THE EFFECT OF THIAMINE DEFICIENCY ON THE GLUCOSE OXIDATIVE PATHWAY OF RAT ERYTHROCYTES*

BY MYRON BRIN,† STEPHEN S. SHOHET, AND CHARLES S. DAVIDSON

(From the Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Departments of Biological Chemistry and Medicine, Harvard Medical School, Boston, Massachusetts)

(Received for publication, March 28, 1957)

Thiamine pyrophosphate has been demonstrated recently to be an essential cofactor for the transketolation of pentose to heptulose in the hexose monophosphate shunt in addition to its well known function in pyruvate utilization by animal tissues (1-4). The observation that the glucose oxidative pathway of mammalian erythrocytes may be studied in vitro in the absence of interfering oxidative phenomena by the addition of methylene blue (5) made it possible to determine the effect, if any, on the transketolase reaction by acute thiamine deficiency, in this tissue.

The data to be presented demonstrate that rat erythrocyte transketolase activity is reduced by the removal of thiamine from the diet. This lends further support to the argument that methylene blue activates the glucose oxidative pathway in erythrocytes, and, in addition, furnishes an assay for evaluation of thiamine nutrition of animals.

EXPERIMENTAL

Male rats, 250 in number, ranging in weight from 80 to 120 gm., were maintained in wire-bottomed cages and fed a purified thiamine-free diet ad libitum. Control animals were raised similarly on a complete ration. All the animals were fed and watered daily and weighed three times weekly. At intervals, blood was withdrawn by cardiac puncture in to a heparinized syringe and prepared for experimental use by centrifuging for 15 minutes at 1500 r.p.m., the plasma and buffy coat being removed and the packed cells diluted to the original volume with a phosphate saline buffer, pH 7.4, NaCl 0.115 M, KCl 0.004 M, MgCl₂ 0.005 M, Na₂HPO₄-NaH₂PO₄ 0.020 M. The hematocrit reading was determined for the blood in each experiment.

* This work was supported in part by the Office of the Surgeon General, Department of the Army, in part by a grant from Merck and Company, Inc., Rahway, New Jersey, and in part by a grant from the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, New York, to Harvard University.

† Present address, Food and Drug Research Laboratories, Inc., Maurice Avenue at 58th Street, Maspeth 78, New York, New York.

1 Diets were obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio, as “thiamine-deficient diet” and “vitamin test diet-complete ration.”
Standard Warburg technique was employed. Small flasks, 10 ml. in total volume, were prepared with a fluid volume of 1.2 ml. composed of 0.2 ml. of 15 per cent KOH in the center well. In the main chamber were placed 0.4 ml. of prepared blood (hematocrit reading approximately 0.4), 0.07 ml. of 0.05 per cent methylene blue (to give a final concentration of 0.0035 gm. per cent), 0.05 ml. of glucose solution (to give a final concentration of 100 mg. per cent), and 0.48 ml. of buffer, pH 7.4. These flasks were incubated at 38° under air for 3 hours, at which time they were removed from the bath. Center well contents were transferred and the C\textsuperscript{14}O\textsubscript{2} was precipitated as the barium salt and counted in a Robinson flow counter. Filtrates were prepared by boiling the diluted flask contents, and these were analyzed for lactate and glucose as previously described (5), and for pentose by the iron-orcinol reaction in the following manner. To 3 ml. of reagent solution (2.5 gm. of orcinol plus 0.5 gm. of ferric alum dissolved in a final volume of 250 ml. with concentrated HCl) in a test tube were added 3 ml. of tissue filtrate. The tubes were agitated and placed in boiling water for 45 minutes. They were read while warm in the spectrophotometer at 685 m\u00b5 against a standard of D-ribose.

All blood samples were incubated separately\textsuperscript{2} with glucose-1-C\textsuperscript{14} and glucose-2-C\textsuperscript{14}. The fraction of the total counts added initially which was recovered as C\textsuperscript{14}O\textsubscript{2} from glucose-1-C\textsuperscript{14} and glucose-2-C\textsuperscript{14} is presented as carbon-1 and carbon-2 for each substrate, respectively.

Results

The rats on the thiamine-deficient diet grew normally for 11 days, when they began to lose weight; they continued to do so for the remainder of the experimental period. Typical data are shown in Fig. 1. Growth was resumed at a normal rate upon the administration of thiamine to the deficient individuals. Although these animals were in poor physical condition by the 10th day, they presented no gross nervous signs until the 3rd or 4th week on the thiamine-deficient diet.

The data which demonstrate that there is a significant biochemical abnormality in erythrocytes obtained from thiamine-deficient animals are shown in Table I. Pentose accumulated progressively in abnormal amounts as the deficiency developed. Red cells obtained from animals fed the deficient diet beyond 40 days accumulated pentose in amounts 2 to 3 times the control value.\textsuperscript{3} Oxygen consumption was depressed

\textsuperscript{2} Glucose-1-C\textsuperscript{14} and glucose-2-C\textsuperscript{14} refer to glucose substrates in which carbon-1 and carbon-2, respectively, were labeled with C\textsuperscript{14}. Carbon-1 refers to the aldehyde carbon of the molecule.

\textsuperscript{3} In one experiment filtrates from a deficient and a control flask were treated with acid phosphatase, deionized, and chromatographed, and the pentose was estimated on the eluate of the chromatogram. Analyzed in this manner, the pentose accumula-
progressively as the deficiency developed, and more lactic acid accumulated in the severely deficient cells.

Further evidence of a biochemical aberration in the utilization of specifically labeled glucose-C\textsubscript{14} in the presence of methylene blue, however, resides in the markedly depressed recovery of C\textsubscript{14}O\textsubscript{2} from glucose-2-C\textsubscript{14}. Whereas the recovery of C\textsubscript{14}O\textsubscript{2} from glucose-1-C\textsubscript{14} was somewhat affected by the deficiency, that from glucose-2-C\textsubscript{14} was depressed in a manner directly related to the duration of the deficiency. At the time growth had ceased, the recovery of carbon-2 was but half and in severely deficient animals it was depressed to but one-seventh the normal amount. These effects were evident before the deflection in the growth curve.

In order to evaluate the specificity of the role of thiamine in relation to the metabolic effects observed in thiamine-deficient erythrocytes, both cells and animals were treated with the vitamin \textit{in vitro} and \textit{in vivo}, respectively.

The data appear in Table II, \textit{A} for the situation in which the parameters were measured in deficient cells, and for aliquots of these same cells, to which was added thiamine hydrochloride. Accordingly, the data are:

\begin{figure}
\centering
\includegraphics{figure1}
\caption{The effect of thiamine deficiency on the growth of rats used in this study. The injection of thiamine intraperitoneally resulted in immediate resumption of the growth of deficient animals.}
\end{figure}
TABLE I

Effect of Thiamine Deficiency on Glucose Oxidative Pathway in Rat Erythrocytes in Presence of Methylene Blue

<table>
<thead>
<tr>
<th>No. of days on diet</th>
<th>Hematocrit reading</th>
<th>Oxygen consumption µl.</th>
<th>Pentose accumulation γ</th>
<th>Glucose residue γ</th>
<th>Lactic acid accumulation γ</th>
<th>Carbon-1 recovery fraction</th>
<th>Carbon-2 recovery fraction</th>
<th>No. of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>41</td>
<td>116 ± 5.3*</td>
<td>126 ± 7.2</td>
<td>337 ± 35</td>
<td>270 ± 59</td>
<td>0.52 ± 0.03</td>
<td>0.139 ± 0.008</td>
<td>26</td>
</tr>
<tr>
<td>1-7</td>
<td>30</td>
<td>116 (81-141)</td>
<td>128 (97-149)</td>
<td>419 (303-494)</td>
<td>0.38 (0.267-0.434)</td>
<td>0.008 (0.086-0.111)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>8-10</td>
<td>35</td>
<td>98 (92-114)</td>
<td>136 (130-144)</td>
<td>364 (243-490)</td>
<td>0.453 (0.394-0.565)</td>
<td>0.073 (0.036-0.115)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>11-15</td>
<td>44</td>
<td>106 (89-117)</td>
<td>177 (141-230)</td>
<td>374 (145-640)</td>
<td>0.462 (0.179-0.759)</td>
<td>0.057 (0.003-0.110)</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>16-29</td>
<td>41</td>
<td>98 ± 4</td>
<td>173 ± 5.2</td>
<td>325 ± 32</td>
<td>409 ± 29</td>
<td>0.49 ± 0.02</td>
<td>0.047 ± 0.003</td>
<td>17</td>
</tr>
<tr>
<td>30-44</td>
<td>49</td>
<td>89 ± 4.5</td>
<td>263 ± 35</td>
<td>415 ± 42</td>
<td>362 ± 21</td>
<td>0.46 ± 0.02</td>
<td>0.030 ± 0.003</td>
<td>29</td>
</tr>
<tr>
<td>45-58</td>
<td>47</td>
<td>84 ± 7</td>
<td>353 ± 38</td>
<td>522 (450-603)</td>
<td>0.45 ± 0.04</td>
<td>0.020 ± 0.004</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of the mean; when there are fewer than eight values, the range is given in parentheses.
presented as paired data and significance may be determined both by the comparison of means and by the evaluation of correlation.

For animals thiamine deficient less than 40 days, the addition of thiamine to the erythrocytes resulted in vitro in a significant depression of the accumulation of pentose ($p = 0.05$) and a significant increase in the recovery of $\text{C}^{14}\text{O}_2$ from glucose-2-$\text{C}^{14}$ ($p = 0.001$); both effects were in the direction of normalcy.

The addition of thiamine to more deficient erythrocytes, from animals on the diet longer than 40 days, resulted in increased oxygen consumption, decreased pentose accumulation, and increased recovery of $\text{C}^{14}\text{O}_2$ from glucose-2-$\text{C}^{14}$, thereby demonstrating a reactivation of transketolase activity. The enhanced recovery of $\text{C}^{14}\text{O}_2$ was significant at the $p = 0.05$ level. A decided correlation was shown in the data for oxygen consumption and the accumulation of pentose when thiamine was added, $r = 0.68$, and 0.54, respectively. Cocarboxylase acted in a fashion similar to that of thiamine in vitro.

### Table II

*Effect of Adding Thiamine to Erythrocytes from Thiamine-Deficient Rats on Glucose Oxidative Pathway in Presence of Methylene Blue*

<table>
<thead>
<tr>
<th>Days on diet</th>
<th>Oxygen consumption, µl.</th>
<th>Micrograms pentose accumulation</th>
<th>Carbon-2 recovery, fraction*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No thiamine</td>
<td>Thiamine added</td>
<td>No thiamine</td>
</tr>
<tr>
<td>20–39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>102 ±3.2</td>
<td>101 ±3.2</td>
<td>188.6 ±9.2</td>
</tr>
<tr>
<td>40–48</td>
<td>86 ±4.6</td>
<td>97 ±5.0</td>
<td>347 ±30.6</td>
</tr>
</tbody>
</table>

B. Thiamine given in vivo‡

| Time after treatment, 0–12 hrs.§ | Oxygen consumption, µl. | 98 ±10.2 | 0.000 ±0.01 | 8 |
| Time after treatment, 1–23 daysǁ | Carbon-2 recovery, fraction* | 175 ±15.1 | 0.060 ±0.013 | 29 |

* The fraction of carbon-14 recovered as $\text{C}^{14}\text{O}_2$ from glucose-2-$\text{C}^{14}$ as substrate.
† In vitro: 50 to 100 µg of thiamine hydrochloride were added to the Warburg flask.
‡ In vivo: 1 mg. of thiamine hydrochloride was injected intraperitoneally at least three times per week.
§ These animals were deficient 20 to 22 days before treatment.
ǁ These animals were on the deficient diet an average of 43 days (20 to 65) before treatment.
In Table II, B are presented data for the situation in which thiamine hydrochloride was administered by injection to animals of a known state of thiamine deficiency (as explained in the footnotes in Table II). These data for treated animals, therefore, were compared for statistical purposes with data for equivalent deficiency states (Table I).

In both the cases presented, namely for cells of animals fed the deficient diet an average of 21 days and studied 12 hours after treatment, and for cells of animals fed the deficient diet an average of 43 days and studied after 23 days of treatment, the accumulation of pentose was depressed \((p = 0.05)\) and the recovery of \(\text{C}^{\text{14}}\text{O}_2\) from glucose-2-\(\text{C}^{\text{14}}\) was increased \((p = 0.001)\); both modifications were in the direction of normalcy.

The chemical aberrations due to thiamine deficiency, associated with the glucose oxidative pathway of rat erythrocytes, viz., an elevated accumulation of pentose, and a depressed oxidation to \(\text{C}^{\text{14}}\text{O}_2\) of the 2nd carbon of glucose, were therefore corrected significantly, although not completely, by treatment of the cells \textit{in vitro} and of the animals \textit{in vivo} with thiamine hydrochloride.

**DISCUSSION**

The present study supports and extends the argument presented previously (5) that methylene blue activates the glucose oxidative pathway in non-nucleated erythrocytes. According to this schema, were transketolase inhibited, pentose would accumulate. Accordingly, the carbon-1 of pentose, formerly the carbon-2 of the initial glucose, would not recycle to hexose phosphate and would not be oxidized to \(\text{CO}_2\). Oxygen consumption would vary in direct relation to the decarboxylation of glucose.

The thiamine-deprived red cell system has satisfied all these criteria. As the deficiency became more severe, more pentose accumulated in the cells and less carbon of the original glucose-2-\(\text{C}^{\text{14}}\) was oxidized to \(\text{C}^{\text{14}}\text{O}_2\). These effects demonstrated a marked depression of transketolase activity. Moreover, severely deficient cells demonstrated less oxygen consumption and an increased accumulation of lactate. These observations are directly in accord with previous studies on the methylene blue effect in erythrocytes (5, 6).

The metabolic defects directly related to transketolase activity which were observed in deficient red cells were modified significantly towards the normal situation when thiamine hydrochloride was added to deficient erythrocytes \textit{in vitro} on the one hand, and to intact deficient animals \textit{in vivo} on the other. The results which were observed in the treated cells serve to emphasize the specific role of thiamine for transketolase activity as measured in the intact erythrocyte.

Though it is worthy of note that complete reversal of the biochemical
effects of the deficiency was not attained, especially in the most severely
deficient cells, one should consider that it has been previously observed
in deficiency states of other vitamins that replacement therapy may not
completely correct the biochemical aberrations (2, 7), though the physical
signs may be alleviated, although often slowly. Whether the residual
nerve effects are due to the lesion in the oxidation of α-keto acids or that in
the transketolation of pentose, or to some other as yet undiscovered pri-
mary or secondary function of thiamine pyrophosphate, remains to be
determined. Although it has been reported that hexose monophosphate
oxidation in brain tissue is minimal (8), this does not preclude the need for
the transketolase enzyme in the utilization of carbohydrate metabolites
necessary for the maintenance of cellular activity. Indeed, the essentiality
of the pentose-transketolase system for the maintenance of cellular in-
tegrity is most strongly emphasized by recent experiments on the main-
tenance of viable red blood cells in storage (9).

From a nutrition standpoint it is significant that the metabolic aberrations
of erythrocyte metabolism caused by thiamine deficiency can be evaluated
on as little as 1 ml. of blood by employing the described techniques.
Previously, the status of thiamine nutrition of an individual has been
evaluated by combining gross examination with chemical studies. This
examination included measuring the urinary excretion of the vitamin, the
thiamine and cocarboxylase levels in blood (10), or the levels of blood
pyruvic and lactic acids before and after exercise (11). According to the
techniques presented in this paper, one can, on as little as 2 ml. of blood,
measure the activity of a thiamine-connected enzyme in intact erythrocytes,
and simultaneously measure the effect of thiamine replacement therapy
with the same cells in order to confirm a thiamine deficiency. The appli-
cation of this technique to human nutrition is now under investigation in
this laboratory, and a preliminary report has been rendered (12).

SUMMARY

The glucose oxidative pathway of thiamine-deficient rat erythrocytes
(in the presence of methylene blue) is slowed markedly at the transketolase
step. In severely deficient erythrocytes, pentose accumulates to levels 3
times the normal level and the recovery of C14O2 from glucose-2-C14 is
depressed to one-seventh the normal level. These biochemical aberrations
appear before growth ceases in the growing rat, and are alleviated signif-
cantly by treatment of the cells in vitro with thiamine hydrochloride or
cocarboxylase or by the intraperitoneal injection of the vitamin in vivo.
The use of this technique for an assay of nutritional status has been indi-
cated.

The authors are grateful to Miss Lee DeCarli, Miss Elsie Puro, and Mr. Felix Oser for technical assistance.

BIBLIOGRAPHY
THE EFFECT OF THIAMINE DEFICIENCY ON THE GLUCOSE OXIDATIVE PATHWAY OF RAT ERYTHROCYTES

Myron Brin, Stephen S. Shohet and Charles S. Davidson


Access the most updated version of this article at http://www.jbc.org/content/230/1/319.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/230/1/319.citation.full.html#ref-list-1