VITAMIN INTERRELATIONSHIPS

II. THIAMINE AND RIBOFLAVIN INTERRELATIONSHIPS IN METABOLISM*

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In Paper I of this series (1) results were reported on the influence of vitamin A deficiency and of a deficiency of various components of the vitamin B complex on the ascorbic acid content of various organs and endocrine glands. It was found that in vitamin A deficiency there was a reduction in ascorbic acid of the heart, kidney, and thymus. In multiple vitamin A depletions there were also losses of ascorbic acid from the adrenals, thyroids, and pituitary. In thiamine deficiency there were significant losses of vitamin C in the lung, kidney, and liver. Repeated thiamine depletions produced additional heavy losses of vitamin C in the kidney, liver, and thymus. In riboflavin deficiency the greatest reduction in ascorbic acid was found in single vitamin depletions. The losses were largely from the liver, kidney, lung, thymus, and thyroids. In pyridoxine deficiency no noteworthy changes occurred in ascorbic acid content of either tissues or glands.

Morgan (2) reported that the administration of nicotinic acid or pantothenic acid alone to dogs receiving ample amounts of all necessary vitamins except those of the "filtrate factors" resulted in their gradual loss of neuromuscular control and sometimes sudden death. Recently Supplee and coworkers (3) found that thiamine and pantothenic acid deficiencies interfere with mobilization of riboflavin in the liver during digestion and assimilation. The influence, however, of pyridoxine deficiency on the riboflavin metabolism of the liver was very slight.

EXPERIMENTAL

Because of the increasing interest in thiamine and riboflavin as oxidative catalysts, a study was undertaken of the possible interrelationship of these vitamins in metabolism. Since such an investigation involved frequent analyses of urine and feces as well as numerous determinations of various tissues and endocrine glands, rapid and reliable methods were necessary for this type of research. For thiamine we found the method of Hennessy and Cerecedo (4), as modified by the staff of the research laboratories.

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of Merck and Company, Inc., (5) quite satisfactory. For the riboflavin content of feces, tissues, and glands we followed the procedures of Conner and Straub (6). For the riboflavin content of rat urine we used our modification of the method of Hodson and Norris (7) for determining the riboflavin content of foodstuffs, details of which will be given later.

With the exception of one group of eight animals, all the experiments were conducted by the paired feeding technique previously used in this laboratory (8); i.e., the control animals were restricted to the same amount of food as was consumed by the pathological litter mates of the same sex. Consequently, the plane of nutrition was eliminated as a possible complicating factor in the study. The following is the composition of the basal ration used for the production of thiamine and riboflavin deficiencies: casein (purified), 1 20; agar-agar, 2; Sure's Salts 1, 4 (9); butter fat, 10; and cerelose, 64. This ration2 was supplemented daily with 20 γ of thiamine, 20 γ of riboflavin, 20 γ of pyridoxine, 6 mg. of choline chloride, and 200 γ of calcium pantothenate. In the experiments on thiamine deficiency no vitamin B_{1} was allowed the avitaminotic rats and in those on riboflavin deficiency none of the latter vitamin was given the pathological animals. Following 2 weeks of thiamine depletion, the avitaminotic rats began to lose weight and generally succumbed 3 to 4 weeks thereafter, the maximum period of experimentation, therefore, being 6 weeks. The results on thirty-two pairs of animals, nineteen pairs of males and thirteen pairs of females, show an average loss of weight per animal during the vitamin depletion periods of 45.7 gm. for the thiamine-deficient rats and a loss of 31.4 gm. per animal for the controls on the same daily food intake, which substantiates our previous conclusions that there is a pronounced tissue catabolism in thiamine deficiency (8).

Riboflavin deficiency was produced in twenty-two pairs of animals, the depletion periods lasting 55 to 100 days. The symptomatology observed was the same as previously reported (10); i.e., rough hair, alopecia, keratitis, conjunctivitis, and premature senility. In addition, we observed that some of the riboflavin-deficient animals, after 5 to 6 weeks depletion, developed "blood-caked" whiskers, reported by Unna (11) to be present in pantothenic acid deficiency. The changes in body weight per animal during the entire vitamin depletion periods were as follows: riboflavin-deficient, -22.5 gm.; restricted controls, +36.5 gm.

Procedures for Thiamine and Riboflavin Determinations in Urine—The urine was collected in amber bottles containing 10 drops of toluene and 0.5 per cent chlorobutanol solution (6), adjusted to a pH of about 1.0 with normal sulfuric acid. The latter was added by being poured over the

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1 Supplied by The Borden Company, New York, under the trade name of Labco.
2 To prevent development of rancidity, this ration was compounded twice weekly.
filter paper in the bottom of the large funnels placed in metabolism cages, a description of which was given in a recent publication (12). In the morning the funnels were washed with an additional 7 cc. of the chloro-butanol solution. The diluted urine was then filtered in 100 cc. graduated cylinders and was brought up to any convenient volume, 20 cc. being that generally used. The urine was now divided for thiamine and riboflavin determinations. Two 5 cc. portions were pipetted into 50 cc. Erlenmeyer flasks for riboflavin determinations and the remaining 10 cc. portion was used for the thiamine determination.

**Thiamine Procedure**—The diluted urine sample was adjusted to a pH of approximately 4.2 with sodium acetate buffer with brom-cresol green as an outside indicator. After the acidity of the urine was adjusted to the desired pH, the urine was transferred from the cylinder to a base exchange tube containing 750 mg. of purified Decalso. From then on the thiamine determination in urine was carried out by the modified procedures described by Merck and Company (5). We used the Pfaltz and Bauer fluorophotometer for both the thiamine and riboflavin determinations. The direct determination of thiamine in urine without adsorption (13) proved undependable in our experience.

**Riboflavin Procedure**—We found by numerous trials that the riboflavin content of rat urine could be determined with the same degree of accuracy when the adsorption on florisil was omitted, which at the same time dispensed with the step of elution with dilute pyridine. 95 to 100 per cent recoveries of riboflavin added to dilute urine were obtained by the following procedure and the results checked with those obtained by adsorption on florisil (6). Our procedure depends on the principle that riboflavin is reduced to a non-fluorescent form by sodium hydrosulfite; so that after such treatment the fluorescence, emanating from impurities or foreign substances in the urine, constitutes the blank. The riboflavin content of the urine is then obtained by subtracting the fluorophotometer reading after reduction from the initial reading before treatment with sodium hydrosulfite and sodium bicarbonate. The following is the technique we used: To 5 cc. samples of urine were added 5 drops of 4 per cent KMnO₄ solution and 2 drops of glacial acetic acid. The mixture was then shaken vigorously for 2 minutes. 5 drops of 3 per cent H₂O₂ solution were then added and the mixture was again shaken until all signs of excess KMnO₄ and H₂O₂ disappeared. Enough distilled water was now added to bring the volume up to 15 cc. Readings were then made in the fluorophotometer which was adjusted so that 0.05 γ of riboflavin per cc. (0.75 γ in 15 cc. of water) gave a galvanometer deflection of 20. After this reading was obtained, 0.2 to 0.4 cc. of a solution of 1 gm. of sodium bicarbonate and 1 gm. of sodium hydrosulfite in 20 cc. of distilled water, kept cool in an ice
bath, was added and another reading was taken. The difference in the two readings gave the fluorescence due to riboflavin. The final reading was converted to micrograms by means of a graph constructed by plotting micrograms of riboflavin versus galvanometer readings. Since the curve is linear, even as low a reading as 0.013 \( \gamma \) per cc. is quite accurate. The amount of riboflavin in the sample is as follows: The reading from the graph \( \times 15 \times \left( \frac{\text{total volume of urine}}{\text{volume used for riboflavin determination}} \right) \) equals micrograms of riboflavin.

**Thiamine and Riboflavin Determinations in Animal Tissues**—The various tissues or organs were pooled from groups of five to six pairs of rats, equal numbers from avitaminotic and control animals. They were covered with 50 per cent ethanol after being cut in thin slices and were dried at 55\(^\circ\) overnight. They were then extracted thoroughly with petroleum ether in Soxhlet extractors, finely ground, and analyzed according to the procedures given above. The petroleum ether extracts of the tissues were found to be entirely free from thiamine and riboflavin. Since at 55\(^\circ\) 6 to 8 per cent moisture was often left in the dried tissues, moisture determinations were made at 103\(^\circ\) and all results were expressed on a moisture-free basis.

Equal amounts of samples were taken of the avitaminotic and control animal tissues. To the tissues were added 0.1 N sulfuric acid. If the samples weighed 800 mg. or more, 75 cc. of acid were added, but if the samples weighed less than 800 mg., 50 cc. were taken. The following volumes of acid were used for the glands pooled from thirty-two animals, for the thymus and adrenals 50 cc., and for the thyroids and pituitary 25 cc. The tissues were autoclaved for 20 minutes at 15 pounds pressure, cooled, the pH adjusted to 4.0 to 4.5, 5 cc. of 10 per cent taka-diastase were added, and the solutions incubated for 2 hours at 45\(^\circ\). After incubation the pH occasionally changed and required readjustment. The solutions were transferred to 100 cc. volumetric flasks and brought up to volume. They were then centrifuged until clear. Aliquots were taken for thiamine and riboflavin determinations and the procedures of Conner and Straub (6) were then followed. We were able to recover 90 to 100 per cent of the thiamine and riboflavin added to autoclaved extracts of the various animal tissues.

**Thiamine and Riboflavin Determinations in Feces**—The thiamine determination of feces was satisfactorily carried out according to the technique adopted for the tissues. The riboflavin determination of feces, however, presented considerable difficulties. The results of the first few weeks were very erratic, frequently the weekly fecal excretion being twice the total intake. The high figures were most probably due to bacterial synthesis, such as Lamoreux and Schumacher (14) observed in the case of the fowl.
Bacterial synthesis in the feces of the rat was recently reported by Wilde-
mann (15). Following numerous failures we finally were able to reduce
the riboflavin excretions in the rat feces to what appeared to be reasonable
figures. The change responsible for reducing the apparent bacterial
synthesis to insignificant proportions consisted in the collection of the
feces under petroleum ether, the solvent used in the next step for removing
fats previous to analysis. Feces were collected daily from each animal in
the metabolism cages in amber bottles which were filled with enough
petroleum ether to cover the feces. At the end of the metabolism period,
usually a week, the feces were removed from the bottles and were placed
in evaporating dishes in the dark room to dry. After drying, the feces
were pulverized in a mortar and fat was extracted with petroleum ether
overnight in Soxhlet extractors. The petroleum ether extracts were free
from thiamine and riboflavin. After fat extraction they were analyzed
by the same procedures as the tissues, the additional precaution taken
being that the extracts of feces were filtered hot to prevent solidification.

\textbf{Thiamine and Riboflavin Interrelationships in Metabolism}

\textbf{Thiamine Deficiency—}The riboflavin and thiamine excretions in the
urine in thiamine deficiency were studied in thirty-five pairs of rats. Four
pairs of animals received food \textit{ad libitum}, while the food of the control rats
of the rest of the groups was restricted to that consumed by the avitamin-
otic animals. The metabolism periods ranged from 7 to 14 days. The
urine was analyzed daily with the exception of Sundays. Monday’s
sample, therefore, covered a 48 hour period. During thiamine depletions
we observed not only a marked reduction of thiamine excretion in the
urine but also a marked disturbance in riboflavin metabolism, as evidenced
by urinary excretions. The increased riboflavin excretions in individual
cases of thiamine-deficient animals varied from 2- to 7-fold compared with
the excretions of the controls. The average weekly riboflavin excretions
during the avitaminotic period for five groups of rats, comprising twenty-
four pairs, were as follows: pathological, or thiamine-deficient, 92.5 \gamma;
restricted controls, 28.3 \gamma. The average weekly thiamine excretions were
1.35 \gamma for the thiamine-deficient animals and 3.84 \gamma for the restricted
controls. No noteworthy differences were found in fecal excretions be-
tween pathological and control animals. These figures, however, were
essential in calculating efficiency of riboflavin and thiamine utilization,
the results of which are submitted in Tables I and II.

In Table III are presented summarized results on the influence of thi-
amine deficiency on the thiamine and riboflavin content of various tissues
and endocrine glands. The data represent average figures on thirty-two
pairs of animals, nineteen pairs of males and thirteen pairs of females.
While the various organs or tissues were analyzed from groups of five to six pairs of animals, the results of which were then averaged, the analyses

**Table I**

Influence of Thiamine Deficiency on Efficiency of Riboflavin Utilization

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>3</td>
<td>12-17</td>
<td>P</td>
<td>140</td>
<td>26.25</td>
<td>113.75</td>
<td>146.98</td>
<td>-33.23</td>
<td>61.5</td>
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<td></td>
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<td>RC</td>
<td>140</td>
<td>31.38</td>
<td>108.62</td>
<td>41.56</td>
<td>66.76</td>
<td>11.8</td>
</tr>
<tr>
<td>4</td>
<td>18-23</td>
<td>P</td>
<td>140</td>
<td>27.92</td>
<td>112.08</td>
<td>98.76</td>
<td>13.32</td>
<td>78.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RC</td>
<td>140</td>
<td>41.00</td>
<td>99.00</td>
<td>21.44</td>
<td>77.56</td>
<td>48.1</td>
</tr>
<tr>
<td>5</td>
<td>24-29</td>
<td>P</td>
<td>140</td>
<td>12.81</td>
<td>127.19</td>
<td>65.02</td>
<td>62.17</td>
<td>71.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RC</td>
<td>140</td>
<td>28.13</td>
<td>111.87</td>
<td>32.22</td>
<td>79.66</td>
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<td>Average</td>
<td></td>
<td>P</td>
<td></td>
<td>22.33</td>
<td>117.87</td>
<td>103.59</td>
<td>14.08</td>
<td>70.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RC</td>
<td></td>
<td>33.50</td>
<td>106.55</td>
<td>31.84</td>
<td>74.71</td>
<td></td>
</tr>
<tr>
<td>2†</td>
<td>7-11</td>
<td>P</td>
<td>350</td>
<td>38.25</td>
<td>311.75</td>
<td>205.16</td>
<td>106.59</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RC</td>
<td>350</td>
<td>38.20</td>
<td>291.80</td>
<td>94.06</td>
<td>197.74</td>
<td>67.7</td>
</tr>
</tbody>
</table>

*P = pathological; RC = restricted control.

† Group 2 was not included in the average, since each animal instead of receiving the regular 20γ daily doses of thiamine, riboflavin, and pyridoxine received 50γ daily of these vitamins.

**Table II**

Influence of Riboflavin Deficiency on Efficiency of Thiamine Utilization

The weekly intake of thiamine was 140γ.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Pair Nos.</th>
<th>Animals*</th>
<th>Weekly fecal excretion</th>
<th>Digestible thiamine</th>
<th>Weekly urinary excretion</th>
<th>Amount of thiamine absorbed</th>
<th>Thiamine utilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-5</td>
<td>P</td>
<td>14.94</td>
<td>125.06</td>
<td>2.66</td>
<td>122.40</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RC</td>
<td>14.58</td>
<td>125.42</td>
<td>2.38</td>
<td>123.04</td>
<td>98.9</td>
</tr>
<tr>
<td>2</td>
<td>6-11</td>
<td>P</td>
<td>9.50</td>
<td>130.50</td>
<td>2.65</td>
<td>127.85</td>
<td>97.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RC</td>
<td>9.25</td>
<td>130.75</td>
<td>2.72</td>
<td>128.03</td>
<td>97.9</td>
</tr>
<tr>
<td>3</td>
<td>12-17</td>
<td>P</td>
<td>9.93</td>
<td>130.07</td>
<td>2.62</td>
<td>137.45</td>
<td>96.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RC</td>
<td>10.18</td>
<td>129.82</td>
<td>2.32</td>
<td>127.50</td>
<td>98.2</td>
</tr>
<tr>
<td>4</td>
<td>18-22</td>
<td>P</td>
<td>6.99</td>
<td>143.01</td>
<td>6.28</td>
<td>136.73</td>
<td>95.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RC</td>
<td>2.91</td>
<td>137.09</td>
<td>5.07</td>
<td>132.02</td>
<td>96.2</td>
</tr>
</tbody>
</table>

*P = pathological; RC = restricted control.

on the glands were carried out on the pooled material collected from all the thirty-two pairs of animals, in order to have a sufficiency for thiamine and riboflavin determinations.
That there is a marked reduction in thiamine content of all the organs and endocrines in thiamine deficiency is quite evident, the greatest losses of vitamin occurring in liver, kidney, heart, and thymus. There are, however, also appreciable losses of riboflavin from the lung, ovaries, and muscles, and small losses from the rest of the body tissues in thiamine avitaminosis. The losses from the muscles, although not pronounced, are most significant, because they represent the largest weight of the carcass of the animal. In sampling for muscle tissue, all the muscles were dissected out, dried, and analyzed as previously described. Therefore, the results are quite representative for the entire animal rather than portions of the various animals used in this study.

The approximate total weights of the glands were as follows: Thymus 270 mg., adrenals 200 mg., thyroids 75 mg., and pituitary 32 mg. The total weight of the thymus glands from the control animals was considerably more but the amount sampled was 270 mg., since this was the total weight of the thymus glands of the avitaminotic rats. While the total

Table III
Influence of Thiamine Deficiency on Thiamine and Riboflavin Content of Various Tissues and Endocrine Glands

The data were obtained from thirty-two pairs of animals, nineteen pairs of males and thirteen pairs of females.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Thiamine Pathological</th>
<th>Control</th>
<th>Change in pathological animals</th>
<th>Riboflavin Pathological</th>
<th>Control</th>
<th>Change in pathological animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \gamma ) per gm.</td>
<td>( \gamma ) per gm.</td>
<td>% per gm.</td>
<td>( \gamma ) per gm.</td>
<td>( \gamma ) per gm.</td>
<td>% per gm.</td>
</tr>
<tr>
<td>Liver</td>
<td>1.55</td>
<td>6.90</td>
<td>-77.5</td>
<td>36.70</td>
<td>41.28</td>
<td>-11.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.09</td>
<td>8.28</td>
<td>-79.6</td>
<td>43.33</td>
<td>48.78</td>
<td>-10.9</td>
</tr>
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<td>Spleen</td>
<td>2.52</td>
<td>6.60</td>
<td>-61.5</td>
<td>15.11</td>
<td>16.10</td>
<td>-6.1</td>
</tr>
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<td>Heart</td>
<td>2.16</td>
<td>9.48</td>
<td>-77.2</td>
<td>34.43</td>
<td>39.13</td>
<td>-12.0</td>
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<tr>
<td>Lung</td>
<td>1.56</td>
<td>3.76</td>
<td>-58.5</td>
<td>8.81</td>
<td>10.39</td>
<td>-15.2</td>
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<tr>
<td>Brain</td>
<td>3.95</td>
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<td>-42.4</td>
<td>7.59</td>
<td>8.13</td>
<td>-6.6</td>
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<td>Testes</td>
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<td>10.63</td>
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<td>5.45</td>
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<td>Ovaries</td>
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<td>8.92</td>
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<td>22.55</td>
<td>29.65</td>
<td>-23.9</td>
</tr>
<tr>
<td>Stomach</td>
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<td>3.50</td>
<td>-59.7</td>
<td>13.02</td>
<td>13.73</td>
<td>-5.2</td>
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<tr>
<td>Small intestines</td>
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<td>2.08</td>
<td>-61.1</td>
<td>8.27</td>
<td>9.23</td>
<td>-10.4</td>
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<td>Large ( \text{&quot;} )</td>
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<td>-62.3</td>
<td>7.77</td>
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<td>-13.6</td>
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<td>-9.6</td>
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<td>Muscles</td>
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<td>-62.0</td>
<td>1.62</td>
<td>2.01</td>
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<td>Thymus</td>
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<td>19.00</td>
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<td>12.64</td>
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<td>Thyroids</td>
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<td>34.72</td>
<td>-4.0</td>
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<td>16.00</td>
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<td>31.25</td>
<td>35.62</td>
<td>-12.3</td>
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<td>Pituitary</td>
<td>4.50</td>
<td>6.00</td>
<td>-25.0</td>
<td>50.82</td>
<td>80.00</td>
<td>-29.6</td>
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</table>
weights seem too small for dependable results, the actual total amounts of riboflavin measured ranged between 0.113 and 1.313 \( \gamma \), which can be measured accurately in the Pfaltz and Bauer instrument. Therefore, the loss of about 30 per cent riboflavin in the pituitary in thiamine deficiency seems significant. The thiamine values in the glands, however, ranged between 0.13 and 0.6 \( \gamma \). The modified Hennessy and Cerecedo procedures (5) call for the determination of approximately 1.0 \( \gamma \) of thiamine for the greatest accuracy. While this was possible for some of the tissues, such as muscle, liver, and kidney, in many tissues we were obliged to read less than 1.0 \( \gamma \) and in urines of thiamine-deficient animals we frequently found only 0.2 \( \gamma \) of thiamine. The results, however, while not quantitative in the cases of low readings, show a relationship between avitaminotic and control animals. Nevertheless, we are convinced that the endocrine glands of the rat, particularly the thyroid and pituitary, need a more sensitive method, such as the microbiological method, for measurements of such minute amounts of vitamins. Our results may therefore be considered suggestive of what may be found with a more sensitive microtechnique.

Since both fecal and urinary excretions of riboflavin in thiamine deficiency are available for four groups of animals, it was possible to calculate the amounts of riboflavin that were digested and absorbed and the percentage that was utilized. Group 3 (Table I) showed a negative balance, while the thiamine-deficient animals of Groups 2, 4, and 5 showed tremendous reduction in efficiency of riboflavin utilization compared with the control animals that received 20 to 50 \( \gamma \) of thiamine daily. The average efficiency of riboflavin utilization for Groups 3, 4, and 5 was as follows: thiamine-deficient 11.2 per cent, restricted controls 70.1 per cent. It is clear from Table I that the greater losses of riboflavin in thiamine deficiency are due mainly to poor absorption. In the case of negative riboflavin balances, some of the riboflavin excreted in the urine undoubtedly originated from losses from the tissues, particularly the muscles.

**Riboflavin Deficiency**—The urinary and fecal excretions of thiamine and riboflavin in riboflavin deficiency were studied in twenty-two pairs of male rats (four groups) during vitamin depletion periods ranging from 31 to 78 days. The animals were, however, sacrificed for their tissues during advanced to terminal stages of deficiency which covered periods ranging from 55 to 100 days. The thiamine and riboflavin content of the various tissues (Table IV), therefore, includes that of animals that were well depleted of riboflavin. Since twenty-two animals did not provide enough material for analyses of the endocrine glands, the latter were not included in this study. That there are marked reductions of riboflavin in all the body tissues in riboflavin deficiency is evident from Table IV. The relatively smaller
losses of riboflavin from the body tissues in riboflavin avitaminosis than the losses of thiamine from the animal organs in thiamine deficiency are due to the fact that the animals in the latter avitaminosis were in a more advanced vitamin-depleted state than the animals in riboflavin deficiency. It is clear from Table II that even in advanced states of riboflavin deficiency, characterized by losses of body weight and general symptomatology, there is absolutely no disturbance in thiamine metabolism.

The results of this investigation may have considerable human application. Since it is now generally recognized that thiamine deficiency is wide-spread in this country (16), it is quite possible that border line riboflavin deficiencies may exist not only from inadequate riboflavin intake but also from poor utilization of the latter vitamin caused by thiamine deficiency. On the other hand, a diet abundant in thiamine may prevent riboflavin deficiency produced by insufficient riboflavin intake. These are, however, problems for the clinicians to solve.

**SUMMARY**

In thiamine deficiency there is a pronounced disturbance in riboflavin metabolism, mainly because of poor absorption. However, in riboflavin deficiency there is no disturbance in thiamine metabolism.

---

**Table IV**

*Influence of Riboflavin Deficiency on Riboflavin and Thiamine Content of Various Tissues*

The data were obtained from twenty-two pairs of male rats.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Riboflavin</th>
<th>Change in pathological animals</th>
<th>Thiamine</th>
<th>Change in pathological animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pathological</td>
<td>Control</td>
<td>per cent</td>
<td>Pathological</td>
</tr>
<tr>
<td>Liver</td>
<td>24.48</td>
<td>53.16</td>
<td>-54.0</td>
<td>5.81</td>
</tr>
<tr>
<td>Kidney</td>
<td>45.74</td>
<td>74.69</td>
<td>-38.7</td>
<td>7.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>16.21</td>
<td>28.64</td>
<td>-43.4</td>
<td>5.37</td>
</tr>
<tr>
<td>Heart</td>
<td>36.62</td>
<td>59.59</td>
<td>-38.5</td>
<td>9.60</td>
</tr>
<tr>
<td>Lung</td>
<td>9.84</td>
<td>19.90</td>
<td>-50.6</td>
<td>3.55</td>
</tr>
<tr>
<td>Brain</td>
<td>6.61</td>
<td>13.15</td>
<td>-49.5</td>
<td>5.91</td>
</tr>
<tr>
<td>Testes</td>
<td>8.48</td>
<td>16.05</td>
<td>-47.2</td>
<td>23.31</td>
</tr>
<tr>
<td>Stomach</td>
<td>8.85</td>
<td>16.56</td>
<td>-58.7</td>
<td>3.64</td>
</tr>
<tr>
<td>Small intestines</td>
<td>7.16</td>
<td>13.16</td>
<td>-45.5</td>
<td>3.15</td>
</tr>
<tr>
<td>Large</td>
<td>6.69</td>
<td>13.94</td>
<td>-52.0</td>
<td>3.35</td>
</tr>
<tr>
<td>Pancreas</td>
<td>9.60</td>
<td>19.78</td>
<td>-51.5</td>
<td>2.98</td>
</tr>
<tr>
<td>Muscles</td>
<td>1.90</td>
<td>3.86</td>
<td>-50.0</td>
<td>0.59</td>
</tr>
</tbody>
</table>
BIBLIOGRAPHY

5. Merek and Company, Inc., Determination of thiamine hydrochloride by the thiochrome method, with the adaptation of the Hennessy and Cerecedo procedure, revised June 6 (1941).
CORRECTIONS

On p. 245, Vol. 146, No. 1, November, 1942, line 5, read much smaller for insignificant.

On p. 246, Tables I and II, next to last column heading, read retained for absorbed.

On p. 249, line 2 from the foot of the page, read retention for absorption.
VITAMIN INTERRELATIONSHIPS: II. THIAMINE AND RIBOFLAVIN INTERRELATIONSHIPS IN METABOLISM
Barnett Sure and Zenas W. Ford, Jr.


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