THE EFFECT OF THIAMINE DEFICIENCY IN RATS ON
THE EXCRETION OF PYRUVIC ACID AND BISULFITE-
BINDING SUBSTANCES IN THE URINE*

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Thiamine deficiency in all species thus far studied affects the
metabolism of pyruvic acid. The lack, until quite recently (1),
of a rapid specific method for estimating pyruvic acid necessi-
tated the use of the rapid but less specific technique introduced by
Clift and Cook (2). Occasionally it has been estimated by iso-
lution as the 2,4-dinitrophenylhydrazone, by the method of Case
(3) or a modification (4) of that method.

The Clift and Cook procedure (2) is based on the fact that
pyruvic acid (as well as other ketones and aldehydes) combines
with bisulfite. Thompson and Johnson (4) were the first to at-
tempt to correlate pyruvic acid with the great increase in bisulfite-
binding substances in the blood of thiamine-deficient rats and
pigeons. Quantitative measurements in the polyneuritic pigeon
of pyruvic acid as the 2,4-dinitrophenylhydrazone demonstrated
that the increase in bisulfite-binding substances was due almost
entirely to pyruvic acid. Less quantitative estimations of pyruvic
acid indicated a similar occurrence in thiamine-deficient rats.
Lu (5) measured pyruvic acid and found increased amounts in the
blood of thiamine-deficient rats, rabbits, and humans.

Sherman and Elvehjem (6) detected no rise in the bisulfite-
binding substances of the blood of thiamine-deficient chicks, but
they found nearly a 3-fold increase in the case of the cloacal ex-

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in Hygiene.

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ercta, the urinary component probably contributing the increased bisulfite-binding substances.

In view of these findings we were led to investigate the pyruvic acid and bisulfite-binding substances content of the urine of rats in various degrees of thiamine deficiency to determine whether an increase in these substances might afford a means of estimating (a) the degree of deficiency and (b) the effect of various factors and substances on the deficient animal. While engaged in this study, Banerji and Harris (7) published a paper describing the rapid and large increase of bisulfite-binding substances in the urine of thiamine-deficient rats and demonstrated that in these animals the decrease was proportional to the amount of thiamine administered. The work reported here confirms and extends their findings; a preliminary note has appeared (8).

EXPERIMENTAL

Urine Collection—Single rats were placed in metabolism cages of the type used in this laboratory for mineral balance studies (10). Each cage consisted of a funnel 11 inches in diameter at the rim. Directly below this large funnel was a sealed bulb supported in a small funnel by means of short projections. The bulb acted to separate the urine from the feces which were deflected into a metal cup, while the urine passed into a flask containing toluene.

The daily urinary output of normal young rats is quite small. Thiamine-deficient rats with the characteristic anorexia excrete even less. To avoid the loss of a goodly proportion of the urine through drying on the sides of the funnels, or the disadvantage of having to place several rats in the same metabolism cage and thus forfeiting individual data, a relatively large volume of urine was secured by incorporating sodium chloride in the diet at high levels. This allowed a daily collection of about 15 ml. of urine from a single rat eating as little as 3 gm. of diet. Loss of urine was relatively small and fairly constant, as shown by creatinine determinations. A 150 gm. rat fed a diet containing 9.4 per cent of sodium chloride may excrete as much as 60 ml. of urine daily.

1 After the preparation of the manuscript of this paper was completed, Harper and Deuel (9) reported on the urinary excretion of pyruvic acid by thiamine-deficient rats. They found an increase as the deficiency progressed and an effect due to the quantity of food, as reported here.
Effect of High Sodium Chloride Intake on Growth of Normal and Thiamine-Deficient Rats—Cowgill et al. (11) found that the administration of large amounts of fluid by mouth to dogs on a vitamin B<sub>1</sub>-deficient diet markedly shortened the time required for the appearance of the anorexia characteristic of a lack of the vitamin. A washing out of the vitamin from the organism through elimination of the excess fluid is offered by these authors as a possible explanation of the results.

The following experiment was performed to determine whether feeding sodium chloride at a high level seriously affects the thiamine requirement, growth, and appearance. Four groups of rats, each containing six animals, were fed the following diets. Group 1 received high salt-low thiamine (Diet 1); Group 2 “normal” salt-low thiamine (Diet 2); Group 3 “normal” salt-adequate thiamine (Diet 3); and Group 4 high salt-adequate thiamine (Diet 4). Diet 2 was essentially Diet 112 of Arnold and Elvehjem (12). It consisted of sucrose 62, casein (acid-washed) 18, factor W = 2 gm. of liver concentrate,<sup>2</sup> autoclaved peanuts<sup>3</sup> 10, autoclaved yeast<sup>4</sup> 4, Salts 51<sup>5</sup> 4, and percomorph oil 2 to 3 drops weekly per rat. The other diets were as follows: Diet 1, 10 gm. of sodium chloride to 100 gm. of Diet 2; Diet 2-B-300, 300 γ of thiamine<sup>6</sup> to 100 gm. of Diet 2; and Diet 1-B-300, 300 γ of thiamine to 110 gm. of Diet 1.

The figures for growth of the different groups are given in Table I.

The large amounts of sodium chloride received by Group 1 did not result in deficiency symptoms appearing any more quickly than in Group 2 receiving a “normal” small amount of the salt. There developed on Diet 1 a rather severe but chronic deficiency which was satisfactory for the needs of this study.

There is no significant difference between the weights of Groups 3 and 4. In both of these groups the animals were apparently

<sup>2</sup>The liver concentrate was obtained from The Wilson Laboratories through the courtesy of Dr. David Klein.

<sup>3</sup>The peanuts were autoclaved 10 hours.

<sup>4</sup>Northwestern yeast, autoclaved 24 hours.

<sup>5</sup>CaCO<sub>3</sub> 1.5, KCl 1.0, NaCl 0.5, NaHCO<sub>3</sub> 0.7, MgO 0.2, Fe citrate 0.5, KH<sub>2</sub>PO<sub>4</sub> 1.7.

<sup>6</sup>The thiamine was furnished by Merck and Company, Inc., through the courtesy of Dr. R. Major.
Thiamine Deficiency

healthy. Histological examination of the organs of the animals in Group 3 showed dilatation of the kidney tubules with no other changes. The same sort of dilatation is seen in kidneys of humans with diabetes insipidus where large quantities of water are being excreted. The kidneys of animals on the thiamine-deficient diet with high sodium chloride content showed no changes, evidently because the anorexia common to this group resulted in a very small urinary excretion. The results of this experiment permitted the conclusion that the use of sodium chloride at a 9.4 per cent level in the diet has no vitriating effects.

**TABLE I**

Comparison of Growth of Rats Receiving Normal and High Amounts of Sodium Chloride with Optimal and Suboptimal Amounts of Thiamine

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Diet No.</th>
<th>Average initial weight</th>
<th>Average weight after 5 wks. on diet</th>
<th>Average weight after 10 wks. on diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 3 * ( \sigma ) ( \varphi ) ( \sigma ) ( \varphi ) ( \sigma ) ( \varphi ) ( \sigma ) ( \varphi ) ( \sigma ) ( \varphi )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1. High salt-low thiamine</td>
<td>43</td>
<td>59*</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>2. “Normal” salt-low thiamine</td>
<td>43</td>
<td>55* ( \dagger )</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>3-B-300. “Normal” salt-optimum thiamine</td>
<td>49</td>
<td>139</td>
<td>111</td>
</tr>
<tr>
<td>4</td>
<td>1-B-300. High salt-optimum thiamine</td>
<td>45</td>
<td>118</td>
<td>99</td>
</tr>
</tbody>
</table>

* One animal died with polyneuritis in the 6th week.
† One animal died with polyneuritis in the 8th week.

**Determination of Bisulfite-Binding Substances**—Satisfactory determinations may be made directly with aliquots of a 24 hour sample of rat urine diluted to 200 ml., although better end-points are obtained when adsorbing agents are previously employed. Experiments with adsorbents indicated that the use of Lloyd’s reagent alone, the procedure applied to human urine (13), was not very satisfactory unless permutit was also used. The bisulfite-binding substances decreased after adsorption treatment, indicating the removal of some of these substances (not pyruvic acid). Increased sodium chloride concentration in rat urine has no effect...
on the determination of bisulfitie-binding substances provided the proper pH is attained.

The principle of the method of Clift and Cook is as follows: Pyruvic acid in acid solution reacts with bisulfite to form an addition compound which decomposes in a more alkaline medium. The liberated bisulfite is then measured with a dilute standard iodine solution, thus giving the concentration of pyruvic acid and other bisulfite-binding substances present.

We determined bisulfite-binding substances in rat urine as follows: 24 hour specimens are diluted to 200 ml. with distilled water. To each 25 ml. aliquot taken, 10 ml. of 0.125 N oxalic acid are added and then 0.6 ml. of approximately 1 M sodium bisulfite solution. After 15 minutes, 1 ml. of 1 per cent starch solution is added and the excess bisulfite is removed by adding 0.1 N iodine 2 to 3 drops in excess. In 4 to 5 minutes approximately 0.01 N sodium thiosulfate solution is used to remove the excess iodine; 0.005 N iodine solution is then added to the first faint color to remove the excess thiosulfate. 5.5 ml. of a clear saturated sodium bicarbonate solution are run in from a burette down the sides of the flask. 0.005 N iodine solution is immediately added dropwise from a microburette at a constant rate until a faint blue color spreads throughout the solution and persists for at least 4 to 5 seconds. The amount of standard 0.005 N iodine is a measure of the bisulfite-binding substances.

Numerous determinations showed that in the normal range of bisulfite-binding substances (in animals receiving adequate thiamine and with restricted food intake, a procedure adopted regularly after the first experiment), the difference in triplicate determinations seldom exceeded 0.05 ml. (1 drop) of 0.005 N iodine, a difference of 5 to 10 per cent. As the bisulfite-binding substances increased in thiamine-deficiency, the error increased slightly, but in 95 per cent of the determinations the differences remained within 0.2 ml. of 0.005 N iodine, still less than 10 per cent of the total bisulfite-binding substances.

In experiments in which it is desired to find the bisulfite-binding substances in rat urine after treatment with the adsorbents the following procedure is used: 125 ml. of the 200 ml. dilution of the 24 hour specimen are taken and 40 ml. of 0.125 N oxalic acid added. The solution is shaken for 4 to 5 minutes with a mixture of 10 gm.
of treated Lloyd's reagent (14) and 5 gm. of permutit, and then filtered. To 25 ml. aliquots of this filtrate 0.6 ml. of approximately 1 M sodium bisulfite solution is added and the procedure continued as described above, with the one difference that only 4 ml. of sodium bicarbonate solution are added. A small blank correction has to be made for bisulfite-binding substances in Lloyd's reagent.

A few workers (15, 16) stress the importance of cooling pyruvate solutions before titrating, although Clift and Cook (2) made no mention of this point. Several experiments in duplicate on aliquots of rat urine not adsorbed indicated that cooling at 10° gave no higher results than when the procedure was carried out at room temperature (25°). However, when the temperature reached 30°, lower values for bisulfite-binding substances were found. It is best, therefore, to keep the urine cool until ready for the final titration.

**Determination of Pyruvic Acid**—Pyruvic acid (Eastman) was redistilled in vacuo, and sodium pyruvate prepared as described by Peters (17) and kept in crystalline form until needed. Determinations as bisulfite-binding substance on the pyruvate gave 82 per cent of the theoretical value.

The estimation of pyruvic acid in urine was made by a slight modification of the method of Lu (1). To a 25 ml. aliquot of the 24 hour specimen of rat urine (diluted to 200 ml.) are added 10 ml. of 10 per cent trichloroacetic acid and the solution is filtered. 2 ml. of the filtrate are measured into a 15 ml. centrifuge tube and 2 ml. of the 2,4-dinitrophenylhydrazine solution are added. Lu's method is followed exactly except that 7 ml. of 1 N sodium hydroxide are added rather than 4 ml. The red color which develops is determined in a Klett-Summerson photoelectric colorimeter with Filter 54. The amount of pyruvic acid present is read from a standard curve constructed by plotting the colorimeter readings against various concentrations of pyruvate. We estimate the accuracy of this method to be within 5 per cent.7

**Diets**—In Experiment 1 the diets all contained 9.4 per cent of sodium chloride. Diet 1, thiamine-low, has been given. Diets

7 Lu (1) states that the method is fairly specific for pyruvic acid, although some other α-keto acids may contribute to some extent, if present, to the colorimeter readings.
l-B-1, l-B-2, and l-B-3 contained 100, 200, and 300 \( \gamma \) of thiamine respectively, per 110 gm. of Diet 1. In Experiment 2 Diet 1-L was used. In this diet the sucrose of Diet 1 was replaced isocalorically by lard.

**Experiment 1. Bisulfite-Binding Substance and Pyruvic Acid in Progressive Thiamine Deficiency**

In this experiment the bisulfite-binding substances were estimated frequently during the time that the rats were restricted to the thiamine-low diet. Each experimental animal was paired with a control which received the same amounts of food supplemented with thiamine. The bisulfite-binding substances of each pair were measured at the same time. In most cases the determinations were made with and without treatment of the urine with the adsorbing agents. In addition, for some days before and after thiamine injection (toward the end of the experiment), pyruvic acid in the unadsorbed urine was measured as the 2,4-dinitrophenylhydrazone. Records of the food intake and weights of the animals were kept.

The results of representative animals are given in Figs. 1 and 2, depicting observations on deficient rats, and in Fig. 3, representing a control animal.

The experiment revealed the following.

In thiamine-deficient animals the bisulfite-binding substances and pyruvic acid in the urine increased greatly. The increase occurred before any symptoms of the deficiency were manifested.

As the deficiency progressed, the bisulfite-binding substances increased.

Within 24 hours after the injection of thiamine into the deficient animals the bisulfite-binding substances and pyruvic acid returned to normal.

There was a definite relation between food intake and bisulfite-binding substances and pyruvic acid. This was clearly indicated with animals receiving adequate thiamine and restricted to different amounts of food. Increasing or decreasing the food intake caused corresponding changes in bisulfite-binding substances and pyruvic acid. However, the changes in bisulfite-binding substances caused by varying the food intake were relatively small compared to the changes produced by the thiamine deficiency, except when
Thiamine Deficiency

the food intake was very high (note the results on the 17th day, Fig. 1). When the deficient animals were given thiamine and access to an abundance of food, their food consumption increased greatly. Thiamine administration resulted in a decrease in the bisulfite-binding substances as normal carbohydrate metabolism was resumed; however, the rise due to increased food intake partly masked the decrease caused by the vitamin. This effect is clearly seen by comparing Figs. 1 and 2. The animal represented by Fig. 1 was allowed food ad libitum after receiving thiamine; that by Fig. 2 had its food intake restricted to the amount eaten before receiving the vitamin; consequently, the effect of the vitamin is much more clearly demonstrated.

These observations prompted the adoption of a procedure wherein the food intake of experimental and control animals was always restricted to the same small amount when determinations were to be made; this eliminated any effect of food intake.

Fig. 1. Bisulfite-binding substances and pyruvic acid in the urine of a thiamine-deficient rat. Bisulfite-binding substances show a progressive rise as the deficiency progresses and a decrease on injection of thiamine (250 μ, subcutaneously at the beginning of the 15th day). This animal was allowed to eat ad libitum after receiving the vitamin. Compare the levels of bisulfite-binding substance and pyruvic acid after thiamine administration with those of Rat 2 in Fig. 2, where the food was limited.
When the food restriction procedure was adopted, it was found that the total bisulfite-binding substances of unadsorbed and adsorbed urine in all cases increased greatly in thiamine deficiency, in some cases reaching levels 10 times higher than the normal controls.

As Figs. 1 and 2 indicate, the bisulfite-binding substances and pyruvic acid both increased greatly in the deficient animals and immediately fell to normal values when thiamine was injected. The pyruvic acid concentration followed closely any changes in the bisulfite-binding level, strongly suggesting that it was the substance being measured as bisulfite-binding. This correlation extended to fluctuations produced by varying the food intake.

In an effort to answer the question of how much of the bisulfite-binding substance was due to pyruvic acid, the bisulfite-binding
substance (usually expressed in this paper as ml. of 0.005 N iodine) of the urine that had been adsorbed was calculated as mg. of pyruvic acid on the basis that 1 ml. of 0.005 N iodine = 0.22 mg. of pyruvic acid. These figures for the bisulfite-binding substance "pyruvic acid" were then compared with the values for pyruvic acid found by the hydrazone method. Table II shows this comparison. In animals receiving adequate thiamine practically all of the bisulfite-binding substance can be accounted for as pyruvate. At the higher concentrations found in deficient animals, the values usually exceeded those of the hydrazone pyruvic acid, but by no more than 10 to 20 per cent. The changes occurring with varying food intake are paralleled by changes in pyruvic acid. It appears, then, that a very great part, if not all, of what is measured as bisulfite-binding substances in urine which has been treated with adsorbing agents is pyruvic acid.

Fig. 2 indicates the differences between the bisulfite-binding levels determined on urine treated with and without adsorbing agents. At low concentrations the differences are quite small; as the bisulfite-binding substances increase, so do the differences.

Fig. 3. Bisulfite-binding substances and pyruvic acid in the urine of a rat receiving adequate thiamine.
As the non-adsorbable pyruvic acid increases, so does some other adsorbable substance or substances. Investigation was made of some substances which might conceivably account for this increase.

**TABLE II**

*Comparison between Levels of Bisulfite-Binding Substances (B.B.S.) (Calculated As Pyruvic Acid) and Pyruvic Acid Determined As 2,4-Dinitrophenylhydrazone*

<table>
<thead>
<tr>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
<th>Rat 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day</strong></td>
<td><strong>B.B.S., pyruvic acid</strong></td>
<td><strong>Hydrolyzed</strong></td>
<td><strong>Pyruvic acid</strong></td>
</tr>
<tr>
<td>Deficient animals</td>
<td>mg.</td>
<td>mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>21</td>
<td>7.8</td>
<td>7.0</td>
<td>89.9</td>
</tr>
<tr>
<td>22</td>
<td>9.1</td>
<td>7.9</td>
<td>86.9</td>
</tr>
<tr>
<td>23</td>
<td>7.6</td>
<td>7.0</td>
<td>92.1</td>
</tr>
<tr>
<td>24</td>
<td>7.6</td>
<td>7.6</td>
<td>100.0</td>
</tr>
<tr>
<td>25</td>
<td>7.0</td>
<td>6.2</td>
<td>88.7</td>
</tr>
<tr>
<td>27*</td>
<td>4.8</td>
<td>4.6</td>
<td>95.9</td>
</tr>
<tr>
<td>Control animals</td>
<td>mg.</td>
<td>mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>Rat 5</td>
<td>1.8</td>
<td>2.2</td>
<td>122.2</td>
</tr>
<tr>
<td>Rat 6</td>
<td>1.4</td>
<td>1.5</td>
<td>107.1</td>
</tr>
<tr>
<td>Rat 7</td>
<td>1.2</td>
<td>1.5</td>
<td>124.9</td>
</tr>
<tr>
<td>Rat 8</td>
<td>3.6</td>
<td>3.5</td>
<td>97.4</td>
</tr>
<tr>
<td>Rat 6</td>
<td>2.9</td>
<td>2.7</td>
<td>98.2</td>
</tr>
<tr>
<td>Rat 7</td>
<td>3.1</td>
<td>3.2</td>
<td>103.2</td>
</tr>
<tr>
<td>Rat 8</td>
<td>41</td>
<td>2.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Rat 7</td>
<td>42</td>
<td>0.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Thiamine administered.

Uric acid binds small amounts of bisulfite, reduces iodine, and is adsorbed on Lloyd's reagent. Quick (18) found that in human pyruvic acid had a very marked stimulatory effect on the excretion of uric acid. Since in thiamine deficiency pyruvic acid concentration in the tissues increases, an increased excretion of uric acid...
or of allantoin might be expected. Investigation disclosed the following. (a) Allantoin does not bind bisulfite nor reduce iodine and cannot be responsible for the increased bisulfite-binding substance of unadsorbed urine. (b) There is no significant difference in the uric acid excretion of thiamine-deficient and control rats. The uric acid was determined by the method of Benedict and Hitchcock (19) and was always less than 0.4 mg. in 24 hours. (c) Creatine, which does not bind bisulfite, was increased in thiamine-deficient animals. However, the increase is not directly attributed to the deficiency, but rather can be accounted for by the degree of starvation induced by the characteristic anorexia.

Experiment 2. Effect of Fat on Bisulfite-Binding Substances of Normal and Thiamine-Deficient Rats

The "sparing action" of fat on thiamine is well known (20-22). Stirn, Arnold, and Elvehjem (22) found that the isocaloric substitution of fat for sucrose allowed polyneuritic rats to grow as well as those given thiamine. It was of interest, therefore, to find the effect of fat on the bisulfite-binding level.

Rats were made thiamine-deficient by being fed Diet 1. Lard was then substituted isocalorically for sucrose in a diet (No. 1-L) fed to the deficient rats as well as to those receiving thiamine. The lard content of Diet 1-L was 36.6 per cent. The carbohydrate content was very low.

The animals were given the same limited amount of food on the day of urine collection and on the preceding day unless otherwise stated. 2 and 2.67 gm. of Diet 1-L have the same caloric value as 3.00 and 4.00 gm., respectively, of Diet 1. The urine was not treated with adsorbents.

The bisulfite-binding substances of five control animals receiving adequate thiamine (first on the high sugar, then on high fat diets) remained within the normal range (below 8 ml. of 0.005 N iodine per 24 hour urine sample). All of the five deficient animals showed very high bisulfite-binding values (25 to 44 ml. of 0.005 N iodine) on the high sucrose, thiamine-deficient diet. All showed some decrease with the feeding of the high fat diet, but the level remained definitely elevated in four of the five until thiamine was

8 Harper and Deuel (9) state, in an objection to the use of the procedure for bisulfite-binding substances, that allantoin binds bisulfite. We do not find this so by our procedure.
given 2 to 3 weeks later. The fifth rat of the deficient group (Rat 26) showed a return to normal values within 2 weeks without thiamine. Sex did not seem to influence the results.

The change from a high carbohydrate to a high fat diet stopped the weight loss in the deficient animals and allowed them to gain in weight and improve in appearance.

The bisulfite-binding values for a normal control animal and for two of the deficient rats are given in Table III. Rat 41 is repre-

**Table III**

*Effect of Fat on Bisulfite-Binding Substances (R.B.S.) of Normal and Thiamine-Deficient Rats*

Bisulfite-binding substances expressed as ml. of 0.005 N iodine per 24 hour urine sample.

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>Amount</th>
<th>B.B.S.</th>
<th>Diet No.</th>
<th>Amount</th>
<th>B.B.S.</th>
<th>Diet No.</th>
<th>Amount</th>
<th>B.B.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-B-3</td>
<td>4.0</td>
<td>4.7</td>
<td>1</td>
<td>4.0</td>
<td>44.0</td>
<td>1</td>
<td>4.0</td>
<td>37.7</td>
</tr>
<tr>
<td>1-L + B1</td>
<td>2.67</td>
<td>3.7</td>
<td>2</td>
<td>1-L</td>
<td>2.67</td>
<td>29.4</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>1-L + B1</td>
<td>2.67</td>
<td>4.5</td>
<td>3</td>
<td>1-L</td>
<td>2.67</td>
<td>30.6</td>
<td>3</td>
<td>1-L</td>
</tr>
<tr>
<td>1-L + B1</td>
<td>2.67</td>
<td>5.2</td>
<td>8</td>
<td>1-L</td>
<td>2.67</td>
<td>32.1</td>
<td>8</td>
<td>1-L</td>
</tr>
<tr>
<td>1-L + B1</td>
<td>2.67</td>
<td>7.9</td>
<td>14</td>
<td>1-L</td>
<td>2.67</td>
<td>30.8</td>
<td>9</td>
<td>1-L</td>
</tr>
<tr>
<td>1-L + B1</td>
<td>2.67</td>
<td>7.8</td>
<td>15</td>
<td>1-L</td>
<td>2.67</td>
<td>25.1</td>
<td>16</td>
<td>1-L</td>
</tr>
<tr>
<td>1-L + B1</td>
<td>2.67</td>
<td>4.7</td>
<td>16</td>
<td>1-L +</td>
<td>2.67</td>
<td>5.0</td>
<td>17</td>
<td>1-L +</td>
</tr>
<tr>
<td>250 γ B1†</td>
<td></td>
<td></td>
<td>250 γ B1†</td>
<td></td>
<td></td>
<td>200 γ B1†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-L +</td>
<td>2.67</td>
<td>4.4</td>
<td>17</td>
<td>1-L +</td>
<td>2.67</td>
<td>4.2</td>
<td>100 γ B1†</td>
<td></td>
</tr>
<tr>
<td>250 γ B1†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The amount of food indicated is the amount allowed on the day in the metabolism cage and the day preceding. On other days the animals were allowed to eat ad libitum.

† Thiamine was injected subcutaneously at the beginning of the 24 hours in the metabolism cage.

sentative of the four deficient animals which showed continued high bisulfite-binding substances on the high fat diet. Rat 26 is the animal showing a return to high normal without thiamine.

**DISCUSSION**

The results presented here, together with those of Banerji and Harris (7), seem to leave no doubt that the level of bisulfite-binding substances and pyruvic acid in the urine of rats is a sensi-
tive indicator of the state of thiamine nutrition. The increased concentration of the specific metabolite accumulating as a result of the specific biochemical lesion may well be taken as a criterion of the deficiency.

The effect of the quantity of food ingested on the amount of bisulfite-binding substances and pyruvic acid excreted possibly can be related to the degree of carbohydrate storage and utilization, although direct evidence for this view is lacking. We have found that with a deficient animal showing a high excretion of bisulfite-binding substances there will be a reduction when food is withheld or continued at the same low level for several days. Increasing the food allowance to a partially fasted normal rat results in an increase in bisulfite-binding substance and pyruvic acid.

The last fact would make it appear that the kidney threshold for pyruvic acid is low. This is borne out by the presence of pyruvic acid in the urine of rats receiving adequate thiamine and only limited amounts of food. Because of the low threshold, any accumulation of pyruvic acid through thiamine deficiency in the body will immediately manifest itself by increased urinary excretion.

On the basis of this view it would be expected that the feeding of any substance that increases the amount of pyruvic acid formed would result in its increased excretion by deficient or normal animals. This is supported by the results of Banerji and Harris (7) who fed lactate to normal and thiamine-deficient rats. There were not only large increases in excretion of bisulfite-binding substances from the deficient animals but also a small increase in the animals receiving optimum amounts of the vitamin. Only a small increase would be expected in normal animals, since they metabolize pyruvic acid very quickly (23).

The relationship between bisulfite-binding substances and pyruvic acid in both blood and urine seems rather clear in thiamine deficiency in rats and pigeons. Most, if not all, of the bisulfite-binding substances can be accounted for as pyruvic acid. However, in human deficiency the relationship is not so clear. Wilkins et al. (24) found a much greater increase in blood bisulfite-binding substance than can be accounted for by the rise in pyruvic acid. Furthermore, Platt and Lu (25) frequently found that blood
pyruvic acid could be restored to normal levels by thiamine administration, while at the same time there was only a slight reduction in the amount of bisulfite-binding substances. Possibly the experimental procedures were at fault, or other deficiencies entered into the picture, or else the human reacts differently from the other species studied.

The effect of fat in improving the physical condition of the deficient rats was as expected from previous work. It would seem reasonable to expect a concomitant quick return to normal of the urinary bisulfite-binding substance when the animals no longer had to metabolize dietary carbohydrate. However, after 2 to 3 weeks on the high fat diet the bisulfite-binding substances were still highly elevated in four of the five deficient animals, although the levels were less than on the high carbohydrate diet. There are certain possible explanations. (a) The small intake of carbohydrate (about 0.12 gm. per rat per day) on Diet 1-L together with that derived from protein may be enough to cause accumulation of bisulfite-binding substance as long as the animal is deficient, even though it gains in weight. (b) When large amounts of fat are being metabolized in the absence of thiamine, intermediary bisulfite-binding products are formed.

The fact that one animal had a return to normal of the bisulfite-binding substances may be an indication that given sufficient time all animals would have behaved similarly.

Banerji (26) has shown that feeding a high fat-thiamine-low diet to rats does not result in increased excretion of bisulfite-binding substances as does a high sucrose-thiamine-low diet. In his experiments, since the animals were transferred from a ration with adequate thiamine to the high fat-low thiamine diet, they had a store of the vitamin in their tissues to begin with. In this important respect, therefore, the experiment differs from that reported here. It would be of great interest to know the thiamine content of rats at the end of an experiment such as that described by Banerji. It may be that the animal retains enough of the vitamin to allow normal excretion of bisulfite-binding substances since very little seems to be required on a high fat diet.

We have confirmed the fact that under the conditions described for determining bisulfite-binding substance acetone is not measured. Acetoacetic acid, however, is reported (2) to bind bisulfite
under similar conditions, although not quantitatively. Despite the long period on the high fat diet, the control animals receiving thiamine showed no elevated bisulfite-binding substance, thus eliminating the possibility that the increase in the deficient animals is caused by a ketosis due to the high fat-low carbohydrate diet alone. The quick response to thiamine indicates that the vitamin deficiency was responsible for the elevated values.

The results reported here together with those of Banerji and Harris (7) invite further investigation on the possibilities for assay procedure based on urinary levels of bisulfite-binding substance or pyruvic acid. In a future paper we will report on the behavior of bisulfite-binding substances in the urine of rats receiving various levels of the vitamin.

SUMMARY

1. Procedures are given for the determination of bisulfite-binding substances in the urine of rats.
2. A rapid increase in bisulfite-binding substances occurs in the urine of rats on a thiamine-low diet, the rise being proportional to the degree of deficiency and occurring before any other symptoms associated with the deficiency. Administration of thiamine brings the level of bisulfite-binding substances to normal within 24 hours.
3. The level of pyruvic acid accounts quite closely for the substance (or substances) measured as bisulfite-binding.
4. The amount of food intake has a marked influence on bisulfite-binding substances and pyruvic acid concentration in urine.
5. The isocaloric substitution of fat for sucrose in the diet fed to thiamine-deficient rats results in improved growth, but only in a partial return to normal in urinary bisulfite-binding substances. In four of five deficient animals the bisulfite-binding substances still remained abnormally high until thiamine was given.

BIBLIOGRAPHY

THE EFFECT OF THIAMINE DEFICIENCY IN RATS ON THE EXCRETION OF PYRUVIC ACID AND BISULFITE-BINDING SUBSTANCES IN THE URINE

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