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Fluoride Content and Mineralization of Red Deer (*Cervus elaphus*) Antlers and Pedicles from Fluoride Polluted and Uncontaminated Regions

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Abstract. Fluoride, calcium, and phosphorus content as well as ash percentage and ash density of primary antlers and pedicle bones were studied in nine yearling red deer stags from a fluoride polluted region in North Bohemia (Czech Republic) and in nine control animals from two uncontaminated areas in West Germany. Fluoride levels in antlers (845 ± 257 mg F^-/kg ash, mean ± SD) and pedicles (1,448 ± 461 mg F^-/kg ash) of the N-Bohemian specimens exceeded that of the controls (antlers: 206 ± 124 mg F^-/kg ash, pedicles: 322 ± 157 mg F^-/kg ash) by factors of 4.1 and 4.5, respectively. Antler and pedicle fluoride concentrations of the deer ($n = 18$) were closely correlated ($r = 0.975$, $p < 0.001$). Analyses of ash percentage and ash density revealed that the antlers of the N-Bohemian deer contained significantly less mineral and were significantly less dense than both their pedicles and the control antlers. In the pooled antler samples ($n = 18$), bone fluoride concentration was negatively correlated with ash density ($r = -0.826$, $p < 0.001$) and ash percentage ($r = -0.759$, $p < 0.001$), whereas non significant, positive correlations existed for the pooled pedicle samples. Ash percentage and ash density of the antlers and their corresponding pedicles were uncorrelated. It is concluded that increased fluoride exposure of deer leads to reduced mineral content and mineral density of antler bone and that it is the rapidity of their growth and mineralization that makes antlers especially susceptible to fluoride action. Due to their ability to accumulate high amounts of fluoride during a defined, limited timespan and the apparently dose-dependent negative effect of fluoride on their density and mineral content, (primary) antlers can be recommended as monitoring tools for studying environmental pollution by fluorides.

Antlers are deciduous bony outgrowths of male deer (and females in *Rangifer*) that develop on top of permanent frontal protuberances, the pedicles. The growth of velvet antlers takes place during a seasonally fixed time-span of some months and constitutes the most rapid formation of a bony structure known in vertebrates, thereby making antlers useful models for the study of bone growth and biomineralization (Goss 1983, 1995; Bubenik 1990; Szuwart *et al.* 1995). After velvet shedding, the dead, hard antlers are retained for some months until casting and the start of new velvet antler growth.

Growing antlers are sinks for calcium, phosphorus, and other constituents of bone mineral and it has been shown that a considerable part of the minerals laid down in (subsequent) antlers is mobilized from the animal's skeleton (Banks *et al.* 1968a, 1968b; Cowan 1968; Hillman *et al.* 1973; Brown *et al.* 1978). In a number of studies, other authors have proven that antler bone, like other calcified tissues, accumulates certain pollutants such as lead, strontium-90, and fluoride (Schultz 1964, 1965; Karstad 1967; Gelbke 1972; Sawicka-Kapusta 1979; Samiullah and Jones 1991; Suttie *et al.* 1985; Walton and Ackroyd 1988; Samujilo *et al.* 1994; Tataruch 1995) and that deer antlers can thus serve as useful indicators of environmental contamination by these substances. By contrast, at present very little is known about whether increased concentrations of certain pollutants in antler bone are associated with changes in other properties of the antlers, such as size, density or mineralization.

The present study compares fluoride concentrations in primary antlers and pedicles of red deer stags (*Cervus elaphus*) from a fluoride polluted region in North Bohemia (Czech Republic) and unpolluted control areas in West Germany. In order to investigate if differences in antler and pedicle fluoride concentrations were associated with variation in bone quality, also calcium and phosphorus content as well as ash percentage and ash density of the samples were determined.

Materials and Methods

Study Areas and Specimens

Primary antlers (unbranched spikes of 7.2 to 33.4 cm length) and pedicles of yearling red deer stags that had been killed during normal hunting operations in the respective areas were analyzed. Nine red deer

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calvaria were available from the region NE of the town Karlovy Vary in N-Bohemia. This area, located on the southern slope of the Ore mountains, is exposed to severe atmospheric fluoride deposition (Valach *et al.* 1993). Major sources of this pollution are thermal power plants burning low quality lignite with high contents of both sulphur and fluoride. According to Valach (1990), fluoride concentration in the lignite varies between 200 and 600 ppm, and average yearly fluoride emissions from coal combustion in the N-Bohemian brown coal belt have been estimated at ca. 12,000 tons (Kotesovec 1990). In 1992, fluoride fallout in the region of the town Chomutov, located in this area, amounted to 366 kg F^-/km^2 (Czerny 1992). Previous studies have shown that wild deer inhabiting the fluoride polluted region NE of Karlovy Vary exhibit both elevated mandibular bone fluoride concentrations and various degrees of dental fluorosis as a result of chronic fluoride intoxication during tooth development (Kierdorf *et al.* 1996a, 1996b, 1996c). In red deer, an increase in the frequency and intensity of the dental lesions was found to be positively associated with an increase in bone fluoride content (Kierdorf *et al.* 1996b). Nine calvaria or complete skulls of red deer yearlings from two hunting districts in the Eifel and Hunsrück mountains (West Germany) served as controls. The two regions are free of major sources of fluoride emissions and have no known history of fluoride pollution.

Sample Collection and Analyses

About 1-cm-thick, full-diameter cross-sectional disks of antler and pedicle bone, respectively, were cut from the cranial outgrowths at levels ca. 1 to 2 cm proximal (pedicle samples) and 3 to 4 cm distal (antler samples) to the antler–pedicle junction, using a diamond saw. In 16 of the specimens, the samples were removed from the left pedicle and antler. In two control skulls, in which the left antler or pedicle had been fractured *intra vitam*, the samples were taken from the right side. After removal, the (dry) samples were weighed on a precision balance. The cut surfaces were then covered with colourless nail varnish (to exclude water from the internal spaces) and the volume of the sections was determined by water displacement. Afterwards, the specimens were ashed in a muffle oven (2 h at 105°C, 24 h at 560°C) and weighed again. Ash percentages (% ash weight) and ash (mineral) densities (g ash per ml) of the samples were calculated from the dry and ash weights and the volume measurements.

Fluoride was measured by a modification of the method proposed by McCann (1968). Samples (5–10 mg) of the ashed specimens were weighed and dissolved by constant stirring for 24 h, in 4 ml of 0.1-M perchloric acid. 0.5 ml of this bone acid solution was then added to 0.5 ml 0.1-M potassium hydroxide and 1 ml total ionic strength adjustment buffer (TISAB, Orion-Research) and used for fluoride determination with a ion-selective combination electrode (model 96-09, Orion-Research). Studies from our laboratory (Pearce *et al.* 1995) and by other authors (Boulton *et al.* 1995) have shown that this analytical procedure is both precise (CV of ca. 2%) and accurate (recovery of >95% in reference samples of known fluoride content). Fluoride levels in the bone sample solutions were within the range of linear relationship between log fluoride concentration and electrode potential. The results of the fluoride determinations are expressed as mg F^- per kg (= ppm) of ashed antler or pedicle bone.

Another 0.5 ml of the bone acid solution was further diluted with 1.0 ml of 0.1-M perchloric acid to form a stock solution for determination of calcium and phosphorus. Calcium was measured by atomic absorption spectrophotometry (Perkin Elmer 2380) using 0.1 ml of the stock solution diluted with 1.5 ml lanthanum. Phosphorus was measured by spectrophotometer (Milton Roy Spectronic 301) using 0.25 ml of the stock solution diluted with 4 ml ascorbic acid-ammonium-molybdate solution (Chen *et al.* 1956). Results of these measurements are expressed as g Ca or P per kg of ashed antler or pedicle bone.

Statistics

Student's two-tailed *t*-tests were applied to test for differences between means of the antler and pedicle bone samples from N-Bohemia and the control regions as well as between means for antlers and pedicles in the two groups. Linear regression analysis and calculation of Pearson's correlation coefficients were performed to reveal the relationship between fluoride concentrations of antlers and pedicles, between fluoride concentrations and ash percentages as well as ash densities in the antler and pedicle samples and between ash percentage and ash density of the antlers and their corresponding pedicles. The value $p < 0.05$ was chosen as the level of statistical significance.

Results

Means and standard deviations for fluoride, calcium and phosphorus concentrations, Ca/P and Ca/F molar ratios, ash percentages and ash densities of the samples as well as the significance levels of the differences between means are given in Table 1. As is revealed by the data for ash percentage and ash density, the antlers from N-Bohemia, which on macroscopic inspection appeared rather porous, were significantly less mineralized and less dense than both their pedicle bone and the control antlers. By contrast, the means for these parameters did not differ significantly between the two pedicle samples. In the controls, the differences in ash percentages and ash densities between pedicles and antlers were lower than in the N-Bohemian sample. Values were again higher in the pedicles, however only the difference in mean ash percentage was of statistical significance. Ash percentages ($r = -0.001$, not significant [ns]) and ash densities ($r = -0.022$, ns) of the antlers and their corresponding pedicles were uncorrelated.

Mean antler and pedicle fluoride content of the N-Bohemian specimens exceeded that of the controls by factors of 4.1 and 4.5, respectively. In all specimens, fluoride concentration in the pedicle was higher than in the corresponding antler, the equation for the linear regression of pedicle fluoride content on antler fluoride content for the pooled samples ($n = 18$) being $y = 1.7314 \times -21.564$ [y = pedicle fluoride content (mg F^-/kg ash), x = antler fluoride content (mg F^-/kg ash)]. Antler and pedicle fluoride levels of the deer were closely correlated ($r = 0.975$, $p < 0.001$, $n = 18$). The differences in fluoride content between the samples were reflected by their calcium/fluoride molar ratios. In the pooled antler samples, bone fluoride content was negatively correlated with ash percentage ($r = -0.759$, $p < 0.001$) and ash density ($r = -0.826$, $p < 0.001$), whereas non significant, positive correlations existed for the pooled pedicle samples ($r = 0.116$, ns, and $r = 0.170$, ns, for the relationships between fluoride level and ash percentage and ash density, respectively). Despite the small sample sizes, the negative relationship between antler bone fluoride concentration and ash percentage was still significant at $p < 0.01$ in the controls and that between antler fluoride content and ash density at $p < 0.05$ in the specimens from N-Bohemia.

Mean calcium and phosphorus concentrations in bone ash were significantly higher in the antlers than in the corresponding pedicles, except for the difference in mean calcium content between control pedicles and antlers, which was just outside the range of statistical significance ($p = 0.051$). Mean calcium/phosphorus molar ratios of the samples ranged between 1.68 and 1.70, and did not differ significantly between antlers and

Table 1. Means and standard deviations (SD) of fluoride, calcium and phosphorus concentrations, Ca/P and Ca/F molar ratios, ash percentages and ash densities in the antlers and pedicles of the deer from N-Bohemia (n = 9) and the control deer from West Germany (n = 9). Significance levels of differences were determined by Student's *t*-tests, ns = not significant

	Fluoride Content (mg F^-/kg ash)	Ca Content (g Ca/kg ash)	P Content (g P/kg ash)	Ca/P Molar Ratio	Ca/F Molar Ratio	% Ash	g Ash/ml
N-Bohemian samples							
A. pedicles							
mean	1,448	393.4	180.8	1.68	140	56.85	0.867
SD	461	14.3	9.1	0.05	42	1.40	0.083
B. antlers							
mean	845	425.0	195.3	1.68	258	47.59	0.559
SD	257	8.8	3.9	0.03	79	3.11	0.089
Control samples							
C. pedicles							
mean	322	404.4	186.2	1.68	721	56.90	0.861
SD	157	19.3	3.7	0.07	305	1.26	0.102
D. antlers							
mean	206	421.0	191.9	1.70	1,229	53.14	0.777
SD	124	13.0	6.0	0.03	546	2.92	0.128
Significance levels (p) of differences between means							
A vs B	<0.01	<0.001	<0.01	0.956, ns	<0.01	<0.001	<0.001
C vs D	0.103, ns	0.051, ns	<0.05	0.530, ns	<0.05	<0.01	0.144, ns
A vs C	<0.001	0.189, ns	0.129, ns	0.854, ns	<0.001	0.935, ns	0.889, ns
B vs D	<0.001	0.458, ns	0.182, ns	0.358, ns	<0.001	<0.01	<0.001

pedicles within a single group or between antlers or pedicles of the two groups.

Discussion

The differences in fluoride concentration of antlers and pedicles between the two red deer samples indicate that the individuals from N-Bohemia had been exposed to much higher levels of environmental fluoride during life than the controls, thus corroborating the results of previous studies on material from this region (Kierdorf *et al.* 1996a, 1996b). In accordance with the findings of other workers (Suttie *et al.* 1985; Walton and Ackroyd 1988; Samujlo *et al.* 1994), our results therefore illustrate that deer antlers (and pedicles) can serve as useful tools for monitoring environmental pollution by fluorides. A major advantage of antlers over other skeletal elements is that the deer do not need to be sacrificed in order to obtain bone samples, since the analyses can be performed on the naturally cast antlers.

It was previously shown for different deer species that increased fluoride exposure during odontogenesis induces a variety of pathologic dental changes that can be used as biomarkers of an elevated fluoride burden on individuals or populations (Kierdorf *et al.* 1993a, 1996a, 1996b, 1996c). As a consequence of a fluoride-induced enamel hypomineralization, decreased resistance of the teeth to masticatory stress and increased dental wear were observed, which in older deer sometimes resulted in completely dysfunctional tooth shapes, presence of tooth fractures and tooth loss following extensive periodontal breakdown (Kierdorf *et al.* 1996a, 1996c). These alterations will inevitably lead to a marked fitness reduction in the affected animals. Moreover, also changes in population structure due to premature death of older individuals can be expected under these conditions.

In male red deer calves, pedicle development normally commences at about 5 to 6 months of age (Li *et al.* 1993). At 10 to 11 months, the primary antlers start to grow from the pedicles. Velvet shedding occurs at about 15 to 16 months and first antler casting at 23 to 24 months (Raesfeld and Reulecke 1988). The yearling stags analyzed in this study had been shot after velvet shedding and prior to antler casting. A rough estimation shows that the time-span for fluoride accumulation by the pedicles had at least doubled that for the primary antlers. However, pedicle fluoride levels in the two samples exceeded those of the antlers 'only' by a factor of 1.7. Our data, thus, indicate a higher rate of fluoride accumulation by antler bone compared to pedicle bone. The capacity of the rapidly growing and highly vascularized antlers (Banks and Newbrey 1983; Li and Suttie 1995; Kierdorf *et al.* 1995a) to take up larger amounts of fluoride within a short time-span has previously been demonstrated by Suttie *et al.* (1985) in white-tailed deer. Addition of 50 ppm fluoride to the diet (containing between 19 and 25 ppm F^-) of two animals resulted in vertebral fluoride concentrations of 7,408 and 7,352 mg F^-/kg ash after two years. The antlers grown by these bucks during the second year of the study contained 5,291 and 5,808 mg F^-/kg ash, respectively. Thus, during a few months period, the growing antlers were able to take up between 71 and 79% of the fluoride accumulated by the vertebrae in two years.

Data on antler and pedicle fluoride concentrations of wild deer are scarce. Karstad (1967) reported values of 134 and 152 mg F^-/kg dry wt, respectively, for the antlers of a 3.5- and a 4.5-yr-old white-tailed buck from an unpolluted region, whereas antler bone levels in deer living near an unspecified industrial complex ranged between 503 and 2,997 mg F^-/kg dry wt. Walton and Ackroyd (1988) studied bone fluoride in antlers and pedicles of 10 roe deer (ages not given) from 3 localities in England and Scotland. In nine of the deer, values ranged between 202 and 471 mg F^-/kg dry wt and did not differ

significantly between pedicles and different parts of the antlers. The tenth animal exhibited much higher fluoride concentrations (1,720 to 2,010 mg F^-/kg dry wt), which where considered anomalous by Walton and Ackroyd (1988). In three Scottish red deer stags (ages not given), these authors found antler fluoride values of 105 to 170 mg F^-/kg dry wt. In 120 red deer antlers (ages of individuals and sampling sites not given) analyzed by Tataruch and Wolfsperger (1995), fluoride values ranged between 40.2 and 579.5 mg F^-/kg ash. When compared with the above results, the antler and pedicle fluoride concentrations of our control sample can be interpreted as reflecting ''normal'' background levels for a central European red deer population. Recently Samuljo *et al.* (1994) reported fluoride concentrations in subsequent antlers and ''skull bones'' of red deer (mean ages of the samples between 3.7 and 5.6 yr) from 4 different regions in West Poland. In the vicinity of a fluoride emission source (chemical fertilizer plant), mean fluoride concentrations of 600.9 and 697.9 mg F^-/kg dry wt were found in antlers and skull bones, respectively. These values are similar to those in the red deer sample from N-Bohemia. Antler and pedicle bone fluoride values of the Polish red deer in areas distant to the source of fluoride emission were significantly lower than those in the vicinity of the fertilizer plant. However, even the lowest mean values of 338.1 and 356.8 mg F^-/kg dry wt recorded for antlers and skull bones, respectively, still clearly exceed the fluoride concentrations found in the red deer from West Germany.

Although as yet no data are available on the amount of fluoride that during antlerogenesis is mobilized from the skeleton and subsequently deposited in the growing antlers, there is circumstantial evidence that this translocation considerably affects antler fluoride concentrations. Karstad (1967) found fluoride values of 503, 886, and 1,906 mg F^-/kg dry wt, respectively, in three antlers cast in consecutive years by a white-tailed buck from a fluoride polluted area. While this author interpreted his data as suggesting increasing fluoride contamination of the environment, the results may alternatively be taken as denoting an increasing contribution of previously skeletally deposited fluoride to the fluoride content of the antlers. The same applies to the fact that in five of the six white-tailed bucks studied by Suttie *et al.* (1985), fluoride concentrations in the antlers grown during the second year clearly exceeded those of the antlers formed in the first year. Based on these results and considering that almost all fluoride in the body is bound to calcium, we conclude that, like in the case of strontium (Schultz 1965; Cowan *et al.* 1968), the fluoride deposited in the growing antlers normally originates from two sources, *viz*, the fraction taken up with the diet and that mobilized from the skeleton. In consequence, especially in large subsequent antlers, bone fluoride concentrations cannot be regarded as a reliable indicator of the amount of fluoride consumed by an individual during the antler growth period. Due to their small size and the still ongoing skeletal growth of the deer during their formation, the contribution of previously skeletally deposited fluoride to antler fluoride levels is probably lowest in primary antlers. We, therefore, assume that fluoride levels in the latter more closely reflect actual dietary fluoride uptake during the period of antler growth. An additional practical advantage of primary antlers is that their value as trophies is rather limited and that they are therefore more readily available for study than subsequent antlers. Hence, analyzing primary antlers is recommended in studies of fluoride exposure of deer, using antlers as monitoring tools.

The analytical results indicate that the main constituent of the mineral phase in all samples was a hydroxyfluorapatite. Mean calcium and phosphorus ash contents of the antlers, although slightly higher, are comparable to those reported by other authors for antlers of different deer species (Bernard 1963; Gelbke 1972; Hyvärinen *et al.* 1977; Miller *et al.* 1985; Tataruch and Wolfsperger 1995). By contrast, no data on the mineralization and density of pedicle bone have been available so far. The higher calcium and phosphorus concentrations in the ash of antlers compared to pedicles points to minor differences in chemical composition between the mineral phase of the two bone types.

The present study disclosed a principal difference between antlers and pedicles with respect to the relationship between the fluoride content of bone and its mineralization and density. Thus, despite the fact that pedicle fluoride values exceeded those of the antlers in all deer, a negative correlation between fluoride level and bone mineral content as well as ash density was observed only in the antlers. Moreover, ash percentage and mineral density did not differ significantly between the two pedicle samples, mean fluoride values of which varied even more than those of the antlers. Based on the comparison of the two antler samples alone, it might be assumed that the lower ash percentage and density of the N-Bohemian specimens were caused by a reduced calcium and phosphorus availability to the animals from this region, where nutrient leaching from soils as a result of acid precipitation has been observed (Valach *et al.* 1993). However, the results of pedicle mineralization and the significant negative correlations between fluoride level and ash percentage (even, if only the controls were considered) as well as mineral density of the antlers do not support such a hypothesis.

By contrast, we interprete the above findings as denoting a negative effect of increased plasma fluoride levels on the processes involved in the formation and biomineralization of antler bone. Our data further indicate that the as yet unknown mechanism(s) by which fluoride causes these changes operate(s) in a dose-dependent manner and apparently already within the range of exposure conditions given in the control animals. In comparing the results for antlers and pedicles, we moreover conclude that it is not the increased fluoride exposure *per se* but this factor in combination with the exceptional intensity of antler growth and the concomitant rapidity of the mineralization process which caused the reduced density and mineral content of the N-Bohemian antlers. According to Muir *et al.* (1985), in red deer approximately 65% of the mineral content of subsequent antlers are laid down during the last ten weeks of antler growth, resulting in a calcium deposition of 5 g per day for hard antlers of 3 kg dry wt. We assume that this process and the concomitant formation of a densely structured and highly mineralized antler cortex, which are under androgenic control (Bubenik *et al.* 1975; Kierdorf *et al.* 1993b, 1995), are especially sensitive to fluoride interference. Further studies are intended to reveal the relative importance of hypomineralization of bone, reduction in the amount of compact compared to trabecular bone, and decrease in the number and/or width of individual trabeculae for the observed differences in mineral content and density between the antler samples.

It is likely that the bone changes induced by fluoride will lead to an impaired biomechanical competence of antlers from deer inhabiting regions with higher levels of environmental fluoride. We, therefore, would expect to find an increased incidence of antler breakage in such populations. The only other study on the effect of pollutants on antler quality so far has been the one by Jop (1979) on roe deer from a forest region in South Poland. He found that between 1922 and 1973 average antler weight in the deer had declined by 32% and argued that this was due to contamination from a large iron and steel works opened in the vicinity of the forest in 1957. As was later shown by Grodzinska *et al.* (1983), this forest area is in fact exposed to an increased deposition of various pollutants, including a high fluoride fallout. However, since no data for pollutant levels in and density or mineral content of the antlers are provided by Jop (1979), the reason(s) for the reduction in antler quality of the roe deer remain(s) unclear.

In conclusion, it can be stated that due to their rather easy accessibility, their ability to accumulate large amounts of fluoride during a fixed time-span and the occurrence of an apparently dose-dependent negative effect of increased fluoride exposure on their density and mineral content, primary antlers are well suited as monitoring tools for studying environmental contamination by fluorine compounds.

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