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The Effects of $CdCl_2$ on the Maternal-to-Fetal Clearance of ^{67}Cu and Placental Blood Flow[1] (41853)

R. J. CHERTOK,[2] B. KULLGREN, AND D. BURBANK

Oak Ridge Associated Universities, Medical and Health Sciences Division, Oak Ridge, Tennessee 37831

Abstract. Copper is an essential element while Cd is an extremely toxic heavy metal of questionable biological usefulness. Cadmium has been reported to interfere with the metabolism of Cu, be teratogenic, and decrease blood flow in the fetal placenta. Because of these reported biological interactions of Cd and Cu, this investigation was conducted to determine the effects of Cd on placental transport of ^{67}Cu and placental blood flow in the guinea pig. All guinea pigs used were 60 ± 1 days pregnant. A placental perfusion technique was used to measure the maternal-to-fetal clearance of ^{67}Cu and $^{3}H_2O$ across the placenta. The clearance of $^{3}H_2O$ served as an indicator of placental blood flow on the maternal side of the circulation. The results indicated that an iv injection of 1 mg Cd/kg body weight resulted in an immediate increase in the clearance of ^{67}Cu which declined over the next 8 min to an elevated level compared to the extrapolated best-fit curve of control values. This iv injection of $CdCl_2$ concomitantly reduced the maternal-to-fetal clearance of $^{3}H_2O$ across the placenta. In conclusion, an acute exposure of the pregnant female to $CdCl_2$ results in an increased maternal-to-fetal clearance of ^{67}Cu and a reduced placental blood flow that can alter the supply of nutrients to the developing embryo or fetus, and therefore modify normal development.

Copper is an essential element, but is also potentially toxic. Because of this dual property, homeostatic mechanisms have evolved to compensate for excesses or deficiencies of copper by controlling intestinal absorption and excretion.

After copper is absorbed, it is transported bound to plasma protein (1) and perhaps to amino acids (2). Shortly thereafter, almost all of the copper is deposited in the liver (3) where approximately 80% is bound to hepatocuprein (4), copper chelatin (5), and metallothionein (6) in the cytosol. The remaining 20% is found in lysosomes (7) or incorporated into other specific copper proteins such as cytochrome C oxidase (8). Copper deficiency is a rare condition because most diets supply a relative abundance of the metal (9). Although the normal infant is born with a store of copper (10), deficiencies have been associated with anemia, neutropenia, and severe demineralization of the bone in premature and full-term infants (11, 12). Similarly, copper toxicosis is rare but does occur where a genetic defect in copper metabolism is present (Wilson's disease) (13).

Cadmium is an extremely toxic heavy metal of questionable biological usefulness (14). In addition to its presence in products such as fungicides, fertilizers, pigments, metal alloys, plastics, coal, and fuel oil, it is also a by-product of recovery processes for zinc, lead, and copper. Cadmium is a cumulative poison, and no apparent homeostatic mechanism operates to maintain body concentrations at an approximately constant level independent of intake (15).

The reported intestinal absorption of Cd is 1 to 8% of the dose (15–19). However, other studies have indicated that absorption can be influenced by dietary constituents such as the essential metallic ions zinc, iron, copper, and calcium (20).

Cadmium has been reported to interfere with the metabolism of copper by reducing its intestinal absorption and increasing its urinary excretion (21). Rats fed 1.5-μg Cd/g diet with a normal copper content (3-μg Cu/g diet) had a reduced ceruloplasmin activity, whereas rats on a diet of 6- or 18-μg Cd/g diet and a normal Cu content had a considerably reduced plasma and liver Cu concentration and serum

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[2] To whom all correspondence should be addressed.

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ceruloplasmin activity (22). Bone malformations from simple Cu deficiency were also noted. Similar effects of Cu deficiency resulting from Cd intoxication have been observed in pregnant ewes and lambs (23).

Cadmium has been reported to be teratogenic in the golden hamster (24) and mice (25). Gerber *et al.* have reported that lead, another heavy metal, reduced placental blood flow but did not change fetal uptake of α-amino isobutyrate in mice (26). Levin and Miller have also reported that Cd decreased blood flow to the fetal placenta 12 to 16 hr and 18 to 24 hr after sc injection of $CdCl_2$ into rats (27).

Because of the reported biological interactions of Cd and Cu, this investigation was designed to determine the effects of Cd on the placental transport of ^{67}Cu and placental blood flow in the guinea pig.

Materials and Methods. Strain 13 guinea pigs from a closed colony maintained at Oak Ridge Associated Universities, Medical and Health Sciences Division, were used for these studies. The guinea pigs were bred, allowed to farrow, and bred again so that the gestational age was known within ±24 hr. All guinea pigs used were 60 ± 1 days pregnant.

The placental perfusion technique used was adapted from that described by Twardock (28) and modified by Kelman (29). The dams were anesthetized with sodium pentobarbital (28 mg/kg) injected ip and Innovar-Vet (Pitman-Moore, 0.44 ml/kg) injected im and secured to a plastic rack in a supine position. A jugular vein was cannulated, and a slow, continuous iv drip of Ringer's lactate solution was maintained throughout the experiment. A carotid artery was cannulated to obtain blood samples at 9- to 10-min intervals. Then the guinea pig was placed in a bath of Ringer's solution maintained at 37°C. A uterine horn was exteriorized by laparotomy and a fetus was exposed by an incision through the uterus and amniotic sac. The uterus, placenta, and fetus were immersed in the Ringer's solution. The umbilical vessels were stripped of connective tissue and ligated near the fetus. Then an umbilical artery was cannulated with a hubless needle fastened to tubing connected to a peristalic pump and a reservoir containing the perfusion fluid (guinea pig serum) maintained at 37°C. An umbilical vein was cannulated to collect the perfusion fluid at 4-min intervals. Perfusion rates were held steady at 0.7 ml/min. Preliminary experiments indicated that perfusion of the placenta at rates greater than 1 ml/min for extended periods (1 to 2 hr) allowed radiolabeled albumin to pass from the perfusing fluid into the maternal circulation. Also, perfusion at a rate of 0.7 ml/min could be maintained for periods as long as 2 hr without an abrupt change in the placental transport of ^3H_2O from dam to fetus. At the start of the perfusion, 100 μCi of ^3H_2O and 20 μCi of $^{67}CuCl_2$ were injected into the dam via the cannulated jugular vein. Perfusate sample collections were started 28 min later to allow time for equilibration of the ^{67}Cu in the animal. Twenty-four minutes after starting the perfusate collection, 1 mg Cd/kg body weight was injected into the cannulated jugular vein. Collection of perfusate samples continued for an additional 28 min.

The data are expressed as the clearance of tritiated water or ^{67}Cu from the maternal to fetal circulation. The formula used for the calculation was

$$\text{Clearance} = P/M \times R,$$

where clearance is milliliters of maternal plasma that contained the amount of ^3H or ^{67}Cu that entered the perfusate in 1 min (ml/min), P is the perfusate concentration of ^3H or ^{67}Cu, M is the maternal plasma concentration of ^3H or ^{67}Cu, and R is the perfusate rate. The M values for ^3H and ^{67}Cu are approximations based on extrapolations between the encompassing plasma concentration values to the mean time of each perfusate collection.

Results. The data from seven animals have been normalized to reduce the animal-to-animal variability associated with placental blood flow and placental transport mechanisms. The data for ^{67}Cu clearance from 4 to 24 min were fitted to the exponential equation $y = ae^{-bx}$ where a is the intercept, b is the slope, and x is time. The data were then normalized to the value at 4 min as determined from the equation. The data for ^3H_2O were normalized in the same manner except that the data were fit to the linear equation $y = a + bx$. It has been demonstrated that the clearance of ^3H_2O is a linear function of perfusate flow rate and has been suggested that variations in ^3H_2O

clearance are the result of variations in the placental blood flow from the maternal circulation (29).

The clearances of ^{67}Cu and $^{3}H_2O$ across the guinea pig placenta from dam to fetus and the effects of an iv dose of $CdCl_2$ (1 mg Cd/kg body weight) are illustrated in Figs. 1 and 2. The clearance of ^{67}Cu declined with time according to the equation $y = ae^{-bx}$ where $a = 0.1942$ and $b = 0.0207$ (Fig. 1). The r^2 value for goodness of fit was 0.965. Twenty-four minutes after the start of perfusate collection, $CdCl_2$ was injected and the clearance of ^{67}Cu increased dramatically. This increased clearance of ^{67}Cu declined for approximately the next 8 min and then assumed a parallel, but elevated, level compared to the best-fit curve extrapolated to 52 min. These data indicated an immediate effect of $CdCl_2$ on the transport properties for ^{67}Cu in the placenta.

The results of $CdCl_2$ injection on the clearance of $^{3}H_2O$ are illustrated in Fig. 2. For the 24 min prior to injection of $CdCl_2$, the clearance of $^{3}H_2O$ was a linear function with time, and the data were fitted to the equation $y = ax + b$, where $a = 0.6454$ and $b = 0.0024$. The r_2 value was 0.7201. Immediately following the injection of $CdCl_2$, there was a decline in clearance of $^{3}H_2O$ for 4 min, after which the clearance essentially followed the same pattern as before the injection of $CdCl_2$. However, the clearance of $^{3}H_2O$ was at a lower level, indicating a reduced maternal blood flow to the placenta.

Discussion. In the experiments reported here, the injection of $CdCl_2$ resulted in an immediate increase in the clearance of ^{67}Cu (Fig. 1) and an immediate decrease in the clearance of $^{3}H_2O$ (Fig. 2). Although the clearance of ^{67}Cu returned to a lower level, it remained elevated compared to the extrapolated values derived from data collected prior to the injection of $CdCl_2$. The increase in ^{67}Cu clearance is even more evident when the decreased load of ^{67}Cu delivered to the placenta as a result of the decreased blood flow is considered. These data indicate $CdCl_2$ has an immediate effect on the placental blood flow as well as an alteration of the transport properties of the placenta for ^{67}Cu.

By injecting Cd directly into the fetus, Levin and Miller (30) have shown that fetal death in the rat is not the result of direct effects of Cd on the fetus. They have also suggested that

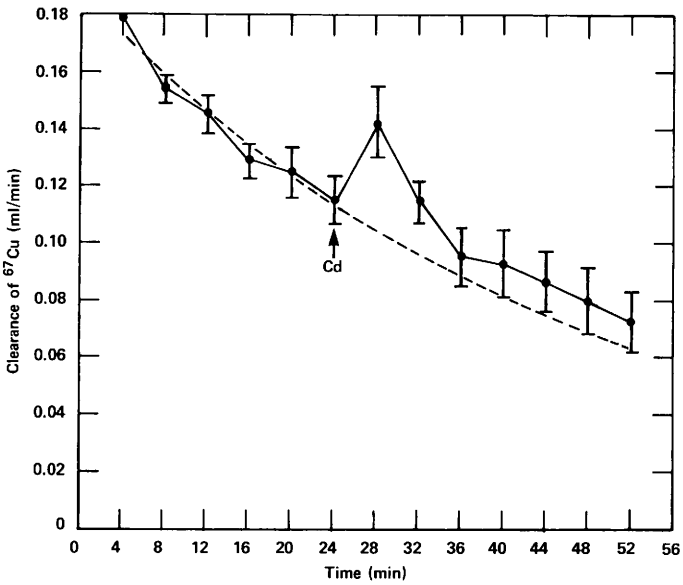

FIG. 1. Clearance of ^{67}Cu (±SEM) as a function of time. Arrow indicates time of iv injection of 1 mg Cd/kg body weight. The broken line represents the data for 0 to 24 min fitted to the equation $y = ae^{-bx}$, where $a = 0.1942$, $b = 0.0207$, and x = time. The value of $r^2 = 0.965$. Each point is the mean of 7 determinations.

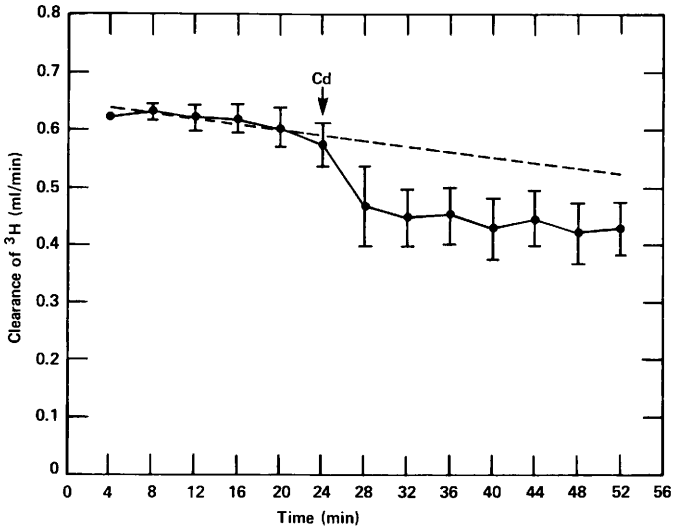

FIG. 2. Clearance of ^{3}H (±SEM) as a function of time. Arrow indicates time of iv injection of 1 mg Cd/kg body weight. The broken line represents the data for 0 to 24 min fitted to the equation $y = ax + b$, where $a = 0.6454$, $b = 0.0024$, and x = time. The value of $r^2 = 0.7201$. Each point is the mean of 7 determinations.

fetal death does not result from renal toxicity of Cd (27).

Gerber *et al.* (26) investigated the effects of dietary lead on placental blood flow and the fetal uptake of α-amino isobutyrate in mice. A microsphere technique was used to measure placental blood flow. Their results indicated lead caused a reduced blood flow to the placenta but did not alter the fetal uptake of α-amino isobutyrate on a per-weight basis.

Levin and Miller (27), also using a microsphere technique, investigated the effects of Cd on the fetal placental blood flow in the rat. On day 18 of gestation, sc injection of 40 μmole of Cd/kg did not statistically reduce the placental blood flow 8 to 10 hr later. However, 12 to 16 hr and 18 to 24 hr after the Cd treatment, the rats experienced a reduced blood flow to the fetal placenta of 40 and 73%, respectively.

Levin and Miller (31) have reported that following sc injection, the placenta accumulated Cd more rapidly than all maternal organs except the liver. They further postulated that the high concentrations of Cd in the placenta might result in cellular damage to the trophoblastic tissue or to the fetal vascular endothelium. Since fetal development depends on a delicately balanced supply of nutrients, an interruption or alteration in this supply could cause a retardation, abnormal growth, or death of the developing embryo or fetus. These experiments indicate that an acute exposure of the pregnant female to $CdCl_2$ results in an increased maternal-to-fetal clearance of ^{67}Cu and a reduced blood flow to the placenta and thus alters the supply of nutrients to the developing embryo or fetus, which could alter the normal progress of development.

1. Bearn AG, Kunkel HG. Metabolic studies in Wilson's disease using Cu^{64}. J Lab Clin Med **45**:623–631, 1955.
2. Neumann PZ, Sass-Kortsak A. The state of copper in human serum: Evidence for an amino acid-bound fraction. J Clin Invest **46**:646–658, 1967.
3. O'Reilly S, Pollycove M, Tono M, Herradora L. Abnormalities of the physiology of copper in Wilson's disease. II. The internal kinetics of copper. Arch Neurol. **24**:481–488, 1971.
4. Mann T, Keilin D. Haemocuprein and hepatocuprein, copper-protein compounds of blood and liver in mammals. Proc R Soc London Ser B **126**:303–315, 1938.
5. Morell AG, Shapiro JR, Scheinberg IH. Copper binding protein of human liver. In: Walsh JM, Cummings JN, eds. Wilson's Disease. Some Current Concepts. Oxford, Blackwell, pp36–42, 1961.
6. Evans GW. Copper homeostasis in the mammalian system. Physiol Rev **53**:535–570, 1973.

7. Sternlieb I, van den Hamer CJA, Morell AG, Alpert G, Gregoriadis G, Scheinberg IH. Lysosomal defect of hepatic copper excretion in Wilson's disease (hepatolenticular degeneration). Gastroenterology **64**:99–105, 1973.
8. Dallman PR. Cytochrome oxidase repair during treatment of copper deficiency: Relation to mitochondrial turnover. J Clin Invest **46**:1819–1827, 1967.
9. Scheinberg IH, Sternlieb I. Copper metabolism. Pharmacol Rev **12**:355–381, 1960.
10. Wilson JE, Lakey ME. Failure to induce dietary deficiency of copper in premature infants. Pediatrics **25**:40–49, 1960.
11. Cordano A, Placko RP, Graham GG. Hypocupremia and neutropenia in copper deficiency. Blood **28**:280–283, 1966.
12. Ashkenazi A, Levin S, Djaldetti M, Fishel E, Benvenisti D. The syndrome of neonatal copper deficiency. Pediatrics **52**:525–533, 1973.
13. Sternlieb I. Evolution of the hepatic lesion in Wilson's disease (hepatolenticular degeneration). Progr Liver Dis **4**:511–525, 1972.
14. Schroeder HA, Nason AP, Tipton JH, Balassa JJ. Essential trace metals in man: Zinc-relation to environmental cadmium. J Chron Dis **20**:179–210, 1967.
15. Cotzias CG, Borg DC, Selleck B. Virtual absence of turnover in cadmium metabolism: ^{109}Cd studies in the mouse. Amer J Physiol **201**:927–930, 1961.
16. Decker CF, Byerrum RU, Happert CA. A study of the distribution and retention of cadmium-115 in the albino rat. Arch Biochem Biophys **66**:140–145, 1957.
17. Richmond CR, Findlay JS, London JF. Whole-body retention of cadmium-109 by mice following oral, intraperitoneal and intravenous administration. U.S. Atomic Energy Commission, University of California. Los Alamos Scientific Publication LA-3610-MS, Los Alamos, New Mexico, pp195–200, 1966.
18. Miller WJ, Blackmon DM, Martin WF. Cadmium absorption, excretion and tissue distribution following single tracer oral and intravenous doses in young goats. J Dairy Sci **51**:1836–1839, 1968.
19. Doyle JJ, Pfander WH, Grebing SE, Pierce JO. Effect of dietary cadmium on growth, cadmium absorption, and cadmium tissue levels in growing lambs. J Nutr **104**:160–166, 1974.
20. Bremner I. Heavy metal toxicities. Quart J Biophys **7**:75–124, 1974.
21. Anke M, Henning A, Schneider HJ, Ludke H, Von Cagern W, Schlegal H. The interrelations between cadmium, zinc, copper and iron in metabolisms of hens, ruminants and man. In: Mills CF, ed. Trace Element Metabolism in Animals. Edinburgh, Livingston Press, p317, 1970.
22. Campbell JK, Mills CF. Effects of dietary cadmium and zinc on rats maintained on diets low in copper. Proc Nutr Soc **33**:15A–17A, 1974.
23. Mills CF, Dalgarno AC. Copper and zinc status of ewes and lambs increased dietary concentrations of cadmium. Nature (London) **239**:171–173, 1972.
24. Holmberg RE, Ferm VH. Interrelationships of selenium, cadmium, and arsenic in mammalian teratogenesis. Arch Environ Health **18**:873–877, 1969.
25. Schroeder HA, Mitchener M. Toxic effects of trace elements on the reproduction of mice and rats. Arch Environ Health **23**:102–106, 1971.
26. Gerber G, Maes J, Deroo J. Effect of dietary lead on placental blood flow and on fetal uptake of α-amino isobutyrate. Arch Toxicol **41**:125–131, 1978.
27. Levin AA, Miller RK. Fetal toxicity of cadmium in the rat: Decreased utero-placental blood flow. Toxicol Appl Pharmacol **58**:297–306, 1981.
28. Twardock AR, Austin MK. Calcium transfer in perfused guinea pig placenta. Amer J Physiol **219**:540–545, 1970.
29. Kelman BJ, Walter BK. Use of perfusion in the study of placental transport of mercury and cadmium. In: Mahlum DD, Sikov MR, Hackett PL, Andrew FD, eds. Developmental Toxicology of Energy-Related Pollutants. Oak Ridge, Tennessee, U.S. DOE/TIC, Conf-771017, p555, 1978.
30. Levin AA, Miller RK. Fetal toxicity of cadmium in the rat: Maternal vs fetal injections. Teratology **22**:1–5, 1980.
31. Levin AA, Miller RK. Fetal toxicity of cadmium in the rat: Time course of placental uptake of Cd. Abstract, Society of Toxicology, 19th Annual Meeting, 1980.

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