The Turnover of Mitochondria in a Variety of Tissues of Young Adult and Aged Rats*

ROBERT A. MENZIES
From the Laboratory of Cellular Biophysics and Aging Research, Department of Biochemistry, Louisiana State University Medical Center, New Orleans, Louisiana 70119

PHILIP H. GOLDF
From the Laboratory of Cellular and Comparative Physiology, Gerontology Research Center, National Institute of Child Health and Human Development, National Institutes of Health, Baltimore City Hospitals, Baltimore, Maryland 21224

SUMMARY

Among the most controversial hypotheses of aging are those which involve the progressive accumulation of error-bearing or altered macromolecules with advancing age. The effect of low levels of error or of alterations in only one or a small number of the many macromolecular mitochondrial components might be amplified by the highly integrated process of mitochondrial biosynthesis and observed as a change in turnover rates. The turnover rates of mitochondria from a variety of tissues of young adult (12-month-old) and aged (24-month-old) rats were measured by following the loss of radioactivity from proteins of purified mitochondrial preparations after initial labeling with 3H-leucine. Mitochondria from liver, brain, heart, and testes appeared to lose label as a homogeneous class with respect to rate. However, mitochondria from kidney, lung, and intestinal mucosa exhibited at least one additional exponential component. No significant differences were found for any tissue between the two age groups. Estimates for the half-lives in days obtained by combining both young and old sets of data are: liver, 9.3; testes, 12.6; heart, 17.5; brain 24.4; small intestine, 0.7 (first) and 17.6 (second); lung, 4 (first) and 16.6 (second); and kidney, 5 (first) and 10.9 (second). It is concluded that these data do not support the concept that errors in macromolecules are accumulated with age.

A number of hypotheses have been advanced to explain the process of biological aging. Among the most intriguing are those which involve the introduction of errors (1-3) or other alterations (4-6) into the transcriptional, translational, or other steps leading to protein synthesis. The most likely type of error is generally thought to be the substitution of an incorrect amino acid for a correct one. The result of such changes would be the formation of macromolecules with reduced or modified biological activity. Wulff et al. (1, 2) have proposed that the progressive accumulation of these error-laden macromolecules with age results in increased RNA and protein turnover to compensate for the functional impairment. Since nearly all cellular particulates exist in a dynamic state of continual degradation and renewal, a route for the incorporation of error-laden macromolecules into them is readily available. Comparison of the turnover rates of a component at different ages of the animal can be used to evaluate the long term stability of the mechanisms controlling its synthesis and degradation. Mitochondria are discrete organelles composed of many groups of diverse macromolecules which must be synthesized and assembled in an ordered relationship to obtain structural and functional integrity. The effect of low levels of error or of alterations in only one or a small number of these macromolecular components might be amplified by the highly integrated process of mitochondrial biosynthesis and observed as a change in turnover rate.

The first report of the measurement of mitochondrial turnover was that of Fletcher and Sanadi (7). They found that four protein and lipid fractions of rat liver mitochondria had almost identical turnover rates and concluded that liver mitochondria turn over as a unit. Subsequently, other laboratories reported data indicating some heterogeneity in the turnover of mitochondrial components in several tissues which raised questions concerning the validity of the unit turnover concept (8-11). However, recent work from a number of laboratories suggests that, although a portion of the mitochondrion, particularly the outer membrane and a number of inducible enzymes, may turn over independently, the inner membrane and associated matrix, which accounts for most of the particle mass, appears to turn over as a unit (12-18). Thus bulk-labeling turnover studies would be expected to reflect the stability of this organelle.

Sanadi and Fletcher (19) have examined the effect of age on mitochondrial turnover and found no large differences. However, their studies were limited to the liver and included only three time points. This paper is a report of a survey study in which we compared the turnover of bulk mitochondrial protein...
from a variety of tissues containing primarily postmitotic cells with those containing large continually renewing cell populations. The effect of senescence was assessed by making these comparisons in young adult (12-month-old) and old (24-month-old) rats.

A preliminary account of part of this work has appeared (20).

**EXPERIMENTAL PROCEDURE**

The animals used in these experiments were male Wistar rats bred and maintained in the animal colony of the Gerontology Research Center (Baltimore). They were fed water and Purina laboratory chow ad libitum. After overnight fasting, 14 young mature adult (12-month-old) rats averaging 459 g and 17 old (24-month-old) rats averaging 471 g were injected intraperitoneally with 0.5 mCi/100 g body weight of 4,5-3H-L-leucine, 18.3 Ci per mmole (Nuclear-Chicago). On a given day, a single animal from each group was killed by decapitation at intervals from 1 to 37 days after administration of the isotope.

The tissues were rapidly excised, rinsed, and chilled in ice-cold 0.9% (w/v) NaCl. They were then individually blotted dry, weighed, and processed as follows. All operations were done at 0° C.

The liver, kidneys, brain, and testes were minced with scissors and homogenized in 5 to 10 volumes of Medium H (0.25 m sucrose, 10 mM Tris-HCl buffer, pH 7.8, and 1 mM MgCl2) in a Potter-Elvehjem type homogenizer fitted with a Teflon pestle. The homogenates were centrifuged at 800 x g for 5 min to remove unbroken cells, nuclei, and cell debris. The pellets were resuspended in 1% (w/v) sodium dodecyl sulfate and assayed for protein by the method of Lowry et al. (21). An aliquot of the purified mitochondrial suspension was solubilized in 1% (w/v) sodium dodecyl sulfate and assayed for protein by the method of Lowry et al. (22) and for radioactivity in a Beckman LS 250 liquid scintillation spectrometer. In some cases, aliquots of the mitochondrial suspensions were precipitated with 0.5 m perchloric acid and extracted twice with 0.2 m perchloric acid and twice with chloroform-methanol (2:1) v/v. The remaining residue had the same specific activity on a protein basis as did the intact mitochondria.

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The regression lines were calculated by using all of the points. Each data point represents an individual animal. The regression lines were calculated by using all of the points. Each data point represents an individual animal. The regression lines were calculated by using all of the points. Each data point represents an individual animal. The regression lines were calculated by using all of the points.


**RESULTS**

The decline of radioactivity (disintegrations per min per mg of protein) of the mitochondrial protein from the various tissues was plotted on a logarithmic scale as a function of time (Figs. 1 to 7). The solid lines are derived from a least squares fit (23) on

![Graph](image)

**TABLE I**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Component</th>
<th>Age group</th>
<th>( k' ) ± 95% confidence limit</th>
<th>Initial specific activity</th>
<th>Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td>Young</td>
<td>(-0.077\ ± 0.0094) 78,700*</td>
<td>9.0</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.073\ ± 0.0067) 79,200*</td>
<td>9.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Testes</td>
<td></td>
<td>Young</td>
<td>(-0.063\ ± 0.0003) 69,200*</td>
<td>11.0</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.052\ ± 0.0072) 65,100*</td>
<td>13.4</td>
<td>13.4</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>Young</td>
<td>(-0.042\ ± 0.0006) 16,300*</td>
<td>18.3</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.038\ ± 0.0047) 17,300*</td>
<td>18.3</td>
<td>18.3</td>
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<tr>
<td>Brain</td>
<td>First</td>
<td>Young</td>
<td>(-0.026\ ± 0.0061) 25,000*</td>
<td>26.8</td>
<td>26.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.030\ ± 0.0073) 27,600*</td>
<td>23.5</td>
<td>23.5</td>
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<tr>
<td>Intestinal mucosa</td>
<td>First</td>
<td>Young</td>
<td>(-0.099\ ± 0.0517) 955,000*</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.080\ ± 0.0204) 549,000*</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>Young</td>
<td>(-0.060\ ± 0.0204) 18,100*</td>
<td>23.5</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.042\ ± 0.0322) 22,100*</td>
<td>16.7</td>
<td>16.7</td>
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<tr>
<td>Lung</td>
<td>First</td>
<td>Young</td>
<td>(-0.17\ ± 0.0422) 36,700*</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.17\ ± 0.0204) 42,300*</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>Young</td>
<td>(-0.054\ ± 0.0005) 36,300*</td>
<td>13.6</td>
<td>13.6</td>
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<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.040\ ± 0.0202) 30,700*</td>
<td>17.3</td>
<td>17.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>First</td>
<td>Young</td>
<td>(-0.11\ ± 0.0422) 41,400*</td>
<td>6.3</td>
<td>6.3</td>
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<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.12\ ± 0.0204) 42,900*</td>
<td>5.6</td>
<td>5.6</td>
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<tr>
<td></td>
<td>Second</td>
<td>Young</td>
<td>(-0.068\ ± 0.0116) 59,800*</td>
<td>11.6</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>(-0.064\ ± 0.0079) 86,600*</td>
<td>10.8</td>
<td>10.8</td>
</tr>
</tbody>
</table>

* Calculated from \( k' \).

+ Calculated from the regression equation.

+ Estimated graphically.

**TABLE II**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>( k' ) ± 95% confidence limit</th>
<th>Average half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>(-0.0742\ ± 0.0050) 78,700*</td>
<td>9.3</td>
</tr>
<tr>
<td>Testes</td>
<td>(-0.0551\ ± 0.0060) 69,200*</td>
<td>12.6</td>
</tr>
<tr>
<td>Heart</td>
<td>(-0.0396\ ± 0.0040) 17,300*</td>
<td>17.8</td>
</tr>
<tr>
<td>Brain</td>
<td>(-0.0283\ ± 0.0050) 17,300*</td>
<td>24.4</td>
</tr>
<tr>
<td>Intestinal mucosa</td>
<td>(-0.040\ ± 0.0020) 25,000*</td>
<td>18.3</td>
</tr>
<tr>
<td>Lung</td>
<td>(-0.042\ ± 0.0014) 27,600*</td>
<td>18.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>(-0.0637\ ± 0.0067) 86,600*</td>
<td>10.8</td>
</tr>
</tbody>
</table>

* Calculated from \( k' \).

+ Calculated from the regression equation.

+ Estimated graphically.
either all of the experimental points (Figs. 1 to 4) or on all of the points from Day 14 onward (Figs. 5 to 7).

No statistically significant differences were found in the apparent decay constants for any tissue between the young (12-month) and old (24-month) age groups (Table I). In addition, with the exception of the first component of the intestinal mucosa and the second component of the kidney, no large differences were observed in the initial specific activities between age groups in any tissue. Since the initial specific activity is obtained from the intercept at the ordinate on a logarithmic scale, its value is very sensitive to biological variability and experimental error. This generally good agreement between the parent decay constants for any tissue between the young (12-month) and old (24-month) age groups suggests that transport rates or barriers, leucine pool sizes, and protein synthetic rates were essentially the same in both. It was therefore considered appropriate to combine both young and old sets of data to calculate a value for $k'$ for a given tissue (Table II).

**Discussion**

**Patterns of Mitochondrial Turnover**—From a comparison of Figs. 1 to 4 with Figs. 5 to 7 it is readily apparent that two general patterns of mitochondrial decay were observed. Mitochondria from liver, brain, heart, and testes appeared to lose label as a homogeneous class with respect to rate. However, mitochondria from kidney, lung, and intestinal mucosa exhibited at least one additional exponential component.

In the intestinal mucosa, the short lived component with a half-life of about 0.7 day (Fig. 5, Table I) is probably the result of epithelial cell turnover and not mitochondrial turnover per se. Values for the half-life of this class of cells average about 1 day (24, 25). The remainder of the loss of label is probably associated with $bona fide$ mitochondrial turnover in the muscle and connective tissue cells of the lamina propria.

A similar situation is evident in the case of the lung (Fig. 6). Here, the short lived component has a half-life of 4 days, which is comparable to the half-life of alveolar cells (26). The long lived component has a half-life of 16.6 days. This is nearly the same as the 17.6 day half-life found for the long lived component in the intestinal mucosa and probably also represents the turnover of mitochondria of muscle and connective tissues.

The mitochondrial fractions from bone marrow and spleen (data not shown) also gave decay curves of the multiple exponential type. In both tissues, the data suggested the presence of at least three components with half-lives ranging from about 1 day to 30 days. The majority of the loss of label was associated with the short lived components and probably represented cellular turnover. No obvious age-associated differences were evident. There was, however, too much variation to warrant further treatment.

Double exponential kinetics seems also to be the case for kidney mitochondria (Fig. 7). This cannot be explained by cellular turnover since Leblond (25) has pointed out that the cells of the kidney turn over very little, if at all. On the other hand, it is possible that the biphasic curve represents two different mitochondrial populations in the kidney. However, Beattie, Basford, and Koritz (8) have reported a water-soluble protein fraction from kidney mitochondria with a half-life of 5.96 days. This was significantly different from the half-life of 8.65 days found for the whole mitochondrion. The value that we obtained for the short lived component is about 6 days (Table I). Thus it appears that in this case the double exponential curve may represent the resolution of mitochondrial components.

Mitochondria from liver, heart, brain, and testes all gave single exponential decay curves. In the liver, heart, and brain, the turnover rates (Table II) observed are in general agreement with most of the values reported in the literature (7, 8, 11-15).

In the testes a half-life of 12.6 days was found (Table II). This probably represents $bona fide$ mitochondrial turnover in spermatogonial, Sertoli, and interstitial cells. However, a contribution due to cell turnover cannot be ruled out. That the sperm sedimented at 1000 $\times$ g in the crude nuclear pellet was confirmed by phase contrast microscopic examination. Except for a few broken tails, there was little or no sperm contamination of the mitochondrial fraction.

**Significance of Apparent Turnover Constant ($k'$)**—Swick, Rexroth, and Stange (10) studied liver mitochondrial turnover by continuously feeding $^{14}$C-carbonate to rats and subsequently isolating the labeled arginine from hydrolysates of the various mitochondrial protein fractions. Because of the high arginine activity of liver, direct reutilization of arginine radioactivity is virtually avoided. The half-lives found were from about 5 to 6 days. Upon labeling heme with $^{14}$C-$\delta$-aminolevulinic acid, Druyan, DeBernard, and Rabinowitz (18) found that rat liver mitochondrial cytochromes $b$ and $c$ had half-lives of 5.5 and 6.1 days. In view of these findings, our values for $k'$ for liver may be underestimated by 30% or more. The values for $k'$ for the other tissues may also be underestimated depending upon the degree of reutilization of labeled leucine in each case. This would also be true for the turnover rates reported by others (8, 15). On the other hand, Flechter and Sanadi (7) used $^{14}$S-methionine and $^{14}$C-acetate to label the proteins and lipids, respectively, of liver mitochondria and found half-lives similar to ours. These values are also virtually identical with those found by Gross, Getz, and Rabinowitz (12) for the turnover of liver mitochondrial DNA and several phospholipids. In those experiments $^{3}$H-thymidine and $^{32}$P$_i$ were used to label the DNA and $^{32}$P$_i$ to label the lipids. These observations do not eliminate recycling of label as a major complicating factor in the interpretation of turnover studies. However, for recycling to be a major factor requires that five different compounds, leucine, methionine, acetate, thymidine, and $P_i$, all must be recycled to about the same extent.

**Effect of Age on Turnover**—The physiological state of the animal might be expected to have important effects on the turnover rates of cellular components. In this regard the differential induction of several mitochondrial enzymes has been documented (27-29). Hirsch and Hiatt (30) have shown that fasting increases the degradative rate and decreases the synthetic rate of rat liver ribosomes. A high protein diet and the administration of prednisolone have both been shown to increase the turnover of liver mitochondrial ornithine aminotransferase and alanine aminotransferase (10), as determined from changes in enzymatic activity. In this paper we have examined the effect of age as a physiological state which may modify the metabolic stability of the mitochondrion. Since senescence or aging involves a general deterioration of the physiological capabilities of the animal, one might expect this to be reflected at the cellular and subcellular levels.

The essence of the error theory (1-3) is that, as the animal undergoes senescence, there is a progressive decrease in the competence and precision of the transcriptional, translational, or other steps involved in protein synthesis. This leads to the progressive accumulation of a pool of error-laden macromolecules with impaired functional capability. Orgel (3) has presented
arguments showing that the effects of errors in RNA and protein involved in metabolic roles other than the processing of genetic information would be neither permanent nor cumulative. Degradation of the offending molecules would remove the errors and all memory would soon be gone. However, errors that lead to altered specificity in an information-handling process would be cumulative and self-accelerating, and would eventually lead to an error crisis threatening the viability of the cell.

If increasing amounts of error-bearing macromolecules are being synthesized in old animals, it would be expected that, although some of them might be completely nonfunctional, a substantial portion would be incorporated into their functional units, in this case, the mitochondria. This would lead to a lowering of the average functional capability per unit of mitochondrial mass. As the cell responds to this decrement, increased synthesis would soon follow to maintain the required functional levels of mitochondria. Increased degradation would then be necessary to re-establish the steady state and prevent the accumulation of large quantities of material. One would therefore predict substantial increases in either turnover or concentration or substantial decreases in functional capability.

Sanadi and Fletcher (9) examined the turnover rates of liver mitochondria from mature adult (12-month-old) and senescent (20- to 22-month-old) rats and found no significant difference between the two age groups. Until now this was the only study reported which considered the effect of age on the metabolic stability of the mitochondrion. Their observation is now confirmed by us and extended to mitochondrial turnover from a variety of functionally dissimilar tissues such as heart, brain, testes, kidney, lung, and intestinal mucosa. It should be noted, however, that the sensitivity of these experiments is such that small differences would not have been statistically significant. On the other hand, one is struck by the relatively small differences between most young and old pairs of turnover constants. We take this to be indicative of our accuracy in estimating a mean turnover rate. We also consider the relatively constant 95% confidence limit to be an estimate of biological variability. For a difference in rate to be physiologically important one would expect it to be greater than the biological variability. Thus it is reasonable to conclude that there is no important difference in the turnover rates between young and old animals.

On the basis of the error theory, as noted above, one predicts an increase in mitochondrial concentration in lieu of changes in turnover rates. Barrows et al. (31, 32) have reported a decrease of about 10% in the succinoxidase activity of kidney and heart homogenates from senescent rats, which they interpret, in the kidney at least, as a reduction in mitochondrial numbers. No changes were seen in the liver. Tauchi et al. (33, 34) have reported that in senescent rat liver the mitochondria per hepatic cell were decreased in number and increased in size without any special qualitative change in ultrastructure. In a previous study from this laboratory (35), mitochondria isolated from rat liver, heart, and kidney showed no significant age-associated decreases in the efficiency of oxidative phosphorylation, control of respiration by ADP, or the maximal oxygen consumption that could be stimulated by ADP. Thus the functional capability of the mitochondria remains intact.

From the foregoing, it is clear that, if the error theory is operative in the tissues studied, there appears to be no measurable compensatory response by the cell at the level of protein synthesis and function. At best there is a hint of deterioration. These observations do not completely rule out the error theory, since both the occurrence of errors and compensation for them may take place at the transcriptional level. We are presently examining this possibility.

REFERENCES

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