Evaluation of the Hereditary Syrian Hamster Cardiomyopathy by ^{31}P Nuclear Magnetic Resonance Spectroscopy: Improvement After Acute Verapamil Therapy

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The relation between metabolic and functional derangement in various cardiomyopathies has not been well characterized. This information was specifically sought in a spontaneous cardiomyopathic model. Metabolic and hemodynamic parameters were obtained in glucose-perfused beating hearts of 180–200-day-old cardiomyopathic Syrian hamsters and age-matched healthy animals. This period in the cardiomyopathic hamster lifetime is intermediary between the necrotic phase and the appearance of heart failure. We used ^{31}P nuclear magnetic resonance spectroscopy to analyze energy metabolites and intracellular pH.

Cardiomyopathic hamsters had significantly higher mole fraction values for inorganic phosphate, lower phosphocreatine mole fraction as well as lower phosphocreatine/inorganic phosphate and adenosine triphosphate/inorganic phosphate ratios. Analysis of pH indicated the presence of regions of increased acidity within the heart of myopathic hamsters. Cardiomyopathic hamsters also had significantly lower left ventricular pressure, coronary flow, and myocardial oxygen consumption.

Separate groups of normal and myopathic hamsters were given verapamil for 24 hours (one injection of 4 mg/kg s.c. followed by 1.2 g/l in drinking water). Verapamil-treated myopathic hamsters had evidence of markedly improved mitochondrial function when compared with untreated animals. Left ventricular pressure and coronary flow rose to normal levels. Replacing glucose by pyruvate in the perfusate of myopathic hamsters results in a marked increase in left ventricular pressure, coronary flow, and oxygen consumption with a moderate rise in phosphocreatine.

Thus, 180–200-day-old cardiomyopathic hamster heart is characterized by evidence of decreased mitochondrial function, by areas of increased acidity within the heart, and by reduced left ventricular function. Verapamil administered for as short a period as 24 hours restored left ventricular pressure, oxygen consumption, and coronary flow to normal values whereas the mole fraction of phosphocreatine and the phosphocreatine/inorganic phosphate ratio rose markedly though remaining lower than in healthy animals. Comparison of data using glucose vs. pyruvate as a substrate indicates that glycolysis is impaired in the heart of cardiomyopathic hamsters. Energy production can be improved by short-circuiting limiting steps in the glycolytic pathway. (*Circulation Research* 1986;59:597–604)

NUCLEAR magnetic resonance (NMR) spectroscopy provides a technique to study *in vivo* myocardial metabolism including high-energy phosphorus compounds.[1,2] The relation of these compounds to myocardial dysfunction in cardiomyopathies is unknown. Nuclear magnetic resonance spectroscopy also permits assessment of changes occurring in response to therapeutic interventions.

The hereditary cardiomyopathic strain of Syrian hamsters, designated UM-X7.1, a derivative of the Bio 14.6 strain,[3] has been used as a model of cardiomyopathy and heart failure.[4–8] The earliest biochemical and morphologic evidence of cardiomyopathy usually appear by 30 days of age.[3,7,9] The cardiac damage is progressive in nature and all affected hamsters die prematurely from cardiac failure at about 250 days of age.[7,8,10] Multiple abnormalities in cellular function have been identified, including defects in the mitochondria, sarcoplasmic reticulum, myofibrils, and sarcolemma. Cardiac performance and myocardial oxygen consumption are markedly reduced.[8,11,12] However, the primary defect(s) starting the cascade of events leading to heart failure and early death has not been identified.

The myocardium of cardiomyopathic hamsters show evidence of calcium overload and it is believed that an imbalance of free calcium plays an important role in the pathogenicity of the disease.[4,5,10,13] Compatible with this theory is the observation that verapamil, a drug with slow calcium channel antagonist properties, is very effective in slowing the progression of the disease and in reducing its severity.[4,5,10,14–16] Neither the duration of verapamil therapy required to improve cel-

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lular and contractile function nor the mechanism of action in preventing myocardial damage are known.

In this study, we evaluated 180–200-day-old cardiomyopathic hamsters. This period in the lifetime of the animals is intermediary between the necrotic phase (ending at about 150 days) and the appearance of heart failure (beginning at about 200 days) and has not been well characterized. Hemodynamic parameters were monitored simultaneously with concentrations of energy-rich phosphorus compounds and intracellular pH (pHi) employing ^{31}P NMR.

The purposes of the study were threefold: to study the functional and metabolic status of 180–200-day-old cardiomyopathic hamsters (i.e., at a stage of cardiac dilatation and incipient heart failure) by ^{31}P NMR spectroscopy; to establish whether short-term administration of verapamil (24 hours) would be sufficient to improve cellular function and hemodynamic performance; and to evaluate whether impaired glycolysis in the isolated, perfused cardiomyopathic hamster heart is one of the factors causing impaired contractile and metabolic function. For this purpose we compared the effect of perfusing the heart with glucose versus pyruvate on ^{31}P NMR spectra.

Materials and Methods

Animals

Syrian hamsters of the UM-X7.1 strain between 180–200 days of age were employed as experimental animals. Age-matched healthy hamsters served as control. Body and heart weight were obtained at the time of death. The hamsters were maintained on normal laboratory diet and drank water *ad libitum*. Twenty-four hours prior to the experiment, the normal and cardiomyopathic hamsters groups were each divided into 2 subgroups. The first subgroup continued to drink normal water whereas verapamil (1.2 g/l) mixed with honey was added to the drinking water of the second group. Animals treated with verapamil also received a single injection of verapamil (4.0 mg/kg s.c.), given 24 hours prior to the experiment. The verapamil-spiked water was not withdrawn prior to death.

Isolated Perfused Heart Studies

The isolated perfused beating heart was perfused by the Langendorff method[12] with a perfusion pressure of 110 mm Hg. The perfusate contained the following (in mM): 117 NaCl, 4.3 KCl, 2.4 $MgCl_2$, 0.1 K_2HPO_4, 25 $NaHCO_3$, 3.5 $CaCl_2$, 0.5 NaEDTA, and 100 units/l of insulin, supplemented with either 15 mM glucose or 10 mM pyruvate. The medium was mixed with 95% O_2 and 5% CO_2. Perfusate temperature was maintained at 35° using counter-current heat exchangers[17] and a thermostat-regulated circulating water bath. Pacing leads designed to prevent noise artifact were inserted at the base of the right ventricle and connected to a Medtronic model 5320 pulse generator for pacing of the heart at a constant rate of 200 beats/min. A cannula was inserted through the left atrium and mitral valve into the left ventricular chamber, then sutured into place. The cannula exiting from the NMR magnet bore was connected to a pressure transducer for left ventricular pressure measurements and recorded on a 4-channel Beckman dynograph.

Arterial and venous oxygen samples were taken before and after placing the heart in the magnet. "Arterial" samples were aspirated from the aortic chamber and "venous" samples were drawn from a catheter introduced into the right ventricular outflow tract for oxygen measurements (Corning model gas analyzer). Coronary flow was measured by collecting the effluent from the right ventricular outflow tract in a volumetric container. Coronary flow was continuously siphoned from the NMR tube while spectra were being taken. Myocardial oxygen consumption was calculated as indicated earlier.[18] The hearts were perfused for 20 minutes with the perfusate including glucose before starting collection of data. This allowed the beating heart to reach steady state with the perfusate.[18] Following accumulation of data the perfusate was switched to a pyruvate-containing substrate (without glucose). Data were again recorded following a 20-minute equilibration period.

^{31}P NMR Spectroscopy

^{31}P NMR spectra of the beating isolated perfused heart were obtained on a 5.6 Tesla, vertical 76-mm bore magnet. The home-built spectrometer was connected to a 1180 Nicolet computer, a pulse programmer, and a high-resolution 20-mm broad band probe. Undecoupled ^{31}P spectra were obtained at 97.3 MHz. Pulse angle was 75°, recycle time 2.25 seconds, and spectral width ± 4000 Hz. Zero ppm was assigned to the resonance position of phosphocreatine (PCr). Transients were accumulated for 20 minutes. For each spectrum the characteristic peaks of inorganic phosphate (Pi), PCr, and phosphate groups of adenosine triphosphate (ATP) were identified[1,2] (Figure 1). The area of each peak was integrated and expressed as "mole fraction" by dividing the integrated values for Pi, PCr and ATP by the sum of the integrated values for all three peaks. The mole fraction does not give absolute values and is not normalized for tissue weight. The ATP/Pi and PCr/Pi ratios are also given. Intracellular pH was initially estimated from the chemical shift of the pH-dependent peak of Pi relative to the peak of PCr.[1,2] The Pi spectrum of cardiomyopathic animals usually showed the presence of one or more smaller peaks at more acidic pH, in addition to one major resonance. To account for these areas of increased acidity we estimated values of pHi in an additional manner. The spectral region containing the Pi and PCr peaks was first expanded. The Pi peak was then divided into 6 equal intervals, beginning at 5 ppm, with 3 equal intervals on either side. Each interval was then integrated and the value expressed as percent of distribution of the Pi peak.

Statistical Analysis

Levene's test of homogeneity of variance was performed on the various sub-groups in each case. When the test was significant, the Kruskal-Wallis multiple

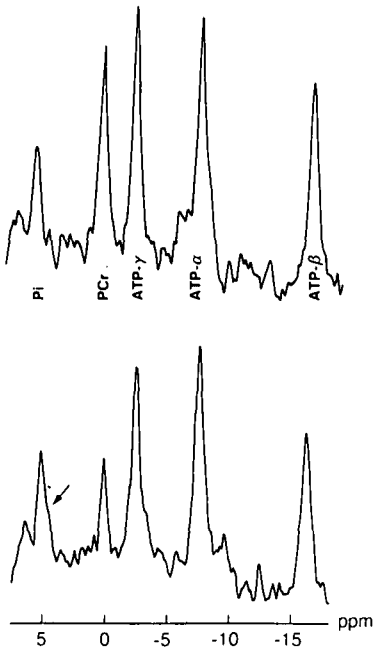

FIGURE 1. *^{31}P nuclear magnetic resonance (NMR) spectra.* Upper panel: *Spectra obtained from a normal hamster heart showing the resonances of inorganic phosphate (Pi), phosphocreatine (PCr), and the three phosphorus nuclei of adenosine triphosphate (ATP). The peak of PCr resonance is assigned the zero part per million (ppm) value. Note the symmetric distribution of the Pi peak and the prominent PCr resonance.* Lower panel: *^{31}P NMR spectra from a cardiomyopathic hamster heart showing a shoulder (arrow) to the right to the peak (i.e., in an area of lower pH). The Pi peak is larger and the PCr peak smaller than in normal animals.*

comparison procedure was used to compare the different sub-group means. Otherwise, the means were compared using analysis of variance and the Newman-Keuls test. The differential effect of glucose vs. pyruvate in the same animals was analyzed using Student's paired *t* test.

Results

Normal Versus Cardiomyopathic Hamsters

Cardiomyopathic hamsters ($n=8$) had a significantly higher mole fraction values of Pi (30.5 ± 7.0 vs. $17.4\pm4.5\%$, $p < 0.05$), lower PCr (21.5 ± 4.3 vs. $38.9\pm4.4\%$, $p < 0.01$) but similar ATP (47.9 ± 5.2 vs. $43.6\pm2.8\%$, NS) (Figure 2, Table 1). Their PCr/Pi ($p < 0.01$) and ATP/Pi ratios ($p < 0.01$) were significantly lower than in normal animals ($n=6$). Cardiomyopathic hamsters had significantly lower left ventricular pressures (46.5 ± 14.5 vs. 175.2 ± 25.0 mm Hg, $p < 0.01$), lower coronary flow (8.9 ± 7.9 vs. 16.9 ± 4.2 ml/min/g wet wt, $p < 0.05$) and lower myocardial oxygen consumption (0.05 ± 0.02 vs. 0.2 ± 0.05 μmol/g dry wt/beat, $p < 0.05$). The mean pHi value calculated from the major Pi peak resonance was lower in the cardiomyopathic group (7.11 ± 0.08 vs. 7.18 ± 0.03), but the difference did not reach statistical significance ($0.05 < p < 0.10$). When distribution of pHi was analyzed using the 6 intervals (described in "Materials and Methods") (Figure 3, top), the third section (corresponding to a chemical shift of 5.0–5.3 ppm, i.e., pHi of about 7.35–7.13) comprised a significantly smaller fraction of the Pi spectrum in the cardiomyopathic hamster ($p < 0.05$). Conversely, the fifth section (corresponding to a chemical shift of 4.4–4.7 ppm, i.e., a pHi of about 6.90–6.62) included a significantly larger fraction of the Pi spectrum in the cardiomyopathic animals when compared to the normal hamsters ($p < 0.01$). The heart weight/total weight $\times 10^{-3}$ ratio was significantly higher in cardiomyopathic than in normal hamsters (5.3 ± 0.7 vs. 3.4 ± 0.5, $p < 0.01$).

Influence of Verapamil on Glucose- and Pyruvate-perfused Normal Hamsters

Verapamil-treated normal hamsters ($n=6$) had a significantly lower mean ATP/Pi ratio ($p < 0.05$) and lower PCr/Pi ratio ($p < 0.01$) when compared to untreated animals. No other significant difference between the two groups was noted (Figure 4, Table 1). There was no significant difference between the verapamil-treated and untreated normal hamsters, pyruvate-perfused ($n=5$) (Figure 4, Table 1).

Influence of Verapamil on Glucose- and Pyruvate-perfused Cardiomyopathic Hamsters

In comparison to untreated cardiomyopathic hamsters, verapamil-treated cardiomyopathic hamsters ($n=10$) had a significantly lower Pi mole fraction (20.8 ± 2.2 vs. $30.5\pm7.0\%$, $p < 0.05$), higher PCr mole fraction (27.9 ± 5.5 vs. $21.5\pm4.3\%$, $p < 0.01$), higher PCr/Pi ratio (1.37 ± 0.36 vs. 0.75 ± 0.27, $p < 0.05$), higher ATP/Pi ratio (2.49 ± 0.35 vs. 1.67 ± 0.51, $p < 0.01$), higher developed left ventricular pressure (154.6 ± 20.2 vs. 46.5 ± 14.5 mm Hg, $p < 0.01$), higher coronary flow (18.0 ± 4.5 vs. 8.9 ± 7.9 ml/min/g wet wt, $p < 0.01$) and higher myocardial oxygen consumption (0.19 ± 0.09 vs. 0.05 ± 0.02 μmol/g dry wt/beat, $p < 0.05$) (Figure 4, Table 1). Mean pHi value calculated from the major

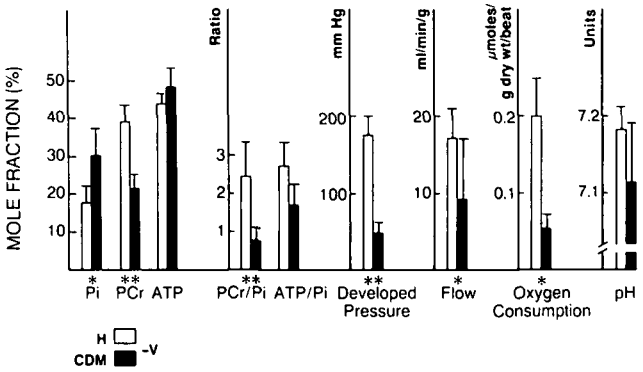

FIGURE 2. *Comparison of ^{31}P NMR spectroscopy and hemodynamic findings in normal (n=6) vs cardiomyopathic hamsters (n=8). Values are given as mean ± one SD. CDM = cardiomyopathic; H = healthy; −V = no verapamil; Flow = coronary flow.*

Table 1. Metabolic and Hemodynamic Parameters in Normal and Cardiomyopathic Hamsters

	Pi (%)	PCr (%)	ATP (%)	PCr/Pi	ATP/Pi	Flow (ml/min/g)
Normal hamsters						
Glucose	17.4±4.5	38.9±4.4	43.6±2.8	2.4±0.88	2.67±0.77	16.9±4.2
Pyruvate	18.5±6.2	39.4±6.8	42.1±1.5	2.49±1.36	2.52±0.93	16.8±3.2
Verapamil/glucose	23.6±3.7	33.6±2.8	42.8±3.3	1.46±0.26	1.86±0.36	16.5±3.4
Verapamil/pyruvate	21.8±3.3	36.6±3.1	41.6±4.0	1.71±0.31	1.96±0.38	17.8±2.1
Cardiomyopathic hamsters						
Glucose	30.5±7.0	21.5±4.3	47.9±5.2	0.75±0.27	1.67±0.51	8.9±7.9
Pyruvate	23.2±3.1	27.5±2.2	50.7±5.1	1.19±0.16	2.17±0.45	14.4±3.6
Verapamil/glucose	20.8±2.2	27.9±5.5	51.3±4.8	1.37±0.36	2.49±0.35	18.0±4.5
Verapamil/pyruvate	22.2±1.9	26.4±4.3	51.3±4.4	1.19±0.23	2.33±0.32	14.9±3.3

Pi = inorganic phosphate; PCr = phosphocreatine; ATP = adenosine triphosphate; % = mole fraction; Flow = coronary flow; MVO_2 = myocardial oxygen consumption; LV pressure = developed left ventricular pressure.
Values are given as mean ± 1 SD.

phosphate peak was similar for the two groups (7.12±0.05 vs. 7.11±0.08, NS). When pHi distribution was analyzed at the 6 specified intervals (Figure 3), the third interval (i.e., pHi 7.35–7.13) comprised a significantly larger portion of the Pi spectrum in the treated compared with untreated cardiomyopathic animals ($p < 0.05$). Conversely, the fifth interval (i.e., pHi 6.90–6.62) comprised a significantly smaller portion of the Pi spectrum in treated animals ($p < 0.01$). This shift in Pi distribution was associated with the presence of a single non-Lorentzian peak in treated animals. There was no other significant difference concerning the other parameters.

No animal in either group was in heart failure as evidenced by the lack of ascitic fluid in the abdominal cavity at the time of death.

Verapamil-treated cardiomyopathic hamsters showed no significant hemodynamic difference from untreated normal animals. They did manifest a significantly lower PCr ($p < 0.05$), lower PCr/Pi ($p < 0.01$) and significantly higher levels of ATP ($p < 0.05$) compared to the normal animals. In pyruvate-perfused cardiomyopathic hamsters there was no significant difference between the group receiving and the group not receiving verapamil (Table 1).

Influence of Pyruvate Versus Glucose

Since only about two-thirds of the glucose-perfused animals were subsequently perfused with pyruvate, the data reported below (using Student's paired *t* test) are slightly different from the data used to assess verapamil effect on the various subgroups (using one way analysis of variance or Kruskal-Wallis test) (Figure 5, Table 1).

Pyruvate Versus Glucose Substrate in the Normal Hamster

No significant change in any variable was noted between normal hamsters perfused with pyruvate versus glucose (Figure 5, Table 1).

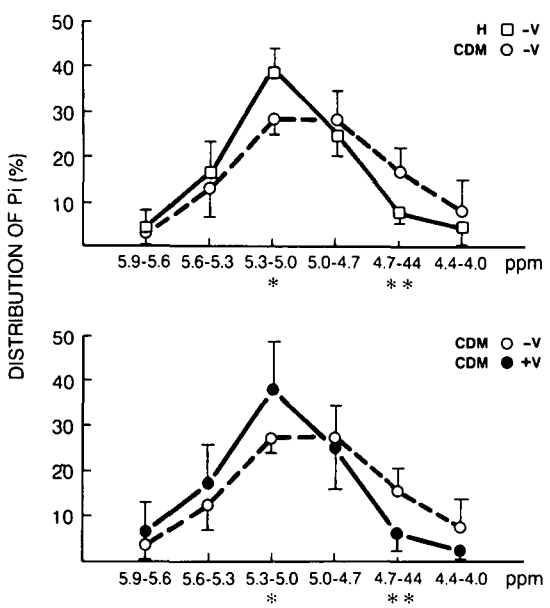

FIGURE 3. *Distribution of Pi according to intracellular pH.* Upper panel: *Comparison between healthy and cardiomyopathic hamsters.* Lower panel: *Comparison between cardiomyopathic hamster, untreated (−V) or treated (+V) with verapamil. ppm = part per million.*

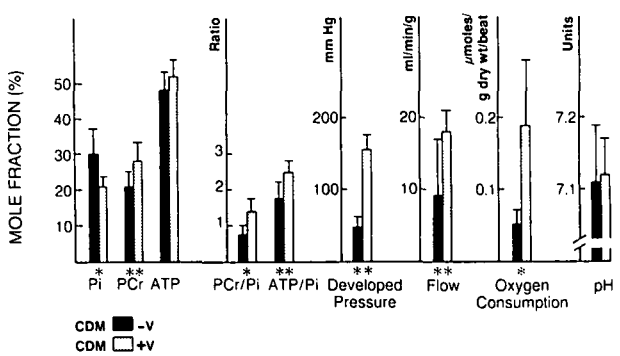

FIGURE 4. *Comparison of ^{31}P NMR spectra and hemodynamic data in untreated (n=8) and verapamil-treated cardiomyopathic hamsters (n=10).*

Table 1. ***(Continued)***

MVO_2 (μmol/g dry wt/beat)	LV pressure (mm Hg)	pH
0.20±0.05	175.2±25.0	7.18±0.03
0.27±0.06	167.8±26.8	7.16±0.02
0.21±0.04	167.0±20.7	7.17±0.06
0.29±0.05	168.8±20.9	7.15±0.03
0.05±0.02	46.5±14.5	7.11±0.08
0.22±0.02	125.0±20.0	7.08±0.05
0.19±0.09	154.6±20.2	7.12±0.05
0.22±0.03	144.2±8.7	7.13±0.06

Pyruvate Versus Glucose in Verapamil-treated Normal Hamsters

The only difference noted was a significantly higher myocardial oxygen consumption in verapamil-treated healthy hamsters when perfused with glucose versus pyruvate ($p < 0.05$) (Figure 5, Table 1).

Pyruvate Versus Glucose in the Cardiomyopathic Hamster

Pyruvate-perfused hamsters had significantly higher PCr (27.5±2.2 vs. 22.8±4.0%, $p < 0.05$), higher developed left ventricular pressures (125.0±20.0 vs. 49.5±6.6 mm Hg, $p < 0.01$), higher coronary flow (14.4±3.6 vs. 6.3±1.3 ml/min/g wet wt, $p < 0.05$) and higher myocardial oxygen consumption (0.22±0.02 vs. 0.05±0.02 μmol/g dry wt/beat, $p < 0.01$) (Figure 5, Table 1). No other significant difference between the examined variables was noted. When compared with pyruvate-perfused normal hamsters, cardiomyopathic animals perfused with pyruvate as a substrate had significantly lower left ventricular pressure ($p < 0.05$) and lower PCr ($p < 0.01$) but otherwise were not different.

Pyruvate Versus Glucose Substrate in Verapamil-treated Cardiomyopathic Hamsters

There was no significant difference between verapamil-treated cardiomyopathic hamsters when perfused with glucose vs. pyruvate (Figure 5, Table 1).

Discussion

^{31}P NMR spectroscopy is a new technique used with increasing frequency for the analysis of heart disease in experimental animals and humans.[1,2,19] An advantage of NMR includes the ability to provide noninvasive sequential information on important phosphorus metabolites and on intracellular pH in the beating heart.

In the first part of this study we have characterized the ^{31}P NMR spectra of isolated glucose-perfused hearts of 180–200 day old cardiomyopathic hamsters. This age period is intermediary between the necrotic stage (peaking at about 100 days) and the florid heart failure stage (evident at 250 days of age).[3,5,7,14] Cardiomyopathic hamster hearts belonging to our age group still show some degree of myolysis. However, marked fibrosis and hypertrophy of the myocardium with dilatation of the cavities and scar calcification are the prominent pathologic features.[13,14] None of our cardiomyopathic animals had evidence of overt congestive heart failure.

Hearts of glucose-perfused cardiomyopathic hamsters were characterized by a marked disturbance in phosphate metabolism as evidenced by an increased Pi mole fraction, a reduced PCr mole fraction, and reduced PCr/Pi and ATP/Pi ratios. A reduced PCr/Pi ratio indicates depressed mitochondrial function.[20] Left ventricular developed pressure and myocardial oxygen consumption were significantly diminished indicating that the energy requirements of cardiomyopathic hearts were reduced. Hence the ^{31}P NMR spectroscopy findings are evidence that the hearts of 180–200 day old cardiomyopathic hamsters were unable to provide appropriate amounts of PCr despite a reduced demand for energy. On the other hand, the decrease in PCr lowers the energy potential and may thereby have caused a reduction in oxygen consumption. The decrease in oxygen consumption indicated a depressed rate of ATP synthesis. The ATP/Pi ratio was reduced in myopathic hamsters with the increased Pi fraction occuring at the expense of the PCr fraction. The normal ATP mole fraction indicated that mitochondrial function, though decreased, provided the energy necessary to preserve ATP levels at a depressed utilization rate. Though the ATP mole fraction as measured by ^{31}P NMR remained normal, this measurement was not normalized for dry weight. Furthermore, we measured only the relative amount of ATP vs. the total free phosphorus detectable by NMR.[1,2]

Other studies of cardiomyopathic hamsters using conventional biochemical techniques have shown that total inorganic phosphate may be reduced in the cardiomyopathic hamster heart[21] but elevated during the heart failure stage.[8] In the presence of a low free phosphorus pool, the ATP fraction might remain normal despite a reduction in absolute ATP levels. Our findings on ATP levels are compatible with reports on cellular function in cardiomyopathic hamsters. Mitochondria of hamsters studied in the intermediary stage,

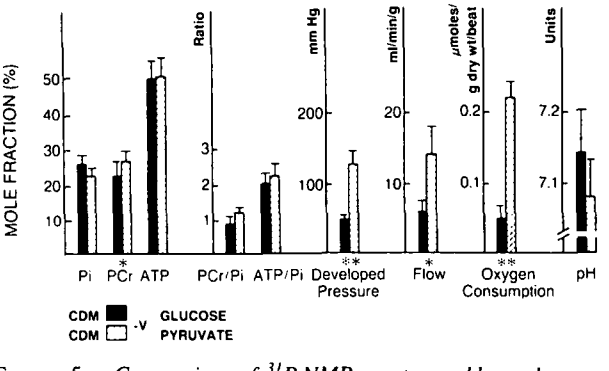

FIGURE 5. *Comparison of ^{31}P NMR spectra and hemodynamic data in glucose vs. pyruvate perfused cardiomyopathic hamsters* (n=5).

i.e., after the necrotic stage but before the appearance of overt heart failure, show only a mild defect in function.[13] Previous studies in myopathic hamsters using conventional biochemical techniques have uniformly shown a reduction in PCr but variable ATP levels, depending on the stage of the disease and the experimental conditions.[12,21,22] Studies of other models of myocardial damage, e.g., during a period of global ischemia in the perfused rat heart, have shown that PCr is lost first whereas ATP levels are not diminished until the PCr level has been decreased by about 80%.[23-25]

Intracellular pH is an important parameter of cellular function. The pH is usually calculated from the chemical shift of the main peak of the Pi resonance.[1,2] When calculated in this manner, the pHi of our cardiomyopathic hamster hearts was slightly lower than the pH of healthy animals, with the difference reaching nearly statistical significance. The Pi spectrum of untreated, glucose-perfused cardiomyopathic hamsters showed an asymmetric distribution, with one or more shoulders shifted towards the PCr peak. Further analysis of the Pi resonance confirmed the visual impression that significantly more Pi was located in areas of reduced pHi. The presence of more than one peak in the Pi resonance reflect either compartmentation of H^+ within the cell or H^+ inhomogeneity in the heart.[23,26] Additional Pi resonance peaks in more acidic pHi areas have been recognized previously. Thus two Pi resonance peaks were noted in the ^{31}P NMR spectra of the perfused, beating rabbit heart made regionally ischemic.[23] This was attributed to signals from tissue orthophosphate at different pHi values in normal and ischemic tissue.[23] Small Pi peaks at acid pH were also noted after graded global ischemia and reperfusion of isolated perfused rat hearts and were attributed to localized areas of tissue necrosis.[23,24] Cellular compartmentation of pHi cannot be assessed by present techniques and cannot be ruled out as the cause for the occurrence of this finding. We feel, however, that tissue inhomogeneity provides a more plausible explanation for the presence of more than one Pi resonance (overlapping), because the muscle lesion in the cardiomyopathic hamster is focal in nature.[7,14] Heterogeneity in pHi would reflect the coexistence of areas of severe muscle damage (with lower pHi) and areas of preserved myocardium (with normal pHi). The exact mechanism for the increased concentration of intracellular hydrogen ions in the glucose-perfused cardiomyopathic hamster heart is unclear. Irrespective of its cause, acidosis probably contributed adversely to the altered cellular metabolism and cardiac performace noted in our animals.[27,28]

Thus, the 180–200-day-old isolated, glucose-perfused cardiomyopathic hamster heart is evidently characterized by a decrease in mitochondrial function which is demonstrated by a depressed myocardial oxygen consumption and a decrease in PCr/Pi and ATP/Pi ratios. It is also characterized by areas of increased acidity within the heart and by reduced left ventricular pressure. The reduction in coronary flow and myocardial oxygen consumption parallels the reduced left ventricular function.

The primary genetic defect causing the Syrian hamster cardiomyopathy has not been identified. The earliest detected biochemical abnormality has been in the sarcolemma: a depression in Na^+, K^+-ATPase activity noted at 25–35 days of age.[9] Evidence of mitochondrial dysfunction is seen at the beginning of the necrotic phase (50 days) and has been related to calcium overload.[10,13,29] Increased intracellular calcium is a prominent feature of the disease and probably contributes importantly to cellular damage and to the appearance of acidosis.[10,29,30] The sequence of events leading to the loss of calcium homeostasis noted in cardiomyopathic hamsters and in other types of heart disease is controversial. Calcium overload could result from primary sarcolemmal defect(s) or from a reduction in energy production by the cell leading to loss of calcium homeostasis.[10,30,31]

In the second part of this study, we evaluated the effect of verapamil. Treatment of cardiomyopathic hamsters with verapamil for 24 hours prior to the experiment significantly improved cellular metabolism. Pi fell whereas the PCr fraction and the PCr/Pi ratio rose, indicating an improvement in mitochondrial function.[20] Furthermore, there was a shift in the Pi resonance asymmetry so that less phosphate was located in areas of low pH. Administration of verapamil also increased left ventricular pressure and myocardial oxygen consumption with appropriate increase in coronary flow. Thus, under the conditions of our experiment, a 24-hour period of therapy with verapamil was sufficient to improve markedly mitochondrial function and to restore the developed left ventricular pressure of cardiomyopathic hamsters to normal values.

Administration of verapamil to 21–30-day-old cardiomyopathic hamsters for periods of 1–10 months prevents the appearance of necrotic lesions, preserves myocardial contractility and mitochondrial function and prolongs life expectancy.[10,15,16] Furthermore, verapamil is effective in improving ventricular function and in preserving the adenine nucleotide pool when administered as a water additive to 180–240-day-old myopathic hamsters with overt congestive heart failure.[8] Beside its protective effect on the cardiomyopathic hamster heart, verapamil protects the myocardium in other experimental conditions, including anoxic injury[32-34] and reperfusion of the ischemic myocardium.[32,35] Conversely, the capacity to lessen myocardial damage in cardiomyopathic hamsters is not unique to verapamil or to the chemically related D-600. Other drugs with totally different pharmacologic properties such as propranolol, low dose of isoproterenol, or dimethyl-prostaglandin E_2 have also been shown to protect the hearts of the cardiomyopathic hamsters.[10,15,36]

The mechanism by which verapamil improves cardiac metabolism, slows the progression of myocardial damage, and prevents early death in the myopathic hamster has been extensively investigated but is not fully elucidated. It was initially theorized that verapamil might reduce the extent of myocardial damage through its slow calcium channel blocking proper-

ties.[10,37,38] Verapamil acts primarily to reduce calcium entry into the cell and therefore prevents the manifestations of intracellular calcium overloading. This may not be the mechanism of action by which verapamil improves cardiac function in the myopathic heart for the following reasons: 1) Calcium overload of the cell is complex and is not totally dependent on the entry of calcium through the slow channels. Calcium entry occurs in large part by exchange for sodium.[38] 2) Accumulation of calcium might occur as a result of impaired energy production by the mitochondria so that the cell is unable to maintain the calcium gradient across the membrane.[30] Verapamil would prevent calcium overload not by directly preventing calcium entry into the cell but by improving cellular metabolism by an undefined mechanism, thereby allowing the cell to unload calcium into the extracellular space. 3) In addition to its inhibitory effect on the slow inward current, verapamil affects the sarcolemma of normal and ischemic hearts. Thus pretreatment with verapamil reduced the loss in Na^+, K^+-ATPase, 5'-nucleotidase, and Na^+–Ca^{2+} exchange rate that occurs during ischemia.[31] Calcium channel antagonists can also inhibit other membrane-mediated events, including ionic currents and receptor processes.[37] 4) Slow calcium channel antagonists having a different molecular structure than verapamil or D-600, such as nifedipine and diltiazem, do not prevent the appearance and progression of the cardiomyopathy.[10] Thus, though verapamil prevents cellular calcium overload, the mechanism for this action is not known and may not be directly related to the slow calcium channel antagonist properties of the drug.

The mechanism for verapamil-related improvement in cellular metabolism is also unclear. Verapamil improves cellular function in a variety of experimental conditions causing injury to the heart.[31–34] Many authors have concluded that verapamil protects the heart from anoxic or postreperfusion injury mainly by reducing contraction strength in the affected area, thereby conserving high-energy phosphate compounds for the cell.[32–34] This proposed mechanism of action of verapamil is not acceptable in our experimental preparation since the administration of verapamil caused an increase in developed left ventricular pressure and in myocardial oxygen consumption. Protective mechanisms not related to reduced mechanical work following verapamil administration have been postulated but are not well defined.[31,34,39] In isolated heart preparations, effects on the peripheral circulation may not be the site of action of verapamil. A primary vasodilator effect on the coronary circulation[40] cannot be excluded but appears less likely for the following reasons. 1) The protective effect of verapamil can be demonstrated in the absence of measurable change in coronary flow during the heart failure stage.[8] 2) The other calcium entry blockers, nifedipine and diltiazem, which are also vasodilators, did not protect against myocardial damage.[10] Also, hydralazine, a potent vasodilator drug, has equivocal effects on myocardial contractility and did not decrease myocardial damage when administered chronically to 21 day old cardiomyopathic hamsters.[16] 3) In other experimental models of heart disease, verapamil protected the myocardium without influencing coronary flow to the affected area.[32,33,41] On the other hand, cardiac performance may be dependent on coronary flow in the studies described here. A decrease in the energy potential and/or an imbalance in the calcium flux may cause the vascular microspasms associated with this model.[40] An increase in the energy potential when the heart is perfused with pyruvate or pretreated with verapamil[8,28] may help establish a normal calcium flux, decrease coronary resistance and microspasms, thus increase coronary flow, stretch the myofibrils, and increase developed pressure.

Whatever its mechanism of action, verapamil administered for as little as 24 hours restored left ventricular pressure, myocardial oxygen consumption, coronary flow, Pi, and ATP/Pi to values similar to those recorded in normal animals. The PCr and PCr/Pi values rose significantly following verapamil administration even though ATP utilization (based on 6 moles ATP $\approx$ 1 mole O_2) increased from 75 to 300 micromoles ATP/g dry wt/min, indicating a large increase in mitochondrial activity.

In the last part of this study we evaluated the status of glycolysis in the isolated perfused cardiomyopathic heart. Glycolysis is slow in the perfused heart and more so in the presence of acidosis.[18,28,42] Therefore a reduced delivery of pyruvate to the mitochondria results. Using pyruvate as a substrate should by-pass limiting steps in the glycolytic pathway and improve the energy state of the heart.[18,28] Indeed, the replacement of glucose substrate by pyruvate was associated with a marked improvement in mechanical performance of the heart and an improvement in mitochondrial function as observed by the increase in PCr and myocardial oxygen consumption. (The rate of glycolysis is especially slow in the perfused heart when glucose is the sole substrate; this may not be a critical factor *in situ*.)

These findings indicate that glycolysis is impaired in the heart of glucose-perfused cardiomyopathic hamsters. Production of energy by oxidation of fatty acids and acetate is also depressed[43] and cannot substitute for the reduced glycolytic rate. Energy production can be improved, at least temporarily, by short-circuiting limiting steps in the glycolytic pathway.

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