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BONE MORPHOMETRICS AND TETRACYCLINE MARKING PATTERNS IN YOUNG GROWING AMERICAN ALLIGATORS (*ALLIGATOR MISSISSIPPIENSIS*)

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ABSTRACT: Nine young American alligators (*Alligator mississippiensis*) were injected at monthly intervals with tetracycline to determine the bone apposition rate and the resorption patterns over a 3-mo period. The periosteal apposition rate increased progressively over the 3-mo period from 2.99 μm/day to 5.94 μm/day. Endosteal apposition rate was much slower with incomplete tetracycline lines being observed on the endosteum. This suggests that most modeling-resorptive activities occur on the endosteal envelope.

Key words: American alligator, *Alligator mississippiensis*, tetracycline marking, periosteal apposition rate, bone resorption patterns, bone growth rates, experimental study.

INTRODUCTION

The bones of American alligators (*Alligator mississippiensis*) are used as an "aging tool" by biologists because the alligator produces annular rings corresponding to hibernation periods (Peabody, 1961; Griffiths, 1962; Enlow, 1969). For this technique to be accurate, the metabolic turnover should be minimal since remodeling would destroy the annular rings. Also, animals with nutritional osteodystrophies from poorly managed animal exhibits would alter these lines if the resorptive mechanisms present in some mammalian species were active (Petter-Rousseaux, 1953; Suzuki, 1963; St. Girons, 1965). To use the alligator for studies of metabolic bone disease, the dynamics of normal bone formation and remodeling should be defined. The objectives of this study were to evaluate the tetracycline dosage (20 mg/kg) for application as a time marker for young alligator bone formation and to evaluate remodeling dynamics using morphometric techniques.

MATERIALS AND METHODS

Nine alligators, 6 mo of age, were hatched and maintained in the Rockefeller Wildlife Refuge, Grand Chenier, Louisiana (30°09′N, 93°19′W). All animals were fed a ground whole nutria diet supplemented with vitamins and minerals (Dawe's Laboratories, Chicago Heights, Illinois 60511, USA) and were housed in tanks maintained at 30 C. Nine alligators were injected intraperitoneally with 20 mg/kg of tetracycline (Pfizer Corporation, 1107 S. Missouri, Lee's Summit, Missouri 64036, USA) four times at 30-day intervals. At the termination of the experiment, the animals were bled using intracardiac puncture and were killed by pithing. Serum calcium and phosphorus levels (Centrifichem 400, Baker Instruments Corporation, Allentown, Pennsylvania 18001, USA) were made to evaluate utilization of Ca/P in the experimental ration.

Bone samples were collected from the femur and stored frozen in a household freezer. Bone cross sections were hand ground, mounted and examined using epifluorescence with excitation by blue light at 360 nm wavelength (E. Leitz, Rockleigh, New Jersey 07647, USA). Each sample taken from the mid-shaft femur was photographed and evaluated using an IBM-PC computer and Bone Morphometric Bioquant II software (R and M Biometrics, Nashville, Tennessee 37209, USA). Direct measurements of complex shaped areas and perimeters are possible. The cross-sectional bone image and metric scale were photographed and printed. The image was then outlined and measured on the digitizing tablet (R and M Biometrics) with a resolution of 0.1 mm. The following determinations were then made: cortex area (mm^2), periosteal apposition rate (μm/day), endosteal area new bone formation (mm^2), and endosteal appositional rate (μm/day).

The initial cortex area represented in Table 1 as day 0 is the mid-shaft cross-sectional area at the start of the experiment. Total area cortex at 1 mo represents the old cortex plus bone formed in the 1-mo period. Apposition rate is defined as the distance between two tetracycline markers expressed as μm/day growth. A total

TABLE 1. Femur morphometric and serum calcium phosphorus values of nine young American alligators which were injected with tetracycline.

Measurement	Time of injection with tetracycline			
	Day 0	1 mo	2 mo	3 mo
Total cortex area (mm^2)	4.01/0.08[a]	4.99/1.11	6.34/1.59	8.66/2.09
Periosteal apposition rate (μm/day)		2.99/1.46	4.12/1.56	5.94/1.46
Total new endosteal area (mm^2)				0.43/0.24
Endosteal apposition rate (μm/day)		1.04/0.34	1.19/0.25	2.23/0.97
Serum Ca (dl/ml)				9.82/0.63
Serum P (dl/ml)				7.15/1.1

[a] Mean value/standard deviation.

of 10 measurements were made at approximately 36° intervals and then averaged.

RESULTS

Morphometric data listed in Table 1 resulted from measurements made from the hand ground fluorescent bone cross-sectional images shown in Figure 1. The dosage of tetracycline was adequate to mark injection times. The cortex area at the start of the experiment was 4.08 mm^2 and 8.66 mm^2 after 3 mo. The first month periosteal appositional rate was 2.99 μm/day increasing to 5.94 μm/day by the third month. A rapid increase in rate of bone formation was shown in this time of normal rapid growth. A minor increase in endosteal appositional rate was seen also. Mean serum calcium/phosphorus concentrations were 9.82/7.15 dl/ml.

DISCUSSION

The use of tetracycline labelling for orthopedic research was reported in 1956 (Andre, 1956). Since that time, numerous studies have emphasized the benefits and limitations of its use (Frost, 1960, 1969; Harris, 1960; Harris et al., 1962; Hattner and Frost, 1962; Hoerman, 1975; Treharne and Brighton, 1979). Distinct measurable lines must be present and at least two injection intervals must be sufficient to produce two lines. The appearance of the line is dependent on the species, dosage of tetracycline and route of inoculation. The dosage used with the alligators was similar to that advocated for humans and dogs, whereas dosages as low as 1 mg/kg have been used in rodents (Thorengren and Hansson, 1981). Distinct sharp lines were produced with the 20 mg/kg dosage. Tetracycline has been reported to retard bone growth at concentrations of 100 mg/kg, which is much higher than the dosage used in this study (Harris et al., 1968). In Table 1 the standard deviation increases as aging occurs. This may reflect a toxic effect of the tetracycline dosage used.

It is essential that the marked bone is hand ground and examined directly using an ultraviolet light source or fixed in absolute ethanol and processed using plastics. Formaldehyde fixation will leach the tetracycline marker (Frost, 1969).

The structure of cortical bone in young alligators is composed of vascular-type fiber bone (Enlow, 1969) which does not develop the typical osteone-haversian canal structures seen in mammalian species. Osteoblastic activity is confined to the periosteal and endosteal areas. Osteocytes are similar to those seen in other species. The "old" cortical bone shows very little uptake of the tetracycline, suggesting that the fiber bone is metabolically inactive.

The periosteal bone formed appeared concentrically uniform. As the animal aged, the apposition rate increased (Table 1). Endosteal apposition rate was much slower than the periosteal and tended to be focal. The presence of incomplete lines in areas where one line previously existed suggests that this tissue is the most metabolically active resorptive portion. As

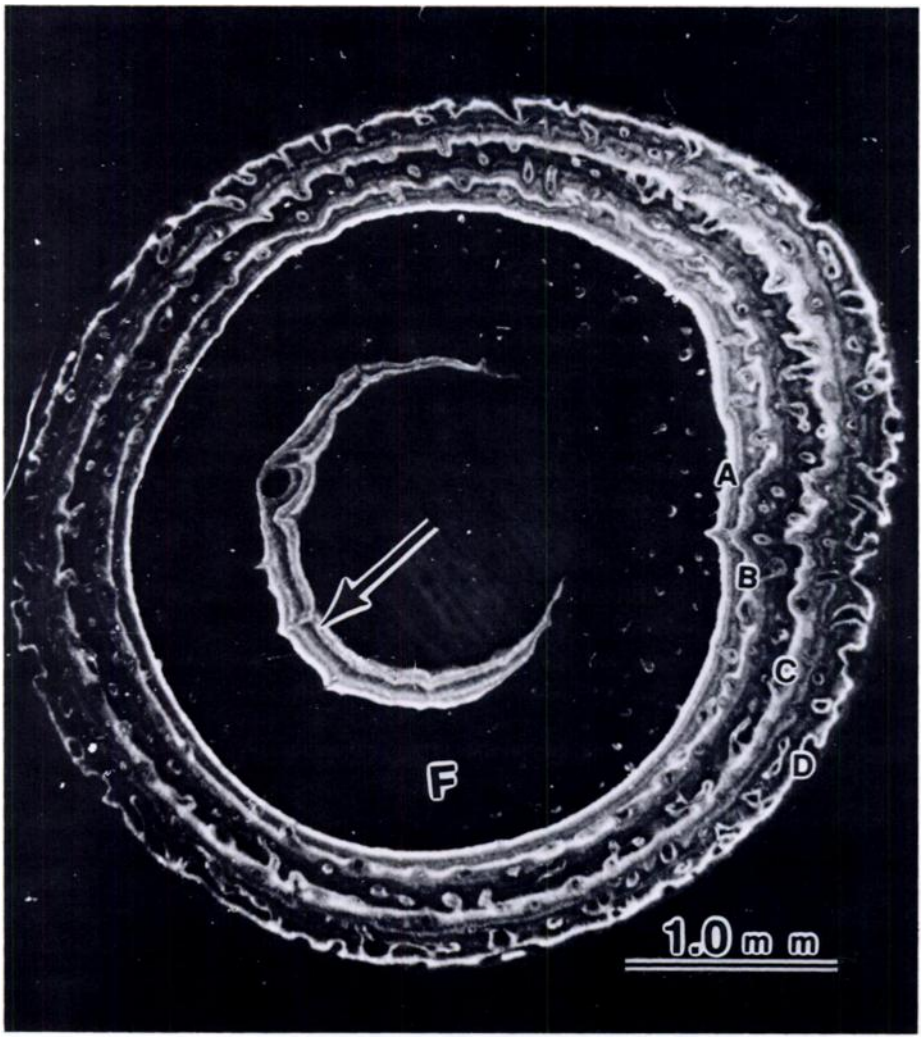

FIGURE 1. Midshaft femur (F) cross section of American alligator demonstrating tetracycline lines: time interval between injection of tetracycline was 30 days (A = day 0, B = day 30, C = day 60, D = day 90). Note the difference between the periosteum (A through D) and endosteal activity (arrow).

growth occurs by periosteal new bone formation, resorption must occur in the endosteum so the bone architecture will remain proportional. In these young alligators, all resorption and remodeling involved only the bone formed by the endosteum. The total mean cortex/endosteal area produced was 4.59/0.43 indicating approximately 10 times faster periosteal bone production rate.

The reliability of the use of the femur cross sections as an aging tool could be determined using this technique of skeletal time marking. Evidence of resorption was not observed from periosteal bone formed in the 3-mo period of this trial since most formed lines were intact. Longer periods involving several successive seasons should be done to accurately define the hibernation affect on the normal "aging" lines. Reported measurements could serve as a basis for assessing changes in the remodeling rates associated with metabolic bone disease.

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