THE SUCCINOXIDASE SYSTEM IN RIBOFLAVIN-DEFICIENT RATS*

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Thus far none of the enzymes which reduce cytochrome c has been isolated from animal tissues. Nevertheless the belief is held that coenzymes I and II are linked to cytochrome by flavoproteins (1, 2) and it has been suggested by Potter (1, 3) that succinic dehydrogenase may be an analogous enzyme. The recent demonstrations by Axelrod and coworkers (4, 5) that the concentrations of the flavoproteins, d-amino acid oxidase and xanthine oxidase, are decreased in the tissues of riboflavin-deficient rats suggested testing the hypothesis that succinic dehydrogenase is a flavoprotein by studying the concentration of this enzyme under conditions similar to those previously employed (4, 5). A positive result, while not establishing the point, would constitute prima facie evidence that the enzyme is a flavoprotein, and would at the same time give added significance to the dietary rôle of riboflavin, since the oxidation of succinic acid is believed to be an essential step in normal carbohydrate metabolism.

EXPERIMENTAL

Weanling male, albino rats were placed on an experimental diet which was designed to produce an uncomplicated riboflavin deficiency.1 The ration was fed ad libitum unless designated otherwise.2

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1 The composition of the diet (designated as Ration B) and the general behavior of the rats are fully described in a previous publication (4).
2 We are indebted to Merck and Company, Inc., Rahway, New Jersey, for generous supplies of thiamine, pyridoxine, riboflavin, pantothenic acid, choline, and nicotinic acid, and to the Abbott Laboratories, North Chicago, Illinois, for the haliver oil used in the present study.
After a depletion period of 10 weeks, fifteen of the rats were sacrificed for the succinoxidase determinations, while the remainder were grouped as indicated in Table I and riboflavin therapy was instituted. These rats were sacrificed at the completion of their respective periods of therapy.

The succinoxidase content of the tissues was determined according to the method of Potter (3) with the following modifications: (a) heart and thigh muscles were minced in an apparatus described by Seevers and Shideman (6) previous to homogenization; (b) the reaction was carried out at 38°; (c) 20 γ of calcium (as calcium chloride) were added to each flask. The addition of calcium was considered necessary, since Axelrod, Swingle, and Elvehjem (7) have demonstrated the marked stimulatory effect of calcium upon the succinoxidase activity of various tissues. 20 γ of calcium produced the maximal effect in the tissues studied. As a routine procedure, the succinoxidase activity of each tissue was determined at levels of 2 and 3 X 10^-8 mole of added cytochrome c. Since the plateau level was reached at 2 X 10^-8 mole per flask, the two determinations served as

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of rats</th>
<th>Riboflavin* therapy</th>
<th>Succinoxidase activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15</td>
<td>None</td>
<td>Liver 99 (54-75)</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>100 γ daily, 12 days</td>
<td>Liver 79 (75-82)</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>100 γ daily, 13 days</td>
<td>Liver 96 (80-112)</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>20 mg. per kilo body weight, 3 days</td>
<td>Liver 94 (80-106)</td>
</tr>
</tbody>
</table>

* After riboflavin therapy was begun, the food intake of each of the rats in Groups III and IV was restricted to 4 gm. of basal ration per day, which is the average daily food consumption by rats in the basal group. Therapy was administered orally to animals in Groups II and III and by subcutaneous injection to those in Group IV.

† The results are reported in terms of the QO₂; i.e., the oxygen uptake per mg. of dry tissue per hour. The range of values is given in parentheses.
duplicates. In the case of heart and thigh muscles the maximal $Q_0$, values were realized when the succinate was added from a Keilin cup at the completion of the equilibration period.

Results

The results obtained are summarized in Table I. It is apparent that significant changes in the succinoxidase content are observed only in the liver. In that tissue, supplementation of the diet with riboflavin results in a marked increase in the succinoxidase activity. This increase is most evident in those rats whose food intake is restricted to that of the basal group. Similar results, i.e. the marked influence of food restriction upon the restoration of enzyme content following vitamin therapy, have been previously noted (4, 5).

The data presented indicate clearly that one or more components of the succinoxidase system of rat liver are affected by the dietary intake of riboflavin. Such results are offered only as indirect evidence in support of the thesis that succinic dehydrogenase is a flavoprotein. The final proof that riboflavin enters into the structure of any of the components of the succinoxidase system must await the isolation in pure state of such components.

SUMMARY

1. The effect of a riboflavin deficiency in the rat upon the succinoxidase content of various tissues was studied.
2. The dietary intake of riboflavin was found to have a definite effect upon the succinoxidase content of liver tissue.
3. The results obtained were taken as prima facie evidence that one or more components of the succinoxidase system are flavoproteins.

BIBLIOGRAPHY

1. Potter, V. R., Medicine, 19, 441 (1940).
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