Anesthesiology
V 39, No 2, Aug 1973

Inhalation Anesthetics and Myocardial Metabolism:

Possible Mechanisms of Functional Effects

*Robert G. Merin, M.D.**

HEART MUSCLE has two properties vital to organ function, automaticity and contractility. Tight controls are exerted on both for the regulation of cardiac function. The energy requirements of the membrane electrical events are minor, however, compared with those of the intracellular contractility mechanism.[1] In consequence, it is unlikely that energy metabolism plays an important role in the effect of anesthetics on myocardial rate and rhythm. Inasmuch as the contractile process in the myocardium requires a large and continuous energy supply, interference in myocardial energy metabolism would appear to be a logical mechanism for the negative inotropic effect of anesthetics. For this reason, this review is not concerned with the metabolic processes involved in cardiac rate and rhythm, but considers the steps in myocardial metabolism at which anesthetics are likely to influence cardiac contractile function.

In the most recent publication on anesthetics and myocardial metabolism,[2] the authors were able to refer to only one study of the effects of anesthetics on myocardial metabolism. Half of the section was devoted to adrenergic effects on myocardial metabolism and the effects of ethanol (hardly a typical anesthetic). Since then there has been more work on the effects of real anesthetics on myocardial metabolism, although at least as many questions are posed as are answered. More importantly, however, we have a much better understanding of the control and mechanisms of myocardial function and metabolism and the basic physiology of the contractile process.

It is worth noting that major contributions to our understanding of the subject have come from work on the perfused rat heart. Unfortunately, the rat heart differs from hearts of higher mammals in several aspects of myocardial function, including a very different intrinsic rate, maximal velocity of shortening and, perhaps most significantly, no increase in contractile force with increase in heart rate (the "rate treppe" or "staircase" effect).[3, 4] Most of the literature on the subject has resulted from *in-vitro* studies of bathed isolated muscles (ventricular, papillary, and atrial) or perfused hearts from rats, dogs, cats, guinea pigs, frogs, and occasionally man. From such work in the perfused rat heart and cat papillary muscle, the direct negative inotropic effect of all potent inhalation anesthetics is apparent.[5, 6] The lack of such an effect in man with cyclopropane, diethyl ether, or fluroxene is probably a result of centrally mediated sympathetic nervous stimulation.[7, 8] Most investigators have assumed that common mechanisms are involved, but in order to elucidate them, lower species and very artificial conditions must be utilized. The *in-vivo* studies have measured myocardial arteriovenous substrate differences in animals of various types and, again occasionally, in humans. However, without myocardial (coronary) blood-flow measurements (which most of the early works did not have[9, 10]) this technique is less meaningful,[11] and, indeed, invalid, unless coronary blood flow is constant.[12] Even with blood-flow measurements, synthetic and storage pathways cannot be measured without the use of tagged molecules. These problems of technique and transfer of data from species to species and from *in vitro* to *in vivo* should be noted.

Correlation of cardiac function and metabolism may best be reviewed by considering the points in the contraction cycle where they may be related. For this purpose, a schema of energetics in cardiac muscle is useful (fig. 1).[13] The energy supply of the fasting heart is *liberated* primarily from fatty acids and

* Associate Professor, Departments of Anesthesiology and Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, New York. Research Career Development Awardee #HL-31752.
Supported in part by USPHS grant #HL-13257.

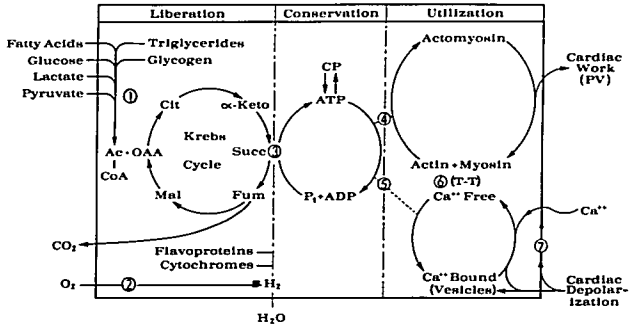


FIG. 1. Schema of energetics in cardiac muscle (adapted after Olson, et al.,[12] with permission of author and publisher).

from lactate delivered in the coronary blood, although glucose, pyruvate, acetate, and triglycerides can be used (fig. 1, ①).[11, 14, 15] Anaerobic glycolysis occurs in the sarcoplasm (cytoplasm of the muscle), producing pyruvate, which under aerobic conditions is converted to acetyl Co-A (Ac-CoA). This acetyl Co-A and the substantially larger quantity from beta-oxidation of fatty acids enter the intramitochondrial tricarboxylic acid (TCA, Krebs) cycle, where the bulk of *energy conservation* as adenosine triphosphate (ATP) and creatine phosphate (CP) occurs. The mitochondrial electron-transport system, which transfers the hydrogen ions released in glycolysis and in the TCA cycle through the flavoproteins and cytochromes to oxygen, couples liberation and conservation of energy (fig. 1, ③). Obviously, oxygen delivery to the mitochondria is essential for TCA cycle function (fig. 1, ②). The critical links between the conservation of energy as ATP and its *utilization* by the contractile process are the ATPases, which are necessary for hydrolysis of ATP to support the energy-dependent processes in the contraction–relaxation cycle of heart muscle (fig. 1, ④ and ⑤), including actin–myosin interactions (fig. 1, ⑥), pumping of calcium into the vesicles of the sarcoplasmic reticulum, and mitochondrial function (fig. 1, ③). The central role of calcium in muscular contraction can be appreciated by noting that not only is actin–myosin binding controlled by sarcoplasmic calcium (fig. 1, ⑥), but the ATPases are also under calcium-ion control (fig. 1, ③ and ⑤). According to current concepts, the link between the electrical depolarization of the cardiac cell membrane and the actual intracellular contractile process is also calcium-mediated (fig. 1, ⑦).

Energy *liberation*, *conservation*, and *utilization* are examined in more detailed below. After each section, the effects of anesthetics are reviewed.

Energy Liberation

Oxygen Supply

Much of the basic theory of the mechanics and biochemistry of cardiac muscle has been based on work in white skeletal muscle, but there is a major metabolic difference between the muscle types.[16, 17, 18] White skeletal muscle function is episodic, occurring mostly in short, high-velocity bursts, while red skeletal and cardiac muscles function at lower velocities and for longer periods. Consequently, white skeletal muscle can work anaerobically for appreciable periods, building up its energy reserve aerobically during rest. In contrast, the heart (and red skeletal muscle) is incapable of sustained anaerobic metabolism but must have oxygen for its energy demands. Anaerobic processes are much less efficient in energy liberation and conservation. For example, one mole of glucose yields only two high-energy phosphate bonds anaerobically,

FIG. 2. *A (above)*, Electron photomicrograph of rat extensor digitorum longus (×17,000) (courtesy of Dr. Clara Franzini-Armstrong). *Arrow*, mitochondrion. *B (below)*, Electron photomicrograph of rat ventricle (×18,000) (courtesy of Dr. Clara Franzini-Armstrong). *Arrow*, mitochondrion.

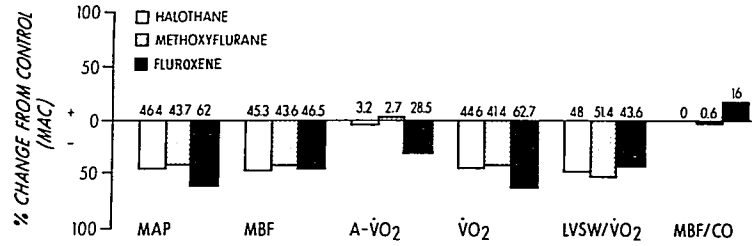


FIG. 3. Myocardial perfusion and oxygenation.

while complete aerobic catabolism of one mole of glucose yields 15 such bonds.[14] The continuous high-energy demands of cardiac muscle can be met only with aerobic metabolism. The activity of the TCA cycle, where most of the aerobic energy liberation occurs, is entirely within mitochondria. A graphic illustration of the difference in oxidative metabolism between white skeletal and cardiac muscle is seen in the distribution of mitochondria in the two muscle types (fig. 2). For these reasons, oxygen kinetics must be considered in relation to metabolism and contractile function.

OXYGEN SUPPLY: EFFECT OF ANESTHETICS

Delivery of oxygen to heart muscle depends upon the oxygen content of the coronary arterial blood and the amount of that blood delivered to the muscle, *i.e.*, the coronary blood flow. The ventilatory depression produced by most anesthetics can result in decreased oxygen concentration in arterial blood, but the depression can be easily controlled and should play no role in the problem being discussed. Although it is possible that anesthetics affect the oxyhemoglobin dissociation curve,[19, 20] the objective evidence is not convincing at present. It appears that if anesthetics interfere with the myocardial oxygen supply, the effect is probably on myocardial (coronary) perfusion.

The anesthetic most widely studied in this regard has been halothane, predominately in the dog. Philipart in Belgium,[21] Eberlein in Germany, [22] Saito in Japan,[23] Weaver in England,[24] and Bagwell [25] and Merin [26] in the United States have all reported strikingly similar dose-related decreases in myocardial blood flow and arterial blood pressure. While holding coronary perfusion pressure constant, Wolff *et al.* found increased coronary vascular resistance and decreased flow with halothane, as compared with diethyl ether.[27] Employing an ingenious technique of counting radioactively tagged embolized microspheres in various organs postmortem, Amory and co-workers reported that coronary perfusion decreased in proportion to the decrement in cardiac output as the delivered halothane concentration increased in the monkey.[28] Both Saito and Eberlein reported conflicting data on coronary A-V oxygen differences during halothane anesthesia. In their initial studies, both investigators indicated that A-V oxygen extraction increased with halothane,[22, 23] while their subsequent papers indicate there was no change or even a decrease.[29, 30]

Saito, Wolff, and Eberlein also tested diethyl ether in their animal preparations.[22, 23, 27] With coronary perfusion maintained by a pump, Wolff's group noted that coronary vascular resistance decreased and coronary blood flow increased as the anesthetic was changed from halothane to diethyl ether. Concomitantly, aortic blood pressure increased. Saito and Eberlein also saw increased aortic pressure and/or cardiac output, together with increased coronary blood flow, with diethyl ether. Both authors commented on the decreased myocardial A-V oxygen difference. Saito *et al.* investigated methoxyflurane and cyclopropane as well.[23] Both drugs decreased cardiac output and coronary flow, but the ratio of coronary flow to cardiac output increased with cyclopropane, as it did with diethyl ether, while a decrease in the ratio was

seen with methoxyflurane and halothane. Both Saito and Eberlein thought that the difference in coronary flow and oxygen extraction might be related to the sympathomimetic effects of diethyl ether and cyclopropane.[7]

We have seen the same degree of myocardial function and blood flow depression with methoxyflurane as with halothane.[31] In contrast to the other investigators, however, with the "sympathomimetic" anesthetic, fluroxene,[8] we found dose-dependent decreases in aortic blood pressure, myocardial blood flow and oxygen uptake (fig. 3).[32] The only difference in these measurements among the three anesthetics we studied was the significant decrease in myocardial A-V oxygen extraction with fluroxene. The stroke work per unit of oxygen consumed decreased with negative inotropic doses of all three anesthetics, while the proportion of the cardiac output delivered to the left ventricle did not change (table 1).

In the reports reviewed above, it appears that myocardial blood flow and oxygen uptake decreased in those instances where ventricular function (and work) was decreased (halothane, methoxyflurane, fluroxene) and increased where ventricular function increased (diethyl ether). Whether this is cause or effect is another problem, however. An estimate of the sufficiency of oxygen supply in relation to oxygen demand in the heart is needed. Some tissues, such as skeletal muscle and liver, are able to increase appreciably the amount of oxygen extracted from arterial blood when flow is diminished. In such tissues an increase in A-V oxygen difference indicates relative insufficiency. The primary mechanism for control of oxygen delivery to cardiac muscle, however, is a change in coronary vascular resistance and hence blood flow, because A-V extraction is very large at resting conditions.[10] Consequently, analysis of coronary arteriovenous oxygen difference alone is of limited value in assessing the adequacy of oxygen delivery to the heart.

The controversy over the relationship between extracellular lactate–pyruvate metabolism and intracellular oxygen supply has been widely discussed over the past decade in the anesthetic literature.[33-36] Although there are serious objections to this use of the relationship,[35] there is some basis for considering the

TABLE 1. Myocardial Efficiency and Myocardial Blood Flow–Cardiac Output

	MAC	2-3 MAC	Per Cent Change
Efficiency			
Left ventricular stroke work/left ventricular O_2 uptake			
Halothane	7.35	3.82*	48
Methoxyflurane	5.46	2.65*	51.4
Fluroxene	7.37	4.15*	43.6
Blood flow–cardiac output Ratio (per cent)			
Halothane	1.52	1.52	
Methoxyflurane	1.69	1.68	
Fluroxene	1.44	1.67	

* $P < 0.01$.

measurements in relationship to myocardial cellular hypoxia.[13, 14, 37-40] The basic premise is inviting.[14] With sufficient oxygen, glucose and lactate are converted to pyruvate, which is then hydrogenated to acetyl CoA, which enters the Krebs (TCA) cycle (fig. 4). The conversion of lactate to pyruvate is dependent on available oxidized nicotinamide adenine dinucleotide (NAD^+). Hypoxia first blocks the terminal cytochrome in the mitochondrial electron-transport system (fig. 5). This results in a buildup of ADP and inhibition of the Krebs cycle with an increase in pyruvate. Hypoxia stimulates cellular membrane transport of glucose, and ADP stimulates glycolysis so even more pyruvate accumulates (fig. 5).[15] Hypoxia converts NAD^+ to NADH which, combined with the excess of pyruvate, drives the pyruvate–lactate reaction further toward lactate formation. In his original paper, Huckabee suggested that the lactate–pyruvate ratio in the blood reflected the NADH/NAD^+ ratio (and hence oxygen availability) intracellularly.[37] Objections to his theory include the effect of the membrane barriers between the two.[35, 41] In a carefully designed and executed study in the perfused rat heart, Opie and Mansford demonstrated that indeed there were major differences in extra- and intracellular levels of lactate and pyruvate, NAD^+ and NADH, and alpha-glycerophosphate and dihydroxyacetone phosphate (one of the shuttle systems by which electrons can be transferred back and

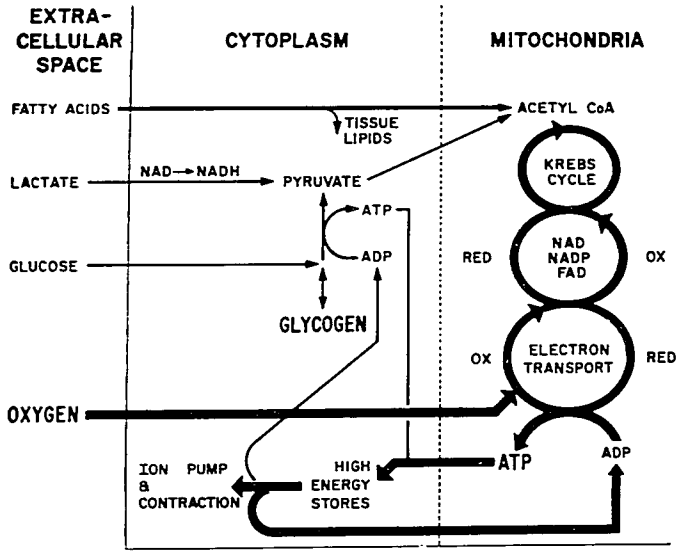


FIG. 4. Aerobic myocardial metabolism (from Scheuer,[11] with permission of author and publisher).

forth between cytoplasm and mitochondria).[28] However, they concluded that if the nutrition of the animal is normal and if no metabolic disease (diabetes) is present, extracellular lactate–pyruvate relationships reflect intracellular NAD⁺/NADH ratios quite well. Inasmuch as the extracellular lactate–pyruvate (L/P) ratios are supposed to be in equilibrium with the intracellular redox pair (NAD⁺/NADH), a steady state is necessary for any conclusions to be drawn from such ratios. While recognizing the problems inherent in use of this relationship, other authors have also felt that the estimate was useful at least qualitatively.[14, 29, 40] It should be noted that this refers only to the total myocardial lactate–pyruvate kinetics, not "excess lactate" or "lactate/pyruvate ratio" alone. The adequately oxygenated heart extracts lactate in relation to the arterial level. Decreased lactate uptake in the face of a rising arterial concentration and/or decreasing pyruvate extraction suggests myocardial cellular hypoxia.

With this background, the effect of anesthetics on this relationship can be explored. In abstract form only, Bagwell reported that the dose-related decrease in contractile force and coronary blood flow produced by halothane was accompanied by negative "excess lactate" values, "indicating that coronary flow was adequate to supply the oxygen needed to maintain normal oxidative metabolism."[25] In a poorly controlled study in open-chest dogs, Tajoli *et al.* noted no change in myocardial lactate and pyruvate extraction with 1 per cent halothane (vaporizer setting), compared with non-anesthetized animals paralyzed with *d*-tubocurarine.[42] With 2 per cent halothane, myocardial extraction and arterial concentrations of both substrates increased. The same group reported similar findings with 1 and 3 per cent methoxyflurane.[43] We have reported that the decrease in myocardial blood flow and oxygen uptake with 2–3 MAC (minimal alveolar anesthetic concentration) halothane or methoxyflurane was accompanied by decreases

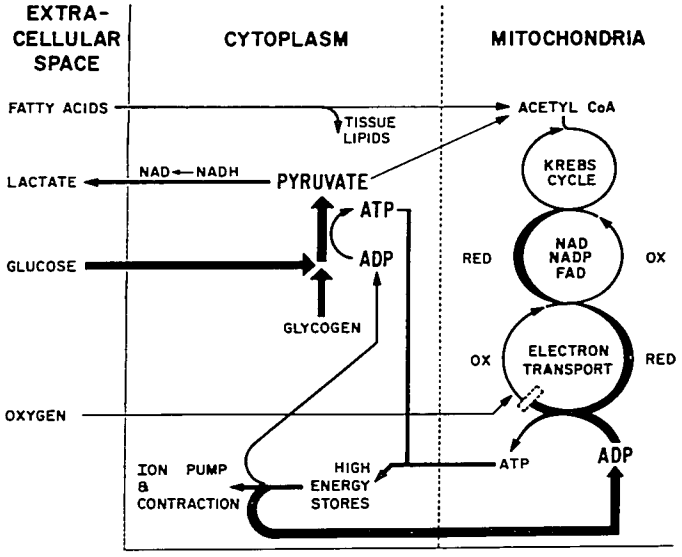


FIG. 5. Anaerobic myocardial metabolism (from Scheuer,[31] with permission of author and publisher).

or no change in myocardial excess lactate with maintenance of lactate uptake in relation to the arterial levels and blood flow (table 2).[26,31] Similar hemodynamic effects of fluroxene were accompanied by very different lactate metabolism.[32] Although the myocardial A-V oxygen difference decreased (fig. 3), the lactate data suggested cellular hypoxia with marked decrease in uptake and even production of lactate in spite of markedly increased arterial lactate values. However, if oxygen delivery actually had been impaired, it is unlikely that A-V extraction would have decreased, unless demand and uptake were uncoupled. Another possibility is the "sympathomimetic" effect of fluroxene. Theye has suggested that alterations in lactate metabolism seen in the whole body and in liver with diethyl ether and cyclopropane are related to sympathetic stimulation of glycolysis.[25] Regan *et al.* have demonstrated such an effect with epinephrine [44] but not with norepinephrine [45] in the dog heart, so the question remains unresolved.

With reference to mechanisms of cardiac depression, Paradise and Griffith reported that high concentrations of halothane and anoxia produced equivalent ventricular depression in the perfused rat heart. The halothane depression was readily reversible and was accompanied by no change in perfusion rate (coronary flow), while the anoxic effects were less readily reversible and caused an increase in perfusion rate.[46] They concluded that the mechanisms of depression were quite different.

In summary, inadequate oxygen delivery does not appear to be a primary mechanism in the myocardial depression produced by anesthetics. Decreased oxygen uptake is more likely a reflection of decreased demand, although the evidence available is less than conclusive.

SUBSTRATE UTILIZATION

The mammalian heart is able to use a variety of fuels to satisfy its large and continuous energy demands. Depending on arterial levels,

oxygen availability, and the hormonal and enzymatic environment, glucose,[9] fatty acids,[47] ketones,[48] lactate,[49] pyruvate,[50] triglycerides [51] and glycogen [52] can provide heart muscle with energy. As mentioned in the previous section, the aerobic Krebs (TCA, citric acid) cycle is the most efficient energy-liberating mechanism available, but, with abundant oxygen, all of the above-mentioned substrates are able to enter into this ubiquitous metabolic pathway. Stated in simplest terms, the heart will extract energy from whichever of these substrates is presented to it in highest concentration. Obviously there are more complex control mechanisms as well.

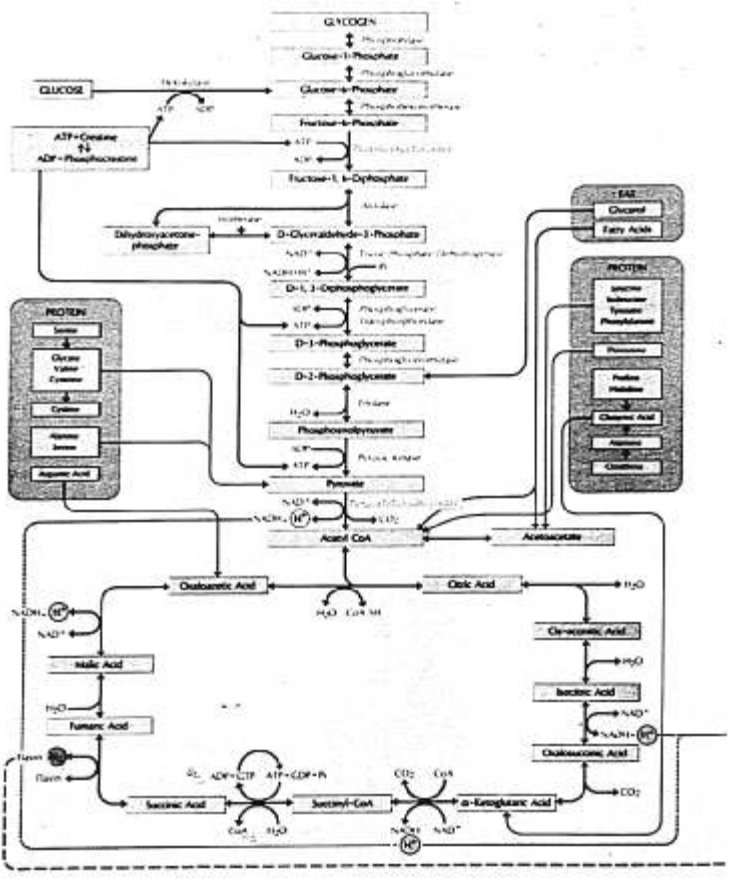


TABLE 2. Myocardial Lactate Metabolism

	MAC	2-3 MAC
Halothane		
Arterial (mg/100 ml)	24.88 ± 4.53	22.54 ± 2.65
A-V (mg/100 ml)	8.21 ± 1.52	10.67 ± 2.39
Uptake (mg/100 g/min)	3.18 ± 0.65	2.54 ± 0.75
Excess (mg/100 ml)	6.1 ± 2.08	−2.12 ± 2.05*
Methoxyflurane		
Arterial (mg/100 ml)	21.85 ± 3.67	17.88 ± 1.6
A-V (mg/100 ml)	10.44 ± 1.98	8.19 ± 0.84
Uptake (mg/100 g/min)	4.79 ± 1.23	2.04 ± 0.3*
Excess (mg/100 ml)	−2.18 ± 1.25	−1.49 ± 1.21
Fluroxene		
Arterial (mg/100 ml)	22.0 ± 3.3	41.3 ± 9.3*
A-V (mg/100 ml)	3.12 ± 1.5 (2/9 neg)	0.96 ± 1.8 (3/9 neg)
Uptake (mg/100 g/min)	1.47 ± 0.17	0.20 ± 0.56

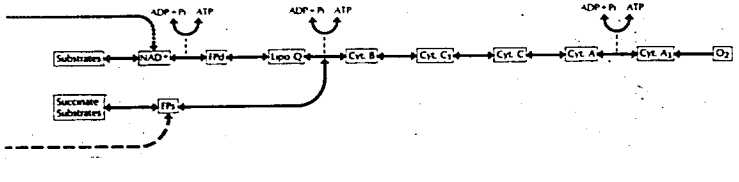
* $P < 0.05$.

GLUCOSE

Normally, transport of glucose across the myocardial cell membrane is the rate-limiting step in myocardial glucose metabolism. This transport is concentration- and insulin-dependent.[53,54] The normal arterial glucose threshold of 60 mg/100 ml (below which there is no glucose uptake) is most likely a result of the insulin dependency,[9,15] although lipid-glycolysis interactions may also play a role (see below). After cellular entry of glucose, the rate-limiting process governing glycolysis is the phosphofructokinase (PFK)-catalyzed conversion of fructose-6-phosphate to fructose-1,6-diphosphate (fig. 6).[55] PFK activity is sensitive to the adenine nucleotides and citrate.[56] When oxygen is plentiful, the TCA cycle keeps the ATP and citrate levels high, inhibiting PFK activity and glycolysis. Subsequent to any event interfering with TCA activity, both ATP and citrate levels decrease; adenosine diphosphate (ADP), adenosine monophosphate (AMP), and inorganic phosphate (P_I) concentrations increase; PFK is activated; glycolytic flux is accelerated. Alkalosis has been shown to increase glycolysis in the perfused rat heart, presumably by PFK activation, since optimal PFK activity occurs at *p*H 8.[57] Hypoxia promotes glycolysis in several ways[55]: cellular glucose transport is greatly increased by oxygen lack[53]; TCA cycle activity is inhibited; ATP and citrate levels decrease; ADP, AMP and P_I levels increase. Although increased work also stimulates glycolysis in skeletal muscle and in the perfused heart,[58] the importance of this effect in cardiac muscle *in vivo* is yet to be determined.

In summary, myocardial glucose utilization is dependent on arterial glucose concentration and plasma insulin level. Hypoxia, or other interference with TCA cycle activity, alkalosis, and possibly increased cardiac work accelerate glycolysis.

FIG. 6 (*left and below*). Pathways of energy liberation and conservation (adapted after Davies,[72] with permission of author and publisher).



Glycogen

Carbohydrate stored as glycogen can be utilized by the heart, although its physiologic role in the heart is relatively unimportant compared with fast (white) skeletal muscle.[18] Cardiac glycogen is synthesized only from glucose because of the absence of fructose 1,6-diphosphatase, which is necessary for the reversal of the glycolytic pathway if pyruvate, lactate or acetyl CoA is to be a glycogen precursor [16] (fig. 6). Insulin stimulates glycogen synthesis, so the fed state favors glycogen buildup. Since cardiac glycogen has been shown to be lower in fed than in fasted hearts, by inference glycogenolysis must be more active in the fed heart as well.[15] The main control point of glycogenolysis is at the phosphorylase system. Catecholamines would be expected to increase glycogenolysis by their known ability to activate phosphorylase through adenyl cyclase and cyclic AMP (see below), but the complexities of adrenergic effects on cardiac metabolism obscure this effect in the intact animal. The whole subject of glycogenolytic control in the heart is unclear at the present. As Opie has stated, "There is no established role for glycogen in the normal cardiac cycle. It is only in extreme conditions such as anoxia that cardiac glycogen is mobilized. . . ." [15]

Lactate

Myocardial lactate usage appears to be regulated by two factors, the arterial level of lactate, and the integrity of pyruvate degradation and oxidative metabolism in the TCA cycle and in the mitochondria (see above).[14] At high arterial concentrations, lactate can provide a substantial contribution to myocardial energy liberation.[59]

Pyruvate

If pyruvate dehydrogenase is not inhibited and if the entry of acetyl Co-A into the TCA cycle is not impaired, heart muscle will use as much pyruvate as it is given.[59] Hypoxia and fatty-acid metabolism can cause both of the above effects and hence are the most common circumstances in which pyruvate use is decreased. Glucose and fructose have been shown to activate pyruvate dehydrogenase and facilitate pyruvate oxidation.[60] The contribution of pyruvate to the cardiac energy supply is minor, however, because of the low circulating arterial levels (0.5–2.0 mg/100 ml).

Fructose

Myocardial transcellular transport of fructose is insulin-independent, but otherwise its degradation is similar to that of glucose. It appears that fructose is used by the heart only in the absence of other fuels.[10, 61]

Fatty Acids

There can be little doubt that free (nonesterified) fatty acids (FFA, NEFA) are important myocardial fuels.[10, 15, 47, 62] Myocardial extraction of saturated fatty acids is inversely proportional to chain length, but the predominant NEFA in the plasma of mammals, the 16-carbon palmitic acid and the 18-carbon oleic acid, are preferentially used over any of the shorter-chain saturated acids.[62] NEFA travels in the blood bound to albumin. As the albumin–NEFA molar ratio approaches unity, myocardial fatty acid extraction decreases.[63] Except in extreme hypoalbuminemia, however, the ratio is unimportant *in vivo*.[15] Transcellular transport appears to be a physical, non-energy-dependent process with a threshold of 0.07 to 0.35 mM, depending on the species.[15, 63] There are at least two intracellular binding sites, a high-affinity locus which is freely exchangeable with the TCA cycle, and a larger, low-affinity site which exchanges more slowly.[64] There is some evidence that NEFA form triglyceride fatty acids (TGFA) before being oxidized, which might account for the low-affinity sites.[65] Nevertheless, TGFA must be hydrolyzed to NEFA and thence, by beta oxidation, to acetyl Co-A before being oxidized.[62] It appears that carnitine (beta-hydroxy gamma-trimethyl ammonium butyrate), which is present in high concentration in heart muscle, is essential for efficient transfer of fatty acid–Coenzyme A complex into the mitochondria for TCA-cycle degradation.[66] Oxidation and uptake of NEFA are more closely correlated than with any other substrate in the heart, although there are other intracellular pathways for NEFA.[62, 67] NEFA oxidation can account for as much as 90 per cent of myocardial oxygen consumption.[10, 15, 62]

Triglycerides

Fat absorbed from the gut is transported primarily as lipoprotein-bound triglyceride fatty acids (TGFA) and broken down to NEFA in the liver.[62] Because TGFA are such a rich energy source, oxidation of 5 mg per minute (less than 10 per cent of the total TGFA passing through the heart) could provide the total cardiac energy requirements.[11] Consequently, quantitation of TGFA extraction *in vivo* has proven difficult, although it has definitely been shown in the perfused heart.[51] Carlson and co-workers recently demonstrated TGFA uptake in resting man by a combination of chemical and radioisotopic measurements.[63] The physiologic import remains unclear. TGFA are also formed from plasma NEFA and are the primary lipid storage form in the heart. They are unquestionably a fuel in the perfused heart.[69, 70] Crass has recently reported that endogenous TGFA oxidation is inversely related to the exogenous NEFA concentration presented to the working perfused rat heart, while the presence of glucose in the perfusate has little effect.[71] It appears that endogenous lipids are utilized only in extremely stressful situations, in a manner similar to the endogenous carbohydrate, glycogen.

Ketones

Acetoacetate and beta-hydroxybutyrate are the principal circulating ketones in plasma and can be utilized by perfused and *in-situ* hearts.[10, 45, 56] Uptake depends on arterial level. As ketone uptake increases, NEFA uptake and glucose uptake decrease. Except in severe starvation or diabetic ketosis, however, arterial levels are low and myocardial ketone utilization is minimal.

Proteins

Although some amino acids may enter into the TCA cycle and liberate energy for cardiac function (fig. 6), this probably only occurs in extreme substrate deficiency (starvation, etc.).[72]

Carbohydrate–Lipid Interaction

I have described some of the interactions between fuels in cardiac muscle in the previous sections. In a series of studies on rat skeletal and cardiac muscle, Randle and co-workers evolved the concept and mechanisms of the "glucose–fatty acid cycle."[73–77] The cycle is based on the inhibitory effects of glucose and insulin on release of NEFA from adipose tissue and on the reciprocal inhibition of glycolysis and pyruvate oxidation in muscle by fatty acids. During carbohydrate deprivation (starvation, hypoinsulinemia, diabetes mellitus), NEFA oxidation is enhanced because the normal stimulation of triglyceride synthesis and the depression of lipolysis in adipose tissue by glucose and insulin are absent. The decreases in plasma glucose and insulin depress cellular membrane transfer of glucose into muscle (including the heart).[54] Further, even in the presence of insulin, the elevated NEFA levels decrease the insulin stimulation of membrane glucose transport,[56] suppress glucose phosphorylation by hexokinase,[54] suppress glycolysis by inhibiting PFK (see above for mechanism),[56] and depress pyruvate entry into the TCA cycle by inhibiting pyruvate dehydrogenase (fig. 6).[75] All of these interactions effectively inhibit glycolysis, and the last interferes with lactate and pyruvate oxidation as well. Although most of the work in perfused and *in-situ* hearts suggests that lipids, ketones and lactate decrease glycolytic flux,[49, 65, 75] there is some evidence that glucose can depress utilization of the other substrates.[79, 80] Olson pointed out as early as 1963 that the multifactorial controls on cardiac fuel utilization made generalizations about the primacy of one fuel over another dangerous.[62] Although it is generally believed that heart muscle preferentially utilizes lipids, particularly NEFA, the evidence is not conclusive.[11]

Hormonal Effects on Myocardial Energy Metabolism

Adrenergic

The sympathetic nervous system plays an important role in controlling myocardial function in awake and anesthetized animals.[7] Although the metabolic effects of adrenergic stimulation have been difficult to categorize, particularly from species to species, increased circulating NEFA levels are a universal manifestation of sympathetic nervous activity. Stimulation of adipose tissue lypolysis is a

constant and well-documented effect of sympathetic (or catecholamine) stimulation which is held to be responsible for the elevated NEFA levels.[51] In an exhaustive review, Himms-Hagen concluded that most of the effects of adrenergic stimulation on lipid metabolism of organ systems could be explained primarily by the increased arterial levels of NEFA.[81] However, glycogenolysis is augmented by sympathetic stimulation, predominantly through activation of glycogen phosphorylase (see below), but also from PFK stimulation. (Both effects are believed to be beta receptor-mediated.) Another systemic effect of sympathetic stimulation could also affect myocardial metabolism. Porte has demonstrated that sympathetic stimulation suppresses pancreatic insulin release through an alpha receptor effect.[52] As discussed before, such an effect would tend to decrease myocardial glucose utilization and favor lipid uptake. However, several investigators have shown myocardial metabolic effects of epinephrine and norepinephrine which are independent of these systemic effects. Regan and co-workers demonstrated that epinephrine stimulated myocardial glycolysis and decreased NEFA extraction,[44] but that norepinephrine decreased glucose and lactate extraction without changing NEFA utilization.[45] Both catecholamines increased TGFA uptake. However, Cowley *et al.* noted increased NEFA oxidation with intracoronary injection of norepinephrine.[82] Glaviano and Masters found that intracoronary norepinephrine stimulated glucose, lactate and NEFA uptake; 1.0 mg/kg propranolol (a large dose) given prior to infusion of norepinephrine markedly decreased NEFA uptake while interfering minimally with carbohydrate metabolism.[84] In a combined *in-vivo* and *in-vitro* study, the same authors produced suggestive evidence that the mechanism of the increased NEFA uptake with norepinephrine involved obligatory esterification of circulating NEFA to TGFA before oxidation (see previous section).[85] According to their hypothesis, beta-adrenergic stimulation would cause activation of a lipoprotein lipase necessary for hydrolyzing TGFA back to NEFA before oxidation of the NEFA could proceed. Beta-adrenergic blockade with propranolol would block this event. Evidence for such a lipase system in the heart has been provided by the work of Christian *et al.*[86]

The metabolic interactions between carbohydrates and lipids probably control the overall metabolic effect of catecholamines on the heart *in vivo*. If the lipid supply is great, NEFA oxidation will be high and the feedback from the TCA cycle via ADP, AMP, P_i and citrate will depress PFK activity and decrease glycolysis. There may be pyruvate output as well because of decreased pyruvate dehydrogenase activity. With decreased NEFA supply, the sympathetic stimulation of glycogenolysis via phosphorylase activation and of glycolysis by PFK stimulation predominate. So there appear to be both direct effects of adrenergic stimulation on myocardial metabolism, probably mediated through glycogenolysis and lipolysis, as well as systemic effects producing increased circulating NEFA, again through lipolysis in adipose tissue as well as through hyperglycemia from hepatic glycogenolysis. The concomitant suppression of pancreatic insulin release would favor lipid uptake by the *in-situ* heart.

In the intact animal, the increase in myocardial oxygen consumption seen with catecholamine administration has been noted to be greater than that dictated by the inotropic effect.[87] In a series of experiments, Mjøs reported that myocardial oxygen consumption was increased in association with elevated NEFA levels, independent of ventricular function changes.[88] By blocking lipolysis with nicotinic acid, he could decrease the elevation of myocardial oxygen uptake produced by beta-adrenergic stimulation without changing the hemodynamic effects.[89] Recently his laboratory has demonstrated that the effect of increased afterload (aortic pressure) on myocardial oxygen consumption was also dependent in part on fatty-acid metabolism.[90] Other studies have suggested that elevated NEFA levels can depress ventricular function [91] and cause ventricular arrhythmias.[92] Apparently, NEFA are capable of interfering with myocardial oxygen utilization and ventricular function, possibly by a detergent effect on membranes.[91]

Adenyl Cyclase–Cyclic AMP

Investigation of the glycogenolytic effect of epinephrine in the liver led to the original

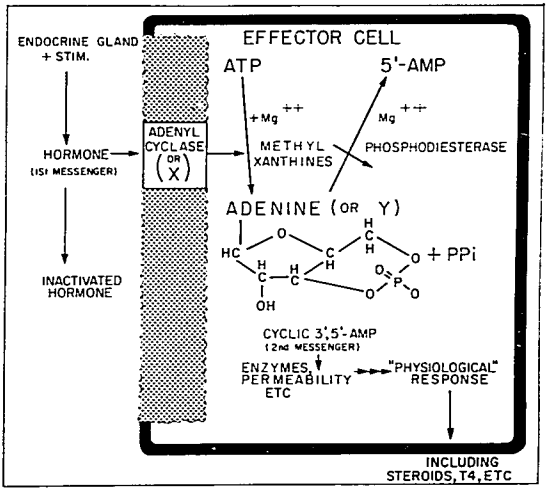


FIG. 7. The second-messenger system, involving adenyl cyclase (from Sutherland et al.[94] with permission of author and publisher).

elucidation of the adenyl cyclase–cyclic AMP mechanism by Sutherland and co-workers.[93] One of the more widely publicized attempts to relate myocardial function and metabolism has been concerned with this interaction between adrenergic stimulation and intracellular cyclic AMP activity. The concept of cyclic AMP as a "second messenger" in translating hormonal effects from blood to intracellular foci has been generally accepted (fig. 7).[94] The hormone stimulates the elaboration of adenyl cyclase, an enzyme found almost exclusively in membrane systems. In the presence of adequate magnesium, adenyl cyclase catalyzes the breakdown of adenosine triphosphate (ATP) to adenosine-3',5'-monophosphate (C-AMP) plus pyrophosphate (PP_i); C (cyclic)-AMP is then broken down to 5'-AMP in the presence of magnesium in a reaction catalyzed by the enzyme phosphodiesterase. The methyl xanthines (caffeine, theophylline, etc.) are phosphodiesterase inhibitors. In the original work of Sutherland, the effect of epinephrine on glycogenolysis was shown to be through activation of adenyl cyclase, producing increased cyclic AMP levels intracellularly.[93] The cyclic nucleotide in turn stimulated phosphorylase b kinase, which catalyzed the conversion of the inactive glycogen phosphorylase b to the active phosphorylase a (fig. 8). Phosphorylase a promoted the degradation of glycogen to glucose-1-phosphate.[95] C-AMP also stimulates a glycogen synthetase kinase, which converts the synthetase from the active I form to the inactive D form.[96] This action, combined with the insulin-suppressing effect of sympathetic stimulation, effectively stops glycogen synthesis. Initially, an attempt was made to correlate the phosphorylase activation and the consequent glycogenolysis in the heart with the positive inotropic effects of beta-adrenergic stimulation,[97] but several studies demonstrated the dissociation between phosphorylase activation and increased contractility.[98, 99] These observations directed attention to the release of cyclic-AMP as the intracellular mediator in a number of hormonal actions. Sutherland's group defined four criteria for acceptance of cyclic AMP as a "second messenger" of any hormone[94]: 1) activation of adenyl cyclase in subcellular preparations; 2) appropriate change of cyclic AMP levels in the intact organ in response to the hormone; 3) poten-

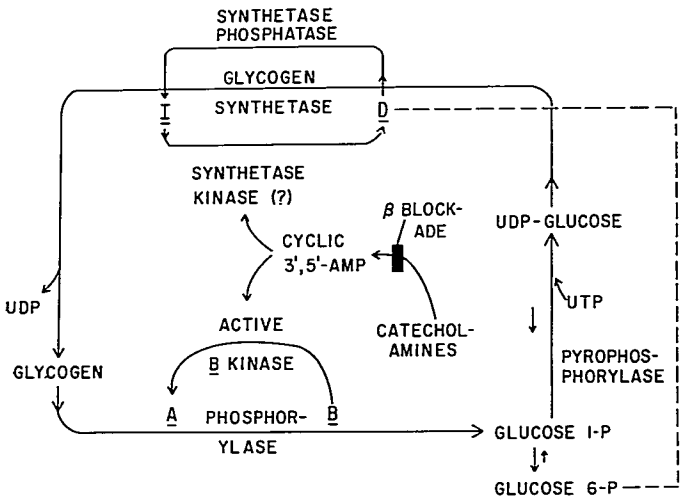


FIG. 8. Myocardial glycogen metabolism (from Mayer,[96] with permission of author and publisher).

tiation of the pharmacologic action of the hormone by phosphodiesterase inhibitors; 4) production of hormonal action by exogenous cyclic AMP. These criteria have been satisfied for the catecholamine activation of liver and cardiac glycogen phosphorylase and for the ACTH-stimulated synthesis of adrenal cortical steroids.[94] The cyclic nucleotide appears to mediate a number of other metabolic reactions as well. Stimulation of phosphofructokinase (PFK) and the lipase of adipose tissue, and decrease in pancreatic insulin secretion, have all been shown to correlate with at least three of the criteria.[100] Although phosphorylase activation is not the mediator of beta-adrenergic inotropy, the criteria of Sutherland appear to have been satisfied for cyclic AMP as the mediator.[101, 102] There are still some doubts about the mechanisms, however.[103-105] It is possible that the inotropic effect of cyclic AMP is not mediated through energy metabolism but rather through an effect on the calcium-accumulating properties of the sarcoplasmic reticulum[101, 106] (discussed in a later section). Although Sutherland's group suggested that adenyl cyclase might be the beta receptor,[107] it is apparent that the enzyme mediates many other hormonal activities.[94] Even in the heart, glucagon, histamine and the prostaglandins stimulate adenyl cyclase and cyclic AMP outside the beta receptor mechanism.[101] Apparently, hormone receptors are located in the cell membrane, but are distinct from adenyl cyclase, which is activated by several types of receptors.[108] Whether the adenyl cyclase–cyclic AMP system is the true mediator of the inotropic effect of various hormones or not, it appears to play an important role in the regulation of myocardial contractile function.

Insulin

Within the decade of the discovery of insulin, all manner of marvelous effects were ascribed to exogenous administration of the hormone, including cardiac inotropic action.[109, 110] Although insulin certainly increases myocardial glucose extraction in the dog and man,[9, 111, 112] intracoronary glucagon-free insulin pro-

duces no inotropic effect in healthy dogs.[112] The positive inotropic effect noted in earlier studies was probably a result of glucagon contamination and/or the sympathetic activation resulting from uncontrolled hypoglycemia.[113] As I have noted, oxygen lack promotes glucose uptake and glycolysis. There has been adequate documentation of the dependence of the hypoxic and ischemic heart on glycolysis.[14, 55, 114-116] Since insulin exerts a major controlling effect on myocardial glucose utilization, the combined use of GIK (*G*lucose to combat the hypoglycemia, *I*nsulin, and potassium (*K*) to prevent hypokalemia) in ischemic heart disease seemed logical. The early work of Sodi-Pallares was directed mainly at arrhythmia prevention,[117] but there was little objective evidence of improvement in cardiac function by GIK infusion until the publication last year of studies in the dog and man. The animal study indicated that GIK significantly reduced the area of infarction and prevented the arterial hypotension and tachycardia produced by ligation of a coronary artery.[118] GIK (with glucagon-free insulin) produced impressive positive inotropic effects in a group of patients with severe left ventricular failure presumed to be caused by ischemic heart disease.[119] An interesting facet of the latter study was the positive inotropic effect of GIK in the control group of patients with functional heart murmurs and no indication of cardiac failure or ischemic heart disease. No hypoglycemia was reported, although only two blood samples were taken. NEFA levels were not reported, so there is little objective information as to whether sympathetic excitation was produced by the insulin. The glucose–fatty acid cycle influence cannot be evaluated, either.[77] The mechanism is obscure. To my knowledge, this is the only reported study in the well-oxygenated intact heart where a positive inotropic effect of insulin has been seen without obvious extraneous causes (drug effects, glucagon contamination, hypoglycemia), although a previous review by the same group intimated that insulin and glucose produced beneficial effects in non-ischemic heart failure, without citing adequate documentation.[120] GIK may well prove to be beneficial in ischemic heart disease, either by increasing the cardiac energy supply or by decreasing the deleterious effects of NEFA.[89-92] However, Opie has cautioned against widespread acceptance of the technique without proper control trials.[121] Although the major effect of insulin on carbohydrate metabolism is probably through acceleration of membrane glucose transport, recently the hormone has been shown to increase pyruvate oxidation in beating heart cells in culture, presumably by activating pyruvate dehydrogenase.[122]

Other Hormones

The myocardial metabolic and functional effects of *thyroid hormone* are difficult to separate from the sympathetic excitation which accompanies hyperthyroidism.[123] It would appear that the hormone does have a direct inotropic effect on cardiac muscle, perhaps mediated through cyclic AMP.[101] The metabolic effects have been reported to include increases in circulating NEFA and in oxidative metabolism, both of which would fit with this finding. The physiologic role of *glucagon* lies in its control of carbohydrate metabolism in the liver.[124] In pharmacologic doses, which far exceed the physiologic amounts secreted by the pancreas, the drug can be a potent inotropic agent, although clinical results have been confusing.[125] The hormone is presumed to act through the adenyl cyclase–cyclic AMP system. In view of the marked variability in the therapeutic effects of glucagon,[125] it is of interest that Epstein and co-workers could correlate the functional effect of glucagon in cat and human papillary muscles with the adenyl cyclase response,[101] even though the inotropic and cardiac metabolic effects have been clearly dissociated.[124] The positive inotropic effect of the *prostaglandins* has also been postulated as depending on adenyl cyclase.[126] Discussion of this protean group of compounds is beyond the scope of this paper.[127]

Effect of Anesthetics

Halothane and Methoxyflurane

Most of the published work on the effects of anesthetics on myocardial metabolism has dealt with energy liberation. In a series of papers based on experiments utilizing atrial muscle strips, initially from rats and subsequently from man (obtained during cardiac surgery), Paradise *et al.* have demonstrated

an interesting correlation between metabolism and function. Gimeno *et al.* had shown that stimulated rat atrial strips could function using glucose, fructose, lactate, pyruvate, acetate, mannose, or butyrate.[128] The highest tensions could be achieved with 16.5 mM glucose, indicating a preference for that substrate by rat atria. Using this information, Paradise and Ko demonstrated that the decrease in developed tension produced by halothane in the stimulated rat atrial preparation could be partially reversed by pyruvate and lactate but not by glucose in any concentration; thus a block in the conversion of glucose to pyruvate was indicated (fig. 9).[129] Inasmuch as one of the prime controls of glycolysis is phosphofructokinase (PFK),[55] this appeared to be a likely site for the block. Shaw and Stadie had demonstrated in skeletal muscle that PFK was inactivated in bicarbonate-free media.[120] Ko and Paradise could see no effect of either glucose or fructose in their bicarbonate and substrate-free medium, confirming that PFK was also inactivated in rat atria. However, the demonstration that fructose (in addition to pyruvate, lactate and acetate) could partially reverse the negative inotropic effect of halothane suggested that PFK was functioning in the halothane-depressed atrium.[121] Consequently, the block must be above the PFK step at the uptake of glucose into the cell (fig. 9, *A*); the phosphorylation of glucose (fig. 9, *B*); or the isomerization of glucose-6-phosphate to fructose-6-phosphate (fig. 9, *C*).

In a separate publication, the same authors confirmed that atrial contractility in a 5-mM glucose medium could indeed be depressed by a bicarbonate-free bath.[122] The fact that the depression could be partially reversed by pyruvate, lactate, and acetate, but not by glucose or fructose, suggested that PFK inhibition was involved. Contractile depression by citrate, a known inhibitor of rat heart PFK,[56] could also be reversed by pyruvate and lactate but not by fructose. However, acetate was relatively ineffective, while glucose was most effective in this preparation. Thus, glucose appeared to be able to influence contractility in the perfused rat atrium in some fashion that bypassed the crucial PFK step. Decreasing calcium ion concentration produced decreases in developed tension, but were only minimally reversed by the highest glucose concentration, negating the idea that the citrate effect involved chelation of calcium. Thus, the glycolytic mechanisms in stimulated rat atria are still somewhat obscure. It is important to note that all this evidence is circumstantial, for no glycolytic intermediates were measured at any time in these studies.

Continuing their studies with atrial appendages from children (taken at cardiac surgery) with a variety of heart diseases, the same investigators demonstrated some differences between the human and rat atrial preparations.[133] Higher glucose and calcium concentrations were necessary to maintain developed tension in the human atria. The human preparation was also less stable than the rat-heart preparation, deteriorating in 60 minutes. Maximum developed tension required 30 mM glucose in the human heart, as contrasted to 16.5 mM glucose in the rat heart. The human atria depressed by a lack of substrate responded very well to 20 mM glucose and pyruvate, but there was little reversal of the depressed tension by lactate, fructose, or acetate, in contrast to results seen with the rat atria.[128] In human atria halothane produced a contractile depression qualitatively similar to that seen in the rat.[134] With the information from the previous study available, only glucose and pyruvate were tested in the human preparation. As in the rat, glucose had no effect on the halothane-depressed tension. Pyruvate not only reversed the depression but produced increases of as much as 50 per cent above the control non-anesthetized state, although the results were extremely variable. Consequently it appeared that a block in glycolysis qualitatively similar to that seen in the rat was contributing to the depressed tension produced in human atria by halothane. The demonstration that decreased tension could be produced by halothane in a glucose-free medium indicated that a block in glucose uptake (fig. 9, *A*) or in phosphorylation (fig. 9, *B*) was not an essential feature of the glycolytic interference produced by the anesthetic.[135] The source of this glycolytic substrate blockade in a glucose-free medium presumably was glycogen (fig. 9, *D*), with block at the hexose

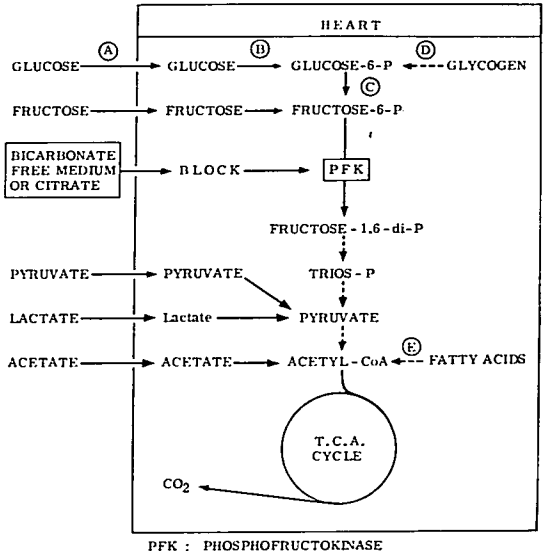


FIG. 9. Glycolytic pathways in atrial muscle (from Paradise,[139] with permission of author).

isomerase conversion of glucose-6-phosphate to fructose-6-phosphate (fig. 9, *C*).

Ko and Paradise had recognized several problems inherent in transferring the results of *in-vitro* rat-atria experiments to the intact animal.[134] The halothane concentrations producing 50 per cent depression in developed tension in all the prior experiments were of the order of the minimal anesthetic alveolar concentrations (MAC), which have been shown to produce little direct myocardial depression in the intact animal.[26, 136, 137] In addition, the predominate fuel for the heart in the fasting state has been assumed to be fatty acids, (fig. 9, *E*),[9, 10, 15, 62] not the carbohydrates involved in these experiments. Technical problems prevented the use of fatty acids in their preparation. Starved rat hearts had been shown to have increased amounts of storage lipids (TGFA).[62] The authors reasoned that these atria would use TGFA and be less sensitive to the glycolytic blocking effect of halothane. The results indeed showed that more halothane (10.8 mg/100 ml) was needed to produce a 50 per cent decrement in developed tension in starved than in fed rat atria (6.5 mg/100 ml).[138] Thus, in the *in-vitro* situation, where presumably lipids were being used for the energy requirements of the atria, more halothane was necessary for equivalent depression, thereby approximating the *in-vivo* situation. The rat atrial model was also used for methoxyflurane, where an effect qualitatively similar to that seen with halothane was observed.[139] The reversal of the methoxyflurane tension depression produced by pyruvate and fructose was even less complete, however, than that seen with halothane. In human atrial appendages obtained from patients during cardiopulmonary bypass, developed tension was markedly increased by pyruvate alone in the absence of anesthetics.[140] As in human atria exposed to halothane, pyruvate completely reversed the tension depression produced by methoxyflurane. In contrast, only a small increment in pentobarbital-depressed tension was produced by pyruvate. Most recently, Morrow and Paradise reported

decreases in rat atrial ^{14}C-labeled glucose uptake without change in pyruvate or fructose uptake by low halothane doses (.25–1.0 mM).[141] Higher concentrations (1.0–2.0 mM) decreased uptake of all three substrates, confirming the observations that the isolated glycolytic block was produced by the low anesthetic doses.

The conclusions from the above studies were that halothane and methoxyflurane decrease atrial developed tension by a block in glycolysis before the PFK-catalyzed conversion of fructose-6-phosphate to fructose-1,6-diphosphate, but that pentobarbital has a different mode of action. The doses of the volatile anesthetics producing this effect were considerably lower than the concentrations producing negative inotropic responses *in vivo*. In atria where endogenous lipids were a significant fuel, the effect disappeared and higher anesthetic doses were needed for depression of contractility. The differences between the substrate effects in rat and human atria observed in these studies might be expected,[3, 4] but one wonders how "normal" these hearts from patients on cardiopulmonary bypass were and whether this also influenced the results. Other investigators have not been able to demonstrate a positive inotropic effect of either pyruvate in halothane-depressed cat papillary muscle[142] or acetate in halothane-depressed dog hearts *in situ*.[143] Thus, the relevance of the nicely demonstrated correlation between metabolism and function in rat and human atria remains puzzling.

At about the same time, our laboratory was working with intact closed-chest dogs.[26] The left ventricle, aorta, right atrium and great cardiac vein were catheterized fluoroscopically. Temperature, hydration and ventilation were maintained constant during MAC and 2–3 MAC halothane anesthesia. Myocardial NEFA uptake decreased significantly with the negative inotropic effect of the high halothane concentration. There was no appreciable myocardial glucose uptake until the arterial concentration reached 100 mg/100 ml in spite of the previously-demonstrated myocardial threshold in the dog (and man) of 60 mg/100 ml.[9] The decrease in glucose uptake appeared to be greater in the depressed hearts. Lactate was utilized and uptake was well maintained in the depressed hearts, while pyruvate uptake declined. The effects of higher arterial glucose levels (213–262 mg/100 ml) on myocardial glucose uptake demonstrated that uptake did not follow arterial levels, as in the normal dog, but resembled uptake in the diabetic dog.[121, 144] Plasma immunoreactive insulin (IRI) levels during halothane anesthesia did not follow blood glucose levels, and were lower during anesthesia than before anesthesia in a different group of dogs (unpublished data). Finally, glucagon-free insulin produced a positive inotropic effect and increased glucose uptake in the halothane-depressed heart in our intact dog preparation[145] (fig. 10). As reported above, insulin's primary action in the heart is to stimulate transcellular glucose transport.[49, 53] At this point in the experiments of Paradise and Ko, membrane glucose transport (fig. 9, *A*) was still a likely site for the glycolytic block produced by halothane in rat atria.[131] Consequently, the effect of halothane in both studies might be to impede membrane transport of glucose. Our low IRI levels combined with the observation of a diabetic glucose-tolerance curve induced by halothane in the dog[2] suggest that a decrease in the pancreatic insulin response to glucose might be responsible for our findings. This blunted insulin response has been confirmed in man with halothane and methoxyflurane.[146]

As mentioned previously, methoxyflurane produced hemodynamic changes similar to those produced by halothane in our dog model.[21] Arterial concentration and myocardial uptake of fatty acids decreased during methoxyflurane-induced cardiac depression, as did uptake of both pyruvate and lactate. Although there was little glucose uptake in either control or depressed hearts, arterial levels were in the low range where minimal uptake might be expected.[9] However, plasma IRI levels were very low during methoxyflurane anesthesia (unpublished data), so that decreased transcellular glucose transport remains a possible mechanism (as with halothane). The effect of insulin on the methoxyflurane depressed heart has not been tested, however.

As Paradise has pointed out,[124, 147] the block in the hexose isomerase step of glycolysis (fig. 9, *C*) should have been present in our

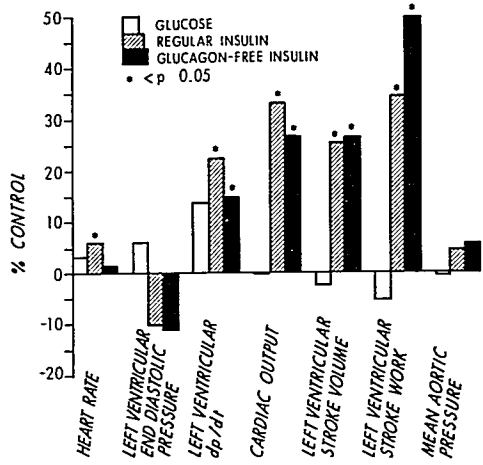


FIG. 10. Cardiodynamic effects of glucose and insulin in the halothane-depressed heart.

control animals anesthetized with MAC anesthesia. Thus, this metabolic defect cannot be responsible for the dose-dependent negative inotropic effect of anesthetics, for the block in glycolysis is virtually complete at *in-vivo* anesthetic concentrations which produce little if any myocardial depression. In addition, only half of the tension reduction produced by anesthetics in the rat atria or less could be reversed by substrates (the human atria behaved differently with pyruvate only). Insulin also produced less than 50 per cent reversal in the intact dog. If interference in glycolytic metabolism by anesthetics is important in the *in-vivo* clinical situation, the myocardial fuel source must be predominantly glucose. (Although glycogenolysis could also be interrupted, myocardial glycogen stores are rapidly exhausted in the working heart.[52]) There are two major circumstances which favor the use of glucose as the primary energy source in the heart: high arterial levels of glucose (accompanied by appropriate insulin secretion) and hypoxia [15] (see above). Although infusions of glucose commonly accompany surgical anesthesia, there is abundant evidence that insulin levels are low and NEFA levels are high under these conditions [146, 148-151]; consequently, a shift to carbohydrate predominance seems unlikely. Hopefully, myocardial hypoxia during surgical anesthesia is rare. As mentioned above, the halothane- and methoxyflurane-depressed dog heart seemed to be well oxygenated. It is possible, however, that the dependence of stimulated rat atrial strips on glucose reflected a relative hypoxia. Brown and co-workers were able to observe light-microscopic changes indicative of poor perfusion of rat ventricle strips and atrial strips more than 16–20 cells wide.[152] Intrinsic contractility [3, 4] and oxygen uptake [91] of the rat heart are considerably greater than in larger mammals. Rat-heart-muscle preparations are very prone to diffusion hypoxia.[153] The rapid rate of stimulation of the rat atrial preparation (200/min) could well lead to hypoxia of the inner core of the muscle and the predominance of anaerobic glycolysis. However, there must be well-oxygenated portions in the outer layers for the pyruvate, lactate, and acetate to liberate energy aerobically and reverse the depression. Recall that any process decreasing TCA cycle activity will also stimulate glycolysis.[77] It may well be that some of the effects of anesthetics on oxidative metabolism

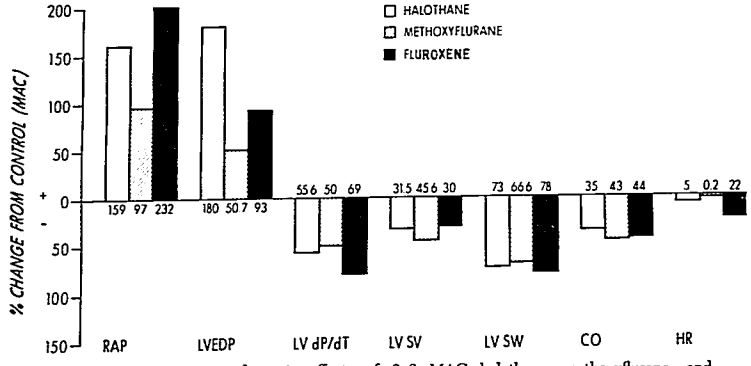


FIG. 11. Comparative dynamic effects of 2–3 MAC halothane, methoxyflurane, and fluroxene. *RAP*, right atrial pressure; LVEDP, left ventricular end-diastolic pressure; LV dP/dT, left ventricular dP/dT; LV SV, left ventricular stroke volume; LV SW, left ventricular stroke work; CO, cardiac output; HR, heart rate.

to be discussed later could contribute to a shift to glycolytic predominance.

The major advantage of the use of myocardial A-V differences in the intact animal is the minimal derangement in physiology produced (compared with perfused hearts, etc.). This advantage is largely lost when an acute experiment using thoracotomy for catheter placement is used. If metabolism and function are to be correlated, both must be measured. In the other published works on the effects of halothane and methoxyflurane on myocardial substrate uptake in the dog.[42, 43] no measure of cardiac function was made. The preparation was an acute thoracotomy without temperature, acid–base, or anesthetic dose measurements. No estimate of coronary blood flow was made. Consequently, it is impossible to correlate the findings in any meaningful fashion.

Fluroxene and Diethyl Ether

The hemodynamic effects of high concentrations of halothane and methoxyflurane in the intact dog are similar and resemble those of halothane in man.[137] The cardiovascular effects of fluroxene have recently been shown to be quite different in man, however.[154] Cardiac output and myocardial function remained stable at 3–4 MAC. As mentioned before, Skovsted and Price reported that the drug behaved like diethyl ether and cyclopropane in the cat, causing increased sympathetic nervous activity.[8] As the explosion hazard is less than with cyclopropane or diethyl ether, we chose to study fluroxene as a representative of the non-cardiac-depressant, sympathomimetic anesthetics in our dog model.[22] To our surprise the hemodynamic effects were entirely similar to those seen with halothane and methoxyflurane (fig. 11). Unlike the latter drugs,[22, 31] fluroxene produced significant metabolic acidosis in our dogs at both MAC (6.5 per cent) and 2–3 MAC (13.8 per cent) (fig. 12). Although it appeared that much of the acidosis was lactic in origin (fig. 13), there was no difference in arterial lactate levels between the animals anesthetized with 73 per cent nitrous oxide and those anesthetized with low concentrations of fluroxene, although MAC fluroxene produced significant metabolic acidosis compared with nitrous oxide (unpublished data). Of the arterial substrate changes between the low and high fluroxene concentrations, only glucose was statistically insignificant (fig. 13). The most striking aspect of the myocardial A-V differences seen with fluroxene was the low extraction of all substrates (table 3). As with halothane, even with high arterial glucose levels there was virtually no

glucose uptake. Negative A-V differences for the other three substrates were seen for the first time in our laboratory with fluroxene. It is tempting to ascribe these effects to sympathetic stimulation. We saw no cardiovascular evidence of such stimulation, however. In addition, fatty-acid levels were not increased by fluroxene in this study, and, in fact, were decreased by higher concentrations. Consequently, we have no objective evidence of sympathetic stimulation in the dog by fluroxene. It is difficult to attribute the high coronary venous pyruvate and lactate levels to increased glycolysis in the face of little or no glucose uptake. This study points up the speculations that are necessary to explain changes in myocardial metabolism on the basis of myocardial A-V differences without more explicit data on concentrations of the metabolic intermediates. One conclusion appears certain, however. There is a marked difference between the myocardial metabolic effects of fluroxene and halothane in the dog.

The pioneering study of Galla *et al.* on the effects of diethyl ether on myocardial substrate utilization in the dog demonstrated a shift in myocardial extraction from predominantly lipid in the awake fasting animal to predominantly carbohydrate during ether anesthesia.[155] This was primarily a function of substrate availability, as glucose, lactate and pyruvate arterial levels increased markedly, while NEFA concentrations decreased. No measurements of myocardial blood flow, body temperature, *p*H, or blood gases were reported. Only animals in which mean aortic blood pressure exceeded

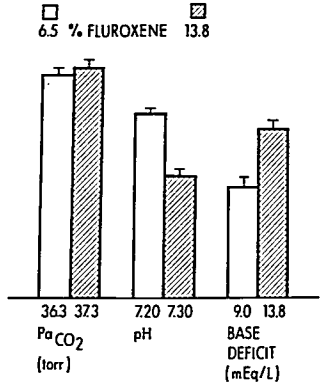


FIG. 12. Acid–base values with MAC (6.5 per cent) and 2–3 MAC (13.8 per cent) fluroxene.

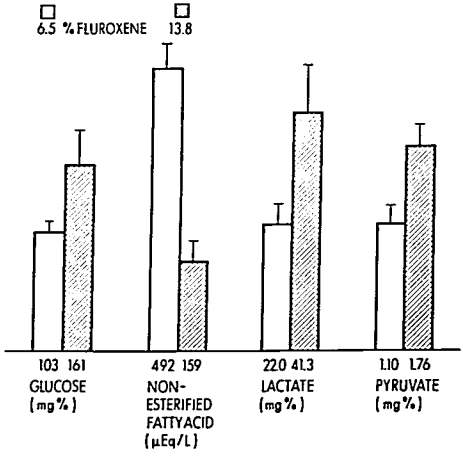


FIG. 13. Arterial substrate concentration with MAC (6.5 per cent) and 2–3 MAC (13.8 per cent) fluroxene.

100 torr were used, and no estimate of myocardial function was made. Consequently the significance of the study to mechanisms of anesthetic myocardial depression is questionable.

Miscellaneous

Measurement of myocardial A-V oxygen and substrate extraction remains the major method of estimating the effects of various interventions on myocardial metabolism in man, although it suffers from the same limitations as in lower animals.[39, 40] Moffitt and co-workers have extensively studied patients undergoing open-heart surgery using this method.[156-161] The authors did not report data from either unanesthetized patients or anesthetized patients not subjected to operation, so no information about the effects of anesthetics alone is available. It is interesting that the insulin response to the massive hyperglycemia produced by the pump prime and the lesser hyperglycemia in the postoperative period was less than might have been predicted. There was suggestive evidence that insulin administration might have improved postoperative cardiac function in association with decreasing ketosis.[161]

Several anesthetics, including chloralose, urethane, diethyl ether, and chloroform, have been reported to have no consistent effect on rat-heart glycogen content.[162] The concentrations of gaseous anesthetics were completely uncontrolled, however, so it is difficult to draw conclusions from the study.

Brown and Crout have suggested that interference in energy liberation could not be an important feature in the negative inotropic effects of anesthetics because they could see papillary muscle depression by anesthetics after pharmacologic blockade of all energy-liberating pathways.[142, 163] Conclusions drawn from these "poisoned" papillary muscles are somewhat suspect. Although it is probable that the basic mechanism of the negative inotropic effect of anesthetics is not an interference in energy liberation, available evidence is not sufficient to prove or disprove this possibility. Radioactive tracer studies of carbohydrates and lipids in both perfused and *in-situ* hearts correlated with evidence of functional depression by anesthetics are necessary. If, indeed, energy liberation is involved, then the energy stores of the heart should be depleted. Tissue levels of ATP and CP must

TABLE 3. Myocardial Substrate Metabolism

	Fluroxene, Alveolar	
	MAC 6.5 Per Cent	2-3 MAC 13.6 Per Cent
Glucose		
Arterial (mg/100 ml)	103 ± 11	161 ± 32
A-V (mg/100 ml)	−4.3 ± 2.9 (6/9 neg)	14.4 ± 21.4 (7/9 neg)
Uptake (mg/100 g/min)	−2.17 ± 1.66	5.51 ± 7.7
Non esterified fatty acids		
Arterial (μEq/l)	492 ± 54	159 ± 29*
A-V (μEq)	58.4 ± 26 (2/8 neg)	14 ± 6.4 (2/8 neg)
Uptake (μEq/100 g/min)	2.39 ± 1.8	0.44 ± .21
Lactate		
Arterial (mg/100 ml)	22.0 ± 3.3	41.3 ± 9.3*
A-V (mg/100 ml)	3.12 ± 1.6 (2/9 neg)	0.96 ± 1.8 (3/9 neg)
Uptake (mg/100 g/min)	1.47 ± 0.77	0.20 ± 0.56
Pyruvate		
Arterial (mg/100 ml)	1.1 ± 0.17	1.76 + 0.20*
A-V (mg/100 ml)	0.16 ± 0.20 (2/9 neg)	0.27 ± 0.12 (2/9 neg)
Uptake (mg/100 g/min)	0.09 ± 0.11	0.11 ± 0.04

* $P < 0.05$.

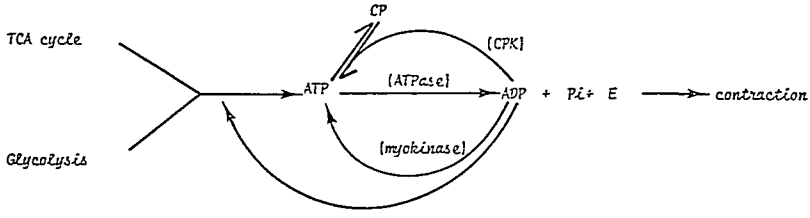


FIG. 14. Myocardial high-energy phosphate kinetics (see text for abbreviations).

also be measured and correlated with the decreased contractility (see below).

Energy Conservation

MITOCHONDRIAL METABOLISM

For more detail on this subject, the reader should consult the review in the Symposium devoted to oxidative phosphorylation.[164] I will briefly review some aspects which I feel are pertinent to the anesthetic effects which have been reported to occur in the heart. As the heart is an aerobic organ and oxidation of substrates takes place within the mitochondria, the mitochondria would be a likely locus for drug effects. Some aspects of mitochondrial metabolism have been discussed above, as TCA cycle activity is located in the organelle. Specific studies of mitochondrial function usually focus on changes in oxygen consumption. One of the TCA cycle intermediates is introduced into the medium containing the mitochondrial preparation, and oxygen uptake is measured. The addition of ADP activates the high-energy phosphorylation mechanism and oxygen uptake accelerates markedly (state 3). Decrease in state 3 oxygen consumption indicates interference with the electron-transfer chain. The location of such a block can be deduced by using different TCA cycle intermediates as substrates (fig. 6A). If the inhibition is before the cytochrome system, oxygen uptake using succinate as a substrate will not be affected, while the glutamate, beta-hydroxybutyrate, etc., oxygen uptake will be decreased. In a like fashion, by using substances known to affect specific enzymes in the transport chain (*e.g.*, rotenone), the locus of action of a drug can be more precisely identified. When TCA substrates are added in the absence of ADP, oxygen uptake is slow (state 4). Increase in oxygen uptake during state 4 mitochondrial respiration is indicative of loss of respiratory control (or uncoupling).[165] Either a block in the electron-transport chain or uncoupling can interfere with the conservation of energy as high-energy phosphates. Another aspect of mitochondrial function which may be related to cardiac metabolism and function is the active transport of cations by the mitochondrial membrane.[11] The physiologic import of cation transport may relate to control of transcellular flux, intracellular compartmentalization, or metabolism.[166] The importance of compartmentalization of calcium in myocardial function is well established.[167, 168] The effect of anesthetics is considered in detail in the discussion of energy utilization.

HIGH-ENERGY PHOSPHATES

The energy liberated from the fuels discussed in the previous sections is conserved (stored) as high-energy phosphate compounds. In muscle, ATP and CP are the predominate storage forms (fig. 14) [11, 169] and exist in a reversible equilibrium. Energy derived from glycolysis and oxidation is tranferred to the high-energy phosphate bond of ATP. Hydrolysis of ATP by an ATPase enzyme frees the energy for use in contraction–relaxation, metabolism, etc. CP serves as a reservoir for the high-energy phosphate, replenishing the ATP, which is the direct source of energy during muscular activity.[170, 171] Consequently, ATP levels vary minimally and CP levels are more labile in heart muscle.[11, 169, 172] The enzyme creatine phosphokinase (CPK) catalyzes rephosphorylation of ADP to ATP via CP (fig. 14). The ADP produced by energy release

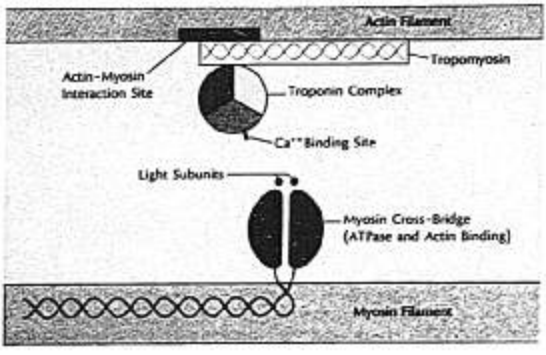


FIG. 15. Schematic representation of contractile proteins (from Katz,[108] with permission of author and publisher).

in muscle can also replenish ATP without further supply from the mitochondrial transport system through myokinase (fig. 14). This enzyme appears to be activated by rather large decreases in ATP, which do not occur in cardiac muscle under normal conditions.[169, 173] Consequently, this system functions only during very low energy availability states. From figure 14 it is apparent that the relationship between ATP synthesis from the heart's energy sources and ATP hydrolysis by the ATPases will determine the level of ATP and CP in the heart. If there is interference with synthesis (hypoxia, a block in energy liberation or conservation), and if ATPase activity is not affected, ATP and CP concentrations decrease. If ATPase activity is depressed without a change in ATP synthesis, ATP and CP concentrations rise. Either event can result in decreased myocardial contractility, although there is evidence that the heart can function satisfactorily with a 50 per cent reduction in high-energy phosphate stores.[11]

EFFECT OF ANESTHETICS

To date there have been only two publications of the effects of anesthetics on cardiac mitochondria. Taylor and co-workers reported the effects of halothane on conformational changes in beef-heart mitochondria.[174] They hypothesized that the electron-microscopic picture of the mitochondrial membrane indicates the energy state of the mitochondrion.[175] Halothane induced beef-heart mitochondria to assume an orthodox non-energized structure seen when mitochondrial oxygen uptake is uncoupled from high-energy phosphate production. Close to 100 per cent of the mitochondria were "uncoupled" with 4 per cent halothane, and the proportion of mitochondria in this state decreased as the halothane dose was lowered. It should be noted, however, that there is controversy as to the validity of the relationship of mitochondrial structure and energy state.[176] Harris *et al.* noted that halothane reversibly depressed NAD-linked electron transport (fig. 6A) in beef-heart mitochondria in a dose-dependent manner.[177] With succinate as a substrate there was minimal depression at equivalent concentrations. Uncoupling was not seen until very high concentrations (>2 mM) of anesthetic were used, and it was irreversible. The same workers reported similar findings with methoxyflurane, fluroxene, chloroform, and diethyl ether, although the latter two depressed succinate-linked respiration as well. (The authors did not specify whether these studies were carried out in beef-heart or rat-liver mitochondria.) It is interesting that the same findings were reported for halothane and methoxyflurane in mitochondria from rat skeletal muscle, although the proportions of red and white muscle studied were not specified.[178] Miller also noted inhibition of NADH-linked mitochondrial electron transport in pigeon, rabbit, beef, and rat hearts by halothane, methoxyflurane, and chloroform, with minimal effects on non-

NADH-linked succinate respiration.[179-181] Unlike Harris, he found no effect of fluroxene on the NADH-linked electron-transport system, although Nahrwold and Cohen showed a dose-related depression of state 3 oxygen uptake by fluroxene in rat-liver mitochondria.[182]

If the effects of anesthetics on electron transfer and oxidative phosphorylation are related to the negative inotropic effect of anesthetics, the levels of ATP and CP in cardiac muscle would be expected to be decreased (if ATPase activity remains unchanged). The only documentation of these levels, to my knowledge, has been in a study of rat hearts.[183] ATP and ADP levels were unchanged by diethyl ether and halothane. High concentrations of ether decreased CP levels, but no data on CP were reported for halothane. Unfortunately, anesthetic dose was poorly controlled and not measured. Respiratory acidosis undoubtedly occurred with the higher anesthetic doses. The "quick-freeze" technique was not employed for the measurement of the high-energy phosphates, so the values are suspect.[184]

In summary, it appears that inhalation anesthetics do interfere with NADH-linked oxidative phosphorylation in the heart but affect succinate-mediated, non-NADH-linked mechanisms minimally. The mechanistic significance of this remains to be determined.

Energy Utilization

The precise way in which cardiac muscle uses the energy liberated and stored is not fully understood. Again, much of the basic work has been done in skeletal muscle and carried over to cardiac muscle, although there are substantial functional and metabolic differences. There can be little doubt that the process of contraction of all muscle involves the making of "cross bridges" between the actin thin filaments and the myosin thick filaments according to the Huxleys' sliding-filament theories.[185, 186] A detailed discussion of the mechanochemistry of cardiac contraction is beyond the scope of this paper.[187]

Contractile Proteins

During diastole, the filamentous contractile proteins, actin and myosin, are dissociated.

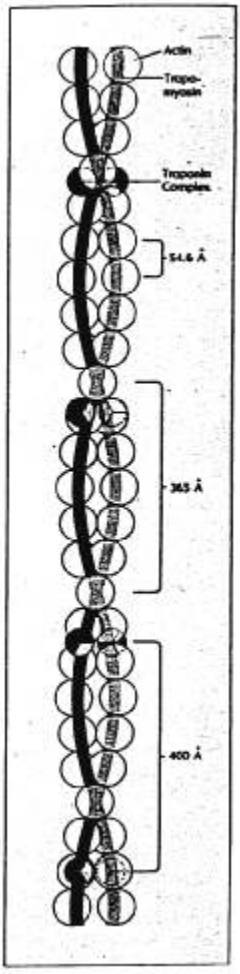


FIG. 16. Schematic representation of actin–tropomyosin–troponin complex (from Katz,[188] with permission of author and publisher).

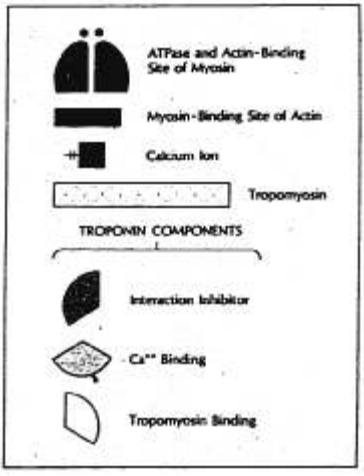


FIG. 17. Symbols for schematic of contractile protein interactions (from Katz,[188] with permission of author and publisher).

The current postulate holds that the regulator proteins, troponin and tropomyosin, are responsible for this inhibition of the actin–myosin cross-bridge formation (fig. 15).[188] Tropomyosin is a long, rod-like protein wound around the actin alpha helix in the opposing grooves (fig. 16). It serves as a framework for the active component of the complex, troponin, and probably participates passively in the cross-bridge inhibition. Troponin is bound to tropomyosin every 400 angstroms along the helix. This active protein has several components, whose structure and function are not entirely clear.[189] Schematically, they may be equated to tropomyosin binding, calcium sensitivity, and cross-bridge inhibition (fig. 17). During diastole, the sarcoplasmic calcium ion concentration is very low ($<10^{-7}$ M) and the troponin–tropomyosin complex inhibits cross-bridge formation (fig. 18). As systole develops, calcium ion concentration rises to a maximum of 10^{-5} M at the time of greatest muscle shortening (fig. 19). The calcium ions are bound to the troponin–tropomyosin complex, releasing the inhibition of cross-bridge formation, and the myosin head attaches to the actin binding site, pulling the filaments toward the middle of the sarcomere (shortening). The ATPase necessary for the hydrolysis of the conserved high-energy phosphate is thought to lie on the myosin head (fig. 17). The activity of myosin ATPase is markedly increased by the actin–myosin association and by the increase in calcium ion concentration. This activation leads to splitting of the Mg-bound ATP to ADP and P_i. Relaxation (dissociation of the actomyosin cross-bridge) is promoted by a rapid decrease in sarcoplasmic calcium ion, with calcium release from the troponin and inhibition of cross-bridge formation again (fig. 20).

Calcium

As oxygen is necessary for TCA-cycle activity, so calcium is essential for the muscle contraction–relaxation cycle. There is little doubt that the link between sarcolemmal electrical depolarization and the formation of the actomyosin cross-bridges is dependent on calcium through the transverse sarcotubules and the longitudinal sarcoplasmic reticulum (SR) (fig. 21).[190] In skeletal muscle, the source of the sarcoplasmic calcium increase in systole

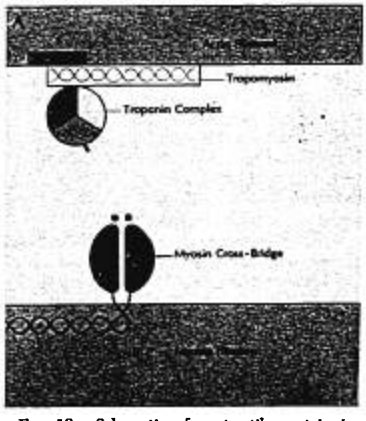


FIG. 18. Schematic of contractile protein interaction during diastole (from Katz,[188] with permission of author and publisher).

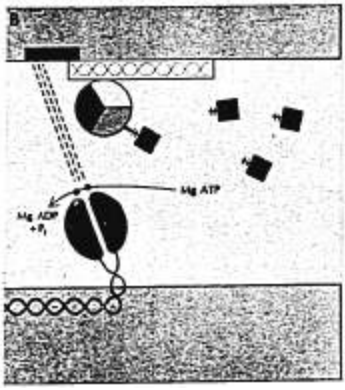


FIG. 19 (*left*). Schematic of contractile protein interaction during systole (from Katz,[188] with permission of author and publisher).

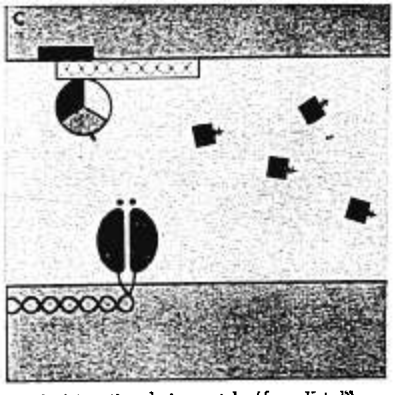


FIG. 20 (*right*). Schematic of contractile protein interaction during early diastole (from Katz,[188] with permission of author and publisher).

and the reservoir to which it returns during diastole is this SR.[190] There is still some controversy about whether the same can be said for cardiac muscle.[191] Skeletal muscle can contract quite nicely in a calcium-free medium, recycling the intracellular calcium, but cardiac muscle is dependent on extracellular calcium for continuing contractility.[192] The sarcoplasmic reticulum in skeletal muscle is more highly developed, has a higher calcium capacity, and is more intimately associated with the myofibrils than that in cardiac muscle (fig. 21).[191] As noted previously, cardiac-muscle mitochondria are more numerous and more closely applied to the myofibrils than those in skeletal muscle. Cardiac mitochondria possess the requisite calcium-accumulating ability to clear the sarcoplasm of calcium during diastole, so they could participate in calcium compartmentalization during relaxation.[192, 193] However, the bulk of the evidence at present suggests that the SR is the major calcium-accumulating mechanism in normal function.[194-196] The origin of the activating calcium in systole still has not been positively identified in the heart,[197] although the SR is the prime candidate.[187, 192, 194] Chidsey's scheme of calcium movement in the myocardium is probably as good a conception as we have (fig. 22).[196] In the normal heart at rest, calcium is present in the extracellular fluid, the SR, and the mitochondria, but not in the sarcoplasm. Membrane excitation increases calcium permeability, and extracellular calcium influxes. This calcium triggers the release of a different intracellular calcium pool (probably from the SR), actin–myosin cross-bridging occurs, and the sarcomere shortens. At the beginning of relaxation (diastole), calcium is rapidly removed from the sarcoplasm, probably into the SR, but possibly into another site (?mitochondria), cross-bridging is inhibited, the sarcomere returns to resting length, and the cycle recurs. Whatever the ultimate calcium compartmentalization turns out to be, there seems to be little question that sarcolemmal calcium transport plays a more important role in excitation–contraction coupling in cardiac than in skeletal muscle.[186]

The active clearance of the sarcoplasmic calcium during early diastole is energy dependent.[195] ATP hydrolysis, catalyzed by calcium-dependent ATPases, fuels the calcium-pumping of both the SR and mitochondria. So calcium mediates excitation–contraction coupling, removes the troponin–tropomyosin

inhibition of actomyosin cross-bridge formation, and activates the ATPases involved in energy utilization for the cross-bridge reaction and the active processes involved in relaxation.

(*Acto*)*myosin ATPase*

In excess of 70 per cent of the total cardiac energy utilization is concerned with the work done and tensions developed by the contractile proteins.[11] Consequently, the ATPase activity of these proteins is a major determinant of energy utilization. The ATPase activity appears to be a basic property of myosin. Actin has no physiologically important activity. Barany has demonstrated that myosin ATPase activity is directly related to the maximum shortening velocity of many types of muscles from a variety of animals, including the very slow ileofibularis of the tortoise, the intermediate cat soleus and rat soleus, and the very rapid mouse extensor digitorum longus.[195] Cardiac myosin falls in the slower group of red muscles.[187] The calcium sensitivity of cardiac myosin ATPase is probably conferred by the tropomyosin–troponin complex, as it is not present in myosin ATPase or deactivated (without tropomyosin and troponin) actomyosin.[199] Consequently, the physiologically active, calcium-regulated ATPase is the associated actomyosin form. Decrease in this activity could impair the energy utilized for the contraction–relaxation cycle and decrease contractility.

Effect of Anesthetics

Although there have been three studies of the effects of anesthetics on (acto)-myosin ATPase, for various reasons the results cannot be considered definitive. Luchi and Kritcher administered nitrous oxide (probably in hypoxic concentrations), diethyl ether, and cyclopropane to dogs, excised the hearts, and measured myosin ATPase activity.[200] They found no change, but during the prolonged extraction of the protein, it is likely that the drug effects were lost, particularly considering that these are gaseous anesthetics. In addition, they were measuring myosin ATPase rather than the actomyosin enzyme, so they could not characterize the effects of the anesthetics on calcium sensitivity, which may be important in drug mechanisms.[199] Brodkin *et al.* looked at actomyosin ATPase activity of rat-heart muscle as influenced by halothane.[201] They dosed the protein *in vitro*, but neither mentioned the partition problem nor measured the drug concentration, so their dose calculations are suspect. The calcium-concentration effect was not tested, and, in fact, was not considered, for they did not buffer calcium in their reaction mixture. In addition, there was probably some contaminant mitochondrial ATPase activity. They needed large doses of halothane (40–100 mM) to see a decrease in ATPase activity, so, aside from the problems in technique, the pharmacologic significance of their findings is doubtful. Although Gorman and Craythorne demonstrated a dose-dependent decrease in cardiac myofibrillar ATPase from cat hearts by methoxyflurane, they did not consider the effect of calcium either.[202] They documented significant mitochondrial ATPase contamination in their preparation (27 per cent). Their reported methoxyflurane concentrations were high (10–40 mM), and they did not consider the partition problem in dosing. There has been no satisfactory documentation of the effects of anesthetics (particularly those with negative inotropic effects) on actomyosin ATPase, the enzyme governing most of the energy utilization during cardiac contraction.

Lain *et al.* studied the effects of chloroform, halothane, diethyl ether and pentobarbital on one of the enzymes thought to control cardiac relaxation, the sarcoplasmic reticulum (SR) ATPase.[203] They also measured SR calcium uptake. Partition factors were considered in calculating dose, but the partition coefficients for their reaction mixtures were not measured, nor was anesthetic concentration. Chloroform and halothane decreased SR calcium uptake and ATPase activity in a dose-dependent manner. The halothane effect was not seen until concentrations higher than those shown to produce depressed contractility (from other investigators' work) were reached, whereas the concentration ranges for depression of both contraction and calcium uptake were similar for chloroform. Diethyl ether had no effect at concentrations which depressed contractility by 80 per cent in a heart–lung preparation (again, reported by other investigators). The decrease in calcium uptake produced by chloro-

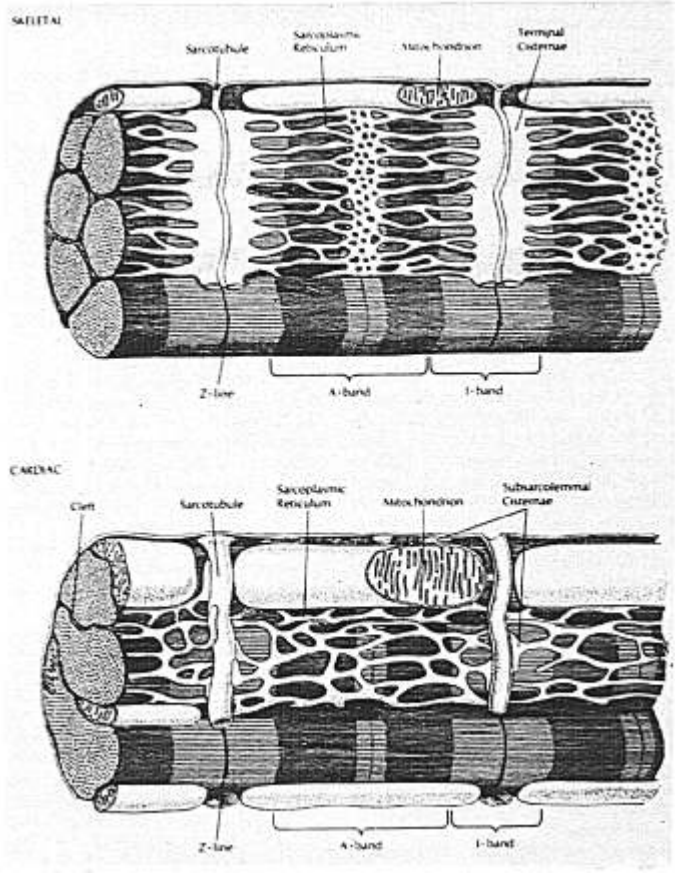


FIG. 21. Schematic of skeletal and cardiac muscle ultrastructure (from Chidsey,[146] with permission of author and publisher).

form and halothane was clearly related to decreased SR ATPase activity. Pentobarbital, on the other hand, uncoupled ATP hydrolysis from calcium uptake. Although calcium uptake was decreased by increasing doses of pentobarbital, ATPase activity actually increased slightly. Thus, the higher the pentobarbital dose, the more ATP had to be split per unit of calcium taken up by the SR. The same group of investigators also reported that amobarbital decreased calcium uptake by increasing calcium binding by SR membrane phospholipids.[204] This could be reversed by ouabain without affecting ATPase activity.

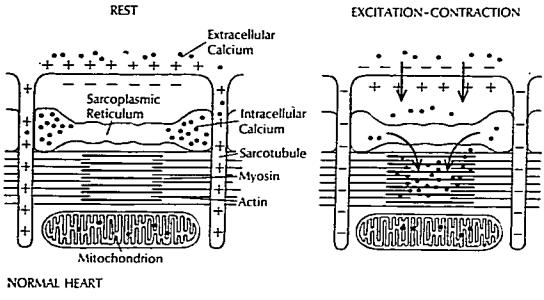


FIG. 22. Schematic of myocardial calcium kinetics (from Chidsey,[195] with permission of author and publisher).

Nayler and Szeto confirmed Lain's and Hess' findings and, in addition, suggested that pentobarbital altered sarcolemmal membrane distribution of calcium.[205] Although there have been no more studies of anesthetic effects on cardiac SR, it is of interest that halothane[206] and diethyl ether[207] have been shown to decrease skeletal muscle SR calcium uptake in a dose-dependent manner. Halothane also decreased ATPase activity. Although diethyl ether stimulated SR ATPase activity, this action was attributed to increase in calcium ion concentration in the reaction mixture, rather than to a direct effect of the anesthetic.[208] Lain and Nayler postulated that decreased calcium uptake during diastole in the SR would result in a smaller pool available for release during systole. Although this mechanism may be valid in skeletal muscle, in view of the lack of knowledge concerning the source of the rapid rise in sarcoplasmic calcium during systole in cardiac muscle, such a mechanism is purely speculative.

Haugaard and co-workers hypothesized along the same line in regard to the mitochondria.[209] They had noted that oligomyocin decreased mitochondrial calcium uptake and retarded relaxation in rat hearts. Miller and Hunter reported that halothane, methoxyflurane, and chloroform markedly inhibited calcium uptake by mitochondria from pigeon and beef hearts in association with the depression of NADH-linked electron transport.[179-181] Fluroxene depressed neither electron transport nor calcium uptake by the mitochondria. The *in-vitro* studies demonstrating direct myocardial depression by all inhalation anesthetics did not include fluroxene,[5, 6] although it has been assumed that the drug behaves like diethyl ether and cyclopropane (which were tested).[8] Sohn *et al.* reported that fluroxene, like other anesthetics, depressed peak developed tension in rat trabeculae carneae in a similar dose-dependent fashion.[210] Maximum shortening velocity (V_{max}) was maintained until very high drug doses were reached, however. Fluroxene appeared to depress the intact dog heart (see above),[22] but no direct measure of contractility was made. Miller hypothesized that the inhibition of calcium uptake in cardiac mitochondria by drugs which produced myocardial depression in intact animals and man suggested a mechanistic connection. If, indeed, fluroxene does not produce direct myocardial depression, then the lack of inhibition of myocardial calcium uptake by fluroxene strengthens this hypothesis. Whether or not fluroxene has a direct action on myocardial contractility different than that of the other anesthetics, coupling the effects on mitochondrial calcium transport and contractility would mean that the mitochondria play an important role in decreasing the sarcoplasmic calcium concentration during diastole and increasing it during systole. The current weight of evidence suggests that SR is more likely to be involved in the calcium

kinetics of the normal contraction–relaxation cycle.

Malsch *et al.*[211] and Price and Davidson [212] have demonstrated the importance of extracellular calcium ion concentration in the negative inotropic effect of halothane on kitten papillary muscle. Malsch's group also reported that relaxation was prolonged during halothane washout, although others have failed to note an effect of halothane on relaxation.[213] If anesthetics did affect SR (or mitochondrial) calcium uptake, then relaxation should be interfered with, inasmuch as this would be the mechanism most likely to be governed by this activity. Price's work merely states that the mechanism(s) by which halothane depresses contractile force is calcium dependent, and allows no further speculation about intracellular events.

Thus, there is suggestive evidence that inhalation anesthetics can affect ATPase activity of the calcium-accumulating sarcoplasmic reticulum and mitochondria in heart muscle. The significance of this in relation to the negative inotropic effect must remain obscure until the kinetics of the calcium flux in the contraction–relaxation cycle is defined. There is no valid information about the effect of anesthetics on the ATPase activity of the contractile proteins.

Overview

Inasmuch as myocardial metabolism has been reviewed in the framework of *energy liberation, conservation* and *utilization*, it may be helpful to consider where the most likely loci for the effects of inhalation anesthetics may be. Olson [214] and Fleckenstein [215] have proposed that heart failure, whether spontaneous (pathologic) or induced (pharmacologic, as with anesthetics), occurs from a defect in energy supply (*liberation* or *conservation*) or *utilization*.

Supply

Factors resulting in an insufficient supply of the cardiac muscle energy sources ATP and CP include: 1) defective oxygen delivery from a) arterial hyoxemia, b) ischemia or c) interferences with transport (carbon monoxide); 2) chemicals which interfere with oxidative and glycolytic phosphorylation (cyanide, 2-4 dinitrophenol, fluoroacetate).[215] 1a) Arterial hypoxia does not play a role in the negative inotropic effect of anesthetics. 1b) Although anesthetics have not been demonstrated to interfere primarily with oxygen transport, an effect on oxyhemoglobin dissociation has not been ruled out. 1c) There is increasing evidence that myocardial-tissue oxygen delivery may depend more on capillary distribution than on coronary blood flow.[216, 217] We have no information about the effect of anesthetics on the cardiac precapillary sphincters which control capillary distribution. 2) Anesthetics can uncouple oxidative phosphorylation, but only at high concentrations, and irreversibly. The reversible partial inhibition of cardiac mitochondrial electron transport associated with anesthetics has not been shown to have a functional effect. If mitochondrial depression interfered with NEFA oxidation, however, then during anesthesia the heart would depend on glycolysis for energy liberation.[77] In consequence, the interference in glycolysis demonstrated by Paradise and co-workers [129-141] and the block in transcellular glucose transport suggested by our laboratory [145] might prove to be important. More precise studies of the latter effects using radioactive tracers are necessary. Definitive investigation of the effects of anesthetics on lipid metabolism is also needed. As an example, inhibition of NEFA oxidation in pathologic failure of guinea-pig hearts has been shown to result from a defect in carnitine (thus interfering with transmitochondrial fatty-acid–CoA transport).[218] Such studies should be done with anesthetics. Decreases in tissue concentrations of CP and possibly ATP and increases in ADP and P_i should also be documented, for confirmation of a defect in energy supply.

Utilization

Fleckenstein maintained that defects in energy utilization in heart failure involved interference with calcium metabolism.[215] His criteria for such a statement included: 1) a negative inotropic effect manifested by decreased isometric tension *in vitro* and decreased stroke volume *in vivo* coupled with increased diastolic volume; 2) no impairment of membrane electrical activity; 3) decreased myocardial oxygen demand; 4) complete and

rapid reversal of all other signs by exogenous calcium; 5) normal or increased myocardial high-energy phosphate content. He documented all of these for several barbiturates and beta-adrenergic antagonists. The positive inotropic effect of digitalis glycosides and catecholamines in these depressed hearts was attributed to calcium release, which seems a likely possibility.[104, 219] All of these criteria have been at least suggested for inhalation anesthetics except the high-energy phosphate content. However, it is interesting to recall that when inhalation anesthetics and barbiturates have been tested in the same system (atrial glycolysis [135] and SR calcium uptake [203]), the metabolic effects have been different. Although the negative inotropic effect of inhalation anesthetics may well involve an effect on calcium fluxes in heart muscle, at the present time, calcium seems to be implicated in almost any process one looks at. As in the case of oxygen, there is still no firm evidence as to cause and effect.

Defective lipid-facilitated membrane calcium transport has been suggested as a mechanism for the inotropic effects of various beta antagonists by Nayler's group.[220] They failed to correlate inotropic effects with actomyosin ATPase activity in the same study. As was the case with the anesthetic investigations of actomyosin ATPase activity,[200, 201] no measure of calcium response was made. Honig and Reddy have shown that beta antagonists specifically shift the calcium response of actomyosin ATPase by affecting the troponin–tropomyosin complex.[221] As mentioned before, this is a possible site for anesthetic effects and needs to be investigated in proper fashion.

At the beginning and throughout this review, I have noted that the transfer of data from species to species, from *in vitro* to *in vivo*, and from skeletal muscle to cardiac muscle must be critically analyzed. I will end by citing a tantalizing bit of very preliminary evidence high-lighting these cautions. There can be no doubt that halothane produces a dose-dependent *negative* inotropic effect on cardiac muscle, *in vitro* and *in vivo*. Strobel (Strobel, G. E., personal communication) has noted in a very preliminary way that halothane produces a dose-dependent *positive* inotropic effect on frog skeletal muscle *in vitro*. C'est la vie!

A computer literature search using the SUNY Biomedical Communication Network through the terminal at the E. G. Miner Library at the University of Rochester School of Medicine was a valuable aid in the preparation of the paper. The author is grafteful to Sally Basch, Jean Latona, and Diane Hilfiker for assistance in preparing the manuscript. The help and encouragement of A. J. Gillies, M.D., are appreciated. Finally, the forbearance, patience and good will of the author's wife and children have been comforting.

References

1. Klocke FJ, Braunwald E, Ross J Jr: Oxygen cost of electrical activation of the heart. Circ Res 18:357–365, 1966
2. Calla SJ: Effects of anesthetic agents on myocardial metabolism, Effects of Anesthesia on Metabolism and Cellular Functions. Edited by JP Bunker, LD Vandam. Pharmacol Rev 17:238–241, 1965
3. Buckley NM, Penefsky ZJ, Litwak RS: Comparative force frequency relationship in human and other mammalian ventricular myocardium. Pfluegers Arch 332:259–270, 1972
4. Henderson AH, Brutsaert DL, Parmley WW, et al: Myocardial mechanics in papillary muscles of the rat and cat. Am J Physiol 217:1273–1279, 1972
5. Paradise RR, Bibbins F: Comparison of the effects of equieffective concentrations of anesthetics on the force of contraction of isolated perfused rat hearts: Correlation with the equieffective anesthetizing partial pressures. ANESTHESIOLOGY 31:349–355, 1969
6. Brown BR, Crout JR: A comparative study of the effects of five general anesthetics on myocardial contractility: I. Isometric conditions. ANESTHESIOLOGY 34:236–245, 1971
7. Price HL: General anesthesia and circulatory homeostasis. Physiol Rev 40:187–218, 1960
8. Skovsted P, Price HL: Central sympathetic excitation caused by fluroxene. ANESTHESIOLOGY 32:210–217, 1970
9. Goodale WT, Hackel DB: Myocardial carbohydrate metabolism in normal dogs with the effects of hyperglycemia and starvation. Circ Res 1:509–517, 1953
10. Bing RJ: Cardiac metabolism. Physiol Rev 45:171–213, 1965
11. Opie LH: Metabolism of the heart in health and disease. Part II, Am Heart J 77:100–122, 1969
12. Zierler K: Theory for the use of arteriovenous concentration differences for measuring metabolism in steady and nonsteady states. J Clin Invest 40:2111–2125, 1961
13. Olson RE, Dhalla NS, Sun CN: Changes in energy stores in the hypoxic heart. Cardiology 56:114–124, 1971/72

14. Scheuer J: Myocardial metabolism in cardiac hypoxia. Am J Cardiol 19:385–392, 1967
15. Opie LH: Metabolism of the heart in health and disease. Part 1. Am Heart J 76:685–698, 1968
16. Opie LH, Newsholme EA: Activities of fructose 1,6-diphosphatase, phosphofructokinase, and phosphoenolpyruvate carboxykinase in white muscle and red muscle. Biochem J 103:391–399, 1967
17. Blanchaer MC: Respiration of mitochondria of red and white skeletal muscle. Am J Physiol 206:1015–1020, 1964
18. Katz AM: Patterns of energy production and energy utilization in cardiac and skeletal muscle, Factors Influencing Myocardial Contractility. Edited by RD Tanz, F Kavaler, J Roberts. New York, Academic Press, 1967, pp 401–416
19. Laver MB: The oxygen dilemma: From lungs to hemoglobin. ANESTHESIOLOGY 32:1–3, 1970
20. Laver MB: A fable of our time: Oxygen transport, or does the emperor have new clothes? ANESTHESIOLOGY 36:105–107, 1972
21. Philippart C: Fluothane et debit coronaire. Acta Anaesthesiol Belg 14:219–237, 1963
22. von Eberlein HJ: Einflub. verschiedever anaesthetica auf coronar widerstand und reaktionsweise des coronarsystems beim hund. Anaesthetist 13:381–384, 1964
23. Saito T, Wakisaka K, Yudate T, et al: Coronary and systemic circulation during (inhalation) anesthesia in dogs. Far East J Anesth 5:105–126, 1966
24. Weaver PC, Bailey JS, Preston TD: Coronary artery blood flow in the halothane-depressed heart. Br J Anaesth 42:678–684, 1970
25. Bagwell EE: Effect of halothane on coronary flow and myocardial metabolism in dogs (abstr). Pharmacologist 7:177, 1965
26. Merin RG: Myocardial metabolism in the halothane-depressed canine heart. ANESTHESIOLOGY 31:20–26, 1969
27. Wolff G, Gradel E, Niederer W: Changes of coronary arteriolar tone, mean coronary flow and aortic pressure under halothane and ether anaesthesia in the dog. Br J Anaesth 40:810–817, 1968
28. Amory DW, Steffenson JL, Forsyth RP: Systemic and regional blood flow changes during halothane anesthesia in the rhesus monkey. ANESTHESIOLOGY 35:81–90, 1971
29. Saito T, Wakisaka K, Okazaki K, et al: Hemodynamic effects of halothane in relation to oxygen supply and halothane uptake of the myocardium and whole body in dogs. Tokushima J Exp Med 13:129–138, 1966
30. vonEberlein HJ: Effects of anesthetics on coronary circulation. Wein Z Inn Med 46: 400–403, 1965
31. Merin RG, Borgstedt HH: Myocardial function and metabolism in the methoxyflurane-depressed canine heart. ANESTHESIOLOGY 34:562–568, 1971
32. Merin RG, Chapman SW: Myocardial dynamic and metabolic effects of fluoroxene in the dog. Abstracts of Scientific Papers, Annual Meeting, American Society of Anesthesiologists, 1972, pp 51–52
33. Greene NM: Inhalation anesthetics and carbohydrate metabolism. Baltimore, Williams and Wilkins, 1963, pp 15–16
34. Greene NM: Carbohydrate metabolism and anesthesia, Metabolism. Edited by JB Stetson. Int Anesthesiol Clin 5 (2):411–426, 1967
35. Theye RA: Effects of halothane, anoxia and hemorrhage upon canine whole-body, skeletal muscle and splanchnic excess lactate production. ANESTHESIOLOGY 35:394–400, 1971
36. Cohen PJ: Metabolic functions of oxygen and biochemical lesions of hypoxia. ANESTHESIOLOGY 37:148–177, 1972
37. Huckabee WE: Relationship of pyruvate and lactate during anaerobic metabolism. V. Coronary adequacy. Am J Physiol 200: 1169–1176, 1961
38. Opie LH, Mansford KRL: Value of lactate and pyruvate measurements in the assessment of the redox state of free nicotinamide-adenine dinucleotide in the cytoplasm of the perfused rat heart. Eur J Clin Invest 1: 295–306, 1971
39. Gorlin R: Assessment of hypoxia in the human heart. Cardiology 57:24–34, 1972
40. Gudbjarnason S: Use of glycolytic metabolism in the assessment of hypoxia in human hearts. Cardiology 57:35–46, 1972
41. Olson RE: Excess lactate and anaerobiosis. Ann Intern Med 59:960–963, 1963
42. Tajoli E, Zotti E, Scalella PL, et al: Il Metabolismo cardiaco del cane durante anestesia con alotano. Acta Anaesthesiol (Padova) 19: suppl 7:31–37, 1968
43. Barusco G, Zotti E, Marzotti A, et al: Il Metabolismo cardiaco del cane durante anesthesia con metossiflurano. Acta Anaesthesiol (Padova) 19: suppl 7:39–42, 1968
44. Regan TJ, Moschos CB, Lehan PH, et al: Lipid and carbohydrate metabolism of myocardium during biphasic inotropic response to epinephrine. Circ Res 19:307–316, 1966
45. Regan TJ, Moschos CB, Oldewurtel HA, et al: Dominance of lipid metabolism in the myocardium under the influence of L-norepinephrine. J Lab Clin Med 70: 221–228, 1967
46. Paradise RR, Griffith LK: Electrolyte content of perfused rat ventricles exposed to halothane or anoxia. J Pharmacol Exp Ther 154:281–288, 1966

47. Scott JC, Finklestein LJ, Spitzer JJ: Myocardial removal of free fatty acids under normal and pathologic conditions. Am J Physiol 203:482–486, 1962
48. Bassenge E, Vendt VE, Schollmeyer P, et al: Effect of ketone bodies on cardiac metabolism. Am J Physiol 208:162–168, 1965
49. Williamson JR: Effects of insulin and diet on metabolism of L(+) lactate and glucose by the perfused rat heart. Biochem J 83:377–383, 1962
50. Williamson JR: Effects of insulin and starvation on the metabolism of acetate and pyruvate by the perfused rat heart. Biochem J 93:97–106, 1964
51. Gousios A, Felts JM, Havel RJ: Metabolism of serum triglycerides and free fatty acids by the myocardium. Metabolism 12:75–80, 1963
52. Neely JR, Whitfield CF, Morgan HE: Regulation of glycogenolysis in hearts: Effects of pressure development, glucagon, and FFA. Am J Physiol 219:1083–1088, 1970
53. Danforth WH, McKinsey JJ, Stewart JT: Transport and phosphorylation of glucose by the dog heart. J Physiol 162:367–384, 1962
54. Morgan HE, Neely JR, Brineaux JP, et al: X. Regulation of glycolytic activity in heart muscle, Regulation of glucose transport, Control of Energy Metabolism. Edited by B Chance, RW Eastabrook, JR Williamson. New York, Academic Press, 1965, pp 347–355
55. Opie LH: Substrate utilization and glycolysis in the heart. Cardiology 56:2–21, 1971/72
56. Newsholme EA: Regulation of phosphofructokinase in muscle. Cardiology 56:22–34, 1971/72
57. Scheuer J, Berry MN: Effect of alkalosis on glycolysis in the isolated rat heart. Am J Physiol 213:1143–1148, 1967
58. Opie LK, Mansford KRL, Owen P: Effects of increased heart work on glycolysis and adenine nucleotides in the perfused heart of normal and diabetic rats. Biochem J 124:475–490, 1971
59. Krasnow N, Neill WA, Messer JV, et al: Myocardial lactate and pyruvate metabolism. J Clin Invest 41:2075–2085, 1962
60. Wieland D, Siess E, Schulze-Wethmar FR, et al: Active and inactive forms of pyruvate dehydrogenase in rat heart and kidney: Effects of diabetes, fasting, and refeeding on interconversion. Arch Biochem Biophys 143:593–601, 1971
61. Opie LH, Shipp JD, Evans JR, et al: Metabolism of glucose-U-C^{14} in the perfused rat heart. Am J Physiol 203:839–843, 1962
62. Evans JR: Importance of fatty acids in myocardial metabolism. Circ Res suppl II:96–106, 1964
63. Evans JR: Cellular transport of long chain fatty acids. Can J Biochem 42:955–969, 1964
64. Kong Y, Friedberg SJ: Rapid intracoronary radiopalmitate injection and myocardial fatty acid oxidation. Metabolism 20:681–690, 1971
65. Shipp JC, Thomas JM, Crevasse L: Oxidation of carbon-14-labelled endogenous lipids by isolated, perfused rat hearts. Science 143: 371–373, 1964
66. Bressler R, Katz R, Wittels B: Role of carnitine in the intracellular translocation of acyl coenzyme-A derivatives. Ann NY Acad Sci 131:207–224, 1965
67. Miller HI, Yum KY, Durham BC: Myocardial free fatty acids in unanesthetized dogs at rest and during exercise. Am J Physiol 220: 589–596, 1971
68. Carlson LA, Kaijser L, Lassers BW: Myocardial metabolism of plasma triglycerides in man. J Mol Cell Cardiol 1:467–475, 1970
69. Crass MF, McGaskill ES, Shipp JC: Effect of pressure development on glucose and palmitate metabolism in the perfused heart. Am J Physiol 216:1569–1576, 1969
70. Crass MF, McGaskill ES, Shipp JC: Metabolism of endogenous lipids in cardiac muscle: Effect of pressure development. Am J Physiol 220:428–435, 1971
71. Crass MF: Exogenous substrate effects on endogenous lipid metabolism in the working rat heart. Biochem Biophys Acta 280:71–81, 1972
72. Davies RE: Biochemical processes in cardiac function. Hosp Pract 5(6):49–56, 1970
73. Newsholme EA, Randle PJ: Effects of fatty acids, ketone bodies, and pyruvate and of alloxan-diabetes, starvation, hypophysectomy and adrenalectomy on the concentration of hexose phosphates, nucleotides and inorganic phosphate in perfused rat hearts. Biochem J 93:641–651, 1964
74. Randle PJ, Newsholme EA, Garland PB: Effects of fatty acids, ketone bodies and pyruvate and of alloxan-diabetes and starvation on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. Biochem J 93:652–664, 1964
75. Garland PB, Newsholme EA, Randle PJ: Effects of fatty acids and ketone bodies, and of alloxan-diabetes and starvation, on pyruvate metabolism and on lactate/pyruvate and L-glycerol 3-phosphate/dihydroxy-acetone phosphate concentration ratios in rat heart and rat diaphragm muscles. Biochem J 93:665–677, 1964
76. Garland PB, Randle PJ: Effects of alloxan-diabetes, starvation, hypophysectomy and adrenalectomy and of fatty acids, ketone bodies and pyruvate on the glycerol output and concentrations of free fatty acids, long

chain fatty acyl-coenzyme A, glycerol phosphate and citrate-cycle intermediates in rat heart and diaphragm muscles. Biochem J 93:678–685, 1964
77. Randle PJ, Garland PB, Newsholme EA, et al: Glucose fatty acid cycle in obesity and maturity onset diabetes mellitus. Ann NY Acad Sci 131:324–333, 1965
78. Evans JR (editor): Structure and function of heart muscle. Circ Res 15:suppl II, 1964
79. Vahouny GV, Katzen R, Entenman C: Myocardial metabolism. II. Role of nutritional state on palmitate and glucose oxidation by isolated perfused hearts. Biochem Biophys Acta 137:181–183, 1967
80. Kaijser L, Lassers BW, Wahlquist ML, et al: Myocardial lipid and carbohydrate metabolism in men during prolonged exercise. J Appl Physiol 32:847–858, 1972
81. Himms-Hagen J: Sympathetic regulation of metabolism. Pharmacol Rev 19:367–461, 1967
82. Porte D: Sympathetic regulation of insulin secretion. Arch Intern Med 123:252–260, 1969
83. Cowley AW, Scott JC, Spitzer JJ: Myocardial FFA metabolism during coronary infusion of norepinephrine in conscious dogs. Am J Physiol 217:511–517, 1969
84. Glaviano VV, Masters TN: Effect of norepinephrine on myocardial metabolism before and after beta receptor blockade. Eur J Pharmacol 7:135–149, 1969
85. Masters TN, Glaviano VV: Effects of norepinephrine and propranolol on myocardial subcellular distribution of triglycerides and and free fatty acids. J Pharmacol Exp Ther 182:246–255, 1972
86. Christian DR, Kilsheimer GS, Pettet G, et al: Regulation of lipolysis in cardiac muscle: A system similar to hormone-sensitive lipase of adipose tissue. Adv Enzyme Regul 7:71–82, 1969
87. Rabb W, Van Lith P, Lepeschin E, et al: Catecholamine induced myocardial hypoxia in the presence of impaired coronary disability independent of external cardiac work. Am J Cardiol 9:455–470, 1962
88. Mjøs OD: Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs. J Clin Invest 50:1386–1389, 1971
89. Mjøs OD: Effect of inhibition of lipolysis on myocardial oxygen consumption in the presence of isoproterenol. J Clin Invest 50:1869–1873, 1971
90. Mjøs OD, Bugge-Asperheim B, Kiil F: Factors determining myocardial oxygen consumption during elevation of aortic blood pressure. Cardiovasc Res 6:23–27, 1972
91. Henderson AH, Craig RJ, Gorlin R, et al: Free fatty acids and myocardial function in perfused rat hearts. Cardiovasc Res 4: 466–472, 1970
92. Oliver MF: Metabolic response during impending myocardial infarction. II. Clinical implications. Circulation 45:491–500, 1972
93. Sutherland EW, Rall TW: Relation of adenosine-3',5'-phosphate and phosphorylase on the actions of catecholamines and other hormones. Pharmacol Rev 12:265–299, 1960
94. Sutherland EW, Robison GA, Butcher RW: Some aspects of the biological role of adenosine 3'-5'-monophosphate (cyclic AMP). Circulation 37:279–306, 1968
95. Mayer SE: Effect of epinephrine on carbohydrate metabolism in the heart, Factors Influencing Myocardial Contractility. Edited by RD Tanz, F Kavaler, J Roberts. New York, Academic Press, 1967, pp 443–455
96. Villar-Palasi C, Larner J, Shen LC: Glycogen metabolism and the mechanism of action of cyclic AMP. Ann NY Acad Sci 185:74–84, 1971
97. Haugaard W, Hess ME: Actions of autonomic drugs on phosphorylase activity and function. Pharmacol Rev 17:27–69, 1965
98. Robison GA, Butcher RW, Øye I, et al: Effect of epinephrine on adenosine 3',5'-phosphate levels in the perfused rat heart. Mol Pharmacol 1:168–177, 1965
99. Mayer SE, Cotten MdeV, Moran NC: Dissociation of the augmentation of cardiac contractile force from the activation of myocardial phosphorylase by catecholamines. J Pharmacol Exp Ther 139:275–282, 1963
100. Sutherland EW, Robison GA: Role of cyclic AMP in the control of carbohydrate metabolism. Diabetes 18:797–819, 1969
101. Epstein SE, Levey GS, Skelton CL: Adenyl cyclase and cyclic AMP. Biochemical links in the regulation of myocardial contractility. Circulation 43:437–450, 1971
102. Ahren K, Hjalmarson A, Isaksson O: Inotropic and metabolic effects of dibutyryl cyclic adenosine 3',5'-monophosphate in the perfused rat heart. Acta Physiol Scand 82:79–90, 1971
103. Shanfeld J, Frazer A, Hess ME: Dissociation of increased formation of cardiac adenosine 3',5'-monophosphate from the positive inotropic effect of norepinephrine. J Pharmacol Exp Ther 169:315–320, 1969
104. Mayer SA: Effects of adrenergic agonists and antagonists on adenyate cyclase activity of dog heart and liver. J Pharmacol Exp Ther 181:116–125, 1972
105. Wastilla WB, Su JY, Friedman WF, et al: Blockade of biochemical and physiological responses of cardiac muscle to norepinephrine by N-tert-butylmethoxamine (butox-

amine). J Pharmacol Exp Ther 181:126–138, 1972
106. Entman ML, Levy GS, Epstein SE: Mechanism of action of epinephrine and glucagon on the canine heart: Evidence for increase in sarcotubular calcium stores mediated by cyclic 3',5'-AMP. Circ Res 25:429–438, 1969
107. Robison GA, Butcher RW, Sutherland EW: Adenyl cyclase as an adrenergic receptor. Ann NY Acad Sci 139:703–723, 1967
108. Levey GS: Solubilization of myocardial adenyl cyclase: Loss of hormone responsiveness and activation by phospholipids. Ann NY Acad Sci 185:449–457, 1971
109. Visscher MB, Muller EA: Influence of insulin on the mammalian heart. J Physiol (Lond) 62:341–348, 1926
110. Bayliss LE, Muller EA, Starling EH: Action of insulin and sugar on the respiratory quotient and metabolism of the heart lung preparation. J Physiol (Lond) 65:33–47, 1928
111. Goodale WT, Olson RE, Hackel DB: Effects of fasting and diabetes mellitus on myocardial metabolism in man. Am J Med 27:212–220, 1959
112. Regan TJ, Frank MJ, Lehan PH, et al: Relationship of insulin and strophanthidin to myocardial metabolism and function. Am J Physiol 205:790–794, 1963
113. Hackel DB: Effect of insulin on cardiac metabolism in normal dogs. Am J Physiol 199:1135–1138, 1960
114. Brachfeld N, Scheuer J: Metabolism of glucose by the ischemic dog heart. Am J Physiol 212:603–606, 1967
115. Weissler AM, Kruger EA, Baba N, et al: Role of anaerobic metabolism in the preservation of functional capacity and structure of anoxic myocardium. J Clin Invest 47: 403–416, 1968
116. Scheuer J, Stezoski SW: Protective role of increased myocardial glycogen stores in cardiac anoxia in the rat. Circ Res 27: 835–849, 1970
117. Sodi-Pallares D, Bisteni A, Medrano GA, et al: The polarizing treatment of acute myocardial infarction. Dis Chest 43:424–432, 1963
118. Maroko PR, Libby P, Sobel BE, et al: Effect of glucose–potassium–insulin infusion on myocardial infarction following experimental coronary artery occlusion. Circulation 45:1160–1175, 1972
119. Majid PA, Sharma B, Meeran MKM, et al: Insulin and glucose in the treatment of heart failure. Lancet II:937–941, 1972
120. Taylor SH, Majid PA: Insulin and the heart. J Mol Cell Cardiol 2:293–317, 1971
121. Opie LH: Metabolic response during impending myocardial infarction. I. Relevance of glucose and fatty acid metabolism in animals. Circulation 45:483–490, 1972
122. Shaw WM, Boder GB: Effect of insulin on pyruvate and glucose metabolism of beating mouse heart cells. J Mol Cell Cardiol 4:485–494, 1972
123. Opie LH: Metabolism of the heart in health and disease. Part III. Am Heart J 77: 383–410, 1969
124. Kones RJ, Phillips JH: Glucagon: Present status in cardiovascular disease. Clin Pharmacol 12:427–444, 1971
125. Glick G: Glucagon: A perspective. Circulation 45:513–515, 1972
126. Klein I, Levey GS: Effect of prostaglandins on guinea pig myocardial adenyl cyclase. Metabolism 20:890–896, 1971
127. Higgins CB, Braunwald E: The prostaglandins. Biochemical, physiological and clinical considerations. Am J Med 53:92–112, 1972
128. Gimeno AL, LaCuara JL, Gimeno AF, et al: Effects of monosaccharides, acetate, butyrate, lactate and pyruvate on the developed tension of isolated rat atria. Proc Soc Exp Biol Med 130:532–539, 1969
129. Ko K, Paradise RR: Effects of substrates on contractility of rat atria depressed with halothane. ANESTHESIOLOGY 31:532–539, 1969
130. Shaw WN, Stadie WC: Two identical Embden-Myerhof enzyme systems in normal rat diaphragm, differing in cytological location and response to insulin. J Biol Chem 234:2491–2496, 1959
131. Paradise RR, Ko K: Effect of fructose on halothane-depressed rat atria. ANESTHESIOLOGY 32:124–129, 1970
132. Ko K, Paradise RR: Effects of substrates on rat atria depressed with bicarbonate-free medium, citrate, or low calcium. Proc Soc Exp Biol Med 134:469–476, 1970
133. Ko K, Paradise RR: Effects of substrates on contractility of isolated human atria. Proc Soc Exp Biol Med 134:386–389, 1970
134. Ko K, Paradise RR: Effects of substrate on halothane-depressed isolated human atria. ANESTHESIOLOGY 33:508–514, 1970
135. Ko K, Paradise RR: Contractile depression of rat atria by halothane in the absence of glucose. ANESTHESIOLOGY 34:152–156, 1971
136. Shimosato S, Li TH, Etsten B: Ventricular function during halothane anesthesia in closed chest dog. Circ Res 12:63–75, 1963
137. Eger EI II, Smith NT, Stoelting RK, et al: Cardiovascular effects of halothane in man. ANESTHESIOLOGY 32:396–409, 1970
138. Ko K, Paradise RR: Effect of halothane on the contractility of atria from starved rats. ANESTHESIOLOGY 34:557–561, 1971

139. Ko K, Paradise RR: Mechanism of the negative inotropic effect of methoxyflurane on isolated rat atria. ANESTHESIOLOGY 36:64–68, 1972
140. Krishna G, Paradise RR: Effects of pyruvate on human atrial muscle contractility depressed by methoxyflurane and pentobarbital. ANESTHESIOLOGY 36:364–368, 1972
141. Morrow RJ, Paradise RR: Halothane inhibition of substrate metabolism in rat atria (abstr). Fed Proc 31:549, 1972
142. Brown BR: Personal communication
143. Smith NT: Personal communication
144. Merin RG: The relationship between myocardial function and metabolism in the halothane-depressed heart. I. The effect of hyperglycemia. ANESTHESIOLOGY 33:391–395, 1970
145. Merin RG: The relationship between myocardial function and metabolism in the halothane-depressed heart. II. The effect of insulin. ANESTHESIOLOGY 33:396–400, 1970
146. Merin RG, Samuelson PN, Schalch DS: Major inhalation anesthetics and carbohydrate metabolism. Anesth Analg (Cleve) 50:625–632, 1971
147. Paradise RR: Personal communication
148. Allison SP, Tomlin PJ, Chamberlain MJ: Some effects of anaesthesia and surgery on carbohydrate and fat metabolism. Br J Anaesth 41:588–592, 1969
149. Clarke RSJ, Johnston H, Sheridan B: Influence of anaesthesia and surgery on plasma cortisol, insulin, and free fatty acids. Br J Anaesth 42:295–299, 1970
150. Horrelt OH, Tarhan S, Moffitt EA: Whole body metabolism during and after abdominal surgery. Can Anaesth Soc J 16:525–537, 1969
151. Oyama T, Takazawa T: Effects of halothane anaesthesia and surgery on human growth hormone and insulin levels in plasma. Br J Anaesth 43:573–579, 1971
152. Brown JW, Christian D, Paradise RR: Histological effects of procedural and environmental factors on isolated rat heart preparation. Proc Soc Exp Biol 129:455–462, 1968
153. Henderson AH, Parmley WW, Gorlin R, et al: Depression of myocardial function by free fatty acids in hypoxia. Circ Res 26:439–450, 1970
154. Cullen BF, Eger EI II, Smith NT, et al: Cardiovascular effects of fluroxene in man. ANESTHESIOLOGY 32:218–230, 1970
155. Galla SJ, Henneman DH, Schweizer HJ, et al: Effects of ether anesthesia on myocardial metabolism in dogs. Am J Physiol 202:241–244, 1962
156. Moffitt EA, Rosevear JW, Townsend CH, et al: Myocardial metabolism in patients having aortic-valve replacement. ANESTHESIOLOGY 31:310–320, 1969
157. Moffitt EA, Rosevear JW, McGoon DC: Myocardial metabolism in children having open-heart surgery. JAMA 211:1518–1524, 1970
158. Moffitt EA, Rosevear JW, McGoon DC: Myocardial metabolism during and after mitral valve replacement. Ann Thorac Surg 10:169–179, 1970
159. Moffitt EA, Rosevear JW, Tarhan S, et al: Myocardial metabolism during and after double valve replacement. Can Anaesth Soc J 18:33–50, 1971
160. Moffitt EA, White RD, Molnar GD, et al: Comparative effects of whole blood, hemodilution, and clear priming solutions on myocardial and body metabolism in man. Can J Surg 14:382–391, 1971
161. Moffitt EA, Rosevear JW, Molnar GD, et al: The effect of glucose–insulin–potassium solution on ketosis following cardiac surgery. Anesth Analg (Cleve) 50:291–297, 1971
162. Panaglia V: Confronto tra gli effetti di alcuni anesthetici generali sul contenuto in glicogeno del miocardio di ratto. Arch Sci Biol (Bologna) 53:235–242, 1969
163. Brown BR, Crout JR: Observation on the mechanism by which anesthetics depress myocardial contractility. Abstracts of Scientific Papers, Annual Meeting, American Society of Anesthesiologists, 1969, pp 18–19
164. Cohen PJ: Effect of anesthetics on mitochondrial function. ANESTHESIOLOGY 39:153–164, 1973
165. Cohen PJ, Marshall BE: Effects of halothane on respiratory control and oxygen consumption of rat liver mitochondria, Toxicity of Anesthetics. Edited by BR Fink. Baltimore, William and Wilkins, 1968, pp 24–34
166. Rasmussen H, Ogata E: Cation flux across the mitochondria membrane as a possible pacemaker of tissue metabolism, Control of Energy Metabolism. Edited by B Chance, RW Estabrook, JR Williamson. New York, Academic Press, 1965, pp 209–216
167. Nayler WG: Calcium exchange in cardiac muscle: Basic mechanism of drug action. Am Heart J 73:379–394, 1967
168. Langer GA: The intrinsic control of myocardial contraction—ionic factors. N Engl J Med 285:1065–1071, 1971
169. Scheuer J, McDonald RH: Current status of myocardial mechanical-energetic relationships. Mt Sinai J Med 37:311–330, 1970
170. Cain DF, Davies RE: Breakdown of adenosine triphosphate during a single contraction of working muscle. Biochem Biophys Res Commun 8:361–366, 1962

171. Infante AA, Davies RE: Adenosine triphosphate breakdown during a single isotonic twitch of frog sartorius muscle. Biochem Biophys Res Commun 9:410–415, 1962
172. Cain DF, Infante AA, Davies RE: Chemistry of muscle contraction: Adenosine triphosphate and phosphorylcreatine as energy supplies for single contractions of working muscle. Nature (Lond) 196:214–217, 1962
173. Kushmerick MJ, Minihan K, Davies RE: Changes in free Pi and ATP in frog sartorius muscles during maximum work and rigor (abstr). Fed Proc 24:598, 1965
174. Taylor CA, Williams CH, Wakabayashi E, et al: Effect of halothane on energized configurational changes in heart mitochondria in situ, Cellular Biology and Toxicity of Anesthetics. Edited by BR Fink. Baltimore, Williams and Wilkins, 1971, pp 117–127
175. Green DE: Conformational basis of energy transductions in membrane systems. Cellular Biology and Toxicity of Anesthetics. Edited by BR Fink. Baltimore, Williams and Wilkins, 1971, pp 79–92
176. Moravec J, Hatt PY: The mechanochemical activity of the mitochondria; an assessment (editorial). J Mol Cell Cardiol 4:91–96, 1972
177. Harris RA, Munroe J, Farmer B, et al: Action of halothane upon mitochondrial respiration. Arch Biochem Biophys 142:435–444, 1971
178. Britt BA, Kalow W, Endrenyi L: Effects of halothane and methoxyflurane on rat skeletal muscle mitochondria. Biochem Pharmacol 21:1159–1169, 1972
179. Miller RN, Hunter FE: Anesthetic induced alteration of calcium uptake by mitochondria—a possible mechanism of decreased myocardial contractility. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, 1971, pp 117–118
180. Miller RN: Effects of methoxyflurane on electron transfer, oxidative phosphorylation and calcium uptake by isolated rat liver and pigeon heart mitochondria. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, 1972, pp 141–142
181. Miller RN: Personal communication
182. Nahrwold ML, Cohen PJ: Effect of forane and fluroxene on mitochondrial respiration. Correlation with lipid solubility and in vivo potency. Anesthesiology 38:437–444, 1973
183. Reinauer H, Hollman S: Einflub der narkoseart suf den gehalt an adeninnucleotiden, lactat and pyruvat in herz, Leber und milz der ratte. Anaesthesist 15:327–332, 1966
184. Wollenberger A, Krause EG, Wahler BE: Orthophosphat und phosphocreatin gehalt des herzmuskils. Naturwissenschaften 45: 294–302, 1958
185. Huxley AF: Activation of striated muscle and its mechanical response. Proc R Soc Lond [Biol] 178:1–27, 1971
186. Huxley HE: Mechanism of muscular contraction. Science 164:1356–1366, 1969
187. Katz AM: Contractile proteins of the heart. Physiol Rev 50:63–158, 1970
188. Katz AM: Contractile proteins in normal and failing myocardium. Hosp Pract 7(10): 57–69, 1972
189. Taylor EW: Chemistry of muscle contraction. Ann Rev Biochem 41:577–616, 1972
190. Sandow A: Excitation–contraction coupling in skeletal muscle. Physiol Rev 17:265–320, 1965
191. Legato MJ: The myocardial cell: New concepts for the clinical cardiologist. Circulation 45:731–735, 1972
192. Ueba Y, Ito Y, Chidsey CA: Intracellular calcium and myocardial contractility. I. The influence of extracellular calcium. Am J Physiol 220:1553–1557, 1971
193. Carafoli E: Mitochondria and the calcium cycle in heart cells, Myocardial Metabolism, Recent Advances in Studies on Cardiac Structure and Metabolism. Volume 3. Baltimore, University Park Press (in press)
194. McCollum WB, Besch HR, Entman ML, et al: Apparent initial binding rate of calcium by canine cardiac relaxing system. Am J Physiol 223:608–614, 1972
195. Schwartz A: Calcium metabolism. Cardiology 57:16–23, 1972
196. Chidsey CA: Calcium metabolism in the failing and normal heart. Hosp Pract 7(8): 65–75, 1972
197. Bailey LE, Ong SD, Queen GM: Calcium movement during contraction in the cat heart. J Mol Cell Cardiol 4:121–138, 1972
198. Barany M: ATPase activity of myosin correlated with speed of muscle shortening. J Gen Physiol 50:197–216, 1967
199. Reddy YR, Honig CR: Ca^{++} binding and Ca^{++} sensitizing function of cardiac native tropomyosin, troponin and tropomyosin. Biochem Biophys Acta 275:453–463, 1972
200. Luchi RJ, Kritcher EM: Drug effects on cardiac myosin adenosine triphosphatase activity. J Pharmacol Exp Ther 158:540–545, 1967
201. Brodkin WE, Goldberg AH, Kayne HL: Depression of myofibrillar ATPase activity by halothane. Acta Anaesth Scand 11:97–101, 1967
202. Gorman HM, Craythorne NWB: Depression of the myofibrillar ATPase activity by methoxyflurane, Progress in Anaesthesiology, Proceedings of the Fourth World

Congress of Anaesthesiologists. Edited by TB Boulton, R Bryce-Smith, MK Sykes, et al. Excerpta Medica Foundation, Amsterdam, 1970, pp 743–747

203. Lain RF, Hess ML, Gertz EW, et al: Calcium uptake activity of canine myocardial sarcoplasmic reticulum in the presence of anesthetic agents. Circ Res 23:597–604, 1968
204. Hess ML, Lain RF, Briggs FN: Influence of amobarbital and ouabain on calcium binding to phospholipids isolated from cardiac sarcoplasmic reticulum. Biochem Pharmacol 18:2075–2079, 1969
205. Nayler WG, Szeto J: Effect of sodium pentobarbital on calcium in mammalian heart muscle. Am J Physiol 222:339–344, 1972
206. Dhalla NS, Sulakhe PV, Clinch NF, et al: Influence of Fluothane on calcium accumulation by the heavy microsomal fraction of human skeletal muscle: Comparison with a patient with malignant hyperpyrexia. Biochem Med 6:333–343, 1972
207. Inesi G, Goodman JJ, Wantanabe S: Effect of diethyl ether on the adenosine triphosphatase activity and calcium uptake of fragmented sarcoplasmic reticulum of rabbit skeletal muscle. J Biol Chem 242: 4637–4743, 1967
208. Fiehn W, Hasselbach W: Effect of diethylether upon the function of the vesicles of the sarcoplasmic reticulum. Eur J Biochem 9:574–578, 1969
209. Haugaard N, Haugaard ES, Lee NH, et al: Possible role of mitochondria in regulation of cardiac contractility. Fed Proc 28: 1657–1662, 1969
210. Sohn YZ, Phear, WPC, Goldberg AH: Effects of fluroxene on isometric and isotonic contractions of isolated heart muscle. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, 1971, pp 197–198
211. Malsch E, Vongvises P, Price HL: Interaction of Ca^{++} and halothane in the normal myocardium. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, 1971, pp 111–112
212. Price HL, Davidson L: Interaction of Ca^{++} and halothane in isolated papillary muscle. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, 1972, pp 91–92
213. Goldberg AH, Phear WPC: Alterations in mechanical properties of heart muscle produced by halothane. J Pharmacol Exp Ther 162:101–108, 1968
214. Olson RE, Piatnek DA: Conservation of energy in cardiac muscle. Ann NY Acad Sci 72:466–479, 1959
215. Fleckenstein A, Doring HJ, Kammermeier H: Experimental heart failure due to inhibition of utilization of high energy phosphates, Coronary Circulation and Energetics of the Myocardium. Edited by G Marchetti, B Taccardi. Basel/New York, S. Karger, 1967, pp 220–236
216. Honig CR, Kirk ES, Myers WW: Transmural distributions of blood flow, oxygen tension and metabolism in myocardium: Mechanism and adaptations, Coronary Circulation and Energetics of the Myocardium. Edited by G Marchetti, B Taccardi. Basel/New York, S. Karger, 1967, pp 31–46
217. Martini J, Honig CR: Direct measurement of intercapillary distance in beating rat heart in situ under various conditions of O_2 supply. Microvasc Res 1:244–256, 1969
218. Wittels B, Spann JF: Defective lipid metabolism in the failing heart. J Clin Invest 47:1787–1794, 1968
219. Langer GA: Effects of digitalis on myocardial ionic exchange. Circulation 46:180–187, 1972
220. Nayler WG, Stone J, Carson V, et al: Effect of beta adrenergic antagonists on cardiac contractions, myofibrillar ATPase activity, high energy phosphate stores and lipid facilitated transport of calcium ions. J Pharmacol Exp Ther 165:225–233, 1969
221. Honig CR, Reddy YC: Effect of catechol analogs on control of actomyosin by native tropomyosin and calcium: The nature of the negative inotropic receptor. J Pharmacol Exp Ther 184:330–338, 1973