# Role of anaerobic metabolism in the preservation of functional capacity and structure of anoxic myocardium

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**Research Article**

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# Role of Anaerobic Metabolism in the Preservation of Functional Capacity and Structure of Anoxic Myocardium

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ABSTRACT Employing an isolated perfused rat heart preparation, we investigated the contribution of anaerobic metabolic energy to the performance, recoverability, and ultrastructure of the heart perfused at 32°C in 5% albumin in Krebs-Ringer Bicarbonate solution. During exposure to anoxia for 30 min, inclusion in the perfusate of the anaerobic substrate, glucose, resulted in marked improvement in electrical and mechanical performance of the heart and in enhanced recovery during the subsequent period of reoxygenation. Lactate production was fivefold greater in the glucose-supported anoxic heart than in the anoxic heart without glucose. Electron microscope sections of the hearts exposed to anoxia in the absence of glucose revealed alterations in mitochondrial morphology and dilatation of the longitudinal tubules. These morphologic changes during anoxia were averted by inclusion of glucose in the perfusion fluid. The data are consistent with the hypothesis that anaerobic energy generation plays a significant role in preserving myocardial function and structure and in promoting recoverability of the anoxic mammalian heart.

## INTRODUCTION

It has been recognized in recent years that mammalian myocardium does not depend solely upon oxygen for its energy needs, and that the myocardium can inded shift for short periods to an anaerobic metabolic pathway (1–4). Very few definitive data deal with the contribution of such anaerobically derived metabolic energy to the electrical and mechanical performance of the heart, to functional recoverability after exposure to anoxia, or to the prevention of intracellular structural alterations accompanying anoxic exposure.

Induction of myocardial hypoxia by simple cessation of coronary perfusion, in vivo or in vitro, does not permit a steady supply of anaerobic substrate to the myocardium. Furthermore, the effects of accumulated end products of anaerobic metabolism are not controlled under these circumstances. Studies with perfused myocardial strips, which avoid these difficulties, do not yield information on spontaneous electrical activity or on electromechanical integration of cardiac function. With these considerations in mind the present studies on the effects of anoxia on the isolated perfused rat heart were initiated. This preparation permits study of electrical and mechanical performance of the isolated heart during relatively prolonged experiments and instantaneous addition or deletion of anaerobic substrate while perfusion is maintained at a constant rate. The studies were designed to determine the degree to which the presence of the anaerobic substrate, glucose, affects the performance, recoverability, and ultrastructure of the perfused anoxic heart.

## METHODS

The hearts used in these studies were removed from male albino (Wistar) rats, weighing from 180 to 235 g. The rats were fed ad lib. with standard Purina laboratory chow until they were sacrificed. The animals were decapitated, the chest opened within 30 sec with a subxiphoid incision, and the heart flooded immediately with ice-cold Ringer's solution. This maneuver produced rapid

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cessation of the heart beat, after which the heart was dissected from the mediastinum with an attached aortic stump 5 mm long. The heart was then mounted on the perfusion cannula which consisted of a No. 17 TW needle with a rounded and smoothed end and notched 3 mm above the tip. The tip of the cannula was placed in the aortic root which was attached firmly via a silk 3-0 ligature applied to the notch in the cannula. The time from extirpation of the heart to in vitro perfusion was 3–5 min.

The perfusion system, as illustrated in Fig. 1, was recirculating in type and consisted of a cardiac perfusion chamber, a perfusion drainage control system, a rotating flask oxygenator, a constant flow pump (Technicon Corporation, Ardsley, N. Y.), a temperature control coil, and a bubble trap. The cardiac chamber, the temperature control coil, and the bubble trap were jacketed and connected via a circulating pump to a thermostatically controlled water bath. The cardiac chamber (7 cm long, 2 cm internal diameter) had two round side openings (8 mm diameter) and a tapered outlet. The chamber was capped by a rubber stopper through which the perfusion cannula and two platinum electrodes with flattened ends entered. The perfusion circuit was completed with tygon tubing. Glass or metal T tubes were inserted at points immediately proximal and distal to the heart chamber and were employed as sampling sites.

The rotating flask oxygenator consisted of a 1 liter round bottom flask held by a three prong device which rotated at a rate of 76 rpm. Three tubes in the rotating flasks provided for inflow and outflow of perfusion fluid and for inflow of gas. The inflow gas filled the chamber above the level of perfusate. This system proved to be superior to a bubbling oxygenator because it yielded a higher concentration of dissolved oxygen, eliminated the problem of foaming when albumin was employed in the perfusion fluid, and permitted rapid change in the concentration of gas dissolved in the perfusate. The Technicon pump operated by peristaltic compression of the tygon tubing passing through it and assured constancy of flow at all levels of perfusion observed in these studies. The temperature control coil was a rewarming chamber which compensated for the change in perfusate temperature as the coil traversed the oxygenator and the pump. The bubble trap prevented intracoronary gas emboli. The bubble trap required venting only while the apparatus was filled before perfusion. A second parallel perfusion circuit was connected to the inflow cannula via a two-way stopcock. This circuit allowed for immediate changes of the composition of the cardiac perfusate without altering the dynamics of the perfusion system. Coronary perfusion was maintained at 10 ml/min. The perfusion fluid was 5% human serum albumin in Krebs-Ringer bicarbonate solution (KRB). The KRB was modified to contain 5.0 mg/100 ml of calcium. The albumin (donated by the American Red Cross) was dialyzed for 24 hr against the KRB. The dialyzed albumin was frozen, and stored, and made up to the final 5% dilution in KRB just before perfusing the heart. The final concentration of calcium in the perfusion fluid was 6.3 mg/100 ml. The solution was gassed with 95% $O_2$, 5% $CO_2$ during aerobic perfusion and with 95% $N_2$, 5% $CO_2$ during anaerobic perfusion. The albumin contributed a source of bound nonesterified fatty acid in concentrations varying from 2300 to 2800 $\mu$eq/liter of perfusate. Perfusion was maintained at 32°C. The volume of perfusate in the system was 200 ml.

Left intraventricular pressure was obtained by introducing a 2 cm length of N. 20 TW needle (the hub of which was removed) through the myocardial wall via an opening in the cardiac perfusion chamber. This needle was attached with a 10 cm length of polyethylene tubing (PE 90) to a P23 DB Statham strain gauge via a second blunt-edged No. 20 TW needle. Each needle was inserted firmly into the ends of the polyethylene tubing leaving a 5–6 cm length of the tubing for pressure transmission. The entire pressure recording system was filled with bubble-free distilled water. The output of the strain gauge amplifier was fed via a coupled resistor-condensor differentiating circuit into a DC channel for constant recording of the first derivative of left ventricular pressure. The maximum rate of rise of systolic pressure (peak systolic dp/dt) was derived from the recording of the first derivative of ventricular pressure. The pressure recording system had a uniform frequency response to 20 cycle/sec. The dp/dt response of the pressure recording system containing the polyethylene tubing was compared to that produced by a No. 20 TW needle coupled directly to a P23 DB strain gauge. In the range of dp/dt from 10 to 3000 mm Hg/sec, the pressure recording system containing the short length of polyethylene tubing between needle and gauge yielded the same measures of dp/dt as those from a system with direct coupling of needle to gauge. A second pressure recording was made from a sidearm in the inflow cannula. In this manner left ventricular pressure, the maximum rate of rise of left ventricular systolic pressure, and coronary perfusion pressure were monitored constantly. The platinum electrodes were lowered to touch the surface of the heart and their ends attached to the bipolar leads of an electrocardiogram. For convenient tracing of both atrial and ventricular activity, one electrode was placed on the right atrial surface, while the second electrode was placed on the left ventricular surface. Continuous recordings of ventricular and perfusion pressure and the electrocardiogram were made by means of a Sanborn 550 multichannel photographic apparatus. In 10 experiments the ventricle was paced electrically at a rate of 235 beats/min. This paced heart rate was slightly higher than the endogenous atrial rate. The pacing stimuli were square wave pulses of 5 msec duration delivered through the platinum electrodes to the ventricular surface at a voltage which was 10% greater than threshold for pacing.

The oxygen consumption of the heart was calculated from oxygen tension measurements of samples of perfusate taken simultaneously from the inflow and outflow conduits to and from the heart. In order to maintain constancy of coronary perfusion at the time when samples were taken for oxygen tension, a constant 2 ml/min flow of fluid from the inflow cannula was maintained. This conduit could be connected directly to the rotating oxy-

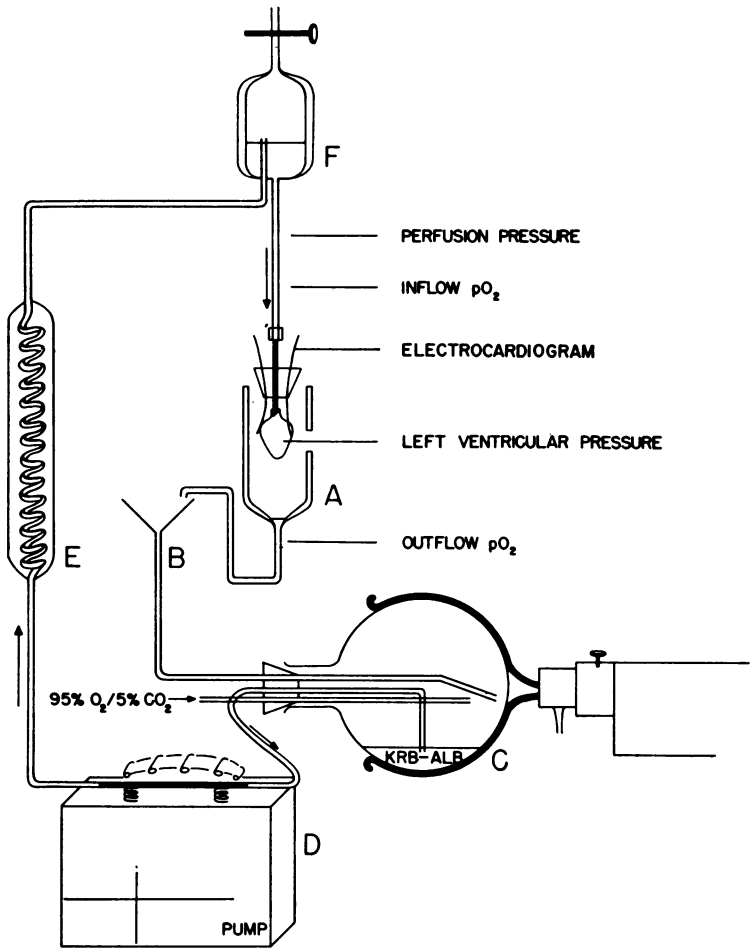


FIGURE 1 Diagram of apparatus for perfusion of isolated rat heart. *A*, cardiac perfusion chamber; *B*, perfusion drainage control system; *C*, rotating flask oxygenator with conduits for inflow, outflow, and gassing; *D*, constant flow pump; *E*, temperature control coil; and *F*, bubble trap.

genator or allowed to flow directly to the oxygen electrode without alteration in cardiac perfusion pressure or flow. Samples were removed from the effluent solution in a similar manner with particular care to maintain temporal equivalence of the inflow and outflow sampling volumes. The oxygen tension of the fluid was measured at 32°C by a rhodium capillary reservoir microelectrode (Instrumentation Laboratories Inc., Watertown, Mass.). The electrode was calibrated before each oxygen tension determination. Oxygen consumption was calculated from the change in oxygen content and the Bunsen coefficient according to the formula: $QO_2 = I - E/760 \times F \times B/$ D.W., where $QO_2$ = oxygen consumption in $\mu$l/hr per mg of dry weight, $I$ = inflow oxygen tension, $E$ = effluent $O_2$ tension, $F$ = coronary perfusion rate, (600 ml/hr in the present study), $B$ = Bunsen coefficient (25 $\mu$l of $O_2$/ml at 32°C), and D.W. = dry weight of heart in mg. The two round openings in the cardiac perfusion chamber permitted free access of the chamber to room air during aerobic perfusion. These openings permitted a more constant chamber gas composition than did a completely closed system. At 32°C, with exposure to 95% $O_2$, 5% $CO_2$, inflow oxygen tension ranged from 550 to 600 mm Hg, while effluent $O_2$ tension ranged from 200 to 300 mm Hg. A correction was made at the termination of the experiment for change in oxygen tension of the effluent drop passing through the chamber. This correction ranged from 19 to 39 mm Hg and varied according to the level of effluent oxygen tension.

Lactate content of the perfusing fluid was determined by the enzyme technique of Scholz et al (5). Pyruvate content was determined by the enzyme method of Bucher and coworkers (6). Total lactate and pyruvate production was calculated from the concentration and volume of the perfusing medium. The dry weight of each heart was derived from the wet weight, and determination of the wet to dry weight ratio in representative samples of heart tissue was obtained at the termination of each experiment.

For the electron microscope studies, we first performed fixation on the whole heart by perfusing the myocardium with 2% glutaraldehyde in phosphate buffer (pH 7.4 and 5°C) at 10 ml/min, using the same perfusion system employed for the physiologic studies (7). At the end of 10 min of perfusion with glutaraldehyde through the coronary bed, small fragments of myocardium were dissected from

the inner wall of the left ventricle. These fragments were then fixed in 2% glutaraldehyde for an additional 1.5 hr period and postfixed with phosphate-buffered (pH 7.4) 1% osmium tetroxide (8). After ethanol dehydration, the tissue was imbedded in DER (Dox Epoxy Resin) mixture (9). The thin sections were stained with uranyl acetate and lead citrate (10). 40 blocks were obtained from each heart. Six to eight blocks, in which the myofibrils were longitudinally oriented, were selected from each heart for study. Approximately 30 thin sections were examined from each of the blocks. The electron micrographs were obtained in a Hitachi HU-11 B-2 electron microscope.

Statistical analyses were performed according to the method of Snedecor (11).

## RESULTS [1]

*Aerobic performance of the isolated rat heart.* The performance characteristics of the isolated rat heart perfused at 32°C in an aerobic (95% $O_2$ 5% $CO_2$) environment in glucose-containing (200 mg/100 ml, 11.1 mM concentration, 14 hearts) and glucose-free albumin KRB (21 hearts) are summarized in Figs. 2 and 3. During 160 min of perfusion the heart maintained sinoatrial rhythm at a rate of 220–240 beats/min. End-diastolic pressure remained constant and below 5 mm Hg during the initial 120 min of perfusion and tended to rise thereafter. Pulse pressure rose gradually during the initial 50 min of perfusion and stabilized in the range of 80–90 mm Hg. Changes of peak systolic dp/dt were parallel to those of ventricular pulse pressure. Perfusion pressure remained at suprasystolic levels and tended to rise gradually throughout the perfusion period. The oxygen consumption remained relatively constant throughout the perfusion period. It is notable that the addition of glucose to the medium during aerobic perfusion produced no significant alteration in ventricular performance (Fig. 3).

In order to test the adequacy of oxygenation provided by this system, we perfused hearts at lower concentrations of oxygen than in the previous study. In 10 studies on the isolated perfused rat heart gassed with 80% $O_2$, 5% $CO_2$, and 15% $N_2$ at 32°C, performance characteristics were virtually identical with those observed in an environment of 95% $O_2$ and 5% $CO_2$. When the hearts were exposed to perfusate equilibrated with 60% $O_2$, 35% $N_2$, and 5% $CO_2$, a variable diminution in ventricular pulse pressure and slowing in sinoatrial rate occurred. These performance data indicate that a gas mixture containing 80% $O_2$ or greater insures adequate oxygen supply in the perfusion system.

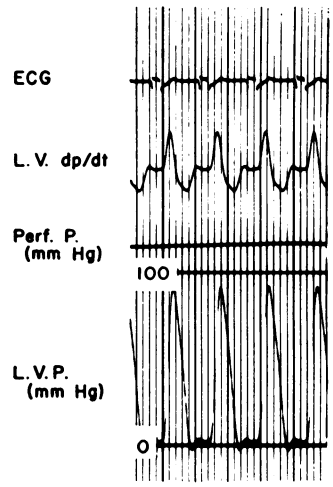


FIGURE 2 Simultaneous recording of the electrocardiogram (ECG), first derivative of left ventricular pressure (L.V. dp/dt), perfusion pressure (Perf. P.), and left ventricular pressure (L.V.P.) after 60 min perfusion of an isolated heart preparation. The 0 and 100 mm Hg calibration lines are shown. Time lines indicate 0.04 sec.

*Effects of anoxia.* The effects of exposure of the isolated perfused rat heart to an anoxic environment (95% $N_2$, 5% $CO_2$) for 30 min, after 60 min of preliminary perfusion in oxygenated glucose-free albumin KRB (95% $O_2$, 5% $CO_2$), are summarized in Fig. 4.

Exposure to anoxic perfusion fluid in the absence of glucose (10 hearts) resulted in a reproducible sequence of electrocardiographic changes consisting initially of sinoatrial slowing, followed by varying degrees of atrioventricular block, and then sinus arrest with a nodal or idioventricular rhythm. 9 of the 10 hearts came to complete electrical and mechanical arrest during the first 20 min of anoxic perfusion. In two of the hearts mechanical arrest of the ventricle preceded total

[1] Material supplementary to this article (six tables) has been deposited as Document number 9759 with the ADI Auxilliary Publications Project, Photoduplication Service, Library of Congress, Washington, D. C. A copy may be obtained by citing the Document number and by remitting $1.25 for photoprints or $1.25 for 35 mm microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.

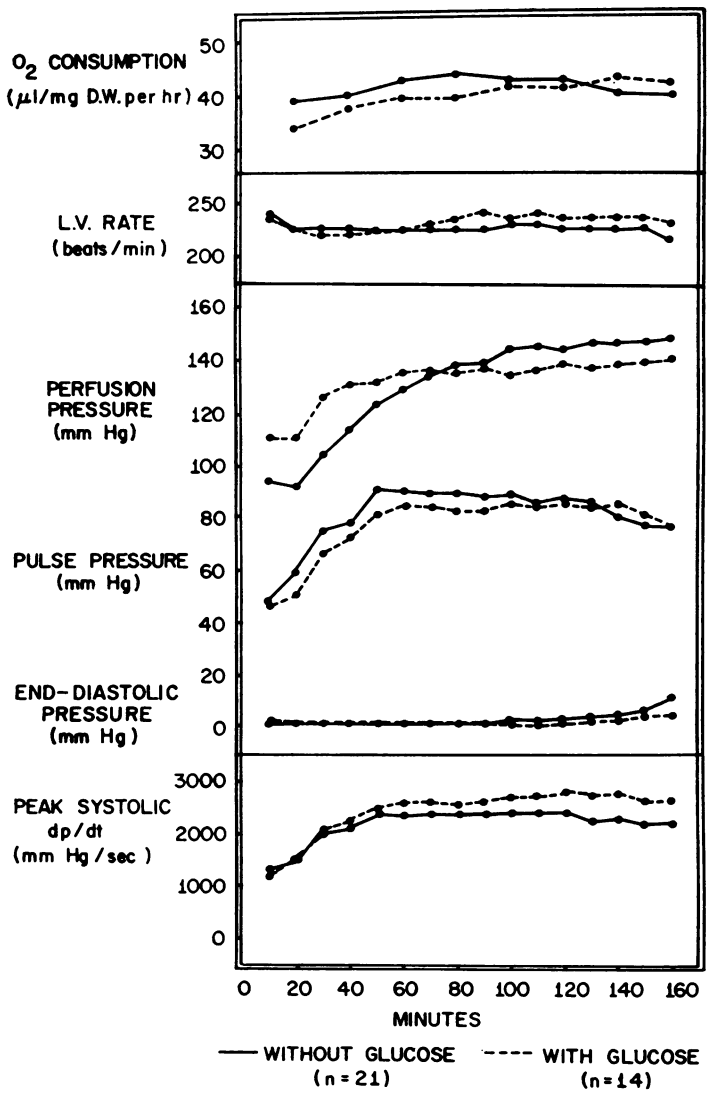


FIGURE 3 Performance of isolated perfused rat hearts under aerobic conditions (95% $O_2$, 5% $CO_2$). Hearts perfused in 5% albumin KRB in absence of glucose (n = 21) are compared to hearts perfused in the same medium containing glucose (200 mg/100 ml, n = 14). Mean data are shown. There is no significant difference between the groups in any performance characteristic.

electrical arrest by 30 sec, and in the remaining hearts mechanical arrest accompanied the cessation of electrical activity. One heart continued to beat from an idioventricular pacemaker site for 30 min. In the course of exposure to anoxia, pulse pressure and peak systolic dp/dt fell progressively; perfusion pressure diminished but remained above systolic levels until the time of mechanical arrest, when intraventricular pressures approached the perfusion pressure.

With exposure to the anoxic perfusion fluid, the presence of glucose (200 mg/ml, 11 hearts) prolonged electrical activity and increased mechanical performance of the isolated heart. Initial sinoatrial slowing and atrioventricular block occurred as in the anoxic hearts without glucose, followed by sinus arrest associated with a persisting nodal or idioventricular pacemaker throughout the 30 min period of anoxia in five of the hearts. An atrial pacemaker with varying atrioventricular block was maintained throughout the 30 min exposure in two hearts. The remaining four hearts progressed from sinus arrest and a nodal or idioventricular rhythm to complete electrical and mechanical arrest after 20 min of anoxic perfusion. All hearts supported by glucose continued to beat during the first 20 min of anoxia. In contrast to the hearts exposed to anoxia in glucose-free perfusate, end-diastolic pressure rose at a slower rate and to lower levels, while pulse pressure and peak systolic

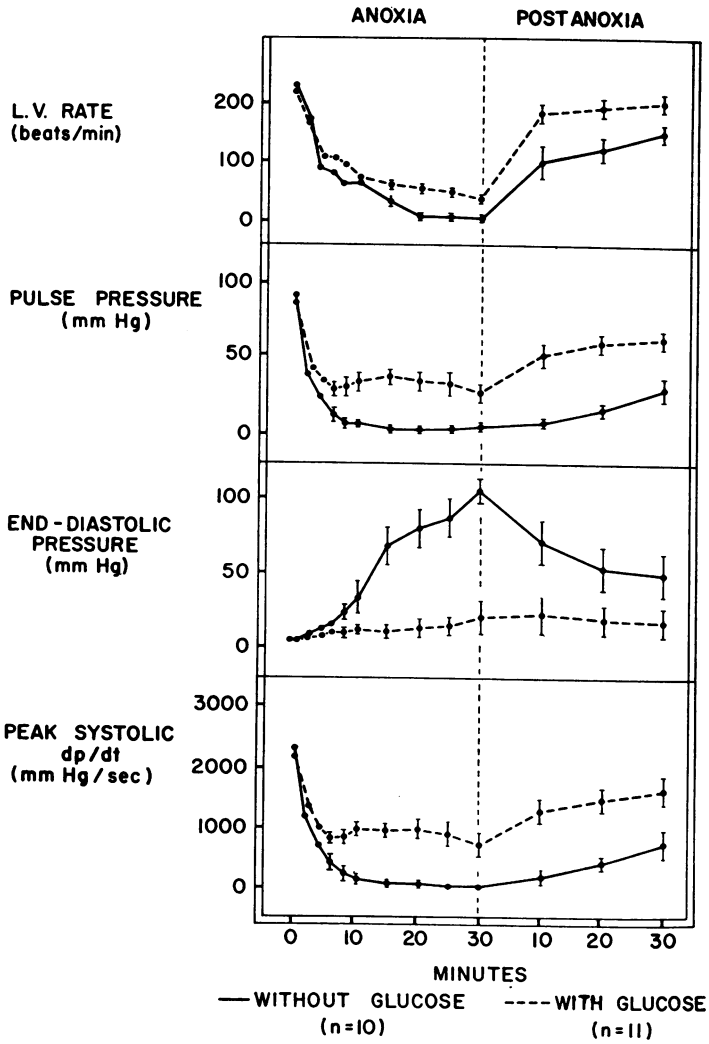


FIGURE 4 Performance of isolated perfused rat hearts during anoxia (95% $N_2$, 5% $CO_2$) and during recovery (postanoxia). Hearts perfused in 5% albumin in KRB in absence of glucose ($n = 10$) are compared to hearts perfused in the same medium containing glucose (200 mg/100 ml, $n = 11$). The mean data are shown. Vertical bars represent $\pm 1$ SE at nonoverlapping points. Peak systolic dp/dt was indeterminate at 25 and 30 min of anoxia in the absence of glucose.

dp/dt fell to a distinctly lesser degree in the hearts supported by glucose in the anoxic medium. Perfusion pressure was diminished throughout the period of anoxic exposure.

During the 30 min of exposure to anoxia, lactate appeared in the perfusing medium (Fig. 5). When the perfusate contained glucose, the mean rise in lactate (97 μmoles, SE 7) during 30 min of anoxia was almost fivefold greater than that observed in glucose-free anoxic perfusion (20 μmoles, SE 5). No measurable pyruvate was detected in the absence of glucose, while slight pyruvate production (2 μmoles, SE 0.8) was observed when glucose was present in the anoxic perfusate.

The recoverability of each heart after exposure to anoxia was tested by returning to oxygenated (95% $O_2$, 5% $CO_2$) perfusion fluid without glucose. During the recovery period, hearts exposed to anoxia with glucose in the perfusion fluid demonstrated a higher ventricular rate, a lower end-diastolic pressure, and a higher pulse pressure and peak systolic dp/dt than anoxic hearts without glucose (Fig. 4). In the control period, the oxygen consumption of the isolated hearts averaged 38.3 (SE 3.4) and 39.7 (SE 2.1) μl/hr per mg of dry weight. With exposure to anoxic perfusion fluid, the oxygen consumption, 20 min after reoxygenation, was lower than the preanoxic control levels. Oxygen consumption was depressed more in the hearts not supported by glucose during the anoxic period than in glucose-supported hearts (mean levels 28.4, SE 2.9 and 34.0, SE 2.3 μl/hr per mg of dry weight respectively for glucose-free and glucose-supported hearts). During

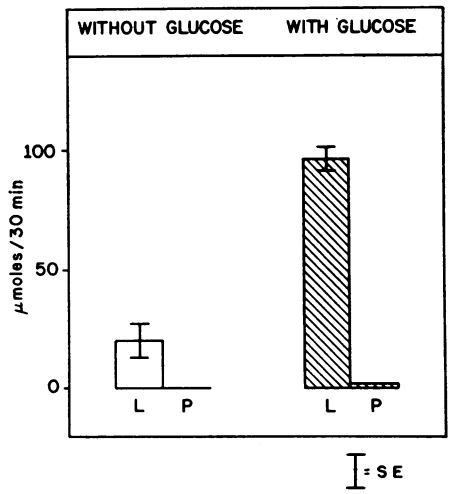


FIGURE 5 Lactate (*L*) and pyruvate (*P*) production by the isolated rat heart during anoxia with (n = 11) and without glucose (200 mg/100 ml, n = 10) in the perfusate.

the recovery period sinoatrial activity returned in most of the hearts of each group (in seven glucose-free and eight glucose-supported hearts). After anoxia, prolonged and irregular QRS waves were noted in all 10 of the hearts exposed to anoxia without glucose. In contrast, in seven hearts exposed to anoxia with glucose, the QRS complex returned to control type, while in four hearts a prolonged and altered configuration of the QRS complex persisted.

In order to exclude changes of ventricular rate as an independent determinant of ventricular performance during and after anoxia, we performed two additional groups of studies in which the hearts were preperfused in oxygenated glucose-free albumin KRB (95% $O_2$, 5% $CO_2$) for 1 hr and paced (235 beats/min) for 10 min before anoxia. The hearts were exposed to anoxia (95% $N_2$, 5% $CO_2$), while the left ventricle continued to be paced at a rate of 235 beats/min throughout the periods of anoxia and recovery. One group of five paced hearts was exposed to anoxia for 30 min by perfusing with albumin KRB containing 200 mg/100 ml of glucose, while the second group

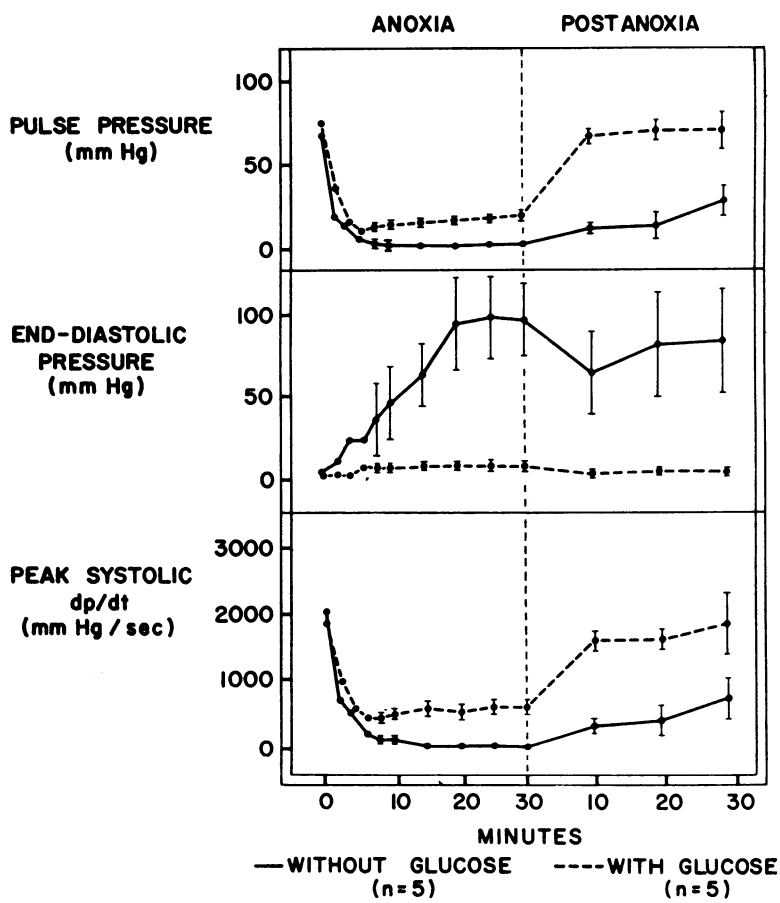


FIGURE 6 Performance of paced (235 beats/min) isolated perfused rat hearts during anoxia (95% $N_2$, 5% $CO_2$) and during recovery (postanoxia). Hearts perfused in 5% albumin in KRB in absence of glucose (n = 5) are compared to hearts perfused in the same medium containing glucose (200 mg/100 ml, n = 5). The mean data are shown. Vertical bars represent ± 1 SE at nonoverlapping points. SE bars are omitted where all hearts reach 0 levels for pulse pressure and peak systolic dp/dt in the absence of glucose.

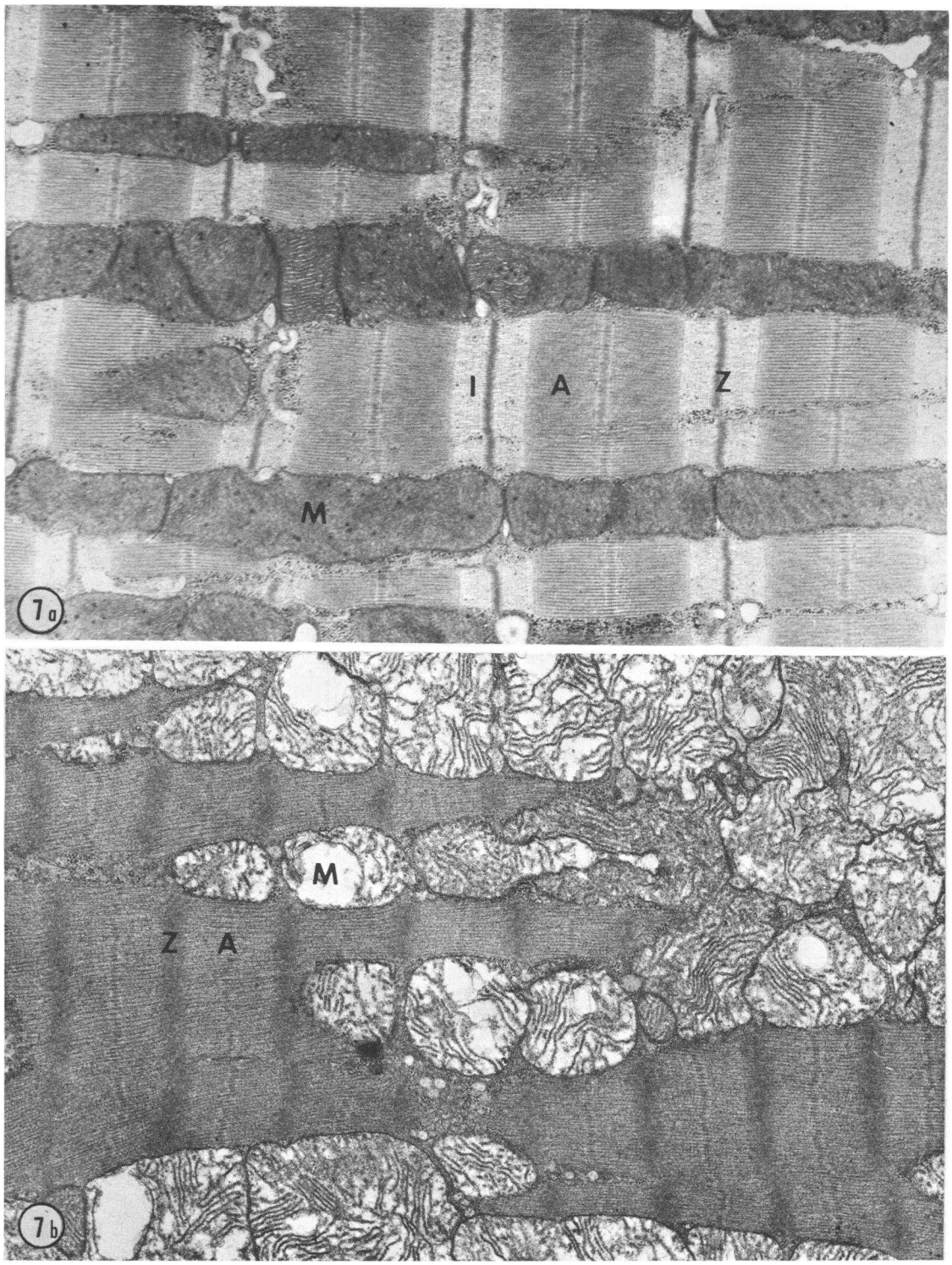


FIGURE 7 (*a*) Low power view of left ventricular myocardium after 90 min of aerobic perfusion in the absence of glucose (× 15,000). Note that the fine structure of the myofibrils is preserved and mitochondria (*M*) show regular cristae and electron-opaque matrix. The transverse tubules (*T*) are seen between the mitochondria. Intramitochondrial microbodies (*i*) are present. *A*, A band; *I*, I band; *Z*, Z line; *L*, longitudinal tubules. (*b*) After 60 min of aerobic perfusion and 30 min of anaerobic perfusion in the absence of glucose. The mitochondria appear enlarged and show decreased electron opacity of the matrix and displacement and separation of cristae. The intramitochondrial microbodies are not seen. Transverse tubules are difficult to recognize. The myofibrils are contracted and demonstrate wide Z areas and absence of I bands.

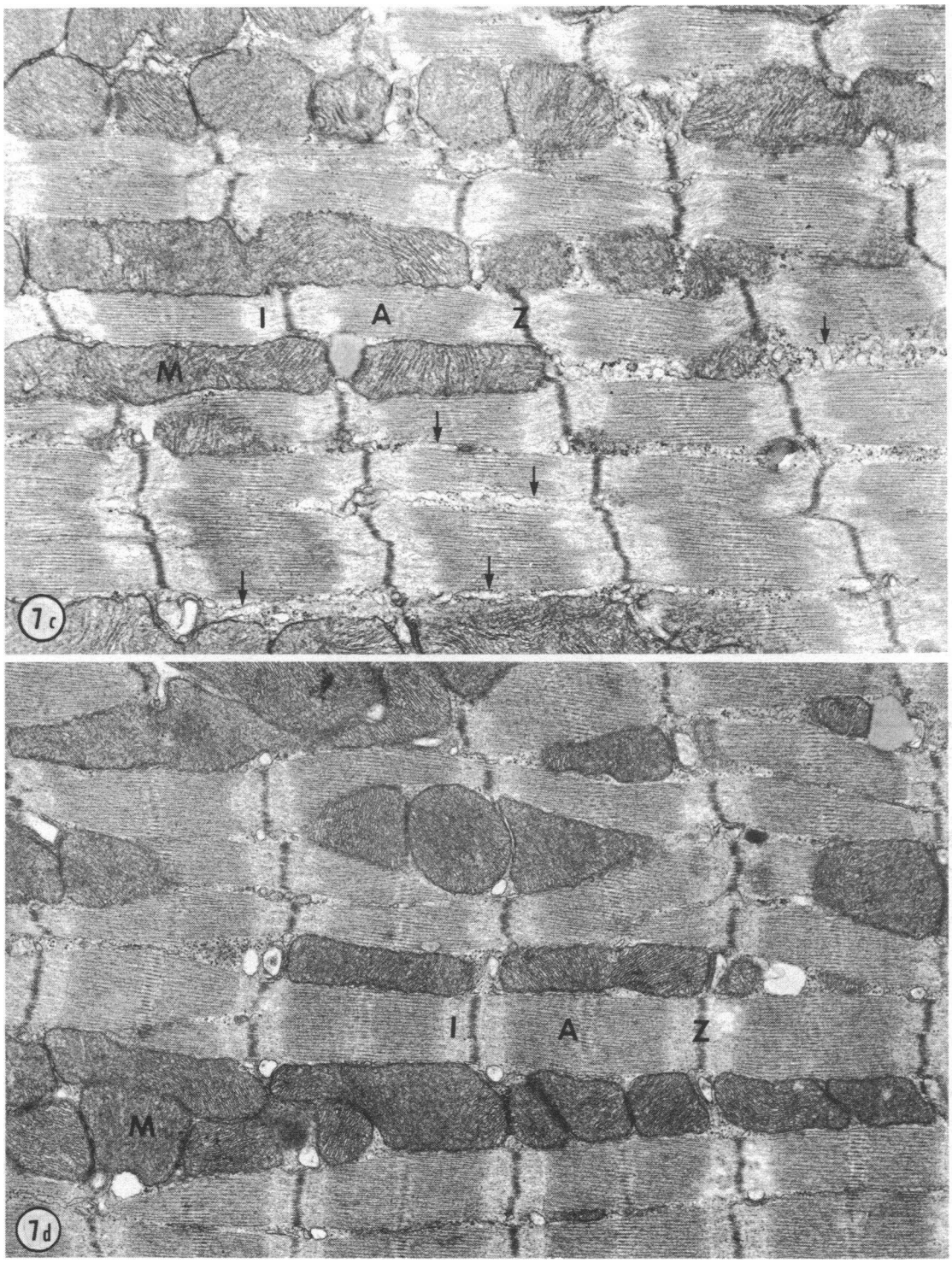


FIGURE 7 (*c*) Low power view of left ventricular myocardium after 60 min of aerobic perfusion and 30 min of anaerobic perfusion in the absence of glucose (× 15,000). The mitochondria in this area are better preserved than in 7 *b*. Several conspicuous areas of dilatation of the longitudinal tubular system are seen (arrows). Myofibrils are not contracted in this section. (*d*) After 60 min of aerobic perfusion and 30 min of anaerobic perfusion in the presence of glucose. The mitochondria, myofibrils, and transverse tubules are well preserved. The intramitochondrial microbodies are diminished in size and number. Longitudinal tubules are not dilated. For abbreviations see Figs. 7 *a* and *b*.

of five hearts was exposed to anoxia for 30 min in glucose-free albumin KRB perfusion fluid. The performance of the paced hearts during exposure to anoxia and during the subsequent recovery period is summarized in Fig. 6. With constant ventricular pacing, end-diastolic pressure, pulse pressure, and peak systolic dp/dt followed a pattern similar to that observed in the presence of spontaneous electrical activity. All five hearts exposed to anoxia in the absence of glucose developed mechanical arrest within 15 min. Mechanical arrest in those hearts was preceded by a period of varying pacemaker block. All five hearts exposed to anoxia in glucose-containing (200 mg/100 ml) perfusion fluid continued to beat at the pacemaker rate (235 beats/min) for the entire 30 min of anoxia.

The recovery of physiologic performance, while pacing was maintained, is illustrated in Fig. 6. During the 30 min recovery period, pulse pressure and peak systolic dp/dt were higher while end-diastolic pressure was lower in the hearts that were supported by glucose throughout the previous exposure to anoxia than in hearts unsupported by glucose. Pacing was established at a 1:1 electrical to mechanical response during the recovery phase of the experiment in all five hearts previously supported by glucose, while varying degrees of pacemaker block were present in the hearts in which glucose support was absent during the anoxic phase.

Electron microscope studies were performed on a total of 16 hearts. One group of four hearts was perfused aerobically for 90 min in glucose-free medium, and another group of four hearts was perfused similarly with glucose-containing perfusion fluid. A third group of four hearts was exposed to anoxia (95% $N_2$, 5% $CO_2$) in glucose-free perfusate for 30 min after 60 min of initial aerobic perfusion. A fourth group of four hearts was perfused similarly to the third group of glucose-containing perfusate during the period of anoxia.

Representative sections of left ventricular myocardium examined from the hearts exposed to aerobic and anaerobic perfusate are shown in Figs. 7 and 8. When aerobic perfusion took place for 90 min in the presence or absence of glucose, cellular fine structures of the myofibrils, mitochondria, and longitudinal and transverse tubules were well preserved. There was little difference in ultrastructure between hearts perfused aerobically with or without glucose. Sarcomere lengths in these sections ranged from 2.1 to 2.4 $\mu$. The mitochondria demonstrated regular cristae and an electron-opaque matrix. Intramitochondrial microbodies were readily observed. The transverse tubules were well defined. The main portions of the longitudinal tubules were narrow, with a diameter ranging from 22 to 28 m$\mu$. Glycogen granules were abundant along the longitudinal tubules and appeared to be slightly increased in the hearts perfused aerobically with glucose when compared to those perfused under similar circumstances in the absence of glucose.

In the hearts perfused anaerobically in the absence of glucose, significant ultrastructural alterations were observed. The muscle cells showed variability in sarcomere length ranging between 1.4 and 2.4 $\mu$. Regions of contracted sarcomeres without I bands were common. A generalized stretching of sarcomeres was not observed despite the elevated diastolic pressures at the time of fixation. Occasional cells showed disruption of the usual discrete alignment of the myofilaments, and there were areas of myofibrillar separation scattered throughout the sarcoplasm. In approximately one-half of the cells of any section, the mitochondria demonstrated marked morphologic changes consisting of a decrease in electron opacity of the matrix, displacement and swelling of the cristae, and disappearance of the dense intramitochondrial microbodies. In the remaining muscle cells the mitochondria were relatively well preserved. In cells with mitochondrial change the transverse tubules were barely recognized, and the tubular membranes appeared approximated. In the cells without mitochondrial change the transverse tubules were readily recognized and appeared to demonstrate no morphologic change. Less frequently observed than the mitochondrial changes was a dilatation of the longitudinal tubular system. The dilatation varied between cells and within the same cell, and these changes in longitudinal tubular structure were distributed independently of the mitochondrial changes and were present in at least 20% of the cells of any section. The dilated lumen of the longitudinal tubules frequently attained a measured diameter of 90 m$\mu$. It was noted that cells demonstrating marked mitochondrial and

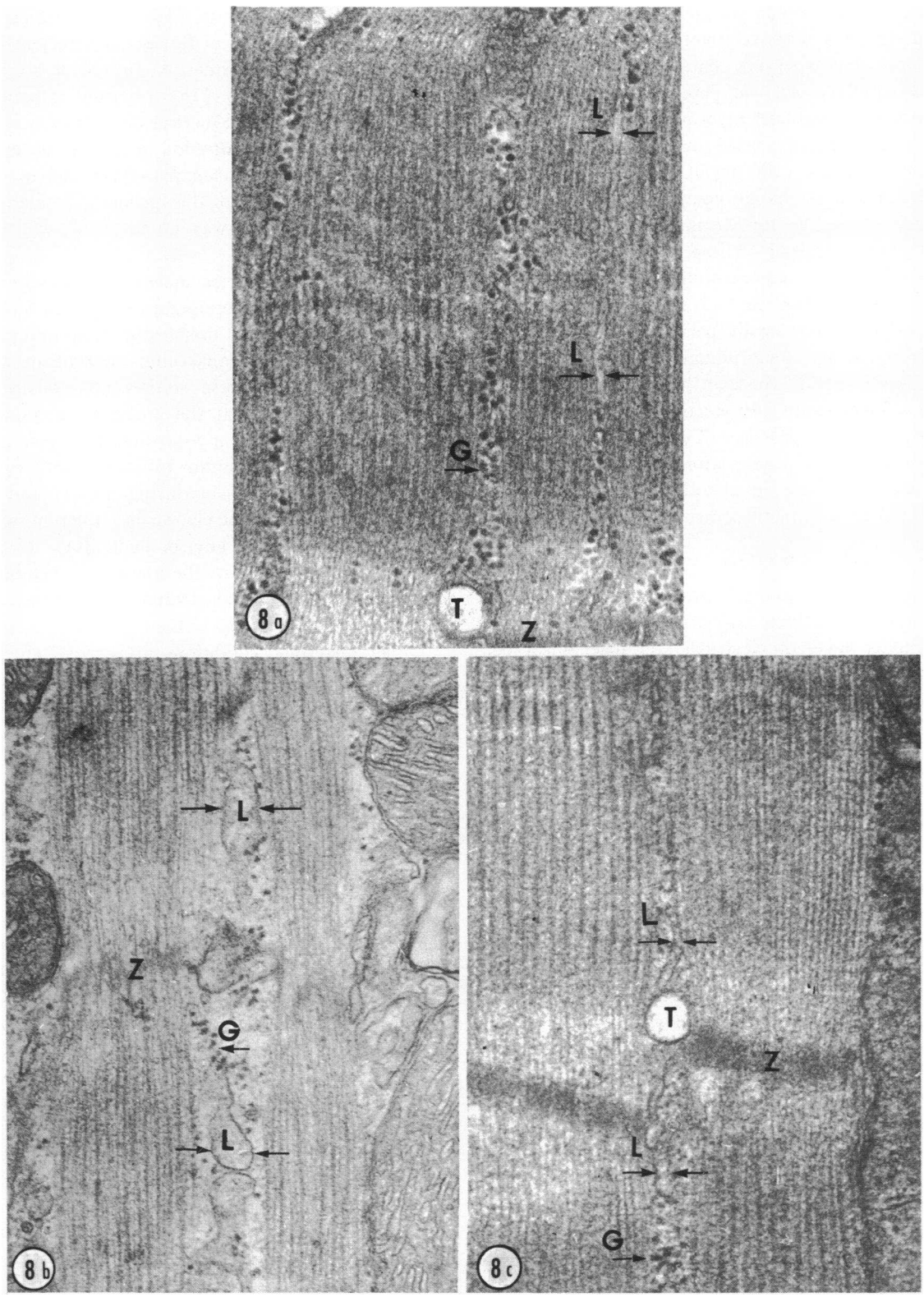


FIGURE 8 High power views of the left ventricular myocardium (× 60,000) with particular emphasis on the ultrastructure of the longitudinal tubules. *G*, glycogen granules; *L*, longitudinal tubule; *T*, transverse tubule; *Z*, Z line. (*a*) After 90 min of aerobic perfusion in the absence of glucose. The longitudinal tubules show a narrow lumen as indicated by arrows. (*b*) After 60 min of aerobic perfusion and 30 min of anaerobic perfusion in the absence of glucose. This section demonstrates marked dilatation of the longitudinal tubules (arrows). (*c*) After 60 min of aerobic and 30 min of anaerobic perfusion in the presence of glucose. The longitudinal tubules are not dilated and are similar in appearance to those seen in the aerobically perfused hearts.

longitudinal tubular change were often in apposition to cells with relatively little change in the tubules or no apparent change in the mitochondria. The capillary bed was patent throughout the anaerobically perfused myocardium.

In the hearts perfused anaerobically in the presence of glucose (200 mg/100 ml), there was little ultrastructural change compared with those perfused aerobically for 90 min. The morphology of the mitochondrial cristae was well preserved throughout the myocardium. The intramitochondrial microbodies were present but less conspicuous than in aerobically perfused hearts. Both the transverse and longitudinal tubules were similar in appearance to those in hearts perfused aerobically for 90 min. Despite the exposure to anoxia for 30 min, the presence of glucose in the perfusate resulted in the preservation of glycogen granules which were seen in abundance along the longitudinal tubules in these hearts.

## DISCUSSION

It is apparent from the present observations that anaerobic metabolic support by glucose permitted a higher level of electrical and mechanical performance of the isolated rat heart during and after recovery from a 30 min exposure to anoxia at 32°C than it did in hearts perfused aerobically for 90 min. Anaerobic metabolic support by glucose also prevented the ultrastructural changes induced by anoxia in the absence of anaerobic support.

Previous studies on the contribution of glucose to the performance of the anoxic mammalian myocardium have not yielded uniform results. In studies on cat papillary muscle, Winbury (12) demonstrated enhanced recovery of contractile force after anoxia in the presence of glucose (5 mM concentration at 38.6°C) but no difference in performance during a period of anoxia of 60 min duration. In studies on the spontaneously beating rabbit atrium, Yang (13) demonstrated enhanced force of contraction and increased atrial rate during anoxia, together with a more rapid and complete recovery in contractile force and atrial rate in hearts exposed to oxygen when glucose (11.1 mM concentration at 31°C) was included in the anoxic perfusion fluid. In the latter studies the effects of glucose were inhibited by iodoacetate or 2-deoxyglucose. Geiger and Hambourger (14) observed that the addition of glucose to the perfused anoxic rabbit heart (1% concentration at 37.5°C) after the onset of functional deterioration did not enhance performance. In recent studies Austen and coworkers (15) reported enhanced ventricular function and increased contractile force in the dog heart when a period of cardiac ischemia induced by aortic occlusion was preceded by infusion of glucose through the coronary circulation. This effect of glucose was noted at 37, 28, and 10°C.

While several variables including glucose concentration, myocardial temperature, species differences, and experimental preparation may influence the relative effects of anaerobic metabolism, the over-all findings of these studies are consistent with the hypothesis that the anaerobic metabolic pathway in mammalian myocardium may generate sufficient energy to maintain functional activity of the contractile mechanism during anoxia and to yield improved return of myocardial function upon reexposure to aerobic environment. It is of interest that such a role of the anaerobic metabolic pathway has been demonstrated in the frog and turtle heart as well (16, 17).

Under anaerobic conditions myocardial energy production is limited to that released in the glycolytic production of lactate. In hearts not supported by glucose the mean lactate production of 20 μmoles/30 min exposure period must be accounted for from the residual myocardial glycogen present after 60 min of aerobic perfusion. The additional lactate production in the presence of glucose in the medium is derived from the latter. Reeves has shown that the work efficiency of the turtle heart calculated on the basis of ATP production was identical under aerobic and anaerobic conditions (17). Regen et al. (18) have reported recently that, after 15 min of anaerobic perfusion in the absence of glucose, the ATP content of rat hearts averaged 2.8 μmoles/g; in the presence of a 5.6 mM concentration of glucose, a comparable group of hearts averaged 13.3 μmoles of ATP/g. Aerobically perfused hearts averaged 17.7 μmoles/g in the absence of glucose and 17.9 μmoles/g in the presence of glucose. The anaerobic production of ATP via the glycolytic conversion of glucose to lactate would thus appear to provide an explanation of the increased ventricular performance and recovery observed in the present investigation.

Exposure of the isolated perfused rat heart to

anoxia was accompanied by suppression of sinus activity and atrioventricular conduction with persistence of a nodal or ventricular pacemaker before complete electrical arrest. A ventricular or nodal pacemaker was distinctly better preserved in the presence of glucose during anoxic exposure. In addition, the functional integrity of the ventricular conduction pathway was better maintained in the anoxic heart supported by glucose as reflected in the more complete restoration of the preanoxic ventricular depolarization complex during the recovery phase of the experiments. It would appear, therefore, that the sinus pacemaker and atrioventricular conduction in the rat heart were more exquisitely sensitive to the effects of anoxia than the ventricular pacemaker sites, and that anaerobic support of electrical activity is most effective at the ventricular level.

The most striking ultrastructural changes on exposure to anoxia in the absence of glucose were the marked morphologic changes in the mitochondria and the dilatation of the longitudinal tubules. It is notable that similar changes in mitochondria and longitudinal tubules have been observed in experimental myocardial ischemia (19–23). The mitochondria are the cellular sites of aerobic energy generation by oxidative phosphorylation. On the basis of studies on striated muscle it has been postulated that the longitudinal tubules are involved in the contraction and relaxation of the myofibrils, acting most probably through a calcium pump mechanism (24–26). The longitudinal tubules have also been demonstrated in striated muscle as sites of intracellular glycolytic dehydrogenases (27). Since the morphologic changes in the mitochondria and longitudinal tubules occurred while the accumulation of end products of anaerobic metabolism was minimized by continuous perfusion of the coronary bed, it is reasonable to conclude that these alterations were primarily related to lack of oxygen. Furthermore, since these alterations were prevented by the presence of glucose and its anaerobic conversion to lactate, it would appear that the primary mechanism for these changes was deficient energy production. Prevention of anoxic morphologic deterioration in the mitochondria and longitudinal tubules by the use of anaerobic glycolytic substrate occurred concomitantly with an enhanced recovery of electrical and mechanical performance of the isolated heart. This finding would suggest that the mitochondria or longitudinal tubules might be important intracellular loci in the irreversible lesion of anoxia.

In myocardial ischemic disease the cellular effects of anoxia are complicated by the additional influence of accumulated end products of anaerobic metabolism, since the perfusion defect is the primary mechanism of the disease. Under such circumstances myocardial glycogen in unperfused areas of myocardium is the major source of anaerobic energy. Glucose as an anaerobic substrate can potentially be provided to areas where myocardial perfusion persists or regenerates. In studies by Sodi-Pallares and coworkers (28) infusion of potassium and glucose in patients with ischemic heart disease diminished the injury potential in the electrocardiogram and stabilized cardiac arrhythmias. While such improvements were attributed primarily to the potassium content of this "polarizing mixture," the anaerobic support by the glucose should be considered at least as a contributing mechanism. It is of interest that older studies on the effects of glucose infusion in patients with congestive heart failure suggested beneficial clinical effects (29, 30). More recent studies demonstrate active anaerobic metabolism of the heart in patients with coronary heart disease (31, 32) and in individuals with heart failure (33). It is possible therefore that metabolic support by glucose may be beneficial in certain clinical circumstances. Further data are needed to clarify such potentially useful clinical effects of glucose.

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