CYTOCHROME C TURNOVER IN SKELETAL MUSCLE

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SUMMARY

The half-life of skeletal muscle cytochrome c has previously been found, by the use of [^{14}C]delta-aminolevulinate, to be approximately 30 days. This turnover value has been re-evaluated by a procedure that does not involve tracer techniques. Transition curves of an approach to steady-state cytochrome c concentrations were determined during: 1) a return from elevated levels following cessation of exercise training, and 2) the decline in cytochrome c following thyroidectomy. The half-lives obtained, 8.1 days and 10.7 days respectively, indicate that this mitochondrial component of skeletal muscle turns over at rates very similar to liver and cardiac muscle.

The half-life of skeletal muscle cytochrome c was recently reported to be approximately 30 days. These data were determined by the loss of [^{14}C]delta-aminolevulinate labeled cytochrome c as a function of time in both growing (1) and nongrowing rats (2). This half-life for skeletal muscle cytochrome c contrasts with the much shorter half-lives obtained by this procedure for liver (3) and cardiac muscle (4). Nonetheless, labeled delta-aminolevulinate has been considered a valuable tracer for cytochromes since it is specifically incorporated into heme (3) and considered not to be reincorporated (5). Furthermore, the use of [^{3}H]delta-aminolevulinate has produced the shortest half-lives for mitochondrial cytochromes in liver and heart muscle (3,4). The half-life of approximately 30 days for skeletal muscle was, therefore, accepted with some confidence. This report, however, describes estimates of skeletal muscle cytochrome c half-life that is considerably shorter than previously reported. The half-life of cytochrome c in mixed skeletal muscle of the rat is found to be approximately 8 days.

METHODS: The turnover time for cytochrome c was determined by following the time course of concentration change during the transition from one

steady-state to another as described by Schimke and Doyle (6). Two independent transition curves were determined. In the first case, a return to normal levels from an elevated cytochrome c content established by prior exercise training was followed. Thirty-four animals (Carworth CFN rats) that were previously trained by treadmill running (1 hr/d, 1 mph, 10% grade, 5 d/wk for 19 wks) were sacrificed at 6 timed intervals after cessation of activity. Treadmill running of this type for 1 hr/d increases the oxidative capacity of the working skeletal muscle by approximately 50% (7). In the second case, the decline from normal levels of cytochrome c following thyroidectomy was determined. Loss of normal thyroid function results in a decrease in oxidative capacity of skeletal muscle to about one-half normal (8,9). For this curve, 29 thyroidectomized rats (Hormone Assay) were sacrificed at timed points during 46 days post-surgery.

Cytochrome c was isolated from the gastrocnemius-plantaris muscle group and quantitatively determined by a modification of the procedure of Druyan et al. (3) as described previously (10). This procedure results in a near quantitative extraction and recovery of purified cytochrome c (80% overall yield). The cytochrome c is free from other cytochromes and yields an oxidized minus reduced absorbance spectrum identical to that reported by Margoliash and Frohwirt (11).

The data were analyzed by a first-order kinetic model (6) using a nonlinear least-squares procedure. The half-life was calculated from the relationship: $t_{1/2} = \ln 2/k$, where k = the derived slope.

RESULTS: The change in cytochrome c concentration for both of the treatments (Figure 1) followed a first-order pattern as expected of degradation processes (6). The least-squares line of best fit for the transition data after cessation of training (Figure 2A) has a slope of 0.085 day^{-1} ($r = -.991$) which corresponds to a half-life of 8.1 days. The predicted cytochrome c concentrations calculated from the line deviated from the observed values by an average of only 1.67% (-1.96% to +4.10% range). The least-squares line of

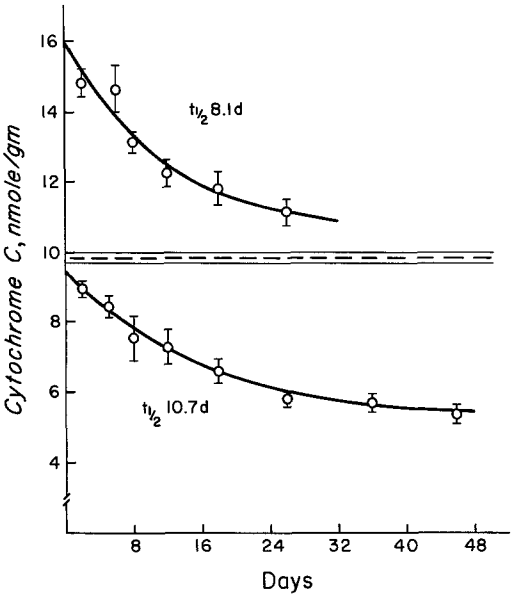

Figure 1. The change in cytochrome c concentration following cessation of exercise training (upper curve) and following thyroidectomy (lower curve). The center dotted line is the mean cytochrome c value for normal animals taken during these studies (N=50). There are approximately 6 and 4 animals per point for the upper and lower curves, respectively. All values are expressed as X ± S.E.

best fit for the transition following thyroidectomy (Figure 2B) had a slope of 0.065 day^{-1} (r = -.986) which corresponds to a half-life of 10.7 days. The predicted cytochrome c concentrations calculated from this line deviated from the observed values by an average of only 1.73% (-2.96% to +2.39% range).

DISCUSSION: The procedure of assessing cytochrome c turnover by following the transition in concentration assumes that upon initiation of the treatment the synthesis rate and degradation rate constant are unchanged throughout the transition period (6). If, however, the treatment alters the synthesis rate or degradation rate constant, it must do so in a manner that is rapid relative to the half-life. From the quality of the first-order curve fit for each transition (i.e., correlation coefficients of .991 and .986) it is probable that both above mentioned conditions were effectively met. Since the factor(s) that elevated cytochrome c levels during exercise training are presumably no

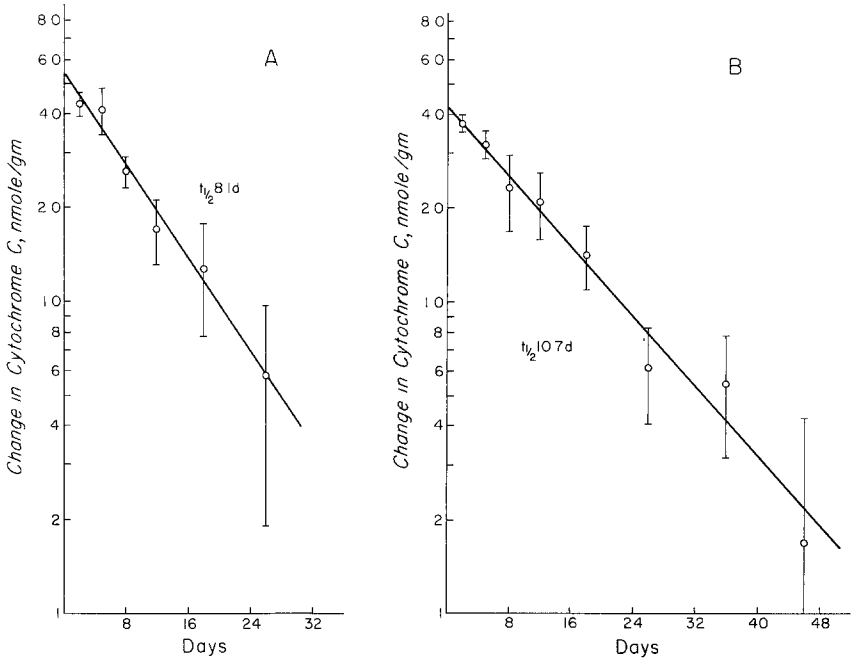

Figure 2. The least-squares lines of best fit for the change in cytochrome c concentration following cessation of exercise training (Panel A) and following thyroidectomy (Panel B). The slope $\pm$ s_{yx} of the regression line in Panel A is .085 $\pm$ .117 (r = -.991) and .065 $\pm$ .189 (r = -.986) for the regression line in Panel B.

longer operative after the cessation of training, the rate constant calculated from the change in cytochrome c should reflect the degradation process of normal adult rats. The half-life of 8.1 days obtained by this procedure is much shorter than the 30 day value obtained previously (1,2) using [^{14}C]delta-aminolevulinate as the tracer for cytochrome c. This discrepancy suggests that the previous data were subject to sample contamination and/or isotope reutilization. The difference in half-lives cannot be accounted for by analytic procedures since the labeled cytochrome c samples were free from other cytochromes and the label was essentially confined to cytochrome c as determined by SDS gel electrophoresis and Sephadex G 25 chromatography. The discrepancy, therefore, is probably due to the *in vivo* handling of the [^{14}C]delta-aminolevulinate label. The reutilization is not a typical amino

acid degradation-reincorporation cycle since [^{14}C]delta-aminolevulinate is specifically incorporated into heme (3). It is possible, however, that there was a slowly turning over pool of heme intermediates in skeletal muscle that were initially labeled during the pulse injection of [^{14}C]delta-aminolevulinate. This pool would presumably have been available as a source of label for cytochrome c. This difficulty with the use of [^{14}C]delta-aminolevulinate as a tracer for cytochrome c must be specific to skeletal muscle, since in our previous studies using [^{14}C]delta-aminolevulinate for skeletal muscle cytochrome c (1,2) the half-life for liver cytochrome c was approximately 6 days, a value expected from the work of others (3,4).

The determination of the time course of cytochrome c change in thryoidectomized animals, admittedly would not yield a half-life appropriate for normal animals, since a slower turnover of mitochondrial proteins is to be expected (12). However, the effect of thyroidectomy is relatively small. Thus, determination of the cytochrome c half-life in these animals would provide confirming data relative to the large discrepancy in half-lives mentioned above. The rapid fall in cytochrome c levels after thyroidectomy (Figure 1) probably reflects the loss of normal thyroid function and is consistent with the rapid half-life of 1.0 day for thyroxine (13). The half-life of 10.7 days is relatively short, but longer than the 8.1 day value found for normal animals (t -test for difference between slopes, $p < .10$). Thus, it appears that a loss of normal thyroid function reduces the degradation rate constant for cytochrome c. Furthermore, it may be concluded that the half-life for skeletal muscle cytochrome c in normal adult rats is approximately 8 days.

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References

1. Terjung, R. L., Winder, W. W., Baldwin, K. M., and Holloszy, J. O. (1973) J. Biol. Chem. 248, 7404-7406.

2. Terjung, R. L., (1974) Physiologist 17, 343.
3. Druyan, R., DeBernard, B., and Rabinowitz, M. (1969) J. Biol. Chem. 244, 5874-5878.
4. Aschenbrenner, V., Druyan, R., Albin, R., and Rabinowitz, M. (1970) Biochem. J. 119, 157-160.
5. Lester, R., and Troxler, R. F. (1969) Gastroenterology 56, 143-169.
6. Schimke, R. T., and Doyle, D. (1970) Ann. Review Biochem. 39, 929-976.
7. Fitts, R. H., Booth, F. W., Winder, W. W., and Holloszy, J. O. (1975) Am. J. Physiol. 228, 1029-1033.
8. Gustafsson, R., Tata, J. R., Lindberg, O., and Ernster, L. (1965) J. Cell Biol. 26, 555-578.
9. Gollnick, P. D., and Ianuzzo, C. D. (1972) Am. J. Physiol. 223, 278-282.
10. Terjung, R. L. (1975) Manuscript Submitted.
11. Margoliash, E., and Frohwirt, N. (1959) Biochem. J. 71, 570-572.
12. Gross, N. J. (1971) J. Cell. Biol. 48, 29-40.
13. Winder, W. W., and Henniger, R. W. (1973) Am. J. Physiol. 224, 1139-1143.