Biology of the Howler Monkey *(Alouatta caraya)*

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Chronological Age Determination as Estimated from Strontium-90 Content of Teeth and Bone

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Introduction

Interpretations of diverse biological functions and specific pathology of free-ranging animals are severely hampered by lack of knowledge of the chronological age of the animal under study. Estimation of age by comparison with known growth rates of animals maintained in captivity is usually inadequate because of ecological, nutritional, and other variables between free-ranging and captive animals. However, the calcification and formation of teeth (NOYES, 1960) and the growth of the lens (LORD, 1959), appear to be factors which are independent of environment in the absence of overt pathology or disease. The crowns of teeth calcify and develop during a finite period of time, and once formed and erupted, are subject to minimal exchange, turnover, or accretion of mineral elements. During the developmental period, the mineral elements of the teeth are in equilibrium with body fluids and indirectly with the diet. The inclusion of bone-depositing tracer materials in the diet, such as strontium-90, represents the incorporation of the substance at the time the teeth are formed (KALCKAR, 1958). Addition of a foreign bone-seeking substance into the diet will, therefore, act as a marker for tooth developement. The 'unnatural' nuclide, strontium-90, has been introduced into the atmosphere and vegetation from fallout caused by atomic testing and worldwide measurable quantities have contaminated naturally occurring foodstuffs since about 1950. Analytical determinations of the quantity of strontium-90 in the teeth of an animal should thus lead to an estimation of the time of tooth formation and hence of chronological age, provided discrimination factors of strontium-90 incorporation relative to that of calcium are known (ROSENTHAL *et al.*, 1966a). It has been well documented that

adult mammals discriminate against dietary strontium-90 relative to calcium by a factor of 0.25 (BRYANT *et al.*, 1958; SCHULERT *et al.*, 1959; ROSENTHAL and HARBOR, 1965; RIVERA, 1965). In newly born mammals, discrimination against strontium has been found to be negligible (LOUGH *et al.*, 1963). Additional findings in man show that strontium discrimination changes from a factor of 1 to a factor of 0.25 during growth and development (ROSENTHAL *et al.*, 1966b).

The correlation between dietary intake of strontium-90 and tooth nuclide concentration has been extensively studied in human deciduous teeth (ROSENTHAL, GILSTER, BIRD, 1963; ROSENTHAL *et al.*, 1964, 1966b) and permanent bicuspids (ROSENTHAL *et al.*, 1966c). These studies show that the Sr-90/Ca content of the tooth (C_T) is linearly related to the dietary Sr-90/Ca intake of the nuclide (C_D) by the equation $C_T = (K)\ C_D$ where K is a constant representing the discrimination factor of strontium relative to that of calcium.

This report describes an approach for estimating the absolute age of free-ranging animals as demonstrated by specific studies with *Alouatta*.

Experimental Methods

Twenty-nine specimens of *A. caraya*, ranging in size and age from newly-born young to mature adults of both sexes, were made available for strontium-90 analysis.

The teeth, including crown and root, were removed from the skull by careful removal of bone matrix with a dental drill. The crowns of the permanent canines, pre-molars, and molars were removed from the root at the cementodentinal junction, were pooled separately, and analyzed by methods previously described in detail (ROSENTHAL, GILSTER and BIRD, 1963) with minor modifications described below. In younger animals, the unerupted and partially erupted permanent dentition was pooled without regard to tooth type, but residual root material was removed. Mandibular bone samples, after removal of the teeth and roots, were analyzed separately.

For the larger monkeys, pooled samples of tooth crown containing more than 350 mg calcium could be analyzed without modification. However, in the smaller monkeys, pooled samples of teeth usually contained between 150–350 mg of calcium and required correction for the analytical strontium-90 error of small samples. The correction factors range between 0.70 to 1.00 depending on the amount of calcium. The correction factors were previously determined by analyzing a graded series of human bone ash samples ranging in calcium content between 50 to 400 mg. Although the various types of tooth crown in an individual animal were analyzed separately, it was found that the strontium-90 content of the different kinds of teeth was similar within the experimental error. Consequently, the average value for all of the teeth for each animal was used for the estimation of age.

In order to relate the strontium-90 content of the teeth to the time of their formation, it is necessary to know the dietary intake of strontium-90, and the discrimination factor at the time of tooth formation. These data are not directly available and indirect methods for estimating the two variables are necessary.

Fortunately, the accumulated deposits of strontium-90 have been estimated by the Atomic Energy Commission of Argentina for the Buenos Aires region, some 1000 km south of the collection site (BENINSON, ELST, and RAMOS, 1964). In order to relate the fallout data with the diet of the herbivorous monkeys, a sample of the dietary vegetation from the islands of the Bella Vista area was obtained in January, 1965. Analysis of the ashed samples, in triplicate, yielded a value of 37.3 pC Sr-90/g calcium. On the basis of this determined value and the accumulated fallout deposition of strontium-90, a probable dietary curve was constructed (Fig. 1). Because of the variations and irregularity of the fallout accumulation curve, a smooth curve was extrapolated between connecting ends of the curve. The true dietary value is presumed to be between the two curves. Furthermore, accumulated fallout was assumed to be absent prior to 1950 when extensive nuclear testing was initiated.

Although definitive studies of the Sr-90/Ca discrimination factors have not been made for different species of animals during growth and development, it is assumed that the metabolism of strontium and calcium would follow the same pattern as that found in man with slight modifications which are due to the difference in time and age for tooth development. During the time of formation of the human bicuspid, the discrimination factor (K) between the diet Sr-90/Ca and tooth Sr-90/Ca was found to be 0.3 (ROSENTHAL *et al.*, 1966b). This value is close to the adult discrimination factor of 0.25 and appears to be valid during the time of development of

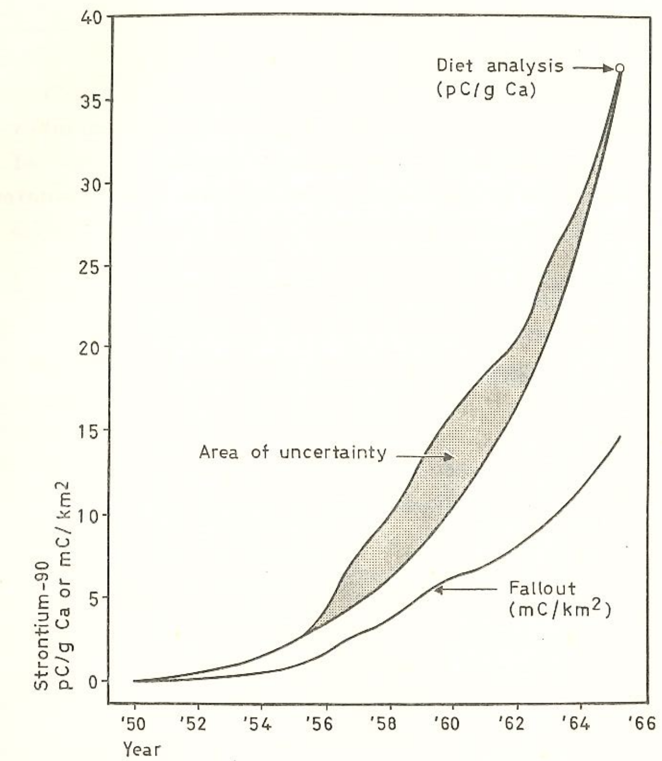

Fig. 1. The accumulated deposition of fallout Sr-90 (mC/km^2) and the estimated dietary content of Sr-90 (pC/g Ca) plotted against the year. See text for details.

all permanent teeth with the possible exception of the late developing human third molar. The estimated diet strontium-90 for older animals, with tooth attrition values between 'I' and 'IV' (see chapter II, this monograph) in which the crown of the permanent teeth was fully erupted, was calculated from the equation $C_T = 0.3\ C_D$. For the smaller monkeys, with 'O' tooth wear classifications, the permanent teeth were partially or entirely unerupted. Because some of these younger animals may still suckle with mother's milk, containing about 1/10 of the mother's dietary Sr-90/Ca intake (REISS, 1961; COMAR, WHITNEY and LENGEMENN, 1955; ROSENTHAL *et al.*, 1964), an intermediate value of 0.2 for the discrimination factor was arbitrarily selected. Admittedly, this factor may be too high, but the degree of suckling relative to the amount of browsing is not known.

Results

The data recorded in Table I demonstrate the strontium-90 content of the teeth obtained for 15 male and 14 female monkeys, the estimated dietary intake of strontium-90 during the time of tooth formation, and the derived age of the animals. The estimated age of all of the animals thus far studied ranges from 1.5 to 12.5 years of age.

The relationship between the strontium-90 content of the tooth crown and the estimated age of the animal, as estimated from the tooth analysis, demonstrates a high degree of correlation (Fig. 2).

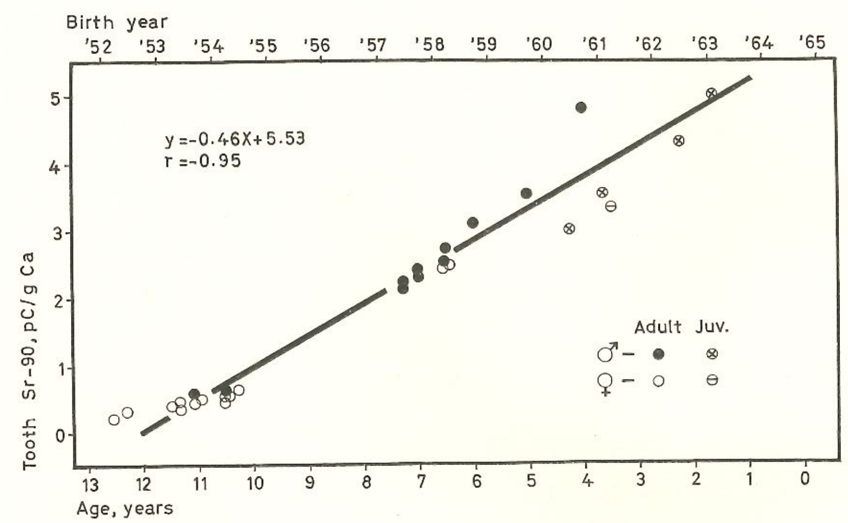

Fig. 2. Strontium-90 content of teeth as a function of the estimated age of *Alouatta.* Note inverse relationship between year of birth and age in years. The line was fitted to the experimental points by the method of least squares.

Separate calculations of correlation coefficients for female animals alone or for male animals alone, including or excluding the juvenile animals, yielded similarly high coefficients.

The body weight of the animals at the time of death demonstrates a typical growth response curve when plotted against the estimated age (Fig. 3). Male *A. caraya* approach an average adult weight of about 7 kg with a range in this sample from 6 to 8.5 kg. Female

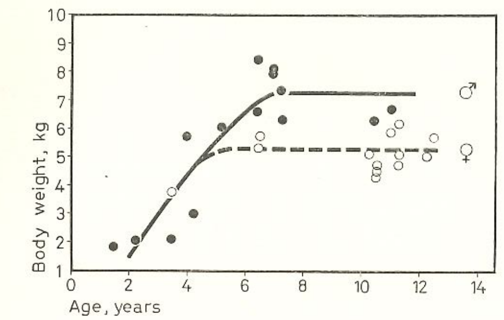

Fig. 3. Body weight as a function of the estimated age of male and female *Alouatta.*

A. caraya are significantly smaller than the males, with an average adult weight of 5 kg and a range of 4.5 to 6 kg. Although sufficient young female animals were absent in this study, the data suggest that maturity in the female animals, as judged by weight, is reached somewhat earlier than in males. This approximates 4.5 to 6 years for the female and 6 to 7 years for the male.

A comparison of the strontium-90 content of the tooth crown with that of the mandibular bone demonstrates that bone, with the exception of one animal, contains significantly higher concentrations of strontium-90 than that found in comparable tooth crowns for the same animals (Fig. 4).

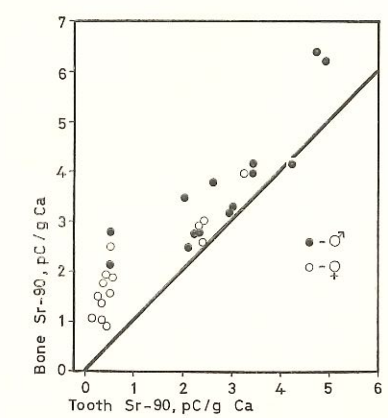

Fig. 4. Relationship between strontium-90 in permanent dentition and mandibular bone of *Alouatta.* The reference line indicates a 1:1 correlation.

Discussion

The studies described in this report cannot be considered definitive because of the unknown strontium-90 content of the diets, and the estimations of discrimination factors. However, the data and the various estimations suggest strongly the plausibility of using tooth strontium-90 analysis as a measure of age of an animal, unobtainable in any other way.

It is somewhat surprising that the data also suggest that none of the *Alouatta* in the collection were more than 12 years of age, while it is well documented that captive monkeys of various species may live as long as 20 to 30 years or more. If the collection is representative of a normal population, the estimated ages indicate that the life span of free-ranging *A. caraya* is relatively short. The largest uncertainty factor is the strontium-90 content of the monkey diet which cannot be directly ascertained for the years between 1950 to 1964. The estimation of the dietary strontium-90 level is, admittedly, an approximation because of the complex nature of the relationships between dietary composition, rates of fallout and rainfall, soil contamination, absorption processes by plants, and the storage of strontium-90 and other mineral elements in edible portions of the vegetation. Furthermore, the relative proportions of the monkey diet with respect to leaf, shoot, seed, or inflorescence is largely unknown. In future studies, the stomach contents would appear to be a more reliable index for strontium-90 content of the diet.

Nonetheless, the data appear to be relatively reliable for a variety of reasons:

(1) The presence of 0.2–0.5 pC Sr-90/g Ca in the tooth crowns of older monkeys suggests that the animals must have been born some time later than 1952 in order to accumulate such amounts of the nuclide. Prior to this time, the fallout in the southern hemisphere was extremely low (BENINSON *et al.*, 1964). It is improbable that a sufficient concentration of strontium-90 would be available in the diet before 1952 to yield the amount of strontium-90 found in the teeth. On this basis alone, all of the Class IV monkeys must have been born between 1952 to 1955, which is in agreement with the estimated age as derived from the tooth strontium-90 determinations.

(2) Two living juvenile *Alouatta*, that were approximately two or three months of age when collected at the time of the expedition, have been raised at the Oregon Regional Primate Research Center. A series of skull x-ray plates demonstrate the rate and degree of calcification of the teeth during growth and development. Because it is well accepted that tooth development and calcification is not influenced to any great extent by environmental conditions, young animals with the same degree of tooth development should be the same age. The skulls of two male juvenile monkeys (No. 148 and No. 246) were also x-rayed prior to strontium-90 analysis of the teeth. A comparison of the degree of tooth development of the living monkeys with those of the analyzed specimens shows that Monkey No. 148 should be about one and a half years of age and Monkey No. 246 should be less than one year of age at the time of collection. The estimated age by strontium-90 analysis of the teeth indicates ages of three and a half and two years and three months, respectively, at the time the teeth were calcified (Table I). The difference between the two kinds of estimates of about two years is remarkably good considering the uncertainty of the parameters used in making the estimations.

(3) The strontium-90 tooth analyses and the estimations derived from the values have not been corrected for the decay rate of strontium-90. Such corrections would increase the tooth content of strontium-90 by 16% during a six-year interval between tooth formation and analysis. This omission is probably not a serious one because the analytical error of strontium-90 analysis is in the order of 25% at best, and the correction for older animals is of doubtful significance. In view of the many uncertainties inherent in this study, it was deemed advisable to avoid such corrections until more data become available, although these variables are recognized.

In view of the difficulty in estimating the absolute age of free-ranging primates and other animals in the absence of a standard for comparison, such as growth rates by tagging studies, estimations to within $\pm$ 2 years is considered to be of considerable value and interest. The uncertainty of the estimates will be decreased with further study and increased knowledge of the complex nature of the transfer of strontium-90 and other nuclides from fallout through the food chain.

The observation that mandibular bone contains higher levels of strontium-90 than the teeth was expected and is due to the greater turnover rate of bone matrix (RIVERA, 1965). During periods of increasing concentrations of strontium-90 in the diet, bone would

Table I. Strontium-90 content of teeth, body weight and estimated age of free ranging *Alouatta caraya*

Monkey No.	Tooth[1] wear	Body weight kg	Tooth Sr-90(C_T) pC/gCa	Est. diet[2] Sr-90(C_D) pC/gCa	Est. age years
			Male		
21	0	3.00	2.95	14.8	4.25
96	0	1.88	4.93	24.6	1.50
148	0	2.17	3.46	17.3	3.50
246	0	2.05	4.28	21.4	2.25
102	I	7.40	2.12	7.1	7.25
151	I	4.12	3.02	10.1	6.00
210	I	5.77	4.76	15.9	4.00
276	I	6.65	2.42	8.1	6.50
182	II	6.33	2.08	6.9	7.25
183	II	6.33	0.57	1.9	10.50
259	II	8.47	2.62	8.7	6.50
107	III	8.00	2.34	7.8	7.00
206	III	8.08	2.25	7.5	7.00
299	III	6.10	3.45	11.5	5.25
233	IV	6.69	0.52	1.7	11.00
			Female		
26	0	3.75	3.26	10.9	3.50
19	I	5.70	2.44	8.2	6.50
238	I	5.34	2.39	8.0	6.50
54	II	5.84	0.44	1.5	11.00
88	II	5.07	0.60	2.0	10.25
156	II	6.10	0.40	1.3	11.25
186	II	4.40	0.53	1.8	10.50
185	II	4.32	0.47	1.6	10.50
100	III	4.68	0.35	1.2	11.25
106	III	5.70	0.19	0.6	12.50
157	III	4.73	0.51	1.7	10.50
160	III	5.00	0.26	0.9	12.25
174	IV	5.47	0.45	1.5	11.00
207	IV	5.00	0.37	1.2	11.25

[1] Tooth wear of first maxillary molar graded arbitrarily.

[2] Diet for animals with 0 tooth wear classification calculated from $C_T = 0.2C_D$. All other calculations from $C_T = 0.3C_D$. See text for details.

contain a higher nuclide content than the teeth that are formed at a lower concentration of strontium-90. Conversely, during periods of decreasing dietary strontium-90, bone might contain smaller amounts of the nuclide as compared to the teeth. Similar observations have been made for human deciduous tooth crowns and roots (ROSENTHAL, 1966b).

The studies described in this report indicate the rationale and approach for estimating the absolute age of free-ranging *Alouatta* based on the strontium-90 content of the teeth with concomitant knowledge concerning the environmental concentrations of the nuclide. The basic hypotheses appear to be valid and applicable for all animals that form a permanent, stable dentition that is not subject to exchange, turnover, or accretion of the nuclide once the teeth are calcified.

Summary

Strontium-90 analysis of permanent teeth was used to determine the chronological age of 29 specimens of *Alouatta caraya*. The method for age determination is based on the permanence of strontium-90 incorporated into the teeth at the time of formation and the linear relationship between the amount of tooth Sr-90/Ca (C_T) and dietary Sr-90/Ca (C_D) expressed by the equation $C_T = K\,C_D$ where K is the discrimination factor of strontium relative to that of calcium. The method is estimated to be reliable to within two years of age.

The data also suggest that free ranging female *Alouatta* reach full growth at about five years of age as compared with male *Alouatta* that reach full growth at about six and a half years of age.

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