CHOLESTEROL METABOLISM IN PANTOTHENIC ACID DEFICIENCY

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Pantothenic acid has been shown to be a constituent of coenzyme A, a coenzyme necessary for acetylation (1–3). While coenzyme A is a general constituent of living organisms, in rat tissues the highest concentrations are found in liver and in adrenal. Most, and possibly all, cellular pantothenic acid seems to be bound in coenzyme A (4, 5). Study of the function of coenzyme A was made possible through its identification as a pantothenic acid derivative.

It had previously been shown by Dorfman et al. (6) and confirmed by Hills (7) that pantothenic acid is concerned with the metabolism of pyruvic acid, primarily in the second or acetate phase of pyruvate oxidation. Additional evidence was provided by Pilgrim et al. (8) who found a decreased rate of pyruvate oxidation in liver from pantothenic acid-deficient rats.

Novelli and Lipmann (9) showed that Lactobacillus arabinosus and Proteus morganii could convert pantothenic acid to coenzyme A, and that the increase in coenzyme A concentration paralleled the respiratory stimulation by pantothenate. Thus, the earlier finding of Dorfman et al. (6) and of Hills (7) that the pyruvic oxidation of pantothenic acid-deficient P. morganii was stimulated by addition of pantothenic acid could be attributed to a formation of coenzyme A from the added pantothenic acid.

Studies made by Novelli and Lipmann (10), as well as those of McElroy and Dorfman (11), support the view that the primary target of coenzyme A is acetate.

The fact that acetate is used in the synthesis of cholesterol was shown by Bloch and Rittenberg (12), while Srere, Chaikoff, and Dauben (13) demonstrated the conversion of acetate to cholesterol by surviving slices of beef adrenal cortex. It seemed possible, therefore, that in the pantothenic acid-deficient animal metabolism of acetate or synthesis of cholesterol might be impaired. The well known effect of cholesterol feeding on the formation of fatty livers was used to study this possibility.

Recent work (14) has shown that adrenal hypofunction, as judged by failure of anoxic anoxia to cause an elevation of blood sugar and liver
glycogen, occurred in pantothenic acid-deficient rats. It therefore seemed advisable to study the effect of cholesterol feeding on the reaction of normal and pantothenic acid-deficient rats to the stress of anoxic anoxia.

Normal and pantothenic acid-deficient rats were fed either a normal or a cholesterol-rich diet, and determinations of liver glycogen, adrenal ascorbic acid, serum cholesterol, liver cholesterol, and liver lipides were made after a test period either at sea level or at a simulated altitude of 20,000 feet. By this means the effect of pantothenic acid deficiency on the ability of the animal to metabolize cholesterol was examined.

In a further effort to elucidate the effect of the deficiency, paired feeding tests were made, as well as parallel observations of riboflavin and pyridoxine deficiencies. The effect of withdrawal of dietary cholesterol or pantothenic acid or both after fatty liver production by a normal diet containing 1 per cent cholesterol was also noted.

**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 composition: vitamin-free casein 22.0, sucrose 66.5, fat 9.0, and salts 2.5. The cholesterol diet had the same composition as the basal diet, with the exception that 1 per cent cholesterol was substituted for an equal amount of fat.

Supplements of crystalline vitamins were given three times a week in amounts to provide per rat the following daily quantities in mg.: thiamine hydrochloride, riboflavin, folic acid, and pyridoxine hydrochloride, each 0.02, calcium pantothenate and p-aminobenzoic acid, each 0.1, niacinamide 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, or, in one series, riboflavin or pyridoxine.

The experimental animals were kept on their respective diets usually for 5 weeks after they were grouped, at the end of which time, in the first experiment, 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.

1 Vitamin test casein procured from General Biochemicals, Inc., Chagrin Falls, Ohio.
2 Primex, a hydrogenated vegetable fat.
The anoxia apparatus was the same as that described originally by Wickson and Morgan (15). At the beginning of the test period 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.

Liver glycogen was precipitated and hydrolyzed according to Good, Kramer, and Somogyi (16), and the resulting reducing solution was titrated with ceric sulfate (17). To determine ascorbic acid in the adrenal glands, a method was devised based on the Bessey modification of the Roe and Kuether method (18, 19). Cholesterol was determined by direct chloroform extraction according to the method of Kingsley and Schaffert (20). Total lipide was determined by extraction of the tissue with petroleum ether in the Soxhlet extractor and weighing of the fatty residue after removal of the solvent.

**Results**

Table I shows the amounts of food eaten, weight gained, and the liver, adrenal, and serum analyses of these rats under normal and anoxic conditions. There was no significant difference on a body weight basis in the amounts of food eaten by the animals on the complete basal diet, on the pantothenic acid-deficient diet, or the cholesterol-rich complete diet, although the total quantities were quite different. However, the rats subjected to the cholesterol-rich pantothenic acid-deficient diet ate slightly more food in proportion to weight than did any other group. The weight gain was strikingly different between the normal and deficient animals, although the addition of cholesterol had no effect. The normally fed animals, both on basal and cholesterol diets, gained nearly twice as much per gm. of food eaten as did the deficient animals.

Cholesterol had little effect on the formation of glycogen during stress in either the normal or deficient rats. There was no increase in liver glycogen as the result of anoxia in animals deficient in pantothenic acid, in contrast with the marked rise in normal animals, thus confirming previous experiments (14). Striking differences occurred in the liver fat of the various groups. The addition of cholesterol to the complete basal diet resulted in liver fat concentrations of 11.2 per cent, as compared with 4.3 per cent in animals on the complete basal diet without cholesterol. This marked effect was not seen in the pantothenic acid-deficient animals. In fact, their liver fat concentrations were only 2.3 and 2.4 per cent, values which are significantly lower than those of the animals on the complete normal diet. In all groups, anoxia caused a decrease in liver fat.

Cholesterol feeding had the same effect on liver cholesterol as on liver fat. The liver cholesterol of the cholesterol-fed control animals was 2.34 per cent, as compared with 0.28 per cent in the controls on the basal
### Table I

Effect of Anoxia on Liver, Serum, and Adrenal Composition of Normal and Pantothenic Acid-Deficient Rats with and without 1 Per Cent Cholesterol in Diet

Four to sixteen rats, usually eight, per group.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Food intake in 5 wks.</th>
<th>Weight gain</th>
<th>Altitude</th>
<th>Liver</th>
<th>Serum</th>
<th>Adrenal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>mg. per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>Normal</td>
<td>262 ± 9</td>
<td>2.3</td>
<td>114</td>
<td>73</td>
<td>0.28</td>
<td>Sea level</td>
</tr>
<tr>
<td>+ cholesterol</td>
<td>281 ± 12</td>
<td>2.4</td>
<td>117</td>
<td>81</td>
<td>0.29</td>
<td>Sea level</td>
</tr>
<tr>
<td>Deficient</td>
<td>172 ± 5</td>
<td>2.6</td>
<td>66</td>
<td>24</td>
<td>0.14</td>
<td>Sea level</td>
</tr>
<tr>
<td>+ cholesterol</td>
<td>180 ± 6</td>
<td>3.1</td>
<td>58</td>
<td>20</td>
<td>0.11</td>
<td>Sea level</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20,000 ft.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20,000 ft.</td>
</tr>
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<td></td>
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<td>20,000 ft.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20,000 ft.</td>
</tr>
</tbody>
</table>
diet. The deficient rats, however, showed almost no increase with cholesterol feeding. They had 0.29 per cent liver cholesterol on the basal diet and 0.42 per cent on the cholesterol diet. There was little difference among any of the groups at sea level, as compared with those under anoxia, although all the figures for the latter groups were lower.

Serum cholesterol increased in the normal rats from 64 mg. per cent on the basal diet to 332 mg. per cent on the cholesterol diet. In the deficient rats it increased from 69 mg. per cent on the basal diet to 123 mg. per cent on the cholesterol diet. Anoxia raised the serum cholesterol of both normal and deficient rats on the basal diet and lowered it in both cholesterol-fed groups.

Although neither the anoxia nor the cholesterol feeding had any significant effect upon adrenal ascorbic acid, the findings confirmed the previous work (14) in that the deficient animals in all groups showed lower adrenal ascorbic acid values and higher adrenal weights than did the normal animals.

In a second experiment the effects of paired feeding and of parallel

### Table II

<table>
<thead>
<tr>
<th>Diet</th>
<th>Feed period</th>
<th>Food intake per wk</th>
<th>Weight gain per wk</th>
<th>Weight gain per gm. food</th>
<th>Liver Lipids per cent wet weight</th>
<th>Liver Cholesterol per cent wet weight</th>
<th>Serum cholesterol mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal + 1% cholesterol</td>
<td>1</td>
<td>35</td>
<td>12.0</td>
<td>0.34</td>
<td>8.5 ± 0.5</td>
<td>1.53 ± 0.08</td>
<td>316 ± 42</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43</td>
<td>19.0</td>
<td>0.44</td>
<td>8.6 ± 0.6</td>
<td>1.73 ± 0.57</td>
<td>265 ± 23</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>45</td>
<td>18.3</td>
<td>0.40</td>
<td>6.7 ± 0.3</td>
<td>1.44 ± 0.07</td>
<td>237 ± 28</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>51</td>
<td>17.2</td>
<td>0.34</td>
<td>7.2 ± 0.5</td>
<td>1.93 ± 0.18</td>
<td>253 ± 40</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>52</td>
<td>14.5</td>
<td>0.28</td>
<td>7.0 ± 0.6</td>
<td>1.84 ± 0.13</td>
<td>215 ± 58</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>45*</td>
<td>11.2</td>
<td>0.25</td>
<td>10.8 ± 0.6</td>
<td>2.73 ± 0.24</td>
<td>232 ± 25</td>
</tr>
<tr>
<td>Pantothenic acid-deficient + 1% cholesterol</td>
<td>5</td>
<td>42*</td>
<td>7.6</td>
<td>0.18</td>
<td>3.4 ± 0.3</td>
<td>0.65 ± 0.08</td>
<td>105 ± 8</td>
</tr>
<tr>
<td>Pantothenic acid-deficient</td>
<td>5</td>
<td>44*</td>
<td>9.4</td>
<td>0.17</td>
<td>3.4 ± 0.3</td>
<td>0.36 ± 0.04</td>
<td>77 ± 7</td>
</tr>
<tr>
<td>Riboflavin-deficient + 1% cholesterol</td>
<td>2</td>
<td>39</td>
<td>5.5</td>
<td>0.14</td>
<td>5.8 ± 0.5</td>
<td>1.59 ± 0.20</td>
<td>126 ± 9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>31</td>
<td>5.0</td>
<td>0.16</td>
<td>7.2 ± 0.4</td>
<td>2.04 ± 0.13</td>
<td>135 ± 16</td>
</tr>
<tr>
<td>Pyridoxine-deficient + 1% cholesterol</td>
<td>4</td>
<td>31</td>
<td>5.0</td>
<td>0.16</td>
<td>7.8 ± 0.8</td>
<td>1.69 ± 0.18</td>
<td>157 ± 8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>32</td>
<td>6.5</td>
<td>0.20</td>
<td>6.9 ± 0.3</td>
<td>1.72 ± 0.11</td>
<td>188 ± 10</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>39</td>
<td>9.0</td>
<td>0.23</td>
<td>7.0 ± 0.4</td>
<td>2.13 ± 0.12</td>
<td>117 ± 10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>33</td>
<td>4.2</td>
<td>0.13</td>
<td>4.0 ± 0.4</td>
<td>1.03 ± 0.18</td>
<td>135 ± 14</td>
</tr>
</tbody>
</table>

* Pair-fed.
riboflavin and pyridoxine deficiencies were noted. The progress of liver lipide deposition was followed in normal animals on the cholesterol diet for 1 to 5 weeks. The values of Table II indicate that the lipide and cholesterol increases in the liver and the increase in serum cholesterol level were nearly as great after 1 week as after 5 weeks on the diet. The rats fed a restricted amount of the normal cholesterol-rich diet, 45 gm. per week, had even higher liver fat, 10.8 per cent, than those on unrestricted intake, 52 gm. of food and 7.0 per cent liver fat. Again the pantothenic

table III
Effect of Withdrawal of Cholesterol or Pantothenic Acid or Both on Rats after Feeding Normal Diet 2 Weeks Plus 1 Per Cent Cholesterol

<table>
<thead>
<tr>
<th>Diet in withdrawal period</th>
<th>With-</th>
<th>Food intake</th>
<th>Weight gain</th>
<th>Liver</th>
<th>Serum cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>drawal</td>
<td>per week</td>
<td>Per gm.</td>
<td>Per cent wet weight</td>
<td>mg. per cent</td>
</tr>
<tr>
<td></td>
<td>period</td>
<td></td>
<td>gm. food</td>
<td>Lipide</td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td>wks.</td>
<td>gm.</td>
<td>gm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal + cholesterol</td>
<td>0</td>
<td>43</td>
<td>0.44</td>
<td>8.6 ± 0.6</td>
<td>1.73 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>62</td>
<td>0.34</td>
<td>10.8 ± 0.4</td>
<td>2.36 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>75</td>
<td>0.25</td>
<td>10.3 ± 0.6</td>
<td>2.41 ± 0.12</td>
</tr>
<tr>
<td>Normal, no cholesterol</td>
<td>3</td>
<td>60</td>
<td>0.33</td>
<td>3.0 ± 0.2</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>89</td>
<td>0.28</td>
<td>3.1 ± 0.1</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>Pantothenic acid-deficient, no cholesterol</td>
<td>3</td>
<td>55</td>
<td>0.29</td>
<td>2.9 ± 0.1</td>
<td>0.42 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>58</td>
<td>0.21</td>
<td>3.2 ± 0.1</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>Pantothenic acid-deficient + cholesterol</td>
<td>3</td>
<td>56</td>
<td>0.30</td>
<td>6.2 ± 0.6</td>
<td>1.64 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>55</td>
<td>0.18</td>
<td>6.1 ± 0.9</td>
<td>1.15 ± 0.26</td>
</tr>
</tbody>
</table>

acid-deficient groups, with and without cholesterol, maintained low liver fat contents, 3.4 per cent, on 42 and 44 gm. of food per week.

The riboflavin-deficient groups which received cholesterol maintained lower food intakes than the normal or pantothenic acid-deficient animals, but accumulated nearly as much liver fat and cholesterol as the normal groups. Their levels of serum cholesterol were not as much elevated, however, as in the latter animals. The same was true of the pyridoxine-deficient rats which were fed cholesterol, except those in the group that was maintained for 4 weeks. These showed a definite decrease in liver lipides and cholesterol. Possibly at this stage of the deficiency a stress condition like that of pantothenic acid deficiency had developed. This deficiency is under further investigation.

In the third experiment a large number of rats was placed at weaning on the normal diet with cholesterol and after 2 weeks divided into four groups. One group was maintained on the same diet, another was given
the normal diet without cholesterol, and two others the pantothenic acid-
deficient diet with and without cholesterol. Some rats from each of these
groups were sacrificed after 3 weeks on these régimes and the rest after 6
weeks. As shown in Table III, the withdrawal of dietary cholesterol
produced normal liver fat and cholesterol and serum cholesterol levels in
3 weeks in both normal and deficient rats. The withdrawal of pantothenic
acid with retention of cholesterol caused a partial loss of liver fat, 6.2 per
cent as compared with 10.8 per cent in the normal plus cholesterol group,
after 3 or 6 weeks. The liver cholesterol, however, was progressively
decreased to 1.64 per cent in 3 weeks and 1.15 per cent in 6 weeks. The
serum cholesterol was not changed in 3 weeks but was reduced to a nearly
normal level in 6 weeks. The progress of the deficiency was apparently
slower at this later age than in the weanlings previously used.

DISCUSSION

It has been reported that pantothenic acid deficiency produces fatty
livers in dogs (21). In the experiments described here, it was found that,
not only did fatty livers not occur in pantothenic acid-deficient rats, but
that these rats were resistant to the liver fat deposition ordinarily caused
by a cholesterol-rich diet. This observation may point to a difference in
utilization of the vitamin or in lipide utilization in the two species.

The deficient rats differed from the normal animals in their blood and
liver levels of cholesterol as well as of liver fat. Whereas the cholesterol-
fed normal rats showed great increases, there was only a small rise in blood
cholesterol in the deficient animals, and almost none in liver cholesterol.
Again, contrary to observations with dogs (22), the deficient rats on the
basal diet had almost the same amount of cholesterol in the serum as did
the controls.

The fact that the pantothenic acid-deficient animals did not develop
fatty livers cannot be ascribed to inadequate intake, since the food con-
sumption on a body weight basis was actually higher in the deficient than
in the normal rats. The propriety of the usual assumption of partial in-
anition in animals on deficient diets, regardless of intake per unit of body
weight, may well be questioned. The pair-fed groups of the second ex-
periment (Table II) illustrate this point. It is clear that mild reduction
of food intake did not lower the fat content of the liver in these normal
cholesterol-fed rats or in the three groups of riboflavin-deficient animals
and in two of the three pyridoxine-deficient groups, all of which had lower
food intakes than the comparable pantothenic acid-deficient animals.

These experiments indicate a disturbed metabolism of cholesterol and
fat in the pantothenic acid-deficient rats. This disturbance may be as-
associated with the coenzyme A function of pantothenic acid and may or
may not be related to choline as it functions in the regulation of liver fat
concentrations. All the diets contained an abundance of lipotropic factors.

The fact that cholesterol feeding had no effect on the resistance of the pantothenic acid-deficient rats to anoxia, as measured by increase in liver glycogen, may indicate that the adrenal hypofunction produced by this deficiency (14) is not primarily due to a lack of cholesterol as such and that the steroid utilized by the adrenal for hormone production may be a cholesterol precursor or else that serum cholesterol is poorly retained by the glands. Certain later experiments support this latter supposition, since the cholesterol content of the adrenals was found to be unchanged by cholesterol feeding.

Popjak (23) advanced the theory that the free cholesterol of the plasma regulates utilization of body fat and synthesis of plasma phospholipides by the liver. The rate of cholesterol synthesis in various species was related to the amount of acetate available for this purpose, possibly under coenzyme A control. When cholesterol is ingested, high levels of plasma cholesterol result with mobilization of body fat from its depots and deposition of surplus fat and cholesterol in the liver.

In pantothenic acid deficiency, cholesterol synthesis from acetate may be operating feebly due to insufficient supplies of coenzyme A. Exogenous cholesterol may be utilized by such an animal efficiently enough to prevent a rise in the plasma cholesterol level with consequent failure of fat mobilization and resulting storage of lipides and cholesterol in the liver. All of the findings of the experiments here reported are consistent with this explanation.

The impairment of adrenocortical function in pantothenic acid deficiency may result from failure of ketosteroid production from specific adrenal steroids due to lack of coenzyme A, a deficit which cannot be made up by exogenous cholesterol.

**SUMMARY**

The feeding of a complete diet containing 1 per cent cholesterol to normal rats resulted in fatty livers and high levels of cholesterol in the liver and the serum. The same cholesterol-rich diet did not produce a rise in liver lipides in pantothenic acid-deficient rats and only small increases in liver cholesterol and serum cholesterol.

This resistance of the deficient rats to formation of fatty livers was not due to curtailment of food intake, since the food consumption of the cholesterol-fed deficient animals on a body weight basis was higher than that of the normal animals. Pair-fed groups likewise had the same response to cholesterol feeding as had unrestricted groups.

Cholesterol feeding had no effect on the response of either normal or deficient rats to the stress of anoxic anoxia equivalent to an altitude of
20,000 feet, when the response was measured by formation of liver glycogen during stress. The previous finding was confirmed that in deficient rats liver glycogen was not raised during stress.

Adrenal ascorbic acid levels were not affected by cholesterol feeding, but the previous observation that in deficient rats adrenal ascorbic acid levels were lower and adrenal weights higher in all groups was confirmed.

Rats fed riboflavin-deficient diets containing cholesterol for 2, 3, or 4 weeks and a similar pyridoxine-deficient diet for 2 or 3 weeks developed typical fatty cholesterol-rich livers and increased serum cholesterol levels. After 4 weeks, however, the pyridoxine-deficient rats exhibited only limited response of this kind.

Removal of pantothenic acid from the diet of rats fed the normal cholesterol-rich diet for 2 weeks resulted in gradual reduction of liver fat and cholesterol and serum cholesterol.

These findings appear to show a specific relationship between pantothenic acid and the metabolism of cholesterol and fat.

These phenomena may result from the rôle of pantothenic acid as part of coenzyme A in the regulation of acetate metabolism and of the synthesis of cholesterol and its analogues.

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