CURRENT CONCEPTS IN CARDIOVASCULAR PHYSIOLOGY

EDITED BY

Oscar B. Garfein

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PREFACE

As understanding of cardiovascular physiology in all its intricacies grows, so does sophistication in interpreting and treating its malfunctions. Today's physician must therefore work constantly to update his or her knowledge of basic cardiac physiology in order to maintain diagnostic and therapeutic capabilities at the highest possible level. Doing so, however, presents a two-fold problem. First, an up-to-date overview of a given area in cardiovascular physiology is hard to come by; moreover, the sheer size and the publishing schedules of some major physiology texts render them obsolete for most of the time between editions. Second, it is difficult, if not impossible, to find a source that is comprehensible to the nonscientist physician. All too often the most valuable discussions in texts and journals presuppose a scientific and technical training greater than that undergone by the practicing cardiologist.

This volume is meant to provide solutions to these problems by presenting discussions of seven different areas related to the field of cardiac physiology. They are written by scientists, each with a special expertise in a particular area of cardiac physiology and all gifted writers interested in communicating their knowledge to clinicians. The choice of topics is mine, made in the belief that they would be the most useful to clinicians dealing with patients suffering from illnesses and not with healthy patients interested in physical fitness. Although the book is directed primarily at clinical cardiologists, cardiovascular surgeons, and trainees in their disciplines, interested internists, medical students, and house officers should also find it useful.

The authors have fashioned truly elegant presentations that are at the same time models of clarity and completeness. Taegtmeyer and Russell have discussed, with originality and insight, the intermediate metabolism of cardiac tissues. Their contribution is also unique for illuminating the delicate ways in which the biochemical pathways and the metabolism are controlled, not to mention the mechanisms whereby physiological events influence biochemical activities and vice-versa.

The myocardial receptors represent the interface between the intracellular machinery governing cardiac muscle performance, coronary vascular activity, the heart's electrophysiological behavior, and its endocrine function and the remain-

ing humors which circulate in the body. Bristow and Port summarize much of what is known of receptor chemistry and physiology, outlining the mechanisms by which these receptors effect changes in intracellular function. In addition, they append a section explaining the techniques used to measure and analyze receptor function.

Levy's description of the complex interplay between the nervous and cardiovascular systems is unique in its approach to the different levels of interactions and integration of the brain, the heart, and the body. Oparil and Katholi have with the utmost lucidity, summed up much of what is now known about the chemical and hormonal factors that regulate, modify, and modulate the cardiovascular system, stressing the physiological and integrative roles played by these humors. A knowledge of coronary artery physiology is fundamental to the intelligent treatment of many patients with heart disease: Hoffman provides a concise, up-to-date and well-constructed view not only of the many influences regulating coronary blood flow that include humoral, neural, intrinsic, vascular, and myocardial factors, but of the physics of flow in general.

Lakatta and Maughan discuss muscle mechanics on every level, from the subsarcomere to the intact, *in-situ* heart. Lakatta has emphasized the biochemical basis of contraction, which is essential to a proper understanding of the subject. Maughan, meanwhile, achieves two feats. One is a survey of all the factors that influence the heart's behavior as a pump; the other, done in concert with Lakatta, is an analysis of the cardiac function at multiple levels, in which parallels are drawn at each level that explain functional equivalency at dimensions varying from angstroms to centimeters.

In too many discussions of cardiac electrophysiology the biophysics of excitable membranes and microscopic and macroscopic anatomic considerations are avoided, leading to misinformation by oversimplification or omissions. Arnsdorf, in his review, covers all the factors determining the heart's electrophysiologic behavior and, with his matrical approach, emphasizes the complex interaction of multiple factors.

The authors have done a superb job of summarizing the most important concepts and current work in their fields. My contribution has been to draw attention to the aspects of their discussions that nonscientists might have difficulty understanding, and I hope I have succeeded. My thanks go to the many people who have helped in the development of the text, including the friends and colleagues who have given of themselves anonymously as well as to the editorial staff of Academic Press. Finally, I am grateful to the many teachers throughout my medical training who stressed the paramount importance of understanding disease as, at least in part, a manifestation of disordered physiology.

Oscar B. Garfein

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CHAPTER 1

Biochemistry of the Heart

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I. CARDIAC METABOLISM

A. Heart Muscle as Site of Energy Transformation

Traditionally, cardiovascular physiologists have paid more attention to coronary circulation and pump function of the heart than to the biochemical processes inside the heart muscle itself. Studies of cardiac metabolism are often fragmented and poorly integrated into other phenomena of cardiovascular function. It is, therefore, useful to remember that the flow of matter does not stop at the cell membrane, but continues inside the cell, where energy-providing substrates are broken down into smaller molecules to provide energy for the mechanical action of the heart. In keeping with the laws of thermodynamics, this process is also termed "energy transformation."

The purpose of this chapter on the biochemistry of the heart is to provide a brief outline of cardiac energy metabolism. To begin with, energy metabolism is perhaps the single most important determinant of cardiac function. Second, such a review on cardiac energy metabolism is timely in light of the renewed interest in intermediary metabolism generated by recent techniques using nuclear magnetic resonance spectroscopy or tracer compounds labeled with positron-emitting radioisotopes which can assess tissue function and metabolism in the intact heart.

Compared with the vast biochemical literature, such a review is necessarily superficial and fragmented. We have limited this chapter to a discussion of energy metabolism, and concepts are presented, rather than too many facts. More detailed information can be found in the excellent texts on general biochemistry (e.g., Lehninger, 1975, 1982) and metabolic regulation (Newsholme and Start, 1973; Newsholme and Leech, 1983), as well as in recent reviews of various aspects of cardiac biochemistry by Neely and Morgan (1974), Randle and Tubbs (1979), Williamson (1979), Vary *et al.* (1981), Liedtke (1981), and Taegtmeyer (1984). For the sake of clarity, entire areas of cardiac biochemistry have been omitted. These include the biochemistry of myocardial cell organelles, membranes, and proteins, as well as such interesting topics as receptors (covered elsewhere in this volume), prostaglandins, and metabolism of the vascular endothelium. It is a sign of the rapid advances in knowledge that even a review can cover only limited aspects of a field.

Fortunately, the functions of metabolism in the heart are few in number and can be understood without detailed knowledge of elaborate chemical formulas or reactions. The main purpose of cardiac metabolism is to provide energy for contraction, ion transport, biosynthesis, and degradation. Like any other living tissue, heart muscle captures and utilizes this energy in the form of ATP. The tissue content of ATP is normally around 20 μmol/g of dry weight. At an O_2 consumption of 4.5 mmol/min/g of dry weight (Taegtmeyer *et al.*, 1980) and a *P–O* ratio (i.e., moles of ADP phosphorylated per mole of O_2 consumed) of 3, heart muscle utilizes 1500 μmol of ATP/min/g of dry weight or 25 μmol ATP/sec/g of dry weight. This simple calculation illustrates that intracellular

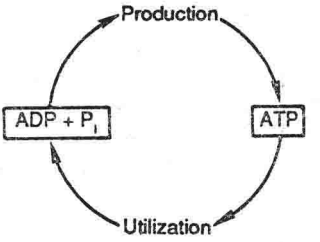

Figure 1. The cycle of cardiac energy metabolism (ATP cycle). Heart muscle captures energy in the form of ATP. Hydrolysis of ATP to ADP and inorganic phosphate (P_i) provides the energy for contraction and ion transport, as well as synthesis and degradation of biomolecules. Stores of ATP in the tissue are small and have to be continuously replenished by oxidative phosphorylation of ADP. To a lesser extent, ATP is also replenished by substrate-level phosphorylation and by the creatine kinase reaction.

stores of ATP are so small that, without replenishment, they would be exhausted within 1 second. Thus, ATP has to be replenished as quickly as it is broken down. The overall concept of this "ATP cycle" is depicted in Fig. 1 and illustrates the important fact that, as long as the heart utilizes ATP, it also has to replenish ATP.

The cyclic process of ATP hydrolysis and resynthesis begins with the contraction of the heart, which drives blood into the aorta and coronary arteries, and ends with the replenishment of the chemical energy stores. Philosophically, the concept of a continuous flux of matter was defined by the Greek philosopher Heraclitus more than 2000 years ago when he coined the phrase "all is in flux." Scientifically, the concept of a dynamic state of living matter has been formulated in the wake of the discovery of metabolic pathways and cycles, and by the discovery of the dynamic state of body constituents by the use of radioisotopes (Schoenheimer, 1942).

The main sources of ATP in heart muscle are oxidative phosphorylation of ADP in the respiratory chain and, to a lesser extent, phosphorylation of ADP either by substrate-level phosphorylation in the glycolytic pathway and the citric acid cycle or by the action of creatine kinase. Since oxidative phosphorylation is quantitatively the main source of ATP and because the key enzymes of oxidative metabolism are located in mitochondria, myocardial cells are well invested with these organelles, which indeed make up more than 20% of the myocardial cell volume.

Oxidative phosphorylation of ADP depends on the production of reducing equivalents (protons) and the passage of electrons along the respiratory chain. Since reducing equivalents are produced in the course of substrate catabolism to acetyl-coenzyme A (CoA) and subsequent oxidation of acetyl-CoA in the citric acid cycle, it is convenient to group the main energy-providing reactions into three stages (Fig. 2). The first stage comprises all reactions leading to acetyl-CoA; the second stage, the oxidation of acetyl-CoA in the citric acid cycle, re-

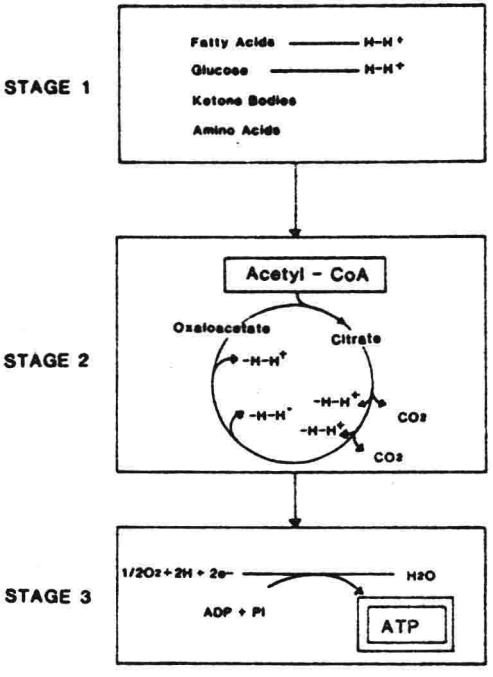

Figure 2. Stages of catabolic reactions in cardiac energy metabolism. Energy-providing substrates are converted to acetyl-CoA, which is, in turn, oxidized in the citric acid cycle. At many stages in their catabolism, the substrates generate reducing equivalents in the form of $NADH^+$ and/or $FADH_2$. Transfer of their electrons to the electron-carrying chain and their reaction with molecular oxygen lead to synthesis of ATP.

sulting in the liberation of CO_2 and reducing equivalents; and the third stage, the flow of electrons down the respiratory chain, leading to the release of free energy, which is conserved in the energy-rich phosphate bond of ATP. This is, of course, a simplified scheme of cardiac metabolism, and before it is discussed in more detail, a brief review of the acquisition of knowledge in cardiac metabolism and the principles of metabolic control is in order.

B. Milestones in Research on Cardiac Metabolism

According to Bing (1976), the first scientific account of cardiac metabolism was given when physiologist Hugo Kronecker presented the findings of his assistant McGuire "On the Nutrition of the Frog Heart" in a lecture before the Physiological Society in Berlin in 1878 (McGuire and Kronecker, 1878). He reported that

the frog heart stopped beating after a short while when perfused with saline alone, whereas mechanical activity could be sustained for longer periods when the perfusate contained blood.

In 1885 Yeo, then Professor of Physiology at King's College in London, provided the first direct demonstration of oxygen uptake by the isolated frog heart when he perfused the heart in a chamber containing hemoglobin, which he analyzed by spectroscopy. Yeo not only observed the reduction of oxyhemoglobin (and respiration) by the heart, but also a faster rate of hemoglobin reduction when the heart was electrically stimulated. "Indeed we may conclude," he summarizes his findings, "that the absorption of a considerable quantity of oxygen is one of the items essential to the life of the tissue. The greater amount of oxygen used by the contracting heart muscle is so constant that it would appear equally certain that during contraction the oxygen requirement of the tissue notably increases." Nearly 20 years later Winterstein (1904) was credited with the first demonstration of an oxygen requirement for the mammalian heart muscle when he showed that isolated rabbit hearts resumed beating as oxygen was readmitted to the perfusion medium after a period of anoxia.

Glucose was the first substrate to be identified as a "source of muscular force" (Muller, 1904), but the early reports of glucose utilization for muscular work were met with skepticism. Locke and Rosenheim (1904, 1907) found that glucose disappeared when perfused through the mammalian heart and concluded that the disappearance of glucose was a fundamental chemical change underlying cardiac activity, rather than a metabolic or fermentative by-process.

During the first part of this century, Lovatt Evans studied many aspects of metabolism of the heart using the heart–lung preparation of Starling (Patterson and Starling, 1914). Aided by improved analytical methods, Evans not only determined oxygen consumption and CO_2 production in mammalian heart (Evans and Starling, 1914), but also measured the utilization of substrates and calculated mechanical efficiency (Evans *et al.*, 1933). The London group was also the first to study the interaction of substrates, as well as the effects of hormones on cardiac metabolism (Evans, 1914; Evans *et al.*, 1933, 1935). In his pioneering work Evans discovered the relationship between heart rate and oxygen consumption, determined the influence of innervation on the heart, and studied the respiratory quotient of the isolated heart. It is of interest that in 1914 Evans was the first to suggest that the energy for cardiac contraction might be derived from more than one source (Evans, 1914).

A major impetus for research in cardiac metabolism came from the work by Richard Bing and co-workers, who measured arteriovenous differences of substrates and products across the heart (for a review, see Bing, 1955). These studies were the result of two major technical advances:

1. The development of a technique for coronary sinus catheterization and its application to the human heart (Bing *et al.*, 1947) made it possible to directly sample blood from the venous bed of the heart.

2. Improved analytical methods became available for more accurate estimations of glucose, lactate, pyruvate, fatty acids, ketone bodies, amino acids, and oxygen consumption.

Using these methods, it was shown either directly or indirectly that the human heart uses the three foodstuffs—carbohydrates, fatty acids, and (under certain circumstances) amino acids—to varying degrees for its energy production. Bing's work suggested that the arterial concentration of carbohydrates affects their relative myocardial usage. As in the heart–lung preparation, the total aerobic metabolism of glucose, lactate, and pyruvate did not account for the total oxygen consumption of the heart, and it was first inferred and then directly proven that the oxidation of fatty acids and ketone bodies made up the balance. Myocardial utilization of fatty acids was noted to be particularly high at high fat intake; the effects of other dietary states or hormones were initially not investigated. While oxidation of ketone bodies accounted for approximately 5% of the total myocardial oxygen consumption in normal individuals, the diabetic dog heart utilized a consistently larger quantity of these fuels (Ungar *et al.*, 1955). The utilization of ketone bodies seemed to be governed by their arterial concentration and the quantity of carbohydrate available (Bing *et al.*, 1954).

Bing *et al.* (1954) further suggested that both human and dog hearts might extract considerable amounts of amino acids from the blood. In fact, after an infusion of a mixture of amino acids, as much as 40% of the total myocardial oxygen consumption could be accounted for by aerobic metabolism of amino acids. The utilization and release of amino acids, especially the uptake of glutamate and the release of glutamine and alanine, by the human heart have since been confirmed (Carlsten *et al.*, 1961; Mudge *et al.*, 1976), although quantitative aspects of amino acids as fuel for respiration in heart muscle are still not well defined.

Nevertheless, the ability of the heart to utilize various substrates illustrates the great versatility of the myocardium in the choice of its fuel supply and can be regarded as an "important factor of safety" (Bing, 1955) in the maintenance of an adequate fuel supply for an organ of vital importance for survival of the total organism.

While studies of arteriovenous differences of metabolites allowed identification of the sources of energy production of the mammalian heart muscle, they provided no insight into the mechanism by which the heart selects its fuels. Answers to this problem could only be provided after (1) improved methods for perfusion of the isolated heart *in vitro* and (2) fast reliable analytical techniques to estimate the concentration of intermediary metabolites became available.

Studies carried out in the 1960s and 1970s were aimed at a more precise understanding of the factors which regulate the transport and metabolism of fuels for respiration. For this purpose the isolated blood perfused cat heart preparation of Langendorff (1895) was adapted for saline perfusion of hearts from other small animals. A recirculating perfusion system for rat hearts was introduced by Bleehen and Fisher (1954) and by Morgan *et al.* (1961). Recirculation of the

medium made the estimation of substrate utilization and metabolite production more accurate. A further advance was the adaptation of the working frog heart preparation of Otto Frank (1895) for the isolated rat heart by Neely *et al.* (1967a). This model made it possible to study for the first time the relationship between cardiac work and metabolism under controlled conditions *in vitro*.

Perfusion of the isolated mammalian heart offers a number of features different from other heart muscle preparations which were developed at about the same time. These preparations include incubated papillary muscles, tissue slices, isolated heart cells, and cell-free suspensions. Advantages of the perfused heart preparation for the physiologist are that the heart continues to beat, substrates and oxygen reach the muscle through its natural vascular bed, the integrity of the plasma membrane is preserved, and the products of metabolism are readily removed. Some disadvantages are that not all substrates or cofactors readily cross the plasma membrane, no distinction can be made between metabolite pools in different cellular compartments, and the production of $^{14}CO_2$ from the oxidation of labeled substrates cannot be measured reliably when bicarbonate-buffered medium is recirculated. Many biochemists have therefore used a variety of techniques in the study of metabolic control, such as tissue suspensions, cell organelles, or isolated enzymes. In an ideal situation the various methods of investigation complement each other and lead to the elucidation of metabolic control mechanisms.

II. CONCEPTS OF METABOLIC REGULATION

A. Bioenergetics, Equilibria, and Rate Constants

Because bioenergetics is at the very center of cardiac metabolism, it is useful to consider the two fundamental principles of thermodynamics, the branch of physical science that deals with energy changes. From casual observation it is readily apparent that energy comes in many forms and that the different forms of energy can be interconverted. It is also common experience that when one form of energy is converted into another, there is also some "loss" of energy to the surroundings (e.g., in the form of heat). This loss of energy can be considerable, and in many machines and biological systems (including the heart) less than 20% of the energy input is recovered as useful work.

Nevertheless, quantitative measurements of the interconversion of energy have shown that, within a given "universe" (i.e., a system and its surroundings), the total amount of energy remains the same. The First Law of Thermodynamics is then the principle of the constancy of energy, and it states that energy can neither be created nor destroyed. The Second Law of Thermodynamics is the principle of entropy and states that a process occurs spontaneously only if it is accompanied by an overall increase in the disorder or entropy of the system and its surroundings. The entire process reaches a steady state with no more net conver-

sions at an equilibrium point. At equilibrium the entropy is maximal under the existing conditions.

We now apply the laws of thermodynamics to a simple biochemical reaction, as it occurs in the myocardial cell. In the reaction

$$A + B \rightleftharpoons C + D \tag{1}$$

the formation of C + D proceeds at a rate proportional to the product of the concentrations of A and B. But this is not the end of the story, because, conversely, the rate of the reversed reaction, C + D to A + B is likewise proportional to the product of the concentrations of C and D. However, from this information we do not know how many molecules of A and B as well as of C and D react in the same unit of time; in other words, we do not know the speed of the two reactions. For this purpose knowledge of the rate constants k_1 and k_2 is required. The term "rate constant" is defined as the absolute number of moles of a substrate reacting per volume and unit of time (e.g., millimoles per liter per minute). The rate constant k_1 is used for the forward reaction, and k_2 is for the backward reaction. At equilibrium the forward and backward reactions are constant, that is,

$$k_1 = k_2$$

or

$$k_1 [A] \cdot [B] = k_2 [C] \cdot [D] \tag{2}$$

or

$$\frac{k_1}{k_2} = \frac{[C] \cdot [D]}{[A] \cdot [B]} = K_{eq} \tag{3}$$

where K_{eq} is the equilibrium constant.

As discussed, biological reaction systems exchange energy with their surroundings, and, in addition to describing the equilibrium position of a reaction, it is therefore also desirable to make predictions about the probability of a reaction occurring in a particular direction. According to the Second Law of Thermodynamics, a reaction is able to proceed only if it leads to an increase in the entropy of the reacting system and its surroundings. A useful numerical value indicating the probability of a process occurring spontaneously has been introduced in the form of the free energy change of the process or ΔG. The term "free energy change" is used to describe the amount of energy available for the performance of work.

At constant pressure and temperature (i.e., the conditions under which cells operate) ΔG is defined as

$$\Delta G = \Delta H - T(\Delta S) \tag{4}$$

where ΔH is the heat given off or taken in when the chemical bonds of the reactants are broken or formed, T is the absolute temperature, and ΔS is the entropy change in the reacting system. Therefore, adding free energy to a system de-

creases entropy, while a reaction liberating energy is associated with increased disorder in the system.

The free energy change in a reaction is related to the concentrations of reactants as described by the equation

$$\Delta G = \Delta G^{01} + RT \ln \frac{[C]\cdot[D]}{[A]\cdot[B]} \tag{5}$$

where ΔG^{01} represents the standard free energy (i.e., all concentrations are 1 *M*, pH 7.0) and R is the universal gas constant.

Because $\Delta G = 0$ at equilibrium, Eq. (5) becomes

$$\Delta G^{01} = -RT \ln \frac{[C]\cdot[D]}{[A]\cdot[B]} \tag{6}$$

and, using Eq. (3),

$$\Delta G^{01} = RT \ln K_{eq} \tag{7}$$

The above derivation shows that, in principle, the standard free energy change of a chemical reaction is nothing but a different way of expressing its equilibrium constant at defined conditions (e.g., pH 7.0, 25°C). Conversely, once the equilibrium constant of a chemical reaction is determined, the standard free energy change can be calculated and expressed as calories per mole of reactant. It is important to remember that ΔG indicates the energy released (−) or consumed (+) by a reaction. The standard free energy changes differ among chemical reactions, as the following examples of some biologically important reactions in hearts muscle show:

Malate	$\rightarrow$ fumarate + H_2	$\Delta G = +0.75$ kcal/mol
ATP + H_2O	$\rightarrow$ ADP + P_i[1]	$\Delta G = -7.3$ kcal/mol
Glucose + 6 O_2	$\rightarrow$ 6 CO_2 + 6 H_2O	$\Delta G = -686$ kcal/mol
Palmitate + 23 O_2	$\rightarrow$ 16 CO_2 + 16 H_2O	$\Delta G = -2338$ kcal/mol

B. Principles of Metabolic Control

With a general idea of the principles of bioenergetics, one is now in a better position to understand the importance of energy transfer from substrates or chemical bonds via ATP to cardiac work. It is a characteristic property of all living cells, including heart muscle, to permit complex chemical reactions to proceed quickly at relatively low temperatures and concentrations in an efficient way. This efficient energy transfer in living cells is probably the result of a long process of evolutionary selection (Baldwin and Krebs, 1981) and occurs through enzyme-catalyzed reactions.

[1] P_i, Inorganic phosphate.