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Research Article



# THE EFFECT OF THIAMINE DEFICIENCY ON HUMAN ERYTHROCYTE METABOLISM[1, 2]

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When mature mammalian erythrocytes are incubated in a glucose medium there is a low level of oxidation, but when methylene blue is added to the reaction mixture oxygen consumption and glucose utilization are greatly increased as a result of activation of the pentose phosphate pathway (1, 2). Non-nucleated erythrocytes possess the enzyme potential to recycle 5-carbon fragments (pentose) to glucose-6-phosphate but not to oxidize glucose-6-phosphate to pentose and carbon dioxide. Methylene blue, by virtue of its ability to carry electrons directly to oxygen, accelerates the mechanism for glucose oxidation, permitting the subsequent nonoxidative reactions of the pentose phosphate pathway to follow. Thiamine pyrophosphate is an essential cofactor for a reaction in this pathway (3, 4) and erythrocytes of thiamine-deprived rats, when incubated with methylene blue, have clearly exhibited impairment of the thiamine-dependent reaction (5–7).

In this report, observations have been extended to human thiamine deficiency. Studies from this laboratory have demonstrated that the opthalmoplegia of Wernicke's encephalopathy is not affected by bed rest, alcohol withdrawal and administration of a purified diet containing ascorbic acid and all of the B vitamins other than thiamine, but that when thiamine alone is added to the purified diet, rapid clearing of ophthalmoplegia ensues (8). It was felt, therefore, that patients with Wernicke's encephalopathy exhibiting extraocular muscle paralysis constituted a clearly defined example of human thiamine deficiency, and erythrocytes of nine patients manifesting this disorder were studied and compared with those from well-nourished controls and with those from a group thought to be deficient of $B_1$ but not exhibiting opthalmoplegia.

## THEORETICAL CONSIDERATIONS

Provided the necessary substrate, enzymes and cofactors are present, the carbon compounds of the pentose phosphate pathway continuously recycle with regeneration of glucose-6-phosphate (9). Oxidation of glucose-6-phosphate and subsequent decarboxylation of 6-phospho-gluconate produces carbon dioxide arising from the first carbon and the residue is a pentose, the first carbon of which was the second carbon of the original glucose molecule:

| | | | | | |
|---|---|---|---|---|---|
| (1) | C | | | | |
| (2) | C | | | (2) | C |
| (3) | C | ⟶ (1) | $CO_2$ + | (3) | C |
| (4) | C | | | (4) | C |
| (5) | C | | | (5) | C |
| (6) | C | | | (6) | C |
| Hexose-phosphate | | | | Pentose-phosphate | |

Subsequent reactions are nonoxidative. Ribulose-5-phosphate, the pentose phosphate resulting from the oxidation of 6-phospho-gluconate, can be converted to xylulose-5-phosphate and ribose-5-phosphate by enzymes (epimerase, isomerase) that are widely prevalent in animal tissues (10). Pentose phosphate may then participate in the transketolase reaction where thiamine pyrophosphate acts as an essential cofactor (3, 4), and in

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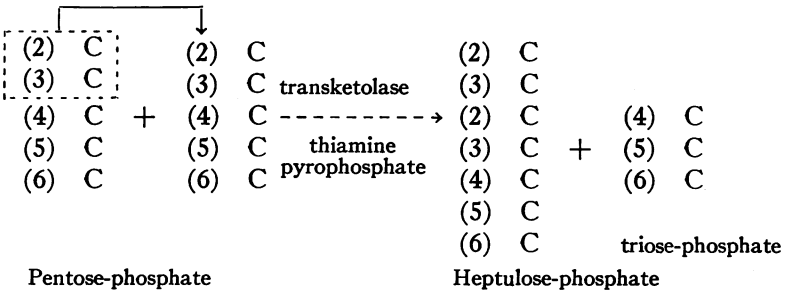
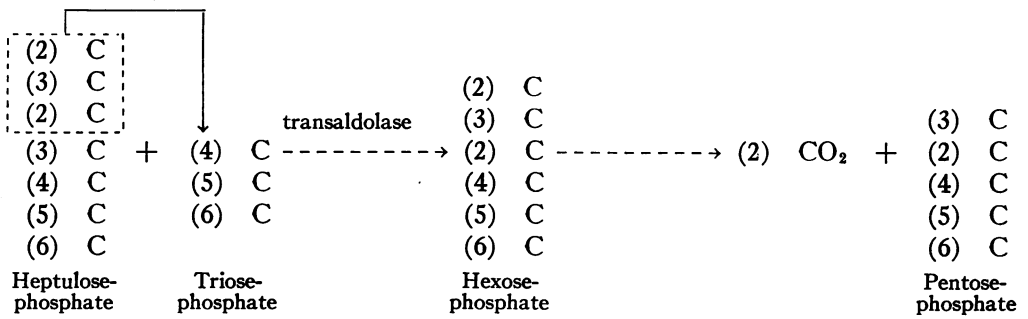
which the active glycolaldehyde portion of xylulose-5-phosphate condenses with ribulose-5-phosphate (11). The product is the seven carbon sugar, sedoheptulose-7-phosphate. The second carbon of the original glucose molecule is now the first carbon of the sedoheptulose:




In the transaldolase reaction (12) the first three carbon atoms of heptulose phosphate condense with the triose phosphate residue regenerating hexose phosphate. The first carbon atom of this new hexose molecule corresponds to the second carbon of the original glucose and $CO_2$ recovered from a second cyclic oxidation is derived from the original second carbon:




In accord with these considerations it has been found that when normal human erythrocytes are incubated with methylene blue and glucose labeled with radioactive carbon ($C^{14}$) in the first position, and, separately, with glucose labeled with $C^{14}$ in the second position, the recoveries of carbon-1 carbon dioxide (C-1-$C^{14}O_2$) and C-2-$C^{14}O_2$ each fall within relatively narrow limits (Table I) (1). The first two carbon atoms of the glucose molecule are those principally involved in the oxidative process conforming to the observation that 95 per cent of radioactive carbon dioxide recovered when erythrocytes and methylene blue were incubated with uniformly labeled glucose could be accounted for by recoveries of radioactive $CO_2$ when glucose labeled only in the first and second positions was used as substrates (1).

Theoretically, thiamine deficiency would interfere with the cycle at the transketolase reaction. Oxidation of hexose phosphate would begin normally and the first carbon of the molecule would be recovered as C-1-$C^{14}O_2$ in the expected amount. Impairment of the transketolase reaction and subsequent lessened regeneration of new hexose phosphate, the first carbon of which would represent the original carbon-2, would result in diminished C-2-$C^{14}O_2$ recovery. As the reaction is altered after the decarboxylation of hexose, pentose would be expected to accumulate. Confirmation of these expected results has been achieved utilizing the erythrocytes of thiamine-deprived rats (5–7).

## MATERIALS AND METHODS

*Patients.* All patients with suspected Wernicke's encephalopathy and/or beriberi brought to the authors' attention after their admission to the Boston City Hospital are included in this study.

*Wernicke's encephalopathy (Table II).* Nine subjects (seven male, two female) were studied. In eight, initial observations were made prior to thiamine administration and one (J.W.) was first studied three

TABLE I

*$C^{14}$ recovery, $QO_2$ and pentose accumulation in erythrocytes of 20 control subjects*

| Patient | Age | Sex | C-1 recovery % counts added | C-2 recovery % counts added | $QO_2$ | Pentose accumulation | Diagnosis |
|---|---|---|---|---|---|---|---|
| | | | | | *µl./3 hrs.* | *µg./flask* | |
| W. B. | 20 | F | 41.6 | 19.3 | 111 | 132 | Normal |
| G. F. | 41 | M | 41.2 | 21.3 | 125 | 120 | Cirrhosis |
| J. B. | 54 | M | 33.9 | 15.7 | 104 | 113 | Cirrhosis |
| R. W. | 19 | F | 37.6 | 18.0 | 123 | 121 | Normal |
| C. D. | 46 | M | 34.1 | 16.6 | 104 | 138 | Normal |
| E. H. | 36 | F | 39.5 | 13.1 | 106 | 158 | Normal |
| D. M. | 26 | F | 31.0 | 14.9 | 103 | 149 | Normal |
| W. B. | 55 | M | 32.6 | 12.7 | 97 | 109 | Cardiac |
| C. B. | 22 | F | 34.6 | 16.5 | 114 | 121 | Enteritis |
| J. C. | 46 | M | 40.1 | 16.6 | 100 | 144 | Penicillin reaction |
| M. D. | 41 | F | 40.7 | 20.2 | 112 | 118 | Cirrhosis |
| J. M. | 60 | M | 35.3 | 17.9 | 110 | 135 | Cirrhosis |
| A. R. | 67 | M | 36.3 | 17.4 | 98 | 135 | Gastritis |
| M. W. | 33 | F | 30.8 | 14.4 | 86 | 129 | Normal |
| J. R. | 54 | M | 34.5 | 13.9 | 102 | 135 | Bronchiogenic carcinoma |
| L. B. | 46 | M | 32.1 | 18.0 | 127 | 154 | Pneumonia |
| S. W. | 33 | M | 34.5 | 15.9 | 102 | 140 | Normal |
| A. B. | 36 | F | 31.5 | 16.6 | 102 | 133 | Normal |
| A. S. | 46 | F | 35.0 | 18.9 | 126 | | Thyrotoxicosis |
| J. S. | 30 | M | 37.8 | 14.1 | 135 | 136 | Normal |
| Mean ± S.D. | | | 35.7 ± 3.5 | 16.6 ± 2.3 | 109 ± 12 | 133 ± 13 | |

hours after thiamine injection. The diagnosis was established by exhibition of weakness or paralysis of both lateral rectus muscles which completely cleared in from two hours to four days after thiamine treatment. Other signs consistent with the Wernicke syndrome (13) included nystagmus in seven (one developing only after return of lateral rectus function), peripheral neuritis in seven, confusion and delirium in eight, and ataxia in all. Four had smooth, red tongues. A diagnosis of portal cirrhosis of the alcoholic type was made on clinical grounds in three patients.

*Suspected thiamine deficiency* (*Table III*). Eight subjects (six male, two female) in this category, all alcoholics, were studied prior to thiamine administration. In five (E.B., J.B., A.K., F.C., C.A.) exhibiting ataxia, peripheral neuritis and confusion, Wernicke's encephalopathy was suspected but eliminated as ophthalmoplegia was absent. One (G.T.) had clinical scurvy, as well as nystagmus and peripheral neuritis. In two subjects with heart failure, edema and peripheral neuritis, beriberi was initially suspected, but was later discarded when other organic heart disease was recognized.

*Control group* (*Table I*). Twenty individuals (11 male, 9 female), 9 of whom were normal laboratory personnel and 11 hospital convalescents, were studied.

*Diet evaluation.* All patients with Wernicke's encephalopathy and suspected thiamine deficiency were chronic alcoholics and had been imbibing heavily before admission. Many had been found in rooms or on the street in states of inebriation and physical collapse. Diet histories were unreliable but it was presumed that all had failed to ingest solid food for at least one week prior to admission. Control subjects had had normal diets for at least several weeks.

*Initial treatment and obtaining of samples.* Intravenous glucose was administered until examination by one of the authors established the diagnosis, at which time blood was obtained for study. Thiamine hydrochloride, 100 mg., was then administered intravenously or intramuscularly and patients were subsequently fed according to clinical indications. Blood samples were obtained periodically after therapy as outlined in Table II, and clinical status ascertained by repeated physical examination. In one case of Wernicke's encephalopathy, thiamine had been given 3 hours before blood was obtained. Samples not immediately studied were refrigerated for a maximum of 8 hours. We have observed no loss of activity in either normal or deficient cells so stored for 12 hours or less.

*Methods.* Heparinized blood was centrifuged at 2,500 rpm for 20 minutes at room temperature. Plasma and buffy coat were removed and packed erythrocytes were diluted to approximately 50 per cent in phosphate saline buffer, pH 7.4 (NaCl, 0.015 M; KCl, 0.004 M; $MgCl_2$, 0.005 M; $Na_2HPO_4$–$NaH_2PO_4$, 0.02 M). Hematocrit was determined for each sample.

Standard Warburg technique was used. Into the outer chamber of 10 ml. Warburg flasks were placed 0.4 ml. prepared erythrocytes, 0.1 ml. 0.05 per cent methylene blue, 0.1 ml. 10 mg./ml. solution of $C^{14}$-labeled glucose,[4] and 0.4 ml. buffer; and into the center well, 0.2 ml. 15 per cent KOH. After equilibration for five minutes the

[4] $C^{14}$-labeled glucose obtained from H. S. Isbell, National Bureau of Standards, Washington, D. C.

TABLE II

*$C^{14}$ recovery, $QO_2$ and pentose accumulation in nine subjects with Wernicke's encephalopathy—Relation to therapy and clinical manifestations*

| Patient | Time | C-1 recovery % counts added | C-2 recovery % counts added | $QO_2$ | Pentose accumulation | Abducens* paralysis | Nystagmus | Peripheral neuritis | Glossitis | Mental disturbance | Portal cirrhosis | Bilirubin | Complications |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *μl./3 hrs.* | *μg./flask* | | | | | | | *mg. %* | |
| E. W. | Pre-Rx | 36.3 | 9.1 | 99 | 191 | ++ | 0 | 0 | + | 0 | 0 | 1.0 | Beriberi |
| 41 | +15 hrs. | 28.0 | 12.9 | 102 | 180 | + | + | | | | | | |
| Male | + 7 days | 29.9 | 10.7 | 107 | 161 | 0 | + | | | | | | |
| S. M. | Pre-Rx | 34.9 | 11.0 | 101 | 179 | +++ | + | + | 0 | + | + | 1.1 | |
| 53 | + 7 hrs. | 29.1 | 12.4 | 106 | 167 | + | + | + | | + | | | Korsakoff psychosis |
| Male | | | | | | | | | | | | | |
| F. E. | Pre-Rx | 31.0 | 9.5 | 74 | 145 | ++ | + | 0 | + | + | 0 | 2.4 | |
| 60 | + 8 hrs. | 27.3 | 11.1 | 83 | 134 | + | + | | | + | 0 | | Persisting confusion |
| Male | | | | | | | | | | | | | |
| T. D. | Pre-Rx | 38.4 | 11.3 | 96 | 147 | ++ | 0 | + | 0 | + | 0 | 1.3 | Delirium tremens; |
| 49 | + 9 hrs. | 34.1 | 14.0 | 105 | 131 | + | 0 | | | + | | | perforated peptic |
| Male | +33 hrs. | 38.7 | 16.8 | 103 | 125 | 0 | 0 | | | + | | | ulcer, operated; |
| | + 6 days | 33.8 | 13.7 | 103 | 123 | 0 | 0 | + | | + | | | Korsakoff psychosis |
| G. F. | Pre-Rx | 39.6 | 13.6 | 101 | 186 | + | + | + | + | + | 0 | 0.9 | |
| 56 | + 6 days | 36.3 | 14.0 | 100 | 216 | 0 | + | + | | + | | | |
| Female | +11 days | 36.5 | 16.8 | 106 | 147 | 0 | + | + | | + | | | Korsakoff psychosis |
| R. S. | Pre-Rx | 41.3 | 6.6 | 88 | 181 | ++ | + | + | 0 | + | 0 | 0.9 | |
| 52 | + 8 hrs. | 31.4 | 12.9 | 108 | 205 | 0 | 0 | + | | + | | | Acute pancreatitis; |
| Female | + 2 days | 33.6 | 13.2 | 106 | | 0 | 0 | + | | + | | | Korsakoff psychosis |
| W. B. | Pre-Rx | 32.7 | 8.8 | 98 | 173 | ++ | 0 | + | 0 | + | + | 6.0 | Hepatic coma |
| 62 | +18 hrs. | 26.8 | 5.8 | 89 | 181 | ++ | 0 | + | | Coma | | | |
| Male | + 4 days | 31.7 | 14.9 | 118 | 137 | + | 0 | + | | + | | | Korsakoff psychosis |
| J. O. | Pre-Rx | 39.1 | 11.7 | 129 | | +++ | + | + | + | + | 0 | 1.7 | Delirium tremens; |
| 53 | +24 hrs. | 28.3 | 12.1 | 119 | | 0 | + | | | + | | | Korsakoff psychosis |
| Male | | | | | | | | | | | | | |
| J. W. | + 3 hrs. | 37.7 | 12.5 | 98 | 144 | + | + | + | 0 | + | + | 4.8 | |
| 68 | +14 hrs. | 38.6 | 17.5 | 118 | 140 | 0 | + | | | + | | | |
| Male | +2½ days | 39.4 | 18.4 | 111 | 150 | 0 | + | + | | + | | | Persisting confusion |

* Symbols indicate: weakness (+), palsy (++), and palsy and internal strabismus (+++).

TABLE III

*$C^{14}$ recovery, $QO_2$ and pentose accumulation in eight patients thought to be thiamine-deficient but not exhibiting ophthalmoplegia*

| Patient | C-1 recovery % counts added | C-2 recovery % counts added | $QO_2$ | Pentose accumulation | Clinical state |
|---|---|---|---|---|---|
| | | | *µl./3 hrs.* | *µg./flask* | |
| E. B. 41 Female | 32.5 | 15.2 | 108 | | Ataxia, nystagmus, peripheral neuritis, cirrhosis |
| J. B. 41 Male | 37.1 | 16.7 | 101 | 162 | Nystagmus, peripheral neuritis |
| G. T. 62 Male | 33.5 | 14.8 | 107 | 143 | Nystagmus, peripheral neuritis, scurvy |
| A. K. 58 Male | 35.9 | 16.3 | 94 | 126 | Confusion, ataxia, nystagmus, barbiturate intoxication |
| F. C. 54 Female | 44.4 | 15.0 | 117 | 178 | Nystagmus, ataxia, peripheral neuritis |
| C. A. 53 Male | 33.7 | 13.6 | 85 | | Seizures, nystagmus, peripheral neuritis |
| W. D. 46 Male | 36.5 | 14.7 | 107 | 144 | Anasarca, CHF*, alcoholic<br>Autopsy: acute bacterial endocarditis |
| A. R. 40 Male | 36.6 | 16.1 | 130 | 122 | Anasarca, CHF, cyanosis, alcoholic<br>Final Dx: chronic cor pulmonale |
| Mean ± S.D. | 36.3 ± 3.7 | 15.3 ± 1.0 | 106 ± 14 | 146 ± 21 | |

* Congestive heart failure.

mixture was incubated at 38° C. for three hours under air. The reaction was terminated by addition of 0.2 ml. 100 per cent trichloracetic acid and, after equilibration, radioactive $CO_2$ absorbed in the center well was precipitated as barium carbonate, plated and counted in a Robinson flow counter. The contents of the outer chamber were transferred and diluted with 10 per cent trichloracetic acid. Filtrates were analyzed for pentose by the orcinol method (14).

Duplicate samples were incubated separately with glucose-1-$C^{14}$ and glucose-2-$C^{14}$, the specific activities of which had been determined by converting to osazones, plating and counting. Specific activity of glucose-1-$C^{14}$ ranged between 4,100 and 17,000 cpm per mg. while that of glucose-2-$C^{14}$ was between 5,500 and 27,000 cpm per mg. The fraction recovered as $C^{14}O_2$ of counts added initially is represented as C-1 and C-2, respectively, and is the arithmetical average of duplicate recoveries.

We have found a linear relationship between three-hour oxygen consumption and final hematocrit of prepared erythrocytes between 45 and 55 per cent, and figures for $QO_2$ were calculated from the arithmetical average of four corrected manometer readings (two incubated with glucose-1-$C^{14}$ and two with glucose-2-$C^{14}$) further corrected to 50 per cent hematocrit.

Erythrocytes obtained before treatment from seven subjects with Wernicke's encephalopathy were incubated with thiamine hydrochloride[5] (0.25 mg.) and cocarboxylase[5] (0.1 mg.) to determine whether the metabolic defect could be corrected *in vitro*. Serum bilirubin concentrations were determined by the method of Ducci and Watson (15).

## RESULTS

When erythrocytes of control subjects were incubated with labeled glucose and methylene blue, carbon-1 recovery ranged between 30.8 per cent and 41.6 per cent and carbon-2 between 12.7 per cent and 21.3 per cent. Minimum $QO_2$ was 86 µl. per three hours and maximum pentose accu-

[5] Purchased from Nutritional Biochemical Corp., Cleveland, Ohio.

TABLE IV

*$C^{14}$ recoveries, $QO_2$ and pentose accumulation of various groups studied**

| | C-1 recovery % counts added | C-2 recovery % counts added | $QO_2$ | Pentose accumulation | Number of experiments |
|---|---|---|---|---|---|
| | | | *µl./3 hrs.* | *µg./flask* | |
| Controls | 35.7 ± 3.5 | 16.6 ± 2.3 | 109 ± 12 | 133 ± 13† | 20 |
| Wernicke's encephalopathy, pretreatment | 36.7 ± 3.6 | 10.2 ± 2.1 (p = <0.01) | 98 ± 15 | 172 ± 18‡ (p = <0.01) | 8 |
| Wernicke's encephalopathy, maximum recovery after treatment | 31.7 ± 4.3 | 13.8 ± 2.1 | 106 ± 11 | 148 ± 21‡ | 8 |
| Wernicke's encephalopathy, pretreatment, thiamine added *in vitro* | 35.5 ± 2.5 | 12.1 ± 1.9 | 107 ± 7 | 173 ± 35§ | 7 |
| Wernicke's encephalopathy, pretreatment, cocarboxylase added *in vitro* | 33.4 ± 3.8 | 11.9 ± 1.9 | 105 ± 8 | 178 ± 38§ | 7 |
| "Suspected" thiamine deficiency | 36.3 ± 3.7 | 15.3 ± 1.0 | 106 ± 14 | 146 ± 21§ | 8 |

* All figures are the mean plus or minus standard deviation.
† Represents 19 experiments.
‡ Represents 7 experiments.
§ Represents 6 experiments.

mulation was 158 µg. per flask (Tables I and IV). There appeared to be no relationship between various individual measurements and age, sex or physical condition of the donor.

Erythrocytes of patients with Wernicke's encephalopathy exhibited evidence of impairment of the transketolase reaction. In seven of eight experiments utilizing red cells obtained prior to therapy, carbon-2 recovery was less than the lowest control value. Mean carbon-2 recovery for this group was 10.2 per cent, significantly less than that of the control (16.6 per cent) (Table IV). In five of seven experiments pentose accumulation was increased and the group mean of 172 µg. per flask was significantly different from the control (133 µg. per flask). The extent of increased pentose accumulation did not correlate with that of depressed carbon-2 recovery. $QO_2$ was slightly diminished and carbon-1 recovery was not influenced. Erythrocytes of J. W., given thiamine two hours before blood was obtained, demonstrated depressed carbon-2 recovery. Observations made from 8 hours to 11 days after therapy revealed a return toward normal metabolic activity in terms of increased carbon-2 recovery, increased $QO_2$, and reduced pentose accumulation in the majority. A decrease in carbon-1 recovery was also noted (Table II and IV). In two instances (G. F., R. S.), however, an increased rather than a diminishing pentose accumulation parallel to increased carbon-2 recovery was observed. Patient W. B., suffering from severe liver disease and hepatic coma, did not have prompt improvement of ophthalmoplegia and his red cells obtained 18 hours after treatment did not demonstrate increased carbon-2 recovery. Four days later, recovery from hepatic coma and clearing eye signs were associated with return toward normal carbon-2 recovery. In all other subjects ophthalmoplegia cleared rapidly.

Other signs attributed to thiamine deficiency were slow in improving or failed to disappear. Of nine patients with Wernicke's encephalopathy, one achieved complete psychiatric recovery, two exhibited persistent confusion and disorientation at time of discharge, and six were transferred to mental hospitals with diagnoses of Korsakoff's psychosis. Nystagmus and peripheral neuritis also tended to persist.

Erythrocytes of eight subjects suspected of thiamine deficiency but not exhibiting opthalmoplegia had no impairment of the transketolase reaction as evidenced by carbon-2 recoveries, $QO_2$ and pentose accumulation in the normal range (Tables III and IV).

In seven experiments where deficient erythrocytes of patients with Wernicke's encephalopathy were incubated *in vitro* with thiamine and cocar-

boxylase, there was only slight increase in carbon-2 recovery and no change in pentose accumulation (Table IV).

## DISCUSSION

An impairment of the transketolase reaction best explains the decreased recycling of carbon-1 of pentose, the carbon-2 of the original glucose molecule, to hexose phosphate and its subsequent recovery as $C^{14}O_2$ in methylene blue activated erythrocytes of patients with Wernicke's encephalopathy. As the first oxidative steps of the pathway were not influenced, recovery of C-1-$C^{14}O_2$ was not appreciably affected whereas that of C-2-$C^{14}O_2$ was significantly reduced. These findings are consistent with the thesis that dietary thiamine deficiency led to insufficient erythrocyte thiamine pyrophosphate as cotransketolase.

Following parenteral administration of thiamine to the deficient patients, a slow return toward normal metabolic activity as evidenced by increasing carbon-2 recovery was observed. Insufficient data are available to determine the time required for complete recovery. Experience with thiamine-deficient rats indicates this to be slow and incomplete (5, 6). Non-nucleated erythrocytes possess a limited capacity to phosphorylate thiamine compared with nucleated cells (16). This suggests that a new generation of erythrocytes must be produced before normal activity can be anticipated. Impaired phosphorlylation of thiamine has also been demonstrated in patients with hepatic cirrhosis (17) and the subject (W.B.) with the most severe liver disease exhibited the poorest response to therapy in terms of both regression of eye signs and improvement in erythrocyte metabolism. Delayed recovery of other coenzymatic functions of thiamine following treatment of patients with Wernicke's syndrome has recently been reported (18).

The failure of *in vitro* incubation of deficient human cells with cocarboxylase and thiamine to result in significantly increased carbon-2 recovery is in contrast to observations with rat erythrocytes in which definite albeit incomplete increased carbon-2 recovery was obtained (5–7). Species differences can be invoked since rat erythrocytes are known to have a greater capacity than human erythrocytes to phosphorylate thiamine to the active pyrophosphate (19). Cocarboxylase added *in vitro* is probably dephosphorylated before entering the erythrocyte (16, 17) and its action would be identical to *in vitro* thiamine. It has also been demonstrated that methylene blue, although stimulating erythrocyte respiration, does not stimulate thiamine pyrophosphate production and indeed may inhibit it (19), and possibly accounts for the failure of complete recovery in thiamine treated rat erythrocytes.

While evidence for reduced transketolase activity in thiamine-deficient erythrocytes is convincing from tracer studies, the supporting pentose data, although suggestive, are less clear cut. Both glucose remaining from the incubating medium and erythrocyte purine nucleosides and nucleotides that enter the trichloracetic acid filtrate give the orcinol reaction and tend to raise the apparent pentose concentratioin. This would tend to be greater in the deficient cells since less glucose is consumed. Moreover, decreases in pentose accumulation did not strictly parallel increased carbon-2 recovery in erythrocytes of treated patients.

Erythrocytes of patients known to have been imbibing heavily, eating poorly and exhibiting physical signs often ascribed to thiamine deficiency, but not suffering from ophthalmoplegia, exhibited no metabolic defect. Moreover, carbon-2 recovery correlated well with resolution of eye signs in the patients with ophthalmoplegia, and the single patient whose signs did not rapidly improve also exhibited persisting erythrocyte abnormality. It is probable that the red cell phenomenon occurs only in the most deficient subjects, *i.e.*, those with ophthalmoplegia, but consideration should be given to the possibility that the rapidly reversible aberration interfering with normal cranial nerve function is related to defective glucose catabolism with red cell and nerve cell being involved simultaneously. Available information indicates, however, that mammalian brain glucose catabolism is primarily by glycolytic pathways (20–22). Since nystagmus, ataxia and peripheral neuropathy have been clearly related to thiamine deficiency (8, 23–25), normal erythrocyte metabolism in patients with these signs but not ophthalmoplegia may be due to differences in severity of deficiency required before one or another coenzymatic function of thiamine is impaired. Furthermore, as slow disappearance of these signs is not unusual, the patients involved may have demonstrated stigmata of an

earlier episode of thiamine deficiency and may have been in a state of relative vitamin adequacy at the time they were studied.

Disturbances of carbohydrate intermediary metabolism recognized in thiamine-deficient states have provided a basis for biochemical evaluation of thiamine deficiency (26). Keto acid concentration in the blood is known to be increased in thiamine deficiency and has been used to indicate the presence of deficiency (27–30). However, these changes have been observed in many diseases apparently unrelated to thiamine deficiency, for example, multiple sclerosis (31), cirrhosis (32), and combined systems disease (33), both in the fasting state and in response to carbohydrate loads. Other investigations of thiamine adequacy have included direct measurement of the blood concentration of the vitamin which has not proved useful (34), and measurement of urinary thiamine excretions which provide only an index of tissue thiamine stores (35).

Application of methylene blue stimulated erythrocyte metabolism to clinical evaluation of thiamine status in man awaits collection and compilation of considerably more data. Defects noted in patients with unequivocal deficiency vary as to degree and are sometimes minimal in individual cases. However, the erythrocyte represents a most available tissue for the study of the thiamine-deficient state and may provide a basis for its clinical measurement.


## SUMMARY

1. Erythrocytes of patients with Wernicke's encephalopathy, when incubated with methylene blue and $C^{14}$-labeled glucose, exhibited failure of the first carbon of pentose (the second carbon of the original glucose) to recycle to hexose and be recovered as radioactive $CO_2$, indicating impaired transketolation of pentose to heptulose.

2. The defect in transketolation was partially reversed after treatment of the patient with thiamine. *In vitro* addition of thiamine or cocarboxylase to deficient erythrocytes did not significantly alter the defect.

3. Erythrocytes of patients with physical signs usually attributed to thiamine deficiency but without ophthalmoplegia exhibited methylene blue stimulated glucose catabolism in no way different from that of well-nourished controls.



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