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Transplacental Movements of Inorganic Lead from Mother to Fetus (40762)[1]

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For more than 100 years lead has been recognized as detrimental to the human fetus, mainly as a result of industrial exposure (1). Many effects of lead on fetal systems have been reported in humans and in rodents used in experimental teratology, but the mechanism of such effects remains enigmatic.

Lead acetate in a dietary concentration of 0.5% is clearly embryotoxic in mice (2, 3), and lead acetate and lead nitrate have also been shown to be embryotoxic in the rat (4, 5). Like many teratogens, lead produces malformations at specific gestational periods. Single lead nitrate doses of as little as 35 mg/kg administered on Day 9 of gestation in the rat produce teratogenic events. From Days 10 to 15 of gestation, lead nitrate is increasingly embryotoxic, but not teratogenic. Central nervous system lesions are produced by as little as 15 mg/kg of lead nitrate on Day 16 of gestation. After Day 16, fetal toxicity decreases (5).

Lead nitrate, chloride, and acetate are all teratogenic in the golden hamster at a dose of 50 mg/kg (6). Further investigations in the golden hamster showed that the teratogenic effect of lead is organ specific. Effects are almost completely restricted to the tail region during the critical stage of embryogenesis in the hamster (Day 9 of gestation) (7). Teratogenic effects of lead at a single dose of 50 mg/kg are also highly strain specific in the golden hamster (8).

A few specific metabolic effects have been identified in lead-intoxicated laboratory rodents. In the mouse embryo, lead intoxication leads to impaired synthesis of heme, which seems to limit their body growth during later gestational periods (9). Administration of lead to pregnant mice also leads to a decrease in placental blood flow, on both a per-embryo and a per-embryo-weight basis. Because decreased blood flow does not affect the uptake of a nonmetabolizable amino acid (α-aminoisobutyrate), it is not apparent whether there is sufficient blood flow reduction to adversely affect the fetus (10). Even though very limited data are available on specific effects of lead on the developing fetus, it is apparent that prenatal lead exposure leads to detrimental behavioral changes postnatally (11).

Although there is little doubt that lead crosses the hemochorial placenta, such investigations are limited in number. Studies of the lead content of human fetuses as well as maternal and fetal blood lead levels show conclusively that the human fetus can be exposed to inorganic lead as a result of maternal lead exposure (12–14). Placental transfers of lead have been measured in the rat by the use of fetal lead uptake. In all cases, substantial amounts of lead were transferred to the fetus after maternal exposure (5, 15, 16). In the rat, lead was found in the fetus within minutes of maternal i.v. injection (5), indicating that lead gains easy access to the fetus. Autoradiographic studies of inorganic lead transfer across the placenta of the golden hamster at Days 7–8 of gestation showed that lead gained access to the fetus within 15 min of maternal i.v. injection. Most of the lead seemed to be present in the yolk sac placenta and not in the chorio-allantoic placenta (17). Thus, it appears that, at least in the golden hamster, the yolk sac placenta constitutes a predominant pathway in the exposure of the fetus to lead.

In this study, we have investigated the clearance of lead from mother to fetus across the hemochorial placenta of the guinea pig, independent of the presence of the fetus.

Materials and methods. Strain 13 guinea pigs were bred, allowed to farrow, and bred

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again so that the gestational age was known within 24 hr. The animals were from a closed colony at the Comparative Animal Research Laboratory in which full-term pregnancies last approximately 68 days. All the guinea pigs used were approximately 60-days pregnant with a range between 59 and 61 days.

The perfusion technique and calculations used in these experiments were identical to those previously used in the study of mercury and cadmium transport across the placenta (18–20). Figure 1 is a schematic diagram of the perfusion system. The pregnant dam, anesthetized with a combination of sodium phenobarbital injected i.p. and Innovar-Vet (Pitman–Moore) injected i.m., was tied in a supine position to a plastic rack. The rack was placed in a glass tray containing mammalian Ringer solution that covered the lower legs and part of the posterior abdomen. One uterine horn was exteriorized through an incision in the lateral abdominal wall and one fetus was exposed through an incision in the uterine wall and amniotic sac. The uterus, placenta, and fetus were immersed in the Ringer's solution, which was heated to 39°C. Connective tissue surrounding the umbilical vessels of the fetus was stripped away, and the vessels were tied off near the fetus. An umbilical artery was cannulated with a hubless needle connected to polyethylene tubing; the cannula was attached to Tygon tubing and passed through a peristaltic pump to a reservoir in the water bath. The venous cannula was elevated to a height sufficient to maintain the venous pressure at approximately normal levels. Outflow from the placenta was passed through the venous cannula and collected in 1-ml aliquots.

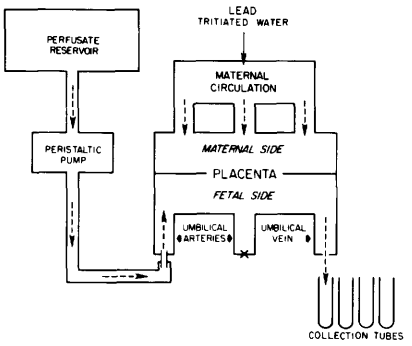

FIG. 1. Schematic diagram of the perfusing system.

After injection of 20 μCi of ^{210}Pb in the form of lead nitrate (specific activity, 33.33 mCi/mg) and 5 μCi of tritiated water into the maternal jugular vein, 0.75-ml maternal blood samples were drawn from a maternal carotid cannula at appropriate intervals. Nine to ten samples were collected in each experiment. Immediately after collection, maternal blood samples were centrifuged, and an aliquot of plasma was obtained. Perfusion pressure, maternal cardiac rate, ECG, blood pressure, and respiratory rate were monitored continuously during each perfusion; abnormal variation of these measurements led to rejection of the data obtained from that animal.

Perfusion rates were varied between 0.48 and 5.08 ml/min. The perfusing solution consisted of sterile, filtered guinea pig serum (pH 7.35). Perfusion samples were collected beginning 30 min after injection of radiolead to avoid sampling during the initial rapid decrease in maternal plasma radiolead. After a single pass, the perfusate was analyzed for radiolead and tritium. Radiochemical analyses were made on triplicate samples of maternal plasma and perfusing solution.

Clearance of tritiated water and radiolabeled lead from maternal to fetal circulations was calculated by the formula:

$$Clearance = P/M \times R,$$

where *clearance* is milliliters of maternal plasma containing an amount of tritium or radiolead equal to that entering the perfusate per minute (ml/min) and P is the tritium or radiolead concentration (μCi/ml) measured in the perfusate. For tritium, M is the mean tritium concentration (μCi/ml), determined from a series of linear approximations of a graph of maternal tritium concentrations vs time. For radiolead, M is the mean radiolead concentration (μCi/ml) of maternal plasma during the collection time of each sample as determined from a series of monoexponential approximations of maternal radiolead concentrations vs time. R is the umbilical flow rate (ml/min).

Data were analyzed by regression

TABLE I. RELATIONSHIP BETWEEN LEAD CLEARANCE AND UMBILICAL FLOW RATE

Dam	Placenta	a^a	b^a	P	r^b	Estimated lead clearance^c ± SE
1	1	0.017	0.007	<0.15	0.73	0.033 ± 0.002
	2	−0.003	0.038	<0.025	0.89	0.091 ± 0.010
2	1	0.015	0.012	<0.025	0.63	0.045 ± 0.005
3	1	−0.013	0.026	<0.01	0.62	0.051 ± 0.012
4	1	0.010	0.013	<0.001	0.95	0.041 ± 0.002
5	1	−0.015	0.024	<0.001	0.95	0.046 ± 0.007
6	1	0.012	0.008	<0.05	0.59	0.032 ± 0.006
	2	−0.003	0.029	<0.001	0.93	0.068 ± 0.008
7	1	0.010	0.013	<0.001	0.70	0.041 ± 0.004
	2	0.001	0.027	<0.001	0.97	0.067 ± 0.007

[a] $Y = a + bX$, where Y = lead clearance and X = perfusion rate.
[b] Correlation coefficient for the regression.
[c] Lead clearance ± SE (milliliters per minute) at a perfusion rate of 2.5 ml/min, as estimated from the regression of lead clearance on perfusion rate.

analysis with a linear model (21). Previous experience has shown that in most experiments tritium clearance is a linear function of umbilical flow rate (18–20). In five of ten perfusions, tritium clearance ceased to be a linear function of umbilical flow rate before the end of the experiment. In such perfusions, only data from those portions of the experiment in which tritium clearance was a linear function of umbilical flow rate are reported.

Results. A total of 213 perfusion samples were collected from ten placentas in seven dams. Clearances of radiolabeled lead presented in Table I correlated significantly with perfusion rate in 9 of 10 perfusions. Table I includes an estimation of radiolead clearance (± SE) from each perfusion at an umbilical flow rate of 2.5 ml/min, which is within the range of the reported umbilical artery flow rate for guinea pigs at 60 days of gestation (22). Results of a typical experiment are shown in Fig. 2. When the data from all perfusions were pooled, radiolead clearance measured 0.047 ± 0.004 ml/min (± SE) at an umbilical flow rate of 2.5 ml/min, as calculated from the regression of radiolead clearance on umbilical flow rate.

The clearance of labeled water was linearly related to umbilical flow rate in all ten experiments during initial time periods. In five of the ten experiments this linear relationship did not remain throughout the entire perfusion period. Changes in maternal blood flow to the placenta are most likely a significant source of variation from the linear relationship between tritium clearance and umbilical flow rate (18–20, 22). When the regression equations in Table II are used to calculate tritium clearances at a constant perfusion rate, tritium clearance

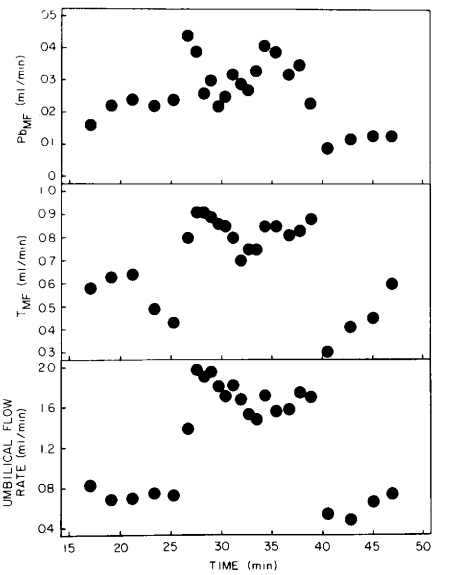

FIG. 2. Results of a typical experiment: Umbilical flow rate, tritium clearances, and lead clearances.

TABLE II. RELATIONSHIP BETWEEN TRITIUM CLEARANCE AND PERFUSION RATE

Dam	Placenta	a[a]	b[a]	P	r[b]	Estimated tritium clearance[c] ± SE
1	1	−0.195	0.436	<0.05	0.89	0.896 ± 0.086
	2	−0.028	0.433	<0.05	0.69	1.055 ± 0.241
2	1	0.981	0.714	<0.001	0.90	2.766 ± 0.108
3	1	0.390	0.460	<0.05	0.55	1.540 ± 0.212
4	1	1.060	0.373	<0.001	0.94	1.972 ± 0.067
5	1	−0.093	0.466	<0.025	0.88	1.072 ± 0.203
6	1	0.090	0.681	<0.001	0.98	1.791 ± 0.037
	2	−0.224	0.983	<0.001	0.96	2.233 ± 0.194
7	1	0.338	0.299	<0.005	0.88	1.086 ± 0.113
	2	0.133	0.277	<0.025	0.80	0.825 ± 0.157

[a] $Y = a + bX$, where Y = tritium clearance and X = perfusion rate.
[b] Correlation coefficient for the regression.
[c] Tritium clearance ± SE (ml/min) at a perfusion rate of 2.5 ml/min, as estimated from the regression of tritium clearance on perfusion rate.

becomes a function of radiolabel supplied by the maternal circulation only. In experiments examining radiocadmium clearance from mother to fetus, we reported a significant linear relationship between the estimated radiocadmium clearance and the estimated tritium clearance at an umbilical flow rate of 2.5 ml/min, which predicted the relationship between the two measurements for individual perfusion well (18). This relationship indicated that a great deal of the variability in cadmium clearance measurements was due to changes in maternal blood flow to the placenta. In the present measurements with lead, regression analysis showed no relationship between the estimated radiolead clearance and the estimated tritium clearance at an umbilical flow rate of 2.5 ml/min ($P < 0.40$), indicating that little of the variability in the lead clearance measurements was due to differences in maternal blood flow to placentas.

Discussion. There is little doubt that inorganic lead gains easy access to the developing fetus of species containing hemochorial placentas. However, the means by which this occurs have only been investigated in a single study with hamsters, in which the primary means of access seemed to be the yolk sac placenta (5). Our data show that lead readily crosses the hemochorial placenta of the rodent. For an umbilical flow rate of 2.5 ml/min, the clearance of lead (0.047 ± 0.004 ml/min) was approximately four times that of inorganic mercury. Clearances of methylmercury, cadmium, and calcium from mother to fetus are approximately three, ten, and five times greater, respectively, than that of lead measured under similar conditions. Clearances of both organic and inorganic mercury are not correlated with umbilical flow rate, while clearance of cadmium from mother to fetus is. With conditions similar to these experiments, mercury (with a relatively low clearance rate) appears to move from mother to fetus by a membrane-limited process (18–20), while cadmium (with a much higher clearance rate) appears to move from mother to fetus by a flow-limited process (18). Lead seems to be somewhat unique in that its clearance is relatively low, but the relationship between lead clearance and umbilical flow leads to the conclusion that lead moves from mother to fetus by a process limited by umbilical flow.

Summary. Placental transport of lead in the guinea pig was measured by perfusing the fetal circulation of placentas (at 60 days of gestation) *in situ*. The clearance of lead from mother to fetus was linearly related to umbilical flow rate and measured 0.047 ±

0.004 ml/min at an umbilical flow rate of 2.5 ml/min. Our data indicate that lead crosses the placenta from the maternal circulation to the fetal circulation at a lower clearance rate than either cadmium or methylmercury but at a greater rate than inorganic mercury.

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