The Acute Toxicity and Teratogenicity of Cadmium in the Pregnant Rat

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Key words: pregnant rat; cadmium; toxicity; distribution; teratogenicity; LD_{50}.

Until the 16th day of gestation the intravenous LD_{50} of Cd^{2+} in the pregnant Wistar-Porton rat is higher, but not significantly different from that (1.8 mg Cd^{2+} per kg body weight) in nulliparous females. At 20 days it is 1.1 mg Cd^{2+} kg per body weight. This decrease is related to the rapid increase in weight of the conceptuses in late gestation and to the retention of most of the dose in the maternal compartment. If the dose is based on body weight at conception, the LD_{50} for the 20-day pregnant rat (1.6 mg Cd^{2+} per kg body weight) and non-gravid female do not differ significantly. Nevertheless, after the same Cd^{2+} dose, hepatic and renal Cd^{2+} concentrations are less in the pregnant than in the non-pregnant animal. The Cd^{2+} concentrations, therefore, do not determine the liver and kidney damage, which is restricted to the pregnant rat. Placentae also accumulate Cd^{2+} and placental haemorrhage follows the injection of the appropriate Cd^{2+} LD_{50} on day 12–20 of gestation. In those animals that die between 16 and 30 h after dosing, haemorrhage and death appear to be correlated. Renal damage, therefore, probably results from haemorrhagic shock. It is not dependent on the transfer of protein-bound Cd^{2+} from the necrotic placentae to the kidney. Between the 8th and 15th day of gestation, Cd^{2+} (1.25 mg per kg body weight) is highly teratogenic. Hydrocephalus is the most frequent abnormality when the dose is given between the 8th and 12th day. Other malformations include eye defects, gastroschiasis and umbilical hernia.

INTRODUCTION

Administration of acute doses of cadmium (Cd^{2+}) at the appropriate stage of gestation has been shown to produce various congenital deformities in hamsters,[1-4] rats[5-7] and mice.[8-11] At the later stages, the maternal animal is extremely susceptible to Cd^{2+} toxicity.[8,12-14] In the rat, for example, s.c. administration of $CdCl_2$ (40 μmol kg^{-1}) between the 17th and 21st day of gestation is followed by extensive bleeding per vaginam after about 6 h.[12] At this time the placenta exhibits marked degenerative changes, chiefly in the pars foetalis.[12] Pronounced histological alterations also occur in the liver and kidneys. Little is known, however, about the effect of pregnancy on the distribution of Cd^{2+} to the maternal organs, and no investigations appear to have been made of the LD_{50} of Cd^{2+} during pregnancy in the rat. Such studies, together with the determination of the teratogenic dose and teratogenic responses to Cd^{2+} in the Wistar-Porton rat, are reported in this paper. A brief account of this work was given at the International Conference on Cadmium (NIH, Bethesda, USA) in June 1978.

EXPERIMENTAL

Analytical and preparative procedures

Tissue samples were digested and analysed for metallic ions as described previously.[15] Gel filtration and preparative electrophoresis were done on columns of Sephadex G75[15] and polyacrylamide gel,[16] respectively. Dilute aqueous protein solutions were concentrated, if necessary, by dialysis against polyethylene glycol. The $^{109}Cd^{2+}$ isotope in solution was measured with 44% efficiency in a Packard γ-counter (Packard Instruments Inc., Caversham, UK). Whole body and organ contents of $^{109}Cd^{2+}$ were determined as described by Magos and Webb.[17] The efficiency of detection was 2% and 15%, respectively.

Animals

Wistar-Porton rats were used: nulliparous adult females (200 ± 10 g body weight), old breeding stock (350 ± 10 g body weight) that had had 4 litters, lactating females with second litters and pregnant animals of proven fertility (i.e. in their second pregnancies). All animals were fed Oxoid Pasteurized Diet (Oxoid Ltd, London, UK). For mating, females were caged individually and kept with one male overnight. If a vaginal plug was found the next morning, this day was regarded as day 0 of pregnancy.[11,18] The pregnant and lactating females were individually caged in polypropylene boxes (45 x 28 x 22 cm) with stainless steel lids. Other animals were housed in groups of four in stainless steel cages (46 x 31 x 22 cm).

LD_{50} determinations

These were made on pregnant rats on day 4, 8, 12, 16 and 20 of gestation, nulliparous and lactating females and animals from the old breeding stock. Four animals from each group were selected at random for use at each dose level (0.6, 1.0, 1.58, 2.5 and 3.98 mg Cd^{2+} per kg body weight). Solutions were prepared from Analar $CdCl_2 \cdot 2\frac{1}{2}$ H_2O (BDH Ltd, Poole, UK) and were given i.v.[19] The animals were housed in a room maintained at 20 ± 2 °C,

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CCC-0260-437X/81/0001-0264$03.00

ventilated to give 15 air changes per hour, with an automatically controlled light period of 12 h.

The LD_{50} values were calculated by Weil's[20] method. Observations were made over a 7-day period, although deaths usually occurred within the first 24 h and seldom after 48 h. All animals that died during working hours were subject to post-mortem examination, and appropriate organs were removed for histology.

Additional studies, in which the dose was based on the initial body weight (i.e. the body weight on the day when the vaginal plug was found), were made with 20-day pregnant rats. The body weights of the nulliparous females that were used as controls for these experiments, were also determined at this time.

Cd^{2+} distribution

Groups of nulliparous and 20-day pregnant rats were injected i.v. with 1.58 mg Cd^{2+} per kg, supplemented in some experiments with $^{109}Cd^{2+}$ (Radiochemical Centre, Amersham, UK; 15 μCi per mg Cd^{2+}). Since this dose exceeded the LD_{50} for the pregnant rats (see Results), a certain selection was inherent because distribution was measured in surviving rats. Animals were killed by decapitation at 5, 15 and 30 min and 1, 4, 8 and 24 h after injection. Samples of blood were collected into heparinized syringes by cardiac puncture. A post-mortem examination was made on each animal, and the liver, kidneys, adrenals, thymus and heart were removed. The uterus was removed from each pregnant animal and the placentae were separated from the foetuses. All organs were weighed and portions taken for histology and chemical analysis. Tissues that were not analysed immediately were frozen in liquid nitrogen and stored at $-20\,^{\circ}C$. In other experiments, 20-day pregnant rats were injected with $^{109}Cd^{2+}$ (15 μCi, 1.05 mg Cd^{2+} per kg body weight) and killed after 8 and 24 h. The placentae were cut into small pieces, washed in saline to remove as much blood as possible, and homogenized in 3 vols 10 mM Tris-HCl buffer, pH 8.0, at 4 °C. The homogenates were centrifuged for 1 h at 105 000 g and portions of the supernatant (each ≡ 1 g wet weight tissue) fractionated by gel filtration. Measurements of placental uptake at various stages of gestation were made at 6 or 8 h after the i.v. injection of 1.25 and 2.49 mg Cd^{2+} per kg.

Effects of Cd^{2+} (1.25 mg per kg body weight i.v.) on embryo-foetal development

These effects were studied in groups of 15 pregnant rats on day 8, 9, 10, 11, 12, 13, 14 and 15 of gestation. The dose was determined by preliminary experiments, where groups of 4-6 rats were given 1.0, 1.1, 1.25, 1.35 and 1.58 mg Cd^{2+} per kg i.v. on the 9th, 11th and 14th day of pregnancy. Groups of control animals (15 per group) of the same gestational ages were injected with equivalent volumes of saline. As the dams were likely to eat any dead, moribund or malformed offspring, all animals were killed by an overdose of ether one day before full term. A complete post-mortem examination was made on each animal, after which the uterus was removed with the ovaries and mounted on a post-mortem board. Corpora lutea on each ovary were counted, and the uterus cut open to display the foetuses. A pair of small artery forceps was placed on the umbilical cord flush with the abdominal wall of the foetus and the cord cut distal to the clamp. Each foetus was blotted to remove amniotic fluid and blood prior to weighing. Placentae were removed from the uterus and examined for any gross abnormalities such as infarcts and abnormal insertion of the cord. The foetuses were examined systematically from head to tail. The presence or absence of a cleft palate was detected by inserting a small pair of curved forceps into the mouth and forcing the jaws apart. After the external examination, half of the foetuses were randomly selected and fixed in Bouin's fluid. The remaining foetuses were fixed in 95% alcohol, for subsequent examination by Wilson's[21] technique and for staining with Alizarin Red S.[22]

Histology

Tissues were fixed in formol-alcohol, mounted in paraffin and sectioned at 5 μm. The sections were stained with haematoxylin-eosin.

RESULTS

Acute toxicity

The LD_{50} of Cd^{2+} for pregnant rats on the 20th day of gestation (1.1 mg Cd^{2+} per kg body weight Table 1) was significantly lower ($P < 0.001$) than that (1.8 mg Cd^{2+}

Table 1. LD_{50} values for Cd^{2+} in nulliparous, pregnant, lactating and old female rats

Animal category	LD_{50} (mg Cd^{2+} per kg body weight)	95% confidence limits
	Body weight measured at time of dosing	
Nulliparous	1.8	1.4-2.2
Lactating	2.5	1.8-3.6
Old breeding stock	2.5	1.8-3.6
Pregnant		
4th day of gestation	2.2	1.8-2.8
8th day of gestation	2.2	1.8-2.8
12th day of gestation	2.5	1.9-3.3
16th day of gestation	2.5	1.9-3.3
20th day of gestation	1.1	[a]
	Body weight measured 20 days before dosing	
Nulliparous	1.8	1.4-2.2
Pregnant		
20th day of gestation	1.6	1.2-2.1

LD_{50} values were calculated by the method of Weil (Ref. 20) from mortality data obtained on groups of 20 (5 × 4) animals.

[a] The mortality data at the 5 dose levels (see Experimental) of this determination were 0, 0, 4, 4 and 4. Calculation of the LD_{50} according to Weil's method (Ref. 20) from these values gives a standard error of zero.

per kg body weight) for nulliparous females. On the 4th-8th and 12th-16th days of pregnancy, however, the LD_{50} values were 2.2 and 2.5 mg Cd^{2+} per kg body weight, respectively. These values were similar to those (Table 1) for lactating females (2.5 mg Cd^{2+} per kg body weight) and animals of the old breeding stock (2.5 mg Cd^{2+} per kg body weight) but were not significantly different from that for the young, non-gravid females. Therefore, increased resistance to Cd^{2+} between the 4th and 16th day of gestation could not be substantiated. It was clear that susceptibility to Cd^{2+} did not increase progressively with advance in gestational age.

The fall in the LD_{50} value between the 16th and 20th day of gestation appeared to be related to the rapid increase in the weight of the conceptuses during this period. Thus, when the Cd^{2+} dose was based on the body weight at the time of conception, or the body weight of the non-gravid condition 20 days before treatment, there was no significant difference between the LD_{50} values (Table 1).

In addition to previously described toxic responses[14] common to gravid and non-gravid animals, there was bleeding per vaginam, due to placental haemorrhage. This occurred in 12, 16 and 20-day pregnant rats 8 h after the i.v. injection of higher doses of Cd^{2+}, and usually resulted in death. In the 20-day pregnant rats, most deaths occurred between 16 and 30 h after dosing. All the animals that died between these times had haemorrhage. Therefore, the 'critical $dose_{50}$' for placental haemorrhage was similar to the LD_{50}.

Sonawane *et al.*[7] observed that the placental concentration of Cd^{2+} increased with both dose and gestational age. Nevertheless, in rats ($n = 3$) injected on the 12th, 16th and 20th day of gestation with the appropriate LD_{50} dose of Cd^{2+} (see Table 1), and killed before apparent haemorrhage (6 h), the mean placental concentrations of the cation were 4.3, 7.1 and 5.1 μg per g wet weight, respectively. It seems therefore that the increased susceptibility of the placenta to Cd^{2+} in late gestation cannot be attributed to greater uptake of the toxic ion by the whole organ.

In contrast with the observations of Parizek,[12] in the present experiments the maternal part of the placenta was affected first and, by 8 h, showed extensive degeneration, whereas the foetal part appeared histologically normal. This agrees with previous observations.[14] By 16-24 h, when vaginal bleeding was severe, both the maternal and foetal parts were destroyed completely. At this time the animals were extremely pale and had laboured breathing. Prior to death, some had violent seizures. The livers of these animals, in contrast with those of nulliparous females that died after a lethal dose of Cd^{2+}, contained many areas of periacinar necrosis of parenchymal cells. Kidney lesions ranged from complete tubular degeneration in some areas to vacuolation and tubular dilation in others, and were observed consistently in pregnant but not in non-pregnant rats.

Organ distribution of Cd^{2+} in nulliparous and 20-day pregnant rats

After the i.v. injection of Cd^{2+} (1.58 mg per kg body weight) the cation cleared rapidly, but not completely, from the blood of both the nulliparous (Table 2) and pregnant rats (Table 3). In the former, the blood concentration decreased to a minimum value within 30 min and remained essentially constant for 24 h (Table 2). In the latter, it appeared to increase between 4 and 8 h. At 24 h it was still greater than at 4 h (Table 3). The Cd^{2+} concentrations in the lungs and heart of pregnant animals also followed this pattern, with transient increases at 8 h (Table 3).

After an acute dose of Cd^{2+} on the 20th day of gestation, the foetal concentration of Cd^{2+} is about 1% of that in the placentae,[23] a small fraction of the total body burden. Thus, under the conditions of the present experiments, in which the pregnant animal was dosed with 1.58 mg Cd^{2+} per kg *total* body weight, the content of Cd^{2+} in the maternal compartment was greater than the body burden of the non-pregnant control. Nevertheless, the organ contents of Cd^{2+} were greater in the pregnant than in the non-pregnant rat (e.g. 327.5 ± 6.4 and 263.7 ± 8.0 μg, respectively, in the liver at 4 h after dosing). The organ weights were also greater by 40% for the liver and kidney. Thus, the organ concentrations, with the exception of the liver at 5 min, were less (Tables 2 and 3). This difference was particularly apparent in the kidney, where the renal Cd^{2+} concentration in the non-pregnant female at 4 h after treatment was about 60% greater than that in the pregnant animal (Tables 2 and 3). Also, in the non-pregnant animal the concentration first increased and then

Table 2. Concentrations of Cd^{2+} in various organs of nulliparous female rats (200 ± 10 g body weight) at different times after the i.v. injection of 1.58 mg Cd^{2+} per kg body weight

Time after injection	Cd^{2+} concentration[a] (μg per g wet weight)						
	Blood	Liver	Kidney	Lung	Heart	Thymus	Adrenal
5 min	7.28 ± 0.13						
15 min	1.10 ± 0.13	20.59 ± 0.94	4.86 ± 0.32	1.51 ± 0.24	1.43 ± 0.18	0.84 ± 0.20	3.99 ± 0.36
30 min	0.62 ± 0.10	27.21 ± 0.36	5.60 ± 0.22	2.49 ± 0.42	2.10 ± 0.20	1.51 ± 0.50	3.73 ± 0.26
60 min	0.67 ± 0.06	25.09 ± 0.40	7.25 ± 0.20	1.93 ± 0.10	2.81 ± 0.16	1.70 ± 0.22	3.70 ± 0.14
4 h	0.69 ± 0.08	24.65 ± 0.06	7.75 ± 0.44	1.79 ± 0.09	2.02 ± 0.24	1.70 ± 0.18	3.30 ± 0.24
8 h	0.69 ± 0.04	27.04 ± 0.44	8.26 ± 0.34	1.75 ± 0.20	1.88 ± 0.20	1.38 ± 0.20	4.92 ± 0.46
24 h	0.62 ± 0.03	26.98 ± 0.40	8.25 ± 0.46	1.60 ± 0.32	2.72 ± 0.18	2.02 ± 0.24	4.75 ± 0.64

[a] Each value represents the mean (± SD) of separate analyses on the tissue from 4 animals.

Table 3. Concentrations of Cd^{2+} in various organs of 20-day pregnant rats (330 ± 10 g body weight) at different times after the i.v. injection of 1.58 mg Cd^{2+} per kg body weight

Time after injection	Cd^{2+} concentration[a] (μg per g wet weight)							
	Blood	Liver	Kidney	Lung	Heart	Thymus	Adrenal	Placenta
5 min	6.17 ± 0.16	17.08 ± 0.42						7.00 ± 0.32
15 min	1.36 ± 0.12	19.04 ± 0.40	3.20 ± 0.30	1.58 ± 0.20	1.36 ± 0.12	1.19 ± 0.18	4.53 ± 0.34	5.30 ± 0.30
30 min	0.87 ± 0.12	22.24 ± 0.36	4.38 ± 0.38	1.40 ± 0.18	1.61 ± 0.20	1.38 ± 0.20	6.16 ± 0.20	5.41 ± 0.28
60 min	0.40 ± 0.08	21.38 ± 0.30	4.35 ± 0.36	1.70 ± 0.24	1.68 ± 0.24	1.76 ± 0.22	6.51 ± 0.32	5.36 ± 0.32
4 h	0.37 ± 0.06	21.25 ± 0.30	4.75 ± 0.40	1.38 ± 0.30	1.35 ± 0.16	1.29 ± 0.30	5.29 ± 0.20	5.64 ± 0.28
8 h	0.91 ± 0.08	24.80 ± 0.32	4.63 ± 0.52	2.41 ± 0.32	2.36 ± 0.30	1.39 ± 0.18	4.97 ± 0.24	5.54 ± 0.30
24 h	0.61 ± 0.04	24.50 ± 0.36	4.05 ± 0.50	2.05 ± 0.28	1.65 ± 0.32	1.15 ± 0.20	3.55 ± 0.20	4.20 ± 0.34

[a] Each value represents the mean (± SD) of separate analyses on the tissue from 4 animals.

Table 4. The distribution of Cd^{2+} between the high molecular weight protein, haemoglobin and metallothionein fractions of the rat placental cytosol at 8 and 24 h after the i.v. injection of 1.05 mg Cd^{2+} (15 μCi $^{109}Cd^{2+}$) per kg body weight on the 20th day of gestation

Time (h) after administration of Cd^{2+}	Cd^{2+} concentration[a] (μg per g wet weight initial tissue)				
	Whole placenta	Cytosol	High molecular weight protein fraction	Haemoglobin	Metallothionein
8	3.20	2.23	1.23	0.51	0.49
24	1.92	1.28	0.63	0.37	0.27

[a] Concentrations are corrected for losses during fractionation. The initial fractionation of the placental cytosol was performed by gel filtration. The haemoglobin fractions were pooled, concentrated and purified further by electrophoresis (see Experimental).

remained constant between 8 and 24 h (Table 2). In the pregnant animal it decreased (Table 3) probably because of the onset of kidney damage.

In the pregnant rat the placental concentration of Cd^{2+} was greater at all times than that in any other organ apart from the liver and, perhaps, the adrenal gland (Table 3). Although, at a constant dose level, the placental concentration of Cd^{2+} in dams with 4, 8 and 13 foetuses varies with the number of placentae (D. Holt and M. Webb, unpublished observations), such variation in the present experiments was small and statistically insignificant, probably because the animals did not have fewer than 9 or more than 13 placentae. From 15 min to 8 h after Cd^{2+} injection the placentae contained a constant Cd^{2+} concentration, equivalent to about 9% of the dose. Between 8 and 24 h, when haemorrhage occurred, this concentration decreased by about 24% (Table 3). Part of the Cd^{2+} accumulated in the placentae is known to be bound as the metallothionein, cadmium-thionein.[24] Under certain conditions this can be extremely nephrotoxic.[25,26] It seemed possible that the liberation of this metalloprotein, with the breakdown of the placentae, might be a causative factor in renal damage. Analysis of the placentae at 8 h after the injection of the Cd^{2+} LD_{50} (1.05 mg Cd^{2+} per kg body weight), however, revealed that although most of the Cd^{2+} retained by these organs was present in the cytosol, the amount bound as the metallothionein (15% of the total Cd^{2+}) was less than that bound by either the high molecular weight proteins (38%) or haemoglobin (16% of the total organ content). Furthermore, the loss of thionein-bound Cd^{2+} (0.22 μg per g wet weight tissue) after the onset of haemorrhage (i.e. between 8 and 24 h; Table 4) was small.

Effects of Cd^{2+} on embryo-foetal development

Maternal animals, dosed with Cd^{2+} (1.0, 1.25, 1.35 or 1.58 mg per kg body weight) between the 8th and 15th day of gestation, appeared normal and healthy with no post-mortem abnormalities in the maternal tissues. Some (10–15%) dead foetuses or foetal readsorptions (mainly early foetal readsorptions) were observed, however, in those that were treated on day 8 to day 11 with 1.25 mg Cd^{2+} per kg body weight. Late readsorptions were apparent when dosing occurred after the 11th day of gestation. On or after the 13th day, the incidence of these readsorptions was particularly high (Table 5). The live foetuses from all Cd^{2+}-treated dams were inactive and pale, smaller in size and weight than the controls ($P < 0.05$; d.f. 98). The two higher doses were highly foeto- (or embryo-) toxic, irrespective of gestation age at the time of injection.

Throughout the 8th to the 15th day of gestation, 70–85% of the litters of the animals treated with 1.25 mg Cd^{2+} per kg had one or more abnormal foetus(es) (Table 5). Day 9 was the most critical with the highest incidence (90%) of deformities. Hydrocephalus was the most

Table 5. Effects of i.v. injection of 1.25 mg Cd^{2+} per kg body weight on different days of gestation in the rat

Day of gestation	Average live foetuses per litter	% dead or reabsorbed foetuses	Average live foetal weight (g)	Abnormal litters and foetuses[a]	Abnormalities (% of total in all live foetuses)					
					Hydrocephalus	Eye defects[b]	Thin abdominal wall	Umbilical hernia	Hydronephrosis	Renal agenesis
8	12	12.5	2.5	10 (96)	37.2	17.8	2.7			
9	13	14.2	2.5	12 (176)	79.9	20.9	7.1			4.1
10	12	15.1	3.1	13 (160)	72.0	14.5	8.5			4.2
11	9	14.7	3.0	13 (112)	47.1	11.5	10.0	4.0	3.2	2.3
12	10	16.4	3.2	11 (96)	13.9	10.2	10.2	5.2	4.5	
13	10	14.1	3.0	12 (64)	5.4	6.0	12.6	6.1	9.0	
14	8	20.0	3.1	12 (52)			24.9	7.0	9.8	
15	7	26.2	2.9	11 (24)			10.4	5.1	4.0	

The maternal animals (15 in each group) were killed and examined on the 20th day of gestation.
[a] The number of abnormal foetuses is given in parentheses.
[b] Anophthalmia and microphthalmia.

prominent abnormality (80% incidence). Eye abnormalities (anophthalmia and microphthalmia) were also maximal after Cd^{2+} administration on the 9th day (Table 5). Injection of Cd^{2+} at later stages of gestation led to gastroschiasis, abdominal hernia, hydronephrosis and renal agenesis. After the 13th day, neither brain nor eye deformities were observed (Table 5). None of the foetuses from Cd^{2+}-treated dams showed skeletal deformities. No teratogenic malformations were observed after the injection of 1.1 mg Cd^{2+} per kg body weight during the critical period of gestation.

Injection of saline into control animals had no effect on foetal mortality or weight, but renal agenesis (1%) and hydronephrosis (2.5%) were observed when saline was given on day 9 or 10 and day 13 or 14 of gestation, respectively. One control foetus had umbilical hernia. None had either hydrocephalus or eye deformities.

DISCUSSION

During the later stages of gestation, pregnant rats have been shown to be highly susceptible to acute Cd^{2+} toxicity[8,12,13] and the present results confirm this difference in terms of the LD_{50} values. The LD_{50}, however, is usually expressed in terms of body weight at the time of the determinations.[27] The toxicological responses to Cd^{2+}, therefore, are difficult to interpret in the pregnant animal because of the weight of the conceptuses. The body weight of the pregnant rat, although only about 15% greater than that of the paired non-gravid control on the 12th day of gestation, increases rapidly between the 16th and 19th day.[28] For comparison, pregnant and non-pregnant rats do not differ in sensitivity to methylmercury, probably because the organic mercury distributes between mother and embryos in proportion to their weights.[28] However, doses of Cd^{2+} based on body weight during late gestation may be excessive for the pregnant animal, since the cation does not readily cross the placenta.[14] Thus, Cd^{2+} appears to be much more toxic to 20-day pregnant rats. Nevertheless, when rats are dosed with Cd^{2+} on the basis of their body weights at the time of conception, the LD_{50} value for the 20-day pregnant animal (1.6 mg Cd^{2+} per kg body weight) does not differ significantly from that for the nulliparous female (1.8 mg Cd^{2+} per kg body weight).

It is clear from the results that when 20-day pregnant rats and non-gravid females are given the same dose of Cd^{2+} (1.58 mg kg^{-1}), on the basis of their body weight, the concentrations of Cd^{2+} are less, but the contents of Cd^{2+} are greater in the livers of the former than of the latter. An appreciable amount of the dose (about 9%) is also retained in the placenta. Probably because of an increased capacity of the liver (the main acceptor organ for the i.v. administered cation) to accumulate Cd^{2+} by virtue of its greater weight, together with the placenta, which provides additional sites of accumulation, the Cd^{2+} concentrations in the lungs, heart, thymus and perhaps the adrenals of the pregnant rat do not differ greatly from those in the non-gravid female. The concentration in the kidneys is appreciably less (Tables 2 and 3). Thus, the damage to the liver and kidneys, which occurs in the pregnant but not in the nulliparous animal in response to this acute dose of Cd^{2+}, cannot be explained by differences in the Cd^{2+} concentrations in these organs. Indeed, simply in terms of the distribution and concentrations of Cd^{2+} in the common body organs, it might be expected that the pregnant animal would be more resistant than the non-pregnant female. Since, under the conditions of these experiments, the converse is true and, in those animals that die between 16 and 30 h after administration of Cd^{2+}, death appears to be correlated with placental haemorrhage, it is probable that haemorrhage is the cause of the damage to the liver as well as the kidneys (see also Ref. 29). A similar inference can be drawn from the observations of Parizek,[13] the toxicity of Cd^{2+} for the maternal rat in late gestation is decreased by surgical removal of the placentae and foetuses, but not by the removal of the foetuses alone. It seems unlikely, however, that the small amount of Cd^{2+} which is eliminated, either as the metallothionein or in other protein-bound forms (Table 4), with the breakdown of the placentae, is a causative factor in kidney damage.

Although few maternal deaths occurred from the i.v. injection of 1.58 mg Cd^{2+} per kg body weight until after the 16th day of pregnancy, this dose caused a high embryo or foetal mortality, irrespective of gestational age. A dose

of 1.35 mg Cd^{2+} per kg was also extremely embryo- and foetotoxic, whereas a dose of 1.1 mg kg^{-1} seemed to be without effect on the conceptuses. An intermediate dose (1.25 mg kg^{-1}), when administered between day 8 and day 12 of gestation, is both embryotoxic and teratogenic. On the 9th day in particular, the most critical in terms of the teratogenic response, some embryos (about 12%) are killed and reabsorbed, whilst those that remain viable give rise to small, anaemic foetuses, about 90% of which are deformed (Table 5). At this gestational age the most frequent teratogenic abnormalities were hydrocephalus and eye defects. Possibly because of species differences in teratogenic response,[30] no malformations of the face, which are characteristic of Cd^{2+} teratogenesis in hamsters,[3] have been observed in the Wistar rats. As teratogenic susceptibility coincides with the rapid period of development and different organs pass through susceptible periods at different times, the syndrome of malformations changes when Cd^{2+} is administered at various gestational ages. Thus, the incidence of hydrocephalus is greatest at day 9 and 10 of gestation whereas the incidence of another common malformation (extreme attenuation of the abdominal wall) is maximal when Cd^{2+} (1.25 mg per kg body weight) is injected into the mother on the 14th day (Table 5).

The increase in foetal mortality with gestational age that is apparent after a single dose of 1.25 mg Cd^{2+} per kg body weight (Table 5) occurs in the absence of placental haemorrhage and seems to be associated with increased transport of the cation to the foetus.[14,23] Consequently, this may be related to the changes in placental size, morphology and permeability[7] during development.

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Received 24 April 1981; accepted 26 May 1981