CHAPTER IV

DISCUSSION

In prenatal toxicology, *in vitro* methods using whole animals are considered to be the useful tools to carry out the investigations. The possibilities of maintaining cells, organs or whole-animals under *in vitro* conditions are, however, limited by time. Thus the information provided by these techniques is primarily restricted to a specific stage of embryonic or fetal development. The present study clearly indicates that mouse embryos of early somite stages grow well under *in vitro* conditions. But the figures for total DNA content and number of somites indicate retarded development as compared with growth *in vivo* (Table 1). This finding is similar to those of Clarkson, Doering and Runner (1969) who reparted that growth rate of mouse fetuses explanted at early somite stages and incubated for 22 hours in culture, was lesser than the control. This reduction in growth might be the result of limitation of nutrient materials in serum or reduction of uptake of nutrient materials from serum. The former hypothesis is strongly supported by the experiment of Clarkson *et al.* (1969) which demonstrated that mouse fetuses developed better in Waymouth's medium with 20 % rat serum than in whole rat serum. The later hypothesis is speculative and still not clear. New (1978) suggested that slowing in growth of mouse embryos in culture is probably related to the absence of a functional allantoic placenta and results from the failure of the ectoplacental cone to develop normally *in vitro*. But at present, the role of the rodent allantoic

placenta in fetal nutrition during organogenesis remains uncertain. Thus, further study on the role of allantoic placenta in relation to the transfer of nutrient materials during organogenesis is a prerequisite before any conclusion can be made.

For toxicological purposes when maintaining the embryos in culture for a long period of time may be less important, whole - embryo culture technique has proved to be useful in toxicological screening. Toxicological studies in whole-embryo cultures have certain advantages in that the effect of a chemical can be investigated without any interference from maternal system. However, the system has the limitation in that the embryos can be maintained for only a fraction of their *in utero* life, and no long-term effects of a chemical can be studied.

In vitro study on the effects of cadmium chloride on mouse embryos cultured during the major parts of organogenesis has demonstrated that cadmium chloride is embryotoxic and teratogenic. A variety of dysmorphogenic effects were observed in embryos cultured in the presence of cadmium chloride at the levels of 2.8 and 3.0 μM for 48 hours (table 8 of the appendix). The dysmorphogenesis observed are generally associated with irregular fusion of the telencephalic and mesencephalic regions, and stunted telencephalic hemispheres (figure 6). Non-closure of the cranial neural tube region was most evident in embryos treated with 2.8 μM and 3.0 μM of cadmium chloride. A reduction in DNA contents was observed in embryos cultured in the presence of cadmium chloride at a concentration as low as 0.5 μM (figure 3). This finding is in agreement with the

results of Webb and Samarawickrama (1981) which reported that maternal administration of cadmium caused a decrease in thymidine incorporation into fetal DNA which persisted for at least 20 hours after treatment. Daston (1982) suggested that decreased thymidine incorporation is attributable to diminished activity of the zinc-dependent enzyme thymidine kinase. Thymidine kinase is essential for DNA synthesis, and diminished activity of this enzyme could result in a decrease in the content of fetal DNA. Decreased DNA synthesis would also be expected to cause a general decrease in fetal growth, which was also observed. Growth parameters for instance, crown-rump lengths, head lengths, somite numbers and morphological score points of embryos treated with 1.0 μM $CdCl_2$ or higher were lower than control values (figure 4 - 5). Results of *in vitro* study suggest a correlation between the diminution of DNA synthesis and the dysmorphogenic effects of cadmium. It is apparent, therefore, that further work is needed to validate the above hypothesis.

The *in vivo* results demonstrated that moderately toxic dose (4 mg. kg^{-1}) of cadmium chloride, given intraperitoneally to pregnant mice, produce fetotoxic and teratogenic effects. Congenital malformations and embryonal or fetal lethality are evoked only on two of the three embryonic days on which it was tested. On the 7th day in particular, was the most critical in terms of teratogenic response. Some embryos (about 63 % per litter) were dead and reabsorbed (table 2), those that remain viable give rise to small fetuses, about 72 % of which are deformed (table 3). At this gestational age the most frequent teratogenic abnormalities were exencephaly and eye defects (figure 11).

Possibly because of species differences in teratogenic reponse (Tuchmann and Duplessis, 1970), no malformations of the face, which are characteristic of Cd^{+2} teratogenesis in hamsters (Ferm, 1971), have been observed in Swiss Albino Mice.

As teratogenic susceptibility coincides with the rapid period of development and different organs pass through susceptible periods at different times, the syndromes of malformations could be changed when administered cadmium chloride at various gestational ages. Thus, the incidence of exencephaly and eye defects are greatest at days 7 and 7.5 of gestation whereas the incidence of another common malformation, i.e., defective ribs and vertebraes and retarded ossification of several bones, mainly skull, is maximum when cadmium chloride is injected into the pregnant mice on the 8.5th day of gestation.

The fetotoxic effects of cadmium chloride as expressed in term of reduced birth weight and crown-rump length of newborn mice depend upon the embryonic age of the exposed embryos. A decreases in the average fetal weight was observed after treatment with a single dose of cadmium chloride only 7.0 or 7.5 of gestation (Figure 10). However, with the exception of insignificant effect on fetal weight following treatment with a single dose of cadmium chloride on day 8.5 of geatation, fetotoxicity as manifested by a decrease in crown-rump length and placental weight were observed in all experimental groups (figure 9 - 10). The data of placental weights (table 13 of the appendix) suggested that the placenta provided protection for the mouse fetus against cadmium accumulation. There

is a correlation of gestational age and placental toxicity. As the gestational age increased the placental weight of the exposed fetus decreased. It is postulated that in early gestational period, the functional placenta of embryo is not established so that cadmium can reach the embryo and exert its embryotoxic and/or teratogenic effects directly on the embryo. After establishment of functional placenta, a significant amount of cadmium is accumulated in the placenta with comparatively little cadmium transfer to the fetus. Large accumulation of cadmium in placenta may interferes with placental development resulted in decreased placental weight. This postulation is supported by Lucis *et al.* (1972) who reported that fetal and neonatal gut and liver concentrated a significant amount of cadmium as a result of *in utero* exposure to cadmium chloride in early gestational period. Sonawane *et al.* (1975) was also showed that placental cadmium concentration increases with the increase of gestational stage of the animals.

Comparison of the results from *in vitro* with *in vivo* study can be used to define the role of cadmium chloride in embryo toxicity and teratogenicity. Those *in vitro* effects correlate closely with the *in vivo* observations. Cadmium chloride causes growth retardation both *in vivo* and *in vitro*; moreover, it induces high incidences of central nervous system defects which are exhibited *in vivo* as exencephaly (figure 11) and *in vitro* as irregular or incomplete neural tube closure (figure 6). Exencephaly is beleived to result from abnormal development of the neural tube during the process of neuralation (Lemire, Beckwith and Warkany, 1978). Irregular or incomplete fusion of the neural tube during organogenesis may be in part responsible for the expression of exencephaly (figure 11).

In summary, the *in vivo* and *in vitro* investigations have shown that cadmium chloride is teratogenic in Swiss Albino mice and produces a variety of birth defects e.g. irregular and incomplete fusion of the neural tube, resulting in high incidences of exencephaly. Its teratogenic action probably involves a direct insult on the developing conceptuses during organogenesis.

The postnatal study revealed no induction of functional impairments in the offsprings. It is difficult to assess the functional impairments in animal experimentation and the present screening methods may not be sensitive enough to detect such the impairments.