The Synthesis and Degradation of Fructose Diphosphate-
Aldolase and Glyceraldehyde 3-Phosphate
Dehydrogenase in Rabbit Liver*

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SUMMARY

Fructose diphosphate aldolase and glyceraldehyde 3-phosphate dehydrogenase have been crystallized from rabbit livers after single or sequential injections of [14C]- and [3H]-lysine. The specific radioactivity of the lysine in the dehydrogenase rises continuously and reaches a maximum during the 5th day after injection, whereas that of the aldolase declines exponentially from the middle of the 1st day. There is sufficient information in the concurrent time curves to permit a numerical solution of the simultaneous equations for the time dependence of activity decay of the free lysine precursor pool and the rate constants for the synthesis and degradation of both proteins, internally corrected for the recycling of isotope. The first order rate constants of degradation of aldolase and the dehydrogenase are 0.62 and 0.23 day⁻¹ corresponding to half-lives of 1.1 and 2.8 days, respectively.

Following an initial rapid decline, the specific activity of the effective free lysine pools tends to plateau at a level intermediate between those of the lysines in aldolase and the dehydrogenase and becomes equal to that of the dehydrogenase when the latter reaches its maximum. During this period, the radioactivity of the free lysine is replenished by the breakdown of the most rapidly metabolized fraction of liver proteins. As measured by the uncorrected raw data, the apparent half-life of lysine-labeled aldolase is 3.86 days and of leucine-labeled aldolase 1.48 days. Leucine is less extensively recycled. In contrast with efforts to obtain true pulse labeling conditions by using a rapidly cleared isotope, the present analysis is facilitated by the use of lysine, an amino acid that is most extensively reutilized.

The synthesis and degradation of enzymes and their control in mammals have been studied extensively and are the subjects of two recent reviews (1, 2). There are large differences in the relative turnover rates of liver proteins, and the turnover of individual enzymes may themselves vary widely as a function of diet or of various kinds of stress. To measure rates in the unperturbed steady state, a suitable enzyme isolation method is required, and it is necessary to follow with time the incorporation and release of a labeled amino acid. The recurrent problem in determining absolute rates is the correction for the recycling of isotope which requires a knowledge of the time course of specific activity of the precursor amino acid pool. [guanido-14C]Arginine exchanges its label rapidly in the free state and has been used to provide recycling corrections (3), but the incorporation efficiency of guanido-labeled arginine is low and variable. Continuous feeding methods have also been employed (4). In the present report, we describe the use of pulse doses of [14C]- and [3H]-lysine to follow simultaneously the labeling of two proteins of rabbit liver. Although lysine is one of the amino acids that is most extensively recycled, the simultaneous differential equations that describe the time curves of labeling of the two proteins may be solved numerically to yield the rate constants of synthesis and decay for both proteins and also the specific activities of the effective precursor-lysine pool.

The liver proteins under investigation are fructose diphosphate aldolase and glyceraldehyde 3-phosphate dehydrogenase. A method has been developed for crystallizing the two enzymes from the same rabbit liver and has been employed to prepare samples for radioassay at time intervals after the injection of isotope. In rabbit skeletal muscle, the dehydrogenase turns over at about half the rates of aldolase and glycogen phosphorylase (5-7). We were interested in knowing whether or not this was a tissue-specific relation and what factors might be involved in determining the relative metabolic stabilities of the proteins.

MATERIALS AND METHODS

Materials—Oxidized and reduced pyridine nucleotides, the barium salt of N-glyceraldehyde 3-phosphate diethylacetal, adenosine triphosphate, and α-glycerophosphate dehydrogenase were obtained from Sigma Chemical Company. Dithiothreitol, the sodium salt of 3-phosphoglycerate, triose phosphate isomerase, and 3-phosphoglycerate kinase from yeast were products of Calbiochem. The cellulose phosphonate was from
BioRad. L-[U-14C]Lysine and L-[U-3H]lysine were obtained from Amer sham/Sepr Ie. The nonionic detergent, WR-1339, was obtained from the Ruge Chemical Co.

**Labeling of Animals**—New Zealand white rabbits, weighing 4 to 5.5 kg, of both sexes from a local inbred strain maintained for the food industry, were kept for several weeks on a Purina rabbit Chow diet, 23% protein. At a standard time of day, measured amounts of labeled amino acid in physiological 0.8% NaCl solution were injected in the marginal ear vein. Only those rabbits that maintained the normal food intake during the experimental period were utilized for enzyme isolation. Animals were also rejected if the administered amino acid did not appear to enter the venous flow quantitatively. When two time points from the same animal were required, the first amino acid injected was labeled with l4C or %.,% at the desired time interval before killing the animal, a large radioactive dose of the same amino acid labeled with the other isotope was administered.

**Enzyme Assay** The aldolase assays were run in 1.0 ml of solution in which the concentrations were as follows: pH 7.5 Tris (chloride), 0.1 M, fructose diphosphate (sodium), 0.02 M, NADH 0.1 mM, a-glycerophosphate dehydrogenase, 0.1 mg ml-1, and triose phosphate isomerase, 0.1 mg ml-1. After addition of rate-limiting amounts of aldolase, the decline of NADH absorption at 340 nm was measured in a Gilford recording spectrophotometer at 25°.

Glyceraldehyde 3-phosphate dehydrogenase was routinely assayed in the reverse reaction direction employing a 3-phosphoglyceroyl phosphate-generating system as previously described (8).

**Counting Procedure**—The concentrations of enzyme solutions, purified to constant specific catalytic and radioactivity, were determined by their 280 nm absorption and known absorption coefficients. Aliquots containing 1 to 3 mg of protein were mixed with 2.0 ml of solubilize (Nuclear Chicago) and 20 ml of scintillation fluid containing, per liter, 5 g of 2,5-diphenyloxazole, 0.1 g of 1,4-bis-2-[(4-methyl-5-phenylxazolyl)]benzene, and 40 ml of ethanol in toluene. Radioactivity was measured with a Tri-Carb liquid scintillation spectrometer. In double labeling experiments, the calculation procedures were checked empirically by the analysis of known mixtures. All data were processed by a computer program.

**Purification of Enzymes**

This method was designed to permit isolation of the two enzymes from the same extract and combines some of the features previously described for the separate isolation of aldolase (9) and the dehydrogenase (10) from liver. One liver, 80 to 100 g, is sufficient for the crystallization of both enzymes. However, the method is more convenient and reproducible when performed on a larger scale. The following directions are for 400 g of tissue and may be scaled up or down.

**Extraction and Alcohol Precipitation**—The livers from anesthetized animals were promptly trimmed, diced, and homogenized in a blender with 2 volumes of cold 25% glycerol. The crude homogenate in a large beaker was suspended in a -10° bath, stirred mechanically, and cooled 95% ethanol from a -10° jacketed reservoir was immediately added at a rate of 20 ml min-1 to give a final ethanol concentration of 27%. Stirring was continued for an additional 20 min. The suspension was then centrifuged at 27,000 X g at -10° for 1 hour. For this purpose, Teflon-lined stainless steel cups of 250-ml capacity were employed. The supernatant solution, strained to remove congealed lipids, was used immediately.

**Chromatography and Crystalization of Aldolase**—The cellulose phosphomonoester used in this step was washed with 95% ethanol and recycled with 0.2 M solutions each of sodium chloride, sodium hydroxide, and potassium phosphate, pH 6.9. The final water wash was done on a column and was continued until the specific conductance of the effluent was less than 30 µhos. Sufficient wet packed resin to make a column (5 x 15 cm) was added to enzyme from Step 1 that had been diluted to 3500 ml with water containing 50 mg of dithiothreitol, and the suspension was stirred slowly for 4 hours or overnight at 4°. The resin was then washed twice with 1-liter portions of water and slurried into the column, washed with 75 ml of water, and eluted by a linear gradient formed from 300 ml of 2 M fructose diphosphate, pH 6.8, in the mixer and 0.1 M potassium phosphate, pH 0.5, in the second chamber. Elution was done at a rate of 10 ml min-1. The elution diagram is shown in Fig. 1. The central portion of the aldolase peak usually emerged at near maximal specific activity and was crystallized at 0.4 saturation of ammonium sulfate, pH 7 at 4°, after concentration of the pooled fractions by ultrafiltration through an Amicon PM 30 membrane. When aldolase only was desired, the phosphate gradient was omitted, and when dehydrogenase only was sought, the fructose diphosphate was omitted.

**Fractionation of Dehydrogenase**—The trailing fractions of the dehydrogenase overlapped hemoglobin and were discarded, entailing a 15 to 30% loss. To the remaining 160 to 200 ml of pooled fractions, NAD was added to a final concentration of 1 mM, and the solution was concentrated to 15 ml by ultrafiltration. The concentrated solution was placed in a 0.25-inch diameter dialysis casing and immersed in a concentrated ammonium sulfate solution calculated to yield a final 0.6 saturation of ammonium sulfate at dialysis equilibrium. NAD and dithiothreitol were present in the outer solution at concentrations of 1 mM. Dialysis was carried out overnight, with oxygen excluded. The precipitate was removed by centrifugation and discarded. Small increments of solid ammonium sulfate to a final 0.64 to 0.65 saturation were added and precipitates were removed. The pH was then raised to about 8.3 with a drop of concentrated ammonium hydroxide, and the enzyme was allowed to crystallize. No significant changes in specific activity were observed after recrystallization. Although the double peak of dehydrogenase in Fig. 1 was always observed in the initial column run, a single symmetrical band was obtained when the purified enzyme NAD complex was added in more conventional fashion to a packed column and rechromatographed in a phosphate gradient. Summaries of the fractiona-
TABLE I
Purification of aldolase and glyceraldehyde 3-phosphate dehydrogenase from 400 g of rabbit liver

<table>
<thead>
<tr>
<th>Step</th>
<th>Total unit a</th>
<th>Total protein mg</th>
<th>Specific activity b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>740</td>
<td>10,000</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>560</td>
<td>13,000</td>
<td></td>
</tr>
<tr>
<td>Chromatography</td>
<td>120</td>
<td>150</td>
<td>1.58</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>78</td>
<td>100</td>
<td>72</td>
</tr>
<tr>
<td>Crystallization</td>
<td>80</td>
<td>51</td>
<td>1.58</td>
</tr>
</tbody>
</table>

a Micromoles of substrate utilized per min. Two moles of NADH are oxidized per mole of aldolase substrate.
b Units per mg of protein.

The large crystals of the two enzymes in Figs. 2 and 3 were grown over a period of a month under controlled conditions.

ENZYME TURNOVER

Table II lists the experimental results with the two proteins over time periods of 5½ to 168 hours after pulse doses of labeled lysine. Of the seven experiments presented, three involve the isolation of both enzymes from the same liver or pooled livers of identically treated animals and two involve double labeling. In four experiments a single enzyme was isolated. When the results are normalized with respect to dose per unit weight, the points fall on the smooth curves of Fig. 4, indicating that the data obtained in the various ways are strictly comparable and that the experimental animals exhibited a relatively uniform metabolic behavior, much more uniform, in fact, than the results (not presented) that were obtained in similar experiments.

**TABLE II**
Specific radioactivities of fructose diphosphate aldolase and glyceraldehyde 3-phosphate dehydrogenase of rabbit liver after pulse doses of labeled lysine

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Number of animals</th>
<th>Time</th>
<th>Dose</th>
<th>Normalized specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hrs</td>
<td>μCi/kg</td>
<td>(dpm/mg) × (μCi/kg)⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aldolase</td>
</tr>
<tr>
<td>1a</td>
<td>1</td>
<td>5.5</td>
<td>22.0 (H)</td>
<td>43.6</td>
</tr>
<tr>
<td>1b</td>
<td>1</td>
<td>78.0</td>
<td>3.3 (14C)</td>
<td>47.4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>19.2</td>
<td>1.00 (14C)</td>
<td>77.1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>19.8</td>
<td>3.32 (14C)</td>
<td>83.1</td>
</tr>
<tr>
<td>4a</td>
<td>4</td>
<td>21.8</td>
<td>6.60 (14C)</td>
<td>19.1</td>
</tr>
<tr>
<td>4b</td>
<td>4</td>
<td>45.8</td>
<td>24.90 (H)</td>
<td>22.3</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>45.0</td>
<td>3.46 (14C)</td>
<td>26.6</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>119.0</td>
<td>2.8 (14C)</td>
<td>36.7</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>168.0</td>
<td>3.33 (14C)</td>
<td>24.3</td>
</tr>
</tbody>
</table>

Fig. 2. Two crystal forms of glyceraldehyde 3-phosphate dehydrogenase NAD complex from rabbit liver. The microplates grown rapidly resemble those of the muscle enzyme.

Fig. 3. Fructose diphosphate aldolase crystallized from rabbit liver.
with rats. It may be noted that one of our initial questions, namely, whether or not aldolase turns over more rapidly than the dehydrogenase in rabbit liver, is answered qualitatively by inspection of Fig. 4. Included in the figure is the calculated time curve of specific activity of the effective free lysine pool of liver. A quantitative treatment of the data is as follows.

For a protein in a steady state of turnover, the rate of change of its specific activity at any time after a pulse dose of a labeled amino acid is given by the expression

\[ \frac{dS}{dt} = k_d P_t - k_s S_t \]

where \( P_t \) and \( S_t \) are the specific activities, respectively, of the precursor pool and of the enzyme at time \( t \); \( k_d \) and \( k_s \) are, respectively, the zero and first order rate constants for synthesis and degradation, and \( C \) is the concentration of the protein. In the steady state

\[ k_s = k_d C \]

The specific radioactivity of the protein rises from 0 to a maximum and then declines as the precursor pool decays. At the maximum, \( dS/dt = 0 \) and

\[ k_d P_t = k_s C(S_t)_{max} = k_d (S_t)_{max} \]

Thus, the specific activity of the precursor pool, in appropriate units, equals the specific activity of the protein at the time that the latter reaches its maximum. Aspects of this particular relation in a general case were pointed out by Zilversmit and coworkers (11) in 1943.

In the present case, we do not use independent measurements of the specific radioactivities of the precursor pool. Direct determinations based upon measurements of free lysine isolated from deproteinized filtrates might be misleading since they would not take into account the possibilities of compartmentation. We, therefore, wished to determine pool activities as a function of time, together with the \( k \) values for the two proteins from the data in Table II and Fig. 4. The concentrations of lysine in aldolase and the dehydrogenase are 105 and 100 residues per molecule of tetramer, respectively, of molecular weight 158,000 and 144,000 (10, 12). Since the weight concentration ratio is 0.98, no correction was made for the lysine content of the proteins, and the unit of specific activity of the precursor lysine pool was taken as disintegrations per min per mg of protein, and the unit of specific activity of the precursor lymph pool as disintegrations per min per average mg of tetramer. Lysine is not readily converted to other amino acids, and hence it is justifiable, for present purposes, to count the whole proteins rather than lysine isolated from hydrolysates and to attribute the results entirely to incorporated lysine. On the basis of results obtained with rabbit muscle (5), it is assumed that the two proteins are synthesized from a common precursor amino acid pool.

One time point in the specific activity of the precursor lysine pool is given by the maximum of the dehydrogenase radioactivity curve at approximately 100 hours. The slope, \( dS/dt \), of the aldolase curve and the specific radioactivity of the aldolase are measurable at this time point, and the rate constant for aldolase degradation is given by \( k_d = (dS/dt)/(P_t - S_t) \). Using the \( k_d \) so derived, \( P_t \) may be calculated for other time points on the aldolase curve and employed in a similar way to obtain the \( k_d \) for the dehydrogenase. The \( P_t \) curve calculated from several suitable time points is plotted in Fig. 4. The derived constants are listed in Table III. The maximum of the aldolase curve is not localized by the data, but since the rate of incorporation in the dehydrogenase declines sharply at or before 54 hours, the aldolase peak activity should occur within a subsequent few hours.

Because of the persistence of label, in the precursor pool, intermediate between the specific activities of aldolase and the dehydrogenase, an exponential decay of the dehydrogenase is not observed, and the observed decay of the aldolase, retarded by recycling, is considerably slower than the calculated rate listed in Table III. Although the results are not unexpected in terms of the known slow rate of isotopic equilibration between the free lysine of liver and plasma demonstrated in rats by Swick (13), it was desirable to check the results with an amino acid that is cleared more rapidly from the intracellular pools of liver. Results obtained with [14C]leucine in aldolase are presented in Table IV. The curve log-specific activity against time is shown in Fig. 5, together with the corresponding plot of the lysine data. The apparent half-life of leucine labeled aldolase, calculated from the slope, is 1.48 days, and the corresponding uncorrected lysine value is 3.86 days. There is thus a considerable reduction in apparent half-life when leucine is em-

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**TABLE III**

<table>
<thead>
<tr>
<th>Degradation*</th>
<th>Concentration, C</th>
<th>Synthesis (( k_s = k_d C ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldolase</td>
<td>Dehydro-</td>
<td>Aldolase Dehydro-</td>
</tr>
<tr>
<td></td>
<td>genase</td>
<td>genase</td>
</tr>
<tr>
<td></td>
<td>(mg/g liver)^b</td>
<td>(mg/g/day)</td>
</tr>
<tr>
<td>0.624</td>
<td>0.247</td>
<td>0.77</td>
</tr>
<tr>
<td>0.77</td>
<td>0.36</td>
<td>0.080</td>
</tr>
</tbody>
</table>

* These correspond to half-lives of 1.11 days for aldolase and 2.8 days for the dehydrogenase.

b Average values based on catalytic activity of homogenate supernatants referred to the activity of the crystallized enzymes. The observed concentration ratios [aldolase]/[dehydrogenase] were 4 ± 1.
obtain an apparent $k_d$ ratio of 3.0 compared with the value of 1.8. At our earliest time point of 51 hours, we employed, but the number obtained remains larger than the calculated value in Table III. Leucine is reutilized but to a lesser extent than lysine.

In the early work on the turnover of the two proteins in rabbit skeletal muscle (5, 6), the relative rates were estimated from time points on the rising portions of the specific activity-time curves. If the time points are sufficiently "early," the negative term in Equation 1 may be neglected, and the total number of counts incorporated at time $t$ is given by the integral

$$\text{CS}_a = k_d \int_0^t p(t)dt$$

(4)

where $p(t)$ is the time function of the specific activity of the precursor pool. The total number ratio of two proteins, $a$ and $b$, synthesized from the same precursor pool is

$$\frac{C_a(t)_{ab}}{C_b(t)_{ab}} = \frac{(k_d)_a}{(k_d)_b} \int_0^t p(t)dt$$

(5)

From the steady state relation, the above ratio reduces to

$$\frac{(S)_a}{(S)_b} = \frac{(k_d)_a}{(k_d)_b}$$

(6)

The degradation constants and hence the half lives of the proteins, $t_1 = \ln 2/k_d$, are thus proportional to the specific activities at early time points on the rising portion of the specific activity curves. At our earliest time point of 51 hours, we obtain an apparent $k_d$ ratio of 3.0 compared with the value of 2.5 from the results in Table III. The corresponding $k_d$ ratio for the enzymes in rabbit skeletal muscle, from specific radioactivities in the rising portion of the time curve, is 1.8. Although the $k_d$ relationship of the two enzymes is preserved in liver and muscle, the $k_d$ ratio is not. As estimated from available data and Equation 2, the aldolase and dehydrogenase, in units of milligrams or micromoles per g of tissue per day, are synthesized at roughly equal rates in muscle whereas in liver the aldolase is synthesized at about 8 times the rate of the dehydrogenase. The differing concentration ratios of these two enzymes in liver and muscle are therefore determined by $k_d$.

The maintenance of the $k_d$ relationship in cell types of quite different structure and function, despite large differences in absolute enzyme concentrations and lifetimes, suggests that the $k_d$ value may be determined by intrinsic properties of the proteins in a mechanism that is quantitatively different but qualitatively similar in both tissues. If, for example, catechol action provided the degradative mechanism, aldolase would be expected to be more susceptible to proteolysis than the dehydrogenase. We have compared the inactivations of the two enzymes by lysates of rabbit liver lysosomes. The results in Table V show that the hydrolyase mixture in the absence of NAD inactivates both proteins but that in the presence of a physiological concentration level of NAD the dehydrogenase is stable but aldolase inactivation is unimpaired. The sugar phosphate substrates at 1 mM concentration have no detectable effect. These purely correlative observations are consistent with the idea that the lifetimes of some, if not all, intracellular proteins are determined by their intrinsic stabilities modulated by physiological fluctuations of stabilizing or destabilizing ligands. Independent regulation of the degradative mechanism, lysosomal or of other general types, is not excluded.

### Table IV

Specific radioactivities of fructose diphosphate aldolase of rabbit liver after pulse doses of [14C]leucine

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Dose (µCi/kg)</th>
<th>Activity (dpm/mg)</th>
<th>Normalized activity ($\text{dpm/mg} \times (\mu\text{Ci/kg})^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48.5</td>
<td>5.91</td>
<td>253.6</td>
<td>42.92</td>
</tr>
<tr>
<td>69.3</td>
<td>6.01</td>
<td>157.7</td>
<td>26.54</td>
</tr>
<tr>
<td>94.0</td>
<td>19.95</td>
<td>366.4</td>
<td>19.92</td>
</tr>
<tr>
<td>115.0</td>
<td>20.0</td>
<td>208.2</td>
<td>10.41</td>
</tr>
</tbody>
</table>

**Fig. 5.** First order decay of labeling in rabbit liver aldolase after pulse doses of [14C]lysine and [14C]leucine.

### Table V

**Inactivation of aldolase and glyceraldehyde 3-phosphate dehydrogenase by lysates of rabbit liver lysosomes**

<table>
<thead>
<tr>
<th>Inactivation</th>
<th>Aldolase</th>
<th>Dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>+NAD</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>-NAD</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>+NAD+L</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>-NAD+L</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

* Measured in 0.04 M potassium chloride, 0.06 M succinate, pH 6.5, at 37° after a 45-min incubation in the presence of 5 mM di-thiothreitol with the exclusion of oxygen. The substrate enzymes were at concentrations of 0.2 mg ml⁻¹, and the dehydrogenase was in the unresolved form containing 2 to 3 mole eq of bound NAD before dilution in the incubation mixture.

### Discussion

The measurement, by the present method, of the absolute rate constants for the synthesis and degradation of specific proteins during an in vivo steady state requires the comparison of two or more proteins of different half-life, formed from the same precursor pool. It is required that at least one of the...
proteins pass through a measurable specific radioactivity maximum following the injection of a labeled amino acid. The reference point in the present case was provided by the dehydrogenase, and a convenient experimental time scale was obtained by the use of lysine, a most extensively recycled amino acid. More experimental points during the first day would have defined a sharper maximum for aldolase since the specific activity of the aldolase lysine at 53 hours was only about half of that observed at 22 hours when the curve was already descending. For a more slowly metabolized protein, such as the dehydrogenase, a more rapidly cleared amino acid would have yielded a sharper activity maximum. Our choice of rabbits rather than rats for these experiments was motivated in part by the previous work on rabbit muscle but primarily by the amount of tissue required for the protein isolation. In our experience, the rabbits also exhibited less individual variation than rats.

A feature of the two-protein method is that it provides an indirect measure of the total course of specific radioactivity of the effective amino acid precursor pool. Subsequent to the completion of this investigation, a paper by Poole (15) appeared in which direct specific activity measurements of free leucine in rat liver were utilized to resolve conflicting apparent half-lives of liver catalase labeled with leucine and with the heme precursor, A-amino levulinic acid. Consideration of the relatively slow pool decay after the first day lowered the apparent half-life of the leucine-labeled protein from 3.5 to 1.8 days, in agreement with the results obtained with A-amino levulinic acid, which apparently satisfies true pulse labeling conditions. Because of a differing rate of leucine pool decay and a shorter half-life of aldolase, the leucine error in our experiments was smaller. The dehydrogenase results are in general accord with Poole's theoretical calculations.

In an investigation parallel with this one, Kuehl and Sumsion (16) compared the relative turnover rates of the same two glycolytic enzymes in rat liver using tritiated leucine as tracer and an immunoprecipitation method for specific protein isolation. Within relatively wide limits of error, the exponential decays corresponded to uncorrected half-lives of 5.4 days for the dehydrogenase and 4.9 days for aldolase. A constant correction factor based upon the relative decay rates of total soluble liver proteins labeled, respectively, with [guanido-14C]arginine and [3H]leucine lowered these values, respectively, to 3.1 and 2.8 days. However, the time interval examined was between 3 and 12 days, a range in which, according to Poole's calculations, differences in decay rate would tend to be obscured.

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The Synthesis and Degradation of Fructose Diphosphate-Aldolase and Glyceraldehyde 3-Phosphate Dehydrogenase in Rabbit Liver
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