CHAPTER IV

EFFECTS OF VITAMIN C DEFICIENCY ON THE METABOLISM OF TESTIS AND EPIDIDYMIS IN GUINEA PIGS

INTRODUCTION

A higher level of ascorbic acid has been correlated with greater metabolic turnover of a tissue including those of the reproductive, since ascorbic acid participates in various biosynthetic reactions as a source of electron energy via the formation of its free radical, monodehydroascorbic acid (Chinoy, 1969, 1970 a, b; 1971, 1972 a, b; 1973; Chinoy et al., 1974 a, b; Sheth, 1976; Kshatriya, 1976; Buch, 1976; Chapters II, IIIA). The role of monodehydroascorbic acid in steroidogenesis through its involvement in the cholesterol metabolism of glands and adrenals has been demonstrated (Fisher, 1962; Guchhait et al., 1963; Biswas, 1969; Biswas and Deb, 1970; Charballeiva et al., 1974). In animals such as guinea pigs which depend on the dietary source of ascorbic acid (Sebrell and Harris, 1967), a deficiency of this vitamin is accompanied by atrophy of testis (Chatterjee, 1967), impaired androgen synthesis (Belavady and Banerjee, 1954), spermatogenic arrest (Biswas, 1967) decreased sperm motility (Buch, 1976), accumulation of lipid in testis and alteration in the levels of nucleic acid and protein (Terroine, 1965). It is also known
that ascorbic acid activates several enzymes and has a synergistic action with hormones like testosterone (Kutsy, 1973; Chapters I and II; Bueh, 1976).

In the light of the foregoing observations, it was thought worthwhile to study the effects of vitamin C deficiency on the metabolism of testis and epididymis of guinea pigs and compare with those of animals fed a vitamin C fortified diet.

MATERIALS AND METHOD

Healthy, adult guinea pigs (Cavia porcellus) weighing between 400-450 gm were used for the experiments. The animals were maintained in an air conditioned animal house (temperature 26±2°C, 14 hours day light) and were supplied food and water ad libitum as mentioned below. The experimental guinea pigs were divided into two groups, each consisting of 12 animals.

Group I - Control: the guinea pigs were fed a vitamin C fortified diet (Hindustan Lever Ltd., Bombay).

Group II - deficient animals: Guinea pigs were fed a vitamin C deficient diet for 21 days continuously. The scorbutic diet was prepared according to the method of Friberg and Lohmander (1970). On the 22nd day of the experiment, the animals were sacrificed by cervical dislocation. The testis and cauda epididymis were excised, blotted free of blood and were used for the biochemical and histochemical studies.
according to the methods given in Chapter I.

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).

Cholesterol: (Pearson et al., 1953).

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

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

Protein: (Gornall et al., 1949).

Histochemical localization of ascorbic acid: (Chinoy, 1969a,b).

A minimum of six replicates were done and the results were analyzed using students 't' test.

RESULTS

Organ weights:

A decrease (P < 0.1) in the weights of the testis and cauda epididymis was observed after 3 weeks of vitamin C deficiency (Table I).

Ascorbic acid and AA-FR special peroxidase:

The concentration of free ascorbic acid was not much altered in the testis and cauda of vitamin C deficient animals. ASG levels were not detectable in testis but were significantly increased (P < 0.001) in cauda of deficient animals. The rate
of AAU in the testis of scorbutic guinea pigs was almost same as in control but in cauda epididymis AAU was significantly enhanced (P < 0.02). The AA-MM complex was not detectable in both the testis and cauda of the vitamin C deficient guinea pigs. An enhancement (P < 0.05) in AA-FR special peroxidase activity was observed in both organs in comparison to control (Table I).

Cholesterol:
Vitamin C deficiency brought about an insignificant increase (P > 0.1) in cholesterol level in the testis but in cauda it was almost same as in control guinea pig (Table I).

Succinate dehydrogenase (SDH):
In vitamin C deficient guinea pigs, the SDH activity of the testis was unaffected, whereas, that of cauda was decreased (P < 0.05) in comparison with control (Table I).

Alkaline and acid phosphatases:
Alkaline phosphatase was activated (P < 0.05) in the testis but decreased significantly (P < 0.01) in the cauda epididymis of vitamin C deficient guinea pigs. On the contrary, the activity of acid phosphatase was reduced insignificantly (P > 0.1) in both the organs of scorbutic guinea pigs (Table I).

Protein:
An increase in the protein concentration was observed in the testis but in cauda epididymis it was significantly (P < 0.1) decreased (Table I).
Histochemical localization of ascorbic acid:

Group I (Control-Guinea pigs fed vitamin C fortified diet):

Testis:

The basement membrane, dividing germinal cells, spermatozoa and interstitial cells showed intense staining for ascorbic acid (Figs. 1 A, B).

Cauda epididymis:

The nuclei of the epithelial cells, stereocilia, spermatozoa and the connective tissue were stained darkly for ascorbic acid (Figs. 2 A, B).

Group II (Vitamin C deficient guinea pigs):

Testis:

The staining pattern was the same as in control testis except that the intensity of staining was comparatively less (Figs. 1 C, D).

Cauda epididymis:

The staining pattern was the same as in group I and the staining intensity was almost similar to that in control (Figs. 2 C, D).

DISCUSSION

The decrease in most of the androgen dependent parameters especially in the cauda epididymis of vitamin C deficient animals suggest that the internal milieu of epididymis is altered corresponding with the probable alteration in the levels
of circulating androgens and testicular steroidogenesis in conformity with the observations of Belavady and Banerjee (1954) and Banerjee et al. (1972). Similarly, a reduction in urinary 17-ketosteroids and accumulation of lipids in testis of scorbutic animals have been reported (Banerjee and Deb, 1951; Ginter et al., 1965; Biswas, 1967; Sebrell and Harris, 1967), together with decreased motility and metabolism of testicular and epididymal spermatosoa (Buch, 1976). The males are more susceptible to vitamin C deficiency than females (Odumosu and Wilson, 1973).

In guinea pigs fed a vitamin C deficient diet for 21 days, mobilization of the stored ascorbic acid was found to occur in both the testis and cauda epididymis. This ascorbic acid turnover was greater in the latter organ, concurrent with an increased storage of the bound vitamin therein. It is probable that in order to maintain the enhanced mobilization of the ascorbic acid in testis and epididymis, the vitamin is transported from other body organs to the reproductive tissues in response to their greater metabolic demand. As guinea pigs are not capable of synthesizing ascorbic acid, whose tissue distribution and synthesis is dependent on testosterone (Dieter, 1969; Majumder and Chatterjee, 1974), the levels of free ascorbic acid are not increased compared to those for the rat exposed to stress conditions (Chapters I to III; Selye, 1950). The results of this study also reveal that
although the ascorbic acid is known to participate in steroidogenesis (Fisher, 1962; Biswas, 1969; Biswas and Deb, 1970; Carballeiva et al., 1974; Deb et al., 1976; Datta and Sanyal, 1976), can stimulate several enzymes (Harrer and King, 1941; Kutsky, 1973) as well as has a synergistic action with testosterone in enhancing the testicular germ cell maturation, anabolic activity and the levels of androgen dependent enzymes (Kutsky, 1973; Chapters I and II), yet the increased mobilization of ascorbic acid is not adequate to maintain the metabolism of the testis and epididymis to control level in scorbutic guinea pigs. Nevertheless, this study clearly elucidates that ascorbic acid has an important role in maintaining the physiological integrity of testis, epididymis and their spermatozoa in conformity with the data of others (Belavady and Banerjee, 1954; Biswas, 1967; Buch, 1976).

SUMMARY

The effects of vitamin C deficiency on the physiological integrity of the testis and cauda epididymis of guinea pigs were investigated. The metabolic disturbances in these androgen dependent organs due to vitamin C deficiency, support the view, that the ascorbic acid and its free radical play a vital role in reproductive functions by their involvement in steroidogenesis and other oxidoreduction reactions as important sources of electron energy.
REFERENCES


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

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

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


<table>
<thead>
<tr>
<th>Organs</th>
<th>Normal Control</th>
<th>Vitamin C deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ORGAN WEIGHTS (gms)</strong></td>
<td>(1.69 \pm 0.10)</td>
<td>(1.41 \pm 0.09)</td>
</tr>
<tr>
<td>Testis</td>
<td>(0.14 \pm 0.05)</td>
<td>(0.31 \pm 0.03)</td>
</tr>
<tr>
<td>Cauda</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **ASCORBIC ACID (AA, ASG, AAU and AA-MM complex rates mg/gm fresh tissue weight)** | | |
| Testis | \(1.52 \pm 0.07\) | \(1.59 \pm 0.07\) |
| ASG | \(0.02 \pm 0.007\) | ND |
| AAU | \(10.36 \pm 0.24\) | \(11.22 \pm 0.69\) |
| AA-MM | \(0.70 \pm 0.05\) | ND |
| Cauda | \(2.47 \pm 0.09\) | \(2.61 \pm 0.36\) |
| ASG | \(0.30 \pm 0.01\) | \(1.09 \pm 0.07\) |
| AAU | \(16.58 \pm 1.00\) | \(24.17 \pm 2.37\) |
| AA-MM | \(2.24 \pm 0.17\) | ND |

| **AA-ER SPECIAL PEROXIDASE ACTIVITY (AA-ER special peroxidase activity/gm fresh tissue weight/20 minutes)** | | |
| Testis | \(10.67 \pm 0.51\) | \(15.60 \pm 2.61\) |
| Cauda | \(12.76 \pm 1.42\) | \(18.43 \pm 2.10\) |

| **CHOLESTEROL (mg %)** | | |
| Testis | \(0.15 \pm 0.03\) | \(0.18 \pm 0.03\) |
| Cauda | \(0.14 \pm 0.006\) | \(0.13 \pm 0.01\) |

| **SUCCINATE DEHYDROGENASE (µg formazan formed/4 hrs/100 mg fresh tissue weight)** | | |
| Testis | \(132.83 \pm 16.53\) | \(129.98 \pm 19.39\) |
| Cauda | \(266.08 \pm 9.98\) | \(229.60 \pm 12.32\) |

| **ALKALINE PHOSPHATASE (U/l)** | | |
| Testis | \(192.83 \pm 16.53\) | \(273.40 \pm 25.36\) |
| Cauda | \(97.60 \pm 1.82\) | \(84.75 \pm 1.63\) |

| **ACID PHOSPHATASE (U/l)** | | |
| Testis | \(142.00 \pm 15.00\) | \(124.05 \pm 10.53\) |
| Cauda | \(469.4 \pm 59.38\) | \(445.30 \pm 40.93\) |

| **PROTEIN (mg/100 mg fresh tissue weight)** | | |
| Testis | \(14.50 \pm 0.40\) | \(16.98 \pm 1.08\) |
| Cauda | \(28.52 \pm 1.90\) | \(22.60 \pm 2.18\) |

ND = not detectable

The values are mean ± S.E.
Figs. 1 A, B
T.S. of testis of guinea pigs fed a vitamin C fortified diet, showing ascorbic acid localization
1 A. X 100
1 B. X 512

Figs. 1 C, D
T.S. of testis of guinea pigs fed a vitamin C deficient diet stained for ascorbic acid
1 C. X 100
1 D. X 390
Figs. 2 A, B
T.S. of cauda epididymis of guinea pigs fed a vitamin C fortified diet stained for ascorbic acid
2 A. X 120
2 B. X 390

Figs. 2 C, D
T.S. of cauda epididymis of guinea pigs fed a vitamin C deficient diet showing the ascorbic acid localization
2 C. X 120
2 D. X 390