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Lead in the Bone and Soft Tissues of Box Turtles Caught Near Smelters

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The amount of lead in modern reptilian bone is unknown, as is how much lead reptilian tissues can accumulate from man-made sources, although cadmium (ROBINSON & WELLS 1975) and pesticides (HOLCOMB & PARKER 1979) amass in chelonian soft tissues after exposure in the wild. We find that, in N. American box turtles (Terrapene carolina) caught 500 m from lead smelters, the bone, blood and soft tissues bear excess lead. The box turtle may live up to 100 years (GRAHAM & HUTCHINSON 1969), has a range of only a few hundred meters (SCHWARTZ AND SCHWARTZ 1974), and the dense chelonian long bones experience a limited, mostly central turnover that leaves a wide cortex with prominent annular rings (CASTANET & CHEYLAN 1979). Hence, the annular pattern of growth in reptilian bone may offer a retrospective means of dating the exposures of long-term resident vertebrates to bone-seeking heavy metals, analogous to that provided by elm trees (SCHROEDER & BALASSA 1961) for vegetative uptake.

MATERIALS AND METHODS

Three box turtles were caught by small streams in woodland south-east of Glover, Missouri and one, north-east of Bixby, Missouri: rural sites of primary lead smelters, and of a study (BAKER et al. 1977) on the heavy metals found in children living up to 8 km away. Four other turtles were caught in a similar habitat, but distant from industry and main roads, 5-15 km outside Morgantown, West Virginia. Both groups shared the same food, water, and outside pen in Morgantown for a period of 30-60 days from their capture to their deaths by an overdose of barbiturate.

Lead content was assayed by atomic absorption spectrophotometry. Tissues were digested in concentrated nitric acid and the lead determined at $\lambda = 217.0$ in a Perkin-Elmer 305-B spectrophotometer equipped with a heated graphite furnace and deuterium arc background corrector and calibrated against a certified lead reference solution (Fisher Scientific). The bone pieces broken out of the shaft had very little marrow. The humeri were scraped clean with surgical blades, the femora with plastic knives. The skin was dorsal, caudal, cranial. Humeral annuli were counted in the sector of their greatest number in hematoxylin-stained cross-sections of paraffin-embedded, decalcified shaft pieces.

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RESULTS AND DISCUSSION

Table 1 shows the lead levels of the liver, kidney, skin, blood, and two long bone shafts, to be significantly higher in exposed animals (E) than in less exposed animals (LE). The LE bone lead values are close to those of non-exposed horses (SCHMITT et al. 1971), cattle and sheep (DOYLE 1979), toads (IRELAND 1977), and those of Mexican forest Indians' teeth (SHAPIRO et al. 1975). Exposed bone lead amounts are less than those in urban Detroit rats (MOUW et al. 1975), ribs and vertebrae of horses living near lead smelters (SCHMITT et al. 1971), and in adult human dentine from urban Philadelphia (SHAPIRO et al. 1975). Lead levels in exposed turtle bone exceed those of bone in deer mice trapped alongside a major Colorado highway (MIERAU & FAVARI 1975), and in toads fed leaded worms for 4–8 weeks (IRELAND 1977).

The lead level of human bone increases with age (BARRY & MOSSMAN 1970; O'CONNOR et al. 1980). CASTANET & CHEYLAN'S (1979) method of matching the numbers of scute rings and appendicular bone annuli for the estimation of age in Mediterranean tortoises has not been previously applied to box turtles. In this study, the number of scute rings and humeral annuli (Table 1) match each other as closely as in CASTANET & CHEYLAN's specimens. The annuli, supported by the scutes, furnish a minimum age, by which to rank the turtles. Confirmation of the reliability of the annuli as indicators of relative and absolute age awaits lengthy study with vital labelling. Table 1 shows that, within E and LE groups, the lead levels in bone, blood and soft tissues hint at a modest correlation with seniority. More importantly there is no significant difference in mean minimum age to account for the much greater amounts of lead in E than LE animals.

Human liver, weight for weight, stores more lead than the kidney (BARRY & MOSSMAN 1970), but although the converse definitely holds for rats (MOUW et al. 1975; MYLROIE & EROGBOGO 1977), deer mice (MIERAU & FAVARI 1975), and toads (IRELAND 1977) the turtle's kidney (E and LE) has only marginally more lead than the liver. The amounts of lead in LE turtle kidneys and livers are slightly less than those of Rana pipiens in areas of rural Vermont (SCHROEDER & TIPTON 1969). Chelonian skin has a weak ability to concentrate lead, leaving three major stores, bone, kidney and liver, to help account for the high blood lead two to three months after removal from exposure. The E lung values are low, but above LE figures, so that inhalation might contribute to the acquisition of lead by E turtles, as could eating items, such as vegetation (SCHMITT et al. 1971) and earthworms (IRELAND & RICHARDS 1977), known to be contaminated by smelters and metallic spoil.

Exposed and LE turtles differed in belonging to subspecies T.c. triungis and T.c. carolina respectively. We believe this factor to be immaterial, because scute ring and annular counts

Table 1 Lead content of box turtle tissues (μg/g wet weight) determined by atomic absorption spectrometry.

TURTLE & SEX	HUMERUS	FEMUR	LIVER	KIDNEY	CARDIAC BLOOD	SKIN	LUNG	BODY WEIGHT(g)	SCUTE RINGS	HUMERAL ANNULI
Cm	113.04	135.86	9.12	23.44	7.66	0.47	0.16	456	17	16
Hm	37.39	48.49	46.95	52.24	2.48	0.24	0.34	396	15	16
Bm	31.11	51.92	15.70	13.09	11.59	0.25	0.19	439	14	15
Dm	25.78	21.73	14.62	8.45	2.17	0.44	0.10	390	10	14
Exposed means & s.d.										
	51.83±41.08	64.50±49.45	21.60±17.15	24.31±19.65	6.00±4.15	0.35±0.12	0.20±0.10	420±32	14±2.9	15.3±0.8
Fm	4.51	5.55	2.21	4.83	0.22	ND	ND	425	21	24
Ef	4.03	2.23	0.63	1.08	0.15	ND	ND	362	18	19
Gf	3.66	2.99	1.00	0.68	ND	0.11	ND	432	20	16
Af	2.54	4.40	0.77	0.55	0.08	0.16	ND	475	11	11
Less exposed means & s.d.										
	3.69*±0.84	3.80*±1.49	1.15*±0.72	1.79†±2.04	0.11*±0.09	0.07†±0.08	-	424±47	17.5±4.5	17.5±4.7

* - means differ at $P < 0.001$, + - at $P < 0.005$, by the equality of variance test or, for skin, Student's t-test. Individual E Values fall outside the tolerance (99%) limits from the LE distribution at the $P < 0.01$ level for the humerus and blood, at $P < 0.05$ for femur, and $P < 0.05$ (95% tolerance limit) for the liver.

ND - is the non-detectable level.

and body weights (Table 1) did not distinguish the groups, and it is toe count and skin coloration that constitute the basis for separating the subspecies. Further studies will, however, use T.c. triungis for another LE group, seek more and older E turtles and other reptiles, and apply microprobe elemental analysis to individual bone annuli and the potentially lead-binding melanin granules in skin (IRELAND et al. 1979).

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