Energy Levels at Systole vs. Diastole in Normal Hamster Hearts vs. Myopathic Hamster Hearts

Richard Sievers, William W. Parmley, Thomas James, and Joan Wikman-Coffelt

From the University of California, San Francisco Cardiovascular Research Institute, and Department of Medicine and Pharmaceutical Chemistry, San Francisco, California

SUMMARY. The following studies were carried out to examine energy metabolites and cardiac performance of the failing heart (hereditary cardiomyopathy) of the Syrian hamster (strain UM-X7.1) perfused either by normal or stress conditions, and to determine whether cyclical changes in energy-related metabolites occurred in the glucose-perfused hearts of both normal and heart failure animals. Hamster hearts from 250-day-old animals with moderate heart failure were removed and perfused either as nonworking hearts (Langendorff method, an afterload pressure of 90 mm Hg and 2.5 mM calcium in the perfusate) or as working hearts with stress conditions [an afterload of 110 mm Hg, high calcium concentrations in the perfusate (3.5 mM), and 10^{-8} M isoproterenol]. Mechanical parameters (developed pressure and max dP/dt) and measurements of oxygen consumption indicated that both contractility and oxygen consumption had fallen 50% in myopathic hearts, compared with those of normal hamsters perfused with either of the two conditions. By means of a specially designed stimulator-triggered freeze clamp, hearts were terminated at systole and diastole, and tissue content of ATP, ADP, AMP, adenosine, phosphocreatine, creatine, pyruvate, lactate, and inorganic phosphate were analyzed. A 50% reduction in cardiac performance of the cardiomyopathic hamster hearts was associated with a corresponding reduction in systolic ATP, adenosine, and phosphocreatine values, while inorganic phosphate and lactate increased. With glucose as the sole substrate, the high energy phosphates, ATP and phosphocreatine, reached maximum values during diastole and minimum values during systole. The phase alternate was observed with creatine, ADP, AMP, and inorganic phosphate. Cyclical changes were less obvious in the cardiomyopathic hearts due to less ATP utilization per cycle. A decrease in high energy phosphates, a low level in the free energy of ATP hydrolysis, augmented levels of lactate, increased inorganic phosphate, and a depressed purine nucleotide pool all of which occur concurrently, may cause the decrease in cardiac performance in the failing heart of the cardiomyopathic Syrian hamster. (*Circ Res 53: 759–766, 1983*)

THE hereditary cardiomyopathic strain of Syrian hamsters, designated UM X7.1, a derivative of the Bio 14.6 strain, has been used as a model of heart failure (Angelakos et al., 1972; Jasmin and Bajusz, 1973; Wrogeman et al., 1973; Ma and Bailey, 1979; Jasmin et al., 1981; Rouleau et al., 1982). Catecholamines may play a major role in the development of the disease. There is a marked increase in urinary norepinephrine during the development of cardiomyopathy (Kobara et al., 1976), and an increased number of norepinephrine binding sites (Karliner et al., 1981). In fact, this cardiomyopathy has been considered as an adrenergic cardiomyopathy by some investigators (Angelakos, 1972), expressing a pronounced sensitivity to epinephrine (Bajusz, 1969; Jasmin et al., 1981). Three general phases in the myopathy have been defined: (1) a necrotic state, (2) a healing stage with hypertrophy and dilation, and (3) a state of continuing dilation and terminal heart failure. Signs of heart failure become evident about the 7th month. Like other heart failure models, the later stages demonstate an imbalance of free calcium (Wrogemann et al., 1973; Jasmin and Bajusz, 1975; Ma and Bailey, 1979), a decrease in cAMP (Wikman-Coffelt et al., 1983b), a shift toward isomyosin V3 (Wikman-Coffelt et al., 1983b), and a decrease in velocity of muscle shortening, as well as peak force (Rouleau et al., 1982).

Since other heart failure models are characterized by a decrease in high energy phosphates (Wikman-Coffelt et al., 1979), a depressed nucleotide pool (Zimmer, 1983), and a decrease in the free energy of ATP hydrolysis (Kammermeier et al., 1982), energy metabolites were analyzed. Hearts were examined in an isolated perfused state during both normal and maximum work performance. Cyclical changes in ATP, PCr, and P_i have been shown to occur in the working heart perfused with glucose as the sole substrate, based on analyses with gated ^{31}P NMR (Fossel et al., 1980), and enzymatically (Krause et al., 1971; Wikman-Coffelt et al., 1983a). This may be due to the slow rate of glycolysis in the heart (Williamson, 1965) and the slow transport of NADH and pyruvate into the mitochondria (Kobayashi and Neely, 1979; Kobayashi and Neely, 1983). Thus, cyclical changes in energy-related metabolites were analyzed in glucose-perfused normal and myopathic hearts to determine possible differences in cyclical

changes. These oscillations may be indicators of rate limiting steps in metabolism during varying cardiac performance.

Methods

Isolated Working Heart and Stimulator-Triggered Rapid Freeze-Clamp

The working heart preparation and termination of metabolic processes at a predetermined phase of the cardiac cycle using the rapid freeze clamp have been described recently in detail (Wikman-Coffelt and Coffelt, 1982). The working heart preparation is a modification of that described by Neely et al. (1967). Innovations in design include counter-current heat exchangers, i.e., one Tygon tube carrying heated fluid surrounds a second tube carrying medium. Medium entered the left atrium and was pumped out the aorta. The medium was a modified Krebs-Henseleit solution containing: 117 mM NaCl, 4.3 mM KCl, 2.5 or 3.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.8 mM K_2HPO_4, 25 mM $NaHCO_3$, 15 mM glucose, and 0.6 mM Na EDTA, as well as 0.01% insulin; 10^{-8} M isoproterenol was added to the working heart studies. The medium was gassed with 95% O_2 and 5% CO_2. The afterload chamber was set at 110 mm Hg, and the preload chamber at 15 mm Hg (Wikman-Coffelt et al., in press). (For the *Langendorff preparation*, the afterload chamber was set at 90 mm Hg; the calcium concentration in the perfusate was 2.5 mM, no isoproterenol was present, and the heart was cannulated only through the aorta.) The isolated heart was perfused for 20 minutes, the preferable time based on preliminary studies (Wikman-Coffelt et al., 1983a, in press) for the perfused heart to reach a stable steady state, before freeze-clamping. The stimulator-triggered freeze clamp bolts on the face of the perfusion apparatus. Specific details of both systems have been described recently (Wikman-Coffelt and Coffelt, 1982; Wikman-Coffelt et al., in press), including measurement of temperature in the heart during "smashing." The center of the heart drops from 37°C to −80°C in 5 msec with the techniques described here. Freezing, for all practical purposes, was instantaneous. Cooling curves indicate the temperature of the heart falls 20,000°C/sec until the heart reaches −80°C or colder (Wikman-Coffelt and Coffelt, 1982). Cooling rate is asymptotic. It takes 23 msec for the heart to reach 90% steady state level of the heat sink. The measured rate of cooling coincides with the calculated rates. Thermal diffusivity in ice is 10^{-2}cm^2/sec. For a wafer 0.3 mm thick, the characteristic time for thermal diffusion—that is, the time for the relative temperature $[(T - T_0)/(T_\infty - T_0)]$ to reach 90% of the steady state level—is the following:

$$\alpha = \frac{(\beta/2)^2}{\delta} = \frac{(.015\ \text{cm})^2}{10^{-2}\ \text{cm}^2/\text{sec}} = 0.0225\ \text{sec}$$

where α = calculated rate of cooling (sec), β = change in tissue thickness (cm), and δ = thermal diffusivity of ice (cm^2/sec).

Indications are that it will take a 0.3 mm thick wafer of ice 22.5 msec to reach −176°C. Observed values give 23 msec (Wikman-Coffelt and Coffelt, 1982).

Physiological Measurements

Golden and Syrian hamsters were divided into two groups for physiological and biochemical analyses of systole vs. diastole. Hearts were terminated at two phases of the cardiac cycle, immediately prior to peak systole, max dF/dt and mid-diastole. The left ventricle was cannulated through the apex for pressure measurements, and pacing wires were inserted in the base of the right ventricle. Pressure recordings were obtained from the left ventricle and aorta with Statham pressure transducers. Oxygen consumption was measured by submerging the heart in a closed container of flowing medium and allowing the coronary flow to drain into the bathing medium, thereby allowing for analyses of arterial-venous differences as well as exchange of gases occurring at the surface of the heart (Wikman-Coffelt et al., 1983a). Details for these measurements have been described previously (Wikman-Coffelt et al., 1983a). Samples were aspirated from above the aorta, from the pump inflow, and from chamber outflow into an gas-tight syringe for determination of oxygen. Arterial and inflow oxygen were between 650 and 700 mm Hg. This high partial pressure was achieved by flushing the media vigorously with 95% O_2 and 5% CO_2 at a cooler temperature and then subsequently raising the temperature as described in earlier reports (Wikman-Coffelt et al., 1983a, in press). Rate of inflow into the chamber was determined by setting the pump speed at a defined rate (60 ml/min), and rate of outflow, by collecting samples in a volumetric flask. Coronary flow was determined by placing a catheter in the right ventricular outflow tract and collecting flow in a volumetric container.

Preparation of Tissue

Preparation of acid extracts were similar to those described by Morgan et al. (1980). The frozen wafers fell off the anvils into liquid nitrogen. A 100-mg sample of the pulverized material was removed, weighed, dried (110°C) and weighed again for assessing wet to dry weight. Three milliliters of frozen pulverized $HClO_4$ (10%) were added to the remaining frozen pulverized tissue. Pulverization under liquid nitrogen continued. The frozen mixture was transferred to a preweighed cold tube and then to a mortar. The weights of the cold tube and acid were used as factors in calculating the tissue weight (wet). The tissue and acid were pulverized under increasing temperature until the mixture thawed. After centrifugation of the mixture (20,000 *g* for 5 minutes), the extract was weighed, neutralized with 5 N KOH, and weighed again for volume determination. The mixture was centrifuged to remove $KClO_4$, and the supernatant stored at −80°C.

Assays

Using the neutralized extract, standard enzymatic assay methods were carried out to determine concentrations of ATP, ADP, phosphocreatine, creatine, pyruvate, and lactate (Bucher et al., 1955; Lamprecht and Trautschold, 1965; Lowry and Passoneau, 1972). Inorganic phosphate was determined as described earlier (Rouleau et al., 1982). Adenosine was analyzed by injecting 10 μl of extract onto a Beckman C18 reverse phase column using a 15-minute gradient of 0–20% methanol in water, 1 ml/min, followed by a 10-minute elution with 20% methanol, 1 ml/min. Detection was by UV absorption at 254 nm with an LDC spectomonitor III; the full scale on the recorder was set at 0 to 0.01 absorption unit for the high concentrations and 0 to 0.001 for the low concentrations. All data were expressed as mean values with standard deviations (±SD). Levels of significance were tested by analysis of variance. Differences were considered significant when $P < 0.05$.

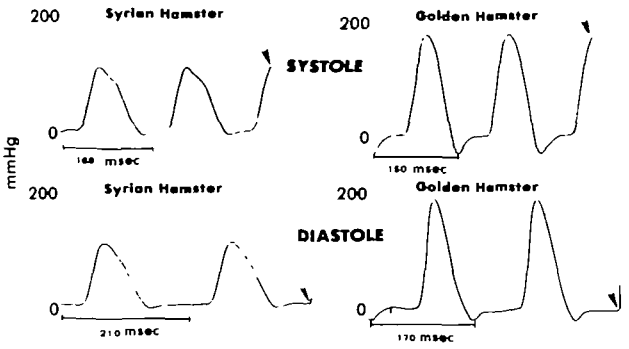

FIGURE 1. *Representative intraventricular (left) pressure tracings of myopathic hamster (Syrian) and normal hamster (Golden) hearts. Arrows indicate the time in the cardiac cycle where the heart was freeze clamped.*

Results

Physiological Studies

Intraventricular pressure tracings for representative myopathic hamster (Syrian) and normal hamster (Golden) hearts are shown in Figure 1. The arrows in the representative tracings indicate points in the cardiac cycle at which the heart was freeze-clamped. The representative tracings shown in Figure 1 demonstrate the lower cardiac performance and the increase in end-diastolic pressure in the myopathic Syrian as compared to the normal Golden hamster. There were no significant differences in the physiological parameters of the groups smashed at systole vs. diastole (Table 1). Significant differences ($P < 0.01$) were observed, however, in developed pressure, dP/dt, and oxygen consumption, but not coronary flow and heart rate between Syrian vs. Golden hamsters when the hearts were perfused either with the nonworking heart (Langendorff method) or the working heart preparation using stress conditions. Furthermore, physiological parameters, including developed pressure, dP/dt, heart rate, coronary flow, and oxygen consumption, were significantly different for the two groups of animals perfused by the working heart vs. the nonworking heart.

Cyclical Changes in Energy Metabolites

Biochemical analyses were carried out during two phases of the cardiac cycle, immediately prior to peak systole, max dF/dt, when the crossbridges were turning over rapidly, and during diastole, when there was minimal crossbridge turnover (Wikman-Coffelt et al., 1979). In the glucose-perfused hearts, ATP and PCr or ATP/ADP and PCr/Cr were significantly different between systole and diastole, (Table 2), whereas, inorganic phosphate, creatine, and ADP were lower during diastole (Table 2). Cyclical changes in high energy phosphates were less obvious in the Syrian hamster, where ATP utilization was diminished, as observed by the decrease in oxygen consumption.

A Comparison of Energy Metabolites with Hearts Perfused by the Langendorff Method vs. that of the Working Heart with Stress Conditions

As observed earlier for rat hearts, the ATP values for normal hamster hearts were significantly lower at systole ($P < 0.01$) with the working heart preparation, compared with those obtained by the Langendorff method, whereas lactate values were higher. Likewise, adenosine, an ATP precursor, was significantly depressed in the hearts perfused with

TABLE 1
Physiological Conditions during Termination of Metabolic Processes for Normal and Myopathic Hamster Hearts

Hamsters	Perfusion procedures	Developed pressure (mm Hg)	End diastolic pressure (mm Hg)	Heart rate (beats/min)	dP/dt (mm Hg/ sec × 10^3)	Coronary flow (ml/min per g wet wt)	Oxygen consumption (μmol/g dry wt per beat)
Control	Working heart						
	Systole	172 ± 13.9	5.1 ± 4.2	292 ± 15	7.93 ± 1.5	23.8 ± 2 1	0.48 ± 0.05
		NS	NS	NS	NS	NS	NS
	Diastole	169 ± 15.6	3.5 ± 2.2	285 ± 22	8.71 ± 0.9	24.6 ± 2.2	0.45 ± 0.04
	Langendorff						
	Systole	93 ± 5.0	3.0 ± 0.8	200 ± 10	4.31 ± 0.7	13 3 ± 1.3	0.26 ± 0 03
Myopathic	Working heart						
	Systole	97 ± 13.2	12.4 ± 3.5	282 ± 40	3.33 ± 1.0	23.7 ± 2.0	0.25 ± 0.05
		NS	NS	NS	NS	NS	NS
	Diastole	101 ± 12.2	14 3 ± 3.3	290 ± 32	3.10 ± 0.9	24.6 ± 1.2	0.21 ± 0.04
	Langendorff						
	Systole	48 ± 11.7	3.9 ± 1.9	200 ± 15	1.66 ± 0.8	12.5 ± 1.8	0.10 ± 0.04

Standard deviation shown $n = 6$ to 9. Working heart: 3.5 mM calcium in the perfusate, afterload pressure 110 mm Hg, and 5×10^{-9} M isoproterenol in the perfusate. Langendorff: 2.5 mM calcium in the perfusate, afterload 90 mm Hg, and no isoproterenol present.

TABLE 2
Energy Metabolites for Normal and Myopathic Hamster Hearts

Hamsters	Perfusion procedures	ATP (μmol/g dry wt)	Phosphocreatine (μmol/g dry wt)	Lactate (μmol/g dry wt)	Inorganic P_i (μmol/g dry wt)	Adenosine (nmol/g dry wt)
Control	Working heart					
	Diastole	27.3 ± 2.7	36.3 ± 3.0	5.4 ± 1.1	14 4 ± 3.0	—
	(*Systole vs diastole*)	$P < 0.05$	NS	NS	$P < 0.001$	
	Systole	24.4 ± 2 2	33.9 ± 4.5	5.8 ± 0.4	21.1 ± 4.5	18 ± 2
	(*Working heart vs. Lang.*)	$P < 0.01$	NS	$P < 0.01$	NS	$P < 0.001$
	Langendorff					
	Systole	28.1 ± 1.3	35.5 ± 3.7	4 4 ± 0.9	21.8 ± 2.9	138 ± 11
Myopathic	Working heart					
	Diastole	10 3 ± 1 6	17.9 ± 4 0	16.5 ± 3.5	32.1 ± 9 8	—
	(*Systole vs. diastole*)	NS	NS	NS	NS	
	Systole	11.2 ± 4 9	19.1 ± 2.3	17.0 ± 2.3	40 1 ± 8.7	8 ± 0.5
	(*Working heart vs. Lang.*)	NS	$P < 0.01$	$P < 0.01$	NS	$P < 0.001$
	Langendorff					
	Systole	12.5 ± 2.3	24 9 ± 4.7	10.0 ± 1.8	42.1 ± 6.6	87 ± 8
Control	Working heart					
	Diastole	32.4 ± 3.2	3.5 ± 0.5	1.2 ± 0.2	8 3 ± 1.4	1.17 ± 0.2
	(*Systole vs. diastole*)	NS	$P < 0.001$	NS	$P < 0$ 001	$P < 0.05$
	Systole	33.9 ± 4 5	5.1 ± 1.0	1.1 ± 0.2	4.8 ± 0.9	0 98 ± 0.2
	(*Working heart vs. Lang.*)	NS	NS	NS	$P < 0.01$	NS
	Langendorff					
	Systole	32.4 ± 3.2	4.3 ± 0.4	1.1 ± 0 3	6.5 ± 0.6	1 10 ± 0.2
Myopathic	Working heart					
	Diastole	23 9 ± 4.4	1.3 ± 0 5	2 2 ± 1.1	9.1 ± 4.0	0.82 ± 0.2
	(*Systole vs. diastole*)	$P < 0.05$	$P < 0.001$	NS	$P < 0.001$	$P < 0.05$
	Systole	29.3 ± 5.3	3.6 ± 0.5	2.4 ± 1.4	3.1 ± 0 8	0.62 ± 0.1
	(*Working heart vs Lang.*)	$P < 0.05$	NS	$P < 0.05$	NS	$P < 0$ 01
	Langendorff					
	Systole	22 8 ± 5.1	3.5 ± 1.1	1.4 ± 0.1	3.6 ± 0.2	1.04 ± 0.1

Working heart (n= 9); Langendorff (n = 6)

stress conditions, compared with Langendorff perfused hearts. For myopathic hearts, the oxygen consumption was 55% less with the Langendorff preparation and the nucleotide pool showed no significant change with reduced cardiac performance, whereas myocardial adenosine, a precursor for purine nucleotides, was severely depressed in the myopathic working hearts. There was, however, a significant increase in PCr ($P < 0.05$) and a significant decrease in lactate ($P < 0.01$) with reduced workload conditions in the myopathic hearts (Table 2).

Differences in Energy Metabolites between Normal and Myopathic Hearts

The high energy phosphate, PCr, and the nucleotides ATP and ADP, as well as adenosine, were significantly lower in the myopathic, compared with the normal hearts, whether they were perfused by the Langendorff method or the working heart. On the other hand, inorganic phosphate and lactate were significantly higher in the myopathic hearts (Table 2). The lactate:pyruvate ratios were also higher in the Syrian hamsters; the lactate:pyruvate values for the Golden hamster (diastole) were 4.0 ± 1.1 and for the Syrian hamster (diastole), 17.3 ± 3.2.

By means of the calculations of Giesen and Kammermeier (1980) and Kammermeier et al. (1982), corrections were made for compartmentalization and bound metabolites, as well as cytosolic water. Using these calculations and values we determined the differences in free energy of ATP hydrolysis between the Golden and Syrian hamsters, and beteen diastole and systole, by two different methods (Table 3). The $\Delta G/d\epsilon_{ATP}$, in both cases, was lower

TABLE 3

Metabolite Values during Systole vs. Diastole in the Cardiomyopathic Syrian Hamster vs. the Golden Hamster

Metabolites Tissue content—Measured value (μmol/g wet wt)		Animal	Cytosolic fraction Calculated value (mmol/liter)	
ATP	3.66	Golden hamster	8.4	Systole
	4.10		9.5	Diastole
	1.68	Syrian hamster	3.5	Systole
	1.55		3.2	Diastole
ADP	0.77	Golden hamster	0.98	Systole
	0.53		0.03	Diastole
	0.54	Syrian hamster	0.90	Systole
	0.20		0.05	Diastole
P_i	3.17	Golden hamster	3.1	Systole‡
	2.16		0.8	Diastole
	6.02	Syrian hamster	9.6	Systole
	4.82		6.8	Diastole
PCr	5.09	Golden hamster	12.7	Systole
	5.45		13.6	Diastole
	2.87	Syrian hamster	7.2	Systole
	2.69		6.7	Diastole
Cr	5.09	Golden hamster	12.7	Systole
	4.83		12.1	Diastole
	4.40	Syrian hamster	11.0	Systole
	3.59		9.0	Diastole
$\frac{\Delta G}{\Delta \epsilon_{ATP}} = \Delta G^{\circ}_{ATP} + RT \ln \frac{(ADP)(P_i)}{(ATP)}$			kJ/mol	
		Golden hamster	50.8	Systole
			63.8	Diastole
		Syrian hamster	45.7	Systole
			54.3	Diastole
$^{*}\frac{\Delta G}{\Delta \epsilon_{ATP}} = \Delta G^{\circ}_{obs(ATP)} + RT \ln \frac{(Cr)(P_i)}{(PCr)(H^+)(K_{CK})}$ † Assumption for diastole: $(ADP)_{free} = \frac{1}{K_{CK}} \frac{(ATP)(Cr)}{(PCr)(H^+)}$				
		Golden hamster	61.2	Diastole
		Syrian hamster	54.4	Diastole

Calculations for cytosolic fractions are taken from Kammermeier et al. (1982). Dry weight values are corrected for the measured 15% dry weight fraction according to our experimental conditions. Values are for nine animals each in the four groups.

Calculations for cytosolic ATP:

$$\frac{\mu\text{mol ATP/g wet weight} - 0.3 \times 5}{2} = \text{mmol/liter cytosolic fraction}$$

5/2 results from a previously calculated cytosolic water content of 40% wet weight (Kammermeier et al., 1982); 0.3 represents the amount compartmentalized and bound (Kammermeier et al., 1982)

Calculations for ADP:

$$\frac{(\mu\text{mol ADP/g wet weight}) - (\text{compartmentalized} + \text{bound}) \times 5}{2}$$

Compartmentalized: 0.35 for Golden hamster and 0.17 for Syrian hamster [based on percent of ADP extracted (Kammermeier et al., 1982)]. Bound: 0.03 (systole), 0.15 (diastole), for Golden hamster; 0.015 (systole), 0.075 (diastole), for Syrian hamster (based on myosin-ADP binding). Thirty percent of the dry weight is myosin, giving 0.18 mmol myosin/liter; 15% myosin ADP binding during systole and 85% during diastole (Nishiki et al., 1978).

Calculations for cytosolic P_i:

$$\frac{(\mu\text{mol/g wet weight})(0.08 \times \mu\text{mol } P_i\text{/g wet wt}) - 1.7 \times 5}{2}$$

0.08 represents 8% bound (Kammermeier et al., 1982); 1.7 is the value for mitochondria P_i (1.3 μmol/g) and extracellular P_i (0.4 μmol/g).

Calculations for PCr and Cr:

$$\frac{(\mu\text{mol/g wet weight}) \times 5}{2}$$

* $\Delta G^{\circ}_{obs(ATP)} = -30.5$ kJ/mol [for 37°C (1 mM Mg^{++})].

† Taken from the creatine kinase equilibrium, since it is at equilibrium during diastole.

‡Calculated assuming ΔpH-dependent P_i distribution across the mitochondrial matrix membrane for ΔpH = 1.0 (Kammermeier et al., 1982).

for the Syrian hamster and lower during systole, compared to the contrast group.

Discussion

When the myopathic Syrian hamster hearts were subjected to either normal or stress conditions during perfusion, they were unable to develop pressures comparable to those of normal hamsters. This depressed cardiac performance was reflected in diminished oxygen consumption. The oxygen consumed per beat was less (approximately one-half) in the Syrian hamster, compared with that of the normal hamster. However, peak systolic pressure and dP/dt were also depressed in the myopathic heart; thus, less oxygen was required for the work performed. With less oxygen required, the myopathic heart extracted less oxygen from a high coronary flow when stressed, rather than reducing coronary flow to meet its demands. There was no significant difference in coronary flow between myopathic and normal hamster hearts, although there was a reduction in the nucleotide pool in the failing hearts. As observed earlier with normal rat hearts (Wikman-Coffelt et al., in press), both normal and myopathic hamster hearts demonstrated coronary autoregulation. Both reduced coronary flow when workload was decreased.

After freeze-clamping and enzymatic analyses of metabolites at max dP/dt and diastole, results indicated that the high energy phosphates, ATP and PCr, were critically depressed during both phases of the cardiac cycle in the Syrian hamster, irrespective of perfusion conditions. The opposite was noted with inorganic phosphate, a product of ATP hydrolysis. The values here were twice as high as in normal hamster hearts. The high lactate:pyruvate ratio and the high inorganic phosphate observed in the Syrian hamster were indicative of a shift in metabolism. A second product of ATP hydrolysis, i.e., ADP, was not augmented—instead, it too was depressed. Due to the influence of ADP on the creatine kinase equilibrium (Lawson and Veech, 1979; McGilvery and Murray, 1974), the low ADP value may have prevented exhaustion of phosphocreatine. The low ADP level helped to partially preserve the free energy of ATP hydrolysis, and indicated a general overall deficiency in the nucleotide pool as observed in other heart failure models (Zimmer, 1983). Whether ADP is calculated from the creatine kinase equilibrium or from direct measurements, including assumptions for compartmentalization and binding, based on published values as described by Kammermeier et al. (1982), the free energy of ATP hydrolysis is considerably lower in hearts of the Syrian hamster, compared with normal hearts. In other studies in which there is a correlation between an oxygen deficiency and a decrease in myocardial function, there is also a corresponding decrease in the free energy of ATP hydrolysis, giving calculated free energy values similar to those reported here for the cardiomyopathic Syrian hamster (Kammermeier et al., 1982; Giesen and Kammermeier, 1980).

Stress conditions were chosen for forcing the heart into maximum energy utilization in order to test the heart's energy reserve, i.e., its ability to replenish the ATP pool. Isoproterenol was used, since it has been shown to cause excess utilization of energy during the resting phase of the cardiac cycle (Gibbs and Gibson, 1972), whereas high calcium and a high afterload were used because they forced the heart into maximal energy utilization during contraction (Neely et al., 1967; Kobayashi and Neely, 1979; Wikman-Coffelt et al., 1983a; 1983b). Under these conditions, adenosine, an ATP precursor, was decreased significantly. Under stress conditions, the myopathic hearts did not develop pressures greater than 120 mm Hg, and the nucleotide pool showed little change. This may have been due to the difference in coronary flow which occurred under the two different perfusion conditions. Based on oxygen consumption measurements (assuming a ~P/O ratio of 3), there was an increased ATP utilization of 0.6 μmol/g dry wt per beat with stress conditions, compared with values obtained with the Langendorff method. This small increase in ATP utilization did, however, cause a decrease in the PCr content and an increase in lactate in the myopathic hearts. This may have been due to the small nucleotide pool and/or the inability for oxidative phosphorylation to keep pace with myocardial demands. As observed here, stress conditions during acute isolated heart perfusion caused a decrease in adenosine. Chronic catecholamine stress which may occur throughout the life of the Syrian hamster (Kobara et al., 1976; Jasmin et al., 1981; Karliner et al., 1981), also induced a depressed nucleotide pool, as well as lower adenosine values, as shown here when the heart was perfused by the Langendorff method. Recovery from a depressed nucleotide pool is slow if not irreversible (Swain et al., 1982). Thus, in myopathic hearts stressed both acutely in vitro and chronically in vivo, adenosine values were markedly low.

Cyclical changes in ATP, PCr, and Pi have been shown to occur in the working rat heart with glucose as the substrate based on analyses made with gated P31 NMR (Fossel et al., 1980) and enzymatically (Krause et al., 1971; Wikman-Coffelt et al., 1983a). This may be due to the slow transport of NADH into the mitochondria (Kobayashi and Neely, 1979) and the slow rate of glycolysis (Williamson, 1965), which cannot keep pace with the energy requirements of a stressed heart. A depressed rate of glycolysis in the glucose-perfused working heart may be due to rate-limiting steps in the metabolic breakdown of sugar, namely, phosphofructokinase (Uyeda and Racker 1965; Oguchi et al., 1980) and the feed-back inhibition of NADH on glyceraldehyde-3-phosphate dehydrogenase (Kobayashi and Neely, 1979; Oguchi et al., 1980), resulting in a slow delivery of pyruvate to the mitochondria (Kobayashi

and Neely, 1983). All working hearts showed marked differences in the ATP:ADP ratios between systole and diastole. These changes are important for demonstrating that the rate of glycolysis may not be fast enough to keep up with energy demands of a stressed heart, or glycolysis may be dependent on the calcium influx following depolarization (Clark et al., 1982). Cyclical changes in high energy phosphates occur in the glucose-perfused heart but not in hearts perfused with pyruvate (Wikman-Coffelt et al., 1983a). In the Syrian hamster, where cyclical changes were small due to a decrease in ATP utilization, and the standard deviation large because of variability in advancement of heart failure, oscillations in energy metabolites were less obvious. These data are important to demonstrate that ATP utilization is reduced during heart failure, and a shift in metabolites is occurring.

Although a decrease in ATP + PCr may not cause a corresponding decrease in muscle mechanics and cardiac performance (Cooke and Bialek, 1979; Donaldson et al., 1981; Giesen and Kammermeier, 1980; Kammermeier et al., 1982; Nishiki et al., 1978), nevertheless, the end products of ATP hydrolysis, the overall depressed nucleotide pool, and their interrelated effects on metabolism, may be effective. Thus, the decrease in contractility and oxygen consumption observed in this heart failure model may be due to interrelated metabolic events working concurrently: a depressed nucleotide pool, a decrease in high energy phosphates, augmented levels of lactate and inorganic phosphate, and a depression in the free energy of ATP hydrolysis. The depressed energy reserve, however, does not appear to be due to a lack in coronary reserve.

We wish to thank Parris Kidd for helping to develop our colony of myopathic Syrian Hamsters, and Robert J. Coffelt for designing and building the perfusion apparatus and freeze-clamp used in these studies.

This work was supported in part by Susan and Don Schleischer

Address for reprints: Joan Wikman-Coffelt, Ph.D., Moffitt Hospital, Rm M1186, University of California, San Francisco, San Francisco, California 94143.

Received February 18, 1983; accepted for publication September 26, 1983.

References

Angelakos ET, Carballo LC, Daniels JB, King MP, Bajusz E (1972) Adrenergic neurohumors in the heart of hamsters with hereditary myopathy during cardiac hypertrophy and failure. Recent Adv Stud Cardiac Struct Metab **1:** 262–278

Bajusz E (1969) Hereditary cardiomyopathy: A new disease model. Am Heart J **77:** 686–696

Bergmeyer HU (1974) Methods of Enzymatic Analysis, edited by HU Bergmeyer. New York, Academic Press, pp 1196–1201, 1404–1464

Bucher T, Czok R, Lamprecht W, Latzko E (1955) *In* Methods of Enzymology, vol 1, edited by SP Colowick, NO Kaplan. New York, Academic Press, pp 253–259

Clark MG, Patten GS, Filsell OH (1982) Evidence for an α-receptor mediated control of energy production in the heart. J Mol Cell Cardiol **14:** 313–321

Cooke R, Bialek W (1979) Contraction of glycerinated muscle fibers as a function of the ATP concentration. Biophys J **28:** 241–258

Donaldson SKB, Bond E, Seeger L, Niles N, Bolles A (1981) Intracellular pH vs Mg^{2+} ATP concentration: Relative importance as determinants of Ca^{2+} activated force generation of disrupted rabbit cardiac cells. Cardiovasc Res **15:** 268–275

Fossel ET, Morgan HE, Ingwall J (1980) Measurement of changes in high energy phosphates in the cardiac cycle by using gated P^{31} nuclear magnetic resonance. Proc Natl Acad Sci USA **77:** 3654–3658

Gibbs CL, Gibson WR (1972) Isoprenaline, propranolol, and the energy output of rabbit cardiac muscle. Cardiovasc Res **6:** 508–515

Giesen J, Kammermeier H (1980) Relationship of phosphorylation and oxygen consumption in isolated perfused rat hearts. J Mol Cell Cardiol **12:** 891–907

Jasmin G, Bajusz E (1973) Polymyopathic et cardiomyopathie herediaire chez le hamster de dyrie. Inhibition selective des lesions du myocarde. Ann Anat Pathol **18:** 49–66

Jasmin G, Bajusz E (1975) Selective prevention by verapamil and other drugs of the hamster hereditary cardiomyopathy. *In* Recent Advances in Myology, edited by WS Bradley, D Gardner-Medium, JN Walton. New York, American Elsevier, pp 413–417

Jasmin G, Proschek L, Cailloux MF (1981) Congestive cardiomyopathy in the Syrian hamster: Possible role of catecholamines in its pathogenesis. *In* Congestive Cardiomyopathy, edited by JF Goodwin, A Hjalmarson, EGJ Olsen. Sweden, AB Hassle, pp 113–120

Kammermeier H, Schmidt P, Jungling E (1982) Free energy change of ATP hydrolysis: A causal factor of early hypoxic failure of the myocardium. J Mol Cell Cardiol **14:** 267–277

Karliner JS, Alabaster C, Stephens H, Barnes B, Dollery C (1981) Enhanced noradrenaline response in cardiomyopathic hamsters: Possible relation to changes in adrenoceptors studied by radioligand binding, Cardiovasc Res **15:** 296–304

Kobara JJ, Riggins RM, Kissinger PT (1976) Abnormal levels of urinary catecholamines in dystrophic mice and hamsters. Proc Soc Exp Biol Med **151:** 168–172

Kobayashi K, Neely JR (1979) Control of maximum rates of glycolysis in rat cardiac muscle. Circ Res **44:** 166–175

Kobayashi K, Neely JR (1983) Mechanism of pyruvate dehydrogenase activation by increased cardiac work. J Mol Cell Cardiol **15:** 369–382

Kobayashi K, Fossel E, Ingwall J (1982) Analysis of creatine kinase reaction in the isolated perfused rat heart using P^{31} NMR saturation transfer (abstr) Biophys J **37:** 123A

Krause E, Bergerdorfer I, Wollenberger A, Bogdanova EV, Babskii EB (1971) Changes in different products of glucose in the frog myocardium during different phases of the cardiac cycle. Dokl Akad Nauk SSSR **199:** 1211–1215

Lamprecht WI, Trautschold I (1965) Determination with hexokinase and glucose-6-phosphate dehydrogenase. Methods of Enzymatic Analysis, vol 1, edited by HU Bergmeyer. New York, Academic Press, pp 543–551

Lawson JW, Veech RL (1979) Effect on pH and free Mg^{2+} on the K_{eq} of creatinine kinase reaction and other phosphate hydrolyses and phosphate transfer reactions. J Biol Chem **254:** 6528–6537

Lowry OH, Passoneau JV (1972) Methods of Enzymatic Analysis, edited by HU Bergmeyer. New York, Academic Press, pp 276–279

Ma TS, Bailey LE (1979) Excitation-contraction coupling in normal and myopathic hamster hearts, I. Identification of a calcium pool involved in contraction. Cardiovasc Res **13:** 499–505

McGilvery RW, Murray TW (1974) Calculated equilibria of phosphocreatine and adenosine phosphates during utilization of high energy phosphate by muscle. J Biol Chem **249:** 5845–5850

Morgan HE, Chua BHL, Fuller EO, Siehl D, (1980) Regulation of protein synthesis and degradation during in vitro cardiac work. Am J Physiol **212:** 804–814

Neely JR, Liebermeister H, Battersby EJ, Morgan HE (1967) Effect of pressure development on oxygen consumption by isolated

rat heart. Am J Physiol **212:** 804–814
Nishiki K, Erecinka M, Wilson DF (1978) Energy relationships between cytosolic metabolism and mitochondrial respiration in rat heart. Am J Physiol Cell Physiol 3: C73–C81
Oguchi M, Gerth E, Fitzgerald B, Park JH (1980) Regulation of glyceraldehyde-3-phosphate dehydrogenase by phosphocreatine and ATP. J Biol Chem **248:** 5571–5576
Rouleau JL, Chuck LHS, Hollosi G, Kidd P, Sievers R, Wikman-Coffelt J, Parmley WW (1982) Verapamil preserves myocardial contractility in the hereditary cardiomyopathy of the Syrian hamster. Circ Res **59:** 405–412
Swain JL, Sabina RL, McHale P, Greenfield JC, Holmes EW (1982) Prolonged myocardial nucleotide depletion after brief ischemia in the open chest dog. Am J Physiol **242:** H818–H826
Uyeda K, Racker E (1965) Coordinated stimulation of hexokinase and phosphofructokinase by phosphate in a reconstituted system of glycolysis. *In* Control of Energy Metabolism, edited by B Chance, RW Estabrook, JR Williamson. New York Academic Press, pp 127–137
Wikman-Coffelt J, Coffelt RJ (1982) A stimulator-regulated rapid freeze clamp for terminating metabolic processes of the heart dring normal physiological working conditions. IEEE Trans Biomed Engr **29:** 448–453
Wikman-Coffelt J, Parmley WW, Mason DT (1979) The cardiac hypertrophy process: Analyses of factors determining pathological vs. physiological development. Circ Res **45:** 697–707
Wikman-Coffelt J, Sievers R, Coffelt RJ, Parmley WW (1983a) The cardiac cycle: Regulation and energy oscillations. Am J Physiol **245:** H354–H362
Wikman-Coffelt J, Sievers R, Coffelt RJ, Parmley WW, (1983b) Biochemical and mechanical correlates at peak systole in myopathic Syrian hamster. *In* Cardiac Adaptation to Hemodynamic Overload, Training, and Stress, edited by R Jacob, RW Bulch, G Kissling. Darmstadt, Steinkopff Verlag, pp 197–203
Wikman-Coffelt Sievers R, Coffelt RJ, Parmley WW (in press) A new apex-ejecting perfused rat heart preparation: Relation between coronary flow and loading conditions. Cardiovasc Res
Williamson JR (1965) Metabolism control in the perfused rat heart. *In* Control of Energy Metabolism, edited by B Chance, RW Estabrook, JR Williamson. New York, Academic Press, pp 353–355
Wrogemann K, Jacobson BE, Blanchaer MC (1973) On the mechanism of a calcium-associated defect of oxidative phosphorylation in progressive muscular dystrophy. Arch Biochem Biophys **159:** 267–278
Zimmer HG (1983) Normalization of depressed heart function in rats by ribose. Science **220:** 81–82

INDEX TERMS: Syrian hamster • Myopathy • Heart failure • Energy levels