EFFECT OF ZINC ON THE TERATOGENIC ACTION AND TISSUE DISTRIBUTION OF CADMIUM IN THE RAT

by

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B.Sc. Universidad Central de Venezuela, 1976

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
M ASTER OF SCIENCE

in
THE FACULTY OF GRADUATE STUDIES
Division of Human Nutrition
School of Home Economics

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
February 1980

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ABSTRACT

The interaction of zinc and cadmium was studied in pregnant rats and their fetuses. Four experimental groups of animals were used, with each group being given one of the following treatments by intraperitoneal injection: (1) cadmium chloride (16µM/kg body weight) with 115m-cadmium, (2) cadmium chloride (16µM/kg body weight) plus zinc chloride (16µM/kg body weight) with 115m-cadmium, (3) zinc chloride (16µM/kg body weight) with 65-zinc, or (4) zinc chloride (16µM/kg body weight) plus cadmium chloride (16µM/kg body weight) with 65-zinc.

In the first experiment the chemicals were administered on day 9 of pregnancy and the animals were sacrificed on day 20 of pregnancy. Fetuses were examined for gross defects and maternal tissues and fetuses were counted for radioactivity in order to determine the distribution of cadmium and zinc.

Gross abnormalities were seen in group 1 (cadmium), where 28.8 percent of the fetuses were malformed. Defects included absent tail (7.7%), dysplastic tail (10.3%), thin abdominal wall (6.4%), dysplastic neck (5.8%), dysplastic eyes (3.2%), exencephaly (1.9%), dysplastic ears (1.3%), dysplastic limbs (0.6%), and stunted body (0.6%). In group 3 (zinc), 2 malformed fetuses both with exencephaly were found in one litter. No malformations were found in groups 2 and 4 (cadmium plus zinc). In addition, fetal weight was significantly decreased in group 1 (cadmium) when compared with groups 2, 3, and 4. There were no differences in numbers of resorption sites, numbers of dead fetuses, or litter size among the four groups.

There were no differences in the maternal tissue
distribution of cadmium between group 1 (cadmium) and group 2 (cadmium-zinc). On the other hand, zinc accumulation was significantly increased in group 4 (zinc-cadmium) compared with group 3 (zinc), in liver, kidney, adrenal, heart, lung, spleen, placenta, fetus, fat, uterus, muscle, and plasma.

In the second experiment pregnant Wistar rats were given treatments 1 (cadmium) and 2 (cadmium-zinc) on day 9, and tissues were sampled on day 10, 11, and 12 of gestation. When animals given cadmium were compared with those given cadmium plus zinc, a significantly greater amount of radioactivity (115m-cadmium) was found in the uterus, uterus plus fetus, fetus, fat, and plasma on day 10 in the cadmium group. On day 11 the only differences found were increased amounts of radioactivity in the adrenals and small intestine. On day 12 radioactivity was increased only in the femur.

When the 4 different sampling days (10, 11, 12, and 20) were compared, it was found that in most tissues the amount of cadmium deposited decreased with time except for the kidney, where cadmium increased, and the liver and spleen where there was no change.

The data suggest that cadmium reaches the fetus in the early hours after injection and is excreted thereafter. Simultaneous administration of zinc did not change cadmium accumulation in maternal tissues but significantly reduced the amount of cadmium reaching the fetus 24 hours post-injection. On the other hand, simultaneous administration of cadmium increased the accumulation of zinc in most of the organs investigated.
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ACKNOWLEDGEMENT

I would like to thank my supervisor Dr. Melvin Lee for his advice and encouragement throughout the whole of this work. I am also grateful to Dr. Kenneth Chan for his interest in the project and for the many useful discussions we had together.

Also acknowledged are Dr. Patricia Gallo and Dr. John Vanderstoep for their helpful suggestions and comments, as well as Mrs. Virginia Green for her help with the statistical analysis of the data and Miss Christina Duke for her collaboration in the production of this manuscript.

Thanks are also due to my husband, Francisco Garcia for his suggestions, patience, and understanding.

Financial support from the Venezuelan Government through FGMA (Fundacion Gran Mariscal de Ayacucho), from the National Research Council of Canada Grant A4692, and a grant from the University of British Columbia Committee on Research, is gratefully appreciated.
CHAPTER I
INTRODUCTION

In recent years the presence of cadmium as an environmental contaminant has aroused concern because of its marked toxicity for humans (Flick et al., 1971), with both acute and chronic poisoning being described. Furthermore, cadmium is of concern because the sources are so widespread in nature. Cadmium is closely related, chemically, to zinc and is found wherever zinc occurs in nature, although the relative amounts of the two minerals vary from place to place. In most mineral deposits and soils the ratio of cadmium to zinc is between 1:100 and 1:1000. Since zinc is an essential nutrient for most forms of life (Underwood, 1962; Bowen, 1966; Schroeder et al., 1967; and Yamagata and Shigematsu, 1970), the close association between cadmium and zinc suggests that probably no naturally occurring material will be completely free of cadmium.

Furthermore, cadmium occurs as an environmental pollutant, because it is a byproduct of some commercial processes. For example, cadmium is obtained as a byproduct in the refining of zinc and other metals. Since it is difficult to separate cadmium and zinc, the former will often be found in small amounts in commercially available zinc compounds (Schroeder et al., 1967).

Environmental cadmium pollution probably has a very long history, as copper, lead, and zinc smelting are old processes. However, the concern with cadmium pollution and toxicity has become more urgent because of the increasing use of cadmium compounds by industry in this century. Not only is cadmium emitted into the atmosphere by mining and smelting (particularly
lead, copper, and zinc smelting), but cadmium is widely used in batteries, alloys, paints, and plastics, as well as in the plating industry and in certain ceramic finishes. The burning of oil and wastes, and the treatment of scrap metals also contribute cadmium to the environment, as does the use of cadmium-containing pesticides. Finally the increasing use of sewage sludge, which may contain a considerable amount of cadmium, for the fertilization of farm land can introduce cadmium directly into the food supply (Friberg et al., 1974).

The growing recognition of the importance of heavy metal pollution of the environment, including cadmium pollution, has focussed attention on the implications for human health. The effect of cadmium on mammalian reproduction and fetal development has been of particular interest. Although the possible role of cadmium in human teratogenesis remains unresolved, it has been recognized for some time that the oral administration of cadmium salts to pregnant mice causes an increase in fetal mortality, a retardation of fetal growth, and an increase in malformations (Schroeder and Mitchener, 1971). Cadmium sulfate, given intravenously, is teratogenic in hamsters, mainly producing malformations of the face (Mulvihill et al., 1970). Given intraperitoneally, it is teratogenic in rats (Barr, 1972), although the spectrum of malformations is quite different from that seen in hamsters. Cadmium as the chloride, acetate, or lactate, was shown to produce placental destruction and fetal mortality when administered subcutaneously to Wistar rats (Parizek, 1964). However, there appear to be some differences in susceptibility among different stocks of the same
strain of rats, as Barr (1973) demonstrated that the incidence and variety of fetal malformations differed in two stocks of Wistar rats given cadmium.

In addition to its teratogenic action, cadmium has been shown to evoke a number of other pathological responses, (Friberg et al., 1974), stimulating interest in the metabolism and tissue distribution of the metal. Cadmium has been found to be primarily concentrated in the liver and kidneys, although other tissues take up smaller amounts (Kotsonis and Klaassen, 1977). The pathological consequences are tissue and organ specific, including testicular atrophy (Parizek and Lahor, 1956), renal dysfunction (Piscator, 1962), hypertension (Perry and Erlanger, 1974), hepatic damage (Friberg, 1955), and anemia (Friberg, 1950). However, the manner in which cadmium exerts its toxic effects is not clearly understood.

There is some evidence that zinc exerts a protective action against pathological teratogenicity of cadmium. Ferm and Carpenter (1967, 1968) demonstrated that when zinc was administered to pregnant rats simultaneously with teratogenic amounts of cadmium, the incidence of fetal malformations was reduced, and Webb (1972) and Sarkar and Mondal (1973) reported that zinc prevents cadmium-induced testicular damage in rats, mice, and pigeons. Ferm and Carpenter (1967, 1968) suggested that the toxic activity of cadmium may be a consequence of its replacement of zinc in metalloenzymes. As zinc has been shown to be essential for normal reproduction and embryological development (Vallee, 1959; Forbes, 1967), its replacement by cadmium may be expected to have deleterious consequences.
Therefore, the research project described here was designed to investigate the relationship between zinc and cadmium in reproduction and development in the rat. Two aspects of this relationship were studied. The teratogenicity of cadmium and zinc and the interaction of the two minerals was examined by administering them individually or together to pregnant rats. Then the effect of simultaneous administration of zinc on the distribution of cadmium in the maternal tissues and fetus and of cadmium on the distribution of zinc in maternal tissues and fetus was examined, using 115m-cadmium and 65-zinc.
Although it has been known for more than a century that cadmium is an extremely toxic element and may constitute a source of damage to human health, it was not until the outbreak of the endemic "Itai-Itai" disease (chronic cadmium poisoning) among the inhabitants of Toyama, Japan that the effects of cadmium on health have attracted special attention in both environmental and medical professions (Ishizaki, 1971).

Since World War II, cadmium in metallic, inorganic, and organic forms has been widely used in metallurgical industries (Dubois and Geiling, 1959) for the production of cooking utensils, batteries, glass, paints, plastics, etc. (Flick et al., 1971). Because of the extensive use of cadmium, increasing awareness has been generated concerning the role of cadmium as an environmental hazard. Many investigators have speculated, based on experimental observations, that cadmium may be intimately associated with various pathological conditions such as certain forms of renal and cardiovascular diseases occurring in man (Piscator, 1962; Perry and Erlanger, 1974).

The biological effects of cadmium, according to Dencker (1975), deserve attention for many reasons. Its use in different industrial processes and products has increased its presence in the environment. Its exceptionally long half-life in the human body, about 10 to 30 years (Kjellstrom et al., 1971), stresses the importance of a better understanding of its action. Cadmium affects specific tissues in the body; for example, the steroid hormone producing organs (Parizek 1957; Kar et al., 1959; Kaul
and Ramaswani, 1970). In late gestation it causes placental necrosis, especially in the pars fetalis, and subsequent fetal death. In the mother, it causes pathological changes with clinical and morphological similarities to human toxemia of pregnancy (Parizek, 1965).

The metabolism of cadmium has been studied by Friberg et al. (1971) after exposure via respiratory, gastrointestinal, or injection routes. The extent of absorption is not known exactly with regard to inhaled or ingested cadmium, but reasonable estimates are about 10% to 40% for the inhaled and 2% for the ingested cadmium. The absorption of ingested cadmium may increase considerably if the diet is deficient in calcium, or protein, or both. There is also considerable individual variations in the absorption of cadmium (Fitzhugh and Meiller, 1941). After a single injection (Piscator and Larson, 1972), cadmium will initially be found mainly in the plasma, but during the first 24 hours after injection a rapid clearance from plasma takes place, so that eventually the concentration in the erythrocytes will exceed that in the plasma (Carlson and Friberg, 1957).

After repeated exposure cadmium will mainly be found in the blood cells, bound to proteins such as metallothionein and hemoglobin. During chronic exposure to cadmium there will be a gradual increase in blood levels of cadmium, but at a certain level a plateau will be reached and no further increase will be seen. When exposure has ceased, the concentration in blood will decrease. Fifty to 75% of the total body burden of cadmium will be found in the liver and kidneys. Immediately after a single
exposure most will be in the liver, but eventually renal levels will exceed liver levels. Repeated exposure to small amounts will also result in the renal concentrations surpassing the liver so liver concentrations will not exceed renal concentrations. The accumulation in the liver and kidneys seems, in large part, to be a result of the storage of cadmium in the cadmium-binding protein, metallothionein (Friberg et al., 1974).

Cadmium is primarily excreted in the feces and the urine, but other routes of excretion include hair, placenta, saliva, and skin.

In addition, the metabolism of cadmium is intimately connected with zinc metabolism. The low molecular weight protein, metallothionein, is able to bind both cadmium and zinc, and these two metals will be transported together. (Bunn et al., 1962). Zinc is an essential metal and many enzymes are zinc dependent. Cadmium seems to have the ability to exchange with zinc in different organs, causing change in enzymatic activity (Friberg et al., 1974). During exposure to cadmium, organ levels of zinc will increase, but the actual mechanisms responsible for this are still not clear (Friberg et al., 1974).

**TERATOGENIC ACTION: GENERAL CONSIDERATIONS**

When experimental mammalian teratology developed in the 1930's, the science of experimental teratology in lower classes of animals was at least 100 years old. Experiments on avian and amphibian eggs were performed successfully during the nineteenth and early twentieth centuries, and many principles and fundamental facts were already known. However, there was doubt
that the results obtained on eggs of lower forms could be applied to mammalian or human situations. It was thought that mammalian embryos and fetuses were so well protected by the maternal organism that they could not be modified by conditions shown to be teratogenic for embryos of lower animals. It was thought that adverse environmental conditions would either kill the mammalian embryo or leave it unharmed (Warkany, 1964).

Modern reproductive studies are concerned with evaluating possible effects on fertility, on the zygote, on its transport, on implantation and development, on the teratogenic potential, on parturition, on the newborn, on lactation, on weaning, on care of the young and on delayed postnatal anomalies (Frohberg, 1977).

A variety of factors influence the outcomes of teratogenicity studies. In teratogenic studies, misleading negative or positive results can be obtained by using the wrong animal species. Methotrexate, which is teratogenic in man and in small rodents, does not cause malformations in rhesus monkeys (Wilson and Fradkin, 1969; Wilson, 1971). Carbaryl is teratogenic in dogs, but not in rats, guinea pigs, rabbits, hamsters, rhesus monkeys, or man. The result obtained in dogs with carbaryl, however, are not relevant to man as the metabolism of carbaryl is different in the two species (Smalley et al., 1968; Weil et al., 1972, 1973).

As differences between species are in part due to differences in the metabolism and pharmacokinetics of drugs, animal species used for teratogenic studies should, if possible, absorb, distribute, metabolize, and excrete the test compound in
a manner similar to that in man. In the case of drugs, their pharmacodynamic properties should be taken into consideration. Adverse pharmacological effects on the maternal animal, like anorexia or sedation, may have an influence on fetal development (Frohberg, 1977). Therefore in all teratologic studies the dams should be subjected to comprehensive clinical and post-mortem examination in order to detect lesions which may impair fetal development.

Although there are some differences in recommendations for teratogenicity testing, as far as duration of treatment and the time of administration are concerned, the relationship must be closely examined, because embryonic differentiation proceeds rapidly during the period of organogenesis (Frohberg, 1977). In general, drugs and chemicals are administered during the period of organogenesis, since the onset of teratogenic susceptibility occurs at about the time the germ layers are formed. In mammals this is several days after conception, about 5 days in hamster and mouse, 8 days in rat, 9 days in rabbit, assumed to be 10 days in monkey, and could be as early as 11 or 12 days in man. Most organs have a period of particular susceptibility to teratogens, and this very likely coincides with early and critical developmental events in that organ (Wilson, 1964).

Furthermore, administration of a teratogen during an earlier period of gestation may cause embryonic death so that malformations likely to appear as a result of later treatment may be hidden. For this reason Wilson (1973) recommended administering suspected teratogens in successive short segments during the period of organogenesis.
In addition, important information may be gained from studies in which the drug concentration is measured in the placenta and in other maternal and fetal tissues to determine whether the pharmacokinetics of a chemical differ in the pregnant and the non-pregnant animal. Physiological substances normally are accumulated in fetal tissue in a similar way as in the maternal tissues. The distribution pattern within the fetus sometimes shows a very strong and selective accumulation in one particular fetal tissue. Tetracycline accumulates selectively in the fetal skeleton, thiouracil in the fetal thyroid gland (Ullberg et al., 1970). The distribution pattern is influenced by chemical properties, such as fat solubility and degree of ionization. Fat soluble compounds generally pass both into the brain and into the fetus, while other drugs may be blocked by placental and perhaps blood-brain barrier. However, some substances seem to cause fetal damage without reaching the fetus (Frohberg, 1974).

In addition, one important point is the dosage. There is a lower range which permits normal development, and a higher range which kills all the embryos, and the mother also if extended far enough. Between these ranges there is a narrow teratogenic zone in which dosage is sufficient to interfere with specific developmental events without destroying the whole embryo (Wilson, 1964).

Therefore, the type of malformations induced by a teratogen depends on the chemical structure, the time of administration and, in part, on the dose. During the early cleavage stages, blastogenesis, the susceptibility of the embryo is normally less
than during organogenesis. However, there are reports showing teratogenicity in early cleavage stages caused by X-rays (Rugh and Grupp, 1959).

A final consideration in teratogenic studies may be genotype, the importance of which has been amply discussed (Fraser, 1961). In simplest terms genotype is important because it determines the inherent susceptibility of an embryo to a given agent at a given time of development.

When organogenesis is completed, the embryo enters the fetal period, which is characterized principally by growth and functional maturation. During this period, teratogenesis, in the strict sense does not occur because embryonic processes can no longer be interrupted or diverted. Any agent sufficiently potent to affect the fetus would either retard growth or cause pathology of the types that occur in postnatal animals. Most of the lesions associated with congenital syphilis should, in this light, be regarded as examples of congenital pathology rather than malformations. The developing rat enters this period about the seventeenth day postconception. Human embryogenesis is completed about the end of the eighth week of intrauterine life and, developmental defects for the most part cannot be produced after this time (Wilson, 1964).

CADMIUM AS A TERATOGEN

Increasing attention is being given to the role that trace elements may play in disease processes. However, relatively little is known about the role that trace elements may assume in mammalian reproduction. Many of the heavy metals normally
present within the body in small quantities are becoming more important constituents of the atmosphere in areas of heavy industrialization and, as such, are unknown factors which may contribute to reproductive problems.

It is interesting to note that some of the cadmium emitted to the air by industries and mines will be inhaled by people and animals, but most of it will be deposited in soil or water. The cadmium deposited in water may then increase the concentrations of cadmium in aquatic organisms. In the event of flooding or irrigation, cadmium in water might also increase the concentrations in soil, in turn causing an increase in cadmium in agricultural products, such as rice and wheat (Friberg et al., 1974). In addition, according to Cherian et al. (1978), the largest sources of cadmium in the normal diet are cereal and meat, especially liver and kidney, where cadmium occurs in the form of metallothionein. Since metallothionein is heat stable (Cherian, 1974), this protein-metal complex is probably not destroyed by cooking and thus may be the main dietary form of cadmium for people who consume these organ meats.

Previous experiments have demonstrated the complex teratogenic interaction of cadmium and zinc (Ferm, 1969). Preliminary studies showed that cadmium concentrations of 7-18μM/kg of body weight were teratogenic in rats when given on day 9, 10, or 11 of gestation, but excess fetal mortality and malformations were not found after administration of cadmium on day 6, 7, 8, or 12 (Ferm, 1969). Cadmium concentrations in excess of 22μM/kg often killed pregnant rats (Webb, 1972). Berlin and Ullberg (1963) were unable to demonstrate that 109-
cadmium crossed the mouse placenta late in gestation, although Ferm et al. (1969) found significant radioactivity in the fetus 24 hours after cadmium was given intravenously to pregnant hamsters on the 8th day of gestation. Therefore, whether or not cadmium reaches the fetus and whether it is concentrated in affected sites is unknown.

Furthermore, Ferm et al. (1969) showed that 96 hours after administration, the concentration of cadmium in the hamster fetus had decreased some 60-fold from the concentration present 24 hours after injection. Thus there was a net elimination of cadmium from the fetus beyond the reduction in concentration due to increase in fetal mass. Appreciable amounts of 109-cadmium were found in the hamster yolk-sac and placenta, and this may bear on the problem of how the fetus eliminates cadmium from its body (Ferm, 1969).

Ferm (1971) stated that cadmium has been shown to induce serious malformations of the face when administered to pregnant hamsters at specific times in development. The time of administration for these facial defects coincided with the beginning of the period of critical embryogenesis. For example, the golden hamster undergoes a very rapid differentiation during the 8th day of gestation. Beginning early on the 8th day it is in an early primitive streak stage and at 8 p.m. on the 8th day 4 to 6 somites have formed. Early on the 9th day of gestation, the neural tube has completely closed and the heart has started to beat. Thus, the hamster embryo telescopes its major stages of differentiation into a 24-hour-period. The site specificity of a number of metal teratogens has been demonstrated and the full
range of deleterious effects of these metals on developing organ systems should be determined. He also stated that these molecular teratogens might well exert their effects via their important roles in the moieties of the metalloenzymes and this may yield some valuable information concerning the morphogenesis of specific malformations.

In addition, Ferm (1971), using hamsters, has reported that the spectrum of malformations changed as the time of the teratogenic insult is shifted to later periods in embryonic development. Thus, facial malformations and exencephaly are found in embryos treated in the early stages of development (beginning on the 8th day) while injections at later stages of development (later on the 8th day) reveal a decided decline in face and head abnormalities with a shift to rib and upper limb defects. Abnormalities of those embryos treated on the 9th day of gestation consisted primarily of rib and limb defects with a change toward defects of the lower limbs. Rib defects were principally those of fusion of adjacent ribs. Also, animals treated in the 9th day of gestation revealed severe abnormalities of the caudal region characterized by fusion of the lower extremities, and sympodia.

Ferm (1971) also found that embryos treated on the 8th day of gestation showed a higher rate of death in utero as well as a higher incidence of developmental malformation among the living embryos. Therefore, he offered two hypotheses to explain the teratogenic events. The first was that the teratogen is nonspecific, the result depending on the organogenetic event at the time of the insult. The second was that certain teratogens
might well prove to be site-specific and induce malformations only in certain organ systems.

Barr, (1973) studying rats found that when the rats were injected with 16 μM/kg body weight of cadmium chloride on three different days of gestation (9th, 10th, or 11th), fetal weight was depressed. Anophthalmia or microphthalmia on one or both sides was most often found after cadmium on day 9, and dysplastic or absent ears were found only after day 9 treatment. Hydrocephalus was observed after cadmium treatment on day 9 or 11. Malformation of the tail, ranging from complete absence to tight curling of the tip was found in a high percentage of the survivors following injection on day 11. In this study the author did not offer an explanation for the teratogenic effect of cadmium, stating that the mechanism of cadmium toxicity in mammals has not been established. The toxicity of cadmium has been shown (Flick et al., 1971) to be inhibited by various agents such as selenium and zinc, leading Barr to suggest that cadmium was competitive with other cations, especially zinc, for specific enzymes.

In another study, Ferm (1969) made an attempt to combine the teratogenic effect of cadmium and lead using pregnant hamsters and found that the teratogenic effects of both cadmium and lead, when injected separately into pregnant hamsters, corresponded well with previous data (Ferm and Carpenter, 1968). Cadmium caused anterior malformations mainly, while lead only caused tail malformations. However, the combination of these agents, revealed that the frequency and severity of the defects in the lip and palate caused by cadmium were reduced in the
presence of lead, while the posterior tail malformations caused by lead appear to be potentiated in the presence of cadmium. Sympodia (union of the feet) was never seen in the animals treated with lead only but did appear with relatively high frequency when cadmium was added to the lead. Therefore, Ferm (1969) speculated that there was a teratogenic effect on embryonic tissues, a block in placental transfer of some essential metabolite, or an induced defect in maternal metabolism which secondarily affects the differentiating embryonic tissue. He suggests that, because of their function in several metalloenzymes, heavy metals may play an important role in embryonic differentiation. Thus, it is possible that under experimental conditions employed by Ferm (1969), cadmium and lead interact additively on certain enzyme systems in the case of the tail malformation. However, lead blocks the effect of cadmium on other enzymes, such as those of the differentiating visceral arch systems, preventing the facial abnormalities.

Holmberg et al. (1969) were also concerned with determining the interrelationship of trace elements as a cause of malformations. They studied the effects of selenium, cadmium, and arsenic on the developing hamster embryos by analysing the fetal mortality and congenital malformations and found that cadmium and arsenic were independently teratogenic. Also selenium, which was not itself teratogenic under similar conditions, did provide significant protection against the malformations induced by cadmium or arsenic when injected simultaneously with either of these teratogens.

Furthermore, Parizek and Lahor (1956) and Parizek (1957,
1960) stated that one single injection of cadmium in doses sufficient to produce complete testicular necrosis in males, would when administrated to pregnant rats, result in a complete destruction of the fetal part of the placenta and death of the fetuses. Parizek (1964) stated that the placental damage was specific for cadmium and was a constant phenomenon already at earlier stages of pregnancy. In addition to this, during the last four days of pregnancy of rats the pathologic changes in the placentas and fetuses were accompanied by additional pathologic changes affecting the maternal organism. During these days of pregnancy the administration of cadmium, in a dose well tolerated by nongravid or post-partum rats, was highly lethal not only for fetuses but for a very high proportion of pregnant rats, producing pathologic changes specific for this period of pregnancy and quite unusual for cadmium intoxication in non-gravid rats.

Parizek (1968) also studied how far the peculiar toxic effects of cadmium during pregnancy could be prevented by the administration of selenium. He injected Wistar rats with cadmium and selenium and found, that in contrast to the pathologic changes observed with cadmium alone, no characteristic changes were observed in the placenta when cadmium salts were injected simultaneously with selenium. Fetuses survived in all rats and no maternal deaths occurred. Hemorrhagic renal necrosis, typical of cadmium intoxication during the last four days of pregnancy, was not observed in rats treated simultaneously with cadmium and selenium. Thus, in agreement with previous preliminary reports (Parizek, 1967), selenium proved to be highly effective in
preventing the peculiar toxic effects of cadmium during pregnancy.

Another area of study has been the interference with facial formation caused by cadmium. Mulvihill et al. (1970) investigated the effect of cadmium in normal and cadmium treated hamsters and found that cadmium has a marked deleterious effect on the head mesoderm of golden hamsters, causing the production of numerous malformations, including unilateral and bilateral cleft lip and palate. Cadmium appears to have marked deleterious effects on cartilage and bone formation. In some animals the vertical cartilaginous nasal capsule was entirely missing. In others it was greatly foreshortened and bifurcated. Bone formation tended to be retarded in all areas. Where clefts were not present, bone formation appeared quite normal. However, they concluded that the mechanism of action of cadmium, whether directly on the differentiating embryonic tissue or indirectly through action on the maternal tissues, remains to be elucidated.

Finally, Chiquoine (1965) investigated the effect of cadmium in the pregnant albino mouse and stated that a single injection of cadmium given to pregnant mice on any day from the 6th to the 17th day of pregnancy results in intrauterine death of the embryos and localized necrosis of the placenta or adjacent decidual tissue. However, he concluded that cadmium did not cause permanent damage to the female reproductive tract.

**CADMIUM AND ZINC DISTRIBUTION IN TISSUES**

The effects of inorganic cadmium in humans and animals have
attracted considerable attention in the last 15 to 20 years. Much of that has been the result of the wide-spread interest generated by the cadmium-induced "Itai-Itai" disease in Japan among people exposed to cadmium-contaminated food (Hagino and Yoshioka, 1961). In the United States the daily human intake of cadmium has been estimated to be 200-500 ug (Schroeder et al., 1967), and the "Standard American Man" has been estimated to have an average body burden of 30 mg of cadmium which may increase with age (Underwood, 1971). A number of toxic effects in humans and animals resulting from chronic cadmium exposure have been reported (Friberg et al., 1974). Testicular atrophy (Alsberg and Schwartz, 1919; Parizek and Lahor, 1956; Gunn and Gould, 1970), hypertension (Schroeder and Vinton, 1962; Perry and Erlanger, 1974), renal dysfunction (Wilson et al., 1941; Friberg, 1950, 1952; Piscator, 1962; Axelsson and Piscator, 1966), central nervous system injury (Gabbiani et al., 1967), anemia (Wilson et al., 1941; Friberg, 1950), and hepatic injury (Friberg, 1955; Stowe et al., 1972; Wagstaff, 1973; Unger and Clausen, 1973; Hadley et al., 1974 Singhal et al., 1974; Johnston et al., 1975) are among the toxic effects attributed to chronic cadmium exposure. Therefore, it should be interesting to investigate how cadmium is distributed in different tissues.

According to Kotsonis and Klaassen (1977), the concentration of cadmium in rats 2 days after administration was highest in the liver, followed by the intestine, kidney, pancreas, heart, lung, testicle, muscle, brain, blood, and plasma. After 14 days most tissues showed approximately a 50% decrease in cadmium with notable exceptions, namely, the kidney,
which had up to a three to four fold increase, the liver, which remained the same at the highest doses, and the intestine, which had up to a 95% decrease. It was of interest to note that in their study only the brain had cadmium concentrations lower than the blood, presumably due to a blood brain barrier.

In addition, after 2 days only 7% of the cadmium in the blood was located in the plasma. Kotsonis and Klaassen (1977), also, stated that the cadmium in the blood quickly concentrates in the erythrocytes, bound probably either to hemoglobin or to a metallothionein-like protein. The high concentration of cadmium in the intestine after 2 days and the dramatic decrease after 14 days may be explained by the presence of an intestinal metallothionein which was subsequently lost during the normal turnover of intestinal epithelial cells.

They also reported that the concentration of metallothionein in the kidney at 2 days after administration of cadmium was unchanged but after 14 days had increased as a function of dose. In contrast, the concentration of metallothionein in the liver at 2 days had increased as a function of dose and was approximately the same after 14 days. Thus, the redistribution of cadmium to the kidney which was observed with time was probably accounted for by the longer time period required for the increase of metallothionein levels in the kidney.

In addition, the unusually long half-life of cadmium, estimated to be 200-300 days in rats (Derbin et al., 1957) was probably due to the presence of metallothionein since most of the cadmium located in the liver and kidney was bound to
metallothionein (Kotsonis and Klaassen, 1977).

Sugawara (1977) also studied the distribution of cadmium in mice but was interested in clarifying the interrelationship between injected cadmium and endogenous zinc in the liver and kidney. He reported that cadmium was detected in both organs as early as 0.5 hours after the cadmium injection and was about 14% and 1% of the injected dose in the liver and kidney, respectively.

Cadmium concentrations increased progressively with time in the liver, at 6 days after injection, the total content of cadmium in the liver reached a maximum level of about 54% of the injected dose. However, there was no significant difference in the concentration of the liver cadmium from the 10 hours group to the 20-day group. In the kidney this concentration increased with time and was about 7% of the injected dose at 20 days after injection. In the kidney, zinc concentration at 20 days increased significantly in comparison with that of the control group. In the liver, zinc concentration was not different from that in the control group up to 10 hours, but after 1 day this concentration increased significantly, and at 6 and 20 days was about two times that observed in the control group.

These results are in agreement with Webb (1972), who also studied the distribution of cadmium in liver and kidney of rats. In addition, Webb examined the question of whether the stored cadmium was mobilized, and thus became toxic to the maternal and the fetal organism during subsequent pregnancy. Virgin female rats were injected subcutaneously with cadmium, which was retained mainly in liver and kidney. When these animals became
pregnant, Webb found that the stored cadmium was not mobilized and thus, in contrast to "free" cadmium, did not induce toxemia or excessive fetal malformations.

Rohrer et al. (1978) examined the distribution of cadmium in zinc-normal and zinc-deficient maternal animals and found that the maternal tissue levels of cadmium increased as the maternal dose of cadmium increased. The increased levels were evident in all tissues from both the zinc-normal and zinc-deficient diets groups. In all cases for the zinc-normal group, the tissues from pregnant rats which received 1.5 mg/kg body weight dose of cadmium contained significantly greater amounts of cadmium than did the same tissues from pregnant rats receiving lower doses of cadmium. A different cadmium accumulation pattern was observed in the maternal tissues of the zinc deficient diet group. No consistent statistical differences existed in the cadmium content of the maternal tissues from different cadmium dose levels. These findings suggested that the cadmium binding capabilities of the maternal tissues may have been influenced by a transitory zinc deficiency.

Pietrzak-Flis et al. (1978) investigated the accumulation and distribution of chronically administered cadmium in two generations of rats and found that, in the first generation dams, at the end of gestation or lactation the cadmium concentration in kidneys was twice the level found in the liver. Also, cadmium concentrations in the brain reached a maximum after 43 days of exposure. In maternal milk, cadmium was detectable on the second day of lactation only in the highest group (5 ppm in the drinking water). Detectable blood cadmium...
levels were found only in adult animals of the 5 ppm exposure group. In the liver and kidney cadmium concentrations increased with time and increased more rapidly during the period 21 to 50 days than from 50 to 130 days of age. Pietrzak-Flis et al. (1978) suggested that these results may indicate that chronic exposure to constant levels of cadmium may result in maximum tissue levels much earlier than would be predicted.

Miller et al. (1975) also studied the effects of normal and zinc deficient diets upon the uptake and distribution of 115m-cadmium in the golden hamster. Cadmium retention in the kidneys of zinc deficient hamsters was found to be significantly lower than in kidneys from normal animals. A trend was noted toward increased retention of cadmium by the whole body and liver of these animals, which the authors stated may explain the relative decrease in the kidneys. Cadmium may have replaced zinc in the liver and other sites where zinc would normally be found, thus resulting in less cadmium being available for transport to the kidneys. In addition, the reduction in zinc intake observed may have resulted in decreased metallothionein production, reducing transport of zinc and cadmium to the kidneys. They also concluded that examination of the replacement of zinc by cadmium in the liver and pancreas and investigation of zinc deficiency effects on metallothionein levels may help explain the decrease in kidney cadmium seen in their investigation.

Cherian et al. (1978) studied the distribution, in the mouse, of cadmium from oral cadmium chloride and cadmium-metallothionein and found that there was no significant difference in the whole body retention of cadmium given in these
two forms during the experimental period of 7 days. However, 56% of the cadmium chloride was found in the liver compared with only 6.5% of the cadmium-metallothionein. In sharp contrast, 63.6% of cadmium accumulated in the kidneys when given in the form of cadmium-metallothionein, compared with only 8.7% when given as cadmium chloride. More cadmium also accumulated in bone, heart, lung, spleen, pancreas, and testes from cadmium chloride than from cadmium-metallothionein oral administration. These results were similar to the body distribution, especially in liver and kidney, after parenteral administration of cadmium chloride and cadmium-metallothionein respectively as reported by Cherian and Shaikh, (1975). They concluded that since the kidney was the main target organ for the chronic toxic effects of cadmium, the increased renal cadmium deposition after oral administration of cadmium-metallothionein may be of significance in deciding the maximum allowable amount of cadmium in food.

ZINC AS A TERATOGEN

Although the effects of many heavy metals on embryonic development in avian systems have been studied, few essential metals have received attention as possible teratogens affecting mammalian embryos. According to Ferm (1968), in fact, few data are available on the effects of essential trace metals on mammalian reproduction, and even less information is available concerning the permeability of the mammalian placenta to heavy metals during gestation. This problem is compounded by the marked variety of placental forms in mammals, as well as the changing structure of the placental membranes during gestation.
Zinc has been reported to produce only a mild teratogenic response. However, Ferm (1968) studying the interrelationship of cadmium, zinc, and copper in embryogenesis in hamsters, reported that the intravenous injection of zinc in amounts greater than 30 mg per kg body weight was lethal for the maternal animals within 24 hours. Dosage levels from 10 to 25 mg per kg body weight were well tolerated by the mother and induced a fetal resorption rate of only 12 percent. Zinc even at higher dosage level, produced few gross malformations, consisting of exencephaly and rib fusion. However, no consistent pattern of malformations was noted in the embryos treated with zinc.

In another study Ferm (1967) investigated the teratogenic effects of cadmium and its prevention by zinc, and stated that when pregnant hamsters were treated with 2 to 6 mg/kg of body weight of zinc on day 8 of gestation, out of 142 fetuses from 12 mothers only 4 were resorbed and only 2 were malformed. Therefore, he stated again that zinc alone provoked a very mild teratogenic response.

Chang et al. (1977) studied the teratogenicity of zinc chloride in doses of 20.5 mg and 25 mg/kg body weight, administered intraperitoneally to mice. They produced significant incidences of skeletal defects. As the dosage of the zinc salt was reduced maternal and fetal toxicity, relative fetal weight reduction, and the incidence of skeletal anomalies were correspondingly decreased. The principal site for the teratogenicity of zinc salts apparently was the skeletal system and with increasing doses the majority of the defects produced
involve the rib cage. Zinc chloride, 20.5 mg/kg body weight, exerted greater toxic effects on the mother and fetuses when administered on day 10 of gestation than on any other day. Ripple ribs caused by zinc first appeared when the injection was given on day 9 of gestation and became more frequent and pronounced after injection on day 11 of gestation. Therefore, they recommended that similar studies be carried out to determine more precisely the relevance of excessive challenge of zinc as an etiologic factor in the development of malformations in mammals. These studies will be very important, because the mechanism of zinc as a teratogen is an open question, as well as its relations to other teratogens, i.e., its species specificity, etc.

**CADMIUM AND ZINC INTERACTIONS**

Zinc is an essential metal. It is a constituent of a number of metalloenzymes, and acts as a cofactor for several other enzymes (Orten, 1966). Zinc is essential for normal growth and development (Halsted et al., 1974). There is a vast literature on zinc deficiency in animals, documenting, for example, impaired growth, testicular atrophy, and parakeratosis (Miller et al., 1958; Robertson and Burns, 1963; Hoekstra, 1969). Some of these effects have also been noted in zinc-deficient humans (Halsted et al., 1972).

Cadmium on the other hand, is not essential, is toxic, and accumulates in the human body with increasing age. It has been estimated that renal damage may occur at cadmium concentrations of over 200 µg/g wet weight in the kidney cortex (Friberg et
In Sweden for example, the average concentration of cadmium in the kidney cortex at 50 years of age has been found to be around 25 µg/g wet weight (Piscator and Lind 1972, Elinder et al., 1976), whereas much higher values have been reported from Japan (Tsuchiya et al., 1972).

In animals, it has been shown that zinc counteracts some of the toxic effects of cadmium (Bunn and Matrone 1966; Banis et al., 1969) and that cadmium enhances the effects of zinc deficiency (Petering et al., 1971). In humans and animals, increased cadmium levels in the kidney have been accompanied by increased zinc concentrations (Schroeder et al., 1967; Anke and Schneider 1971; Piscator and Lind 1972; Hammer et al., 1973; Piscator 1974). It has been postulated (Piscator and Lind, 1972) that this zinc increase is related to the increased concentration of renal metallothionein, which contains equimolar amounts of cadmium and zinc. A concentration of cadmium in kidney cortex exceeding 200 µg/g wet weight may give rise to tubular proteinuria. Friberg et al. (1974), Parizek et al. (1957) and Gunn et al. (1961) demonstrated that simultaneous subcutaneous administration of zinc and cadmium to rats protected against the severe testicular injury observed when cadmium was given alone, Gunn et al. (1963) reported also that cadmium-induced testicular tumors were prevented by the administration of zinc. Schroeder and Buckman (1967) showed that the increased blood pressure in rats given cadmium could be reduced by injection of zinc chelate. A marginal zinc intake by rats gives rise to high cadmium absorption and retention compared to animals given excess of zinc (Campbell et al.,
1978). Furthermore, even a low intake of cadmium aggravates the symptoms of zinc deficiency in rats (Petering et al., 1971).

In a number of in vitro studies, cadmium has been shown to decrease the activity of zinc-dependent enzymes (Immehoch et al., 1969; Vallee, and Ulmer, 1972). It has been proposed that the toxicity of cadmium, at least partly, can be explained by a competition between cadmium and zinc at cofactor sites in enzymes requiring zinc, resulting in decreased activities of these enzymes (Vallee, and Ulmer, 1972). Thus, the tubular proteinuria which occurs at high renal cadmium concentrations might be explained by decreased activities of certain zinc requiring enzymes, such as alkaline phosphatase and leucineaminopeptidase, which are thought to be engaged in the tubular reabsorption of proteins (Wachsmut and Torhorst, 1974). Reduced activities of these enzymes in kidney has been observed in pigs and rats perorally exposed to cadmium (Cousins et al., 1973; and Washko et al., 1975).

In higher mammals, such as man, horse, and pigs zinc concentrations in renal cortex have been shown to increase on a equimolar basis with the increase of cadmium up to a cadmium level of about 50 to 70 ug/g above this level the increase of zinc is less pronounced (Piscator et al., 1975; Elinder et al., 1977; Elinder et al., 1978). The basal level of zinc in renal cortex of humans and horses has been estimated to be about 25 ug/g (Piscator et al., 1972). The increase of zinc at low cadmium levels is believed to be a compensation for the increase of cadmium, a mechanism probably involving the production of a form of metallothionein which binds both zinc and cadmium in a
molar ratio of 1:1 (Nordberg et al., 1972). The mechanism behind the less marked increase of zinc in relation to cadmium at high concentrations is still not determined. It may be attributable to the synthesis of other forms of metallothionein with a higher ratio of cadmium to zinc or possibly to a relative deficiency of zinc in kidney (Elinder and Piscator, 1978).

In addition, investigators (Gunn et al., 1961; Ferm and Carpenter, 1967, 1968), have suggested that cadmium exerts its toxic effects through the inactivation of zinc metalloenzymes, as a result of its exchange with zinc. The administration of additional zinc enables it to retain its binding sites in the enzymes and thus, prevents the binding sites from being filled by cadmium. It is interesting to note the results of an experiment carried out by Dixon and Compher (1977), where zinc metalloenzyme was shown to be essential for the regeneration of the forelimb of the adult newt. Cadmium inhibited regeneration, presumably by interfering with zinc metalloenzymes. Therefore, they suggested that zinc was necessary for normal regeneration to occur, and its replacement in metalloenzymes could be deleterious.

Furthermore, it is well known that in the liver and kidney, zinc is increased by the injections of cadmium. This increase might be due to the induction of metallothionein synthesis in both organs, (Suzuki, 1972). However, the origin of this increased zinc is still under discussion. Winge et al., (1975) attributed the accumulation of zinc in part to depletion of blood zinc. Faeder et al. (1977), proposed increased dietary uptake as an explanation of the high zinc absorption from the
duodenum, due to low molecular weight chelate complexes in the intestine. Sugawara et al. (1978) tried to define this problem using rats and concluded that cadmium binding protein does not influence zinc binding to the low molecular weight factor. The rapid accumulation of liver zinc following injection of cadmium may be due not to uptake of dietary zinc but to supply of exchangeable zinc from other organs, and partly to depletion of plasma zinc.

Brown and Chatel (1978) investigated the interactions of cadmium and zinc in ducks from a heavily polluted area of British Columbia (Canada) and stated that competition of zinc and cadmium for similar binding sites in tissue was well established. It has been shown that toxic effects of cadmium do not occur until the binding capacity of metallothionein is exceeded and cadmium occurs in the high molecular weight protein pool (Winge et al., 1974; Irons and Smith, 1976). Their studies indicate that cadmium may appear in the high molecular weight pool, not only when the binding capacity of metallothionein was exceeded, but also when there was a deficiency of zinc in the high molecular weight protein pool. Since toxic effects of cadmium occur when it appears in the high molecular weight protein pool, it was likely that deficiencies of zinc increase toxic effects of cadmium by permitting an increase of cadmium in the high molecular weight protein pool.

Considerable interest has been generated concerning metallothionein because of its distinctive properties. This low molecular weight cytoplasmic metalloprotein is characterized by a high cysteine sulfur content which accounts for its unusually
high binding properties for zinc, cadmium and mercury (Richards and Cousins, 1976). Originally metallothionein was isolated and characterized from liver and kidney derived from animals of unknown nutritional history (Kagi et al., 1960; Nordberg et al., 1972). This precluded defining any role that this binding protein may have in the metabolism of nutrient minerals. To avoid this problem large doses of metals, for example zinc, cadmium and mercury have been injected or fed to animals prior to metallothionein isolation (Cousins et al., 1974; Chen et al., 1974; Weser et al., 1973). However, such an approach, although valuable for preparative purposes, can only serve a limited role in the elucidation of the function or functions of metallothionein in vivo because the normal population may never be exposed to such large amounts of metals (Richards and Cousins 1976).

Several functions have been postulated for metallothionein, largely based on studies that employed injected doses of zinc or cadmium. These include roles in absorption (Richards and Cousins 1975a), hepatic storage (Richards and Cousins 1975a, b), and detoxication of heavy metals (Squibb, 1974; Shaikk and Lucis, 1972). Repeated administration of low doses of cadmium produce tolerance to the subsequent injection of higher, usually lethal, doses of the metal (Kimura et al., 1974). This tolerance has been attributed to the protective effect of metallothionein, which was synthesized in response to exposure to cadmium. Metallothionein has a high affinity for the binding of cadmium and has been suggested as a possible means of detoxication of the metal (Kimura et al., 1974).
Probst et al. (1977) have presented data to support the protective role of metallothionein in mice. These workers have demonstrated a dose-response relationship between administered cadmium and hepatic concentrations of the protein. They also found that the LD 50 value for subsequently administered cadmium was directly related to hepatic metallothionein concentration.

In another study, Ghafghazy and Mennear (1973) investigated the effects of acute and subacute administration of cadmium on carbohydrate metabolism in mice. These workers reported that a single dose of the metal produced a significant reduction in pancreatic insulin secretion, whereas repeated administration did not. They suggested that this apparent tolerance was a reflection of the presence of metallothionein in the pancreatic beta-cell.

The regulation of intestinal zinc absorption is an important aspect of zinc homeostasis. The nature of this mechanism is still not clearly defined, but Starcher (1969) reported the presence of a metallothionein in the chick intestine which was capable of binding radionuclides of copper and zinc. It was proposed that this protein functioned directly in the transport of these metals from the intestinal lumen to the plasma.

Chen et al. (1977) showed that zinc accumulated entirely in metallothionein in livers of rats fed diets containing 1000 and 2000 ppm of dietary zinc for 2 weeks, providing further evidence for a relationship between metallothionein and zinc metabolism. Thus, metallothionein was proposed to play a role as a storage protein for zinc.
Although the exact biological role of this protein is not well understood, its physical and chemical characteristics are consistent with a role in a wide range of biochemical and physiological mechanisms such as catalysis, transport, storage, immune phenomena and heavy metals detoxication.

**CADMIUM IN HUMAN HEALTH**

As the last point in this review, it will be interesting to mention some of the studies which relate cadmium toxic effects and human health. In 1950 Friberg showed that one of the main symptoms in chronic cadmium poisoning was proteinuria. Results of investigations of cadmium-poisoned men (Friberg, 1957) have shown that in chronic poisoning kidney damage arises, especially in the tubules. As has been pointed out by Butler and Flym (1958), however, proteinuria does not necessary occur in all tubular damage but only damage to special parts of the tubules. Therefore there may be several reasons for the tubular damage caused by cadmium, according to Piscator (1962). Probably what happens is that cadmium, which is especially deposited in the kidneys, inhibits enzyme systems which are necessary for reabsorption. In agreement with this hypothesis Clarkson and Kench (1956) found an increased excretion of amino acids in cadmium workers, which could be caused by decreased reabsorption. Another possibility (Friberg, 1950) is that protein deposition in the tubules from increased protein concentration of the glomerular filtrate was a result of increased production of protein in the body due to the toxic action of cadmium.
Another harmful effect of cadmium for humans has been suggested by Schroeder (1966) and Carroll (1966), who said that abnormal levels of cadmium may be a causative factor in heart failure and death. Hawley and Kopp (1975) induced cardiac problems in rats by dosages bracketing the ranges for cadmium found in human blood. Voors and Shuman (1976) investigated, by means of autopsy studies, the hypothesis that cadmium exposure is associated with cardiac problems, and found a positive correlation between liver concentrations of cadmium and death from heart disease. However, their results are not in accordance with those of Tipton (1960) who studied a different population and found no similar association. They did not give any explanation for the difference in results. However, their sample population was very small (28 patients) and even though they studied smoking habits, which seems to be a major source of the body cadmium burden (Lewis et al., 1972; Shuman et al., 1974), no association was noted between reported cigarette smoking and death from heart disease or between smoking and cadmium liver concentration.

It is important to keep in mind the fact that hypertension has been associated with high kidney levels of cadmium in certain studies (Morgan, 1969) cannot be used to form conclusions concerning causality. The data of most of these studies, according to Morgan (1969) were obtained by analysis of kidneys from people who died from vascular disease, and the interpretation of the data is subject to the usual difficulties met in this type of epidemiological analysis, i.e., conclusions are ambiguous, and are based on the association of
cardiovascular disease with dustfall data (Friberg, 1950) or with cadmium concentration in air (Friberg, 1974).

In certain areas of Japan where there has been a considerable exposure to cadmium for decades, hypertension has not been associated with cadmium exposure (Nogawa and Kawano, 1969). In addition, there are no reports showing that workers exposed to cadmium have a higher prevalence of hypertension than other groups (Tsuchiya, 1971).

Therefore, according to Friberg (1974) the available data do not support the hypothesis that cadmium is causally associated with cardiovascular disease in man. However, there are reasons to study the question further. The findings, particularly in the animal studies, but even in the epidemiological studies in human beings, merit further attention with particular emphasis upon the mechanisms of cadmium induced hypertension, because the reasons for differing results reported by various investigators are not clear.

Lauwerys et al. (1978) undertook a survey among 500 pregnant women living in different areas of Belgium, in order to evaluate the extent of exposure of heavy metals (lead, mercury and cadmium), during fetal life, their possible biological effects, and the epidemiological factors which may influence the intensity of exposure. They found that the cadmium accumulation in the newborn blood was 50 percent lower than in the mother's blood. This suggests that the placenta plays a barrier role for the transfer of cadmium. However, Baglan et al. (1974) who analyzed 100 maternal and fetal blood samples from persons living in Tennessee, found, values 10 times higher than those
found by Lauwerys et al. (1978). They explained that the discrepancy was probably due to methodological differences rather than to varying degrees of cadmium exposure of the population studied, since it is now generally recognized that the normal concentration of cadmium in the blood of the general population is below 1.0μg/100 ml (Lauwerys et al., 1976; Ulander and Axelson, 1974).

Further, Buchet et al. (1973) continued the previous studies of Lauwerys et al. (1978) of the possible influence of various epidemiological factors (residence, age, smoking habits, drinking habits, occupation) on the exposure of the pregnant women and their newborns to some heavy metals, including cadmium. They stated that of the parameters studied, only smoking habits had an influence on the amount of cadmium found in the blood of the mother. The distribution of blood-cadmium levels in mothers was significantly different between smokers and non-smokers. The high values of blood-cadmium in smokers were consistent with the presence of this pollutant in cigarette smoke. However, that increased blood-cadmium due to cigarette smoking was not associated with a similar increase in blood-cadmium in the newborns. This was explained by the observation that cadmium concentration in the placenta was on the average 25 percent higher in smokers than in non-smokers (Roels et al., 1978). Since cadmium is a well-known inducer of metallothionein, it is possible that induction of metallothionein synthesis in the placenta was responsible for the higher cadmium accumulation in the smoker's placenta and hence for a decreased transfer of the metal to the newborn.
Roels et al. (1978) reported another study with the same group of women in which placental levels of heavy metals (among them cadmium) were analyzed, with the aim of finding out whether they were related to the levels in maternal and cord blood and whether the different epidemiological factors cited above could also influence the metal accumulation in placenta. The most significant observation made during this study was the marked accumulation of cadmium in placenta (in comparison with its levels in maternal blood). This accumulation was related to the role of the placenta as a barrier for the transfer of cadmium to the fetus. This observation however, did not allow them to conclude that cadmium was not hazardous to the fetus. Accumulation of cadmium in the placenta could alter its functions and thus be embryotoxic. Indeed, as previously reported, Parizek, (1965) has shown that after maternal cadmium injections of 2 to 4 mg/kg the placenta could be rapidly damaged, leading to death of the embryos in utero. However, there was no evidence that the slight accumulation of cadmium in placenta found in the human population had any deleterious action on the pregnancy.

Finally, among the various epidemiological factors studied by Roels et al. (1974), only smoking was found to increase cadmium accumulation in placenta (approximately 3 percent). It must, however, be recognized that in the general population the diet constitutes the main source of cadmium intake and dietary habits of the pregnant mothers were not investigated during this survey. In addition, in their study the diet of the mother or the ingestion of a particular food by the mother (like liver or
kidney) in large quantities may be a factor which influenced the accumulation of cadmium in the placenta or in other maternal tissues, even though this possibility may be remote.

Therefore, the present study, based on the above information, was concerned with two objectives. The first was to investigate the effects of zinc on the teratogenic action of cadmium. The second was to examine the interaction in the distribution of the metals, by administering them together or individually to pregnant rats. Then the effects of zinc on cadmium distribution in the maternal tissues and fetus, or cadmium on zinc distribution in the maternal tissues and fetus were evaluated, using $^{115m}$-cadmium or $^{65}$-zinc.
CHAPTER III
MATERIALS AND METHODS

Young adult female Wistar rats (purchased from Canadian Breeding Laboratories, Quebec), weighing about 250 to 350 g, were kept overnight with males (1 male with 3 females). The following morning the males were separated from the females and vaginal washings were examined for sperm. The presence of sperm indicated day zero of pregnancy. The pregnant rats were caged separately, with Purina Rat Chow and water supplied ad libitum. The animal room had fluorescent lights with a lighting cycle of 12 hours light, from 600 to 1800, and 12 hours darkness, from 1800 to 600 and the temperature of the room was 72±2 degrees Fahrenheit.

Wistar rats were used for these experiments because in preliminary studies it was found that they were more sensitive than some other strains of rats to the teratogenic action of cadmium. In addition, the greater variety of malformations produced in Wistar rats makes this strain more useful for studying the teratogenic effects of cadmium. (Parzyck et al., 1978).

EXPERIMENT 1

Treatment of Experimental Animals

On day 9 of pregnancy each rat was injected intraperitoneally, under light ether anaesthesia, with one of
the following:

1) Cadmium chloride, 16uM/kg body weight in saline containing 10-15uCi carrier-free 115m-cadmium chloride.

2) Cadmium chloride plus zinc chloride, 16uM/kg body weight of each, in saline containing 10-15uCi carrier-free 115m-cadmium chloride.

3) Zinc chloride, 16uM/kg body weight in saline containing 10-15uCi carrier-free 65-zinc chloride.

4) Zinc chloride plus cadmium chloride, 16uM/kg body weight of each, in saline containing 10-15uCi carrier-free 65-zinc chloride.

All solutions were prepared to a concentration such that each animal received approximately 1 ml of solution. After the injection of each rat, a standard was prepared, consisting of the same amount of solution as was given to the rat.

Day 9 was chosen for the day of injection on the basis of the studies of Barr (1973) and Parzyck (1978), in which it was demonstrated that a greater number and variety of malformations occurred following injection of cadmium on day 9 than occurred following injection of cadmium on day 8, 10, or 11. This was confirmed in preliminary studies in this laboratory, but the

Radioisotopes were obtained from New England Nuclear Corp., as carrier-free products.
data are not included here. The effectiveness of cadmium given on day 9 may be expected as this is during the period of most rapid organogenesis in the rat (Barr, 1973; Holmberg et al., 1969; Frohberg, 1974; Wilson, 1964).

The amount of cadmium to be injected was selected on the basis of preliminary studies which showed that a cadmium dose of 16μM/kg of the body weight was teratogenic when given on day 9 of pregnancy. Amounts of cadmium in excess of 22μM/kg of body weight killed many pregnant rats (Barr, 1973), and 32μM/kg of body weight killed all pregnant dams.

In addition, it has been reported (Ferm and Carpenter, 1967, 1968) that the protective effect of zinc against the teratogenic effects of cadmium was noted when administered in equal amounts simultaneously with cadmium or 15 minutes to 6 hours after the injection of cadmium. Therefore, in groups 2 and 4 (cadmium-zinc and zinc-cadmium groups) zinc chloride was injected simultaneously with cadmium chloride.

**Examination and Collection of Tissues**

The four groups of animals (9 to 12 in each group) were sacrificed on day 20 of pregnancy. The rats were anesthetized with ether and blood was drawn directly from the heart with a heparinized syringe. Approximately 5 cc of blood was obtained from each animal and placed in 3 tubes. One tube was used for the measurement of whole blood radioactivity, the second was centrifuged and the plasma was removed for radioactive counting, and the third sample was used for determination of the hematocrit (Brown, 1976).
The thorax of the animal was opened and the heart was removed for counting. The abdominal wall was then opened and the whole uterus was dissected, from ovary to ovary. The uterine wall was opened and each embryonic sac separated and examined. The empty uterine wall was examined carefully for resorption sites, which were recorded for each animal.

Each embryonic sac was opened and the fetus separated from the placenta. The fetuses were then cleaned, weighed, and fixed in Bouin's solution\(^1\), each fetus was placed in an individual counting tube. One of the placentas, chosen at random, from each animal was then cleaned in distilled water, weighed and placed in a counting tube. The rest of the placentas from each animal were pooled and, after cleaning in distilled water and weighing, were placed in counting tubes. The same thing was done for the embryonic sacs and for the umbilical cords. In addition, the number of fetuses, both dead and alive, was recorded. The living fetuses were carefully examined for gross external malformations, and these were recorded.

The whole liver, the heart, the lungs, the left kidney, both adrenals, the spleen, the upper part of the small intestine, (approximately 3 cm), about 1 g of fat from the abdominal cavity, the left femur, approximately 1 g of muscle from the left lower limb, the whole brain, and approximately 3 cm of uterus were then dissected from the mother, washed in distilled water, and weighed. Each tissue was placed in a

\(^1\)Bouin's solution contained 25 cc saturated picric acid, 25 cc of formalin (40% formic acid), and 5 cc of glacial acetic acid.
EXPERIMENT 2

After analyzing the results of the first experiment it was found that even though there was a large range of malformations in group 1, some small amount in group 3, and none in groups 2 and 4, almost no radioactive cadmium was found in the fetuses of the 115m-cadmium treated groups and there were very small differences in the distribution of cadmium in the 115m-cadmium treated animals. Therefore, it was decided to carry out a second experiment based on the hypothesis that cadmium goes to the fetus in the early hours after injections and is then excreted. This hypothesis is supported by reports that cadmium crosses the placenta and causes damages in the pars fetalis of the placentas of treated animals (Parizek et al., 1968; Ferm et al., 1969; Bruce et al., 1977; Lucis et al., 1971).

Therefore, young adult female Wistar rats weighing about 250 to 350 g were mated as described above.

Treatment of Experimental Animals

At 1100 on day 9 of pregnancy each pregnant rat was injected intraperitoneally with a solution of either cadmium chloride plus 115m-cadmium chloride, or cadmium chloride plus zinc chloride plus 115m-cadmium chloride, prepared as described above.

Six groups of animals, 5 animals per group, were established. Three groups (groups 5, 7, and 9) were injected with cadmium chloride plus 115m-cadmium, and sacrificed between
1000 to 1200 on days 10, 11, and 12 respectively of pregnancy; and the other 3 groups (6, 8, and 10) were injected with cadmium chloride plus zinc chloride plus 115m-cadmium chloride, and sacrificed between 1000 to 1200 on days 10, 11, and 12 of pregnancy.

**Examination and Collection of Tissues**

All animals were anesthetized with ether and sacrificed as described above. The manner of treating the tissues was as described in the first experiment, except for the treatment given to the uterus and fetus. The uterus was dissected from ovary to ovary and the uterine wall was opened, the embryonic sacs were separated and counted as a unit with fetus and placentas. Because of the small size of these tissues at these early stages of pregnancy it was very difficult to identify the different parts. However, in this case most of the uterus was cleaned, weighed and placed into a counting tube. In addition, the terminal end of the right horn of the uterus, with one embryo, was dissected together as a unit and placed in a tube for counting and the new tissue was called "Uterus plus Fetus".

In this experiment it was not possible to identify and keep a record of the live fetuses, dead fetuses, resorption sites or number of malformations due to the small size and similar appearance of all embryos at these early stages of gestation.

**COUNTING OF RADIOISOTOPES**

Tubes containing the radioactive tissues were placed in a Picker Autowell Gama Spectrometer for the measurement of 115m-
cadmium and 65-zinc. In the case of 115m-cadmium the spectrometer was set to record disintegrations with energies between 0.820 and 1.1 MeV and for 65-zinc disintegrations with energies between 0.06 and 0.07 MeV were recorded. Each tissue was counted twice for 5 minutes, together with the standards of the injected dose. The standards were prepared for each animal at the time of injection.

Counts (115m-cadmium and 65-zinc) per minute per gram were calculated for each tissue, using the wet weight of each sample for the calculations. Then the counts as percent of the total amount injected into each animal were also calculated. This was calculated from the standards.

**STATISTICAL ANALYSIS**

Statistical analyses were performed using a standard program (Statistical Package For The Social Sciences, SPSS) at the Computer Center of the University of British Columbia. Means, standard errors, and standard deviations were calculated. Student's t test was employed in order to compare means of pairs of experimental groups. One-way Analysis of Variance and Multiple Range tests were used to compare the variables "Hematocrit", "Fetal Weight", and "Malformations" among the four treatment groups in Experiment 1. Two-way Analysis of Variance was used to compare the two treatment and three treatment periods in Experiment 2.

For statistical analysis all the fetuses from each dam were pooled, so that the means of fetal weight, counts per minute per gram, and percent of injected dose were calculated for each dam
and reported as "Fetus".

Furthermore, because the only difference in the procedures employed in these two experiments was the day of pregnancy on which the animals were sacrificed, it was possible to compare the 10 groups statistically. Analysis of variance and multiple range tests were used in comparing the following variables: the hematocrit of the 10 groups; the maternal tissues and the fetuses of the groups treated with cadmium chloride plus 115m-cadmium or the groups treated with cadmium chloride plus zinc chloride plus 115m-cadmium.
CHAPTER IV
RESULTS

Gross external malformations were seen at external examination of the fetuses. In the cadmium treated group, out of 12 litters, 10 contained some malformed fetuses. In the zinc group out of 10 litters only 1 litter contained 2 malformed fetuses, whereas no malformations were found in the cadmium-zinc groups.

In the cadmium group 28.8 percent of the fetuses presented some kind of abnormalities (Table I). Dysplastic tail and absent tail were the predominant defects, followed by thin abdominal wall, dysplastic neck, dysplastic eye, exencephaly, dysplastic ears, dysplastic limb, and stunted body. In the zinc group both malformed fetuses were exencephaly.

In addition, several other parameters of fetal health were examined (Table II). They were: resorption sites, dead fetuses, litter size and fetal weight. It was found that the cadmium treated fetuses were significantly smaller than the cadmium-zinc treated and zinc treated fetuses. When the other three parameters were compared in the 4 different treated groups no significant difference was found.

Another parameter studied was the maternal hematocrit. A significant difference was found between the zinc and zinc-cadmium groups, where cadmium seems to have caused the volume percent of cells to be smaller. However, this result is probably not meaningful, since no differences were found between the cadmium and cadmium-zinc groups. Also the variances between the means of these 4 groups were small (Table III).
TABLE I

Effect of Zinc on Cadmium-Induced Malformations, and Cadmium on Zinc-Induced Malformations of Fetuses of Wistar Rats Injected on Day 9 and Examined on Day 20 of Gestation

Percent and Number of Malformed Fetuses

<table>
<thead>
<tr>
<th>Malformation</th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
<th>Zinc</th>
<th>Zinc-Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Fetuses</td>
<td>156</td>
<td>109</td>
<td>129</td>
<td>110</td>
</tr>
<tr>
<td>Total Malformed</td>
<td>28.84(45)</td>
<td>0</td>
<td>1.55(2)</td>
<td>0</td>
</tr>
<tr>
<td>Absent Tail</td>
<td>7.69(12)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dysplastic Tail</td>
<td>10.25(16)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Exencephaly</td>
<td>1.92(3)</td>
<td>0</td>
<td>1.55(2)</td>
<td>0</td>
</tr>
<tr>
<td>Dysplastic Neck</td>
<td>5.76(9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dysplastic Ears</td>
<td>1.28(2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thin Abdominal Wall</td>
<td>6.41(10)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dysplastic Limbs</td>
<td>0.64(1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dysplastic Eyes</td>
<td>3.20(5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stunted Body</td>
<td>0.64(1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

( ) = Number of Malformed Fetuses
TABLE II

Effect of Zinc on Cadmium-Induced Characteristics, and Cadmium on Zinc-Induced Characteristics of 20 Day Old Fetuses of Wistar Rats

<table>
<thead>
<tr>
<th></th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
<th>Zinc</th>
<th>Zinc-Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Fetuses</td>
<td>156</td>
<td>109</td>
<td>129</td>
<td>110</td>
</tr>
<tr>
<td>No. of Litters</td>
<td>12</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Resorption Sites</td>
<td>1.17±0.61*</td>
<td>0.78±0.32</td>
<td>1.30±0.42</td>
<td>1.22±0.36</td>
</tr>
<tr>
<td>Dead Fetuses</td>
<td>0.17±0.11</td>
<td>0.11±0.11</td>
<td>0.10±0.10</td>
<td>0.44±0.24</td>
</tr>
<tr>
<td>Litter Size</td>
<td>13.00±0.70</td>
<td>12.11±0.63</td>
<td>12.90±0.64</td>
<td>12.22±0.79</td>
</tr>
<tr>
<td>Fetal Weight (g)</td>
<td>2.89±0.06*</td>
<td>3.26±0.11</td>
<td>3.47±0.08</td>
<td>3.21±0.11</td>
</tr>
</tbody>
</table>

1=Mean±Standard Error
2=Significant difference (p<0.05) from other groups
3=Mean number per litter
4=Average of litter means
TABLE III

Effect of Zinc and Cadmium on the Hematocrit of Wistar Rats Injected on Day 9 and Sampled on Day 20 of Gestation. All Chemicals were Injected at 16μM/kg of Body Weight.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of Animals</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>12</td>
<td>32.2 ± 0.81</td>
</tr>
<tr>
<td>Cadmium-Zinc</td>
<td>9</td>
<td>32.0 ± 0.9</td>
</tr>
<tr>
<td>Zinc</td>
<td>10</td>
<td>33.4 ± 0.4</td>
</tr>
<tr>
<td>Zinc-Cadmium</td>
<td>9</td>
<td>31.0 ± 0.52</td>
</tr>
</tbody>
</table>

1=Mean±Standard Error
2=Significant difference (p<0.05) from the zinc group
Table IV shows the recovery of 115m-cadmium, as percent of the injected dose, in several tissues of animals given cadmium chloride alone or cadmium chloride plus zinc chloride. In these tissues, total organ weight was recorded, thus, the figures indicate total organ recovery. There were no significant differences between the cadmium and cadmium-zinc groups for any tissue except the fetuses. However, this was probably not a meaningful difference, as the recovery in the cadmium-zinc group was only 2 or 3 counts per minute, compared with zero in the cadmium group.

The largest accumulations of 115m-cadmium were found in the liver (49.52% in the cadmium group and 49.47% in the cadmium-zinc group), followed by the kidney (3.19% in the cadmium group and 3.51% in the cadmium-zinc group), where the addition of zinc slightly, but not statistically significantly, increased the amount of radioactivity deposited in this organ by day 20 of gestation. Only small amounts of radioactive cadmium were found in all the other organs examined as shown in Table IV.

Table V lists the percent of injected dose recovered per gram of tissue, since the total tissue weight was not known, the results are expressed per gram of tissue, as percent of dose administered. In the fat and uterus the accumulation of 115m-cadmium was significantly greater (p<0.05) in the cadmium group than in the cadmium-zinc group. For the other tissues the differences between the two groups were not statistically significant.

Table VI shows the recovery of 65-zinc, as percent of the injected dose, in several tissues of animals given zinc chloride
### TABLE IV

Effect of Zinc on Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 20 Of Gestation. All Chemicals were Injected at 16μM/kg of Body Weight.

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent of Injected Dose Recovered</td>
<td>12</td>
</tr>
<tr>
<td>Liver</td>
<td>49.5253 ± 1.086¹</td>
<td>49.4702 ± 1.086</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.1938 ± 0.145</td>
<td>3.5173 ± 0.052</td>
</tr>
<tr>
<td>Brain</td>
<td>0.0096 ± 0.002</td>
<td>0.0170 ± 0.004</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.0238 ± 0.003</td>
<td>0.0214 ± 0.003</td>
</tr>
<tr>
<td>Heart</td>
<td>0.1296 ± 0.006</td>
<td>0.1569 ± 0.015</td>
</tr>
<tr>
<td>Lung</td>
<td>0.1625 ± 0.012</td>
<td>0.1614 ± 0.010</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.2850 ± 0.011</td>
<td>0.2809 ± 0.010</td>
</tr>
<tr>
<td>Femur</td>
<td>0.0476 ± 0.005</td>
<td>0.0612 ± 0.007</td>
</tr>
<tr>
<td>Placenta</td>
<td>0.0055 ± 0.001</td>
<td>0.0064 ± 0.002</td>
</tr>
<tr>
<td>Placentae</td>
<td>0.1140 ± 0.011</td>
<td>0.1012 ± 0.006</td>
</tr>
<tr>
<td>Umbilical cord</td>
<td>0.0021 ± 0.001</td>
<td>0.0012 ± 0.001</td>
</tr>
<tr>
<td>Embryonic sac</td>
<td>0.0223 ± 0.004</td>
<td>0.0212 ± 0.004</td>
</tr>
<tr>
<td>Fetus</td>
<td>0.0000 ± 0.000</td>
<td>0.0015 ± 0.001²</td>
</tr>
</tbody>
</table>

¹=Mean±Standard Error

²=Significant difference (p<0.05) from corresponding group without zinc
TABLE V

Effect of Zinc on Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 20 of Gestation. All Chemicals were Injected at 16μM/kg of Body Weight.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>0.0133 ± 0.002*</td>
<td>0.0170 ± 0.004</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>0.2852 ± 0.021</td>
<td>0.2372 ± 0.017</td>
</tr>
<tr>
<td>Fat</td>
<td>0.2966 ± 0.106</td>
<td>0.0487 ± 0.010²</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.1888 ± 0.012</td>
<td>0.1397 ± 0.009²</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0114 ± 0.002</td>
<td>0.0071 ± 0.002</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.0017 ± 0.001</td>
<td>0.0008 ± 0.001</td>
</tr>
</tbody>
</table>

¹=Mean±Standard Error

²=Significant difference (p<0.05) from corresponding group without zinc
### TABLE VI

Effect of Cadmium on Zinc Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 20 of Gestation. All Chemicals were Injected at 16μM/kg of Body Weight.

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Zinc</th>
<th>Zinc-Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>3.3568 ± 0.229¹</td>
<td>6.4016 ± 0.334²</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.3324 ± 0.022</td>
<td>0.5614 ± 0.031²</td>
</tr>
<tr>
<td>Brain</td>
<td>0.2612 ± 0.015</td>
<td>0.2940 ± 0.012</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.0063 ± 0.001</td>
<td>0.0147 ± 0.002²</td>
</tr>
<tr>
<td>Heart</td>
<td>0.1537 ± 0.009</td>
<td>0.1914 ± 0.010²</td>
</tr>
<tr>
<td>Lung</td>
<td>0.2115 ± 0.010</td>
<td>0.2983 ± 0.012²</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.1220 ± 0.008</td>
<td>0.1748 ± 0.010²</td>
</tr>
<tr>
<td>Femur</td>
<td>0.3001 ± 0.016</td>
<td>0.3079 ± 0.025</td>
</tr>
<tr>
<td>Placenta</td>
<td>0.0590 ± 0.004</td>
<td>0.0778 ± 0.003²</td>
</tr>
<tr>
<td>Placentae</td>
<td>0.6067 ± 0.039</td>
<td>0.7826 ± 0.066²</td>
</tr>
<tr>
<td>Umbilical cord</td>
<td>0.0295 ± 0.003</td>
<td>0.0338 ± 0.003</td>
</tr>
<tr>
<td>Embryonic sac</td>
<td>0.2557 ± 0.018</td>
<td>0.3114 ± 0.028</td>
</tr>
<tr>
<td>Fetus</td>
<td>0.5064 ± 0.034</td>
<td>0.6470 ± 0.030²</td>
</tr>
</tbody>
</table>

¹=Mean±Standard Error

²=Significant difference from corresponding group without cadmium (p<0.05)
alone or zinc chloride plus cadmium chloride. In this tissues, as in the case of Table IV, total organ weight was known, so the figures indicate total organ recovery. There were significant differences (p<0.05) between the zinc and zinc-cadmium groups in the liver, kidney, adrenal, heart, lung, spleen, placenta, and fetus, where the addition of cadmium increased the amount of 65-zinc deposited in those organs.

As in the cadmium groups, the liver was the organ in which the largest amount of 65-zinc was accumulated (3.35% in the zinc group and 6.40% in the zinc-cadmium group). This was followed by the kidney (0.33% in the zinc group and 0.56% in the zinc-cadmium group). Radioactivity was found in all tissues examined.

Table VII lists the percent of injected dose recovered per gram of tissue, since the total tissue weight was not known the results were expressed per gram of tissue, as percent of dose administered. In fat, uterus, muscle, and plasma, the accumulation of 65-Zinc was significantly greater (p<0.05) in the zinc group than in the zinc-cadmium group. The differences between the two groups for whole blood and small intestine were not statistically significant.

Due to the fact that gross external malformations were found in the cadmium group when the animals were injected on day 9 and sampled on day 20 of gestation, but no radioactivity was detected in the fetuses, a second experiment was carried out in order to determine whether cadmium reaches the fetus in the early hours after injection. The animals were injected with cadmium chloride plus 115m-cadmium, or with cadmium chloride and
### TABLE VII

**Effect of Cadmium on Zinc Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 20 of Gestation.** All Chemicals were Injected at 16μM/kg of Body Weight.

<table>
<thead>
<tr>
<th></th>
<th>Zinc</th>
<th>Zinc-Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of Animals</strong></td>
<td><strong>10</strong></td>
<td><strong>9</strong></td>
</tr>
<tr>
<td>Whole Blood</td>
<td>0.0507 ± 0.003¹</td>
<td>0.0574 ± 0.004</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>0.1865 ± 0.015</td>
<td>0.2144 ± 0.010</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0162 ± 0.002</td>
<td>0.0285 ± 0.002²</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.1825 ± 0.010</td>
<td>0.2209 ± 0.011²</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.1080 ± 0.007</td>
<td>0.1267 ± 0.005²</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.0033 ± 0.000</td>
<td>0.0081 ± 0.001²</td>
</tr>
</tbody>
</table>

¹=Mean±Standard Error

²=Significant difference (p<0.05) from corresponding group without cadmium
zinc chloride plus 115m-cadmium on day 9 (as described above) and sampled on day 10, 11 and 12 of gestation.

A significant difference in 115m-cadmium (p<0.05) was found between the cadmium and cadmium-zinc groups on day 10 in the uterus, uterus plus fetus, and fetus. In all these tissues the amount of 115m-Cadmium was less in the cadmium-zinc group than in the cadmium group. No differences were found in the other tissues (Table VIII).

With respect to those tissues where recovery was calculated as percent of injected dose per gram of tissue, a significant difference was found in the fat and plasma (Table IX), where recovery was less (p<0.05) in the cadmium-zinc groups than in the cadmium group.

When the animals were sampled on day 11 the amount of radioactivity recovered in the adrenals and in the small intestine was significantly less in the cadmium-zinc group than in the cadmium group (Tables X and XI).

In the case of the animals sampled on day 12 the only significant difference found was in the femur, where the amount of cadmium that was recovered was less in the cadmium-zinc group than in the cadmium group (Tables XII and XIII).

The cadmium treated group was also studied using one-way analysis of variances for days of gestation (10, 11, and 12). It was found that the amount of 115m-cadmium deposited in the kidney expressed as percent of injected dose recovered increased with time, and in the uterus decreased with time as shown on Tables XIV and XV. However, when one-way analysis of variance was carried out
TABLE VIII

Effect of Zinc on Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 10 of Gestation. All Chemicals were Injected at 16μM/kg of Body Weight.

Percent of Injected Dose Recovered

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>48.5201 ± 4.252</td>
<td>46.6721 ± 1.327</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.1737 ± 0.148</td>
<td>2.2039 ± 0.168</td>
</tr>
<tr>
<td>Brain</td>
<td>0.0185 ± 0.003</td>
<td>0.0167 ± 0.003</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.0405 ± 0.010</td>
<td>0.0186 ± 0.003</td>
</tr>
<tr>
<td>Heart</td>
<td>0.1760 ± 0.004</td>
<td>0.1763 ± 0.009</td>
</tr>
<tr>
<td>Lung</td>
<td>0.1938 ± 0.022</td>
<td>0.1975 ± 0.011</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.2700 ± 0.018</td>
<td>0.2685 ± 0.025</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.7795 ± 0.150</td>
<td>0.3226 ± 0.076</td>
</tr>
<tr>
<td>Femur</td>
<td>0.0862 ± 0.006</td>
<td>0.0748 ± 0.008</td>
</tr>
<tr>
<td>Uterus+Fetus</td>
<td>0.0870 ± 0.007</td>
<td>0.0451 ± 0.007</td>
</tr>
<tr>
<td>Fetus</td>
<td>0.0152 ± 0.001</td>
<td>0.0094 ± 0.001</td>
</tr>
</tbody>
</table>

1=Mean±Standard Error

2=Significant difference (p<0.05) from corresponding group without cadmium
**TABLE IX**

Effect of Zinc on Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 10 of Gestation. All Chemicals were Administered at 16μM/kg of Body Weight

<table>
<thead>
<tr>
<th></th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of Animals</strong></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Whole Blood</strong></td>
<td>$0.0242 \pm 0.004^1$</td>
<td>$0.0193 \pm 0.004$</td>
</tr>
<tr>
<td><strong>Small Intestine</strong></td>
<td>$0.5731 \pm 0.104$</td>
<td>$0.4960 \pm 0.024$</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td>$0.0867 \pm 0.018$</td>
<td>$0.0290 \pm 0.004^2$</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td>$0.0233 \pm 0.003$</td>
<td>$0.0148 \pm 0.003$</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td>$0.0068 \pm 0.001$</td>
<td>$0.0031 \pm 0.001^2$</td>
</tr>
</tbody>
</table>

$^1$=Mean±Standard Error

$^2$=Significant difference (p<0.05) from corresponding group without zinc
**TABLE X**

Effect of Zinc on Cadmium Distribution in Organs of Wistar Rats Injected on Day 9 and Sampled on Day 11 of Gestation. All Chemicals were Injected At 16μM/kg of Body Weight.

<table>
<thead>
<tr>
<th></th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Liver</td>
<td>50.9920 ± 2.1491</td>
<td>48.6335 ± 0.559</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.1210 ± 0.009</td>
<td>2.2162 ± 0.194</td>
</tr>
<tr>
<td>Brain</td>
<td>0.0313 ± 0.013</td>
<td>0.0527 ± 0.014</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.2999 ± 0.004</td>
<td>0.0198 ± 0.0012</td>
</tr>
<tr>
<td>Heart</td>
<td>0.1714 ± 0.007</td>
<td>0.1579 ± 0.012</td>
</tr>
<tr>
<td>Lung</td>
<td>0.2519 ± 0.040</td>
<td>0.1941 ± 0.025</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.3185 ± 0.033</td>
<td>0.2823 ± 0.022</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.6178 ± 0.062</td>
<td>0.3706 ± 0.061</td>
</tr>
<tr>
<td>Femur</td>
<td>0.0697 ± 0.007</td>
<td>0.0549 ± 0.011</td>
</tr>
<tr>
<td>Uterus+Fetus</td>
<td>0.0736 ± 0.011</td>
<td>0.0448 ± 0.007</td>
</tr>
<tr>
<td>Fetus</td>
<td>0.0139 ± 0.003</td>
<td>0.0086 ± 0.002</td>
</tr>
</tbody>
</table>

1=Mean±Standard Error

2=Significant difference (p<0.05) from corresponding group without cadmium
TABLE XI

Effect of Zinc on Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 11 of Gestation. All Chemicals were Injected at 16μM/kg of Body Weight.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>0.0332 ± 0.009(^1)</td>
<td>0.0245 ± 0.005</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>0.5958 ± 0.052</td>
<td>0.3826 ± 0.027(^2)</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0825 ± 0.034</td>
<td>0.0288 ± 0.004</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0271 ± 0.006</td>
<td>0.0137 ± 0.003</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.0039 ± 0.002</td>
<td>0.0023 ± 0.001</td>
</tr>
</tbody>
</table>

\(^1\)=Mean±Standard Error

\(^2\)=Significant difference (p<0.05) from corresponding group without zinc
TABLE XII

Effect of Zinc on Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 12 of Gestation. All Chemicals were Injected at 16μM/kg of Body Weight.

Percent of Injected Dose Recovered

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Liver</td>
<td>53.8880 ± 2.5531</td>
<td>47.4111 ± 2.171</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.7313 ± 0.197</td>
<td>2.5588 ± 0.039</td>
</tr>
<tr>
<td>Brain</td>
<td>0.0485 ± 0.014</td>
<td>0.0433 ± 0.014</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.0174 ± 0.003</td>
<td>0.0206 ± 0.004</td>
</tr>
<tr>
<td>Heart</td>
<td>0.1693 ± 0.014</td>
<td>0.1818 ± 0.006</td>
</tr>
<tr>
<td>Lung</td>
<td>0.2221 ± 0.020</td>
<td>0.1815 ± 0.011</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.2925 ± 0.021</td>
<td>0.2403 ± 0.030</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.3324 ± 0.082</td>
<td>0.4404 ± 0.076</td>
</tr>
<tr>
<td>Femur</td>
<td>0.0741 ± 0.001</td>
<td>0.0593 ± 0.003</td>
</tr>
<tr>
<td>Uterus+Fetus</td>
<td>0.0770 ± 0.031</td>
<td>0.0583 ± 0.011</td>
</tr>
<tr>
<td>Fetus</td>
<td>0.0086 ± 0.003</td>
<td>0.0101 ± 0.003</td>
</tr>
</tbody>
</table>

1=Mean±Standard Error

2=Significant difference (p<0.05) from corresponding group without cadmium
TABLE XIII

Effect of Zinc on Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 12 of Gestation. All Chemicals were Injected at 16μM/kg of Body Weight.

Percent of Injected Dose Recovered per Gram of Tissue

<table>
<thead>
<tr>
<th></th>
<th>Cadmium</th>
<th>Cadmium-Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>0.0328 ± 0.004(^1)</td>
<td>0.0292 ± 0.003</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>0.3480 ± 0.050</td>
<td>0.2844 ± 0.014</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0433 ± 0.013</td>
<td>0.0391 ± 0.010</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0141 ± 0.005</td>
<td>0.0176 ± 0.001</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.0030 ± 0.002</td>
<td>0.0036 ± 0.001</td>
</tr>
</tbody>
</table>

\(^1\)Mean±Standard Error
TABLE XIV

Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 10, 11, and 12 of Gestation. Cadmium Chloride was Injected at 16μM/kg of Body Weight.

Percent of Injected Dose Recovered

<table>
<thead>
<tr>
<th>Sampling Day</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Liver</td>
<td>48.5201±4.2522*</td>
<td>50.9920±2.1489</td>
<td>53.8880±2.5529</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.1737±0.1476</td>
<td>2.1210±0.0089</td>
<td>2.7313±0.1970*</td>
</tr>
<tr>
<td>Brain</td>
<td>0.0185±0.0026</td>
<td>0.0313±0.0126</td>
<td>0.0485±0.0140</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.0405±0.0098</td>
<td>0.0299±0.0037</td>
<td>0.0174±0.0027</td>
</tr>
<tr>
<td>Heart</td>
<td>0.1760±0.0040</td>
<td>0.1714±0.0073</td>
<td>0.1693±0.0140</td>
</tr>
<tr>
<td>Lung</td>
<td>0.1930±0.0224</td>
<td>0.2519±0.0399</td>
<td>0.2221±0.0205</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.2700±0.0180</td>
<td>0.3185±0.0328</td>
<td>0.2925±0.0209</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.7795±0.1504</td>
<td>0.6178±0.0620</td>
<td>0.3324±0.0818*</td>
</tr>
<tr>
<td>Femur</td>
<td>0.0862±0.0056</td>
<td>0.0697±0.0072</td>
<td>0.0741±0.0013</td>
</tr>
<tr>
<td>Uterus+ Fetus</td>
<td>0.0870±0.0072</td>
<td>0.0736±0.0106</td>
<td>0.0770±0.0308</td>
</tr>
<tr>
<td>Fetus</td>
<td>0.0152±0.0009</td>
<td>0.0139±0.0035</td>
<td>0.0086±0.0029</td>
</tr>
</tbody>
</table>

1=Mean±Standard Error
2=Significant difference (p<0.05) from day 11 group
3=Significant difference (p<0.05) from day 10 group
TABLE XV

Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 10, 11, and 12 of Gestation. Cadmium Chloride was Injected at 16µM/kg of Body Weight.

Percent of Injected Dose Recovered per Gram of Tissue

<table>
<thead>
<tr>
<th>Sampling Day</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>0.0242±0.0043</td>
<td>0.0332±0.0085</td>
<td>0.0328±0.0039</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>0.5731±0.1041</td>
<td>0.5958±0.0524</td>
<td>0.3480±0.0496</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0067±0.0181</td>
<td>0.00825±0.0336</td>
<td>0.00333±0.0126</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0233±0.0033</td>
<td>0.0271±0.0058</td>
<td>0.0141±0.0050</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.0068±0.0009</td>
<td>0.0039±0.0018</td>
<td>0.0030±0.0018</td>
</tr>
</tbody>
</table>

1=Mean±Standard Error
on the cadmium-zinc group for days of gestation (10, 11 and 12), no significant differences were found in those tissues when recovery was expressed as percent of injected dose recovered in the whole organ (Table XVI). When the same analysis was done on those organs in which recovery is expressed as dose recovered per gram of tissue, a significant difference was found in the small intestine, where it appeared that with the addition of zinc the amount of cadmium recovered in this organ decreased with time (Table XVII).

Maternal hematocrit, litter size, and fetal weight were also studied, as parameters that reflect overall fetal health. No significant differences were found between days or between groups (Table XVIII).

Finally, one-way analysis of variance by days was used to compare days 10, 11, 12, and 20 in the cadmium and the cadmium-zinc groups for various tissues.

In the case of the liver no significant differences were found between days or between groups. Approximately 50% of the injected dose reached the liver 24 hours post-injection and this remained unchanged until day 20 of gestation. Furthermore, the treatment (cadmium versus cadmium-zinc) did not change this pattern, as can be seen in Figure I.

The kidney presented a different pattern. In both treatment groups the amount of cadmium deposited in this organ increased significantly with time (Figure II).

In the brain the amount of cadmium was less on day 20 than at day 12 in the cadmium treated animals, but no difference was found in the cadmium-zinc group (Figure III).
TABLE XVI

Effect of Zinc on Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 10, 11, and 12 of Gestation. All Chemicals were Injected at 16uM/kg of Body Weight.

Percent of Injected Dose Recovered

<table>
<thead>
<tr>
<th></th>
<th>Sampling Day</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>11</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>No. of Animals</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>46.67±1.3241</td>
<td>48.63±0.5588</td>
<td>47.41±2.1708</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>2.20±0.1678</td>
<td>2.21±0.1937</td>
<td>2.55±0.0395</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.01±0.0025</td>
<td>0.05±0.0136</td>
<td>0.04±0.0144</td>
<td></td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.02±0.0027</td>
<td>0.02±0.0013</td>
<td>0.02±0.0040</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.17±0.0093</td>
<td>0.16±0.0123</td>
<td>0.18±0.0057</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0.19±0.0113</td>
<td>0.19±0.0251</td>
<td>0.18±0.0112</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.26±0.0250</td>
<td>0.28±0.0221</td>
<td>0.24±0.0299</td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>0.32±0.0757</td>
<td>0.37±0.0606</td>
<td>0.44±0.0757</td>
<td></td>
</tr>
<tr>
<td>Femur</td>
<td>0.07±0.0085</td>
<td>0.05±0.0107</td>
<td>0.05±0.0028</td>
<td></td>
</tr>
<tr>
<td>Uterus+Fetus</td>
<td>0.04±0.0073</td>
<td>0.04±0.0069</td>
<td>0.05±0.0109</td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>0.009±0.0012</td>
<td>0.008±0.0081</td>
<td>0.010±0.0034</td>
<td></td>
</tr>
</tbody>
</table>

1=Mean±Standard Error
### TABLE XVII

Effect of Zinc on Cadmium Distribution in Different Organs of Wistar Rats Injected on Day 9 and Sampled on Day 10, 11, and 12 of Gestation. All Chemicals were Injected at 16μM/kg of Body Weight.

Percent of Injected Dose Recovered per Gram of Tissue

<table>
<thead>
<tr>
<th>Sampling Day</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>0.0193±0.0041</td>
<td>0.0245±0.0047</td>
<td>0.0292±0.0029</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>0.4960±0.0238</td>
<td>0.3826±0.0266</td>
<td>0.2844±0.0142</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0290±0.0036</td>
<td>0.0228±0.0041</td>
<td>0.0391±0.0104</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0148±0.0033</td>
<td>0.0137±0.0031</td>
<td>0.0176±0.0010</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.0031±0.0010</td>
<td>0.0023±0.0009</td>
<td>0.0036±0.0011</td>
</tr>
</tbody>
</table>

\(^1=\)Mean±Standard Error

\(^2=\)Significant difference (p<0.01) from corresponding 10, and 11 groups.
<table>
<thead>
<tr>
<th>Day</th>
<th>Hematocrit</th>
<th>Litter Size</th>
<th>Fetal Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cadmium</td>
<td>Cadmium-Zinc</td>
<td>Cadmium</td>
</tr>
<tr>
<td>Day 10</td>
<td>39.500±0.387</td>
<td>39.800±0.583</td>
<td>36.400±1.077</td>
</tr>
<tr>
<td></td>
<td>14.000±1.304</td>
<td>10.000±3.130</td>
<td>13.200±1.281</td>
</tr>
<tr>
<td></td>
<td>0.043±0.003</td>
<td>0.045±0.006</td>
<td>0.055±0.008</td>
</tr>
<tr>
<td>Day 11</td>
<td>36.400±1.030</td>
<td>38.400±1.122</td>
<td>5.800±1.393</td>
</tr>
<tr>
<td></td>
<td>11.400±2.293</td>
<td>0.038±0.020</td>
<td>0.110±0.025</td>
</tr>
</tbody>
</table>

*Mean±Standard Error*
Fig. 1 Accumulation of 115m-Cadmium in liver of animals given Cadmium or Cadmium+Zinc on day 9 of gestation.
Fig. II Accumulation of 115m-Cadmium in kidneys of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 10.
Fig III Accumulation of 115m-Cadmium in brains of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 12.
Fig. IV Accumulation of 115m-Cadmium in adrenals of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 10.
In the adrenals the amount of cadmium decreased significantly with time in the cadmium treated group. (Figure IV), however, it appeared unchanged in the cadmium-zinc group.

In the heart, also, the amount of cadmium decreased with time in the cadmium treated animals, where a significant difference was found between day 10 and 20 of gestation, but no differences were found in the cadmium-zinc group (Figure V).

The amount of cadmium in the lung increased slightly but not statistically significantly between day 10 and 11, and decreased significantly thereafter in the cadmium treated animals, however the changes in the cadmium-zinc group were not significant (Figure VI).

In the spleen no significant change was found among days in either group, or between the two treatments (cadmium and cadmium-zinc), as is shown in Figure VII.

In the femur there was a significant difference between day 10 and 20. The amount of cadmium deposited in the femur was less on day 20 in the cadmium treated animals, whereas no differences were found in the cadmium-zinc group (Figure VIII).

In the case of the uterus, the analysis compared only days, 10, 11, and 12 because the day 20 values for this organ were calculated as percent of injected dose per gram of tissue. Nevertheless, a significant difference was found in the cadmium group where the amount of cadmium deposited decreased with time. In the cadmium-zinc group, although the amount of cadmium increased with time, the change was not significant. In addition, the amount of cadmium accumulated in the uterus 24 hours post-injection was significantly greater in the cadmium
Fig. V Accumulation of 115m-Cadmium in hearts of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 10.
Fig. VI Accumulation of 115m-Cadmium in lungs of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 10.
Fig. VII Accumulation of 115m-Cadmium in spleens of animals given Cadmium or Cadmium+Zinc on day 9 of gestation.
Fig. VIII Accumulation of $^{115m}$-Cadmium in femurs of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different ($p<0.05$) from day 10.
Fig. IX Accumulation of 115m-Cadmium in uterus of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 10, ** = significantly different (p<0.05) from corresponding group with Zinc.
group than in the cadmium-zinc group (Figure IX).

The tissue called "Uterus plus Fetus" was analysed in the same manner as the uterus and no differences were found in either treatment group, comparing different days. However, as in the case of the uterus the amount of 115m-cadmium recovered was significantly greater 24 hours post-injection in the cadmium group than in the cadmium-zinc group (Figure X).

When the fetuses were studied, it was found that the amount of 115m-cadmium recovered was significantly decreased in both groups with time. In addition, the amount of 115m-cadmium that reached the fetus 24 hours post-injection was significantly greater in the cadmium group than in the cadmium-zinc group (Figure XI).

The amount of 115m-cadmium found in the blood was significantly greater on day 11 or 12 than on day 20 in the cadmium group but no differences were found in the cadmium-zinc group (Figure XII).

In the small intestine the amount of 115m-cadmium recovered decreased with time in both groups. There was a significant decrease in the cadmium group between day 10 or 11 and 20; and between days 11 and 12. In the cadmium-zinc group the main difference was found between day 10 and 11 (Figure XIII).

In fat the amount of 115m-cadmium increased in the cadmium group between day 12 and 20, but the difference was not significant. In the cadmium-zinc group no change was detected (Figure XIV).

In the muscle a significant difference was found in the cadmium group between day 11 and day 20, the amount of 115m-
Fig. X Accumulation of 115m-Cadmium in uterus plus fetus of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, ** = significantly different (p<0.05) from corresponding group with Zinc.
Fig. XI Accumulation of 115m-Cadmium in fetuses of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 10, ** = significantly different (p<0.05) from corresponding group with Zinc.
Fig. XII Accumulation of 115m-Cadmium in blood of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 11 and 12.
Fig. XIII Accumulation of $^{115m-}\text{Cd}$ in small intestine of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different ($p<0.05$) from day 10, ** = significantly different ($p<0.05$) from day 11.
Fig. XIV Accumulation of 115m-Cadmium in fat of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from corresponding group with Zinc.
Fig. XV Accumulation of 115m-Cadmium in muscles of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 11, ** = significantly different (p<0.05) from day 12.
cadmium being smaller on day 20. In the cadmium-zinc group a significant difference was found between day 12 and day 20, again the amount of 115m-cadmium being smaller on day 20 (Figure XV).

In the plasma, as well, the amount of 115m-cadmium decreased with time in the cadmium group, being significantly less on day 20 than on day 10 of gestation. However, the changes in the cadmium-zinc group were not significant (Figure XVI).

The maternal hematocrit was also analysed in the same manner and it was found that there was a significant decrease in the maternal hematocrit with time in both groups (Figure XVII).
Fig. XVI Accumulation of 115m-Cadmium in plasma of animals given Cadmium or Cadmium+Zinc on day 9 of gestation, * = significantly different (p<0.05) from day 10.
Fig. XVII Effect of Cadmium or Cadmium+Zinc in the maternal hematocrit of animals injected on day 9 of gestation, * = significantly different (p<0.05) from day 10.
CHAPTER V
DISCUSSION

The present results show that cadmium causes gross external fetal malformations when it is administered to pregnant Wistar rats on day 9 of gestation. Even though all of these animals were injected intraperitoneally at approximately the same time (in the early hours of the 9th day of gestation) the fetuses presented a wide range of malformations that included defects of the head, the neck, the abdominal wall, the limbs, the tail, and the whole body. Within a litter some fetuses were significantly smaller than the rest of the littermates, which indicates differential effects.

It is not known whether these toxic manifestations are the result of the action of cadmium on the maternal tissues or on the placenta, or whether they represent a direct action of cadmium on the fetus, or a combination of these factors. Although the placenta is an effective barrier against the transfer of small amounts (less than 4µM/kg body weight) of maternally administered cadmium (Berlin and Ullberg, 1963), larger (more than 10 µM/kg body weight) amounts will enter the fetus (Ferm et al., 1969). It should be noted that the latter (16µM/kg body weight) was the case in these studies.

The wide range of malformations seen in these experiments may be explained by a general hypotheses proposed by Ferm (1971), to account for the action of so-called "broad-spectrum teratogens". He suggested that these teratogens are non-specific, that is to say, the result depends primarily on the organogenetic event at the time of the insult. Thus, a wide
spectrum of malformations should result from any teratogenic stimulus given during the time of critical embryogenesis. Therefore, these would be "non-specific" in their effects. It was shown in Figure XI that 115m-cadmium was present in the fetuses in an appreciable amount even 72 hours post-injection. This could explain that it was found a broad spectrum of defects, even though only one injection was administered to these animals, cadmium was present in the embryonic structures, at least, during three important days of organogenesis, when the development of the rat embryo goes from the heart primodium to the posterior limb bud stage (Schneider, and Norton, 1979).

Nonetheless, an alternative hypothesis (Ferm, 1971) states that certain teratogens might well prove to be site specific and induce malformations only in certain developing organ systems. This mechanism (site-specific teratogens) would suggest that a specific organ-teratogen relationship exists which could best be explained by interference with a particular enzymatic event of development. The metals should be good examples of site-specific teratogens, for they enter into a variety of rather specific enzymatic reactions (Ferm, 1971).

The fact that, when the animals were examined within a single litter the same kind of malformation was found in several fetuses, may be evidence for the specificity of the teratogenic effect of cadmium. That is to say, that when thin abdominal wall was the defect found in the first fetus examined in one particular litter; that was the predominant defect in the whole litter. However this may not be the case, since specificity can be explained in another way.
This fact could better be explained by variation in the actual time of mating of the animals. For some animals the time of injection, considered to be the early hours of the 9th day of gestation, might actually have been the late hours of the 8th day or the later hours of the 9th day of gestation. Therefore, the actual defect found could be the result of the organogenetic event that was taking place in the moment of injection, and it did not depend on the specificity of the teratogen.

In addition, the maternal-embryonic relationship may be disturbed since it was found that 115m-cadmium accumulates in the placenta, embryonic sac, and the umbilical cord. This decidual and placental uptake may indicate some interference with embryonic nutrition and be the cause of significantly smaller fetuses found in the cadmium-treated group. This is supported by the fact that some massively damaged placentas were found in the cadmium-treated group. These placentas were similar to the ones that Parizek (1964) described as "...a clot of blood".

Nonetheless, the placenta serves as an efficient barrier to the transfer of cadmium from mother to fetus, as evidenced by the relatively high placental and low fetal concentrations of cadmium found in these experiments. An explanation for this has been offered by Lucis et al. (1972), who found that cadmium in the placenta was bound to macromolecules, so that the transfer across the placenta is restricted or inhibited.

The results of this study, do not support the hypotheses of Parizek (1964) and Dencker (1974), that cadmium interferes with the steroid producing organs and that this may cause an
interruption of pregnancy, since, there was no difference in the mean number of resorption sites in the 4 treatment groups. That is to say, although cadmium does not cause an increase in resorptions it may still affect the steroid producing organs, since it has been mentioned above that placental damage was seen.

However, as Dencker (1974) stated, a possible enzyme affinity for cadmium may influence the normal maternal-embryonic relationship, disturbing metabolic pathways and resulting in inhibited implantation, and an increase in malformations.

Thus, teratogenic effects of specific chemical agents in mammalian development may be attributed to one of three general actions, or perhaps to a combination of them. First, there may be an alteration of some factors in the maternal system which secondarily affects embryonic differentiation (Ferm et al., 1969). As an example of this possibility, the teratogenicity of cadmium might be due to altered protein structure, as reported by Kench and Sutherland (1966). In cases of human cadmium intoxication, if the protein structure is altered it is possible that the embryo will show external malformations. Second, cadmium teratogenicity may be related to the blocking of placental transfer of some essential material necessary for normal embryonic differentiation. Third, the teratogenic effect of cadmium may be due to a direct effect of this metal upon specific embryonic tissues (Ferm et al., 1969).

A review of the literature on the action of cadmium on the reproductive process can only lead to the conclusion that while the effect is clearly documented, the actual mechanism of action
of cadmium ions is very much an open question.

These investigations have also shown the protective effect of zinc on cadmium-induced malformations, since no external defects were found in the cadmium-zinc treated animals. However, the results do not agree with Ferm et al. (1969) who reported that the placental transfer of cadmium was not affected when zinc was administered to pregnant animals together with cadmium. It was shown here that at 24 hours post-injection the amount of cadmium in the fetuses of the cadmium-zinc treated group was significantly less than that in the cadmium treated animals, even though this difference was not statistically significant 48 or 72 hours after injection.

However, the means by which zinc protects against teratogenesis cannot be related to a complete block in the transfer of cadmium across the placenta since the present data shows that the simultaneous injection of an amount of zinc equimolar to that of cadmium does not completely prevent the transfer of cadmium from maternal to embryonic tissues although the teratogenic effect of cadmium was abolished.

It is possible that zinc replaces cadmium at certain embryonic sites. Thus, although cadmium transfer is not impeded, those sites specifically related to the production of a teratogenic lesion by cadmium could be occupied by zinc, cadmium being bound elsewhere in the embryo at non-specific sites (Ferm et al., 1969).

In addition, as Ferm (1967) stated, the protective effect of zinc could be related to a critical physiological cadmium:zinc ratio. The importance of this has been
demonstrated, for example, by Schroeder (1967), who has shown that high renal ratios of cadmium:zinc are associated with arterial hypertension in rats and that this hypertension can be reversed by zinc.

Further investigations on the permeability of the mammalian placenta to heavy metals and their localization in specific differentiating embryonic tissues are necessary in order to identify the exact mechanisms of these site specific malformations as well as the complex interaction of these teratogenic agents with protective agents.

When the distribution of 115m-cadmium was examined in the maternal organs, with and without the addition of zinc, it was found that zinc did not change the patterns of cadmium distribution except in the case of the fat, uterus, small intestine, and femur. That is to say in general the simultaneous administration of zinc does not change the pattern of cadmium distribution and accumulation in rat tissues. In the case of fat, small intestine, and femur the results may not be very meaningful since differences were found in only one group of animals for each of the tissues (Tables V, VII, IX, XI, and XII).

It is interesting to notice that for most of the organs examined the accumulation of 115m-cadmium decreased with time, but the accumulation of 115m-cadmium in fat increased with time. Therefore, it may be that cadmium is to some extent not excreted from the system, but is deposited in fatty tissue. The addition of zinc seems to decrease significantly the amount of cadmium accumulated in fatty tissue, however it appears that zinc does
not change the accumulation of cadmium in most of the organs examined, so that cadmium may be excreted out of the system.

The addition of zinc also decreases the amount of $^{115m}$-cadmium deposited in the uterus, and this may be the reason why the amount of cadmium that reaches the fetuses is smaller in the cadmium-zinc group than in the cadmium group.

On the other hand, when the other two groups, (zinc and zinc-cadmium) were examined the opposite situation was found, the addition of cadmium seems to increase the amount of zinc deposited in most organs.

This could be explained by competition of zinc and cadmium for similar binding sites in tissue, which is well established. It is also known that cadmium exerts toxic effects via its effect on zinc metalloenzymes (Horvath, 1976), and that high levels of zinc prevent or reduce the toxic effects of cadmium (Flick et al., 1971).

In addition, it has been shown that, in the liver and kidney, endogenous zinc is increased by the injection of cadmium (Sugawara et al., 1978). This increase might be due to the induction of metallothionein synthesis in both organs (Suzuki, and Yoshikawa, 1972). The origin of this increased zinc is still under discussion, but Winge et al. (1975) attributed the accumulation of zinc, in part, to depletion of blood zinc. However, the present study does not agree with that explanation since the amount of $^{65}$-zinc found in blood was slightly but not significantly increased by the addition of cadmium.

Sugawara et al. (1978) suggested that the rapid accumulation of organ zinc following the administration of
Cadmium may be not due to uptake of dietary zinc but to a redistribution of exchangeable zinc from other organs. This explanation also seems difficult to understand since, in the present study the accumulation of administered 65-zinc significantly increased in most of the organs studied. Therefore, at the present moment the mechanism by which the accumulation of zinc increases in the presence of cadmium seems to be not very well understood.

The data presented in this study reveal that after intraperitoneal injection, 115m-cadmium is predominantly concentrated in the liver, presumably bound to metallothionein, (Lucis et al., 1972; Webb, 1975; Kotsonis and Klaassen, 1975). This pattern does not change by 24, 48, 72 hours, or 11 days after injection. After 11 days most tissues showed a decrease even though not significant in their content of 115m-cadmium. The exception to this were kidney and fat, which had an increase; the spleen which remained the same, and the small intestine which had a significant decrease.

It is of interest to note that the 115m-cadmium content of the plasma was very low, which indicates that the concentration of 115m-cadmium found in whole blood was in the cells (erythrocytes) presumably bound to a protein like hemoglobin (Carson and Friberg, 1975; Nordberg et al., 1971). This also agrees with the results of Lucis et al. (1972) who stated that in experimental animals cadmium is rapidly cleared from the circulation and deposited intracellularly so that only traces of cadmium remain in blood plasma or serum. This may be one of the factors which plays a role in the low transplacental passage of
this element, together with the fact that the rat placentas also contain intracellular cadmium-binding macromolecules (Lucis et al., 1972).

The brain had a lower 115m-cadmium concentration than did the plasma, possibly due to the blood-brain barrier. The high concentration of cadmium in the intestine 48 hours post-injection, and the dramatic decrease after 11 days may be explained by the presence of an intestinal metallothionein which is subsequently lost during the normal turnover of intestinal epithelial cells (Richards, and Cousins, 1975; Kotsonis and Klaassen, 1977).

Finally, several issues illustrated by these experimental data need to be considered. First, as Petering (1978) stated, we need to be more concerned about the nutritional factors which may play a role in the host response to ingestion of heavy metals, as they may be extremely important, both in experimental and clinical evaluation of the toxicity of cadmium and other toxic metals.

It has been suggested that the acute ingestion of cadmium may lead to alterations of zinc metabolism. This is an important issue, since it brings into focus the importance of a good nutritional status as a host defense mechanism. It also indicates a possible important preventive measure which needs further study, since cadmium exposure will be with us for a long time (Petering, 1978).

In addition, even though the levels of the two metals used in these experiments exceed the usual levels of environmental contamination, two important points should be kept in mind. The
first is that there is always the possibility of accidental exposure to high levels of one of these metals during early human gestation. Secondly, there also may be marked species variation in the teratogenic response to a given teratogen, the example of thalidomide being a case in point. This compound, in relatively low therapeutic doses, caused several malformations in the human embryos, but it took extremely high levels of the same compound to produce malformations in experimental animals. Therefore, it is possible that a similar situation may exist, and until individual species sensitivity to various teratogens is determined all potential teratogens should be carefully evaluated (Holmberg, and Ferm, 1969).


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