This is to certify that the

thesis entitled

EMBRYOTOXICITY OF CISPLATIN IN RATS AND MICE

presented by

KIT ANN KELLER

has been accepted towards fulfillment
of the requirements for

M.S. degree in Zoology

S.K. Agarwal

Major professor

Date 6.8.82

EMBRYOTOXICITY OF CISPLATIN IN RATS AND MICE

By

Kit Ann Keller

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree

MASTER OF SCIENCE

Department of Zoology

1982

ABSTRACT

EMBRYOTOXICITY OF CISPLATIN IN RATS AND MICE

By

Kit Ann Keller

The antitumor agent, cisplatin [cis-dichlorodiammineplatinum (II)] was tested for possible teratogenic and embryolethal effects on Wistar rats and Swiss Webster mice. Rats were given a single ip injection of 0.3, 1.0, 2.5 or 3.0 mg/kg cisplatin on day 6, 8, 11, or 14 of gestation. Mice were given a single ip injection of 0.3, 3.0, 6.0, 8.0 or 13.0 mg/kg on day 8 only. The embryonic LD_{50}'s in the rat were 2.88, 1.28, and 1.0 mg/kg for day 6, 8, and 11, respectively. There was no significant increase in embryolethality at any of the given doses on day 14. The embryonic LD_{50} for mice was 5.24 mg/kg. An increase in the incidence of growth retardation or gross malformations was not noted in the surviving fetuses. Cisplatin is highly embryolethal in rats and mice at dosages well below adult therapeutic levels. This embryolethality is gesational stage-specific with the highest mortality corresponding to the period of rapid DNA replication in early organogenesis.

To my mother
who taught me that
the only limits to achievement
are those of your own creation

ACKNOWLEDGMENTS

I wish to express my deepest gratitude to Dr. S.K. Aggarwal, my research advisor and friend, for his immutable patience and consideration. This thesis would not have been possible without his support.

I also want to extend my appreciation to Drs. R. Band and J.E. Trosko, who served on my guidance committee and Dr. J.L. Gill for his help and advice on the statistical analysis of my data.

Lastly, I'd like to thank Jim SanAntonio and Mary Lynn Bajt for their warm friendship and conversation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Cisplatin, [cis-dichlorodiammineplatinum (II)] is the first of the metal coordination complexes and a class of potent, broad spectrum anticancer drugs (50,45) to be put into use in cancer chemotherapy. The drug has been used by itself and in combination with other drugs for the treatment of ovarian and testicular carcinomas. It has also displayed variable, but significant, activity in the treatment of carcinomas of the head and neck, urinary bladder, prostate and pediatric osteogenic sarcomas (44,45). The drug is not free from its side effects including renal toxicity, gastrointestinal toxicity (anorexia and diarrhea), nausea, vomiting, mylosuppression, ototoxicity (tinnitus and high frequency hearing loss), decrease in serum electrolytes (hypomagnesia), peripheral neuropathy and toxicity to the hemopoietic organs (45,51,56). The major limiting factors in cisplatin therapy are its nephrotoxicity and the severe nausea and vomiting. Nephrotoxicity can be ameliorated by slow intravenous infusion of the drug, with concomitant hydration and diuresis and is no longer considered dose-limiting (44). Various antiemetics have been used in an attempt to counteract the nausea and vomiting but have proven to be ineffective in most instances (44). However, Δ-tetrahydrocannabinol (THC), a synthetic extract of cannabis has been used with some success (48). THC, by itself, has been shown to be embryolethal and teratogenic in various laboratory animals (3,13,14,42).

Although cisplatin is now available for the treatment of various cancers, the precise mechanism of action of cisplatin and its

cytotoxicity is still unknown. Similarities between the mechanism of action of this drug and bifunctional alkylating agents have been suggested in many studies (32,46,47). Cisplatin concentrates in the nucleus of mammalian cells (47), binds to DNA (43,57) and inhibits its synthesis (32,37,55) indicating its similarities to radiomimetic agents (57). Further, it is both mutagenic (61) and carcinogenic (30). Studies of cisplatin distribution in animals show that the initial concentration is highest in the excretory organs (27,28,31). However, after 4 days, the highest concentration is found in the ovaries, uterus, and skin (31). In addition, cisplatin has been shown to induce atrophy of the testes and prostate in dogs and monkeys (51). A more recent study shows that cisplatin induces spermatocyte and spermatid death in mice. However, stem cells seem to be unaffected (33).

All the available alkylating agents now in clinical use, which cross-link DNA similar to cisplatin, have been shown to produce developmental defects in the frog (4), chick (16,39), mouse (7,11,22,23,41), rat (5,6,20,34,38,59) and rabbit (1,15). There is also evidence that polyfunctional alkylating agents may be teratogenic in humans as well (10,19,52,53,54). Only one study on cisplatin embryotoxicity in mice has been reported in the literature (29). It is, therefore, important that we further investigate the effects of this drug on the reproductive system and pregnancy, especially since children and women of childbearing age are being treated with this drug.

The effects of a single dose of cisplatin on fetal development in Wistar rats and Swiss Webster mice were evaluated in this study. This

should provide a baseline of information for any further studies using cisplatin in humans, as well as other laboratory animals.

MATERIALS AND METHODS

Wistar rats weighing 250-325 gm (obtained from Charles River Breeding Lab, Willington, MA) and Swiss Webster mice weighing 25-35 gm (obtained from Spartan Breeding Lab, Haslett, MI), bred and reared in our own laboratory, were used throughout the experiment. The animals were allowed free access to tap water and Wayne laboratory animal food (Allied Mills, Inc. Chicago) at all times, except for pair fed controls, which were limited to the food intake of their corresponding drug treated counterparts. All animals were maintained under a 12 hour light-dark cycle at 23°C. Timed pregnancies were obtained by placing proestrus, virgin females with a male (male/female ratio of 1:3 for rats and 1:5 for mice) at the beginning of the dark cycle. The females were then removed at the end of the dark period and examined for the presence of vaginal plug and sperms in the vaginal smears. This was regarded as day 1 of pregnancy, while taking mid-dark cycle (3:00 A.M.) as the time of fertilization. A record of weight and food intake was kept throughout the experiment in order to monitor maternal toxicity.

Cisplatin was obtained from Johnson Matthey Research Laboratories (Sonning, U.K.). Dose solutions in 0.75% saline were prepared immediately before use. Rats were given a single ip injection of 0.3, 1.0, 2.5 or 3.0 mg/kg cisplatin on day 6, 8, 11 or 14 of gestation. Mice were given a single ip injections of 0.3, 3.0, 6.0, 8.0 or 13.0 mg/kg on day 8 only. All injection were given at approximately 9:00 A.M.

Dams were killed on day 20 (rats) or day 18 (mice), 2 days prior to parturition, by cervical dislocation. The uterus was removed and the number of live, dead and resorbed fetuses was recorded. In the case of early and total litter resorption, implantation sites were not always apparent and the whole uterus was placed in 10% ammonium sulfide for 10 min. for such detection (26). In most dams the site of resorption was clearly recognizable by the presence of metrial glands, large stalks of blood vessels in the uterine wall and darkened regions within the uterine endometrium. The fetuses were removed, weighed to the nearest milligram and externally examined for any deformities under a dissecting scope. Two thirds were randomly placed in Bouin's fluid and autopsy examinations were performed using the Wilson razor section technique (60). The remaining one third were fixed in 95% ethanol, cleared in 0.7% potassium hydroxide and stained in alizarin red-S for skeletal examination (8). Small samples of placental and uterine rat tissues were fixed in Bouin's fluid and processed for routine light microscopy. Photomicrographs were taken using a Zeiss-photomicroscope II loaded with plus-X film.

Changes in maternal body weights were calculated by subtracting the body weights on the day of conception plus the total litter weight from the weight on day of sacrifice. Analysis of differences between control and drug-treated animals in regard to changes in the average maternal body weight and food intake were made by the student's t-test. The mean fetal body weights of drug-treated animals were compared with those of the litters from control dams by student's t-test. Analysis of differences between control and drug-treated litters in regard to average percent of intauterine deaths and skeletal

variations was made using arcsin square root percentage transformations. The distribution of resorption sites within the uterus was analysed by a split-plot anova. LD_{50}'s were calculated using the average percentage of fetal deaths (12).

RESULTS

Maternal Toxicity Due to Cisplatin

Maternal weight gain/loss and daily food intake during the gestation period was used as a criterion for any maternal toxicity due to the drug. Both the weight gain/loss (TABLE 1) and daily food intake (FIGURES 1 and 2) were affected in a dose dependent fashion. None of the dosages of cisplatin used in this present study were found to be lethal to the dams and none appeared seriously ill.

RATS: Changes in the weight of dams treated day 6 were negligible, except for the animals in the highest dose (3.0 mg/kg) treatment group (TABLE 1). On treatment days 8, 11, and 14 the dose dependent pattern was much more evident. The decreases in weight gain, compared to their respective controls, on day 8 and 11 - the two most embryotoxic days of gestation, were correlative. The maternal weight of the day 8, pair fed control groups both corresponded to the weight of a cisplatin treated group a dose lower than their treated counterparts.

The food intake of the 0.3, 2.5 and 3.0 mg/kg, day 8, cisplatin treated dams decreased when compared to saline treated controls (FIGURE 1). The 0.3 mg/kg treated animals showed a significant (27%) decrease on the day of treatment, but within 24 hr. food intake resumed to normal. At the higher (2.5 and 3.0 mg/kg) dosages food intake

decreased approximately 64% and 91%, respectively, and did not approach normal levels until the 18th day of gestation.

MICE: Maternal weight gain did not exhibit any significant decrease until a 6.0 mg/kg dosage or higher of cisplatin was administered (TABLE 1). As with the experimental rats, the decrease in maternal weight of the pair fed control group corresponded to a dosage group which was lower than its treated counterpart.

Food consumption was not as greatly affected by the cisplatin treatments in the mice as it was with the experimental rats (FIGURE 2). The only significant decrease in food intake occured with the 13.0 mg/kg treated dams, which appeared in a sporadic pattern throughout gestation.

Embryolethal effects

Cisplatin was highly embryolethal in both rats and mice, causing fetal death and resorption when administered during early gestation at dosages well below the adult therapeutic dose levels (FIGURE 3).

RATS: Table 2 summarizes the effects of a single ip injection of cisplatin in pregnant rats on day 6, 8, 11 or 14 of gestation. The frequency of fetal death or resorption generally occurred in a dose-dependent pattern.

Day 6 treatment: Cisplatin did not induce 100% resorption in any treatment dam with the exception of one female, administered 3.0

mg/kg, which contained a completely resorbed litter. The average frequency of resorption of the remaining females in the 3.0 mg/kg treatment group showed a significant (67.11%) increase when compared to the control. The three lower dosages (0.3, 1.0 and 2.5 mg/kg) did not induce any statistically significant increase in embryo mortality. The embryonic LD_{50} on day 6 was calculated to be 2.88 mg/kg (95% confidence limit: 2.04 to 4.07).

Day 8 treatment: The highest (3.0 mg/kg) dosage of cisplatin induced 100% resorption in all the dams. In the 2.5 mg/kg dosage group, five out of eight females had completely resorbed litters. The remaining three dams in the treatment group showed a significant increase (76.46%) in the frequency of resorption. The average percent of resorption also increased significantly (15.31%) in the 1.0 mg/kg treated group when compared to the day 8 control rate of 6.31%. Seven of the eight females treated with 1.0 mg/kg cisplatin had only 1-3 resorptions per animal which fall within the range of normal. Whereas, the remaining dam had 66.67% of her litter resorbed, showing a high susceptibility to the drug as compared to the rest of her treatment group. Pair fed controls of the 2.5 and 3.0 mg/kg treatment groups did not show any significant increase in embryomortality. The embryonic LD_{50} on day 8 was calculated to be 1.28 mg/kg (95% confidence limit: 1.10 to 1.51)

Day 11 treatment: The 11th day of gestation proved to be the most susceptible period to cisplatin treatment. Both the 2.5 and 3.0 mg/kg dosages induced 100% litter resorption in all the dams treated. In the 1.0 mg/kg dosage group there was a significant increase (51.19%) of resorption compared to the control. The embryonic LD_{50} on day 11 could not be calculated statistically though estimated to be near 1.0 mg/kg dose range.

Day 14 treatment: There was no significant increase in embryolethality at any of the given dosages when cisplatin was administered on day 14 of gestation. The embryonic LD_{50} was calculated to be much larger than an adult LD_{100} dosage and one can assume an adult/embryonic LD_{50} ratio of 1:1 in this case.

MICE: An embryolethal dose-response pattern was also evident in the experimental mice (TABLE 3). The three highest dosages (6.0, 8.0 and 13.0 mg/kg) induced 100% resorption of the litters in all the dams treated. The 3.0 mg/kg cisplatin treated group exhibited a large variance in the frequency of resorption. Two of the six dams had complete resorption of their litters, while another two showed no resorptions at all. When the average percent of resorption is calculated, without including these completely resorbed litters, it proves to be comparable to the control value. The pair fed controls of the 13.0 mg/kg cisplatin treated group had a significant increase (49.18%) in resorption of approximately half the value of their treated

counterparts. The embryonic LD_{50} was calculated to be 5.25 mg/kg cisplatin (95%) confidence limit: 2.88 to 9.55).

Intrauterine distribution of resorption sites

The intauterine positions of the resorption sites were evaluated in order to determine if any distribution pattern exists with cisplatin induced fetomortality (TABLE 4).

RATS: There was no apparent pattern of distribution in the location of resorption sites between any of the groups, control or treatment (TABLE 4).

MICE: All of the resorption sites in the control and 3.0 mg/kg treatment groups were located in the middle and cervix regions of the uterus (TABLE 4). With 3.0 mg/kg the distribution of resorption sites is spread evenly throughout the uterus. The pair fed control group distribution was not significantly different from the control group distribution, however, a small percentage of the resorptions were found in the ovarian region.

Average fetal body weights

RATS: A small, but significant decrease in fetal body weights was evident in the 1.0, 2.5 and 3.0 mg/kg cisplatin treatment groups administered on day 6 of gestation (TABLE 2). The day 14, 1.0 and 2.5 mg/kg treatment groups also showed a statistically significant decrease compared to the control. However, it should be noted that the testing for significant differences in average fetal weight was complicated by

the fact that the average fetal weight of the control group for treatment day 8 was found to be significantly different from the control values on day 6, 11 and 14. The reason for this disparity could be attributed to differences in the time of fertilization over the 12 hour mating period. This deviation of the control weight does not appear to reflect on the fetal weights of the cisplatin treated groups on day 8. The groups which show a decrease in average fetal weights are well within the range of the low, day 8, control average. In light of these results it is not possible to conclude that there was definite decrease in average fetal body weights in any of the treatment regimes.

The incidence of growth retarded fetuses (more than 3 standard deviations below the control average) was not significantly increased (TABLE 2). The three cases in which there was only a single surviving fetus from an otherwise destroyed litter (2 in the day 8, 2.5 mg/kg treatment group and 1 in the day 11, 1.0 mg/kg treatment group) each was stunted.

MICE:In the cisplatin treatment groups that had surviving fetuses (0.3 and 3.0 mg/kg) a small, but significant, decrease in fetal body weight was observed (TABLE 3). The pair fed control group showed a larger decrease in fetal body weight than the cisplatin treated animals. Stunted fetuses were not found in any group, treatment or control.

Malformations and incidence of skeletal variations

In this present study there was only one rat and one mouse fetus with gross malformations. There was, however, an increase in skeletal variations (24, 21) including "rudimentary" and "extra" 14th ribs (FIGURE 4) and cleft vertebral centra (bipartite or dumbell shaped) (FIGURE 5).

RATS: One rat fetus, a lone survivor of a resorbed litter, from the day 8, 2.5 mg/kg treatment group displayed clubbed appendages, hypognathous, spina bifida occulta and a retarded, twisted tail (FIGURE 6).

The frequency of skeletal variations between the control groups varied from 27.27% to 0% (TABLE 5). The increase in the frequency rate, though significant in some groups, was quite sporadic in its distribution among the treatment regimes. Only the day 8 and day 14 treatment groups appeared to follow any sort of dose-response pattern. The day 11 treated survivors (1.0 mg/kg cisplatin) showed no skeletal variations at all. The highest dosage groups on day 8 and day 14 treatment with any survivors (2.5 and 3.0 mg/kg, respectively) were the only groups to show at least one skeletal variation in every litter.

MICE: One mouse fetus, form the 3.0 mg/kg treatment group, displayed exencephaly and cleft lip and palate (FIGURE 7).

No cleft vertebral centra were seen in any of the fetuses. The occurrance of "rudimentary" and "extra" 14th ribs appeared in every group, control and treatment (TABLE 5). A significant increase from

the control frequency was seen in the 3.0 mg/kg treatment group.

Histology of the rat uterus and placenta

The uterine tissue of the drug-treated animals appeared unaffected at the light microscope level. No lesions or necrotic areas were noted in the muscular wall of the uterus. Early resorption sites were distinguishable as an undifferentiated cell mass which stained more intensely with hematoxylin and eosin than the surrounding tissue (FIGURE 8). At later resorption sites fetal forms were still recognizable in some cases, but most of the mesenchymal elements had degenerated and the mass of debris was infiltrated with leucocytes (FIGURES 9, 10, 11).

The 20 day placentas of animals treated with the two lower dosages (0.3 and 1.0 mg/kg) of cisplatin were comparable to the placentas of saline-treated controls. At the higher (2.5 and 3.0 mg/kg) dosages, however, increases in the thickness of the placental basal zone, with concomitant decreases in the proportion of the labyrinth, were evident. This is probably due to a massive increase in the number of vacuolated "glycogen cells" (FIGURES 12, 13, and 14). In both the control and treated placentas these vacuolated "glycogen cells" have undergone extensive cytolysis, and in some instances are detectable as pools of nuclear and cellular debris. Giant cells, which normally form a complete layer between the basal zone and the decidua basalis (FIGURE 12) are fewer and sparsely distributed in the drug-treated placentas (FIGURE 13). No morphological changes were noted in the placental labyrinth.

TABLE 1. Mean maternal body weight gain

Day	# of dams	Dose (mg/kg)	Maternal weight (gm ± S.D.) Day of mating	Day of treatment	Day of killing	Wt. change[1] (%)	Wt. change minus fetal wt. (%)
RAT:							
6	5	normal	288 ± 27	308 ± 29	395 ± 30	+37	+27
	3	0.3	303 ± 12	317 ± 16	412 ± 29	+36	+26
	3	1.0	296 ± 19	309 ± 18	391 ± 10	+32	+23
	3	2.5	307 ± 28	325 ± 27	396 ± 31	+30	+25
	3	3.0	289 ± 6	302 ± 10	328 ± 24	+13	+11[2]
8	8	normal	255 ± 76	298 ± 30	376 ± 33	+47	+38
	8	0.3	252 ± 66	293 ± 18	363 ± 25	+44	+33
	8	1.0	255 ± 13	276 ± 14	343 ± 26	+35	+26
	8	2.5	252 ± 77	292 ± 16	304 ± 32	+21	+20[2]
	3	N-F(2.5)[3]	283 ± 35	273 ± 24	360 ± 31	+27	+26[2]
	4	3.0	275 ± 23	313 ± 31	299 ± 24	+ 9	+ 9[2]
	3	N-F(3.0)[3]	281 ± 34	304 ± 36	346 ± 47	+23	+22[2]
11	3	normal	296 ± 13	324 ± 20	403 ± 9	+36	+25
	3	1.0	281 ± 16	309 ± 10	346 ± 31	+23	+18
	3	2.5	316 ± 25	347 ± 30	338 ± 20	+ 7	+ 7[2]
	3	3.0	296 ± 14	327 ± 15	306 ± 23	+ 3	+ 3[2]
14	3	normal	306 ± 19	350 ± 24	416 ± 22	+36	+25
	3	1.0	285 ± 33	329 ± 34	371 ± 46	+30	+20
	3	2.5	282 ± 40	332 ± 42	347 ± 44	+23	+13[2]
	3	3.0	296 ± 50	336 ± 50	354 ± 63	+20	+11[2]
MICE:							
8	5	normal	32 ± 4	34 ± 3	50 ± 2	+ 56	+ 31
	4	0.3	31 ± 4	34 ± 4	48 ± 5	+ 55	+ 27
	6	3.0	29 ± 2	32 ± 2	43 ± 10	+ 48	+ 28
	5	6.0	32 ± 2	35 ± 3	35 ± 8	+ 9	+ 9[2]
	4	8.0	30 ± 4	34 ± 5	32 ± 4	+ 7	+ 7[2]
	4	13.0	27 ± 2	31 ± 3	24 ± 3	- 11	- 11[2]
	3	N-F(13.0)[3]	32 ± 5	35 ± 5	39 ± 2	+22	+ 8[2]

[1] Percent of weight at day of mating.

[2] Significantly different ($P < .05$) from control: statistical significance calculated by student's "t" test on average weight of females on day of killing minus litter weight & weight on day of mating.

[3] Saline treated animals given corresponding amounts of food of dye & clonidin treated animals. Dose in parentheses.

Figure 1. Graph showing the average maternal food intake from day 8 to day 20 of gestation of normal and day 8 cisplatin-treated rats. Each point represents the average of at least three animals.

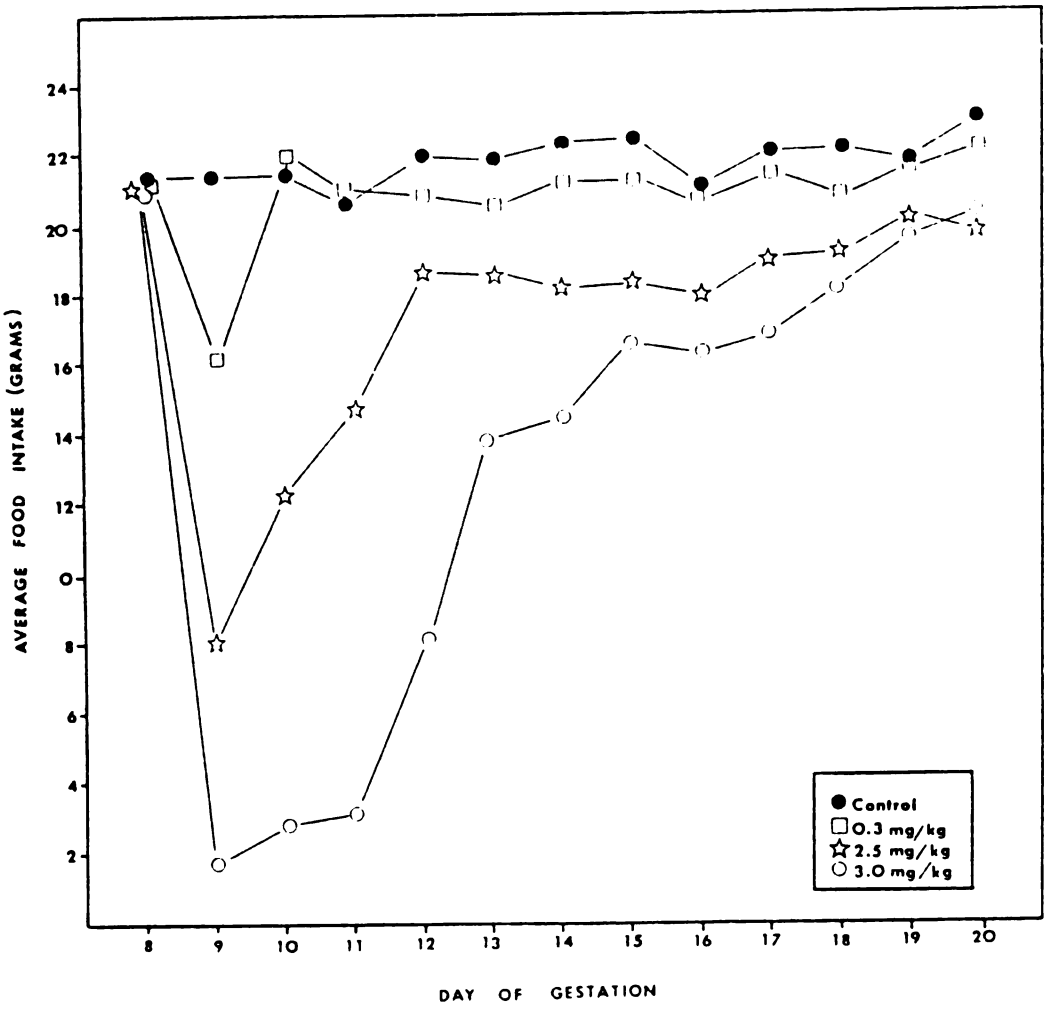

AVERAGE FOOD INTAKE (GRAMS)
24
22
20
18
16
14
12
0
8
6
4
2
8
9
10
11
12
13
14
15
16
17
18
19
20
DAY OF GESTATION
Control
0.3 mg/kg
2.5 mg/kg
3.0 mg/kg

Figure 2. Graph showing the average maternal food intake from day 8 to 18 of gestation of normal and day 8 cisplatin-treated mice. Each point represents the average of at least three animals.

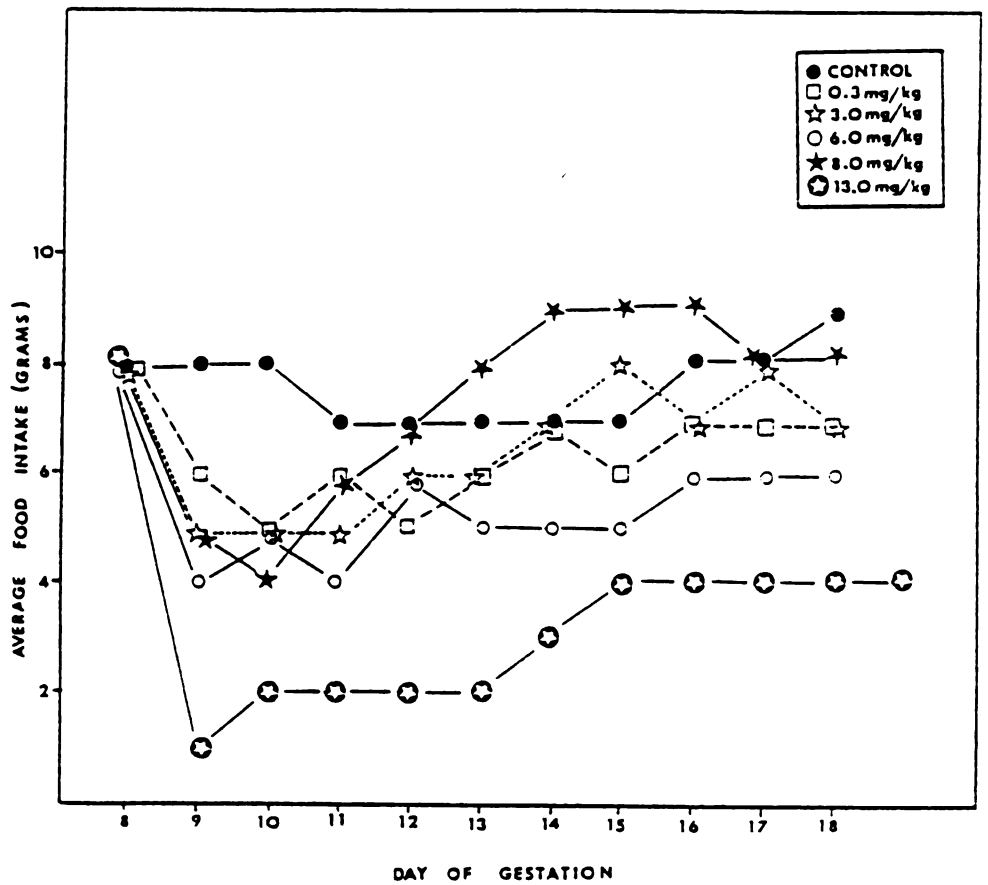

CONTROL
0.3 mg/kg
3.0 mg/kg
6.0 mg/kg
8.0 mg/kg
13.0 mg/kg
AVERAGE FOOD INTAKE (GRAMS)
10
8
6
4
2
8
9
10
11
12
13
14
15
16
17
18
DAY OF GESTATION

Figure 3. Macrophotograph showing a normal (right) and day 8 cisplatin-treated (3.0 mg/kg) left pregnant rat dissected on day 11 of gestation. Note the resorbed embryos (arrows) in the drug-treated dam.

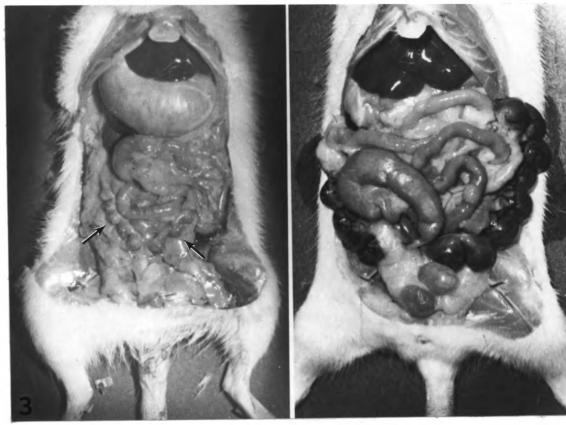

3

TABLE 2. Effects of a single dose of cisplatin given to pregnant rats on day 6-14 of gestation[1]

DAY 6

	Control	0.3 mg/kg	1.0 mg/kg	2.5 mg/kg	5.0 mg/kg
Number of dams	5	3	3	3	3
Ave. # of implants per dam[2]	15.80 ± 2.49	16.00 ± 4.00	15.67 ± 1.15	16.67 ± 0.58	17.68 ± 1.53
Total implantation sites	79	48	47	50	53
Ave. fetal mortality per dam[2]	2.40 ± 1.14	2.33 ± 1.15	2.00 ± 1.73	5.34 ± 2.31	13.68 ± 4.04
Total fetal mortality	12	7	6	16	41
% fetal mortality[3] ( )[4]	15.12	15.42	13.33	22.60	79.63[5] (67.11)[5]
Ave. live fetuses per dam[2]	13.40 ± 2.61	13.67 ± 4.16	13.67 ± 2.52	11.34 ± 2.08	4.00 ± 4.58
Total live fetuses	67	41	41	34	12
% live fetuses ( )[4]	84.88	84.58	86.67	77.40	20.37 (32.89)
dams with resorptions/ total dams[6]	5/5	3/3	3/3	3/3	3/3
Dams with resorptions only/ total dams[6]	0/5	0/3	0/3	0/3	1/3
Ave. fetal body wt. (gm ± S.D.)[2]	2.14 ± 0.31	2.23 ± 0.16	2.00 ± 0.21[5]	1.90 ± 0.26[5]	1.77 ± 0.20[5]
Number of stunted fetuses[7]	0	0	1	1	0

TABLE 2. Continued

DAY 8						
Control	0.3 mg/kg	1.0 mg/kg	2.5 mg/kg	N-F(2.5)[8]	3.0 mg/kg	N-F(3.0)[8]
8	8	8	8	3	4	3
11.38 ± 4.32	14.13 ± 1.89	12.29 ± 3.90	14.00 ± 1.73	15.60 ± 0.17	11.00 ± 7.21	11.00 ± 7.21
91	113	99	114	42	63	33
0.50 ± .70	1.00 ± 0.93	2.00 ± 2.67	12.00 ± 4.20	1.33 ± 0.58	15.60 ± 0.17	1.67 ± 2.08
5	8	16	103	4	63	5
6.31	7.73	15.13[5]	91.17[6](76.46)[5]	9.44	100.00[5]	12.22
10.75 ± 4.29	13.13 ± 2.59	8.00 ± 4.14	1.38 ± 3.11	12.67 ± 1.53	0.00 ± 0.00	9.33 ± 6.51
86	105	83	11	38	0	28
93.69	92.27	84.82	8.83 (23.54)	90.56	0.00	87.78
5/8	6/8	5/8	8/8	3/3	4/4	2/3
0/8	0/8	0/8	5/8	0/3	4/4	0/3
1.85 ± .53	2.06 ± 0.50	2.24 ± 0.36	2.06 ± 0.36	2.15 ± 0.16	---	2.34 ± 0.11
2	3	1	2	1	---	0

TABLE 2. continued

DAY 11				DAY 14			
Control	1.0 mg/kg	2.5 mg/kg	3.0 mg/kg	Control	1.0 mg/kg	2.5 mg/kg	3.0 mg/kg
3	3	3	3	3	3	3	3
16.00 ± 1.73	12.67 ± 3.51	15.00 ± 2.00	16.68 ± 1.15	15.67 ± 1.53	16.33 ± 1.53	15.00 ± 3.00	14.67 ± 2.52
48	38	45	50	47	49	45	44
0.67 ± .58	7.00 ± 7.00	15.00 ± 2.00	16.68 ± 1.15	1.67 ± 0.58	1.00 ± 0.00	0.33 ± 0.58	2.34 ± .58
2	21	45	50	5	3	1	7
4.34	51.19[5]	100.00[5]	100.00[5]	10.89	6.27	1.85	16.17
13.40 ± 2/61	5.67 ± 5.03	0.00 ± 0.00	0.00 ± 0.00	14.00 ± 2.00	15.33 ± 1.53	14.67 ± 2.52	12.33 ± 2.52
67	17	0	0	42	46	44	37
95.66	48.81	0.00	0.00	89.11	93.73	98.15	83.86
2/3	3/3	3/3	3/3	3/3	3/3	1/3	3/3
0/3	0/3	3/3	3/3	0/3	0/3	0/3	0/3
2.17 ± 0.16	2.31 ± 0.55	---	---	2.23 ± 0.18	2.00 ± 0.28[5]	1.91 ± 0.34[5]	2.23 ± 0.31
0	1	---	---	0	0	0	1

[1] Pregnant Wistar Rats given a single ip injection of cisplatin at the doses indicated on day 6, 8, 11 or 14 of gestation. Fetuses were obtained by autopsy on day 20 of gestation.

[2] Results given as mean ± S.D.

[3] The average percent affected fetuses per litter. Fetal mortality taken as number dead and resorbed.

[4] In parentheses; excluding litters with resorptions only.

[5] Significantly different [P < .05] from control.

[6] Resorptions = resorbed and live fetuses; resorption only = totally resorbed or dead fetuses (no live fetuses).

[7] Growth retardation; less than 3 S.D. below control average

[8] Saline treated animals given corresponding amounts of food of the day 8 cisplatin treated animals. Dose in parentheses.

TABLE 3. Effects of a single dose of cisplatin given to pregnant mice on day 8 of gestation[1]

	Control	0.3 mg/kg	3.0 mg/kg	6.0 mg/kg	8.0 mg/kg	13.0 mg/kg	N-F (13.0)[2]
Number of dams	5	4	6	5	4	5	3
Ave # of implants per dam[3]	9.00 ± 2.00	10.25 ± 0.96	9.67 ± 4.55	10.60 ± 2.51	11.00 ± 1.80	10.50 ± 1.70	11.67 ± 2.08
Total implantation sites	45	41	70	53	44	42	35
Ave. fetal mortality per dam[3]	1.20 ± 1.30	11.67 ± 0.58	4.5 ± 5.09	10.60 ± 2.51	11.00 ± 1.80	10.50 ± 1.70	5.67 ± 3.05
Total fetal mortality	6	5	27	53	44	42	17
% fetal mortality[4] ([5])	12.99	11.87	40.93 (11.40)	100.00	100.00	100.00	49.18
Ave. live fetuses per dam[3]	7.80 ± 1.90	9.00 ± 0.82	7.17 ± 5.78	0.00 ± 0.0	0.0 ± 0.0	0.00 ± 0.0	6.00 ± 3.61
Total live fetuses	39	36	43	0	0	0	18
% live fetuses[5] ( )	87.01	88.13	59.07 (88.60)	0.00	0.00	0.00	50.82
Dams with resorptions/ total dams[7]	3/5	3/4	4/6	5/5	4/4	5/5	3/3
Dams with resorptions only/ total dams[7]	0/5	0/4	2/6	5/5	4/4	5/5	0/3
Ave. fetal body wt. (gm ± S.D.)[6]	1.03 ± 0.21	.95 ± .09	.94 ± .22	---	---	---	.74 ± .20[6]
Number of stunted fetuses[8]	0	0	0	---	---	---	0

1 Pregnant Swiss Webster mice given a single ip injection of cisplatin at the doses indicated. Fetuses were obtained by autopsy on day 18 of gestation.

2 Saline treated animals given corresponding amounts of food of the day 8, 13.0 mg/kg treat animals.

3 Results given as mean ± S.D.

4 The average percent affected fetuses per litter. Fetal mortality taken as number dead and resorbed.

5 In parentheses; excluding litters with resorptions only.

6 Significantly different ($P < .05$) from control

7 Resorptions = resorbed and live fetuses; resportion only = totally resorbed or dead fetuses (no live fetuses).

8 Growth retardation; less than 3 S.D. below average.

TABLE (4) LOCALIZATION OF RAT AND MICE FETOMORTALITY ACCORDING TO INTRAUTERINE POSITION[1]

RAT: Day	Dose (mg/kg)	# Resorptions/ Total # Implants	INTRAUTERINE POSITION[2] U_1	O_2	O_3	M_2	M_1	M_3	C_3	C_2	C_1	#RESORPTIONS PER POSITION / # OF IMPLANTATION SITES PER POSTION (%) O	M	C	# RESORPTIONS PER POSITION / TOTAL RESORPTION (%) O	M	C
6	NORMAL	12/79	2/10	1/10	1/6	0/9	1/9	1/9	1/6	1/10	4/10	15.38	7.41	23.08	33.33	16.67	50.00
	0.3	7/48	1/6	2/6	1/3	0/6	1/6	0/6	0/3	0/6	2/6	26.67	5.56	13.33	57.14	14.29	28.57
	1.0	16/44	0/6	1/6	1/3	0/6	0/2	1/6	0/3	0/6	3/6	11.11	7.14	16.67	33.33	16.67	50.00
	2.5	16/50	3/6	1/6	4/5	2/6	2/4	0/6	1/5	1/6	2/6	47.06	25.00	23.53	50.00	25.00	25.00
	3.0	25/37	2/4	2/4	3/4	3/4	3/5	4/4	2/4	3/4	3/4	43.75	76.92	50.00	28.00	40.00	32.00
8	NORMAL	3/82	1/11	0/12	0/4	0/10	0/8	0/10	0/4	1/12	1/11	3.70	0.00	7.41	33.33	0.00	66.67
	0.3	8/14	3/14	1/16	0/8	1/14	1/10	0/14	0/8	2/16	0/14	10.23	5.26	5.26	50.00	25.00	25.00
	1.0	14/92	3/11	2/13	1/5	2/11	0/12	1/11	0/5	2/13	3/11	20.69	8.82	17.24	42.86	21.43	35.71
	2.5	14/25	2/3	2/3	1/2	2/3	0/3	1/3	1/2	2/3	3/3	62.50	33.33	75.00	35.71	21.43	42.86
	N-F (2.5)[4]	4/40	4/5	0/5	0/3	0/5	0/4	0/5	0/3	0/5	0/5	30.77	0.00	0.00	100.00	0.00	0.00
	N-F (3.0)[4]	5/31	2/4	0/4	0/2	0/4	1/3	0/4	0/2	0/4	2/4	20.00	9.09	20.00	40.00	20.00	40.00

Table 4. continued

11	NORMAL	2/48	1/6	1/6	0/3	0/7	0/4	0/3	0/3	0/60	0/6	13.33	0.00	0.00	100.00	0.00	0.00
	1.0	10/24	0/4	1/4	0/0	3/3	1/2	2/3	0/0	2/4	1/4	12.50	75.00	37.50	10.00	60.00	30.00
14	NORMAL	5/47	1/6	1/6	1/6	0/5	1/5	1/5	0/6	0/6	0/6	10.75	13.33	0.00	60.00	40.00	0.00
	1.0	3/49	1/6	0/6	0/3	0/7	0/5	1/7	0/3	0/6	1/6	6.67	5.26	6.67	33.33	33.33	33.33
	2.5	1/45	0/6	0/6	1/3	0/5	0/5	0/5	0/3	0/6	0/6	6.67	0.00	0.00	100.00	0.00	0.00
	3.0	7/44	2/6	3/6	0/3	0/6	0/2	1/6	0/3	1/6	0/6	33.33	7.14	6.67	71.43	14.29	14.29
MICE:																	
8	NORMAL	5/40	0/5	0/8	0/0	2/5	0/4	2/5	0/0	1/8	0/5	0.00	28.57	7.69	0.00	80.00	20.00
	0.3	4/35	0/6	0/6	0/0	1/4	0/3	1/4	0/0	1/6	1/6	0.00	18.18	16.67	0.00	50.00	50.00
	3.0	6/49	1/8	1/8	0/1	1/6	0/3	1/6	0/1	2/8	0/8	11.76	13.33	11.76	33.33	33.33	33.33
	N-F [4]																
11	(13.0)	11/27	1/5	0/5	0/0	1/2	2/3	1/2	0/0	3/5	3/5	10.00	57.14	60.00	9.09	36.36	54.55

1. Horns with complete resorptions or less than 4 implantation sites not included.

2. # Resorptions/total # of implantation sites for that position. Intrauterine positions assigned according to the following: (modified from Naubeat, Et. Al. 1980).

   # Fetuses per Horn

   4: O_2 M_2 M_3 C_2

   5: O_1 O_2 M_1 C_2 C_1

   6: O_1 O_2 M_2 M_3 C_2 C_1

   7: O_1 O_2 M_2 M_1 M_3 C_2 C_1

   8: O_1 O_2 O_3 M_2 M_3 C_3 C_2 C_1

   9: O_1 O_2 O_3 M_2 M_1 M_3 C_3 C_2 C_1

   10: O_1 O_2 O_3 M_2 M_1 M_3 C_3 C_2 C_1

   11. O_1 O_2 O_3 M_2 M_1 M_3 C_3 C_2 C_1

   12: O_1 O_2 O_3 M_2 M_1 M_3 C_3 C_2 C_1

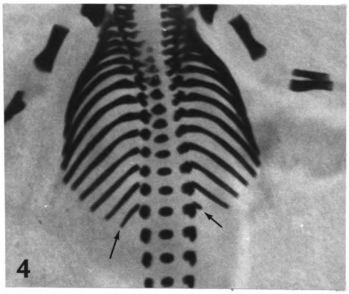
   (O = OVARY M = MIDDLE C = CERVIX)

3. Analysed with a split-plot anova.

4. Saline treated animals feed corresponding food intake of day 8 cisplatin treated animals. Dose in parentheses.

Figure 4. Macrophotograph showing a normal mouse fetus with a "rudimentary" and "extra" 14th rib (arrows).

Figure 5. Macrophotograph showing a normal rat fetus with a bipartite and dumbell shaped vertebral centra (arrows).


4

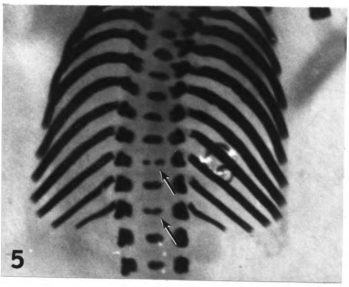
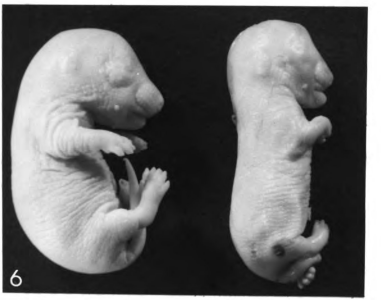

5

Figure 6. Macrophotograph showing a normal (left) and malformed (clubbed appendages, hyponathous, spina bifida occulta and retarded and twisted tail) (right) rat fetus.

Figure 7. Macrophotograph showing a normal (left) and malformed (exencephaly and cleft lip and palate) (right) mouse fetus.


6

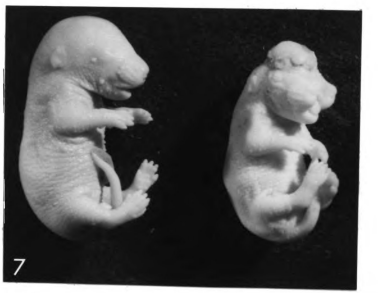

7

TABLE 5. Incidence of skeletal variations[1]

	Day of Gestation	Dose (mg/kg)	# Fetuses Examined	% Affected Fetuses/Total	# Litters Affected/ Total Litters
RAT:	6	control	17	11.76	2/5
		0.3	10	50.00[2]	2/3
		1.0	10	50.00[2]	2/3
		2.5	8	37.50	3/3
		3.0	3	0.00	0/3
	8	control	22	13.64	3/8
		0.3	29	13.79	4/8
		1.0	9	47.37[2]	6/8
		2.5	3	100.00[2]	2/2
		N-F(2.5)[3]	10	0.00	0/3
		N-F(3.0)[3]	9	0.00	0/3
	11	control	12	0.00	0/3
		1.0	4	0.00	0/3
	14	control	11	27.27	2/3
		1.0	12	25.00	2/3
		2.5	9	33.00	2/3
		3.0	8	62.50	3/3
MICE:	8	control	15	13.33	2/5
		0.3	16	18.75[2]	2/4
		3.0	16	50.00	4/4
		N-F(13.0)[3]	7	0.00	0/3

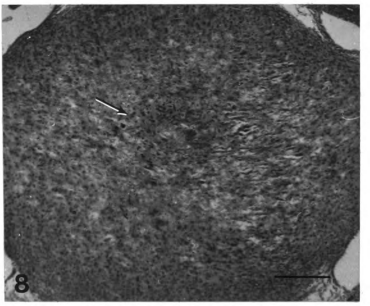
1. Skeletal variations include "rudimentary" (less than half the length of the 13th rib) and "extra" (at least half the length of the 13th rib) 14th rib and cleft vertebral centra (bipartitle or dumbell shaped). Note: mice show only "rudimentary" and "extra" 14th ribs.

2. Significantly different ($P < .05$) from controls.

3. Saline treated animals given corresponding amounts of food of the day 8 cisplatin treated animals. Dose in parentheses.

Figure 8. Photomicrograph showing an early embryo resorption site (arrow) in a cisplatin-treated rat uterus. Hematoxylin and Eosin. X 77. Bar = 0.2 mm.

Figure 9. Photomicrograph showing a late embryo resorption site in a cisplatin-treated rat uterus. Note that the fetal form is still distinguishable. Hematoxylin and Eosin. X 110. Bar = 0.2 mm.


8

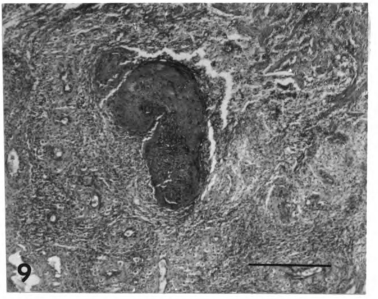
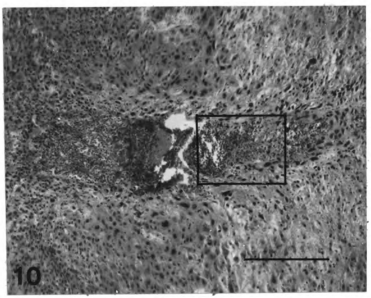

9

Figure 10. Photomicrograph showing a late embryo resorption site in a cisplatin-treated rat uterus. The area enclosed byt he rectangle is shown in figure 11. Hematoxylin and Eosin. X 121. Bar = 0.2 mm.

Figure 11. Photomicrograph of an enlarged area of the same embryo resorption site in figure 10. Note infilration of the site by leucocytes (arrow). Hematoxylin and Eosin. X 196. Bar = 0.1 mm.


10

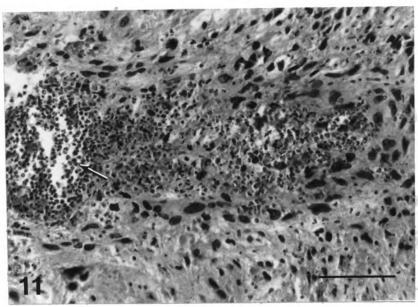
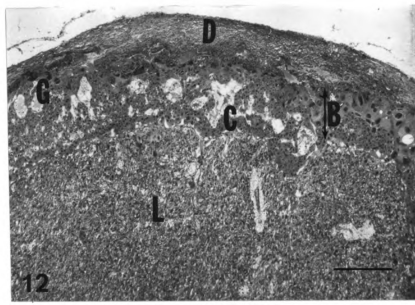

11

Figure 12. Photomicrograph of a normal day 20 rat placenta showing the giant cells (G), basal zone (B), vacuolated "glycogen cells" (C), the labyrinth (L) and the decidua basilis (D). Hematoxylin and Eosin. X 77. Bar = 0.2 mm.

Figure 13. Photomicrograph of a day 20 placenta from a cisplatin-treated rat. Note the increase in the size of the basal zone (B) and the increase in the number of vacuolated "glycogen cells" (C). Decidua basilis (D). Hematoxylin and Eosin. X 77. Bar = 0.2 mm.


D
G
C
B
L
12

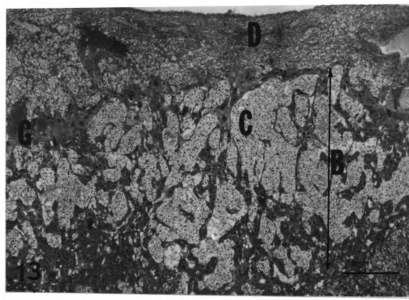

D
G
C
B

Figure 14. Photomicrograph of the basal zone of a day 20 placenta from a cisplatin-treated rat. Note the vacuolated "glycogen cells" (C) and the small basophilic cells (B). Hematoxylin and Eosin. X 490. Bar = 0.03 mm.

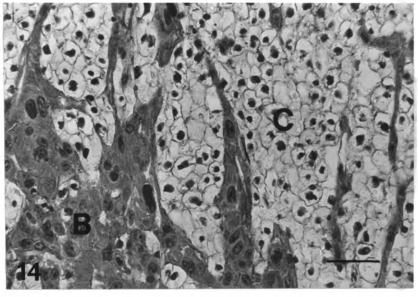

C
B
d4

TABLE 6. Comparison of adult and embryonic LD_{50} doses of cisplatin and various alkylating agents in rats.

Agents	Estimated acute LD_{50} (mg/kg) Adult	Embryonic[1]	Estimated adult/embryonic LD_{50} (mg/kg)
Cisplatin	7.60 - 12.00	1.00	7.60 - 12.00
Nitrogen mustard[2]	2.00	0.70	2.86
Triethylene melamine[2]	1.25	0.55	2.27
Triethylene thiophosphoramide[2]	8.00	5.00	1.60
Chlorambucil[2]	24.00	10.00	2.40
Cyclophosphamide[2]	40.00	14.00	2.86

1 Determined on most susceptible day of gestation

2 LD_{50}'s obtained from previously published work (6,38).

DISCUSSION

Cisplatin is a bifunctional agent which causes interstrand/intra-strand DNA cross-linking in mammalian cells (32, 36, 37, 55). It's high cytotoxicity and antitumor activity is seen only with the drug in a bifunctional state (45). For this reason, inhibition of DNA replication due to these cross-linking effects has been proposed to be cisplatins major mechanism of action. It is, therefore, not surprising that cisplatin induced embryolethality shows gestational stage-specificity. The rate of DNA replication in the embryo increases 1000-fold over 2 or 3 days of early organogenesis and around day 11 (mice) or day 13 (rat) of gestation this increase slows down to two fold or lower (40). The highest embryolethality induced by cisplatin corresponds to this period of induced DNA replication. In addition, it has been shown that cell replication can also be prevented, even after DNA synthesis is already completed, by the inhibition of cytokinesis (2).

Therapeutic cross-linking agents show a fairly consistent relationship between the maternal and the embryonic LD_{50}'s. In Table 6 the embryocidal effects of cisplatin and some of the commonly used alkylating agents are compared. The adult and embryonic LD_{50}'s of the various compounds were obtained from previously published work (7, 38). These results indicate that cisplatin is approximately 2 to 4 times more potent embryotoxin in comparison with classical alkylating agents. These differences could be attributable to actual differences in the mechanism of cytotoxic and embryotoxic action or to differences in pharmokinetic factors. The compound's

stability in an aqueous solution protein binding, metabolism, excretion and movement across the palcenta are all involved in the determination of the concentration of the drug which reaches the developing embryo. Once the drug reaches the site, embryonic cell permeability, intracellular factors, such as the distribution of nucleophilic sites and the cells ability to repair a particular type of injury determines the final effect of the drug.

The placenta is no longer considered a barrier to chemical agents in the maternal blood. Even large and/or highly charged molecules have been shown to cross the placenta, though at a fairly slow rate (17, 58). Therefore, the critical factor is not whether cisplatin crosses the placenta, but rather, is it transfered at a fast enough rate to reach levels damaging to the embryo before maternal blood levels drop. This rate cannot be determined with the limited data available. However, in a study on placental transfer of ^{191}Pt (35), There were indications of placental binding or accumulation, though some ^{191}Pt was present in all the fetuses examined. Thus, cisplatin could possibly accumulate at a high enough concentrations to directly effect the embryo. This is supported by the histological examinations performed in this study which suggest that the site of toxic action of the cisplatin is the developing fetal mesenchyme. Decidual and placental tissues appeared to be normal in most cases. The increase in placental "glycogen cells" seen with cisplatin treatment is unlikely to cause adverse effects and is probably the result of maternal hyperglycemia which is often seen with cisplatin treatment (9, 18, 58).

The findings in this study indicate that cisplatin induces a high percentage of fetal mortality in dosages well below the adult therapeutic levels. These results are comparable to a previous study of cisplatin embryotoxicity in Swiss Webster mice in which a dose of 13.0 and 3.0 mg/kg (kp) on day 8 of gesation induced 100% and 31% fetal mortality, respectively (29). However, 3 surviving fetuses were reported in a 8.0 mg/kg treatment group, whereas in this present study even a lower dose of 6.0 mg/kg cisplatin induced 100% litter resorption. In addition, minor skeletal abnormalitites were found in their control and drug-treated fetuses which were not noted in the fetuses in this experiment, including zig-zag sternebrae and undefined vertebral malformations. More litters need to be examined in order to draw any conclusions, though these results suggest possible intra-strain differences in the two experimental mice populations, which may account for the disparity in lethality between the two studies. Further difference can be due to different sources of cisplatin resulting in differences in the efficacy and toxicity of the drug.

As a group, alkylating agents are potent embryotoxins, inducing intrauterine death and congential malformations in many species, when administered during early gestation (7). Cisplatin does not appear to conform to the pattern of embryotoxicity exhibited by these compounds. These DNA cross-linking agents generally have a wide dose range producing malformations and uniformly produce their most salient defects from day 11 to day 13 gestation. The characteristic developmental defects that are induced at high frequencies by these compounds were not noted in this study, with the exception of the two

fetuses mentioned. With so few incidences of malformations it is difficult to conclude whether these were due to a direct action of the drug or a spontaneous occurrance of a preexisting genetic mutation, perhaps precipitated by the drug or maternal stress.

Moderate maternal toxicity, manifested by weight loss and anorexia, was evident with the higher dosages of cisplatin. This toxicity was dose related and, as evident by the pair fed controls, entailed more than just weight loss due to decreased food consumption. The additional weight loss was most likely due to fluid loss, as a result of diuresis and/or diarrhea, a common effect of cisplatin. Mice have been shown to be much more tolerant to cisplatin treatment with an adult LD_{50} reported between 12.0 and 18.0 mg/kg, as compared to rats with an LD_{50} reported between 7.6 to 12.0 mg/kg (25, 29, 51). This difference in susceptibility was also evident in this study. A six-fold increase in dosage was necessary to produce the same embryolethality in mice as in the rat, on comparable days of gestation.

REFERENCES

1. Adams, C.E., Hays, M.E., and Lulwak-Mann, C. (1961). The action of various agents upon the rabbit embryo. J. Embryo. Exp. Morph. 9:468-491.

2. Aggarwal, S.K. (1974). Inhibition of cytokinesis in mammalian cells by cis-dichloro-diammine-platinum (II). Cytobiol. 8:395-402.

3. Banerjee, B.N., Galbreath, C., and Sofia, R.D. (1975). Teratologic evaluation of synthetic -tetrahydro-cannabionol in rats. Teratology 11:99-101.

4. Bodenstein, D. (1974). Effects of nitrogen mustard on embryonic amphibian development. I. Ectodermal effects. J. Exp. Zool. 104:311-341.

5. Brock, N. and VonKreybig, T. (1964). Experimenteller Bertag zur prufung Teratogenes wirhinger von Arzneimitten an der Laboratorumsratte. Sonder druck aus, Naunyn-Schmiedebergs, Arch. Exp. Path. Pharmak. 249:117-123.

6. Chaube, S., Kury, G. and Murphy, M.L. (1967). Teratogenic effects of cyclophosphamide in the rat. Cancer chemother. Rep. 51:363-376.

7. Danforth, C.H. and Ceter, E. (1954). Nitrogen mustard as a teratogenic agent in the mouse. Proc. Soc. Exp. Biol. Med. 86: 705-707.

8. Dawson, A.B. (1926). A note on the staining of the skeleton of cleared specimens with alizarin red-S. Stain Technol. 1:123-124.

9. Diamont, Y.Z. and Shafrir, E. (1978). Placental enzymes of glycolysis, gluconeogenesis and lipogenesis in the diabetic rat and in starvation. Diabetologia. 15:481-485.

10. Diamond, I., Anderson, M., and McCredie, S. (1960). Transplacental transmission of Busulfan (Myleran) in a mother with leukemia. Pediatrics. 25:85-90.

11. Didcock, K.A., Jackson, D., and Robson, J.M. (1956). The action of some nucleotoxic substances on pregnancy. Brit. J. Pharmacol. 11:437-441. it Analysis, 3rd Ed., Cambridge Univ. Press, Cambridge, England.
12. Finney, D.J. (1971). Probit Analysis, 3rd Ed., Cambridge Univ. Press, Cambridge, England.

13. Fleischmann, R.W., Hayden, D.W., Rosenkrantz, H.m and Braude, M.C. (1975). Teratologic evaluation of -tetrahydrocannabinol in mice including a review of the literature. Teratology. 12:47-50.

14. Geber, W.F., and Schramm, L.C. (1969). Effect of marijuana extract on fetal hamsters and rabbits. Toxicol. Appl, Pharmacol. 14:276-282.
15. Gerlinger, P.J. (1964). Action de cyclophosphamide injecte a la mere ser le realisation de la forme du corps des embryons lapin. C.R. Soc. Biol. (Paris) 158:2154-2157.

16. Gerlinger, P.J. Ruch, J.V., and Calvert, J. (1963). Action du cyclophosphamide sur la formation de l'embryon. C.R. Soc. Biol. (Paris). 157:173-176.

17. Ginsburg, J. (1971). Placental transfer. Ann. Rev. Pharmacol. 11:387-408.

18. Goldstein, R.S., Noordewier, B., Bond, J.T., Hook, J.B., and Mayor, G.H. (1981). cis-Dichlorodiammineplatinum nephrotoxicity: Time course and dose response of renal functional impairment. Toxicol. Appl. Pharmacol. 60:163-175.

19. Greenberg, L.H., and Tanaka, K.R. (1964). Congenital anomalies probably induced by cyclophosphamide. J. Am. Med. Assoc. 188:423-426.

20. Haskins, D. (1948). Some effects of nitrogen mustard on the development of external body form in the fetal rat. Anat. Rec. 102:493-511.

21. Howe, W.L. and Parson, P.A. (1967). Genotype and environment in the determination of minor skeletal variants and body weight in mice. J. Embryol. Exp. Morphol. 17:283-292.

22. Jurand, A. (1959). Action of triethanomelamine (TEM) on early and later stages of mouse embryos. J. Embryol. Exp. Morph. 7:526-539.

23. Kageyama, M. and Nishimura, H. (1961). Developmental anomolies in mouse embryos induced by triethylenemelamine (TEM). Acta. Med. Univ. Kioto. 37:318 (abstract).

24. Kimmel, C.A. and Wilson, J.G. (1973). Skeletal deviations in rats: Malformations or variations? Teratology 8:309-316.

25. Kociba, R.J. and Sleight, S.D. (1971). Acute toxicologic and pathologic effects of cis-dichlorodiamnineplatinum (II) (NSC-119875) in the male rat. Cancer Chemother. Rep. 55:1-8.

26. Kopj, R., Lorenz, D., and Solewski, E. (1964). Der Einfluss von Thalidomid auf die Ferlilitat von Ratten in Generationsversuch uber zwei Generationen. Naunyn Schmiedeberg, Arch. Exp. Path. 247:121-135.

27. Lange, R.C., Spencer, R.P., and Harder, H.C. (1972). Synthesis and distribution of a radiolabeled antitumor agent: cis-Diamminedichloroplatinum (II). J. Nucl. Med. 13:328-330.

28. Lange, R.C., Spencer, R.P., and Harder, H.C. (1973). The antitumor agent cis-$Pt(^{NH}_{3})\ Cl_2$: Distribution and dose calculations for ^{195m}Pt. J. Nucl. Med. 14:191-195.

29. Lazar, R., Conran, P.C., and Damjanov, I. (1978). Embryotoxicity and teratogenicity of cis-diamminedichloroplatinum. Experientia. 35:647-648.

30. Lepold, W.R., Miller, F.C., and Miller, J.A. (1979). Carcinogenicity of antitumor cis-platinum (II) coordination complexes in the mouse and rat. Cancer Res. 39:913-918.

31. Litterst, C.L., Gram, T.E.., Dedrick, R.L., Leroy, A.F., and Guario, A.M. (1976). Distribution and deposition of platinum following intravenous administration of cis-diamminedichloroplatinum (II) (NSC-119875) to dogs. Cancer Res. 36:1633-1640.

32. Mansy, S., Rosenberg, B., and Thomson, J.A. (1973). Binding of cis-and trans-dichlorodiammineplatinum (II) to nucleosides. I. Location of the binding sites. J. Am. Chem. Soc. 95:1633-1640.

33. Meistrich, M.L., Finch, M., Cunha, M.F., Hacker, U., and Au, W.W. (1982). Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. Cancer Res. 42:122-131.

34. Monie, I.W. (1961). Chlorambucil induced abnormalities of the urogenital system of rat fetuses. Anat. REc. 139:145-153.

35. Moore, W. Jr., Hysell, D., Crocker, W., and Stara, J. (1975). Biological fate of a single administration of ^{191}-Pt in rats following different routes of exposure. Environmental Res. 9:152-158.

36. Munchausen, L.L. (1974). The chemical and biological effects of cis-dichlorodiammineplatinum (II), an antitumor agent, on DNA. Proc. Natl. Acad. Sci. USA. 71:4519-4522.

37. Muchausen, L.L. and Rahn, R.O. (1975). Biologic and chemical effects of cis-dichlorodiammineplatinum (II) (NSC-119875) on DNA. Cancer Cemother. Rep. 59:643-646.

38. Murphy, M.L., DelMoro, A., and Lacon, C.R. (1958). The comparitive effects of five polyfunctional alkylating agents on the rat fetus with additional notes on the chick. Ann. N.Y. Aca. Sci. 68:762-781.

39. Murphy, M.L., Dagg, C.P., and Karnofsky, kD.A. (1957). Comparison of teratogenic chemicals in the rat and chick embryos: An exhibit with additives for publication. Pediatrics. 19:701-714.

40. Nrubert, D., Barrach, H.J., and Merker, H.J. (1980). Drug-induced damage to the embryo or fetus. In: Drug-Induced Pathology, (Grundmann, E., Ed.), Springer Verlag, New York, pp. 241-331.

41. Nishimura, H. and Takagaki, S. (1959). Congenital malformations in mice induced by nitrogen mustard. Acta. Med. Univ. Kioto. 36;20-26.

42. Pace, H.B., Davis, M.W., and Borgen, L.A. (1971). Teratogenesis and marijuana. In: Marijuana: Chemistry, Pharmacology and Patterns of Social Use. Ann. N.Y. Acad. Sci. 191:123-131.

43. Pascoe, J.M. and Roberts, J.J. (1974). Interactions between mammalian cell DNA and inorganic platinum compounds.. I. DNA interstrand cross-linking and cytotoxic properties of Platinum (II) compounds. Biochem. Pharmacol. 23:1345-1357.

44. Prestayko, A.W. (1981). Cisplatin and analogues: A new class of anticancer drugs. In: Cancer and Chemotherapy, vol. III. Crook, S.T. and PLrestayko, A.W., Eds. Academic Press, New York pp. 133-154.

45. Prestayko, A.W. and Crooke, S.T., and Carter, S.K. (1980). Cisplatin: Current Status and New Developments. Academic Press, New York.

46. Roberts, J.J. and Pascoe, J.M. (1972). Cross-linking of complementary strands of DNA in mammalian cells by antitumor platinum compounds. Nature (London). 235:282-284.

47. Rosenberg, B. (1973). Platinum coordination complexes in cancer chemotherapy. Naturwissenschaften. 60:399-406.

48. Rosenberg, B. (1977). Noble metal complexes in cancer chemotherapy. In: Inorganic and Nutrition Aspects of Cancer (Schranze, G.U., Ed). Plenum Press, New York, pp. 129-150.

49. Rosenberg, B., VanCamp, L. Grimley, E.B., and Thomson, A.J. (1967). The inhibition of growth or cell division in Escherichia coli by different ionic species of platinum (IV) complexes. J. Biol. Chem. 242:1347-1352.

50. Rosenberg, B., VanCamp, L., and Trosko, J.E. (1969). Platinum compounds: a new class of potent antitumor agents. Nature (London). 222:385-386.

51. Schaeppi, J., Heyman, A., Fleischman, R.W., Rosenkrantz, H., Illevski, V., Phelan, R., Cooney, D.A., and Davis, R.D. (1973). cis-Dichlorodiammineplatinum (II) (NSC-119875): Preclinical toxicologic evaluation of intravenous injection in dogs, monkeys and mice. Toxicol. Appl. Pharmacol. 25:230-241.

52. Shaw, E.B. and Steinbach, H.L. (1968). Aminopterin induced fetal malformations. Am. J. Dis. Chold. 115:477-482.

53. Sholton, D. and Monie, I.W. (1963). Possible teratogenic effect of chlorambucil on a human fetus. J. Am. Med. Assoc. 186:74-79.

54. Sokal, J.E., and Lessman, E.M. (1960). Effects of cancer chemotherapeutic agents on the human fetus. J. Am. Med. Assoc. 172:1765-1771.

55. Stone, P.J., Kelman, A.D., and Sinex. F.M. (1974). Specific binding of antitumor drug cis-$Pt(NH_3)_2Cl_2$ to DNA rich in quanine and cytosine. Nature. 251:736-737.

56. Talley, R.W., O'Bryan, R.M., Glutterman, J., Brownlee, R.W., and McCredie, K.B. (1974). Clinical evaluation of toxic effects of cis-platinum(II)diamminedichloride. Recent Results Cancer res. 48:160-166.

57. VanDerberg, H.W. and Roberts, J.J. (1976). Inhibition by caffine of post-replication repair in chinese hamster cells treated with cis-platinum(II)diamminedichloride: The extent of platinum binding to template DNA in relation to size of low molecular weight nascent DNA. Chem-Biol. Interact. 12:375-390.

58. Villee, C.A. (1967). Biochemical aspects of the mammalian placenta. In: The Biochemistry of Animal Development, (Weber, R., Ed.). Academic Press, New York.

59. VonKreybig, T. (1965). Extremilalsmissbildungen in tierexperiment. Arzeimittel-Forsch. 15:1213-1217.

60. Wilson, J.G. (1965). Methods for administering agents and detecting malformations in experimental animls. In: Teratology, Principles and Techniques, (Wilson, J.G., Ed.). Univ. Chicago Press, Chicago, pp. 262-277.

61. Zwelling, L.A., Bradley, L.O., Scharkey, N.A., Anderson, T., and Kohn, K.W. (1979). Mutagenicity, cytotoxicity and DNA crosslinking in V79 chinese hamster cells treated with cis- and trans-PT(II) diamminedichloride. Mut. Res. 67:271-280.