CHAPTER III A

METABOLIC SIGNIFICANCE OF ASCORBIC ACID IN VASECTOMIZED RATS

INTRODUCTION

Vasectomy is one of the most widely used contraceptive methods in males especially in India, since it involves a simple operative procedure and no hospitalization. However, there are conflicting reports regarding its effects on the male reproductive system. Studies on rodents, dogs, bulls, and human beings have revealed that vasectomy has no adverse effects on pituitary weight, gonadotrophin activity, histology of genital organs, spermatogenesis, sperm metabolism, androgenicity of testes, secretory functions of accessory organs and fertility (Kar et al., 1965; Flickinger, 1972, 1973, 1975; Ewarts and Coffey, 1973; Basterday et al., 1973; Welland et al., 1972; Lohiya and Dixit, 1974; Johnsonbaugh et al., 1975; Neaves, 1974, 1975; Meenakshi et al., 1976; Bedford, 1976; Sheth, 1976; Buch, 1976). On the contrary, several other workers have reported a decline in organ weights, transitory degenerative changes in testis, structural changes in spermatocytes and epididymis, disturbances in spermatogenesis, reduced sperm counts, a decline in androgen levels concomitant with a reduction in the amount of urinary 17-ketosteroids and the formation of spermatic granuloma in the epididymis and vas deferens at
the site of ligation (Laumas and Uniyal, 1967; Kubota, 1969; Igboeli and Rakha, 1970; MacMillan et al., 1968; Alexander, 1972, 1973; Sacher and Schilling, 1973; Vare and Bansal, 1973; Sackler et al., 1973; Brueschke et al., 1974; Deerick et al., 1974; Kinson and Layberry, 1975; Chatterjee, et al., 1976). Significant quantitative changes in some of the contents of human seminal plasma in vasectomized males have also been reported (Mun et al., 1972; Gregoire and Moran, 1973; Brummer, 1973). Procedural variations, different species, age of the animal and duration of operation have been suggested as responsible for the differences in the results obtained (Flickinger, 1972), whereas Neaves (1974) suggested that factors other than procedural differences were responsible for the controversial data. Meenakshi et al. (1976) have shown that conventional and defective vasectomy did not affect the pituitary weight, its gonadotrophin activity or urinary 17-keto steroids.

The protective and beneficial role of ascorbic acid (AA) in rat and guinea pigs for maintaining the metabolism of their testis, epididymis, spermatozoa and secretory functions of sex accessory organs via the involvement of the free radical of ascorbic acid, monodehydroascorbic acid has been highlighted earlier (Geth, 1976; Buch, 1976; Chinoy unpublished observations; Chapter II). The present study was therefore an attempt to
elucidate and substantiate the role of AA in vasectomized rats by biochemical and electron spin resonance studies.

**MATERIALS AND METHODS**

Healthy, adult male albino rats (*Rattus norvegicus*) of the Holtzman strain, weighing about 200-250 gm and maintained at a temperature of 26±2°C and 14 day light hours were utilized for the experiments. The animals were supplied with standard food (Hindustan Lever Ltd., Bombay) and water *ad libitum*.

**Surgical treatments:**

The animals were vasectomized by scrotal incision under light ether anaesthesia using semisterile conditions. The vas deferens was exposed, severed at the proximal part and the cut ends were ligated separately with silk thread in order to prevent recanalization. The experimental animals were distributed 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>Bilaterally vasectomized for 10, 20, 30 and 48 days</td>
<td>48</td>
<td>11th, 21st, 31st and 49 day respectively</td>
</tr>
<tr>
<td>III</td>
<td>Bilaterally vasectomized and given ascorbic acid extraneously (100 mg orally/animal/day) for 10, 20 days respectively</td>
<td>48</td>
<td>11th and 21st day respectively</td>
</tr>
</tbody>
</table>
The animals were autopsied after the respective treatments and their testis and epididymides (Caput and Cauda) were excised, blotted free of blood, cleared of fat body and connective tissue and weighed. Sperm suspensions were prepared from testis and cauda epididymis and used for assessing sperm numbers and motility according to the method of Prasad et al. (1972). The following estimations were carried out as per details described in Chapter I.

Free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU) and ascorbic acid-macromolecule complexing (AA-MM) : (Chinoy et al., 1974).

Ascorbic acid free radical (AA-PR) forming special peroxidase: (Chinoy, 1973).

Cholesterol : (Pearson et al., 1953).

Succinate dehydrogenase (SDH) : (Kun and Abood, 1949).

Alkaline and acid phosphatase : (Belfield and Goldberg, 1971).

Protein : (Gornall et al., 1949).

Electron spin resonance studies (ESR):

Weighed samples of cauda epididymis from the rats of groups I to III were used and the sperm suspensions were prepared. One ml aliquots of the sperm suspension were transferred to a standard quartz flat cell (E-248) and the ESR
spectra were recorded on a Varian E-4 Spectrometer at a
temperature of 25±2°C. The following spectrometer settings
were maintained constant throughout the experiments.

Field set: 3390 gauss
Scan range: 40 gauss
Scanning time: 30 minutes
Modulation frequency: 100 gauss
Receiver gain: 6.03 x 10^4
Microwave power: 10 dB.

Under the above constant spectrometric settings the
AA-FR 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 the students 't' test.

RESULTS

Sperm counts:

In 10, 20, 30 days post vasectomized rats, sperm counts
were decreased significantly (P < 0.001) as compared to
controls but by 48 days and by administration of ascorbic acid
to vasectomized rats, recovery occurred to normal level in
testicular sperm suspension only (Table I).
Motility:

50% and 21% motile sperms (as compared to 80% in normal) were observed in 10 and 48 days vasectomized animals, whereas at 20 and 30 days no motile sperms were present. Ascorbic acid administration to vasectomized rats brought about a 10% recovery in number of motile spermatozoa by 20 days (Table I) in comparison to 10 days and later stages of vasectomy.

**TABLE I**

Sperm counts (million/ml suspension) and motility (%) of control, vasectomized and vasectomized + ascorbic acid (AA) treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intact Control</th>
<th>Vasectomized</th>
<th>Vasectomized + AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 days</td>
<td>20 days</td>
<td>30 days</td>
</tr>
<tr>
<td>Testicular sperm</td>
<td>7.00±</td>
<td>3.00±</td>
<td>5.00±</td>
</tr>
<tr>
<td>suspension</td>
<td>1.00</td>
<td>1.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Cauda epididymal</td>
<td>60.00±</td>
<td>31.00±</td>
<td>33.00±</td>
</tr>
<tr>
<td>sperm suspension</td>
<td>6.00</td>
<td>4.00</td>
<td>1.80</td>
</tr>
<tr>
<td><strong>Motility (%)</strong></td>
<td>80</td>
<td>50</td>
<td>No motile sperms</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
A reduction in the weights of testis and epididymides occurred throughout the period of vasectomy. The decrease was significant \( (P < 0.001) \) at 30 days in testis. Administration of ascorbic acid to vasectomized rats (10, 20 days) brought about an almost complete recovery in organ weights (Table II).

**TABLE II**

Weights (in gm) of testis and epididymides of intact control, vasectomized and vasectomized + AA treated rats

<table>
<thead>
<tr>
<th>Organs</th>
<th>Intact control</th>
<th>Vasectomized</th>
<th>Vasectomized + AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 days</td>
<td>20 days</td>
<td>30 days</td>
</tr>
<tr>
<td>Testis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caput</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.15</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.

**Free ascorbic acid (AA):**

The levels of free ascorbic acid were on the whole increased or remained same as in normal in all the organs investigated. Administration of AA to vasectomized animals
brought about a significant ($P < 0.05$) recovery and or increase ($P < 0.001$) in the levels of free AA in all tissues (Fig. 1).

**Ascorbigen (ASG):**

ASG levels increased ($P < 0.001$) in testis (10, 30 days) and caput (10 days) but decreased in cauda. Ascorbigen levels recovered to normal values or increased in all tissues of vasectomized animals fed ascorbic acid except that it was not detectable in cauda by 20 days (Fig. 1).

**Ascorbic acid utilization (AAU):**

The rate of AA utilization increased ($P < 0.01$) in all tissues in rats of group II. However, the increase was more pronounced in epididymis than in the testis. Administration of ascorbic acid to vasectomized rats (Group III) brought about a recovery or increase ($P < 0.02$) in AAU.

**Ascorbic acid macromolecule complex (AA-MM):**

The rate of AA-MM complexing increased ($P < 0.02$) in testis and epididymis by vasectomy. However, by ascorbic acid administration to vasectomized animals AA-MM complex recovered or increased ($P < 0.02$) than in normal (Fig. 1).

**AA-FR special peroxidase:**

The activity of AA-FR special peroxidase decreased ($P < 0.05$) in testis, epididymis by 10 days of vasectomy. By 20 and 30 days
it increased as compared to 10 days. Ascorbic acid administration to vasectomized rats (20 days) restored the enzymic level in all tissues in comparison to 10 days of treatment (Fig. I).

**Cholesterol:**

The levels of cholesterol increased on the whole in all tissues of vasectomized animals ($P < 0.001$). However, in rats of group III (vasectomized + AA); its concentrations were by and large restored or increased (Table III).

**TABLE III**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Intact control</th>
<th>Vasectomized</th>
<th>Vasectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 days</td>
<td>20 days</td>
<td>30 days</td>
</tr>
<tr>
<td>Testis</td>
<td>0.11 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Caput</td>
<td>0.10 ± 0.01</td>
<td>0.14 ± 0.001</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.14 ± 0.03</td>
<td>0.25 ± 0.02</td>
<td>0.23 ± 0.02</td>
</tr>
</tbody>
</table>

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

The concentration of SDH decreased ($P < 0.001$) after 10 days of vasectomy in testis, and epididymis but by 20 and 30 days the enzymic levels were elevated in comparison to those of 10 days. Administration of ascorbic acid to vasectomized rats restored the enzymic activity significantly ($P < 0.01$) in epididymis, whereas, in the testis no recovery was observed (Fig. 2).

Alkaline phosphatase:

The activity of alkaline phosphatase on the whole increased ($P < 0.02$) in all organs throughout the study except by 20 days. The extraneous feeding of ascorbic acid to vasectomized rats brought about a recovery except that in caput a decrease was observed (Fig. 2).

Acid phosphatase:

The activity of acid phosphatase was on the whole ($P < 0.001$) increased throughout the experimental period except that decrease ($P < 0.02$) was observed in epididymides by 20 days. Ascorbic acid administration to vasectomized animals brought about a recovery in enzyme activity in testis and cauda but not in caput (Fig. 2).

Protein:

Protein levels remained unaltered by 10, 20 and 30 days of vasectomy. Ascorbic acid administration increased ($P < 0.02$)
the protein concentration in the epididymides but not in testis (Fig. 2).

Electron spin resonance study:

A doublet of MREIA with a line width of 1.8 gauss and a g-value of 2.004 was obtained in cauda epididymal sperm suspensions of rats of groups I to III respectively. The free radical concentration was found to be higher in epididymal sperm suspensions of both groups II and III in comparison to those of group I (control) (Figs. 3, 4).

DISCUSSION

The results of the present study revealed an overall reduction in a number of androgen sensitive parameters, such as weights of testis and epididymides, SDH and AA-ER special peroxidase activities, diminished sperm counts and percentage of motile spermatozoa during the initial period (10 and 20 days) of vasectomy. However, since within 30 days a recovery in most of these parameters was observed, is suggestive of a transient reduction in circulating levels of testosterone and androgenicity of testis during the early period of vasectomy. The results also show that vasectomy did not affect spermatogenesis since normal sperm counts were noted by 48 days of vasectomy, although motility did not recover (Buch, 1976). This study decreased sperm counts in vasectomized rats (group II).
together with an enhanced acid/alkaline phosphatase activity indicate resorption of non-motile spermatozoa (Linnets and Amann, 1968) and their ingestion by leucocytes (Bedford, 1976).

It is clear from the results that ascorbic acid utilization is increased in the testis and epididymides concurrent with an active mobilization of the bound ascorbiger to the free form. The utilization occurs by the enhanced formation of ascorbic acid free radical, monodehydroascorbic acid (MDHA), (as shown by biochemical and ESR data), which by virtue of possessing an unpaired electron, has stronger reducing properties than AA and is known to participate in several oxide-reduction reactions including steroidogenesis in testis and adrenals and cholesterol oxidation (Fisher, 1962; Guchhait et al., 1963; Biswas, 1969; Biswas and Deb, 1970; Kutsky, 1973), as a source of electron energy (Kern and Racker, 1954; Yamazaki et al., 1959; Staudinger et al., 1961; Gorbunova, 1956; Chinoy, 1970a, b; 1971, 1972 a, b; 1973). It is therefore suggested that the overall increase in AA turnover in earlier stages (10 and 20 days) of vasectomy is probably a response to overcome the effects of stress (Selye, 1950) and to restore the metabolism of testis, epididymis, their spermatozoa and testicular androgenicity within 30 days via the involvement of ascorbic acid in androgenesis (Biswas, 1969; Biswas and Deb, 1970; Kutsky, 1973; Datta and Sanyal, 1976). The recovery of almost all androgen sensitive parameters including cholesterol
levels together with the enhanced rate of formation of ascorbic acid-free radical, MDHA (ESR data) in vasectomized rats fed ascorbic acid extraneously (Group III) for 10 and 20 days, substantiates the important metabolic significance of ascorbic acid indirectly by (i) increasing androgenicity via its role in steroidogenesis or by (ii) a direct activation of enzymes (Harrer and King, 1941; Sobrell and Harris, 1967; Kutsky, 1973) and due to (iii) the synergistic action of AA with endogenous testosterone in increasing the anabolic activity (Buch, 1976; Kutsky, 1973) and maturation of testicular germ cells (Kutsky, 1973). A similar beneficial influence of vitamin C in maintaining the secretory activity of accessory reproductive glands of vasectomized rats fed AA has been elucidated by Sheth (1976).

It is evident from this study that vasectomy produces no adverse effects on the reproductive functions in agreement with the observations of others (Weiland et al., 1972; Easterday et al., 1973; Johnsonbaugh et al., 1975; Meenakshi et al., 1976; Sath, 1976; Buch, 1976), as ascorbic acid has a beneficial role in restoring and maintaining the physiological integrity of testis, epididymis and their spermatozoa. It is therefore suggested that ascorbic acid be administered to vasectomized human males in countries like India where mass scale vasectomies are carried out. This simple inexpensive treatment may prove useful in avoidance of most of the
disturbances in the physiology of reproductive organs of operated volunteers and will make the contraceptive purpose of vasectomy more efficacious, meaningful and applicable.

SUMMARY

Biochemical and electron spin resonance (ESR) spectroscopic investigations were carried out to study the effects of short term bilateral vasectomy and the administration of ascorbic acid to vasectomized rats on the metabolism of the testis, epididymides (Caput and cauda) and their sperm suspension. The biochemical and ESR data suggest a beneficial role of ascorbic acid in (i) restoration of the altered metabolism of the reproductive organs and spermatozoa and (ii) maintenance of their functional status by suppressing the metabolic disturbances in vasectomized animals, thereby making the contraceptive purpose of vasectomy more effective and applicable.
REFERENCES


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

10. Chapter II. Effects of an anti-androgen, cyproterone acetate on metabolism of testis and epididymides of male albino rats.


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-RE) forming special peroxidase activity in testis, caput and cauda epididymis

ND = not detectable
1 = Normal intact control
2 = Bilaterally Vasectomized -
   10, 20 and 30 days respectively
3 = Bilaterally vasectomized + ascorbic acid treated - 10 and 20 days respectively
Fig. 2

The activities of succinate dehydrogenase (SDH), alkaline, acid phosphatases, and protein levels in testis, caput and cauda epididymis

1 = Normal intact control
2 = Bilaterally vasectomized -
   10, 20 and 30 days respectively
3 = Bilaterally vasectomized + ascorbic acid treated - 10 and 20 days respectively
- CONTROL
S3 IO DAYS
GO 20-DAYS
G3J 30 DAYS
20 T
IO |
O ....

TESTIS

CAPUT

CAUDA

HRS

SDH

\( \frac{\text{Formazan Formed/100 mg Fr. Tissue Weight}}{\text{Hrs}} \)

ACID ALKALINE PHOSPHATASES

\( \frac{\text{Units}}{100 \text{ mg Fr. Tissue Weight}} \)

PROTEIN

\( \frac{\text{mg/100 mg Fr. Tissue Weight}}{100 \text{ mg Fr. Tissue Weight}} \)

CONTROL
20 DAYS
10 DAYS
30 DAYS

1 2 3
TESTIS

1 2 3
CAPUT

1 2 3
CAUDA
Fig. 3

Spin concentration of ascorbic acid free radical, monodehydroascorbic acid in cauda epididymal sperm suspensions of control, vasectomized and vasectomized + ascorbic acid (AA) treated rats.
Fig. 4

A doublet of monodehydroascorbic acid in cauda epididymal sperm suspensions of control, vasectomized, and vasectomized + AA treated rats obtained by E.S.R. spectrometry.
INTRODUCTION

Unilateral or bilateral vas ligation or occlusion does not affect the spermatogenic function of testis, its weight or Leydig cell structure (Swanson and Hafs, 1969; Segal, 1972; Poddar et al., 1973). Moreover, increased androgenicity of testis and enhanced fructose, alkaline phosphatase content of accessory sex organs were observed (Thakur et al., 1972; Chinoy et al., 1973, 1974; Poddar et al., 1973; Sheth, 1976). On the contrary, degenerative changes of seminiferous tubules, loss of sperm motility and fertilizing ability, changes in the epididymal milieu and decreased urinary 17-ketosteroid output occur indicating impaired androgenesis due to occlusion or ligation of the vas (Glover, 1962; Smith, 1962; Barack, 1968; Igboeli and Foote, 1968; Prasad et al., 1972; Lubicz-Nawrocki et al., 1973; Sackler et al., 1973; Vare and Bansal, 1974). All these controversial data on the effects of ligation or occlusion of the vasa efferentia and vas deferens on reproductive functions prompted a reinvestigation into the effects of bilateral vasa efferentia ligation (VEL), vas deferens ligation (VDL) and total ligation (VDL+VEL) on some androgen dependent enzymes and metabolites of testis and
epididymides.

MATERIAL AND METHODS

Healthy, adult albino rats (*Rattus norvegicus*) weighing between 200-250 gm were utilized throughout the experiments. They were maintained on standard feeds (Hindustan Lever Ltd., Bombay) and water *ad libitum* in an air conditioned animal house (temperature 26±2°C) with 14 hours day light. The animals were divided into the following groups:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>No. of animals used</th>
<th>Day of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact normal control</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Bilaterally vasoligated (VDL)</td>
<td>12</td>
<td>11th</td>
</tr>
<tr>
<td>III</td>
<td>Bilaterally vasa efferentia ligated (VEL)</td>
<td>12</td>
<td>11th</td>
</tr>
<tr>
<td>IV</td>
<td>Bilaterally VDL+VEL (total) ligated</td>
<td>12</td>
<td>11th</td>
</tr>
</tbody>
</table>

Surgical procedure:

The rats of groups II to IV were operated under mild ether anaesthesia by scrotal approach. The vas deferens, vasa efferentia or both were ligated as required. The ligature was placed as close to the origin of vasa efferentia as possible in the case of VEL and VDL+VEL. The vas deferens was ligated at its proximal portion.
After the respective treatments the animals were autopsied by cervical dislocation. The testis, caput and cauda epididymides were removed, blotted free of blood, weighed and used for biochemical studies as per details described in Chapter I.

Free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU) and ascorbic acid macromolecule complexing (AA-MM): (Chinoy et al., 1974).

Ascorbic acid free radical (AA-FR) forming special peroxidase. (Chinoy, 1973).

Succinate dehydrogenase (SDH): (Kun and Abood, 1949).

Alkaline and acid phosphatases: (Belfied and Goldberg, 1971).

Protein: (Gornall et al., 1949).

A minimum of six replicates were done for each parameter and treatment. The results were analyzed statistically by student's 't' test.

RESULTS

Organ weights:

No change in the weights of the organs was noted by VDL and VEL. On the contrary, the weights of cauda and caput decreased \( (P < 0.001) \) in totally (VDL + VEL) ligated rats in comparison to control (Table I).
Weights (in gm) of testis and epididymides of normal, VDL, VL, and VDL + VEL ligated rats

<table>
<thead>
<tr>
<th>Organs</th>
<th>Normal</th>
<th>VDL</th>
<th>VEL</th>
<th>VDL + VEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>1.38± 0.01</td>
<td>1.62± 0.06</td>
<td>1.49± 0.08</td>
<td>1.39± 0.10</td>
</tr>
<tr>
<td>Caput</td>
<td>0.20± 0.01</td>
<td>0.20± 0.03</td>
<td>0.19± 0.002</td>
<td>0.10± 0.01</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.15± 0.02</td>
<td>0.17± 0.02</td>
<td>0.21± 0.02</td>
<td>0.08± 0.01</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.

Free ascorbic acid (AA):

No alteration in the levels of free AA were observed in testis by the three treatments. In epididymis, the levels of free AA increased by VEL and VDL + VEL, but declined by VDL (Fig. 1).

Ascorbigen (ASC):

The levels of ascorbigen decreased significantly (P < 0.001) in all the organs of rats of groups II to IV as compared to those of group I (Fig. 1).

Ascorbic acid utilization (AAU) and ascorbic acid macromolecules (AA-MM) complexing rates:

The rates of ascorbic acid utilization and AA-MM complex...
were not affected by all treatments in the testis and cauda, whereas in caput it was increased \((P < 0.001)\) (Fig. 1).

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

There was an overall significant decrease \((P < 0.001)\) in the enzymic activity in all three organs throughout the study. The decrease was more pronounced by VDL (Fig. 1).

**Succinate dehydrogenase (SDH):**

A significant \((P < 0.001)\) decrease in the activity of SDH was observed in all the three organs throughout the study. However, the effects of VEL and VDL + VEL were more pronounced on cauda epididymal SDH activity (Fig. 2).

**Alkaline phosphatase:**

An overall reduction in the levels of alkaline phosphatase was noted in testis. However, the decrease was more pronounced by VDL + VEL \((P < 0.05)\). On the contrary, there was an activation of enzyme in the epididymides except that a slight decrease was noted in cauda by VEL (Fig. 2).

**Acid phosphatase:**

The enzymic activity was decreased in all the three organs throughout the study except that an increase was noted in the testis and cauda by VDL (Fig. 2).

**Protein:**

A decrease in the protein concentration was found in the testis by VDL and VEL and in cauda by all surgical treatments.
However, protein levels were elevated in epididymis especially by VDL and VDL + VEL (Fig. 2).

DISCUSSION

It has been reported earlier (Chapter III A) that short term bilateral vasectomy had no permanent adverse effects on testicular and epididymal metabolism except that some transient metabolic disturbances were observed during the initial stages. However, ligation at different levels of epididymis is claimed to cause testicular damage associated with disruption of spermatozoa and alterations in epididymal function (Glover, 1962; Smith, 1962; Prasad et al., 1972; Buch, 1976). In the present study also, a decrease in a number of androgen sensitive parameters (SDH, protein, acid phosphatase and AA-FR special peroxidase) was observed in all the experimental groups which suggests: (i) the impaired function and hormonal activity of testis, in conformity with the work of Sackler et al. (1972) and (ii) an alteration in the epididymal milieu due to the absence of normal flow of testicular fluid with sperms as in VEL and total ligation. However, the weights of the organs remained either almost same as in normal or insignificantly increased which may be due to elevated hydrostatic pressure (Sackler et al., 1973) or as a consequence of differential sensitivity of the different androgen sensitive parameters to androgen levels.
An enhanced alkaline phosphatase (present study) especially in the epididymides suggests the increased sperm absorption (Linnetz and Amann, 1968) and therefore explains the decrease/absence of spermatozoa in all these conditions (Buch, 1976).

The ascorbic acid metabolism was on the whole enhanced with greater mobilization and utilization of the bound form via non-enzymic pathway. The significance of enhanced ascorbic acid turnover has been highlighted in Chapters I, II and III A.

In conclusion, the present study shows that VEL and VDL+VEL have more pronounced inhibitory effects than VDL on the metabolism of testis and epididymides. However, Thakur et al. (1972) and Sheth (1976) did not observe decreased secretory activity of accessory reproductive organs in ligated rats. This discrepancy may be due to the higher threshold requirement of androgens for the maintenance of the physiological integrity of testis and epididymides than those needed by the sex accessories (Prasad et al., 1973; Gupta et al., 1974; Karkun et al., 1974; Dinakar et al., 1974 a, b; Sheth, 1976).

SUMMARY

The metabolism of testis, caput and cauda epididymides were significantly altered in vas deferens ligated (VDL), vasa efferentia ligated (VEL) and totally ligated (VDL+VEL) rats, although their weights were unaltered. The enhanced ascorbic acid metabolism especially in the epididymides in conditions such as VEL and VDL + VEL may be a response of the organs to overcome the stress due to ligation.
REFERENCES


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


5. Chapter III A. Metabolic significance of ascorbic acid in vasectomized rats.


The concentrations of free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU), ascorbic acid macromolecule (AA-MM) and ascorbic acid free radical (AA-FR) forming special peroxidase activity in testis, caput, and cauda epididymis of rats
ND = not detectable
1 = Normal intact control
2 = Bilaterally vas deferens ligated (VDL) - 10 days
3 = Bilaterally Vasa efferentia ligated (VEL) - 10 days
4 = Totally ligated (VDL + VEL) - 10 days

AA-FR.SPECIAL AA-MMC AAU ASG A A
£o
A U  CONTROL 
H3 VDL 
E  VEL  
£ 2 3 VDL+VEL
*lx.og
o 
o
Ao 
o 
«
0 ... 
(Si
O  O
Mg/gm FR.TISSUE Wt./HOUR Mg/glTl FR. TISSUE Wfc
PEROXIDASE ACTIVITY/<
IT] 
FR.TISSUE Wfc ./ 20 MiN.

AA-FR.SPECIAL AA-MMC AAU ASG A A
£o
A U  CONTROL 
H3 VDL 
E  VEL  
£ 2 3 VDL+VEL
*lx.og
o 
o
Ao 
o 
«
0 ... 
(Si
O  O
Mg/gm FR.TISSUE Wt./HOUR Mg/glTl FR. TISSUE Wfc
PEROXIDASE ACTIVITY/<
IT] 
FR.TISSUE Wfc ./ 20 MiN.

AA-FR.SPECIAL AA-MMC AAU ASG A A
£o
A U  CONTROL 
H3 VDL 
E  VEL  
£ 2 3 VDL+VEL
*lx.og
o 
o
Ao 
o 
«
0 ... 
(Si
O  O
Mg/gm FR.TISSUE Wt./HOUR Mg/glTl FR. TISSUE Wfc
PEROXIDASE ACTIVITY/<
IT] 
FR.TISSUE Wfc ./ 20 MiN.

AA-FR.SPECIAL AA-MMC AAU ASG A A
£o
A U  CONTROL 
H3 VDL 
E  VEL  
£ 2 3 VDL+VEL
*lx.og
o 
o
Ao 
o 
«
0 ... 
(Si
O  O
Mg/gm FR.TISSUE Wt./HOUR Mg/glTl FR. TISSUE Wfc
PEROXIDASE ACTIVITY/<
IT] 
FR.TISSUE Wfc ./ 20 MiN.

AA-FR.SPECIAL AA-MMC AAU ASG A A
£o
A U  CONTROL 
H3 VDL 
E  VEL  
£ 2 3 VDL+VEL
*lx.og
o 
o
Ao 
o 
«
0 ... 
(Si
O  O
Mg/gm FR.TISSUE Wt./HOUR Mg/glTl FR. TISSUE Wfc
PEROXIDASE ACTIVITY/<
IT] 
FR.TISSUE Wfc ./ 20 MiN.
The activities of succinate dehydrogenase (SDH), alkaline, acid phosphatases and protein levels in testis, caput and cauda epididymis of rats
1 = Normal intact rats
2 = Bilaterally vas deferens ligated (VDL) - 10 days
3 = Bilaterally vasa efferentia ligated (VEL) - 10 days
4 = Totally ligated (VDL + VEL) - 10 days