

Thiamine Therapy in Relation to the Glucose Oxidative Pathway in Human Red Cells

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THE metabolic breakdown of glucose can occur through three different reactions. In one of these, the hexomonophosphate or direct glucose oxidative pathway,¹ glucose-6-phosphate is the hexose source. This requires two cofactors, one of which is triphosphopyridine nucleotide (TPN), to initiate the reactions. Tissue cells, particularly the erythrocyte, have the capability of utilizing glucose in the direct glucose oxidative pathway, dependent upon the availability of TPN. This cofactor can be made available from other biochemical sources or, as demonstrated by Brin and Yonemoto,² methylene blue added to the red cells, in an *in vitro* system, can produce sufficient TPN to activate the shunt.

After the initial activation, hexoses are metabolized to a pentose form and then to other carbon byproducts. Studies by Brin,³ Wolfe^{4,5} and co-workers, using biochemical determinations and radioactive isotope changes in distribution, demonstrated that red cells, from animals which had experimental thiamine deficiencies induced by diet or thiamine analogues and from patients with thiamine deficiencies, could metabolize glucose to the pentose step but further reaction was impaired. The pentose reaction requires transketolase and thiamine pyrophosphate as cofactors,¹ and apparently this step is dependent upon adequate concentration of the thiamine enzyme cofactor for completion. Otherwise there is an accumulation of pentose.^{3,5} In this investigation, alterations in pentose levels were used for rapid study of changes produced in relation to thiamine medication as given to hospital inpatients.

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METHODS AND MATERIALS

Heparinized blood samples were drawn from 202 inpatients on the regular hospital diet. In all subjects the blood sugar, serum protein and hematocrit were within normal limits. After the blood was centrifuged at 2,500 r.p.m. for twenty minutes, the plasma and buffy coats were removed and the packed cells were diluted with an equal volume of phosphate saline buffer pH 7.4 (NaCl 0.115 M, KCl 0.004 M, MgCl₂ 0.005 M, Na₂HPO₄-NaH₂PO₄ 0.020 M). Samples in triplicate were prepared by the technic of Brin^{2,3} and Wolfe^{4,5} using 0.4 ml. of prepared red cells, 0.1 ml. of 0.05 per cent methylene blue, 0.1 ml. of 2 per cent glucose and 0.4 ml. of phosphate saline buffer. These samples were incubated at 38°C. for three hours and the reaction stopped with trichloroacetic acid. The contents were brought to a boil and then filtered. Two ml. of the filtrate was added to 2 ml. of iron orcinol solution (2.5 gm. orcinol, 0.5 gm. ferric alum in 250 ml. volume of conc. HCl). After thirty minutes in a boiling water bath, samples were transferred hot to curvettes and read in a Beckman DU and/or a Coleman Jr. spectrophotometer against D-ribose standards at 685 m μ . Internal standards were prepared by adding known quantities of pentose to the prepared specimens and blanks.

Further studies were made on prepared samples which contained the following as added to five separate specimens in each category: 0.1 to 0.2 ml. TPN 30 gamma/ml., 0.1 to 0.4 ml. thiamine 100 gamma/ml., and 0.1 to 0.4 ml. thiaminase 50 units/ml. obtained from Bracken fern extract. Five additional patients were given 5 gm. D-xylose orally, and pentose determinations on blood and urine were made hourly for five hours.

Effects of thiamine medication on pentose levels in the red cell were studied by following the erythrocyte levels in normal control subjects, patients with infectious hepatitis and four patients with early bronchogenic carcinoma. Thiamine was administered by three different routes: intramuscularly, 50 mg. twice a day; intravenously, 50 mg. and orally



TABLE I
Pentose Levels in Red Cells of Normal Patients

Normal Patients	Pentose Level*
117	129.0 ± 5.4 (Coleman)
85	134.0 ± 5.1 (Beckman)
202	130.0 ± 5.1 Range 118.4-145.2
	Average

* Microgram/sample of prepared blood ± standard deviation consisting of 0.4 ml. prepared red cells, 0.1 ml. methylene blue, 0.1 ml. glucose, and 0.4 ml. buffer.

TABLE II
In Vitro Red Cell Pentose Levels* (controls and after addition of TPN, thiaminase and thiamine)

	TPN	Thiaminase	Thiamine
Control level . . .	130 ± 5.1	130.2 ± 5.2	136.0 ± 5.7
Control + 0.1 ml. †	146.5	140.5	134.0
Control + 0.2 ml.	152.0	176.0	132.0
Control + 0.3 ml.	181.0	132.9
Control + 0.4 ml.	192.0	133.4

* As in Table I.

† Measured increments replacing buffer volume to maintain constant volume of 1 ml.

in separate dosage courses of 5, 25, 50 mg. daily; 50 mg. daily; 50 mg. twice daily; and 50 mg. three times a day.

RESULTS

Pentose levels (Table I) determined on red cell samples obtained from normal patients were found to range from 118.4 to 145.2 mmg. with an average level of 130 mmg. (standard deviation of 5.1 mmg.) which is comparable to the figures of 133 ± 13 mmg. found by Wolfe.⁵ Addition of TPN increased the control level of 130 mmg. to 152 mmg. (Table II). Thiaminase, by inactivating thiamine, raised the control level of 130.2 mmg. to 192 mmg. Direct addition of thiamine demonstrated little change in pentose levels. Patients receiving xylose orally demonstrated maximum absorption at two hours, raising an average control level of 139.0 mmg. to 161.0 mmg. with an associated positive urine pentose test. The curve, as determined by the iron-ornicol reaction, was similar to the curve found by Christiansen⁷ using the method of Roe and Rice⁸ (Table III).

TABLE III
Absorption Studies Determined Hourly on Red Cells and Urine after Intake of Oral D-xylose, 5 gm.*

	Control Level	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
Red cell	139.0 ± 5.4	147.0	161.0	155.4	154.0	150.4
Urine	Negative	-	+	-	-	-

* As in Table I.

The control levels (Table IV) in the normal patients receiving thiamine averaged 138.5 ± 4.5 mmg. (Table IV). After thiamine was given for seven to ten days, the level was depressed to an average of 112.8 mmg., with minimal changes at later intervals. Comparison of the effect of various dosages did not demonstrate correlation with the amount given, and the depression of pentose levels reached the same range by ten days. The use of 5 mg. of thiamine orally did not seem to affect the pentose level. The intramuscular doses produced a depression to a level of 110.2 ± 4.5 mmg. Patients with carcinoma had an initial level of 145.2 mmg. which was depressed to $114.0 \pm$ mmg. while on thiamine therapy.

COMMENTS

Red cells of normal subjects were studied to determine the presence of pentose as formed in the glucose oxidative pathway and the relation of this level to thiamine activity. The source of this pathway is glucose-6-phosphate, and entry from this step into the other reaction of the shunt can be augmented by the addition of TPN as demonstrated by Touster¹ and Siperstein⁹ and the findings here, and by activating the pathway with methylene blue as in the method of Brin and Wolfe.²⁻⁴ In part of the pathway, the pentose reaction (which requires transketolase and thiamine pyrophosphate as cofactors) has been found to be hindered by lack of thiamine in the body. This can be produced by thiamine deficiency,^{3,5} or by the action of thiamine analogues on thiamin,⁴ or by using thiaminase as in this study. All of these result in an elevation of the red cell pentose level.

Thiamine, as administered to patients in this study, in dosages greater than 25 mg. orally

TABLE IV
Pentose Levels after Thiamine Therapy
(Results in microgram/sample of prepared blood \pm standard deviation)

Dosage (mg.)	Onset	4 days	7 days	10 days	14 days
<i>A. Normal Patients—Sixteen Patients Each Dosage</i>					
5 daily orally	136.1 \pm 5.5	130.0	131.5	129.4	
25 daily orally	130.9 \pm 5.3	124.3	120.2	115.5	113.1
50 daily orally	134.5 \pm 5.0	125.0	118.4	114.0	111.3
50 twice a day orally	140.0 \pm 4.5	128.5	112.8	112.9	112.6
50 three times a day orally	133.9 \pm 4.9	122.2	114.1	112.0	111.5
50 twice a day intramuscularly	138.0 \pm 4.3	117.5	111.0	110.2	110.1
50 intravenously*	128.6 \pm 5.8	3 hr.	103.1	24 hr.	119.9
<i>B. Hepatitis Patients—Twelve Patients</i>					
50 twice a day orally	Over periods of 45–60 days averaged 105.4 \pm 4.7				
<i>C. Carcinoma Patients—Four Patients</i>					
50 twice a day orally	145.2 \pm 6.0	131.5	122.1	114.0	114.3

* Five patients in this category.

given daily, lowered the red cell pentose level to an average of 112.8 mmg. Thiamine, *in vitro*, did not alter the level. The depression occurred most rapidly with intravenous therapy, somewhat slower by the intramuscular route, and slowest by the oral route. All subjects eventually attained the same degree of depression regardless of the route or dosage. The difference in effect of thiamine, *in vivo* and *in vitro*, probably represents the metabolic requirement for the active enzyme form, thiamine pyrophosphate. The depression in the pentose level after thiamine therapy reflected the increased intracellular level.

Pentose levels in patients with infectious hepatitis were slightly more depressed than in normal patients after medication. This difference may merely reflect a slightly increased intracellular content or utilization of thiamine. The pattern of depression of the pentose level after thiamine therapy in patients with infectious hepatitis and carcinoma was otherwise similar to that in normal patients.

Measurement of thiamine can be accomplished directly by chemical study or bioassay, or indirectly by measuring some prod-

uct of metabolism which is altered by thiamine. Since the pentose reactions in the glucose oxidative pathway were altered inversely by thiamine, determination of human red cell pentose levels would seem to represent an indirect method of measuring thiamine activity.

SUMMARY

In the metabolism of glucose, the hexomono-phosphate or direct glucose oxidative pathway involves the formation of pentoses which require the presence of thiamine pyrophosphate for further reaction. The pentose level was studied in human red cells. Normal patients had values of 130.0 \pm 5.1 μ g. This level could be increased by *in vitro* addition of TPN, which apparently facilitated the pentose shunt, and thiaminase which inactivated thiamine. Thiamine therapy depressed the pentose level to an average of 112.8 \pm 5.5 μ g. regardless of the route or dosage over 25 mg. Similar levels were found in patients with infectious hepatitis and lung carcinoma. These studies demonstrated that human red cell pentose determinations varied inversely with alterations of thiamine activity.

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