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Distribution of Mercury in Neonatal Guinea Pigs after Exposure to Mercury Vapor

Minoru Yoshida, Hiroshi Satoh,[1] Haruhiko Aoyama, Sakae Kojima, and Yukio Yamamura

Department of Public Health, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyame-ku, Kawasaki 213 and [1]Department of Hygiene and Preventive Medicine, Hokkaido University School of Medicine, Sapporo 060, Japan

The mammalian fetus and neonate is well known to be particularly susceptible to the toxic effects of heavy metals and other chemical substances and subsequently the potentially adverse effects appear in the developing individuals (Kacew et al. 1984). Exposure of the neonate to toxic metals occurs in the living environment through nursing and intake of metal-containing foods and water, and respiration in contaminated atmospheres.

Significant exposure of the neonate to mercury compounds, such as mercuric mercury and methylmercury, is often a potential hazard; higher gastrointestinal absorption of these compounds in suckling animals results in the more accumulation and retention of mercury than in the adult (Jugo 1979a; Sandstead et al. 1982). Elemental mercury vapor is also absorbed to an even greater extent than the inorganic mercury. Waffarn et al. (1979) reported that neonates in nursery incubators were exposed to mercury vapor generated from broken liquid mercury thermometers. However, it is still unknown how the toxic effects and metabolism of mercury vapor in immature animals differ from that in adults. The purpose of this study is to clarify the accumulation and distribution of mercury in neonatal animals after exposure to mercury vapor.

MATERIALS AND METHODS

Hartley strain guinea pigs in the late gestation period were purchased from the Saitama Agriculture Cooperative Association for Laboratory Animal (Saitama, Japan). They were housed at the Laboratory Animal Center of our university until they gave birth. Within 12 hours after

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Send reprint requests to Dr. M. YOSHIDA at the above address.

parturition, the dam and neonates were exposed to mercury vapor for 120 min in an exposure chamber as described previously (Yoshida et al. 1987). Mercury concentration in the air was measured once at 30 min by the air sampling method and was determined to be between 8 and 10 mg Hg/m^3. Immediately after the exposure, the dam and neonates were injected with an anesthetic dose of ketaminine hydrochoride and blood samples were drawn directly from the heart into heparinized glass tubes. Both the dam and neonates were killed by exsanguination and the brain, heart, lung, liver and kidneys were removed. Erythrocytes were separated from plasma.

The mercury concentration in the tissues and blood were determined by the reductive vapor-atomic absorption method after digestion with nitric acid in a Uniseal decomposition vessel (Uniseal Ltd., Israel) (Pan S-K et al. 1980). The activity of catalase in the blood and liver were determined according to the UV spectrophotometric method described by Aebi (1974). The metallothionein level in the liver was determined by the mercury-binding assay with a slight modification by Naganuma et al. (1987). Gel filtration of the liver supernatant was performed as follows: Maternal or neonatal liver was homogenized in a Teflon Potter-Elvehjem homogenizer with 0.25 M sucrose-50 mM Tris-HCl buffer, pH 7.4. The 25% (w/v) homogenate was centrifuged at 105,000 x g for 60 min. Four milliliters of soluble fraction was applied to a column of Sephadex G-75 and eluted at $4^{o}C$ with 50 mM Tris-HCl buffer at pH 7.4 containing 0.1 M NaCl. Fractions of 5.4 ml were collected at a flow rate of 24 ml/h.
The data were analyzed statistically using Wilcoxon's T-test.

RESULTS AND DISCUSSION

Elemental mercury that crossed the pulmonary membranes was rapidly oxidized into mercuric ions in the red blood cells. Nielsen-Kudsk et al. (1969) has reported that catalase in the red blood cells mainly participated in the oxidation of elemental mercury. Later investigations showed that the catalase-hydrogen peroxide intermediate, complex I, was important as the primary pathway in this process (Halback et al. 1978; Magos et al. 1978). Table 1 shows the activity of catalase in the blood and liver of dams and neonates of both control and exposed groups. In the blood the catalase activity is lower in neonates compared to the mothers. Neonatal liver also showed much lower activity of catalase, about one fourth that of in the mothers.

Table 1. Catalase activity of blood and liver in mother and neonate.

	Blood[a]		Liver[a]	
	Mother	Neonate	Mother	Neonate
Control group	100±68 (n=3)	52±42 (n=6)	315± 62[b] (n=3)	62±14 (n=6)
Exposed group	93±54 (n=3)	74±50 (n=11)	365±135[b] (n=3)	89±43 (n=11)

The number of animals is in parentheses.
Values are expressed as mean ± standard deviation.
a: $sec^{-1}.g^{-1}.ml$.
b: Averages of mothers and neonates of both control and exposed groups were significantly different by Wilcoxon's T-test ($p<0.01$).

Table 2 gives the mercury content in the blood after exposure to mercury vapor for two hours. The blood mercury concentration was calculated from the plasma and erythrocyte values and the hematocrit.

Table 2. Mercury exposure levels and mercury concentration in blood of mothers and neonates.

	Exposure levels (mg/m^3)	Mercury concentration (ng Hg/ml)			Ratio of E/P
		Whole blood	Plasma	Erythrocyte	
1 Mother	8	168	87	280	3.2
Neonate(n=4)		281±33	253±57	311± 56	1.2
2 Mother	8	184	148	226	1.5
Neonate(n=4)		302±51	275±42	334± 98	1.2
3 Mother	10	237	127	409	3.2
Neonate(n=3)		379±58	357±79	406±139	1.1

The number of animals is in parentheses.
Values are expressed as mean ± standard deviation.

Though lower activities of catalase occurred in neonatal blood and liver, the mercury concentration in the neonatal whole blood was approximately 64% higher than that in the mother. The mercury levels in the plasma of neonates were also two to three times higher than the concentrations in maternal plasma, but the erythrocytes levels were similar. So the differences could not be explained by the differences in the activities of catalase. As numerous investigators have already pointed out (Halback et al. 1978; Magos et al.

1978), the oxidation of elemental mercury may be due to the catalase-hydrogen peroxide intermediate, complex I, rather than the activity of catalase determined by the method used in this study.

The ratios of mercury concentration in erythrocytes to plasma in the column on the right-hand side of Table 2 was approximately 2.6 (1.5-3.2) for mothers and 1.2 (0.5-2.1) for neonates. An increased accumulation of mercury is found in neonatal plasma. Mercury levels in neonate plasma were also two times higher than the concentrations in maternal plasma but the erythrocyte levels were similar. In general, plasma is considered to be the main mediating pathway for the transport of metals. Therefore, the difference in plasma mercury levels between mother and neonate indicates the difference in transportability of mercury through the body.

Table 3. Distribution of mercury in organs of mother and neonate after mercury vapor exposure.

	Mercury concentration (ng Hg/g)				
	Brain	Lung	Heart	Liver	Kidney
1 Mother	87	7590	258	128	7390
Neonate(n=4)	119± 9	8260±1460	511±30	397±61	6080±912
2 Mother	141	5030	388	312	8120
Neonate(n=4)	194±32	8310±1220	590±94	329±35	3260±595
3 Mother	220	7000	1160	338	13500
Neonate(n=3)	250±10	13800±2110	1200±63	315±22	5000±191

The number of animals is in parentheses.
Values are expressed as mean ± standard deviation.

The mercury concentration in the major organs of the mother and neonate is shown in Table 3. Neonatal mercury concentrations in the brain were approximately 28% higher than those in the mother, and 58% higher in lung and 64% in heart. On the other hand, the accumulation of mercury in the kidneys was lower in neonates than in mothers. In the liver, mercury concentrations in the neonates were slightly higher or similar to those in the mothers. Thus, the distribution pattern of mercury was not different in mothers and neonates, but the mercury concentration in the tissues proved to be markedly different. Jugo et al. (1979b) and Webb et al. (1982a) reported that the accumulation of mercury, when Hg^{2+} was given orally or intraperitoneally, was higher in the brain, liver, and whole blood, but not in the kidneys, in neonatal rats

than in adults. The mercury concentration in the neonatal kidneys was much lower than in the maternal kidneys. Although there was a difference in the manner of mercury exposure, their findings coincided with ours. The lower kidney uptake of mercury in the neonate has been explained by the functional immaturity of kidneys at parturition, perhaps a low rate of the glomerular filtration. Therefore, the different accumulation of mercury in organs of the neonate and adult may depend on the differences in the renal function and the transportability from blood to tissues.

In the liver of developing mammals, a high concentration of metallothionein is known to exist in association with zinc and copper. The metallothionein has not only a function in copper and/or zinc homeostasis, but also in detoxification of a toxic heavy metals (Cherian et al. 1978; Webb et al. 1982b).

Table 4. Metallothionein concentration in liver of mother and neonate.

	Mother	Neonate
	(nmol Hg bound/g wet wt.)	
Control group	26± 7 (n=3)[a]	72±23 (n=6)
Exposed group	31±21 (n=3)[a]	133±95 (n=11)

The number of animals is in parentheses.
Values are expressed as mean ± standard deviation.
a: Averages of mothers and neonates of both control and exposed groups were significantly different by Wilcoxon's T-test ($p<0.05$).

Table 4 shows the metallothionein concentrations in maternal and neonatal liver of control and mercury-exposed guinea pigs. In the control and exposed group, metallothionein levels were significantly higher in neonatal liver than in maternal liver. However, there was no significant difference between the control and exposed groups in the metallothionein levels in neonatal liver.

A typical Sephadex G-75 elution profile of the liver-soluble fraction in a mercury vapor-exposed mother (the upper panel) and neonate (the lower panel) is shown in Fig. 1. In the profile of the dam, mercury was mostly associated with a high molecular weight protein which was eluted at the void volume of the column. Chromatograms of the neonatal liver were quite different from the mother's: large mercury peaks

appeared in the fractions having a molecular weight of 10,000-12,000, corresponding to the molecular weight of metallothionein. Since it is unclear whether a large amount of mercury bound to a metallothionein-like protein in the neonatal liver affects the retention of mercury in various tissues during development, this matter is also of importance from the point of view of toxicological implication in developing mammals.

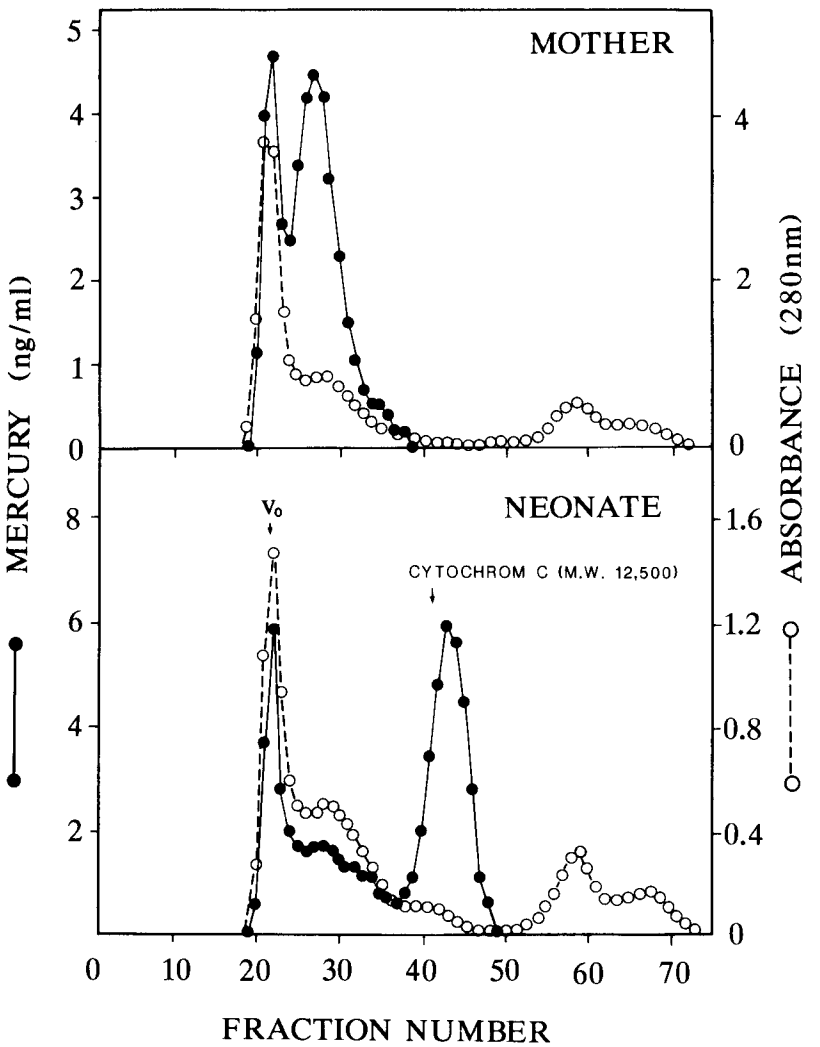

Fig. 1. Sephadex G-75 gel chromatography of mercury in the soluble fraction in the liver from mother (the upper panel) and neonate (the lower panel) of guinea pigs exposed to mercury vapor.

Although pulmonary ventilation is different in maternal and neonatal guinea pigs, there was a higher uptake of mercury in the brain, the critical organ for mercury vapor, in the neonate under the same mercury vapor exposure. The findings suggest that if organisms are accidentally exposed to a high concentration of mercury vapor, the central nervous system is more severely damaged in neonates than in adults.

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