THE EFFECT OF PANTOTHENIC ACID DEFICIENCY ON ACETYLATION IN RATS*

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Lipmann et al. have shown (1) that coenzyme A1 contains large amounts of pantothenic acid. Since this coenzyme has been shown to be essential for in vitro acetylations of sulfanilamide (3, 4) and choline (5), it seems significant to determine whether acetylation in animals depends upon an adequate intake of pantothenic acid.

Methods

The ability of normal and pantothenic acid-deficient rats to acetylate a foreign substance was compared by injection of p-aminobenzoic acid (PAB) and the subsequent measurement of the acetylated PAB excreted. Young male rats weighing around 100 gm. or mature rats averaging over 300 gm. were used in various experiments. Those selected for pantothenate deficiency were given a purified diet free of calcium pantothenate containing 73 per cent glucose, 18 per cent vitamin-free casein, 4 per cent corn oil (Mazola), 4 per cent Salts IV (6), 1 per cent cod liver oil, and the following vitamins2 per 100 gm.: thiamine chloride 400 γ, riboflavin 800 γ, pyridoxine hydrochloride 400 γ, nicotinic acid 4000 γ, and choline chloride 100 mg. Control animals received 2.5 mg. of calcium pantothenate per 100 gm. of ration. For acetylation measurements, a larger series was run with 1 mg. of PAB given intraperitoneally per animal, and a smaller series with 2.5 mg. of PAB. The urine of the following 24 hours was analyzed for total and free PAB by the method of Bratton and Marshall (7). Bound PAB was calculated by the difference and expressed as per cent which was bound of the total excreted during the 24 hours. Blank runs showed no natural interference with the test at the concentrations used. Repeated PAB

1 This name has recently been used to identify the coenzyme by Kaplan and Lipmann (2).
2 The crystalline vitamins used in this study were kindly supplied by Merck and Company, Inc., Rahway, New Jersey, and the glucose and corn oil by the Corn Industries Research Foundation, New York.

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injections were made on the same animals with a minimum of 3 days between subsequent doses.

Response of deficient animals to the missing vitamin was determined on five rats which had been on the deficient diet for 2 months, on which consistently low acetylation values had been obtained. Individual amounts of 50 γ, 200 γ, 500 γ, or 1 mg. of calcium pantothenate were injected intraperitoneally in the same solution with the PAB dose. Following the 50 and 200 γ doses, the animals were allowed to return to the previous low acetylation values. These same animals were then given the 500 γ and 1 mg. doses. Finally, both deficient animals and controls were given 1 mg. injections of calcium pantothenate every 3 days. Pantothenic acid excretions were measured at various times on urine samples by microbiological assay, by the method of Hoag et al. (8).

Results

Table I summarizes the acetylation data on ten young rats initially weighing around 100 gm. each. An average of nearly 90 per cent of each PAB dose was excreted in 24 hours by these animals, regardless of dose size, supplement, or diet. Normal animals showed an average of 68.3 per cent acetylated PAB out of the total excreted. No marked change in acetylation was observed due to weight increase of the normals, although over the entire period of the experiment (over 4 months) the values showed a slight drift upward as their weight increased from around 100 gm. to well over 300 gm. (Fig. 1). Thus near the end of 4 months, the control animals bound 72.7 per cent PAB (Series 3, Table I), as compared to the over-all average of 68.3 per cent for the entire 4 months. Since the acetylations obtained after 24 hour fasting (74.9 per cent bound) were measured near the end of the experimental period, this average has been compared with the 72.7 per cent acetylation value.

The ability of the pantothenic acid-deficient animals to acetylate PAB was definitely below that of the control animals after 2 weeks on the diet, when the determinations were first made. The average acetylation of 50.0 per cent of 1 mg. doses then remained fairly constant, except for occasional radical variations. With 2.5 mg. of PAB, acetylations dropped to 36.7 per cent. With this dose, the addition of 3 per cent sodium acetate to the diet caused a significant increase in acetylation to 43.5 per cent. A similar rise with 3 per cent sodium acetate on 1 mg. doses of PAB was observed, but the great variation among the animals (s.e. = ±3.8 for a 7.3 per cent increase) leaves doubt as to its significance. Added acetate had no effect on the acetylation by normal animals, and 2.5 mg. of PAB were acetylated equally as well as 1 mg.

There was no apparent tendency for a continued fall in acetylation
**Table I**

*Acetylation of p-Aminobenzoic Acid by Rats on Normal and Pantothenic Acid-Deficient Diets*

<table>
<thead>
<tr>
<th>Series No.</th>
<th>Treatment</th>
<th>PAB dose mg.</th>
<th>Normal Trials</th>
<th>No. of animals</th>
<th>Acetylation* per cent</th>
<th>Pantothenic acid-deficient Trials</th>
<th>No. of animals</th>
<th>Acetylation* per cent</th>
<th>Decrease per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>1</td>
<td>51</td>
<td>4</td>
<td>68.3 ± 0.78</td>
<td>6</td>
<td>54</td>
<td>50.0 ± 1.2</td>
<td>26.8</td>
</tr>
<tr>
<td>1</td>
<td>1% NaOAc diet</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>68.9 ± 2.9</td>
<td>6</td>
<td>6</td>
<td>50.0 ± 2.9</td>
<td>27.4</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>70.8 ± 1.3</td>
<td>6</td>
<td>6</td>
<td>57.3 ± 3.8</td>
<td>19.1</td>
</tr>
<tr>
<td>1</td>
<td>3% &quot; &quot;</td>
<td>2.5</td>
<td>3</td>
<td>3</td>
<td>66.3 ± 1.0</td>
<td>6</td>
<td>6</td>
<td>66.7 ± 1.6</td>
<td>44.6</td>
</tr>
<tr>
<td>2</td>
<td>3% NaOAc diet</td>
<td>2.5</td>
<td>6</td>
<td>3</td>
<td>65.1 ± 3.3</td>
<td>12</td>
<td>6</td>
<td>43.5 ± 1.9</td>
<td>33.1</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>72.7 ± 1.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>24 hr. fasting</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>74.9 ± 1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 mg. Ca pantothenate injec</td>
<td>1</td>
<td>12</td>
<td>3</td>
<td>74.4 ± 0.64</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Per cent bound ± standard error of the mean.

Fig. 1. The effect of pantothenic acid deficiency and of various treatments on acetylation of p-aminobenzoic acid by rats. PAB doses were 1 mg., except where indicated, and were administered 24 hours before the acetylation value plotted. 1, 2.5 mg. of PAB dose; 2, 24 hour fasting; 3, 50 γ of calcium pantothenate intraperitoneally; 4, 200 γ of calcium pantothenate intraperitoneally; 5, 500 γ of calcium pantothenate intraperitoneally; 6, 1 mg. of calcium pantothenate intraperitoneally; 7, 1 mg. of calcium pantothenate intraperitoneally with each PAB dose given deficient animals; 8, 1 mg. of calcium pantothenate intraperitoneally with each PAB dose given normal animals.
ability as the pantothenic acid deficiency progressed and the animals lost weight. This lower plateau is consistent with the observations of Olson and Kaplan, who have found that the coenzyme A content of liver follows a somewhat similar course during pantothenic acid deficiency, falling to approximately one-fourth the normal concentration. The combined evidence thus indicates that, under the conditions of these experiments, the ability to acetylate PAB is a function of the coenzyme A concentration.

In Table II are shown the responses obtained when the deficient rats were injected with various levels of calcium pantothenate. Doses of 200 or 500 mg of the vitamin gave slight but inconsistent increases in acetylation. These were not significant on the few data available. 1 mg doses, however, gave an immediate response to normal (69.5 per cent). This figure was not further increased by repeated doses. Normal animals injected with 1 mg doses of calcium pantothenate acetylated 74.4 per cent, compared to the 72.7 per cent obtained in immediately preceding runs (Series 3, Table I). Fig. 1 summarizes the per cent acetylation by normal and deficient animals as a function of time, illustrating the marked changes which occurred when the various treatments were given. For clarification in demonstrating the response of deficient animals given various amounts of calcium pantothenate, two curves are shown for the rats on the pantothenic acid-deficient diet.

Pantothenic acid excretion falls very rapidly within the 1st week on the deficient diet. Deficient animals showing low acetylation ordinarily excrete well under 1 mg per day. Following injection of the vitamin, up to 50 per cent of a given dose may be lost during the first 24 hours. Acety-

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Table II

<table>
<thead>
<tr>
<th>Ca pantothenate injected</th>
<th>Trials</th>
<th>No. of animals</th>
<th>Acetylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td></td>
<td></td>
<td>per cent*</td>
</tr>
<tr>
<td>1</td>
<td>33</td>
<td>5</td>
<td>70.7 ± 1.1</td>
</tr>
<tr>
<td>1 mg.</td>
<td>33</td>
<td>5</td>
<td>70.7 ± 1.1</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>2</td>
<td>54.0 ± 1.6</td>
</tr>
<tr>
<td>200</td>
<td>3</td>
<td>3</td>
<td>61.1 ± 5.2</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>3</td>
<td>49.3 ± 1.4</td>
</tr>
<tr>
<td>500 mg.</td>
<td>2</td>
<td>2</td>
<td>50.0 ± 1.2</td>
</tr>
</tbody>
</table>

* Per cent bound ± standard error of the mean.
† Compared to normal animals as 68.3 per cent (Table I).
lations did not return to the low value characteristic of the deficiency for 2 weeks or more, in spite of no further source of pantothenate.

DISCUSSION

The data in Table I show that the normal rats acetylated 18 per cent more of a 1 mg. dose of PAB than those on a pantothenic acid-deficient diet. This amounts to more than a 25 per cent decrease, due to the absence of the vitamin. With a larger dose of PAB (2.5 mg.) the deficient animals acetylated 29.6 per cent less than the controls, or a decrease of 44.6 per cent of the normal. Injection of 1 mg. of calcium pantothenate into deficient animals immediately raised their acetylating ability to normal, although single doses of 500 γ or less failed to give complete recovery in any animal. The extra 1 mg. to control animals already receiving a supposedly adequate level of the vitamin in the diet resulted in a slight increase in acetylation which may prove significant with more trials.

Apparently a considerable amount of pantothenic acid (up to 1 mg.) is needed at one time at the site of PAB acetylation (presumably the liver (9, 10)) for the immediate formation of sufficient coenzyme A to initiate a normal reaction after depletion. In view of the rapid loss of pantothenic acid in the urine following administration of injected doses, it would appear that the yield of coenzyme A is very small. Indeed, Novelli and Lipmann (11) have shown that bacteria yield but 4 to 8 per cent coenzyme A from a given amount of added pantothenate. On the other hand, animals retain remarkably good acetylation values (above 40 per cent), even after severe deficiency symptoms appear. This may be due in part to the relatively low PAB dose of 1 mg. given. Dose size unquestionably has much to do with the amount acetylated, as measured in the 24 hour urine. Bloomberg (9) reports that human subjects acetylate 100 per cent of a 25 mg. dose or less of PAB in 24 hours, while they bind only 70 to 75 per cent of a 100 mg. dose. Martin and Rennebaum (12) report values of but 3 per cent in 24 hour urines of rats given 1 mg. of sulfanilamide per gm. of body weight. Benigno (13) found rabbits to acetylate 76 per cent of a dose of 0.03 gm. of PAB per kilo of body weight in 12 hours, while Doisy and Westerfield (14), using 1 gm. doses in 2 kilo rabbits, found 30 to 40 per cent bound in 48 hour urines. Our control animals acetylated a 2.5 mg. dose not significantly different from 1 mg. (66.3 versus 68.3 per cent). In contrast, the deficient animals bound only 36.7 per cent as compared to 50.0 per cent of the 1 mg. dose, although with the larger dose the animals actually coupled twice as much acetate.

The addition of acetate to the diet did not produce any marked change in the ability of normal animals to acetylate either the 1 mg. or 2.5 mg. dose of PAB. Short periods of starvation were also without effect. How-
ever, with the deficient animals, the addition of acetate allowed considerable improvement. This was especially true at the higher dose of PAB when more acetate was needed. This effect may indicate that acetate formation is abnormal in pantothenic acid-deficient animals as well as the enzyme which makes use of it, whereas in normal animals the low doses of PAB used apparently do not cause any appreciable strain on the body system for acetate or coenzyme A formation or function.

By use of D-labeled sodium acetate, Bloch and Rittenberg (15) have now well demonstrated that acetate acts directly in acetylating PAB and is perhaps the sole directly contributing substance (cf. also (3)). Current workers, however, do not agree on the effect of added acetate on acetylation ability of an animal (12). Much of such disagreement may be due to dose size, as already indicated, to the animal used, or to the use of blood in preference to urine for analysis. Martin and Rennebaum (12) have shown the lack of correlation between blood and urine analyses. These factors may explain completely the failure of these authors (12) to show lowered acetylations in pantothenic acid-deficient rats. Their observed decreased acetylations in thiamine and in riboflavin deficiency may also be accounted for, since both these vitamins are known to function in enzymes which lead to formation of acetate. It is possible that, when large doses of PAB or sulfanilamide are given, the stress is primarily upon those systems which supply acetate, whereas with small doses failure of the acetylating mechanism may be shown more clearly. The present findings indicate that pantothenic acid functions in the acetylating mechanism of the rat, presumably through coenzyme A of Lipmann and collaborators.

**SUMMARY**

1. Normal rats were found to acetylate 70 per cent of the amount excreted in 24 hours after a 1 mg. or 2.5 mg. dose of \( p \)-aminobenzoic acid administered intraperitoneally.

2. Rats rendered pantothenic acid-deficient acetylated only 50 per cent of a 1 mg. dose and 37 per cent of a 2.5 mg. dose.

3. Simultaneous injection of 1 mg. of calcium pantothenate to deficient animals immediately returned their acetylation to normal.

4. The effect of added acetate, 24 hour fasting, and the size of the dose on the degree of acetylation have also been investigated.

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**BIBLIOGRAPHY**


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