Effect of Thiamine Deficiency on the Swelling, Electron Transport, and Oxidative Phosphorylation of Rat Heart Sarcosomes*

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The decrease in the rate of oxygen uptake by isolated heart sarcosomes from rats fed thiamine-deficient diets proceeds significantly faster with pyruvate than with α-ketoglutarate in the early stages of deficiency. There is very little if any decrease in the oxygen uptake with malate even in the terminal stage. Unlike the rates of oxygen uptake, the P:O ratios (with pyruvate and α-ketoglutarate) of thiamine-deficient sarcosomes are not different from the controls during the entire course of progressive deficiency. The inhibition of hypotonicity-initiated swelling of heart sarcosomes by pyruvate plus fumarate combination, after an appreciable temporary increase at 1–2 weeks of thiamine deficiency, decreases successively following feeding of severely thiamine-deficient diets for over 3 weeks. At 1–2 weeks there is also a considerable temporary decrease of the rate of $NADH_2$ oxidation in $NADH_2$–cytochrome *c* reductase assay systems. Both the increase of inhibition of swelling and the decrease of the $NADH_2$ oxidation rate correlate with a temporary increase of respiratory control ratios. The enhancement of the $NADH_2$–cytochrome *c* reductase activity produced by 5 μmoles α-glycerophosphate and by 10 μmoles β-hydroxybutyrate is identical and constant up to 1 week of thiamine deficiency, then it increases considerably up to about 3–5 weeks.

Thiamine deficiency produces well-delineated biochemical lesions of carbohydrate metabolism leading to increase of pyruvate and lactate in the blood as shown by Peters (1936). Later work by Lohmann and Schuster (1937) and by Peters (1948, 1953) has demonstrated that this increase is due to the fact that thiamine enters into the composition of cocarboxylase and, thus, its absence constitutes a block to the channeling of pyruvic acid into the citric acid cycle. However, the depletion of thiamine leads also to a block within the citric acid cycle since cocarboxylase is required for α-ketoglutaric acid decarboxylation as well. Impairment of carbohydrate metabolism at other points results because of the requirement of transaldolase and transketolase for cocarboxylase (Horecker and Smyrniotis, 1953; Racker *et al.*, 1953). Other expectable consequences are a decrease of ATP synthesis derived from protein catabolism *via* the glutamate → α-ketoglutarate pathway, and an incomplete utilization of acetyl-CoA originating from fatty acid catabolism. These biochemical alterations are especially interesting in heart tissue since a morphological consequence of thiamine deficiency is cardiac hypertrophy (Yoshitoshi *et al.*, 1961).

This paper reports investigations on heart sarcosomes from rats during progressive thiamine deficiency. The following parameters were studied: The comparative decrease of the rate of oxygen uptake, and the respiratory control and the P:O ratios (using pyruvate, α-ketoglutarate, glutamate, or malate as substrate); inhibition of sarcosomal swelling by pyruvate plus fumarate combination; sarcosomal $NADH_2$[1]–cytochrome *c* reductase and diaphorase activities; and the effect of α-glycerophosphate and β-hydroxybutyrate on the latter activities.

Experimental Procedures

Feeding and Care of Animals.—Male Sprague-Dawley rats, housed two in a cage and weighing 380–450 g at the beginning of the experiment, were fed a modification (Arcos *et al.*, 1961) of the 18% casein plus dextrose base semisynthetic diet of Miller *et al.* (1957), which contained here no thiamine (called hereafter "thiamine-devoid diet") or 0.7 mg thiamine/kg (called hereafter "thiamine-low diet"). The controls received, for at least 2 weeks before sacrifice, the same diet containing 3 mg thiamine/kg, which is a level sufficient to allow normal growth of young rats and to maintain adult rats without deficiency symptoms. Food and water were *ad libitum*.

Swelling and Oxidative Phosphorylation.—Sarcosomes were isolated, the swelling tests were carried out, and oxygen uptake was measured as described previously (Arcos and Argus, 1964). However, in the present studies on the inhibition of swelling by pyruvate plus fumarate (both at 1×10^{-2} M) the cells were tilted at frequent regular intervals for oxygenation of the medium. Furthermore, in the manometric oxygen uptake experiments shown in Table I the amount of fluoride was now reduced to 40 μmoles. When pyruvate (20 μmoles) was used as substrate in the latter experiments, fumarate (2 μmoles) was also present. For the determination of the extent of inorganic phosphate incorporation, at the termination of the oxygen-uptake measurement the flask was quickly removed from the bath and placed in ice, and the reaction was stopped immediately with 2 ml ice-cold 20% trichloroacetic acid. The precipitated proteins were removed by centrifugation, and the orthophosphate content of the protein-free supernatant was determined by the method of Lowry and Lopez (1946). Initial phosphate level was determined in control flasks in which the protein was precipitated immediately following equilibration. The respiratory control ratios were determined in separate experiments with ADP as acceptor (using sarcosomes from 110 mg tissue/flask) following the procedure of Holton *et al.* (1957).

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[1] The following abbreviations were used: Tris, trishydroxymethylaminomethane; NAD (previously known as DPN), nicotinamide-adenine dinucleotide; and $NADH_2$ (previously known as DPNH), reduced nicotinamide-adenine dinucleotide.

TABLE I

COMPARATIVE OXIDATIVE RATES AND P:O RATIOS OF RAT HEART SARCOSOMES WITH PYRUVATE AND α-KETOGLUTARATE DURING THIAMINE DEFICIENCY[a]

	Weeks of Feeding	Pyruvate[b] (μl O_2/mg/hr)	Probability[c]	P:O	α-Ketoglutarate (μl O_2/mg/hr)	Probability[c]	P:O
Control	0	86.0		2.80	63.5		2.81
Thiamine Devoid	1	81.4	$0.05 > p > 0.02$	2.74	64.6	$0.70 > p > 0.60$	2.78
	2	63.7	$p < 0.001$	2.69	55.8	$0.05 > p > 0.02$	2.88
	3	42.6	$p < 0.001$	2.76	43.3	$p \cong 0.001$	2.87
	4				31.4	$p < 0.001$	2.67
Thiamine Low	1	71.7	$0.01 > p > 0.001$	2.74	62.6	$0.70 > p > 0.60$	2.79
	2	76.9	$0.01 > p > 0.001$	2.75	60.9	$0.30 > p > 0.20$	2.87
	3	74.0	$p \cong 0.001$	2.61	57.9	$0.05 > p > 0.02$	2.78
	4	57.3	$p < 0.001$	2.66	49.2	$p < 0.01$	2.64
	5	53.7	$p < 0.001$	2.60	46.3	$p \cong 0.001$	2.83
	6	48.2	$p < 0.001$	2.74	43.2	$p < 0.001$	2.83
	7	36.2		2.80	38.7		2.78
	8	33.0		2.76	39.7		2.77

[a] Oxygen uptake was measured manometrically, at 30°, for 20 minutes, using the following reaction medium: 50 μmoles phosphate buffer (*p*H 7.4), 10 μmoles $MgCl_2$, 6 μmoles ATP, 40 μmoles NaF, 0.03 μmole cytochrome *c*, 20 μmoles substrate, 2 mg hexokinase, 10 mg glucose, sarcosomes corresponding to that present in 200 mg fresh normal tissue,and 0.25 M sucrose to complete to a final volume of 3 ml. Oxygen uptake is expressed as μl of O_2 per mg trichloroacetic acid–precipitated, acetone-extracted, dry particulate weight per hour. Orthophosphate was determined by the colorimetric method of Lowry and Lopez (1946). All values in the table are averages of three experiments. [b] 2 μmoles fumarate were also present. [c] Based on the null hypothesis for the true difference between the respiratory rates of the experimental and the control groups.

$NADH_2$ Oxidation.—The activity of $NADH_2$–cytochrome *c* reductase was measured (at 340 mμ) following an adaptation of the procedure of Zins *et al.* (1959). The determinations were carried out in 3-ml volumes of 0.05 M potassium phosphate buffer, or of 0.44 M sucrose plus 0.05 M potassium phosphate buffer, both at *p*H 7.4. The final reaction mixtures contained in a total volume of 3.10 ml: 0.04 μmole of cytochrome *c*, 127 μg of $NADH_2$, and an amount of sarcosomes corresponding always to that present in 15 mg of fresh normal heart tissue. Five μmoles of α-glycerophosphate or 10 μmoles of β-hydroxybutyrate were added when the effect of these compounds was studied. Diaphorase activity was measured (at 600 mμ) in 3-ml volumes of 0.02 M Tris buffer, or of 0.44 M sucrose plus 0.02 M Tris buffer (both at *p*H 7.4), following an adaptation of the procedure of Edelhoch *et al.* (1952) and Mahler *et al.* (1952). The final reaction mixtures contained in a total volume of 3.2 ml: 0.12 μmole of the dye, 500 μg of $NADH_2$, and an amount of sarcosomes corresponding always to that present in 30 mg of normal heart tissue. In both the $NADH_2$–cytochrome *c* reductase and the diaphorase assay, absorbancy changes were recorded at 30-second intervals, at 23°, in a Beckman DU spectrophotometer fitted with thermospacers. It was ascertained that the activity of the sarcosomes is always constant and directly proportional to their concentration. For this reason the volume of sarcosomal stock suspension added in these determinations was adjusted so that the concentration of sarcosomes in the test systems was constant at the various stages of thiamine deficiency. The levels of both types of $NADH_2$ oxidase activities were calculated from the slopes of the linear segments of the rate curves, by means of $NADH_2$ and 2,6-dichlorophenolindophenol reference curves.

RESULTS

Increase of the Heart Weight–Body Weight Ratios.—During the course of feeding both thiamine-deficient diets the rate of body-weight loss was appreciably greater than the rate of heart-weight loss, resulting in an increase in the ratio: (heart weight/body weight) × 10^3. The following average values were obtained for this ratio: control rats, 3.23; rats on thiamine-low diet for 8 weeks, 3.91 ($p < 0.001$); rats on thiamine-devoid diet for 4 weeks, 3.97 ($p < 0.001$). The hearts of the rats were noticeably flabby after about 6 weeks on the thiamine-low diet and after about 2 weeks on the thiamine-devoid diet. The rats could not be maintained on the thiamine-low diet for more than 10 weeks or on the thiamine-devoid diet for more than 5 weeks.[2] These results are in agreement with the observations of Yoshitoshi *et al.* (1961).

Comparative Oxidative Rates; the P:O *and Respiratory Control Ratios.*—Comparison of the oxidative rates with pyruvate and α-ketoglutarate, as shown in Table I, indicates faster depletion of cocarboxylase at the pyruvate than at the α-ketoglutarate level in the early stage of thiamine deficiency. The decrease of these rates is due to the depletion of cocarboxylase and does not appear to be related to other possible effects of thiamine deficiency in the sarcosomes. In fact, results not shown in the table indicate that with malate (20 μmoles) as substrate the respiratory rate was altered only to a very small extent, even at the terminal stage of deficiency, following feeding of the thiamine-devoid diet for 4 weeks. The average oxygen uptake was 49.8 μl/mg/hour in the normals and 47.3 μl/mg/hour in the experimentals ($p \cong 0.10$).

In contrast to the respiratory rates, the P:O ratios were not affected during the entire course of thiamine deficiency when using either pyruvate or α-ketoglutarate as substrate (Table I). Also with malate as substrate the P:O ratios were unaffected when the rats were maintained on the thiamine-devoid diet for 4 weeks (average P:O = 2.81).

[2] Thiamine depletion was accompanied by a gradual decrease of the rectally measured body temperature (using a "Tele-therm" thermocouple probe, Yellow Springs Instrument Co., Yellow Springs, Ohio). In the rats receiving the thiamine-devoid diet there was a successively increasing difference between the A.M. and P.M. temperatures, the latter temperatures being always the higher. No such differences were apparent in the controls or in the rats fed the thiamine-low diet. The average rectal temperature of control rats was 37.7°, measured either A.M. or P.M. The average rectal temperatures of deficient rats were: with the thiamine-devoid diet at 5 weeks, A.M., 33.1°, P.M., 35.0°; with the thiamine-low diet at 9 weeks, both A.M. and P.M., 36.8°.

TABLE II
RESPIRATORY CONTROL RATIOS OF RAT HEART SARCOSOMES DURING FEEDING THIAMINE-DEVOID DIET[a]

Weeks of Feeding	Glutamate	Pyruvate[b]	α-Ketoglutarate
0 (control)	2.65	1.78	1.40
1	2.86	1.82	1.39
2	2.22	1.41	1.17
3	2.45	1.23	1.23

[a] For the determination of the respiratory control ratios the oxygen uptake was measured manometrically up to 30 minutes (at 30°) in the presence and absence of ADP as acceptor. The composition of the reaction mixture in a total volume of 3 ml was that of Holton *et al.* (1957); the additional substrates investigated here were also at the level of 20 μmoles. The values in the table are averages of four experiments for the control and of three experiments for the thiamine-deficient rats. [b] In the presence of 2 μmoles of fumarate as "sparker."

The respiratory control ratios followed at weekly intervals up to 3 weeks of feeding the thiamine-devoid diet (using glutamate, pyruvate, and α-ketoglutarate as substrate) are given in Table II. There is at 1 week a small but well-detectable increase with glutamate, very little if any with pyruvate, and none with α-ketoglutarate. Beyond 1 week the respiratory control ratios decrease below the control with all three substrates. Unfortunately these experiments had to be terminated before other systems for the determination of the respiratory control ratios could be investigated.

Change in Inhibition of Sarcosomal Swelling by Substrates.—The simultaneous presence of pyruvate and fumarate causes a statistically significant ($0.02 < p < 0.05$) inhibition of hypotonicity-initiated swelling of heart sarcosomes, while there is no statistically significant inhibition with pyruvate alone ($0.60 < p < 0.70$) or with fumarate alone ($0.30 < p < 0.40$). The use of the pyruvate-plus-fumarate substrate combination corresponds to maximal loading of the oxidative system (Aisenberg and Potter, 1955). Inhibition of sarcosomal swelling by the two substrates indicates the resynthesis of ATP from the inorganic phosphate and ADP present in small equimolar amounts (resulting from the hydrolysis of ATP carried in the particles through isolation), since ATP inhibits and reverses the swelling of liver mitochondria (Raaflaub, 1953; Lehninger, 1959), and of heart sarcosomes (Nakamura *et al.*, 1961; Arcos and Argus, 1964).

Figure 1 shows the effect of thiamine deficiency on the inhibition, produced by the pyruvate-plus-fumarate combination, of hypotonicity-initiated swelling of heart sarcosomes. The curves represent the variations, during the course of thiamine depletion, of the ratios: per cent decrease of absorbancy in the simultaneous presence of pyruvate-plus-fumarate over the per cent decrease in the absence of the two substrates, both at the same time interval of feeding. Similar swelling ratios have been used previously with liver mitochondria to express the change in the utilizability of glutamate as citric cycle substrate during feeding a hepatocarcinogenic azo dye (Arcos *et al.*, 1961).

Figure 1 indicates that, following a compensatory response at about 1–2 weeks of thiamine deficiency, there is a successive statistically significant increase of the ratios indicating decreased inhibition of swelling (thus decreased ability of the sarcosomes to utilize pyruvate for ATP synthesis). This increase of the ratios is faster for the thiamine-devoid than for the thiamine-low diet, which is consistent with the more rapid thiamine depletion when feeding the former diet. The decrease of substrate-produced inhibition is

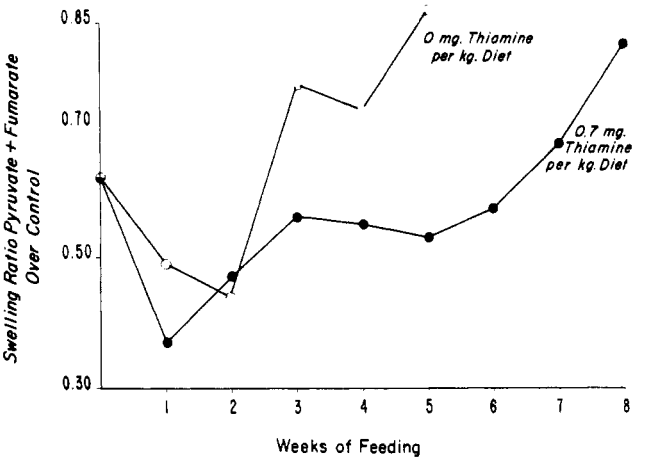

FIG. 1.—Effect of progressive thiamine deficiency on the inhibition by pyruvate-plus-fumarate of hypotonicity-initiated swelling of rat heart sarcosomes in 0.04 M sucrose buffered with 0.03 M Tris, at *p*H 7.4. Both substrates were at the concentration of 1×10^{-2} M. The swelling ratios were calculated by dividing the per cent decrease of absorbancy at 40 minutes in presence of the substrate combination with the per cent decrease of absorbancy at 40 minutes in the absence of the substrates (control), both at the same time interval of feeding. The probabilities for true differences between the ratio at 0 week and the ratios at the maximum and minimum points of the curves are: between 0 and 2 weeks, $0.02 < p < 0.05$; between 0 and 5 weeks, $0.05 < p < 0.10$ (0 mg thiamine/kg diet). All points represent averages obtained with five rats.

caused by thiamine deficiency and may not be attributed to leakage of NAD from the particles. Decreased inhibition is, in fact, not influenced by the addition of 0.07 μmole of this nucleotide to the test systems, which is a level below the minimum concentration required for swelling-inducer action of NAD (Arcos and Argus, 1964).

Change in the Rate of Electron Transport.—Figure 2 shows the $NADH_2$–cytochrome *c* reductase and diaphorase activities in a 0.44 M sucrose medium (buffered with phosphate) during the time course of thiamine depletion. The figure shows that, with both the thiamine-devoid and the thiamine-low diets, there is a temporary 40% decrease in the $NADH_2$–cytochrome *c* reductase activity at 1 week. The normal activity level is then regained and maintained, with both diets, between 3 and 5 weeks. Animals maintained on the thiamine-low diet beyond 5 weeks showed a gradual decrease in the $NADH_2$–cytochrome *c* reductase activity until death.

Addition of either 5 μmoles of α-glycerophosphate (Fig. 2) or of 10 μmoles of β-hydroxybutyrate produces comparable increases in the rate of $NADH_2$ oxidation in all stages of thiamine deficiency. The increase produced by the two compounds corresponds to 26% with sarcosomes from normal rats, and to 20–25% at 1 week of deficiency. From then on, however, there is an increasing degree of enhancement produced by both compounds, corresponding to 58% with the thiamine-devoid diet at 3 weeks and to 53% with the thiamine-low diet at 5 weeks. There is subsequently a gradual decrease of the enhancement produced by the two compounds in animals maintained on the thiamine-low diet.

When the assay is carried out in strongly hypotonic 0.05 M phosphate buffer without sucrose (Fig. 3), however, the extent of temporary decrease at 1 week of deficiency is only 20% from a much higher normal level. In this test system activity greatly exceeds, between 3 and 5 weeks, the normal level. The results

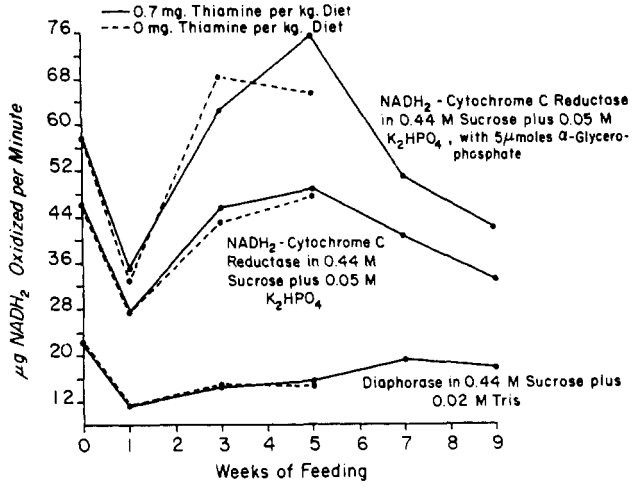

FIG. 2.—Change in $NADH_2$–cytochrome *c* reductase and diaphorase activities of rat heart sarcosomes in 0.44 M sucrose during the time course of feeding thiamine-deficient diets. For assaying the former (at 340 mμ), the system contained 127 μg $NADH_2$, 0.04 μmole cytochrome *c*, an amount of sarcosomes present in 15 mg normal fresh tissue, and, when stated, 5 μmoles α-glycerophosphate. For the latter (assayed at 600 mμ) it contained 500 μg $NADH_2$, 0.12 μmole 2,6-dichlorophenolindophenol, and an amount of sarcosomes present in 30 mg of normal fresh tissue. Both systems were at *p*H 7.4. Activities were calculated from the rate curves by means of reference curves of $NADH_2$ and of the dye. The probability for the true difference is, at 0 week, between the activities with and without α-glycerophosphate, $p \leqq 0.05$. For the difference between 0 week and the minimum at 1 week, in the 0.44 M sucrose–0.05 M K_2HPO_4 system, $p < 0.01$ for both the 0 mg and the 0.7 mg thiamine/kg diets. For the *increase* of the enhancement of the $NADH_2$ oxidation rate by α-glycerophosphate: the probability for the difference between the percentage increases due to α-glycerophosphate at 1 week and at 3 weeks (0 mg thiamine/kg diet) is $0.02 < p < 0.05$, and for the difference between 1 week and 5 weeks (0.7 mg thiamine/kg diet) $0.001 < p < 0.01$. All points represent averages obtained with five to six rats.

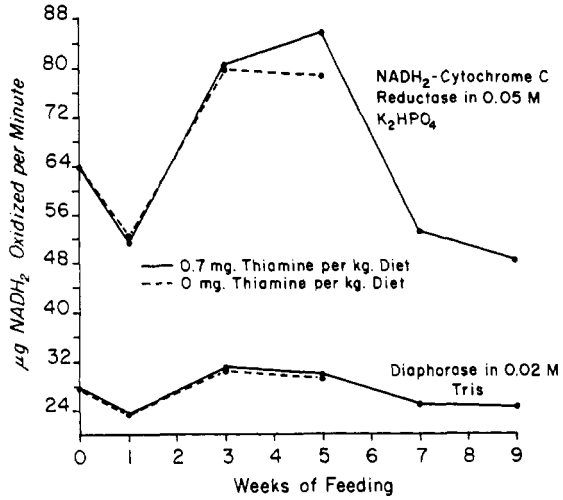

FIG. 3.—Change in the $NADH_2$–cytochrome *c* reductase and diaphorase activities of rat heart sarcosomes in hypotonic media during the time course of feeding thiamine-deficient diets. Except for the absence of sucrose the experimental conditions were identical to those described in the legend to Fig. 2. All points represent averages obtained with five to six rats.

in Figure 2 are in agreement with those of Boxer and Devlin (1961) who found that "intact" heart sarcosomes, unlike liver mitochondria, oxidize exogenous $NADH_2$ at a high rate as measured polarographically; this finding was now confirmed in manometric experiments. The ability of the sarcosomes to oxidize $NADH_2$ at a high rate is not an artifact of the isolation procedure. This is indicated by the fact that hypotonic treatment or conditions considerably enhance in sarcosomes both the rate of polarographically measured oxygen uptake (Boxer and Devlin, 1961) and the rate of spectrophotometrically measured $NADH_2$ oxidation by $NADH_2$–cytochrome *c* reductase (compare Figs. 2 and 3).

Paralleling the observations with $NADH_2$–cytochrome *c* reductase, the percentage decrease of diaphorase activity at 1 week thiamine deficiency is considerably greater in the medium with sucrose (Fig. 2) than in Tris alone (Fig. 3).

DISCUSSION

Change in the Inhibition of Swelling and $NADH_2$ Oxidation Rate at 1–2 Weeks.—The temporary decrease of the swelling ratios (Fig. 1) and of the oxidative rates of $NADH_2$ (Figs. 2 and 3) represents a peculiar biochemical response. These phenomena appear to result from a temporary increase of the respiratory control[3] for the following reasons: (a) Relative mitochondrial size reflects the level of high-energy intermediates and phosphates present (e.g., Ernster, 1958; Lehninger, 1962) and ATP-induced contraction always involves ATP hydrolysis (Lehninger, 1962), because the maintaining or shrinking of the particle volume against the osmotic gradient is supported by the free energy thus released. Therefore the increased inhibition of sarcosomal swelling (decreased swelling ratios) at 1–2 weeks by the pyruvate-plus-fumarate combination should be interpreted as the result of a more efficient utilization of the substrates in the deficient than in the control sarcosomes for maintaining sarcosomal volume and energy level. (b) There is no change in the efficiency of ATP synthesis *per se* since the P:O ratios are not different from the controls throughout the entire course of progressive thiamine deficiency (Table I). (c) Since phosphate acceptor is not present in the $NADH_2$–cytochrome *c* reductase assay system, "tightening" of coupling should result in the slowing of electron transport, which was the actual finding (Fig. 2). (d) When the $NADH_2$–cytochrome *c* reductase assay was carried out in hypotonic conditions (in the absence of sucrose), the percentage decrease at 1 week was reduced to less than half although the 0-week level was much higher (Fig. 3). This is consistent with the well-known phenomenon that hypotonicity "loosens" respiratory control.

Direct determination of the respiratory control ratios did not permit a clear-cut substantiation of this increase, although with glutamate the increase at 1 week, albeit small, was clearly demonstrable. Beyond 1 week there is a decrease of the ratios which can be expected following the results in Figures 1–3. It must be remembered that, depending on the experimental conditions, the respiratory control ratios of mitochondrial particles may vary substantially (e.g., Holton *et al.*, 1957). Thus, it is possible that for a clear-cut demonstration of the "tightening" of respiratory

[3] Consistent with the increased efficiency of substrate utilization in the initial stage of thiamine deficiency is the observation in this laboratory that there is no decrease in myocardial contractile force (measured under 8 g tension) in rats maintained on thiamine-devoid diet over 1 week. Decrease begins at the end of the second week and, although initially small, rapidly reaches 50–75% by 4–5 weeks (Earl E. Aldinger, personal communication).

control in the early stage of thiamine deficiency specific conditions may be required and, furthermore, that the determination of the ratios may have to be carried out at shorter time intervals to detect optimum response.

An alternative possibility which could account for the increased inhibition of swelling (Fig. 1) is an increase of the permeability of the sarcosomes toward pyruvate and fumarate at 1–2 weeks. However, this same increased permeability should also result in a corresponding increase of the Q_{O_2}, which was not actually found (Table I). Moreover, the temporary decrease of the $NADH_2$ oxidation rate (Figs. 2 and 3) would then require an opposite postulation, namely, a temporary decrease of sarcosomal permeability toward $NADH_2$ during the same period of thiamine deficiency.

Effect of α-Glycerophosphate and β-Hydroxybutyrate on Electron Transport.—The enhancement by these compounds of the rate of sarcosomal $NADH_2$ oxidation (Fig. 2) is possibly the result of providing additional pathways for the transfer of reducing equivalents from the exogenous nucleotide to the electron transport chain via sarcosome-bound glycerophosphate oxidase or hydroxybutyric dehydrogenase. Since soluble extraparticulate glycerophosphate dehydrogenase appears to be required for the functioning of the "glycerophosphate shuttle" (Boxer and Devlin, 1961), it is possible that functional components of the cell sap are tightly retained on the surface of the sarcosomes. Actually, experiments not detailed here show that the ability of α-glycerophosphate to enhance the rate of oxidation of $NADH_2$ could not be abolished by repeated washing of the sarcosomes. The increased enhancement of the rate of $NADH_2$ oxidation by α-glycerophosphate and by β-hydroxybutyrate does not appear to be related to possible effects of these compounds on sarcosomal permeability. Indeed, neither α-glycerophosphate nor β-hydroxybutyrate was found to affect sarcosomal swelling noticeably at the concentrations used. However, the final demonstration of the increased activity of the two "shuttles" during an intermediate phase of thiamine deficiency will have to await the determination of the activities of myocardial soluble α-glycerophosphate dehydrogenase, and of the sarcosome-localized glycerophosphate oxidase and hydroxybutyric dehydrogenase in the whole time-course of thiamine deficiency.

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