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Passage of Cadmium Across the Perfused Guinea Pig Placenta[1] (39877)

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There is extensive documentation in the literature which implicates cadmium as a causative or contributing agent in human diseases including renal dysfunction, osteomalacia, and hypertension. Very little is known, however, about chronic exposures to low levels of cadmium such as we might find in cadmium-polluted environments. Since background levels of cadmium are steadily increasing with growing usage of the metal, it seems especially important to gain more knowledge of the effects of cadmium on mammalian systems (1).

The widespread belief that the placenta constitutes an "effective barrier" against the transfer of cadmium at low concentrations to the developing fetus is based primarily on reports of low accumulations of cadmium in fetuses of several species (1-5). For example, at 12-days gestation fetuses from rats previously dosed with 0.1-0.6 mg of Cd/kg accumulated between 0.0001 and 0.0095% of the maternal dose, while placentas accumulated 0.063-0.206% (6). Yet high doses of cadmium (0.88-30 mg/kg maternal body weight) have been teratogenic or fetotoxic to hamsters (7, 8), rats (4), and mice (9), as well as the cause of fetal death by a peculiar necrosis in the fetal vessels of the placenta (10). Despite existing literature, only limited data are avialable on cadmium transport across the placenta since most studies of cadmium transfer to the fetus are confounded by an inability to separate fetal uptake and placental transport. We have examined transport of cadmium in the maternal-fetal direction across the perfused placenta of the guinea pig, a species with placental structure histologically similar to man.

Materials and methods. Strain 13 guinea pigs were bred, allowed to farrow, and bred again so that gestational age was known within 24 hr. The animals were from an inbred colony at the Comparative Animal Research Laboratory in which full-term pregnancies last approximately 68 days. All of the guinea pigs used were approximately 60 days pregnant with a range between 59 and 61 days.

The perfusion technique used in these experiments is similar to one previously described (11). A schematic diagram of the perfusion system is shown in Fig. 1. The pregnant dam was anesthetized with a combination of sodium pentobarbital injected ip and Innovar-Vet injected im. The anesthetized dam, tied to a plastic rack in a supine position, was placed in a glass tray containing mammalian Ringer's solution that covered the lower legs and part of the posterior abdomen. The Ringer's solution and perfusing solution were maintained at 39° during each experiment.

The lateral abdominal wall was incised, one uterine horn was exteriorized, and an incision was made through the uterine wall and amniotic sac, exposing one or two fetuses. The uterus, placentas, and fetuses were immersed in the Ringer's solution. Connective tissue surrounding the umbilical vessels of one fetus was stripped away, the vessels were tied off near the fetus, and an umbilical artery was cannulated with a hubless needle connected to polyethylene tubing. The arterial cannula was attached to Tygon tubing and passed through a peristaltic pump and then to a reservoir placed in the water bath (11). The venous cannula was elevated to a height that maintained the venous pressure at approximately its normal level (12). Outflow from the placenta passed through the venous cannula and was collected in 3-ml portions. After injection of 100 μCi of ^{115}Cd (sp act 0.42 mCi/mg) and 5 μCi of tritiated water into the maternal jugular vein, 0.75-ml maternal blood samples were drawn from the carotid cannula at

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10-min intervals; five to nine 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; if any of these measurements varied abnormally, the data from that animal were discarded.

Perfusion rates were varied between 1.15 and 3.50 ml/min. Perfusion samples were collected from 15 min after injection of radiocadmium so that sampling during the initial rapid decrease in maternal plasma radiocadmium was avoided. After a single pass, the perfusate was analyzed for radiocadmium and tritium. The perfusing solution consisted of sterile, filtered guinea pig serum with a pH of 7.35. Radiochemical analyses were made on triplicate samples of maternal plasma, whole blood, and perfusing solution.

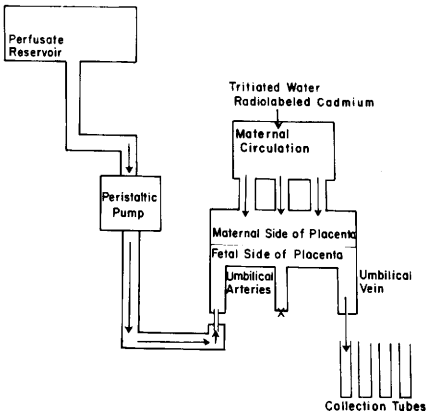

FIG. 1. Schematic diagram of the perfusing system.

Clearance of tritiated water and radiolabeled cadmium from maternal blood was calculated by the following formula:

$$Clearance = (P/M)\ R,$$

where *clearance* is milliliters of maternal plasma containing an amount of radiocadmium or tritium equal to that entering the perfusate per minute (milliliters per minute), and P is the tritium or radiocadmium concentration (microcuries per milliliter) measured in the perfusate. For tritium, M is the average tritium concentration (microcuries per milliliter) as determined from a series of linear approximations of a graph of maternal tritium concentration versus time. For radiocadmium, M is the average radiocadmium concentration (microcuries per milliliter) of maternal plasma during the collection time of each sample as determined from a series of monoexponential approximations of maternal radiocadmium concentrations versus time. R is the rate of flow of the perfusion sample (milliliters per minute).

Results. A total of 145 perfusion samples was collected from 10 placentas in six dams. Clearances of radiolabeled cadmium, presented in Table I, correlated significantly with perfusion rate in 9 out of 10 perfusions. Included in Table I is an estimate of radiocadmium clearance (±SE) from each perfusion at a flow rate of 2.5 ml/min. This flow

TABLE I. RELATIONSHIPS BETWEEN CADMIUM CLEARANCE AND PERFUSION RATE.

Dam	Placenta	a[a]	b[a]	P	r[b]	Range of perfusion rate (ml/min)	Estimated Cd clearance[c]
1	1	0.187	0.044	>0.05	0.28	1.76-2.95	0.300 ± 0.068
1	2	−0.056	0.130	<0.001	0.67	1.78-2.91	0.271 ± 0.017
2	1	0.043	0.025	<0.005	0.49	1.15-3.47	0.106 ± 0.008
2	2	0.099	0.018	<0.05	0.31	1.68-3.44	0.144 ± 0.011
3	1	−0.122	0.289	<0.001	0.62	1.89-3.50	0.601 ± 0.041
3	2	0.065	0.410	<0.001	0.85	1.27-2.11	1.089 ± 0.044
4	1	0.128	0.030	<0.01	0.57	1.76-2.83	0.202 ± 0.008
5	1	−0.109	0.164	<0.001	0.91	1.38-3.19	0.302 ± 0.011
6	1	0.069	0.159	<0.001	0.72	1.70-2.46	0.466 ± 0.034
6	2	0.049	0.036	<0.05	0.50	1.40-2.00	0.137 ± 0.028

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

TABLE II. RELATIONSHIP BETWEEN CLEARANCES OF LABELED WATER AND PERFUSION RATE.

Dam	Placenta	a^a	b^a	P	r^b	Estimated tritium clearance[c]
1	1	0.196	0.633	<0.01	0.47	1.78 ± 0.15
1	2	−1.075	0.948	<0.001	0.84	1.30 ± 0.10
2	1	1.376	0.123	>0.05	0.13	1.44 ± 0.14
2	2	1.129	−0.223	<0.001	0.68	0.57 ± 0.06
3	1	0.113	0.833	<0.001	0.83	2.20 ± 0.08
3	2	−1.246	1.349	<0.001	0.80	2.13 ± 0.33
4	1	−1.741	1.306	<0.001	0.96	1.52 ± 0.13
5	1	−1.094	1.010	<0.001	0.86	1.43 ± 0.09
6	1	0.573	0.188	<0.001	0.68	1.04 ± 0.27
6	2	0.053	0.098	>0.05	0.29	0.30 ± 0.36

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

rate was chosen as a reference point since it is within the range of reported umbilical artery flow rates in guinea pigs at 60-days gestation (13) and is easily attainable in most perfusion experiments. When the data from all perfusions were pooled, radiocadmium clearance was 0.361 ± 0.003 ml/min (±SE) at 2.5 ml/min as calculated from the regression equation of radiocadmium clearance on perfusion rate.

As previously reported (11, 14) the clearance of labeled water was linearly related to perfusion rate in almost all perfusions (8 of 10). Changes in maternal blood flow to the placenta are most likely a significant source of variation from the linear regression in the measurements summarized in Table II. When the regression equations in Table II are used to calculate tritium clearances at a constant perfusion rate, tritium clearance becomes a function of radiolabel supplied by the maternal circulation only. There was a significant ($P < 0.005$) linear relationship between the estimated radiocadmium clearances and the calculated tritium clearances at a perfusion rate of 2.5 ml/min which predicted the relationships between the two measurements for individual perfusions well ($r = 0.65$). The linear relationship between radiocadmium clearance and tritium clearance at 2.5 ml/min indicates that much of the variability in the measurements of cadmium clearance was due to changes in maternal blood flow to the placenta and/or differences in placental exchange surfaces. The use of clearance of tritiated water as a baseline measurement to allow for variability in maternal blood flows and/or differences in placental exchange surface areas is similar in principle to the use of clearance of antipyrine proposed by Dancis *et al.* (15). When the data from all perfusions were pooled, the clearance of labeled water measured 1.51 ± 0.07 (±SE) at 2.5 ml/min.

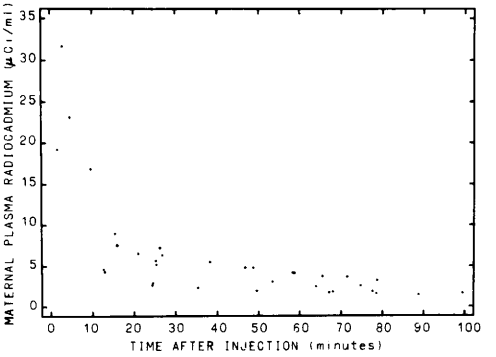

FIG. 2. Maternal plasma cadmium as a function of time after injection.

Radiocadmium was rapidly removed from the maternal circulation. Figure 2 represents the pooled data from all experiments measured in microcuries of radiocadmium per milliliter of maternal plasma as a function of time after cadmium injection.

Discussion. Our data show that the widely accepted premise that there is a strong "placental barrier" to the movement of cadmium across the placenta may not be correct. The clearance of cadmium, 0.36 ± <0.01 ml/min (±SE), at a perfusion rate of 2.5 ml/min was more than two times as large as the clearance of methylmercury (11), 0.15 ± 0.01 ml/min (±SE), and larger than

the clearance of calcium (12), 0.27 ml/min, measured under similar conditions. The differences in observed clearances between cadmium and methylmercury are equally apparent when they are compared to the baseline measurements used in both studies. In this study, the clearance of cadmium (0.36 ml/min) at 2.5 ml/min was 24% of the clearance of labeled water (1.51 ml/min) at 2.5 ml/min when the data from all perfusions were pooled. In a previous study, the clearance of methylmercury (0.15 ml/min) was 10% of the clearance of labeled water (1.51 ml/min) at 2.5 ml/min when the data from all perfusions were pooled. Since considerable amounts of both methylmercury (16) and calcium (17) accumulate in fetuses late in gestation, it is apparent that there is no strong "placental barrier" to these materials and thus there is most likely little barrier to cadmium movements across the placenta.

Cadmium clearance was linearly related to perfusion rate, unlike methylmercury and calcium clearances, which are independent of perfusion rate (11; Twardock, personal communication). In this sense, cadmium clearance appeared to be more nearly like the clearance of labeled water, which was also linearly related to perfusion rate.

Even though the cadmium present in the maternal circulation readily crossed the guinea pig placenta, it is apparent from the literature that the fetus accumulates little cadmium from an iv dose. Figure 2 shows that cadmium, introduced by iv injection into the maternal blood stream, is quickly removed from maternal plasma. This cadmium is most likely sequestered in the liver and kidney by the protein, metallothionien, as has been shown in other studies (18; cited review articles). The implication of these data is that, although the fetus is normally exposed to very little cadmium, any pathological condition such as disease or malnutrition which might affect maternal metallothionien could expose the developing fetus to greatly increased amounts of cadmium.

Summary. By perfusing the fetal circulation of placentas (61- to 63-days gestation) *in situ,* we were able to separate fetal uptake from placental transport of cadmium in the guinea pig. Our data indicate that cadmium crosses the placenta from the maternal circulation to the fetal circulation with relative ease. Since cadmium from an iv dose was rapidly removed from the maternal circulation, low levels of transfer to the fetus reported in the literature for many animals are more likely due to low levels of circulating maternal plasma cadmium than to the presence of a strong "placental barrier" to cadmium.

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