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Turnover of ^{13}C in Cellular and Plasma Fractions of Blood: Implications for Nondestructive Sampling in Avian Dietary Studies

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The assay of naturally-occurring stable isotopes of carbon and nitrogen in avian tissues to infer diet offers a number of advantages over conventional dietary investigations (Hobson and Sealy 1991, Hobson and Clark 1992a, b). For example, because stable-isotopic compositions of a consumer's tissues can be related ultimately to those in its diet (reviewed by Peterson and Fry 1987), this technique provides a

time-integrated estimate of assimilated and not just ingested foods. Furthermore, because the turnover of stable isotopes in a tissue is related to the metabolic activity of that tissue (Tieszen et al. 1983), the isotopic analysis of several tissues from the same consumer can provide both short- and long-term dietary information (e.g. Hobson and Sealy 1991). In addition, the establishment of diet-tissue isotopic fractionation factors (i.e. isotopic differences between diet and tissues) and isotopic turnover rates for various avian tissues allows quantitative predictions of diet (Hobson and Clark 1992a, b). For tissues that are not metabolically active (e.g. feathers or other keratin-based structures), stable-isotope ratios may also form a record of past dietary information (Hobson and Clark 1992a; see also Schell et al. 1983).

There are, of course, several dietary questions that cannot be addressed adequately using stable isotopes and, in many cases, the stable-isotope approach will augment rather than replace conventional dietary techniques, such as stomach sampling (Hobson and Clark 1992a, b). However, another important advantage to using the stable-isotope technique is its potential for nondestructive sampling. Schaffner and Swart (1991) recently showed how measurements of stable-carbon and oxygen isotopes in egg-shell fragments taken from nest sites can provide information on the foraging locations of female seabirds. Stable-isotopic concentrations in feathers may reflect diet during the period feathers are grown (Hobson and Clark 1992a), and Mizutani et al. (1990) analyzed feathers to confirm the relative importance of freshwater and marine foods in a wild population of cormorants (*Phalacrocorax carbo*).

While feathers and egg shells provide convenient materials for nondestructive isotopic assays, the interpretation of isotopic data from these sources may be complicated by the fact that it is often not clear to what extent isotopes present in feathers and egg shells are derived from diet or stored nutrients (Hanson 1962, Austin and Fredrickson 1987, Krapu 1981, Ankney et al. 1991). Also, these materials can only provide dietary information during a relatively short portion of a bird's life cycle. In cases where birds may be captured, blood may be a much more useful material for nondestructive isotopic sampling of individuals. The isotopic assay of serial blood samples from the same individual would provide a convenient way to monitor that individual's diet through time. Moreover, because blood can be separated into cellular and plasma fractions, components known to differ in their rates of protein turnover (e.g. Waterlow et al. 1978), the potential exists for dietary information based on two different periods of integration to be obtained from the same blood sample. Hobson and Clark (1992a, b) recently reported isotopic fractionation factors and turnover rates established for whole-blood samples of birds. Here, we report the results of an experiment designed to determine the isotopic turnover rates of diet-derived ^{13}C associated with the plasma and cellular fractions of the blood of American Crows (*Corvus brachyrhynchos*). This is the first estimate of these turnover rates for birds.

Methods.—We held nine two-year-old crows (raised from the nestling stage in captivity) in a large outdoor aviary provided with a shelter, natural perches, and food and water *ad libitum*. Prior to our study, crows were maintained for 10 months on a diet composed of pelletized dog food, poultry grower, and chicken eggs. Before tests began, crows were switched to a wheat-based ration of turkey grower mixed with gelatin and water to form a palatable, homogenous diet. They were maintained exclusively on this diet for 38 days to reduce interindividual variability in blood-isotope signatures prior to testing. Crows were then weighed and blood samples taken from three randomly selected birds. We then switched all crows from the wheat-based diet to a nutritionally similar food composed of ground corn and commercial turkey grower mixed with gelatin and water. Corn has a C-4 photosynthetic pathway and so has a carbon-isotopic signature that is more enriched in ^{13}C compared to C-3 plants such as wheat. Therefore, corn can be used as a natural dietary tracer to determine isotopic turnover in various tissues (Hobson and Clark 1992a). Following the diet switch, we collected blood from two randomly selected crows at 2-, 4-, 8-, 16-, and 27-day intervals. After 45 days on the corn-based diet, we stopped sampling, weighed all birds, and removed blood from three crows. During the experiment, blood was collected from seven crows twice, but at least 15 days elapsed between consecutive sampling ($\bar{x}$ = 30 days, range 15–43 days). Crows gained an average of 6% in body mass over the period on the corn-based diet (initial mass, $\bar{x}$ = 404 ± SD of 31 g; final mass, $\bar{x}$ = 428 ± 40 g; gain, $\bar{x}$ = 24 ± 13 g), an increase likely reflecting lipid deposition and regrowth of feathers. These birds were not growing and their modest mass gain was expected during this premigration period. Therefore, the isotopic turnover rates estimated will be dominated by effects of maintenance metabolism rather than growth (Fry and Arnold 1982).

In all cases, blood was taken from the brachial artery, immediately transferred from syringes to storage vials, and centrifuged (5,000 rpm for 7 min) within 30 min. Cellular and plasma fractions were then stored separately and frozen within 2 h at −20°C. Prior to isotopic analysis, each fraction was freeze dried. Samples were loaded into pyrex combustion tubes with 1 g of CuO and then combusted at 550°C for at least 2 h. Isotopic analyses were performed on a VG-SIRA 12 mass spectrometer (Isotech, Middlewich, England). Carbon-13 concentrations are reported in δ notation as parts per mil (‰) deviation from the Peedee Belemnite (PDB) standard as follows:

$$\delta^{13}C = 1{,}000[(R_{sample}/R_{standard}) - 1], \quad (1)$$

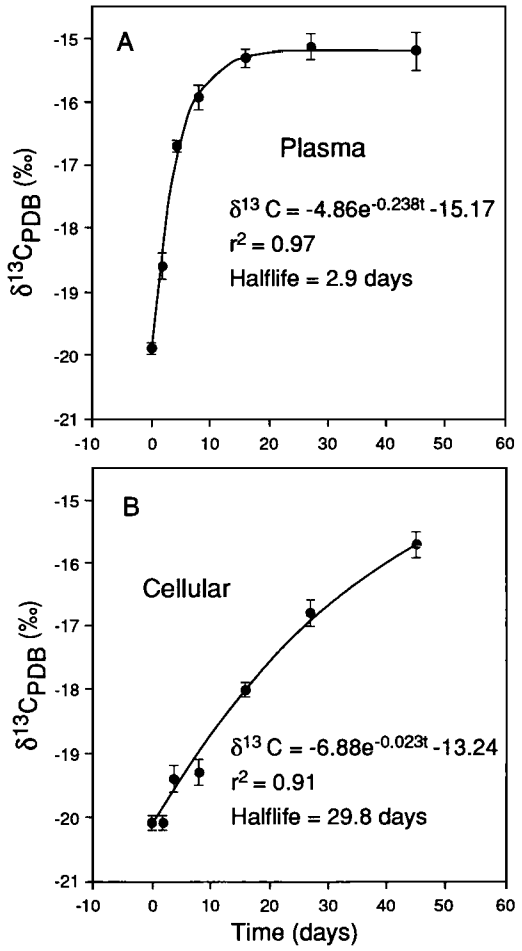

Fig. 1. Patterns of change in stable-carbon isotope ratios of American Crow blood fractions ($\bar{x} \pm$ SD). Sample sizes are $n = 2$, except for first and last data points, where $n = 3$. Data for each fraction fitted with exponential equation as shown.

where R is $^{13}C/^{12}C$. Measurement precision on a lentil standard was ±0.1‰ (SD).

Results.—Stable-carbon isotopic concentrations of wheat- and corn-based diets were −20.6 ± 0.2‰ ($n = 5$) and −15.5 ± 0.4‰ ($n = 5$), respectively. For the plasma fraction of crow blood, the mean difference between initial and asymptotic $\delta^{13}C$ values (4.7‰) resembled closely the mean difference in $\delta^{13}C$ between diets (5.1‰). Consistent with the incorporation of isotopically enriched carbon from the corn-based diet, stable-carbon isotope values of blood fractions shifted toward more positive values over the course of the experiment (Fig. 1).

Patterns of carbon turnover in crows resembled exponential models and so, for each blood fraction, we fitted the isotopic data to equations of the form $Y = a + be^{ct}$ using the PROC NLIN procedure of SAS (SAS Institute 1985). In the equation, Y represents the $\delta^{13}C$ value of the tissue in question, a and b are parameters determined by initial and asymptotic conditions, c is the turnover rate of carbon in the tissue, and t is the time in days since the diet switch. This equation provided a good fit for both blood fractions (Fig. 1). We calculated half-lives of tissue carbon as $\ln(0.5)/c$.

Discussion.—Plasma $\delta^{13}C$ values were in equilibrium with dietary values at the beginning and end of the experiment and, therefore, estimates of $\delta^{13}C$ isotopic fractionation between diet and plasma were obtained. Our estimate of 0.5 ± 0.3‰ for the mean $\delta^{13}C$ plasma-diet fractionation factor must be considered only an approximation, however, and further studies designed to determine fractionation values for ^{13}C and other isotopes would be useful. Hobson and Clark (1992b) provided estimates for whole blood-diet fractionation factors ranging from −0.3 ± 0.8‰ for gulls (*Larus delawarensis*) raised on a fish diet to 1.2 ± 0.6‰ for quail (*Coturnix japonica*) raised on a grain-based diet.

The short half-life of 2.9 days for dietary-derived ^{13}C in blood plasma is close to the value of 2.6 days found for liver by Hobson and Clark (1992b) in similar investigations using captive quail. This finding is consistent with the fact that plasma proteins are synthesized mainly in the liver (Schoenheimer 1949, Waterlow et al. 1978). The isotopic measurement of blood plasma, thus, should yield short-term dietary information reflecting an integration over about a week. The much slower turnover rate of the cellular fraction of blood (half-life = 29.8 days) was expected since blood cells are known to have greater longevity than constituents of plasma (Schoenheimer 1949). The isotopic analysis of this blood fraction should yield dietary information integrated over about two months. However, longer-term studies may better refine estimates of stable-isotopic turnover rates in the cellular fraction of avian blood. Hobson and Clark (1992a) determined a diet-derived ^{13}C turnover value of 11.4 days for whole blood of quail. As expected, this value is intermediate between those for plasma and cellular components.

Because crows were held in a large outdoor aviary, we suspect that their metabolic rates were similar to the field metabolic rates of wild crows. However, it is possible that wild or captive birds with higher metabolic demands than our captive birds may show faster turnover rates than those calculated here (see Hobson and Clark 1992a). Also, the relative proportions of various blood components may be influenced by species or hormonal condition (Sturkie 1976). Further studies are required to determine how these factors may influence isotopic turnover in each tissue of interest (Hobson and Clark 1992a). We also caution that the isotopic turnover rates we have determined for both blood fractions represent averages of all carbon constituents in these fractions. Several (minor) plasma proteins may, for example, have much slower

turnover rates than the average value we have derived.

We have shown that stable-isotope concentrations in avian blood reflects diet and that by analyzing both the plasma and cellular fractions of blood both short- and long-term dietary information may be obtained. These results indicate that, when birds can be captured alive, destructive sampling through the use of liver and muscle samples for isotopic assay is not necessary. For those studies concerned with estimates of longer-term dietary integrations, typically provided through the analysis of bone collagen (e.g. Hobson and Sealy 1991, Hobson and Montevecchi 1991), it may instead be possible to use other, slow-growing tissues such as subsamples of the epidermis (see King and Murphy 1990). We also encourage further research into the use of tissue biopsies for isotopic analysis. The advent of high-sensitivity mass spectrometers requiring samples on the order of a few milligrams certainly makes this feasible.

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