A relationship between pantothenic acid and the adrenal gland has been evident since the earliest work with this vitamin was done. Morgan and Simms (1) found pathologic changes in the adrenals of rats on diets free from "filtrate factor," and reported (2) that the achromotrichia of rats maintained on these diets could be at least partly reversed by extracts of the thyroid and of the adrenal cortex. Daft and Sebrell (3) and Nelson (4) likewise described a hemorrhagic adrenal necrosis in rats on deficient diets, which they later showed (5, 6) could be cured by synthetic pantothenic acid. Further evidence for the existence of a relationship between pantothenic acid and the adrenal gland was provided by Ralli and Graef, who found (7) that, when grayed pantothenic acid-deficient rats were adrenalectomized, the new hair growing in was well pigmented. Analyses of melanin content of rat hides showed (8) that the deficient diet produced a decrease in extractable melanin, while adrenalectomy in deficient rats resulted in an increase which could be inhibited by desoxycorticosterone acetate (DOCA).

Cytological studies by Deane and McKibbin (9) showed the adrenal cortices of pantothenic acid-deficient rats to be enlarged and the zonae reticularis and fasciculata progressively drained of ketosteroids. These changes were interpreted by the authors to be indications that pantothenic acid deficiency acts as an "alarming agent" for the rat, causing the release of adrenocorticotropic hormone (ACTH) which stimulates the adrenal to secrete cortical hormone until exhaustion occurs. Ashburn (6) has also suggested that an adrenal hypofunction may exist in pantothenic acid-deficient rats. The work of Gaunt et al. (10), who observed a lethargic diuresis in pantothenic acid-deficient rats when water was given by stomach tube and a decreased resistance to water intoxication, provided evidence which is consistent with this theory. This subject has been recently reviewed (11).

If it is true that pantothenic acid deficiency imposes a continuous stress on the adrenal cortex, then, according to Selye (12, 13), in the final stages

* Present address, Child Research Council, University of Colorado Medical School, Denver, Colorado.
of the deficiency exhaustion of the adrenal should occur and the deficient animals should react as though adrenalectomized, particularly if exposed to a secondary stress. Anoxic anoxia was chosen as the secondary stress to investigate this hypothesis and to determine any deviations of carbohydrate metabolism in the intact pantothenic acid-deficient rat. Liver glycogen, blood glucose, adrenal ascorbic acid levels, and adrenal weights were recorded in normal and deficient rats at sea level and 20,000 feet of simulated altitude.

**EXPERIMENTAL**

Female rats of the Long-Evans-Wistar strain were given the purified pantothenic acid-deficient diet when their young were 15 days of age. At 21 days of age, the young were weaned, placed in individual metabolism cages, and grouped according to weight, litter, and sex. The basal diet had the following per cent composition: vitamin-free casein\(^1\) 22.0, sucrose 66.5, fat\(^2\) 9.0, and salts\(^3\) 2.5.

Supplements of crystalline vitamins were given three times per week in amounts to provide the following daily quantities in mg.: thiamine hydrochloride, riboflavin, folic acid, and pyridoxine hydrochloride, each 0.02, calcium pantothenate and \(\beta\)-aminobenzoic acid, each 0.1, nicotinic acid amide 0.066, inositol 2.5, biotin 0.002, and choline 5.0. Vitamin A, vitamin D, and tocopherols were supplied separately in the amounts of 1000 i.u., 100 i.u., and 3 mg. per rat per week. The deficient groups received the vitamins listed with the exception of pantothenic acid.

The experimental animals were kept on their respective diets for 5 weeks after they were grouped, at the end of which time some were subjected to fasting for 24 hours at sea level and others under reduced oxygen tension, 349 mm. of Hg, corresponding to 20,000 feet of elevation. During this test period they received no water. They were then anesthetized with sodium amytal and sacrificed.

The anoxia apparatus consisted of a series of 1 quart jars connected to the evacuating system by copper tubes sealed in the lids, as described by Wickson and Morgan (14). At the beginning of the test periods the rats were placed in the glass jars, and the pressure was reduced gradually. The rats taking the test period at sea level were placed in jars under the same conditions except that there was no reduction in pressure.

Blood glucose was determined by the method of Giragossintz, Davidson, and Kirk (15). Liver glycogen was precipitated and hydrolyzed according

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\(^1\) Vitamin test casein procured from General Biochemicals, Inc., Chagrin Falls, Ohio.

\(^2\) Primex, a hydrogenated vegetable fat.

\(^3\) Hubbell, R. B., Mendel, L. B., and Wakeman, A. J. (24).
to Good, Kramer, and Somogyi (16), and the resulting reducing solution was titrated with ceric sulfate (15). To determine ascorbic acid in the adrenal glands, a method was devised based on the Bessey modification of the Roe and Kuether procedure (17, 18).

A difficulty arose in these and other experiments with pantothenic acid-deficient animals in that no reliable, easily determined criterion of the

**Table I**

*Effect of Stage of Pantothenic Acid Deficiency upon Response of Rats to Anoxia*

<table>
<thead>
<tr>
<th>Group</th>
<th>Time on diet (wks.)</th>
<th>Liver glycogen, mg. per cent fresh liver</th>
<th>Blood glucose, mg. per cent</th>
<th>Adrenal ascorbic acid, mg. per cent</th>
<th>Adrenal weights, mg. per cent body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2</td>
<td>±12* 104 ±193 61</td>
<td>At sea level 312 28</td>
<td>97 At 20,000 ft. 301 32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>±16 99 ±441 64</td>
<td>At sea level 413 25</td>
<td>104 At 20,000 ft. 399 25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>±16 113 ±211 64 At sea level 353 24</td>
<td>±12 At 20,000 ft. 21 29</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>±3 86 ±391 64</td>
<td>At sea level 245 34</td>
<td>±6 At 20,000 ft. 364 25</td>
<td></td>
</tr>
<tr>
<td>Pantothenic acid-deficient</td>
<td>2</td>
<td>±40 143 ±51 69</td>
<td>At sea level 247 30</td>
<td>±14 At 20,000 ft. 336 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>±18 177 ±37 66</td>
<td>At sea level 254 32</td>
<td>±13 At 20,000 ft. 303 32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>±5 131 ±20 64 44</td>
<td>At sea level 226 32</td>
<td>±5 At 20,000 ft. 222 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>±15 148 ±52 66</td>
<td>At sea level 360 35</td>
<td>±6 At 20,000 ft. 324 25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>±19 148 ±31 66</td>
<td>At sea level 360 35</td>
<td>±9 At 20,000 ft. 324 25</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± standard error.
† One rat.

stage of the deficiency has been found. Graying of fur and failure of growth are apparently not indicative of the same degree of deficiency, nor do they occur simultaneously and consistently in animals subjected to the deficiency for equal periods. A preliminary experiment was made, therefore, with sixteen groups of animals to determine the period of depletion during which the most marked differences between the normal and deficient animals could be seen. The results indicated that even after 2 weeks of depletion the fasting liver glycogen of the deficient rats was greater than that of the normal animals, but these levels could not be raised under anoxia as successfully. The largest differences between de-
icient and normal rats were seen after 5 weeks of deficiency. Similarly, smaller increases in blood sugar levels were produced under anoxia in the deficient rats than in the normal at each period studied, but the greatest divergence occurred after 5 weeks (Table I). The values for adrenal ascorbic acid were generally low in the deficient groups and the adrenal weights significantly increased. Since the mortality among the deficient groups beyond 5 weeks was excessive, the deficiency period of 5 weeks was chosen for the later work.

In the next experiment, one group of the deficient rats was given 1 mg. of pantothenic acid in saline, injected intraperitoneally immediately before the 24 hour test period. In a second experiment, one group of deficient rats was given similarly 5 mg. of pantothenic acid. Another group of deficient rats was injected subcutaneously with 2 ml. of water-soluble adrenal cortical extract (ACE)⁴ in three doses during the 24 hours preceding the test period. Still another deficient group received subcutaneously 1 ml. of DOCA⁶ in oil in three doses during the 24 hours preceding the test period. One group of rats on the control diet received food limited to the amount eaten by the deficient animals. These are called hereafter "inanition controls."

Results

The results are summarized in Table II. In Experiments I and II, the pantothenic acid-deficient rats showed no rise in liver glycogen during the stress period of anoxia, although at sea level the deficient groups had higher values than did the normal. The normal rats increased their liver glycogen under reduced oxygen tension 600 to 700 per cent.

The blood sugar values reflected the same condition, with a significant rise after anoxia in the controls and a slight but probably significant one in the deficient animals.

Although the anoxia had no consistent effect on either the adrenal ascorbic acid or the adrenal weights of any group, the deficient animals in all groups showed lower adrenal ascorbic acid values and increased adrenal size compared with the normal animals. These findings, along with the raised liver glycogen of the deficient rats at sea level, may mean that the deficient rats at sea level were under a primary physiological stress which was exaggerated by the secondary anoxia stress.

Administration of 1 mg. of pantothenic acid to the deficient rats just preceding the test in Experiment I (Table II) enabled them to increase their liver glycogen about 100 per cent only, but the blood sugar reached

⁴ Cortin, adrenal cortical extract in 70 per cent alcohol, generously supplied by Dr. D. J. Ingle of the Research Laboratories of The Upjohn Company, Kalamazoo, Michigan.

⁶ A product of Roche-Organon Inc., Nutley, New Jersey.
the normal level under anoxia. In Experiment II, however, administration of 5 mg. of pantothenic acid to the deficient rats did not produce a rise in liver glycogen after anoxia and only a mild rise in blood sugar.

**Table II**

*Liver Glycogen, Blood Sugar, Adrenal Ascorbic Acid, and Adrenal Weight As Affected by Pantothenic Acid Deficiency and Anoxia*

Seven to fifteen rats in each group.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Group</th>
<th>Average weight before test</th>
<th>Liver glycogen, mg. per cent of fresh liver</th>
<th>Blood sugar, mg. per cent</th>
<th>Adrenal ascorbic acid, mg. per cent</th>
<th>Adrenal weight, mg. per 100 gm. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>At sea level</td>
<td>At sea level</td>
<td>At sea level</td>
<td>At sea level</td>
<td>At sea level</td>
</tr>
<tr>
<td>I</td>
<td>Normal</td>
<td>129 gm.</td>
<td>125 gm.</td>
<td>97</td>
<td>832</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1</td>
<td>±258</td>
<td>±6</td>
<td>±12</td>
<td>±16</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>74</td>
<td>70</td>
<td>178</td>
<td>202</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±21</td>
<td>±21</td>
<td>±8</td>
<td>±25</td>
<td>±22</td>
</tr>
<tr>
<td></td>
<td>given 1 mg. pantothenic acid</td>
<td>75</td>
<td>73</td>
<td>174</td>
<td>402</td>
<td>69</td>
</tr>
<tr>
<td>II</td>
<td>Normal</td>
<td>135</td>
<td>134</td>
<td>140</td>
<td>868</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±25</td>
<td>±115</td>
<td>±8</td>
<td>±15</td>
<td>±25</td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>59</td>
<td>67</td>
<td>227</td>
<td>221</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±25</td>
<td>±30</td>
<td>±15</td>
<td>±10</td>
<td>±30</td>
</tr>
<tr>
<td></td>
<td>given 5 mg. pantothenic acid</td>
<td>65</td>
<td>66</td>
<td>221</td>
<td>208</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±20</td>
<td>±60</td>
<td>±12</td>
<td>±12</td>
<td>±15</td>
</tr>
<tr>
<td></td>
<td>Deficient, given ACE</td>
<td>62</td>
<td>71</td>
<td>242</td>
<td>637</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±27</td>
<td>±155</td>
<td>±9</td>
<td>±16</td>
<td>±16</td>
</tr>
<tr>
<td></td>
<td>Deficient, given DOCA</td>
<td>68</td>
<td>66</td>
<td>158</td>
<td>229</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±15</td>
<td>±22</td>
<td>±9</td>
<td>±12</td>
<td>±34</td>
</tr>
<tr>
<td></td>
<td>Inanition controls</td>
<td>73</td>
<td>73</td>
<td>738</td>
<td>1226</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±303</td>
<td>±374</td>
<td>±12</td>
<td>±13</td>
<td>±37</td>
</tr>
</tbody>
</table>

This difference between the two sets of data probably indicates that the second group of animals was in a later stage of the deficiency than the first. The fact that the deficient rats (without added pantothenic acid) in Experiment I were able to produce a significant increase in blood sugar level after anoxia, while those in Experiment II had a much smaller rise,
tends to support this concept. It may be that, after a certain stage in the deficiency is reached, so much damage has occurred to the adrenal gland that pantothenic acid cannot be immediately utilized. Since the blood sugar after anoxia in both experiments reached almost normal levels when pantothenic acid was given, it is conceivable that in Experiment II utilization of pantothenic acid in the carbohydrate metabolism was just beginning, and, because of the mobile character of the blood sugar picture, was immediately apparent there, while in Experiment I utilization was possible earlier and therefore was manifested also in the glycogen levels.

Administration of adrenal cortical extract enabled the deficient rats to raise their liver glycogen and blood sugar under anoxia as well as did the normal groups, strongly indicating that it is a lack of these hormones in pantothenic acid deficiency which produces the failure of carbohydrate metabolism under stress. Addition of desoxycorticosterone acetate, on the other hand, did not produce a rise in liver glycogen or blood sugar.

The inanition controls had very high levels of liver glycogen and blood glucose both with and without anoxia. The semistarvation itself, in this case, was a stress strong enough to raise liver glycogen above the normal. This rise in liver glycogen under anoxia proved that the failure of the deficient rats could not be ascribed to a lack of caloric intake. The inanition controls showed no increase in blood sugar under anoxia, but this was probably due to the previously high level, which could result only in glycosuria if still further raised.

**DISCUSSION**

That morphological damage occurs in the adrenal cortices of pantothenic acid-deficient rats has been well established (1, 3–6, 9). Our experiments demonstrate the deranged adrenal function produced by this deficiency, as evidenced by inability of the deficient rat to raise liver glycogen and blood sugar under anoxia. The deficient rats responded to anoxia as do adrenalectomized animals (19–22), but when given ACE before the test period reverted to the normal. This is strong evidence in favor of the theory that pantothenic acid deficiency produces adrenal hypofunction.

At the same time, these deficient animals had lower adrenal ascorbic acid values and higher adrenal weights than did the normal. According to the experiments of Long and his coworkers (23) lower values for adrenal ascorbic acid should indicate an increased activity of the adrenal cortex, rather than the decreased response indicated by the carbohydrate content of these animals. Long's experiments were, however, largely of short duration, whereas in this case there was a 24 hour period of stress superimposed upon the 4 to 5 week period of the deficiency itself. In the experiments described in this paper, adrenal ascorbic acid values may thus be
regarded, not as indicative of the reaction of the adrenal cortex to the secondary stress of anoxia, but rather as a measure of the effect of the deficiency state as a whole upon this gland. Since the other findings lead us to believe that the animals were in the "stage of exhaustion" (12, 13), it would be reasonable to assume that the lowered values of adrenal ascorbic acid of the deficient rats indicate that these animals were under a stress to which the normal controls were not subjected and this stress can be only that of the deficiency itself. A corroborative finding was the higher liver glycogen values of the deficient rats at sea level, although this may be ascribed to underfeeding, as in the case of the inanition controls.

Somewhat similar results were obtained in an earlier study of riboflavin deficiency with the same techniques (14). Riboflavin-deficient rats also failed to raise their carbohydrate levels under anoxic stress, but this ability was fully restored when 0.1 mg. of riboflavin was injected just previous to the test. Pair-fed normal animals reacted with exaggerated gluconeogenesis as did those observed in the present study. Apparently in riboflavin deficiency immediate repair of the carbohydrate mechanism can be effected if the vitamin is given, whereas in advanced pantothenic acid deficiency this ability has been lost. The fact that adrenal cortical extracts can instantly restore the mechanism in such animals points to the adrenal cortex as the site of the defect. Without pantothenic acid the gland appears to lose its ability to produce the gluconeogenic corticosteroids and to undergo structural changes not readily reparable.

SUMMARY

When pantothenic acid-deficient rats were subjected to lowered oxygen tension (349 mm. of Hg) equivalent to an altitude of 20,000 feet for a period of 24 hours, they were unable to raise liver glycogen or blood sugar levels as were normal rats. Adrenal ascorbic acid values were also lower in the deficient animals, while adrenal weights were increased.

Administration of adrenal cortical extract to the deficient animals before the stress period enabled them to produce the normal carbohydrate response. This was not true of desoxycorticosterone acetate.

Injection of pantothenic acid into the deficient rats before the stress period was effective in producing the normal response only when the animals had not reached the final stages of adrenal exhaustion.

These findings are interpreted as demonstrating that pantothenic acid deficiency imposes a stress on the adrenal cortex, resulting in exhaustion of the gland and adrenal hypofunction.

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