THE INFLUENCE OF VITAMIN E DEFICIENCY ON THE METABOLISM OF SODIUM FORMATE-C₁⁴ AND GLYCINE-1-C₁⁴ BY THE RABBIT

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Several recent reports from this laboratory have implicated vitamin E in the metabolism of the nucleic acids. It was shown that vitamin E-deficient rabbits excrete extra quantities of allantoin (1) and that the livers from such animals exhibit greatly increased xanthine oxidase activities (2). Finally it has been shown that vitamin E deprivation in the rat leads to an increased incorporation of formate into nucleic acids (3). The combined results of these experiments have been interpreted to indicate that the turnover rate of nucleic acids is increased in vitamin E deficiency.

The present report is concerned with the influence of vitamin E deficiency on the metabolism of glycine and formate by the rabbit. The results indicate that vitamin E may be somewhat specifically concerned with formate metabolism.

EXPERIMENTAL

White New Zealand rabbits, weighing initially about 600 gm., were given the same purified diet previously described (1). Controls received this diet with oral supplements of 4 mg. of α-tocopherol acetate per kilo of body weight two times weekly. Vitamin E-deficient rabbits were selected for experiment when they exhibited signs of muscular dystrophy. These signs included a urinary creatine-creatinine ratio of approximately 3:1 (4) and difficulty in righting itself after the rabbit was placed on its side. This condition occurred after from 3 to 4 weeks of feeding. Each experiment with a deficient rabbit included a control which had received the diet with vitamin E supplementation for the same period of time as the deficient animal.

For the experiments in which the incorporation of the radioactive substrates into tissue nucleic acids and proteins was studied, the rabbits were injected intraperitoneally with either 10 µc. of glycine-1-C₁⁴ (specific activity, 0.58 mc. per mmole) or sodium formate-C₁⁴ (2.85 mc. per mmole) per 100 gm. of body weight. The animals were killed 4 hours later and the various organs and tissues were immediately frozen in a dry ice-acetone mixture.

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In the first experiments the tissues were fractionated by the Schneider procedure (5). An aliquot of the nucleic acid fraction was evaporated for counting and another aliquot was wet ashed for phosphorus determination. The protein fraction was dried and counted and corrected to infinite thinness. All samples were counted with an end window Geiger tube with a window thickness of 2 mg. per sq. cm. Three rabbits in each group (a total of twelve rabbits) were used in these experiments.

In other experiments, bone marrow and skeletal muscle from two control rabbits and two vitamin E-deficient rabbits which had been injected with formate-C\textsuperscript{14} were pooled separately. Nucleic acids were extracted with hot 10 per cent NaCl and fractionated into ribonucleic (RNA) and deoxyribonucleic (DNA) acid fractions by the alkaline digestion method (6), according to the procedure previously described for rat liver (3).

The next experiments were designed to compare the incorporation of formate and glycine into acid-soluble and nucleic acid purines. The combined viscera from two control rabbits and from two vitamin E-deficient rabbits receiving either glycine-1-C\textsuperscript{14} or sodium formate-C\textsuperscript{14} were pooled separately. After homogenization with an equal volume of cold H\textsubscript{2}O, the tissue was extracted two times with 5 volumes of cold 10 per cent trichloroacetic acid (TCA). The acid-soluble nucleotides contained in these extracts were hydrolyzed with H\textsubscript{2}SO\textsubscript{4} as described by Heinrich and Wilson (7). The purines were precipitated as the copper salts (8) and free purines were regenerated with H\textsubscript{2}S\textsubscript{2}. The pH of the purine solution was adjusted to 7, and an equal volume of saturated picric acid was added. The adenine picrate was subjected to several recrystallizations and counted as such. The tissue residue remaining after extraction of acid-soluble material was extracted with hot 10 per cent NaCl. The sodium nucleotides, which were precipitated with cold 95 per cent ethanol, were hydrolyzed with H\textsubscript{2}SO\textsubscript{4}, and guanine sulfate and adenine picrate were prepared and purified as described by Plentl and Schoenheimer (9). Self-absorption curves were prepared for adenine picrate and guanine sulfate, and all counts were corrected to infinite thinness.

In order to study the oxidation of formate-C\textsuperscript{14} and glycine-1-C\textsuperscript{14} to CO\textsubscript{2}, control and vitamin E-deficient rabbits were injected with 2.5 \textmu c. per 100 gm. of body weight of either of these substrates and placed in desiccators connected with a gas train. Expired CO\textsubscript{2} was collected in 5 N NaOH, and then precipitated and counted as barium carbonate. A self-absorption curve for barium carbonate was prepared and the counts were corrected to infinite thinness. There were two or three rabbits in each group.

Since knowledge of relative pool size is important in experiments \textit{in vivo} such as these, the concentration of free glycine in various tissues of control and vitamin E-deficient rabbits was determined. The tissues were ex-
tracted with cold 10 per cent TCA and the glycine content of the extract was determined by a chemical procedure (10). There were three rabbits in each group.

**Results**

The data presented in Table I show that vitamin E deficiency resulted in a greatly increased incorporation of formate into the nucleic acids of all tissues studied except liver. The increase was most marked in skeletal muscle and bone marrow. Skeletal muscle is known to be severely affected by vitamin E deficiency in the rabbit. Since vitamin E-deficient rabbits exhibit granulocytosis (11), it is not surprising that the incorporation of formate into bone marrow nucleic acids was greatly increased. The lack of effect in liver may indicate that, during the relatively short feeding periods employed, the liver content of vitamin E was not severely reduced.

In striking contrast to the results with formate, vitamin E deficiency did not result in an increased incorporation of glycine-1-C¹⁴ into nucleic acids. Actually vitamin E-deficient rabbits incorporated less glycine into nucleic acids than did the controls.

Vitamin E deficiency influenced the incorporation of the two radioactive substrates into proteins in a manner quite similar to the effect observed in nucleic acids. In the data presented in Table II, the deficient rabbits incorporated much more formate into the protein of skeletal muscle and bone marrow than did the controls. When glycine-1-C¹⁴ was injected, the deficient animals incorporated less of the label into the protein of most tissues than did the controls.

Since vitamin E deficiency appeared to have its greatest effect on the incorporation of formate into the nucleic acids of skeletal muscle and bone marrow, these two tissues were fractionated into RNA and DNA fractions.

### Table I

**Specific Activities of Nucleic Acids**

The results are expressed as counts per minute per micromole of P.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Formate-C⁴ injected</th>
<th>Glycine-1-C⁴ injected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vitamin E-deficient</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>5.3</td>
<td>41.9</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>39.5</td>
<td>271.3</td>
</tr>
<tr>
<td>Small intestine</td>
<td>18.0</td>
<td>82.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>8.6</td>
<td>35.5</td>
</tr>
<tr>
<td>Liver</td>
<td>29.1</td>
<td>20.4</td>
</tr>
</tbody>
</table>
The results are given in Table III. With both tissues there was a greater incorporation of formate into DNA than into RNA. In the case of bone marrow this may reflect the incorporation of formate into thymine (12). Vitamin E deficiency led to an increased incorporation of formate into both types of nucleic acid. This is similar to the result observed in rat liver (3).

The data in Table IV show that formate and glycine were incorporated into nucleic acid adenine and guanine to the same extent in the normal animals. In the normal animals injected with formate, the specific activity of acid-soluble adenine was not significantly different from that of nucleic acid adenine. When glycine-1-C\textsuperscript{14} was injected, the specific activity of the acid-soluble adenine in the normal animal appeared to be somewhat higher than the nucleic acid adenine. The specific activities of both acid-soluble and nucleic acid purines following formate injections were much higher in the deficient animals. In agreement with the other experiments, vitamin E deficiency resulted in a decreased incorporation of glycine-1-C\textsuperscript{14} into purines.

The data given in Table V show that the vitamin E deficiency resulted
in a slightly decreased conversion of formate to CO$_2$ and in an increased oxidation of glycine-1-C$^{14}$ to CO$_2$.

### Table IV

**Specific Activities of Nucleic Acid and Acid-Soluble Purines**

The results are expressed as counts per minute per mg.

<table>
<thead>
<tr>
<th></th>
<th>Formate-C$^{14}$ injected</th>
<th>Glycine-1-C$^{14}$ injected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vitamin E-deficient</td>
</tr>
<tr>
<td>Nucleic acid guanine sulfate</td>
<td>8.5</td>
<td>100.0</td>
</tr>
<tr>
<td>&quot; adenine picrate</td>
<td>7.9</td>
<td>51.0</td>
</tr>
<tr>
<td>Acid soluble &quot; &quot;</td>
<td>9.0</td>
<td>212.0</td>
</tr>
</tbody>
</table>

### Table V

**Specific Activities of Expired CO$_2$**

The results are expressed as counts per minute per micromole of barium carbonate.

<table>
<thead>
<tr>
<th>Interval after injection (hrs.)</th>
<th>Formate-C$^{14}$ injected</th>
<th>Glycine-1-C$^{14}$ injected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vitamin E-deficient</td>
</tr>
<tr>
<td>0-1</td>
<td>11.6</td>
<td>9.0</td>
</tr>
<tr>
<td>1-2</td>
<td>6.1</td>
<td>5.7</td>
</tr>
<tr>
<td>2-3</td>
<td>3.5</td>
<td>2.6</td>
</tr>
<tr>
<td>3-4</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>4-5</td>
<td></td>
<td>1.5</td>
</tr>
</tbody>
</table>

### Table VI

**Concentration of Free Glycine in Rabbit Tissues**

The results are expressed as mg. per 100 gm., wet weight.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Vitamin E-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle</td>
<td>37</td>
<td>42</td>
</tr>
<tr>
<td>Liver</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>Whole blood</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

Vitamin E deficiency did not consistently affect the concentration of free glycine as indicated by the data presented in Table VI.

### DISCUSSION

Perhaps the most important point to be made from these experiments is that vitamin E deficiency in rabbits led to a greatly increased incorpora-
tion of formate into nucleic acid purines, but resulted in a decreased incorporation of glycine into purines. This differential effect of the deficiency cannot be the result of changes in pool sizes. The fact that the specific activities of the CO$_2$ expired following the injection of either precursor were not markedly affected by vitamin E deficiency indicates that there was no marked change in the glycine or formate pool. Also, since quite early in purine biosynthesis glycine and formate appear to be combined (13), any change in the pool size of subsequent intermediates should affect the incorporation of both glycine and formate in a similar manner.

The results obtained in these experiments may be explained on the basis of an exchange reaction of the 2 position of the purine ring without complete degradation of the molecule. Such an exchange has been demonstrated in experiments in vitro (14). The observation (Table IV) that vitamin E deficiency had a greater effect on the incorporation of formate into acid-soluble adenine than on the incorporation into nucleic acid adenine would suggest that this exchange occurs at the nucleotide level. Vitamin E may somehow control such an exchange reaction, possibly through an effect on the synthesis or activity of coenzymes derived from folic acid.

**SUMMARY**

Control and vitamin E-deficient rabbits were injected with sodium formate C$^{14}$ or glycine 1-C$^{14}$. The deficient animals incorporated much more formate into nucleic acids and protein than did the controls. There was an increased incorporation of formate into both ribonucleic acid and deoxyribonucleic acid of skeletal muscle and bone marrow as a result of vitamin E deficiency. The vitamin E-deficient rabbits incorporated less glycine-1-C$^{14}$ into nucleic acids and protein than did the controls. The concentration of free glycine in tissues and the oxidation of formate to CO$_2$ were not consistently affected by vitamin E deficiency. The deficient animals oxidized more glycine-1-C$^{14}$ to CO$_2$ than did the controls.

It is suggested that in the vitamin E-deficient rabbit there may be an exchange of the carbon of the 2 position of the purine ring which does not involve a degradation of the entire molecule. Vitamin E may control such an exchange reaction, possibly through an effect on coenzymes derived from folic acid.

**BIBLIOGRAPHY**

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