CHAPTER III

Effect of Ascorbic Acid on Adrenal and Testis of Pantothenic Acid Deficient Rats and on the Synthesis in vitro of L-Ascorbic Acid in Liver
Introduction

Studies on the steroidogenic response in nutritional deficiencies or excess is of considerable interest in the present time. Endocrine disturbances form an essential part in the clinical picture of malnutrition. The correlation between the clinical manifestations of malnutrition and those of altered endocrine function are evidenced by the frequency of serious sexual disturbances, pigmentation of the skin, lowered metabolic rates, gynecomastia, asthenia, hypotension and other symptoms which could be attributed to dysfunction of the thyroid, the adrenals, the pituitary or the gonads in undernourished individuals. Among the nutritional factors a great majority of the vitamins directly or indirectly influence the steroidogenic function of adrenal and gonads.

The importance of pantothenic acid in steroid metabolism is thought to be due to the fact that it constitutes a moiety of coenzyme A(1) which is known to be a factor in the synthesis of cholesterol, the precursor of steroids, from acetate (2). Deficiency of pantothenic acid causes adrenal hypofunction (3-9) and excessive amount of pantothenic acid improves the capacity of animals to withstand stress (10). Deb et al. (7) studied the adrenocortical activity in pantothenic acid deficient rats. Hypofunction of the adrenal cortex was indicated by the diminished ascorbic acid and total cholesterol content of the adrenal glands, diminished blood sugar and glycogen value of the liver, diminished
urinary excretion of 17-ketosteroids, and relative lymphocytosis and eosinophilia in deficient rats. Goodman (5) confirmed this finding by directly measuring 17-ketosteroid concentration of adrenal venous blood. Studies of the enzymes concerned has not been carried out by any investigator.

Vitamin C has been observed to play an important role in adrenal cortical function and cholesterol metabolism. Excessive amount of ascorbic acid improves the capacity of animals to withstand stress (11) and reduce the cholesterol depletion of the adrenal following stress (12). The role of this vitamin in cholesterol metabolism has been reported by several workers (13-15) and a voluminous literature has been piled up to establish the inter relationship between these two factors. As pantothenic acid is intimately related to cholesterol metabolism by its occurrence in coenzyme A, study on the integrated role of these two vitamins in relation to steroidogenesis is of interest for a number of years. But the mechanism of action of ascorbic acid on the process of steroidogenesis is not yet clear and as the coenzyme A plays a number of roles in metabolism, the inter relationship between these two vitamins is not yet established.

Testes is also a major steroid producing organ, and therefore the functional activity of this organ is also controlled both by pantothenic and ascorbic acid. Barbordiak et al. (16) reported testicular degeneration in animals
deprived of pantothenic acid. Similar atrophy has been observed by Fidanza et al. (17). Pantothenic acid in excess stimulates the reproductive activity of animals (18, 19) indicating an involvement of the vitamin in reproduction. On the other hand involvement of vitamin C in the same process has been suggested by several investigators (20–24). But no attempt has been made to find out the combined or integrated role of these two vitamins on gonadal steroidogenesis.

As reviewed by Reid (25) ascorbic acid has some interrelationship with the metabolism of other vitamins. Since the tissue distribution and urinary excretion of ascorbic acid decreased in deficiency of pantothenic acid, it was suggested that this vitamin is in some way involved in the biosynthesis of ascorbic acid in rats (26). Chatterjee and Kar (27) noted a diminution in the rate of ascorbic acid synthesis in liver of pantothenic acid deficient rats, which was again restored by feeding pantothenic acid to the deficient animal. Dumm and Ralli (28) showed that decreased urinary excretion of ascorbic acid in pantothenic acid deficient rats is again increased following addition of ascorbic acid to the deficient diet.

The preventive and curative role of ascorbic acid on deficiency of B vitamins has been studied by Terroine (29). Inclusion of ascorbic acid in the pantothenic acid deficient diet delayed or suppressed the appearance of deficiency signs (30–32) and also an alteration in the coenzyme A
content of liver (Causi and Romano, 33). Terroine (34) also confirmed the higher concentration of pantothenic acid in the livers of animals given ascorbic acid, compared with deficient control rats. Considerable literature has accumulated indicating an inter relationship between the metabolism of pantothenic acid and ascorbic acid, but has not gained the universal acceptance. Lenti et al. (35) failed to demonstrate any change in the adrenal ascorbic acid following a single high dose of sodium pantothenate in normal animals. Fidanza (36) also could not find any significant effect of pantothenic acid deficiency on the ascorbic acid content of the tissues.

The results obtained by different investigators have not led to definite conclusion regarding any functional inter relationship existing between these two vitamins. Although considerable amount of work has been done on the biosynthesis of adrenal corticoids in pantothenic acid deficiency, yet very little work has been done after supplement of ascorbic acid to them. Moreover literature is rather scanty on the studies of the enzymes present in the adrenal, either in pantothenic acid deficient animals or in ascorbic acid supplemented pantothenic acid deficient animals.

The importance of both pantothenic acid and ascorbic acid in maintenance of normal gonadal function has been discussed (16-24). Some workers emphasized on the role of ascorbic acid in maintenance of normal reproductive activity in absence of pantothenic acid (37) but none of them could
give a clear idea on the matter. Though some work has been done in the case of adrenal, almost no work was done on the enzymes present in the testes in either pantothenic acid deficient animal or ascorbic acid supplemented pantothenic acid deficient animal.

The rate of ascorbic acid synthesis in the liver of pantothenic acid deficient rats has been studied (27) but the effect of ascorbic acid on the same process under the similar condition has not been studied yet.

Rats require pantothenic acid from the external source. So the deficiency condition can be produced in them by withdrawing the vitamin from the diet.

The use of analogues to study the effects of nutritional deficiency of the vitamins is now well accepted. Several authors have reported the action of omega methyl pantothenic acid as an antimetabolite of pantothenic acid (38-41). Kimura and Ariyama (42) showed that this antimetabolite can produce severe pantothenic acid deficiency in rats.

In the present investigation an attempt has been made to study the steroidogenesis in adrenal and testes of rats on a pantothenic acid deficient diet and also the role of ascorbic acid supplementation on the same process under similar experimental condition. To produce the deficient condition, a diet deprived of pantothenic acid and supplemented with calcium salt of omega methyl pantothenic acid was used. This antimetabolite
was used to produce the deficient condition within a short period of time and the experiments were carried out with the adrenal and testes of pantothenic acid deficient rats with and without ascorbic acid supplementation. The results were compared with the pair-fed control animals. The functional activity of the steroid producing organs has been evaluated by studying the localization of the enzyme $\Delta^5-3\beta$-OHD, one of the principal enzymes involved in the process of steroidogenesis histochemically. Localization of neutral lipid, and succinic dehydrogenase by histochemical technique in addition to biochemical estimation of both cholesterol and ascorbic acid were also performed in the experimental and control groups of rats to have a clearer idea on the functional activity of the steroid producing organs.

The rate of ascorbic acid synthesis in liver was also studied in both the above experimental conditions and the results were compared with the pair fed control animals to see if any change occurs in it.

**Materials and Methods**

One hundred male albino rats weighing 25-30 g were taken for study. They were maintained on standard laboratory diet as mentioned in the section "Materials and Methods" (Chapter II). After the animals were kept for a few days in such condition they were divided into three groups of equal average body weight. Then the treatments were started.
Animals of one of the groups were maintained on the same laboratory diet and considered as the control group. In case of the other two groups, calcium pantothenate was omitted from the diet and replaced by 0.6 g of calcium-$\omega$-methyl-pantothenate, an analogue of pantothenic acid, per 100 g diet. One of these two groups received an intraperitoneal injection of 50 mg ascorbic acid ('Redoxon'-Roche) daily and was considered as ascorbic acid treated pantothenic acid deficient group. The other one received equal volume of saline and was taken to be pantothenic acid deficient group. The control animals were also treated with equal volume of saline. Both the control animals and the ascorbic acid supplemented pantothenic acid deficient animals were pair fed with the pantothenic acid deficient animals. The signs of pantothenic acid deficiency started appearing from the 11th or 12th day of the treatment. The animals were sacrificed after 24 hr. fasting on the 24th day of the treatment. The ascorbic acid treated pantothenic acid deficient animals were sacrificed 24 hr. after the last injection administered on the 23rd day. Adrenals and testes from the left side of each of the animals were considered for the present study. The wet weights of adrenals, testes, prostrate and seminal vesicle were recorded.

1. Histology of the organ

Adrenal, testes and prostrate from the animals were fixed in carnoy's fluid for histological study. Routine histological sections were prepared, stained with Haematoxylin
2. **Histochemical localization of enzyme activities in the adrenal cortex and testes**

Fresh frozen sections of both adrenal and testes were cut at 20 μ in a cryocut at -20°C. The sections were placed on coverslips and then incubated at 37°C in the appropriate media for demonstrating the activities of SDH (43) and Δ5-3β-OHD (44). Following incubation the tissue sections were fixed in 10% neutral formol and then rinsed in distilled water. The sections were mounted in gum apathy for microscopic observation. The control sections were incubated in the corresponding media from which the substrates were omitted.

3. **Histochemical demonstration of neutral lipid**

The histochemical distribution of neutral lipid was studied according to Kay and Whitehead (45).

4. **Biochemical estimation of total ascorbic acid and total cholesterol in testes and adrenal gland**

Biochemical estimation of ascorbic acid in the above tissues was done according to Roe and Kuscher (46). Cholesterol was estimated by adopting the modified method of Sperry and Webb (47).

5. **Studies on the synthesis of L-ascorbic acid in vitro**

The synthesis of L-ascorbic acid *in vitro* by liver homogenate was studied by using D-glucuronolactone as
substrate as employed by Mukherjee, Kar, Sasmal and Chatterjee (48).

6. In two sets of experiments the two experimental groups of animals were allowed to survive till their natural death to see if there is any change in the life span of pantothenic acid deficient animal following ascorbic acid treatment.

**Results**

Pantothenic acid deficiency syndrome started appearing from the 11th or 12th day of the experiment. The animals deprived of pantothenic acid showed retardation of growth. No marked difference could be noted in the histological picture of adrenal cortex either in pantothenic acid deficient animals or in ascorbic acid treated pantothenic acid deficient animals. The histology of testes in pantothenic acid deficiency shows degeneration and arrest of spermatogenesis, but in ascorbic acid treated pantothenic acid deficient animals a lesser degree of degeneration was noted (Figs. 1, 2, 3). A flattened epithelial lining of the prostrate gland together with presence of lesser secretion in the lumen was noted in the pantothenic acid deficient animal, but in ascorbic acid treated pantothenic acid deficient animal this histological appearance shows an improvement (Figs. 4, 5, 6).

The activity of $\Delta^5-3\beta$-OHD was found to be localised in the adrenal cortical cells also in the interstitial cells.
of the testes (Figs. 7,8,9,10,11,12). The SDH activity was localised in the cells of adrenal cortex and both in the interstitial and tubular cells of the testes (Figs.13,14,15, 16,17,18). Pantothenic acid deficiency caused a significant diminution in both $\Delta^5$-3$\beta$-OHD and SHD activity in adrenal and testes when compared to those of control animals. Ascorbic acid treatment in pantothenic acid deficient animals caused an increase in the activities of these enzymes in both the organs, when compared to the deficient group.

Marked decrease was noticed in the neutral lipid distribution of adrenal cortex in pantothenic acid deficient animals. Though there was a slight increase in the neutral lipid in ascorbic acid treated pantothenic acid deficient animal, it was still far lesser than normal (Figs.19,20,21). The neutral lipid distribution in the testes of both pantothenic acid deficient animal and ascorbic acid treated pantothenic acid deficient animal decreased when compared to pair-fed control, but no significant difference could be noted between the two experimental groups.

Biochemical estimation of ascorbic acid showed an increase in its concentration in the adrenal in both the experimental groups in comparison to the pair-fed control, though the weight of the glands decreased (Table 2). The ascorbic acid concentration of testes remained unchanged but their weights decreased in the experimental conditions (Table 3).
The total cholesterol concentration of adrenal and testes decreased markedly in pantothenic acid deficiency, when compared to the pair fed control, whereas it remained unaltered in ascorbic acid treated pantothenic acid deficient animals (Tables 2, 3).

The rate of ascorbic acid synthesis by the liver in pantothenic acid deficient animals significantly increased when compared to the control animals. In the ascorbic acid treated pantothenic acid deficient group the rate of ascorbic acid synthesis is similar to that of the control group (Table 4). The protein content of liver remained unaltered (Table 4).

The weight of the prostrate and seminal vesicle reduced in animals deprived of pantothenic acid, ascorbic acid treatment could not prevent this reduction in weight compared to controls (Table 1). The ascorbic acid treated pantothenic acid deficient animals showed a longer span of life than the deficient animals alone. All the animals of the deficient group died on or before the 27th day of the treatment, whereas the ascorbic acid treated pantothenic acid deficient animals survived 33 days or more.
Table 1

Effect of exogenous vitamin C treatment on the body and organ weights in rats deprived of pantothenic acid
(The data are means ± S.E.M.)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of animals</th>
<th>Body weight</th>
<th>Adrenal * wt. (mg/100 g. b.wt.)</th>
<th>Testes * wt. (mg/100 g. b.wt.)</th>
<th>Prostate wt. (mg/100 g. b.wt.)</th>
<th>Seminal vesicle wt. (mg/100 g. b.wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair-fed control + saline (A)</td>
<td>16</td>
<td>40.25 ± 2.22</td>
<td>76.0 ± 6.34</td>
<td>28.48 ± 2.92</td>
<td>676.11 ± 20.31</td>
<td>72.58 ± 6.45</td>
</tr>
<tr>
<td>Pantothenic acid deficient + saline (B)</td>
<td>16</td>
<td>42.55 ± 1.99</td>
<td>59.62 ± 3.71</td>
<td>21.12 ± 1.33</td>
<td>524.94 ± 50.95</td>
<td>49.94 ± 7.56</td>
</tr>
<tr>
<td>Pantothenic acid deficient + Vitamin C (C)</td>
<td>16</td>
<td>42.01 ± 2.34</td>
<td>48.87 ± 4.06</td>
<td>22.68 ± 1.22</td>
<td>410.04 ± 49.83</td>
<td>43.60 ± 5.29</td>
</tr>
</tbody>
</table>

'P' values between groups
A & B                      P < 0.05  P < 0.02  P = 0.01  P < 0.05  P < 0.05
A & C                      P < 0.001  P > 0.05  P < 0.001  P < 0.01  P < 0.001

* Weight of both glands.
Table 2

Effect of exogenous vitamin C treatment on total cholesterol and ascorbic acid content of adrenal gland in pantothenic acid deficient male rats

(The data are means ± S.E.M.)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of animals</th>
<th>Adrenal weight* (mg/100 g b. wt.)</th>
<th>Adrenal cholesterol (mg/100 mg tissue)</th>
<th>Adrenal ascorbic acid (mg/100 g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair-fed control</td>
<td>10</td>
<td>26.38 ± 3.12</td>
<td>5.06 ± 0.48</td>
<td>115.63 ± 11.51</td>
</tr>
<tr>
<td>Saline (A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantothenic acid deficient</td>
<td>10</td>
<td>20.42 ± 1.53</td>
<td>3.63 ± 0.23</td>
<td>168.93 ± 19.34</td>
</tr>
<tr>
<td>Saline (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantothenic acid deficient</td>
<td>10</td>
<td>21.98 ± 1.38</td>
<td>5.35 ± 1.25</td>
<td>267.83 ± 35.68</td>
</tr>
<tr>
<td>Vitamin C (C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

'P' values between groups

A & B: P < 0.05
A & C: P > 0.05

* Weight of both adrenals.
<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of animals</th>
<th>Testes weight* (mg/100 g b.wt)</th>
<th>Testes cholesterol (mg/100 mg tissue)</th>
<th>Testes Ascorbic acid (mg/100 g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair-fed control + Saline (A)</td>
<td>10</td>
<td>627.12 ± 22.57</td>
<td>4.91 ± 0.17</td>
<td>30.17 ± 3.38</td>
</tr>
<tr>
<td>Pantothenic acid deficient + Saline (B)</td>
<td>10</td>
<td>490.55 ± 58.02</td>
<td>3.42 ± 0.09</td>
<td>27.24 ± 1.56</td>
</tr>
<tr>
<td>Pantothenic acid deficient + Vitamin C (C)</td>
<td>10</td>
<td>411.70 ± 77.59</td>
<td>4.92 ± 0.18</td>
<td>34.10 ± 2.23</td>
</tr>
</tbody>
</table>

'P' values between group

<table>
<thead>
<tr>
<th></th>
<th>A &amp; B</th>
<th>A &amp; C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.027</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

* Weight of both testes.
Table 4

Effect of exogenous vitamin C on the synthesis in vitro L-ascorbic acid by liver tissue of pantothenate deficient rats.

(The data are means ± S.E.M.)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of animals</th>
<th>L-ascorbic acid synthesized (u moles/g tissue)</th>
<th>L-ascorbic acid synthesized (u moles/g tissue)</th>
<th>Liver protein (mg/100 mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair-fed control</td>
<td>8</td>
<td>337.61 ± 27.88</td>
<td>17.42 ± 1.47</td>
<td>19.65 ± 1.63</td>
</tr>
<tr>
<td>Saline (A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantothenic acid deficient</td>
<td>8</td>
<td>484.70 ± 38.14</td>
<td>23.58 ± 1.13</td>
<td>20.48 ± 1.39</td>
</tr>
<tr>
<td>Saline (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantothenic acid deficient</td>
<td>8</td>
<td>342.88 ± 41.75</td>
<td>18.29 ± 1.77</td>
<td>19.44 ± 1.79</td>
</tr>
<tr>
<td>Vitamin C (C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

'P' values between groups

A & B  P < 0.01  P < 0.01  P > 0.05
A & C  P > 0.05  P > 0.05  P > 0.05
Discussion

Studies on pantothenic acid deficiency in weanling rats have been reported by many investigators. But it has been demonstrated that adult rats can hardly be subjected to such deficiency (49,50). The activity of omega-methyl pantothenic acid as a pantothenic acid anti-metabolite has previously been reported (38,41,51,52). Kimura and Ariyama in 1962 (42) observed that the antagonist omega-methyl pantothenic acid can produce pantothenic acid deficiency syndrome in adult rats also. Rats treated with the antagonist exhibit in two to three weeks external signs which are deemed indicative of pantothenic acid deficiency. The acetylation capacity of para-aminobenzoic acid decreased to a great degree.

In the present investigation, the calcium salt of omega-methyl pantothenic acid was used along with pantothenic acid deficient diet and the deficiency condition was produced within 24 days. Studies with omega-methyl pantothenic acid by Goodman (5) clearly indicate that this antagonist impairs biosynthesis of adrenal corticosteroids. The reversibility of this impairment by pantothenic acid clearly indicates that it is due specifically to interference by the antagonist with pantothenic acid metabolism. The importance of pantothenic acid in adrenal function is for the fact that it constitutes a moiety of coenzyme A (1) which is known to be a factor in the synthesis of steroids from acetate (2). The testes is the other important steroid synthesizing organ and this vitamin has
been observed to play an important role in the function of this organ also. Testicular degeneration in deficiency of pantothenic acid has been reported by several investigators (16,17,53). The functional role of pantothenic acid in testes may be similar as in the case of adrenal because it has been observed by Fidanza et al. (17) that the degenerative changes in testes due to deficiency of pantothenic acid are also reversible by supplement of the vitamin.

In the present investigation retardation of growth was noted in pantothenic acid deficient animals. Supplement of ascorbic acid could not help in maintenance of the lost weight (Table 1). It was also observed that though supplement of ascorbic acid could not maintain the growth of the animals, it in some way, played a beneficial role in pantothenic acid deficiency, as, such treatment improved the life span of the animals. Terroine (34) also pointed out that the growth of rats given ascorbic acid is not quite as good as that of the control rats receiving pantothenic acid. There was a marked decrease in the weights of adrenal, testes, seminal vesicle and prostrate in animals deprived of pantothenic acid compared to control (Table 1). A further insignificant decrease in weight of the testes, prostrate and seminal vesicle was noted in ascorbic acid supplemented group, but the weight of the adrenal remained unchanged. Moreover, the weight of the adrenal in ascorbic acid supplemented pantothenic acid deficient animal was less than control but the difference was not significant.
Pantothenic acid deficiency in rats has been reported as leading to pathological changes in several organs. The most often reported damage concerns the hemorrhagic necrosis of adrenal glands (54-57). But no significant change in the histology of the adrenal gland in the experimental conditions was noted in the present investigation, in comparison to the pair fed control. Barboriak et al. (16) also could not find any hemorrhagic necrosis in the adrenal of pantothenic acid deficient animals when the deficiency condition was produced by using bis-(N-pantoyl-β-aminoethyl) disulphide an antagonist of pantothenic acid. According to the opinion of Goodman (5) the failure of omega-methylpantothenic acid to cause adrenal necrosis might have been due to brief period of administration. Even if complete blockage of the synthesis of an indispensable compound was effected by the antagonist, a minimum period of time would have to elapse before existing stores of the essential compound were depleted below the level required for cell viability.

Probably the most widely used indices of adrenocortical function are the concentration of cholesterol and ascorbic acid in the gland (58). In the present experiment a significant decrease in adrenal cholesterol was noted in pantothenic acid deficient animals (Table 2). This result is in close agreement with the observation of the other investigators (9,55,59,60). This decrease in adrenal cholesterol content may be due to diminished cholesterol synthesis under this condition,
as it has been observed by several investigators that cholesterol synthesis is diminished in absence of pantothenic acid (9,61,62). Pantothenic acid deficiency in rats is accompanied by adrenal hypofunction (3-7,9,63). This insufficiency may be caused by diminished endogenous synthesis of cholesterol, the precursor of steroid hormones, as indicated by the lower total cholesterol contents of the adrenals. Following ascorbic acid treatment to the deficient animals in the present investigation, the cholesterol content of the gland came back to control level. As the ascorbic acid treatment was started from the first day of experiment, it can be stated that this vitamin in some way helps to maintain the cholesterol synthesizing process in the adrenal gland.

The effect of pantothenic acid deficiency on the adrenal ascorbic acid concentration in rats does not seem quite conclusive, perhaps because of the different degrees of pantothenate deficiency in the animals studied. One group of investigators have reported that adrenal ascorbic acid is reduced in pantothenate deficiency (7,61,64). Whereas another group of workers, failed to demonstrate any significant change in the adrenal ascorbic acid concentration under the similar condition (36,65). In contrast to the observation of all these workers, in the present investigation an increase in adrenal ascorbic acid has been observed (Table 2). This increase in ascorbic acid concentration may be due to non-functional accumulation of ascorbic acid in hypofunctioning adrenal; because adrenal undergo hypofunctioning under this deficiency state (3-7,9,63).
Higher concentration of ascorbic acid has been observed in resting state (66) of the gland. Following ascorbic acid treatment the concentration of this vitamin increased further, this may be due to increased storage.

The significant reduction in neutral lipid (Figs. 19, 20, 21) distribution along with simultaneous fall in $\Delta^5-3\beta$-OHD (Figs. 7, 8, 9) activity and SDH (Figs. 13, 14, 15) activity in the pantothenic acid deficient rat adrenal cortex, supports the postulation that adrenal cortical function is retarded in this condition. When compared to the deficient group the neutral lipid distribution was higher both in the zona glomerulosa and zona fasciculata of the ascorbic acid supplemented animals. Simultaneous increase in $\Delta^5-3\beta$-OHD activity and SDH activity indicates improvement in the functional activity of the organ in the ascorbic acid supplemented group when compared to the deficient group alone.

There are evidences of relative insufficiency of the adrenal cortical hormone in deficiency of pantothenic acid (3, 4). It has been demonstrated that the adrenals of pantothenic acid deficient rats, when incubated in vitro, secrete a reduced amount of $\Delta^4$-3-ketosteroids (63). Longwell et al. (6) found reduced corticosterone output in rats fed with a pantothenic acid deficient diet supplemented with omega-methyl pantothenic acid. Direct studies by Goodman (5) confirmed this observation and in addition he demonstrated that the decrease
in corticosteroid synthesis preceded the development of adrenal necrosis.

Evidence has accumulated to indicate an interrelationship between the metabolism of pantothenic acid and ascorbic acid. The excretion of ascorbic acid significantly decreased in rats on diets deficient of pantothenic acid (26,28). Following the period of deficiency, the addition of pantotenate to the diet was again associated with an increased excretion of ascorbic acid (28). Daft (31) was the first to report a vitamin sparing action of ascorbic acid. He observed that supplements of 2% ascorbic acid to a pantothenic acid free diet prevented the development of a pantothenic acid deficiency in rats. The action of ascorbic acid did not protect all rats. Some died with symptoms of pantothenic acid deficiency (31,32). These results have been confirmed by several investigators (30,67,68). Daft and coworkers have investigated the mechanisms involved in the sparing action by ascorbic acid and have reproduced their early observations (69). Glucuronolactone also has been found to be active in sparing pantothenic acid (67). As reviewed by Reid (25), ascorbic acid is known to be involved in (i) the maintenance of tissue levels of other vitamins, (ii) the oxidation of vitamins in the tissues, and (iii) the sparing action of vitamins in deficiency states.

In the present investigation the results obtained from the study of the adrenals in pantothenic acid deficient animals are in close agreement with the observation of the
previous investigators, leading to the conclusion that deficiency of pantothenic acid causes adrenal hypofunction. The results obtained from studies on the adrenals of ascorbic acid supplemented pantothenic acid deficient animal supports the probability of the existence of an inter relationship between the metabolism of these two vitamins. It can be suggested from the results of the present experiment that ascorbic acid plays a beneficial role on the functional activity of adrenal gland even in absence of pantothenic acid and also increases the life span of the deficient animals.

Role of pantothenic acid in reproduction and fertility is well recognised. It has been demonstrated by Nelson and Evans (70) that institution of pantothenic acid deficiency as late as the day of mating resulted in failure of implantation, resorption or defective formation of litters. Baurrnfeind and Norris (71) noted that egg hatchability is greatly reduced even before the hens demonstrate any visible deficiency symptoms. Barboriak et al. (16) and Fidanza et al. (17,53) noted testicular degeneration in deficiency of pantothenic acid.

In the present set of experiments, spermatogenic arrest and cellular degeneration in both the tubular and the interstitial cells were noted in deficiency of pantothenic acid. In comparison to this group the testes of ascorbic acid supplemented pantothenic acid deficient animal showed less degenerative changes though the spermatogenic arrest was still present (Figs. 1,2,3). The weight of the testes, seminal
vesicle and the prostrate decreased in pantothenic acid deficiency and decreased further following ascorbic acid treatment (Table 1). The cholesterol content of the testes also significantly reduced in deficiency state and was almost similar to control in ascorbic acid supplemented pantothenic acid deficient animals (Table 3). The ascorbic acid concentration remained almost unchanged in both the experimental groups (Table 3). Though the beneficial effect of ascorbic acid on gonadal function is well accepted, the mechanism of action of ascorbic acid on testicular function and the ascorbic acid storing capacity of testes are not known clearly. Owing to this, it is difficult to explain the results of ascorbic acid estimation of testes, in the present experiment.

Evidences obtained from the histological observations indicate that supplement of ascorbic acid prevents the degeneration of testes to some extent, caused by deficiency of pantothenic acid but it cannot help to maintain spermatogenesis. Spermatogenesis is the result of a balanced co-ordinated functions of releasing factors, gonadotropins and the gonadal steroids. Factors affecting any one of these will affect spermatogenesis also.

The histochemical localization of \( \Delta^{5-3\beta}-\text{OHD} \) (Figs. 10,11,12), and SDH (Figs.16,17,18) activities, of testes also supports the above postulation. But no significant change could be noted in neutral lipid distribution. Both the enzyme activities were markedly decreased in pantothenic acid
deficiency. The enzyme activities in the ascorbic acid supplemented pantothenic acid deficient group were far lesser than those of the control animals but slightly more than in the deficient group, indicating improvement in the functional activity of the organ.

There is no direct evidence indicating that pantothenic acid deficiency causes inhibition of androgen synthesis in the testes, but considerable evidences have accumulated to demonstrate adrenal cortical hypofunction in pantothenic acid deficiency (3-7,9,63). Cholesterol is the precursor of both adrenal cortical hormones and the androgens of testes. The reduced adrenal steroidogenesis in deficiency of pantothenic acid has been attributed to decreased cholesterol synthesis in such condition (9,61). Thus the gonadal hypofunction in deficiency of pantothetic acid may also be attributed to diminished synthesis of gonadal steroids due to inavailability of sufficient precursor cholesterol. Ascorbic acid supplementation either influences directly the testes and prevents it from degeneration and also helps to maintain the cholesterol level, or it influence gonadotrophin secretion from pituitary which in turn influence the testes. Role of ascorbic acid in regulating pituitary gonadotrophin has previously been reported (24).

The androgens of the testes play two major functions. One is control of spermatogenesis and the other is the maintenance of the secondary sex organs. In deficiency of pantothenic acid, both of them are affected, which indicates gonadal
hypofunction. Supplement of ascorbic acid also failed to cause any change in either spermatogenesis or the weight of the secondary sex organs. But the degenerative changes in testes were less in this group in comparison to deficient animal. So it can be stated that whatever may be the mechanism of action of ascorbic acid, it can maintain the structure of the testes to some extent in deficiency of pantothenic acid.

As the $\Delta^5$-3/β-OHD is the key enzyme in the process of androgen synthesis, the decrease in the enzyme activity supports the postulation that pantothenic acid deficiency causes inhibition of the process. This inhibition of the process might be due to lowered cholesterol synthesis, or degeneration and atrophy of the interstitial cells; or lowered secretion of pituitary gonadotropins. The slight increase in the enzyme activity in the ascorbic acid supplemented group indicates an improvement in the functional activity under this condition. This observation also supports the previous postulation regarding the role of ascorbic acid in absence of pantothenic acid.

The functional activity of any organ depends upon its structural integrity. Pantothenic acid deficiency causes degeneration and atrophy of both the interstitial cells and the tubular cells and thus causes diminished androgen synthesis and arrest of spermatogenesis respectively. Supplement of ascorbic acid prevents these cells from degeneration to some extent and maintains the enzyme activity partially. Similarly, the lowered SDH activity in the testes of deficient group indicates retarded
metabolism in the organ. Slight increase in enzyme activity in the ascorbic acid supplemented group supports the postulation that supplement of ascorbic acid restores the metabolic processes to some extent in deficiency of pantothenic acid.

Since the tissue distribution and urinary excretion of ascorbic acid decrease in deficiency of pantothenic acid (26) it has been suggested that this vitamin is, in some way, involved in the biosynthesis of ascorbic acid. In contrast to the observation of Chatterjee and Kar (27) a significant increase in the rate of ascorbic acid synthesis was noted in liver of animals deprived of pantothenic acid, which came back to normal following ascorbic acid treatment.

The decreased urinary excretion of ascorbic acid in deficiency of pantothenic acid has been attributed to decreased synthesis of ascorbic acid (26). It may also be owing to the increased utilization of the vitamin by the body in the deficiency state, because it has been observed by Daft and Schwarz (32) and Terroine (34) that manifestations of pantothenic acid deficiency were markedly reduced in rats by the addition of 2% ascorbic acid to the diet. These evidences indicate that ascorbic acid requirement of the body increases in deficiency of pantothenic acid. The increase in the rate of L-ascorbic acid synthesis in vitro (Table 4) in deficient animal can thus be explained. As the requirement of ascorbic acid is increased in the body under the deficient condition, the enzyme system present in the liver microsomes tries to cope with the need,
and the ascorbic acid synthesizing process is stimulated. But the extra amount of ascorbic acid synthesized is probably not sufficient to maintain the adrenal cortical and gonadal function at control level. So, though the rate of ascorbic acid synthesis is significantly increased, the adrenal and testes are still hypofunctioning. When ascorbic acid is treated externally, there is no need to synthesize the extra amount of the vitamin in the body. So the rate of L-ascorbic acid synthesis in vitro in pantothenic acid deficiency comes down to normal rate following ascorbic acid treatment. But Chatterjee and Kar (27) studied the rate of ascorbic acid synthesis in deficiency of pantothenic acid and noted a 70% decrease. Addition of the vitamin in vitro or the coenzyme was of no effect on the rate of ascorbic acid synthesis but addition of the particular vitamin to the deficient diet restored the synthetic power. It has also been observed that in vitro addition of different anti-vitamin does not inhibit the synthesis. Chatterjee and Kar (27) suggested that the loss in the synthetic ability of the enzymes under deficiency conditions is not for the decrease in the amount of cofactors only, but is primarily due to the derangement of the microsomes where the enzymes responsible for ascorbic acid synthesis are present. The difference between the previous observation (27) and the present observation may be due to the difference in the methodology followed to produce the deficiency conditions. Previous workers produced the deficiency condition by feeding the deficient diet for a prolonged period of time whereas, in the_
present experiment the deficiency condition was produced by using the antimetabolite omega-methyl pantothenic acid for only 24 days. The anatomical changes are preceded by prolonged and sustained biochemical changes. It is possible that when deficiency is produced by deficient diet alone, longer time is needed to get the condition and the microsomes are deranged causing inhibition of the synthetic ability, though the need of ascorbic acid is still present. But when the condition is produced with the help of the anti-vitamin, the deficiency condition appears prior to any damage done to the liver microsomes. So the rate of ascorbic acid synthesis is increased in deficiency condition to cope with the need of the body. The values of liver protein (Table 4) also supports this postulation. The protein content of liver remained unchanged in both the experimental conditions, indicating that the liver do not undergo degeneration when pantothenic acid deficiency is produced by feeding omega-methyl pantothenic acid. Morgan and Yudkin (72) suggested that there are four possible ways by which the requirements of the B vitamins might be reduced by dietary carbohydrate and similar substances (a) They might promote the synthesis of the vitamins in the tissues; that is they might increase the endogenous supply of vitamins. (b) They might reduce the metabolic need of the vitamins by the tissues (c) They might increase the absorption of dietary supplies of the vitamins. (d) They might promote the synthesis of the vitamins in the alimentary canal. Among these four possibilities we can disregard the first and third
possibilities as because the rats cannot synthesize pantothenic acid and the vitamin content was nil in the diet supplied to them. The fourth possibility can also be neglected because in the present investigation, the supplement of ascorbic acid was given intramuscularly. So the possibility that ascorbic acid reduce the metabolic need of the vitamins is applicable in this case. This supports the view that there may be a inter relationship between the action of ascorbic acid and pantothenic acid.

Summary

Role of pantothenic acid and ascorbic acid on steroidogenesis and reproduction has been studied by several investigators. Substituent role of vitamin C in absence of pantothenic acid has also been pointed out. But the steroidogenic capacity of adrenal and gonads in pantothenic acid deficient, and ascorbic acid supplemented pantothenic acid deficient animals has not been studied thoroughly. In the present experiment an attempt has been made to study the steroidogenic activity of adrenal and testes in animals deprived of pantothenic acid and also in ascorbic acid supplemented pantothenic acid deficient animals.

To evaluate the functional activity of the steroid producing organs studies has been carried out biochemically and histochemically.
The results of the present investigation indicate that in deficiency of pantothenic acid the body weight and also the weights of adrenal, testes, prostrate and seminal vesicles, decreased. Treatment of ascorbic acid in animals deprived of pantothenic acid could not maintain the weights. Cholesterol content of both adrenal and testes decreased significantly in deficiency of pantothenic acid. Ascorbic acid supplementation in deficiency of pantothenic acid helped to maintain the cholesterol level of these glands. Ascorbic acid level of the adrenal was high in absence of pantothenic acid, which was higher in the ascorbic acid supplemented group. However, ascorbic acid concentration of the testes remained unchanged in both deficient and ascorbic acid supplemented deficient group.

Study of the rate of ascorbic acid synthesis in liver, showed an interesting observation. Significant increase in the rate of ascorbic acid synthesis in animals deprived of pantothenic acid indicates that requirement of vitamin C increased in deficiency state. When supplied externally, the need of ascorbic acid is less and the rate of synthesis in the liver comes back to normal level.

Histological studies and histochemical demonstration of the SDH and $\Delta^5-3\beta$-OHD, and neutral lipid supports the assumption that ascorbic acid improves the function of adrenal and testes in absence of pantothenic acid.
References


