A STUDY OF URINARY LACTIC ACID LEVELS IN HUMANS

I. INFLUENCE OF THIAMINE AND PYRITHIAMINE

OTTO W. WENDEL¹

Department of Chemistry, Bethany College, Bethany, West Virginia, U.S. A.

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Since the early studies by Peters and co-workers (1—4) there has been considerable interest in the role of thiamine in pyruvic and lactic acid metabolism. Elevations in urinary lactic acid have been observed in various pathological conditions such as pneumonia, acute yellow atrophy of the liver, grand mal epilepsy, and the eclampsia of pregnancy. In the human, thiamine deficiency generally leads to anorexia and nausea followed by neurological symptoms and cardiac hypertrophy.

Sherman and Elvehjem (5) have shown that thiamine avitaminotic animals have increased levels of pyruvic and lactic acids in certain tissues. These workers (6) have also shown that an accumulation of lactic acid appears to result from the inhibition of lactic dehydrogenase by high concentrations of pyruvic acid. Oxidation of lactate and pyruvate is essential for the normal activity of brain tissue, and since pyruvate is the ultimate source of the acetyl group of acetyl choline, it follows that any interference with the oxidative decarboxylation of pyruvic acid may be expected to produce neurological dysfunctions as evidenced by progressive ataxia, spastic paraplegia, and hyperesthesia.

Rosenwald (7) while studying the dog, and Fornaroli (8) the rat, found urinary lactic acid increased when the animals were maintained on a thiamine deficient diet. Wildemann and Kremer (9) were the first investigators to study the depressive influence of thiamine on urinary lactic acid in the human. On a diet of noodles, Akiya and Motohashi (10) found that the pH of urine was elevated following ingestion of either citric or acetic acid. Two hours after oral administration of either acid the amount of lactic acid in the urine was markedly decreased; tartaric or lactic acids were less effective in this regard. The authors suggest that citric or acetic acid in some manner affects the progress of the tricarboxylic acid cycle, probably effecting more efficient oxidation of pyruvic acid, with an attendant decrease in lactic acid formation and subsequent elevation of urine pH. In a recent study Murata (11) employing himself as an experimental subject, found that the

¹ Present address: Division of Laboratories and Research, New York State Department of Health, New Scotland Avenue, Albany 1, New York

level of urinary lactic acid could be significantly reduced by administration of thiamine, either orally or intravenously.

Woolley and White (12) showed that mice developed a thiamine deficiency when pyrithiamine was fed. They found that the process was reversible when sufficient thiamine was included in the diet.

The present study was directed toward the further elucidation of the role of thiamine in pyruvate metabolism by determining alterations in urinary lactic acid and serum lactic dehydrogenase levels following the oral administration of thiamine, and its antimetabolite, pyrithiamine.

EXPERIMENTAL

Materials and Methods

1. Experimental Subjects

Twenty young males (ages 21–22) previously maintained for four months on a somewhat standardized institutional diet, were employed throughout the study.

Control Phase — A control study was performed prior to the initiation of each of three experimental phases. This was done in order to determine whether the prior experimental period produced any residual effects. The control procedure consisted of fasting the subjects for 24 hours, followed by the administration of 500 g of starch wafer prepared from washed potato starch. Fractional urine samples were then collected over a 24-hour period. This procedure was repeated twice during each control phase. One week elapsed between all experimental phases.

Thiamine Phase — At the completion of the control study the subjects were separated in random fashion into two groups, fasted for 24 hours, and fed the starch wafer diet. After the ingestion of the wafers, subjects in Group I received 15 mg of thiamine; Group II received a placebo containing inulin. Urine samples were collected as indicated above.

Pyrithiamine Phase — Subjects, randomly separated into two groups were fed the wafer diet. Group I then received 15 mg of thiamine orally; Group II received 40 mg of pyrithiamine.

Combined Thiamine-Pyrithiamine Phase — The subjects were again randomly separated, however this time into five groups and treated as follows:

Group	Pyrithiamine	Thiamine
	<i>mg</i>	<i>mg</i>
I	0	0
II	15	10
III	30	10
IV	60	10
V	120	10

2. Sample Collection

In all four phases urine samples were collected at 2 hour intervals over a 24-hour period. Lactic acid determinations were made on each two hour fraction until the eighth hour sample. The fractions were then combined and a lactic acid determination made on the twenty-four hour composite sample.

Blood samples were obtained during the four phases of the study and four and eight hours following the ingestion of the starch wafers.

3. Chemical Analysis

Urinalysis—All urine samples for pH determination were obtained under mineral oil in order to prevent escape of carbon dioxide. The pH of the urine was detaminated with a Beckman pH meter. Urinary lactic acid was determined by a modification of the method of Friedemann *et al.* (13, 14).

Blood Analysis—Lactic dehydrogenase was determined by the method of Wacker *et al.* (15). The determinations were read in a Bausch and Lomb "Spectronic 20" Colorimeter at 340 m μ .

RESULTS

In a series of four control experiments, the maximum concentration of urinary lactic acid appeared in the fourth hour collection. This observation coincides with the results of Murata (11) and Akiya and Motohashi (10). The 24-hour sample has a concentration markedly lower than that observed in any of the fractions obtained during the first hours.

TABLE I
Pre-fasting 24 Hour Urinary Lactic Acid Levels in Humans Maintained on an Unregulated Diet.

	Total urine volume	Total lactic acid
	ml	mg
Control Series I		
Phase 1	1097	52.3
Phase 2	1046	62.4
Phase 3	966	49.4
Average	1036	54.8
Control Series II		
Phase 1	1178	49.6
Phase 2	1204	52.5
Phase 3	1017	54.7
Average	1133	52.3
Control Series III		
Phase 1	1276	55.6
Phase 2	1114	53.5
Phase 3	1048	48.4
Average	1146	52.5

A control series was run prior to each experimental series. The phases indicate three individual determinations made in each series.

A concentration maximum for lactic acid was obtained at four hours in the control series, therefore it might be expected that if thiamine were to influence lactic acid metabolism, it would be observed at that time. To the contrary, the lactic acid concentration was found diminished in the sixth hour fraction. Murata (11) has suggested that thiamine may influence water metabolism which these results also tend to indicate.

TABLE II
Influence of Starch Wafer Diet on Urinary Lactic Acid Levels Following a 24-Hour Fast

		Lactic acid level, hours following water ingestion					Total urine volume	Total lactic acid
		2	4	8	12	24		
		<i>mg/100 ml</i>					<i>ml</i>	<i>mg</i>
Control	Series I							
	Phase 1	8.34	9.33	5.42	3.83	2.38	986	50.9
	Phase 2	7.76	8.14	5.71	3.86	2.84	1013	51.5
	Phase 3	7.21	7.76	4.88	2.15	2.87	954	58.2
	Average	7.77	8.15	5.34	3.28	2.70	984	53.5
Control	Series II							
	Phase 1	6.31	7.86	5.14	4.33	2.14	1137	49.6
	Phase 2	7.74	8.43	6.37	3.81	2.10	1041	53.7
	Phase 3	6.43	7.97	7.15	3.81	3.14	916	55.2
	Average	6.83	8.04	6.33	3.71	2.46	1031	52.8
Control	Series III							
	Phase 1	6.96	8.41	5.14	4.06	2.16	1048	55.1
	Phase 2	7.33	9.13	6.07	4.14	2.48	1213	58.9
	Phase 3	6.86	7.84	5.18	3.81	2.44	1146	56.9
	Average	7.05	7.05	5.48	4.00	2.36	1135	54.4

A control series was run prior to each experimental series. The phases indicate three individual determinations made in each series.

Pyridithiamine intake resulted in an appreciable increase in urinary lactic acid in the fourth hour samples, apparently having no direct influence on water metabolism as was observed with thiamine. This is believed to be so since the 24-hour composite samples showed a marked change in concentration when compared with the control series.

Pyridithiamine in dosages up to and including 30 mg were found to be ineffective in inducing an increase in the lactic acid concentration of those subjects receiving 10 mg of thiamine. Subjects receiving either 60 or 120 mg of pyridithiamine exhibited a marked rise in lactic acid excretion.

The urinary pH was consistently lower in those subjects not receiving thiamine and those receiving pyridithiamine. Likewise, serum lactic dehydrogenase levels decreased when thiamine was lacking or its activity inhibited.

TABLE III
Influence of Thiamine and Pyrithiamine on Urinary Lactic Acid Concentration

	Lactic acid level, hours following wafer ingestion					Total urine volume	Total lactic acid
	2	4	8	12	24		
<i>mg/100 ml</i>						<i>ml</i>	<i>mg</i>
Thiamine-treatment Group							
Subject 1	2.88	1.61	1.23	2.85	2.94	1358	64.2
2	3.11	1.94	1.40	3.04	3.61	1514	62.8
3	2.68	1.55	1.18	2.67	4.68	1468	55.6
4	4.49	2.16	1.36	3.14	3.58	1341	61.1
5	3.86	1.68	1.06	3.08	3.61	1268	65.1
6	3.68	1.75	1.21	2.81	3.17	1190	59.6
7	5.41	2.24	1.81	4.68	4.91	1554	75.8
8	3.42	1.55	1.13	3.81	4.06	1506	62.6
9	3.68	1.86	1.23	3.61	4.17	1414	50.9
10	2.17	1.83	1.01	2.43	3.18	1253	44.5
Average	3.57	1.82	1.26	3.21	3.80	1387	60.22
Pyrithiamine-treatment Group							
Subject 1	2.95	8.67	6.64	5.68	1.88	1017	117.5
2	3.64	8.41	6.81	5.81	2.71	986	146.3
3	2.43	9.46	7.02	5.41	2.43	1114	184.5
4	1.89	10.21	6.78	4.36	1.93	1048	154.3
5	2.41	12.34	7.14	6.17	1.38	1217	124.6
6	2.66	9.84	6.59	4.14	1.45	1081	129.1
7	3.02	10.78	7.43	5.17	1.69	1016	159.3
8	1.99	8.90	6.58	5.36	2.81	893	165.4
9	2.09	11.81	8.17	6.17	2.54	974	138.6
10	2.34	10.02	8.03	6.48	2.07	1018	140.5
Average	2.54	10.04	7.11	5.47	2.08	1036	146.0

DISCUSSION

Wildemann and Kremer (9) observed that the increase in urinary lactic acid observed following the administration of glucose to humans could be diminished by oral administration of thiamine. However, these workers examined only the influence of thiamine on the 24-hour urine sample. The data presented in this study suggest that the fasted subject utilizes the starch wafer diet two to three hours following ingestion and excretes a concentration maximum of lactic acid during the fourth and sixth hour. The influence of thiamine is detected only through the sixth hour. The lactic acid concentration found in the composite sample is not significantly different from the control sample which may be accounted for by a dilution due to increased mobilization of bound water, or increased water intake. The influence of thiamine was detected in the sixth hour fractional sample which would be expected.

Thiamine functions in the biological system as a precursor to thiamine pyrophosphate which functions as cocarboxylase, and in the formation of lipothiamine pyrophosphate. The carboxylase catalyzed reactions of interest in this study were those associated with the conversion of pyruvic acid to acetyl coenzyme A and oxaloacetic acid, or when thiamine is lacking, to lactic acid.

TABLE IV
Urinary pH Values Under Various Experimental Conditions

Experimental condition	Pre-fasting	Hours following starch wafer ingestion						
		2	4	6	8	24	Composite	
Control Series I								
Phase 1	5.8	6.3	5.7	5.6	5.4	5.8	5.8	
Phase 2	6.3	6.8	5.3	5.8	5.4	5.6	5.5	
Phase 3	6.8	6.6	5.8	5.4	5.1	5.3	5.4	
Control Series II								
Phase 1	6.6	6.8	6.7	6.8	5.7	5.4	6.3	
Phase 2	6.4	6.1	5.8	5.4	5.6	5.2	5.8	
Phase 3	5.9	5.6	5.4	5.4	4.9	5.2	5.3	
Control Series III								
Phase 1	6.1	5.9	5.7	5.4	5.5	5.8	5.6	
Phase 2	6.3	6.0	5.9	5.7	5.7	5.9	5.8	
Phase 3	6.6	5.8	6.1	6.3	6.5	6.5	6.4	
Thiamine Series	6.5	6.9	7.1	7.3	6.8	6.7	6.9	
Pyrithiamine Series	6.3	6.0	5.5	5.1	4.7	5.5	4.9	
Pyrithiamine- Thiamine Series								
Py 0 mg Th 0 mg	6.4	6.3	6.1	5.8	5.9	5.7	5.9	
15 10	6.1	6.5	6.5	6.4	6.3	6.6	6.4	
30 10	6.2	6.6	6.7	6.8	7.0	6.8	6.6	
60 10	6.3	6.4	6.6	6.9	7.0	6.9	6.7	
120 10	6.1	6.0	6.2	5.7	5.1	4.9	5.1	

When pyruvate metabolism is accelerated by thiamine, lactic acid is diminished. An increase in pyruvic acid results in an inhibition of lactic dehydrogenase which is necessary for the conversion of pyruvic acid to lactic acid, and *visé versa*. The experimental data presented support this concept. If the wafer diet were continued together with thiamine supplementation, one would expect a continued depression in the lactic acid concentration.

In an attempt to specifically counteract the activity of thiamine, the antivitamin, pyrithiamine, was employed. In studying mice fed a thiamine-free diet, Woolley and White (12) found none of the characteristic symptoms of a thiamine-deficient diet. When pyrithiamine was simultaneously fed, many of the characteristic symptoms of the deficiency were noted. The effects of the pyrithiamine were found to be reversed by supplementation with thiamine. Woolley and Merrifield (16) have suggested that pyrithiamine may affect other thiamine-dependent systems than carboxylase, since animals dying as a result of administration of pyrithiamine showed normal levels of liver cocarboxylase. The present data suggest that pyrithiamine either inhibits the biosynthesis of cocarboxylase or prevents the combination of cocarboxylase with the apoenzyme to produce the decarboxylating enzyme. Woolley (17) believes the latter to take place.

In the present study, pyrithiamine did not antagonize cocarboxylase activity until 60 mg was administered to subjects receiving 10 mg of thiamine,

TABLE V
Serum Lactic Dehydrogenase (LDH) Levels

Experimental condition	LDH ^a level (<i>units/ml</i>), hours following starch wafer ingestion		
	2	6	24
Control Series I			
Phase 1 (10)	46	78	96
Phase 2 (10)	55	81	93
Phase 3 (10)	47	80	91
Control Series II			
Phase 1 (10)	49	79	96
Phase 2 (10)	61	86	89
Phase 3 (10)	58	83	94
Control Series III			
Phase 1 (10)	60	76	95
Phase 2 (10)	56	88	91
Phase 3 (10)	49	84	89
Thiamine Series (10)	46	38	40
Pyrithiamine Series (10)	47	98	118
Pyrithiamine-Thiamine Series			
Py 0 mg Th 0 mg	49	77	88
15 10	51	74	79
30 10	46	81	89
60 10	44	93	107
120 10	56	103	131

^a Units of LDH activity/ml = $\frac{\Delta \text{O.D.} \times 1000}{\text{minutes}}$

Readings are made at 340 m μ .

whereas 40 mg of pyrithiamine, when given to subjects not receiving thiamine, caused a marked increase in lactic acid excretion. The data suggest that enough residual thiamine pyrophosphate is present after a 24-hour fast to maintain pyruvate metabolism for several hours. Over an extended period one would expect to observe an increase in lactic acid excretion, since no additional cocarboxylase would be synthesized due to inhibition of thiamine pyrophosphate synthesis by pyrithiamine. This view is contrary to that held by Woolley (17).

SUMMARY

1. Urinary lactic acid excretion rates were determined on human males maintained on a starch wafer diet. Lactic acid levels reached a maximum four hours following ingestion of the diet.
2. Thiamine supplementation resulted in a marked diminution in the urinary lactic acid six hours following administration.
3. Pyrithiamine administration resulted in a marked increase in urinary lactic acid excretion four hours following ingestion.
4. Pyrithiamine in a 60 mg dosage was found to negate the influence of 10

mg of thiamine on pyruvate metabolism.

5. Urinary pH changes were consistently decreased with increases in lactic acid excretion. Serum lactic dehydrogenase was decreased in those subjects deficient in thiamine.

6. The data tend to support the concept pyriethiamine blocks the biosynthesis of thiamine pyrophosphate.

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