Mitochondrial Turnover in Animal Cells

HALF-LIVES OF MITOCHONDRIA AND MITOCHONDRIAL SUBFRACTIONS OF RAT LIVER BASED ON [14C]BICARBONATE INCORPORATION*

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The half-lives of proteins of mitochondria and a well characterized set of mitochondrial subfractions have been estimated using two methods based on incorporation of a minimally reutilized amino acid precursor, [14C]bicarbonate. The apparent average rate constants of protein degradation were measured on rat liver homogenate, mitochondria, and distinct outer compartment, inner membrane, and matrix compartments. The half-lives were found to be 3.3 days for liver homogenate, 3.8 days for mitochondria, 2.8 days for outer compartment, 3.8 days for inner membrane, and 3.9 days for matrix. The value obtained for inner membrane is not significantly different from that of matrix, but both are significantly greater than outer compartment.

In an alternative method, [14C]bicarbonate and [3H]leucine were used together to compare the relative rates of degradation of these subcompartments. The double label procedure yielded relative half-lives which were again in the order inner membrane equal to matrix and greater than outer compartment. When the absolute values of these rates were extrapolated from the information obtained in the double and single precursor studies, the extrapolated values agreed well with those obtained by direct measurement.

The half-lives measured here in mitochondria and mitochondrial subfractions were based on [14C]bicarbonate metabolism and were lower than values reported with highly reutilized precursors. When bicarbonate was used as the first precursor in a double label study, it gave results consistent with single label incorporation studies.

Results presented here are consistent with a model of mitochondrial turnover in animal cells in which outer compartment is degraded independently of the inner compartment. The latter, consisting of inner membrane and matrix, may be degraded as a unit.

Definitive models of mitochondrial biosynthesis and degradation have yet to be established. Some controversy exists as to whether mitochondria are degraded as whole units or by independent processes acting on different mitochondrial subcomponents. Demonstration of similar half-lives for mitochondrial subfractions would suggest unit degradation, whereas evidence of a heterogeneity of half-lives implies independent processes.

Fletcher and Sanadi (1) first showed that cytochrome c and three poorly characterized liver mitochondrial subfractions had similar half-lives. Beattie et al. (2) examined mitochondria and five mitochondrial subfractions and found that all six had equivalent half-lives in liver, but not in kidney and brain, mitochondria. Similarly, Gross et al. (3) found mitochondrial DNA and some mitochondrial phospholipids to have equal half-lives in liver, but not in heart or brain. These studies suggested that in liver at least, mitochondria were degraded as complete units, although the actual measured half-life varied from study to study.

In contrast to these findings, DeBernard et al. (4) and Satav et al. (5), in unrelated approaches, found outer membrane to turn over more rapidly than inner membrane-matrix. Druyan et al. (6) and Aschenbrenner et al. (7) examined outer membrane cytochrome b and inner membrane cytochromes b, c, and heme a. The outer membrane component had the shortest half-life, while the others seemed to turnover synchronously. Indirect evidence that mitochondrial proteins may have heterogeneous half-lives was offered by Swick et al. (8) and Glass and Doyle (9), using two different procedures. Both found that the measurement of a rate constant of degradation for whole mitochondria was dependent on the length of time the study was conducted, which theoretically should not be the case for a substance with a single rate constant.

There are many reasons for the continuing lack of agreement between such studies. The major disparities are due to the artifacts caused by precursor reutilization and precursor remaining in the system for a long time. This can lead to overestimation of half-lives, apparent non-first order disappearance of label, and the creation or removal of differences in the relative rates of degradation of subfractions. Double label studies, which yield end point information without indicating the shape of the curve in between time points, are especially subject to such errors if the primary precursor is highly reutilized (10). The relationship between rates of degradation of components can also be obscured by inappropriate periods of study. If different half-lives exist in a protein population, the study must be long enough to discriminate between them. Finally, the choice of subfractions to be studied and their purity can affect the observations made.

In an effort to develop a better understanding of mitochondrial degradation, mitochondrial turnover was examined in this study using techniques which minimize the above artifacts. [14C]Bicarbonate has recently been recommended as the precursor of choice for incorporation studies in liver cells due to its pulse-like administration and very low probability of reutilization (10-13). Therefore, this precursor was used to measure the half-lives of mitochondria, outer compartment,
**Mitochondrial Turnover in Rat Liver**

inner compartment, and matrix in a single label study. These subcompartments were chosen as optimal because they are easily and reproducibly obtained, and their purity and distinctness can be characterized by biochemical and enzymological assay. Information obtained with these functional compartments can be characterized by biochemical and enzymological assay. Information obtained with these functional compartments can be differentiated according to arbitrary criteria.

The double label technique first proposed by Arias et al. (14) was employed as an alternative method of comparing the rates of degradation of mitochondrial and subcompartments, using bicarbonate as the first precursor. The study was conducted for varying lengths of time to ensure adequate discrimination between half-lives.

A comparison of the rates of degradation of outer compartment and well separated inner membrane and matrix proteins, based on [14C]bicarbonate incorporation, allows new insight into the possible models of mitochondrial degradation.

**EXPERIMENTAL PROCEDURE**

**Materials.** All chemicals were purchased from the relevant sources or were of the highest purity grade. The following enzymes were used: monoamine oxidase, ATPase, glutamate dehydrogenase, and glutamic oxaloacetic transaminase (all from Boehringer Mannheim), diaphorase, and succinate dehydrogenase (both from G. Heidelberg, Germany), and 3-[14C]amino-2-phenylpropionate (50-100 mCi/mmol) and 5-[3H]leucine (25 mCi/mmol) from New England Nuclear Corporation. 3-[14C]amino-2-phenylpropionate and 5-[3H]leucine are used as final isotopic activity of 500-1000 mCi/ml and 7 to 8. Labelled precursors were administered intragastrically in a volume of 0.5 ml and dose injected into the tail. (C57 male rats, 100-150 g were obtained from Charles River Breeding Laboratories, Wilmington, Mass.)

**Protein Preparation.** For the "bicarbonate-labeled" animal, a generous supply of liver mitochondria were isolated. In brief, the liver was homogenized in 10 vol of 0.25 M sucrose, 5 mM Tris-chloride, pH 7.4, and centrifuged at 10,000 g for 20 min. The supernatant was removed and the mitochondrial fraction was washed once with the same medium as above. The mitochondrial material was resuspended in water and centrifuged (100,000 g) for a second time. All fractions were collected as described above (21) and stored at -78°C. The "bicarbonate-labeled" material was used for the "first label" experiments.

For the "leucine-labeled" animal, the liver mitochondrial fraction was isolated from the same animal, centrifuged at 100,000 g for 1 h. The "leucine-labeled" mitochondria were added to the "bicarbonate-labeled" mitochondrial fraction to a final concentration of 50-100 mg protein per ml. The mitochondrial fraction was resuspended in water, centrifuged (100,000 g) and stored at -78°C. The "leucine-labeled" mitochondria were used for the "second label" experiments.

**Preparation of Mitochondria and Subfractions.** The "high yield" mitochondria were suspended in 0.25 M sucrose, 5 mM Tris-chloride, pH 7.4, and centrifuged at 10,000 g for 20 min. The supernatant was removed and the mitochondrial fraction was washed once with the same medium as above. The mitochondrial material was resuspended in water and centrifuged (100,000 g) for a second time. All fractions were collected as described above (21) and stored at -78°C. The "bicarbonate-labeled" material was used for the "first label" experiments.

For the "leucine-labeled" animal, the liver mitochondrial fraction was isolated from the same animal, centrifuged at 100,000 g for 1 h. The "leucine-labeled" mitochondria were added to the "bicarbonate-labeled" mitochondrial fraction to a final concentration of 50-100 mg protein per ml. The mitochondrial fraction was resuspended in water, centrifuged (100,000 g) and stored at -78°C. The "leucine-labeled" mitochondria were used for the "second label" experiments.

**ATEX indicator (18)**

**Mitochondria and subfractions were prepared and enzymes assayed as indicated under "Experimental Procedures." All measurements are given as mean ± standard deviation. No. the number of animals, is in parentheses. Results were obtained on four to eight independent experiments. Enzyme activities are expressed as nanomoles of substrate/min/mg.

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**TABLE I**

**Characterization of Mitochondria and Subfractions**

<table>
<thead>
<tr>
<th>Mitochondria and subfractions were prepared and enzymes assayed as indicated under &quot;Experimental Procedures.&quot; All measurements are given as mean ± standard deviation. No. the number of animals, is in parentheses. Results were obtained on four to eight independent experiments. Enzyme activities are expressed as nanomoles of substrate/min/mg.</th>
<th>Wet wt. (g)</th>
<th>Mitochondrial protein/wet wt (mg/g)</th>
<th>Mitochondrial protein/liver protein (mg/g)</th>
<th>Acceptor control ratio</th>
<th>Specific activities of marker enzymes in mitochondrial fraction and subfractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondria</td>
<td>1060 ± 320</td>
<td>700 ± 266</td>
<td>10.4 ± 3.2</td>
<td>6.7 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Outer compartment</td>
<td>744 ± 246</td>
<td>258 ± 83</td>
<td>43.1 ± 14.5</td>
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<td></td>
</tr>
<tr>
<td>Inner membrane</td>
<td>398 ± 134</td>
<td>1114 ± 348</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix</td>
<td>1536 ± 475</td>
<td>46 ± 25</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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*a* ND, none detected.

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1 The "Experimental Procedures" are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a magnifier. Five full-size photographs are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 81M-232, cite author(s), include a check or money order for $1.60 per set of photographs. Full size photographs are also included in the microfilm edition of the Journal that is available from Waverly Press.
mitochondrial turnover in rat liver is indicated in Table II. Arylsulfatase A, a lysosomal matrix enzyme, urate oxidase, which is a peroxisomal enzyme, and glucose 6-phosphatase, a mitochondrial enzyme, are present to different degrees in the mitochondrial fraction. Preliminary treatment of mitochondria with digitonin sufficient to remove all of these activities without disrupting the outer membrane (17) does not significantly increase the specific activities of the remaining mitochondrial enzymes (data not shown). Therefore, the total protein contributed by these organelles to the mitochondrial fraction is not believed to be significant. All of the measurable activities of these three markers found in the mitochondrial fraction were recovered in the outer compartment; inner membrane and matrix were essentially free of them.

On the basis of these three types of studies, the mitochondria used here were found to be structurally intact and subsequently well separated into three distinct subcompartments.

Determination of Rates of Degradation of Mitochondrial Fractions—The apparent rate constant of degradation, $k_d$, can be measured by administering a labeled protein precursor and following the disappearance of label from protein over time. $[^{14}C]$NaHCO$_3$ was employed as the precursor in such a single isotope incorporation study. Rats were injected with bicarbonate and sacrificed at 1, 3, 6, and 8 days. The appro-

**Table II**

| Presence of marker enzymes of nonmitochondrial origin in mitochondrial fractions |
|---|---|---|---|
| Arylsulfatase A | Urate oxidase | Glucose-6-phosphatase |
| Activity | % | Activity | % | Activity | % |
| Liver homogenate | 9 ± 1 | 100 | 1.6 ± 0.2 | 100 | 211 ± 102 | 100 |
| Mitochondria | 32 ± 6 | 44 ± 12 | 2.0 ± 0.8 | 14 ± 4 | 331 ± 188 | 26 ± 13 |
| Outer compartment | 104 ± 24 | 46 | 5.0 ± 1.3 | 16 | 887 ± 170 | 25 |

Fig. 1. Determination of rates of degradation of liver homogenate and mitochondrial fractions with $[^{14}C]$bicarbonate. Rats were injected intraperitoneally with 250 μCi of $[^{14}C]$NaHCO$_3$ in 0.25 ml. Three animals were killed at 1, 3, 6, and 8 days. Fractions were prepared and radioactivity of protein measured as indicated under "Experimental Procedures." $k_d$ and $T_{1/2}$ were calculated by linear regression analysis as indicated under "Experimental Procedures." A, liver homogenate; B, mitochondria; C, outer compartment; D, inner membrane; E, matrix.
private fractions were isolated at each time point. The perchloric acid-precipitable radioactivity was measured on liver homogenate, mitochondria, outer compartment, inner membrane, and matrix. Results are presented graphically in Fig. 1, A-E. $k_d$ was determined by linear regression analysis.

The decrease in In (specific radioactivity) appears linear over this time interval. The $k_d$ for inner membrane is not significantly different from that of matrix. The $k_d$ of outer compartment is different from both the $k_d$ of inner membrane ($p < 0.01$) and that of matrix ($p < 0.05$).

For a heterogeneous mixture of proteins of varying half-lives, the apparent or average $k_d$ for the mixture can be calculated from the following (28):

$$k_d = \frac{\sum k_m}{M}$$

(4)

where $n =$ number of proteins, $k_i =$ individual $k$, $m =$ mass of individual protein, and $M =$ total protein mass. It has been estimated that the mitochondrial protein mass is 15% outer compartment, 18% inner membrane, and 67% matrix (29). Substitution of these values and the respective measured $k_d$ values into Equation 4 yields a $k_d$ of 0.192, which compares well with the value of 0.185 measured here for total mitochondrial protein.

**Determination of Relative Rates of Degradation**—For the double label method to have validity, it must be shown that both precursors are incorporated initially in the same ratio, for all fractions, in the absence of protein degradation. Two fractions may incorporate a precursor at different specific activities; but the ratio of incorporation of two different precursors must be the same for all fractions examined. In other words, all fractions must have equal accessibility to both precursors. Fractions must not be able to discriminate between either the isotopic labels or the precursors. When different precursors are used together, purified proteins may have unequal initial ratios depending on their amino acid compositions. However, such a cause for difference is not likely when comparing mixtures of many proteins because the mixtures would be expected to have the same average amino acid composition.

When [3H]leucine and [14C]bicarbonate were injected simultaneously, the ratios of isotopes incorporated into protein were as shown in Table III. The animals were sacrificed at $1/2$ h to avoid significant degradation of labeled protein. Analysis of variance revealed no significant difference between ratios of incorporation of isotopes by any fraction ($p(F) > 0.1$). When the same experiment was performed and animals were sacrificed at $1 1/2$ h, there was still no difference (not shown). The final ratio obtained was solely a function of the initial ratio of injected precursors. Thus, it was established that subsequent differences in ratio between fractions would be due solely to differences in their $k_d$ values and not to incorporation differences caused by transport or other phenomena.

**Comparison of Relative Rates of Degradation of Mitochondrial Subfractions with the Double Label Method**—With the double label method, optimal differentiation between protein half-lives is attained when the interval between administrations of precursors is at least one half-life of the protein in question (27). The data obtained here (Fig. 1) indicated half-lives in the range of 2.5–3.5 days; others have found mitochondrial half-lives of 5–7 days (7, 9). In order to allow for either range, precursors were administered over intervals of 3.5, 6, and 9 days.

Table IV shows the ratios obtained from an initial injection of [14C]bicarbonate, followed at 5, 6, or 9 days with [3H]leucine. The order of ratios of the fractions and, therefore, of their $k_d$ values was the same at each interval. The In (3H/14C) of liver homogenate was greater than that of outer compartment, which was always greater than that of mitochondria, which was greater than those of inner membrane and matrix. The In (3H/14C) of these last two appeared equivalent. This relative order of $k_d$ values was in good agreement with the $k_d$ values determined from the data in Fig. 1, except that liver homogenate showed a larger $k_d$ than outer compartment with the double label method for unknown reasons.

Glass and Doyle (9) demonstrated a linear relationship between "ln R," where $R$ is the ratio of isotope incorporation, and the measured $k_d$ for many liver cell components. They suggested that a "standard curve" could be composed of known $k_d$ values versus experimental "ln R." The slope of this line equals the proportionality constant in Equation 3. Given any component's "ln R," its $k_d$ could be extrapolated from the curve.

In order to normalize the data in Table IV and to see how well calculated $k_d$ values would compare with measured $k_d$ values (Fig. 1), such a curve was constructed. For a single liver, the In (3H/14C) values found for liver homogenate and mitochondria were plotted against their respective $k_d$ values of 0.208 and 0.185. The In (3H/14C) for each of the subfractions was then plotted and the corresponding $k_d$ values extrapolated from the line. An example is shown graphically in Fig. 2 for a single liver from a 5-day interval. This analysis was repeated on the ratios obtained from each of the six livers shown in Table IV. The actual calculations were made from a line determined by linear regression, rather than graphic analysis. The results of such transformations are presented in Table V. There was excellent agreement between directly measured

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**Table III**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>$^{3}_H/^{14}_C$</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{3}_H$</td>
<td>$^{14}_C$</td>
</tr>
<tr>
<td>Liver homogenate</td>
<td>1.00 ± 0.10</td>
<td>1062 ± 61</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>1.00 ± 0.05</td>
<td>400 ± 28</td>
</tr>
<tr>
<td>Outer compartment</td>
<td>1.00 ± 0.05</td>
<td>730 ± 33</td>
</tr>
<tr>
<td>Inner membrane</td>
<td>1.00 ± 0.05</td>
<td>322 ± 17</td>
</tr>
<tr>
<td>Matrix</td>
<td>1.00 ± 0.05</td>
<td>326 ± 40</td>
</tr>
</tbody>
</table>

**Table IV**

Administration of precursors at varying intervals

Each of six rats received 500 pCi of [14C]NaHCO$_3$ in 0.5 ml. After the indicated interval, 60–70 pCi of [3H]leucine in 0.5 ml were injected and the rats were killed $1/2$ h later, except in the 5-day interval at $1/2$ h. Fractions were prepared and In (3H/14C) calculated as indicated under "Experimental Procedures." Each column contains values from a single animal.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>ln ($^{3}_H/^{14}_C$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 days</td>
</tr>
<tr>
<td></td>
<td>6 days</td>
</tr>
<tr>
<td></td>
<td>9 days</td>
</tr>
<tr>
<td>Liver homogenate</td>
<td>1.49 ± 0.26</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>0.99 ± 0.14</td>
</tr>
<tr>
<td>Outer compartment</td>
<td>1.23 ± 0.24</td>
</tr>
<tr>
<td>Inner membrane</td>
<td>0.71 ± 0.09</td>
</tr>
<tr>
<td>Matrix</td>
<td>0.70 ± 0.08</td>
</tr>
</tbody>
</table>
and extrapolated values for the $k_d$ values of inner membrane and matrix. Outer compartment had a slightly lower $k_d$ by extrapolation, but was still significantly greater than either of the other two subcompartments. Furthermore, the same $k_d$ was obtained for each fraction regardless of whether the interval between precursors was 5, 6, or 9 days. Thus, results obtained with the double label method agree well with data obtained after administration of $[^{14}C]$bicarbonate alone.

**DISCUSSION**

We have attempted to minimize the anomalies of many mitochondrial turnover studies by using well separated and characterized mitochondria and mitochondrial subcompartments and $[^{14}C]$bicarbonate as the protein precursor. Bicarbonate is rapidly incorporated into liver cell proteins via the urea cycle. Little is recycled from other tissues. Arginine, glutamate, and aspartate, the amino acids into which bicarbonate is predominantly incorporated, are minimally reutilized by animals on complete diets (13, 30). Therefore, this precursor should yield shorter half-lives than those measured with most amino acids.

The half-life of 3.3 days measured here for liver proteins of homogenate is in agreement with the 2.8–2.9 days measured by Chee and Swick (31) with $[^{14}C]$NaHCO$_3$, but lower than the 5 days obtained with $[^{14}C]$leucine (9). We obtain a mitochondrial half-life of 3.7 days, considerably shorter than the 10 days found with $[^{38}S]$methionine (1), or 7–10 days with $[^{3}H]$leucine (2, 5, 7), and slightly below the 5–7 days measured with $[^{14}C]$guanidinoarginine (7, 14).

For a mixture of proteins degraded at different rates, but exhibiting “pseudo-first order” disappearance of tracer, the slope of decay curve should equal the average $k_d$ for the whole population. The value measured experimentally may differ from the true average, depending on the length of the study period and the rate of utilization of the label. The interval at which the experimentally measured “average $k_d$” best equals the true average $k_d$ has been determined empirically by Garlick et al. (28). It is the time needed for label incorporation to decrease to 10% of the initial value of 3–4 half-lives of the mixture. This is only true for systems in the steady state, where tracer is administered in a pulse and minimally reutilized. We believe these conditions have been met closely as is currently possible by using $[^{14}C]$bicarbonate. The $k_d$ values measured here should be very close to the true average $k_d$ of the whole protein population of each subcompartment.

In the present study, the outer compartment ($T_{1/2} = 2.8$ days) was found to be degraded significantly more rapidly than the matrix ($T_{1/2} = 3.9$ days) or the inner membrane ($T_{1/2} = 3.8$ days). This agrees qualitatively with DeBernard et al. (4), who did not separate matrix from inner membrane, and with Druyan et al. (6), who found that outer membrane cytochrome $b_5$ had a half-life of 4.4 days, while inner membrane cytochromes $b$ and $c$ had half-lives of 5–6 days.

We find the rate of degradation of inner membrane proteins to be no different from that of the matrix proteins. These compartments were shown to be well separated by several criteria and not detectably contaminated by other marker enzyme activities. This finding is in agreement with that of Gear (32), who in a careful study of mitochondrial enzyme activities during liver regeneration suggested synchronous turnover of matrix and inner membrane.

All of the above results were confirmed when degradation was measured in an alternate manner, using the double label method first proposed by Arias et al. (14). This technique was modified to allow the use of two different precursors, $[^{14}C]$bicarbonate and $[^{3}H]$leucine, because of the advantages already cited. The same conditions initially described (10, 14, 27) must be met, as follows.

1) The system should be in a steady state for the length of the experiment and the proteins examined must be undergoing “first order” degradation. 2) The time interval chosen, $t$, must be one to three half-lives of the most rapidly turning over component for maximal discrimination. 3) The first precursor must be administered as a pulse relative to the interval of the experiment and must not be reutilized. 4) Measurements should be made shortly after the administration of the second precursor when its incorporation is at or approaching maximal. 5) The $\ln (P_2/P_1)$ must be determined when $t = 0$, i.e. when both precursors are administered simultaneously and must be the same for all fractions.

Liver metabolism was assumed to be in a steady state during the intervals of observation; however, Zak et al. (27) have shown that even when the protein pool is increasing by 20% per day, the double label ratio change is small. The time intervals of 5, 6, and 9 days were chosen to allow maximal discrimination between fractions with half-lives on the order of 2–6 days, which is the range predicted by the majority of reports. The longer interval is especially necessary to ensure that the similarity between the matrix and inner membrane ratios is not an artifact. Bicarbonate is maximally incorporated within 1 h after injection (13, and data not shown), and its low probability of reutilization has been discussed. $[^{3}H]$Leucine is also maximally incorporated within 40–60 min (33), after which it remains at a plateau for some time. Except for the 5-day interval study, the animals in these experiments were sacrificed ½ h after leucine administration. The equivalence.
of incorporation of both precursors by all fractions has been demonstrated in Table III. The importance of this control is emphasized since different precursors were used. This control is often omitted from double label studies.

Results from the double label method agree qualitatively and quantitatively with those obtained from the single label study, with the exception of the outer compartment. The $k_d$ for outer compartment predicted by the double label extrapolation is slightly lower than that directly measured.

Some information on mitochondrial degradation is suggested by these studies. In the absence of significant recycling, and with a pulse administration, first order disappearance of tracer from a system implies random removal. If mitochondria were degraded according to "life span" kinetics, the curve of ln (specific radioactivity) versus time would ideally plateau and then slope down, although this can be complicated by reutilization artifacts (for review see Ref. 10). Mitochondria, outer compartment, matrix, and inner membrane plots (Fig. 1) all indicate apparent random disappearance of tracer, suggesting random breakdown of mitochondria. This in turn suggests that either all mitochondria are equivalent, or, if there are "younger" mitochondria, they are as likely to be degraded as "older" mitochondria. In either case, mitochondrial degradation may be more a function of positional differences within the cell (e.g. proximity to a lysosome), rather than mitochondrial quality or size. The outer compartment may be turned over more rapidly than the remainder of the mitochondrion because its accessibility renders it more subject to "attack." The shorter half-life of outer compartment also suggests the action of different or additional degradation processes specific for this subcompartment.

Acknowledgments—We are grateful to T. Baroody for technical assistance, Dr. J. Lipsky for statistical analyses, and Drs. K. Schwerzmann, J. Wehrle, and M. Amzel for helpful discussions.

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