THE EFFECTS OF THIAMINE DEPRIVATION, AND OXYTHIAMINE- AND PYRITHIAMINE-TREATMENT ON CARDIAC FUNCTION AND METABOLISM IN THE RAT1,2

D. James B. SUTHERLAND,2 August W. JAUSSI, and Clark J. GUBLER

Department of Zoology and Graduate Section of Biochemistry, Brigham Young University, Provo, Utah 84601
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In an attempt to relate the cardiac involvement symptoms of thiamine deficiency with biochemical changes, determinations were made of activities of pyruvate and 2-ketoglutarate dehydrogenases and levels of pyruvate, lactate, creatine phosphate, ATP, ADP and AMP in the heart, and pH, PO2 and PCO2 in the blood, and to correlate these with heart weight, heart rate and electrocardiogram patterns at various stages of thiamine deficiency induced by thiamine deprivation, oxythiamine treatment or pyrithiamine treatment. Pair-fed controls were used in order to rule out effects due to the inanition unavoidably associated with the deficiencies. The bradycardia and cardiac hypertrophy could not be related causally to blood acid-base changes, high levels of blood pyruvate and lactate, or deficiency of tissue energy parameters, creatine phosphate, ATP or ADP. The development of bradycardia was shown to be due to thiamine lack or antagonism in the early stages of deficiency and not to inanition. However, the marked drop in rate in the terminal stages was, to a great degree, due to semistarvation. Bradycardia was shown to persist in deficient hearts during perfusion in vitro. Most of the changes in the electrocardiogram shown earlier were found to be due to inanition. The slowing of the heart rate appears to have no cause and effect relationship to the conduction processes reflected in the electrocardiogram. The appearance and severity of both bradycardia and cardiomegaly parallel the decrease in activities of pyruvate and 2-ketoglutarate dehydrogenases in the heart.

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3 Present address: Department of Pharmacology, School of Medicine, University of Ottawa, Ottawa, Ontario, Canada.
Acute thiamine deficiency in the rat results in death within 4 to 5 weeks after removal of vitamin B₁ from the diet (1). Terminal symptoms include anorexia with associated growth failure or weight loss (2), bradycardia and cardiomegaly (3), electrocardiogram abnormalities (3, 4), adrenal hypertrophy (5, 6), elevation of blood pyruvate and lactate levels (7) and, under proper conditions, neurological disturbances, ataxia and convulsions (8–10).

The biochemical basis for the overt signs of thiamine deprivation has been primarily attributed to reduced tissue levels of thiamine diphosphate, the active coenzyme for pyruvate dehydrogenase (11, 12), 2-ketoglutarate dehydrogenase (13, 14) and transketolase enzymes (15–17), although an additional role independent of its coenzyme function has been suggested within the central nervous system (18, 19).

The present study was designed to determine whether a correlation could be established between the order of appearance and severity of the observed symptoms of cardiac dysfunction and some biochemical lesions. This objective was aided by the use of two thiamine antagonists, oxythiamine (OTh) and pyrithiamine (PTh), which produce different thiamine deficiency symptoms (5). OTh results in marked anorexia and elevation of blood pyruvate, but no neurological disturbances have been observed. PTh, on the other hand, produces no elevation of blood pyruvate but is characterized by severe neurological disturbances (ataxia and convulsions). Since a prominent symptom of thiamine deficiency is anorexia and associated growth failure, appropriate pair-fed controls were used to help establish which symptoms were related to thiamine deprivation and which were due to the associated semi-starvation.

**MATERIALS AND METHODS**

*Care and treatment of animals.* Adult male rats of the Sprague-Dawley strain,*2* weighing between 90 and 120 g were housed on arrival in individual cages in a temperature controlled room (23°C) and fed commercial laboratory pellets*3* for 3 days after which they were divided into the experimental groups and fed either our basal thiamine-deficient diet (1) or a commercial thiamine-deficient diet.*4* When the experimental diet was started the rats were supplemented with daily subcutaneous injections of 0.2 ml of 0.9% saline per 100 g body weight containing the following additions: Normal control (NC)*1* and pair-fed control (PFC) groups received 10 µg of thiamine; the thiamine-deprived (ThD) groups

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*Abbreviations used in this paper are: NC, normal control; PFC, pair fed controls; OTh, oxythiamine; PTh, pyrithiamine; EEG, electrocardiogram; PDH, pyruvate dehydrogenase complex; 2-KGDH, 2-ketoglutarate dehydrogenase complex; CP, creatine phosphate; HMS, hexose monophosphate shunt; bpm, beats per minute.

*1* Obtained from Sprague-Dawley, Inc., Madison, Wisconsin.

*2* Purina Laboratory Chow, Ralston Purina Co., St. Louis, Missouri.

*3* Nutritional Biochemicals Corp., Cleveland, Ohio.
received no supplement; the oxythiamine group (OTh) received 10 μg of thiamine plus 2 mg of oxythiamine; and the pyrithiamine group (PTh) received 10 μg thiamine and 50 μg of pyrithiamine. The food intake of pair-fed control animals was regulated so that the weight gained or lost was the same as for the treated member of the pair.

Electrocardiography. Electrocardiographic tracings were recorded by the method of Beinfeld and Lehr (20). The rats were lightly anesthetized with ether after which the fore and hind limbs were placed through four holes in an insulated board into glass baths containing 10% sodium chloride. The apparatus was enclosed in a grounded Faraday cage. The electrodes from the appropriate baths for recording the standard lead II were connected by shielded cables to a dual-trace differential amplifier, contained in a storage Oscilloscope.*5 Permanent records of the electrocardiograms (ECG) were made on film.*6

Ether anesthetization occasionally produced transient changes in the ECG, especially in deficient animals. When these changes were observed anesthesia was discontinued until a characteristic tracing was restored. ECG's were generally recorded during the pause following the expiratory phase of respiration since the isoelectric line was most stable during this interval.

Pyruvate dehydrogenase and 2-ketoglutrate dehydrogenase assays. Rats were killed by decapitation on the 0, 2, 7, 14, 19, 21, 27 and 28 days of treatment. Immediately after excision, the hearts were rinsed in 0.154 M KCl in 1 mM EDTA, pH 7.4 at 0°C, freed of extraneous tissue, weighed and minced with scissors. A

![Reaction vessel for determination of pyruvate dehydrogenase and 2-ketoglutamate dehydrogenase activities.]

*5 Model 564, Tektronix Inc., Beaverton, Oregon.
*6 Tektronix Oscilloscope Camera, Model C-27, Tektronix Inc., Beaverton, Oregon.
10% homogenate (w/v) was prepared by homogenizing the minced tissue in a glass-Teflon Potter-Elvehjem type tissue grinder for 2 min at 0°C with 9 volumes of 0.154 M KCl in 1 mM EDTA, pH 7.4. The homogenate was immediately assayed for pyruvate (PDH) and 2-ketoglutarate (2-KGDH) dehydrogenase activities by a modification of the methods of REINAUER et al. (21) and GUBLER (1). A suitable aliquot (0.25 ml) of the 10% homogenate was added to the inner reaction vial (Fig. 1) which contained the following in micromoles (total volume 0.5 ml at 37°C): 15 of phosphate buffer, pH 7.4; 10 of MgSO₄; 60 of nicotinamide; 5 of NAD⁺; 3 of ATP; 2 of sodium fumarate; 33 of K₃Fe(CN)₆; and either 100 of sodium pyruvate containing approximately 0.125 μCi (277,500 dpm) of 1-¹⁴C-pyruvate for the PDH assay or 5 of 2-ketoglutarate containing 0.25 μCi (555,000 dpm) of 2-ketoglutarate-U-¹⁴C for 2-KGDH assay. The timed reaction was started by sealing the smaller reaction vial, by means of the rubber stopper, into the larger liquid scintillation vial which contained 0.5 ml of 0.1 M p-(diisobutylcresoxyethoxyethyl) dimethyl benzyl ammonium hydroxide (Hyamine 10X)*7 in methanol, a carbon dioxide absorbent. The sealed scintillation vial was incubated for 15 min at 37°C in a Dubnoff shaking metabolic incubator. The reaction was terminated by adding 0.25 ml of 2 N sulfuric acid to the inner reaction vial via the attached syringe. The sealed vial was then shaken at medium speed for 30 min at 4°C to allow for complete absorption of the released radioactive carbon dioxide by the alkaline solution. The inner vessel was then removed and 10 ml of BRAY’s scintillation fluid (22) was added to the Hyamine 10X solution in the bottom of the scintillation vial. The counts per minute (cpm) of the absorbed ¹⁴CO₂ was determined by counting the sample in a liquid scintillation counter.

The activity of the dehydrogenase complexes, measured in μmoles of carbon dioxide liberated per hour per gram of wet heart weight, was calculated as follows:

\[
\text{Decarboxylase activity (μmoles/hour/gram)} = \frac{\text{Tissue cpm} - \text{Blank cpm}}{\text{cpm/μmole of substrate}} \times C
\]

\[
C = \frac{1}{\text{Time(hr)} \times \text{Weight(g)}}
\]

The conversion factor C equalled 160 since 0.025 g of wet heart tissue was assayed for 15 min.

**Determination of CP, ATP, ADP and AMP.** Pentobarbital anesthetized rats (4.5 mg pentobarbital/100 g body wt., i.p.) were artificially respirated with 100% oxygen through an endotracheal tube while the thoracic cavity was opened to expose the heart. A plastic collar was placed between the heart and the artificially respirated lungs and then the heart was compressed between the jaws of heart tongs cooled to the temperature of liquid nitrogen. The 1–2 mm wafer thin heart was then chipped free of excess frozen blood and tissue. The frozen weight of the heart was then used as the wet weight. At no time was the heart allowed to

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thaw, and between transfers the tissue was stored at the temperature of liquid nitrogen.

The heart was then ground to a fine powder with a porcelain mortar and pestle half-immersed in a liquid nitrogen bath. Frozen 6% perchlorate was added in the amount of 3.25 ml per g of heart tissue. The frozen perchlorate and heart tissue were then ground together to a fine powder, the mixture was transferred to a 12 ml graduated glass centrifuge tube and stirred at high speed on a mixer until thawed after which it was centrifuged at 1,100 g for 15 min at 0°C.

The precipitate was transferred to a pre-weighed plastic weighing boat and dried to a constant weight at 40°C. This value was used for the dry heart weight. The supernatant fraction was neutralized with 5 M K₂CO₃ solution using 0.01 ml of 50% methyl orange as the indicator. The neutralized solution was allowed to stand for 10 min at 0°C to allow the potassium perchlorate precipitate to settle out before the clear supernatant solution was assayed.

Creatine phosphate (CP) and adenosine-5'-triphosphate (ATP) were assayed by the spectrophotometric method of LAMPRECHT and STEIN (23), and adenosine-5'-diphosphate (ADP) and adenosine-5'-monophosphate (AMP) by the spectrophotometric method of Adam (24).

Pyruvate and lactate determinations. Tissue pyruvate and lactate levels were determined on additional aliquots of the deproteinized extracts prepared for the nucleotide assays. Myocardial pyruvate and L-lactate were determined spectrophotometrically by the enzymatic methods of BüCHER, et al. (25) and HOHORST (26), respectively. The change in absorption (ΔA₃₄₀ nm) due to the conversion of NAD⁺ to NADH or of NADP⁺ to NADPH was measured spectrophotometrically. Thermospacers surrounding the cell compartment of the spectrophotometer maintained the reaction mixture at 37°C.

Perfusion of the isolated rat heart. The apparatus for the perfusion of the isolated rat heart was similar to that described by ZACHARIAH (27). The experimental animal was anesthetized with ether and the ECG and heart rate recorded as previously described. The heart was excised and immediately attached via the ascending aorta to the perfusion apparatus. The retrograde flow through the aorta supplied the coronary arteries with oxygenated (5% CO₂ in O₂) perfusate at 37°C at a hydrostatic pressure of 75 ± 2 cm H₂O (55 ± 1.5 mmHg). The heart was first perfused with Krebs-bicarbonate (27) containing half the recommended concentrations of calcium and magnesium (see Table 5). The pH, PO₂ and PCO₂ of the medium were 7.40, 1245 mmHg and 36 mmHg, respectively, as measured on a pH/PCO₂/PO₂ analyzer according to the instructions of the manufacturer. After 5 min the perfusion medium was changed to a Krebs-bicarbonate containing 0.13 mM pyruvate and 4.02 mM lactate. After an additional 5 min the heart was again perfused with control medium followed by a Krebs-bicarbonate solution containing 0.39 mM pyruvate and 8.04 mM lactate. The force and rate of contrac-

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*8 Corning Digital 160, Corning Scientific Instruments, Medfield, Massachusetts.
tion at the apex of the myocardium was measured with a microdisplacement
myograph transducer in line with a recorder\textsuperscript{9} at the end of each 5 min perfusion
period. The transducer was mounted on a stand with a vertical fine adjustment
mechanism and the tension on the heart was periodically adjusted to maintain
2 g of tension during diastole. The heart, with the apex pointing up, was bathed
in perfusate surrounded by a 37°C constant temperature water jacket.

* Determination of the acid-base balance status. The acid-base balance of
thiamine deficient rats (terminal condition; after 4 1/2 weeks) and that of young
control animals of the same body weight were determined as follows: A rat was
anesthetized with sodium pentobarbital (4.5 mg/100 g body weight, \textit{i.p.}). As
soon as the animal was sufficiently anesthetized to allow an abdominal incision to
be made, 2 ml of blood were withdrawn anaerobically from the abdominal aorta
at a point anterior to the renal artery with a heparinized syringe. Determination
of blood pH, PO\textsubscript{2} and PCO\textsubscript{2} were performed as above.

Hematocrit determinations were done on a sample of the same blood used
for acid-base studies. Heparinized capillary tubes containing the arterial blood
were centrifuged 10 min in a micro-capillary centrifuge after which the hematocrit
was determined.

Differences between the means of the normal control groups and the experi-
mental groups were evaluated for statistical significance using "Student’s t-
distribution" \textsuperscript{(28)}. Differences between a pair-fed control group and the experi-
mental group were determined by the "paired observation t-test." The critical
regions for the 1% and 5% levels of significance were determined by using a
two-tailed test. Values were expressed as mean± standard error or as percent of
control values.

\textbf{RESULTS}

Normal control, thiamine-deprived, oxythiamine- and pyrithiamine-treated
groups were prepared several times during the course of the study. At each
repetition the various groups displayed a characteristic growth pattern (Fig. 2).
The normal control group gained weight continuously throughout the course of
the experimental period. The thiamine-deprived rats gained weight at a normal
rate until about the ninth day and then lost weight steadily so that by the 25th
day they had regressed to their starting weight. When thiamine deprivation
was continued to the 35th day, the rats were as much as 30 g below starting weight
and neurological symptoms (impaired righting reflex, drowsiness and ataxia)
were occasionally observed.

The oxythiamine-treated rats showed a small weight gain up to the 7th day
but gradually lost weight thereafter and by the 21st day they appeared emaciated
with arched back and unkempt fur. A characteristic symptom of the terminal

\textsuperscript{9} Physiograph type PMP-4A, E. and M. Instrument Co., Houston, Texas.
Fig. 2. Growth curves for rats on experimental thiamine deficiencies. Normal control (●—●) rats were given 10 μg thiamine in 0.2 ml saline/100 g body weight/day subcutaneously; thiamine-deprived (○—○), 0.2 ml saline/100 g body weight/day; oxythiamine (▲—▲), 10 μg thiamine plus 2 mg oxythiamine in 0.2 ml saline/100 g body wt/day; pyrithiamine (△—△), 10 μg thiamine plus 50 μg pyrithiamine in 0.2 ml saline/100 g body weight/day.

stage was priapism, but no polyneuritic symptoms were observed. The pair-fed control rats to this group were on a reduced but constant diet of 4–8 g per day, and, except for their size, appeared normal.

Table 1. Effect of experimental treatments on heart weight and water content.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Wet heart weight (g)</th>
<th>Dry heart weight (g)</th>
<th>% Heart water</th>
<th>Wet heart weight in mg. % of body wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5</td>
<td>250 ± 16*</td>
<td>0.967 ± 0.026</td>
<td>0.279 ± 0.009</td>
<td>71.2</td>
<td>381 ± 16</td>
</tr>
<tr>
<td>Thiamine-deprived</td>
<td>6</td>
<td>150 ± 18</td>
<td>0.635 ± 0.048</td>
<td>0.183 ± 0.017</td>
<td>71.2</td>
<td>427 ± 24</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td>&lt; .01</td>
<td>&lt; .01</td>
<td>N.S.</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Oxythiamine-treated</td>
<td>7</td>
<td>125 ± 11</td>
<td>0.574 ± 0.068</td>
<td>0.157 ± 0.015</td>
<td>72.6</td>
<td>468 ± 54</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td>&lt; .01</td>
<td>&lt; .01</td>
<td>N.S.</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Pyrithiamine-treated</td>
<td>8</td>
<td>136 ± 7</td>
<td>0.627 ± 0.047</td>
<td>0.184 ± 0.012</td>
<td>70.7</td>
<td>460 ± 26</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td>&lt; .01</td>
<td>&lt; .01</td>
<td>N.S.</td>
<td>&lt; .01</td>
</tr>
</tbody>
</table>

* Mean ± standard error.
Pyrithiamine-treated rats gained weight normally for the first 10 days, then leveled off at about the 14th day before losing weight very rapidly. By the 19th day, ataxia and convulsions were always observed. Pair-fed control rats to the pyrithiamine-treated group were on reduced food intake from the 10–14th day and from the 15th day on received no food until they were sacrificed on the 19th day.

Terminal body and heart weights in Table 1 are compared for the various groups. In line with the much smaller body weights in the experimental groups, the total heart weights are also significantly smaller than in the ad lib controls. However, when calculated as per cent of body weight as in the last column, all three deficient groups showed a significant cardiac hypertrophy. That this is not due to edema is shown by the unchanged water content. The hypertrophy was confirmed in a second experiment using pair-fed controls (Table 2). Most

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. rats</th>
<th>Total heart weight (g)</th>
<th>Ventricular weight (g)</th>
<th>Atrial weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair-fed control</td>
<td>7</td>
<td>0.433±0.054*</td>
<td>0.373±0.050</td>
<td>0.060±0.006</td>
</tr>
<tr>
<td>Thiamine-deprived</td>
<td>7</td>
<td>0.480±0.043</td>
<td>0.409±0.050</td>
<td>0.071±0.009</td>
</tr>
<tr>
<td>*p value</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>10</td>
<td>0.504±0.044</td>
<td>0.426±0.046</td>
<td>0.078±0.004</td>
</tr>
<tr>
<td>Oxythiamine-treated</td>
<td>10</td>
<td>0.559±0.036</td>
<td>0.475±0.041</td>
<td>0.083±0.007</td>
</tr>
<tr>
<td>*p value</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>8</td>
<td>0.553±0.027</td>
<td>0.466±0.023</td>
<td>0.087±0.008</td>
</tr>
<tr>
<td>Pyrithiamine-treated</td>
<td>8</td>
<td>0.569±0.044</td>
<td>0.480±0.036</td>
<td>0.089±0.013</td>
</tr>
<tr>
<td>*p value</td>
<td></td>
<td>&lt;0.05</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* Mean±standard error in grams.

of this hypertrophy in all three deficient groups was accounted for by an increase in ventricular weight. This is somewhat at variance with an earlier study (3) which showed a significant increase in atrial weight in the OTh-treated rats. The reason for this difference is not known unless it lies in the better control in the present study by the use of pair-fed controls. In Fig. 3 a nomogram has been constructed by plotting body weight against heart weight. From this, one can see that the pair-fed (partially starved) rats had lower heart weights than the ad lib controls. This makes the actual cardiac hypertrophy in the deficient groups even more pronounced.

As shown in Figs. 4, 5 and 6, bradycardia of thiamine-deprived and antagonist-treated animals first became apparent by the second week. This decrease in heart rate was significantly (*p < 0.05*) different from both normal and pair-fed control values. The thiamine-deprived group (Fig. 4) showed a 14% reduction in heart rate from the second to the fourth week, and by the end of the fifth week a 36% decrease was observed. Four of the pair-fed control rats to the deficient group
had died by the 35th day. The remaining four were living on 2–3 g of diet per day and had lost 30 g from their starting weight. The average heart rate of these rats was $306 \pm 8$ bpm and was significantly ($p<0.01$) greater than the deficient group of the same day ($232 \pm 23$ bpm), but lower than the normal control group ($360 \pm 11$ bpm).

Oxythiamine-treated rats showed the smallest reduction in heart rate (Fig. 5). The pair-fed control animals to the OTh-group had normal heart rates up to the 14th day, but by the 25th day they had an average heart rate of $360 \pm 18$ bpm which was not significantly different from the oxythiamine-treated group of the same day ($320 \pm 41$ bpm).

Bradycardia became very pronounced in the pyrithiamine rats once the polyneuritic symptoms began to occur (Fig. 6). Pair-fed control rats to the pyrithiamine group were starved between the 14 and 19th day so that they would lose weight as rapidly as the pyrithiamine animals and were near to the point of death due to starvation on the 19th day. The heart rate of these moribund pair-fed controls was $268 \pm 61$ bpm which was not significantly different from the ataxic pyrithiamine rate ($245 \pm 38$ bpm).
The results of electrocardiography of the various experimental groups using standard lead II are reported in Table 3. On the 28th day of thiamine deprivation no significant change in any of the measured parameters was found. By the 35th day most of the thiamine-deprived rats were moribund and a significant change

Table 3. Lead II electrocardiogram intervals in normal and experimental rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>Heart rate bpm</th>
<th>P-wave interval msec</th>
<th>P-R interval msec</th>
<th>QRS interval msec</th>
<th>R-T interval msec</th>
<th>T wave msec</th>
<th>P-T interval msec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0-35</td>
<td>376±18b</td>
<td>16±2</td>
<td>52±6</td>
<td>17±2</td>
<td>28±4</td>
<td>50±8</td>
<td>80±9</td>
</tr>
<tr>
<td>Thiamine-deprived</td>
<td>28</td>
<td>327±27b</td>
<td>14±2</td>
<td>49±8</td>
<td>20±5</td>
<td>36±6</td>
<td>55±9</td>
<td>75±2</td>
</tr>
<tr>
<td>Thiamine-deprived</td>
<td>35</td>
<td>232±23b,c</td>
<td>15±2</td>
<td>64±8</td>
<td>23±3b,c</td>
<td>36±4b</td>
<td>52±8</td>
<td>100±10b</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>35</td>
<td>306±8</td>
<td>15±4</td>
<td>61±5b</td>
<td>18±2</td>
<td>38±2</td>
<td>62±12</td>
<td>99±13b</td>
</tr>
<tr>
<td>Oxythiamine</td>
<td>28</td>
<td>320±41b</td>
<td>14±2</td>
<td>47±6</td>
<td>16±2</td>
<td>34±8</td>
<td>52±8</td>
<td>81±12</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>28</td>
<td>360±18</td>
<td>17±4</td>
<td>46±4</td>
<td>16±1</td>
<td>39±7b</td>
<td>70±8b</td>
<td>85±15</td>
</tr>
<tr>
<td>Pyrithiamine</td>
<td>19-21</td>
<td>245±38b</td>
<td>13±3</td>
<td>53±9</td>
<td>19±2</td>
<td>39±7b</td>
<td>41±12</td>
<td>92±16</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>21</td>
<td>268±61</td>
<td>18±2</td>
<td>53±7</td>
<td>18±2</td>
<td>44±10b</td>
<td>57±10</td>
<td>95±13</td>
</tr>
</tbody>
</table>

a Mean±SE
b p < .05 when compared to normal controls.
c p < .05 when compared to pair-fed controls.
in the P-R, QRS, R-T, and P-T intervals was recorded when compared to normal control animals. However, the pair-fed control rats were also moribund due to semi-starvation and showed similar changes in the P-R, R-T and P-T intervals. No change was observed in the QRS interval as was the case in the thiamine-deprived group. The oxythiamine-treated rats showed no significant (p>0.05) ECG changes during the course of the whole experiment. When pyrithiamine treatment was carried to the point of ataxia, the P-wave was significantly (p<0.05) reduced in duration and the R-T interval lengthened. A comparison of pair-fed control animals indicates that prolonged semi-starvation has a more pronounced effect on the ECG than complete starvation.

The R-wave amplitude in terminal thiamine-deprived, OTh- and PTh-treated groups was 0.68, 0.50, and 0.30 mvols respectively, but only the thiamine-deprived group differed significantly from the normal control value of 0.38 mvols (p<0.05). The thiamine-deprived animals also showed occasional increases in S-wave amplitude which were associated with S-T segment depression or elevation.

Progressive changes in cardiac PDH and 2-KGDH activities expressed as percent of normal control values are also represented in Figs. 4, 5 and 6. The Th-deprived group (Fig. 4) showed a slight decline in PDH activity by the first week but a significant reduction (p<0.05) did not occur until the second week.
By the fourth week, PDH activity in the Th-deprived group was only 19% of the normal control value of $129 \pm 5 \mu$moles $^{14}$CO$_2$ liberated/g tissue/hour. Cardiac 2-KGDH activity in the Th-deprived group did not show a significant drop in activity until the third week. However, by the fourth week enzymatic activity was only 20% of the normal control activity of $99 \pm 5 \mu$moles (Fig. 4).

The oxythiamine-treated rats showed 63% of the normal control values for PDH by the end of the first week and 32% ($41 \pm 3 \mu$moles) by the fourth week. Pair-fed control animals to the oxythiamine-treated group had values comparable to normal control values (Fig. 5). The OTh group, as was the case for PDH activity, was the first to show a significant ($p<0.05$) reduction in 2-KGDH activity by the second week (Fig. 5). During the terminal stages of OTh-treatment, enzymatic activity fell to $50 \pm 1 \mu$moles or 50% of the normal control value of $99 \pm 5$.

The pyrithiamine-treated rats did not show a significant ($p<0.05$) decline in activity until the second week. By the 19th day ataxia and convulsions were observed yet the PDH activity was still 61% of the normal control level (Fig. 6). The pair-fed control animals to the PTh group showed 84% of the normal control values on the 19th day. No statistically significant decreases in 2-KGDH activity in whole heart preparations from PTh-treated rats was noted until ataxia was observed. Under these conditions enzymatic activity fell to $71 \pm 11 \mu$moles.
Fig. 7. Terminal cardiac pyruvate dehydrogenase (PDH) and 2-ketoglutarate dehydrogenase (2-KGDH) activities in 27 day normal control (NC), 27 day thiamine-deprived (ThD), 21 day oxythiamine-treated (OTH) and 19 day pyrithiamine-treated (PTH) rats. The vertical bars denote the means of 5 rats per group while the vertical lines show the standard error.

or 71% of control values (Figs. 6 and 7). The terminal levels of PDH and 2-KGDH in the heart are shown in Fig. 7. Both activities were significantly reduced in all three types of deficiency.

Terminal myocardial levels of creatine phosphate (CP) and the adenosine phosphates (ATP, ADP and AMP) are presented in Table 4. Levels of CP at various periods of deficiency are also plotted in Figs. 4, 5 and 6. Terminal levels of CP were elevated in both OTh- and PTh-treated rats, but this did not appear

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. rats</th>
<th>CP</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>Pyruvate</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5</td>
<td>3.64±0.43*</td>
<td>4.09±0.38</td>
<td>1.01±0.01</td>
<td>0.14±0.05</td>
<td>1.6±0.3</td>
<td>94±31</td>
</tr>
<tr>
<td>Th-deprived</td>
<td>6</td>
<td>3.86±0.62</td>
<td>4.08±0.46</td>
<td>0.87±0.05</td>
<td>0.12±0.02</td>
<td>2.1±0.7</td>
<td>159±51</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTH-treated</td>
<td>6</td>
<td>4.71±0.62</td>
<td>4.22±0.23</td>
<td>0.83±0.08</td>
<td>0.11±0.02</td>
<td>4.3±1.3</td>
<td>113±36</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH-treated</td>
<td>7</td>
<td>5.31±0.61</td>
<td>4.25±0.25</td>
<td>0.91±0.11</td>
<td>0.14±0.03</td>
<td>2.6±1.1</td>
<td>104±45</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Each value represents the mean±SE of a minimum of 5 rats per group expressed in μmoles/gram wet (frozen) heart weight.
Table 5. Effect of pyruvate and lactate on rate and force of contraction of isolated perfused hearts from normal and thiamine-deprived rats.

<table>
<thead>
<tr>
<th>Treatment of in vitro conditions</th>
<th>Group</th>
<th>No. rats</th>
<th>Heart rate (bpm)</th>
<th>Force of contraction (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mean change&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Change from in vivo to in vitro</td>
<td>NC</td>
<td>16</td>
<td>398±4.2</td>
<td>-62±4.3</td>
</tr>
<tr>
<td></td>
<td>ThD</td>
<td>18</td>
<td>358±8.6</td>
<td>-68±9.3</td>
</tr>
<tr>
<td>p value&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
<td>N.S.</td>
</tr>
<tr>
<td>Change from Krebs&lt;sup&gt;d&lt;/sup&gt; to</td>
<td>NC</td>
<td>16</td>
<td>336±17.2</td>
<td>-6±1.0</td>
</tr>
<tr>
<td>Krebs-N&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ThD</td>
<td>18</td>
<td>289±10.0</td>
<td>-5±0.9</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>Change from Krebs to perfusate</td>
<td>NC</td>
<td>16</td>
<td>278±11.5</td>
<td>+9±1.0</td>
</tr>
<tr>
<td>Krebs—D&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ThD</td>
<td>18</td>
<td>269±13.3</td>
<td>-9±0.9</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td>N.S.</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean±SE

<sup>b</sup> p value—paired t-test, each animal served as its own control.

<sup>c</sup> p value—unpaired t-test, the ThD group compared to NC group.

<sup>d</sup> Modified Krebs (19) contained the following in 1 M concentrations: 0.119 NaCl, 0.0047 KCl, 0.0013 CaCl<sub>2</sub>, 0.0012 K<sub>2</sub>HPO<sub>4</sub>, 0.0006 MgSO<sub>4</sub> and 0.025 NaHCO<sub>3</sub> (gased with 100% CO<sub>2</sub> for one hour).

<sup>e</sup> Krebs-N contained additional pyruvate (0.13 mM) and lactate (4.02 mM).

<sup>f</sup> Krebs-D contained 0.39 mM pyruvate and 8.04 mM lactate.
until the terminal stages. No change was observed in the levels of ATP, ADP or AMP in any of the treated groups unless the terminal drop in ADP in the OTh-treated rats can be considered of more than borderline significance.

An increase in cardiac pyruvate concentration was observed in both the thiamine-deprived and antagonist-treated rats (Table 4), but only those in the antagonist-treated groups were significantly increased ($p<0.05$). A statistically significant ($p<0.05$) increase in L-lactate was seen only in the thiamine-deprived group (Table 4).

As is evident from Table 5 perfusion of isolated hearts from normal or thiamine-deprived rats with 0.13 mM pyruvate plus 4.04 mM lactate produced less than a 2% variation in the heart rate. Increasing the concentration of pyruvate and lactate in the perfusate to that found in the blood of severely thiamine deficient rats resulted in less than 3% decrease in the heart rate. On the other hand, perfusion of hearts from both the thiamine-treated and deprived groups with perfusion medium containing either low or high levels of pyruvate and lactate resulted in at least a 25% reduction in the force of contraction. The rate (beats per minute) of the isolated rat heart was considerably lower than the heart rate of intact animals. However the mean decrease from the in vivo level was comparable in both the thiamine control and deficient groups (Table 5). Therefore, the bradycardia of the four week thiamine deficient animal was found to persist in the isolated heart.

A group of ten severely thiamine deficient rats (4.1/2 weeks) and seven normal animals were found to have comparable arterial blood pH values of $7.353\pm0.061$ and $7.388\pm0.062$ (mean $\pm$ SE), respectively ($p>0.05$). Likewise, the respective blood $P_{O_2}$ and $P_{CO_2}$, expressed in mmHg, were found to be similar in the two groups of animals $59.9\pm2.9$ and $35.9\pm3.1$ and $59.7\pm3.5$ and $34.6\pm4.8$, respectively ($p>0.05$). However, the arterial hematocrit of the vitamin B$_1$ deprived group was $45\pm2$ (%), which was significantly ($p<0.01$) higher than the control value of $39\pm1$.

**DISCUSSION**

The present study clearly demonstrates that thiamine deprivation and pyrithiamine treatment result in an increase in the heart weight relative to body weight. This hypertrophy was found not to be due to edema since the water content of the cardiac muscle in the various experimental groups was not significantly different. The observed cardiomegaly in the thiamine-deprived and antagonist-treated rats was associated with a slower heart rate. The development of bradycardia during the early stage of thiamine deficiency was shown to be the result of thiamine lack and not due to gradual starvation. However, the precipitous drop in heart rate seen during the agonal stage of thiamine deprivation was found to be partly the result of semi-starvation, since pair-fed control rats also showed a sharp drop in heart rate at ap-
proximately the same time. Drury et al. (29) came to a similar conclusion from their pair-fed study in relation to thiamine deprived rats. Cheney et al. (3) showed that oxythiamine treatment produced bradycardia a week or more before the onset of acute symptoms, but pyrithiamine treatment did not produce bradycardia until convulsions were observed. Gurtner (30) observed bradycardia after oxythiamine treatment but not after pyrithiamine treatment. However, he prepared his rats slightly differently from Cheney et al. and the present study and therefore his results are not directly comparable.

Some evidence for the slowing of the heart rate in thiamine-related cardiomyopathies was found in the rat electrocardiogram. However, ECG irregularities were principally noted only during the terminal stages of thiamine deficiency. Many of the changes were due to the moribund condition of the animal since pair-fed rats also showed many of these abnormalities. Nevertheless, thiamine deficiency in the rat was found to cause a lengthening of the QRS complex and depression of the S-T segment, as well as an increase in the amplitude of the R-wave, (Table 3). However, these changes were not observed until long after bradycardia was apparent which would indicate no direct cause and effect relationship. Additionally, the absence of any definite alteration in the conduction velocity of the rat myocardium as interpreted from the ECG at the time of the onset of bradycardia, suggests that the slowing of the heart rate occurs earlier in the contractile process, probably at the pace-maker level (sino-atrial node). Supportive evidence for the conclusion that no consistent change in the ECG is responsible for the observed bradycardia comes from some earlier studies. Drury et al. (29) found no change in the ECG of thiamine-deprived or pair-fed rats until a moribund condition resulted. Hundley et al. (31) found prolonged P-R interval in 7 out of 20 acutely thiamine-deficient rats. Yoshitoshi et al. (32) reported a 17.6% occurrence of arrhythmia in severely deficient rats.

The present study clearly demonstrates that myocardial PDH and 2-KGDH activities are markedly decreased during thiamine deprivation. Similarly, the two thiamine antagonists, oxythiamine and pyrithiamine were shown to produce a significant decrease in the activities of these two thiamine diphosphate-dependent enzymes. Decreased PDH activity results in an increase in cardiac pyruvate and lactate levels. However, the accumulation of these end products of glycolysis within heart tissue was not as great as expected. Possibly, during thiamine deficiency, pyruvate enters the tricarboxylic acid cycle via carbon dioxide fixation rather than by decarboxylation. The finding of Benevenga et al. (33) that thiamine deficient calves incorporated more pyruvate into amino acids than normal calves supports this concept. Hence, the possible contribution of increased pyruvate carboxylase activity in the metabolism of thiamine deficient cardiac tissue should be examined.

Although cardiac PDH activity in thiamine deficient animals has been
extensively studied and has been shown to be significantly decreased \((1, 21, 34)\), the dependence of cardiac metabolism on this enzyme to satisfy its energy requirements might not be as extensive as previously thought. It has been shown that when fatty acids and glucose are perfused together through an isolated rat heart, the fatty acids are preferentially oxidized \((35)\). Insight into how this response might occur was initially provided by the observation of \textit{Lin et al.} \((36)\) that the activity of PDH multienzyme-complex is controlled by a phosphorylation-dephosphorylation reaction of one of its components, pyruvate decarboxylase. Subsequent work carried out by \textit{Wieland} and his colleagues \((37, 38)\) on isolated rat hearts perfused with glucose, demonstrated that as much as \(70\%\) of the total measurable PDH activity was in the active (dephospho) form. However, the addition of fatty acids, ketone bodies or acetone to the perfusate reduced the metabolically active form to \(30\%\). Similarly, overnight fasting decreased the active form of cardiac PDH to less than \(15\%\). Because of these considerations, the \(18\%\) total measurable PDH activity in ThD cardiac tissue, as reported in the present study, might possibly be sufficient for adequate cardiac function. Nevertheless, whether all of this remaining PDH activity is in the active form remains to be clarified. The 2-KGDH enzyme complex is analogous in the mechanism of action and cofactor requirements to that of PDH \((39)\). However, the mechanism of regulation of enzymatic activity is not yet fully understood, although cyclic AMP mediation has been suggested \((40)\). Since 2-KGDH is an important enzyme in the Krebs cycle, decreased 2-ketoglutarate metabolism would be expected to impair cardiac energy synthesis from carbohydrate, fatty acid and amino acid sources. It was therefore decided to relate the enzymatic activity of this enzyme to the energy content of the myocardium in which thiamine diphosphate requirement of 2-KGDH had been impaired. It was found that by the fourth week of thiamine-deprivation cardiac 2-KGDH was only \(20\%\) of the control value. However, the myocardial CP, ATP, ADP and AMP content of thiamine deficient rats was not significantly different from that of normal rats. These results indicate that neither reduced PDH nor 2-KGDH activity have an appreciable effect on cardiac energy synthesis. The possibility exists that, like pyruvate, 2-ketoglutarate might be metabolized by other thiamine independent enzymes. In the brain a shunt around the 2-KGDH step by way of gamma-aminobutyrate has been demonstrated \((41)\). However, the contribution of this pathway in the thiamine deficient heart remains to be investigated. \textit{McCandless et al.} \((34)\), in determining the energy status of the thiamine deficient heart, found the ATP content down \(16.8\%\) and \(34.1\%\) during the 4th and 5th weeks, respectively. Unfortunately, no values for cardiac CP were reported since they felt their values were too variable for valid conclusions. Additional support for the suggestion that decreased PDH and 2-KGDH activity does not necessarily adversely affect cardiac energy levels comes from the use
of thiamine antagonists. The present study showed that oxythiamine- and pyrithiamine-treatment both resulted in significant increases in cardiac CP stores even though PDH and 2-KGDH activities were significantly depressed. INOUE et al. (42) also found the CP content in thiamine deficient brains to be unchanged, while the ATP content increased. HOLOWACH et al. (43) reported that pyrithiamine treatment of the mouse brain resulted in an increase of CP and ATP concentrations. In the liver, pyrithiamine treatment doubled the ATP content even though PDH and 2-KGDH activities were 25% and 50% of the normal values. SCHENKER et al. (44) found the renal cortex ATP level normal, while medullary content was decreased during severe thiamine deficiency. These results indicate that reduced PDH and 2-KGDH activities do not reduce the amount of high energy phosphates stored in the rat heart. The bradycardia of thiamine deficiency was not demonstrated to be the result of reduced energy for contraction since, in the case of oxythiamine- or pyrithiamine-treated hearts, an actual increase in the available energy was observed. It is doubtful that reduced energy for contraction is the etiology of bradycardia in the rat since the thiamine deficient heart in human beriberi (8) and the anesthetized dog (45) are able to beat at a higher rate for long periods of time indicating that even during severe thiamine deficiency cardiac metabolism is able to satisfy the energy requirements of a rapidly beating heart.

The transketolase activity of the hexose monophosphate (HMS) shunt is also dependent on the coenzyme thiamine diphosphate. However, OPIE (46), in reviewing the role of the HMS shunt in cardiac metabolism, indicated that the shunt was either quiescent or only moderately active in the myocardium. In addition, MCCANDLESS et al. (34) observed that the giving of thiamine to thiamine-deprived rats reversed the cardiac symptoms within 24 hr but the transketolase activity remained at 40% of control values for up to 6 days after recovery. In light of these considerations, the role of transketolase in the development of cardiac dysfunction in thiamine deficiency was not investigated in the present study.

Bradycardia, as observed in the thiamine deficient rat, has also been attributed to the accumulation of pyruvate and lactate in the blood and within the myocardium (47-49). In the present study, perfusion of rat hearts from both normal and 4-week thiamine deficient animals with neutralized perfusion medium containing pyruvate and lactate levels similar to those reported for severely thiamine deficient rats resulted in only a slight alteration in heart rate that was not of the same magnitude as seen in the deficient animal. However, the force of contraction was significantly decreased in both groups.

The bradycardia of the thiamine deficient animal was found to persist in vitro and in the absence of active nervous innervation, which suggests that bradycardia of thiamine deficiency is myocardial in origin. However,
the possibility that neural transmitter substances were not sufficiently cleared from the myoneural junction during the control perfusion of 5 min is not excluded. An attempt to perfuse hearts of 5-week deficient animals that were in ataxia proved unsuccessful. The shock of excision and attachment to the perfusion apparatus either resulted in fibrillation, tachycardia or severe bradycardia. These findings suggest that elevated blood pyruvate and lactate levels are not the direct cause of bradycardia in thiamine deficiency.

The pH, PCO₂, and PO₂ determinations on the arterial blood of severely deficient rats were made to see if the increase in blood pyruvate and lactate had altered the acid-base balance, but no significant change from control values were noted. These findings therefore justified the use of neutralized perfusate solutions. The arterial hematocrit of severely thiamine deficient rats when compared to that of control animals of the same body size but of a younger age was found to be significantly increased. Future studies relating the appearance of bradycardia and cardiomegaly with hematocrit changes are needed to assess more meaningfully the possible role of this response and the etiology of cardiac dysfunction in thiamine deficiency.

In summary, then, the appearance and degree of severity of bradycardia of thiamine deficiency was found to be correlated with the changes in enzymatic activities of cardiac PDH and 2-KGDH. However, no direct relationship between the heart rate and the energy content of the myocardium could be demonstrated. Additionally, no relationship between the tissue levels of pyruvate and lactate in the heart and the rate of the heart beat could be established. Perfusion of either normal or thiamine deficient hearts with either high or low levels of pyruvate and lactate was found to have no relationship to the in vitro heart rate. The heart changes observed in these studies include confirmation of cardiomegaly and ECG irregularities. The use of two thiamine antagonists helped confirm the relationship between the degree of cardiac dysfunction and the coenzyme activity of thiamine.

REFERENCES