RELATIONSHIP BETWEEN CARBOHYDRATE AND LIPID METABOLISM AND THE ENERGY BALANCE OF HEART MUSCLE[1]

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INTRODUCTION

Utilization of carbohydrate and fatty acids is strongly influenced by rates of consumption and production of high energy compounds in cardiac muscle. In well-oxygenated hearts, fatty acid has been identified as the preferred substrate by both in vivo and in vitro studies (for review see 146). Availability of fatty acid suppresses glucose utilization by inhibition of several steps in the glycolytic pathway. Since a variety of long- and short-chained fatty substrates are inhibitory and since the effect is abolished in hearts perfused under anaerobic conditions, oxidation of the fatty acid appears to be required for this effect. When energy utilization of well-oxygenated hearts is increased, consumption of fatty acids and/or glucose is accelerated. Under these conditions, fatty acids remain the preferred oxidative substrate.

Availability of oxygen to the heart can be restricted either by lowering or reducing to zero the oxygen tension of the perfusate, thereby inducing hypoxia or anoxia, or by restricting the flow of perfusate containing high oxygen tensions to induce ischemia. In hypoxic or anoxic hearts, fatty acid oxidation is suppressed and glycolysis is stimulated. The acceleration of flux through glycolysis may be as much as 10- to 20-fold. On the other hand, ischemia results in only a transient increase in glycolytic flux that is followed within 3–4 min by inhibition. Fatty acid oxidation is suppressed in ischemic hearts leading to accumulation of long-chained CoA derivatives and increase in triglyceride levels.

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The purpose of this article is to identify steps in the pathways of glycolysis and fatty acid oxidation that serve important rate-controlling functions. Effects of cardiac work, hypoxia, anoxia, and ischemia on these reactions are described and an attempt is made to identify regulatory factors responsible for the change in utilization of carbohydrate and fatty acid. Effects of insulin on glucose and glycogen metabolism are mentioned, but, in general, hormonal control of these pathways is not included in the scope of this review. We focus on regulation of the production of acetyl-CoA from glucose and fatty acids. Control of the oxidation of this intermediate through the citric acid cycle is discussed only briefly. The primary purpose of the review is to relate increased energy consumption to accelerated utilization of carbohydrates and fatty acids and to contrast the effects of energy deprivation on glycolysis in anoxic and ischemic hearts.

METHODS FOR THE STUDY OF REGULATION OF METABOLIC PATHWAYS IN INTACT TISSUES

Preparations of Heart Muscle Available for Study

Adequate control of substrate and hormone levels, oxygen tension, rates of ventricular pressure development, and coronary flow has been most effectively achieved in isolated perfused hearts (133, 145), frequently of rats. Perfused tissue offers many advantages over heart slices, ventricular or atrial strips, papillary muscles, and homogenates for studies of the regulation of carbohydrate and fatty acid metabolism. In the perfused heart, substrates, hormones, and oxygen are delivered to the cells via an intact capillary circulation. Sufficient tissue is available to simplify measurements of substrate uptake and levels of metabolites. Since cell membranes are intact, regulation of substrate utilization by changes in membrane permeability is retained. In well-oxygenated preparations, ventricular performance is stable for periods in excess of 3 hr. The major limitation of the perfused heart as compared to heart cells in culture (81, 205) involves the period over which experiments are feasible. Although isolated hearts have been maintained for weeks, experiments exceeding several hours are not practical on a large scale. This limitation is not of great importance for studies of most aspects of carbohydrate and lipid metabolism, but does make studies of enzyme induction or of reparative processes difficult.

Isolated hearts are most often perfused by the Langendorff technique (see 143). Modifications of this method allow recirculation of a small volume of perfusate, usually Krebs-Henseleit bicarbonate buffer, from which substrate disappearance can be estimated. In addition, perfusate can be allowed to flow through the heart a single time to provide an unchanged supply of substrate and to prevent accumulation of metabolic products. In these latter experiments glucose utilization and fatty acid oxidation can be estimated by measuring conversion of specifically-tritiated glucose and ^{14}C-fatty acid to $^{3}H_2O$ and $^{14}CO_2$. In the Langendorff preparation perfusate is supplied over a range of pressures by retrograde flow down the aorta. Unless special efforts are made to drain the left ventricle, intraventricular pressure increases to levels slightly above the perfusion pressure with each beat. Pressure development

depends upon a limited degree of ventricular filling, arising either from the Thebesian circulation or from incompetence of the aortic valve due to use of low viscosity perfusate. Intraventricular pressure development in the Langendorff preparation can be increased by raising perfusion pressure. Increased pressure development is associated with increased oxygen consumption and accelerated utilization of carbohydrate and lipid. Since coronary flow is a linear function of perfusion pressure and ventricular pressure development, these parameters cannot be dissociated in this preparation. Anoxia or hypoxia is induced by exposing the perfusate to gas mixtures containing either zero or reduced oxygen tension.

The major disadvantage of the Langendorff preparation is the inability to vary levels of external cardiac work during perfusion. This difficulty was overcome by development of a working heart apparatus in which both the left atrium and aorta are cannulated (133, 143). Perfusate is supplied to the atrium over a range of left atrial filling pressures and outflow resistance is varied by changing the height of the hydrostatic pressure head against which the heart is required to pump. In this preparation ventricular pressure development is increased either by raising left atrial filling pressure or outflow resistance and results in increased consumption of oxygen, carbohydrates, and fatty substrates. The major advantage of the working heart preparation is the ability to correlate changes in ventricular function with metabolic events. This feature is of particular importance in studies of drug or hormone action or in following the course of ventricular function during anoxia.

As noted above, coronary flow is linearly related to ventricular pressure development in the Langendorff preparation. This also holds true for the working heart model. As a result of this relationship, these preparations are not well-suited for investigation of the effects of ischemia on ventricular function and metabolism. In each case, a reduction in coronary flow was associated with lower levels of ventricular pressure development and energy utilization. Since these changes are parallel, reduced delivery of oxygen is partially compensated for by reduced O_2 demand. A preparation suitable for studies of cardiac ischemia was developed by modifying the working heart model (147). Flow of perfusate into the left atrium was maintained, but a ball valve was inserted in the outflow tract, immediately above the aorta. The valve allowed ejection of perfusate during systole, but prevented flow into the coronary ostia during diastole. As a result, aortic diastolic pressure in the area immediately above the aortic valve was reduced from 60 to 20 mm Hg and coronary flow fell by 60%. Initially, peak systolic aortic pressure was maintained despite a reduction in coronary flow. After 4–6 min, however, ventricular failure began and progressively intensified. If a minimal perfusion pressure was not provided, ventricular failure was irreversible after about 30 min. On the other hand, maintenance of a coronary perfusion pressure of 25 mm Hg produced a reduced, but fairly stable, level of ventricular function for at least 1 hr.

The Langendorff, working, and ischemic models of the perfused rat heart allow for detailed studies of the effects of increased ventricular pressure development, cardiac work, hypoxia, anoxia, and ischemia on utilization of carbohydrate and fatty acids. The Langendorff model is easiest to use and is suitable for studies of hormone

action, increased ventricular pressure development, and anoxia in situations in which correlation with changes in cardiac work is not required. The working model is needed for studies of the effects of external work on metabolism while the ischemic model allows studies simulating myocardial infarction.

Although the isolated perfused heart is a convenient tool for studies of cardiac metabolism, evaluation of the physiological significance of regulatory mechanisms described in this preparation requires experiments in intact animals. Assessment of metabolic changes in the heart, in vivo, rests upon determinations of A-V differences of substrates and metabolic products, estimation of oxidation of radioactive substrates that are added to the arterial blood, and analysis of metabolite levels in rapidly frozen tissue. Estimation of substrate utilization by measurements of A-V differences is complicated, in some instances, by the small changes that occur, and, in others, by simultaneous utilization and production of the substrate. A-V differences of glucose across the heart are quite small and estimates of the rates of utilization are imprecise (214). Simultaneous uptake and production of substrate is particularly apparent when utilization of fatty acid is calculated (118, 139). The coronary circulation supplies blood both to cardiac muscle and adipose tissue in the epicardial surface and around the major coronary vessels. In some instances, release of free fatty acid (FFA) from adipose tissue and muscle exceeds uptake by the muscle, resulting in higher venous than arterial levels of FFA. Estimation of the rate of uptake and oxidation of [^{14}C] fatty acid supplied to the arterial inflow has been used to circumvent these difficulties.

Detection of metabolic changes in abnormal areas of cardiac muscle may be masked if the area is surrounded by larger areas of normal tissue. Resort to biopsy of the affected area for estimation of metabolite levels may allow for detection of abnormal areas in some instances. The major advantage of in vivo studies is the opportunity to explore regulatory mechanisms in a physiological setting that have been defined in isolated hearts.

Identification of Rate-Controlling Reactions in Carbohydrate and Fatty Acid Metabolism

In general, two approaches have been used for identification of rate-controlling reactions in intact cells (for review see 194). These methods include 1. correlation of levels of metabolites in a pathway with rates of flux through the pathway and 2. detection of reactions whose levels of substrates, products, and cofactors within the tissue are far removed from levels expected from the thermodynamic equilibrium of the reaction. Meaningful conclusions regarding the importance of a reaction in controlling flux through a pathway can be derived by these methods only if the substrates and products are contained within a single compartment of the cell. In this regard, detection of rate-controlling steps in fatty acid oxidation is complicated by compartmentalization of long- and short-chained CoA derivatives between the mitochondrial and cytoplasmic spaces. In these circumstances, increased levels of a substrate within one compartment can mask reduced levels within the other compartment and can lead to incorrect conclusions regarding the rate-controlling function of reactions consuming the substrate.

Correlation of metabolite levels with flux rates allows for detection of "cross-

over" points within a pathway (32). For example, acceleration of glycolytic flux in anoxic muscle is usually associated with depletion of the substrates of phosphofructokinase, F-6-P, and ATP, and accumulation of the products, F-1,6-P and ADP. If the metabolite levels of the glycolytic pathway are taken as unity in well-oxygenated tissue, the substrate levels in the anoxic heart fall below 1 while product levels exceed this value. As a result, the metabolite levels are said to "cross-over" the control values at the level of the phosphofructokinase reaction. These findings, in association with an increased glycolytic flux, are interpreted to indicate that the phosphofructokinase reaction is accelerated in anaerobic tissue.

Studies of Rate-Controlling Enzymes

Enzymatic steps within the pathway of glycolysis or fatty acid oxidation may be identified as rate-controlling as outlined above or may be suggested to be the site of regulation by possessing a number of activators and inhibitors of enzyme activity. It should be emphasized, however, that complex regulation of an enzyme does not establish the physiological role of the catalyst as a regulatory factor.

In studies of the physiological significance of factors controlling an enzymatic activity, it is important to assay activity in the presence of physiological levels of substrates, activators, and inhibitors. For example, the physiological significance of regulation of phosphorylase *b* by ATP, AMP, and P_i (135) can be understood only if the enzyme is assayed in the presence of relatively high levels of ATP and low levels of AMP and P_i. In these circumstances, it is apparent that ATP is strongly inhibitory and that the remaining activity is modulated by changes in AMP and P_i.

An additional problem in assessing the significance of regulatory phenomena that are detected in assays of pure enzymes involves use of enzyme concentrations far below those encountered in the cell. For example, citrate synthase is strongly inhibited by ATP when assayed in dilute solution, but the nucleotide is without effect when enzyme concentration is increased to values approximating those in the intact cell (215). Another similar situation is encountered in assessing the physiological significance of the inhibition of adenine nucleotide translocation across the mitochondrial membrane by long-chain acyl-CoA (169, 208). This metabolite is strongly inhibitory when added to mitochondrial suspensions in concentrations near those found in the heart. However, long-chain CoA derivatives are strongly bound to protein, and the mitochondrial concentration in these experiments was far below that encountered in intact cells. Under these circumstances, the ratio of long-chain CoA to mitochondrial protein was much higher than encountered in the cell. These considerations introduce uncertainties in assessing the physiological significance of the regulatory mechanism.

This section has outlined methods for study of regulation of carbohydrate and fatty acid metabolism in heart muscle. Perfusion techniques allow for close control of substrate levels, oxygen supply, and mechanical activity of the heart. Reactions controlling utilization of these substrates can be identified by measurements of metabolites and flux rates. Mechanisms of enzymatic regulation can be investigated using physiological levels of the purified protein, substrates, activators, and inhibitors.

GENERAL FEATURES OF THE CONTROL OF CARBOHYDRATE UTILIZATION

Utilization of glucose and glycogen by heart muscle is regulated at a number of steps in conversion of these substrates to acetyl-CoA. These steps are described in this section (Figure 1). An increased flux of substrate through a reaction can arise either from a mass action effect due to higher concentrations of one of the substrates or from activation of the enzymatic catalyst. The following reactions have been identified from either cross-over plots or studies of purified enzymes as being subject to

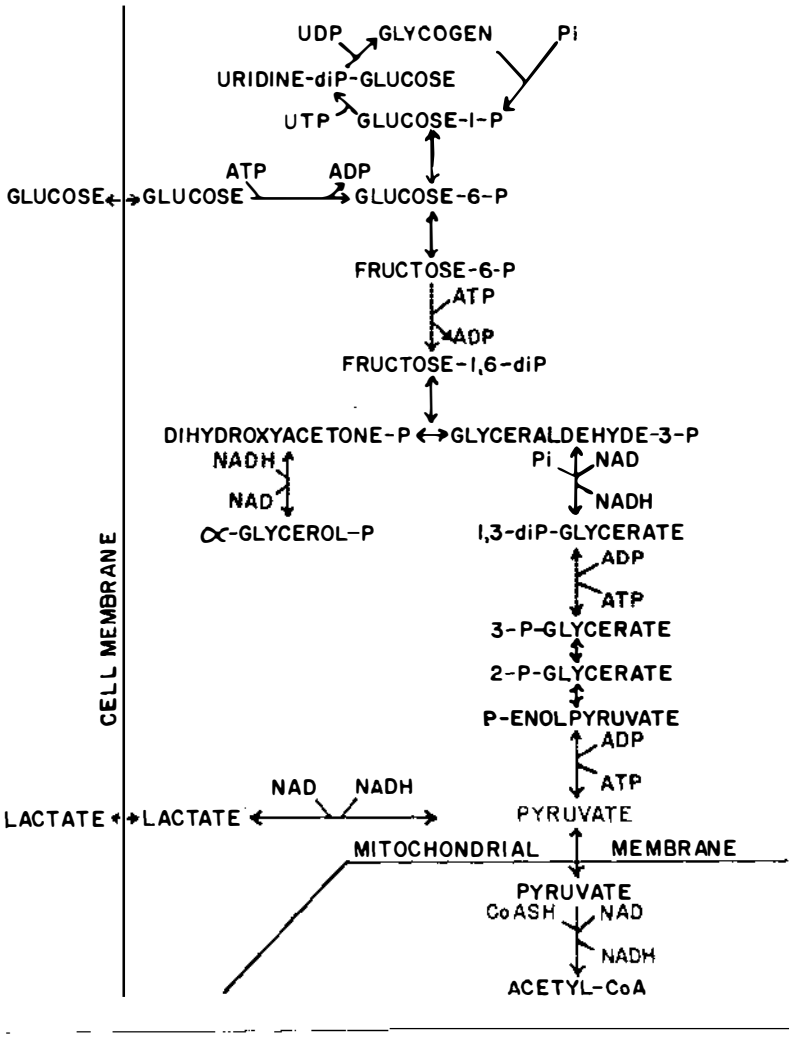

Figure 1 Pathway of glucose and glycogen metabolism in the heart.

activation or inhibition: glucose transport, hexokinase, phosphorfructokinase, glyceraldehyde-3-P dehydrogenase, pyruvate kinase, pyruvate dehydrogenase, glycogen synthetase, and glycogen phosphorylase.

Glucose Transport

Kinetic data on glucose transport through muscle membranes are compatible with the hypothesis that the transport is carrier-mediated, not energy-requiring, and leads to equilibration of sugar concentrations inside and outside the cell (for reveiw see 138). Transport has been studied in greatest detail in erythrocytes. In both muscle and red cells the process has been found to be saturable at increasing sugar concentrations, to be stereospecific, to demonstrate competitive inhibition between pairs of sugars, and to show countertransport. The simple "carrier" model of sugar transport accounts for the kinetic properties of the process in muscle, insofar as these properties have been investigated. In human erythrocytes, more complex models have been proposed, as discussed in recent reviews (114, 138).

Transport regulation is poorly understood on a biochemical basis. A variety of agents are known to accelerate the process in heart muscle, including insulin, growth hormones, epinephrine, anoxia, and increased ventricular pressure development, but the substance that directly modulates the activity of the carrier in these circumstances has not been identified. In each case, kinetic studies have established that the carrier-mediated, stereospecific component of sugar entry, as distinguished from a diffusion component, is the process that is accelerated.

In avian erythrocytes, the rate of transport in anoxic cells is inversely related to cell levels of ATP and directly related to accumulation of low energy compounds (227). If ATP is added to the incubation medium of these cells, however, the nucleotide accelerates transport rate. This effect is contrary to the effect expected if ATP were responsible for the aerobic inhibition of transport. In recent experiments (228), NADH has been found to accelerate transport when added to the incubation medium of these cells. The effect of this nucleotide is consistent with a role as an activator of transport in anaerobic cells.

In several of the studies in heart muscle that we discuss, insulin was added to accelerate transport. After addition of the hormone, intracellular free glucose accumulates and the major rate-controlling step of glucose uptake shifts from transport to glucose phosphorylation. Effects of insulin on transport have been reviewed recently (132).

Phosphorylation of Glucose

Phosphorylation of glucose by ATP (glucose + MgATP $\longrightarrow$ glucose-6-P + ADP, Mg) is the first step in glycolysis in mammalian cells, including heart muscle. The reaction is catalyzed by hexokinase and is essentially irreversible under physiological conditions; the equilibrium constant at pH 6.0 in the presence of excess Mg^{2+} was found to be 155 (for review see 196). Glucose-6-phosphatase, the enzyme that catalyzes the reverse reactions in liver, is absent from heart muscle.

Hexokinase in mammalian tissues exists in 4 molecular forms (97). Three of these forms, type I, II, and III, are low K_m enzymes that are found in muscle; but type IV, the high K_m glucokinase, is found in liver. Type II hexokinase accounts for more

than 50% of total activity in heart muscle; the activity of this form is reduced markedly in hearts from diabetic rats (47, 98). Although the isozymes have different K_m values for glucose and ATP, the physiological implications of a shift in isozyme pattern is unknown. All of the isozymes have a molecular weight of about 95,000 (78).

Mammalian hexokinase is found in both the soluble and mitochondrial fractions of tissue homogenates. Partition between these fractions depends upon the composition of the homogenization medium. When 50 m*M* tris buffer is used, approximately 30% of the enzyme activity is soluble (55). Inclusion of ions at concentrations simulating those found in the intracellular water increases the proportion of soluble enzyme to about 70% (98). Since the enzyme that is bound to mitochondria is less sensitive to inhibition by glucose-6-P, partition of enzyme between the soluble and mitochondrial fractions is of importance in correlating rates of glucose phosphorylation in intact hearts with the kinetic properties of the enzymatic activity in homogenates. In addition to ionic strength, glucose-6-P, ATP, ADP, and AMP can release enzyme from mitochondria while Mg^{2+} favors rebinding (196). This finding suggests that partitioning of enzyme between the soluble and mitochondrial fractions may vary depending upon rates of glucose metabolism. This possibility was explored by addition of physiological levels of glucose-6-P, nucleotides, and P_i to brain homogenates (181). Variations in the concentrations of these factors, in the physiological range, had only small effects on mitochondrial binding.

The reaction mechanism of hexokinase appears to involve random addition of substrates (181, 196). Release of products is an important factor in the overall rate with release of glucose-6-P following release of MgADP (103). A number of factors regulate the hexokinases of rat heart, including concentrations of ATP, ADP, AMP, and P_i (55). The K_m for glucose averages about 50 μM and the K_m for ATP is 0.5 m*M*. The dissociation constant for inhibition, K_i, of ATP hydrolysis by glucose-6-P is approximately 0.04 m*M*, while the K_i for inhibition of glucose phosphorylation by glucose-6-P is approximately 0.2 m*M*. Since hearts perfused with buffer containing high perfusate levels of glucose and insulin have intracellular levels of substrates that are 15 to 70 times their K_m values, variation in rate is achieved primarily by enzyme inhibition. The intracellular level of glucose-6-P is the most important factor in determining the rate, accounting for more than 90% of the restraint on glucose phosphorylation. The glucose-6-P inhibition is moderated by tissue levels of ATP (69) and P_i (197). A similar dependence of the rate of glucose phosphorylation on glucose-6-P inhibition was reported earlier in human erythrocytes (195).

Recent studies of Kosow & Rose (104) revealed that the onset of the glucose-6-P inhibition was a biphasic phenomenon. Approximately half of the enzyme was immediately inhibited while restraint of the remaining portion developed with a half-time of 12 or 130 sec for the soluble and bound portions of the enzyme, respectively. The authors suggest that only a portion of the enzyme exists in a form that is immediately inhibited by glucose-6-P and that the delay involves a shift of the remaining enzyme to the inhibition-sensitive form. This property is suggested to allow for rapid build-up of the products ADP and glucose-6-P. ADP signals a burst of O_2 consumption while production of fructose-6-P from glucose-6-P provides adequate levels of substrate for activation of phosphofructokinase.

Regulation of hexokinase activity occurs primarily through changes in levels of the product glucose-6-P. Estimations of the rate in intact cells from data obtained with purified enzymes is complicated by the presence of three forms of the enzyme in heart muscle and by binding of the catalyst to mitochondria. Kinetic properties of the various types of hexokinase have not been studied in detail, the extent of mitochondrial binding of hexokinase in intact cells is uncertain, and the changes in kinetic proerties of the bound as compared to soluble enzyme are incompletely explored.

Phosphofructokinase

Since conversion of glucose-6-P to fructose-6-P, catalyzed by phosphoglucoisomerase, is not an important rate-controlling step in glycolysis, the next site of regulation in the glycolytic pathway is the phosphorfructokinase reaction (fructose-6-P + MgATP $\longrightarrow$ fructose-1,6-diP + MgADP). Regulation at this site was first suggested by Cori (40) in relation to his studies of the effect of work on muscle metabolism. Subsequently, the reaction has been identified as an important rate-controlling step in many cell types, as reviewed recently (15, 120). In the present paper, attention is limited to the physiological role of phosphofructokinase in controlling glycolysis. In this regard, the following factors appear to be of importance: 1. the isozyme of phosphofructokinase that is present, 2. the intracellular pH, and 3. the intracellular concentrations of fructose-6-P, high and low energy phosphates, fructose-1,6-diP, citrate, ammonium ion, and cyclic AMP.

Three isozymes of phosphofructokinase have been identified and labeled A, B, and C (219). Heart and skeletal muscle contain the A isozyme while liver and erythrocytes contain the B form. Other tissues contain both forms and have a complete set of 5 hybrids of the A and B monomers. Brain contains a third monomer, C, which was identified by its lack of reaction to antisera to either the A or B isozymes. The C isozyme has not been purified. Differences in the regulatory behavior of the A and B isozymes have been noted. The B form is sensitive to inhibition by 2,3-diphosphoglycerate, is more sensitive to inhibition by ATP, but less sensitive to inhibition by 3-P-glycerate. The B form is less sensitive to activation by AMP and ADP (100).

Early attempts to purify phosphofructokinase were handicapped by instability of the enzyme. Addition of fructose-1,6-diP stabilized the heart enzyme (120) and led to purification of the protein. The fully active form of phosphofructokinase has a molecular weight of 360,000. Higher molecular weight forms appear which depend on enzyme concentration, pH, and the presence of substrates and modifiers. The specific activity of the more aggregated forms is the same as the form with a molecular weight of 360,000 (1). The enzyme can be dissociated into a relatively inactive dimer at mildly acid pH and can be reassociated at alkaline pH in the presence of fructose-6-P and fructose-1,6-diP (120). In the presence of 6.5 *M* guanidine HCl, the enzyme appears to dissociate into 6 subunits of approximately 67,000 mol wt each (15). In the studies of Kemp & Krebs (101), one mole of fructose-6-P and three moles of ATP were bound per 90,000 g. Later experiments of Mansour (120) indicated that 3.6 moles of ATP and 1.8 moles of fructose-6-P were bound per 90,000 g.

Intracellular pH would appear to have a large effect on phosphofructokinase activity. Assay of the purified heart enzyme at pH 6.9 revealed a sigmoid relationship between enzyme activity and concentration of fructose-6-P. At this pH, the enzyme is markedly sensitive to allosteric regulation. At pH 8.2, the enzyme exhibits Michaelis-Menten kinetics, has increased activity, and is not subject to regulation (120, 218). The effect of pH is particularly great over the physiological range from pH 6.8 to 7.3.

Allosteric control of phosphofructokinase activity is extensive and provides for large changes in catalytic activity. High and low energy phosphates modify activity. ATP and creatine-P inhibit activity while ADP, AMP, and P_i activate the enzyme. The apparent K_i for ATP varies from 0.08–0.3 mM when fructose-6-P concentrations are in the physiological range. The activators are of special importance since their intracellular levels increase in anoxic or ischemic muscle. Newsholme (150) has pointed out the amplification that is achieved by the combined effects of reduced levels of ATP and creatine-P and increased levels of ADP, AMP, and P_i. The amplification mechanism involves adenylate kinase ($2\ ADP \rightleftharpoons ATP + AMP$). Since the equilibrium constant of this enzyme is 0.44, a 15% reduction in ATP levels increases AMP about 5-fold. The effect of this amplification is to markedly increase phosphofructokinase activity with a small fall in ATP.

Fructose-1,6-diP, the product of the reaction, is also a powerful activator. The enzyme binds two moles of this metabolite per 100,000 g (206). One of these sites has a dissociation constant of 0.23 μM and the other of 56 μM. Binding to the low affinity site probably accounts for allosteric regulation at pH 6.9, while binding at the high affinity site affects catalytic activity even at pH 8.2. Removal of fructose-1,6-diP by addition of fructose diphosphatase or aldolase inhibits activity (54). At pH 6.9 this inhibition could be reversed by allosteric activators, while at pH 8.2 only fructose-1,6-diP would overcome the inhibition. The importance of the tight binding of activators is emphasized by the increase in activity that occurs in epinephrine-treated muscle. When phosphofructokinase is extracted from such muscle, it is less sensitive to inhibition by ATP or caffeine (121). This change in behavior is believed to be due to more extensive binding of activators such as fructose-1,6-diP or cyclic-AMP, and not to phosphorylation of the protein.

Citrate inhibits phosphofructokinase and provides a signal coupling oxidation of fatty substrates to glycolysis (120, 177). Citrate is contained within both the mitochondrial and cytoplasmic fractions of the cell. If citrate concentrations in the cytoplasm increase to a greater extent than in the whole heart, compartmentalization of this intermediate could represent an additional mechanism for amplification of phosphofructokinase control (150).

The final substance that may exert a physiologically important control over phosphofructokinase activity in heart muscle is NH_4^+. In hearts perfused with buffer lacking insulin, ammonia production is increased approximately 10-fold (131). This increase is inhibited by addition of the hormone. The dissociation constant for binding for NH_4^+ to phosphofructokinase is 0.33 mM, a value within the physiological range (3). In contrast to other activators, phosphofructokinase exhibits qualita-

tively the same type of allosteric control in its presence. The increased activity is accounted for by a decreased K_m for ATP and fructose-6-P and an increased K_i for citrate and ATP.

Phosphofructokinase is a major control point in the glycolytic pathway that directs glucose residues into either glycogen or production of pyruvate. The enzyme is subject to a complicated series of controls involving energy levels of the cell, substrate concentration, tricarboxylic acid cycle intermediates, and products of amino acid and nucleotide degradation.

Glyceraldehyde-3-P Dehydrogenase

Activation of phosphofructokinase leads to accumulation of fructose-1,6-diP and, in some circumstances, to restriction of glycolytic rate by reactions further down the pathway. The next reaction, catalyzed by aldolase, is the conversion of fructose-1,6-diP to dihydroxyacetone phosphate and glyceraldehyde-3-P. Although the mass action ratio of products and substrates of aldolase in the perfused heart is displaced somewhat from equilibrium (232), this displacement has been suggested to be due to binding of dyhydroxyacetone-P to the enzyme rather than to restraint on flux at this step. Similarly, isomerization of the triose phosphates by triose-P isomerase (dihydroxyacetone-P $\rightleftharpoons$ D-glyceraldehyde-3-P) is not thought to represent an important regulatory reaction in glycolysis. On the other hand, the ratio of dihydroxyacetone-P/glyceraldehyde-3-P in heart muscle does not reach the equilibrium ratio of 22 (232). Evidence for incomplete equilibration has also been obtained in ascites tumor cells and adipose tissue (196). In the perfused heart, however, the product of the mass action ratios of the aldolase and triose-P isomerase reactions is close to the equilibrium value (232), supporting the contention that these are not important regulatory reactions.

Activation of phosphofructokinase appears to shift the control of glycolytic rate to glyceraldehyde-3-P dehydrogenase in hearts perfused under anoxic or ischemic conditions. Evidence that the dehydrogenase may be rate controlling is based on accumulation of fructose-1,6-diP and triose-P without accumulation of intermediates further down the pathway. Several properties of the enzyme appear to account for this behavior (38, 196). 1. The kinetic constants for all reactants are low. When the enzyme is assayed at low concentrations (10^{-9} *M*) at pH 7.4, the K_m for NAD and glyceraldehyde-3-P is 90 and 2.5 μM, respectively, while the K_i for NADH and 1,3-diP-glycerate is 0.3 and 1.0 μM, respectively (71). Since NADH and 1,3-diP-glycerate are competitive with respect to glyceraldehyde-3-P, they may be more effective in setting the intracellular level of glyceraldehyde-3-P than in restricting rate, provided NAD^+ is available. On the other hand, the low K_i for NADH compared to the high K_m for NAD indicates that product inhibition may be an important factor in restricting glycolytic rate in cells with impaired ability to reoxidize NADH. 2. The concentration of enzyme is high relative to either the concentration of substrates or products. The high level of enzyme has been proposed to be due to the fact that the pH optimum is about 2 units above the physiological intracellular pH and that the enzyme is inhibited by ATP (39, 64). 3. At pH 7, and

in the presence of 1.5 m*M* P_i, the ratio of (NADH) (1,3-diP-glycerate)/(NAD) (glyceraldehyde-3-P) would be expected to be about 10^{-3} (196). Since the functional ratio of NADH/NAD may be about 10^{-3}, approximately equal concentrations of glyceraldehyde-3-P and 1,3-diP-glycerate would be expected. When NADH accumulates, glyceraldehyde-3-P levels would rise.

These factors indicate that glyceraldehyde-3-P dehydrogenase within the cell operates under suboptimal conditions due to the intracellular pH and inhibition by ATP. The rate appears to be controlled by product inhibition. In aerobic cells, the level of 1,3-diP-glycerate is probably the more important inhibitor, while in ischemic or anoxic muscle, NADH would assume the dominant regulatory role.

Pyruvate Kinase

The next step in the glycolytic pathway that has been suggested to be a regulatory site is pyruvate kinase (P-enolpyruvate + ADP + H^+ ⇌ pyruvate + ATP). The intermediate reactions catalyzed by phosphoglycerate kinase (1,3-diP-glycerate + ADP ⇌ 3-P-glycerate + ATP), phosphoglycerate mutase (D-2-glycerate ⇌ P-enolpyruvate + H_2O) have not been inplicated as regulatory reactions. The finding that the mass action ratio of products and substrates of pyruvate kinase in the perfused heart apparently was displaced from the equilibrium ratio by 2 orders of magnitude suggested that this enzyme might restrict glycolytic rate (232). The apparent displacement was based on whole tissue levels of ADP and ATP and estimates of intracellular pH and free Mg^{2+} concentration. Restriction of the steady state rate of glycolysis at this step has not been demonstrated in heart muscle.

Muscle contains a form of pyruvate kinase that is distinct from the liver enzyme (for review see 99). The muscle enzyme is inhibited by ATP by competition at the ADP site. Inhibition results in accumulation of metabolites preceding this step, including 1,3-diP-glycerate, a powerful inhibitor of glyceraldehyde-3-P dehydrogenase. Recently, the muscle enzyme has been found to be inhibited in an allosteric manner by phenylalanine. Since this inhibition is relieved by alanine that is present in much higher concentration in heart muscle, the physiological significance of this regulatory mechanism is unclear. It should be noted that the muscle enzyme, as contrasted to liver pyruvate kinase, is not inhibited by fructose-1,6-diP.

Pyruvate Dehydrogenase

Pyruvate produced from glycolysis may be converted to acetyl-CoA, lactate, or alanine in heart muscle. The first two products account for most of the pyruvate. Conversion of pyruvate to lactate as compared to acetyl-CoA is determined by the levels of NADH and acetyl-CoA and the activity of pyruvate dehydrogenase (pyruvate + CoASH + NAD^+ ⟶ acetyl-CoA + CO_2 + NADH + H^+).

Pyruvate dehydrogenase is a multienzyme complex consisting of three enzymes that are directly involved in pyruvate metabolism plus two other enzymes involved in modification of pyruvate dehydrogenase activity (for review see 187). The molecular weight of the complex is approximately 4 million. The reactions are catalyzed by the following enzymes.

1. Pyruvate dehydrogenase [pyruvate + TPP (thiamine pyrophosphate) $\longrightarrow$ 1-hydroxyethyl-TPP + CO_2].
2. Dihydroxylipoyl transacetylase [(*a*) 1-hydroxyethyl-TPP + lipoic $acid_{oxid} \longrightarrow$ acetyllipoic $acid_{red}$ + TPP; (*b*) acetyllipoic $acid_{red}$ + CoASH $\longrightarrow$ acetyl-CoA + lipoic $acid_{red}$].
3. Dihydroxylipoyl dehydrogenase [lipoic $acid_{red}$ + $NAD^+ \longrightarrow$ lipoic $acid_{oxid}$ + NADH + H^+].
4. Pyruvate dehydrogenase kinase [pyruvate dehydrogenase *a* + ATP $\longrightarrow$ pyruvate dehydrogenase *b*].
5. Pyruvate dehydrogenase phosphatase [pyruvate dehydrogenase *b* $\longrightarrow$ pyruvate dehydrogenase *a* + P_i].

The enzymes are linked in the complex by noncovalent bonds. A subcomplex consisting of the transacetylase and dihydroxylipoyl dehydrogenase, a flavoprotein, can be dissociated. The transacetylase has a molecular weight of about 1 million and consists of 24 identical chains, each containing one molecule of covalently-bound lipoic acid. The pyruvate dehydrogenase portion of the complex has a molecular weight of 154,000. The flavoprotein has a molecular weight of 112,000 and contains 2 moles of FAD. The pyruvate dehydrogenase complex appears to be organized to permit efficient interaction of the individual enzymes.

Activity of the pyruvate dehydrogenase complex is subject to regulation by a phosphorylation-dephosphorylation sequence involving pyruvate dehydrogenase kinase and phosphatase. The kinase is tightly bound to the transacetylase portion of the complex, while the phosphatase is loosely attached. In the presence of Ca^{2+} ions the phosphatase is bound tightly (179). The binding is inhibited by EGTA.[2] The site of phosphorylation is a serine residue on the pyruvate dehydrogenase component. Phosphorylation decreases activity while dephosphorylation restores it. Both the kinase and phosphatase are Mg^{2+} dependent, but the phosphatase requires a Mg^{2+} concentration 10 times greater than the kinase for optimal activity. It has been suggested that the kinase and phosphatase are regulated by the intramitochondrial concentration of free Mg^{2+}, which is dependent on the ratio of ATP/ADP. Pyruvate protects heart pyruvate dehydrogenase from inactivation by the kinase.

Activity of pyruvate dehydrogenase is inhibited by the products of the reaction, acetyl-CoA, and NADH (185). Initially it was thought that product inhibition accounted for restraint of the enzyme in hearts oxidizing fatty substrates. More recently, Wieland and associates (229, 230) have reported that the enzyme is converted from the active to inactive form in hearts of starved or diabetic rats and that the active form is restored by refeeding and insulin treatment. In fed rats, ~70% of total activity was in the *a* form, but only 15% was active in hearts of diabetic or starved rats. Similarly, perfusion of rat hearts for 15 min with buffer containing fatty acids or ketone bodies resulted in a fall of the *a* form from 58 to ~30%. These experiments suggest that the catalytic activity of pyruvate dehydrogenase in the heart is controlled both by product inhibition and interconversion of enzyme forms.

[2]EGTA represents ethylene glycol-bis-(β-aminoethyl ether)-N,N'-tetraacetic acid.

Enzymes Involved in Turnover of Glycogen

Glycogen synthesis and degradation occur by separate pathways and each pathway is subject to a complicated series of control mechanisms. The enzymes involved and their mechanisms of control have been recently reviewed (61, 77, 112, 222). It is beyond the scope of this article to more than outline the reactions and to indicate the mechanisms of regulation.

GLYCOGEN SYNTHESIS Three steps are involved in the conversion of glucose-6-P into glycogen. These are catalyzed by phosphoglucomutase (glucose-6-P $\rightleftharpoons$ glucose-1-P), uridyl transferase [UTP + glucose-1-P $\rightleftharpoons$ uridine-diP-glucose (UDPG) + pyrophosphate], and glycogen synthase (UDPG + glycogen$_n$ $\longrightarrow$ glycogen$_{n+1}$ + UDP). Regulation of glycogen synthesis occurs at the synthase step.

Glycogen synthase occurs in two forms: *a* and *b*. Conversion of one form to the other depends on a phosphorylation-dephosphorylation mechanism. The *a* form is dephosphorylated. Its affinity for UDPG is increased by glucose-6-P, but the maximal rate of the reaction is unaffected. This form is relatively insensitive to inhibition by ATP and other nucleotides. Synthase *a* is converted to synthase *b* by phosphorylation of the protein. This reaction is catalyzed by a cyclic-AMP-dependent protein kinase (222). The *b* form is essentially inactive in the absence of glucose-6-P and the activation is inhibited by ATP and other nucleotides. Synthase *b* is reconverted to the *a* form by synthase phosphatase. Inhibition of the phosphatase by glycogen results in a progressively lower percentage of synthase in the *a* form as polysaccharide is stored. By this mechanism, glycogen shares in the regulation of its synthesis.

GLYCOGEN BREAKDOWN Only the phosphorylase reaction is required for conversion of a glucose residue from glycogen to glucose-1-P (glycogen$_{n+1}$ + P_i $\longrightarrow$ glycogen$_n$ + glucose-1-P). Phosphorylase also occurs in two forms, *a* and *b*. The *a* form is the phosphorylated form of the enzyme and is active in the absence of AMP. It is converted to the *b* form by phosphorylase phosphatase. Activity of the *b* form is dependent upon AMP for activity, and the activity is inhibited by ATP and glucose-6-P. Reconversion to the *a* form is catalyzed by phosphorylase kinase. Activity of the kinase also is regulated by a phosphorylation-dephosphorylation mechanism and is increased by low levels of Ca^{2+}. The cyclic-AMP-dependent protein kinase is responsible for activation of phosphorylase kinase.

In heart muscle, turnover of glycogen is regulated both by hormones and nonhormonal factors. Epinephrine and glucagon activate adenyl cyclase, increase cyclic-AMP levels, and result in conversion of phosphorylase to the *a* form and synthase to the *b* form. Insulin increases the fraction of synthase in the *a* form. The major nonhormonal factors involved in regulating turnover of glycogen are the intracellular levels of 5'-AMP, ATP, glucose-6-P, P_i, and glycogen itself.

INTEGRATED CONTROL OF CARBOHYDRATE METABOLISM

As discussed in the introduction to this paper, studies of the control of carbohydrate utilization in intact tissues depend upon identification of rate-controlling reactions

by comparison of the mass action ratios of substrates and products under various conditions with the equilibrium ratio and by cross-over plots. In some instances the levels of metabolites are too low for reliable analysis by existing techniques. In these cases, the contribution of a pair of reactions has been considered together. This situation pertains to the glyceraldehyde-3-P dehydrogenase and phosphoglycerate kinase steps where the level of 1,3-diP-glycerate is usually too low to be measured (232). Additional factors complicating detection of nonequilibrium and rate-controlling reactions are compartmentalization of metabolites of the glycolytic pathway into two or more pools or binding of metabolites to muscle proteins. ATP and ADP are distributed between the cytoplasmic and mitochondrial spaces in heart muscle. In addition, ADP is bound to actin (127). Glucose-6-P in diaphragm muscle has been suggested to be divided into separate compartments for glycogen synthesis and for conversion to pyruvate (6, 110). Since inorganic phosphate binds to muscle proteins (75, 83), the intracellular concentration calculated from whole tissue analyses probably overestimates the concentration available either as a substrate for phosphorylase or glyceraldehyde-3-P dehydrogenase or as an activator of phosphofructokinase and hexokinase. On the other hand, studies of carbohydrate utilization in heart muscle are simplified by the absence of glucose-6-phosphatase, fructosediphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase (42, 164). Although the hexosemonophosphate shunt is potentially active in heart muscle (37), the bulk of the carbohydrate appears to be utilized via the main glycolytic pathway.

Regulation of Carbohydrate Utilization in Well-Oxygenated Hearts

Both in vivo and in the isolated perfused rat heart, fatty substrates are used preferentially to glucose and glycogen (for review, 146). In the heart of man or the unanesthetized dog, 35–75% of oxygen consumption can be accounted for by oxidation of plasma FFA (113, 118, 124, 139). Similarly, in the perfused rat heart 80–100% of oxygen consumption can be accounted for by uptake of short- or long-chain fatty substrates. If an exogenous supply of fatty substrates is not provided, the heart uses endogenous stores of triglyceride in preference to exogenous glucose.

In well-oxygenated hearts that are developing low levels of ventricular pressure, glucose transport is the major restriction to utilization of exogenous glucose and glycogen breakdown is restrainted at the phosphorylase reaction (130, 134). In addition, the mass action ratios of four reactions in the glycolytic pathway are displaced from the equilibrium ratio by 1 order of magnitude or more. These include hexokinase, phosphofructokinase, glyceraldehyde-3-P dehydrogenase, and pyruvate kinase (232).

The rate of glucose transport in well-oxygenated hearts depends upon the levels of insulin bound to the tissue, the sensitivity of transport to stimulation by insulin, and the availability of fatty substrates (for review see 138). Transport is inhibited in hearts of diabetic rats due to low levels of insulin, but, in addition, transport is relatively insensitive to stimulation by insulin (for review see 132). Decreased insulin sensitivity is related to high plasma levels of growth hormone, glucocorticoids, and fatty acids. In hearts of hypophysectomized-diabetic rats the basal transport rate

remains low due to insulin deficiency, but sensitivity to stimulation by insulin is restored by removal of the pituitary. Perfusion of rat hearts with buffer containing fatty substrates reduces insulin sensitivity (141), while inhibition of fatty acid oxidation with α-bromopalmitate restores insulin sensitivity of diabetic hearts to normal (26, 183). The inhibitory effect of fatty acid is evident when either entry of glucose or nonmetabolized analogs is measured. Inhibition of transport by fatty acid is sufficient to prevent accumulation of intracellular free glucose despite inhibition of intracellular glucose phosphorylation, except when high levels of insulin are present.

Glycogen breakdown is restrained in the well-oxygenated heart in a number of ways. 1. Phosphorylase is almost entirely in the *b* form due to low activity of phosphorylase kinase (180). 2. Activity of the *b* form is restrained by high tissue concentrations of the inhibitors ATP and glucose-6-P, and low concentrations of the activator 5'-AMP (135). 3. The remaining activity of the *b* form is controlled by tissue levels of inorganic phosphate. Intracellular concentrations of P_i are below the K_m of the inhibited enzyme for this substrate.

The combined effects of fatty acid oxidation are to markedly reduce glucose utilization and to divert most of the substrate that is taken up into glycogen. This effect is particularly apparent in hearts of alloxan diabetic rats. The plasma of these animals contains high levels of FFA and the heart contains increased stores of triglyceride (185). Although glucose uptake by these hearts is markedly restricted (129), the glycogen content of the diabetic heart is twice normal. Increased accumulation of glycogen is explained by a profound inhibition of phosphofructokinase by citrate (177). High levels of citrate accumulate in hearts oxidizing fatty substrates (73, 176). As a result of the inhibition of phosphofructokinase, higher tissue levels of fructose-6-P and glucose-6-P accumulate and shift the pathway of glycogen turnover toward storage of polysaccharide.

Regulation of the portion of the glycolytic pathway between intracellular glucose and acetyl-CoA can be studied more directly by removing the restraint imposed on these reactions by sugar transport. Addition of insulin leads to accumulation of measurable levels of intracellular free glucose and a shift in the control of glycolytic rate to reactions within the cell (62, 130). In these circumstances, the rate of glucose phosphorylation can be expressed as a function of intracellular glucose concentration. As discussed above, the activity of hexokinase is regulated primarily by the intracellular concentration of glucose-6-P. The intracellular level of this intermediate depends upon the activity of phosphofructokinase. Addition of fatty acids to the perfusion medium of isolated hearts restrains glucose phosphorylation due to inhibition of this enzyme (185). Similarly, hearts removed from diabetic rats have a markedly impaired rate of glucose phosphorylation that is associated with high tissue levels of glucose-6-P, fructose-6-P, and ATP, and low levels of fructose-1,6-P (129, 152, 175, 186, 191). Impairment of glucose phosphorylation contributes to the delay in return of blood sugar to normal following administration of large doses of insulin to severely diabetic rats. A period of 4–24 hr is required for return of phosphorylation to normal following insulin treatment (137). Hearts removed from hypophysectomized diabetic rats do not have an impaired rate of glucose phospho-

rylation, but a block can be induced by treatment of the animal with growth hormone and glucocorticoids (137). Return of inhibition is associated with increased tissue levels of citrate and a cross-over point at the phosphofructokinase reaction (152, 191).

The final site at which fatty acid restrains glycolysis is at the pyruvate dehydrogenase reaction. Acetyl-CoA and NADH, products of the reaction, inhibit the enzyme and lead to conversion of a larger fraction of the pyruvate to lactate (185). Additions of a variety of fatty substrates to the perfusate inhibit pyruvate uptake by the rat heart.

Skeletal muscle also uses fatty acids as a major substrate for oxidative metabolism (28, 84, 85, 118, 182). In patients subjected to a prolonged fast, carbohydrate utilization by skeletal muscle is nil (27). This reduction represents an essential homeostatic mechanism to spare glucose for utilization by the brain and other tissues that are more dependent upon it as a fuel for oxidation. A number of studies have attempted to determine whether preferential utilization of fatty acid in skeletal muscle of fasting or diabetic animals is related to the low plasma levels of insulin and the accompanying slow rates of glucose transport, or whether fatty acids inhibit glycolysis in this tissue as they do in heart muscle (12, 91, 92, 94, 204). A number of factors other than absence of an effect of fatty acid may account for inability to demonstrate these inhibitory effects. 1. Since most of the skeletal muscle preparations that were used were not perfused, access of the fatty acid to the muscle cells cannot be assured. Inhibition of glucose uptake in the diaphragm by ketone bodies has been observed (166, 185). 2. In skeletal muscle, the capacity of glucose phosphorylation is relatively great as compared to the capacity of transport. Even when transport is stimulated by addition of insulin, intracellular free glucose accumulates only if phosphorylation is restrained by a reduction in the incubation temperature (174). 3. Effects of fatty acid on glucose transport in isolated muscle preparations that are contracting have not been investigated. In future studies, it would appear useful to use skeletal muscle preparations from trained rats in which the capacity for fatty acid oxidation is increased (7, 126). This possibility is suggested by the observation that citrate levels increase to a more limited extent in skeletal than heart muscle following fat feeding (4).

To recapitulate, glycolysis in well-oxygenated heart muscle is retrained at the level of glucose transport, hexokinase, phosphofructokinase, and pyruvate dehydrogenase. Factors involved in this restraint are tissue levels of insulin and triglycerides and the availability of fatty substrates in the perfusate.

Effects of Increased ATP Consumption on Carbohydrate Utilization

An increase in ATP and O_2 consumption in heart muscle accompanies increased ventricular pressure development or increased heart rate. An increase in pressure development arises either from an increased end-diastolic volume due to higher atrial filling pressure or higher resistance in the aortic outflow tract (133, 143, 159). In addition, increased utilization of ATP and O_2 is induced by addition of inotropic agents such as epinephrine or glucagon (13, 63, 188). In some instances, particularly

in epinephrine-treated hearts, the demand for oxygen exceeds the supply (63). In these cases, the effects of increased ATP utilization is complicated by effects of insufficient O_2 supply.

When hearts are perfused with substrate-free buffer, increased demand for oxidative substate results in rapid utilization of endogenous stores of triglyceride and glycogen (45, 141). Decreased tissue concentrations of glucose-6-P, together with increased levels of inorganic phosphate, account for an accelerated rate of glycogen breakdown by phosphorylase *b* (47, 148). Conversion of phosphoyrlase *b* to *a* is not a prominent component of the effect. Although ATP utilization is increased, tissue levels of ATP were unaffected by the increase in ventricular pressure development until depletion of polysaccharide was nearly complete.

When glucose is present in the perfusate, an increase in ventricular pressure development accelerates glucose uptake and oxidation (44, 133). This effect is accounted for by more rapid rates of both glucose transport and phosphorylation (133, 142, 144, 163). Acceleration of transport involves an increase in maximal transport rate and can be demonstrated with glucose and the nonmetabolized analogs, L-arabinose and 3-O-methylglucose. The biochemical mechanism by which increased pressure development is transduced into faster transport is unknown. Although an increase in ventricular pressure development makes transport more sensitive to the stimulatory effects of insulin (133), the hormone is not required for the effect on transport since an increase in work by hearts of diabetic rats raised glucose uptake to normal (30).

Although transport is accelerated in hearts that develop higher levels of ventricular pressure, measurable levels of intracellular free glucose do not accumulate (144), indicating that glucose phosphorylation accelerates in concert with glucose transport. Similarly, a 4-fold increase in the rate of flux through phosphofructokinase is accompanied by an increase in fructose-1,6-P but not in glucose-6-P, indicating that phosphofructokinase is accelerated along with transport and hexokinase (142, 148, 163). Factors accounting for the activation of phosphofructokinase in hearts developing increased ventricular pressure are the subject of controversy. Opie et al (163) reported that ATP, creatine-P, and citrate concentrations fell while ADP, AMP, and P_i increased in hearts developing higher levels of ventricular pressure. Although the changes could account for activation of phosphofructokinase, they were not observed in the earlier work of Neely et al (141) or in later experiments (142) in which an attempt was made to reproduce Opie's results. It is our opinion that factors accounting for activation of phosphofructokinase in hearts developing increased rates of ventricular pressure remain to be defined. In support of this opinion, increased work did not reduce high energy phosphates in a rat heart-lung preparation (125).

Provision of both palmitate and glucose to hearts developing higher levels of ventricular pressure results in preferential use of the fatty acid (141). Similarly, acetate is used in preference to glucose when this pair of substrates is supplied (142). Preferential utilization of fatty acid involves inhibition of phosphofructokinase, phosphorylase, and glucose transport. Inhibition of phosphofructokinase is ac-

counted for by higher tissue levels of citrate and creatine-P (148) and leads to higher concentrations of glucose-6-P. Slower rates of glycogen utilization are accounted for by inhibition of phosphorylase *b* and activation of glycogen synthetase by glucose-6-P. The 6- to 8-fold acceleration of glucose transport, resulting from increased ventricular pressure development in hearts that are provided only glucose, is completely inhibited by inclusion of fatty acid in the perfusate (141). The inhibitory effect also is seen when transport of 3-O-methylglucose is measured. A variety of fatty substrates, including acetate and β-hydroxybutyrate, as well as palmitate, are inhibitory. When hearts are perfused under anaerobic conditions, the inhibitory effects of fatty acid on glucose transport do not occur. These observations indicate that oxidation of fatty acid is required for the effect of this substrate.

Exposure of the heart to epinephrine or glucagon increases the force and frequency of contraction and results in increased utilization of oxygen and glucose (107, 175, 231). Accelerated uptake of glucose persists after the period of rapid glycogenolysis has passed and results in a large increase in lactate production. Inhibition of the inotropic effect of epinephrine with dichloroisoproterenol abolishes the glycogenolytic effect of epinephrine and partially inhibits the stimulation of glucose uptake. The rapid phase of glycogenolysis in glucagon- or epinephrine-treated hearts is associated with conversion of a large fraction of phosphorylase to the *a* form and with increased tissue content of cyclic AMP (41, 192, 233). Faster rates of glycolysis involves activation of glucose transport and phosphorylation and phosphofructokinase (233). Epinephrine increases tissue levels of AMP, ADP, creatine, and inorganic phosphate while the level of ATP falls. These changes account for activation of phosphofructokinase. During the period of maximal phosphofructokinase activity, the major restriction of glycolysis is shifted to glyceraldehyde-3-P dehydrogenase (233).

Increased ATP utilization that is associated with increased ventricular pressure development accelerates glucose uptake and glycogenolysis unless fatty substrates are supplied to the heart. Faster rates of carbohydrate utilization involves activation of glucose transport, hexokinase, phosphofructokinase, and phosphorylase *b*. The activities of hexokinase and phosphorylase *b* are controlled by intracellular levels of glucose-6-P. Factors regulating glucose transport and phosphofructokinase in these experiments remain to be defined. If the need for oxygen exceeds its delivery, the heart may become relatively hypoxic, as indicated by depletion of high energy phosphates and accumulation of low energy products.

Effects of Decreased ATP Production on Carbohydrate Utilization

The most common cause of decreased ATP production in heart muscle is restriction of oxygen supply. Reduced oxygen availability results from decreased or zero oxygen tension in the perfusate (hypoxia or anoxia) or from decreased flow of well-oxygenated perfusate through the coronary vessels (ischemia). In some experimental situations, ischemia is defined as reduction of blood flow to zero (108, 236), but in pathological situations in man, minimal flow often remains (95). In many earlier studies hypoxia and anoxia were investigated as models of ischemic damage to the

heart. As will be discussed, however, hypoxia and anoxia markedly accelerate consumption of glucose and glycogen while ischemia inhibits utilization of these substrates. In future work, these conditions must be clearly distinguished.

In the Langendorff or working heart preparations, hypoxia and anoxia are induced by perfusing the tissue with buffer containing reduced or zero oxygen tensions (136, 151, 234). Coronary flow in these preparations is directly related to the perfusion pressure which is maintained by a peristaltic pump. In order to investigate the effects of ischemia, modifications of the working heart technique were introduced, as discussed earlier in this paper, which allow for dissociation of aortic and coronary flow (147). These models have the advantage that perfusion of the entire heart can be reduced in a controlled fashion. Rates of glucose and glycogen consumption can be determined and levels of metabolites within the pathway can be measured.

Other models of ischemia that have been studied include transection of the aorta (108, 236) and ligation of major coronary vessels (20). Aortic transection results in an abrupt and total cessation of coronary flow and allows for investigation of factors regulating consumption of glycogen stores. This model does not provide for investigation of utilization of exogenous glucose or release of metabolic products from the heart. Ligation or constriction of major coronary vessles most closely simulates myocardial ischemia in man, but mechanisms of control of substrate utilization are difficult to investigate. The problems arise from inclusion of ischemic muscle within a mass of normal muscle which performs more work as a result of the occlusion.

Perfusion of hearts with a buffer containing glucose and gassed with N_2:CO_2 results in a 10- to 20-fold increase in glucose uptake and a rapid rate of glycogen breakdown. Acceleration of glucose transport, hexokinase, phosphorylase, and phosphofructokinase accounts for the increase in flux (41, 136, 151, 175, 191, 234). Reactions in the glycolytic pathway beyond phosphofructokinase accelerate in response to higher levels of substrate. The increase in catalytic activity of these reactions can be accounted for on the basis of the properties of the purified enzyme, with the exception of membrane transport (55, 135, 177). As noted above, however, anoxia stimulates the carrier-mediated component of sugar entry. Acceleration of phosphofructokinase is accounted for by increased intracellular levels of activators of the enzyme, ADP, AMP, and P_i, and decreased levels of the inhibitors, ATP and creatine-P. Increased activity leads to depletion of fructose-6-P and accumulation of fructose-1,6-P, another activator of the enzyme. Acceleration of hexokinase results from decreased levels of glucose-6-P, a powerful inhibitor, together with increased levels of P_i, a competitive inhibitor of the glucose-6-P inhibition. Activation of phosphorylase involves conversion of a portion of the enzyme to the *a* form and increased activity of phosphorylase *b* (41, 236). Conversion to the *a* form is partially blocked by depletion of endogenous stores of catecholamines or by addition of β-adrenergic blocking agents (236). The catecholamine-dependent portion of phosphorylase *b* to *a* conversion correlates with increased tissue levels of cyclic AMP and increased activity of phosphorylase kinase (105). The activity of phosphorylase *b* is increased by higher tissue levels of AMP and P_i, an activator and substrate of the enzyme, respectively, and decreased levels of ATP and glucose-6-P, inhibitors of the enzyme (135).

Although glycolytic flux is accelerated in anoxic hearts, the increase in rate generates only about 10–15% of the ATP that is formed in well-oxygenated tissue (79, 93, 108). In the anaerobic heart, ATP consumption decreases as a result of ventricular failure which occurs when only about 20% of the ATP is depleted. Failure has been suggested to result from a fall in intracellular pH which interferes with uptake and release of Ca^{2+} from the sarcoplasmic reticulum (140), or from failure of Ca^{2+} to bind to the contractile proteins (96).

The failure of glycolytic flux to increase sufficiently to supply optimal levels of ATP is due to restraint at the glyceraldehyde-3-P dehydrogenase step (234). Restriction of flux through this step could be accounted for by high tissue levels of NADH or 1,3-diphosphoglycerate, powerful product inhibitors of the enzyme. As noted above, levels of 1,3-diphosphoglycerate are below the level of detection in extracts of muscle. Since the levels of 3-P-glycerate and 2-P-glycerate are not increased in anaerobic muscle, it is likely that the level of 1,3-diphosphoglycerate is also unchanged by the shift to an anaerobic environment. If this is the case, intracellular levels of NADH would represent the major restraint on glyceraldehyde-3-P dehydrogenase. Intracellular NADH levels reflect the availability of hydrogen acceptors, such as dihydroxyacetone phosphate and pyruvate, and the rapidity with which the reduced products, α-glycerol phosphate and lactate, are released from the cell. High levels of α-glycerol phosphate accumulate in anoxic muscle, presumably as a result of low membrane permeability. Lactate efflux is more rapid, but intracellular levels of this product increase 2- to 3-fold in anaerobic hearts (199). It would appear that the glycolytic rate could be enhanced in anaerobic muscle if washout of lactate could be accelerated or if substrate levels of another hydrogen acceptor were provided. Deterioration of mechanical function is delayed in these hearts if increased concentrations of glucose and/or insulin are provided or if the heart contains more glycogen (86, 88, 202, 223, 224).

In contrast to the sustained increase in glycolytic flux in anaerobic mucle, induction of ischemia results in a transient increase in glycolytic flux which is followed by inhibition (199). Inhibition of glycolysis occurs even through intracellular levels of ADP, AMP, and P_i are increased.

The transient acceleration of glycolytic flux principally involves consumption of the glycogen stores (199, 236). In the aortic transection model, acceleration of glycolysis is associated with conversion of phosphorylase *b* to *a* with increased tissue levels of cyclic AMP (237). Comparable measurements are not available at present for the ischemic rat heart. During this phase of increased glycolytic flux, levels of glycose-6-P and fructose-6-P increase in hearts perfused in the absence of insulin. When glycolysis is accelerated by addition of the hormone, control levels of the hexose monophosphates are raised and their intracellular levels are unchanged during the transient acceleration of glycolysis induced by ischemia. In both the presence and absence of insulin, intracellular levels of fructose-1,6-P, glyceraldehyde-3-P, and dihydroxyacetone-P are increased, indicating activation of phosphofructokinase. This activation can be accounted for by higher tissue levels of ADP, AMP, and P_i and reduced levels of ATP and creatine-P.

As the reduction in blood flow is maintained, the glycolytic rate falls below that seen in well-oxygenated hearts and is about one tenth the rate encountered with

anoxia (199). Inhibition of the glycolytic rate is not overcome by addition of insulin to the perfusate. In this circumstance, the rate-controlling step appears to be glyceraldehyde-3-P dehydrogenase (unpublished observations). This conclusion is based on the presence of high levels of glyceraldehyde-3-P, low levels of the metabolites beyond this step, and an impaired rate of glycolysis. Restraint at this step appears to involve high levels of NADH. In the ischemic muscle, lactate accumulation is more marked than in anaerobic hearts. Along with the accumulation of lactate, α-glycerol phosphate levels increase about 10-fold above control values. Increased accumulation of lactate in ischemic as compared to anaerobic muscle is poorly understood, but involves impaired extracellular washout of this product due to reduction in coronary flow, the effect of pH on levels of undissociated lactate (89), and, possibly, the effects of ischemia on the membrane transfer of lactate. Interventions to improve glycolytic rate in ischemic muscle should be directed toward accelerating release of lactate from the muscle or toward provision of other hydrogen acceptors for reoxidization of NADH.

A number of factors could be suggested to account for the marked difference in glycolytic rate in ischemic as compared to anoxic muscle. Increased accumulation of lactate in the ischemic muscle could decrease intracellular pH more markedly than in anoxic muscle. Decreased extracellular pH decreases glycolytic rate in the isolated rat heart, while increased pH accelerates glycolysis (50, 160, 203). Phosphofructokinase is partially sensitive to pH in the physiological range (218). Estimation of intracellular pH from the distribution of dimethadione (29), however, indicates that intracellular pH decreases only slightly in ischemic muscle while the extracellular pH falls by about 0.6 pH units (unpublished observations).

A second explanation for the difference between anoxia and ischemia may involve the effect of a slow washout of lactate on the intracellular level of NADH. In this case, glycolytic rate would be controlled by removal of the reduced product of glycolysis. If ischemia is less severe, glycolytic rate could increase and finally approach that seen in anaerobic muscle (20, 46, 162). Inhibition of glycolysis in ischemic muscle most likely involves changes in both intracellular pH and lactate concentrations. It should be noted that glucose can be toxic to cells if reoxidation of NADH does not occur. Under these circumstances, ATP is consumed for production of fructose-1,6-diP without achieving ATP synthesis. The effect is apparent in rapidly glycolyzing cells, such as those of tumors, and forms the basis for the antitumor action of compounds such as oxamic acid (76). These considerations should be kept in mind when attempts are made to restrict damage to ischemic muscle by provision of high glucose levels and insulin.

GENERAL FEATURES OF THE CONTROL OF FATTY ACID AND LIPID METABOLISM

The heart is capable of utilizing both albumin-bound free fatty acids and complex lipids from the blood. The pathways of fatty acid metabolism in cardiac muscle are illustrated in Figure 2. The first step in this pathway is the uptake of fatty acids from

plasma proteins. This process appears to occur by passive diffusion of fatty acids from binding sites on plasma proteins to binding sites on the cell surface proteins. A soluble cytosolic protein which binds fatty acids has been described and may function in distributing fatty acids within the cells (157). Plasma triglycerides are hydrolyzed to fatty acids and glycerol before utilization can occur. This process is catalyzed by lipoprotein lipase that is located on the capillary endothelium. There is some evidence that plasma glycerides may be taken up intact or as partial glyc-

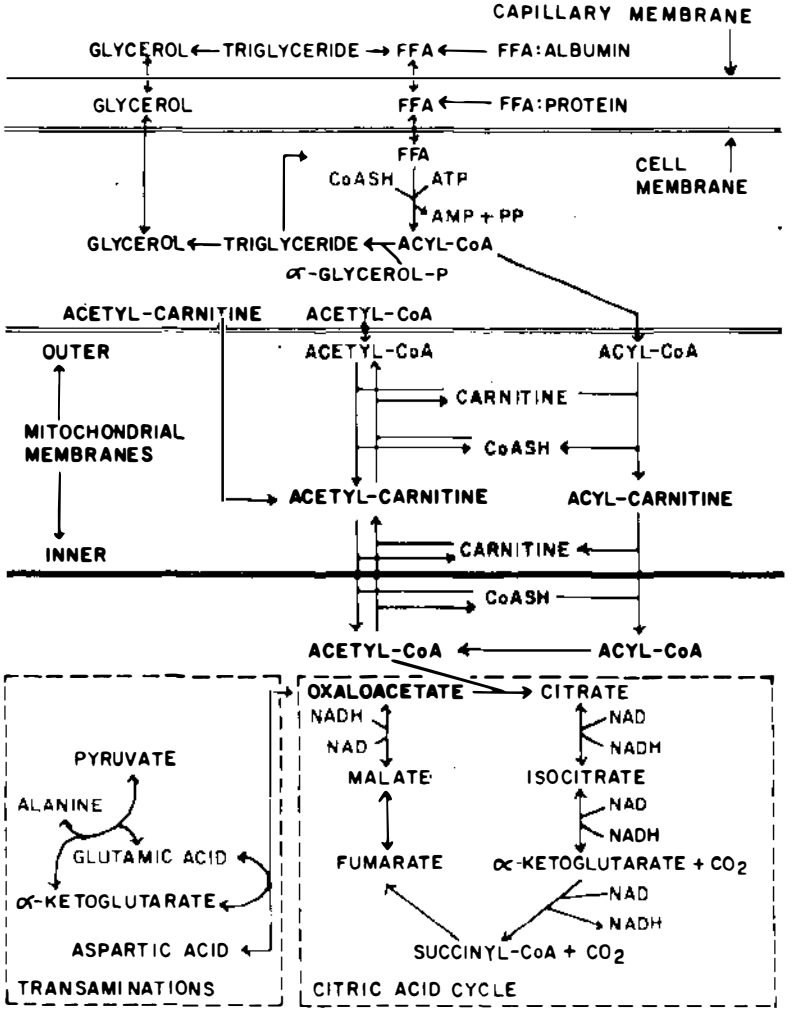

Figure 2 Pathway of fatty acid metabolism in the heart.

erides, especially following myocardial injury from toxic doses of catecholamines (190). Inside the cells, fatty acids are activated to fatty acyl-CoA before further metabolism can occur. Activation is catalyzed by a group of enzymes known as acyl-CoA synthetases. The activated fatty acid can either be esterified to triglycerides and phospholipids or oxidized to CO_2 and H_2O. Acyl-CoA hydrolases are also present that can break down acyl-CoA to fatty acid and CoASH. Tissue lipids can be hydrolyzed to fatty acids and glycerol. Hydrolysis of triglycerides occurs in a stepwise manner with removal of one fatty acid at a time. The process is catalyzed by a series of lipases and the triglyceride lipase appears to be under hormonal control. The fatty acids released from tissue lipids can be reactivated and oxidized.

In heart muscle, oxidation of long-chain fatty acids is completely dependent on the presence of carnitine. The acyl unit from acyl-CoA is transferred to carnitine by carnitine-acyl CoA transferases that are located between the inner and outer mitochondrial membranes. The acyl unit is translocated through the inner mitochondrial membrane and transferred to CoASH inside the mitochondrial matrix by a second carnitine-acyl CoA transferase located on the inner membrane. Carnitine and its acyl derivatives are located exclusively in the cytosolic compartment, whereas CoASH and its acyl derivatives are compartmentalized in both the cytosol and mitochondrial matrix. The inner membrane does not appear to be permeable to either CoASH, carnitine, or their acyl derivatives.

Intramitochondrial acyl-CoA is oxidized to acetyl-CoA by the β-oxidation system. The acetyl-CoA that is produced is oxidized by the citric acid cycle or transferred to cytosolic carnitine and CoASH by carnitine-acetyl CoA transferases similar to the long-chain acyl transferases.

A number of reviews on fatty acid metabolism have been published. For detailed discussions of many aspects of this subject, the reader is referred to these references (14, 22, 23, 57, 65, 146, 161, 221).

Rate-Controlling Reactions in Fatty Acid Metabolism

The rate-controlling steps in fatty acid metabolism have not been as well established as they have for carbohydrate metabolism. The rate of utilization is dependent on both the plasma concentration of fatty acids and on the energy demands of the tissue. The availability of plasma fatty acids, as well as alternative substrates, may control the rate of utilization. In addition, lipoprotein lipase appears to be under hormonal control and its activity varies with the nutritional and hormonal status of the animal (193). At any plasma concentration, however, the rate of fatty acid utilization from the plasma or from tissue lipids depends on the energy requirements of the tissue.

The intracellular steps that limit utilization of fatty acid will be discussed in the following sections on individual enzyme systems. However, it is important to point out that the steps that have been reported to limit utilization by isolated mitochondria may be different than those that function in the intact tissue. Many of the conclusions about rate-controlling steps have been derived from studies of the capacity of enzyme systems, i.e. acyl-CoA synthesis or acyl transfer and β-oxidation, in isolated mitochondria. As pointed out by Bremer & Wajtozak (22), the in

vitro capacity of an enzyme system gives little indication of its role as a rate-controlling reaction in vivo. Some of the problems encountered in studying regulation of fatty acid utilization, as mentioned earlier in this review, include the fact that long-chain fatty acids and long-chain acyl-CoA and acyl-carnitine derivatives are only slightly soluble in water, form micelles (241), and bind extensively to soluble and membrane proteins (171). Many of the mitochondrial studies have been conducted with dilute solutions of mitochondria and in some cases without added proteins (22). Therefore, the ratio of long-chain fatty substrates to protein was very different from the ratio in the intact cell. Consequently, concentrations of substrates that have been found to give maximal rates of utilization with isolated mitochondria cannot be expected to behave similarly in the in vivo condition. On the other hand, studies with intact tissue involve measurements of the whole cell content of metabolic intermediates. Reliable methods for distinguishing between cytosolic and mitochondrial matrix metabolites have not been developed. The concentration of unbound fatty acids in the cell has not been measured due to uncertainties of fatty acid binding to proteins in the interstitial and intracellular compartments. In intact cells, one is forced to deal with minimal or maximal possible levels of substrates rather than with concentration of the unbound substrate.

Mass Action Effect of Increased Plasma Fatty Acids

The rate of fatty acid utilization depends to a large extent on the fatty acid:albumin molar ratio in the plasma (58, 161, 213). With an increase in this ratio, fatty acids bind to sites with less affinity and the rate of uptake by the cells is increased. The rate of uptake is not a linear function of plasma concentration, however, and at high concentrations, cellular binding sites and the capacity of the cells to metabolize fatty acids is saturated. With isolated perfused rat hearts developing low levels of ventricular pressure, a concentration of about 0.5 m*M* palmitate bound to 3% albumin gives maximal rates of uptake (165). Since this is a higher fatty acid:albumin ratio than is normally found in vivo, the rate of uptake in intact animals could be expected to vary with the plasma concentration up to about 0.8 m*M* palmitate. With increased cardiac work and increased ATP consumption, a higher fatty acid:albumin ratio was required to saturate uptake.

Hydrolysis and Uptake of Plasma Lipids

This reaction (plasma triglycerides $\longrightarrow$ glycerol + 3 fatty acids) occurs on the capillary endothelium prior to uptake of glyceride fatty acid by the heart (193). It is catalyzed by lipoprotein lipase (clearing factor lipase), 80% of which is associated with the capillary endothelium and is released with heparin. Activity of clearing factor lipase in adipose tissue is high in fed animals when triglyceride fatty acid is taken up for storage. This activity is decreased in fasted or diabetic animals when mobilization of adipose tissue lipid is rapid. Insulin induces a rise in the activity, and epinephrine, ACTH, growth hormone, and thyroid-stimulating hormone decrease the activity. Although the hormonal effects on lipoprotein lipase are opposite to those on the tissue hormone-sensitive triglyceride lipase, both effects appear to be mediated through changes in cyclic AMP. In heart muscle, as contrasted to

adipose tissue, the activity of lipoprotein lipase increases after a 10–24 hr fast and during diabetes in association with accelerated triglyceride oxidation (17). This activity also increased in heart following epinephrine treatment (119) and exercise (154).

Fatty Acid Activation

This general reaction (ATP+fatty acid+CoASH $\rightleftharpoons$ acyl-CoA+AMP+PP_i)[1] accounts for activation of fatty acids in all mammalian tissues (221). It requires Mg^{2+} and is stimulated by other cations, especially K^+. The overall reaction is reversible and involves formation of an enzyme-bound acyl adenylate intermediate (9). The acyl-CoA synthetases that catalyze this general reaction appear to have a broad range of specificity with respect to the fatty acid substrate. Enzymes specific for short, medium, and long-chain fatty acids have been described (2, 10, 22, 65, 221). Acetyl-CoA synthetase is found in all tissues and this enzyme also activates propionate. Enzymes that activate butyrate and propionate but not acetate (butyryl-CoA synthetase) are present in heart mitochondria. The medium-chain fatty acids are activated by octanoyl-CoA synthetase. Longer chain acids, C_{12} to C_{22} both saturated and unsaturated, are activated by palmityl-CoA synthetase, so named because its activity is greatest with palmitate as substrate. At the present time it is not clear whether these activities represent a single enzyme for each group of fatty acids or complexes of different enzymes with greater substrate specificity.

Most of the acyl-CoA synthetases require ATP, but about 3% of the activity in liver is GTP-dependent (117, 170, 198). This latter activity does not appear to be present in heart (23). The GTP-specific enzyme activates short, medium, and long-chain fatty acids. Kinetic studies with partially purified long-chain acyl-CoA synthetase indicate that the K_m values for the forward reaction are 42, 4650, and 50 μM for palmitate, ATP, and CoASH, respectively (8). For the reverse reaction, the K_m values were 2600, 630, and 890 μM for palmityl-CoA, AMP, and PP_i, respectively. In homogenates of heart muscle the K_m for CoASH was about 30 μM (unpublished observations).

The intracellular distribution of activating enzymes varies somewhat from tissue to tissue. In heart muscle, butyryl-CoA synthetase appears to be located in the mitochondrial matrix, whereas acetyl-CoA synthetase is distributed about equally between matrix and cytosol (10). Medium-chain fatty acids appear to be activated exclusively in the mitochondrial matrix (2), which probably accounts for the fact that mitochondrial oxidation of these fatty acids does not depend on the presence of carnitine (65). Palmityl-CoA synthetase activity in heart muscle is located exclusively on the sarcoplasmic reticulum and outer mitochondrial membrane (23, 49, 65).

Physiological regulation of acyl-CoA synthetases by factors other than availability of substrates (fatty acid or CoASH) has not been clearly established. The total activity in various tissues is greater than that required to support the highest rates of fatty acid oxidation and conversion to tissue lipids (22, 167). In vivo, however, regulation of enzyme activity could make this step rate-controlling. Regulation of palmityl-CoA synthetase by a soluble protein in a manner analogous to regulation

of phosphorylase by protein kinase has been proposed (60). Addition of a protein fraction from the supernatant of various tissues was found to activate the microsomal enzyme from liver. Since a high palmitate:albumin molar ratio (6:1) was used in these studies, the effect of the protein fraction could have been to bind fatty acids and palmityl-CoA and to prevent inhibition of the enzyme by these substances. In more recent studies, addition of the supernatant protein fraction did not stimulate synthetase activity (8, 116).

The ATP-dependent enzyme is inhibited by several factors, including α-bromopalmitate, high concentrations of erucate, and atractyloside (5, 168, 171, 173, 210). Palmityl-CoA inhibits the liver enzyme by increasing the K_m for CoASH; this inhibition can be reversed by addition of more CoASH (168). Palmityl-CoA synthetase activity in heart muscle is inhibited by long-chain acyl-CoA, and this inhibition can be relieved by addition of albumin (unpublished observations). Product inhibition of fatty acid activation may have a physiological role in regulating rates of fatty acid utilization in intact hearts. High concentrations of palmitate (0.5–1.2 mM bound to 3% albumin) result in accumulation of long-chain acyl-CoA and acetyl-CoA and greatly reduced levels of CoASH in isolated rat hearts (165). It was proposed that fatty acid activation was limited under these conditions by low levels of CoASH. An increase in the K_m of the enzyme for CoASH caused by accumulation of palmityl-CoA would amplify the effect of low CoASH levels. In support of this, acceleration of fatty acid activation by increased cardiac work was associated with higher tissue levels of CoASH and reduced levels of acyl-CoA.

AMP inhibits palmityl-CoA synthetase activity (5, 172), but accelerates activation and oxidation of short- and medium-chain acids, ketone bodies, and citric acid cycle intermediates (56). As pointed out in studies of the AMP inhibition of palmityl-CoA synthetase, high levels of AMP are generally associated with activation of energy-generating pathways. Inhibition of fatty acid activation by AMP would be contrary to this effect. It must be considered, however, that tissue levels of AMP in heart muscle rarely, if ever, increase in well-oxygenated hearts, but increased levels are associated with conditions in which fatty acid oxidation is impaired, i.e. ischemia, hypoxia, or anoxia. Adenosine also inhibits the enzyme (48). Therefore, inhibition of fatty acid activation by AMP and adenosine in oxygen-deficient hearts may have an energy-sparing effect and may limit the undesirable accumulation of long-chain acyl-CoA derivatives in the tissue.

Triglyceride Homeostasis

Tissue lipids appear to be in a dynamic state at all times. The steady-state levels of tissue triglycerides depend on the relative rates of esterification and lipolysis. Esterification exceeds the rate of lipolysis under some conditions (excess availability of fatty acids and under anoxic or ischemic conditions) and net synthesis of triglycerides occurs. Triglycerides can serve as a source of substrate for energy metabolism in hearts deprived of exogenous fatty acid (45, 52, 158). The rate of lipolysis is accelerated by increased cardiac work or treatment of the tissue with catecholamines (35, 45, 106, 141). Under these conditions, the rate of lipolysis exceeds esterification and the tissue levels of triglycerides decrease.

ESTERIFICATION

(*a*) acyl-CoA + α-glycerol-P ⟶ 1-acylglycerol-3-P (lyso-phosphatidic acid) + CoASH

(*b*) 1-acylglycerol-3-P + acyl-CoA ⟶ 1-2-diacylglycerol-3-P (phosphatidic acid) + CoASH

(*c*) 1-2-diacylglycerol-3-P ⟶ 1-2-diglyceride + P_i

(*d*) 1-2-diglyceride + acetyl-CoA ⟶ triglyceride + CoASH

Acylation of glycerol-3-P occurs in a stepwise manner with addition of one acyl group at a time from long-chain acyl-CoA. Addition of each acyl group appears to be catalyzed by separate enzymes (128). The first acylation is catalyzed by glycerol-3-P acyltransferase, and when saturated acyl-CoA substrates are used it is specific for position one on glycerol-3-P. Monoacylglycerol-3-P acyltransferase catalyzes acylation of the second position forming phosphatidic acid. Lyso-phosphatidic acid and phosphatidic acid have been isolated during liver microsomal and mitochondrial synthesis of complex lipids (200). Phosphatidic acid is an intermediate in the synthesis of both phospholipids and triglycerides. Phosphatidic acid phosphatase catalyzes formation of 1,2-diglyceride. A diglyceride acyl-transferase adds the third acyl group to form triglyceride. These esterification enzymes are found in microsomal, mitochondrial, and lysosomal fractions of cell homogenates.

Little is known about the regulation of lipid synthesis in heart muscle. Exogenous ^{14}C-fatty acids are rapidly incorporated into triglycerides (216). Tissue levels of neutral lipids are higher in hearts from diabetic or fasted animals and can be elevated by perfusion of isolated hearts with buffer containing palmitate (141, 185). These increases are associated with higher tissue levels of fatty acid and fatty acyl-CoA (72, 165). The amount of lipid, the rate of fatty acid conversion to trigylcerides, and the tissue levels of fatty acyl-CoA are increased in ischemic and anoxic myocardium (57, 149, 201). These data imply that the rate of esterification depends on the availability of fatty acid, the rate of fatty acid oxidation, and the tissue levels of acyl-CoA. On the other hand, no correlation could be demonstrated between tissue levels of acyl-CoA and rates of esterification in adipose tissue (51) or liver (200). The activity of palmityl-CoA synthetase in liver mitochondria was some 5–6 times higher than that of glycerol-3-P acyltransferase, suggesting that the activity of the latter enzyme was rate-limiting for triglyceride synthesis (200). If this situation pertains to heart, the increase in neutral lipids that occurs with increased availability or decreased oxidation of fatty acids may not be related solely to higher levels of fatty acyl-CoA. It is of interest that isolated hearts perfused with palmitate utilized endogenous triglycerides at a slower rate and have a lower lipase activity than hearts perfused with glucose (43), indicating that decreased lipolysis may account for much of the net increase in tissue lipids.

Much of the increase in neutral lipids that occurs during oxygen deficiency or exposure to high levels of fatty acids may be related to availability of glycerol-3-P. Tissue levels of glycerol-3-P increase in anoxic (234) and ischemic (unpublished observations) hearts. Increased triglyceride formation in diabetic hearts and in isolated hearts perfused with fatty acid may also be related to increased production of glycerol-3-P (53, 72). Heart muscle contains very little glycerol kinase activity

(153). Since ^{14}C-glucose is incorporated into glyceride glycerol but not to glyceride fatty acid (53, 122), glycolysis appears to be the source of glycerol-3-P for glyceride synthesis.

LIPOLYSIS

(*a*) Triglyceride $\longrightarrow$ diglyceride + fatty acid
(*b*) Diglyceride $\longrightarrow$ monoglyceride + fatty acid
(*c*) Monoglyceride $\longrightarrow$ fatty acid + glycerol

Triglycerides are sequentially hydrolyzed to fatty acids and glycerol. Removal of the first fatty acid by triglyceride lipase is rate-limiting (52). For this reason, most of the neutral lipid present in heart muscle is triglyceride; the ratio being 13:0.1:0.6 for tri-, di-, and monoglycerides, respectively.

Regulation of lipase activity in heart appears to be similar in many respects to that of the adipose tissue enzyme (35). The rate of lipolysis, as measured by glycerol release, is accelerated in hearts removed from diabetic or fasted animals and subsequently perfused in vitro (72, 185), and is stimulated by catecholamines in association with elevated tissue levels of cyclic AMP (35). Unlike the adipose tissue system, however, insulin does not block the rise in cyclic AMP and the increase in glycerol release induced by isoproterenol (35). The mechanism of the accelerated glyceride utilization that is associated with increased cardiac work in isolated hearts perfused without exogenous fatty acid may involve decreased tissue levels of fatty acid and fatty acyl-CoA (165). Since fatty acids are inhibitory to lipase activity (43), increased lipolysis may result from a faster removal of product.

Acyl Transfer and Translocation and the Function of Carnitine

1. acyl-CoA_{out} + $carnitine_{out}$ $\rightleftharpoons$ acyl-$carnitine_{out}$ + $CoASH_{out}$
2. acyl-$carnitine_{out}$ + $CoASH_{in}$ $\rightleftharpoons$ acyl-CoA_{in} + $carnitine_{out}$

These general reactions account for the transfer of long-, medium-, and short-chain acyl units between acyl-CoA and acyl-carnitine in the extramitochondrial compartment (reaction 1) and between cytosolic acyl-carnitine and CoASH within the mitochondrial matrix (reaction 2) (65, 67). "In" and "out" in the above reactions refer to the space within the inner mitochondrial membrane and the combined intermembrane space and cytosol, respectively. These reactions are catalyzed by carnitine-acyl CoA transferases. Transferase systems that are specific for long-chain (carnitine-palmityl CoA transferase), medium-chain (carnitine-octanoyl CoA transferase), and short-chain fatty acids (carnitine-acetyl CoA transferase) have been described (65, 212).

Fatty acids that are activated in the extramitochondrial space, regardless of chain length, apparently cannot penetrate the inner mitochondrial membrane without first being transferred from acyl-CoA to carnitine by reaction 1 above (22, 65). Short- and medium-chain fatty acids can be activated within the matrix by the acyl-CoA synthetases located within this space and their subsequent oxidation is carnitine independent (23, 65). However, oxidation of long-chain fatty acids by heart muscle is completely dependent on the presence of carnitine. A second transferase enzyme is located on or within the inner mitochondrial membrane (66, 80, 90, 156, 220) and,

in some manner, this enzyme transfers the acyl group from acyl carnitine across the membrane and reforms acyl-CoA inside the mitochondrial matrix. As originally proposed by Fritz & Yue (67), the acyl-carnitine derivatives penetrate the inner membrane and transfer of the acyl unit occurred within the matrix space. However, it is now thought that the inner membrane is impermeable to CoASH, acetyl-CoA, carnitine, acetyl-carnitine, and possibly to long-chain acyl-CoA and carnitine derivatives (24, 80).

Yates & Garland (239, 240) modified Fritz's original model to allow for the membrane location of transferase II and for a permeability barrier to both CoASH and carnitine. The model now includes a bimodal distribution of carnitine-acyl CoA transferase with one activity located either on the outer mitochondrial membrane or between the inner and outer membranes (transferase I). Transferase II is more closely associated with the inner membrane and catalyzes the anisotropic translocation of acyl units from cytosolic acyl-carnitine to matrix CoASH without the acyl-carnitine molecule actually penetrating the inner membrane. Two transferase activities with somewhat different properties have been isolated (25, 34, 225). Recently, data has been presented which indicate that long-chain acyl-carnitine does in fact penetrate mitochondrial membranes and that penetration occurs along a hydrogen ion concentration gradient (115). Since free carnitine does not readily penetrate biological membranes, acyl transfer within the mitochondrial matrix would, however, require a mechanism for transporting carnitine back into the cytosol.

The transferase reactions are freely reversible and, based on the measured ratios of substrates and products, are maintained at or near their equilibrium positions in intact tissues (22). The equilibrium constants in the direction of acyl-carnitine synthesis is 0.45 for carnitine-palmityl CoA transferase (155). The equilibrium constant toward acetyl-CoA synthesis is 0.6 for carnitine-acetyl CoA transferase (68). The reported K_m values for the various substrates of carnitine-palmityl CoA transferase range from 5.5 to 45 μM for CoASH, 10 to 30 μM for palmityl-CoA, 45 to 2100 μM carnitine, and 40 to 140 μM for palmityl carnitine (21, 102).

Synthesis of palmityl-carnitine was reported to be rate-limiting for fatty acid oxidation in liver mitochondria (207) but more recent studies have shown that either the capacity to translocate acyl units or the rate of β-oxidation limits the maximum rate of oxidation by isolated mitochondria (22, 167). Mitochondria from heart muscle have the highest capacity to oxidize acyl-carnitine of any tissue studied (16); Fritz (65) concluded that synthesis of palmityl-carnitine was rate-limiting for fatty acid oxidation in muscle mitochondria. However, in isolated perfused hearts the rate of acetyl-CoA oxidation through the citric acid cycle limited the rate of fatty acid utilization at low levels of ATP utilization (142, 165). When oxidative phosphorylation and fatty acid oxidation were stimulated by increased cardiac work, the tissue levels of fatty acyl-carnitine increased in association with decreased levels of fatty acyl-CoA and acetyl-CoA, indicating that oxidation of acyl-carnitine limited maximal rates of fatty acid utilization at fast rates of ATP consumption. Acyl translocation and transfer to matrix CoASH, rather than β-oxidation, appeared to be the

limiting step. This conclusion was based on the observation that oxidation of octanoate, which can bypass the carnitine-dependent translocation step, maintained high tissue levels of acetyl-CoA.

Physiological regulation of long-chain acyl transferases has not been clearly established. Carnitine-palmityl CoA transferase is inhibited by all of its substrates (22, 65). Palmityl-CoA inhibits by increasing the K_m for carnitine; this inhibition may function in vivo to divert acyl units from oxidation to synthesis of triglyceride. Accumulation of palmityl-CoA and inhibition of acyl-CoA synthetase could also function to limit fatty acid activation.

β-Oxidation

1. Acyl-CoA + $FAD^+ \rightleftharpoons$ α-β-unsaturated acyl-CoA + $FADH_2$
2. α-β unsaturated acyl-CoA + $H_2O \rightleftharpoons$ β-hydroxyacyl-CoA
3. β-hydroxyacyl-CoA + $NAD^+ \rightleftharpoons$ β-ketoacyl-CoA + NADH + H^+
4. β-ketoacyl-CoA + CoASH $\rightleftharpoons$ acyl-CoA (–2 carbons) + acetyl-CoA

These four reactions account for the stepwise degradation of fatty acyl-CoAs to acetyl-CoA by the β-oxidation system (221). The enzymes that catalyze these reactions are located in the mitochondrial matrix in close association with the inner membrane (11). In mammalian cells, fatty acids appear to be degraded exclusively by the β-oxidation system (92). The above reactions are catalyzed by acyl-CoA dehydrogenases, enoyl-CoA hydrase, β-hydroxyacyl-CoA dehydrogenase, and thiolase, respectively. Fatty acyl-CoAs with chain lengths from C_4 to C_{20} are readily oxidized by this system. Of the acyl-CoA dehydrogenases, enzymes specific for short-, medium-, and long-chain acyl-CoAs have been isolated (221). Free acyl-CoA intermediates apparently do occur during oxidation of long-chain acyl-CoAs, and under specific conditions shorter chain intermediates have been isolated (217). In isolated mitochrondria, β-hydroxyacyl-CoA can be made to accumulate when the NADH/NAD ratio is high (22). This intermediate can also be transferred to carnitine with accumulation of β-hydroxyacyl-carnitine.

Regulation of β-oxidation in intact cells appears to occur through variations in the levels of substrates (acyl-CoA, NAD^+, and FAD^+) (22). Although the thiolase reaction is reversible, the equilibrium constant is greatly in favor of acetyl-CoA formation (221), suggesting that accumulation of acetyl-CoA would not have a large influence on the overall rate of β-oxidation except through reduced levels of matrix CoASH. The rate of β-oxidation is geared to flux through the citric acid cycle and to the rate of oxidative phosphorylation. The maximum rate is achieved with an energy charge of 0.65 (92). A high NADH/NAD ratio inhibits the β-hydroxyacyl-CoA dehydrogenase and, based on the appearance of β-hydroxyacyl-carnitine, β-hydroxyacyl-CoA accumulates (22). The presence of succinate inhibits the overall rate of β-oxidation in isolated mitochondria apparently by competing for FAD^+, although some decrease in fatty acyl-CoA does occur. A reduction in mitochondrial acyl-CoA by high levels of carnitine or by binding of extramitochondrial acyl-carnitine to albumin shifts the transferase reaction toward formation of acyl-carnitine and lowers the rate of β-oxidation. Similarly, lower levels of long-chain

acyl-CoA and reduced rates of oxidation followed addition of 4-pentenoic acid, whose CoA derivative is oxidized only slowly and accumulates in the mitochondria (70).

In isolated rat hearts perfused with high levels of palmitate, the rate of β-oxidation is geared directly to the rate of oxidative phosphorylation and may be limited at low rates of energy use by the concerted action of high NADH/NAD ratios and the accumulation of acetyl-CoA (146). With increased oxidative phosphorylation as a result of elevated cardiac work, the NADH/NAD ratio decreased, flux through the citric acid cycle increased, and β-oxidation accelerated. Since succinate inhibited β-oxidation in isolated mitochondria by competing for available FAD^+ and CoASH (22), it is of interest that the level of succinyl-CoA decreased as the concentration of palmitate was raised in hearts performing low levels of work, and more of the total CoASH was shifted to formation of long-chain acyl-CoA and acetyl-CoA (146). With increased cardiac work and acceleration of the citric acid cycle and β-oxidation, the reverse shift occurred and the levels of succinyl-CoA increased. These observations indicate that the concentration of succinyl-CoA per se does not greatly influence the rate of either β-oxidation or the activity of citrate synthetase (211) in intact tissue. The level of succinyl-CoA appears to be determined by the rate of oxidative phosphorylation, the tissue level of acetyl-CoA, and flux through the first portions of the citric acid cycle.

Acetyl Transfer and Translocation

Transfer of acetyl units between CoASH and carnitine and translocation between the mitochondrial matrix and cytosol occurs by a two-enzyme system similar to that for long-chain acyl units (see section on acyl transfer above). Carnitine acetyl-CoA transferases have been characterized in heart muscle (65, 68, 123). This enzyme system, like the long-chain acyl transferase, appears to function at or near its equilibrium position under most conditions (165, 178). Palmityl-CoA inhibits acetyl transferase by competing with carnitine (33). In the perfused rat heart, the mass-action ratio for these enzymes is shifted toward synthesis of acetyl-CoA when the concentration of exogenous palmitate is high (165). This effect is associated with higher levels of fatty acyl-CoA and possibly results from acyl-CoA inhibition of acetyl transferases. The shift could function to limit β-oxidation by increasing acetyl-CoA and decreasing CoASH in the mitochondrial matrix.

Since acetate does not represent a major physiological fuel and since a large fraction of the total acetyl-CoA synthetase activity is located in the mitochondrial matrix, the physiological function of carnitine-acetyl CoA transferase is not clear. The activity of this enzyme system is high in tissue with large oxidative capacity (23, 65). It has been proposed that one function of acetyl translocation may be storage of excess high energy acetyl units produced by β-oxidation as acetyl-carnitine and acetyl-CoA in the cytosol (178). Rapid transfer of acetyl units from the cytosolic compartment would buffer large changes in matrix acetyl-CoA. A more recent proposal suggests that this enzyme system may function to couple the rates of cytosolic fatty acid activation and acyl transfer to the rate of oxidation (165). By this mechanism, excess acetyl units produced in the mitochondrial matrix would be

transferred to cytosolic carnitine and CoASH. The lower levels of these substrates in the cytosol could limit fatty acid activation and acyl transfer. With increased oxidative phosphorylation and increased utilization of acetyl-CoA by the citric acid cycle, acetyl units could be rapidly transferred back into the matrix making more CoASH and carnitine available in the cytosol.

INTEGRATED CONTROL OF FATTY ACID METABOLISM

Increased Availability of Fatty Acids

The in vivo control of fatty acid metabolism by the heart encompasses regulation of plasma levels of fatty acids, triglycerides, and other substrates by extramyocardial tissues as well as local control of specific enzyme systems involved in myocardial fatty acid utilization. As indicated previously, the rate of fatty acid uptake depends to a large extent on the fatty acid:albumin molar ratio, and within physiological concentrations, the rate of uptake is directly proportional to the level of plasma fatty acid (165). Also, the increased activity of lipoprotein lipase makes more fatty acid available from plasma glycerides for uptake by the heart when fatty acid is mobilized from adipose tissue. Much of the control of myocardial fatty acid utilization is, therefore, exerted by whole body regulation of fatty acid mobilization and synthesis in adipose tissue and liver (193). Thus, in the postabsorptive state, in diabetes or fasting when plasma levels of insulin are low and fatty acid mobilization is rapid, the rate of fatty acid uptake, oxidation, and storage by heart muscle is increased.

In the presence of a high fatty acid:albumin ratio, the rate of acetyl-CoA utilization through the citric acid cycle limits fatty acid oxidation and the tissue levels of acetyl-CoA, acetyl-carnitine, acyl-CoA, and acyl-carnitine increase, while the levels of free CoASH and carnitine decrease (165). Tissue lipids also increased in hearts perfused with high concentrations of palmitate (43, 141). Unlike liver and adipose tissue, cardiac muscle normally accumulates relatively little triglycerides even when excess fatty acids are available. The restricted accumulation of tissue lipids and the limited rate of fatty acid uptake at high perfusate concentrations of fatty acid may be due to 1. control of the activating enzymes by low levels of CoASH and perhaps by increased levels of acyl-CoA and 2. control of β-oxidation by low levels of CoASH and high levels of NADH (146, 165). Flux through the citric acid cycle is known to be geared to oxidative phosphorylation by changes in the levels of high energy phosphates and NADH (74, 111, 184). Both the level of high energy phosphates and the NADH:NAD ratio increase in hearts oxidizing palmitate as compared to those oxidizing glucose (146). With saturating levels of exogenous palmitate, flux through the citric acid cycle is limited by the dehydrogenase reactions and the level of acetyl-CoA increases about 10-fold. This rise in acetyl-CoA is accompanied by a 50% increase in fatty acyl-CoA, an 8-fold increase in acetyl-carnitine, and a 3-fold increase in acyl-carnitine. Since acetyl-carnitine is located in the cytosol and is in equilibrium with both the cytosolic and matrix pools of acetyl-CoA through the carnitine-acetyl CoA transferase system, the rise in acetyl-carnitine probably indicates that much of the increase in acetyl-CoA occurred in the cytosol. An associated decrease in succinyl-CoA indicates that matrix CoASH is

preferentially converted to acetyl-CoA. The levels of both CoASH and carnitine decrease to values below those found to give optimal rates of fatty acid activation and acyl transfer in tissue homogenates (22, 65, 165).

Collectively, these data are interpreted to indicate that limited flux through the citric acid cycle resulted in higher levels of acetyl-CoA and lower levels of CoASH in both the cytosol and mitochondrial matrix. These changes function as a feedback control to limit fatty acid activation in the cytosol and β-oxidation in the matrix. The low levels of carnitine may also limit fatty acyl transfer, suggesting that the small increase in fatty acyl-CoA occurs in the cytosol. If so, fatty acyl-CoA inhibition of acyl-CoA synthetase activity associated with lower levels of CoASH could account for the limited rate of fatty acid uptake.

Effects of Fatty Acid Utilization on Production of Inhibitors of Glycolysis

Limited flux through the citric acid cycle in hearts oxidizing fatty acids accounts for increased production of inhibitors of glycolysis (19, 185) and preferential oxidation of fatty acids. Production of acetyl-CoA from fatty acids is associated with production of excess NADH both from β-oxidation and the citric acid cycle. Since oxidative phosphorylation utilizes NADH only in responses to ATP hydrolysis, the $NADH/NAD^+$ ratio increases and the rates of dehydrogenase reactions, principally isocitric dehydrogenase and a β-ketoglutarate dehydrogenase (111, 146, 184), are geared to the rate of oxidative phosphorylation. With limited flux through the dehydrogenases and continued production of acetyl-CoA, the rate of citrate and isocitrate synthesis exceeds flux through the rest of the cycle and the levels of these metabolites increase (19, 185). This so-called "unspanning" (184) of the cycle results in lower levels of oxaloacetate which then limits the rate of citrate synthesis and leads to accumulation of acetyl-CoA. Since lower perfusate levels of fatty acid are required to cause accumulation of citrate than of acetyl-CoA, inhibition of the citric acid cycle by high NADH is more marked than inhibition of β-oxidation (146, 165). The extra carbon involved in accumulation of citrate and isocitrate comes from a shift in the aspartate-oxaloacetate transamination reaction (19). However, only a limited amount of aspartate is available, and with limited production of oxaloacetate from within the cycle, citrate synthesis and oxidation of acetyl-CoA are ultimately controlled by the rate of oxaloacetate formation. As discussed earlier, this mechanism accounts for preferential utilization of fatty acids by inhibition of phosphofructokinase due to higher levels of citrate, inhibition of pyruvate oxidation by increased NADH and acetyl-CoA, and inhibition of glucose transport by as yet unknown factors. It also should be noted that control of the citric acid cycle via coupling to oxidative phosphorylation simultaneously results in regulation of fatty acid and carbohydrate utilization. As noted above, fatty acid oxidation is controlled at the fatty acid activation and β-oxidation steps by low levels of CoASH.

Effects of Increased ATP Utilization

Increasing the rate of energy utilization by raising the level of cardiac work accelerated the rate of palmitate uptake and oxidation (44, 141, 165). The sequence of events in the effect of work on fatty acid uptake appears to be increased oxidative

phosphorylation, lower levels of NADH, and acceleration of the citric acid cycle (146, 165). With increased flux through the cycle, tissue levels of acetyl-CoA, fatty acyl-CoA, and acetyl-carnitine decrease while the levels of CoASH, carnitine, and fatty acyl-carnitine increase. These changes are associated with lower levels of tissue fatty acids, faster rates of fatty acid oxidation, activation, and uptake. In addition, the reversed flux of acetyl units from the cytosol into the matrix is initiated by increased utilization of acetyl-CoA through the citric acid cycle and leads to elevated levels of cytosolic CoASH and carnitine. These changes increase rates of fatty acid activation and acyl transfer to carnitine, lead to higher levels of fatty acyl-carnitine, and lower levels of tissue fatty acid and accelerated fatty acid uptake. With increased oxidative phosphorylation, the rate of fatty acid oxidation appears to be limited by acyl translocation from acyl-carnitine across the inner mitochondrial membrane (165).

When low levels of exogenous palmitate are present, an increase in CoASH with increased cardiac work cannot account for stimulation of fatty acid activation and uptake. In these hearts, however, there is a small decrease in fatty acyl-CoA. The extent to which this decrease could contribute to activation of acyl-CoA synthetase is unknown. If it is assumed that much of the tissue acyl-CoA is bound to proteins and that the observed decrease occurs in the cytosolic compartment, the decrease in the unbound concentration would be large and could account for a faster rate of fatty acid activation.

Certain other substrates when present in the blood in sufficiently high concentrations can decrease the rate of uptake and oxidation of fatty acids. Pyruvate and ketone bodies have been reported to reduce oxidation (14, 59). The mechanism of this effect is not clear, but may be related to the ability of these substrates to compete for available pools of CoASH and NAD^+. Glucose does not decrease the rate of palmitate uptake, indicating that sufficient quantities of pyruvate cannot be produced by glycolysis (161). The presence of high concentrations of pyruvate increase C^{14}-palmitate conversion to tissue lipids (59). This observation indicates that pyruvate competes more effectively for matrix CoASH than for cytosolic CoASH and leads to inhibition of β-oxidation. Erucic acid, an ingredient of margarine in some countries, inhibits fatty acid oxidation and results in increased conversion of fatty acids to triglycerides in heart muscle (36).

Endogenous triglycerides can serve as a source of fatty acids for energy metabolism, whereas phospholipids appear to function primarily as structural lipids and contribute little, if any, fatty acids for energy metabolism. Utilization of tissue lipid is evident in hearts perfused without exogenous substrate or with glucose (44, 52, 141, 158). High levels of tissue triglycerides accumulate in hearts following injury from toxic doses of norepinephrine (190). This increase in tissue lipid only occurs when exogenous lipid is present and is not related to high tissue levels of fatty acids. However, fatty acid oxidation is decreased under these conditions, and, although tissue levels of fatty acyl-CoA were not measured, these levels may have been elevated.

Accelerated rates of triglyceride utilization occur in hearts treated with isoproterenol (35) and in hearts performing increased cardiac work if exogenous fatty acids are not present (43, 141). The contribution of endogenous lipids to energy

metabolism is also increased in hearts perfused with glucose and acetate when the work load is raised (142). The effect of catecholamines on triglyceride utilization appears to be mediated by higher levels of cyclic-AMP and activation of the tissue lipase. The effect of increased cardiac work is associated with lower tissue levels of fatty acids and fatty acyl-CoA (165). Thus accelerated utilization of tissue lipid with increased cardiac work may result from lower levels of the products of lipolysis.

Fatty Acid Metabolism in Oxygen Deficiency

With a decrease in oxygen supply, oxidation of fatty acids by the heart sharply declines (57, 161, 201). Fatty acid uptake is either reduced or remains unchanged, but a large fraction is converted to tissue lipids. In association with the reduced rate of fatty acid oxidation in ischemic rat hearts, the tissue levels of fatty acyl-CoA increases 3-fold (149) and α-glycerol-P increases by more than 15-fold (unpublished observation). Increased levels of both substrates for glyceride synthesis may account for the accelerated conversion of fatty acid to tissue lipids. The rate of fatty acid synthesis in the heart is normally slow, but under hypoxic conditions conversion of ^{14}C-acetate to long-chain fatty acids is increased (82, 226). The rate of fatty acid synthesis is directly proportional to lactate production (82), indicating that lipid synthesis is related to the availability of reducing equivalents.

Other than the inability to synthesize ATP, the metabolic and functional consequences of reduced fatty acid oxidation in hypoxic or ischemic hearts are currently a controversial subject. The presence of high levels of fatty acids has been reported to decrease myocardial contractility (87) and to increase arrhythmias (109), but these observations have been disputed (189). Fatty acids have been reported to be uncouplers of oxidative phosphorylation (18) and to increase oxygen consumption by the heart (31). However, oxygen consumption is increased by only about 13% in isolated rat hearts perfused with 1.2 m*M* palmitate bound to 3% albumin, as compared to hearts perfused with glucose alone (165). This is the theoretical increase that would be expected from a shift from oxidation of glucose with a P/O ratio of 3.2 to oxidation of fatty acid with a P/O ratio of 2.8; that is, for the same rate of synthesis of ATP or its energetic equivalents, more oxygen must be consumed when fatty acids are substrates.

High levels of fatty acids and fatty acyl-CoA derivatives are known to inhibit many enzymes in a nonspecific manner (171). In addition, fatty acyl-CoAs inhibit some enzymes in a specific fashion. Inhibition of fatty acyl-CoA synthetase by palmityl-CoA was mentioned earlier, and inhibition of adenine nucleotide transferase by fatty acids and several acyl-CoA derivatives has been reported (169, 208, 235). It was suggested that inhibition of nucleotide translocation by acyl-CoAs may have a function in regulation of cardiac metabolism (209). In aerobic hearts perfused with high levels of palmitate, tissue levels of fatty acyl-CoA increased only about 50% (165). This lack of acyl-CoA accumulation results from preferential conversion of available cytosolic CoASH to acetyl-CoA. Acetyl-CoA is not an inhibitor of adenine nucleotide transferase (169). Therefore, rapid conversion of long-chain acyl-CoAs to acetyl-CoA and transfer to the acetyl units to cytosolic CoASH may represent an important protective function of the carnitine-acetyl-CoA transferase in heart muscle. In ischemic hearts, however, the reduction in fatty acid oxidation

is accompanied by lower tissue levels of acetyl-carnitine and acetyl-CoA (149), presumably due to inhibition of β-oxidation, and by increased levels of fatty acyl-CoA and fatty acyl-carnitine. In addition, the carnitine-acyl-CoA transferase activity of mitochondria isolated from chronically ischemic myocardium is greatly reduced and some reduction is seen even after 30 min of ischemia (238).

SUMMARY

Utilization of carbohydrate and lipid in heart muscle is closely coupled to the energy needs of the heart. When the substrates are present together, fatty acid is used in preference to glucose. Inhibition of glycolysis is accounted for by inhibition of glucose transport, hexokinase, phosphofructokinase, and pyruvate dehydrogenase. Addition of high levels of insulin can overcome the transport block, but inhibition of intracellular glucose metabolism remains. Increased ventricular pressure development accelerates glucose transport, hexokinase, and phosphofructokinase if only the sugar is provided as exogenous substrate. When fatty acid is also present, it remains the preferred substrate. Restriction of oxygen availability by reduction of perfusate oxygen tension to zero markedly accelerates glucose and glycogen utilization. On the other hand, restriction of oxygen availability by reduction of coronary flow inhibits glycolysis. In both cases, levels of fructose-1,6-P are increased, but in the ischemic tissue, disposal of this intermediate is restrained at the glyceraldehyde-3-P dehydrogenase step, probably as a result of high levels of NADH. In the ischemic muscle, high levels of lactate accumulate, suggesting that reoxidation of NADH may be limited by transport of reducing equivalents out of the heart.

The rate of fatty acid uptake and oxidation by aerobic heart muscle is controlled primarily by 1. availability of exogenous fatty acid, 2. the rate of acetyl-CoA oxidation by the citric acid cycle at low rates of energy utilization, and 3. the rate of acyl translocation across the inner mitochondrial membrane at high rates of energy utilization. The presence of a high activity of carnitine-acetyl-CoA transferase appears to have an important function in coupling rates of fatty acid activation and acyl transfer to the rate of oxidative phosphorylation and utilization of acetyl-CoA by the citric acid cycle. The rate of flux through the dehydrogenase reaction of the citric acid cycle ultimately determines the rates of oxidation of both carbohydrate and fatty substrates and adjusts the rate of utilization of each substrate to the availability of the other.

Endogenous triglycerides can be used for energy metabolism. The rate of utilization depends on availability of exogenous fatty acid, the presence of hormones such as catecholamines and insulin, and on the level of cardiac work. The biochemical mechanisms that control the rates of synthesis and lipolysis are poorly understood.

Fatty acid metabolism in hypoxic and ischemic hearts is characterized by reduced rates of oxidation and uptake, higher levels of fatty acyl-CoA and α-glycerol phosphate, and increased conversion of fatty acids to tissue lipids. Tissue levels of acetyl-CoA and acetyl-carnitine decrease while levels of CoASH, carnitine, fatty acyl-CoA, and fatty acyl-carnitine increase. Higher tissue levels of fatty acids, fatty acyl-CoA, and fatty acyl-carnitine may be detrimental to myocardial metabolism and function. This point, however, requires further investigation.

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