohol, and tobacco use and reproductive history. Data regarding the labor and delivery were recorded after delivery.

Another venous blood sample from the mother and a blood sample from the umbilical cord were collected at delivery for PbB analysis. Babies with one or more of the following exclusion criteria were removed from the data set analyzed below:

1. Birth weight under 2000 g
2. Gestation age under 36 weeks
3. 5-min Apgar under 6
4. Multiple birth
5. Major congenital anomaly

Data on the current pregnancy was updated after delivery. Additional information was gleaned from hospital records.

Not all participants in the Mexico City Prospective Lead Study contributed data to the infant cry study. The infant cry work did not get under way until about 40% of the total patients had given birth. Some infants born after this date were not tested because of early hospital discharge or resistance of mothers to have their babies tested at 2 days of life, and because of failed appointments or occasional down time for apparatus repair at 2, 15, and 30 days of life.

Table 1 compares PbB values and other subject characteristics between the group that contributed infant cries and the group that were not tested for infant cries in our study sample. All maternal PbB levels were significantly higher in the group with infant cry recordings. Socioeconomic status (SES) was also significantly higher in the group with recordings. There were no other significant differences between groups on the other characteristics tested.

Pregnant women participating in the infant cry study were young, with small families, of lower or lower-middle socioeconomic status, with infrequent or nonexistent drug, alcohol and tobacco use, all residing within the Valley of Mexico during their pregnancies. Their pregnancies were uneventful and the deliveries were without serious complications. Exclusion criteria ensured essentially healthy infants.

Data reported here were collected in Mexico City where the principal sources of lead were lead-glazed pottery, lead in canned foods, and lead in air and dust from gasoline (8,14, 15,28,29). Use of low temperature ceramic ware for cooking, serving, and storing food and drink has been a tradition in Mexico for more than 3000 years, although lead-glazed pottery was introduced only after the Spanish Conquest. Lead-glazed ceramic ware still enjoys widespread use among the general population, with an incidence of between 40% and

TABLE 1

COMPARISON OF CASES WITH AND WITHOUT ANY INFANT CRY RECORDINGS IN STUDY SAMPLE

	With Recording		Without Recording	
	Mean	SEM	Mean	SEM
Blood lead (μg/dL)				
12 wks	9.1*	(0.5)	7.3	(0.3)
20 wks	8.0†	(0.4)	6.3	(0.3)
28 wks	8.7‡	(0.4)	6.2	(0.4)
36 wks	8.2*	(0.4)	6.8	(0.4)
Delivery	9.8‡	(0.5)	7.4	(0.4)
Cord	7.8	(0.5)	7.8	(0.6)
Maternal age (years)	27.3	(0.4)	27.7	(0.4)
Gravidity	2.5	(0.1)	2.5	(0.1)
Parity	1.8	(0.1)	1.9	(0.1)
Birth weight (g)	3193	(32)	3184	(25)
Gestational age (weeks)	40.0	(0.1)	39.9	(0.1)
SES (1–9)	4*		3	

All measures except SES are means (blood lead is geometric mean); SES is median. Numbers in parentheses are SEM. Lead SEM is geometric SEM and is approximate, as geometric SEM is asymmetric about the geometric mean and is represented here by an average of the plus and minus value. Parity includes study delivery. All statistical tests except SES are t tests; SES is Mann–Whitney U. Comparisons were between groups. Conversion factor for μg/dL to μmol/L is 0.0483.

$*p < 0.05$; $\dagger p < 0.01$; $\ddagger p < 0.001$.

50% in urban areas (40% of this sample reported use during pregnancy). The importance of lead in canned foods has been recently reduced due to the nearly complete conversion of the Mexican canning industry to solderless methods of production, although imported cans with lead solder can still be found in local markets. Lead in air in Mexico City has been falling because the mid-1980s, as lead in gasoline has been systematically reduced. Further details of measured lead sources in the study sample can be found in reference 26.

Blood Collection and PbB Analysis

All blood samples for lead analysis were collected after thorough cleaning of the puncture site in Becton-Dickinson purple-top Vacutainers containing EDTA and refrigerated at 4°C until shipping to Environmental Sciences Associates Laboratories, Inc. (ESA Labs), Bedford, MA for analysis. ESA Labs is one of the reference laboratories for the Centers for Disease Control Blood Lead Proficiency Testing Program and is a participant in the New York State Health Department Quality Control Program.

Duplicate analyses of each sample were made by the method of anodic stripping voltammetry. All samples with mean duplicate values less than 5 μg/dL (0.24 μmol/L) were reanalyzed in duplicate by atomic absorption spectrometry with graphite furnace and the original values discarded. Mean values of the duplicate analysis were used as the index of lead exposure. Additional details on the reliability of the PbB measurements can be found in ref. 26.

Baby Cry Recording and Analysis

The apparatus, recording, and analysis were according to previously published protocols (10). We recorded cries at 2, 15, and 30 days after birth. The baby was placed on his back with the microphone 15 cm from his mouth. A heel flick was used to elicit the cry, which was recorded for 30 s.

Cries were recorded with a Realistic SCT-35 cassette tape recorder and a Realistic Cardioid Dynamic Microphone. The operator pressed a foot pedal at the moment of stimulus application to provide a mark on the tape for synchronizing data analysis. Recorded cries were low-pass filtered at 10 KHz and were digitized by computer at 20,000 samples/s. A Fast Fourier Transform calculated the log magnitude spectrum for consecutive 25 ms blocks of the cry utterance (defined as a cry sound lasting at least 0.5 s).

The three a priori selected cry parameters were constructed as follows from the first three utterances:

Median fundamental frequency. The median frequency of the lowest band of frequencies of the log magnitude spectrum was calculated for each of the first three cries and a mean value obtained. This measure represents the basic vibration frequency of the vocal fold.

Percent nasal cry. The duration of the characteristic band of frequencies representing nasal cries (located between the first and second formant, between 2 and 3 KHz) for each cry was divided into total duration of the cry and expressed as percent. The mean percent of the values for the first three cries were used.

Number of cries. The number of voiced utterances in the first 30 s after the heel flick were counted.

Statistical Analysis

Pearson correlations were calculated for each of the three parameters on each of the 3 testing days against the natural log lead value for each period of pregnancy. A total of 54 correlations were calculated.

Similar bivariate and univariate statistics were calculated between the cry parameters and covariates (Table 2). Selection

TABLE 2

BIOMEDICAL AND DEMOGRAPHIC VARIABLES USED IN ANALYSES

Maternal Variables	Infant Variables
Maternal age	Fetal suffering
Gravidity+	Apgar (1- and 5-min)
Parity	Physical measures (birth weight, gestational age, head circumference+ chest circumference+, superior segment length+, total length)
Problems in previous pregnancy+	Sex+
Hypertension, present pregnancy+	Infant feeding (breast, bottle, both)
Albumin in urine, present pregnancy+	
Socioeconomic (marital status+, occupation, education+, income)	
Maternal nutrition+	
Maternal alcohol, drug, and cigarette use	
Maternal weight gain	
Delivery variables	
Delivery mode (cesarian, forceps, vaginal)	
Delivery anesthetic and medications	
Duration of labor	

+Variables significantly associated with one or more of the infant cry measures in multiple regression analyses ($p < 0.10$)

of covariates was based on experimental evidence or theoretical grounds that implicated them in either infant development or maternal lead exposure. Those variables with significant associations at $p < 0.10$ with the cry parameters were retained for possible entry into a multiple regression analysis.

Stepwise forward multiple regression with backward elimination (entry and elimination criteria were $p < 0.10$ and $p > 0.10$, respectively) was used to construct an empirical model of the baby cry parameter with the significant covariates but without lead. The goal of the analysis was to account for as

TABLE 3

REGRESSION AND MULTIPLE REGRESSION MODEL RESULTS OF BLOOD LEAD UPON ACOUSTIC CRY PARAMETERS

				90% Conf. int.			
	r*	Partial r†	Coefficient‡	Lower	Upper	Model prob.§	N¶
% Nasal Cry							
2 Days							
cord PbB	−0.35	−0.39	−16.47	−25.88	−7.05	0.005	51
15 Days							
12 wk PbB	−0.22	−0.21	−10.75	−19.68	−1.83	0.048	87
cord PbB	−0.17	−0.18	−6.84	−12.68	−0.99	0.055	117
Median Fundamental Frequency (Hz)							
15 Days							
mat. PbB at Birth	0.16	0.17	20.64	2.04	39.25	0.068	124
mat. PbB at Birth with Cry Energy**	0.16	0.16	18.66	1.19	36.13	0.079	124
30 Days							
cord PbB	0.15	0.24	25.69	8.37	43.02	0.016	109
cord PbB with Cry Energy	0.15	0.23	24.88	7.46	42.29	0.020	109
Number of Cries							
15 Days							
36 wk PbB	−0.26	−0.26	−3.79	−6.09	−1.49	0.007	108
30 Days							
20 wk PbB	−0.15	−0.16	−1.99	−3.86	−0.12	0.080	117
28 wk PbB	−0.26	−0.27	−3.44	−5.33	−1.54	0.003	120
36 wk PbB	−0.30	−0.30	−3.94	−6.02	−1.86	0.002	105

*Simple regression; †Partial r in multiple regression; ‡Coefficient is from multiple regression model and reflects change in dependent variable with each natural log unit increment in blood lead; §Probabilities are two-tailed; ¶N's for different multiple regression models are not equal because not all mothers gave blood samples at all pregnancy periods and not all infants were tested on all days.

**Mean cry energy, to the extent that it is associated with subglottal pressure or voice volume, is a potential control variable for fundamental frequency and analyses were run with and without cry energy to control for its effect.

much variance of the baby cry parameter as possible before testing for the effect of lead. Lead variables with significant bivariate associations with the baby cry parameter of interest were tested for significant entry into the multiple regression last.

The Lagrange multiplier test was used to test the significance of control variable coefficient change when the lead variable was added to the multiple regression model. This test essentially uses the residuals of the control variables against the cry parameter without lead in the model as the dependent variable in the model with lead added. A significant effect of the control variable on the residuals indicates a significant change of the coefficient of the variable on the addition of lead to the model.

Forward multiple regression, without elimination, and backward multiple regression models were also used for each lead variable and cry parameter. All control variables were made available for these analyses without regard to their univariate or bivariate associations with the cry parameter. Such analyses reduced the possibility of eliminating a potentially confounding variable that could account for much of the variance of a lead variable found significant in the stepwise models.

As only 50 subjects provided baby cry measures on all 3 days of testing, statistical analysis reported here was limited to cross-sectional testing on each testing day, or pairwise comparisons across days. All statistical analyses were performed with Statgraphics Plus.

RESULTS

All three a priori selected baby cry measures were associated with prenatal or perinatal PbB. The first column of Table 3 shows simple Pearson correlation coefficients between natural log PbB and the measures. Figures 1, 2, and 3 show nonlinear regressions of selected associations between each infant cry parameter and PbB in a log-linear model.

Percentage nasal cry at 2 and 15 days significantly decreased with increasing maternal PbB at 12 weeks of pregnancy and cord PbB. Median fundamental frequency at 15

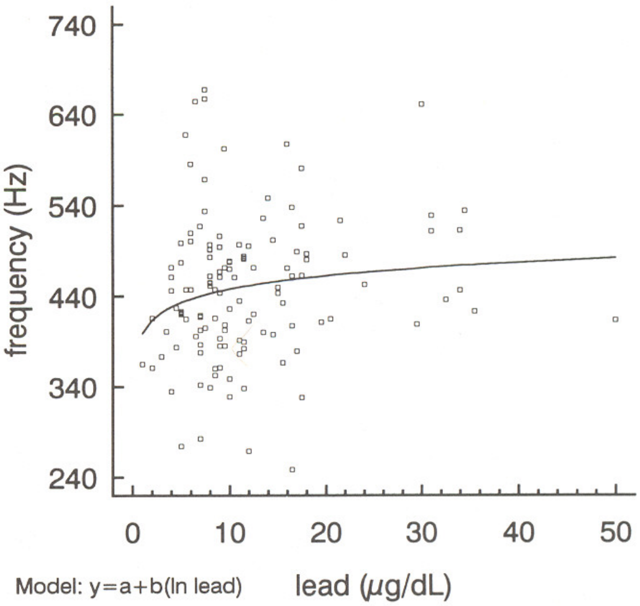

FIG. 2. Natural logarithmic regression between maternal blood lead at delivery and median fundamental frequency at 15 days of life. Frequency is the mean of the median frequency for the first three cry utterances.

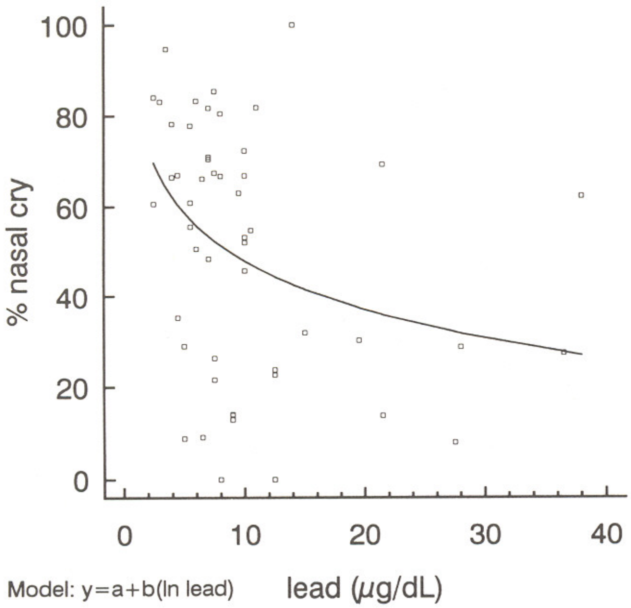

FIG. 1. Natural logarithmic regression between umbilical cord blood lead and percentage nasal cry at 2 days of life. Percentage is calculated as the mean of the first three cry utterances. This figure and the others use the cry parameter unadjusted for covariates. See Table 3 for regression coefficients. Conversion factor for μg/dL to μmol/L = 0.0483.

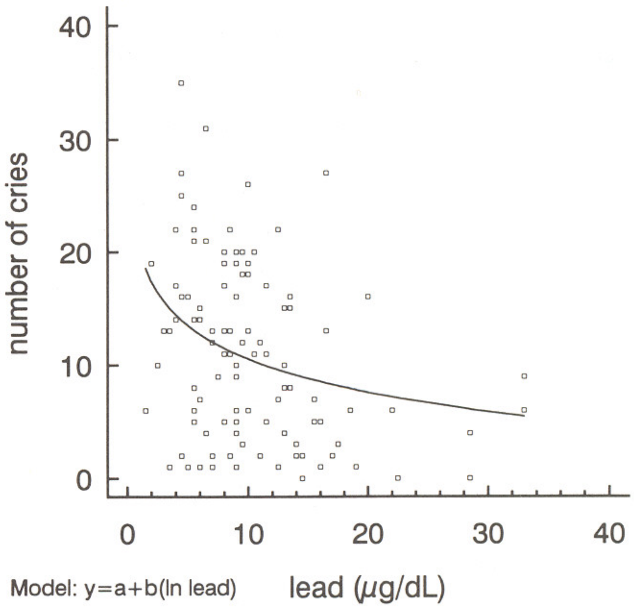

FIG. 3. Natural logarithmic regression between maternal 36-week blood lead and number of cries (minimum 0.5 s voiced utterance) in the 30-s recording period after heel flick.

days increased with increasing maternal PbB at delivery and that frequency at 30 days increased with increasing cord PbB. Number of cries at 15 days decreased with increasing maternal PbB at 36 weeks of pregnancy and the same measure at 30 days decreased with increasing maternal PbB at 20, 28, and 36 weeks of pregnancy.

Table 3 also shows the partial regressions, coefficients, confidence limits, and probabilities of the same baby cry measures adjusted for covariates and confounders against maternal and cord PbB as determined by stepwise multiple regression analysis, entering the PbB measure last. The adjusted coefficients were not significantly different from the slopes calculated from the simple correlations.

Table 4 shows a complete multiple regression model for the median fundamental frequency at 30 days and umbilical cord PbB with and without the lead variable entered last. This cry parameter was selected because the coefficients of the previously entered control variables changed the most after the lead variable entry. The bottom model of Table 4 shows the results of the Lagrange multiplier test for assessing the significance of the change in coefficients of the control variables after adding lead. No coefficient changed significantly.

Table 5 shows a similar analysis conducted on number of cries at 30 days and maternal 20-week lead, the weakest adjusted cry parameter association with lead. Lagrange multiplier tests on the other models showed no significant change in any coefficient.

The additional variance accounted for by adding lead last to the stepwise regression models varied from a low of 1.5% for median fundamental frequency at 15 days with maternal lead at delivery, to 7.2% for number of cries at 30 days with maternal lead at 36 weeks, to a high of 12.4% for percentage nasal cry at 2 days with umbilical cord lead. All percentage additional variance contributed by adding lead to the models was calculated by using model R^2 adjusted for degrees of freedom. Use Tables 4 and 5 to calculate additional proportion of variance accounted for by lead for other models.

Forward multiple regression without elimination and backward multiple regression models, both using all possible control variables regardless of significance level on the prior univariate and bivariate tests against the cry parameter, produced little effect on the size of the lead coefficient, nor on its significance level in any model, when compared to the stepwise model (analyses not shown).

DISCUSSION

Subjects with and without baby cry records differed primarily in maternal PbB levels. The major factor determining the presence of an infant cry record in the entire study group was date of birth, as data collection of infant cries did not start until 40% of the patients had delivered. The other factors determining presence or absence of an infant cry record (see

TABLE 4

FORWARD STEPWISE MULTIPLE REGRESSION MODEL

Independent Variable	Coefficient*	SE	p†
Median Fundamental Frequency (Hz)—30 Days Without Lead			
Constant	548.0	137.5	0.0001
Mean cry energy (arbitrary units)	0.01	0.00	0.0634
Chest circumference (cm)	−8.64	3.53	0.0159
Sex (female)	25.70	14.98	0.0891
Single mother	−62.73	34.98	0.0759
R-SQ. (ADJ.) = 0.114 SE = 74.21 MAE = 53.32			
109 observations fitted			
Median Fundamental Frequency (Hz)—30 Days With Lead			
Constant	526.4	134.8	0.0002
Mean cry energy (arbitrary units)	0.01	0.00	0.0891
Chest circumference (cm)	−10.02	3.50	0.0051
Sex (female)	28.80	14.71	0.0530
Single mother	−69.56	34.35	0.0455
ln umbilical cord lead (μg/dL)	24.88	10.49	0.0196
R-SQ. (ADJ.) = 0.152 SE = 72.61 MAE = 52.40			
109 observations fitted			
Residuals of Model Without Lead (Hz) (Lagrange Multiplier Test)			
Constant	−21.6	134.8	0.8731
Mean cry energy (arbitrary units)	−0.00	0.00	0.8458
Chest circumference (cm)	−1.37	3.50	0.6957
Sex (female)	3.09	14.71	0.8339
Single mother	−6.83	34.35	0.8428
ln umbilical cord lead (μg/dL)	24.88	10.49	0.0196
R-SQ. (ADJ.) = 0.006 SE = 72.61 MAE = 52.40			
109 observations fitted			

*Change in value of dependent variable with each unit change of independent variable; †Significance level of change in coefficient of independent variables with addition of lead to the first model in residual model only.

TABLE 5

FORWARD STEPWISE MULTIPLE REGRESSION MODEL

Independent Variable	Coefficient	SE	p
Number of Cries – 30 Days Without Lead			
Constant	31.6	5.7	0.0000
Unmarried couple	−7.2	2.8	0.0111
Albumin in urine	−3.6	1.6	0.0278
R-SQ. (ADJ.) = 0.092 SE = 7.508 MAE = 6.278			
117 observations fitted			
Number of Cries – 30 Days With Lead			
Constant	35.8	6.2	0.0000
Unmarried couple	−7.2	2.8	0.0098
Albumin in urine	−3.6	1.6	0.0257
ln maternal lead at 20 weeks	−2.0	1.1	0.0802
R-SQ. (ADJ.) = 0.108 SE = 7.4 MAE = 6.2			
117 observations fitted			
Residuals of Model Without Lead (Number) Lagrange Multiplier Test			
Constant	4.3	6.2	0.4907
Unmarried couple	−0.1	2.8	0.9840
Albumin in urine	−0.0	1.6	0.9900
ln maternal lead at 20 weeks	−2.0	1.1	0.0802
R-SQ. (ADJ.) = 0.001 SE = 7.4 MAE = 6.2			
117 observations fitted			

Method section) were more or less scattered evenly throughout the remaining birth dates. Maternal lead showed a highly significant secular trend upward throughout the full study period (26) that was not attributable to a secular trend in PbB analysis accuracy over the same time interval. As mothers delivering later in the study had higher PbB levels than mothers delivering earlier, the difference in PbB between mothers whose infants contributed cry data and those that did not was almost entirely due to the temporal pattern of data capture in the infant cry study. Whereas the overall higher PbB levels in the infant cry group may have made it easier to detect the lead effects reported here, the range of lead values in the infant cry group was similar to the range in the group without cry measures.

Not all mothers contributed a usable blood sample at each target period during the pregnancy. The great majority of missing PbB values were due to failure to arrive for the appointment or arriving before or after the plus and minus 2-week acceptable range around the target date. This was particularly acute for the 12- and 36-week blood test. Other failures to obtain a PbB value were due to blood clotting, breakage during shipping, or inadequate blood sample volume. In an analysis reported elsewhere (26) there were no significant differences in PbB levels nor in a number of demographic variables between subjects with and without a complete record of PbB during the pregnancy. Whereas the missing data contributed to lower power for statistical tests conducted at periods of pregnancy with fewer PbB measurements there was no indication of systematic bias of results due to this factor.

Mean and range of blood lead levels of our subjects were within the values reported from other prospective lead studies (1,2,7,9,11), although the sources of lead differed among studies. The magnitude of exposure of our subjects was similar to that found in a wide range of urban environments, including industrial cities and towns.

Given the reported associations between baby cry parameters and maternal drug and alcohol use mentioned above, we specifically looked at relationships between these substances, as potential confounders, and baby cry in our sample. Because any maternal prescription drug use, and regular other drug use and alcohol use during pregnancy were among the exclusion criteria for this data set, it was not surprising to find no significant association between even occasional alcohol use and the baby cry parameters on the univariate and bivariate control variable screening tests. Only 21% (n = 33) of the mothers of the children measured in this study reported any alcohol drinking during pregnancy. Of those drinking during pregnancy 73% (n = 24) drank less than once every month and the remainder only once a month. None of the mothers in the study reported any drug use during the pregnancy. The amount of alcohol and drug use among study subjects here was considerably less than among subjects in baby cry studies specifically testing alcohol or drug effects.

One might think that the strict exclusion criteria applied to both mothers and babies born in the study would produce conservative estimates of lead effects upon baby cry parameters. Indeed, the results reported here apply specifically to healthy babies born to healthy mothers. The exclusion criteria, however, really serve to eliminate a range of conditions that might produce changes in the baby cry that could obscure or be confounded with changes produced by lead. The exclusion criteria, as well as statistical control of other variables, serve to reduce the chance that an association between lead and baby cry might be through an intermediate variable, e.g., a direct lead effect on infant size at birth and an effect of infant size on baby cry.

Whereas the stepwise regression models by their nature reduce colinearity among independent variables, and by our method of preselection of control variables with univariate and bivariate tests increase the parsimony of the models, the

TABLE 6

CRY PARAMETERS BY BLOOD LEAD CATEGORY

	Low PbB	Mid PbB	High PbB
% Nasal cry—2 days	62.4	46.4	44.5
Cord lead*	4.5	7.7	17.4
n†	15	17	19
% Nasal cry—15 days	52.1	44.1	43.3
12-week maternal lead	5.0	9.2	16.0
n	24	36	28
% Nasal cry—15 days	53.3	44.2	43.3
Cord lead	3.7	7.2	18.4
n	31	48	39
Med. Fund. Freq. (Hz)—15 Days	431.2	454.7	459.1
Maternal delivery lead	5.2	9.3	20.5
n	35	49	43
Med. Fund. Freq. (Hz)—30 days	430.0	454.8	447.5
Cord lead	4.0	7.5	19.0
n	32	42	35
Mean number of cries—15 days	17.5	14.2	10.7
36-week maternal lead	4.3	7.9	15.9
n	28	45	35
Mean number of cries—30 days	12.9	12.4	9.1
20-week maternal lead	4.3	7.9	15.4
n	36	41	40
Mean number of cries—30 days	12.6	10.8	8.6
28-week maternal lead	4.7	8.3	15.9
n	31	45	44
Mean number of cries—30 days	14.3	11.6	7.5
36-week maternal lead	4.8	8.9	16.8
n	32	38	35

*Lead values are arithmetic means in μg/dL (conversion factor to μmol/L = 0.0483); †Number of subjects in each blood lead category.

stepwise method may inadvertently exclude important confounding variables whose presence could reduce the lead effect to insignificance or alter its coefficient. Finding little change of the lead coefficient or its significance with forward or backward multiple regression models with the full complement of control variables, even with the reduced degrees of freedom that the additional independent variables produce, increases our confidence that the nature and size of the lead effects described are not artifactual.

Percentage nasal cry decreased with increasing cord PbB, as predicted from our interpretation of the earlier veterinary results. The velum is elevated to seal the buccal cavity from the pharynx and is normally raised during swallowing. Paralysis or weakening of the mechanisms altering food and air flow among pharyngeal and esophageal passages could be expected to interfere with respiratory function and might be associated with the suffocation-induced death in the animals exposed to lead. As no association of PbB with reduced percentage of nasal cry was found for 30-day-old infants, the findings suggest that lead exposure in utero is associated with a short-term weakening of mechanisms responsible for raising the velum in infants to 15 days after birth.

Although we predicted no direction of effect of PbB upon median fundamental frequency, the positive correlation found here is the same as found with infants exposed to cocaine in utero (3,19) and in infants born to heavy marijuana smokers (18). In cocaine exposed infants part of the drug effect on median fundamental frequency was mediated through drug related reductions in birth weight. We found that decreased linear measurements of infant size at birth (superior segment length and chest circumference, against cry at 15 and 30 days, respectively) were better predictors of increased median fundamental cry frequency than birth weight, but the presence of infant size in the multiple regression did not alter the apparent lead effect on the cry measure. Unlike cocaine, the effect of lead on fundamental frequency of the cry is not mediated, even in part, through any lead effect on the gross physical size of the infant.

The fundamental frequency of the cry is determined by the vibration frequency of the vocal folds. Tension of the vocal folds, altered by activity of the XIth nerve which innervates the larynx, is the principal determinant of vibration frequency. Another important influence is subglottal pressure (32) and is responsible for the rise in voice pitch frequently observed with increased voice volume. We made no direct measurement of subglottal pressure, but we did measure the related variable mean cry energy. Although increasing cry energy was associated with higher fundamental frequency in our data set, including cry energy in the models did not significantly alter the lead effect (compare partial correlations for median fundamental frequency with and without cry energy at each age in Table 3). Lead apparently alters fundamental frequency of the cry by affecting the activity of structures innervated by the XIth nerve, though our data can not rule out a direct effect of perinatal lead exposure on vocal fold length.

Fewer cries per trial were noted in babies born to mothers with higher lead levels during the second half of their pregnancies. Reduction of cry number was also noted in babies born to cocaine using mothers (3). This measure might be considered a general index of risk for poor outcome. Fewer cries per trial may indicate lower sensitivity to the eliciting stimulus or

TABLE 7

CORRELATIONS WITHIN CRY MEASURES ACROSS DAYS

	Day 15	Day 30
Percent nasal cry		
Day 2	0.078	0.035
	(61)	(56)
	0.553	0.796
Day 15		0.450
		(129)
		0.000
Median fundamental frequency		
Day 2	0.240	0.145
	(60)	(55)
	0.065	0.290
Day 15		0.457
		(128)
		0.000
Number of cries		
Day 2	0.164	0.279
	(61)	(59)
	0.205	0.032
Day 15		0.190
		(135)
		0.027

Correlation, sample size in parentheses, probability.

a reduced state of arousal in general rather than to specific noxious effects on the vocal apparatus.

We constructed Table 6 to obtain another view of the data. We arbitrarily divided the subjects into three groups based on their lead levels. The group was divided into low, mid, and high PbB by selecting low, middle, and high thirds of the lead distribution for each cry parameter at each period of pregnancy. To keep the lead categories mutually exclusive we defined low and high PbB by selecting subjects with lead values below and above the 33rd and 66th percentile, respectively, and mid PbB by selecting those with lead values at and above the 33rd and at and below the 66th percentile. We did not consider splitting tied PbB values that would have resulted in equal *n* for each group, so the *n* for the mid PbB group is almost always the largest of the three. We then calculated the mean value of the cry parameter associated with that PbB category and the category's mean PbB value for all the significant lead-cry parameter relationships. The mean lead values showed the expected logarithmic increase with increase in lead category. All cry parameters but the relationship between median fundamental frequency at 30 days and cord lead showed either an increasing or decreasing monotonic function with PbB category. Furthermore, for most of the measures, the greatest change in cry parameter occurred between the low and mid PbB group, as can also be seen in the figures. The range of highest cutoff PbB value for the mid PbB group was from 9 to 12 μg/dL, depending on the lead variable and cry parameter. The table suggests that considerable effect of lead on infant cry can be measured at or below 12 μg/dL.

Unfortunately, the present state of knowledge regarding the acoustic parameters of infant cry does not permit us to assign cutoff values to define what should be considered normal and abnormal cries. As more work is done to establish the predictive validity of these measures, we may eventually arrive at the point where we can calculate odds ratios for the effects of various pre- and perinatal insults.

There is increasing evidence that lead exposure alters auditory function in children (6,23,25,31). Our laboratory has recently demonstrated an effect of pre and perinatal lead exposure on brainstem auditory evoked responses (BAER) in some of the same infants reported on here (27). As the pattern of development of the baby cry likely depends on intact auditory function, we measured the relationship between the BAER and the characteristics of the baby cry to try to determine if any of the associations between lead and BAER could be mediated by altered auditory function, especially at 15 and 30 days of life. The Vohr et al. (33) study showed a relationship between toxic exposure (hyperbilirubinemia) and concurrent alterations of BAER and baby cry parameters.

Twenty-one babies contributing to the 15-day baby cry data had BAER measurements at nearly the same time. The latencies of peak III and peak V were negatively correlated with percent nasal cry ($r = -0.405, p = 0.068; r = -0.459, p = 0.036$, respectively). BEAR conduction time correlations involving these peaks (I–III and I–V conduction times, $r = -0.514$, $p = 0.017$; $r = -0.544$, $p = 0.011$, respectively) with percent nasal cry were even stronger.

Although it is tempting to hypothesize that prenatal and perinatal lead exposure alters function of the structures innervated by many cranial nerves (VIII, X and XI) simultaneously to explain the relationships among lead, cry, and BAER, another explanation is possible. Except for the reduction of percentage nasal cry with cord lead at 2 days of life, the remainder of our results were found only in infants at 15 and 30 days. By itself, this result could be explained by the reduced power to detect significant lead effect produced by the small *n* in our sample at 2 days. However, the generally low values of the correlations within cry measures across days (Table 7) suggest that considerable individual change in infant cry behavior within each subject took place from 2 to 30 days. The changes in cry parameters with age could represent maturation, in which various aspects of cry behavior come more and more under willful control. As at least some modulation of infant cry with increasing age requires auditory feedback, the BAER results together with the age pattern of lead effect on cry suggest that some changes in 15- and 30-day cry characteristics associated with lead in this article might be secondary to lead-induced alteration in auditory function.

Regardless of the origin of the effect of lead on baby cry, its presence alters the principal means of communication the infant has with her caretaker. Subtle acoustical alterations in infant cry may expose the infant to further risk of developmental delay due to its impact on the social interaction between the caretaker and the baby.

Acoustic analysis of baby cry may provide an early marker for specific CNS damage produced by several toxic agents. The present results suggest that it is a sensitive and selective index for measuring the effect of pre- and perinatal lead exposure.

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