The Mechanism of Kidney Transamidinase Reduction in Vitamin E-Deficient Rabbits*

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A recent investigation by Van Pilsum and Wahman (1) revealed low kidney transamidinase activities in vitamin E-deficient rabbits. One logical mechanism to explain this finding is suggested by the observation that dietary creatine depresses kidney transamidinase activity in rats (2, 3). If the creatinuria of vitamin E deficiency depresses glycococamine synthesis by kidney transamidinase while creatine synthesis in the whole animal is normal (1) or increased (4, 5), it would indicate that feedback depression of kidney transamidinase by creatine does not regulate creatine synthesis. It thus seems important to establish the cause of the reduced kidney transamidinase activity in vitamin E-deficient rabbits. Accordingly, we have investigated the mechanism of kidney transamidinase depression by vitamin E deficiency. The evidence obtained supports the hypothesis that the depression is secondary to excessive creatine excretion. A direct effect of vitamin E on transamidinase could not be demonstrated.

EXPERIMENTAL PROCEDURE

New Zealand rabbits of both sexes, initially weighing approximately 500 g each, were given a vitamin E-deficient purified basal diet (6), and as prophylaxis for coccidiosis certain animals received 0.04% sodium sulfaquinoxaline in the drinking water for 3-day periods alternating with 2-day periods of untreated water. The control rabbits were given oral supplements of 8 mg of dl-a-tocopheryl acetate in corn oil per kg of body weight three times weekly. After 3 to 4 weeks, when all the vitamin E-deficient rabbits were severely dystrophic, kidney transamidinase activities were measured by the procedure of Van Pilsum et al. (7). The Sims method for developing the Saksugeli color was used (8). Three of the control animals were fed creatine as 5% of the purified diet (creatin-fed control rabbits) for 5 days before kidney transamidinase assay. The Polin procedure (9), with minor modifications, was used to measure creatine and creatinine concentrations of bladder urine of these creatine-fed control animals as well as of many other control and vitamin E-deficient rabbits at the time the animals were killed.

Several attempts were made to demonstrate a direct effect of vitamin E on kidney transamidinase. In some experiments, transamidinase activity was measured after kidneys from vitamin E-deficient rabbits had been homogenized in the presence of a-tocopherol. In other experiments, 1:1 mixtures of kidney homogenates from vitamin E-deficient and control rabbits were assayed for transamidinase activity and compared to the activities of the homogenates before mixing. Finally, the rate of recovery of transamidinase activity after the intraperitoneal injection of 41.7 mg of d-a-tocopheryl polyethylene glycol 1000 succinate (equivalent to 10 mg of a-tocopherol)1 into vitamin E-deficient rabbits was measured.

The relative importance of the kidney in glycococamine synthesis by the rabbit was estimated by following the incorporation of glycine-1-C14 into skeletal muscle creatine by nephrectomized and sham operated control rabbits. Both groups of rabbits were subjected to a combination of ether and sodium pentobarbital anesthesia, laparotomy, and the manipulation of the kidneys, except that the kidneys were left intact in the sham operated animals. After the animals regained consciousness, two sham operated and two nephrectomized rabbits were each given an intravenous injection of 100 μC of glycine-1-C14 (1 mc per mmole) per kg of body weight. One sham operated and one nephrectomized rabbit were given an intravenous injection of 20 μC of glycococamine-2-C14 (1.05 mc per mmole) per kg of body weight. All animals were killed 4 hours after injection of the radioactive material and skeletal muscle creatine was isolated for counting as the creatine zinc chloride salt (5). In several instances, muscle creatine was also isolated as the potassium creatinine picrote (10), so that the two methods of isolation could be compared. The radioactivity of liver proteins obtained by the Schneider fractionation procedure (11) was determined in each case to prove the adequacy of the injections. All samples were counted in metal planchets with an end window Geiger tube, and the observed counts were corrected to infinite thinness.

RESULTS

A summary of the kidney transamidinase activities and the creatine to creatinine ratios of the various groups of animals is presented in Table I. It is apparent that the vitamin E-deficient rabbits had as significant creatinuria as did the control rabbits which were fed creatine. Both creatine feeding and vitamin E deficiency reduced kidney transamidinase activity, whereas sodium sulfaquinoxaline had no effect on transamidinase levels. The relationship of creatine excretion to transamidinase activity is shown in Fig. 1, in which kidney transamidinase activity is plotted against the creatine to creatinine ratio of bladder urine. Transamidinase activity at first falls rapidly with increasing creatine excretion and reaches a minimum at a creatine to creatinine ratio of 3.5.

1 The d-a-tocopheryl polyethylene glycol 1000 succinate was kindly supplied by Dr. Stanley R. Ames of Distillation Products Industries.

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The recovery of kidney transamidinase activity lagged behind the return of creatine excretion to normal in the vitamin E-deficient rabbits which were injected with d-a-tocopheryl polyethylene glycol succinate (Table II). Other attempts to demonstrate a direct effect of vitamin E on the transamidinase were likewise unsuccessful. Homogenizing 7, 8, or 44 mg of dl-a-tocopherol per g of tissue directly with kidneys from vitamin E-deficient rabbits failed to increase the transamidinase activity. Similarly, mixing kidney homogenates from control and vitamin E-deficient rabbits gave only additive results (Table III), as would be expected if the reduced transamidinase activity were due to low enzyme concentration rather than absence of cofactors or presence of inhibitors.

Considerable extrarenal synthesis of glycocyamine by rabbits is indicated by the data in Table IV. The specific activity of muscle creatine of the nephrectomized rabbits was about 50% of that of the sham operated rabbits when glycine-1-C\textsubscript{14} was the creatine precursor. When glycocyamine-2-C\textsubscript{14} was the creatine precursor, nephrectomy did not influence the specific activity of the muscle creatine. Liver protein specific activity was similar in the sham operated and nephrectomized rabbits, with glycocyamine contributing negligible radioactivity to protein.

### Table II

**Recovery of kidney transamidinase activity in vitamin E-deficient rabbits**

Severely dystrophic rabbits were each given 41.7 mg of d-or-tocopheryl polyethylene glycol 1000 succinate intraperitoneally. One animal was killed at each time interval.

<table>
<thead>
<tr>
<th>Hours after injection</th>
<th>Transamidinase activity*</th>
<th>Urinary creatine creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.2†</td>
<td>4.5†</td>
</tr>
<tr>
<td>4</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>7.8</td>
<td>0.45</td>
</tr>
<tr>
<td>79</td>
<td>13.3</td>
<td>0.33</td>
</tr>
<tr>
<td>120</td>
<td>14.5</td>
<td>0.27</td>
</tr>
</tbody>
</table>

* Number of pmoles of glycocyamine formed per hour per g wet weight of kidney.
† Average for the vitamin E-deficient group shown in Table I.

### Table III

**Effect of mixing homogenates from control and vitamin E-deficient rabbits**

For these assays of transamidinase activity, the incubation mixture consisted of 20 pmoles of glycine, 20 pmoles of L-arginine, and either 0.5 ml of 4% kidney homogenate from control or vitamin E-deficient rabbits or a mixture of 0.25 ml from a vitamin E-deficient and 0.25 ml from a control rabbit in a total volume of 1.5 ml of 0.067 M phosphate buffer pH 7.4.

<table>
<thead>
<tr>
<th>Glycocyamine formed</th>
<th>Control</th>
<th>Vitamin E-deficient</th>
<th>1:1 mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmoles/g wet wt. kidney/hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5</td>
<td>6.3</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>27.0</td>
<td>6.0</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>20.5</td>
<td>8.4</td>
<td>13.4</td>
<td></td>
</tr>
</tbody>
</table>

### Table IV

**Creatine formation in nephrectomized rabbits**

<table>
<thead>
<tr>
<th>Operation</th>
<th>C\textsubscript{14}-labeled precursor</th>
<th>Muscle creatine specific activity*</th>
<th>Liver protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>creatine chloride</td>
<td>potassium creatinine picrate</td>
<td>c.p.m./mg</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>Glycine</td>
<td>7.1</td>
<td>9.6</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>Glycine</td>
<td>4.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Sham</td>
<td>Glycine</td>
<td>15.4</td>
<td>15.2</td>
</tr>
<tr>
<td>Sham</td>
<td>Glycine</td>
<td>13.0</td>
<td>12.6</td>
</tr>
<tr>
<td>Sham</td>
<td>Glycocyanine</td>
<td>305</td>
<td>1</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>Glycocyanine</td>
<td>337</td>
<td>1</td>
</tr>
</tbody>
</table>

* Counts per minute per μmole of creatine.

**DISCUSSION**

The low kidney transamidinase activity of the vitamin E-deficient rabbits confirms the report of Van Pilsum and Wahman...
Kidney Transamidinase in Vitamin E-Deficient Rabbits

Kidney transamidinase activity was measured in vitamin E-deficient, control, and creatine-fed control rabbits. Vitamin E-deficient rabbits exhibited as low kidney transamidinase activities as did creatine-fed control rabbits. The transamidinase activity in vitamin E-deficient rabbits which were given parenteral dl-a-tocopherol polyethylene glycol 1000 succinate remained low until after the creatinuria had ceased. Addition in vitro of dl-a-tocopherol or of normal kidney homogenate to kidney homogenates from vitamin E-deficient rabbits did not increase the enzyme activity of the latter. Thus it appears that the reduced enzyme activity is not the direct result of vitamin E deficiency, but that it can best be explained by feedback depression of transamidinase activity by the excess creatine presented to the kidneys.

Considerable extrarenal synthesis of glycyocamine is indicated by the incorporation of glycine-1-14C into muscle creatine of nephrectomized rabbits. Either an increase in extrarenal synthesis of glycyocamine (in vitamin E deficiency) or a normal excess of kidney transamidinase (which is reduced in the deficiency) could account for the presence of normal or increased creatine synthesis in spite of reduced kidney transamidinase activity.

Acknowledgments—We are indebted to Dr. J. F. Diehl for furnishing many of the control and vitamin E-deficient rabbits and to Mrs. Hope Lehman and Mr. Sam Maben for valuable technical assistance.

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