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The Influence of Zinc on the Ontogeny of Hepatic Metallothionein in the Fetal Rat[1] (41234)

V. L. CHARLES-SHANNON,[2] L. B. SASSER, D. K. BURBANK, AND B. J. KELMAN[3]

Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830

Abstract. The ontogeny of hepatic metallothioneins (Mt) in fetal tissue as related to dietary and hepatic Zn was investigated. Sixty 6-month-old female rats were divided into two groups and given either double-distilled water or water containing 700 μg of Zn per milliliter. Dams from each group were killed on 16, 19, or 21 days of gestation, and maternal and fetal livers were removed. Mt content of the tissue was estimated by Piotrowski's Hg-saturation method. Results established the presence of an endogenous hepatic Mt in the fetal rat as early as 16 days of gestation. We further demonstrated a marked progressive increase in fetal Mt from Day 16 through gestation accompanied by a decrease in maternal hepatic Mt. It is suggested that Zn increased fetal Mt by inducing fetal synthesis, redistributing fetal Mt, or increasing Mt transport to the fetus, because both fetal and maternal hepatic Mt were increased. Fetal hepatic Mt concentration was several times greater than maternal Mt at corresponding stages of gestation. Mt may serve to either ensure adequate storage of Zn or Cu for fetal development or protect the fetus against metal toxicity, but the significance of these high endogenous levels of fetal Mt are not clear at this time.

Metallothioneins (Mt) are low-molecular-weight cytoplasmic proteins that bind heavy metals and whose synthesis is induced in mammalian tissues by certain heavy metals. The physiological function of Mt has not been definitely established (1, 2), but several roles have been proposed since the initial identification of Mt in 1957 (3). Mt is able to bind and store such nutrients as Zn and Cu (4–6), and, in addition, may also act as regulator proteins in Zn and Cu absorption and metabolism (7, 8). Several investigators have proposed that Mt may play a role in the detoxification of heavy metals or offer protection from their toxic effects (9–14).

Hepatic Mt concentrations are greater in the newborn than in the adult rat (15–18) but approach adult levels within 4 weeks of age. High levels of Mt have also been found in fetal hepatic tissues associated with Cd, Cu, and Zn (19–24). Bell (24) reported much greater concentrations of a Mt-like protein in fetal liver than in maternal liver and suggested that the association of endogenous Zn with this protein indicates that fetal Mt may be involved with the regulation or storage of fetal hepatic Zn. Zinc status has been shown to influence the incorporation of Zn and Cd into Mt (25). Williams *et al.* (26) indicated that few attempts have been made to delineate those stages of pregnancy at which marginal deficiencies of essential metals may have the most profound effect on the development of the fetus. Because Zn is readily transferred across the placenta and is intimately involved in the physiological processes of Mt synthesis, we have investigated the ontogeny of hepatic Mt in fetal tissue as related to dietary and hepatic Zn.

Materials and Methods. Six-month-old female Sprague-Dawley rats, averaging approximately 265 g in weight, were caged with a male overnight. Pregnancy was confirmed the following day by the presence of copulation plugs and this day was designated as Day 0 of gestation. The animals

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[2] Oak Ridge Associated Universities Faculty Participant at the Comparative Animal Research Laboratory, Oak Ridge, Tenn. 37830.

[3] Present address: Battelle, Pacific Northwest Laboratories, P.O. Box 999, Richland, Wash. 993522.

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were housed in galvanized wire cages (four per cage) and provided *ad libitum* a Zn-adequate commercial diet containing 50 ppm Zn. Sixty pregnant rats were divided into two groups and given either double-distilled water (controls) or water containing 700 μg of Zn per milliliter as $ZnCl_2$ (treated) beginning Day 0 of gestation. Water consumption by treated animals was recorded daily and Zn intakes were calculated. An accumulative average of 235, 380, and 590 mg of Zn was consumed per rat in the Zn-treated group from the drinking water at 16, 19, and 21 days gestation, respectively. Dams from each group were sacrificed on 16, 19, or 21 days of gestation after methoxyflurane anesthesia. Cesarian sections were performed and the fetuses and chorioallantoic placentas were removed following hysterectomy; maternal livers were also removed. The fetuses were decapitated and the fetal livers were removed. Fetal livers and placentas were pooled for each litter. All tissues were frozen immediately with ethanol and dry ice and stored at −120°C until analysis.

Mt content of the tissues was estimated by a modified Hg-saturation method (27, 28). This method is based on Mt's high affinity for Hg and its stability when treated with trichloroacetic acid (TCA). The TCA precipitates most of the tissue debris and non-Mt-bound Hg, leaving Hg-bound Mt in solution. Thus, we measured the Hg-binding capacity of the nonprecipitable tissue homogenate. This was not pure Mt but was an estimate of the 6,000-molecular-weight thionein and had properties common with those previously reported for Mt; i.e., its synthesis was induced by Zn, had an apparent molecular weight of 10,000, showed an absorption maximum at 254 but not at 280, and was heat and acid stable (19).

To determine the appropriate amount of Hg to add to the tissue homogenates, Hg-saturation curves were plotted using the relationship between Hg addition and Hg uptake by the TCA supernatant (Figs. 1 and 2).

Maternal livers, fetal livers, and placentas were homogenized in 1.15% KCl (7 ml/g of tissue). Three and one-half milliliters of maternal homogenate was added to a test tube containing 280 μg of ^{203}Hg (2 μg/μl). One milliliter of 10% TCA was added to the solution to precipitate the protein and excess Hg, leaving the Mt-bound Hg in the supernatant. Fetal livers and placentas were treated in the same manner as maternal livers but because of limited tissue only 1 ml of homogenate was added to 80 μg of ^{203}Hg and precipitated with 0.3 ml of 10% TCA. In both cases the concentration of the Hg added was 80 μg/g of tissue homogenate. After centrifugation for 20 min at 105,400*g* the supernatant was counted for ^{203}Hg in a deep-well gamma-scintillation counter and the data were expressed as Hg-binding capacity (HgBC) of the nonprecipitable fraction (μg Hg/g wet tissue).

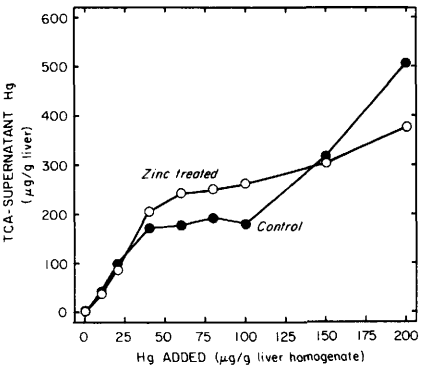

FIG. 1. Mercury saturation of the TCA supernatant following the addition of increasing amounts of ^{203}Hg to the liver homogenate of fetal rats at 21 days of gestation.

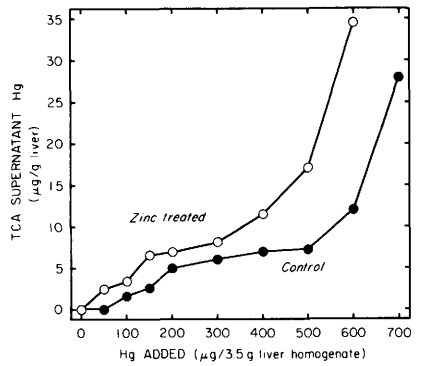

FIG. 2. Mercury saturation of the TCA supernatants following the addition of increasing amounts of ^{203}Hg to the liver homogenate of pregnant rats at 21 days of gestation.

The supernatant was also chromatographed on Sephadex G-75 columns previously calibrated with ovalbumin, ribonuclease A, and bacitracin. The columns were eluted with a citrate buffer at pH 2.2. Zinc concentration was determined in maternal livers, fetal livers, and placentas by atomic absorption spectrophotometry after nitric acid digestion. Quantities of 16-day-old fetal liver were insufficient for Zn analysis. Data were subjected to a two-way analysis of variance and differences between means were determined by the Student–Newman–Keul multiple-range test (29).

Results. Zinc treatment did not affect litter size, maternal or fetal liver weight, or chorioallantoic placenta weights (Table I). No teratological events were found upon gross examination; however, eight resorption sites were found in the Zn-treated group and six in the control group. Two animals were not pregnant when killed. As expected, the fetal liver and placenta increased in weight with advancing gestation but the maternal liver weight did not change (Table I).

A typical Hg-saturation curve for fetal liver homogenates is illustrated in Fig. 1 for 21-day control and Zn-treated fetuses. The Hg-saturation plateau of both treatments occurred between 40 and 100 μg of added Hg per gram of tissue. This is the region where sufficient Hg was added to react with all the Mt without saturating the proteins that were precipitated by the TCA. Similar results were found at 16 and 19 days gestation. The Hg-saturation plateaus of the maternal liver were similar to those of the fetal liver, although the Hg-binding capacity was much less (Fig. 2).

A typical gel filtration profile of fetal and maternal liver cytosol is shown in Fig. 3. Practically all of the Hg was associated with the 10,000-molecular-weight fraction, indicating that the assay is quite specific—a finding that is in agreement with data reported by Kotsonis and Klaassen (28). Thus, assuming Mt binds 7 moles of Hg/mole, Hg-binding capacity can be equated to total Mt, although it must be remembered that the preparation is not pure (2).

Zinc content and HgBC of maternal liver, fetal liver, and placenta are listed in Table II. Fetal liver HgBC (μg Hg/g tissue) was significantly greater ($P < 0.01$) than maternal liver HgBC for both treatment groups and at all stages of gestation. Maternal liver Zn concentrations were also lower ($P < 0.01$) than fetal liver Zn concentrations. However, total Hg-binding capacity (THgBC) and total Zn content of fetal liver was less ($P < 0.01$) than that of maternal liver at all stages of gestation because of decreased liver mass. Placental Zn concentrations were lower ($P < 0.01$) than either maternal or fetal liver Zn and placental HgBC was barely detectable.

Maternal liver HgBC of Zn-treated dams was significantly greater ($P < 0.01$) than that of control dams. Maternal liver HgBC of control rats decreased ($P < 0.05$) with advancing gestation, but liver HgBC of rats given supplemental Zn in their drinking water was increased ($P < 0.05$) at 21 days of gestation. Liver Zn concentration of control dams decreased ($P < 0.05$) with advancing stages of gestation, but the concentration in Zn-treated dams decreased ($P < 0.05$) only at 21 days of gestation.

Fetal liver HgBC of control and Zn-treated animals were the same at 16 days of gestation, but at 19 and 21 days the fetal liver HgBC of Zn-treated animals was greater ($P < 0.01$) than that of the controls. Fetal liver HgBC and fetal liver Zn concentration of both groups increased ($P < 0.01$) with advancing gestation. The THgBC of fetal liver increased with advancing gestation similar to HgBC, but was affected by Zn treatment ($P < 0.01$) only at 21 days gestation. Total fetal hepatic Zn also increased with advancing gestation ($P < 0.01$) but the slight increase due to Zn treatment was not significant ($P > 0.05$).

The HgBC of placental tissue was very small, but the concentration was greatest at 16 days of gestation and least at 19 days of gestation in both groups. No significant difference in placental Zn was observed between treatments, but placental Zn was greatest at 16 days of gestation.

Discussion. These data establish the presence of an endogenous hepatic Mt in the fetal rat as early as Day 16 of gestation and extend the data of earlier studies on Mt in the perinatal period. The data further

TABLE 1. LITTER SIZE, AND WEIGHT OF MATERNAL LIVER, FETAL LIVER, AND PLACENTA FROM ZINC-TREATED AND CONTROL RATS AT DAYS 16, 19, AND 21 OF GESTATION

Treatment	Day of gestation	Number of animals	Litter size (fetuses/litter)	Tissue weight (g/organ)			
				Maternal liver	Fetal liver	Placenta
Control	16	14	9.6 ± 0.9[a]	12.8 ± 0.5	0.074 ± 0.009	0.343 ± 0.025
	19	8	9.5 ± 1.0	13.4 ± 0.7	0.150 ± 0.003	0.674 ± 0.017
	21	8	11.0 ± 1.0	14.4 ± 0.9	0.277 ± 0.013	0.866 ± 0.013
Zinc treated	16	13	12.6 ± 0.6	12.9 ± 0.6	0.061 ± 0.008	0.331 ± 0.021
	19	8	11.6 ± 1.6	12.5 ± 0.2	0.144 ± 0.004	0.647 ± 0.031
	21	7	9.6 ± 1.6	12.9 ± 0.6	0.275 ± 0.033	0.857 ± 0.012

[a] Mean ± *SE*.

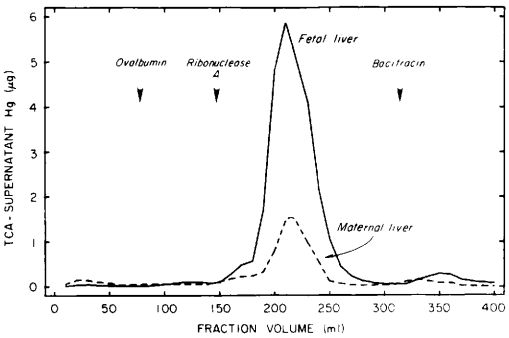

FIG. 3. Sephadex G-75 gel filtration profiles of TCA supernatant of fetal and maternal liver homogenates at 21 days of gestation.

demonstrated a marked progressive increase in fetal Mt from Day 16 through gestation. This increase may have been a response to an increase in fetal zinc.

Zn in the drinking water of pregnant rats may have induced the synthesis of fetal Mt and altered the distribution of Mt within the fetus or increased its transport to the fetus from the dam because both fetal and maternal hepatic HgBC were increased by Zn treatment. It is well known that Zn will induce Mt synthesis in the adult liver (6, 8, 30), but it is not clear if the increased Mt in the liver of these fetuses was of maternal or fetal origin. We have found that Cd (a strong inducer of Mt synthesis in the adult) injected directly into the 18-day-old rat fetus does not result in increased fetal Mt (31). Likewise, Cd injected subcutaneously into pregnant rats failed to induce the synthesis of fetal hepatic Mt (32).

Fetal hepatic HgBC was several times greater than maternal HgBC at corresponding stages of gestation. This elevated fetal Mt may be related to the elevated hepatic Zn of the fetus as compared to that of the dam, but the magnitude of this hepatic Zn difference was much less than the magnitude of difference in Mt. A good relationship existed between HgBC and Zn concentration in both maternal and fetal liver, although it was not as strong for THgBC and total Zn. Better correlations would be expected if Zn content of Mt rather than the concentration of the entire liver were used. The amount of other metals associated with Mt, such as copper, may be another variable influencing this relationship.

TABLE II. ZINC CONTENT AND HG-BINDING CAPACITY OF LIVER AND PLACENTA FROM PREGNANT AND FETAL RATS AT VARIOUS STAGES OF GESTATION

	Day of gestation	Maternal liver*		Fetal liver**		Placenta*	
		Control	+ Zn	Control	+ Zn	Control	+ Zn
HgBC (μg/g)	16	9.24 ± 0.65^{a}	13.1 ± 1.0^{d}	44.1 ± 2.4^{a}	51.2 ± 3.5^{a}	2.25 ± 0.33^{a}	3.11 ± 0.49^{e}
	19	6.90 ± 0.35^{b}	12.5 ± 2.3^{d}	119.4 ± 1.3^{b}	132.3 ± 5.7^{d}	1.19 ± 0.23^{b}	1.49 ± 0.27^{c}
	21	$4.70 \pm .074^{c}$	18.0 ± 2.3^{e}	148.7 ± 11.0^{c}	200.3 ± 10.6^{e}	$1.71 \pm 0.59^{c,d}$	1.95 ± 0.25^{d}
THgBC (μg)	16	118 ± 8.4^{a}	168 ± 13.4^{d}	3.29 ± 0.16^{a}	3.13 ± 0.21^{a}	0.771 ± 0.051^{a}	1.02 ± 0.071^{b}
	19	92 ± 4.7^{b}	157 ± 14.7^{d}	17.9 ± 0.22^{b}	19.5 ± 0.86^{b}	0.803 ± 0.031^{a}	$0.964 \pm 0.055^{a,b}$
	21	67 ± 10.1^{c}	231 ± 15.1^{e}	41.2 ± 3.13^{c}	55.0 ± 2.93^{d}	1.51 ± 0.23^{c}	1.67 ± 0.094^{c}
Zn (μg/g)	16	32.3 ± 0.9^{a}	30.7 ± 1.2^{a}	—	—	12.7 ± 0.5^{a}	12.8 ± 0.2^{a}
	19	27.2 ± 1.5^{b}	31.3 ± 0.7^{a}	45.5 ± 1.7^{a}	55.7 ± 2.4^{c}	11.4 ± 0.2^{b}	11.6 ± 0.2^{b}
	21	25.8 ± 1.8^{c}	28.2 ± 0.6^{b}	65.5 ± 2.2^{b}	72.5 ± 2.3^{d}	11.6 ± 0.6^{b}	11.3 ± 0.2^{b}
Total Zn (μg)	16	411 ± 12.1^{a}	395 ± 15.3^{a}	—	—	4.36 ± 0.17^{a}	4.25 ± 0.06^{a}
	19	364 ± 13.2^{b}	$391 \pm 9.2^{a,e}$	6.83 ± 0.25^{a}	8.00 ± 0.36^{a}	7.71 ± 0.16^{b}	7.49 ± 0.44^{b}
	21	344 ± 15.7^{c}	$374 \pm 15.5^{b,e}$	18.7 ± 0.70^{b}	20.4 ± 0.73^{b}	10.70 ± 0.51^{c}	9.70 ± 0.16^{c}

* Values in each grouping followed by the same superscript are not different ($P < 0.05$).
** Values in each grouping followed by the same superscript are not different ($P < 0.01$).

It is interesting that maternal hepatic HgBC decreased with advancing gestation in controls but not in Zn-treated dams, whereas fetal hepatic HgBC increased in both groups. Since maternal liver Zn also declined with advancing gestation in the controls, the Zn content of the diet may not have been sufficient to meet fetal demands without drawing on reserves of the maternal liver. If this is the case, then the significance of fetal Mt may be to regulate or retain Zn for fetal development.

Others (8, 33, 34) have found that the amount of Zn transported to the fetus increased with advancing gestation. Evans and Reis (35) noted that the rate of Zn turnover during pregnancy and lactation was twice as great as that in the nonpregnant mouse. This mobilization of Zn from the dam and increased uptake by the fetus coincides with the progressive increase in HgBC in the fetal rat liver during gestation. Mobilization of the maternal Zn stores may be related to the increasing demands of the fetus for Zn, which would suggest that fetal Mt is synthesized in response to increased fetal Zn.

Mt may have a significant role in the regulation or storage of Zn for fetal development, especially if available Zn from the maternal system is low, or for postnatal requirements during the nursing period when dietary Zn may be low. Further investigation is required to define the role of Mt in the fetal system regarding Zn metabolism. Mt may also be important in protecting the fetus against metal toxicity.

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