

Using pentosidine as a biomarker of age in Bald Eagles (*Haliaeetus leucocephalus*) and captive psittacines

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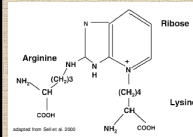
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Introduction

The phenomenon of aging is progressive, irreversible, and universal to all living things. One unavoidable outcome of the aging process is the modification of proteins by sugars, which can lead to the formation of advanced glycation endproducts. The cumulative nature in skin collagen and inherent fluorescence of one such endproduct, pentosidine (Ps), make it a valuable biomarker of aging that can readily be measured in the laboratory (Sell et al. 1996). For the past few years, we have been examining the validity of using the accumulation of Ps in the skin collagen of different avian species as a method to estimate chronological age (Chaney et al. 2003; Fallon et al. 2006 A.B). Because birds display few external morphological indicators of age after reaching adulthood, alternative aging techniques need to be developed.

Study 1 examines the correlation between age and the accumulation of Ps in skin collagen of wild Bald Eagles (*Haliaeetus leucocephalus*), and compares this correlation to established relationships of other species. In Study 2, we examine the viability of using small skin biopsies taken from live birds to assess Ps concentrations.



Objectives

Study 1

- Establish the correlation between Ps and age in wild Bald Eagles
- Examine correlational differences in Ps accumulation between Bald Eagles and established relationships of two other species: Ruffed Grouse (*Bonasa umbellus*) and Double-crested Cormorant (*Phalacrocorax auritus*)

Study 2

- Examine correlation between Ps and age in captive psittacines
- Establish the validity and practicality of using the technique on skin biopsies taken from live birds



Materials and Methods

Study 1

Twenty-four known age Bald Eagles were found dead and collected from 2002-2006 in the state of Maine by employees of the Maine Department of Game and Inland Fisheries and/or BioDiversity Research Institute, all working under permits issued by the U.S. Fish and Wildlife Service. Carcasses were kept frozen for a variable length of time prior to skin sample collection. Approximately 3 grams of skin were removed from the breast of each bird at necropsy and kept frozen in saline or distilled water until assayed.

Forty mg skin samples were assayed for Ps concentration using HPLC and an in-line fluorescence detector. Collagen content was measured via spectrophotometric hydroxyproline assay.

Study 2

A single 6 mm full thickness skin biopsy was taken from the breast of eleven macaws of various species (Blue and Gold, Green-winged, Blue-throated, Military, and hybrid) and two Moluccan Cockatoos. Biopsies were collected by Dr. Susan Clubb while birds were maintained on isoflurane anesthesia.

Results

Study 1

Regression analysis revealed a linear increase of Ps concentrations with age in Bald Eagles ($y = 0.1669x + 0.6007$ $r^2 = .89$, $p < 0.0001$) as seen in Figure 1, with no significant sex differences detected. This linear relationship was similar to that found previously for Double-crested cormorants ($y = 0.146x + 7.3301$ $r^2 = 0.76$) but was significantly different ($p < 0.01$) than that found for Ruffed Grouse ($y = 0.8063x + 2.1542$, $r^2 = 0.80$) (Fallon et al 2006 B; Fallon et al. unpublished data).

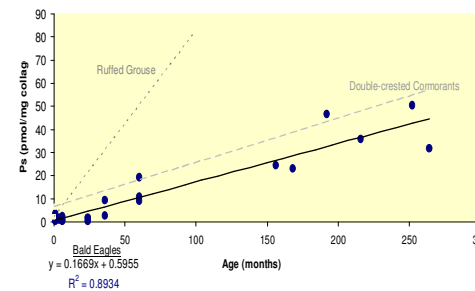


Figure 1. Pentosidine concentration as a function of age in Bald Eagles (●), Double-crested Cormorants (long dashes) and Ruffed Grouse (short dashes).

Study 2

We found no significant correlation between age and Ps accumulation when results from both macaws and cockatoos are included in the analysis. However, when the two cockatoos are excluded from the data, a strong linear and curvilinear relationship is evident ($y = 0.0559x + 14.086$ $r^2 = 0.7518$ and $y = -0.0002x^2 + 0.1646x + 4.3714$ $r^2 = 0.9279$) as seen in Figure 2. Both cockatoos showed much lower levels of Ps than macaws of similar age.

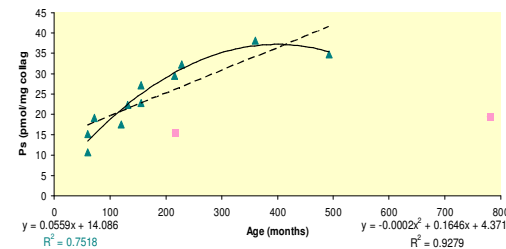


Figure 2. Pentosidine concentration as a function of age in various macaw species (●) and two Moluccan Cockatoos (■).



Conclusions

Study 1

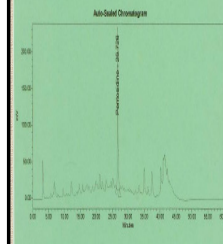
- Pentosidine accumulation is linearly correlated with age in Bald Eagles.
- Bald Eagles accumulate Ps at a rate similar to Double-crested Cormorants but more slowly than Ruffed Grouse. Moreover, the similarity of rate of accumulation between cormorants and eagles suggests that some extrapolation may be made between species.
- Pentosidine in skin collagen may be used as a tool to estimate chronological age in Bald Eagles.

Study 2

- For the first time, this technique has been used on skin biopsies taken from live birds.
- These data suggest a different rate of accumulation between macaw species and Moluccan Cockatoos
- As this technique is refined, it will provide valuable data on age-related population demographics, help target depredation action against appropriate individuals, and may benefit species survival programs.

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