road km N of Los Planes on Gualaca/Chiriquí Grande road, USNM 607627–607629; Fortuna, GML 068767.

C. o. punctulatus: VERAGUAS: Cordillera del Chucú, USNM 62013 (syntype); Santa Fé, AMNH 187972; Chitra, AMNH 246541, 246552, 246553, 246556, 246561. COCLÉ: Cascajal, USNM 150875.

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Effect of Handling Time and Freezing on Catabolic Enzyme Activity in House Sparrow Pectoralis Muscle

TIMOTHY P. O'CONNOR[1] AND TERRY L. ROOT[2]

[1]*Department of Biology, University of Michigan, Ann Arbor, Michigan 48109, USA; and*

[2]*School of Natural Resources, University of Michigan, Ann Arbor, Michigan 48109, USA*

Activities of certain catabolic enzymes have been used as indicators of the metabolic capacity of a tissue (Marsh 1981). For example, citrate synthase (CS) activity has been used to indicate the capacity for oxidation of acetyl-CoA in the citric acid cycle, and β-hydroxyacyl CoA-dehydrogenase (HOAD) has been employed as an indicator of tissue capacity for fatty acid oxidation (e.g. Bass et al. 1969, Marsh 1981, Wickler 1981, Marsh and Dawson 1982, Yacoe et al. 1982, Olson 1987, 1990, Carey et al. 1989). In some studies enzymatic-activity determinations have been carried out on freshly dissected tissues (e.g. Bass et al. 1969, Marsh 1981, Marsh and Dawson 1982, Yacoe et al. 1982, Carey et al. 1989). In others, tissues were dissected out of the organism, frozen, stored at approximately −70°C, and then later thawed and analyzed (e.g. Olson 1987, 1990, Olson et al. 1988). The latter protocol allowed investigators to capture and dissect organisms in the field, and then store tissues for subsequent analyses. One of the variables inherent in such a protocol is the handling time between tissue dissection and freezing. We refer to this period as the "time to freezing."

Another variable is the extent to which freezing affects the enzyme activities. Srere (1969) reported that more CS can be extracted from frozen than fresh tissue. In order to address these variables, we examined the relationship between time to freezing and the activity of both CS and HOAD in the pectoralis muscle of the House Sparrow (*Passer domesticus*). We also compared the enzymatic activities of freshly dissected tissues with those of tissues that had been frozen for storage.

We mist netted 19 House Sparrows in Ann Arbor, Washtenaw County, Michigan in October 1989. Within 4 h of capture, individuals were sacrificed by thoracic compression, and pectoralis muscles were quickly removed. Once removed each muscle was arbitrarily placed into one of six experimental groups. Each group was composed of five pectoralis samples, with muscles from the same individuals being placed in different groups. Five of the experimental groups included muscles that were frozen, while the sixth group included freshly dissected muscles. The freshly dissected muscles were homogenized immediately, and the homogenate was then sonicated in order to lyse

the cells. Enzyme activities were measured according to the procedure below. The five frozen muscle groups included muscles that were, upon removal, placed in cryogenic tubes and held at room temperature (ca. 20°C) for 5, 15, 30, 45, or 60 min after dissection, and then were quick frozen with liquid nitrogen. These frozen samples were stored at −70°C for one to five months prior to enzyme activity determinations. Olson et al. (1988) found that neither CS nor HOAD activities were affected by four months of storage at −70°C. Both CS and HOAD assays were performed at 25°C using a Gilford 260 spectrophotometer coupled to an Apple IIe microcomputer. Citrate synthase (E.C. 4.1.3.7) activity was determined at 412 nm according to Olson's (1990) modification of the protocol of Srere (1969), while HOAD (E.C. 1.1.1.35) activity was measured at 340 nm following Olson's (1990) modification of the method of Bass et al. (1969). Duplicate or triplicate trials were performed on all samples for both enzymes. Only those samples for which at least two replicates were within 20% of each other were included in our analyses. Total-protein concentration of the homogenates was determined with the Folin phenol reagent method of Lowry et al. (1951) for calculation of enzyme activities on a per unit protein basis.

Regression lines were computed by the least-squares method, and an F-test was used to determine if the slopes differed significantly from zero. The enzyme activity of frozen samples was compared to that of freshly dissected samples by means of an independent t-test. All statistics were computed with the SYSTAT 5.0 package (SYSTAT, Inc., Evanston, Illinois), and significance was accepted at the 0.05 level.

Neither CS nor HOAD activity showed sign of change with time to freezing (Fig. 1). The relationship between activity (A) and time to freezing (T) for each enzyme is best described for CS by

$$A = 193.1 - 0.19T, \quad (1)$$

and for HOAD by

$$A = 18.9 + 0.07T. \quad (2)$$

The slopes of the regression lines do not differ significantly from zero for CS ($P > 0.50$) or HOAD ($P > 0.28$).

An ANOVA indicated that the variation among the five groups of frozen tissues was not significant for either CS ($P > 0.69$) or HOAD ($P > 0.48$). Therefore, the enzyme-activity values from all frozen samples were grouped together, and a grand mean was calculated. This mean was then compared to the mean obtained from freshly dissected tissues. For CS, the grand mean activity of all the frozen samples (n = 24) was 187.46 ± SE of 6.16 μmoles·(min·g)$^{-1}$, while the mean activity of the freshly dissected samples (n = 4) was 170.98 ± 7.74 μmoles·(min·g)$^{-1}$. The difference between these values was not significant (P = 0.30). The grand mean of the HOAD activity of all frozen samples (n = 16) was 21.19 ± 1.34 μmoles·(min·g)$^{-1}$. The mean activity for the freshly dissected muscles (n = 4) was 19.54 ± 1.20 μmoles·(min·g)$^{-1}$. Again, the difference was not significant (P = 0.56).

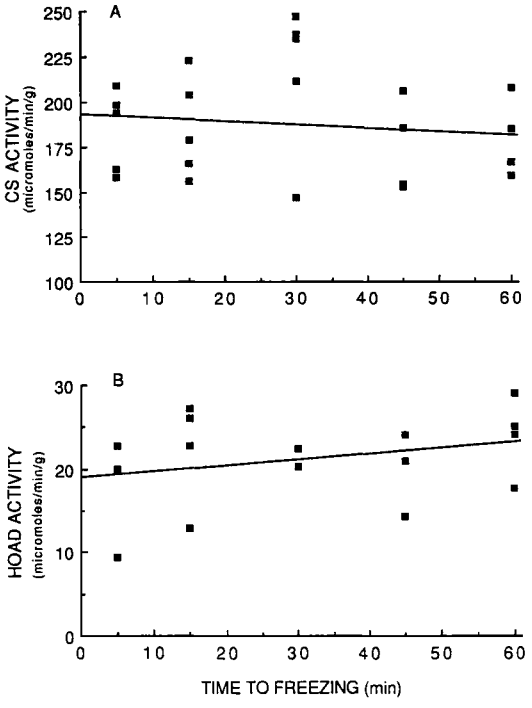

Fig. 1. Relationship between time to freezing and enzyme activity in House Sparrow pectoralis muscle. Each point represents enzyme activity for one House Sparrow pectoralis. Both (A) citrate synthase (n = 24) and (B) β-hydroxyacyl CoA-dehydrogenase (n = 16) activities expressed as μmoles·(min·g)$^{-1}$. See text for regression-line equations.

After determining the total protein concentration of the muscle homogenates, enzyme activities were recalculated. The grand mean activities of frozen samples and mean activities of fresh samples were as follows: CS_{frozen} = 0.81 ± 0.02 μmoles·(min·mg protein)$^{-1}$; CS_{fresh} = 0.75 ± 0.04; $HOAD_{frozen}$ = 0.092 ± 0.005; $HOAD_{fresh}$ = 0.088 ± 0.005. Such recalculations did not affect the results, however, in that no significant differences were found either within the frozen samples, or between fresh and frozen samples for CS or HOAD.

One of the most important experimental techniques of ecological physiologists is the application of biochemical analyses to free-living, rather than captive, study organisms. This often involves drawing blood or dissecting out muscles in the field, and then freezing samples for subsequent analysis. In this paper we have examined how variability in handling time between muscle dissection and freezing affects the activity of two catabolic enzymes. We can conclude from our results that in House Sparrows neither CS nor HOAD activity declines significantly when time to freezing varies between 5 and 60 min. We

must, however, caution investigators that the generality of our findings is unknown. Metabolic enzymes other than avian CS and HOAD may be affected by time to freezing values of less than 60 min. Therefore, we recommend that tissues be frozen as soon as possible after dissection and that they be kept as cold as possible until they are frozen. Investigators in the field can place tissues in cryogenic tubes and then immerse the tubes into liquid nitrogen for transport back to the laboratory. Should liquid nitrogen be unavailable, tissue samples can be frozen in foil on dry ice.

According to Srere (1969), more CS can be extracted from frozen than fresh tissue, presumably due to the cell lysing that results from freezing. Since both CS and HOAD are mitochondrial enzymes, we expected the activity of both enzymes to be higher in samples that had been frozen. Although the activity of both enzymes was higher in frozen than fresh tissue, the differences were not significant for either CS or HOAD. Consequently, we believe that the sonication of the muscle homogenate involved in the enzyme-activity determination protocol results in thorough cell lysing and that freezing the muscle samples does not significantly enhance the cell-lysing process.

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DNA Fingerprinting in Avian Behavioral Ecology: Two Cultures Arise

Patricia Adair Gowaty[1] and H. Lisle Gibbs[2]
[1]*Department of Zoology, University of Georgia, Athens, Georgia 30602, USA; and*
[2]*Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1, Canada*

As each of us learns more and more about less and less, it seems inevitable that students of avian mating systems will occupy two cultures. Like C. P. Snow's two cultures—sciences and liberal arts—we are developing different languages, different methods, and seemingly different questions. The two cultures that now study avian mating systems are represented by those who are importing molecular-genetic tech-