CHAPTER II

EFFECTS OF AN ANTI-ANDROGEN, CYPROTERONE ACETATE ON THE METABOLISM OF TESTIS AND EPIDIDYMIDES OF MALE ALBINO RATS

INTRODUCTION

Cyproterone acetate (CA) is one of the most potent anti-androgens which inhibits the action of androgens at both the nuclear and cytosol receptor sites in target organs (Brandes, 1974). CA affects the pituitary release of LH (Johnson and Naqvi, 1969) and testicular functions (Ogasa and Yoshin, 1970; Neumann, 1971/1972; Stern and Murphy, 1972; Hovatta, 1972; Heinert and Taubert, 1973). The synthesis of testosterone is reduced due to the inhibition of the enzyme systems (Grants and Stitch, 1973) and thereby a decrease in plasma levels of androgens occur in CA treated animals and human volunteers (Brotherton and Barnard, 1974; Murray et al., 1975; Roy et al., 1976; Koch et al., 1976). On the contrary, several studies on rodents and primates have shown that microquantities of CA released from subcutaneously implanted silastic capsules cause transient infertility in these animals (Prasad et al., 1971/72, 1973 a,b; Prasad, 1973; Gupta et al., 1974 a; Bose et al., 1975) by selectively inhibiting epididymal function without altering the secretory activities of accessory reproductive organs and libido. This is probably due to a
different threshold requirement of androgens for maintenance of the functional integrity of different reproductive organs (Dinakar et al., 1974 a, b; Gupta et al., 1974 b; Sheth, 1976; Chapter I).

It is known that (i) CA inhibits testosterone synthesis (Grants and Stich, 1973; Roy et al., 1976), (ii) ascorbic acid is involved in steroidogenesis in gonads and adrenals (Fisher, 1962; Biswas and Deb, 1970; Kutsky, 1973; Datta and Saryal, 1976), (iii) the biosynthesis and tissue distribution of this vitamin is androgen dependent (Dieter, 1969; Majumder and Chatterjee, 1974; Chapter IX), (iv) enhanced utilization of AA occurs in androgen deprived conditions (Chapter I), (v) AA and testosterone propionate have a synergistic effect on epididymal metabolism (Chapter I), and (vi) AA has a beneficial influence in maintaining the secretory activity of accessory reproductive glands and the metabolism of epididymal spermatozoa in CA treated animals (Sheth, 1976; Buch, 1976). Therefore the present study was undertaken to investigate the effects of CA on endogenously produced and exogenously administered androgens, the recovery pattern after withdrawal of CA treatment as well as the influence of ascorbic acid on reproductive functions of CA treated rats in the light of the foregoing data.
MATERIALS AND METHOD

Sexually mature albino rats (Rattus norvegicus) weighing between 200-250 gm were used for the study. The animals were maintained under conventional laboratory conditions (temperature 26±2°C and 14 day light hours) on standard diet (Hindustan Lever Ltd., Bombay) and water supplied ad libitum. The animals were divided into the following groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of animals used</th>
<th>Day of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal intact control</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Normal + Cyproterone acetate (CA) treated</td>
<td>30</td>
<td>8th &amp; 16th</td>
</tr>
<tr>
<td></td>
<td>Normal intact rats were given an intramuscular injection of CA (Schering A.G. Berlin) 5 mg/rat for 7 and 15 days continuously. CA was made up in a vehicle (Benzyl benzoate:castor oil (1:25), made according to the method of Walsh and Gittes, 1970).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Normal + Vehicle treated</td>
<td>12</td>
<td>8th &amp; 16th</td>
</tr>
<tr>
<td></td>
<td>Normal intact animals were given only vehicle (0.2 ml/rat) for 7 and 15 days.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Normal + CA + ascorbic acid (AA) treated</td>
<td>30</td>
<td>8th &amp; 16th</td>
</tr>
<tr>
<td></td>
<td>Intact animals were given CA as in group II and in addition, a daily dose of ascorbic acid (100 mg/rat) was fed orally for 7 and 15 days respectively</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
V CA recovery for 1 month
The animals were administered CA for 7 days and then the injections were discontinued. The animals were utilized after 1 month of CA withdrawal.

VI Castration + CA + TP treated rats
Bilaterally castrated animals were given CA as in group II and testosterone propionate (TP) (200 µg/day/rat) was simultaneously injected intramuscularly for 7 and 15 days.

VII Castration + Vehicle + TP treated rats
Bilaterally castrated rats were given the vehicle as in group III and the same amount of TP as in group IV for 7 and 15 days.

VIII Castration + CA + TP + Ascorbic acid treated rats
Bilaterally castrated animals were given CA and TP for 15 days as in group VI and in addition, ascorbic acid was fed orally (100 mg/rat/day) for 15 days.

After the respective treatments, the animals were autopsied and the testis, caput and cauda epididymides were removed, blotted free of blood, weighed and used for the biochemical estimations and ESR studies. The details of the following methods are given in Chapter I.
Free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization and ascorbic acid macromolecule complexing (AA-MM): (Chinoy et al., 1974).
Ascorbic acid free radical (AA-ER) forming special peroxidase: (Chinoy, 1973).
Cholesterol: (Pearson et al., 1953).
Succinate dehydrogenase (SDH): (Kun and Aboud, 1949).
Acid and alkaline phosphatase: (Seldfield and Goldberg, 1971).
Protein: (Gornall et al., 1949).

**Fertility test:**

The cauda epididymal sperm suspension of rats treated with CA and CA + AA (groups II and IV) was prepared in a known volume of normal physiological saline solution according to the method of Buch (1976) and injected into the uterine horn of the anaesthetized proestrous females. The epididymal sperm suspension of CA treated animal was injected into the left horn and that of CA + AA treated rats into the contralateral right horn. The cervical ends of the uterus were ligated in order to prevent the loss of sperms. On the fourth day after insemination, the females were autopsied, the uterus was removed and flushed with normal saline solution. The suspension from each of the horns was examined separately for the presence of fertilized ova under a light microscope.
Electron spin resonance spectrometry:

The sperms from the weighed samples of cauda epididymis of normal intact control, CA treated and CA + AA treated (15 days) animals (groups I, II, IV) were gently released into 0.9 % saline solution. The sperm suspension was transferred to a standard quartz flat cell (E-248) and the ESR spectrum was recorded on a Varian E-4 ESR spectometer at a temperature of 25±2°C. The spectrometer settings were as follows:

1. Field set - 3390 gauss
2. Scan range - 40 gauss
3. Modulation frequency - 100 gauss
4. Scanning time - 30 minutes
5. Receiver gain - 6.03 x 10^4
6. Microwave power - 10dB

Under the above constant spectrometric settings the ascorbic acid free radical signal height was taken as a measure of its concentration and was expressed as spin concentration in arbitrary units.

A minimum of six replicates were done for each tissue and parameter and the results were statistically analyzed using student's 't' test.
RESULTS

Organ weights:

A significant decrease (P < 0.001) occurred in the weights of testis, cauda and caput epididymis of the CA treated, CA + TP and CA + TP + AA treated rats of groups II, VI and VIII. On the contrary, the weights recovered to almost control level in testis and caput of rats of group IV. The weights of cauda however were same as in CA treated rats. A complete recovery in organ weights occurred by one month after CA withdrawal in all tissues as compared to control (Table I).

TABLE I

Weights (in gm) of testis and epididymides in Intact control, Intact + CA, CA + AA, CA withdrawal, castration + CA + TP and Castration + CA + TP + AA treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Testis</th>
<th>Caput</th>
<th>Cauda</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact control</td>
<td>1.38±</td>
<td>0.20±</td>
<td>0.15±</td>
</tr>
<tr>
<td>II</td>
<td>Intact + CA</td>
<td>0.63±</td>
<td>0.08±</td>
<td>0.07±</td>
</tr>
<tr>
<td></td>
<td>(7 days)</td>
<td>0.04±</td>
<td>0.01±</td>
<td>0.005±</td>
</tr>
<tr>
<td></td>
<td>(15 days)</td>
<td>0.03±</td>
<td>0.002±</td>
<td>0.004±</td>
</tr>
<tr>
<td>IV</td>
<td>Intact + CA + AA</td>
<td>0.85±</td>
<td>0.10±</td>
<td>0.07±</td>
</tr>
<tr>
<td></td>
<td>(7 days)</td>
<td>0.04±</td>
<td>0.001±</td>
<td>0.005±</td>
</tr>
<tr>
<td></td>
<td>(15 days)</td>
<td>0.04±</td>
<td>0.007±</td>
<td>0.007±</td>
</tr>
<tr>
<td>V</td>
<td>Ca-Withdrawal</td>
<td>1.53±</td>
<td>0.23±</td>
<td>0.17±</td>
</tr>
<tr>
<td></td>
<td>(1 month)</td>
<td>0.02±</td>
<td>0.02±</td>
<td>0.01±</td>
</tr>
<tr>
<td>VI</td>
<td>Castration + CA + TP</td>
<td>-</td>
<td>0.06±</td>
<td>0.06±</td>
</tr>
<tr>
<td></td>
<td>(7 days)</td>
<td>-</td>
<td>0.004±</td>
<td>0.018±</td>
</tr>
<tr>
<td></td>
<td>(15 days)</td>
<td>-</td>
<td>0.09±</td>
<td>0.10±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.001±</td>
<td>0.005±</td>
</tr>
<tr>
<td>VIII</td>
<td>Castration + CA + TP + AA</td>
<td>-</td>
<td>0.07±</td>
<td>0.05±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.01±</td>
<td>0.01±</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
**Free ascorbic acid (AA):**

The levels of free ascorbic acid in caput and cauda of CA treated rats groups II and VI (CA + TP) were either increased or were almost same as in control rats but in testis a significant ($P < 0.001$) decrease was noted. By CA withdrawal, AA levels recovered to control values in testis and cauda but increased ($P < 0.05$) in caput. CA + ascorbic acid administration caused an overall elevation in AA in all organs as compared to those of control (Fig. 1).

**Ascorbigen (ASG):**

In the rats of group II, ASG levels increased in testis (7 days) and caput (7 and 15 days) but were reduced in cauda and testis (15 days). In CA + TP treated castrates, ASG was not detectable in caput and cauda epididymides. ASG increased ($P < 0.001$) in the testis by CA withdrawal but was again not detectable in caput and cauda. No recovery in ASG levels of epididymides was observed by CA + AA treatment, whereas in the testis, an increase ($P < 0.001$) was noted by 15 days (Fig. 1).

**Ascorbic acid utilization (AAU):**

The rate of utilization was by an large increased ($P < 0.02$) by all treatments except that an insignificant decrease was noted in the testis by CA administration (group II) and in cauda by CA withdrawal (Fig. 1).
Ascorbic acid macromolecule (AA-MM) complexing rat:

The rate was on the whole decreased in the testis, caput and cauda by CA administration (group II). In CA + TP treated rats (group VI) however, a decrease by 7 days and a significant (P < 0.001) increase by 15 days was noted in both caput and cauda. CA + AA treatment brought about an overall enhancement in all organs while CA withdrawal (group VII) restored the AA-MM complex rate to control level in testis and caput only (Fig. 1).

Ascorbic acid free radical (AA-FR) forming special peroxidase:

The AA-FR special peroxidase activity decreased in all organs (P < 0.001) by CA treatment to rats of groups II and IV respectively. However, CA + ascorbic acid administration brought about a recovery in enzymic activity of testis and caput. In cauda the level was the same as in the rats of group II. By CA withdrawal, enzymic recovery was noted only in testis (Fig. 1).

Cholesterol:

CA administration to intact rats (group II) brought about an elevation in cholesterol (P < 0.001) except that a decrease was observed in testis and cauda by 15 days. CA + TP administration to castrates also increased (P < 0.001) the cholesterol content of caput (7 and 15 days) and of cauda.
(P < 0.001) (15 days). Similarly, CA + AA treatment for 7 days was found to elevate cholesterol levels in all organs (P < 0.05, < 0.001) in comparison to controls whereas, recovery to control values was noted in cauda and testis by 15 days of treatment (Table II).

**TABLE II**

Cholesterol concentration (mg %) in testis and epididymides of Intact control, Intact + CA, CA+AA, Castration+CA+TP treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Testis</th>
<th>Caput</th>
<th>Cauda</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact control</td>
<td>0.11±</td>
<td>0.10±</td>
<td>0.14±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>II</td>
<td>Intact + CA (7 days)</td>
<td>0.17±</td>
<td>0.13±</td>
<td>0.23±</td>
</tr>
<tr>
<td></td>
<td>(15 days)</td>
<td>0.07±</td>
<td>0.12±</td>
<td>0.12±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.008</td>
<td>0.006</td>
<td>0.007</td>
</tr>
<tr>
<td>IV</td>
<td>CA + AA (7 days)</td>
<td>0.74±</td>
<td>0.15±</td>
<td>0.20±</td>
</tr>
<tr>
<td></td>
<td>(15 days)</td>
<td>0.10±</td>
<td>0.18±</td>
<td>0.16±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>VI</td>
<td>Castration+CA+TP (7 days)</td>
<td>-</td>
<td>0.39±</td>
<td>0.15±</td>
</tr>
<tr>
<td></td>
<td>(15 days)</td>
<td>-</td>
<td>0.16±</td>
<td>0.36±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
Succinate dehydrogenase (SDH):

There was a significant \( P < 0.001 \) decrease in the SDH activity of testis and epididymides in rats of groups II, VI and VII. The administration of ascorbic acid in vivo to rats of groups IV and VIII (CA + AA; Castration + CA + TP + AA) was found to restore the SDH activity significantly \( P < 0.001 \) in epididymides to almost control level. However in testis the enzyme did not recover. Similar results were obtained by CA withdrawal (Fig. 2; Table III).

Since treatment with vehicle (Table III) caused marked changes in SDH activity, normal intact rats were taken as controls instead of vehicle treated rats in further experiments.

Alkaline phosphatase:

The enzymic activity significantly \( P < 0.001 \) decreased in the testis and epididymides in animals of groups II and VI (CA treated and castration + CA + TP) except that an increase was observed in caput (15 days CA treatment) and cauda (7 days CA + TP).

Extraneous administration of ascorbic acid (15 days) to rats of groups IV and VIII brought about a significant \( P < 0.001 \) increase or recovery in enzymic activity of caput and cauda as compared to those of groups II and VI respectively. However, by CA withdrawal no recovery was noted (Fig. 2, Table III).
Acid phosphatase:

The enzyme activity decreased on the whole in the epididymis and testis in animals of groups II and VI respectively (intact + CA; Castration + CA + TP) except that an increase was observed in testis (15 days, group II) and in cauda (7 and 15 days, group II).

Acid phosphatase significantly increased (P < 0.001) in testis and caput of rats treated with CA + AA (group IV) by 15 days but in cauda the enzyme did not recover. The extraneous administration of AA to castrated + TP + CA treated rats (group VIII) was more effective in restoring enzymic levels in both caput and cauda than in rats of group IV. By CA withdrawal (group V) a recovery was noted in testis and cauda but not in caput (Fig. 2, Table III).

Protein:

The levels of protein were by and large increased or else were unaltered in comparison to control except for a decrease in caput by 15 days CA treatment (group II) (Table IV).

Fertility test:

At autopsy, the right uterine horn which received sperm suspension from CA + AA treated animals showed increased vascularization in comparison to the left horn which was inseminated with sperms of CA treated rats. However, fertilized ova were not observed in both the horns.
ESR studies:
A doublet of ascorbic acid free radical, monodehydro-ascorbic acid (MDHA) with a line width of 1.8 gauss and a g value of 2.00 was obtained in cauda epididymal sperm suspensions of control, CA treated and intact + CA + AA treated rats. The spin concentration was significantly higher (P < 0.001) in CA and CA + AA treated rats than in controls (Figs. 3,4).

### TABLE IV

Protein concentration (mg/100 mg fresh tissue weight) in testis, caput and cauda epididymides of control, intact + CA, castration + CA + TP treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Testis</th>
<th>Caput</th>
<th>Cauda</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact control</td>
<td>12.00 ± 0.94</td>
<td>17.15 ± 0.07</td>
<td>22.09 ± 0.50</td>
</tr>
<tr>
<td>II</td>
<td>Intact + CA</td>
<td>20.00 ± 1.00</td>
<td>46.50 ± 6.16</td>
<td>31.50 ± 3.94</td>
</tr>
<tr>
<td></td>
<td>(7 days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(15 days)</td>
<td>15.59 ± 1.54</td>
<td>6.16 ± 0.46</td>
<td>23.08 ± 7.44</td>
</tr>
<tr>
<td>VI</td>
<td>Castration + CA + TP</td>
<td>-</td>
<td>32.15 ± 0.93</td>
<td>22.61 ± 2.99</td>
</tr>
<tr>
<td></td>
<td>(7 days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(15 days)</td>
<td>-</td>
<td>35.65 ± 1.86</td>
<td>39.45 ± 1.49</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
DISCUSSION

From the results it is evident that CA treatment causes a decline in the weights of testis and epididymides, accumulation of cholesterol in testis, a decrease in activities of a number of their androgen dependent enzymes and thereby alters the metabolism of these organs. Roy et al. (1976) and Koch et al. (1976) have also reported inhibitory influence of CA on several parameters of male fertility. These changes are attributed to a decreased androgen synthesis and its circulating levels by CA treatment (Grants and Stitch, 1973; Brotherton and Barnard, 1974; Murray et al., 1975; Roy et al., 1976; Koch et al., 1976), since CA is known to inhibit the release of pituitary LH (Johnson and Naqvi, 1969; Bloch and Davidson, 1969). That CA decreases androgen synthesis is substantiated by the recovery of several androgen sensitive parameters of the human semen in volunteers who were administered androgen along with CA (Roy et al., 1976).

Since the physiological integrity of epididymides depends on the levels of circulating androgens as well as the normal flow of testicular fluid with sperms (Gustafsson, 1966; Rajalakshmi and Prasad, 1971), androgen deprivation by CA alters the internal milieu of epididymis and renders it hostile
for survival, motility and fertilizing ability of spermatozoa (Prasad et al., 1970; Rajalakshmi et al., 1971; Prasad et al., 1973; Gupta et al., 1974; Arora et al., 1975). Therefore, the 40% sluggishly motile cauda epididymal spermatozoa in CA treated rats failed to impregnate the females (Buch, 1976; this study). This is in conformity with the work of Frasad and his collaborators, Roy et al. (1976) and Koch et al. (1976) and elucidate that, alteration in the physiological milieu of epididymis via androgen deprivation is one of the important anti-androgenic effects of CA. However, the data on CA withdrawal treatment suggests that the alterations are transient and reversible within one month of cessation of treatment (group V), in agreement with the work of others (Prasad, 1971/1972; Prasad, 1973a; Rajalakshmi et al., 1971). On the other hand, Schenck et al. (1975), Elger and Von-Berswordt-Wallrabe (1973) and Back and Shenton (1976) have failed to induce infertility by CA in rats, whereas Buch (1976) reported a more pronounced inhibitory effect of CA on epididymal sperm motility than on their metabolic turnover. This discrepancy may be due to the different doses, modes of administration of CA with or without a particular vehicle, the age and strain of animals, duration of the study and different androgen sensitivity of various reproductive organs and their biochemical constituents (Rajalakshmi and Prasad, 1975).
An increase in acid phosphatase activity and protein levels in cauda epididymis especially in animals of group II, is probably indicative of enhanced phagocytosis of spermatozoa (Linnetz and Amann, 1968) and increased synthesis of specific lysosomal enzymes which are involved in the autolytic process (Rajalakshmi and Prasad, 1975). Buch (1976) also observed a reduction in the epididymal sperm counts in CA treated rats indicating active sperm disposal.

Increased mobilization and utilization of ascorbic acid (AA) after CA treatment is probably in response to the decreased androgen levels and the concurrent increase in histamine (Assaykeen and Thomas, 1965; Orr and Quay, 1975). The role of AA in the detoxification of histamine in androgen deprived castrated rats and the inverse relationship between histamine and testosterone have been elucidated in Chapter I. The enhanced utilization of AA occurs via the formation of its free radical, monodehydro ascorbic acid (MDHA) (Figs. 3, 4), which by virtue of possessing unpaired electrons is an important source of electron energy for various oxido-reduction reactions such as: (i) steroidogenesis in gonads and adrenals by the oxidation of cholesterol and stimulation of hydroxylases involved in hormonogenesis (Fisher, 1962; Guchhait et al., 1963; Ghosh and Bhattacharyya, 1967; Sih and Whitlock, 1968; Biswas and Deb, 1970; Chinoy, 1970a, b; 1971, 1972 a, b; 1973;
Kutsky, 1973; Carballeiva, et al., 1974), (ii) for maintaining sperm metabolism (Chinoy and Buch, 1976) and (iii) the secretory activity of accessory reproductive organs (Sheth, 1976).

The recovery or an increase of most of the androgen dependent parameters together with increase in MDA concentration of testis and epididymis in CA treated rats fed ascorbic acid in vivo (groups IV, VIII), suggests the restoration of androgen levels, epididymal milieu and testicular androgenicity. Similarly, the secretory activity of sex accessory glands and metabolism of spermatozoa were restored in CA + AA treated animals but not the sperm motility (Sheth, 1976; Buch, 1976). It is evident from this data that the androgen levels are restored in CA + AA treated rats indirectly by ascorbic acid and its involvement in steroidogenesis and by the direct administration of androgen to CA treated male volunteers in the study of Roy et al. (1976). That ascorbic acid brings about the recovery of androgen sensitive enzymes by its synergistic action with testosterone in increasing anabolic activity (Kutsky, 1973; Chapter I), cannot be ruled out. It is apparent from the foregoing data that ascorbic acid has a beneficial and protective role in maintaining the testicular and epididymal functions in CA treated rats with no improvement in the CA-induced decreased motility and fertilizing ability of spermatozoa. It is therefore suggested.
that an extraneous dose of ascorbic acid be administered to human volunteers on CA treatment in order to make the contraceptive purpose of CA more efficacious.

In conclusion, (i) the transient alterations brought about in testicular and epididymal functions by CA are due to changes in androgen levels and are reversible, (ii) although CA affects both the endogenously produced and exogenously administered androgens, a more pronounced effect was observed on the latter, (iii) the effects of ascorbic acid administration to CA treated rats were comparatively more significant than those of CA withdrawal, since (iv) ascorbic acid not only restores the androgen levels via the formation of its free radical MDHA which is involved in steroidogenesis, but also by its synergistic action with testosterone.

SUMMARY

The effects of an anti-androgen, cyproterone acetate (CA) on the metabolism of the testis and epididymides of sexually mature albino rats were investigated. This study indicates that CA inhibits the anabolic affects of endogenously produced and exogenously administered androgens. These effects were transient and were reversible within one month after withdrawal of CA treatment. The overall physiological integrity of the epididymides was not affected by CA due to
protective and beneficial influence of ascorbic acid and its free radical via their utilization and involvement in various biosynthetic reactions including steroidogenesis in the testis. This hypothesis is substantiated by electron spin resonance spectrometric studies.
REFERENCES


11. Chapter I. Synergistic action of testosterone and ascorbic acid for maintenance of epididymal functions.

12. Chapter IX. Hormonal control of tissue distribution and metabolism of ascorbic acid in male rats.


TABLE III

Activities of SDH (μg formazan formed/4 hrs/100 mg fresh tissue weight), alkaline and acid phosphatases (U/l) in testis and epididymides of intact control, castrated + CA + TP, Castrated + CA + TP + AA, Intact control + vehicle, castrated + Vehicle + TP treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intact control</th>
<th>Castrated + CA + TP (15 days)</th>
<th>Castrated + CA + TP + AA (15 days)</th>
<th>Intact control + Vehicle (15 days)</th>
<th>Castrated + Vehicle + TP (15 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>326.77± 29.76</td>
<td>-</td>
<td>-</td>
<td>168.67± 1.41</td>
<td>-</td>
</tr>
<tr>
<td>Caput</td>
<td>199.00± 5.59</td>
<td>ND</td>
<td>135.75± 10.15</td>
<td>55.40± 3.91</td>
<td>119.05± 14.31</td>
</tr>
<tr>
<td>Cauda</td>
<td>249.00± 15.05</td>
<td>150.70± 13.93</td>
<td>339.80± 18.93</td>
<td>126.40± 2.21</td>
<td>190.35± 7.57</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caput</td>
<td>82.43± 35.35</td>
<td>95.50± 4.69</td>
<td>310.40± 5.36</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cauda</td>
<td>217.66± 6.89</td>
<td>117.50± 8.00</td>
<td>256.10± 43.84</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caput</td>
<td>898.66± 21.24</td>
<td>501.00± 81.70</td>
<td>839.63± 20.70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cauda</td>
<td>612.58± 44.95</td>
<td>296.40± 22.40</td>
<td>649.05± 38.57</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The values are mean ± S.E. ND = Not detectable
Fig. 1

The concentrations of free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU), ascorbic acid macromolecule (AA-MM) complexing and ascorbic acid free radical (AA-FR) forming special peroxidase activity in testis, caput and cauda epididymides of rats.

ND = not detectable
1 = normal intact control
2 = cyproterone acetate (CA) treated - 7 and 15 days
3 = CA withdrawal - 30 days
4 = CA + ascorbic acid (AA) treated - 7 and 15 days
5 = Castration + CA + testosterone propionate (TP) treated - 7 and 15 days
TESTIS CAPUT CAUDA

AA-FR SPECIAL
AA-MMC AAU

PEROXIDASE
PEROXIDASE ACTIVITY/20 MIN.

AA

ASG

Mg/gm. FR. TISSUE Wt.

AA-U

Mg/gm. FR. TISSUE Wt./HOUR

AA-VMC

Mg/gm. FR. TISSUE Wt./20 MIN.

AA-FR SPECIAL

PEROXIDASE ACTIVITY/20 MIN.

TESTIS 1 2 3 4 5
CAPUT 1 2 3 4 5
CAUDA 1 2 3 4 5

CONTROL CA-REC

7 DAYS
15 DAYS
The activities of succinate dehydrogenase (SDH), alkaline and acid phosphatases in testis, caput and cauda epididymides of rats

ND = not detectable

1 = Normal intact control
2 = CA treated - 7 and 15 days
3 = CA withdrawal - 30 days
4 = CA + AA treated - 7 and 15 days
5 = Castration + CA + TP treated - 7 and 15 days
Fig. 3

Spin concentration of ascorbic acid free radical, monodehydroascorbic acid in cauda epididymal sperm suspensions of control, CA and CA + AA treated rats.
A doublet of monodehydroascorbic acid in cauda epididymal sperm suspensions of control, CA and CA + AA treated rats obtained by Electron Spin Resonance Spectrometry.

Fig. 4