

Control of Maximum Rates of Glycolysis in Rat Cardiac Muscle

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SUMMARY The maximum rate of glucose utilization by isolated rat hearts was approximately 18 $\mu\text{mol/g}$ dry weight per minute. This rate was observed in aerobic hearts that were developing high levels of ventricular pressure. This same rate has been reported for anoxic hearts. In both conditions, stimulation of glycolysis resulted in increased cytosolic NADH/NAD ratios and the rate of disposal of glycolytically produced NADH appeared to limit the maximum glycolytic rate. In aerobic hearts, oxidation of glucose and lactate increased linearly as developed ventricular pressure was raised from 60 to 150 mm Hg, but then plateaued. Oxygen consumption and pyruvate oxidation, on the other hand, continued to increase linearly over a wide range of cardiac work. The observation that substrates that produce NADH in the cytosol (glucose and lactate) showed limited rates of utilization, whereas pyruvate oxidation was linearly related to oxygen consumption, indicates that disposal of cytosolic NADH limits maximum stimulation of glycolysis. With maximum stimulation, the rate of glyceraldehyde-3-phosphate dehydrogenase appeared to determine the overall rate of the glycolytic pathway. The rate of this enzyme was probably restricted by an increased cytosolic NADH/NAD ratio. Glycolytic production of pyruvate was not fast enough to match the rate of its utilization by the citric acid cycle under any condition studied, and with glucose as the only exogenous substrate, synthesis of citrate was limited by availability of acetyl-CoA. Anaerobic production of ATP from glycolysis never accounted for more than 7% of the normal aerobic requirements for energy. We conclude that glycolytic rate in cardiac muscle is not sufficient to support high rates of oxidative metabolism. *Circ Res* 44: 166-175, 1979

GLUCOSE can serve as a major substrate for energy production in heart muscle, but no condition has been reported in which its oxidation accounts for 100% of oxidative metabolism. The mechanisms for this limited ability to oxidize glucose are not fully understood. Overall control of glucose utilization occurs by synchronized activation or inhibition of several steps in the glycolytic pathway (Opie, 1968; Neely and Morgan, 1974; Newsholme and Randle, 1964). The rate-limiting step for utilization of exogenous glucose is glucose transport when insulin is not present, phosphofructokinase (PFK) when insulin is present in aerobic hearts or glyceraldehyde-3-phosphate dehydrogenase in anoxic or ischemic hearts where the rates of transport and PFK are stimulated (Rovetto et al., 1975; Williamson, 1966). Glucose transport and PFK are also stimulated in aerobic hearts by increased cardiac work (Neely et al., 1969 and 1976), and in this case, some step further down the glycolytic pathway limits glucose utilization. As a consequence of the limited rate of glycolysis, glucose oxidation is not rapid enough to meet the energy needs of the heart, and endogenous lipids are used (Neely et al., 1970; Crass et al., 1971; Evans, 1964; Denton and Randle, 1967).

Glycolysis normally produces only about 30% of the acetyl-CoA oxidized by the citric acid cycle (Opie, 1968; Neely and Morgan, 1974; Bing, 1965). When glucose is the only exogenous substrate available for isolated hearts, this value can be increased to about 70%. From published data, glycolysis does not appear to be capable of supplying adequate amounts of acetyl-CoA for oxidative metabolism and for maintenance of tissue levels of citric acid cycle intermediates. In other words, the citric acid cycle is substrate-limited when glucose is the only substrate provided. In these hearts, tissue levels of acetyl-CoA and high energy phosphates are low and high energy phosphate stores decrease to a greater extent with increased cardiac work than in hearts performing equivalent work but receiving a combination of glucose and fatty acids as substrate (Neely et al., 1976). When noncarbohydrate substrates such as pyruvate or fatty acids are present, tissue levels of citric acid cycle intermediates are high, and oxidation of these substrates can account for 100% of the oxygen consumption.

The purpose of the present study was to determine the maximum rates of glucose utilization in aerobic cardiac muscle and to determine the mechanisms that restrict glycolytic production of pyruvate with increased oxidative metabolism. High rates of glycolysis were achieved by perfusing hearts with buffer containing 15 mM glucose as the only carbon substrate and insulin 25 mU/ml, to stimulate glucose transport. Oxidative rates were increased by raising ventricular pressure development from 60 to 200 mm Hg.

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Methods

Hearts were removed from male Sprague-Dawley rats (250–350 g) and perfused by the working heart technique as described earlier (Neely et al., 1967). The perfusate was Krebs-Henseleit bicarbonate buffer gassed with a 95% O₂, 5% CO₂ mixture and containing substrates as listed in the figures and tables. Ventricular pressure development was adjusted to the desired level by raising left atrial filling pressure and increasing the aortic resistance by closing a screw clamp on the aortic outflow tube. The perfusate was allowed to pass through the heart only once. Rates of glycolysis were determined by measuring the rate of production of ³H₂O from 2-³H-glucose (Neely et al., 1972). Glucose oxidation was estimated as ¹⁴CO₂ production from U-¹⁴C-glucose. Rates of lactate and pyruvate oxidation were measured as ¹⁴CO₂ production from 1-¹⁴C-pyruvate or 1-¹⁴C-lactate. When rates of oxygen consumption or CO₂ production were measured, the pulmonary artery was cannulated and samples of venous perfusate were collected without exposure to air. Rates of oxygen uptake and CO₂ production were calculated from the arterial-venous concentration difference and rate of coronary flow. Lactate and pyruvate production were estimated by measuring the appearance of these compounds in the perfusate. Tissue levels of glycolytic intermediates and metabolites of the citric acid cycle were determined on 6% (wt/vol) perchloric acid extracts of hearts quick-frozen by clamping with Wollenberger clamps cooled in liquid nitrogen. Assays were performed on a fluorometer by standard enzymatic methods as described by Bergmeyer (1974).

Extracellular space was calculated from the distribution of ¹⁴C-sorbitol, and intracellular space was calculated by subtracting the sorbitol space from total tissue water. Intracellular concentrations of glucose were calculated by subtracting the amount of glucose present in the sorbitol space (using the concentration in the perfusate to represent extracellular concentration) from the whole tissue content and dividing by the intracellular volume.

Ventricular pressure development and heart rate were recorded from a sidearm on the aortic cannula by use of a Statham P23Db pressure transducer connected to a Beckman dynograph recorder.

Results

The rate of oxygen consumption by cardiac muscle is known to be directly related to the peak pressure developed during systole and to heart rate. In the present study, intrinsic heart rate ranged between 200 and 250 beats/min, and cardiac work was increased by raising peak systolic pressure and cardiac output. The total amount of pressure developed by the ventricle per minute was calculated as the product of heart rate and peak systolic pressure. This product was linearly related to the rate of oxygen consumption over the range of cardiac

work studied (Fig. 1). The relationship between work output and oxygen consumption was similar when either glucose or pyruvate was present as exogenous substrate. These data demonstrate that it is possible to vary the rate of oxidative metabolism over a wide range in the isolated heart by increasing mechanical work, and this range of cardiac work was used to study the effects of oxidative rates on glucose utilization.

Oxidation of carbon substrates would be expected to increase linearly with oxygen consumption and, as reported earlier (Neely et al., 1976), oxidation of fatty acids did follow this pattern. However, when glucose was the only exogenous substrate available, its utilization was linearly related to oxygen consumption over only part of the work range studied (Fig. 2). Both glycolytic rate and glucose oxidation leveled off at the higher levels of pressure development even though oxygen consumption continued to increase. The maximum rate of glucose utiliza-

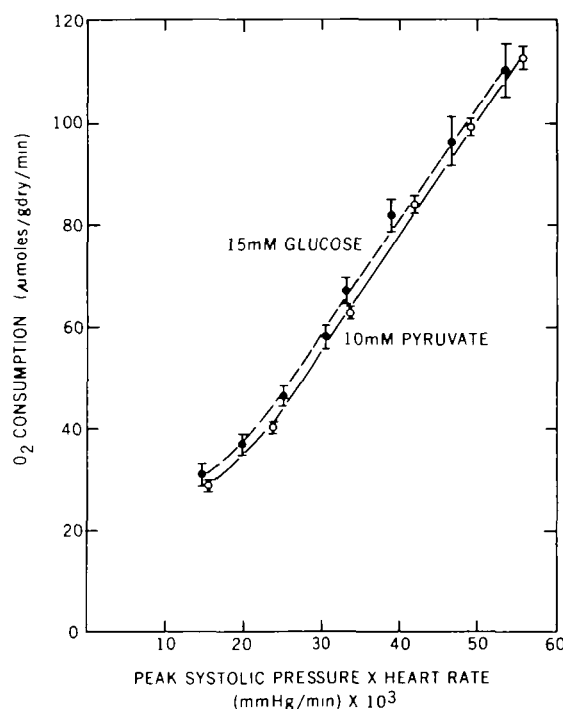


FIGURE 1 Effects of cardiac work and substrate on oxygen consumption. Peak systolic pressure was increased from 60 to 200 mm Hg. Heart rate averaged 240 beats/min. Ventricular function is expressed as the product of peak systolic pressure and heart rate. The perfusate contained either glucose (15 mM) plus insulin (25 mU/ml) or pyruvate (10 mM). Hearts were perfused for 10 minutes as a Langendorff preparation with an aortic pressure of 60 mm Hg and then for 5 minutes as a working preparation at each level of work. Consumption of O₂ increased to a new steadystate rate within 1 minute following an increase in work. The average rate during the last 4 minutes of perfusion at each workload was taken as the rate of oxygen consumption. Each line represents the mean \pm SEM for eight hearts.

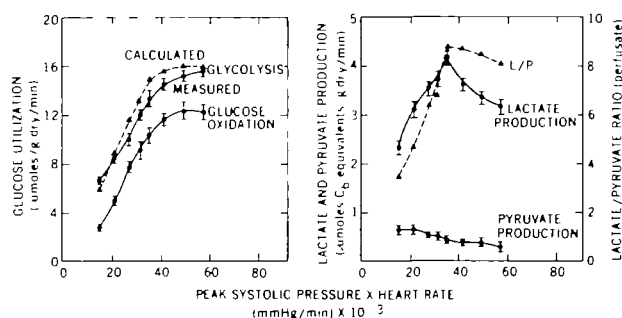


FIGURE 2 Effects of pressure development on rates of glycolysis, glucose oxidation, and lactate and pyruvate production. Cardiac work was increased in increments as described in Figure 1. The perfusate contained glucose (15 mM) and insulin (25 mU/ml). The perfusate was not recirculated. Glycolytic rates were measured as $^3\text{H}_2\text{O}$ production from ^3H -2-glucose. In a separate group of hearts, glucose oxidation was measured as $^{14}\text{CO}_2$ production from U- ^{14}C -glucose. Lactate and pyruvate production was estimated from the release of these compounds into the perfusate. The calculated rate of glycolysis was determined on the hearts used for measurement of glucose oxidation as the sum of glucose oxidized plus one-half of lactate and pyruvate production. Each point represents the mean \pm SEM for eight hearts.

tion was reached when the product of pressure and heart rate was about 40×10^3 or when peak systolic pressure was about 150 mm Hg. At the highest level of cardiac work, glucose oxidation could account for only 60% of the oxygen consumed. Presumably, the remaining oxygen was used to oxidize tissue stores of triglycerides.

This limited rate of glucose oxidation could have been due either to a limited capacity of glycolysis to produce pyruvate or to a limited rate of pyruvate oxidation by the mitochondria. However, the data presented in Figure 2 indicate that glycolytic production of pyruvate was rate-limiting. This conclusion is based on the observation that lactate production increased over the same range of cardiac work where glucose utilization increased, but production declined at the higher levels of pressure development. Pyruvate production decreased as pressure was increased. This decrease in production of both lactate and pyruvate suggests that the rate of oxidation of pyruvate by the citric acid cycle was greater than the rate of its production by glycolysis, and indicates that glucose oxidation was limited by some step in the glycolytic pathway.

An attempt to identify this rate-limiting step was made by crossover analysis of glycolytic intermediates. Tissue levels of glycolytic intermediates were determined in hearts that were developing either 60 or 180 mm Hg peak systolic pressure. These levels of pressure correspond to values of 15 and 45×10^3 , respectively, for the product of pressure and rate. Glycolytic rate was increased about 2-fold over this range of cardiac work (Fig. 2), and

the higher workload represented a level at which glycolysis was maximally stimulated. The percent changes in the concentration of intermediates that resulted from increasing pressure development are shown in Figure 3. The decrease in intracellular glucose and glucose-6-phosphate (G6P) associated with a rise in fructose-1,6-diphosphate indicated that PFK was stimulated as glycolysis was accelerated by increased work. Although intracellular glucose decreased, its concentrations at all levels of cardiac work (Table 1) were greater than the K_m (50 μM) of hexokinase for glucose, suggesting that glucose transport and the concentration of intracellular glucose did not limit glycolytic rate. The cellular levels of G6P and glucose decreased progressively as work and glycolytic rate were increased (Table 1), suggesting that the rate of PFK was accelerated in proportion to the work performed. Acceleration of this step probably accounted for increased glycolysis as the workload was raised. The data shown in Figure 3 also indicate that, with stimulation of PFK, the slowest step in glycolysis became that catalyzed by glyceraldehyde-3-phosphate dehydrogenase. This conclusion is based on the observed increase in levels of substrates for the reaction (fructose-1,6-diphosphate, dihydroxyacetone phosphate, and glyceraldehyde-3-phosphate) and on the associated decrease in the levels of intermediates that are products of the reaction (phosphoenolpyruvate and pyruvate). With current

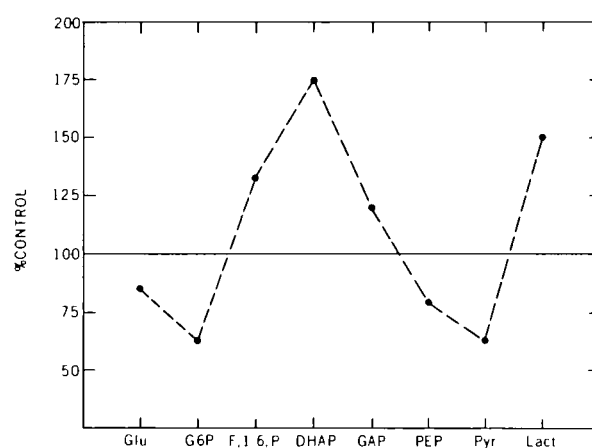


FIGURE 3 Effects of increased cardiac work on glycolytic intermediates. Hearts were perfused with buffer containing glucose (15 mM) and insulin (25 mU/ml). The control hearts (solid horizontal line) were perfused for 20 minutes, with peak systolic pressures of 60 mm Hg (the lowest value for product of pressure and rate in Figures 1 and 2). Hearts in the high-work group (dashed line) were perfused for 20 minutes, with peak pressures of 180 mm Hg (next to highest value in Figures 1 and 2). The 180 mm Hg pressure level corresponds to a level of work at which glycolysis was maximally stimulated. The data for the low-work group are expressed as 100%. Data for the high-work group are expressed as percent of the values for the low-work group.

TABLE 1 *Effects of Increased Ventricular Pressure Development on Intracellular Glucose and Glucose-6-phosphate (G6P) Levels*

Peak systolic pressure (mm Hg)	Extracellular glucose (mM)	Intracellular glucose (mM)	G6P (mM)
60	14.7 ± 0.1	8.9 ± 0.18	0.41 ± 0.009
100	14.7 ± 0.1	8.7 ± 0.5	0.35 ± 0.014
150	14.6 ± 0.1	6.8 ± 0.5	0.32 ± 0.04
180	14.6 ± 0.1	6.6 ± 0.3	0.28 ± 0.008

Hearts were perfused for 10 minutes as a Langendorff preparation with an aortic pressure of 60 mm Hg and then switched to working conditions with the level of peak systolic pressure shown for 5 minutes. The perfusate contained glucose (15 mM) and insulin (25 mU/ml). The values represent the mean + SEM for six hearts at each pressure.

techniques, it is difficult to obtain accurate values for tissue levels of 1,3-diphosphoglycerate. Without these data it is impossible to state conclusively that glyceraldehyde-3-phosphate dehydrogenase, rather than one of the other reactions between glyceraldehyde-3-phosphate and phosphoenolpyruvate, was the rate-controlling enzyme under these conditions. However, the dehydrogenase is known to be regulated by a number of factors, and there are few data to suggest specific control of the other enzymes in intact tissue.

Glyceraldehyde-3-phosphate dehydrogenase from both skeletal (Bloch et al., 1971; Furfine and Velick, 1965) and cardiac (Mochizuki and Neely, 1978) muscle is strongly inhibited by NADH, and the data presented below suggest that maximum stimulation of glycolysis was limited by an increase in the cytosolic NADH/NAD ratio. The perfusate lactate-pyruvate ratio increased progressively as ventricular pressure was raised from 60 to 150 mm Hg, and then leveled off as glycolysis became maximally stimulated (Fig. 2). The intracellular lactate-pyruvate ratio also increased at the higher cardiac workloads (Table 2). These data suggest that accelerated production of cytosolic NADH was associated with a rise in the level of NADH and that its level may have increased to a critical concentration

such that further increases in the rate of glyceraldehyde-3-phosphate dehydrogenase were prevented.

The NADH/NAD ratio might be expected to decrease rather than increase with acceleration of oxidative metabolism and electron transport, and whole tissue levels of NADH did show a slight decrease (Table 3). However, it is possible that cytosolic and mitochondrial NADH/NAD ratios may change in opposite directions. NAD does not penetrate the inner mitochondrial membrane, and reducing equivalents must be transported from the cytosol to the mitochondrial matrix by the malate-aspartate shuttle (Safer et al., 1971). An increase in the rate of this shuttle is probably initiated by a rise in cytosolic or a decrease in mitochondrial NADH/NAD ratio. This ratio in mitochondria did appear to decrease with increased cardiac work as indicated by calculated mitochondrial levels (Table 3) and by the lower malate-oxaloacetate and isocitrate- α -ketoglutarate ratios (Table 2). These data indicate that, with glucose as the only exogenous substrate available, increased oxidative metabolism lowered the mitochondrial NADH/NAD ratio, whereas stimulation of glycolysis increased the ratio in the cytosol. Thus, with rapid rates of glycolysis, cytosolic production of NADH may be faster than the rate of its reoxidation.

NADH produced by glycolysis can be reoxidized by three pathways (Fig. 4). These include: (1) the conversion of pyruvate to lactate by lactate dehydrogenase, (2) the transport of reducing equivalents into the mitochondria via the malate-aspartate shuttle, or (3) the α -glycerophosphate oxidase system (Safer et al., 1971). The first pathway was not very important in the aerobic hearts used in the present study. Since glucose was the only exogenous substrate provided, pyruvate formed by glycolysis was the major substrate for oxidation by the citric acid cycle, and it, therefore, was not available for conversion to lactate. The decrease in lactate production at the high workloads (Fig. 2) indicated that pyruvate dehydrogenase competed more effec-

TABLE 2 *Effects of Increase Pressure Development on Tissue Levels of Substrates and Products for Dehydrogenase Reactions*

Peak systolic pressure (mm Hg)	Intracellular concentration (mM)								
	Lact	Pyr	Lact/Pyr	Malate	OAA	Malate/OAA	Isocit	α KG	Isocit/ α KG
60	0.87 ±0.01	0.064 ±0.008	13.5 ±2	0.199 ±0.013	0.019 ±0.001	10.3 ±0.6	0.022 ±0.001	0.090 ±0.006	0.25 ±0.02
100				0.134 ±0.008	0.013 ±0.0005	10 ±0.6	0.019 ±0.001	0.116 ±0.006	0.17 ±0.009
150				0.111 ±0.002	0.011 ±0.0004	9.8 ±0.4	0.019 ±0.001	0.127 ±0.007	0.15 ±0.008
180	1.07 ±0.08	0.031 ±0.002	34 ±2	0.102 ±0.005	0.101 ±0.004	9.7 ±0.4	0.015 ±0.001	0.108 ±0.008	0.14 ±0.005

Hearts were perfused as described for Table 1. Lact = lactate; Pyr = pyruvate; OAA = oxaloacetate; Isocit = isocitrate; α KG = α -ketoglutarate.

TABLE 3 Calculated Changes in Cytosolic and Mitochondrial NAD and NADH with Increased Work

Peak systolic pressure (mm Hg)	Measured levels (μmol/g dry wt)			Calculated levels (μmol/g dry wt)					
	Whole tissue			Cytosolic			Mitochondrial		
	NAD	NADH	NADH/NAD	NAD	NADH	NADH/NAD	NAD	NADH	NADH/NAD
60	3.19 ± 0.09	0.290 ± 0.2	0.031	2.07	0.0051	0.0044	1.12	0.285	0.255
180	3.14 ± 0.08	0.201 ± 0.1	0.064	1.93	0.0073	0.0038	1.21	0.194	0.161

Whole tissue levels of NAD and NADH were measured as described in Methods. These values were used to calculate cytosolic and mitochondrial levels of NAD and NADH as follows: Mitochondria isolated from heart muscle contain 5.0 nmol total NAD(H)/mg protein (LaNoue et al., 1972), and heart muscle contains 280 mg mitochondrial protein/g dry tissue (Wenger et al., 1968). Therefore, 1.40 μmol total NAD(H)/g dry tissue are located in mitochondria. When this value is subtracted from the NAD(H) levels measured on whole tissue (NAD + NADH above), 2.08 and 1.94 μmol/g dry tissue are left in the cytosolic space at 60 and 180 mm Hg pressure, respectively. The cytosolic NADH/NAD ratios calculated from the lactate-pyruvate ratio using a value of 1.11×10^{-4} as K_m for lactate dehydrogenase (Williamson et al., 1967) were 0.0024 and 0.0038 at 60 and 180 mm Hg, respectively. Using these values for cytosolic NAD + NADH and the NADH/NAD ratio, NAD and NADH levels in the cytosol were calculated. The calculated cytosolic levels were subtracted from the measured whole tissue levels of NAD and NADH to obtain the mitochondria levels. These calculations assume that there is no exchange between the cytosolic and matrix pools of total NAD (NAD + NADH) and that the measured levels of NAD and NADH were in solution freely accessible to lactate dehydrogenase. The latter assumption probably overestimates the free concentrations, since these cofactors are known to bind extensively to the dehydrogenases (Bloch et al., 1971). Nonetheless, the calculated values should indicate direction of change.

tively for the available pyruvate than did lactate dehydrogenase. This preferential oxidation of pyruvate probably occurred because the intracellular pyruvate concentration of 0.03–0.06 mM (Table 2) was below the K_m of lactate dehydrogenase for pyruvate ($K_m = 0.14$ mM). Therefore, lactate production accounted for only a small percent of the pyruvate formed, and the reducing equivalents produced by glycolysis must have been transported into the mitochondria. The α -glycerophosphate oxidase system is not functional in cardiac muscle because of low levels of α -glycerophosphate. Thus, transport of reducing equivalents by the malate-aspartate shuttle probably accounted for reoxidation of NADH. The rise in cytosolic NADH/NAD ratio indicated that the rate of transport into the mitochondria could not keep pace with NADH production by glycolysis.

If the rate of hydrogen transport into the mitochondria does limit glycolysis, lactate oxidation, which also produces NADH in the cytosol, should

reach a maximum rate similar to that for glucose. Pyruvate, on the other hand, does not produce cytosolic NADH, and its oxidation should be greater than that for either lactate or glucose. Thus, rates of oxidation of lactate and pyruvate were determined. Oxidation of these substrates increased rapidly after pressure development was raised from 60 to 180 mm Hg and appeared to reach a new steady state within 1 minute (Fig. 5). The rate for

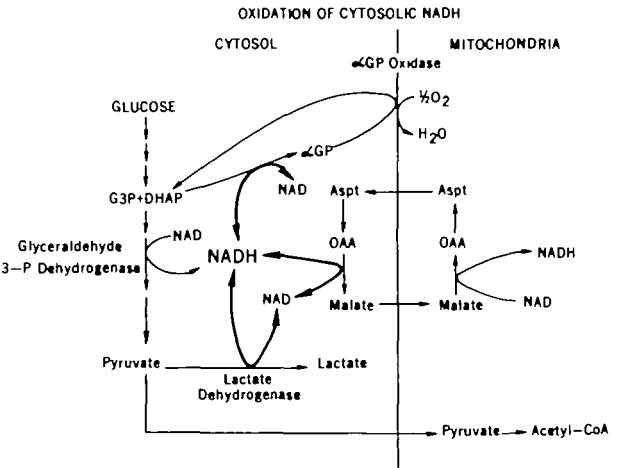


FIGURE 4 Pathways for oxidation of cytosolic NADH. Glycolytically produced NADH can be oxidized by lactate dehydrogenase, α -glycerophosphate dehydrogenase, and malate dehydrogenase.

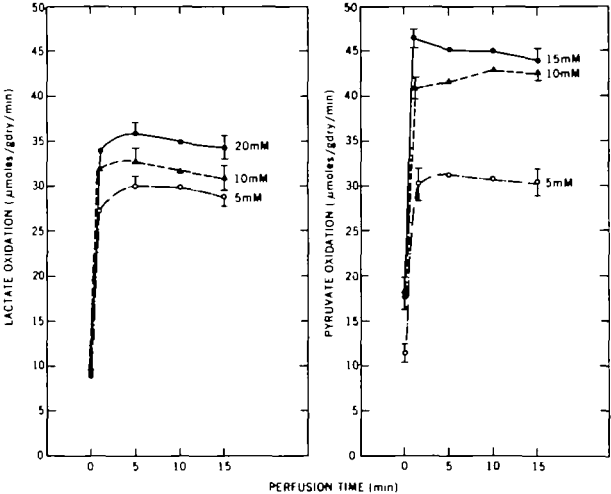


FIGURE 5 Effects of substrate concentration and pressure development on lactate and pyruvate oxidation. Hearts were perfused for 10 minutes with perfusate containing the concentration of substrates indicated in the figure. During this time, peak systolic pressure was 60 mm Hg. Rates of oxidation as estimated by $^{14}\text{CO}_2$ production from $1\text{-}^{14}\text{C}$ -lactate (left panel) or from $1\text{-}^{14}\text{C}$ -pyruvate (right panel) reached a steady state within 5 minutes. The low-work oxidation rate is shown for values obtained at the end of 10 minutes (zero time values in the figure). Pressure development was increased to 180 mm Hg at this time, and oxidation rates were followed for an additional 15 minutes. Each curve represents data from six hearts, with standard errors of the mean shown by vertical lines.

lactate was increased only slightly when the perfusate concentration was raised from 5 to 10 and 20 mM. Pyruvate oxidation was greater at 10 or 15 mM than at 5 mM. Since 10 mM concentrations of both substrates appeared to saturate the uptake process, the rates of oxidation of each substrate as a function of cardiac work was determined using 10 mM concentrations in the perfusate (Fig. 6). Lactate oxidation was somewhat faster than glucose oxidation at the highest workloads, but oxidation of both these substrates reached a maximum rate at about the same level of cardiac work. Oxidation of pyruvate, on the other hand, was greater than that of either lactate or glucose at all levels of cardiac work, and its oxidation increased in a linear relation to oxygen consumption. Since both of the substrates that produce NADH in the cytosol showed limited rates of oxidation, disposal of cytosolic-reducing equivalents would appear to be important in determining the maximum rates of their utilization.

If the maximum rates of utilization of glucose and lactate are limited by the rate of mitochondrial removal of cytosolic NADH, then the maximum

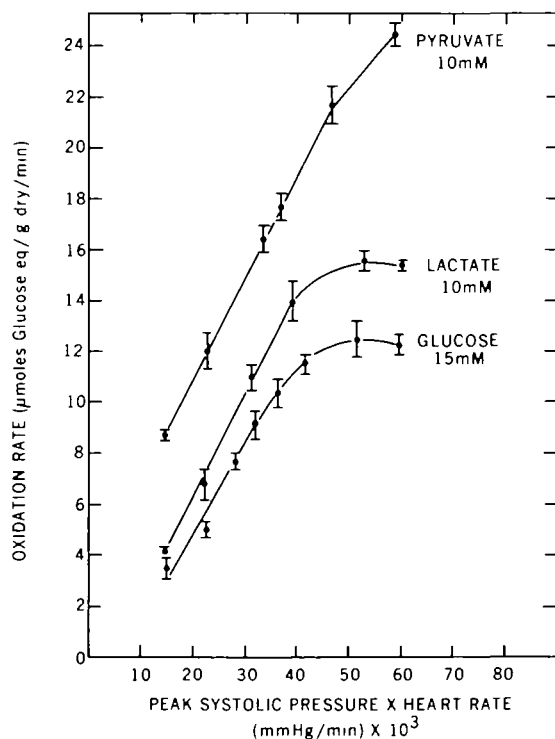


FIGURE 6 Effects of cardiac work on rates of oxidation of glucose, lactate, and pyruvate. The perfusate contained either $U\text{-}^{14}\text{C}$ -glucose plus insulin (25 mU/ml), $1\text{-}^{14}\text{C}$ -lactate, or $1\text{-}^{14}\text{C}$ -pyruvate at the concentrations shown. Cardiac work was increased in steps and maintained at each level for 5 minutes. Rates of oxidation were determined between the 4th and 5th minutes in each case. Lactate and pyruvate oxidation rates are expressed as glucose equivalents. Each point represents the mean \pm SEM for eight hearts.

rate of cytosolic production of NADH in the intact tissue should be similar to the maximum rates of the malate-aspartate shuttle as determined on isolated mitochondria. When extrapolated to intact tissue, reported values for hydrogen transport in isolated mitochondria indicate that the maximum rate for intact tissue should be about 20–29 μmol NADH transported per gram of dry tissue per minute (LaNoue and Williamson, 1971; Digerness and Reddy, 1976). The rates of cytosolic NADH production in the intact tissue used in the present study agree quite well with these extrapolated values (Fig. 7). The maximum rates with glucose and lactate as substrate were about 26 and 34 $\mu\text{mol/g}$ dry weight per minute, respectively. The negative rate of cytosolic NADH production with pyruvate as substrate reflects the rate of lactate production.

Most of the ATP synthesized with either substrate present occurred from oxidation of NADH produced in the mitochondria. At the lowest level of cardiac work used, NADH production in the mitochondria from oxidation of pyruvate, lactate, and glucose was 73, 41, and 23 $\mu\text{mol/g}$ dry weight per minute, respectively (Fig. 7). At the highest level of cardiac work, the mitochondrial rates increased to about 195, 120, and 100, respectively. Total production from these substrates, i.e., the sum of the cytosolic and mitochondrial rates, was 190, 158, and 126 $\mu\text{mol/g}$ per minute, respectively. Assuming a one-to-one relationship between atoms of oxygen used and NADH oxidized, the total production of NADH from all substrates as calculated from rates of oxygen consumption (Fig. 1) was about 60 and 220 $\mu\text{mol/g}$ per minute, at the lowest

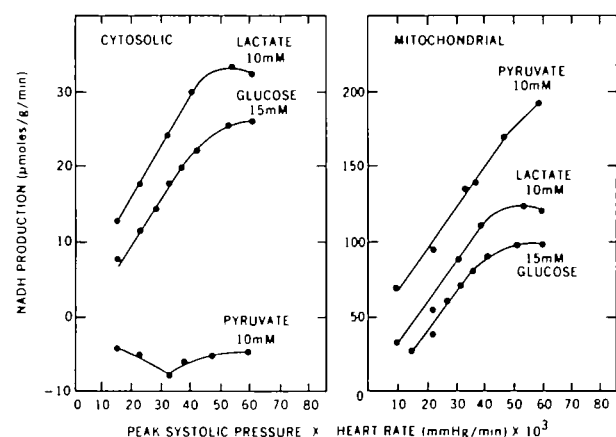


FIGURE 7 Calculated rates of NADH production from glucose, lactate, and pyruvate utilization. Rates and production of NADH in the cytosol were calculated from the data in Figures 2 and 6 as follows: for glucose = $(2 \times \text{glycolytic rate}) - \text{lactate production}$; for lactate = $\text{lactate oxidation} + \text{pyruvate production}$; for pyruvate = $\text{lactate production}$. The rates for mitochondrial production were calculated as follows: for glucose = $8 \times \text{glucose oxidation}$; for pyruvate = $4 \times \text{pyruvate oxidation}$; for lactate = $4 \times \text{lactate oxidation}$.

TABLE 4 Effects of Exogenous Substrate and Peak Systolic Pressure Development (PSP) on Tissue Levels of Citric Acid Cycle Intermediates

Substrate	PSP	Tissue metabolites ($\mu\text{mol/g}$ dry tissue)			
		Citrate	Isocitrate	α -Ketoglutarate	Malate
Glucose	60	0.84 ± 0.06	0.059 ± 0.001	0.123 ± 0.01	0.53 ± 0.03
	180	0.64 ± 0.03	0.041 ± 0.003	0.170 ± 0.01	0.27 ± 0.01
Lactate	60	1.7 ± 0.14	0.161 ± 0.008	0.281 ± 0.021	1.255 ± 0.03
	180	1.3 ± 0.06	0.139 ± 0.003	0.226 ± 0.009	0.68 ± 0.04
Pyruvate	60	7.2 ± 0.11	0.378 ± 0.007	1.54 ± 0.02	6.4 ± 0.04
	180	6.5 ± 0.16	0.327 ± 0.009	1.34 ± 0.05	5.4 ± 0.27

Hearts were perfused for 10 minutes as a Langendorff preparation with an aortic pressure of 60 mm Hg and with 15 mM glucose as substrate. They were then switched to working conditions with peak systolic pressures of either 60 or 180 mm Hg for 10 minutes. The substrates were either glucose (15 mM) plus insulin (25 mU/ml), pyruvate (10 mM), or lactate (10 mM). Values for each condition represent the mean \pm SEM, for six to eight hearts.

and highest workloads, respectively. From these data, it appears that production of pyruvate from glucose and lactate was inadequate to support mitochondrial oxidative rates, and the remaining mitochondrial NADH required to support oxygen consumption must have been derived from oxidation of endogenous lipids.

The low rate of mitochondrial production of NADH from oxidation of glucose and lactate indicated that these substrates could not supply adequate amounts of pyruvate to run the citric acid cycle. This conclusion was supported by measurement of tissue levels of several citric acid cycle metabolites (Table 4). In general, the levels of citric acid cycle intermediates corresponded to the rate of oxidation of the substrate present, i.e., the levels were highest with pyruvate and lowest with glucose. Generally, levels of these intermediates decreased with increased cardiac work and faster rates of flux through the cycle.

Assuming a P/O ratio of 3 and using the rates of oxygen consumption as shown in Figure 1, total oxidative production of ATP increased from 185 to 674 $\mu\text{mol/g}$ per minute over the range of cardiac workloads used in the present study (Fig. 8). Oxidation of pyruvate could account for at least 100% of this ATP at all levels of work. With most workloads, oxidation of pyruvate, as assessed by $^{14}\text{CO}_2$ production from $1\text{-}^{14}\text{C}$ -pyruvate, accounted for more than 100% of oxygen consumption, indicating that a small amount of the pyruvate that was converted to acetyl-CoA was subsequently diverted to metabolites other than CO_2 . Oxidation of lactate accounted for 100% of the ATP produced at most of the workloads studied. At the higher levels of work, however, lactate was incapable of supplying carbon to meet the metabolic needs of the tissue and, at the highest workload, only 83% of the ATP produced could be accounted for by lactate oxidation. Oxidation of glucose was less than that required to support oxygen consumption at all levels of cardiac work. Glucose accounted for about 60% of the carbon substrate oxidized at both the lowest and the highest work, but at intermediate levels of pressure development (100–120 mm Hg) this percentage increased to almost 100. Anaerobic production of ATP by glycolysis increased from 13 to 33

$\mu\text{mol/g}$ per minute as cardiac work was raised, but never contributed more than 7% of the total ATP produced.

Discussion

The perfusion conditions used in the present study were designed to yield maximum glycolytic rates. High concentrations of glucose and insulin were used to achieve maximum stimulation of glucose transport, other exogenous substrates were omitted to prevent glycolytic inhibition (Neely et al., 1969; Garland et al., 1963; Randle et al., 1970), and high levels of ventricular pressure development were imposed to accelerate ATP hydrolysis. The maximum rate achieved under these conditions was about 16 $\mu\text{mol/g}$ dry weight per minute. It is interesting that this maximum rate in aerobic hearts is

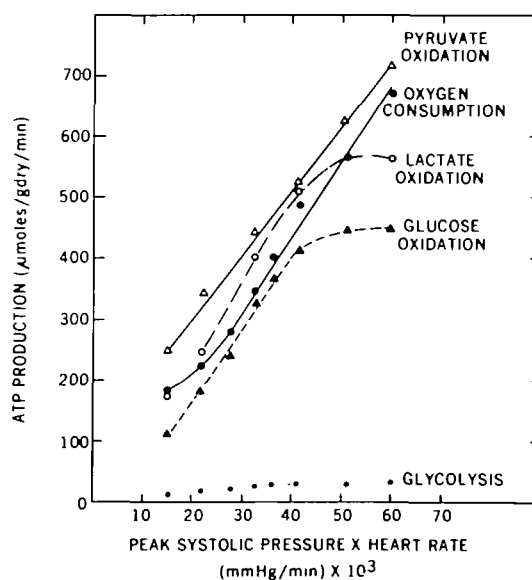


FIGURE 8 Calculated rates of ATP production. These rates were calculated from data presented in Figures 1, 2, and 6 as follows: from oxygen consumption = $6 \times \text{O}_2$ consumption; from glucose oxidation = $36 \times$ glucose oxidation; from lactate oxidation = $(18 \times \text{lactate oxidized}) + (3 \times \text{pyruvate produced})$; from pyruvate oxidation = $15 \times \text{pyruvate oxidized}$; from glycolysis = $2 \times$ glycolytic rate.

almost the same as that reported for anoxic hearts (Williamson, 1966; Rovetto et al., 1973), and is faster than the maximum rate (about 11 $\mu\text{mol/g}$ per minute) seen in ischemic hearts (Rovetto et al., 1975; Neely et al., 1975).

These rates of glycolysis could produce ATP at maximum rates of about 32 $\mu\text{mol/g}$ per minute, which would account for only about 7% of the normal aerobic requirement. It is unlikely that ATP production by substrate level phosphorylation in glycolysis is ever of major quantitative importance in aerobic hearts and it can compensate for only a small percent of the loss of oxidative production of ATP in anoxic or ischemic hearts. Thus, the quantitative importance of glycolysis in energy metabolism is not ATP synthesis, but rather the production of pyruvate which is subsequently oxidized by the citric acid cycle. Oxidation of the pyruvate formed by glycolysis yields the equivalent of 30 mol of ATP for each mole of glucose and, under normal physiological conditions, oxidation of glucose contributes about 30% to the total energy requirements of the tissue.

The rather low contribution of glycolysis to ATP production is due in large part to inhibition of glucose transport and PFK by oxidation of fatty acids (Newsholme and Randle, 1964; Neely et al., 1969; Opie, 1968). PFK is the rate-controlling step of glycolysis under most physiological conditions, and acceleration of this step accounts for faster rates of glycolysis in oxygen-deficient tissue. In addition, stimulation of PFK with increased mechanical work accelerates glycolytic rate in aerobic hearts receiving only glucose as substrate. Available data suggest that when PFK is stimulated, the slowest step in the glycolytic pathway is shifted to oxidation of triose phosphates by glyceraldehyde-3-phosphate dehydrogenase. This conclusion is supported by the observation that acceleration of PFK in anoxic and ischemic hearts results in increased tissue levels of fructose-1,6-diphosphate, dihydroxyacetone phosphate, and glyceraldehyde-3-phosphate (Rovetto et al., 1975; Williamson, 1966). In the present study, acceleration of PFK by increased cardiac work also resulted in increased levels of these glycolytic intermediates. Thus, the rate of glyceraldehyde-3-phosphate dehydrogenase may set the upper limit to which glycolysis can be stimulated under any condition.

The mechanism that limits glycolysis when the pathway is fully stimulated is not known, but an increase in the cytosolic NADH/NAD ratio is common to all conditions in which glycolysis is accelerated. Increased NADH is a well-known consequence of oxygen deficiency in heart muscle (Opie, 1968; Rovetto et al., 1975; Williamson, 1966). In the present study, cytosolic NADH/NAD ratios increased even though the oxygen supply appeared to be adequate. The rise in NADH in aerobic hearts may occur because an acceleration of PFK results in higher tissue levels of glyceraldehyde-3-phos-

phate, which in turn increases the rate of glyceraldehyde-3-phosphate dehydrogenase. Increased flux through this step apparently produces NADH faster than it can be oxidized. Since NADH is known to inhibit glyceraldehyde-3-phosphate dehydrogenase (Bloch et al., 1971; Furfine and Velick, 1965), an increase in cytosolic NADH/NAD could prevent further stimulation of this enzyme by the higher levels of glyceraldehyde-3-phosphate.

Cytosolic NADH can be reoxidized in cardiac muscle by three reactions (Fig. 4): (1) Lactate dehydrogenase oxidizes NADH, but the lactate that is formed must be exported from the cell. This is the major pathway in oxygen-deficient tissue, and most of the glucose utilized in anoxic or ischemic tissue appears as lactate (Rovetto et al., 1973). (2) α -Glycerophosphate dehydrogenase can convert dihydroxyacetone phosphate to α -glycerophosphate using NADH. In many tissues, the α -glycerophosphate can be reoxidized to dihydroxyacetone phosphate by a mitochondrial α -glycerophosphate oxidase. However, in cardiac muscle, the level of α -glycerophosphate is normally below the K_m of this enzyme system, and NADH probably is not oxidized by this route (Safer et al., 1971). (3) The malate-aspartate shuttle (LaNoue et al., 1973; Safer, 1975) is the most important means of oxidizing NADH in aerobic hearts. This system transports reducing equivalents into the mitochondria as malate where they can be transferred to mitochondrial NAD by malate dehydrogenase.

All three pathways for oxidation of cytosolic NADH are potentially operative in aerobic hearts, and it might be expected that cytosolic NADH would not accumulate with acceleration of glycolysis. The fact that NADH does increase in the cytosol is supported by several observations. Isolated hearts that receive only glucose as substrate always produce lactate (Opie, 1968; Rovetto et al., 1973). This occurs even though the pyruvate produced from glycolysis is needed for oxidative metabolism, and levels of citric acid cycle intermediates are low. With insulin treatment, glycolysis is accelerated, and although the contribution of glucose to oxidative metabolism is increased, a larger percent of the pyruvate formed is converted to lactate (Neely et al., 1972; Randle et al., 1970). In the present study, acceleration of oxidative metabolism by an increase in cardiac work resulted in faster rates of glycolysis, which were also accompanied by increased production of lactate. These observations suggest that, even in aerobic tissue with low rates of glycolysis, some of the NADH formed in the cytosol is used to convert pyruvate to lactate, and when glycolysis is accelerated, lactate production is also increased. Therefore, the rate of mitochondrial removal of cytosolic NADH does not occur in a 1:1 relationship to glycolytic production.

Why should aerobic cardiac muscle convert pyruvate to lactate, which is exported from the cell, and divert NADH from oxidative metabolism under

conditions in which the pyruvate is needed to fuel the citric acid cycle and the reducing equivalents are needed to run electron transport? This situation may exist, because any time glycolytic production of NADH is accelerated, transport of reducing equivalents into the mitochondria as malate also must be increased. The rate of malate transport is proportional to its concentrations (LaNoue and Williamson, 1971). If malate dehydrogenase operates in the cell near its thermodynamic equilibrium, an increase in malate to achieve faster transport must be associated with an increase in either NADH or oxaloacetate. Since oxaloacetate levels were lower than the K_m of malate dehydrogenase for this substrate, accelerated transport would require a higher level of cytosolic NADH to establish the necessary concentration gradient of malate for increased inward movement. Therefore, the rate of mitochondrial removal of NADH is probably increased secondarily to a rise in cytosolic NADH. This rise in cytosolic NADH can also shift the lactate hydrogenase reaction toward formation of lactate. Increased production of lactate would therefore occur any time glycolysis is accelerated, even if adequate oxygen is available. Production of lactate therefore does not necessarily reflect oxygen deficiency. In contrast to isolated hearts, the heart in vivo normally takes up lactate (Opie, 1968; Bing, 1965). This probably occurs because the in vivo heart receives adequate amounts of fatty acids from the blood, has high levels of citric acid cycle intermediates (especially citrate), and has low rates of glycolysis due to inhibition of glucose transport and PFK. Thus, glycolytic production of NADH is low, and lactate can be converted to pyruvate.

If the maximum rate of glycolysis is set by the rate of removal of cytosolic NADH, it is curious that this rate should be approximately the same in anoxic and aerobic hearts. In anoxic hearts, the mitochondrial pathways for oxidation of NADH are inoperative, and production of lactate is the only pathway for continued reoxidation. If there is a 1:1 relationship between NADH produced by glycolysis, reoxidation by lactate dehydrogenase, and washout of lactate from the cells, NADH should not accumulate. The rise in NADH that has been observed in anoxic hearts may occur by two mechanisms: (1) Since tissue pyruvate remains below the K_m (0.14 mM) for lactate dehydrogenase (Williamson, 1966; Rovetto et al., 1973), acceleration of lactate production would require increased levels of NADH. In addition, higher levels of lactate are required to establish a concentration gradient for lactate efflux from the cells and, as lactate levels increase, NADH would have to increase even more to maintain accelerated lactate production. The rise in lactate to stimulate efflux, and in NADH to increase lactate production, would be expected to occur in proportion to the increase in glycolysis. (2) In anoxic tissue, mitochondrial NADH would be

expected to increase, and some NADH may be transported to the cytosol. The malate-aspartate shuttle is thought to operate only to transport reducing equivalents into the mitochondria (Bremer and Davis, 1975), but in the present study, formation of lactate at a rate of 5–7 $\mu\text{mol/g}$ per minute in hearts receiving pyruvate as substrate suggests that mitochondrial reducing equivalents can be transported to the cytosol. Thus, at high rates of glycolysis, NADH could increase to levels that inhibit glyceraldehyde-3-phosphate dehydrogenase and restrict further increases in glycolytic rate.

Oxidation of cytosolic NADH in ischemic hearts is complicated by slow coronary flow and lack of washout of cellular lactate (Rovetto et al., 1973). The rise in lactate-pyruvate ratio is greater than in anoxic hearts and the rate of glycolysis is lower. The extent of NADH accumulation in this case depends on how fast the lactate is removed from the tissue, and the level of NADH apparently becomes high enough in ischemic hearts to inhibit glycolysis at a glycolytic rate that is lower than in anoxic hearts.

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