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Effects of lead exposure on pregnancy outcome and the fetal brain of squirrel monkeys

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LÖGDBERG B, BERLIN M, SCHÜTZ A. Effects of lead exposure on pregnancy outcome and the fetal brain of squirrel monkeys. *Scand J Work Environ Health* 13 (1987) 135–145. Eleven pregnant squirrel monkeys were perorally exposed to lead during the latter two-thirds of pregnancy [mean blood lead 0.54 µg/ml (2.61 µmol/l), range 0.39–0.82 µg/ml (1.88–3.96 µmol/l)], at a dosing regime producing no maternal toxic symptoms. Lesions similar to lead encephalopathy and growth retardation of the fetal cerebrum were seen in some of the offspring, as well as neurological and behavioral symptoms at adult age. Cerebral lead levels in offspring (an abortion, stillborns, a sacrificed full-term fetus, and a neonatal death) were between 0.1–0.7 µg/g. Pre- and perinatal mortality, and prematurity, was increased, and the size of the offspring at birth was reduced. The head circumference tended to be reduced postnatally. Zinc protoporphyrin in blood was determined in four of the pregnant monkeys; the mean values were between 0.58–1.05 µg/ml (0.93–1.68 µmol/l). These findings represent the first clearly prenatal brain damages described for experimental peroral lead exposure.

Key terms: intrauterine growth retardation, macrencephaly, micrencephaly, neurotoxicology, prenatal, primate, stillbirth, zinc protoporphyrin.

Epidemiologic studies have indicated a correlation between cerebral damage — eg, hyperactivity, mild mental retardation, visual and motor disturbances — and low to moderate lead exposure prenatally or early postnatally (10, 28, 31, 60). (For a review, see reference 53.) However, due to the sociohereditary factors involved, a causal relationship remains a controversial hypothesis. Animal experiments with prenatal exposure to inorganic lead perorally or via the air — ie, the routes of exposure which are relevant to humans — have failed to produce any cerebral lesions. Clearly prenatal cerebral lesions — dysraphisms, hemorrhage and hydrocephalus — have only been described following lead administration to chicken (in ovo) and small rodents (parenteral), in which high blood and tissue lead concentrations were produced (7).

Rodents are inadequate as animal models for mimicking mild mental and neurological retardation found in primates, as they lack the latter's corresponding period of gestation during which important neurodevelopmental events occur. For instance, rodents have no subpial granular layer (observations to be published) and possibly no radial glial cells of the special type described for primates (42). These cell populations may be critical for the addition of neuroblasts in late primate, including human, cerebrogen-

esis. Certain structural malformations of the cerebrum, which often form the basis of mental retardation in humans, eg, polymicrogyria, have not been described in rodents. The placental structure of subhuman primates is more similar to that of man than is the placental structure of rodents (3, 47).

For these reasons, primates are likely to be more suitable models for the study of dose-effect relationships in humans. The purpose of the present study was to investigate the effects of lead exposure of pregnant monkeys on the fetus and its cerebrum and to correlate the effects with different biological indicators of lead dose. Future reports will describe neuropathological, behavioral and physical effects on the offspring, as well as placental effects.

Material and methods

Animals

Data from 31 adult female squirrel monkeys (*Saimiri sciureus*) of Roman-arch type with black and yellow fur, and their offspring, were used in this study. They were caught wild in 1978, at the ages of 1–4 years and were transported to our colony, where they have been used successfully in breeding since 1980 without any intercurrent diseases except for minor staphylococcal skin infections. They normally give birth to one baby per year. Medical records have been kept for all the monkeys from the time of their arrival at the colony. Their body weights at the start of the present study were about 700–800 g. Since our stock of fertile females is limited, we decided to titrate suitable lead doses in a pilot experiment with five male monkeys.

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Housing and diet

All 31 monkeys (the lead-exposed monkeys and the nonexposed monkeys used for breeding) were maintained in large stainless steel cages, about $5 \times 2 \times 2.3$ m, in groups of four to six per cage. Sufficient space for normal activity was available. The temperature in the rooms was kept at 23°C and the relative humidity at $60 \pm 5\%$. Lights were on from 0700 to 1900. The diet of the monkeys consisted of pellets (Nafag Affenfutter, Gossau, Switzerland) ad libitum, amounting to about 80–100 g per monkey daily, supplemented with folic acid (Folacin, Kabi-Vitrum, Stockholm, Sweden) in the drinking water, and half an apple per day during the winter half of the year. Vitamin E and selenium (Tokocel®, Agrivet, Uppsala, Sweden) were added to the water once a month. Two to three intramuscular injections of 20 mg of iron (Imposil®, Agrivet, Uppsala, Sweden) were given between the gestational age of 9–18 weeks to prevent iron deficiency. No other pharmaceuticals were given during pregnancy.

Timing of pregnancies

Of the 31 monkeys, 11 were exposed to lead. (The monkey with pregnancy 12 — see table 3 in the Results section — was the same as that with pregnancy 8, reexposed after a one-year latency.) The pregnancies were timed to ± 3.5 d by limiting matings to one week every month, with diagnostic abdominal palpations after a few weeks. The births in both the lead-exposed and the unexposed groups occurred in the summer or late spring.

Exposure to lead acetate

The duration of the lead exposure was from around nine weeks of gestation until the end of the pregnancy (normally about 21 weeks). Analyses of a series of fetal brains from squirrel monkeys (unpublished results) indicated that the cerebral development at nine weeks of gestation is comparable to that at about 12 weeks' gestation in humans, a time at which the second wave of neuroblasts aimed for the human cerebral cortex is still migrating (44). According to others (48) and our own experience, the risk for spontaneous abortion is much smaller from about this age onward, compared to just a few weeks earlier, and thus the probability of selecting resistant fetuses or placentas is minimized if exposure begins at this age.

Lead acetate salt $[\text{Pb}(\text{Ac})_2 \times 3\text{H}_2\text{O}]$ was administered perorally. About half of the daily dose was dissolved in 50 ml of distilled and millipore-filtered water (Milli-R04, Millipore Corporation, Bedford, Massachusetts, United States). (The filtering reduced the pH of the water from 7.0 to 5.9–6.0.) The monkeys had access to this lead solution for about 8 h starting at 0730. During this time they were kept separated in different sections of the large cage. In or-

der to keep the ingested dose constant, this time was varied by about ± 3 h to compensate for varying rates of drinking. The other half of the dose was pulverized and applied (ie, dissolved) on a freshly cut surface of pieces of apple, which were given to the monkeys twice daily — just before and just after the 8-h access to the water dose. For the remaining 16 h tap water with Folacin was given ad libitum. In some instances the proportions between the doses in water and apple were different.

Pilot exposure

Five male squirrel monkeys were perorally exposed to lead acetate at doses designed to produce blood lead concentrations of 0.55–1.0 $\mu\text{g}/\text{ml}$. This pilot study was carried out to determine the dosing regime for the pregnant monkeys. In two of the male monkeys, the blood lead (PbB) levels accidentally rose above 1.0 $\mu\text{g}/\text{ml}$ up to 1.6 $\mu\text{g}/\text{ml}$. Toxic symptoms, which included watery diarrhea, anorexia and physical weakness, quickly appeared above about 1.2 $\mu\text{g}/\text{ml}$; cardiac arrhythmia was noted in one monkey. Such symptoms did not occur among the lead-exposed pregnant females.

Blood lead analyses and other observations from the lead-exposed monkeys during pregnancy

Weekly blood samples of about 1 ml were withdrawn from the vena saphena with a plastic syringe, after the fur in the area had been carefully shaved and the skin area cleaned with 70 % ethanol, and then transferred into lead-free heparinized glass tubes. The blood samples were taken just before the morning doses were administered. The lead concentration of the blood was determined, the same day, at the Institute of Occupational Medicine in Lund, by a flame atomic absorption spectrometric (AAS) method (20) which was adapted to 1-ml samples. Each sample was weighed before the analysis. The detection limit of the method was estimated to be 0.05 $\mu\text{g}/\text{ml}$.

Hemoglobin and, for pregnancies 9–12, the zinc protoporphyrin levels were determined for the same blood samples. The hemoglobin level was determined spectrophotometrically and the zinc protoporphyrin value was determined with a hematofluorometer (AvivTM, Lakewood, New Jersey, United States), after the blood sample was mixed with air in the test tube. Additional gassing with pure oxygen was tested, but it did not influence the results. At about 14–17 weeks of pregnancy, the blood indices were determined for pregnancies 9–12, and for five pregnancies in the control group, with the use of coulter counting.

At the time of the blood sampling, the lead-exposed monkeys were weighed and scrutinized for toxic or other abnormal symptoms. The monkeys had been handled extensively prior to pregnancy (ie, in connection

with the collection of blood and urine samples and weighing), and they were accustomed to these routines. Little stress was seen during the handling, and no pharmaceutical sedation was required.

Normal values for the pregnancy and birth indices were obtained during the pregnancies of the lead-exposed monkeys prior to lead exposure, and from a group of unexposed pregnant monkeys in our colony. This latter group was comparable to the lead-exposed group as to age, weight, previous pregnancy outcome, housing and diet, etc. (See table 1 and data given earlier in the Material and Methods section.) The lead-exposed fetuses and newborns were also compared with a series of fetuses of different ages, collected earlier, from pregnancies timed to ± 0.5 d or ± 3.5 d, in order to help determine if the lead-exposed offspring with low birth weights were small for their gestational age.

Organ lead analyses

Tissue pieces from the abortion and the perinatal deaths, as well as from five consecutive births among unexposed controls and an unexposed fetus collected at a hysterotomy, were taken fresh or after perfusion of the vascular tree with an aldehyde fixative in phosphate buffered saline. The phosphate buffered saline was obtained from Suprapur chemicals with a maximum lead content of 0.01 ppm. The lead content of the final fixation solution was below the detection limit (ie, about <0.03 $\mu\text{g}/\text{sample}$, the sample volumes being a few milliliters).

Organ lead levels were determined according to a method used earlier for blood and food samples (43). The samples were mineralized and analyzed by flame AAS after the extraction of lead as lead dithiozonate into methyl isobutyl ketone. For the small samples obtained in this study the reagent volumes were reduced, and the lead was extracted with 1.5 ml of methyl isobutyl ketone. The detection limit was estimated at 0.03 μg per sample. The lead levels in the blood samples analyzed by this method ranged, with few exceptions, within $\pm 5\%$ of the calculated mean value for about 90 participating laboratories in the UK External Quality Assessment Scheme.

Very small samples, ie, blood samples from the offspring and breast milk samples, were analyzed by graphite furnace AAS by the technique of standard addition. The detection limit was estimated at 0.003 $\mu\text{g}/\text{sample}$, with sample volumes of 100–200 μg .

Triplet samples, about 1–2 g each, of cerebral and placental tissues were taken, and one sample from each triplet was analyzed as a control by Dr Markus Stoeppeler at the Institut für Chemie, Kernforschungsanlage, University of Jülich, Federal Republic of Germany. Other tissues were analyzed in duplicate at our laboratory only. The lead concentrations are given as micrograms per milliliter for tissue fluids and liquids, and as micrograms per gram for solid tissues (1 $\mu\text{g} = 4.83$ nmol).

Table 1. Pregnancy indices for a five- to six-year period, including all values for the normal control group, and pre-exposure values for the lead-exposed group. (N = number of pregnancies)

	Number of monkeys	Offspring mortality (prenatally or day 0–1 postnatally)	Body weight before pregnancy	
			Mean	Standard error of the mean
Normal control group	20	8 % (N = 60)	740 g	15 g
Lead-exposed group before lead exposure	11	7 % (N = 18)	736 g	23 g

Cerebral size and pathology

The cerebrums were weighed following fixation, and a coronal tissue slice was excised by hand with a microtome. One cutting surface extended from the motor cortex to immediately anterior of the mesencephalon. After dehydration and paraffin embedding of the slices, histological sections were prepared from this surface. These sections were stained with Ehrlich's hematoxylin and eosin and evaluated for fresh hemorrhages. The light microscopical appearance and size of the cerebrums were compared with those of brains from five newborns, collected during the same period of time from the unexposed control group.

Other measurements and statistical analyses

The body weights of the mothers and offspring and the head circumferences of the offspring were also determined. The body weights, cerebral weights and cerebral lengths were compared with control values with the use of Student-t statistics. Regression analyses were made of the relation between the lead and hemoglobin levels of the blood.

Neurobehavioral parameters were measured from video recordings, which were taken of lead-exposed and unexposed offspring during object manipulation in a standardized situation. These methods will be published in detail elsewhere.

Results

Doses and blood lead levels

To maintain the individual PbB levels at 0.55–1.0 $\mu\text{g}/\text{ml}$ in the male monkeys, 5–20 mg of lead/kg of body weight was given daily. With the aim of reaching the same PbB values in the pregnant monkeys, a few of them were initially given the same relative doses.

The resulting blood lead concentration leveled off however around 0.3–0.45 $\mu\text{g}/\text{ml}$ only (figure 1, monkeys 1 and 2). Only with daily doses of around 50–100 mg/kg did the blood lead rise further towards the desired values.

Figure 1. Lead concentration in the blood (Pb-B) as a function of gestational age for 12 lead-exposed squirrel monkey pregnancies. An incident occurred with the preexposure blood samples from pregnant monkeys 9–12 in that the test tubes were damaged, the result being cracks in the glass. This occurrence could explain the unexpectedly high PbB values. No such obvious risk for contamination occurred with the other preexposure blood samples. All the PbB values of each individual monkey were corrected for variation in hemoglobin by multiplying them by the mean hemoglobin value/simultaneous hemoglobin value. (Filled circles = termination of pregnancy, $1 \mu\text{g/ml} = 4.826 \mu\text{mol/l}$).

The maternal PbB values (for all the lead-exposed pregnancies) and the zinc protoporphyrin values (for pregnancies 9–12) and lead doses throughout the pregnancies are shown in figures 1 and 2. The average PbB

Figure 2. Zinc protoporphyrin in blood (ZPP-B) as a function of gestational age for four pregnant squirrel monkeys exposed to lead. ($1 \mu\text{g/ml} = 1.597 \mu\text{mol/l}$).

values ranged between 0.39–0.82 (mean = 0.54) $\mu\text{g/ml}$, the mean hemoglobin values ranged between 112–161 (mean = 133) g/l, and the mean zinc protoporphyrin values for the four pregnancies ranged between 0.58–1.05 $\mu\text{g/ml}$ (table 1). The normal PbB values were about 0.04–0.09 $\mu\text{g/ml}$. As more than 90 % of the lead in blood is in the erythrocytes, the individual PbB values were standardized to the mean hemoglobin values in order to compensate for any variation in hematocrit due to possible small variations in the blood sampling technique. (See the text of figure 1.) The standard error of the mean of the change in the PbB level due to this standardization was 0.067.

Effects on the pregnant mothers

No clinical toxic symptoms of the type experienced in the pilot exposure of males or other clinical signs of disease were noted among the pregnant females during the lead exposure. Low mean hemoglobin values were noted for three of the pregnant monkeys (table 3, monkeys 4, 9 and 11). Coulter counting on pregnancies 9–12, at about 14–17 weeks of pregnancy, showed the erythrocytes to be normocytic and normochromic, while the hematocrit in pregnancies 9 and 11 was decreased, compared to that of the controls (table 2). However, the low hemoglobin levels for monkeys 4, 9 and 11 rose to normal (143–144 g/l) four, one, and one week, respectively, after the end of pregnancy/exposure. In general, the individual hemoglobin values of the monkeys in the lead-exposed group, measured four weeks after the end of pregnancy, were found to be similar to values measured just prior to the lead exposure (mean 110 %, range 91–126 %). Regression analyses between the PbB and hemoglobin values, with various time latencies (one, two, three and four weeks), were performed for a few monkeys. These analyses indicated that the hemoglobin level was inversely related to the PbB concentration, with a latency of one week, for some monkeys. The zinc protoporphyrin level increased more slowly than the

Table 2. Selected blood indices, at about 14–17 weeks of pregnancy, for a subgroup of the unexposed and the lead-exposed monkeys.

	Hemoglobin (g/l) ^a		Hematocrit (volume %)		Mean corpuscular volume (fl)		Mean corpuscular hemoglobin concentration (weight/volume %)		Mean corpuscular lead concentration ^c (μg/ml)
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Normal control pregnancies (N = 5)	144	135–154	41.6	38–45	60.5	57.6–62.5	34.6	33.9–35.3	..
Lead-exposed pregnancies									
Number 9	99	.	28.7	.	60.5	.	34.7	.	1.50
Number 10	135	.	39.2	.	61.7	.	34.6	.	2.09
Number 11	89	.	26.3	.	59.3	.	34.0	.	2.24

^a 1 g/l = 15.5 μmol/l.

^b Erythrocyte.

^c Calculated as (mean blood lead concentration (table 3) × 100)/hematocrit value (1 μg/ml = 4.826 μmol/l).

Table 3. Important lead dose indicators and effects on the pregnancy and the fetal brain during lead exposure of pregnant squirrel monkeys. All the blood lead values are corrected for variation in hemoglobin by multiplying them with the mean hemoglobin value/simultaneous hemoglobin value for each individual monkey.

Pregnancy number	Blood lead concentration (μg/ml) ^a		Mean zinc protoporphyrin concentration in blood (μg/100 ml) ^b	Mean hemoglobin (g/l) ^c	Mean blood lead concentration/mean hemoglobin value × 1000	Length of gestation (d)	Birth weight ^d (g)	Cerebral characteristics			Offspring mortality
	Mean	Standard error of the mean						Lead concentration (μg/g)	Weight ^e (grams)	Petechial hemorrhages ^f	
1	0.39	0.14	..	123	3.2	151.5	96	..	..	..	Live
9	0.43	0.09	68	117	3.7	140.5	96	0.24	13	+	Stillbirth
3	0.43	0.27	..	139	3.1	143.5	90	..	..	..	Live
2	0.44	0.13	..	130	3.4	151.5	109	..	..	..	Live
5	0.44	0.17	..	127	3.5	148.5	95	0.58	15	—	Stillbirth
4	0.47	0.29	..	112	4.2	101.5	28.5	0.11	..	—	Abortion
7	0.56	0.13	..	127	4.4	148.5	90	0.19	21	—	Stillbirth
6	0.58	0.25	..	149	3.9	149.5	82.5	0.69	23	+ +	Neonatal death (12 d)
11	0.59	0.17	93	113	5.2	145.5	80	0.16	12.5	+	Sacrificed after Caesarean section
8	0.59	0.18	..	138	4.2	153.5	96	..	..	..	Live
12	0.79	0.14	58.5	161	4.9	147.5	105	..	..	..	Live
10	0.82	0.16	105	155	5.5	140.5	95	0.15	14	+ +	Neonatal death (14 h)

^a 1 μg/ml = 4.826 μmol/l.

^b 1 μg/100 ml = 15.97 nmol/l.

^c 1 g/l = 15.5 μmol/l.

^d Body weights are fresh weights.

^e Cerebral weights are weights after formalin fixation.

^f Graded in extent as — = none, + = moderate number, + + = plentiful.

PbB concentration after the lead exposure, and it reached plateau values about 30–45 d after the PbB level (figure 2).

The water and food consumption of the lead-exposed pregnant monkeys was 50–100 % (150–300 ml water/monkey, daily) and 85–100 %, respectively, of that of the unexposed pregnant controls. The body weights determined at corresponding times (0–8 weeks) before and after pregnancy did not differ significantly between the lead-exposed group [740 (SEM 15) g before and 752 (SEM 27) g after] and the unexposed controls [N = 20, 736 (SEM 23) g before and

711 (SEM 46) g after]. The weight of monkey 4 decreased the last few weeks before the manifest abortion. The placenta of this abortion was largely hyalinized.

Nine of the lead-exposed monkeys were reintroduced into breeding after six months; eight of these (77 %) became pregnant again within six months, which is similar to the yearly rate of reproduction among unexposed controls (67 % for the same year, a mean of 78 %/year over a six-year period). The PbB values at the beginning of the new pregnancies were between 0.1–0.2 μg/ml.

Table 4. Tissue lead concentrations ($\mu\text{g/g}$ wet weight) in the offspring of squirrel monkeys exposed to lead during pregnancy. Also included are three lead values for placentas (monkeys 4, 10 and 11) and four blood lead values from mothers, taken one day after the blood lead values of their offspring (stillbirths/neonataly dead offspring 5, 7, 9 and 10). The calcium content of some of the specimens is given in parentheses as the percentage of fresh weight.

Tissue	Offspring number					
	4	5	7	9	10	11
Placenta	4.65 (0.01 %)	..	..	..	3.2	4.5
Amnion fluid	0.048 (0.008 %)	..	..	..	..	..
Blood ($\mu\text{g/ml}$) ^a	..	0.61	0.71	0.70	0.45	..
Blood, mother	..	0.64	0.74	0.88	0.77	..
Tibia	24 (3.17 %)	35	..	..	..	..
Lung	0.69	0.10	..	..	..	..
Liver	8.97	2.85	..	..	..	..
Kidney	0.36	0.40	..	..	..	..

^a 1 $\mu\text{g/ml}$ = 4.826 $\mu\text{mol/l}$.

Effects on the pregnancy and the offspring

The outcome of the 12 lead-exposed pregnancies is shown in table 3. One monkey, number 11, was delivered by Caesarean section at 145.5 days' gestation, which approximated the mean gestational length of the group. (See the following text.) In the remaining 11 pregnancies, 45 % of the developing offspring died during pregnancy or on the first day after birth. This rate should be compared with the 7–8 % mortality of unexposed offspring over the the same developmental period (table 1). All six spontaneously deceased offspring were male, as were three of the five that survived.

The mean birth weight of the lead-exposed offspring (with the abortion, number 4, and the Caesarean section, number 11, excluded) was significantly reduced [95.5 (range 82.5–109) g] in comparison with the mean of 105 (range 80–120) g of an unexposed group over a time period of six years ($P < 0.05$). The mean gestational length was 147.5 (range 140.5–153.5) d. We have not obtained a reliable normal control value for gestational length from our breeding group for comparison. See the Discussion section for further comments.

Cerebral effects

The cerebrums of offspring 6, 10, and 11 exhibited multiple characteristic petechial, pericapillary hemorrhages, especially in the white matter (table 3). Several endothelial changes were seen (unpublished results). The cerebrum of offspring 6 also showed a massive edema, with gross pallor, flattening of the gyri, and microscopic vacuolation of the neuropil.

With the exclusion of the offspring of monkeys 6 (twelve days old and with macroscopic edema) and 4 (the abortion), the other five cerebrums from the dead offspring in the lead-exposed group were reduced both

in mean weight (15.1 g) (table 3), in comparison with the mean control weight of 18.7 (range 18–20) g ($N = 5$) (formalin-fixed ($P < 0.05$), and in mean antero-posterior length [41 (range 36–46) mm] in comparison with the control length of 46.6 (range 44–48) mm ($N = 5$) ($P < 0.05$).

Tissue lead concentrations

The lead concentrations in the tissues of the dead offspring are shown in table 3 (cerebrum) and table 4 (other tissues). High lead concentrations were found in the placenta and in the fetal skeleton, when compared with the level in the cerebral tissue. The four PbB values from fresh stillbirths and a neonatal death (offspring 5, 7, 9, and 10) amounted to a mean of 85.5 (range 65–95) % of the PbB levels of their respective mothers 1 d later.

Table 3 includes lead concentrations in the cerebrum as determined by our laboratory. The German control analyses were, on the average, 102.5 (range 58–120) % of our values. Cerebral tissue from two of the five newborn controls, and also from an unexposed 21-week-old squirrel monkey fetus, was prepared and analyzed according to the procedure already mentioned for the lead-exposed offspring. These samples of cerebral control tissue contained 0.02, 0.02, and < 0.015 μg of lead/g of wet weight, respectively. Fresh cerebral tissue, prepared from unexposed newborn and fetal rats and analyzed in our laboratory, had lead concentrations between 0.015–0.008 $\mu\text{g/g}$ of wet weight.

Lead analyses of milk from mothers 7 and 8 were performed 11 and 21 d after the births, respectively. Duplicate samples were taken with an interval of 1 d. The lower of the two resulting values was used, since the higher values were obviously a result of contamination. The lead concentration of the milk was 63.8 % (0.3/0.47 $\mu\text{g/g}$) and 60.6 % (0.2/0.33 $\mu\text{g/g}$), respectively, of the simultaneous PbB value of the mothers.

Other measurements

The mean head circumference, at a postnatal age of 14 weeks, for the five surviving lead-exposed offspring was 15.8 (range 15–17) cm, compared to 16.8 (range 16–18) cm ($N = 10$) for a group of offspring born over the same period of time in the unexposed control group. This reduction was not statistically significant. However, those offspring with the highest maternal mean PbB values [offspring 8 (female) and 12 (male)] had the smallest head circumferences (about 15 cm).

A preliminary, tentative evaluation of neurobehavioral effects in the lead-exposed offspring, measured in ongoing testing, is included in figure 3 in the Discussion section.

Discussion

The results of our study show that lead levels of 0.11–0.69 $\mu\text{g/g}$ in fetal brain and 0.39–0.82 $\mu\text{g/ml}$ in maternal blood in the squirrel monkey are associated with increased mortality and cerebral damage in the offspring.

Lead doses and blood and tissue lead concentrations

It should be noted that some of the variation in the PbB values seen in figure 1 is not compatible with the half-time of lead in blood which we observed for each individual monkey the first month after the end of exposure (the group mean for the half-time being about one month), and it is therefore likely to reflect errors in blood sampling and analyses.

The fact that higher lead doses were required to increase the PbB to high subclinical values in several pregnant monkeys, compared with those of the male monkeys of the pilot study, and the tendency of the PbB concentration to level off, can be interpreted in several ways. First, it could mean that these pregnant females, at that stage of pregnancy, had a comparatively lower intestinal absorption of lead than the males and thereby indirectly protected the fetus. On the other hand, the higher doses per kilogram of body weight for the pregnant females could actually correspond to a higher uptake of lead per kilogram of body weight, which, however, is quickly redistributed from the blood stream. If this was the case, lead could either be excreted in high concentrations in the urine or be transferred to other tissues, ie, those of the mother and maybe, to some extent, to those of the rapidly growing placenta/fetus, which could thereby accumulate large amounts of lead.

The placenta and the fetus, especially the skeleton, held high lead concentrations already at 14.5 weeks of gestation, following five weeks of lead exposure (table 4, offspring 4). The pattern of lead distribution in the fetal tissues, with high concentrations in the skeleton and liver (table 4), mimics that seen in lead-exposed human fetuses of comparable age (22, 25). The fact that the PbB levels in the stillbirths (offspring 5, 7, and 9) and the offspring that died the first day after birth (offspring 10) were similar to the lead levels in the blood samples collected 1 d later from their mothers (see table 4) strongly suggests an extensive transplacental passage of lead, at least during late pregnancy. This finding is in accordance with results obtained in human studies (1, 2, 6, 8, 12, 16, 18, 21, 23, 27, 37, 41, 52, 61).

In a general female population PbB levels decrease progressively throughout pregnancy, possibly partly due to the deposition of blood lead in the placenta and the fetus (1, 2). Increased lead concentrations have been noted in human stillbirths (5) and in the placentas of human stillbirths and neonatally dead (56).

Lead concentrations in breast milk in the present study were about 60 % of the maternal PbB values during the weeks immediately after birth, both for a mother with a stillbirth (offspring 7) and for a mother with a surviving offspring (number 8). This source of lead could not have affected offspring 10, since it did not consume any milk during its 14 h of postnatal life.

Effects on the pregnant mother

The normal weights of the mothers after birth, and the almost normal food consumption during pregnancy in the lead-exposed group, excludes general under- or malnutrition as a probable cause of the reported effects. This finding is contrary to that of studies with smaller experimental animals, eg, rats, in which the very high PbB values required to obtain detectable lesions in the developing nervous system (3–5 $\mu\text{g/ml}$) have often been associated with severe malnutrition, uncertainty thereby being introduced as to the cause of the neurological effects. (See, eg, reference 59.) Considering the corresponding decrease in hematocrit and the normality of other blood indices, the low hemoglobin values of monkeys 4, 9, and 11 were probably the result of hemodilution. The normal rate of reproduction which followed the end of lead exposure indicates that the fertility of the exposed females was not affected negatively.

Effects on pregnancy and the fetal brain

Pre- and perinatal mortality of the offspring. The clinically manifest abortion of monkey 4 occurred at 14.5 weeks of gestation. The peak PbB value of about 0.7 $\mu\text{g/ml}$ (two weeks prior to this), the decrease in maternal body weight, and the hyalinization of the placenta indicate that the toxic insult could have occurred some time before the clinically manifest abortion. A similar latency has been documented in clinical studies (35).

The pre- and perinatal mortality rate in this study is comparable to that in previous studies of women exposed to industrial lead — printing, pottery — either directly or indirectly, through their husbands. (See reference 39 for references of studies between 1901–1930. There is a paucity of quantitative data on lead exposure in these early studies.) More recently, Wilson (58) reported on a case concerning a woman with an intrauterine fetal death at 33 weeks of gestation, probably due to a high lead concentration in the drinking water. Her PbB value was $0.55 \pm \text{about } 0.2 \mu\text{g/ml}$ during the first few weeks after the abortion. Valentino et al (54) reported that a woman lead worker with a history of one abortion and two intrauterine deaths (the last of these occurring at 38 weeks of gestation) had a PbB level probably somewhere between 0.5–0.6 $\mu\text{g/ml}$ at the time of the abortion.

Reduced birth weight and prematurity. The mean gestational length of 147.5 d (21 weeks) in the lead-exposed group was slightly lower than the mean length of 152 (SD 3.9) d which has been reported for a large unexposed group of captive Bolivian squirrel monkeys (48). However, comparisons with a series of unexposed squirrel monkey fetuses of known ages (unpublished results) showed that the foot length, body weight, and cerebral weight of the lead-exposed offspring were small at birth for the length of gestation (ie, similar to normal fetuses of about 20 to 21, 20 and 18 to 21 or more weeks of age, respectively). This finding indicates a prenatal growth retardation which does not spare the cerebrum. It should be noted that the high cerebral weights of offspring 6 and 7 could have been related to cerebral edema.

Cerebral effects. The characteristic gross and microscopic cerebral lesions had features in common with those of encephalopathy seen in human infants perorally exposed to lead (4, 32, 33, 36). Such lesions have not previously been convincingly linked to prenatal lead exposure. However, the two brains with high cerebral weights, ie, with a "macrencephalic" condition (offspring 6 and 7), are perhaps similar in nature to the form of macrocephaly (and macrencephaly), combined with seizures, which was described by Rennert (38). He found this form, often congenital, among children whose parents had been intoxicated by lead in pottery glazing.

Reduced cerebral weight, ie, mild "micrencephaly," as was seen in the rest of the deceased lead-exposed newborns ($P < 0.001$, if offspring 6 and 7 are excluded), has not been reported previously with peroral prenatal lead exposure. However, it has recently been produced by the intraperitoneal administration of lead to guinea pigs (11). Indications of brain-growth retardation were also found in a study on chickens (17) and, indirectly, in the often reported general fetal growth retardation (= fetal or neonatal body weight reduction) following lead exposure. The reduction of the head circumference of the surviving offspring in the present study was not statistically significant, but it corresponded well with the magnitude of the prenatal maternal mean PbB values.

Other studies of cerebral effects following prenatal lead exposure

Clinical studies on humans. Only three cases of well-documented prenatal lead exposure in association with suspected cerebral damage, diagnosed on the basis of mental or neurological symptoms in the child, have been reported (13, 34, 45). In the case reported by Palmisano et al (34) the cerebral effects may have been due to prenatal ethanol exposure. In these three cases, the maternal PbB values during pregnancy were 0.60–0.76 $\mu\text{g/ml}$, and the neonatal PbB values of the children were 0.50–0.66 $\mu\text{g/ml}$, ie, within the range

of the mean PbB values found in the present study. The exposure to lead was thought to have occurred partly or wholly during the last trimester. No neuropathology seems to have been reported in these or other cases of human prenatal lead exposure, with the possible exception of the cases presented in Rennert's study (38).

Studies on experimental animals. The results from animal experiments have not been used to any large extent in assessments of risks to the human fetus, eg, in evaluations of the aforementioned epidemiologic hypothesis that low or moderate lead levels in prenatal tissue may cause cerebral damage. Few of these studies have reported the PbB concentrations. Almost no studies at all have reported brain or placental lead concentrations, and no study has described pathological effects on the placenta. Pathological effects on the cerebrum have only been described in studies with parenteral or in ovo administration of the lead, resulting in very high lead concentrations in the blood and tissue (26, 46).

Furthermore, most of the animal models would appear to be inadequate for the purpose of testing the hypothesis that such lead levels can cause negative effects on higher mental or neurological functions in the human child, eg, mild mental retardation or disturbances in fine hand movements. Rodents and most other animals a priori lack the neurodevelopmental processes critical for the creation, as well as the retardation, of these sophisticated forms of neurobehavioral functions, which exist only in primates. (See also the Introduction.)

Studies on subhuman primates. Tachon et al (49) daily administered 5 mg or 1 mg of lead acetate/kg intramuscularly to 11 pregnant monkeys (*Macaca irus*). The 5-mg dose induced abortion and was lethal to the mother. No fetal effects were detected macroscopically, and no PbB values were reported at this dose. With a daily dose of 1 mg/kg, PbB values of 1.16 (SD 0.49) $\mu\text{g/ml}$ were measured in the mother — concentrations which were lethal to the mother after birth — and PbB values of 1.39 (SD 0.51) $\mu\text{g/ml}$ were measured in the newborn. Much higher peak values immediately following the injections cannot be excluded. "Erythrodiapedesis" was noted in the cerebrums of the offspring before or just after birth, but no further characterization was given. This occurrence may be pathological and of a similar nature as the hemorrhages noted in the present study.

Levin & Bowman (24) daily administered lead acetate doses of 3, 6, or 9 mg/kg (averages) to eight pregnant rhesus monkeys (*Macaca mulatta*) via drinking water, from three months prior to conception and throughout pregnancy. On the basis of the body weights of female *Macaca mulatta* (between 4.4–10.7

kg, according to reference 30) the absolute daily lead dose per monkey can be calculated to be up to 21.6–52.6 mg, ie, about half of the doses administered in the present study. The monkeys were divided into two subgroups, with mean PbB values of 0.40 $\mu\text{g}/\text{ml}$ and 0.54 $\mu\text{g}/\text{ml}$, respectively, during pregnancy. The authors did not report any data on the individual prenatal blood lead values of the mothers. No abortions were noted, and the offspring did not show any adverse effects on health, body weight, or behavior. The organ lead levels and neurohistology of the offspring were not studied.

The following factors could explain the differences between the study of Levin & Bowman (24) and the present study: the different species of monkey used and the fact that our monkeys were caught wild (55), possible differences in prenatal PbB curves, and possible adverse effects in their study on the unfertilized egg or lethal effects on the early embryo, which would act to "sort out" lead-sensitive individuals. Furthermore, some experimental data on rodents suggest that the toxic effects of lead can be reduced by prior low-level lead exposure (40).

Pathogenesis of effects and dose-effect relationships

Pathogenesis. The cerebral damages that occurred were probably the result of a direct lead effect on the cerebral vessels and the cerebral parenchyma, either by itself or in combination with secondary effects via damage of other organs such as the placenta. The tissue distribution of lead, being low in the cerebrum and high in the placenta, and a severe retardation of placental growth (unpublished results) suggest the latter hypothesis. The lead-exposed pregnant monkeys were handled more than those in the unexposed group, with more weighings and blood samplings, but this

difference is unlikely to be involved in the pathogenesis of the cerebral effects.

Blood lead concentration. The blood indices indicated that the anemia, which was seen in a few of the lead-exposed pregnancies, was mainly due to hemodilution. If the maternal PbB values from the pregnancies are corrected for possible variation in hemodilution (ie, mean PbB value/mean hemoglobin value in table 3 or the mean corpuscle lead concentration in table 2), they seem to correlate better with the mortality and severity of cerebral damage of the offspring. (See table 3.) However, there was an individual variation in sensitivity to lead in the blood among the monkey pregnancies, as illustrated by the different fetal effects in pairs of pregnancies with similar PbB means and PbB curves: numbers 1 and 2, 7 and 8, and 10 and 12 (figure 1, table 3 and figure 3).

Cerebral lead. In the earlier mentioned cases of encephalopathy in human infants perorally exposed to lead (see the heading "Cerebral Effects" earlier in the Discussion section), and in early postnatal lead encephalopathy in experimental animals (14, 50), lead levels in brain tissue have been reported to be between 1.5–25 $\mu\text{g}/\text{g}$. The brain lead concentrations in the present prenatal exposure study were, however, only between about 0.1 and 0.7 $\mu\text{g}/\text{g}$. The brain lead concentrations for the unexposed squirrel monkey fetuses and newborns were around 0.01 $\mu\text{g}/\text{g}$ or lower, a value similar to that found in humans (19) and rats. Exposure of inbred rats, early postnatally, with resulting brain lead concentrations, PbB values, and brain : blood lead ratios within or slightly above the

Figure 3. Tentative summary of the relation between prenatal lead dose (from table 3) and cerebral effects. The cerebral effects are numbered according to severity as follows: 0 = reduction of aggressive behavior, 1 = 0 + other behavioral disturbances (hyperactivity or seizures), 2 = 0 + neurological disturbances (visuomotor) and mild microcephaly, 3 = mild microcephaly and pre- or perinatal death, 4 = 3 + hemorrhages, 5 = 3 + plentiful hemorrhages, 6 = macrocephaly (edema or suspected edema). (1 μg Pb/ml blood = 4.826 μmol Pb/l blood, 1 μg zinc protoporphyrin/ml blood = 1.597 μmol zinc protoporphyrin/l blood)

ranges in the present study has not produced any morphological effects (9, 15, 29).

These results suggest that the fetal brain is even more sensitive to lead than the infant brain, which is considered to be more sensitive than the adult brain. Furthermore, a species difference in cerebral lead sensitivity is suggested by the clinical and experimental data; the sensitivity seems to be higher in primates, including man, than in rodents.

Zinc protoporphyrin in the blood. The zinc protoporphyrin values, measured in pregnancies 9–12, seemed to correlate with the cerebral effects (figure 3 and table 3). The offspring with the two highest maternal zinc protoporphyrin values (offspring 11 and 10; peak values 1.2 and 1.4 $\mu\text{g}/\text{ml}$, respectively; see figure 2) showed characteristic encephalopathic lesions in the brain. The offspring with the lowest maternal value (offspring 12, peak value 0.7 $\mu\text{g}/\text{ml}$) has not yet reached the age for neurobehavioral testing.

The zinc protoporphyrin values measured in our study are similar to those determined among fertile women workers in a Swedish industry between 1977 and 1979 (57), in which the mean values ranged from 0.58 to 0.83 $\mu\text{g}/\text{ml}$ and a small group of the women had peak values between 1.2 and 3.3 $\mu\text{g}/\text{ml}$. The same hematofluorometer was used as in the present study.

Final comment

A no-effect lead level for the fetus could not be found within the dose range used in the present study, with exposure during the last two-thirds of gestation. It cannot be excluded that the fetus may be even more sensitive to lead during early gestation. Furthermore, the fetus is not only at risk following lead exposure of the mother during pregnancy, since lead which has accumulated in a fertile, nonpregnant woman can probably be mobilized at pregnancy, with increased PbB and fetal damage as a result (51). An adequate animal model, perhaps the squirrel monkey, might produce information essential to the assessment of risks incurred from lead exposure of the human fetus.

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