5. DISCUSSION

5.1 Significance of the Parameters Studied

The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells and it has been used as a crude measure of the damage to spermatogenesis (Schlappack et al., 1988). A strong correlation exists between weight of the testis and the number of germ cells (Sinha Hikim et al., 1989). The reduction in the weight of the testis has been shown to occur due to the loss of germ cells (Setchell and Galil, 1983). In the present study weight of the testis was taken to assess the maintenance of testicular functions.

The weights of the accessory sex organs are dependent on the availability of androgens as castration causes reduction of weights due to absence of testosterone. Increase in the availability of testosterone leads to hypertrophy and consequent increase in the weights of accessory sex organs. The organ size may even exceed that of its pre-castrational state with the administration of higher dose of testosterone (Neumann and Steinbeck, 1974). The weights of the accessory sex organs in castrated rats have been widely used as a bioassay for androgenic and antiandrogenic compounds (Neumann and Steinbeck, 1974). The measurement of the weights of the accessory sex organs in intact rats has been shown to reflect the estimation of the cumulative effect of biologically active testosterone over a period of time (Mathur and Chattopadhyay, 1982). In the present study the weights of epididymis, seminal vesicles and ventral prostate were taken to assess the bioavailability of androgen and the cumulative effect of androgenic activity.
The size of the kidneys of the castrated mice decreased by 30 to 50% in comparison to that of the intact animals. Treatment with testosterone propionate restored the weights to normal range (Kochakian, 1960). The activity of β-glucuronidase in kidney has been reported to be influenced by androgen (Fishman, 1951). Administration of testosterone has been reported to result in 2- to 5-fold increase in the activity of kidney β-glucuronidase after 6 days of treatment (Mowszowicz et al. 1974). Kidney β-glucuronidase has been shown to be under the influence of androgens (Bardin et al., 1978). Availability of testosterone has been reflected by the increased activity of kidney β-glucuronidase in rats (Mathur and Chattopadhyay, 1982, 1986). In the present study the weight of the kidney and the activity of renal β-glucuronidase were determined to assess the effect of androgenic activity on kidney.

Alkaline phosphatase is a brush border enzyme involved in mediation of membrane transport (Goldfisher et al., 1964) and transphosphorylation (Sastry et al., 1978). The brush border membrane and microsomes on a subcellular level in the proximal tubules of kidney have been reported to be the target sites for cisplatin-induced nephrotoxicity (Hannemann et al., 1991; Yanase et al., 1992). Alkaline phosphatase has been used as a marker enzyme to assess the physiological status of brush border membrane in renal tubules (Yanase et al., 1992). In the present study the activity of renal alkaline phosphatase was determined to assess the integrity of brush border membrane in kidney.

Testosterone plays an important role in maintaining spermatogenesis, accessory sex organs and secondary sexual characters. The pituitary hormones, follicle stimulating hormone and luteinizing hormone are responsible for the stimulation of spermatogenesis and steroidogenesis respectively (Greep and Fevold, 1937). In the
present study the serum levels of testosterone, follicle stimulating hormone and luteinizing hormone in serum were determined to assess the availability of these hormones in extracellular fluid for the action on their target sites.

The two key enzymes involved in the biosynthetic pathway of testosterone are 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase. The activity of delta-5-3β-hydroxysteroid dehydrogenase has been shown to respond to hCG in neonatal interstitial cells in culture (Meidan et al., 1985). Stimulation of 3β-hydroxysteroid dehydrogenase activity in testes of immature, hypophysectomized rats by administration of hCG in vivo has been reported (Murono and Payne, 1979). A suppression in the activities of 17β-hydroxysteroid dehydrogenase has been reported in the rat testis after administration of hCG or testosterone (Inano et al., 1973). The activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase have been used to study the testicular steroidogenesis of rats in different experimental conditions (Srivastava and Srivastava, 1991; Ghosh et al., 1990, 1991, 1995). Determination of activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in Leydig cells reflected the status of steroidogenesis in testis.

Glucose metabolism in Sertoli cells plays an important role in the maintenance of the adjacent germinal cells. Secretion of lactate and pyruvate by Sertoli cells has been reported to regulate and maintain spermatogenesis (Grootegoed and de Boer, 1989). Sertoli cells have been reported to support metabolically the germ cells by the FSH-stimulated secretion of pyruvate and lactate, which are the exclusive energy source for spermatocytes and spermatids (Jutte et al., 1981a, 1982, 1983). Determination of concentrations of lactate and pyruvate were performed to assess the functional status of Sertoli cells which play an important role in spermatogenesis.
Histometric studies on the testis provide the strongest evidence to see the difference between control and experimental tissues (Russell et al., 1990). A positive relationship exists between the tubular diameter and the spermatogenic activity of the testis (Sinha Hikim et al., 1989). Tubular diameter measurements have been reported to discriminate between varying levels of spermatogenic damage (Russell et al., 1990). Johnson et al. (1983) have shown that late spermatocytes can be used to evaluate daily sperm production which indicate the cell loss during meiotic divisions and also during spermiogenesis. In order to assess the status of spermatogenesis diameters of seminiferous tubule and its lumen were measured along with determination of the number of preleptotene primary spermatocytes, pachytene spermatocytes and Sertoli cells.

DNA contents in various stages of spermatogenesis vary. The amount of DNA in the primary spermatocyte, secondary spermatocyte and spermatid were present in the ratio of 4:2:1 (Davson and Segal, 1975). Protein and RNA synthesis occur both in somatic cells and in germ cells. In the present study quantitative determination of DNA indicated the rate of cell division and subsequent maturation. Determination of the levels of RNA and protein showed the resultant effect of intracellular synthetic and breakdown processes.

5.2 Treatment

Cisplatin is one of the widely used chemotherapeutic agents for testicular cancers. Cisplatin-based regimens have been used as the first line of therapy for disseminated testicular cancers and cause permanent remissions (Rozencweig et al., 1981).
In the present study cisplatin (3 mg/day/Kg body weight) was administered intraperitoneally to adult and immature rats for 3 alternate days in order to see the effects of cisplatin on testicular functions. Similar dose regimen was administered in mouse in different experimental conditions (Teicher et al., 1991). The administered dose would not cause acute toxicity in rats as the LD$_{50}$ for cisplatin has been reported to be 11 mg/Kg body weight in rats (Meistrich et al., 1982). No mortality was recorded during experimentation. However, approximately 20% loss in the weights of the animals was recorded during treatment.

Cisplatin is most effective when administered either intraperitoneally or intravenously. After intraperitoneal administration, concentrations of cisplatin in tissues were 2.5- to 8-fold greater than those achieved after intravenous administration (Pretorius et al., 1981). Intraperitoneal administration of cisplatin has been reported to increase the exposure of the peritoneal cavity to cisplatin approximately 10 to 15 fold as compared to that of systemic circulation (McClay, 1990).

Administration of testosterone in doses between 5 and 25 mg/day for 3 days were needed for the quantitative maintenance of spermatogenesis (Sharpe et al., 1988). Requirement of 15 mg testosterone propionate/day has been shown to achieve normal testicular testosterone levels in hypophysectomized rats (Stevens and Steinberger, 1983). In the present study cisplatin (3 mg/day/Kg body weight) along with testosterone propionate (15 mg/animal/day for the adult and 3 mg/animal/day for the immature rats) were administered intraperitoneally to adult and immature rats for 3 alternate days in order to see the protective effects, if any, exerted by testosterone supplementation on the cisplatin induced testicular damage.
The animals were sacrificed on the 7th, 15th and 35th days of the experiment in order to see the short and long term effects of cisplatin, with or without testosterone supplementation, on testicular functions of adult and immature rats. The immature rats killed on the 7th day of the experiment were in pre-pubertal stage (32±2 days) while the rats killed on the 15th day of the experiment were in pubertal stage (40±2 days) and the rats killed on the 35th day of the experiment were post-pubertal (60±2 days).

In vitro studies on the effect of cisplatin on Leydig and Sertoli cells were done by incubating these cells with varying doses of cisplatin alone (1 x 10^-2 to 1 x 10 ug/ml medium) or along with testosterone (1 uM).

5.3 Effect of Cisplatin on Body and Organ Weights

Administration of cisplatin to adult and immature rats caused a significant decline in the body weight as compared to the corresponding groups of control animals (Table 1 & 11). A similar decrease in the body weight has been shown in rats and mouse following cisplatin treatment (Vawda and Davies, 1986; Huang et al., 1990). The decrease in the body weight reflected the decreased nitrogen balance which may be due to the decrease in the levels of serum testosterone. Testosterone has been shown to exert a strong anabolic action which has a stimulatory effect on nitrogen balance and body weight (Kochakian, 1975).

Adult as well as immature rats, treated with cisplatin, showed a decrease in the weights of the testes when compared to their corresponding groups of control animals (Fig. 1 & 2). However, the decline was not significant in the adult rats. The reduction in the testicular weights could be due to the germinal cell loss by direct inhibition of cisplatin on the spermatogenic compartment or an indirect effect through inhibition of
testosterone production. The present findings are in agreement with the earlier studies in which reduction of weight of testis has been correlated with the loss of germ cells (Lu and Meistrich, 1979; Meistrich et al., 1982; Setchell and Galil, 1983; Sinha Hikim et al., 1989).

The weights of epididymis and other accessory sex organs such as seminal vesicles and ventral prostate decreased significantly in adult (Fig. 1) and immature (Fig. 2) rats following cisplatin treatment as compared to the corresponding groups of control animals. The strong inhibition of the weights of the epididymis, seminal vesicles and ventral prostate may be either due to the decrease in the levels of serum testosterone or a direct action of cisplatin on the accessory sex organs. It has been reported that the accessory sex organs are androgen dependent and thus reflect the availability of androgen over a period of time (Hunt et al., 1978). The decrease in the weight of the accessory sex organs indicated the atrophy of glandular tissue along with the reduction in secretory activity and reflected the cumulative effect of androgenic activity.

5.4 Effect of Cisplatin on β-glucuronidase and Alkaline Phosphatase Activities in Kidney

Cisplatin treatment to adult and immature rats caused a significant decrease in the weight of kidneys (Fig. 1 & 2) as well as in the activity of renal β-glucuronidase as compared to the corresponding groups of control animals (Table 4 & 14). These effects may be due to the decreased availability of testosterone following cisplatin administration. Kidneys have the potential to respond to androgen stimulation for growth and metabolic activities similar to the accessory sex organs (Kochakian, 1977).
FIG. 1. EFFECTS OF CISPLATIN ON THE WEIGHTS OF TESTES (T), EPIDIDYMIDES (E), SEMINAL VESICLES (SV), VENTRAL PROSTATE (VP) AND KIDNEYS (K) OF ADULT RATS (MEAN ± SD)

<table>
<thead>
<tr>
<th>DAYS OF EXPERIMENT</th>
<th>ORGAN WEIGHTS (mg/100g body weight)</th>
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<tbody>
<tr>
<td>7</td>
<td>T, E, SV, VP, K</td>
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<tr>
<td>15</td>
<td>T, E, SV, VP, K</td>
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<tr>
<td>35</td>
<td>T, E, SV, VP, K</td>
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</table>

- CONTROL
- TREATED

* P < 0.05
FIG. 2. EFFECTS OF CISPLATIN ON THE WEIGHTS OF TESTES (T), EPIDIDYMIDES (E), SEMINAL VESICLES (SV), VENTRAL PROSTATE (VP) AND KIDNEYS (K) OF IMMATURE RATS (MEAN ± SD)

ORGAN WEIGHTS (mg)

DAYS OF EXPERIMENT

CONTROL
● TREATED
+ P<0.05

7
15
35
Renal β-glucuronidase activity has been suggested as a bioassay of serum testosterone levels in rats (Malarvizhi and Mathur, 1996).

The decreased activity of renal alkaline phosphatase in adult and immature rats following cisplatin treatment as compared to the corresponding groups of control animals (Table 5 & 15), is indicative of inhibition of cellular processes by direct action of cisplatin on the brush border membrane along the proximal tubules of kidney. The brush border membrane and microsomes on a subcellular level in the proximal tubules of kidney have been reported to be the target sites for cisplatin-caused nephrotoxicity (Hannemann et al., 1991; Yanase et al., 1992). The decreased activity of renal alkaline phosphatase in rats following cisplatin treatment has been reported in different experimental conditions (Reeves et al., 1990; Aggarwal, 1993; Bogin et al., 1994).

5.5 Effect of Cisplatin on Nucleic Acid and Protein Contents of Testis

Administration of cisplatin to adult and immature rats did not cause significant alterations in the DNA, RNA and protein contents of testis (Table 2 & 12). These findings are in agreement with the earlier studies in which protein, RNA and DNA contents of the testis were not affected nor there was any change in the rate of protein synthesis in mouse treated with cisplatin (Vawda and Davies, 1986).

5.6 Effect of Cisplatin on the Levels of Serum Testosterone

The levels of serum testosterone were significantly suppressed in adult and immature rats following cisplatin treatment as compared to the corresponding groups of control animals (Fig. 3 & 4). A decrease in the intratesticular testosterone concentration was noted in rats following cisplatin administration (Huang et al., 1990).
Subclinical hypoandrogenism has been reported in the patients receiving cisplatin based chemotherapy (Drasga et al., 1983; Lange et al., 1983). Suppression of testosterone production in rats following cisplatin administration has also been reported (Carreau et al., 1988; Pogach et al., 1989; Vawda, 1989).

The decrease in the serum testosterone levels in adult and pubertal, post-pubertal rats could be either due to the diminished responsiveness of Leydig cells to LH and/or the direct inhibition of testicular steroidogenesis. While in the immature animals the levels of testosterone are already low as in pre-pubertal stage testosterone is not needed in high amounts (Jegou and Sharpe, 1993).

5.7 Effect of Cisplatin on Steroidogenic Enzymes in Leydig Cells in vitro

The activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase decreased significantly in the Leydig cells cultured with cisplatin as compared to their corresponding controls (Fig. 5). It may reflect a direct effect of cisplatin on Leydig cells, causing decreased steroidogenesis. Cisplatin has already been shown to affect the Leydig cell and its testosterone production (Jacobson et al., 1978; Maines and Mayer, 1985). The activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase were also decreased significantly in Leydig cells cultured in the presence of cisplatin and testosterone (Table 19), which reflects that the effect of cisplatin could not be reversed by addition of testosterone. On the other hand, testosterone might also contribute to the inhibition of the pathway leading to its own synthesis.

Implantation of testosterone-estrodiol filled silastic capsules has been reported to cause a reduction in the volume of smooth endoplasmic reticulum in the Leydig cells
FIG. 3. SERUM TESTOSTERONE LEVELS IN ADULT RATS TREATED WITH CISPLATIN (CDDP) OR CISPLATIN WITH TESTOSTERONE PROPIONATE (CDDP + TP) (MEAN ± SD)

CONTROL

TREATED

* P < 0.05

DAYS OF EXPERIMENT

SERUM TESTOSTERONE (pg/ml)

CDDP 7

CDDP + TP 7

CDDP 15

CDDP + TP 15

CDDP 35

CDDP + TP 35
FIG 4. SERUM TESTOSTERONE LEVELS IN IMMATURE RATS TREATED WITH CISPLATIN (CDDP) OR CISPLATIN WITH TESTOSTERONE PROPIONATE (CDDP+TP) (MEAN ± SD)

- CONTROL
- TREATED
* P<0.05

DAYS OF EXPERIMENT

CDDP

CDDP+TP

7

15

35
FIG. 5. 3β-HYROXYSTEROID DEHYDROGENASE (3β-HSD) & 17β-HYDROXYSTEROID DEHYDROGENASE (17β-HSD) ACTIVITIES IN LEYDIG CELLS CULTURED WITH VARYING DOSES OF CISPLATIN (MEAN ± SD)

3β-HSD ACTIVITY (in mole NAD converted mg-l-protein min-1)

17β-HSD ACTIVITY (in mole NADPH converted mg-l-protein min-1)

CISPLATIN/ml MEDIUM

100 10ng 100ng 1μg 5μg 10μg

* P < 0.05
and the ability of the testis to secrete testosterone in vitro (Ewing et al., 1977). Administration of dehydrotestosterone to hypophysectomized rats has been shown to inhibit the 3β-hydroxysteroid dehydrogenase activity in vivo and in the cultured testicular cells from adult rats with dehydrotestosterone in vitro (Fanjul et al., 1992, 1993).

5.8 Effect of Cisplatin on the Levels of Serum LH

The levels of serum LH increased significantly following cisplatin treatment both in adult and immature rats as compared to the corresponding groups of control animals (Fig. 6 & 7). The elevated levels of LH in rats and mouse following chemotherapy have been reported (Debeljuk et al., 1973; Schilsky et al., 1980). Administration of LHRH to the adult men after combination chemotherapy for Hodgkin's disease have been reported to cause increase in the levels of serum FSH and LH with decreased testosterone (Mecklenburg and Sherins, 1974; Jacobson et al., 1978).

The increased levels of LH along with decreased levels of serum testosterone in adult, pubertal and post-pubertal rats reflected decreased steroidogenic ability of the testis in the cisplatin treated-rats. The decreased activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in Leydig cells cultured with cisplatin suggested a direct action of cisplatin on Leydig cells (section 5.7). Thus the increased levels of LH may be caused by the decreased state of steroidogenesis in testis as was also reflected by decrease in serum testosterone (section 5.6).

In immature rats in the absence of high amounts of testosterone, elevated levels of LH may be required to differentiate Leydig cells without stimulating testosterone
FIG. 6. SERUM LH LEVELS IN ADULT RATS TREATED WITH CISPLATIN (CDDP) OR CISPLATIN WITH TESTOSTERONE PROPIONATE (CDDP+TP) (MEAN ± SD)

- CONTROL
- TREATED

* P < 0.05

DAYS OF EXPERIMENT

CDDP CDDP+TP

50 30 10
SERUM LH (µg/ml)

15 7
FIG. 7. SERUM LH LEVELS IN IMMATURE RATS TREATED WITH CISPLATIN (CDDP) OR CISPLATIN WITH TESTOSTERONE PROPIONATE (CDDP+TP) (MEAN ± SD)

CONTROL

TREATED

P < 0.05

DAYS OF EXPERIMENT

7

15

35

SERUM LH (ng/ml)
production (Sharpe, 1993) as the animals were still in pre-pubertal stage at the time of collection of blood.

5.9 Effect of Cisplatin on the Levels of Serum FSH

The levels of serum FSH increased significantly after cisplatin administration in adult and immature rats as compared to the corresponding groups of control animals (Fig. 8 & 9). The increase in the levels of serum FSH in adult, pubertal and post-pubertal rats could be due to the impairment of spermatogenesis by direct effect of cisplatin on the spermatogenic compartment as reflected by decrease in the number of Sertoli cells, preleptotene and pachytene spermatocytes (section 5.10). It may also be through inhibition of testosterone production (section 5.6). The present findings are consistent with the earlier reports demonstrating increase in serum FSH levels following damage to the seminiferous epithelium (Debeljuk et al., 1973; Gomes et al., 1973; Main and Setchell, 1980). FSH was elevated when germinal aplasia had occurred in patients receiving chemotherapy (Chapman et al., 1979). The elevation of serum FSH after chemotherapy has been reported as a marker of testicular germinal aplasia (Schilsky et al., 1980). Seminiferous tubules have been shown to be the site for exerting feedback regulation for FSH secretion (van Thiel et al., 1972). The decrease in the total number of Sertoli cells may cause an increase in serum FSH levels due to a decreased secretion of inhibin (de Jong and Robertson, 1985).

Thus the increase in the levels of serum FSH in adult, pubertal and post-pubertal rats could be due to the germ cell loss in the spermatogenic compartment or damage of Sertoli cells, thereby affecting the feedback regulation of FSH secretion. Whereas in immature rats the increased levels of FSH may be responsible for
FIG. 8. SERUM FSH LEVELS IN ADULT RATS TREATED WITH CISPLATIN (CDDP) OR CISPLATIN WITH TESTOSTERONE PROPIONATE (CDDP + TP) (MEAN ± SD)

- CONTROL
- TREATED
+ P < 0.05

SERUM FSH (ng/ml)

<table>
<thead>
<tr>
<th></th>
<th>CDDP</th>
<th>CDDP + TP</th>
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DAYS OF EXPERIMENT

7 7 7
FIG. 9. SERUM FSH LEVELS IN IMMATURE RATS TREATED WITH CISPLATIN (CDDP) OR CISPLATIN WITH TESTOSTERONE PROPIONATE (CDDP+TP) (MEAN±SD)

CONTROL
TREATED
* P < 0.05

SERUM FSH (ng/ml)

CDDP  CDDP+TP
7  15  35

DAYS OF EXPERIMENT
stimulating the initiation and expansion of early spermatogenesis (Means et al., 1976; Russell et al., 1987).

5.10 Effect of Cisplatin on Histometric Parameters

The diameter of seminiferous tubules and seminiferous tubular lumen significantly decreased after cisplatin treatment in adult rats as compared to the corresponding groups of control animals (Table 6). It may be due to a direct action of cisplatin on the seminiferous tubules or an indirect effects through inhibition of testosterone production. The tubular diameter has been correlated with the spermatogenic activity of the testis (Sinha Hikim et al., 1989).

The number of preleptotene primary spermatocytes, pachytene spermatocytes and Sertoli cells were significantly decreased in adult rats following cisplatin treatment as compared to their corresponding groups of control animals (Table 7). The present findings are consistent with several earlier reports in which damage of seminiferous epithelium and germinal cell loss occurred following cisplatin treatment (Vawda and Davies, 1986; Vawda, 1994).

In the present study the reduction in the number of preleptotene primary spermatocytes, pachytene spermatocytes and Sertoli cells could be due to the direct effect of cisplatin on the seminiferous tubular compartment. It could also be due to the decreased intratesticular concentration of testosterone due to decreased steroidogenesis (section 5.7). Suppression of testosterone production and intratesticular testosterone have been reported in rats following cisplatin treatment (Pogach et al., 1989; Huang et al., 1990).
5.11 Effect of Cisplatin on the Levels of Lactate and Pyruvate in Sertoli Cells *in vitro*

The concentrations of lactate and pyruvate decreased significantly in Sertoli cells cultured with cisplatin alone as compared to their corresponding controls (Fig. 10). These observations are also indicative of direct action of cisplatin on Sertoli cells and their metabolic functions. It indicated that Sertoli cells were not able to support the germ cells metabolically and thereby impairing spermatogenesis. The concentrations of lactate and pyruvate were also decreased significantly in Sertoli cells cultured with cisplatin and testosterone. Testosterone has been reported to have no effect on the production of lactate by Sertoli cells *in vitro* (Jutte et al., 1983). It indicated that the protective effect of testosterone supplementation in cisplatin-induced testicular damage was not at the level of glucose metabolism by Sertoli cells.

5.12 Effect of Co-administration of Cisplatin and Testosterone Propionate in Adult Rats

Administration of cisplatin along with testosterone propionate to adult rats caused a significant decrease in the body weight as compared to the control group of animals (Table 8). A decrease in the body weight was also noted in adult rats following cisplatin treatment (section 5.3). It is thus suggested that co-administration of testosterone propionate along with cisplatin could not reverse the effect of cisplatin on the body weight.

The weight of the testis showed a slight increase in adult rats after cisplatin with testosterone propionate treatment as compared to the control group of animals (Fig. 11). The slight increase in the weight of the testis may reflect a direct effect of
FIG. 10. LACTATE AND PYRUVATE CONCENTRATIONS IN SERTOLI CELLS CULTURED WITH VARYING DOSES OF CISPLATIN

(MEAN ± SD)

- LACTATE
- PYRUVATE

* P < 0.05
exogenous testosterone on the testis. Direct effect of dehydrotestosterone on the testis has been shown to increase the testicular weight and sperm production (Ahmad et al., 1973; Harris et al., 1977).

The significant increase in the weights of epididymis and other accessory sex organs such as seminal vesicles and ventral prostate in adult rats following cisplatin along with testosterone propionate treatment (Fig. 11) may also be due to a direct action of exogenous testosterone on its target sites. A single injection of testosterone propionate to the animals castrated for 40 days showed a quick response in cell hypertrophy within 23 h and mitotic activity at 35 h (Burkhart, 1942).

Treatment of cisplatin along with testosterone propionate to adult rats caused an increase in the weight of kidney (Fig. 11) along with an increase in renal β-glucuronidase activity (Table 4) which is also indicative of direct action of exogenous testosterone on kidney. Testosterone administration has been reported to result in 2- to 5-fold increase in the activity of renal β-glucuronidase after 6 days of treatment (Mowszowicz et al., 1974).

The levels of serum testosterone increased significantly in adult rats after administration of cisplatin along with testosterone propionate as compared to the control group of animals (Fig. 3). Serum levels of LH and FSH decreased significantly in adult rats following treatment of cisplatin with testosterone propionate as compared to the corresponding groups of control animals (Fig. 6 & 8). The increased levels of serum testosterone along with decreased levels of LH and FSH in serum on the 7th day of the experiment can be due to the presence of exogenous testosterone and its feedback regulation at hypothalamic/hypophyseal level. While on the 15th and 35th days, the exogenous testosterone could not be available in the blood
FIG. 11. EFFECTS OF CISPLATIN & TESTOSTERONE PROPIONATE ON THE WEIGHTS OF TESTES (T), EPIDIDYMIDES (E), SEMINAL VESICLES (SV), VENTRAL PROSTATE (VP) AND KIDNEYS (K) OF ADULT RATS

(MEAN ± SD)

ORGAN WEIGHTS (mg/100g body weight)

T  E  SV  VP  K

15 35

DAYS OF EXPERIMENT

CONTROL
TREATED
P < 0.05
due to its short half life. However, the increased concentration of serum testosterone may be due to the restoration of testicular functions by testosterone supplementation along with cisplatin. Experimental disruption of spermatogenesis has been shown to cause morphological and functional changes in Leydig cells along with an increase in the plasma levels of LH and FSH indicating a local mechanism of controlling Leydig cell functions (Saez et al., 1991). Administration of cisplatin along with testosterone propionate to adult rats did not show any significant change in the diameters of seminiferous tubules and its lumen as compared to the corresponding groups of control animals (Table 6). There was no change in the number of preleptotene primary spermatocytes, pachytene spermatocytes and Sertoli cells after administration of cisplatin along with testosterone propionate as compared to the corresponding groups of control animals (Table 7). These findings reflected a protective effect of testosterone propionate when given along with cisplatin. The withdrawal of experimental testosterone in adult rats has been reported to reduce the lumen size to nearly half of its original size (Ghosh et al., 1992). Degeneration of pachytene primary spermatocytes and step 7 and step 19 spermatids have been reported within 6 days in experimentally induced testosterone depletion in vivo (Sharpe et al., 1990).

Administration of testosterone propionate along with cisplatin to adult rats could reverse the effects of cisplatin possibly by direct action of exogenous testosterone on its target sites on the 7th day of the experiment. While on the 15th and 35th days of the experiment, the exogenous testosterone could not be available in the blood due to its conversion to metabolic end products. However, it may be possible that the testosterone supplementation along with cisplatin could recover the cisplatin-induced Leydig cell dysfunction.

The decrease in the activity of renal alkaline phosphatase in adult rats following
cisplatin treatment along with testosterone propionate (Table 5) could be due to an inhibition of cellular processes by direct action of cisplatin on the brush border membrane of kidney which could not be restored by testosterone supplementation.

Administration of cisplatin along with testosterone propionate to adult rats did not cause any significant change on the DNA, RNA and protein contents of testis as compared to the corresponding groups of control animals (Table 9).

5.13 Effect of Co-administration of Cisplatin and Testosterone Propionate in Immature Rats

Administration of cisplatin along with testosterone propionate to immature rats caused a decrease in the body weight as compared to the corresponding groups of control animals (Table 16) which reflected that the exogenous testosterone could not restore the effect of cisplatin on body weight. The decrease in the weight of testis in immature rats on the 7th day following administration of cisplatin along with testosterone propionate (Fig. 12), may be due to a direct action of cisplatin on pre-pubertal testis. While on the 15th and 35th days of the experiment, the decreased weights of testis may be indicative of lower levels of intratesticular testosterone caused by cisplatin co-administration with testosterone propionate. Lower levels of intratesticular testosterone thus were not able to stimulate initiation of early spermatogenesis, when the animals were at pubertal and post-pubertal stages.

The weight of epididymis and other accessory sex organs such as seminal vesicles and ventral prostate increased significantly in immature rats on the 7th day following cisplatin along with testosterone propionate treatment as compared to the corresponding groups of control animals (Fig. 12). It may be due to a direct effect of
FIG. 12. EFFECTS OF CISPLATIN AND TESTOSTERONE PROPIONATE ON THE WEIGHTS OF TESTES (T), EPIDIDYMIDES (E), SEMINAL VESICLES (SV), VENTRAL PROSTATE (VP) AND KIDNEYS (K) OF IMMATURE RATS (MEAN ± SD)

CONTROL
TREATED
+ P < 0.05

ORGAN WEIGHTS (mg)

T E SV VP K T E SV VP K T E SV VP K

DAYS OF EXPERIMENT
7 15 35
exogenous testosterone on the accessory sex organs. However, the decrease in the weights of the accessory sex organs after 15 and 35 days of the experiment would reflect a state of decreased testosterone levels as the exogenous testosterone could not be available in the blood due to its short half life. Testosterone has been reported to be essential for the development and maintenance of specific reproductive tissues such as testis, epididymis, seminal vesicles and ventral prostate (Mooradian et al., 1987).

Administration of cisplatin along with testosterone propionate to immature rats resulted in an increase in the weight of the kidney (Fig. 12) along with an increase in renal β-glucuronidase activity on the 7th day (Table 14). These findings could also be due to the direct effect of exogenous testosterone. However, the decrease in the weight of the kidney after 15 and 35 days of the experiment could reflect a state of decreased testosterone levels as the exogenous testosterone could not be available in the blood due to its short half life.

Administration of cisplatin along with testosterone propionate to immature rats caused a significant increase in serum testosterone levels on the 7th day, while the serum testosterone levels were significantly decreased on the 15th and 35th days after the treatment (Fig. 4). The increased levels of serum testosterone on the 7th day reflected the availability of exogenous testosterone in the serum, while on the 15th and 35th days, exogenous testosterone could not be available in the blood due to its conversion to metabolic products. However, the decreased concentration of serum testosterone indicated that the Leydig cells dysfunction caused by cisplatin could not be recovered by testosterone supplementation along with cisplatin when the animals were at pubertal and post-pubertal stages, respectively.

Serum levels of LH and FSH were significantly decreased in immature rats on
the 7th day, while the serum levels of LH and FSH increased significantly on the 15th and 35th days after treatment of cisplatin along with testosterone propionate as compared to the corresponding groups of control animals (Fig. 7 & 9). The decrease in the levels of serum LH and FSH on the 7th day could be due to the feedback regulation by exogenous testosterone at hypothalamic/hypophyseal level. While on the 15th and 35th days of the experiment, exogenous testosterone could not be available in the blood due to its short half life. However, the increase in the levels of serum LH and FSH after 15 and 35 days of the experiment could be due to the onset of feedback regulation of pituitary hormones because of low testosterone or the impaired spermatogenesis as the Leydig cell dysfunction caused by cisplatin in pubertal and post-pubertal rats could not recover by testosterone supplementation along with cisplatin. Male patients receiving chemotherapy during puberty have been reported to have profound effect on both germ cell production and Leydig cell function which resulted in elevated levels of serum FSH and LH with low levels of serum testosterone (Sherins et al., 1978).

The decrease in the activity of renal alkaline phosphatase was noted in immature rats following treatment of cisplatin with testosterone propionate as compared to the control group of animals (Table 15). It could be due to the inhibition of cellular processes by direct action of cisplatin on the brush border membrane of kidney which was not restored by testosterone supplementation.

Administration of cisplatin along with testosterone propionate to immature rats did not cause any significant effects on the DNA, RNA and protein contents of testis (Table 17).

In the present study cisplatin has been shown to create a situation in which
testicular steroidogenesis was inhibited along with disruption in the spermatogenic compartment. It is suggested that in addition to a direct effect of cisplatin on Leydig cells there could also be a local regulatory role of seminiferous tubules on the Leydig cell functions. Cisplatin does not directly act on hypothalamus/pituitary, but acts through a feedback regulation from testis. Testosterone supplementation has been demonstrated to exert a protective effect on cisplatin-induced damage to the testicular functions. It may be due to the fact that testosterone reinforces the Sertoli cell barrier which gets disrupted following cisplatin administration. It needs further studies to ascertain whether similar situation exists in humans also. Further studies in this direction would indicate if testosterone supplementation in the patients treated with cisplatin would give them protective effects against reproductive toxicities.