CHAPTER 1

GENERAL REVIEW OF THE LITERATURE

Introduction

Chemically, cadmium stands between zinc and mercury. Like other

heavy metals, cadmium has long been known to be toxic. However, this

was not regarded with much concern until relatively recently, when it

was realized that apart from the occasional acute intoxication, long-term

exposure to low concentrations is cumulative and may be fatal. This

applies not only to high-risk occupations associated with metallurgical

processes, but also, with spreading of industrial pollution and general

population.

The hazards of cadmium have attracted attention which increased

since the rock-soil-plant-animal-man relationship became better known.

Increasing industrial pollution may endanger life by the cumulative

toxic effects of cadmium. Major sources of pollution are mining and

smelting, metal coating, welding, incineration of refuse, fossil fuels

and use of sewage sludge as fertilizer. Cadmium intoxication usually

results from ingestion or inhalation, but the pathogenesis is not well

understood. With chronic intoxication, the critical organ is kidney,

where one third of body cadmium is accumulated. Apart from diseases

recognized in man, a large number of pathological conditions have been

produced in experimental animals, including congenital abnormalities,

ovarian, testicular and brain lesions, as well as interference with the

immune system. Their full significance for man is not yet known. Toxi-

cologic interest in cadmium has grown steadily over the years and, most

recently, has been intensified by the discovery that cadmium can cross

the placental barrier and interferes with embryonic development (Webb and Samarawickrama, 1981). This finding has caused concern among the public heath authorities about the hazards of toxic injury from cadmium to the offspring of exposed populations in both occupational and community situations. Obviously, the Literature on the toxic effects of cadmium is quite voluminous, which precludes comprehensive review of the subject in this thesis. Thus, in the following sections, only the relevant embryotoxic effect of cadmium is described in some details while rather brief accounts on other aspects of cadmium toxicology and the theoretical background of mammalian whole-embryo culture system are presented.

Natural Occurrence of Cadmium

Cadmium is ubiquitous in the earth crust in very low concentrations and is quite evenly distributed, about 0.15-0.2 μg g^{-1} in igneous rock (Winter, 1982). The soil reflects cadmium content of rock from which it is derived. Some sedimentary rocks and their soils may have higher concentrations of cadmium depending on the amount of organic matter incorporated, in which cadmium could be accumulated through the food chain. Pacific island phosphorites is a good example, from which superphosphate is made, with up to 90 μg g^{-1} (William and David, 1973). Excessive use of superphosphate with accumulated cadmium may thus contribute to soil pollution. Fossil fuels, particularly diesel and other mineral oils, may also contain higher concentrations of cadmium.

Industrial Use of Cadmium

Cadmium is used for metal coating in a manner similar to zinc. It is more expensive than zinc, but gives a better quality coat. It is

usually applied by electrolysis, occasionally by dipping and spraying. Another major use is pigments in paints, enamels and plastics, usually based on cadmium sulphide which gives colours from yellow to maroon. Cadmium as salts of fatty acids is used increasingly as a stabilizer for PVC and related plastics for protection from radiation and oxidation; and more recently, in nickel-cadmium batteries. In lesser quantities, cadmium is used as a constituent of solders, for semiconductors and as a hardening agent for copper (Winter, 1982).

Sources of Environmental Pollution

The most important source of pollution is mining and smelting. Cadmium has a relatively high vapour pressure. Any metallurgical process involving heating at high temperatures will evaporate cadmium adding to atmosphere and create environmental pollution. With large lead smelters for instance, leaf samples 15 km. away and 40 km. downwind have shown abnormally high cadmium levels (Little and Martin, 1972; Buchauer, 1973). Motor fuels are a main reason for high atmospheric cadmium levels in large cities. In Los Angeles and Tokyo, cadmium levels of 0.004 μg.m^{-3} and 0.01 μg.m^{-3} respectively, have been measured (Winter, 1982)

Incineration of domestic refuse in an industrial society with much cadmium in pigments and plastics can be a significant source of atmospheric pollution that settles on plants and soil (Fleischer *et al.*, 1974). Another souce of pollution is sewage when used as fertilizer. Its cadmium content tends to be significantly higher in industrial cities, compared with residential areas where it is mainly derived from food residues, and domestic and storm water. There is a positive correlation between the amount of cadmium in the sludge and the degree of industrialization in the catchment areas of the treatment plants (Sommers, 1977).

Cadmium in Plants

Cadmium from soil is taken up by plants through their roots and is deposited on the leaves from aerial fallout. The majority of cadmium content from aerial fallout remains on the leaf surface and cadmium retention is higher with rough and hairy surfaces (Little, 1973). The amount of cadmium in plants varies with species. Unless there is dust from recent fallout on the leaves, seeds tend to contain more cadmium than leaves. Rice normally contains a mean of $0.029 \, \mu g \, g^{-1}$ cadmium (Masironi et al., 1977), while in highly polluted areas $0.72-4.17 \, \mu g \, g^{-1}$, with a mean of $2.50 \, \mu g \, g^{-1}$, were recorded (Hise and Fulkerson, 1973). Cadmium in australian wheat grains ranged from 0.012 to $0.036 \, \mu g \, g^{-1}$ (Hise and Fulkerson, 1973; Williams and David, 1977).

Some species of plants contain exceptionally high concentrations of cadmium. Lounamaa (1956) found $120 \, \mu g \, g^{-1}$ ash weight in lichens, which was about ten times that in herbs and tree leaves. Edible mushrooms may contain a surprisingly high amount of cadmium. Concentration of more than $10 \, \mu g \, g^{-1}$ wet weight have been recorded (Lorenz et al., 1978).

Cadmium uptake of plants increases not only with increased soil cadmium content, but also with the type of soil and particularly its acidity. Acid soil makes cadmium and other heavy metals more available to plants (Lagerwerff, 1971; Maclean, 1976; Williams and David, 1976). Soil is acidified by SO_2 derived from burning coal and mineral oils with high sulphur content which may be significant in areas around large power stations.

From data available, cadmium apparently does not accumulate in natural eco-systems to the extreme levels reported for other heavy metals. Excessive cadmium is toxic to plants, causing stunting,

reduced chlorophyll-content, chlorosis, wilting and necrosis.
Susceptibility varies with species and some,like spinach and soybeans,
are more sensitive, while others,like tomatoes and cabbage, are more
resistant (John, 1973; Bingham and Page, 1975).

Pathological Effects of Acute Intoxication

Occasionally there may be an acute oral intoxication from solder
in water pipes, taps or refrigeration devices, or from unwashed
contaminated hands (Cartensen and Poulsen, 1973). Nausea, vomiting,
diarrhoea, abdominal cramps, headache and salivation were found in acute
intoxication. In fatal cases, there is shock due to loss of fluid and
death within 24 h. Alternatively, death may be caused within one or two
weeks by acute renal failure and cardiopulmonary depression. The single
no-effect dose in man is estimated to be 3 mg and the lethal dose
350-8900 mg (Lauwreys, 1977).

The first case of inhaled cadmium toxicity was reported by Sovet
in 1858, when three persons polishing silver with cadmium carbonate
were exposed to clouds of cadmium dust. Nowadays, inhalation of large
quantities of cadmium causing acute intoxications occurs usually with
metallurgical processes involving heating, such as welding, soldering
and smelting. Welders appear to be a particularly high-risk group
(Friberg, 1978).

Clinically, there is little discomfort at the time of exposure.
However, several hours later bronchial and pulmonary irritation develop
with dry throat, dizziness, weakness, chills, fever, chest pain and
dyspnoea. If the exposure is fatal, death is caused by delayed pulmonary
oedema (Hammond, 1980). Friberg et al. (1974) estimated the fatal dose
to be 5 mg m^{-3} of cadmium oxide fume for 8 h. Lesser doses have been
linked with interstitial pneumonia (pneumonitis). The no-effect level

after long-term inhalation is still speculative, but has been estimated as 20-50 μg m^{-3} of respirable cadmium oxide and acid-soluble cadmium dust (Lauwreys, 1977).

The pathogenesis of lung lesions is not well understood, but Limited observations indicate pulmonary changes of interstitial pneumonia similar to those caused by inhalation of NO_2 (Evans et al., 1973), or inhalation of ozone (Plopper et al., 1973) and oxygen (Kapanchi et al., 1969) in high concentrations. It is hypothesized that alveolar macrophages become excessively stimulated and damaged, and release excessive amounts of enzymes from their lysosomes, whose digestive capacity may damage the pulmonary tissue (Samarawickrama and Webb, 1978).

Pathological Effects of Chronic Intoxication

While acute intoxications with cadmium are rare, it is now realized that chronic intoxications are far more important. Whichever way cadmium is administered,it eventually finds its way to the kidneys, where one third of all the body burden may be found, predominantly in the cortex. The organ with the next highest concentration, but always lower than in the kidney, is the liver.

It appears that both plants and animals have not evolved an efficient system for eliminating cadmium because of the very low concentration of this metal in the environment. In animal, cadmium is excreted mainly through the intestines by desquamation of epithelial cells (Valberg, Sorbic and Hamilton, 1976). Although cadmium is toxic to virtually every system of the body, kidneys are the critical organ for cadmium poisoning. The first signs may occur after a long interval

following cessation of exposure. It is estimated that under normal conditions in an unpolluted environment, in man kidneys have accumulated 50 μg g^{-1} wet weight by the age of 50 years (Friberg *et al.*, 1974). The critical concentration appears to be 200 μg g^{-1} when kidney failure sets in. The first functional change is glycosuria, followed by abrupt diuresis and aminoaciduria, low molecular weight proteinuria, aciduria and hypercalciuria. Deficient kidney function is also revealed by using concentration tests and inulin clearance tests. Kjellstrom *et al.*(1977) and Friberg *et al.*(1974) reported that after 10 years of occupational exposure to 25 μg m^{-3}, kidney cortex may accumulate cadmium at the critical concentration of 200 μg g^{-1}.

The morphological details of kidney damage, like damage to other organs, have so far not been fully described in man. Some conclusions can be drawn from experimental animals, although information is still sketchy and there are differences between species. The primary damage is probably in the tubules and may be recognized within 24 hours after cadmium injection (Murakami and Webb, 1981). In rats and rabbits, desquamation of epithelial cells of the proximal convoluted tubules have been reported, as well as vacuolation and granulation of residual cells. Kawai *et al.* (1979) injected rats subcutaneously with 0.5 mg kg^{-1} day^{-1}, 6 days a week for 25 weeks, kidneys showed only slight swelling of the tubular epithelium, apart from minute casts in the proximal tubules. However, during the next 10-15 weeks, the epithelium of the proximal convoluted tubules became atrophic and after 22 weeks interstitial nephrosclerosis developed.

After dosing rabbits with cadmium, Kawai *et al.* (1979) found first non-specific lesions, mainly cloudy swelling in the proximal

convoluted tubules, which disappeared after a few months, followed one year after by tubular atrophy and interstitial oedema. After 19 months, amyloidosis developed with marked glomerular involvement. More recently, the characteristic of morphological changes of glomerulonephritis were reported in rats induced by long-term oral exposure to cadmium. This indicates damage caused by cadmium which may incite an antigen-antibody reaction which characterizes of glomerulonephritis (Joshi *et al.*,1981).

Liver tends to contain the second highest concentration of cadmium, and a variety of lesions have been described in a number of experimental animals (Stowe *et al.*,1972), including single parenchymal cell necrosis, focal necrosis, congestion, vacuolation of Kupffer cells, lymphocytic infiltration, fibrosis, biliary hyperplasia, desquamation of lining cells and changes in the smooth and rough endoplasmic reticulum. The pathogenesis of cadmium hepatotoxicity is not clear. It appears that functional changes as indicated by liver function tests precede morphological changes (Cook *et al.*, 1974), and the critical determining factor of cadmium toxicity is not the administered dose, but rather its tissue concentration (Colucci *et al.*, 1975).

Cadmium and Testicular Changes

The testes appear to be the most susceptible organ to cadmium toxicity, although their cadmium concentration is the least of all organs with the exception of blood (Gunn and Gould, 1970). Following parenteral injection of a soluble cadmium salt, there was a very rapid progression of changes in the testes and proximal part of the epididymis which included haemorrhagic necrosis followed by atrophy and permanent sterility. The nuclei of the spermatozoan precursors were disrupted,

while the mature spermatozoa were less affected (Gabbiani et al.,1974; Lee and Dixon, 1973). These changes were seen in rats, mice and other scrotal mammalian species, but not in non-scrotal animals such as hedgehog and shrew (Dryden and McAllister, 1970), and birds (Lofts and Murton, 1967).

Lesions could be prevented and normal mating reactions as well as fertility could be preserved with maintenance doses of testosterone, indicating that these changes were secondary to the loss of androgen. Changes in the accessory glands were mainly atrophy and consistent with what could be expected with loss of androgen production (Chandler and Timms, 1976; Saksena et al., 1977). Although the mechanisms of testicular lesions have not been fully clarified, they appear to be caused primarily by damage to the testicular endothelium and circulatory failure (Chiquoine, 1964; Schlaepfer, 1971).

Cadmium and Ovarian Changes

Ovarian changes have been produced by cadmium in prepubertal rats (Kar et al.,1959), hyperaemia of ovaries, atresia of follicles and haemorrhage into the follicular cavity, followed by recovery after about one week. The ovaries of adult rats were not affected.

Placental Transfer of Cadmium

A variety of animal species including mice (Wolkowski, 1974), hamsters (Ferm et al.,1969), and rats (Sonawane et al.,1975) have been used for investigation. Early study by Berlin and ullberg (1963), using autoradiography, demonstrated an uptake of cadmium in mice placenta after intravenous injection of labelled cadmium (^{109}Cd). No cadmium accumulation in the fetuses was observed. Because of the extreme

concentration difference between the mother and the fetus it was

suggested that whole-body autoradiography may not be sensitive enough

to detect the extremely small amounts of cadmium in fetal tissues

(Wolkowski, 1974). Later studies with mice which were injected (i.v.) with

^{109}Cd on the twentieth day of gestation showed that cadmium could cross

the placenta and reach the fetus. Neonatal mice showed a cadmium content

of approximately 0.09 % of the maternal dose as determined by whole-body

scintillation counting (Tanaka et al., 1972). Placental transfer of

cadmium has also been demonstrated in neonatal hamsters with ^{109}Cd

administered on the eighth day of gestation (Ferm et al., 1969).

A 60-fold decrease in embryo-cadmium content was detected between day 9

and day 12 of gestation. These authors suggested that the developing

yolk sac might be actively removed some of the cadmium from the fetus.

This suggestion, however, needs to be confirmed by future experimentation.

The protective function of the yolk sac was not observed in mice where

the yolk sac ceases to exist in late gestational stage resulting in an

increased fetal mortality (Wolkowski, 1974)

It was found that fetal and neonatal gut and liver concentrated

a significant amount of cadmium as a result of in utero exposure to

cadmium in early gestational peroid (Lucis et al., 1972). Sonawane

et al., (1975) showed that cadmium may cross the placenta at any time

of the pregnancy. The placental and fetal cadmium concentrations also

increase with the dose of cadmium exposure and with the gestational

stage of the animals (Sonawane et al., 1975). Ahokas and Dilts (1979)

also demonstrated that only a small amount of cadmium reached the

embryo prior to the formation of a functional placenta. After

establishment of placenta, large accumulation of cadmium was detected

in the placenta with comparatively little cadmium transferred to the

fetus. However, the placental cadmium transfer increased with the
dose of cadmium exposure (Ahokas and Dilts, 1979). In an attempt to
determine the extent of placental barrier to cadmium transfer, Kelman
and Walter (1977) measured the blood cadmium concentrations on either
side of perfused guinea pig placentas. They found that cadmium was
cleared very rapidly from the maternal blood, and that this clearance
was linearly related to perfusion rate. It was suggested that the low
maternal fetal cadmium transfer is influenced by factors such as maternal
metallothionein function or the maternal plasma cadmium levels and may
not be related to the effectiveness of the placental barrier (Kelman
and Walter, 1977).

Recent studies indicated that a cadmium-binding protein (CBP)
found in the maternal system or in the placenta may play an important
role in the fetotoxicity induced by cadmium. The synthesis of this CBP
is believed to be zinc dependent. Investigation by parizek *et al.*(1968)
demonstrated that zinc-deficient rats displayed greater fetotoxicity
upon exposure to cadmium. However, no significant difference in fetal
cadmium content was observed between control or zinc deficient animals
(Rohrer *et al.*, 1978). Thus the CBP may exert its protective effect on
the fetus by complexing with the cadmium at the maternal or placental
level without reducing the placental cadmium transfer or the fetal
cadmium content. The precise protective mechanism, however, has not yet
been described.

Pathological Effects of Cadmium on the Placenta

Using pregnant albino rats, Parizek (1964) first reported that
a single subcutaneous injection of 0.04 mM (4.5 mg) $CdCl_2kg^{-1}$body weight
given to the animal on gestational day 17 to 21 resulted in degenerative
changes of the placenta(pars fetalis) and hemorrhage within 24 hours
of administration.

Despite the removal of fetuses from the womb prior to cadmium injection, the remaining placenta *in situ* still exhibited vascular degeneration upon cadmium administration. Chiquoine (1965) performed a subsequent experiment with mice to examine the sensitivity of the placenta to cadmium toxicity in relation to the time of pregnancy. Animals were injected subcutaneously with 6.7 mg $CdCl_2kg^{-1}$body weight between gestational day 1 and 17. It was found that injection of cadmium on days 1 to 5 of pregnancy resulted in normal fetuses at parturition. Gross signs of placenta and decidual necrosis and hemorrhage were observed in animals injected with the same dose of cadmium after the thirteenth day of pregnancy. Hemorrhage within the uterus and embryonic death were found in these animals. Between day 6 and 12 of pregnancy, similar alteration of the uterine vascular system and microscopic changes in the embryos showed a varying degrees of autolysis and degeneration. It was concluded that a single injection of cadmium chloride given to pregnant mice on any day from the sixth to the seventeenth of pregnancy results in intrauterine death of the embryos and localized necrosis of the placenta or adjacent decidual tissue. Despite the acute and rapid placental necrosis and fetal death, no irrevocable harm was reported in the maternal animals.

Webb (1970) also reported consistent placental necrosis and intrauterine embryonic death in rats which were injected subcutaneously with 2.5 mg $CdCl_2kg^{-1}$body weight on day 11, 15,17, and 18 of pregnancy. Preinjection of cadmium prior to mating produced only minimal effect on the pregnancy. It was concluded that preexisting stores of cadmium, accumulated before pregnancy, are not mobilized by the maternal animal to produce any significant damage to the developing fetus.

Teratogenic Effects of Cadmium

A number of studies have demonstrated that cadmium interferes with prenatal mammalian development and may cause a wide range of malformations (Ferm and Carpenter, 1968; Ferm, 1971; Barr, 1973). Despite various factors such as strain of animals, route of administration, dose of cadmium given, and period of gestation that may influence the teratogenic effects of cadmium, facial malformations seem to be a consistant and prominent finding in cadmium-induced teratology (Mulvihill et al., 1970; Ferm, 1971; Barr, 1973).

Mulvihill et al. (1970) investigated the facial malformation in hamster fetuses induced by intravenous administration of cadmium sulfate (0.44 mgkg^{-1})on day 8 of pregnancy. Delayed ossification of the palatine shelves as well as the absence or bifurcation of the cartilaginous nasal system was found in the 14-day fetuses. Other skeletal defects (ribs,limbs, and skull) were also observed in rat and mouse embryos after cadmium exposure (Barr, 1973; Ferm, 1971; Gale and Ferm, 1973). Besides skeletal anomalies, Barr (1973) reported a markedly attenuated abdominal musculature, undescended testicles, and deformities of eyes and ears of rat fetuses exposed to 1-2 mgCdCl$_2$ kg^{-1} on day 9-11 of gestation.

From the above studies, it is not fully recognized whether cadmium acts directly on the fetus or indirectly through alteration of maternal metabolism or nutritional function of placenta. It is therefore important to evaluate the toxic activities of cadmium under various experimental conditions. The most importance is the study of its adverse effects on embryonic tissue, which is known to be very sensitive to chemical injuries.

Problem and Outline of Plan

In recent years, techniques for the culture of postimplantation rodent embryos have been refined to a point where simple and reproducible methods support growth and development which approximate that of embryos *in vivo* (see New, 1978, for review). The possibility of maintaining viable mammalian conceptuses *in vitro* is particularly attractive to toxicologists since it removes the complex influences of the maternal environment upon toxicogenic activity. Postimplantation embryo culture has been utilized in several investigations of the derect embryotoxicity of agents (New, 1978; Kochhar, 1980; Schmid *et al*.,1981; Schmid *et al*., 1982). Recent applications include the detection of dilantin teratogenic activity in human serum (chatot *et al*.,1980) and the demonstration of cyclophosphamide bioactivation to an ultimate teratogen (Fantel *et al*., 1979). The aim of the work to be described in the present thesis is, therefore, to evaluate the relative embryotoxicity of cadmium chloride with the postimplantation embryo culture system and compare its effects with those induced *in vivo*. For these purposes, in the first series of experiments we investigated the *in vitro* and *in utero* growth and development of mouse embryos, *in vitro* dose response relationship of cadmium chloride, and the influence of cadmium chloride on growth and development of mouse embryos *in vitro*. In the second series of experiments, we investigated: *in vivo* dose response relationship of cadmium chloride, the adverse effects of cadmium chloride administered during organogenesis-phase of embryonic development *in utero*, and the effects of cadmium chloride on postnatal growth and development.