STUDIES ON THE ISOLATED GAS-PERFUSED CAT HEART

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Lloyd P. Gabel

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ABSTRACT

Isolated cat hearts were perfused with warm, moist 95% oxygen-5% carbon dioxide by a modification of the Langendorff technique. Other hearts, perfused with substrate-free Krebs-Henseleit solution in a recirculating system, served as controls.

Gas-perfused hearts beat more forcibly, produced more work, and failed at a slower rate than did the controls under conditions of constant temperature, perfusion pressure and heart rate.

Both preparations were rapidly depleted of their carbohydrate stores and then utilized endogenous lipids, primarily phospholipids, as their source of energy during prolonged perfusion. The rate of phospholipid utilization was found to be a function both of the number of beats which occurred during the perfusion period and the isometric resting tension. This utilization rate was independent, however, of the heart rate, isometric developed tension, duration of perfusion, and the extent of failure.

Gas-perfused hearts, in contrast to their controls, showed a high incidence of spontaneous contractile alternation. This was sometimes accompanied by electrical alternation. A lower threshold to the arrhythmia-inducing effects of raised perfusion pressure was also observed during gas perfusion. Data obtained by the use of a procedure which presumably extracts electrolytes from the extracellular space during gas-perfusion indicated that the myocardial cells lost potassium and gained sodium at the expense of the extracellular space. These ionic shifts reached equilibrium within three hours of the initiation of gas perfusion. The extent of these shifts ~~were~~ was not significantly influenced by prior treatment of the hearts with "therapeutic" concentrations of ouabain.

Reactivity to injections of histamine, acetylcholine, adrenaline and isoproterenol was similar in liquid- and gas-perfused hearts. The actions of single injections of agents which were not susceptible to oxidation or enzymatic degradation (such as cocaine and pronethalol) remained constant in gas-perfused hearts unless the drugs were removed by short periods of liquid perfusion.

The greater contractile force and delayed onset of spontaneous failure observed in the gas-perfused heart could not be explained on the basis of any physiological or biochemical differences when comparisons were made to the relatively short-lived liquid-perfused preparation. A hypothesis was forwarded, based upon an early suggestion by A.J. Clark, that liquid-perfused hearts fail because their perfusate removes a factor which is necessary for the maintainance of optimum contractile force. It was reasoned that if this were so, the factor would accumulate in the extracellular space of gas-perfused hearts and should be obtainable in a concentrated form by perfusion with small volumes of liquid perfusate.

It was found that intermittent perfusion with small volumes of liquid (5.0 ml) at thirty-minute intervals caused rapid failure of gas-perfused hearts. These "washings" were recovered quantitatively and were found to exert a slight positive inotropic action on failed, isolated atria. Treatment of the washings with ammonium sulphate caused the precipitation of a material which was strongly inotropic to the test preparation. Pre-treatment of gas-perfused hearts with relatively low concentrations of ouabain (1×10^{-10} to 1×10^{-9} g/ml) decreased in a dose-related fashion both the quantity and specific activity of the recovered material. That this effect was related to the inotropic activity of the glycoside was demonstrated by the difference in potency

between digitoxin and dihydrodigitoxin. Relating these results to liquid-perfused hearts, it is suggested that spontaneous failure occurs in these preparations because of perfusate removal of an inotropic factor, and that the digitalis glycosides act to correct this by interfering with this removal.

This thesis is dedicated to my family, my wife Frances, Jonathan and David.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

"A systematic study of the changes in the energy transformation of the heart occurring in failure is feasible only in the isolated organ where the variables of circulatory dynamics cannot only be measured but where they can be controlled". A. Wollenberger, 1949

The literature concerning the physiology, pharmacology and biochemistry of the myocardium may be divided into two main classes: One in which the heart is studied *in situ* and the other in which it or some part is removed and maintained *in vitro*. In the former instance, the heart may be considered to be performing its usual function of perfusing itself and the body, while it is being influenced simultaneously by autonomic tone, arterial and venous pressure, and blood-borne factors such as hormones, metabolic substrates and end-products. Studies using intact preparations are presumed to yield information about the heart under conditions similar to those encountered in the normal life of the organism. By the same token, the very fact that the heart is operating within its usual environment means that the results of these experiments are influenced by a host of factors which may vary not only between laboratories but between individual animals and within the same animal. It is not uncommon to find reports in the literature with diametrically opposed results concerning a particular aspect of cardiac function. For example, Murrow (1) reported that the inotropic effect of ouabain is not affected by reserpine, whereas Tanz (2) concluded that reserpine prevented the ouabain - induced augmentation of contractility. These studies differed in choice of preparation, in that Murrow's data was obtained from *in vivo* experiments whereas Tanz studied the isolated papillary muscle.

Experiments with isolated tissues permit close control of those physical and chemical factors which influence the outcome of the experiment. By the selection of the proper saline medium or perfusion system,

one is able to maintain a more stable environment in regard to such factors as pH, pO_2, pCO_2, temperature, electrolyte concentration, osmolarity and drug or substrate concentrations. However, a review of the literature will reveal that even with these supposed advantages, reports of conflicting results still appear. For example, Kreisberg and Williamson (3), found that ouabain increased the uptake and utilization of glucose by perfused rat hearts, whereas Zacharia (4) found the uptake and utilization in the same preparation was unchanged.

The ability to control the supportive environment has fostered the proliferation of so many possible sets of experimental conditions that direct comparison between reports is again often impossible. One may cite the review by Lockwood (5) in which over 100 variations of Ringer's solution are described. The study in this report, unfortunately, offers no remedy to this situation but instead contributes further confusion to the field by suggesting a new technique for the maintainance of isolated hearts.

The Isolated Perfused Heart:

Langendorff in 1895 first described a preparation in which an isolated beating frog heart was maintained for prolonged periods by perfusing the tissue with either oxygenated serum or saline solution (6). Contractile force was measured either with a lever connected by a string to the unanchored portion of the heart or by determining the pressure developed within the ventricular chamber. Langendorff also showed that this preparation could be applied to mammalian hearts and determined the effects of temperature on the strength of contraction and the heart rate (7,8). A.J. Clark and co-workers, between 1913 and 1937, reported a series of studies in which the Langendorff preparation was used to examine the effects of ions, lipids, narcotics and metabolic poisons on the contractility and oxygen consumption of the frog heart (9-14). These studies culminated in the publication in 1938 of a monograph describing their work regarding the metabolism of the frog heart (15). Beside their monumental contributions to the understanding of cardiac metabolism, Clark and his co-workers pioneered the use of a perfusion system in which the perfusate was recycled after passage through the heart. Thus only a relatively small volume of perfusate was necessary and oxygen and substrate utilization could be determined by sampling the perfusate before and after passage through the heart.

The Langendorff preparation has since served as an extremely useful tool in studies concerned with the effect of various physical and chemical changes in the perfusate upon contractility, heart rate and metabolism. It has been applied to the study of avian, reptilian, amphibian and mammalian hearts. The isolated heart can be made to perform external work by pumping perfusate against a resistance (16). In this modification the heart perfuses

its vasculature. Work can also be determined by the lifting of a weight attached to the apex. The competence of the heart may be determined by imposing standard perfusate pressure loads. Cardiac efficiency can be measured by relating the power output to the minute oxygen consumption (efficiency = Power/O_2 consumption).

Perhaps the most outstanding problem to which the Langendorff preparation has been applied is the study of cardiac failure. Following cannulation and perfusion of the heart, the strength of contraction, although initially great, diminishes gradually until it reaches a level too low to be useful. The etiology and prevention of this gradual decline has been the subject of many reports. The term "failure" is generally applied to the gradual decline in isometric or isotonic contractility of hypodynamic preparations or to a decreased ability to perform external work under conditions of contant heart rate, imposed load and outflow resistance.

The removal or isolation of the heart from the animal appears to guarantee its eventual failure. The nature and composition of the perfusate is the prime determinant of the time for which an isolated heart remains essentially stable. The use of blood, which logically represents the ideal perfusate, simply delays the onset of spontaneous failure. The isolated heart perfused with blood from a donor animal in a recirculating system (17), and to a lesser extent the dog or rat heart-lung preparation (18,19), all demonstrate a long stable period of contractility before failure ensues.

When saline solutions are to be used for perfusion, the regulation of factors such as ionic and osmolar content, temperature, pH, oxygen and substrate content become matters of prime concern. The perfusate used must contain oxygen and organic substrates in quantities which provide a normal supply at the experimental coronary flow rates which are encountered.

The flow rate must also be sufficient to remove metabolic end-products whose accumulation is considered to be detrimental to myocardial performance.

Cardiac metabolism and contractile mechanism are a means of converting potential chemical energy into useful mechanical work. These functions may be subdivided into three general compartments (20): Uptake and/or storage of substrates such as oxygen, glucose and lipid for eventual use; conversion or conservation of substrate potential energy into the readily available potential chemical energy of high energy phosphate bonds in adenosine triphosphate (ATP) and creatine phosphate (CP); (21) and conversion of the energy of these phosphate bonds into mechanical power. These compartments, which may be delineated in terms of cellular structures such as membrane, mitochondrion, or myofibril, are interdependent. Alterations in each may be demonstrated during the onset and progression of heart failure. External manipulation to cause modification of the function of any one compartment can be shown to affect all of them. By the same token, any spontaneous alteration in the function of one compartment due to unknown factors which may be causative to heart failure should be reflected by measurable changes in the other compartments.

Oxygen and the Isolated Heart

The heart is incapable of contracting a significant oxygen debt (22) in spite of a small capacity for oxygen storage in the form of myoglobin (23). Thus the supply of oxygen can be a major determinant of the longevity and work production of an isolated preparation. Saline solutions equilibrated with oxygen at atmospheric pressure contain approximately 2 ml per 100 ml perfusate (24). The oxygen requirement for hypodynamic dog or cat hearts is 3-5 ml/100 g wet wt./min (18,25,26,27).

Since the heart is capable of extracting almost all the oxygen content of the perfusate (28) it is obvious that the minimum perfusate flow under these conditions must be 150-250 ml/100 g wet wt./min. This is generally equalled or surpassed at perfusion pressures of 30-60 mm Hg, (29,30). The isolated rat heart, which has an oxygen consumption approximately 3-4 times that of the dog or cat heart (27), has been shown to receive more than adequate supplies of oxygen when perfused with saline solutions as judged by metabolic criteria such as $A\text{-}V_{O_2}$ differences, lactate production and glycogenolysis (27,31,32,33). Efforts to increase oxygen carriage of saline perfusate by the addition of haemoglobin (34) or erythrocytes (16, 35,36) have therefore been superfluous. These improvements contribute neither to the contractility nor to the longevity of the preparations. Furthermore, Douglas and co-workers (35,37) have shown that the inotropic effects of drugs which increase the oxygen consumption are similar when either plain or enriched media is used. They also noted that catecholamine-induced increases in oxygen consumption were similar in both instances but, as would be expected, drug induced increases in coronary flow were greater in hearts perfused with unenriched medium. Blinks and Koch-Weser (24) reviewed the literature pertaining to this question, and decided that although saline perfusates could support hypodynamic preparations, the oxygen supply would be inadequate were external work loads to be imposed. The oxygen requirement of working hearts is at least 2-3 times that of hypodynamic preparations (25,38,39) and it is in situations in which work is produced that the addition of haemoglobin or erythrocytes to the perfusate becomes useful. The type of work performed also bears directly upon the oxygen consumption, as work done against a large pressure or load increases the oxygen consumption more than does work in which large volumes of per-

fusate are pumped against a relatively small pressure (40).

The rapid onset of spontaneous failure in isolated hearts perfused with saline solutions is accompanied by a decrease in oxygen consumption (10,11,41,42,43). Since contractile force and oxygen consumption decline in parallel, no change in efficiency occurs. Wollenberger (44) suggested that the oxygen supply might be the limiting factor in this instance. However, the recent studies of Zacharia (4) and Fisher and Williamson (27) show that increasing the coronary flow of isolated rat hearts by the addition of pentaerythritol tetranitrate prevents the fall in oxygen consumption, but not the decline in contractility. Spontaneous failure is delayed in hearts perfused with blood or artificial media which contain plasma or serum (45-51) and is then characterized by stable oxygen consumption during failure (10,11,52,53). Thus, failure can be accompanied by either decreased or unaltered efficiency, when judged solely by contractile strength and oxygen consumption.

During hypoxic or anoxic periods, contractile force diminishes, myocardial lactate (but not pyruvate) ion accumulates and the intra-cellular contents of ATP and CP fall precipitously (54-57). Myocardial glycogen stores are depleted rapidly during hypoxia and recovery of these stores is not always observed upon re-oxygenation (56,58). The restoration of contractile force following return to aerobic conditions depends to a large extent upon the species examined and the presence or absence of glucose. Winbury (59) has shown that although glucose does not alter the loss of contractility in anoxic cat papillary muscle, its presence during anoxia allows recovery of contractility following restitution of the aerobic state. It has been shown that cat ventricular muscle will contract during anoxia when stimulated by ouabain (60), epinephrine, or calcium (61) only

if glucose is present. Glucose can support the beating of the anoxic frog heart, but not that of the anoxic rabbit heart (62). Winbury's observations (59) would indicate that during anoxia, glucose permitted the maintenance of cellular integrity, if not contractile activity, of the cat papillary muscle. MacLeod and Daniel (63) confirmed Winbury's observation, and reported that large concentrations of glucose protected against the effects of anoxia on the membrane potential. Bing (64) and Bing and Michal (65) observed on increase in lactate production during anoxia, and suggested that anaerobic metabolism occurred. Certainly the beating of the anoxic frog heart would further support this idea, at least for amphibian hearts. Winbury (28) in reviewing the literature concerning anaerobic metabolism concluded that anaerobic glycolysis was an important source of energy during anoxia.

It is of interest to note that the Langendorff preparation is often used in studies concerning the pharmacological effects of drugs on the tone of the coronary vasculature. Commonly, fibrillation is induced in order to negate the effect of rhythmic contractility on coronary flow (66). The oxygen consumption during fibrillation is often 3-5 times that observed when normal work loads are imposed (38,67). The fibrillating Langendorff preparation may therefore be hypoxic. Valid conclusions cannot be made concerning the <u>in vivo</u> activity of a compound on the basis of this type of experimental data. Conclusions drawn from studies in which isolated hearts are arrested by increases in perfusate potassium content may also be questionable, because Whalen and Weddle (68) have shown that increased extra-cellular potassium content causes increased oxygen consumption in isolated rat ventricle.

Fawaz and co-workers (55,69) have reported that the ability

of the heart-lung preparation to oxygenate blood diminishes with time. Reports concerning spontaneous failure in this preparation (44) attended by decreased myocardial lactate utilization and increased glucose uptake may, in fact, be complicated by an unnoticed state of hypoxia.

Organic Substrates and the Isolated Heart

The heart derives its energy from the metabolism of exogenous organic materials. The hypodynamic and the working perfused hearts have been used in many studies of uptake and utilization of substrates. The isolated heart can utilize a host of organic substrates including carbohydrates, ketones, lactic and pyruvic acids, lipids and amino acids when these are present in the perfusate either singly or in combination (4,30, 32,58,70-78). The relative proportions to which each is utilized are dependent not only upon their concentration and molecular structure (76,79) but also on heart rate, cardiac work and temperature (5,58,73,75,78,80, for recent review, see Winbury (28). The previous dietary state of the donor animal can also influence the pattern of substrate consumption in the isolated preparation (28). The uptake of substrates by isolated hearts remains unchanged following the onset of spontaneous failure (44, 81). Reports that such failure may be secondary to decreased glucose uptake due to insulin loss 44) can be discounted, since addition of this hormone to the perfusate restores the normal rate of glucose uptake but does not influence the progression of failure (4).

The heart contains a relatively large store of glucose (400-500 mg %) in the form of glycogen, which serves as a readily oxidizable source of energy during periods of increased work, hypoxia, or in the absence of exogenous substrates (23).

The glucose utilization of hypodynamic hearts is approximately one-fifth of that observed when work loads are imposed. Neely, Liebermeister and Morgan (82) concluded that carbohydrate is used to a small extent in non-working heart, but its presence becomes of prime importance during periods of work. The literature concerning the eventual conversion of the potential energy of these substrates to ATP and CP via glycolysis and oxidative phosphorylation has been reviewed (83,84). Matsumoto and co-workers have recently demonstrated the presence of the pentose pathway in ventricular muscle, this source of energy being absent in atrial tissue (85,86). Olson (23,79) has determined that the isolated heart, although utilizing both glucose and free fatty acids (FFA), uses carbohydrate to a much greater extent. In the absence of glucose 75% of the extracted FFA was oxidized to CO_2, the remainder entering into myocardial lipid stores. The presence of carbohydrate reduced the magnitude of FFA uptake by 50%, with the fraction taken up almost entirely entering the lipid stores.

The relatively inefficient anaerobic breakdown of glucose to pyruvate via glycolysis yields but 2 moles of ATP per mole glucose. The subsequent conversion of pyruvate to CO_2 (via mitochondrial oxidative phosphorylation) yields the bulk of the total of 38 glucose-derived high energy bonds (83). It would seem logical that the highly efficient oxidation of either endogenous or exogenous lipids (1 mole palmitic acid yields 138 moles ATP) should be sufficient to furnish the necessary energy for contraction in the absence of glucose. Yet, in the absence of glycolytically derived ATP, work capacity diminishes.

The ATP contents of cardiac and other tissue may be considered to be in three interdependent compartments - the membrane fraction, the

cytoplasmic pool and the mitochondrial pool. ATP utilized at membrane and sarcoplasmic reticular sites is important in the operation of the sodium pumping mechanism (87) and the removal of calcium from actomyosin (relaxing factor activity) (88). The ATP utilized at this site originates in the cytoplasmic fraction. There is strong evidence that cytoplasmic and mitochondrial ATP production are separate processes which are linked by the transfer of phosphate from mitochondrial ATP to cytoplasmic ADP by an adenylate kinase present in the wall of the mitochondrion (89). Webb (90) showed that inhibition of glycolysis by fluoride ion greatly decreased contractility while causing only minimal decreases in cellular ATP content. Inhibition of mitochondrial respiration by cyanide or malate caused no greater decrease in contractility but very much greater decreases in ATP content. He suggested that ATP derived from glycolysis (cytoplasmic pool) is necessary for the efficient utilization of mitochondrial ATP by the contractile apparatus.

The presence of glucose in the perfusate has been shown to delay the onset of failure in cat heart muscle (91) and to maintain the cellular content of ATP and CP at normal levels throughout failure (91, 92,93,94). The failed heart cannot utilize lactate (44), an ion which is normally avidly removed from blood or perfusate and metabolized to pyruvate and CO_2 (23,65). Excessive lactate production causes increased intracellular levels of this ion which are considered to be injurious (44), possibly due to the release of intra-cellular cathepsins (95).

Spontaneous failure in the absence of substrates differs in many respects from the failure which occurs in their presence. During short periods of exposure to substrate-free fluid, glycogen is rapidly consumed (27, 96-99), but contractility remains essentially normal (90).