CHAPTER IV

Effect of Pantothenol on Adrenal and Testis of Control and Scorbutic Guinea Pigs
Introduction

There exists a close interdependence between the activities of the endocrine glands and some of the vitamins. Among the vitamins, ascorbic acid and pantothenic acid have been found to play important role in steroidogenesis. But their mechanism(s) of action on steroidogenesis is not yet clear. Pantothenic acid is involved in cholesterol synthesis in view of its occurrence as a component of coenzyme A which following acetylation is incorporated into cholesterol (1-12).

Fall in cholesterol and ascorbic acid in adrenals has been observed in scorbutic guinea pigs and these changes have been repeatedly rediscovered (13-18). Pirani (19) is of opinion that deciciency of vitamin C produces non-specific stress and he assumed that the changes in the adrenal in this disease is due to cortical stimulation by the anterior pituitary. According to Stepto et al. (20) adrenals are hypertrophied during scurvy. Deb (21) has noted accumulation of sudan positive materials in the interstitial cells of testis during ascorbic acid deficiency. A hypofunction of gonads under the same condition has been reported by Cavazos et al. (22,23) and Biswas and Deb (24). Administration of pantothenic acid in excess, produces after a certain period of time, haemorrhage in adrenals (25) of rats and fall in cholesterol content one or
six hour later following the injection (26). Similar treatment in rats produces stimulation of both gametogenic and endocrine function of testes (27,28) in rats. In the present investigation an attempt has been made to study the effect of pantothenic acid on adrenal and testes of normal and vitamin C deficient guinea pigs and thus to find out the inter relationship, if any, between the action of pantothenic acid and ascorbic acid.

A detailed study of the adrenal cortical and testicular activity was, therefore, found necessary in normal and vitamin C deficient animals with or without pantothenic acid. Cholesterol and ascorbic acid are possibly related to the formation of steroids, as is evident from the fact that injections of ACTH of the pituitary in rats and guinea pigs lowered both cholesterol and ascorbic acid contents of adrenal gland (29, 30). The fact that cholesterol content of the gland varies with its functional state was also shown by Noach and Van Rees (31). Biswas (32,33) and Biswas and Deb (34) reported the importance of ascorbic acid in the metabolism of male reproductive organs. So both the cholesterol and ascorbic acid content of adrenal and testes were estimated.

To have an idea about the morphology of the organ under different experimental conditions, the histology of the organ was studied. Many investigators studied the morphology of the adrenal (35,36) and testes (35,37-40) under differential experimental conditions and found that histological picture of the
glands varies with its functional state. Decrease in size of seminiferous tubules, lack of spermatogenesis, vacuolation of sertoli cells and the diminution in the size of interstitial cells have been reported by Zubiran and Gomez-Mont (35) in human under chronic malnutrition. Banerjee and Dravid (41) observed inhibition in spermatogenesis, with diminution in the size of seminiferous tubules, and degenerative changes in the leydig cells in vitamin B\textsubscript{12} deficient rats. The weight of the accessory sex organs on the other hand reflects the amount of androgenic hormones in the testicles (42). Degeneration and atrophy of the germinal epithelium was noted by Lindsay and Medes (43) and the pathological changes of the Leydig cells accompanied by spermatogenic arrest was noted by Kocen and Cavazos (44) in scorbutic guinea pigs. These findings were also noted by Biswas and Deb (24) and Mukherjee and Banerjee (45).

Cellular physiology of an organ can be better understood by histochemical demonstration of different enzymes and other cytoplasmic constituents in it, rather than by routine staining procedure. Sayers et al. (46) noted an intimate relationship between the density of sudanophilic materials and the concentration of cholesterol in adrenals. High accumulation of these substances in the adrenal indicates inadequate secretion and subnormal functioning of the organ (46,47). Decrease in cholesterol content of adrenal is said to be owing to the increased demand of cortical hormones (48).
Accumulation of high amount of sudanophilic substances in the interstitial cells of testes has been noted in scurvy by Biswas and Deb (49).

Studies of SDH and \(\Delta^5-3\beta\)-OHD activities have been carried out to study the functional activity of adrenal cortices (50). Lautenschlaeger (51) observed rise and fall in SDH activity with concomitant fall and enhancement respectively in \(\Delta^5-3\beta\)-OHD activity in guinea pig adrenal cortex following metopirone treatment which interferes in steroid hormone synthesis.

Deb (21) has observed histochemical abnormalities in both spermatogenic and interstitial cells during ascorbic acid deficiency. Dey et al. (52), and Biswas (53) have studied SDH and \(\Delta^5-3\beta\)-OHD activities in testes to study the functional status of the organ. Dey et al. (54) have also studied the LAP activity in the guinea pig testes under different experimental conditions and correlated the enzyme activity with the proliferation of the interstitial cells.

In the present investigation the above parameters have, therefore, been studied to get an idea about the functional activities of the adrenal and testes in guinea pigs following pantothenal treatment in presence or absence of ascorbic acid.
Materials and Methods

One hundred male guinea pigs of average weight 200-225 g were selected and were maintained on the standard laboratory diet of gram and grass for 10 days. The animals were then divided at random into four groups and were fed with a scurbutogenic diet \((55)\) from the day of experiment. Each animal of groups I and II received an intramuscular injection of 5 mg ascorbic acid (Redoxon; 'Roche'), those of groups II and IV received an intramuscular injection of 500 \(\mu\)g pantothenol (Bepanthane; 'Roche') and the group III animals received an intramuscular injection of 0.1 ml physiological saline, daily for 20 days. All the animals were fed with a concentrate of vitamins A, D and K orally, every alternate day. The animals of groups I, II and IV were pair fed with the animals of group III. The symptoms of scurvy started appearing in the group III animals from the 14th day. The scurbutic animals which survived till the 21st day were sacrificed with the animals of all other groups, after 24 hours fasting.

Adrenal, testes and prostrate and seminal vesicle were dissected out and weighed. The left adrenal and testis of each animal was taken up for study. The glands of six animals from each group were fixed in Carnoy's fluid for histological studies. One piece of prostrate from each animal was also fixed in Carnoy's fluid for the same purpose.
1. Measurement of tubular area

The outlines of the semi-niferous tubules were drawn on a white paper using a Camera Lucida and keeping the magnification constant in all the cases. The areas of the tubules drawn were measured by using a planimeter.

2. Histochemical localization of enzyme activities in the adrenal cortex and testes

Fresh frozen sections of both testes and adrenal 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 (56), and Δ⁵-

\[ \Delta^5 - \text{OHDH} \]

(57). Following incubation the tissue sections were fixed in 10% neutral formal and then rinsed in distilled water. LAP (58) activity was localized in testes only. The sections were mounted in gum-apathy for microscopic examination. 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 localized according to Kay and Whitehead (59).

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

The biochemical determination of ascorbic acid in the tissues were done according to Roe and Kuether (60). The
estimation of cholesterol was determined by adopting the modified method of Sperry and Webb (61).

Results

A fall in the weight of the testes has been observed in the scorbutic animals compared to controls (Table 1). Pantothenic acid caused a reduction in the weight of this primary male sex organ either with or without ascorbic acid. A difference in the histological picture in the four groups of animals were noted (Figs. 1, 2, 3, 4). The testes from the control animals showed normal spermatogenic cells and Leydig cells. Presence of only the spermatogonia and spermatocytes in the testes of pantothenic acid treated control animals indicated arrest of spermatogenesis. There was proportionate increase of Leydig cells compared to control. Severe degenerations of testes, as evidenced by massive sloughing of tubular elements, together with arrest of spermatogenesis was noted in scorbutic animals. A distension of Lamina Propria was noted in scorbutic testes. An increase in the interstitial cells was observed in scorbutic animals treated with pantothenic acid, although the degenerative changes in spermatogenic elements can still be noted.

From the Table 2 it can be noted that in comparison to control animals there is marked reduction in the average tubular area in all the three experimental conditions (Figs. 5, 6, 7, 8).
Compared to control guinea pigs, those supplemented with an additional pantothenic acid, showed a slight insignificant increase in the weight of the prostate and seminal vesicle (Table 1). The secondary sex organs of the scorbutic animals, which had the minimum weights, also showed a rise in weight on pantothenic acid treatment, but the increased weight was observed to be still lower than that of control animals. Control animals either with or without pantothenic acid had similar histological picture of prostate. Scorbutic animals showed complete absence of secretion in the lumen of this gland, the epithelial cells having been transformed into cubical type. An improvement in the histological appearance of prostate has been observed in these animals on treatment with pantothenic acid, which was, however, far from normal appearance (Figs. 9, 10, 11, 12).

Scorbutogetic diet alone caused an increase in the weight of the adrenals compared to those of either control animals or pantothenol treated control animals. The last two groups showed no change in between them (Table 1). The adrenal was hypertrophied and haemorrhagic in the scorbutic group, but in the pantothenic acid treated scorbutic group such haemorrhage was absent and the histological appearance of the cells showed an improvement (Figs. 13, 14, 15, 16).

The histochemical studies of adrenal and testes showed some differences in effects. A slight increase in neutral lipid was noted in the testes of pantothenol treated control
animals. It increased markedly in the testes of scorbutic animals and increased further following pantothenol treatment in them (Figs. 17, 18, 19, 20). No change in neutral lipid was noted in adrenal of pantothenol treated control animals. It decreased markedly in the adrenal of scorbutic guinea pigs which again of scorbutic guinea pigs which again increased slightly following pantothenol treatment (Figs. 21, 22, 23, 24).

In the testes, the SDH activity increased, following pantothenol treatment. A fall in enzyme activity was noted in scorbutic condition which increased slightly in pantothenol treated scorbutic group (Figs. 25, 26, 27, 28). SDH activity in adrenal was similar as in the testes (Figs. 29, 30, 31, 32).

The \( \Delta^5-3\beta \) -OHD activity in testes remained unchanged in pantothenol treated control animals, decreased in scorbutic and again increased slightly in pantothenol treated scorbutic group (Figs. 33, 34, 35, 36). But the localization of enzyme activity was changed following pantothenol treatment. In the control animals and the scorbutic animals the \( \Delta^5-3\beta \) -OHD activity was localized in both interstitial cells and tubular cells. Following pantothenol treatment more enzyme was localized in the tubular cells than in the interstitial cells. An increase in the enzyme activity was noted in the adrenal of pantothenol treated control animal. The activity decreased markedly in scorbutic adrenal and increased slightly again in pantothenol treated scorbutic group (Figs. 37, 38, 39, 40).
The activity of LAP was studied in testes only. Activity of this enzyme increased markedly in pantothenol treated control animals, decreased in scorbutic testes, and increased again, slightly in comparison to scorbutic, when the animals were treated with pantothenol (Figs. 41, 42, 43, 44).

A fall in ascorbic acid and an increase in cholesterol have been noted in the scorbutic testes (Table 3). Treatment with pantothenic acid caused an insignificant increase in both ascorbic acid and cholesterol concentrations in the scorbutic animals. Such treatment caused no alteration in either ascorbic acid or cholesterol in control animals.

Compared to controls with and without pantothenic acid, a fall in ascorbic acid and cholesterol has been noted in the scorbutic and pantothenic acid treated scorbutic adrenals (Table 4). Treatment with pantothenic acid alone in control animals, depleted cholesterol from the adrenal gland leaving the ascorbic acid content unaltered. Same treatment in vitamin C deficient animals did not cause any alteration in either of the above constituents.
Description of Figures

Fig. 1. Histology of testes from control guinea pig. Presence of different stages of spermatogenesis and mature sperms shows normal functioning of the organ (From X 400).

Fig. 2. Control testes on treatment with pantothenic acid shows presence of only first few stages of spermatogenesis, indicates arrest of spermatogenic function. There is proportionate increase in number of Leydig cells in comparison to normal. Compare with Figure 1 (From X 400).

Fig. 3. Severe degeneration of testes in both spermatogenic and Leydig cells in absence of vitamin C (From X 400).

Fig. 4. Testes of vitamin C deficient animals on treatment with pantothenic acid shows degeneration of tubular cells similar to scorbutic animals. Appearance of the interstitial cells are, however, better than in scorbutic animals. Compare with Figure 3 (From X 400).
Description of Figures

Fig. 5. Showing the areas of the tubules of control animals drawn with the help of a camera lucida.

Fig. 6. Camera leucida drawing showing marked decrease in the tubular size following pantothenol treatment in control guinea pigs. Compare with Figure 5.

Fig. 7. A decrease in tubular size in the testes of scorbutic animal. Compare with Figure 5.

Fig. 8. Tubular areas drawn from the slide prepared for histological study of testes of pantothenol treated scorbutic animal. Figure showing marked decrease in tubular size in comparison to control animals.

Fig. 9. Histology of control prostate of guinea pig (From X 400).

Fig. 10. Histology of prostate from pantothenol treated control guinea pig, showing normal appearance (From X 400).

Fig. 11. Absence of secretory material from the lumen and replacement of columnar epithelial lining with cubical, indicates complete hypofunctioning of the gland in vitamin C deficient guinea pigs (From X 400).

Fig. 12. Presence of small amount of secretory material in the lumen and appearance of the lining epithelium indicates an improved condition of the gland in scorbutic animals when treated with pantothenic acid. Compare with Figure 11 (From X 400).
Description of Figures

Fig. 13. Histology of adrenal from control guinea pig (From X 192).

Fig. 14. Histology of adrenal from pantothenol treated control animal. No significant change in comparison to control (From X 192).

Fig. 15. Histology of the adrenal of scorbutic guinea pig showing hypertrophy and haemorrhage in the gland. Compare with Figure 13 (From X 192).

Fig. 16. Absence of haemorrhage from the adrenal of pantothenol treated scorbutic animals, showing improvement in adrenal morphology. Compare with Figure 15 (From X 192).
Description of Figures

Fig. 17. Distribution of neutral lipid in the testes of control guinea pig (From X 96).

Fig. 18. A slight increase in neutral lipid distribution in the testes of control guinea pigs following pantothenol treatment. Compare with Figure 17 (From X 96).

Fig. 19. A marked increase in neutral lipid distribution in testes of animals, deprived of vitamin C. Compare with Figure 17 (From X 96).

Fig. 20. A slight increase in neutral lipid distribution in the interstitial cells of testes in pantothenol supplemented ascorbic acid deficient animal in comparison to scorbutic group. Compare with Figure 19 (From X 96).
Description of Figures

Fig. 21. Distribution of neutral lipid in the adrenal cortex of control guinea pig (From X 96).

Fig. 22. No significant change in neutral lipid distribution of testes in control animals treated with pantothenol (From X 96).

Fig. 23. A fall in neutral lipid in the adrenal of scorbutic guinea pigs. Compare with Figure 21 (From X 96).

Fig. 24. A slight increase in neutral lipid in adrenal of pantothenol treated scorbutic animals in comparison to scorbutic group. Compare with Figure 23 (From X 96).
Description of Figures

Fig. 25. SDH activity in testes of control guinea pig (From X 96).

Fig. 26. Showing marked increase in enzyme activity, specially in the tubular cells in pantothenol treated control animals. Compare with Figure 25 (From X 96).

Fig. 27. Testes of ascorbic acid deficient guinea pig showing decrease in SDH activity. Compare with Figure 25 (From X 96).

Fig. 28. Slight increase in SDH activity specially in the tubular cells of guinea pigs deprived of vitamin C and supplemented with pantothenol. Compare with Figure 27 (From X 96).
Description of Figures

Fig. 29. SDH activity in the adrenal cortex of control male guinea pig (From X 96).

Fig. 30. Section of adrenal showing increase in SDH activity in pantothenol treated control guinea pig. Compare with Figure 29 (From X 96).

Fig. 31. A fall in SDH activity in adrenal of guinea pig deprived of ascorbic acid. Compare with Figure 29 (From X 96).

Fig. 32. Section of adrenal from pantothenol treated scorbutic guinea pig showing increase in SDH activity in comparison to scorbutic group. Compare with Figure 31 (From X 96).
Description of Figures

Fig. 33. Section of testes showing $\Delta^5$-3\(\beta\)-OHD activity in control guinea pig (From X 96).

Fig. 34. Shows no significant change in $\Delta^5$-3\(\beta\)-OHD activity of testes in pantothenol treated control guinea pig (From X 96).

Fig. 35. Testes of scorbutic guinea pig showing a decrease in the $\Delta^5$-3\(\beta\)-OHD activity. Compare with Figure 33 (From X 96).

Fig. 36. A slight increase in $\Delta^5$-3\(\beta\)-OHD activity in testes pantothenol treated scorbutic guinea pig. Compare with Figure 35 (From X 96).
Description of Figures

Fig. 37. $\Delta^5-3\beta$-OHD activity in the adrenal cortex of control male guinea pig (From X 96).

Fig. 38. Increased activity of $\Delta^5-3\beta$-OHD in the adrenal cortex of pantothenol treated control guinea pig (From X 96).

Fig. 39. Diminution in $\Delta^5-3\beta$-OHD activity in the adrenal cortex of scorbutic guinea pig (From X 96).

Fig. 40. Adrenal cortex of pantothenol treated scorbutic guinea pig showing an increase in $\Delta^5-3\beta$-OHD activity in comparison to scorbutic animal (From X 96).
Table 1

Effect of exogenous pantothenol on body and organ weights of normal and scorbutic guinea pigs
(The data are means + S.E.M.)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of animals</th>
<th>Body weight Before treatment (g)</th>
<th>Body weight After treatment (g)</th>
<th>Adrenal weight* (mg/100 g b. wt.)</th>
<th>Testes weight* (mg/100 g b. wt.)</th>
<th>Prostate weight (mg/100 g b. wt.)</th>
<th>Seminal vesicle weight (mg/100 g b. wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + Saline (I)</td>
<td>21</td>
<td>205.1 ± 21.2</td>
<td>251.7 ± 15.2</td>
<td>64.8 ± 2.75</td>
<td>133.64 ± 6.4</td>
<td>127.75 ± 9.7</td>
<td>117.91 ± 12.7</td>
</tr>
<tr>
<td>Control + Pantothenol (II)</td>
<td>20</td>
<td>200.8 ± 19.7</td>
<td>238.1 ± 22.4</td>
<td>60.6 ± 2.73</td>
<td>96.60 ± 9.4</td>
<td>148.05 ± 12.9</td>
<td>142.56 ± 13.6</td>
</tr>
<tr>
<td>Vitamin C deficient (III)</td>
<td>19</td>
<td>207.7 ± 18.8</td>
<td>198.1 ± 21.8</td>
<td>92.4 ± 5.84</td>
<td>93.57 ± 11.6</td>
<td>86.32 ± 11.6</td>
<td>96.21 ± 9.4</td>
</tr>
<tr>
<td>Vitamin C deficient + Pantothenol (IV)</td>
<td>19</td>
<td>210.3 ± 19.8</td>
<td>201.9 ± 24.5</td>
<td>93.2 ± 6.2</td>
<td>61.14 ± 7.2</td>
<td>111.83 ± 17.2</td>
<td>101.8 ± 10.4</td>
</tr>
</tbody>
</table>

'P' values between groups I & II P > 0.05 P > 0.05 P < 0.01 P > 0.05 P > 0.05
I & III P < 0.05 P < 0.001 P < 0.01 P < 0.02 P > 0.05
I & IV P > 0.05 P < 0.001 P < 0.001 P > 0.05 P > 0.05
III & IV P > 0.05 P > 0.05 P < 0.05 P > 0.05 P > 0.05

* Weight of both glands.
Table 2

Effect of pantothenol on the tubular area of the testes of normal and scorbutic guinea pigs.
(The data are means ± S.E.M.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>No. of animals</th>
<th>Total number of tubules measured</th>
<th>Area in square cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>7</td>
<td>105</td>
<td>10.56 ± 0.55</td>
</tr>
<tr>
<td>II</td>
<td>Control + Pantothenol</td>
<td>8</td>
<td>200</td>
<td>2.83 ± 0.63</td>
</tr>
<tr>
<td>III</td>
<td>Scorbutic</td>
<td>8</td>
<td>200</td>
<td>3.72 ± 0.89</td>
</tr>
<tr>
<td>IV</td>
<td>Scorbutic + Pantothenol</td>
<td>8</td>
<td>200</td>
<td>4.71 ± 1.13</td>
</tr>
</tbody>
</table>

'P' values between Groups I and II

I and III  \( P < 0.001 \)
I and IV  \( P < 0.001 \)
III and IV  \( P > 0.05 \)
### Table 3

Effect of pantothenol on average cholesterol and ascorbic acid content of testes in normal and scorbutic guinea pigs

(The data are means $\pm$ S.E.M.)

<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 (µg/100 mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + Saline (I)</td>
<td>12</td>
<td>154.70 ± 9.2</td>
<td>2.75 ± 0.24</td>
<td>47.90 ± 7.3</td>
</tr>
<tr>
<td>Control + Pantothenol (II)</td>
<td>15</td>
<td>100.27 ± 11.3</td>
<td>2.29 ± 0.2</td>
<td>48.70 ± 5.4</td>
</tr>
<tr>
<td>Vitamin C deficient + Saline (III)</td>
<td>12</td>
<td>101.96 ± 10.2</td>
<td>3.75 ± 0.41</td>
<td>26.92 ± 6.9</td>
</tr>
<tr>
<td>Vitamin C deficient + Pantothenol (IV)</td>
<td>12</td>
<td>72.14 ± 8.0</td>
<td>4.59 ± 0.8</td>
<td>34.95 ± 6.4</td>
</tr>
</tbody>
</table>

*P* values between groups

- I & II $P < 0.01$ $P > 0.05$ $P > 0.05$
- I & III $P < 0.001$ $P < 0.05$ $P < 0.05$
- I & IV $P < 0.001$ $P < 0.05$ $P > 0.05$
- III & IV $P < 0.05$ $P > 0.05$ $P > 0.05$

* Weight of both glands
Table 4

Effect of pantothenol on average cholesterol and ascorbic acid content of adrenal in normal and scorbutic guinea pigs

(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 (µgm/100 mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + Saline (I)</td>
<td>12</td>
<td>63.5 ± 2.8</td>
<td>4.95 ± 0.45</td>
<td>77.5 ± 7.1</td>
</tr>
<tr>
<td>Control + Pantothenol (II)</td>
<td>15</td>
<td>61.6 ± 3.1</td>
<td>3.61 ± 0.22</td>
<td>86.2 ± 5.4</td>
</tr>
<tr>
<td>Vitamin C deficient + Saline</td>
<td>15</td>
<td>88.5 ± 6.7</td>
<td>2.26 ± 0.24</td>
<td>31.8 ± 6.1</td>
</tr>
<tr>
<td>(III)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin deficient + Pantothenol (IV)</td>
<td>13</td>
<td>80.1 ± 6.9</td>
<td>2.45 ± 0.21</td>
<td>33.5 ± 8.7</td>
</tr>
</tbody>
</table>

'P' values between groups I & II: P > 0.05, P < 0.02, P > 0.05
I & III: P < 0.05, P < 0.001, P < 0.001
I & IV: P < 0.05, P < 0.001, P < 0.001
III & IV: P > 0.05, P > 0.05, P > 0.05

* Weight of both glands.
Discussion

The results of the present investigation indicate testicular hypofunction and adrenal hyperfunction in scorbutic guinea pigs. Treatment with pantothenol in control guinea pigs potentiates adrenal function. However, under similar condition, arrest of spermatogenesis and proliferation of interstitial cells were noted in the testes. Treatment of scorbutic animals with pantothenol improved to some extent the morphology of both adrenal and the interstitial cells of testes.

There is a close interdependence between nutritional factors and endocrine function. Of the nutritional factors studied to date, pantothenic and ascorbic acid have been shown to affect the adrenal cortex more consistently. Several investigators (13-20, 24, 62-66) have shown that both pantothenic acid and ascorbic acid deficiency interferes with the normal physiological function of adrenals and gonads. Although the ascorbic acid content of the steroid producing organs has provided a good index for the measurement of steroidogenesis, the actual mechanism of action of ascorbic acid is not well defined. Cholesterol is the major precursor of the steroids. Pantothenic acid is involved in cholesterol formation in view of its occurrence as a component of coenzyme A which following acetylation is incorporated into cholesterol (1-12). So there is possibility that pantothenic acid might influence steroidogenesis by influencing the formation of the precursor.
Mitolo and Manfredi (67) have reported that pantothenic acid increases the life span of the animals in vitamin C deficiency, but there is no report if the functional alteration due to deficiency of one vitamin can be corrected by the other. The diet supplied to ascorbic acid deficient guinea pigs in the present experiment contained all the essential nutrients as in a basal diet, except vitamin C. So it was sufficient, as regards the pantothenic acid requirement. However, in these deficient guinea pigs a decrease in acetylation reaction has been noted by Lahiri and Banerjee (68). As pantothenic acid is involved in coenzyme A synthesis, the purpose of the present study was to see if the abnormal physiological functions of adrenals (13-20) and testes (21-24) due to ascorbic acid deficiency could be corrected with an extra amount of pantothenic acid and whether similar extra amount of this vitamin affects the adrenals and gonads of control animals in the same manner.

Cholesterol metabolism is seriously affected in absence of ascorbic acid. The radioactive study on the biosynthesis of cholesterol in scorbutic guinea pigs by feeding labeled acetate was reported by Becker et al. (69) who showed that biosynthesized cholesterol contained higher specific activities in scorbutic animals. In 1951, Oesterling and Long (15), and Banerjee and Deb (16) reported a reduction of adrenal cholesterol in scurvy. But Lahiri and Banerjee (68) also observed that the total body cholesterol increase significantly in scorbutic guinea pigs. The observation by Willis (70)
showed that atherosclerosis developed quickly even without cholesterol diet in scorbutic guinea pigs. Administration of ascorbic acid could cure the early lesions of atherosclerosis, but the advanced lesions were more resistant to reversal. From the literature it seems that ascorbic acid has an important role in the regulation of cholesterol metabolism in animal tissues.

By the use of isotopically labeled acetate, it has been shown that acetic acid is the sole source of carbon atoms for cholesterol synthesis, and the steps involved in the synthesis are also established (71,72). The first step of the cholesterol synthesis is acetylation of coenzyme A. The occurrence of pantothenic acid in coenzyme A and the role of the particular coenzyme in acetylation reaction (1-12) have been well established. In ascorbic acid deficiency although coenzyme A content of tissues remains unaltered, a reduction in acetylating capacity has been noted by Lahiri and Banerjee (68). They suggested that ascorbic acid is in some way concerned in acetylation reaction in the body. Decrease in ascorbic acid and cholesterol content of adrenals in scurvy are repeatedly discovered by several investigators (13-19). According to Stepto et al. (20) adrenals are hypertrophied during scurvy. Pirani (19) is of opinion that deficiency of vitamin C produces non-specific stress. When the adrenal cortex is stimulated by stressful conditions, it responds by secreting an extra amount of steroid hormones which initiate a variety of physiological reactions.
Pantothenic acid, as part of coenzyme A, plays a critical role in the oxidative metabolism of both carbohydrate and fatty acids and is also involved directly in cholesterol synthesis. Cholesterol is the major precursor of steroid hormones. So factors which influence cholesterol metabolism are supposed to influence the metabolism of steroid hormones also.

Pantothenol treatment in control male guinea pigs caused a marked fall in the weight of the testes. It also decreased in vitamin C deficient animals and following pantothenol treatment under this condition, a further decrease in weight of the organ was noticed. Decrease in the weight of the testes due to atrophy, in tyrosine fed rats have been observed by Biswas and Deb (73). But testes has two functions, spermatogenic and endocrine. The spermatogenic function of testes is carried out by the seminiferous tubules under the influence of follicle stimulation hormone (FSH), while the androgenic function is carried out by the interstitial cells of Leydig under the influence of interstitial cell stimulating hormone (ICSH) of pituitary. Because of this, the decrease in testes weight might be due to atrophy of the whole organ or due to functional inhibition of either seminiferous tubules or the Leydig cells. As the weight of the testes decreased markedly in all the three experimental conditions, a detailed study of the organ was carried out to know exactly the cause of such an effect. Fidanza and his group (28,74-76) studied the effect of excess pantothenic acid on the weight of the
primary and secondary sex organs and suggested that pantothenic acid in excess causes functional and anatomical modification of the gonads in rats. But in the present set of experiments on guinea pigs, pantothenol treatment caused a marked decrease in the weight of the testicles (Table 1). This difference in response between rats and guinea pigs may be due to two different vertebrates studied.

The cholesterol and ascorbic acid concentration of testes remained unaltered in pantothenol treated control animals (Table 3). In this testes of scorbutic animals the ascorbic acid content was very low and cholesterol concentration was high. This increase in cholesterol concentration in scorbutic guinea pigs has previously been observed by Biswas (77) who has attributed this accumulation to diminution in the functional activity of the organ. The cholesterol concentration showed an insignificant increase in testes of scorbutic guinea pigs following pantothenol treatment, indicating that this vitamin might possibly cause an increase in cholesterol synthesis, but in the absence of vitamin C this cannot be utilized for the synthesis of steroid hormone.

Abnormal testes shows different picture anatomically and morphologically. Zubiran and Gomez-Mont (35) reported decrease in size of seminiferous tubules, lack of spermatogenesis, vacuolization of Sertoli cells and diminution in the size of interstitial cells in human under chronic malnutrition.
Banerjee and Dravid (41) have observed inhibition in spermatogenesis, with diminution in the size of seminiferous tubules, and degenerative changes in the Leydig cells in vitamin B₁₂ deficient rats. Degeneration and atrophy of the germinal epithelium have been reported by Lindsay and Mades (43) and also by Biswas and Deb (24,49) in scorbutic guinea pigs. Kocen and Cavazos (44) noted pathological changes of the Leydig cells accompanied by spermatogenic arrest under the same condition. In the present set of experiments the histological changes in the testes of scorbutic guinea pigs were similar to the observation of previous workers and support the postulation that testes is atrophied in absence of vitamin C. In pantothenol treated control animals the histological picture of the testes was found to be similar to cryptorchid animal. Arrest of spermatogenesis along with proliferation of the interstitial cells has been noted in cryptorchid testes (78) and also in pantothenol treated control animals. According to some authors the changes as observed in cryptorchid testes might be due to changes in temperature (79), but this has not gained universal acceptance. The effect of pantothenol on inhibition of spermatogenesis in guinea pig without affecting the interstitial cells has not been reported previously. This might be due to either a direct effect on the germinal cells or an indirect one mediated through inhibition of follicle stimulating hormone. In the pantothenol treated scorbutic testes, an improvement in the morphological picture of interstitial cells in comparison to scorbutic group has been noted, though
the degenerative changes in the seminiferous tubules remained similar, indicating differential involvement of this vitamin on gametogenic and endocrine component of testes (Figs. 1, 2, 3, 4).

From Table 2 it can be noted that there is a marked decrease in the size of the seminiferous tubules in all the three experimental conditions (Figs. 5, 6, 7, 8) indicating inhibition of gametogenic activity. From the morphological picture and measurement of the size of the seminiferous tubule it can be postulated that the spermatogenic activity was reduced in all the three experimental conditions.

When estimated biochemically the cholesterol concentration of the control testes following pantothenol treatment remained unchanged whereas histochemical observation shows an increase in sudan positive materials. This increase might be due to increase in neutral lipid other than cholesterol. The distribution of sudanophilic lipids was markedly increased in the scorbutic testes. An accumulation of these lipids in the testes has previously been observed by Biswas and Deb (49). They have suggested it to be due to decreased spermatogenesis and androgen synthesis of the organ. The distribution of neutral lipid further increased in testes of pantothenol treated scorbutic animals (Figs. 17, 18, 19, 20). Possibly by the same mechanism as in the control animals, pantothenol increases neutral lipid in the scorbutic testes also. But due to absence of vitamin C, steroids cannot be synthesized and the lipid has been found to be accumulated.
SDH is an important enzyme of the citric acid cycle. Kormano et al. (30) observed an increased SDH reaction in the interstitial cells of the cryptorchid rats. The results of the present investigation supports the view that there is a cryptorchid like change in the pantothenol treated scorbutic animal because there was an increase in the enzyme activity in this condition also. Biswas and Deb (49) have previously noted a decrease in SDH activity along with testicular atrophy in scorbutic testes. The results of the present experiment is in close agreement with the observation of the above workers and support the postulation that reduction in the enzyme activity was due to vitamin C deficiency in the system. Hypo-function of interstitial cells due to tyrosine toxicity in rats, which was postulated to be due to reduced biosynthesis of ascorbic acid, was also accompanied with a reduction in SDH (39). The increase in SDH activity in the interstitial cells in pantothenol treated scorbutic animals in comparison to scorbutic was possibly due to stimulatory effect of the vitamin on the production of androgenic hormone in the experimental condition studied (Figs. 25, 26, 27, 28).

The enzyme $\Delta^5\text{-}3\beta\text{-OHD}$ occupies the key position in the process of steroid synthesis (81). According to Biswas and Deb (82) the decrease in $\Delta^5\text{-}3\beta\text{-OHD}$ activity in testes indicates impairment of steroid hormone synthesis. The enzyme activity did not change in pantothenol treated control animal showing unaltered endocrine activity under this condition. The
marked decrease in scorbutic condition might be attributed to lack of gonadotropin, in view of the fact that gonadotropin level decreases in scurvy (24) and decreased gonadotropins causes inhibition of this enzyme activity (32). Following pantothenol treatment in scorbutic animal, there was slight increase in the enzyme activity. This increase indicates that pantothenol stimulates to some extent, in an unknown way, possibly, the interstitial cells of Leydig to produce androgens in absence of vitamin C.

Leucine Aminopeptidase (LAP) in the guinea pig testes shows a precise specificity in its localization pattern, being present only in the testicular Leydig cells. Dey et al. (54) studied the LAP activity of testes in different experimental conditions and observed a close correlation between the enzyme activity and the proliferation and functional activity of the intertubular Leydig cells. They noted an increase in the enzyme activity in the interstitial cells of the ectopic testes, and decrease in the estrogenized group with atrophy of Leydig cells. Thus the increase in enzyme activity in the pantothenol treated control animals might be attributed to proliferation of the Leydig cells in this condition. The decrease in enzyme activity in scurvy is due to atrophy of the Leydig cells. Pantothenol treatment in scorbutic animal caused an increase in enzyme activity in comparison to the scorbutic group. Thus it is evident that pantothenol plays some stimulatory role selectively on the interstitial cells of Leydig.
The weight of the accessory sex organs increases or decreases according to the amount of androgenic hormones in the testicles (42). Detailed information about the structure of prostrate gland is also available (83). The epithelium of the normally functional gland is of a higher columnar type. The nucleus occupies only the bottom of the cell. The ultimate effect of castration is to convert the epithelium of the whole prostrate to a low cuboidal type, with the nucleus occupying most of the cell. In the present investigation the weight of the prostrate increased, though not significantly, in the pantothenol treated control guinea pigs (Table 1). However, the columnar epithelium of the luminar lining of the prostrate and the glandular secretion were similar to the control group. The decrease in weight of prostrate (Table 1), flattening of the columnar epithelium to cubical type and lack of secretion in the lumen support the observation that there is inhibition in the androgenic activity of testes in scurbutic condition. Following pantothenol treatment in scurbutic animals, slight increase in the prostrate weight was noted. This indicates an improvement in the function of the interstitial cells in this condition in comparison to scurbutic group. The appearance of the histological picture of the prostrate was also improved (Figs. 9, 10, 11, 12).

Cholesterol and ascorbic acid seems to be related to the formation of adrenal steroids, as is evident from the fact that injections of adrenocorticotropic hormone of the pituitary in rats and guinea pigs also lowered ascorbic acid
along with cholesterol content of adrenal gland (29,30). It is possibly owing to this reason, the most widely used indices for the measurement of adrenocortical function are measurement of the concentration of both cholesterol and ascorbic acid of the gland (84). Ralli and Dumm (85) postulated that the pantothenate content of the diet can modify the concentration of cholesterol and possibly of ascorbic acid in the rat adrenal. In pantothenic acid deficiency, both adrenal cholesterol and ascorbic acid concentration decreased (66). There is also evidence that diets contained large amounts of pantothenate increase the adrenal cholesterol concentration in rats (85). In the present set of experiments, a fall in adrenal cholesterol concentration in pantothenol treated control animals, has been observed although the weight and ascorbic acid content of the gland remained unaltered (Table 4). It is difficult to explain this result in view of the fact that adrenal cholesterol in rat is increased by pantothenate treatment (85). Only explanation may be that, the rats and guinea pigs belong to different species. Fidanza and Constable (86) could not find any significant change in adrenal cholesterol in guinea pigs treated with pantothenol for ten days. In another study Fidanza and Lenti (26) noted a sharp fall in adrenal cholesterol in rats either one hour or six hour after treatment with sodium pantothenate. They related the effect to an enhanced activity of adrenal glands in the utilization of cholesterol as the precursor of steroid hormone. To determine,
whether this phenomenon is related to that of stress, the
behaviour of adrenal ascorbic acid, following this treatment
was also studied (87). As the ascorbic acid content of the
gland remained unaltered, stress as a cause of this pheno-
menon was excluded. In the present experiment the ascorbic
acid concentration of adrenal remains unaltered, while choles-
terol concentration decreased significantly (Table 4) follow-
ing pantothenol treatment for 20 days. But it is known from
the previous works that stimulation of adrenal gland is
followed by decrease in both ascorbic acid and cholesterol
(29,30). It can not be explained properly why in such condi-
tion of enhanced steroidogenesis ascorbic acid did not indi-
cate any change.

In the scorbutic animals, adrenal cholesterol and
ascorbic acid concentration decreased markedly with simulta-
neous increase in the weight of the gland (Table 4). These
results are in close agreement with those of the previous
workers (13-19). According to many of them the changes noticed
in the adrenals in scurvy can be explained by assuming that the
adrenal cortex is stimulated by the anterior pituitary due to
non-specific stress of scurvy. As in the case of pantothenol
treated control animal, there was no significant change in
the weight or ascorbic acid content of the adrenal in panto-
thenol treated scorbutic group, in comparison to scorbutic
animals. In this case the cholesterol content also remained
unaltered. It is not known whether pantothenol increases
cholesterol synthesis in adrenal of guinea pig or not. Even
if this treatment increases cholesterol synthesis in the adrenal of scorbutic guinea pigs, it is immediately utilized by the gland to synthesize more steroids and apparently no change can be noted in ascorbic acid or cholesterol concentration of the gland.

From the histological study of adrenal cortex no change could be found in the pantothenol treated control animal, whereas hypertrophy and haemorrhage was noted in the scorbutic group. In pantothenol treated scorbutic group though the adrenal is still hypertrophied, signs of haemorrhage is absent. This indicates that pantothenol helps the adrenal to cope with the demand of cortical hormones by the body and prevents it from atrophy (Figs. 13, 14, 15, 16).

Sayers et al. (46) noticed an intimate relationship between the density of sudanophilic material and the concentration of cholesterol in adrenal. High accumulation of these substances in the adrenal indicates inadequate secretion and subnormal functioning of the organ (46, 47). Decrease in cholesterol content of adrenal is said to be due to increased demand for cortical hormone (48). In the present set of experiments there was no change in the sudanophilic material in the adrenal of pantothenol treated control animal, whereas it was found to be greatly decreased in scorbutic adrenal supporting the view that adrenal is stimulated during scurvy. In the pantothenol treated scorbutic group there was a slight increase in sudanophilic lipid in the adrenal in comparison
to the scorbutic. It can be noted that although a decrease in cholesterol concentration of control animal following pantothenol treatment has been observed, no change could be found in the distribution of sudanophilic lipid. No change could be found in the cholesterol concentration of adrenal in pantothenol treated scorbutic guinea pig in comparison to scorbutic, yet there was slight increase in the distribution of sudanophilic lipids (Figs. 21, 22, 23, 24).

The disparity between biochemical and histochemical findings cannot be properly explained. Although sudanophilia is an index of the cholesterol concentration in the organ, it stains neutral lipids other than the above mentioned steroid. The limitations of this histochemical findings as a valid qualitative index cannot be ruled out also.

Several investigators have employed localization of SDH (50) and $\Delta^5-3\beta$-OHD (50) in adrenals to study the functional activity of the organ. In the present set of experiment the SDH activity was found to be markedly increased in adrenals of pantothenol treated control animals. It decreased in scorbutic adrenal and increased slightly in the pantothenol treated scorbutic group in comparison to scorbutic. This enzyme might be taken as indicative of the activity of the TCA cycle (88). The enzyme in the present set of experiment indicates that TCA cycle is slowed down in scorbutic condition. This result is in close agreement with the result of Banerjee and Kawishwar (89), who observed an inhibition of krebs cycle in scurvy.
When the normal animals are treated with pantothenol the increase in the SDH activity indicates a stimulation of Krebs cycle. In the pantothenol treated scorbutic adrenal there is a marked increase in enzyme activity indicating a better functioning of TCA cycle under this condition, though ascorbic acid is absent. The influence of pantothenic acid on TCA cycle has been emphasized by several investigators (85). Dorfman et al. (90) and Hills (91) observed that pantothenic acid increased the rate of oxidation of pyruvic acid by suspensions of *proteus morgani* grown in a pantothenate free medium. 

A suggestion of a similar relation in animals came from the work of Pilgrim et al. (92) who showed that pantothenic acid deficiency decreased the rate of oxidation of pyruvate in rat liver. In 1947, Novelli and Lipmann (93) confirmed the work of Dorfman et al. (90) and Hills (91) and demonstrated that in bacteria an increase in the concentration of coenzyme A paralleled the stimulation of pyruvate oxidation by pantothenate. Shive et al. (94) suggested that the pantothenate deficient cells are unable to convert pyruvic acid and oxaloacetic acids to citrate, and thus enable pyruvate to be oxidized in the TCA cycle. The increase in SDH activity in the pantothenol treated scorbutic guinea pig in the present experiment may be due to stimulation of TCA cycle. According to Banerjee and Kawishwar (89) the decrease in SDH activity in scurvy is due to inhibition of Krebs cycle. Thus the enhancement of the enzyme activity following pantothenol treatment in scorbutic guinea pig might be due to stimulation of inhibited TCA cycle by pantothenol (Figs. 29,30,31,32).
\( \Delta^5-3\beta\text{-OHD} \) is one of the principal enzymes involved in the process of steroidogenesis. The activity of this enzyme along with the study of other constituents can be taken as indices to study the functional activity of the steroid producing glands. Following pantothenol treatment in control guinea pigs there was an increase in the enzyme activity indicating that the adrenal function is enhanced under this condition. The marked decrease in the enzyme activity of the gland in scurvy in this experiment should not be attributed to diminished activity of the organ, because both ascorbic acid and cholesterol were depleted from the gland. It has been observed previously that urinary excretion of 17-ketosteroids (95, 96) are markedly increased in scurvy and there is a diminution in both cholesterol and sudanophilic lipids from the gland, indicating hyperfunctioning of the adrenal. From the result obtained in the present investigation, it can be assumed that the decrease in enzyme activity is due to reduced content of substrate, i.e. cholesterol content, in view of the fact that the rate of enzyme activity is directly proportional to the substrate concentration (96). There was an increase in the enzyme activity in the pantothenol treated scorbutic adrenal when compared to the scorbutic group, though the cholesterol content remained unaltered (Figs. 37,38,39,40). The morphology of the gland was also better than that of the scorbutic group. From this result it can be postulated that pantothenol treatment in scorbutic guinea pig increases the cholesterol synthesis of the gland. This increase in precursor causes
enhancement of the enzyme activity resulting in increased steroid synthesis. Thus the cholesterol formed is immediately utilised by the adrenal to synthesize more steroids to cope with the stress of scurvy. Due to this reason the enzyme activity increases keeping the cholesterol content unaltered. But this is a mere postulation from these studies.

From all these above evidences it can be concluded that pantothenol treatment in control guinea pigs stimulates the functional activity of the adrenal. Same treatment in scorbutic guinea pigs improves the functional activity of the adrenal to some extent and helps it to cope with the stress due to deficiency of vitamin C. The role of pantothenic acid to cope with stress has been shown by many investigators (85). The results of this observation support the view of those workers.

Testes shows a different picture following pantothenol treatment both in control and scorbutic guinea pigs. In the control animals pantothenol causes an inhibition of spermatogenic activity, though the endocrine activity of the Leydig cells is stimulated to some extent. Deficiency of vitamin C causes diminution in both the gametogenic and endocrine functions of the organ. Pantothenol treatment in scorbutic guinea pigs stimulates the interstitial cells to some extent but the gametogenic function remains unaffected.
Summary

Effect of ascorbic acid in absence of pantothenic acid has been discussed in the previous Chapter (Chapter III). In the present experiment an attempt has been made to study the role of pantothenic acid in absence of ascorbic acid. Guinea pigs cannot synthesize ascorbic acid and due to this reason they can be made deficient in this vitamin. So the role of pantothenic acid has been studied in normal and vitamin C deficient guinea pigs.

To evaluate the adrenal cortical and testicular function histochemical and biochemical techniques have been followed.

The results indicate that pantothenol treatment in normal guinea pigs causes alteration in both adrenocortical and testicular function by some mechanism which cannot be properly explained. Steroidogenesis both adrenocortical and gonadal and also spermatogenesis were seriously affected in absence of vitamin C. Adrenal cortex showed hyperfunction whereas testes showed hypofunction in such condition. Pantothenol treatment during vitamin C deficiency caused improvement in the morphology of the adrenal and also in the cellular activity of both adrenal and testes.

It can be postulated that possibly pantothenol treatment caused improvement in the endocrine activity of both adrenal and testes by supplying more of the precursor, cholesterol. But this treatment cannot maintain spermatogenesis.
References


