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Acute and Chronic Effects of Cisplatinum upon Testicular Function in the Rat

HOSEA F. S. HUANG,* LEONARD M. POGACH,† ESWARI NATHAN,† AND WILLIAM GIGLIO†

*From the *Department of Surgery, Section of Urology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey and the †Department of Medicine, East Orange Veterans Administration Medical Center, East Orange, New Jersey*

One of the side effects of cisplatinum-based chemotherapy is the impairment of spermatogenic function. In order to understand the mechanisms responsible for this side effect, the present study examined the short- and long-term effects of five daily injections of 2 mg/kg cisplatinum upon the functional normality of Leydig cells and Sertoli cells in intact adult rats, and their relationship with the status of spermatogenesis. Results of the present study demonstrate that cisplatinum treatment resulted in a progressive but reversible loss of germ cells from the seminiferous epithelium. Although testicular testosterone contents reduced transiently after the administration of cisplatinum, these testosterone levels are otherwise sufficient to support complete spermatogenesis. Thus, the cisplatinum-induced germinal regression cannot be accounted for by hypoandrogenism. The testicular ABP contents of the drug-treated rats remained unchanged during the treatment period, decreased transiently 30 days after the treatment, and returned to normal 120 days after treatment. A decrease in epididymal ABP content was also noted 10 and 30 days after the drug treatment. These observations suggest that Sertoli cell functions were affected by cisplatinum treatment. The effects of cisplatinum upon Sertoli cells were further demonstrated by the dose-dependent suppression of the production of ABP, lactate, and estradiol in cultured Sertoli cells. In addition, cisplatinum administration resulted in a reversible decrease in pituitary weights and an irreversible decrease in seminal vesicle weights. These results further demonstrate the toxic effects of cisplatinum upon various aspects of the male reproductive system.

Key words: cisplatinum, testis, spermatogenesis, Sertoli cell, seminal vesicle

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Cisplatinum, a platinum coordinated compound, is one of the most commonly used chemotherapeutic agents in clinical oncology. Despite its effectiveness in the suppression of cancer cells in various organs or tissues (Rosenberg et al, 1965; Roberts et al, 1982), this drug has various side effects including nephrotoxicity (Corden et al, 1985), peripheral neuropathy (Hill et al, 1975; Walsh et al, 1982) and, in men, reversible azoospermia (Drasga et al, 1983; Lange et al, 1983; Loehrer et al, 1984). While its inhibition of nucleic acid synthesis is apparently responsible for the anti-tumor action and the regression of the seminiferous epithelium, cis-

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Correspondence to: Dr. H. F. S. Huang, Department of Surgery Section of Urology, UMDNJ-New Jersey Medical School, 185 S. Orange Avenue, Newark, NJ 07103.

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platinum may also affect spermatogenesis through its action upon Leydig cell testosterone production (Maines and Mayer, 1985) or Sertoli cells (Pogach et al, 1989). The present study examined the short and long term effects of acute doses of cisplatinum upon pituitary–testicular hormonal axis and Sertoli cell functions, and their correlation with the status of spermatogenesis. Furthermore, the effects of cisplatinum on Sertoli cell secretory functions were examined in cultured Sertoli cells as the first step toward understanding the cellular and molecular action of this drug on Sertoli cells.

Materials and Methods

In Vivo Experiment

Mature Sprague-Dawley male rats (300 to 350 g; Taconic Farm, Taconic, NY) were maintained in an air-conditioned, light-controlled animal room and were given Purina rat chow and water ad libitum. Animals (n = 5 to 6 per group) were given one or five daily sc injections of cisplatinum (Cisplatin, Bristol Myers Corp, Syracuse, NY; 2 mg/kg body weight) and sacrificed 24 hours later, or given five daily injections and sacrificed 10, 30, or 120 days later. This dose was selected based on the dosage of 80 to 120 mg/m^2 of body surface used in the treatment of testicular neoplasms (Einhorn and Donahue, 1977). Control animals received 1 ml of saline injections. In order to alleviate the potential nephrotoxicity of this drug, all animals received ip hydration of 20 ml of saline 1 hour before each drug administration (Corden et al, 1985). This was continued once daily for 5 days after the last drug injection. At the end of the experiment, the animals were anesthetized with ether and 3 to 5 ml blood samples were taken from the retro-orbital sinus within 5 minutes. Animals were subsequently sacrificed by an overdose of ether. Testes, epididymes, pituitary glands were dissected and weighed immediately. The complex of seminal vesicle and prostate glands were also removed. Subsequently, the seminal vesicle (with its content) was separated from the prostate tissue and weighed. Special care was taken to avoid the rupture of the seminal vesicles. The weight of the coagulated fluid was added to that of the respective tissue if the vesicle was ruptured during the dissection.

One third of one testis from each animal was fixed in Bouin's solution and processed for histology. The remaining testicular tissue was frozen on dry ice and stored at −70°C for subsequent testosterone and androgen binding protein assay. The second testis from each animal was used immediately for the collection of interstitial fluid (ITF) and seminiferous tubular fluid (STF), using the methods of Turner et al (1984). The validity of this procedure had been reported previously (Huang et al, 1989).

In Vitro Experiment

Sertoli cells were isolated from 13- to 15-day-old male rats (Camm Research Lab, Wayne, NJ) and cultured by the method described by Mather and Phillips (1984) with minor modification. In brief, the decapsulated testicular tissues were exposed to 1 mol/L glycine/2 mmol/L EDTA in PBS in the presence of DNAase I and soybean trypsin inhibitor for 10 minutes. This process removes and destroys the Leydig cells. The tubules were subsequently subjected to collagenase and hyaluronidase digestion. The Sertoli cell clumps (3×10^6 cells) were plated and cultured in a 1:1 mixture of DMEM and Ham's F-12 culture medium supplemented with FSH (NIH-S-8, 10 μg/ml), testosterone (1 μmol/L), and a mixture of insulin/transferrin/selenlium (Sigma biochemical, St. Louis, MO, 10 μl/ml) in a 32°C humidified CO_2 incubator. Forty-eight hours after the plating, the cultures were treated with Tris buffer for 2 minutes to remove the contaminating germ cells (Galdieri et al, 1981). The cells were subsequently cultured in the same medium in the presence of 1×10^{-6}–1×10 μg of cisplatinum per ml medium. The medium was collected at 48 hour intervals and stored at −30°C for the measurement of ABP, lactate, and estradiol. At the end of the experiment, Sertoli cells were trypsinized and cell number per well was determined with a coulter counter (Coulter Electronic, Inc, Hialeah, FL).

Spermatogenesis

The paraffin sections were stained with periodic acid-Schiff's reagent and counter-stained with hematoxylin (Preece, 1972). The stages of the seminiferous epithelium were determined by the development of the PAS positive acrosome of spermatids (Leblond and Clermont, 1952).

Hormone Measurement

The pituitary gland from each animal was sonicated in 1 ml of phosphate buffered saline (pH 7.4) for gonadotropin measurement. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were determined by double antibody radioimmunoassay as described previously (Huang and Hembree, 1979) using NIADDK rat FSH RP-2, Rat FSH I-5, and anti rat FSH S-11 for the FSH assay, and rat LH RP-2, rat LH I-6, and anti-rat LH S-7 for the LH assay. The sensitivity (95% binding) of the assay was 2 and 0.4 ng/tube for FSH and LH, respectively. The intra-assay coefficient of variation was approximately 8% for both FSH and LH.

Testosterone concentrations in serum, STF, and ITF were determined by RIA in respective ether extracts as described previously (Huang and Boccabella, 1988) using antiserum provided by Radioassay System Laboratory (Carson, CA) with approximately 18% cross reactivity with DHT. Testicular testosterone was determined in the ether extract of 60 to 100 mg of decapsulated testicular tissue homogenized in 1 ml phosphate buffered saline (pH 7.4) without chromatography. After correcting for recovery (90 to 95%) and tissue weights, the results were expressed as per testis or per gram testis. The sensitivity of the testosterone assay was 2.25 pg/tube. The intra- and inter-assay coefficients of variation were 5 and 12%, respectively. Estradiol in Sertoli

cell culture medium was determined by RIA in ether extract of the spent medium using antiserum provided by Radioassay System Laboratory. The sensitivity of the assay was 4 pg/tube with an intraassay coefficiency of variation of 6%.

Androgen Binding Protein Measurement

For the androgen binding protein (ABP) assay the weighed testicular tissue and one epididymis from each animal were homogenized with polytron; the cytosols were obtained by centrifugation as described previously (Huang and Boccabella, 1988). ABP concentrations in tissue cytosols were determined by RIA (Gunsalus et al, 1978) using the reagents provided by NIADDK. The sensitivity of this assay was 0.4 μlEq of GMB-E-1 (Gunsalus et al, 1978) per tube. The intra-assay coefficient of variation was less than 5%. The results were corrected for the tissue weights and buffer volume and were expressed as per organ or per gram tissue. ABP concentrations in serum, STF, ITF, and culture medium were also measured by the same assay system.

Lactate Assay

Lactate concentrations in Sertoli cell culture medium were measured by enzymatic method, as described previously (Huang et al, 1989).

Statistics

Analysis of variance was used to detect the significance of the treatment effect among groups. When the treatment effect was significant, the Scheffe's multiple range test was used to identify the difference between groups.

Results

Organ Weights

Both testicular and epididymal weights of the mature rats dropped significantly 10 days after the cessation of drug treatment ($P < 0.01$), and continued to decline for at least another 20 days (Table 1). By the end of the recovery period, the testicular and epididymal weights of the treated animals had been restored to 77 and 72% of the control values, respectively.

A significant decrease in pituitary and seminal vesicle weights ($P < 0.01$) occurred following cisplatinum administration. The effect on the pituitary was first noted 10 days after the last drug treatment; these changes were reversible (Table 1). The decrease in seminal vesicle weight was first noted as early as 24 hours after the five daily injections and persisted throughout the experimental period (Table 1). Expression of these organ weights as per kg body weight revealed that the decrease in the organ weights was not related to the decrease in body weight (data not shown).

Spermatogenesis

In normal animals, the stage VI epithelium contains type B spermatogonia, pachytene spermatocytes, and step 6 and 16 spermatids (Fig 1). As type B spermatogonia divide to form preleptotene spermatocytes, other cell types synchronously differentiate as well. The presence of mature spermatids at the luminal edge of the stage VII–VIII epithelium illustrates the completion of spermatogenesis (Fig 2). Ten days after the last cisplatinum injection, degeneration of germ cells was noted in many of the seminiferous tubules. The pachytene spermatocytes in stages V or VI of the epithelium were decreased in number while other cell types were numerous (Fig 3). Concomitantly, preleptotene spermatocytes in many of the stages VII or VIII epithelium were absent and pachytene spermatocytes were also reduced in number (Fig 4). In addition, leptotene spermatocytes in stages X–XI were absent, whereas a small number of mature spermatids were retained or phagocytosed (Fig 5, 6). Twenty days later, the maximum loss of spermatogenic cells had been reached. Although elon-

Table 1. *Effects of cisplatinum (2 mg/kg) upon organ weights (mean ± SEM mg)*

Number of injections	Days after treatment		Body weight	Testis	Epididymis	Pituitary	Seminal vesicle
1	1	Control*	341 ± 6	1625 ± 34	457 ± 13	8.3 ± 0.5	1078 ± 72
		Cisplatinum	343 ± 6	1610 ± 43	443 ± 15	7.2 ± 0.5	998 ± 71
5	1	Control	364 ± 10	1649 ± 45	477 ± 15	9.9 ± 0.6	1181 ± 42
		Cisplatinum	288 ± 10†	1691 ± 105	447 ± 19	9.0 ± 0.8	660 ± 26†
5	10	Control	415 ± 8	1808 ± 23	487 ± 12	11.0 ± 0.1	1223 ± 61
		Cisplatinum	230 ± 11†	1398 ± 68†	391 ± 19†	6.2 ± 0.2†	239 ± 28‡
5	30	Control	441 ± 17	1749 ± 58	549 ± 23	11.1 ± 1.0	1338 ± 185
		Cisplatinum	324 ± 19†	1091 ± 72‡	368 ± 39†	8.7 ± 1.0†	512 ± 131‡
5	120	Control	563 ± 22	1841 ± 65	605 ± 29	14.1 ± 0.7	1518 ± 222
		Cisplatinum	313 ± 32†	1432 ± 174†	436 ± 63†	10.1 ± 0.8	566 ± 252‡

* n = 5 to 6 rats per group.
† $P < 0.05$.
‡ $P < 0.01$.

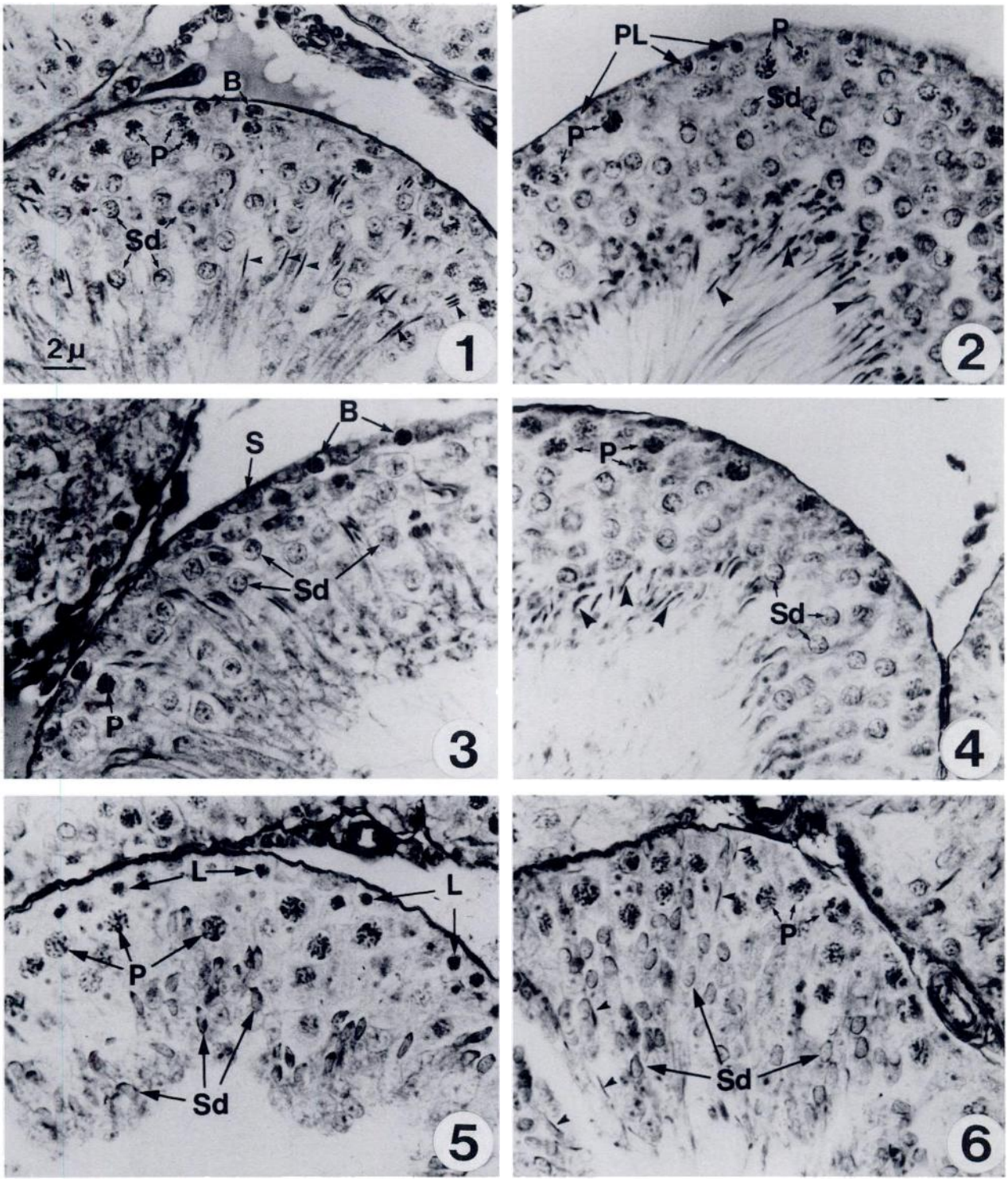
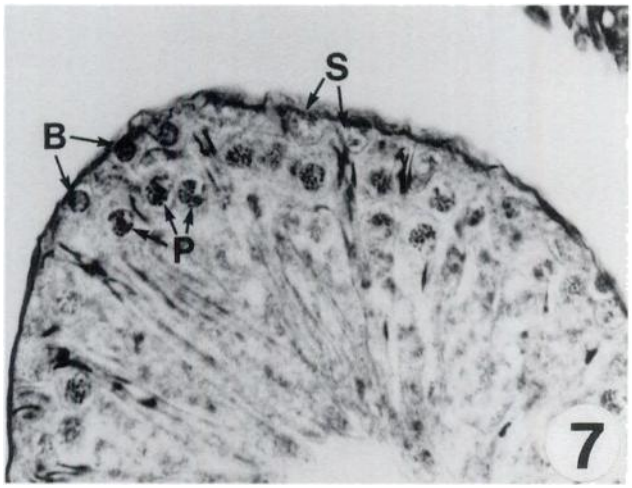
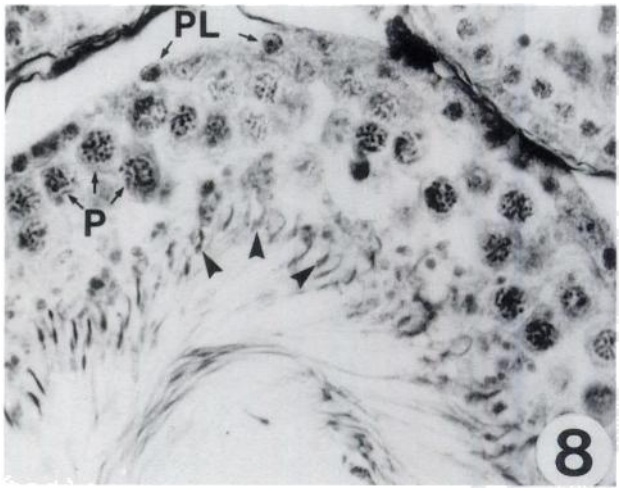
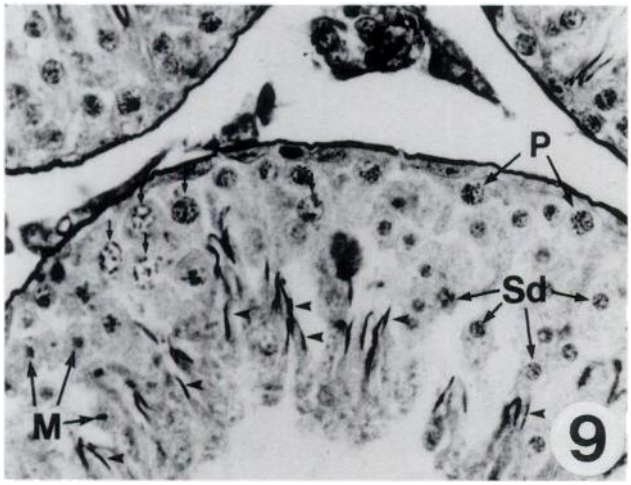
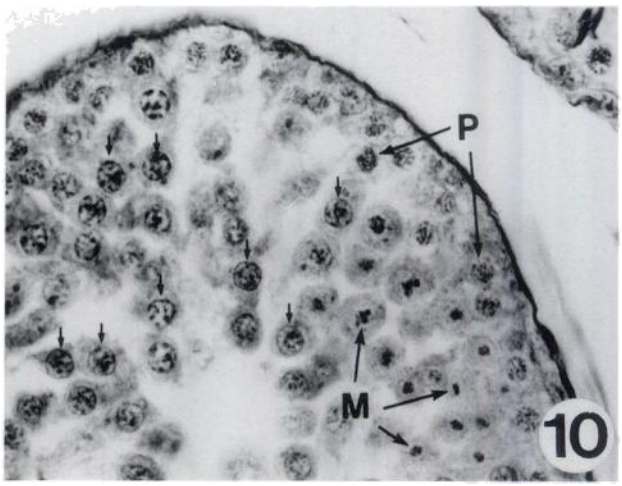


Fig 1.—Portion of a stage VI tubule of a control rat showing the presence of type B spermatogonia (B), pachytene spermatocytes (P), step 6 spermatids (Sd), and step 16 spermatids (arrow heads) (×600). **Fig 2.**—Portion of a stage VII–VIII tubule of a control rat showing the presence of preleptotene spermatocytes (PL), pachytene spermatocytes (P), step 7 spermatids (Sd), and mature spermatids (arrow heads); (×600). **Fig 3.**—Portion of a stage VI tubule from a rat treated with cisplatinum and sacrificed 10 days after the last injection. Note that pachytene spermatocytes (P) were reduced in number or absent in some areas while other cell types remained. S = Sertoli cell nucleus. See Fig 1 for the key to other cell types (×600). **Fig 4.**—Portion of a stage VII tubule from a rat treated with cisplatinum and sacrificed 10 days after the last injection. Note that the preleptotene spermatocytes were absent from the basement compartment of the epithelium. See Fig 2 for the key to other cell types (×600). **Fig 5.**—Portion of a stage X–XI tubule of a control rat showing the presence of leptotene spermatocytes (L), pachytene spermatocytes (P), and elongating spermatids (Sd) (×600). **Fig 6.**—Portion of a stage IX–X tubule from a rat treated with cisplatinum and sacrificed 10 days after the last injection. Note the absence of leptotene spermatocytes. In addition, the presence of elongated spermatids (arrow heads) suggests failure or delay in spermiation. See Fig 5 for the key to other cell types (×600).









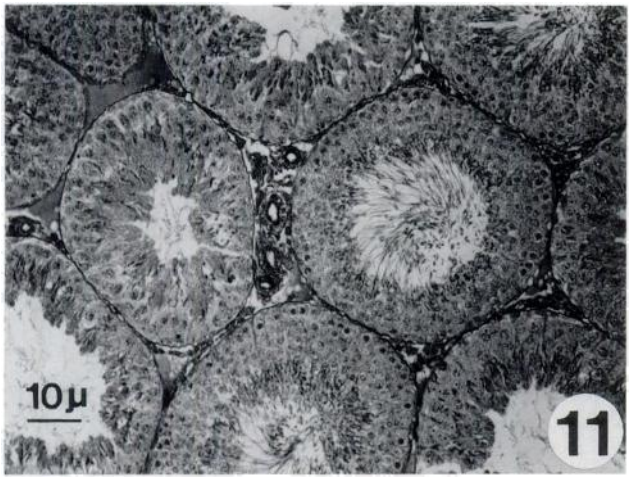


Fig 7.—Portion of a stage VI tubule from a rat treated with cisplatinum and sacrificed 30 days after the last injection. Note that the step 6 spermatids were absent. See Fig 1 for key to other cell types (×600). **Fig 8.**—Portion of a stage VII tubule from a rat treated with cisplatinum and sacrificed 30 days after the last injection. Note that the step 7 spermatids are absent. See Fig 2 for the key to other cell types (×600). **Fig 9.**—Portion of a stage XIV–I tubule of a control rat showing the presence of young pachytene spermatocytes (P), secondary spermatocytes (arrows), the second meiotic figure (M), some newly formed spermatids (Sd), and step 14 and 15 elongated spermatids (arrow heads) (×600). **Fig 10.**—Portion of a stage XIV–I tubule from a rat treated with cisplatinum and sacrificed 30 days after the last injection. Note that despite the presence of young pachytene spermatocytes (P), secondary spermatocytes (arrows), and the second meiotic figure (M), step 14 and 15 elongated spermatids are absent (×600). **Fig 11.**—A rat sacrificed 120 days after the last cisplatinum injection. The presence of complete cellular association, and mature spermatids at the luminal edge of stage VII epithelium illustrates the restoration of qualitatively normal spermatogenesis (×120).

gated spermatids were seen in many of the stages VI–VIII epithelium, these tubules were devoid of young spermatids (Fig 7, 8). At this time, meiosis appeared normal and the production of spermatids continued. However, elongated spermatids were absent in many of the stage XIV epithelium (Fig 9, 10). Nevertheless, mature spermatids were observed in all animals examined. Qualitatively normal spermatogenesis was observed in the cisplatinum treated animals after 120 days of recovery (Fig 11).

Hormonal Concentrations

The pituitary contents of FSH and LH in control animals measured at various times ranged between 1544 to 2229 ng/gland and 944 to 1265 ng/gland, respectively. These parameters were not affected by cisplatinum administration (Table 2). Serum concentrations of FSH and LH of the cisplatinum treated rats did not differ from those of the control animals (FSH: 25 to 37 ng/ml; LH: 2 to 3 ng/ml) at any time point (Table 2). Furthermore, testosterone concentrations in serum, ITF, and STF of the treated rats did not differ from those of the controls (serum: 1.7 to 2.3 ng/ml; ITF 42.9 to 73.1 ng/ml; STF 36.4 to 40.8 ng/ml) at all times (Table 2). However, testicular testosterone contents of these rats were suppressed by 30% ($P < 0.05$) 1 and 10 days after the last dose of cisplatinum (Fig 12). This suppression was still evident, though less pronounced, after 30 days of recovery. A significant decrease in testosterone concentration per gram of testis was only noted 1 day after the 5-daily injections ($P < 0.05$, Fig 12).

Androgen Binding Protein (ABP)

Testicular ABP content of the mature rats was not affected by cisplatinum treatment except on the 30th day of recovery (Fig 13). However, due to the decrease in testicular weight, the ABP concentration per gram of tissue tended to be higher in the treated animals during the recovery period. Epididymal ABP content and concentration also remained unchanged during the treatment period. It was suppressed significantly during the first month of the recovery period ($P < 0.05$, Fig 14), but returned to 80% of the control values by the end of the experimental period.

In control animals, the average ABP concentrations of STF, ITF, and serum ranged between 28,000 to 39,000, 16,000 to 22,000 and 77 to 167 ng/ml, respectively. Cisplatinum treatment did not affect these ABP concentrations throughout the experimental period (Table 3).

In Vitro Effects of Cisplatinum on Sertoli Cells

Addition of cisplatinum to culture medium suppressed the production of ABP, lactate, and estradiol by cultured Sertoli cells ($P < 0.01$, Fig 15). The effective dose for ABP suppression was 10^{-3} μg/ml, and the sensitivity of ABP responses to cisplatinum was approximately 100 times greater than those of lactate and estradiol.

Discussion

The present study demonstrates that administration of cisplatinum to adult male rats resulted in acute damage to spermatogenic functions, but this effect was reversible. This is in agreement with clinical observations (Drasga et al, 1983; Lange et al, 1983; Berthelsen et al, 1984; Meistrich et al, 1989) and animal experiments (Meistrich et al, 1982).

The absence of preleptotene and leptotene spermatocytes 10 days after cisplatinum injections, and

Table 2. *Effects of cisplatinum on gonadotropins and testosterone*

Number of injection	Days after treatment		FSH		LH		Testosterone		
			Serum (ng/ml)	Pituitary (ng/gland)	Serum (ng/ml)	Pituitary (ng/ml)	Serum (ng/ml)	ITF (ng/ml)	STF (ng/ml)
1	1	Control*	25 ± 2	2229 ± 348	2.3 ± 0.2	978 ± 83	2.6 ± 0.4	64.7 ± 2.5	40.4 ± 4.2
		Cisplatinum	22 ± 2	1583 ± 184	2.7 ± 0.3	938 ± 144	2.9 ± 0.7	64.7 ± 4.5	41.5 ± 4.6
5	1	Control	25 ± 3	1549 ± 83	2.4 ± 0.3	1265 ± 198	1.7 ± 0.4	73.1 ± 4.3	38.6 ± 4.4
		Cisplatinum	28 ± 3	1363 ± 113	2.8 ± 0.3	1304 ± 110	2.2 ± 0.3	72.0 ± 3.9	42.7 ± 4.1
5	10	Control	33 ± 2	1730 ± 135	3.3 ± 0.1	944 ± 145	2.2 ± 0.3	56.5 ± 4.0	38.3 ± 3.6
		Cisplatinum	39 ± 4	1270 ± 106	3.3 ± 0.1	915 ± 182	2.3 ± 0.5	67.8 ± 3.4	45.1 ± 4.4
5	30	Control	37 ± 9	2129 ± 558	2.3 ± 0.4	944 ± 163	1.9 ± 0.4	42.9 ± 5.0	36.4 ± 4.2
		Cisplatinum	21 ± 6	2135 ± 546	2.2 ± 0.3	1264 ± 289	1.1 ± 0.5	51.6 ± 6.3	41.0 ± 8.2
5	120	Control	36 ± 7	2183 ± 345	2.7 ± 0.5	1054 ± 188	2.3 ± 0.6	61.2 ± 3.7	40.8 ± 3.5
		Cisplatinum	28 ± 5	1628 ± 210	3.1 ± 0.6	1449 ± 206	1.9 ± 0.7	61.6 ± 3.8	40.3 ± 4.0

* n = 5 to 6 rats per group.

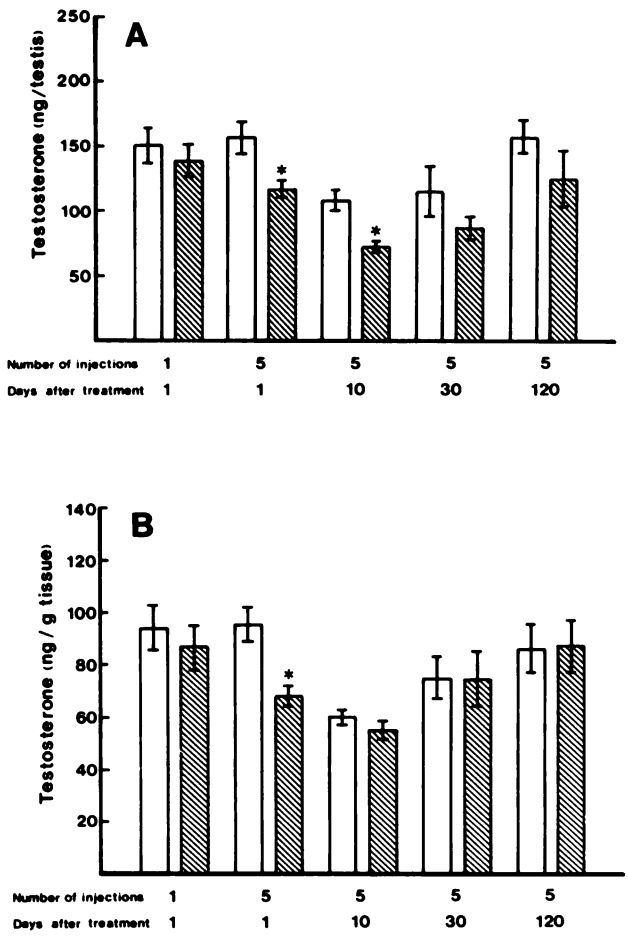


Fig 12.—Testicular testosterone content (A) and concentration (B) of rats sacrificed at various times after cisplatinum (CP) injections. Results are expressed as mean ± SEM. *P < 0.05 versus control. n = 5 or 6. □ = control rats, ▨ = cisplatinum-treated rats.

step 5–8 spermatids 30 days after cisplatinum injections are consistent with the reported killing of types A2-B spermatogonia (Meistrich et al, 1982). In addition, the absence of steps 14–15 spermatids 30 days after drug administration is indicative of the killing of preleptotene spermatocytes. Since these cells reside in the basement compartment of seminiferous tubules and are active in nucleic acid and protein synthesis, direct drug effects on these cells is likely. However, since cisplatinum causes the disruption of the Sertoli cell tight junctions (Pogach et al, 1989) and is capable of entering the adluminal compartment (Meistrich et al, 1982), the absence of the cell types mentioned above could result from cell death during meiosis, spermiogenesis due to the direct drug effects on the germ cells, or an abnormal tubular environment. The presence of phagocytosed or retained spermatids 10 days after drug injection, an indication for abnormal spermiogenesis, supports this possibility.

Though the previously mentioned lesions were noted in all animals, none of the lesions occurred in all tubules of the respective stages. This variation among tubules may allow some germ cells to differentiate to completion. Since cisplatinum disrupts Sertoli cell tight junctions (Pogach et al, 1989), the presence of mature spermatids in these animals suggests that the intactness of the Sertoli cell tight junctions may not be essential for the completion of spermatogenesis. Alternatively, the cisplatinum treatment perhaps did not disrupt the tight junctions in all tubules, thus leaving some germ cells to differentiate to completion.

Subclinical hypoandrogenism is common in patients receiving cisplatinum based chemotherapy (Drasga et al, 1983; Lange et al, 1983). A defect in the cytochrome P-450 system had been postulated to account for the cisplatinum induced hypoandrogenism (Maines and Mayer, 1985). Suppression of testosterone production in rats following cisplatinum administration had also been reported

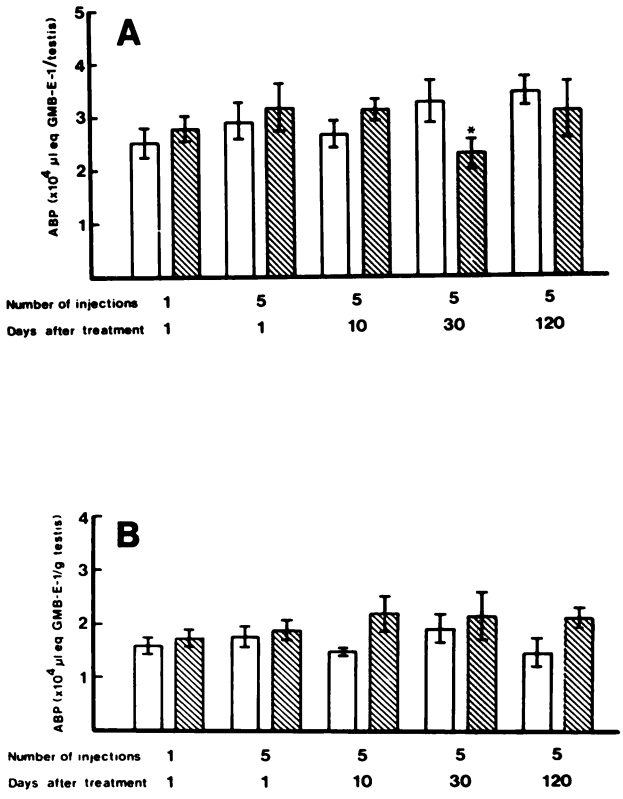


Fig 13.—Effects of cisplatinum administration upon testicular ABP content (A) and concentration (B) of adult rats. Mean ± SEM. *P < 0.05. □ Control rats, □ cisplatinum-treated rats. n = 5 or 6.

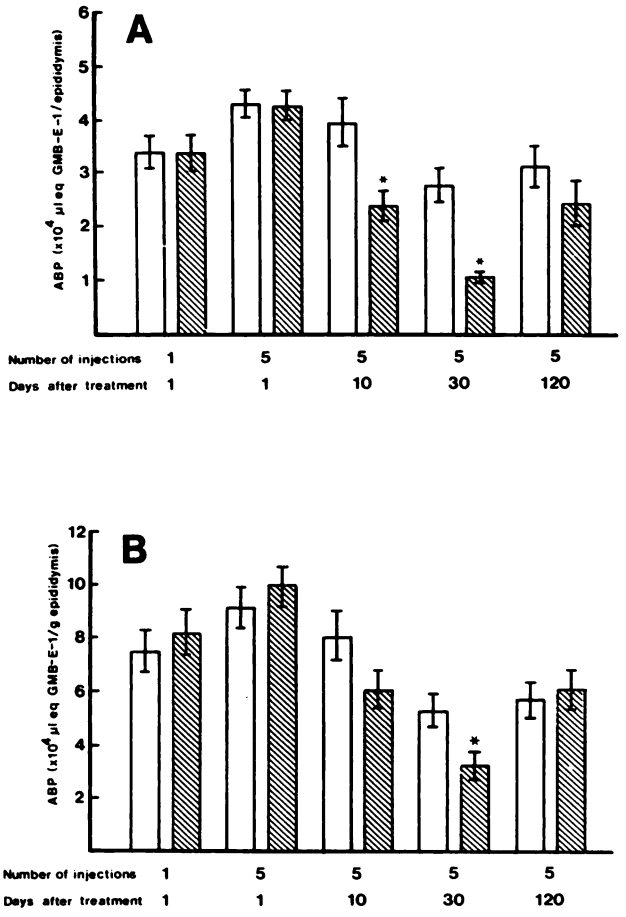


Fig 14.—Effects of cisplatinum administration upon epididymal ABP contents (**A**) and concentration (**B**) of adult rats. Results are expressed as mean ± SEM. *P < 0.05. □ = control rats, □ = cisplatinum-treated rats. n = 5 or 6.

(Pogach et al, 1989). In the present study, a decrease in the testicular testosterone content and concentration was noted 24 hours after 5-daily injections, consistent with the previous study (Pogach et al, 1989). However, this change apparently was not sufficient to cause a decrease in serum testosterone which was noted in the previous experiment. Although testicular testosterone contents of the treated animals remained subnormal during the recovery period, testosterone concentration per gram tissue was not different from those of the controls. Since testosterone concentrations in ITF and STF remained normal throughout the experimental period, testosterone secretion by Leydig cells and its distribution within the testis were probably not affected by the drug treatment. Thus, the subnormal testosterone contents noted during the recovery period may reflect a decrease in the accumulation of testosterone due to a decrease in the mass of the testicular tissue. Alternatively, a decrease in testosterone production may have been compensated for by a decrease in the production of interstitial fluid and/or seminiferous tubular fluid. Since testosterone concentrations in the testis of the treated animals would be otherwise sufficient to support complete spermatogenesis (Huang and Boccabella, 1988; Zirkin et al, 1989), the cisplatinum-induced regression of the seminiferous epithelium cannot be accounted for by hypoandrogenism.

The disruption of the Sertoli cell tight junctions in rats following cisplatinum administration (Pogach et al, 1988) demonstrates that Sertoli cells are susceptible to this drug. This notion is supported by the current findings of acute and dose-dependent effects of cisplatinum on the production of ABP, lactate, and estradiol in cultured Sertoli cells. Since the production of these substances involves overall cellular activities, and the dose of cisplatinum that suppressed the production of all these substances approximated that used in the in vivo study, the spermatogenic lesions

Table 3. *Effects of cisplatinum on the distribution of androgen binding protein in serum, ITF, and STF**

Number of injections	Days after treatment		Serum	ITF	STF
1	1	Control†	167 ± 17	16,610 ± 1730	31,976 ± 3670
		Cisplatinum	233 ± 27	12,366 ± 1641	29,886 ± 2858
5	1	Control	124 ± 17	15,946 ± 2044	27,606 ± 1845
		Cisplatinum	83 ± 19	14,835 ± 969	37,463 ± 3628
5	10	Control	85 ± 11	21,874 ± 3615	38,980 ± 3464
		Cisplatinum	72 ± 12	17,603 ± 1262	36,903 ± 2740
5	30	Control	77 ± 12	18,616 ± 943	38,064 ± 5220
		Cisplatinum	82 ± 12	14,972 ± 1815	40,052 ± 4276
5	120	Control	112 ± 11	18,604 ± 2045	28,424 ± 1395
		Cisplatinum	76 ± 9	20,790 ± 1117	26,904 ± 1925

* Expressed as the mean ± SEM µlEq GMB-E-I/ml.
† n = 5 to 6 rats per group.

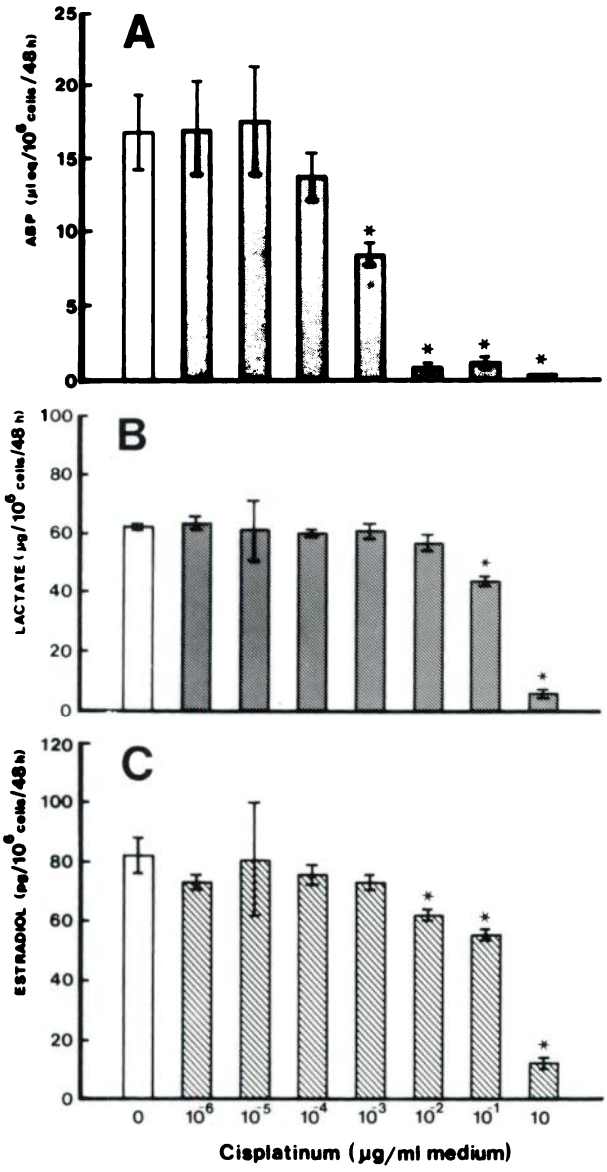


Fig 15.—Effects of cisplatinum upon Sertoli cells in vitro. Sertoli cells of 13 to 15-day-old rats were cultured at 32°C in DMEM/Ham's F-12 medium supplemented with FSH, testosterone, insulin, transferrin, and selenium. Cisplatinum (10^{-6}–10 µg/ml) was added on the third day of the culture, and media were collected at 48 hour intervals. ABP, lactate, and estradiol concentrations were measured by the methods described in Materials and Methods. Results are expressed as mean ± SEM. n = 3 wells per group. **(A)** = ABP, **(B)** = lactate and **(C)** = estradiol.

noted in the in vivo experiment may be attributed, at least in part, to the drug effects on Sertoli cells.

Because the number of Sertoli cells per testis remain relatively unchanged in adult animals, testicular ABP content has been used as an indicator for the normalcy of Sertoli cells. In addition, testicular ABP is released bidirectionally into STF and transported to the epididymis, or into circulation through ITF (Gunsalus et al, 1980). Assessment of ABP concentrations in STF, ITF, serum, and epididymis will reveal the normality of the partitioning of testicular ABP which may be relevant to the status of the seminiferous epithelium (Gunsalus et al, 1978; Sharpe, 1988). The presence of normal ABP concentrations in STF, ITF, and serum in treated rats suggests that the production of ABP and its distribution within testis were not affected. This is consistent with the previous observation (Pogach et al, 1989), but is different from the results of the in vitro experiment. Since the Sertoli cell cultures contained less than 5% of germ cell contaminants, whereas germ cells remained abundant in the testes during the treatment period, the discrepancy between the in vivo and in vitro Sertoli cell responses to cisplatinum perhaps can be attributed to the differences in the Sertoli cell–germ cell associations (Mather et al, 1983; Galdieri et al, 1984; Le Magueresse et al, 1986). A decrease in testicular ABP at the time of maximal germinal regression supports this notion. Moreover, a decreased epididymal ABP content was noted while the ABP levels in the STF remained unchanged. This could result from a decrease in tubular fluid production, a failure in the fluid transporting mechanism, or an increase in ABP degradation in the epididymis as a result of insufficient testosterone inflow (Mather et al, 1983).

Sertoli cell tight junctions may be involved in the regulation of the bidirectional release of testicular ABP (Gunsalus et al, 1980). Since cisplatinum disrupts the Sertoli cell tight junctions (Pogach et al, 1989), an increase in serum and ITF ABP concentrations was expected. Failure to observe these changes suggests that the intactness of these junctions may not be necessary for normal bidirectional release of testicular ABP. This notion is consistent with observations in vitamin A deficient rats (Huang et al, 1989). Recent studies suggest that the biochemical properties of ABP and other Sertoli cell proteins may dictate the mode of their own release into different compartments of the testis (Cheng et al, 1986; Sharpe, 1988). Examination of the effects of cisplatinum upon the distribution of different Sertoli cell proteins and their biochemical properties in STF and ITF may facilitate our understanding of the drug effects on the cellular activities of Sertoli cells and their subsequent effects on spermatogenesis.

The decrease in the weights of the seminal ves-

icle and pituitary gland following cisplatinum administration illustrate another aspect of the toxic effects of this drug on male reproduction. Since serum testosterone levels remained unchanged throughout the experimental period, cisplatinum may affect the seminal vesicle directly, perhaps through hormone–target cell interaction or the expression of seminal vesicle proteins. Alternatively, cisplatinum may affect the metabolism of an androgen which is important for the functional normality of accessory glands (Coffey, 1988). Since these changes persisted for at least 3 months after the cessation of drug treatment, permanent damage to the seminal vesicle is likely. On the other hand, a delayed and reversible decrease in pituitary weights may be secondary to the drug effects on certain feedback mechanisms. Since pituitary FSH and LH content remained unchanged, the gonadotrophs were capable of responding to endogenous GnRH and other feedback signals. Further experimentation is need to comprehend the mechanisms responsible for cisplatinum-induced changes in pituitary and accessory glands.

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