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Effects of Maternal Exposure to Six Heavy Metals on Fetal Development

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In recent years, the opportunity of maternal exposure to various chemicals in the workplace and the general environments have increased becaus of an increase in the number of woman working in industry. In many exposure cases, the fetus and the neonate are at greater risk than the adult (US EPA 1977). However, the studies published in the literature largely deal with the adult and not the fetus or neonate. Therefore, the biochemical changes leading to fetal toxicity are not known almost. Under these circumstances, UNEP/ILO/WHO (1984) published the booklet entitled "Principles for evaluating Health Risks to Progeny associated with Exposure to Chemicals During Pregnancy".

Humans are exposed to various metals by many ways in their daily lives. For instance, Chilsom (1980) reported that the children residing near ore smelters were exposed to a combination of lead, zinc, copper, cadmium and arsenic. Even in workplace, when the workers handle one metal, they are also exposed to other metals at the same time although the concentration of the latter may be low (Tsuchiya 1977). However emphasis has been put on the study of a single toxic metal probably because greater effort is demanded to study the effects of more than one metals at a time.Therefore, the knowledge regarding the implication of interactions between metals has remained fragmentary (Bonner et al. 1981;Kalia et al. 1984[a,b];Rehman and Chandra 1984;Singh et al. 1979). We previously studied the relationship of seven heavy metals between maternal and fetal samples collected from normal pregnant Japanese women and positive correlations of mercury, lead(Pb), cadmium(Cd), and manganese(Mn) contents were found between maternal and cord blood whereas they were not found as to essential metals such as copper(Cu), zinc(Zn) and iron(Fe) (Tsuchiya et al. 1984). We also pointed out the more serious influence of heavy metals on the neonates than on their mothers as the results of both the higher contents and the correlative behavior of toxic metals in cord blood.

In the present investigation, an attempt has been made to ascertain the maternofetal relationship found in the human

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pregnancy by the use of experimental animals as the first step of the study on the fetotoxicity resulted from the maternal exposure to combined heavy metals.

MATERIALS AND METHODS

Nulliparous 12 weeks old female rats of Wistar strain were equally divided into two groups of five rats so that mean body weights of both groups were nearly the same (Control group 239 $\pm$ 7 gm.; Treated group 240 $\pm$ 8 gm.). Two ml of solution containing Cd, Mn, Cu, Zn and Fe all in chloride form and 0.5 ml of lead acetate solution were intraperitoneally administered into the rats of treated group separately in order to avoid the precipitation. This injection was made once prior to mating and comprised Pb 1 mg per kg body weight(1 mg/kg), Cd 0.5 mg/kg, Mn 2.5 mg/kg, Cu 0.5 mg/kg, Zn 1 mg/kg and Fe 2 mg/kg. Control rats received the administration of equimolar hydrochlic and acetic acids in similar manner. All rats were fed with tap water and the commercial laboratory diet (Oriental,K.K.) ad libitum and the consumption of them and body weights were monitored. For mating, two or three females were placed with one 16 weeks old male rat of the same strain in each cage for four days just after the administration.

Twenty days after the administration, all rats were sacrificed by collecting blood from the heart using heparinized syringe under diethyl ether anaesthesia. Red and white blood cell counts and hemoglobin concentration were determined by Coulter automatic cell counter Model S and hematocrit value was measured by means of heparinized microcapilary tubes. The δ-aminolevurinic acid dehydratase (ALAD) of the erythrocyte was determined by the revised European standard method and glutamic oxaloacetic transaminase (GOT) by the Reitman-Frankel method. The fetus, placenta, heart, lung, spleen, stomach, intestine and femur were removed and washed with deionized water. After the water was wiped out by paper filter, wet weights of the tissues were recorded. Dry weight of them were also recorded after drying at 100 ^{o}C for 24 hrs. Wet digestion of the samples was carried out as previously described (Tsuchiya et al. 1984) for the distribution measurement of the administered metals. Data were subjected to statistical analysis performing Student' t-test and differences were considered significant when p was less than 0.05.

RESULTS AND DISCUSSION

To investigate the effects of concurrent exposure to six heavy metals on the maternal rats themselves, maternal body weight and intakes of food and water were monitored but no differences in them were found between control and treated groups.On the other hand, between pregnant and nonpregnant rats in respective groups there found significant difference in body weight for the last one week (Figure 1). This difference was greater in the control group than in the treated group and this is attributed to the

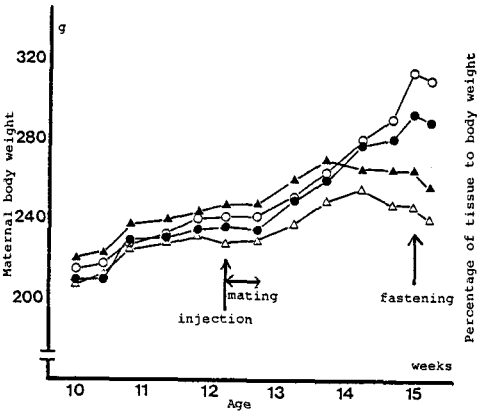


Figure 1. Mean maternal body weight gain

○ : pregnant rat in control group
△ : nonpregnant rat in control group
● : pregnant rat in treated group
▲ : nonpregnant rat in treated group

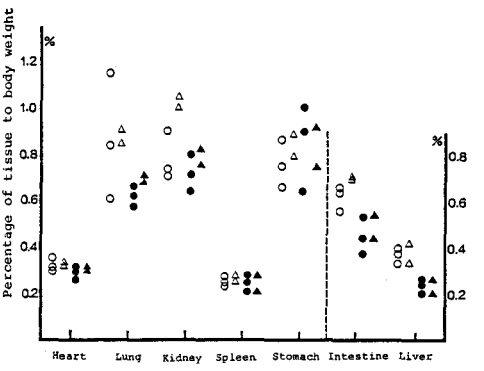


Figure 2. Wet weights of tissue relative to body weight

○ : pregnant rat in control group
△ : nonpregnant rat in control group
● : pregnant rat in treated group
▲ : nonpregnant rat in treated group

difference in fetal weight between both groups described later. Among the hematological and serum biochemical parameters in Table 1, red blood cell count, hemoglobin concentration and hematocrit showed a tendency to decrease by pregnancy in both control and treated groups as shown in Table 2 while these differences were not tested for significance because of small sample size in each subgroup. The administration tended to depress ALAD activity whereas it tended to increase GOT activity on the other hand. But the differences were not significant because of large intra-group deviation and small sample size.

Figure 2 shows the ratios of wet tissue weight to body weight separately by administration and pregnancy. There found the tendency that nonpregnant rats had higher ratios of lung and kidney than pregnant rats had whether they were administered or not. Among the other tissues, the ratios of heart, liver and intestine were significantly depressed by the administration after consideration of pregnant rats together with nonpregnant ones. The administered heavy metal levels in maternal blood and

Table 1.—Comparison of Hematological and Biochemical Parameters between Control and Treated Groups

	Control		Treated	
	Mean*	(Range#)	Mean	(Range)
Red blood cell (10^4/mm^3)	606	(489 ~ 763)	681	(570 ~ 802)
White blood cell (/mm^3)	6380	(4800 ~ 7800)	8520	(5800 ~ 11300)
Hemoglobin (g/100 ml)	12.7	(10.2 ~ 15.5)	14.2	(13.2 ~ 16.1)
Hematoclit (%)	38	(31 ~ 48)	43	(38 ~ 52)
ALAD (μmol/min/ml·RBC)	6.7	(3.6 ~ 10.0)	3.8	(2.0 ~ 7.2)
GOT (Karmen)	74	(65 ~ 80)	131	(95 ~ 196)

* : All numbers of samples except GOT(4 samples in each group) are five respectively.
# : Minimum ~ Maximum

eight tissues, placenta and fetus are presented in Table 3. Declines of zinc and iron contents in blood and iron content in a spleen were recognized by pregnancy as shown in Table 2, though the significance of difference was not tested for the same reason as the hematological parameters in the table. Among the metal contents, it is a noteable feature that Cd accumulated in all the tissues studied here. It is the only metal that transferred across a placenta to a fetus while Pb, Mn, Zn and Fe also accumulated in the placenta. In liver, significant increases in Pb, Cd and Mn contents were found whereas homeostasis was kept as to essential metals such as Cu, Zn and Fe. The similar tendency was found in femur too. Only Cd content in maternal blood remained high though the other metal contents were not significantly high probably because the blood was collected 20 days after the administration.

Pregnancy rate, litter size and wet weights of fetus and placenta are compared between control and treated groups in Table 4. Three rats of five in each group became pregnant after four days of mating. Therefore the pregnancy rates of both groups equally became 60 %. There was no significant difference in mean litter size of respective three pregnant rats between control (13.0 $\pm$ 1.4) and treated groups (12.3 $\pm$ 1.5). But as to the weight of fetus as an index of fetal development, there was drastically significant difference between both groups. Namely, the mean wet weight of fetuses in the treated group (0.97 gm.) was approximately halved that in the control group (2.23 gm.) by the administration. Similarly, the mean wet weight of placentae in the treated group (0.32 gm.) was reduced to about one half of that in the control group (0.56 gm.). The both differences were significant at the level of 0.1 %.

In this study, the intraperitoneal injection was applied to avoid

Table 2.—The parameters reduced by Pregnancy

		Nonpregnant	Pregnant
Red blood cell (10^4/mm^3)	C §	725, 763	489, 502, 550
	T ‖	745, 802	570, 627, 663
Hemoglobin (g/100 ml)	C	15.0, 15.5	10.2, 10.8, 12.1
	T	14.7, 16.1	13.2, 13.3, 13.5
Hematoclit (%)	C	45 , 48	31 , 32 , 36
	T	43 , 52	38 , 40 , 42
Zn in blood (μg/g·wet)	C	5.6, 6.0	3.8, 4.7 , 4.7
	T	6.5, 6.9	3.9, 5.2 , 5.7
Fe in blood (μg/g·wet)	C	460, 515	364, 366 , 382
	T	475, 557	355, 361 , 414
Fe in spleen (μg/g·wet)	C	862,1250	325, 698 , 738
	T	842,1089	486, 758 ,773

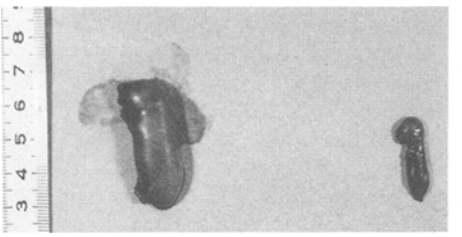
§ : Control group
‖ : Treated Group

Table 3.—The Distribution of the administered metals in Fetus, Placenta and Maternal Tissues

μg /g·wet weight

		Pb	Cd	Mn	Cu	Zn	Fe
Fetus	C§	0.078 ± 0.029#	0.0018 ± 0.0007	0.151 ± 0.054	1.51 ± 0.19	13.2 ± 2.1	45 ± 8.3
	T‖	0.085 ± 0.033	0.0037 ± 0.0021***	0.124 ± 0.030*	1.41 ± 0.23*	11.0 ± 1.4***	43 ± 7.7
Placenta	C	0.092 ± 0.058	0.0035 ± 0.0021	0.074 ± 0.024	1.12 ± 0.36	8.8 ± 1.0	73 ± 17
	T	0.295 ± 0.368**	0.0317 ± 0.0123***	0.088 ± 0.028*	1.15 ± 0.27	10.2 ± 1.7***	91 ± 35**
Blood	C	0.082 ± 0.018	0.0040 ± 0.0012	0.040 ± 0.0051	1.21 ± 0.21	5.0 ± 0.86	417 ± 67
	T	0.116 ± 0.038	0.059 ± 0.018**	0.045 ± 0.0066	1.36 ± 0.18	5.6 ± 1.2	432 ± 85
Heart	C	0.073 ± 0.011	0.0032 ± 0.00097	0.23 ± 0.045	3.4 ± 0.13	14.2 ± 1.5	56 ± 8.5
	T	0.092 ± 0.016	0.0809 ± 0.0138***	0.22 ± 0.022	4.3 ± 0.31**	14.2 ± 1.6	56 ± 6.5
Lung	C	0.082 ± 0.020	0.0043 ± 0.0028	0.11 ± 0.025	0.81 ± 0.13	10.2 ± 0.84	43 ± 9.5
	T	0.199 ± 0.067*	0.75 ± 0.43**	0.11 ± 0.011	1.29 ± 0.11***	12.2 ± 0.84**	65 ± 6.6**
Kidney	C	0.222 ± 0.245	0.017 ± 0.016	0.32 ± 0.057	2.7 ± 0.63	13 ± 1.9	48 ± 16
	T	0.854 ± 0.457*	3.9 ± 0.77***	0.41 ± 0.058	4.5 ± 1.3*	20 ± 2.1***	62 ± 18
Liver	C	0.060 ± 0.022	0.0064 ± 0.0015	1.88 ± 0.52	3.2 ± 0.34	25 ± 5.5	165 ± 79
	T	0.92 ± 0.42*	8.7 ± 1.7***	10.1 ± 0.86***	3.5 ± 0.73	31 ± 4.6	168 ± 47
Spleen	C	0.15 ± 0.084	0.0031 ± 0.0031	0.16 ± 0.027	1.05 ± 0.13	17 ± 2.1	775 ± 333
	T	0.40 ± 0.13**	0.85 ± 0.16**	0.16 ± 0.0047	1.27 ± 0.07*	19 ± 1.3	790 ± 216
Femur	C	0.27 ± 0.15	0.0006 ± 0.0006	0.49 ± 0.52	2.9 ± 0.51	94 ± 4.3	39 ± 19
	T	5.01 ± 1.55**	0.199 ± 0.120*	1.06 ± 0.25	3.1 ± 0.31	89 ± 8.6	42 ± 8.4
Stomach	C	0.24 ± 0.12	0.0040 ± 0.0024	0.29 ± 0.16	1.08 ± 0.19	12 ± 1.3	15 ± 0.82
	T	0.56 ± 0.40	0.28 ± 0.050***	0.31 ± 0.11	1.01 ± 0.10	12 ± 1.6	25 ± 5.7*
Intestine	C	0.88 ± 0.022	0.022 ± 0.011	7.1 ± 3.0	1.49 ± 0.55	19 ± 8.3	29 ± 11
	T	1.82 ± 0.76	0.29 ± 0.097**	8.7 ± 3.3	2.27 ± 0.99	29 ± 12	52 ± 16*

Significance level of difference from control(* : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$)
§ : Control group
‖ : Treated group
: Mean ± Standard deviation



Control Treated

Figure 3. Comparison of fetal size between control and treated groups

the interactive effect on the intestinal absorption of the metals (Hamilton and Valee 1974;Theil and Calvert 1978). The administered dose of each metal had been subjected to be one-hundredth of LD_{50}, but we decided the dose referring to the following reports on the toxicity of the metals , LD_{50} of which had not been clarified. The LD_{50} of lead acetate is reported to be 130 mg/kg·body weight (71 mg/kg as Pb) when it is administered intraperitoneally to rats (Windholz 1983). Chandra et al. (1983) found no effect on the fetal weight by 5 mg Pb/kg or 6 mg Mn/kg of daily intraperitoneal administration separately throughout gestation, but they observed significant decrease in fetal weight by the combined exposure to them. Rehman (1984) intraperitoneally administered Pb, Cu and Zn (8 mg each/kg) respectively to the rats of Charles Foster strain and found the inhibition of ALAD activity by Pb and Cu while the activity was increased by Zn. Giavini et al. (1980) treated intraperitoneally Sprague Dawley rats on day 3 of gestation with 3 mg/kg $CdCl_2$ (1.8 mg Cd/kg), 50 mg/kg $Pb(NO_3)_2$ (31 mg Pb/kg) and 7.5 mg/kg $CuSO_4$ (3.0 mg Cu/kg) respectively and recognized the embryotoxicity only in the case of Cu. This result seems to be related to the prevention of pregnancy by Cu inserted into the uterine lumen. As to the acute toxicity of the intraperitoneal injection of ferric chloride, LD_{100} is reported to be 260 mg/kg (53.7 mg Fe/kg) in mice (Suzuki 1977).

Compared to the doses in these reports, each dose in the present investigation was small and seems to have no effect.But the maternal concurrent exposure to six heavy metals resulted in the depression in fetal development as seriously as shown in Figure 3

Table 4.-Comparison of Parameters on Pregnancy between Control and Treated Groups

	Control	Treated
Pregnancy rate (%)	60 (3/5)	60 (3/5)
Litter size	13.0	12.3
Wet weights of fetuses (g)	2.23 ± 1.09	0.97 ± 0.41***
Wet weights of placentae (g)	0.56 ± 0.17	0.32 ± 0.15***

*** : $p < 0.001$

at the level where maternal health itself was not affected. The difference in fetal development may be suspected to resulted from the difference in the day of gestation because the start of pregnancy was not identified in the present study by finding the vaginal sperm during four days of mating. But judging from the distribution of mean fetal weight per litter, the suspicion can be dispeled. For the mean fetal wet weight of each litter varied from 1.4 gm. to 3.7 gm. in the control group and this variation seems to be owing to the difference in the day of gestation. Similar variation was observed in the treated group whereas the variation ranged from 0.60 gm. to 1.4 gm.. So the ranges of of both groups did not overlap each other at all. This intra-group variation indicates that the difference in the fetal weight between both groups resulted from the difference not in the start of pregnancy but in the fetal development.

In view of the distribution of the administered metals presented in Table 3, it seems possible that Cd played an important role in depressing the fetal development. But judging from the report by Rohrer et al.(1979), the interaction of the other metals seems necessary for the depression. They administered intraperitoneally to the Holzman rats at early stage of gestation with 1 or 2 mg Cd/kg and found no depression in the fetal weight though they found it by the administration late in pregnancy. However, further studies are needed to clarify the mechanism of depression in fetal development after the maternal combined exposure to six heavy metals. The ratios of mean metal contents in the tissues of the treated group to that of the control one are shown in Table 5. By this table, it is made clear that all ratios of essential metals such as Cu, Zn and Fe did not exceed two whereas those of Pb, Cd and Mn exceeded it in liver and so on. This difference in the behavior may be one of keys to solving the problem.

Table 5.—Ratios of Mean of Treated Group to that of Control Group

	Pb	Cd	Mn	Cu	Zn	Fe
Fetus	1.09	2.06**	0.82*	0.93*	0.83***	0.96
Placenta	3.21**	9.06***	1.19*	1.03	1.16***	1.25
Blood	1.41	14.8**	1.13	1.12	1.12	1.04
Heart	1.26	25.3***	0.96	1.26**	1.00	1.00
Lung	2.43*	174***	1.00	1.59***	1.20**	1.51
Kidney	3.85*	229***	1.28	1.67*	1.54***	1.29
Liver	15.3*	1360***	5.37***	1.09	1.24	1.02
Spleen	2.67**	274**	1.00	1.21*	1.12	1.02
Femur	18.6**	332*	2.16	1.07	0.95	1.08
Stomach	2.33	70.0***	1.07	0.94	1.00	1.67*
Intestine	2.07	13.1**	1.23	1.52	1.53	1.79*

Significance level of difference from control group (* : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$)

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