al., 1982; Samarawickrama and Webb, 1981), produces resorptions (Samarawickrama and Webb, 1981; Machemer and Lorke, 1981; Gale and Layton, 1980) and causes fetal or embryonic death (Ferm, 1971; Levin and Miller, 1980; Rohrer et al., 1979). Fetal growth retardation (Hastings et al., 1987; Ahokas et al., 1980; Baranski et al., 1983; Baranski, 1987) and congenital malformations (Gale and Ferm, 1973; Chernoff, 1973; Barr, 1973) in the offspring of exposed rats have been the most widely reported adverse reproductive effects.

The passage of maternally administered Cd to offspring is different depending on the developmental stage. Cd administered to pregnant animals is possibly transferred to fetus via placenta, and to neonates through milk. At low to moderate doses during pregnancy, the placenta acts to restrict the entry of Cd into the fetus (Sonawane et al., 1975; Whelton et al., 1993). However, Cd can cross the placental barrier if the dose is sufficiently elevated (Sonawane et al., 1975). The transfer of Cd to milk is also restricted (Bhattacharyya, 1983; Pitrzak-Flis et al., 1978). Of the small amount cadmium passed from the dams to offspring during gestation and lactation, the major portion has been shown to be transferred during lactation in mice (Whelton et al., 1993). Moreover, Cd level in the brain increases after neonatal exposure to 50 ppm Cd (Gupta et al., 1993), which is different from the result of gestational exposure. However, the entry of Cd to neonatal brain has not been fully elucidated at lower dose of Cd during lactation.

The mechanism of Cd-mediated fetotoxicity is not fully understood. However, there are some data suggesting that toxic effects of Cd may be mediated by altered Zinc (Zn) and Copper (Cu) metabolisms (Baranski, 1986; Kuhnert et al., 1987; Sorell and Graziano, 1990; Suzuki et al., 1990). Adequate availability of both Zn and Cu is essential for normal growth and development. Insufficient Zn availability in fetal or early postnatal life is teratogenic (Hurley and Swenerton, 1966), retards growth (Sandstead et al., 1972; Beach et al., 1980) and alters cognitive function (Sandstead et al., 1972). Fetal or neonatal Cu deficiency is also teratogenic (Keen et al., 1982), reduced brain catecholamine levels (Morgan and O'Dell, 1977; Feller and O'Dell, 1980) and decreases myelination in the central nervous system (Dipaola et al., 1974; Zimmerman et al., 1976). Gestational exposure to oral Cd over 50 ppm in drinking water resulted in decreased Zn and Cu content in fetal liver (Baranski, 1987; Sorell and Graziano, 1990; Sowa and Steibert, 1985; Roelfzema et al., 1988, 1989), brain (Baranski, 1987; Sowa and Steibert, 1985), kidney and intestine (Sowa and Steibert, 1985), as well as in whole body (Pond and Walker, 1975; Petering et al., 1979) and in these tissues of neonates (Baranski, 1986) or adult offspring (Baranski, 1986; Roelfzema et al., 1989). However, both increased (Waalkes et al., 1982) and unaltered (Sowa et al., 1982) concentrations of Zn and Cu in the fetus have been reported following maternal Cd exposure. Moreover, little is known about the effects of perinatal Cd exposure on offspring at the level lower than those used in the previous studies.

Recently, estrogen-like activity of cadmium has been suggested by Garcia-morales *et al*(Garcia-Morales et al., 1994), who showed that Cd mimicked the effect of estrogens by decreasing the level of estrogen receptor mRNA and transcription of the ER gene. In addition, female offspring, exposed *in ureto* to Cd, experienced an earlier onset of puberty (indicated by an earlier vaginal opening) and an increase in the epithelial area and the number of terminal end buds in the mammary glands (Johnson et al., 2003). This showed that only two injections of Cd to dams at a dose of 0.5 or 5 μg/kg body weight can be enough to affect the reproductive organs of offspring. On the other hand, effects of Zn and/or Cu conditions on sexual maturation and reproductive development have not been reported until now.

Therefore, this study aimed to investigate the effects of Cd at a dose of 1 or 10 ppm in drinking water during pregnancy and early lactation period on the concentrations of Cd, Zn and Cu in the brain, kidney and liver of mouse neonates. The doses were chosen because very few have examined the effects of Cd in the range exceeding 10 ppm in drinking water. In addition, we measured these metals at two time points (at birth and after ten days interval) to describe the effects along the developmental stage. We also evaluated the effects of perinatal Cd exposure with these doses on vaginal opening and estrus cycle for the first time.

Materials and methods

Animals and treatments

Forty female C57Bl/6J Jcl mice of initial body weight 17-22 g were purchased from a commercial breeder (CLEA Japan, Inc.). After acclimatization for two weeks, female mice were mated with males (body weight 20-25 g) of the same strain (1:1). The onset of pregnancy was confirmed by the presence of plug in the vagina (defined as gestational day (GD) 0). Mating male mice were removed and 18 pregnant mice were housed individually in disposable cages (CLEA Japan, Inc.) on sawdust bedding and had free access to chow with reduced amounts of estrogen and phytoestrogen; 17ß-estradiol was less than 0.05 μg/kg, genistein was less than 0.5 mg/kg, and coumestrol was less than 1 mg/kg (NIH07-PLD; based on an open formula of National Institute of Health, CLEA Japan, Inc.). Rooms were kept at 23 ± 2 ℃ with a cycle of 12 h of light and 12 h darkness; the lights came on at 0700.

Pregnant mice were divided into three groups. The animals of the treatment groups received 1 (n=6) or 10 (n=6) ppm Cd as cadmium chloride in drinking water from GD 0 to postnatal day (PND) 10 (the day of birth was defined as PND 0), respectively. The control group (n=6) received only distilled water. From PND 10, animals of all groups were given distilled water to drink. Each cage was checked every morning for the presence of newborns and the day of parturition, the number of living pups, the number of stillborn pups (defined as dead pup when we newly found parturition), sex ratio and the body weights per litter were recorded. Pups were left with each dam until weaning at PND 21. At weaning, the littermates were separated and housed by sex. Female offspring were housed individually after vaginal opening. All pups were weighed every 10 days until PND70. We also checked the number of death during this period.

All experimental protocols were approved by the Animal Care Committee of the Graduate School of Medicine of the University of Tokyo.

Sexual maturation

After weaning, all female offspring (n=5, 12 and 5 for control, 1 ppm and 10 ppm group, respectively, and n=1-5/dam) were checked for vaginal opening every day. The day of and the body weight at vaginal opening were recorded.

Beginning with PND 50, vaginal smears were taken daily for fifteen days, and estrus cycle was monitored. The days from diestrus to metestrus was defined as the duration of one cycle. One cycle with more than six days was defined as longer cycle. If a set of smear from giver mouse did not show the order of diestrus-proestrus-estrus-metestrus, this mouse was judged to be without cyclicity. Thus, individual female offspring was classified into one of the three categories; without cyclicity, longer duration of one cycle and normal cyclicity (other than without cyclicity and longer duration of one cycle). Data with plural cycles per individual during the observation period were averaged within the individual for statistical analysis.

Metal measurements

One male and one female pups from each litter were sacrificed at PND 0 and 10 under diethyl ether. Brain, kidney and liver were removed and weighed. All dams were sacrificed at PND 21 likewise, then cerebrum, cerebellum, brainstem, kidney and liver were removed and weighed. Samples were ashed with 60% nitric acid and 60% perchloric acid (2:1) at 110 ℃ for 5 h. Ashed samples were diluted with distilled water. Tissue Cd, Zn and Cu concentration was determined with a Inductively Coupled Plasma Mass Spectrometer (Hewlett Packard - 4500 ICP-MS, USA). Calibration curves were made using ICP multi-element standard solution for MS (MERCK,

Germany). Three replicas were performed per sample. Relative standard deviation was less than 5%. As reference material, standard reference material 1577b bovine liver (National Bureau of Standards) was analyzed together with the samples. Results from the analysis of the reference materials were well within ± 10% of the certified values. Reagent blanks were processed with each set of samples. Detection limits for tissue were 2 ng/g for Cd, 35 ng/g for Zn and 8 ng/g for Cu. Some samples were under detection limit on Cd measurement, and the values for those samples were applied the half of the detection limit when statistical analysis was performed.

Statistical Analysis

Frequencies were analyzed with Pearson chi-square test. Values of averaged parameters were statistically evaluated with a one-way ANOVA followed by Dunnett test. In comparison of the metal concentrations of offspring between the two time points, Paired *t*-test was used. *P* values less than 0.05 were considered statistically significant.

Results

Reproductive performance of the dams

The weight gain in dams during pregnancy was not different among groups. Table 1 shows the data on the reproductive performance of the dams. All these parameters were not significantly different among groups. However, the number of death after birth tended to increase in the Cd-treated groups.

Growth and sexual maturation of offspring

There were no significant differences in the body weight grain between groups both in male and female offspring until PND 70.

Sexual maturation and function are shown in Table 2. Though there was no statistical difference, the vaginal opening tended to be later than the control group by one and two days in 1 and 10 ppm Cd group, respectively. Body weight at vaginal opening did not differ between groups. Two female offspring in 10 ppm Cd group did not show cyclicity, resulting in a significant difference in the frequency of offspring without cyclicity (p=0.024 by Pearson chi-square test). We excluded these two offspring from analysis on the duration of one cycle. No significant difference was found in the duration of one cycle between groups.

Metal concentrations in dams

Tissue concentrations of Cd in dams are shown in Fig. 1. Cd concentration in the kidney [$F(2, 12)=9.14$, $p<0.0001$] and liver [$F(2,12)=33.54$, $p<0.0001$] was higher in 10 ppm Cd group compared with the other groups (Fig. 1A), while no between-group difference was found in the brain regions examined (cerebrum, cerebellum and brainstem) (Fig. 1B). Neither Zn nor Cu concentrations in the examined tissues differed among the groups.

Metal concentrations in offspring

Fig. 2 shows the metal concentrations of the neonatal tissues on PND 0. Between-group difference in Cd concentration was significant in the brain [$F(2, 27)=5.62$, $p=0.009$] and marginally significant in the liver (Fig. 2A). Zn concentration in the kidney [$F(2, 30)=3.49$, $p=0.043$] and the liver [$F(2,29)=4.48$, $p=0.019$] was different among groups, while it was not in the brain (Fig. 2B). Cu concentrations in the tissues of offspring did not differ among groups (Fig. 2C).

Fig. 3 shows the metal concentrations of offspring at PND 10. Difference in Cd concentration was found in the kidney [$F(2, 17)=4.05$, $p=0.036$] and liver [$F(2,17)=3.78$, $p=0.049$], and the difference was marginal in the brain (Fig.

3A). No between-group difference in Zn concentrations were found in the tissues examined (Fig. 3B). Cu concentration in the kidney [$F(2,17)=4.02$, $p=0.037$] and the liver [$F(2, 17)=3.80$, $p=0.043$] showed the difference among groups (Fig. 3C). In the liver, Cu concentration was significantly lower in the offspring of 10 ppm group than control. No such difference among the groups was found in the brain. Neither sex differences in the concentrations of these metals were recognized.

Changes of the metal concentrations were recognized in the neonatal tissues between PND0 and PND10. In the brain, Cd and Cu concentrations were significantly decreased from PND0 to PND10 in control group ($p=0.032$ and $p=0.023$, respectively) but not in the Cd-treated groups, while Zn concentrations were decreased in all three groups ($p=0.014$, $p=0.002$ and $p=0.042$ for control, Cd 1 ppm and 10 ppm group, respectively). In the kidney, only the Cd concentration in the control group was decreased ($p=0.035$). In the liver, Zn concentration was significantly decreased only in the control group ($p=0.005$), whereas Cu concentrations were decreased in all the groups ($p=0.005$, $p=0.014$ and $p=0.022$ for control, Cd 1 ppm and 10 ppm group, respectively).

Discussion

In the present study, perinatal exposure to low-dose Cd increased Cd concentrations in the brain of offspring brain at PND0, as well as the kidney and the liver at PND10. The Cd exposure also increased hepatic and renal Zn concentrations in the offspring at PND0, a novel finding suggesting the effect of Cd may not be solely leading to the deficiency of this trace elements as shown with many reports employing higher doses of Cd. Cu concentration in Cd exposed offspring was higher in the kidney and lower in the liver. Furthermore, we suggest the possibility that the onset of puberty can be delayed in female offspring by the Cd exposure, and this is the first report to show the effect of perinatal Cd exposure on estrus cycle of female offspring.

Cd concentration in the brain of Cd-exposed offspring was significantly higher than that of control at PND0, and had a higher tendency at PND10. The latter agreed with several previous studies showing the increased levels of Cd in the brain of offspring after neonatal exposure (Gupta et al., 1993; Choudhuri et al., 1996; Valois and Webster, 1987; Wong and Klaassen, 1980, 1982). However, studies employing a wide range of dose (up to 180 ppm in drinking water) and varying exposure paradigms have not found elevated levels of Cd in the fetal brain (Baranski, 1987; Sowa and Steibert, 1985; Murthy et al., 1986). This is explained by the placental barrier to restrict the entry of Cd to the fetus (Sonawane et al., 1975). Cd enters the brain most freely right from birth with entry decreasing until access is basically restricted around PND21 (Hastings and Miller, 1998). This can be accounted for, in some part, by the formation of a well-developed blood-brain barrier with age. Lucis *et al.* (Lucis et al., 1972) reported that Cd penetrates the blood barrier with more ease in fetal rats than in adults. Considering together, our result suggests that although the transfer of Cd from mother to offspring via placenta is restricted, Cd can still cross the placenta and accumulate in the brain at birth even at low level. Even though the development of blood-brain barrier with age is a factor for the increased exclusion of Cd (Hastings and Miller, 1998), brain Cd level at PND10 was still higher due to an immature blood-brain barrier.

Cd concentrations in the kidney and the liver of offspring did not differ among groups at PND0, which is consistent with the previous study using 5 ppm Cd in drinking water during gestation (Sorell and Graziano, 1990). However, when dams were given 50-180 ppm Cd in drinking water during gestation, fetal liver at GD20 showed the higher concentration of Cd (Baranski, 1987; Sorell and Graziano, 1990 ; Roelfzema et al., 1989). On the other hand, Cd concentrations in these tissues were higher in Cd-exposed groups at PND10. Although Cd transfer via milk has reported to be low in mice and rats (Lucis et al., 1972), the present study showed the importance of lactational transfer of Cd at such low level; this is consistent with the results of preceding studies (Whelton et al., 1993; Petersson et al.,

2000).

Zn concentrations in the kidney and in the liver were higher in 10 ppm Cd group than control at PND0. This result was inconsistent with the previous studies dosing over 50 ppm Cd, which reported decreased Zn and Cu content in fetal kidney (Sowa and Steibert, 1985) and liver (Baranski, 1987; Sorell and Graziano, 1990; Sowa and Steibert, 1985; Roelfzema et al., 1988). In rats, the maternal Cd exposure produced a decrease in fetal hepatic metallothionein (MT) levels with decrease in Zn level/concentration (Waalkes and Bell, 1980; Sasser et al., 1985). On the other hand, hepatic Zn increases in the rabbit fetus following maternal Cd exposure, where simultaneous increase in MT was observed (Waalkes et al., 1982). Although we have not determined whether the increase in Zn concentration in neonatal organs were related with the induction of MT, this result for the first time suggested that the response of mice were similar to that of rabbits. Although the fundamental cause of such species difference is not known, this should be clarified with further researches. Furthermore, Zn concentration in the liver of control offspring decreased from PND0 to 10. Previous studies also found the decrease in hepatic Zn concentration with age after birth in rodents (Reis et al., 1991). On the other hand, Zn concentrations in the liver of exposed offspring did not change during this period, suggesting that maternal low-dose Cd exposure promotes Zn retention in the liver of offspring.

Cd accumulation did not affect the Zn and Cu concentrations in the kidney and liver of the dams. This is inconsistent with some of the previous studies using higher Cd doses (i.e., 50-200 ppm Cd in drinking water), in which increases in hepatic and/or renal Zn (Sorell and Graziano, 1990; Pond and Walker, 1975; Chmielnicka and Sowa, 1996) or Cu (Chmielnicka and Sowa, 1996) concentration have been noted in pregnant rats. On the other hand, as discussed above, the increased Zn concentrations in the kidney and the liver of offspring were observed in the absence of significant Cd accumulation in these tissues at PND0. Christley and Webster (Christley and Webster, 1983) demonstrated that Cd does not necessarily have to be present in tissue at detectable levels to produce toxicity. Furthermore, studies involving Cd exposure during gestation usually demonstrated alterations in essential trace metals even though there was no increase in Cd (Hastings and Miller, 1998). Therefore, it is suggested that undetectable difference of Cd level between the Cd-exposed and control groups might affect Zn metabolism in the kidney and the liver of the neonates, and/or that alteration of Zn metabolism by Cd are more sensitive in fetuses/neonates than adults.

Finally, we found a tendency that the day of vaginal opening was later in female offspring born to Cd-treated dams. Furthermore, two female offspring of the 10 ppm Cd group did not show estrus cyclicity at all. Salvatori *et al.* (Salvatori et al., 2004) also showed a delay of vaginal opening in rats prenataly exposed to Cd. On the other hand, an earlier onset of vaginal opening was reported in rats with much lower dose (Johnson et al., 2003). This suggests that Cd has estrogen-like activity, because *in utero* exposure to estrogens or estrogen-like substances causes early onset of puberty (Hilakivi-Clarke et al., 1997; Rothschild et al., 1988). However, female rats prenataly exposed to genistein had delayed puberty onset, and those exposed to diethylstillbestrol had atypical vaginal cycles (Levy et al., 1995). They suggested that prenatal exposure to estrogen-like substances including genistein might cause a delay in puberty onset by an inhibitory or androgenizing effect on the hypothalamo-hypophysial axis, which controls estrogen production leading to initiate vaginal opening. Moreover, disrupted estrus cycle and anovulation were shown to be characteristic responses to estrogen exposure in rodents during critical periods of neuroendocrine differentiation (earlier than 5 to 10 days of age) (Gellert, 1978). Therefore, our data supported the notion that perinatal low-dose Cd exposure would perturb the reproductive-endocrine function. In this study, we could not find the clear relations between vaginal opening/estrus cycle and Zn/Cu concentrations.

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References

Ahokas, R.A., Dilts, P.V. Jr, Lahaye, E.B., 1980. Cadmium-induced fetal growth retardation: protect effect of excess dietary zinc. Am. J. Obstet. Gynecol. 136, 216-221.

Baranski, B., 1986. Effect of maternal cadmium exposure on postnatal development and on tissue cadmium, copper and zinc concentrations in rats. Arch. Toxicol. 58, 255-260.

Baranski, B., 1987. Effect of cadmium on prenatal development and on tissue cadmium, copper, and zinc concentrations in rats. Environ. Res. 42, 54-62.

Baranski, B., Stetkiewick, I., Sitarek, K., Szymczak, W., 1983. Effects of oral, subchronic cadmium administration on fertility, prenatal and postnatal progeny development in rats. Arch. Toxicol. 54, 297-302.

Baranski, B., Stetkiewick, I., Trzcinka-Ochocka, K., Sitarek, K., Szymczak, W., 1982. Teratogenicity, fetal toxicity and tissue concentration of cadmium administered to female rats during organogenesis. J. Appl. Toxicol. 2, 255-259.

Barr, M. Jr., 1973. The teratogenicity of cadmium chloride in two stocks of wistar rats. Teratology 7, 237-242.

Beach, R.S., Gershwin, M.E., Hurley, L.S., 1980. Growth and development in postnatally zinc-deprived rats. J. Nutr. 110, 201-211.

Bhattacharyya, M.H., 1983. Bioavailability of orally administered cadmium and lead to mother, fetus, and neonate during pregnancy and lactation: An overview. Sci. Total Environ. 28, 327-342.

Chernoff, N., 1973. Teratogenic effects of cadmium in rats. Teratology 8, 29-32.

Chmielnicka, J., Sowa, B., 1996. Cadmium interaction with essential metals (Zn, Cu, Fe), metabolism metallothionein, and ceruloplasmin in pregnant rats and fetuses. Ecotoxicol. Environ. Saf. 35, 277-281.

Choudhuri, S., Liu, W.L., Berman, N.E., Klaassen, C.D., 1996. Cadmium accumulation and metallothionein expression in brain of mice at different stages of development. Toxicol. Lett. 84, 127-133.

Christley, J., Webster, W.S., 1983. Cadmium uptake and distribution in mouse embryos following maternal exposure during the organogenic period: a scintillation and autoradiographic study. Teratology 27, 305-312.

Dipaola, R.V., Kanfer, J.N., Newberne, P.M., 1974. Copper deficiency and central nervous system myelination in the rat: Morphological and biochemical studies. J. Neuropathol. Exp. Neurol. 33, 226-236.

Feller, D.L., O'Dell, B.L., 1980. Dopamine and norepinephrine in discrete areas of the copper-deficient rat brain. J. Neurochem. 34, 1259-1263.

Ferm, V.H., 1971. Developmental malformations induced by cadmium. Biol. Neonate 19, 101-107.

Frery, N., Nessman, C., Girard, F., Lafond, J., Moreau, T., Blot, P., Lellouch, J., Huel, G., 1993. Environmental exposure to cadmium and human birthweight. Toxicology 79, 109-118.

Gale, T.F., Ferm, V.H., 1973. Skeletal malformations resulting from cadmium treatment in the hamster. Biol. Neonate 23, 149-160.

Gale, T.F., Layton, W.M., 1980. The susceptibility of inbred strains of hamsters to cadmium-induced embryotoxicity. Teratology 21, 181-186.

Garcia-Morales, P., Saceda, M., Kenney, N., Salomon, D.S., Kim, N., Salomon, D.S., Gottardis, M.M., Solomon, H.B., Sholler, P.F., Jordan, V.C., Martin, M.B., 1994. Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. J. Biol. Chem. 269, 16896-16901.

Gellert, R.J., 1978. Kepone, mirex, dieldrin, and aldrin: estrogenic activity and the induction of persistent vaginal estrus and anovulation in rats following neonatal treatment. Environ. Res. 16, 131-138.

Gupta, A., Gupta, A., Murthy, R.C., Chandra, S.V., 1993. Neurochemical changes in developing rat brain after pre- and postnatal cadmium exposure. Bull. Environ. Contam. Toxicol. 51, 12-17.

Hastings, L., Choudhury, H., Petering, H.G, Cooper, G.P., 1987. Behavioral and biochemical effects of low-level prenatal cadmium exposure in rats. Bull. Environ. Contam. Toxicol. 20, 96-101.

Hastings, L., Miller, M.L., 1998. Developmental Neurotoxicity of Cadmium. In: Slikker, W., Chang, L. (Eds.), Handbook of Developmental Neurotoxicology. Academic Press, N.Y., pp. 517-538.

Hilakivi-Clarke, L., Clarke, R., Onojafe, I., Raygada, M., Cho, E., Lippman, M., 1997. A maternal diet high in n - 6 polyunsaturated fats alters mammary gland development, puberty onset, and breast cancer risk among female rat offspring. Proc. Natl. Acad. Sci. U.S.A. 94, 9372-9377.

Hurley, L.S., Swenerton, H., 1966. Congenital malformations resulting from zinc deficiency in rats. Proc. Soc. Exp. Biol. Med. 123, 692-697.

Johnson, M.D., Kenney, N., Stoica, A., Hillakivi-Clarke, L., Singh, B., Chepko, G., Clarke, R., Sholler, P.F., Lirio, A.A., Foss, C., Reiter, R., Trock, B., Paik, S., Martine, M.B., 2003. Cadmium mimics the *in vivo* effects of estrogen in the uterus and mammary gland. Nat. Med. 9, 1081-1084.

Keen, C.L., Lonnerdahl, B., Hurley, L.S., 1982. Teratogenic effects of copper deficiency and excess. In: Sorenson, J.R.J. (Ed.), Inflamatory diseases and copper. Humana Press, Clifton, pp. 106-121.

Kuhnert, B.R., Kuhnert, P.M., Debanne. S., Williams, T.G, 1987. The relationship between cadmium, zinc, and birth weight in pregnant women who smoke. Am. J. Obstet. Gynecol. 157, 1247-1251.

Kuhnert, B.R., Kuhnert, P.M., Zarlingo, T.J., 1988. Associations between placental cadmium and zinc and age and parity in pregnant women who smoke. Obstet. Gynecol. 71, 67-70.
Levin, A.A., Miller, R.K., 1980. Fetal toxicity of cadmium in the rat: maternal vs fetal injections. Teratology 22, 1-5.
Levy, J.R., Faber, K.A., Ayyash, L., Hughes, C.L. Jr., 1995. The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. Proc. Soc. Exp. Biol. Med. 208, 60-66.
Lucis, O.J., Lucis, R., Shaikh, Z.A., 1972. Cadmium and zinc in pregnancy and lactation. Arch. Environ. Health 25, 14-22.
Machemer, L., Lorke, D., 1981. Embryotoxic effect of cadmium on rats upon oral administration. Toxicol. Appl. Pharmacol. 58, 438-443.
Morgan, R.F., O'Dell, B.L., 1977. Effect of copper deficiency on the concentrations of catecholamines and related enzyme activities in the rat brain. J. Neurochem. 28, 207-213.
Murthy, R.C., Ali, M.M., Chandra, S.V., 1986. Effects of in-utero exposure to cadmium on the brain biogenic amine levels and tissue metal distribution in rats. Ind. Health 24: 15-21.
Petering, H.G, Choudhury, H., Stemmer, K.L., 1979. Some effects of oral ingestion of cadmium on zinc, copper and iron metabolism. Environ. Health Perspect. 28, 97-106.
Petersson, Grawe, K., Oskarsson, A., 2000. Cadmium in milk and mammary gland in rats and mice. Arch. Toxicol. 73, 519-527.
Pitrzak-Flis, Z., Rehnberg, G.L., Favor, M.J., Cahill, D.F., Laskey, J.W., 1978. Chronic ingestion of cadmium and/or tritium in rats. I. Accumulation and distribution of cadmium in two generations. Environ. Res. 16, 9-17.
Pond, W.G, Walker, E.F., 1975. Effect of dietary Ca and Cd level of pregnant rats on reproduction and on dam and progeny tissue mineral concentrations. Proc. Soc. Exp. Biol. Med. 148, 665-668.
Reis, B.L., Keen, C.L., Lonnerdal, B., Hurley, L.S., 1991. Longitudinal changes in the mineral composition of mouse milk and the relationship to zinc metabolism of the suckling neonate. J. Nutr. 121, 687-699.
Roelfzema, W.H., Roelofsen ,A.M., Herber, R.F., Peereboom-Steg, J.H., 1988. Cadmium and zinc concentrations in fetal and maternal rat tissues after parenteral administration of cadmium during pregnancy. Arch. Toxicol. 62, 285-290.
Roelfzema, W.H., Roelofsen, A.M., Leene, W., Peereboom-Steg, J.H., 1989. Effects of cadmium exposure during pregnancy on cadmium and zinc concentrations in neonatal liver and consequences for the offspring. Arch. Toxicol. 63, 38-42.
Rohrer, S.R., Shaw, S.M., Lamar, C.H., 1979. Cadmium fetotoxicity in rats following prenatal exposure. Bull. Environ. Contam. Toxicol. 23, 25-29.
Rothschild, T.C., Calhoon, R.E., Boylan, E.S., 1988. Effects of diethylstilbestrol exposure in utero on the genital tracts of female ACI rats. Exp. Mol. Pathol. 48, 59-76.
Salvatori, F., Tarassi, C.B., Salzgeber, S.A., Spinosa, H.S., Bernardi, M.M., 2004. Embryotoxic and long-term effects of cadmium exposure during embryogenesis in rats. Neurotoxicol. Teratol. 26, 673-680.
Samarawickrama, G.P., Webb, M., 1981. The acute toxicity and teratogenicity of cadmium in the pregnant rats. J. Appl. Toxicol. 1, 264-269.
Sandstead, H.H., Gillespie, D.D., Brady, R.N., 1972. Zinc deficiency: Effect on brain of the suckling rats. Pediatr. Res. 6, 119-125.
Sasser, L.B., Kelman, B.J., Levin, A.A., Miller, R.K., 1985. The influence of maternal cadmium exposure or fetal cadmium injection on hepatic metallothionein concentrations in the fetal rat. Toxicol. Appl. Pharmacol. 80, 299-307.
Shiverick, K.T., Salafia, C., 1999. Cigarette smoking and pregnancy. I: ovarian, uterine and placental effects. Placenta 20, 265-272.
Sonawane, B.R., Nordberg, M., Nordberg, G.F., Lucier, G.W., 1975. Placental transfer of cadmium in rats: influence of dose and gestational age. Environ. Health Perspect. 12, 97-102.
Sorell, T.L., Graziano, J.H., 1990. Effect of oral cadmium exposure during pregnancy on maternal and fetal zinc metabolism in the rat. Toxicol. Appl. Pharmacol. 102, 537-545.
Sowa, B., Steibert, E., 1985. Effect of oral cadmium administration to female rats during pregnancy on zinc, copper, and iron content in placenta, fetal liver, kidney, intestine, and brain. Arch. Toxicol. 56, 256-262.
Sowa, B., Steibert, E., Gralewska, K., Piekarski, M., 1982. Effect of oral cadmium administration to female rats before and/or during pregnancy on the metallothionein level in the fetal liver. Toxicol. Lett. 11, 233-236
Suzuki, K.T., Tamagawa, H., Takahashi, K., Shimojo, N., 1990. Pregnancy-induced mobilization of copper and zinc bound to renal metallothionein in cadmium-loaded rats. Toxicology 60, 199-210.
Valois, A.A., Webster, W.S., 1987. Retention and distribution of cadmium in the mouse brain: an autoradiographic and gamma counting study. Neurotoxicology 8, 463-469.
Waalkes, M.P., Bell, J.U., 1980. Depression of metallothionein in fetal rat liver following maternal cadmium exposure. Toxicology 18, 103-110.
Waalkes, M.P., Thomas, J.A., Bell, J.U., 1982. Induction of hepatic metallothionein in the rabbit fetus following maternal cadmium exposure. Toxicol. Appl. Pharmacol. 62, 211-218.
Whelton, B.D., Toomey, J.M., Bhattacharyya, M.H., 1993. Cadmium-109 metabolism in mice. IV. Diet vs. maternal stores as a source of cadmium transfer to mouse fetuses and pups during gestation and lactation. J. Toxicol.

Environ. Health 40, 531-546.
Wong, K.L., Klaassen, C.D., 1980. Tissue distribution and retention of cadmium in rats during postnatal development: minimal role of hepatic metallothionein. Toxicol. Appl. Pharmacol. 53, 343-353.
Wong, K.L., Klaassen, C.D., 1982. Neurotoxic effects of cadmium in young rats. Toxicol. Appl. Pharmacol. 63, 330-337.
Yu, H.S., Tam, P.P.L., Chan, S.T.H., 1985. Effects of cadmium on preimplantation mouse embryos in vitro with special reference to their implantation capacity and subsequent development. Teratology 32, 347-353.
Zimmerman, A.W., Matthieu, J.M., Quarles, R.M., Brady, R.O., Hsu, J.M., 1976. Hypomyelination in copper-deficient rats. Arch. Neurol. 33, 111-119.

Table 1.Effect of Cd exposure to the dams during pregnancy and early lactation period on reproductive performance

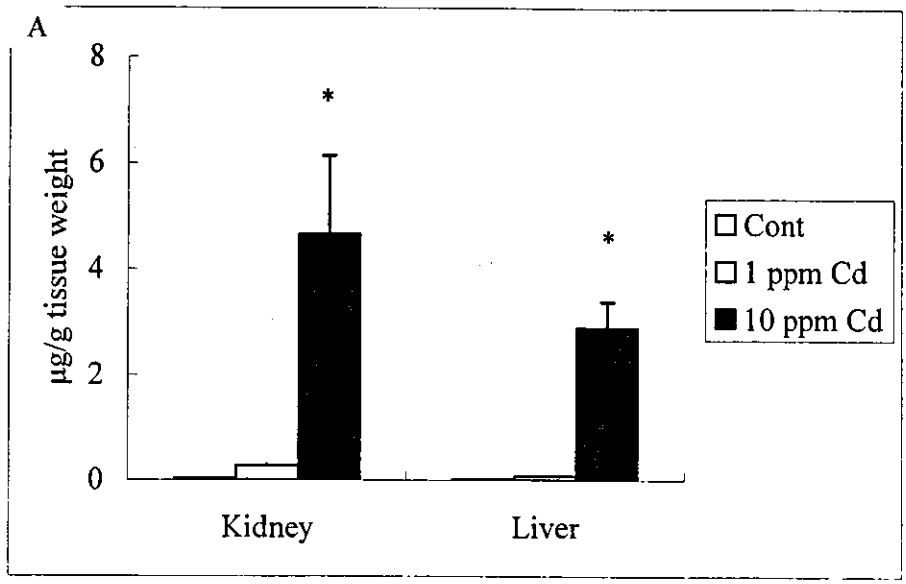
	Control	1 ppm Cd	10 ppm Cd
Period of gestation (days)	19.7 ± 0.5	18.8 ± 0.2	21.1 ± 1.1
Litter size	5.8 ± 1.1	7.7 ± 0.5	5.8 ± 0.7
Sex ratio (males to females)	1.29 ± 0.25	1.02 ± 0.39	1.00 ± 0.52
Birth weight (g)	1.303 ± 0.016	1.298 ± 0.009	1.300 ± 0.018
Livebirth (n)	35	46	34
Stillbirth (n)	3	2	1
Death after birth (n)	6	11	13

Values are expressed as mean ± SE for period of gestation, litter size, sex ratio and birth weight.
n=6 for control, n=6 for 1 ppm Cd group and n=6 for 10 ppm Cd group.

Table 2.Effect of Cd exposure to the dams during pregnancy and early lactation period on sexual maturation and function of female offspring.

	Control	1 ppm Cd	10 ppm Cd
Day of vaginal opening (PND)	30.2 ± 1.2	31.4 ± 0.7	32.4 ± 1.2
Body weight at vaginal opening (g)	15.00 ± 0.10	15.41 ± 0.28	15.52 ±0.49
Duration of one cycle (days)	5.23 ± 0.32	5.72 ± 0.50	5.29 ± 0.49
Estrous cycle			
No. of offspring with normal cyclicity (	3	10	2
No. of offspring with longer cycle (n)	2	2	1
No. of offspring without cyclicity (n)	0	0	2*

Values are expressed as mean ± SE for day of vaginal opening, body weight at vaginal opening and duration of one cycle.
n=5 for control, n=12 for 1 ppm Cd group and n=5 for 10 ppm Cd group.
*; $p<0.05$ compared with control



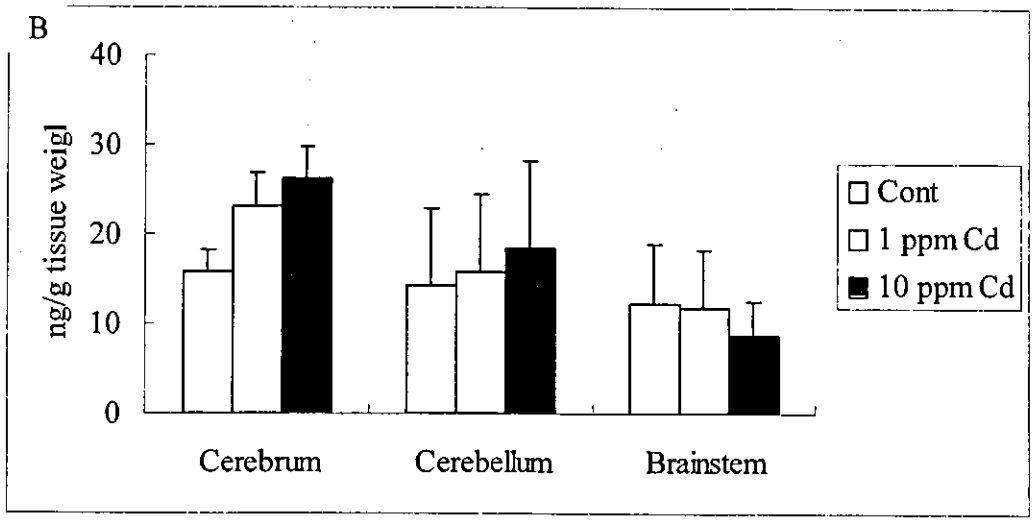


Fig 1. Effect of Cd exposure to the dams during pregnancy and early lactaion perios on Cd concentration in the kidney, the liver (A) and the brain (B) of dams.

Data are expressed as mean ± SE.

*; $p<0.0001$ compared with control.

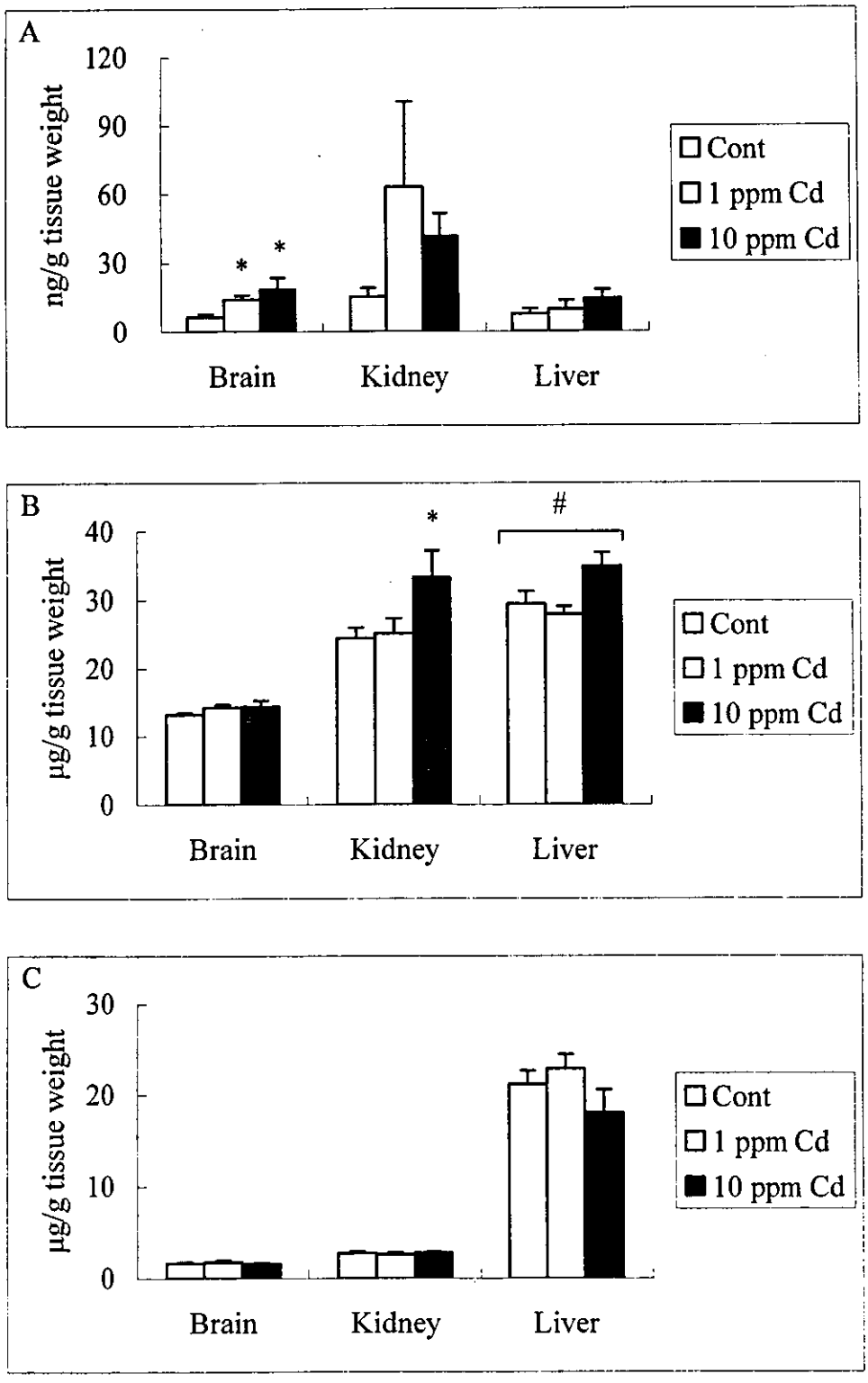


Fig 2. Effect of Cd exposure to the dams during pregnancy and early lactation period on Cd (A), Zn (B) and Cu (C) concentrations in tissues of offspring at PND0.

Data are expressed as mean ± SE.

#; $p<0.05$ different from each other by one-way ANOVA

*; $p<0.05$ compared with control

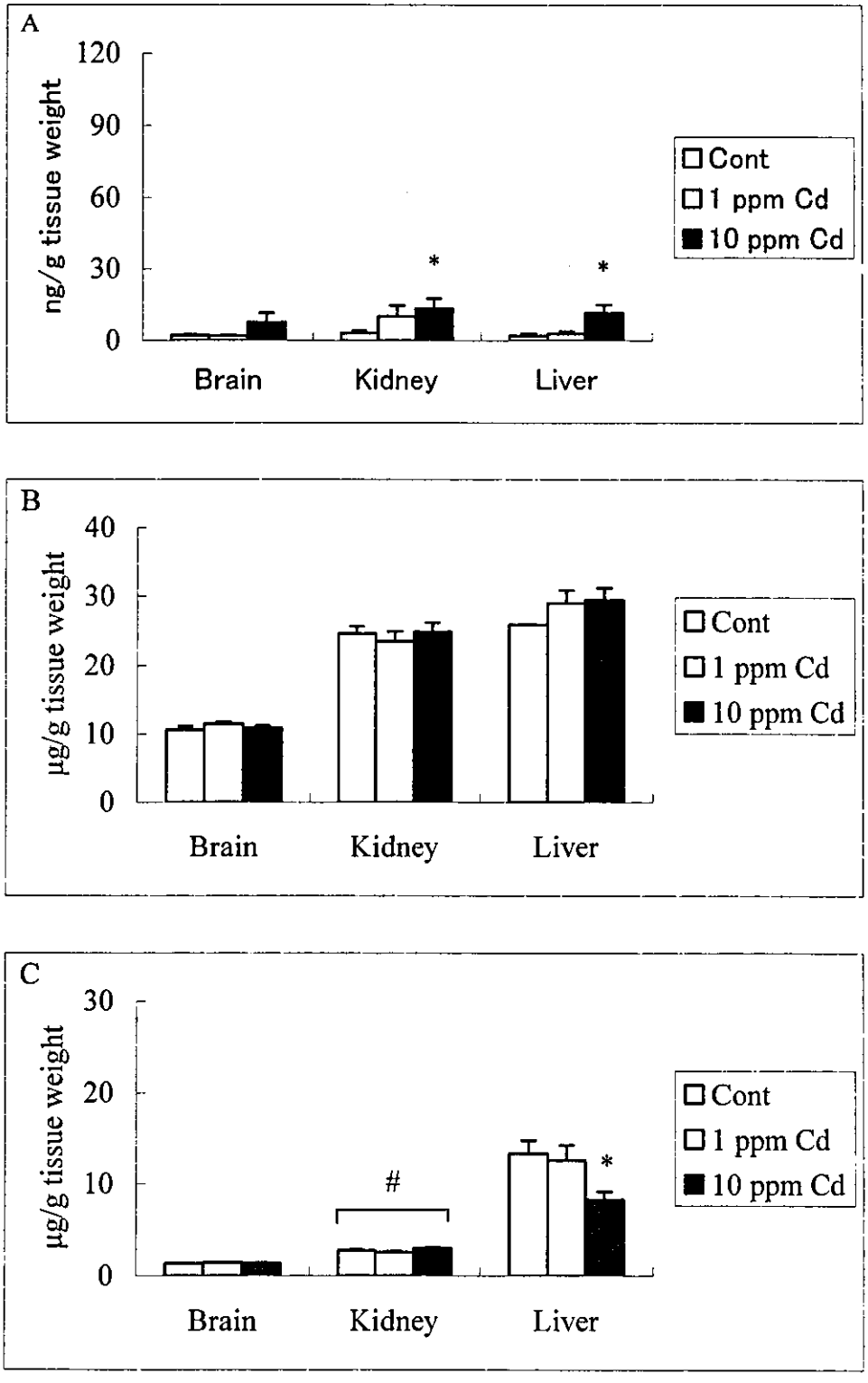


Fig 3. Effect of Cd exposure to the dams during pregnancy and early lactation period on Cd (A), Zn (B) and Cu (C) concentrations in tissues of offspring at PND10.

Data are expressed as mean ± SE.

#; $p<0.05$ different from each other by one-way ANOVA

*; $p<0.05$ compared with control

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総括研究報告

甲状腺機能阻害剤の周生期投与による新生仔マウス臓器中微量元素の変動

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研究要旨

前年度までに、カドミウムが発達毒性を示すことを示唆する複数のデータを得ているが、前年度吉田（克己）氏らが示したように、詳細は不明ながら甲状腺機能への影響が認められた．したがって、カドミウムの発達毒性の少なくとも一部が甲状腺機能への影響を介したものである可能性が考えられる．前項で述べたように、カドミウムは必須微量元素の代謝に影響を及ぼし、甲状腺機能と微量元素には関係があることも示されているが、成長発達が盛んな新生仔期において、甲状腺機能と微量元素の関係を調べた報告はきわめて限られている。そこで、本研究ではマウスの妊娠 12 日目から仔の出生後 10 日目（PND10）にわたり MMI（methimazole）0.008、0.025、あるいは 0.08%(v/w) を含む飲料水を摂取させることにより甲状腺機能を低下させ、PND10 に母仔の脳、腎、肝中の銅、亜鉛濃度を ICP-MS を用いて測定した。MMI の投与によって、胎児期及び新生仔期における体重増加は量依存的に減少し、母仔ともに解剖時において血漿中 T_4 濃度は低下していた。T_4 の反応と MMI の投与量との関係は母と仔では異なっており、MMI に対する感受性は仔の方が高いと示唆された。

新生仔では、肝・腎ともに銅濃度は有意に低下し、亜鉛濃度は有意に上昇した。特に肝の銅濃度は、高濃度群において対照群の 1/4 にまで低下した。これら新生仔における変動は、本研究よりも甲状腺機能低下の程度がより大きい条件で実施された成獣における微量元素の変動とは反対であった。以上より、周生期における甲状腺機能の低下が新生仔臓器中微量元素に及ぼす影響は、成獣と異なること、それらの変化の方向は、低濃度のカドミウム曝露と同じであることが示された。

1．緒言

銅と亜鉛は必須微量元素であり、ヒトの生存にとって不可欠である。銅の生体内での影響は多様であり、様々な銅酵素として働いている[1]。亜鉛の生体内での影響もまた多様であり、300 以上の亜鉛酵素が存在している[2]。哺乳類では 30%以上の亜鉛が核内に存在し[3]、多くの亜鉛を含んだたんぱく質が遺伝子発現に関与している[4]。ヒトを含む哺乳類では正常な成長と発達に、銅と亜鉛が必要であることが示されており、胎児期や新生児期の銅欠乏は、催奇性[5]、脳中カテコールアミン量の減少[6,7]、中枢神経系における髄鞘形成の低下[8,9]を示す。また、胎児期や新生児期の亜鉛欠乏も催奇性を引き起こし[10,11]、成長阻害[10]や認知機能の変化[12]をもたらす。

ヒトの甲状腺機能低下症では、血清中の銅及び亜濃度は低下することが知られている[13,14]。実験動物では、妊娠マウスの甲状腺機能をプロピルチオウラシル（propylthiouracil: PTU）投与によって低下させたとき、母体の脳、腎、肝の銅濃度が上昇したことが報告されている[15]。ただし、この実験ではPTU 投与によって、母マウスのチアノーゼと新生仔の高い死亡率が観察された。また、甲状腺機能を低下させたラットでは、肝と腎の亜鉛濃度が低下する例も報告されている[16]。このように、甲状腺機能と微量元素には関係があることが示されているが、その詳しい仕組みはまだ明らかになっていない点が多い。

甲状腺ホルモンの作用は多様であり[17]、成体においてはエネルギー代謝の制御などに関連するが、発達期の脳においては、成長と分化を制御する因子である[18]。甲状腺ホルモンと亜鉛はともに成長を制御し、成長ホルモンと相互作用することが知られている。しかし、成長発達が盛んな新生仔期

において、甲状腺機能と微量元素の関係を調べた報告は極めて限られている。

メチマゾール（methimazole: MMI）は、甲状腺濾胞細胞において甲状腺ペルオキシダーゼを阻害し、甲状腺ホルモンの前駆物質であるチログロブリンのチロシン残基ヨウ素化を抑制する、代表的なチロキシン合成阻害剤である[19]。実験動物において、MMI は PTU とともに、甲状腺機能低下モデルを作るのによく用いられる[20]。妊娠マウスに MMI を飲料水に溶かして投与すると、MMI は胎盤を容易に通過し、胎児と母の血中濃度比がほぼ 1 になる[21]。MMI は乳汁分泌性（乳汁／血漿濃度比 1.0。c.f. PTU : 0.1）もある[22,23,24,25]。

本研究では、周生期マウスに MMI を投与して甲状腺機能を低下させ、新生仔期に脳、腎、肝中の銅と亜鉛濃度を測定することにより、甲状腺機能が臓器中微量元素に与える影響を、成長と関連させ明らかにすることを目的とした。

2．対象と方法

2.1. 動物及び MMI 周生期投与

妊娠 1 日目のマウス(C57BL/6J)を日本クレア社から 17 匹購入した。餌は NIH-07PDL（オリエンタル酵母工業社製。成分《日本食品分析センター調べ》：水分 6.5%、粗たんぱく質 26.6%、粗脂肪 6.5%、粗繊維 1.7%、粗灰分 6.8%、可溶無窒素物 51.9%）を与えて飼育した。飼育室の温度は 23±2℃、湿度は 50±10%、明暗サイクルは 12 時間（明：7:00～19:00、暗：19:00～7:00）に保った。

17 匹のマウスは投与する MMI の濃度によって 4 群に分けた。4 群はそれぞれ、対照群（n=6）、低濃度群（0.008% (w/v) ; n=4）、中濃度群（0.025 ; n=4）、高濃度群（0.080 ; n=4）とし、マウスは GD1～10（妊娠後の日数；プラグ確認日を GD0 とした）の体重増加量の平均値が等しくなるよう割り付けた。MMI は精製水（milliQ による）中に溶かし、GD12 から PND10（出産後 10 日目；出産確認日を PND0 としている）まで各母マウスに投与した。MMI を含む飲料水は適宜再調製して補充した。PND0 に一腹の産子数及びその雌雄の数を記録した。PND1 に母マウス 1 匹あたりの仔を雄雌それぞれ 2 匹ずつ合計 4 匹にそろえた。ただし、新生仔の雌雄どちらかが 2 匹に満たない場合は合計 4 匹になるようにそろえ、合計 4 匹を満たない場合はそのままとした。新生仔の体重測定時

(PND0,3,6,10) に確認した仔数が、PND0 で記録された新生仔数より減少していた場合、その差を食殺数として記録した。

2.2. 母及び仔マウスの解剖

すべての母及び仔マウスの体重を GD1,10,12,14,16,18 及び PND0,3,6,10 に測定した。

PND10 にすべての母及び仔マウスを解剖し、脳、肝、腎、及び血液を採取した。母、仔 (雄)、仔 (雌) マウスの各臓器重量を測定し、微量元素の測定に備え-80℃で保存した。血液は 15 分間遠心分離 (15,000 rpm、4℃) し、血漿を T_4 (チロキシン) 測定まで-80℃で保存した。この際、仔の血液は同じ母マウスから産まれた全ての仔のものをプールした。

2.3. 血漿 T_4 (チロキシン) の測定

母及び仔マウスの血漿 T_4 は enzyme immunoassay (EIA) kit (Dainabot 社 (東京) 製。IMx T_4 ダイナパック) を用いて測定した。本測定は、東北大学大学院医学系研究科 腎・高血圧・内分泌内科 森弘毅先生に依頼して、東北大学において行われた。

2.4. 組織中微量元素濃度の測定

母、仔 (雄)、仔 (雌) マウスの各臓器中の銅と亜鉛の含有量は ICP-MS (Inductively Coupled Plasma - Mass Spectrometry : 誘導結合プラズマ質量分析法) を用いて測定した。ICP-MS は無機元素分析装置であるため、試料は湿式灰化した後測定を行った。

湿式灰化は以下の手順で行った。各臓器から組織 100 mg を採取し、硝酸と過塩素酸の 2:1 の混酸を加えた。試料を 90℃に加熱して 20 分その温度を維持した後、110℃で 5 時間加熱した。加熱灰化した試料は冷却した後、milliQ 精製水で希釈して保存し、ICP-MS を用いた測定に備えた。

ICP-MS 装置は HP-4500 (Hewlett-Packard 社製) であり、イットリウムを内標準として添加し、絶対検量線法によって銅と亜鉛を定量分析した。ICP マルチエレメントスタンダード XXI MS 用 (メルク社製) を用いて検量線を作成し、NIST-SRM (National Institute of Standards & Technology, Standard

Reference Material) 1577b Bovine Liver（ゼネラルサイエンスコーポレーション社製。成分：銅 160±8 μg/g、亜鉛 127±16 μg/g）を標準資料として測定し、銅、亜鉛とも保証値の範囲内であることを確認した。

2.5.統計分析

統計パッケージとして JMP version 5.1.1 を用いた。本研究で測定した各指標について、「MMI 投与量」を効果とする一元配置分散分析を行った。また、仔から得られた各指標については「MMI 投与量」と「性差」を効果とする二元配置分散分析を行った。有意水準はいずれも 0.05 とした。

3．結果

3.1. 母及び仔マウスの体重変化

GD1,12,18 において体重に有意差はなかったものの、MMI を投与開始した GD12 から GD18 までの体重増加量で群間に有意差（$p<0.05$）があった。これは、図 1 で示されるように、MMI 投与後 2 日間の体重増加の群間差（$p<0.01$）によるもので、この 2 日間における高濃度群の増加量は対照群と比べて有意に小さかった($p<0.05$; Dunnett)。[図 1]

出産日は GD19 で 5 匹、GD20 で 10 匹、GD21 で 2 匹であった。17 匹中 1 匹（0.080%群）のマウスが仔を全て食殺し、食殺は全て PND0 から PND3 の間に起こった。表 1 で示されるように、一腹の産子数に有意差はなかったが、MMI 投与量によって出産日が異なる傾向が見られた。食殺は中濃度群と高濃度群で観察され（$p<0.05$）、高濃度群の食殺数は対照群と比べて有意に多かった($p<0.01$; Dunnett)。[表 1]

図 2 に示されるように、新生仔の体重増加量は MMI の量依存的に小さくなった。その結果、PND0 の新生仔の体重に有意な差はないものの、PND10 の体重には有意差（$p<0.05$）があった。PND10 における高濃度群の平均体重は対照群の 89%であり、有意に小さかった($p<0.05$; Dunnett)。[図 2]

3.2. 母及び仔マウスの臓器重量

母マウスでは肝重量と腎重量が MMI の量依存的に増加した。また脳重量は、母仔ともに MMI

の低用量では対照群と比べて小さいが、投与濃度の増加に伴い大きくなるというパターンが見られ、仔では投与量の異なる群間で脳重量に有意差があった（$p<0.05$）が、性差は見られなかった。その他の仔の臓器では、重量には群間の差が見られなかった。母マウスの肝重量は群間に有意差があり（$p<0.01$）、高濃度群は対照群と比べて有意に大きかった($p<0.01$; Dunnett)。

3.3. 血漿中 T_4 濃度

母及び仔マウスの血漿 T_4 量はそれぞれ群間で有意差があった。図 3 に示すように母マウス（$p<0.01$）ではMMIを投与した群全てで血漿 T_4 濃度は低下し、仔マウス（$p<0.001$）では量依存的に低下した。母マウスでは投与群全ての血漿 T_4 濃度が対照群に比べて有意に低下し($p<0.05$、0.01、0.05 ; Dunnett)、仔マウスでは中濃度群と高濃度群の血漿 T_4 量が対照群に比べて有意に低下した($p<0.01$、0.001 ; Dunnett)。[図3]

3.4. 組織中微量元素濃度の変動

母及び仔マウスの各組織中の銅と亜鉛の濃度は表 2 のようになった。[表2]

母マウスの各組織中の銅及び亜鉛の濃度に群間差は見られなかった。一方、仔マウスの組織中銅濃度は、投与群の脳、肝（図 4)、腎（図 5）で MMI の量依存的に低下し、脳及び腎では雌のほうが高かった。肝（$p<0.0001$）及び腎（$p<0.05$）中において、投与量の異なる群間で銅濃度に有意差が見られ、腎（$p<0.05$）において性差が有意であった。また、高濃度群の肝中銅濃度が対照群に比べて有意に小さかった($p<0.05$; Dunnett)。脳中の銅濃度は投与量の異なる群間で差は見られなかったが、腎と同様に性差が見られた（$p<0.001$)。[図4] [図5]

仔マウスの組織中亜鉛濃度は、脳においては、投与量の異なる群間での差も、性差も見られなかった。肝及び腎（図 6）において MMI の量依存的な増加が見られ、腎では群間差が有意であった（$p<0.05$）が、性差は見られなかった。[図6]

4．考察

母及び仔マウスの血漿中 T_4 濃度から判断すると、MMI は低濃度群でも甲状腺機能を低下させており、モデルとしての有効性が示されたと考えられる。甲状腺機能低下によって周生期の成長が阻害されたことは、新生仔体重増加の減少や、妊娠中 MMI 投与開始直後の高濃度群における体重増加の一時的な抑制などとも整正された結果である。

T_4 の反応と MMI の投与量との関係は母と仔で異なっていた。新生仔では T_4 が MMI の量依存的に低下したが、母体では量依存性を認めなかった。ラット成獣を様々な濃度の MMI に暴露した実験[20]では、飲料水中 MMI 濃度と血清中 MMI 濃度は相関したが、甲状腺中の MMI 濃度は、飲水中 MMI 濃度の増加に対して頭打ちとなった。また、ラット甲状腺で 50%のヨウ素結合抑制を示した投与濃度は、本研究の低濃度群より低い 0.003%（飲料水中）であった。マウスにおいても、本研究の投与濃度では甲状腺における作用が頭打ちとなり、母マウスの血漿中 T_4 濃度にも投与群間の差が見られなかった可能性がある。ただし、マウス成獣において MMI の投与により血漿中 T_4 をほぼ枯渇させた例もあることを考えると、血漿中 T_4 濃度が頭打ちになった理由は他にもあると考えられる。これに対し、仔マウスの血漿中 T_4 濃度が MMI の量依存的に低下したのは、MMI に対する感受性が異なるためと考察される。既に述べた先行研究[22,23,24,25]によると、MMI の血漿／乳汁濃度比が 1.0 であると報告されており、これに基づけば、乳汁中の MMI の濃度は飲料水中 MMI 摂取量に相関している。また、血清中の MMI 濃度は飲料水中の濃度と比べて極めて低い（約 1/25）ことが知られているため[20]、乳汁中 MMI 濃度は、飲料水中の濃度より低かったと考えられる。従って、仔マウスは、その血清中 MMI 濃度は親マウスよりも低いと予想されるにかかわらず、親マウスと同程度（約 3/4）の T_4 濃度低下を示したことになり、MMI に対する感受性が高いと示唆される。このことは、仔マウスの血清中 MMI 濃度を実際に測定することによって検証する必要がある。

ラット成獣の臓器中微量元素は甲状腺機能低下状態において、銅が脳、肝、腎において増加し、亜鉛は肝、腎において減少する。このとき、PTU を投与したラットで、母体のチアノーゼと新生仔の高い死亡率が観測されており、肝、腎の亜鉛を測定したラットでは血清中 T_4 濃度は対照群の半分であった。本研究のモデルは、これらの先行研究よりも甲状腺機能低下の程度が弱かったことが、母マウスの臓器中微量元素に差が表れなかった原因と考えられる。

新生仔マウスのいずれの臓器においても銅濃度は低下し、肝、腎において亜鉛濃度が上昇した。この結果は、甲状腺機能低下の程度がより大きい条件下で行われたラット成獣と反対の結果である。また、新生仔マウスとラット成獣いずれにおいても銅と亜鉛の増減が反対に変動した。このことから甲状腺ホルモンは、臓器微量元素に影響を与える際に、この二つの元素を反対に変動させると予想される。新生仔のみで変化が認められたことは、上述したT_4の効果の違いが関連しているのかもしれない。

新生仔の肝における、高濃度群の銅濃度の低下は特に顕著であった。MMI は Cu^{2+}の強力なキレート剤であり、セルロプラスミンやチロシナーゼなどの銅酵素（酸化酵素）を阻害する[26]。このことから、肝中の銅濃度の低下は甲状腺機能への影響を介したものではなく、MMI の局所的なキレート効果による可能性も考えられる。しかし、血清 MMI 濃度の低いと推測される新生仔のみで銅濃度が低下したことから、キレート剤としての作用が肝中の銅濃度の変動原因であるとは考えにくい。また、MMI のキレート剤としての作用によって、生体内の銅量が減少したという報告も見あたらない。銅変動の他の理由としてメタロチオネイン（metallothionein: MT）が考えられる。MT は金属結合たんぱく質であり、生理的には亜鉛･銅を結合していて、重金属をはじめとする有害化学物質、ストレスなどの因子によって誘導されることが知られている[27]。また、前述したラットの甲状腺機能低下モデルでは、肝 Zn の減少とともに、肝の MT 濃度が低下した。このことから、新生仔の肝の銅濃度が低下したのは、MT の減少による可能性も考えられる。

今回、脳、腎において銅濃度の変動に性差が見られたことから、これらの臓器では甲状腺ホルモンと性ホルモンの相互作用があると予想される。ただし、PND10 におけるマウスの微量元素の性差に関する報告は見あたらず、そのメカニズムは不明である。

5．結論

本研究において、周生期における甲状腺機能の低下が新生仔組織中微量元素に及ぼす影響は成獣と異なることが示唆された。また、臓器中の微量元素において、銅と亜鉛の変動が反対になることが示唆された。しかし、今回の実験だけでは詳しい仕組みがわからないので、更なる研究が必要である。