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RADIOCARBON DATING OF FOSSIL EGGSHELL

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ABSTRACT. Controlled feeding experiments demonstrate that the ^{14}C content of the carbonate fraction of fossil avian eggshell should reliably reflect the ^{14}C activity of feed and contemporary atmospheric CO_2 regardless of amounts of ^{14}C depleted scratch injested. Consideration of biochemical pathways and exchange rates across lung membranes leads to the possibility that the carbonate fraction may, in some instances, be slightly ^{14}C depleted. ^{14}C dates on eggshell carbonate should require little or no correction. As with marine shell carbonate, normal soil-forming processes may alter the surficial carbon isotopes in eggshell carbonate. ^{14}C dates on the protein fraction should be even more reliable than those on the carbonate fraction, but only if special precautions or separation techniques exclude non-indigenous carbon from the sample. Original protein contents are likely to be too low for conventional ^{14}C dating techniques.

INTRODUCTION

Avian eggshells occur in the Pleistocene and Holocene fossil record, often with sufficient diagnostic morphology and/or stratigraphic association to make ^{14}C dating of eggshell critical in testing hypotheses (Williams, 1981; Haynes, in press). For all ^{14}C dated material, either explicit or implicit assessments of two basic assumptions in the dating method must be made: 1) the initial ^{14}C activity of the dated material, and 2) the sample as a closed system with respect to carbon. We assess both assumptions with regard to eggshells, and report experiments on initial ^{14}C activity of chicken eggs.

PHYSIOLOGY AND BIOCHEMISTRY OF EGGSHELLS

Eggshells contain carbon in two basic fractions: carbonate and organic matter, primarily protein. For quantitative reasons, the carbonate fraction is more attractive for conventional ^{14}C dating than the protein fraction, which is comparatively small. The protein carbon is more likely to obey the closed system assumption, and direct ion counting technology

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will utilize this fraction most effectively. Thus, both sources of carbon are of interest in ^{14}C dating. We evaluated each fraction for its source of carbon and the factors controlling its initial ^{14}C activity.

Fossil eggshells of interest to paleontologists and paleoecologists are from Pleistocene species, especially extinct ratites, such as the moas of New Zealand, the elephant birds of Madagascar, and the dromornithids (not ratites) of Australia. We assume that the egg physiology of modern avian species are analogous to extinct species.

Taylor (1970) and Taylor and Stringer (1965) summarized the physiology of eggshell formation. Chicken eggshells consist of columns of calcite ($CaCO_3$) perpendicular to the shell's surface. Column diameters are typically 0.05 mm in diameter and separated by a thin proteinaceous film. The protein constituents are synthesized in the liver from food-product metabolites and should, therefore, carry the ^{14}C label of nutrient organic carbon. The shell $CaCO_3$ carbon derives from the dissolved inorganic carbon in the blood plasma. A likely mechanism involves the enzyme carbonic anhydrase catalyzing the reaction

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+.$$

The bicarbonate ion equilibrates with carbonate ion by

$$HCO_3^- = CO_3^{=} + H^+.$$

Calcium ions (Ca^{++}) from the plasma and $CO_3^{=}$ ions are attracted to the shell protein onto locations spaced at their approximate interionic distances in calcite. The protein may then act as a template for the nucleation of eggshell calcite. Other mechanisms are possible, but all derive the carbon from inorganic carbonate ions dissolved in blood plasma.

Before ^{14}C dating of shell carbonates can be interpreted, the derivation of the plasma carbonate must be known. It could originate from respiration of organic foodstuff, in which case, like the eggshell protein, the fresh egg carbonate natural ^{14}C label would be the same as that in the chicken's organic nutrient, when corrected for istopic fractionation. But chickens habitually ingest small pebbles, often limestone or shell, which help pulverize grains in their gizzards prior to digestion. Thus, it is possible that some carbon labeled with the ^{14}C of ingested mineral carbonate makes its way to the plasma-dissolved inorganic carbon. If this happens, and the mineral carbonate contains little or no ^{14}C, as could be the case in limestone, the initial ^{14}C activity of the shell carbonate might be lower than that of the shell organic matter. An erroneously old ^{14}C age on the shell carbonate would result.

It would seem that this "limestone" effect on the shell carbonate ^{14}C age should be minimal for two reasons: 1) a large, probably major portion of the plasma-dissolved inorganic carbon must be the product of respiration of organic nutrients, and 2) CO_2 exchange between plasma and the atmosphere through the lung membranes tends to bring plasma into isotopic equilibrium with the atmosphere. Since we found sufficient uncertainty about the effects on ^{14}C dates of mineral carbonate "scratch" in avian diets, we carried out a series of experiments to maximize the probability of ^{14}C date bias, and then looked for the effect.

EXPERIMENTAL APPROACH

We measured ^{14}C activities and $^{13}C/^{12}C$ ratios on both carbonate and organic fractions on four sets of eggs from chickens that were fed controlled diets. We also measured carbon isotope contents of the feed. Two groups of chickens received feed high in limestone $CaCO_3$ (ca 22%) while two groups received feed low in limestone $CaCO_3$ (ca 11%). The eggs were collected in each case after 5 to 8 (early group) and 20 to 25 (late group) days on the special feed.

RESULTS AND DISCUSSION

Table 1 shows the carbon isotope analysis results. As expected, the mineral carbonate in the feeds contained no measurable ^{14}C. All other carbon fractions in the experiment,

TABLE 1. Carbon isotopic analyses of eggs and feed

	Sample no.	Fraction	Description		$\delta^{13}C$ PDB (‰)	^{14}C% modern (normalized to $\delta^{13}C$ = 25‰)
Eggs	1058A	organic	early group	fed high $CaCO_3$ feed	-	243 ± 14*
	1058B	carbonate			-	166 ± 2
	1059A	organic	first group		-	**
	1059B	carbonate			+ 2.6	161 ± 2
	1060A	organic	early group	fed low $CaCO_3$ feed	-29.8	170 ± 4
	1060B	carbonate			+ 3.1	160 ± 2
	1061A	organic	late group		-	**
	1061B	carbonate			+ 2.4	168 ± 2
Feed	1062A	organic	high $CaCO_3$ content		-19.7	165 ± 2
	1062B	carbonate			+ 2.3	< 2.5
	1063A	organic	low $CaCO_3$ content		-	163 ± 2
	1063B	carbonate			+ 2.2	< 2.5

*Sample very small, highly diluted for analysis
**Too small for ^{14}C analysis

when normalized to the same $\delta^{13}C$ value, had essentially the same ^{14}C activity of contemporary atmosphere at the time of the feeding (1969). In our experiment, "dead" mineral carbonate was absorbed by chickens and had no measurable effect on the initial ^{14}C content of eggshell carbonate or organic matter.

One curiosity is the $\delta^{13}C$ values of the eggshell carbonate all slightly positive (enriched in ^{13}C) with respect to PDB, and similar to other chicken eggshells we measured. This is closer to the value expected for isotopic equilibrium in atmospheric CO_2 than expected for food respiration products. Interestingly, the $\delta^{13}C$ values Folinsbee et al (1970) show for chicken eggshell range from -13°/oo to -7°/oo. Similarly, the $\delta^{13}C$ values for Aepyornis reported in Folinsbee et al (1970), Berger et al (1975), and in this study fall in the -14°/oo range. These more negative values may represent varying degrees of isotopic equilibrium attainment in chicken lung membranes. Isotopic equilibrium can be tested by measuring $\delta^{13}C$ in blood bicarbonate.

A single comparison of $\delta^{13}C$ values in eggshell carbonate and chicken blood revealed the eggshell to be 9.6°/oo more enriched in ^{13}C, too great for either equilibrium or kinetic explanations (Turner, 1982). Further such tests are needed.

POST-DEPOSITIONAL EFFECTS

Time and subaerial processes will affect both the calcite and protein fractions of fossil eggshells, similar to effects on carbonate in molluscan shells. Original protein will gradually disintegrate into smaller fragments and wash away. Rate of disappearance of protein will depend on soil conditions and thickness of shell fragments. Soil organic matter will tend to adsorb onto calcite surfaces, thus affecting the ^{14}C age of total organic matter.

Soil process may alter ^{14}C dates on eggshell calcite by surficial exchange of carbonate or deposition of $CaCO_3$. A number of studies conclude isotopic alteration of calcite. Accurate ^{14}C dating of marine shells requires removal of outer layers of calcite: ^{14}C dating of Foraminifera tests (Eriksson and Olsson, 1963), fine-grained carbonate material subjected to subaerial processes (Long, unpub ^{14}C data), and $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratio alteration studies in calcitic sediments (Gross, 1964).

APPLICATIONS TO DATING FOSSIL EGGSHELLS

We now consider the applicability of this study on chicken eggs to avians in general and large extinct birds in particular. Williams (1981) reported a ^{14}C date on modern Emu eggshell of

560 ± 70 BP (SUA-1069). He did not note whether this date is ^{13}C normalized, nor did he measure ^{14}C in diet. This could mean that larger birds may incorporate small but significant amounts of mineral carbon in their eggshells and, as suggested above, plasma-dissolved inorganic carbon may not entirely equilibrate isotopically with atmospheric CO_2. The $^{13}C/^{12}C$ ratio may prove to be an indicator of such isotopic equilibrium.

Clearly, more analyses on modern large bird eggshells are needed, but evidence so far suggests that fossil eggshell carbonate dates cannot be more than a few hundred years too old if properly pretreated to remove surficial carbonate. Ideally, carbonate dates should be compared with shell protein or amino acid ^{14}C dates on fossil eggs. The latter could be done by isolating specific organic compounds and performing ^{14}C measurements by direct ion counting accelerator techniques.

CARBON ISOTOPE ANALYSIS ON FOSSIL EGGSHELL

Recently, we analyzed several fossil eggshells for ^{14}C and ^{13}C. In two cases we were able to analyze both calcite and organic matter fractions (table 2). Note that the carbonate-organic matter pairs, A-2515, 2516 and A-3106, 3105 are within

TABLE 2. Carbon isotope analyses on fossil eggshell

Lab no.	Bird	Fraction dated	^{14}C date*	$\delta^{13}C$ (PDB) (‰)	Loc, ref
A-2515	Ostrich	Carbonate	6270 ± 50	- 3.4	Egyptian Sahara, Haynes (in press)
A-2516	Ostrich	Organic residue from A-2515	6660 ± 320	- 19.8	Egyptian Sahara, Haynes (in press)
A-2517	Ostrich	Carbonate	4780 ± 50	- 4.4	Egyptian Sahara, Haynes (in press)
A-2518	Ostrich	Carbonate	6290 ± 150	- 4.4	Egyptian Sahara, Haynes (in press)
A-3106	Ostrich	Carbonate	8280 ± 60	- 5.6	Egyptian Sahara, Haynes (in press)
A-3105	Ostrich	Organic residue from A-3106	8680 ± 450	- 21.6	Egyptian Sahara, Haynes (in press)
A-2833	Aepyornis	Carbonate	7450 ± 150	- 12.3	Madagascar (coll by P S Martin)
A-2834	Aepyornis	Carbonate	1550 ± 90	- 14.6	Madagascar (coll by P S Martin)

*Normalized to $\delta^{13}C = -25$‰

one standard deviation of agreement. Although surficial carbonate was removed from 2515 and 3106 before analysis, exchange with more recent carbon possibly affected its ages to a minor degree, thus producing a fortuitous agreement. We consider it

more likely that the eggshell carbonate and organic matter had similar ^{14}C activity when the egg was laid, and the $\delta^{13}C$ signals a significant trend toward isotopic equilibrium during shell growth.

It does not seem likely that diet is an important factor in controlling the $\delta^{13}C$ of eggshell carbonate for the following reasons: 1) The experimental eggs (table 1) showed ca 32°/oo enrichment of ^{13}C in carbonate over the organic fraction. 2) One fossil ostrich egg carbonate (table 2) is ca 16°/oo enriched over its organic matter. 3) Aepyornis eggshell carbonate seems to be in the -13 to -15°/oo $\delta^{13}C$ range, which is likely to be less than 16°/oo enriched with organic matter. 4) Other chicken egg $\delta^{13}C$ values on carbonate/organic matter pairs analyzed (Long, unpub data) are +3.2, -21.2, and +2.2, -27.0.

CONCLUSIONS

1) Fossil avian eggshell carbonate ^{14}C dates should reliably represent the date of organic nutrients the bird consumed if the carbonates undergo laboratory pretreatments which are standard for other carbonate samples. 2) Although ingestion of "old" carbonate may affect the ^{14}C content of eggshell, we find no compelling evidence that it has. 3) The $^{13}C/^{12}C$ of eggshell carbonate is evidently not solely controlled by diet. It may be related to kinetic or equilibrium processes at the lung membrane, but our evidence cannot yet make this distinction.

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