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Factors Influencing Placental Transfer of Methylmercury in Man

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A case of fetal methylmercury (MeHg) poisoning was first described in Sweden (Engleson and Herner 1952) and the pathological findings from the autopsy of cases of fetal Minamata disease owing to environmental MeHg pollution, were reported in Japan (Takeuchi et al. 1964). Fetal MeHg poisoning was also reported in U.S.A. (Snyder 1971) and in Iraq (Amin-Zaki et al. 1974;Choi et al. 1978). Placental transfer and fetal toxicity of MeHg have been demonstrated experimentally in various species of animals (Berlin and Ullberg 1963;Moriyama 1967;Yang et al. 1972; Reynolds and Pitkin 1975). Mercury level in the cord blood was usually higher than that in the mother (Tejning 1968;Suzuki et al. 1971;Mitani et al. 1976). The difference in hemoglobin (Hb) concentration between newborns and their mothers (Suzuki et al. 1971), placental aging and decreased separation of maternal and fetal circulation (Olson and Massaro 1977) and lipid solubility of MeHg (Mansour et al. 1974) have been considered as the possible causes of higher mercury concentration in the fetal blood.

This report deals with the factors influencing placental transfer of MeHg in man and has a special emphasis on hematologic factors including erythrocyte count, Hb concentration and the binding affinity of Hbs to MeHg.

MATERIALS AND METHODS

Paired samples of cord blood from newborns and the blood from their mothers were collected at the delivery at the Hospital of Asahikawa Medical College during the period from December 1981 to November 1982. Except for a few cases of premature birth most samples were obtained from full term deliveries (Table 1).

Blood samples were stored at 5°C in a refrigerator for not more than 24 hr after delivery before assay. Hematocrit (Ht) and Hb were measured prior to erythrocyte (RBC) separation. Ht was determined by the capillary tube method, and Hb concentration was measured by Drabkin's cyanmethemoglobin method.

RBC were separated by centrifugation and rinsed 3 times with large volume of 0.85% NaCl. Four types of examinations, i.e. MeHg uptake to RBC, MeHg release from RBC, determination of intracellular distribution of MeHg in RBC and binding affinity of MeHg to Hb, were performed on RBC samples.

RBC were rinsed once with large volume of 15 mM Tris-HCl buffer solution together with 0.85% NaCl and 1 mM glucose (=TBS, pH 7.3), and packed cells (PC) were obtained by centrifugation at 3000 rpm for 10 min. Each 20 µl of PC was suspended in 3 ml of a mixture of MeHg and bovine serum albumin (BSA, Sigma) solution in TBS (16 mg BSA/ml). Methylmercury chloride was contained in the BSA solution at a concentration of 10^{-5} M. Radioactive ^{203}Hg-CH_3HgCl was added to 10 nCi/10 nmol/ml BSA solution. These suspensions were incubated at 25°C with constant gentle stirring and were centrifuged at various time intervals after incubation. Radioactivity in the aliquot of supernatant and in the remained suspension was measured with LKB-Wallac 1280 Ultrogamma. The amount of MeHg bound to RBC and the proportion of MeHg in RBC to the total amount of MeHg in the suspension were calculated.

MeHg release from RBC of a newborn and the mother was examined simultaneously at 25°C. MeHg concentration in TBS was 10 nmol/ml.

Table 1. Hematologic Examinations of Paired Blood Samples

No.	Maternal Blood		Cord Blood		Parturition	Newborn	
	Ht(%)	Hb(g/dl)	Ht	Hb		Sex	B.W.(g)
1	42.0	14.2	48.0	15.9	normal	F	3,365
2	42.0	13.8	44.5	14.7	normal	F	3,420
3	43.0	14.2	51.5	16.0	42 weeks	M	2,750
4	42.0	13.8	42.0	13.8	normal	M	3,435
5	37.5	12.6	45.0	17.8	37 weeks	M	2,360
6	39.0	13.2	46.0	14.7	normal	F	3,730
7	43.0	14.1	NT*	NT	normal	F	2,910
8	NT	NT	NT	NT	35 weeks	M	1,900

* NT: Not tested because of partial hemagglutination.

Intracellular distribution of MeHg in RBC of cord blood and the mother was determined with gel-filtration by Sephadex G-25 of stroma-free hemolysate.

Hb was prepared from stroma-free hemolysate with gel-filtration by Sephadex G-25, and the binding affinity of MeHg to Hb was determined with ultrafiltration by Amicon MPS-1 kit system and Scatchard plots.

Details of the procedures except for MeHg uptake to RBC were described previously (Doi and Tagawa 1983).

RESULTS AND DISCUSSION

Hematologic examination of the blood samples from newborns and their mothers revealed that newborns almost always had a higher Ht and a higher Hb concentration than their mothers (Table 1).

The capacity and rate of MeHg uptake to RBC was approximately equal in any paired sample of newborn and mother (Fig. 1). No significant difference was found in the mean uptake of MeHg to RBC between newborns and their mothers at any stage of the experiment.

MeHg was detected in two fractions of gel-filtrates of stroma-free hemolysate which were constituted of Hb and a low molecular weight substance in both the newborn's and the mother's RBC. The proportion of MeHg bound to Hb and a low molecular weight substance was approximately equal between the hemolysates of the newborn and the mother (Table 2).

At least two types of binding sites were suggested on both the mother's and the newborn's Hbs based on Scatchard plots (Fig. 3). A summary of the binding analyses for 3 pairs of mother's and newborn's Hbs was shown in Table 3. The number of primary and secondary sites (N_1 and N_2)

Table 2. Intracellular Distribution of MeHg in RBC of Maternal and Cord Blood

Sample No.	Maternal Blood		Cord Blood	
	Hb(%)*	LMW(%)#	Hb(%)	LMW(%)
1	45	55	49	51
3	48	52	48	52
4	44	56	50	50
7	47	53	47	53

*, #: Proportion of MeHg in the fraction of Hb(*) or low molecular weight substance(#) to the total amount of MeHg in the hemolysate.

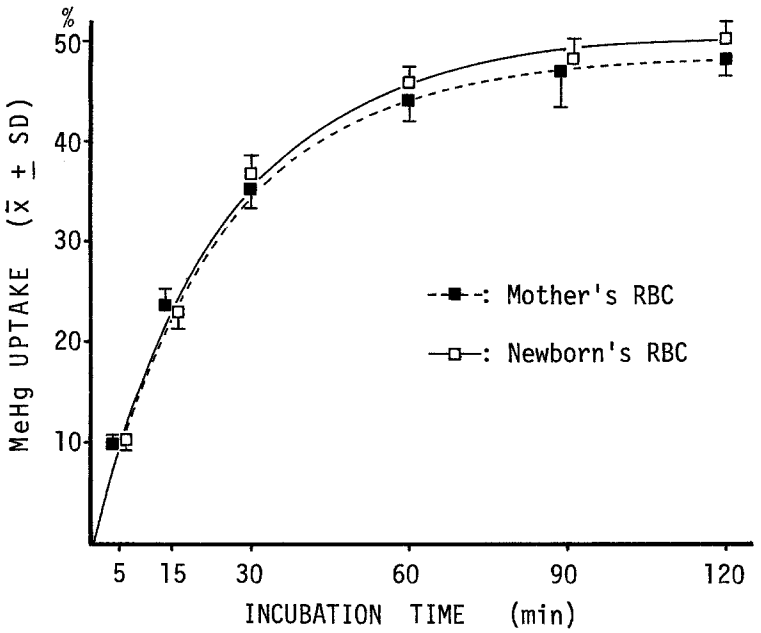

Figure 1. Uptake of methylmercury chloride to RBC of newborns and their mothers. Eight pairs of cord blood and maternal blood were used in the experiment.

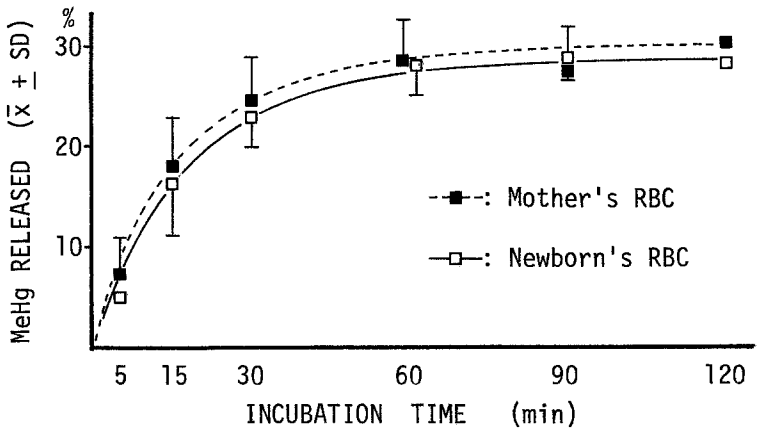

Figure 2. Release of methylmercury chloride from RBC of newborns and their mothers. Five pairs of cord blood and maternal blood were used in the experiment.

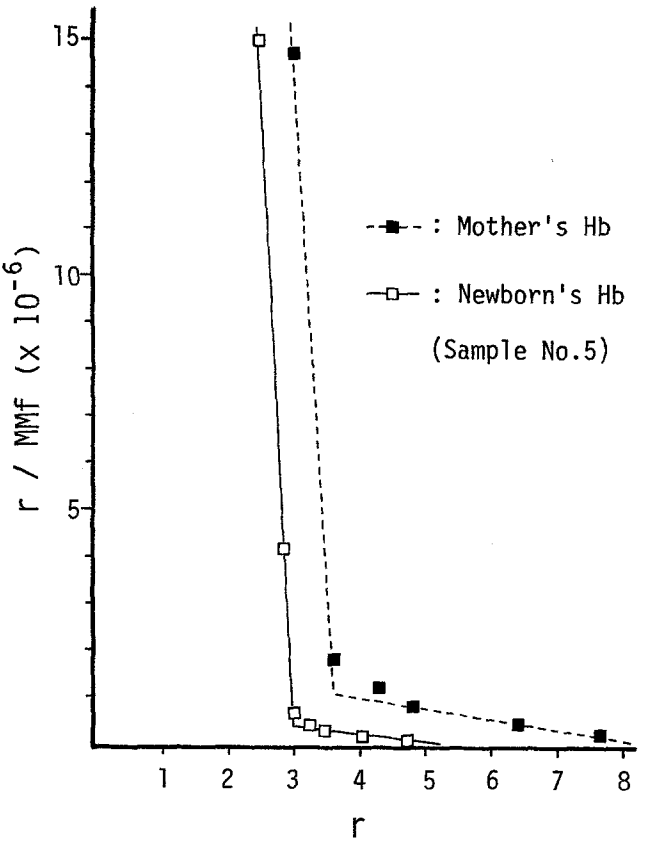

Figure 3. Scatchard plots for methylmercury chloride to Hbs of a pair of a newborn and his mother. "r" shows moles of MeHg bound per mole of Hb. MMf means molar concentration of free MeHg in Hb-MeHg mixture. Final concentration of Hb was 5 x 10^{-6} M in the mixture.

Table 3. Summary of Binding Experiments of Methylmercury Chloride to Hbs of Newborns and Their Mothers

Sample No.		N_1	K_1 (x 10^6)	N_2	K_2 (x 10^4)
3	M	3.7	8.8	8.1	15.2
	C	3.3	11.7	5.7	13.4
5	M	3.7	20.3	8.4	21.6
	C	3.0	26.4	5.2	15.3
6	M	3.7	18.8	7.7	20.8
	C	3.5	12.6	6.6	9.6

M,C: Maternal or cord blood; N_1,N_2: Number of primary or secondary site; K_1,K_2: Binding constant for primary or secondary site.

on the mother's Hb was larger than the number on the newborn's Hb, though the binding constants for the primary and secondary sites (K_1 and K_2) were approximately equal between both Hbs.

We should consider at least three classes of factors; chemical properties of MeHg, physiological and biochemical factors in the developing fetus and in the pregnant woman.

Molecular weight, lipid/water solubility ratio and ionization constant are generally known as the factors limiting the rate of diffusion of an agent through the placenta. Suzuki et al. (1967) reported that methylmercury acetate was able to transfer across the placenta of the mouse more easily than mercuric chloride and phenylmercury acetate. Autoradiographic findings by Berlin and Ullberg (1963) agree with the results of Suzuki et al. (1967). An exceedingly high affinity to sulfhydryl groups is the most important characteristics of mercury compounds including MeHg. A strong binding of alkylmercury compounds to Hbs (Takeda et al. 1968;Fang and Fallin 1976) and the characteristic behavior of MeHg to RBC of various species of animals (White and Rothstein 1973; Naganuma et al. 1980) are expressions of this chemical property. Doi and Tagawa (1983) revealed the decisive role of cysteinyl residues on the Hb molecules as the binding sites of MeHg.

In the present study, no significant difference was found between newborns and their mothers in MeHg uptake to RBC and in MeHg release rate from RBC, notwithstanding the higher binding affinity of mother's Hb than that of newborn's Hb to MeHg.

Newborn's Hb usually consists of 80% of fetal Hb (Hb F), 20% of adult Hb (Hb A) and a trace amount of minor components (Hb A_2 etc), whereas Hb A constitutes 97% of the total Hb in normal adults (Wintrobe et al. 1981). Hb F is a tetramer composed of two α-chains and two γ-chains and is signified as $\alpha_2\gamma_2$. Hb A is a tetramer signified as $\alpha_2\beta_2$. The α- and γ-chain have only one cysteinyl residue at positions 104 and 93 from the N terminal respectively. The β-chain has two cysteinyl residues at positions 93 and 112 (Schroeder et al. 1963;Dayhoff et al. 1972). Consequently Hb F has 4 cysteinyl residues on a tetramer and Hb A has 6 cysteinyl residues on a tetramer. Cysteinyl residues at position 104 on the α-chain and at position 112 on the β-chain are located in the $\alpha_1\beta_1$ contact junction, while cysteinyl residues at position 93 on both the β- and γ-chain are located outside of the contact junction (Perutz et al. 1968). These structural differences in the Hb molecules are thought to be the main causes of difference in the binding affi-

nity of Hbs for MeHg.

The present results suggest that the binding affinity of mother's Hb for MeHg plays minor role in the placental transfer of MeHg, and reconfirmed the importance of Hb concentration and RBC count on both (fetal and maternal) sides of the placenta as previously suggested by Suzuki et al. (1971).

Mitani et al. (1976) and Nishima et al. (1979) insist that the higher MeHg concentration in the cord blood than in the maternal blood cannot be explained by the higher Hb concentration in the cord blood alone. We ought to consider other factors as well for explaining the higher MeHg concentration in the cord blood. It appears to be indisputable that Hb concentration in the cord blood is the most important hematologic factor including the binding affinity of Hbs.

Hb synthesis and erythropoiesis start in the early embryonic stage, but the increase of RBC count is very slow in the first trimester and usually exceed the level of mother at term (Wintrobe et al. 1981). Moreover, fetal blood flow increases in the various organs including the brain in the later gestational stages (Rudolph 1969). Some amino acids such as cysteine and histidine have high affinity constants for MeHg (Simpson 1961). Amino acids are transferred actively through the placenta from the maternal plasma to the fetal plasma, and their concentrations are usually higher in the fetal plasma than in the maternal plasma (Young 1976). Gestational anemia is common in pregnant women especially in the developing countries, and severe anemia was frequently observed in the mothers of fetal MeHg poisoning patients in Iraq (Amin-Zaki et al. 1974;Choi et al. 1978). The contributions of these factors to the placental transfer of MeHg should be elucidated in future.

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