Acknowledgments
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References

Myocardial LDH Isozyme Distribution in the Ischemic and Hypoxic Heart
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SUMMARY Small myocardial specimens were obtained from 12 patients undergoing coronary reconstructive surgery and from 12 patients undergoing surgical correction for cyanotic congenital heart defects. The specimens were analyzed for LDH isozyme distribution. A control analysis was performed on myocardial specimens obtained at the time of surgical correction for acyanotic congenital heart defects in seven patients with normal coronary arteries. There was a 42% increase in the proportion of A subunits in the hearts of coronary patients as compared to controls. This represented a shift toward an anaerobic isozyme distribution. There was no change in the percentage of A units from the hearts of cyanotic patients as compared to acyanotic hearts of the same age.

Cardiac muscle from patients with coronary vascular disease had an altered LDH subunit composition. Such an alteration was not present with chronic systemic hypoxia. These deficiencies may or may not be related to differing local metabolic responses to the two conditions. However, in the clinical situations, ischemic heart muscle may be oxygen deprived to the point of lactic acid production while hypoxic heart muscle usually is not. Consequently, these findings may represent a compensatory cellular mechanism which provides for continued energy production during chronic ischemia by enhancing glycolysis.

NORMALLY, ENERGY DELIVERY IN HEART MUSCLE proceeds by aerobic metabolism. However, when the heart is required to work under clinical conditions of chronic oxygen deprivation, we have observed that satisfactory or even excellent cardiac contractions are often main-

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degree of reproducibility;1 2) by working with ratios, the problems of complete tissue extraction inherent in absolute values were eliminated; and 3) the analysis could be easily performed on the small pieces of tissue usually available.

Lactate dehydrogenase is distributed in virtually all vertebrate cells in five isoenzymic forms. The physiochemical analysis of LDH shows that it is a tetramer composed of four equal sized subunits. These tetramers can be dissociated into the A + B (or M + H) polypeptides. Combinations of these two monomeric subunits into the tetramers generates the standard LDH patterns of isozymes: 2

LDH-1  LDH-2  LDH-3  LDH-4  LDH-5
A4B0  A3B1  A2B2  A1B3  A0B4

Tissues relying primarily on aerobic metabolism such as the heart have a preponderance of LDH-1 or B subunits whereas cells which have a great capacity for anaerobic metabolism, such as skeletal muscle, have predominantly LDH-5 or A subunits (fig. 1). Since the relative amounts of these isozymes is a function of their rates of synthesis and degradation, it is conceivable that protracted cellular hypoxia could induce shifts in the relative concentrations of these isozymes so as to facilitate anaerobic metabolism.

Method

In this report, the definition for chronic was a duration of reduced oxygen supply sufficient to produce either a local or systemic compensatory response. In the case of ischemia, this was seen as intercoronary collateral development and in hypoxia as an increased hematocrit.

To study ischemia, small pieces of muscle which had been excluded by cardiotomy sutures in the antero-apical area of the left ventricle were examined in 12 patients undergoing aorto-coronary artery saphenous vein bypass grafting. The samples were taken immediately after going on bypass and before the aorta had been cross clamped. Care was taken to avoid the inclusion of fat or fibrous tissue in the sample. The definition of ischemic heart disease, for the purpose of this report, was radiographically demonstrable occlusions of 90% or greater in the proximal portions of all three major coronary arteries and angina refractory to medical management. All patients had normally contracting ventricles and no patient was in congestive heart failure. The hearts were of normal size as judged by preoperative chest X-ray and observation in the operating room. At the time of surgery, no evidence of scar formation could be found in any heart.

To study hypoxia, specimens of myocardium were obtained from 12 patients varying in age from two days to six years. The specimens were removed from the right ventricular outflow tract at the time of corrective surgery for cyanotic tetralogy of Fallot and from multiple areas of the ventricular myocardium immediately after death following attempted correction or palliation of other cyanotic congenital heart defects. The definition of cyanosis, for the purposes of this study, was an arterial pO2 of less than 60 mm Hg. To serve as controls for both groups, myocardial specimens, removed from the outflow tract of the right or left ventricle, were examined in seven patients ranging in age from four weeks to 52 years who underwent surgical correction for acyanotic tetralogy of Fallot, pulmonic stenosis, and idiopathic hypertrophic subaortic stenosis. In the control group, the pO2 was in the normal range and there was no demonstrable coronary artery disease.

The samples were used immediately or stored at −60°C for a maximum of a week. During this time, as determined by a separate control experiment, there were no changes in either absolute amount of enzyme or isozyme distribution. The specimens were homogenized in 0.1M phosphate buffer at pH 7.0 and the soluble proteins separated by centrifugation at 34,000 × g. Aliquots of the supernatant were studied by electrophoresis on cellulose acetate membranes in a Beckman Microzone Cell Model R-101, using a Tris-borate-EDTA buffer system at pH 8.6 for 30 minutes at 350 volts. Following electrophoresis the membranes were stained for LDH at 22°C for 20 minutes by a tetrazoil staining procedure. 4 The membranes were fixed, cleared, and dried. Quantitative measurements of each isozyme were then made by spectrophotometric scanning of the stained isozymes. The optical density peaks were used to determine the percentage contribution of each LDH isozyme to the total activity in the sample. Knowing the molecular composition of each isozyme, the total contribution of each isozyme was calculated. Due to the small amounts of tissue which were usually available, total enzyme concentrations were not measured.

Results

The percentage of each subunit, A or B, for the ischemic and the control group of patients age five to 52 years, is presented in table 1. Means are expressed ± the standard error of the mean (SEM) and the significance of difference between the means calculated by Student’s t-test. The mean percentage of A monomers in the total LDH sample for control hearts was 11.8% (SEM ± 0.72) while the percentage for ischemic hearts was 16.8% (SEM ± 1.25) (P < 0.05). This
represented a mean increase of 42% in A subunits in ischemic heart tissue when compared to controls. Figure 2 shows a typical example of the comparison between optical scanograms of ischemic and control hearts. As acetate strips do not photograph well, a comparison zymogram between an ischemic and normal, fresh postmortem heart was run on starch gel and is shown in figure 3.

The percentages of A + B subunits for hypoxic hearts are shown in table 2. This data is similarly presented in graphic form in figure 4 and shows the rapid decrease in percentage of A units within the first year of life. Table 3 shows the percentage of A + B subunits in hypoxic hearts as compared with A + B subunits of nonhypoxic hearts of similar age groups. Again, within the first month, the percentage of A units in both groups is high. At two months, the percentage of A units of both groups has started to decrease and at five years the percentage of A units of both groups is less than 12%. Table 4 lists the percentage of each A + B subunit for hypoxic hearts at 12 months or older in comparison with control hearts one year of age or older. Again, the average percentage of A monomers for the controls was 11.8% (SEM ± 0.72) while the percentage for the hypoxic hearts was 12.8% (SEM ± 1.17). There was no significant difference between these two groups by Student's t-test.

**TABLE 1. The Percentage of A and B Subunits of LDH in Control Hearts over One Year of Age and Ischemic Hearts**

<table>
<thead>
<tr>
<th>Patient</th>
<th>%A Control group</th>
<th>%B Control group</th>
<th>Patient</th>
<th>%A Ischemic group</th>
<th>%B Ischemic group</th>
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</thead>
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<tr>
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<td>90.4</td>
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<td>2</td>
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<td>2</td>
<td>18.5</td>
<td>81.5</td>
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<td>12.3</td>
<td>87.7</td>
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<td>17.4</td>
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<td>87.2</td>
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</tr>
<tr>
<td>7</td>
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<td>7</td>
<td>18.0</td>
<td>82.0</td>
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<tr>
<td>8</td>
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<td>86.6</td>
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<tr>
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<td>73.0</td>
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<td>10</td>
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<td>84.4</td>
<td></td>
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</tbody>
</table>

**TABLE 2. The Percentage of A and B Subunits of LDH in Hypoxic Children's Hearts in Relation to Age**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>pO2</th>
<th>%A</th>
<th>%B</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>39</td>
<td>43.4</td>
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<td>2 d</td>
<td>41</td>
<td>40.4</td>
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<tr>
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<td>3 d</td>
<td>25</td>
<td>33.5</td>
<td>66.5</td>
</tr>
<tr>
<td>4</td>
<td>3 w</td>
<td>45</td>
<td>24.4</td>
<td>75.6</td>
</tr>
<tr>
<td>5</td>
<td>2 m</td>
<td>28</td>
<td>19.2</td>
<td>80.8</td>
</tr>
<tr>
<td>6</td>
<td>1 y</td>
<td>42</td>
<td>10.1</td>
<td>89.9</td>
</tr>
<tr>
<td>7</td>
<td>4 y</td>
<td>36</td>
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<td>88.8</td>
</tr>
<tr>
<td>8</td>
<td>5 y</td>
<td>40</td>
<td>17.8</td>
<td>82.2</td>
</tr>
<tr>
<td>9</td>
<td>5 y</td>
<td>57</td>
<td>15.3</td>
<td>84.7</td>
</tr>
<tr>
<td>10</td>
<td>5 y</td>
<td>50</td>
<td>9.1</td>
<td>90.9</td>
</tr>
<tr>
<td>11</td>
<td>5 y</td>
<td>42</td>
<td>11.9</td>
<td>88.1</td>
</tr>
<tr>
<td>12</td>
<td>6 y</td>
<td>47</td>
<td>14.3</td>
<td>85.7</td>
</tr>
</tbody>
</table>

Abbreviations: d = days; w = weeks; m = months; y = years.

**FIGURE 2. Optical scan showing ischemic heart LDH pattern at top and control heart LDH pattern below.**
surgically precluded hydroxyproline and microscopic analysis. To help minimize this possibility, care was taken to study tissue only from normally contracting hearts and to study only that tissue which appeared grossly normal. Provided obvious areas of scar tissue are excluded, evidence indicates that the heart muscle-to-collagen ratio remains unchanged regardless of ischemia, hypertrophy, age or sex. Histologic studies of diseased and normal hearts also indicate that the muscle fiber to capillary ratio remains at 1:1 regardless of age or pathology. Accordingly, it was felt that, under the restrictions set by clinical studies, the specimens examined portrayed as accurate a picture as possible of the LDH composition of heart muscle cells. Regarding similarity of the LDH profile of different portions of the ventricle, our analysis of muscle specimens removed from the normal, fresh postmortem human heart demonstrate that LDH profiles of portions of the right ventricle, left ventricle, and interventricular septum are indistinguishable. An example of this analysis is presented in figure 5. The percentage of A units from these hearts was 12.9%. There is no statistical difference between this previously reported study and the control group of this series.

During anaerobic glycolysis, the conversion of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate requires the removal of hydrogen ions from glyceraldehyde-3-phosphate. These hydrogen ions are attached to the coenzyme nicotinamide adenine dinucleotide (NAD) and so converts oxidized NAD to reduced NADH. In order for glycolysis to proceed in the absence of oxygen, NADH must itself be oxidized so that a fresh supply of NAD is again free to accept hydrogen ions from glyceraldehyde-3-phosphate. This occurs at the last step of the glycolytic pathway, i.e., the conversion of pyruvate to lactate. This reaction, which is mediated by LDH, delivers the hydrogen ions on NADH to pyruvic acid with the formation of lactic acid and oxidized NAD (fig. 6). The purpose of lactic acid, therefore, is to provide a temporary reservoir for storage of hydrogen ions until such a time that oxygen again becomes available. The purpose of LDH is to preside over this reservoir, carefully regulating the formation and accumulation of lactic acid so that a continued supply of energy is assured, yet in a way that maintains pH within the narrow limits of cell tolerance. Since the energy requirements and pH tolerances of various cells differ, depending upon their function, so do their LDH isozyme patterns. For example, skeletal muscle and liver can tolerate a relatively acidic

**Table 3** The Percentage of A and B Subunits of Normally Oxygenated and Hypoxic Children's Hearts of Similar Age Groups

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>pO₂</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3 w</td>
<td>45</td>
<td>24.4</td>
<td>75.6</td>
</tr>
<tr>
<td>13</td>
<td>4 w</td>
<td>80</td>
<td>28.8</td>
<td>71.2</td>
</tr>
<tr>
<td>5</td>
<td>2 m</td>
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<td>19.2</td>
<td>80.8</td>
</tr>
<tr>
<td>14</td>
<td>2 m</td>
<td>62</td>
<td>15.7</td>
<td>84.3</td>
</tr>
<tr>
<td>10</td>
<td>5 y</td>
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<td>11</td>
<td>5 y</td>
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<td>5 y</td>
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<td>6 y</td>
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<td>7 y</td>
<td>89</td>
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<td>87.7</td>
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</table>

See table 2 for abbreviations.

**Discussion**

If the overlying cardiac pathology resulted in myocardial replacement by capillary and fibrous tissue, the muscle cell LDH profile might be obscured by the LDH contribution from these other cells. The small amounts of tissue removed...
medium with little difficulty and require the capability of producing or disposing of large amounts of lactate. This is reflected in their high concentration of LDH-A monomers (fig. 1). On the other hand, if the normal heart could easily and quickly convert large amounts of pyruvic acid to lactic acid, one might expect that arrhythmias, congestive heart failure, and sudden death would occur commonly in healthy individuals. Hence, the myocardium produces tetrarsers which contain predominantly LDH-B subunits. These tetragrams are effectively inhibited by large accumulations of lactic acid and thus normally convert little pyruvate to lactate.9, 10

The question is, why does the superimposition of chronic oxygen deprivation on the heart’s normal mechanism of glucose utilization cause an LDH shift in ischemia but not in hypoxia?

Response to Hypoxia

Zinkham, Blanco, and Kupcyk11 have shown that in fetal life, the myocardium contains a greater percentage of A units than does the heart following birth. Hypoxic conditions exist in utero, and as Shelley12 has demonstrated, the fetal heart is much richer in glycogen that the infant or adolescent heart. Also, energy requirements of the fetal heart are probably low enough that cardiac lactic acid production does not occur to the extent that would be deleterious to cardiac action. It follows, therefore, that in embryonic development, an efficient system for glucose utilization would be helpful. At birth, however, an adequate oxygen supply becomes readily available and increased demands are placed on the heart by crying and feeding, etc. Concurrently with this, the LDH isozyme pattern shifts rapidly to the aerobic form, composed primarily of B subunits.

It is interesting to note, as demonstrated in table 2 and figure 4, that the isozyme pattern of hypoxic infants and children also shows a rapid shift to the aerobic form following birth and that the percentage of A subunits in hypoxic children one year of age or greater is not statistically different from the percentage of A subunits seen in control hearts. There are two plausible reasons that might explain why the normally oxygenated and hypoxic heart shows similar LDH isozyme distributions. First, because of prior gene programming, the LDH pattern will shift, within a specific time frame, to a predetermined level of A unit activity no matter what the external environment, or secondly, despite a low arterial PO2, the myocardial cells are not hypoxic. The first interpretation may be too rigid to explain biological complexity and adaptability but, the second possibility draws support from recognized aspects of hypoxic cardiac physiology.

Rudolph has shown that the coronary A-V O2 difference between children with cyanotic and acyanotic heart disease remains the same. Accordingly, the myocardial oxygen utilization in Rudolph’s two groups was almost identical, i.e., approximately 11 mg/min/100 g heart tissue.13 In this situation, the extra oxygen carrying capacity of cyanotic blood was being provided by an increased hematocrit. An additional aid, during hypoxia, may be alterations in the red cell 2,3-diphosphoglycerate content so as to favor oxygen release.14 Further evidence for an adequate oxygen supply is

![Figure 5. Optical scan showing LDH pattern from various portions of the right and left ventricle in the normal heart.](http://circ.ahajournals.org/)

provided by Schewer et al., who showed that the myocardial lactate extraction of cyanotic and acyanotic children is the same.14 Hence, as the hearts of cyanotic children usually are not producing lactic acid, anaerobic metabolism through the glycolytic pathway cannot be an important contributor of energy.

Response to Ischemia

Myocardial ischemia implies a reduction in arterial flow sufficient to cause a switch from aerobic to anaerobic
metabolism and the resultant production of lactic acid. While experimental evidence for this occurring under controlled laboratory conditions is overwhelming,\textsuperscript{16,17} clinical evidence is not as striking. Exercise or atrial pacing studies of patients with coronary artery disease usually show either myocardial lactic acid production or a decrease in lactic acid extraction.\textsuperscript{18-20} Why lactic acid production is not a uniform finding is difficult to say but is probably related to inexact coronary sinus sampling sites,\textsuperscript{21} dilution of coronary sinus lactate, and/or delay in establishing arteriovenous lactate equilibrium following exercise. On the basis of the strong experimental evidence, the altered lactate metabolism found in patients with coronary artery disease, and the possible relationship of lactic acid to angina,\textsuperscript{22,23} it was accepted that the heart muscle tissue in these studies was chronically exposed to mild or moderate reductions in arterial flow adequate to cause a shift from aerobic to anaerobic metabolism but inadequate to kill the cells.

With the exception of its effect on the kidney, ischemia does not directly elicit a systemic response. Therefore, maintaining energy delivery in ischemic organs becomes a problem at the organ level. In the case of the heart, this probably occurs first by collateral development and contribution of blood from the left ventricular lumen.\textsuperscript{24} When these measures become inadequate, a change may occur in the energy delivery mechanism at the cellular level. Since ischemia implies decreased delivery of substrate as well as oxygen, the stimulus is provided to facilitate a more efficient utilization of available glucose. When ischemia is chronic, time is allowed for the synthesis of enzymes which may facilitate energy delivery under the new environmental conditions. As glycolysis cannot proceed without a method of hydrogen ion disposal, a shift toward the anaerobic pattern of LDH isozymes might be anticipated.

The synthesis of LDH subunits is under genetic control, however, and while the above reasoning is logical with regard to environmental physiology, it raises a difficult biological question. That is, can a mature, nonreplicating cell reprogram its genetic function under the influence of a powerful, prolonged external stress in order to adapt to the stress? This question is raised to acknowledge its presence and an answer is not intended by this report. While the mechanism through which the myocardium from patients with vascular disease alters the LDH subunit composition is unknown, the genetic regulation of cell function may not be so fixed as to preclude accommodation to prolonged, stressful stimuli. A point of considerable interest is that the anaerobic shift occurs only to the extent that glucose utilization is enhanced, but not to the point that cardiac function is overtly jeopardized by excessive lactic acid production.

In summary, these data indicate that the method for adaptation to systemic oxygen deprivation occurs by systemic compensatory mechanisms which maintain oxygen delivery to the tissues at normal levels. This leaves undisturbed the cell's normal mechanism of energy delivery. When environmental conditions are imposed which preclude systemic compensation, our evidence indicates that a basic change occurs at the cellular level. This is expressed as an

\[ \text{D-glucose} \rightarrow \text{D-fructose-1, 6-diphosphate} \]
\[ \rightarrow \text{dihydroxyacetone phosphate} \]
\[ \rightarrow \text{NAD} \longrightarrow \text{D-glyceraldehyde-3-phosphate} \]
\[ \rightarrow \text{NADH} + \text{H}^+ \]
\[ \rightarrow \text{1, 3-diphospho-D-glycerate} \]
\[ \rightarrow \text{2-phospho-D-glycerate} \]
\[ \rightarrow \text{L-Lactate} \rightarrow \text{[LDH]} \rightarrow \text{pyruvate} \]
\[ \rightarrow \text{pyruvate-dehydrogenase complex} \]
\[ \rightarrow \text{acetyl CoA} \]
\[ \rightarrow \text{[citric acid cycle]} \]
\[ \rightarrow \text{CO}_2 + \text{H}_2\text{O} \]

\textbf{Figure 6.} Energy pathway for glycolysis. Scheme shows the points where NAD is reduced to NADH, and where NADH is oxidized to form NAD and lactic acid. The latter step is mediated by LDH.
alteration in the anaerobic enzyme complement which may favor glucose metabolism.

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G L Hammond, B Nadal-Ginard, N S Talner and C L Markert

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