Regulation of Lactate Dehydrogenase Levels in the Mouse*

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The number of lactate dehydrogenase molecules per cell equivalent in normal adult mouse tissues is: heart muscle $1.13 \times 10^9$, skeletal muscle $3.6 \times 10^9$, liver $3.94 \times 10^9$, and kidney $3.91 \times 10^9$. These steady state levels of lactate dehydrogenase reflect a balance between synthesis and degradation. To study the relative role of the two processes the half-lives of lactate dehydrogenase in these four mouse tissues were determined by means of a combination of the double isotope labeling technique and enzyme purification by oxamate-aminohexyl-Sepharose 4B affinity chromatography. This technique makes possible the measurement of lactate dehydrogenase turnover in single mouse organs and allows for the comparison of isotope decay between different tissues without being affected by differences in the size of the amino acid pools.

All the lactate dehydrogenase isozymes in the same tissue were found to have the same half-life. The half-lives were: heart 8.2 days, skeletal muscle 43.0 days, liver 4.3 days, and kidney 6.1 days. The rates of lactate dehydrogenase synthesis in these tissues were calculated to be 110, 67, 74, and 52 molecules per cell per s, respectively. From these data and from the proportion of A and B subunits in each tissue, the rates of synthesis of each subunit were calculated. From the results it is concluded that the different steady state lactate dehydrogenase levels, a phenotypic expression of the lactate dehydrogenase genes, reflect mainly the differing rates of degradation since the rates of synthesis are very similar; thus the regulation of the quantitative expression of the lactate dehydrogenase genes in the mouse is largely post-translational.

The different amount and proportions of A and B subunits of lactate dehydrogenase (EC 1.1.1.27) in different cell types produce a very characteristic isozyme pattern, which is almost as distinctive for each cell type as the so called "differentiated proteins." In mammals these isozymes follow a binomial distribution suggesting a random combination of the subunits (1, 2). These different levels and tissue-specific patterns could be produced either by differences in the rate of synthesis, differences in rate of degradation, or a combination of both. The rate of synthesis is the end result of all translational and post-translational events, while the rate of degradation is clearly a post-translational process. For this reason, to understand the regulation of the phenotypic expression of the lactate dehydrogenase genes it is of interest to determine the turnover of this enzyme in different tissues. The published reports on the turnover rate of lactate dehydrogenase in the rat liver are conflicting (3-9), with calculated half-lives for the liver isozymes differing in more than 1 order of magnitude (3, 9). The discrepancies reflect the lack of adequate methodology for accurate measurement of protein turnover (see Refs. 10 and 11), as well as the use of an experimental animal that does not reach steady state. On the other hand, it has been reported that different isozymes in the same tissue have different half-lives (3-6, 9). Because the binormality of the isozyme pattern is preserved, this is only possible if there is a continuous dissociation and reassociation of the tetrameric molecules in the cell. This is contrary to what would be expected by the kinetics of subunit association (12), and it has been demonstrated that this dissociation and reassociation does not occur in vivo (13) or in vitro (14). For this reason in this study we have measured lactate dehydrogenase turnover in different mouse tissues at steady state in an attempt to determine the relative roles of pre- and translational versus post-translational phenomena in the regulation of lactate dehydrogenase gene expression. A methodology has been used that we believe solves some of the problems involved in comparing protein turnover between different tissues. At the same time, we have re-examined the question of homogeneous or heterogeneous turnover of the different isozymes in the same tissue.

MATERIALS AND METHODS

G57Bl/6J adult males obtained from the Jackson Laboratory (Bar Harbor, Maine) were maintained on a standard diet for the duration of the experiment. L-(4,5-3H)leucine, 50 Ci/mmol, and L-[U-14C]leucine, 570 mCi/mmol, were from New England Nuclear. All other reagents and material were obtained from commercial sources and were of the highest quality available.

Lactate dehydrogenase activity was determined at 25° by methods previously described (19). Protein concentration was measured by the method of Lowry et al. (16), and DNA was determined by the method of Burton (17) with calf thymus DNA as a standard.

Cellulose polyacetate electrophoresis of lactate dehydrogenase isozymes was performed as described by Shaklee (18). Scanning and quantification of the isozymes was done with a Beckman model RB Analytical recording densitometer. The optical density peaks were used to determine the percentage contribution of each isozyme to the total activity in the sample, and knowing the molecular composition of each isozyme, the total contribution of each subunit could be calculated. Acrylamide disc gel electrophoresis was performed as...
Regulation of Lactate Dehydrogenase Levels

Lactate dehydrogenase purification from single mouse organs by oxamate-aminohexyl-Sepharose 4B

Tissues were homogenized and run on an oxamate-aminohexyl-Sepharose 4B column as described in the text.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Lactate Dehydrogenase</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homogenate</td>
<td>Recovered</td>
</tr>
<tr>
<td>Heart</td>
<td>24.4 (±2.5)</td>
<td>23.2 (±1.9)</td>
</tr>
<tr>
<td>Muscle (1 g)</td>
<td>636.6 (±31)</td>
<td>627.8 (±34)</td>
</tr>
<tr>
<td>Liver</td>
<td>260.8 (±30)</td>
<td>258.5 (±25)</td>
</tr>
<tr>
<td>Kidney</td>
<td>77.2 (±15)</td>
<td>74.7 (±3.7)</td>
</tr>
</tbody>
</table>

*a* Two kidneys used in each experiment.  

Fig. 1 (left). Polyacrylamide gel electrophoresis of mouse lactate dehydrogenase (LDH) purified by the oxamate-aminohexyl-Sepharose 4B procedure. Electrophoresis was carried out in 7% gels, in Tris/glycine buffer, pH 8.3 (16). The gels were run for 3½ h at 5 mA/gel. One of the gels was stained for lactate dehydrogenase activity by the tetrazolium method (21) and the other for total protein with Coomassie brilliant blue. There is a perfect coincidence between lactate dehydrogenase activity and protein bands.  

Fig. 2 (right). Sodium dodecyl sulfate-acrylamide slab gradient gel stained for proteins showing the different steps of lactate dehydrogenase (LDH) purification by aminohexyl-oxamate-Sepharose 4B. Eight per cent to 20% linear gradient gels were prepared according to Studier (22) with some modifications. This is a discontinuous gel system with a Tris/HCl electrode buffer system, pH 8.3. The gels were run for 10 h at 15 mA. The lactate dehydrogenase bands are visible in the extract but absent from the wash. There are two bands of lactate dehydrogenase corresponding to the two subunits with the B subunits migrating behind the A subunits. There is no evidence of contaminating proteins.

appreciably increase the yield of lactate dehydrogenase. As shown in Table II the levels of enzyme were very stable in the four tissues examined suggesting that the animals were at steady state.

Binomial Distribution and Steady State Concentration in Adult Mouse Tissues

Between 75 and 80% of the mass of skeletal muscle and heart muscle tissues is contributed by the muscle cells themselves (23); nevertheless, the muscle tissues as well as the other two tissues studied contain a heterogeneous cell population. The zymogram pattern of each one represents the combination of the patterns of the different cell types present in the tissue. Heart and kidney are richer in B subunits while
liver and skeletal muscle are richer in A subunits. If lactate dehydrogenase subunits are allowed to polymerize at random, that is, if they are located in the same cell, they will produce a binomial isozyme distribution. If the subunits are located in different types of cells containing different proportions of the two subunits, then they will produce binomial isozyme distributions in each cell but not in the tissue as a whole. In Table III, the observed distribution of the lactate dehydrogenase isozymes is reported along with the expected value, assuming random subunit combination. For heart, liver, and skeletal muscle the observed and expected values are very similar, thus implying that the different cell types in each tissue have virtually the same isozyme distribution or that a single cell type is making an overwhelming contribution to the lactate dehydrogenase pattern. In any event, these three tissues behave as if they were homogeneous cell populations. On the other hand, the observed isozyme distribution in kidney departs significantly from the expected and reflects the contribution of at least three cell types predominantly located in the cortex, medulla, and papilla (Fig. 3). These results do demonstrate that the methods used are sensitive enough to detect heterogeneity in cell populations if individual components differ substantially in their isozyme makeup.

The size of the cells in these different tissues varied greatly and the catalytic activity of the A and B subunits are also different, A being about twice as active as B. Therefore, the amount of enzyme activity and the number of molecules of each subunit per cell equivalent was calculated. From Table IV the different phenotypic expressions of the lactate dehydrogenase genes when located in different cell

### Table II

**Lactate dehydrogenase activity in mouse tissues**

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
<th>28</th>
<th>32</th>
<th>36</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU</td>
<td>21 ± 2</td>
<td>540 ± 23</td>
<td>256 ± 20</td>
<td>74 ± 6</td>
<td>21 ± 2</td>
<td>570 ± 32</td>
<td>220 ± 23</td>
<td>68 ± 5</td>
<td>22 ± 1</td>
<td>610 ± 40</td>
<td>291 ± 31</td>
</tr>
<tr>
<td>IU</td>
<td>23 ± 1</td>
<td>520 ± 31</td>
<td>235 ± 25</td>
<td>70 ± 5</td>
<td>20 ± 0.5</td>
<td>574 ± 28</td>
<td>260 ± 19</td>
<td>83 ± 7</td>
<td>22 ± 2</td>
<td>523 ± 36</td>
<td>257 ± 16</td>
</tr>
<tr>
<td>IU</td>
<td>23 ± 1.5</td>
<td>608 ± 41</td>
<td>283 ± 25</td>
<td>65 ± 5</td>
<td>24 ± 3</td>
<td>618 ± 39</td>
<td>268 ± 31</td>
<td>80 ± 6</td>
<td>24 ± 2</td>
<td>531 ± 27</td>
<td>291 ± 21</td>
</tr>
<tr>
<td>IU</td>
<td>24 ± 3.5</td>
<td>623 ± 43</td>
<td>212 ± 13</td>
<td>68 ± 5</td>
<td>26 ± 2</td>
<td>603 ± 43</td>
<td>212 ± 13</td>
<td>68 ± 5</td>
<td>24 ± 2</td>
<td>523 ± 36</td>
<td>199 ± 8</td>
</tr>
</tbody>
</table>

**FIG. 3.** Lactate dehydrogenase isozyme pattern of three different areas of mouse kidney. Kidney cortex, medulla, and papilla were dissected and homogenized as described under "Materials and Methods." Vertical starch gel electrophoresis was performed according to Boyer et al. (24) with an EDTA/boric acid/Tris buffer, pH 8.6. The gel was run at 4° for 15 h at 200 V, sliced horizontally, and stained for lactate dehydrogenase activity (21).

### Table III

**Relative distribution of lactate dehydrogenase isozymes in mouse tissues**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>A*</th>
<th>B*</th>
<th>Lactate dehydrogenase-1</th>
<th>Lactate dehydrogenase-2</th>
<th>Lactate dehydrogenase-3</th>
<th>Lactate dehydrogenase-4</th>
<th>Lactate dehydrogenase-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>35.0</td>
<td>65.0</td>
<td>17</td>
<td>17.8</td>
<td>38.3</td>
<td>38.5</td>
<td>32.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>96.0</td>
<td>4.0</td>
<td>1.0</td>
<td>0.9</td>
<td>14.0</td>
<td>14.1</td>
<td>85.0</td>
</tr>
<tr>
<td>Liver</td>
<td>98.0</td>
<td>2.0</td>
<td>4.0</td>
<td>7.5</td>
<td>96.0</td>
<td>92.2</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>42.0</td>
<td>58.0</td>
<td>22.1</td>
<td>11.3</td>
<td>26.0</td>
<td>32.8</td>
<td>25.8</td>
</tr>
</tbody>
</table>

* Percentage of A subunit.
* Percentage of B subunit.
* Obs., observed percentage of a given isozyme.
* Exp., expected percentage of isozyme if distribution were binomial.
types are very apparent. For example, there are 30 times more A subunits per skeletal muscle cell than per liver cell, and 54 times more B subunits in a heart muscle cell than in a liver cell.

Relative Turnover Rate of Different Lactate Dehydrogenase Isozymes

To determine whether the different isozymes in the same tissue have the same or different half-lives, the relative turnover rates of the different isozymes from heart and kidney were measured as described previously. The results are shown in Figs. 4 and 5 and in Table V. From Figs. 4 and 5 it is evident that all the radioactivity detected in the gels correspond to the lactate dehydrogenase bands. This provides additional evidence that the enzyme obtained from the oxamate column is virtually pure. The similar $3^\text{H}/^{12}\text{C}$ ratios of all the isozymes in heart and kidney shown in Table V indicate that in both organs the different isozymes are produced and degraded at the same rate. It was not possible to study the relative turnover rate of $\text{A}_2\text{B}_1$ from liver and skeletal muscle because this isozyme represents less than 5% of the total lactate dehydrogenase in the organ and the disintegrations per min of the corresponding section of the gel were never significantly greater than background. In itself the low radioactivity of $\text{A}_2\text{B}_1$ in liver and skeletal muscle excludes a very rapid turnover rate for this isozyme.

From these results it can be stated that the turnover rates of the different isozymes from heart and kidney are equal or very similar. From a methodological point of view it seems satisfactory to assume that lactate dehydrogenase from a given organ behaves as if it were a homogeneous cell population.

Turnover of Lactate Dehydrogenase from Different Tissues

Reproducibility of Results — Because in all cases $[^{14}\text{C}]$-labeled and $[^{3}\text{H}]$-labeled leucine were injected at the same time and the disintegrations per min for each organ should be similar in all experiments if the animals were at steady state. This fact provides an ideal internal control for the validity of the experiments. When $[^{14}\text{C}]$- and $[^{3}\text{H}]$-labeled leucine were injected at the same time and the disintegrations per min for each organ should be similar in all experiments if the animals were at steady state. This fact provides an ideal internal control for the validity of the experiments.

### Table IV

<table>
<thead>
<tr>
<th>Subunits of lactate dehydrogenase per cell in adult tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of lactate dehydrogenase subunits per cell was determined from the measured enzyme activity and DNA content of the tissue, taking into consideration the tetrameric structure of the enzyme, a molecular weight per subunit of 35,000, and the specific activities measured for mouse lactate dehydrogenase A4 and B4. One international unit of A4 and B4 have 1.955 x $10^{10}$ and 3.585 x $10^{10}$ subunits, respectively. The size of the mouse genome was assumed to be 7 x $10^{12}$ g of DNA (19).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Number of lactate dehydrogenase subunits per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Heart</td>
<td>$1.13 \times 10^8$</td>
</tr>
<tr>
<td>Muscle</td>
<td>$3.60 \times 10^8$</td>
</tr>
<tr>
<td>Liver</td>
<td>$3.94 \times 10^7$</td>
</tr>
<tr>
<td>Kidney</td>
<td>$3.91 \times 10^7$</td>
</tr>
</tbody>
</table>

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Fig. 4. Distribution of radioactivity in mouse heart lactate dehydrogenase isozymes. Heart lactate dehydrogenase from mice injected with 30 $\mu$Ci of $[^14]\text{C}$-leucine and 150 $\mu$Ci of $[^3]\text{H}$-leucine with a 7-day interval was purified by the oxamate-aminohexyl-Sepharose 4B method and run on a 7.5% acrylamide disc gel (18 mm, inner diameter). After electrophoresis the gel was sectioned in 1-mm slices, dissolved with 0.3 ml of 30% H$_2$O$_2$, and counted as described under "Materials and Methods." All radioactivity in the gel corresponds to the isozyme bands.

Fig. 5. Distribution of radioactivity in mouse kidney lactate dehydrogenase isozymes. The procedures and the kidneys of the animals described in Fig. 4 were used.

### Table V

$[^3]\text{H}/[^{14}\text{C}]$ Ratio of lactate dehydrogenase isozymes from mouse heart and kidney

Two mice were injected with 150 $\mu$Ci of $[^{14}\text{C}]$-labeled leucine and 4 days later with 30 $\mu$Ci of $[^{3}\text{H}]$-labeled leucine. Four hours after the $[^{14}\text{C}]$-labeled leucine injection the animals were killed, and the lactate dehydrogenase from hearts and kidneys purified by aminohexyl-oxamate affinity chromatography. The purified lactate dehydrogenase was separated in 7% acrylamide gels and stained for lactate dehydrogenase activity. The isozyme bands were cut out, digested with H$_2$O$_2$, and the radioactivity measured.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Isozyme</th>
<th>$[^3]\text{H}$ dpm</th>
<th>$[^{14}\text{C}]$ dpm</th>
<th>$[^3]\text{H}/[^{14}\text{C}]$ dpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>LDH-1*  (B$_1$)</td>
<td>970</td>
<td>115</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>LDH-2 (A$_1$B$_1$)</td>
<td>865</td>
<td>107</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>LDH-3 (A$_1$B$_2$)</td>
<td>265</td>
<td>31</td>
<td>8.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>LDH-5 (A$_2$)</td>
<td>203</td>
<td>17</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>LDH-4 (A$_1$B$_2$)</td>
<td>389</td>
<td>29</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>LDH-3 (A$_1$B$_1$)</td>
<td>853</td>
<td>62</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>LDH-2 (A$_2$B$_1$)</td>
<td>1240</td>
<td>93</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>LDH-1 (B$_2$)</td>
<td>1845</td>
<td>135</td>
<td>13.6</td>
</tr>
</tbody>
</table>

* LDH, lactate dehydrogenase.
animals killed 4 h later, the $^{3}$H/$^{14}$C ratio was constant and similar among different tissues, both with respect to lactate dehydrogenase and total protein (data not shown). Similarly, the $^{3}$H counts per min in lactate dehydrogenase from each organ obtained at 4-day intervals for 40 days after the administration of $^{14}$Cleucine were very similar, with less than 10% variation between animals or between time points (data not shown). The consistency of the $^{3}$H counts per min gives support to the notion that the rate of lactate dehydrogenase synthesis was similar in all mice throughout the experimental period. Table VI summarizes a complete experiment in which $^{14}$C- and $^{3}$Hleucine were injected at the same time. From this table, it can be seen that the apparent relative turnover of lactate dehydrogenase in the different tissues is similar to the relative turnover of total protein in the same tissue. The apparent relative rates are liver > kidney > heart > skeletal muscle, but these figures could be a reflection of either real differences in turnover or in the size of the leucine pool.

To eliminate the influence of the size of the leucine pool the half-life of lactate dehydrogenase and total protein was determined from the changing $^{3}$H/$^{14}$C ratio when the two isotopes were injected at different intervals.

**Determination of Lactate Dehydrogenase Half-life**—All animals were injected with 30 $\mu$Ci of $^{14}$Cleucine at Day 0. At 4 day intervals four animals were injected with 150 $\mu$Ci of $^{3}$Hleucine and killed 4 h later. Then, the interval between the administration of the two isotopes ranges from 0 to 40 days. Fig. 6 shows the increasing $^{3}$H/$^{14}$C ratio of lactate dehydrogenase purified from mouse heart, liver, skeletal muscle, and kidney. Because the $T_{1/2}$ is determined from the $^{3}$H/$^{14}$C ratio, different leucine pools in different tissues should not affect the results. The consistency of the decay curve obtained with changing interval supports the validity of the approach used, and its linearity is in agreement with the assumption that protein degradation is a first order reaction.

Assuming that protein synthesis is a zero order reaction, at the steady state, then $K_{a} = K_{d}E$, where $K_{a}$ is the constant rate for synthesis, $K_{d}$ is the constant rate for degradation, and $E$ is the titer of enzyme at the steady state. It was shown previously that the half-life for both subunits in a tissue is the same (Table V). Because $T_{1/2} = \ln 2/K_{d}$ by knowing the amount of each subunit in the different tissues, $K_{a}$ and $K_{d}$ for each subunit can easily be calculated.

The $K_{a}$ and $K_{d}$ calculated from the data of Fig. 6 and Table IV are shown in Table VII where the constant rate for synthesis has been expressed in molecules produced per s per cell equivalent.

It is evident from this table that the rate of degradation of lactate dehydrogenase in the four tissues studied is very different, with half-lives ranging from 4.3 days for liver to 43.0 days for skeletal muscle. On the other hand, the rate of synthesis is very similar in all four tissues, with only a 2-fold difference between heart muscle with 110 molecules per cell per s and kidney with 52 molecules per cell per s. The same similitude is observed when each subunit is considered separately; this is especially true for the $A$ subunits where a maximum 4-fold difference exists between liver and kidney. The differences among cells in the synthesis of $B$ subunits are greater but still less than the differences in the amount of this subunit present in the different cell types.

Table VIII shows the half-life of total protein for the same
organ. The turnover rate of the total protein and the turnover rate of the lactate dehydrogenase are similar, with the exception of kidney where the turnover rate of total protein is greater.

From these experiments it can be concluded that the rate of degradation of lactate dehydrogenase in different organs is very different and closely related to the total protein turnover in the organ. The rate of synthesis in different cell types is very similar when expressed in molecules per cell. There is a better agreement between lactate dehydrogenase levels in a tissue and the half-life of the enzyme in the same tissue than between enzyme levels and rates of synthesis. Nevertheless, some significant differences in the rates of synthesis between different tissues still remain when each subunit is considered individually, especially for the B subunit.

**DISCUSSION**

**On Methodology—**To study the mechanisms regulating the phenotypic expression of any gene in different cell types at the level of protein synthesis and degradation, it is necessary to apply a methodology that will permit comparison of the results obtained for different tissues or cells in the same organism. Two main requirements must be fulfilled in order to compare the results from different tissues, and these have been met by the present investigation. First, a method of purification that yields the same recovery of a particular protein from all cells or tissues studied without significant contamination by other radioactive proteins must be applied. Lactate dehydrogenase purification by oxamate-aminohexyl-Sepharose 4B fulfilled this requirement by giving consistent and complete recovery of the enzyme without any evidence of contamination by other proteins, with obvious advantages over the immunoprecipitation (3-6) and molecular hybridization (25) procedures. Second, a method of determining the rate of synthesis and degradation must be used that allows for the comparison of results obtained from different tissues with different amino acid pool sizes. No technique has been described as yet which completely solves this problem, and with all pulse-labeling methods the apparent half-life obtained is probably too long due to amino acid reutilization (26).

Nevertheless, the modification of the double label technique of Arias et al. (27) used here, where the rate of synthesis and degradation were calculated from the half-life and enzyme levels at steady state, minimizes this problem. Because the $T_{1/2}$ was determined from the ratio of the two isotopes in the same tissue, the size of the amino acid pool only affects the determination of the $T_{1/2}$ to the extent that it influences amino acid reutilization. Other techniques that seem to reduce this problem either cannot be used in many tissues as is the case of L-$\text{L}$guanido-$\text{C}^{15}$|arginine (28), or present other problems (10) when used for proteins with a long half-life, as is the case of the labeling to steady state (29) and precursor-product techniques (29-31).

Three lines of evidence suggest that the methodology used in this investigation give as close an estimate of the true half-life as any of the methods available. (a) The data are internally consistent. The turnover calculated from the $T_{1/2}$ correlates well with the synthesis estimated from the $\text{H}^{3}$ counts after a 4-h pulse where reutilization is not a problem (compare Table VI with Table VII). With both approaches the relative turnover of lactate dehydrogenase per cell is liver > kidney > heart > muscle. This strongly suggests that the determined $T_{1/2}$ is close to the actual half-life. (b) The linearity of most of the $\text{H}^{3}/\text{C}^{14}$ ratio, obtained with different intervals between isotope administrations, suggests that either reutilization is not a major problem with the intervals used, or that it is uniform during the major part of the decay curve. The absence of linearity of the decay curve when the interval between injections is 2- to 3-fold longer than the $T_{1/2}$ is due to the low specific activity of the protein at this stage, when the method loses sensitivity (8). This is contrary to the intervals recommended by Zak et al. (31). (c) The values reported here are in close agreement with the values obtained in the rat by other methods, i.e. 3.3 to 6.6 days for total liver protein (7, 8, 27), 3.5 to 6 days for liver lactate dehydrogenase (7, 8, 25), and 5.4 to 8 days for cardiac myosin (31-33). Nevertheless, the figures presented here probably are minimum values for the rate of synthesis and degradation, especially for peripheral tissues as skeletal muscle (26).

**Turnover of Individual Isozymes—**It has been suggested that the unit of degradation of oligomeric proteins is the subunit and not the oligomer (see Ref. 34). There are a few reports of different subunits in the same molecule turning over at different rates (3-6, 9, 35-37). These reports suggest that these multimeric molecules are in a continuous dynamic state of dissociation and re-association in vivo with a consequent exchange of subunits. In the same context, different degradation rates for lactate dehydrogenase isozymes in the same tissue have been reported (3-6, 9). According to Fritz et al. (3-6) the A-rich enzymes in cardiac muscle turn over at a faster rate than the B-rich isozymes, while the contrary occurs in skeletal muscle and liver. They invoked a continuous dissociation and re-association of the tetramer with subunit exchange to explain their results. Inconsistencies in their different reports (3-6) and the lack of a binomiality of their isozone patterns makes theirs results very difficult to interpret. Moreover, this hypothesis, which is not supported by our results, is incompatible with a binomial distribution of isozymes. As it has been shown here a binomial distribution does exist in the animal. If the different isozymes are in a continuous equilibrium they should have the same apparent half-life at steady state. Some of the reasons for the discrepancies in the results have been pointed out (38).

In contrast with these reports we have shown here that, at least in the mouse, there is a single rate of degradation of all lactate dehydrogenase isozymes in the same tissue, and previously we demonstrated an absence of subunit exchange between lactate dehydrogenase tetramers in vivo (13). The same type of result has been reported for aldolase isozymes in chick brain (39). From our results and also those of Lebherz (39) it seems clear that at least for lactate dehydrogenase and aldolase the degradative machinery of the cell does not discriminate between the two types of subunits, and that the

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$T_{1/2}$ (days)</th>
<th>$K_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>11.20</td>
<td>0.0617</td>
</tr>
<tr>
<td>Muscle</td>
<td>45.3</td>
<td>0.0153</td>
</tr>
<tr>
<td>Liver</td>
<td>3.9</td>
<td>0.178</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.8</td>
<td>0.119</td>
</tr>
</tbody>
</table>

**Fig. 6** for lactate dehydrogenase.
unit of degradation of these enzymes is the tetramer and not the subunit.

Steady State Levels and Lactate Dehydrogenase Turnover — From this study and others (3, 9) it is apparent that the lactate dehydrogenase genes have different degrees of expression in every cell type. In some cases, these differences are quite impressive for both A and B genes. There are 32 times more A subunits per skeletal muscle cell equivalent than per kidney cell, and 52 times more B subunits in heart than in liver cells. These steady state intracellular levels are a function of both the rate of synthesis and the rate of degradation. Nevertheless, with the limitations inherent to the determination of protein turnover mentioned above, which allows only for the measurement of apparent rates of synthesis and degradation, the results presented here show that despite the different amounts of enzyme per cell equivalent in the four tissues investigated, the apparent rates of total lactate dehydrogenase synthesis per cell equivalent are very similar. They range from 52 to 110 molecules/cell/"s" in kidney and heart, respectively. Most of the differences in steady state levels seems to be the result of the characteristic apparent $T_{1/2}$ of the molecule in each tissue. Even though these values can only be approximate, their internal consistency, and the close correlation of lactate dehydrogenase turnover with the turnover of the total protein of the organ (determined either from the decay curve or from isotope incorporation during a pulse label), they strongly suggest that post-transcriptional events are quantitatively more important than the combination of all events up to and including translation, to determine lactate dehydrogenase steady state levels. On the other hand, because of the similar half-lives of all lactate dehydrogenase isozymes in the same tissue, the different amounts of A and B subunits in the same cell must be produced by differences in the rate of synthesis. We are presently investigating whether this is due to different amounts of the respective mRNA or different efficiencies of translation.

Very little data are available on turnover rates of lactate dehydrogenase, or any other protein, in different tissues. Nevertheless, the results that have been reported for a few enzymes, with the exception of the already mentioned report of Fritz et al. (3), are similar to those for lactate dehydrogenase in the mouse. Rat aldolase has a half-life of 4.9 days in liver (7) and 20 days in skeletal muscle (40). Rat alanine aminotransferase has a half-life of 3 days in liver and 20 days in skeletal muscle (41). On the other hand, kidney and liver catalase is degraded at the same rate (42, 43). Recently very heterogeneous half-lives for lactate dehydrogenase in different rat tissues have also been reported (9), with values comparable to those reported here for the mouse. The calculated half-life of 4.3 days for liver lactate dehydrogenase in the mouse agrees well with the 4 to 6 days obtained for lactate dehydrogenase-5 in the rat (7-9, 25), and the turnover rate of 74 molecules per liver cell per s reported here for the mouse is very similar to the rate of 84 molecules per liver cell per s in the rat as calculated by Lindy (25), suggesting similar regulatory mechanisms in the two species.

It has been suggested that protein turnover in vivo is correlated with the in vitro stability, susceptibility to proteolytic degradation of the protein (44-46), and tightness of cofactor binding (47). In this respect, it is known that lactate dehydrogenase B binds more tightly to the co-factor and is also more resistant to heat denaturation. However, the A and B subunits in the same tissue have the same rate constant for degradation as we have shown. More important, the same enzyme has very different stabilities depending on the cell in which it is located. Such a discrepancy between in vitro and in vivo stabilities has also been found for other enzymes (46). The results presented here suggest that the micro-environment of the protein is important in determining its stability in vivo and plays a fundamental role in the production of the different phenotypes of the lactate dehydrogenase genes.

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