

HORMONE LEVELS AND ANTLER DEVELOPMENT IN WHITE-TAILED AND SIKA FAWNS

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Abstract—1. Overall mean values of testosterone (T), androstenedione (A), thyroxine (T_4), calcium (Ca), phosphorus (P), and alkaline phosphatase (AP) were (T) 2.56 ± 2.44 ng/ml, (A) 3.16 ± 2.58 ng/ml, (T_4) 8.22 ± 4.18 μ g/dl, (Ca), 10.88 ± 0.65 mg%, (P) 8.03 ± 0.68 mg%, and (AP) 81.89 ± 19.45 IU/l in white-tailed fawns and (T) 3.69 ± 2.76 ng/ml, (A) 18.26 ± 17.58 ng/ml, (T_4) 4.41 ± 1.59 μ g/dl, (Ca) 10.08 ± 0.80 mg%, (P) 9.42 ± 1.69 mg% and (AP) 95.35 ± 22.65 IU/l in sika fawns.

2. High T titers correlated with antler button growth, and A titers peaked as buttons hardened in both groups.

3. Higher T_4 levels in late fall and early winter may have had a synergistic role for button growth in both groups.

4. Generally higher P levels in sika fawns and relatively higher Ca levels in white-tailed fawns might be species dependent.

5. However, relatively constant Ca and P in both groups represented mineral homeostasis.

6. The mineralization role of AP activity was evident in both groups.

INTRODUCTION

The annual regeneration of antlers by male deer has been found to be a useful model for the study of mineral metabolism and human bone disease (Cowan *et al.*, 1969). Antlerogenesis causes a severe mineral drain on the deer's skeleton which leads to an annual physiological osteoporosis (Meister, 1956; Banks *et al.*, 1968a, b). While the endocrine control of the antler cycle in adult deer has been studied exhaustively, little attention has been given to the initiation of the first antlers in fawns (Sempere & Boissin, 1982).

White-tailed deer (*Odocoileus virginianus*) fawns grow small "buttons" rather than antlers their first year, while sika deer (*Cervus nippon*) fawns grow small but complete antlers their first year (Brown, personal observation). Thus these species of young animals offer somewhat different models for the comparative study of the antler growth phenomenon. This project was conducted to examine the hormone titers and other blood parameters of white-tailed and sika fawns in relation to their antler development.

MATERIALS AND METHODS

Five white-tailed and three sika male fawns were captured in the early summer of 1978 in south Texas. Ages of the fawns at capture were estimated to be between three days and two weeks. While the exact ages of the fawns were not known, they were assumed to be of identical ages for comparative purposes.

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The fawns were maintained in individual 5×5 m covered pens, bottle fed on goat's milk, and weaned onto a pelleted ration of 18.0% protein at approximately four months of age. Beginning in October, blood samples were taken bi-weekly for one year by venepuncture. During the first half of the study, the deer were manually restrained. After 6 months, they were tranquilized with xylazine hydrochloride (Rompun) for handling. Concurrent studies showed that this tranquilizer had no effect on the blood parameters measured (Faulkner *et al.*, 1979). Blood samples were centrifuged, and the serum was stored at -20°C for further analysis.

Testosterone (T), androstenedione (A), and thyroxine (T_4) titers were measured by radioimmunoassay. All assays utilized rabbit-anti-bovine antibodies which did not cross react with other hormones. The sensitivity of the T and A assays was 10 pg, and the T_4 assay, 0.1 mg. Day to day variation of all assays was 10%. Levels of calcium (Ca), inorganic phosphorus (P), and alkaline phosphatase (AP) activity were determined spectrophotometrically.

All data were analyzed using analysis of variance to determine annual changes of each parameter. Pearson's correlation coefficient was calculated with a SPSS program (Gill, 1978) to determine correlations between all bi-weekly data both year-round and in different antler growth stages in both species.

RESULTS

Antler development

In the white-tailed fawns, the development of "buttons" (Fig. 1) began in the middle of October. They remained in velvet until the beginning of February when the velvet was rubbed off. The buttons remained hard until the middle of April when they were cast. New antler growth was initiated soon after casting. Rapid growth occurred during the next two months, and the first antlers remained in velvet until the end of the study the following September.

In the sika fawns, antler spikes, rather than buttons appeared the first year. The velvet spikes began grow-

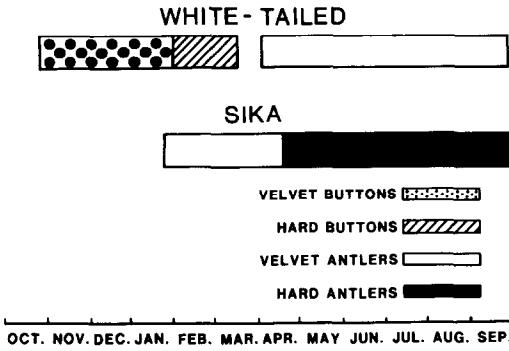


Fig. 1. Comparison of antler development in South Texas white-tailed and sika fawns.

ing at the beginning of January, and the velvet was rubbed off in April. The hardened spikes remained throughout the study period.

Annual cycles of blood parameters

Elevated T levels (Fig. 2) were found from November to February in the white-tailed fawns. Titters remained low the rest of the year, except for a brief surge in March. In the sika fawns, T levels slowly increased to a peak (7.57 ng/ml) in January and decreased through the winter. Unlike the white-tails, the sikas' T titers increased again in the spring and peaked (13.70 ng/ml) in May.

Androstenedione titers (Fig. 3) peaked in both November and March (9.05 and 9.60 ng/ml, respectively) in the white-tailed fawns. In the sika fawns, A levels peaked in October, decreased, and then were elevated throughout the summer. The elevated titers were far higher than those of the white-tailed fawns.

White-tailed T₄ titers (Fig. 4) peaked in December (24.85 µg/dl), then declined steadily throughout the remainder of the study. In the sika fawns, T₄ increased and peaked (10.73 µg/dl) in November.

After two months, the titers decreased gradually to the nadir (2.40 µg/dl) in May. A slight increase was seen over the course of the summer. Thyroxine levels in the white-tailed fawns were consistently higher than those in sika fawns.

Serum calcium (Fig. 5) and P levels (Fig. 6) remained relatively constant throughout the year in both groups. However, Ca levels were slightly higher in the white-tailed fawns, while P levels were higher in sika fawns.

Alkaline phosphatase activity (Fig. 7) in the white-tailed fawns increased gradually throughout the winter and peaked (110.24 IU/l) in March. It then dropped sharply to the nadir (42.5 IU/l) in August. In the sika fawns, AP activity decreased to the nadir (55.5 IU/l) in December, then increased through the winter and peaked (126.00 IU/l) in March. Activity remained relatively high in the summer with a drop in late August. Alkaline phosphatase activity was higher in sika fawns in the summer and in the white-tailed fawns in the winter.

Comparison of annual means

There were no significant differences ($P > 0.05$) between the overall mean values of any of the parameters examined (Table 1). Overall, T titers were fairly close in the two species. Androstenedione was considerably higher in the sika fawns, but the high SE over the year in both species precluded statistical significance. Similarly, T₄ titers were higher in white-tails than in sikas, but both species had high SE over the year. Calcium and P levels were very close between the groups. Alkaline phosphatase activity was higher in sika fawns, but again a large SE in both groups prevented a statistical difference.

Correlation coefficients between blood parameters

In the white-tailed fawns T levels were significantly ($P < 0.05$) correlated with P and AP ($r = 0.44$ and 0.58 , respectively). Androstenedione levels were significantly ($P < 0.01$) correlated with Ca, P, and AP

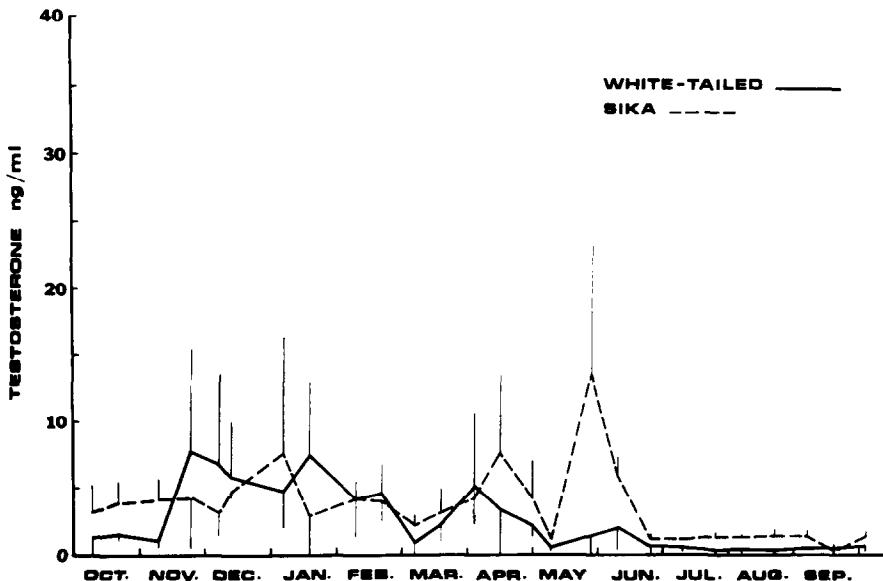


Fig. 2. Annual cycle of testosterone in male white-tailed and sika fawns.

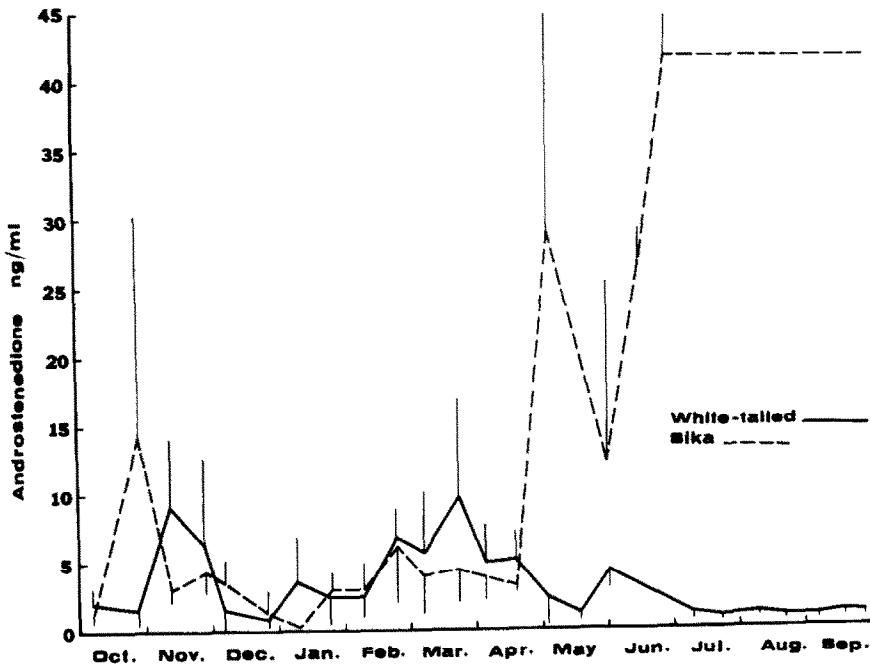


Fig. 3. Annual cycle of androstenedione in male white-tailed and sika fawns.

activity ($r = 0.52, 0.53, \text{ and } 0.64$, respectively). Thyroxine levels were significantly ($P < 0.01$) correlated with T, Ca, P, and AP activity ($r = 0.56, 0.52, 0.54$, and 0.44 , respectively). Calcium levels were significantly ($P < 0.01$) correlated with P and AP activity

($r = 0.47 \text{ and } 0.50$, respectively), while P levels were correlated ($P < 0.001$) with AP activity ($r = 0.73$).

In the sika fawns, T levels were significantly ($P < 0.01$) correlated with A, Ca, and P levels ($r = -0.50, 0.60, \text{ and } 0.40$, respectively). Androstene-

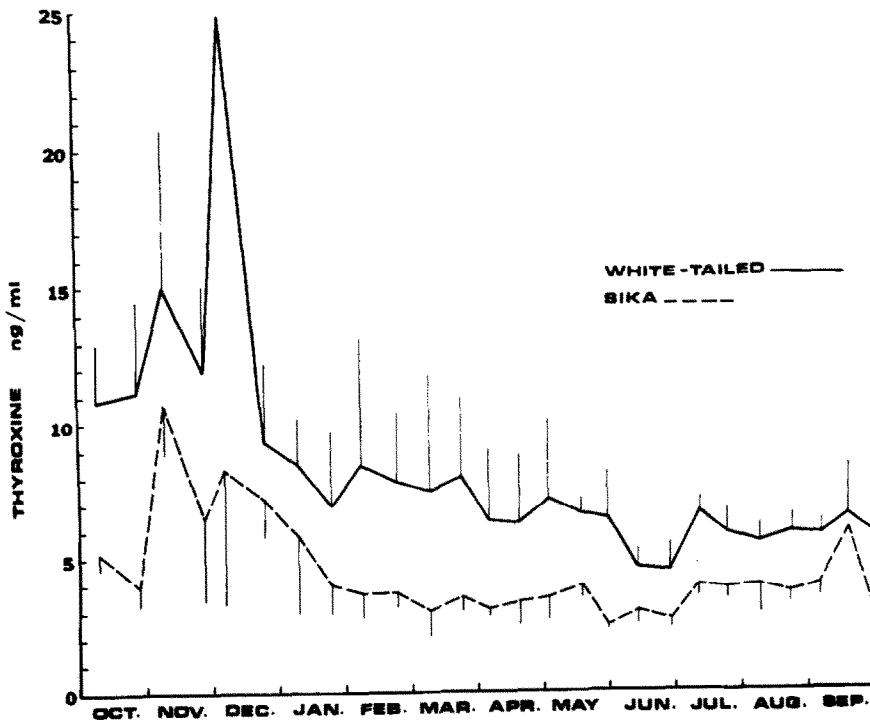


Fig. 4. Annual cycle of thyroxine in male white-tailed and sika fawns.

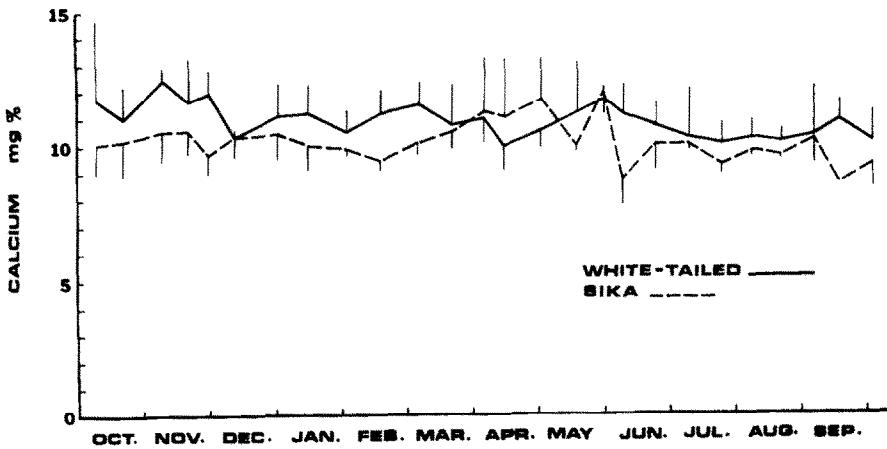


Fig. 5. Annual cycle of calcium in male white-tailed and sika fawns.

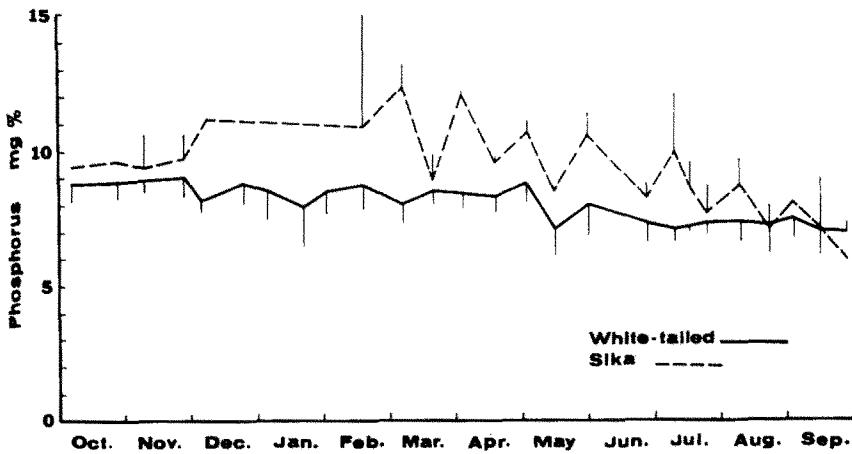


Fig. 6. Annual cycle of phosphorus in male white-tailed and sika fawns.

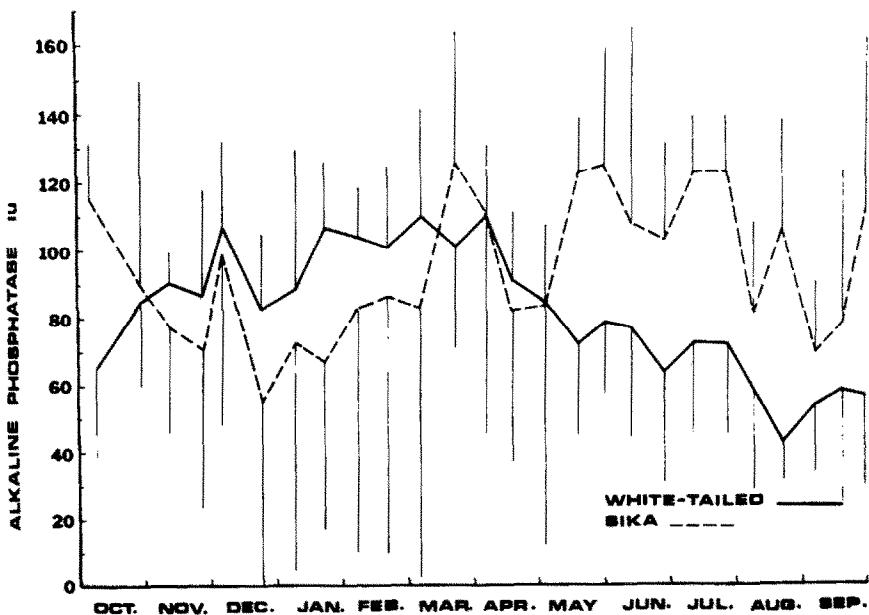


Fig. 7. Annual cycle of alkaline phosphatase activity in male white-tailed and sika fawns.

Table 1. Mean annual values of blood parameters in five male white-tailed and three male sika deer fawns

	White-tailed		Sika	
	\bar{X}	SE	\bar{X}	SE
Testosterone (ng/ml)	2.56	2.44	3.69	2.76
Androstenedione (ng/ml)	3.16	2.58	18.26	17.58
Thyroxine ($\mu\text{g}/\text{dl}$)	8.22	4.18	4.41	1.59
Calcium (mg%)	10.88	0.65	10.08	0.80
Phosphorus (mg%)	8.03	0.68	9.42	1.69
Alkaline Phosphatase (IU/l)	81.89	19.45	95.35	22.65

dione levels were correlated with Ca and P ($r = -0.47$ and -0.70 , respectively). Thyroxine levels were significantly ($P < 0.05$) correlated with AP activity ($r = -0.49$). Calcium levels were correlated with P levels ($r = 0.49$).

In the antler initiation stage of the white-tails (March–May), T_4 levels were significantly ($P < 0.01$) correlated with P and AP ($r = 0.80$ and 0.74 , respectively). Androstenedione levels were correlated with AP ($r = 0.86$) while P levels were correlated with AP ($r = 0.69$). In the antler initiation stage of the sika fawns (October–January) none of the blood parameters were significantly ($P > 0.05$) correlated indicating an independent relationship among all of these parameters studied.

In the white-tailed antler velvet stage (May–September) A levels were significantly correlated with Ca and AP ($r = 0.72$ and 0.67 , respectively) and AP was correlated with Ca and P ($r = 0.60$ and 0.68 , respectively). In the sika velvet stage (January–April) neither parameter was significantly ($P > 0.05$) correlated with other data, indicating an independent relationship in velvet stage among all of these parameters.

During the period of hardened spikes in the sika fawns, (April–September) T_4 levels were significantly ($P < 0.05$) correlated with AP ($r = -0.66$) and T levels were significantly ($P < 0.001$) correlated with A ($r = -0.997$), indicating an opposite trend of T and A in this stage.

DISCUSSION

In the annual hormone cycles of adult deer, high T levels are generally associated with rut, rubout, and mineralization of the velvet antlers (Brown, 1975). In our white-tailed fawns, higher T titers occurred in November, before the buttons rubbed out. However, no rutting behavior was observed. In addition, the T peak in March correlated with the further development of the pedicle, prior to the initiation of the first set of antlers (Fig. 1). This is similar to the T peak found by Lincoln (1971) in red deer (*Cervus elaphus*) fawns. T levels were not elevated, however, during the stage of antler growth in our yearlings. In the sika fawns, T was also elevated just before velvet spike growth. Rutting behavior was also not observed, and although T levels declined after June, the deer maintained their hardened antlers afterward.

In the white-tails, A titers peaked similarly to T, in November and March, and also followed the T trend the remainder of the year (Fig. 2). It is not known if A had an additive effect to T in these deer, but it is

certainly possible. In the sikas, however, A peaked briefly in October, well before velvet spike growth, then declined and became elevated in the spring and summer. This prolonged period of elevated A titers during the period of hard antlers may explain why the antlers were not cast when the T titers were low. It also suggests that A may be more important than T in the antler growth cycle in fawns of this species.

T_4 levels in the white-tailed fawns were mostly significantly ($P < 0.05$) higher month by month than those in sika fawns, suggesting a higher metabolic activity of the white-tails than of the sikas. Both white-tailed and sika fawns had higher T_4 levels in the winter and lower T_4 levels in the summer (Fig. 3). This is consistent with our findings in yearling white-tailed deer (Chao & Brown, unpublished data). Higher T_4 titers during the winter are consistent with results found in roe deer fawns (Sempere & Boissin, 1981) and support the concept of a synergistic role for T_4 in the initiation and growth of antlers in both species of fawns. Initial higher T_4 levels in all of the fawns might have been due to their young age. (Bubenik & Bubenik, 1978). The short-term decrease of T_4 in both groups in early summer (June) might have been the consequence of higher ambient temperatures.

Relatively constant Ca and P levels in both fawn groups was expected, and represents the compensatory function of the parathyroid–calcitonin system (Hall, 1978). However, consistently higher P levels in the sika fawns and relatively high Ca levels in the white-tail fawns might be species dependent. In the sika deer, however, both Ca and P values were higher than those reported by Dhindsa *et al.* (1975).

In the white-tailed fawns, AP activity was higher from January to April, during rub-out and while the buttons were hard. AP activity decreased when the first set of antlers was growing from late spring through the summer and early fall. This is in contrast to the study of Graham *et al.* (1962) which found that antler growth in the late spring and summer was marked by an increase in serum AP activity. In the sika fawns, the different antler growth cycle was reflected by differences in AP activity. The spikes were initiated in January and hardened in April, the latter following high AP activity. High AP activity (Fig. 6) was maintained as the sika fawns retained their hardened spikes. The postulated mineralization role of AP proposed by Fleisch & Neuman, (1961) and Molello *et al.* (1963), was thus demonstrated in this study.

Correlations between blood parameters

In the white-tailed fawns, a significant ($P < 0.01$) correlation between T and T_4 levels suggests a relationship between the gonadal and thyroidal systems. This relationship, however, was not evident in the sika fawns. Similar T_4 trends were found in all fawns. However, the different antler growth cycles in the two species were reflective of different T cycles, indicating possibly different relationships between T_4 and T. Thyroxine might be involved in Ca and P homeostasis via AP activity in white-tailed deer, but this might not be true in sika fawns, as evidenced by little correlation between T_4 and Ca or P and a negative correlation between T_4 and AP activity.

During the antler initiation period of the white-tailed fawns, significant correlations between T_4 levels

and P or AP indicated a close relationship between those parameters. High T_4 levels during this stage might also suggest the synergistic role of T_4 in the initiation of antler growth. In the sika fawns, this relationship was very weak. However, higher T_4 levels in this stage does not exclude a synergistic role of T_4 on the initiation of antler growth.

During the antler velvet stage in the white-tailed fawns, the significant correlation between AP activity and both Ca and P indicated that AP might be involved in the Ca and P transport system as it is in other animals (Russell *et al.*, 1972; Devgun *et al.*, 1981). In the sika fawns, the lack of a significant correlation indicated a weak relationship between these parameters.

It is obvious from these studies that the endocrine control of the antler cycles of these two species differs. We can offer no logical explanation at present for the difference in the correlations between parameters between the two species. Results were no doubt affected by relatively small numbers of animals and fairly high standard errors. The lack of statistical correlations between parameters in the sika deer leads one to question the biological significance of the stronger correlations found in the white-tails. The fact that the white-tails go through a button stage as fawns, while the sikas grow spikes more similar to antlers, indicates that the latter species may have a more mature endocrine system at this early age. On the other hand, the sikas may in fact employ other hormones, as yet not measured, to control their antler cycles in a manner different from the white-tails. Due to these differences in juvenile antler development and endocrine levels one must be cautious in future comparisons of these species.

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REFERENCES

- BANKS W. J., EPLING G. P., KAINER R. A. & DAVIS R. W. (1968) Antler growth and osteoporosis (a) I. Morphological and morphometric changes in the coastal compacta during the antler growth cycle. (b) II. Gravimetric and chemical changes in the coastal compacta during the antler growth cycle. *Anat. Rec.* **162**, 387–405.
- BROWN R. D. (1975) Some aspects of the endocrine control of antler growth in white-tailed deer (*Odocoileus virginianus*). Ph.D. thesis. The Pennsylvania State University.
- BUBENIK G. A. & BUBENIK A. B. (1978) Thyroxine levels in male and female white-tailed deer (*Odocoileus virginianus*). *Can. J. Physiol. Pharm.* **56**, 945–949.
- COWAN R. L., HARTSOOK E. W. & WHELAN J. B. (1969) Deer antler growth: an ideal test for the study of bone metabolism. *Sci. Agric.* **17**, 3–4.
- DEVGUN M. S., PATERSON C. R. & MARTIN B. T. (1981) Seasonal changes in the activity of serum alkaline phosphatase. *Enzyme* **26**, 301–305.
- DHINDSA D. S., COCHRAN T. H., CASTRO A., SWANSON J. R. & METCLAFE J. (1975) Serum biochemical and electrophoretic values from four deer species and from pronghorn antelope. *Am. J. Vet. Res.* **36**, 1455.
- FAULKNER L. W., ZAMORA L., JIMENEZ M. & BROWN R. D. (1979) The effect of xylaxine hydrochloride on some blood parameters in white-tailed deer. NIH-MBS Symposium. Atlanta, Georgia, April 19. (Abstract E-3).
- FLEISCH H. & NEUMAN W. F. (1961) Mechanism of calcification: role of collagen, polyphosphates and phosphatase. *Am. J. Physiol.* **200**, 1296–1300.
- GILL J. L. (1978) *Design and Analysis of Experiments in the Animal and Medical Science*. Vol. 2, Iowa State Univ. Press, Ames.
- GRAHAM E. A., RAINEY R. K., ALBERT E., HOUGHTON E. H. & MOYER C. A. (1962) Biochemical investigations of deer antler growth. Part I. Alterations of deer blood chemistry resulting from osteogenesis. *J. Bone and Joint Surg.* **44A**, 482–488.
- HALL B. (1978) Components of skeletal growth I. Hormones—the growth of antlers. pp. 213–215 In B. Hall (ed). *Development and Cellular Skeletal Biology*. Academic Press, New York.
- HILLMAN, J. R., DAVIS R. W., ABDELBAKI Y. Z. (1973) Cyclic bone remodeling in deer. *Cell Tiss. Res.* **12**, 323–330.
- LINCOLN G. A. (1971) Puberty in a seasonally breeding male—the red deer stag (*Cervus elaphus*). *J. Reprod. Fert.* **25**, 41–54.
- MEISTER W. (1956) Changes in histological structure of the long bones of white-tailed deer during the growth of antlers. *Anat. Rec.* **124**, 709–724.
- MOLELLO J. A., EPLING G. P. & DAVIS R. W. (1963) Histochemistry of the deer antler. *Am. J. Vet. Res.* **24**, 573–579.
- RUSSELL R. G. G., MONOD A., BONJOUR J. P. & FLEISCH H. (1972) Relation between alkaline phosphatase and Ca^{+2} -ATP in Ca transport. *Nature, Lond.* **240**, 126.
- SEMPERE A. J. & BOISSIN J. (1982) Neuroendocrine and endocrine control of the antler cycle in roe deer. In *Antler Development in Cervidae* (Edited by Brown R. D.). Caesar Kleberg Wildlife Research Institute, Kingsville. In Press.