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Annual Testosterone Rhythm in the Black Bear (Ursus americanus)[1]

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ABSTRACT

Thirty-one blood samples were obtained from wild male black bears at different times of the calendar year. The samples were assayed for serum testosterone and the results were correlated with testicular size, time of year and nutritional status. A significant annual testosterone rhythm was found with high values during March through mid-July (mean 153 ng per 100 ml) and low values from mid-July through November (mean 47 ng per 100 ml, $P<0.001$). Testicular length was also greater during March through mid-July (mean 6.9 cm) than mid-July through November (mean 5.8 cm, $P<0.005$). *Five* animals were studied in the spring while still in their dens after having spent the winter isolated and not feeding. Despite a mean 19 percent loss in body weight and a winter of social isolation, serum testosterone was found to be elevated in these animals (mean 161 ng per 100 ml). This suggests that the annual testosterone cycle of this species is independent of nutritional and social factors.

INTRODUCTION

Annual testosterone rhythms have been described in a number of species including the ram (Sanford et al., 1974), white-tailed deer (McMillin et al., 1974), stallion (Berndtson et al., 1974), rock hyrax (Neaves, 1973), caribou (Whitehead and McEwan, 1973), and rhesus monkey (Plant et al., 1974). These annual rhythms can be considered adaptive mechanisms which help synchronize breeding to ensure optimal survival of the species. For large mammals in the northern hemisphere, this would dictate that the peak of the annual testosterone rhythm occurs at a time which allows the young to be born in the spring.

The reproductive physiology of the black bear (*Ursus americanus*) provides interesting examples of other adaptive mechanisms to accomplish the same goal (Erickson et al., 1964). In North America, bears breed in June or early July. Total gestation lasts about seven months. However, through a strategy of delayed implantation, embryonic growth does not begin until five months after breeding. The period of active uterine growth lasts only about six weeks. The cubs are born in a very immature state in late January or early February. At this time they are in the protective environment of the winter den. The cubs are then nursed in the den from a birth weight of less than 500 grams to a weight of more than 2500 grams when they leave the den with the mother in the spring. Thus, the strategies of delayed implantation and a period of nursing in the den combine to prepare the cubs for their first exposure to the environment in the spring.

A second intriguing aspect of the reproductive biology of the black bear is the annual gonadal cycle of the male. Adult male black bears spend the winter isolated in dens, not eating or drinking. Every year the testes of adult male bears increase in size and show active spermatogenesis coincident with the breeding season (Erickson et al., 1964). It has been shown that this increase in size and spermatogenesis begins in late winter while the bears are still in their dens (Erickson et al., 1964).

Because of the intriguing reproductive biol-

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ogy of this species a study was undertaken of the serum testosterone in wild adult male black bears to determine if this species possesses an annual testosterone rhythm synchronized with the breeding season. A seasonal study of serum testosterone in this species could also provide important information on factors controlling annual testosterone rhythms. A number of factors are known to influence serum testosterone including nutrition (Grewal et al., 1971) and exposure to receptive females (Purvis and Haynes, 1974). If serum testosterone were found to increase while the animals were still isolated in their dens and not eating, nutrition and exposure to receptive females could be excluded as factors responsible for the annual testosterone rhythm.

MATERIALS AND METHODS

Serum samples were obtained from wild free-ranging hears in the Superior National Forest of northern Minnesota. The bears were captured in barrel traps or foot snare sets baited with meat. After capture the bears were tranquilized (Seal et al., 1970) and removed from the trap. Blood was obtained from the femoral vein and the animals were weighed and baculum measurements taken. Testicular size was estimated by palpating the longest axis of the testis and measuring this distance with a calipers. The ages of some animals were known; for other(s) it was estimated from annuli in the cementum of first premolars as described by Willey (1974). Before release the animals were tagged for future identification. Some of the animals were fitted with lightweight collars containing radio transmitters. This allowed tracking of the animals and locating them in their winter dens. Blood samples were obtained in winter by tranquilizing the animals in their dens using a pole syringe. Some animals were also recaptured in the summer, allowing serial measurements. Due to prevailing field conditions blood was not obtained on every capture and testicular and baculum measurements were not always obtained.

Serum testosterone concentration was measured by radioimmunoassay using a method previously described by McMillin et al. (1974). One milliliter of serum was used in the nonbreeding period and 0.5 ml of scrum was used in the breeding period. The water blank with this method is zero and the blank using ether extracted bear scrum is also zero. The interassay variability of this method is 7 percent and the sensitivity using these dilutions is less than 20 ng percent.

Statistical significance was determined using the Student's *t* test. All calculations were done on a HP 65 calculator.

RESULTS

Serum samples for testosterone assay were obtained from 31 captures of 21 different wild bears.

Figure 1 is a histogram summarizing the mean monthly serum testosterone and testicular diameter values observed. From this histogram the seasonal variation in serum testosterone and testicular size is apparent. Unfortunately, samples were not obtained for all months. Therefore, the precise timing of the annual testicular and testosterone rhythms could not be determined. However, by grouping the data (Table 1) it is possible to determine the significance of the seasonal variation. The mean serum testosterone was significantly higher in animals captured preceding or during the period of reproductive activity than during reproductive inactivity (153 ng percent vs. 47 ng percent, $P<0.001$).

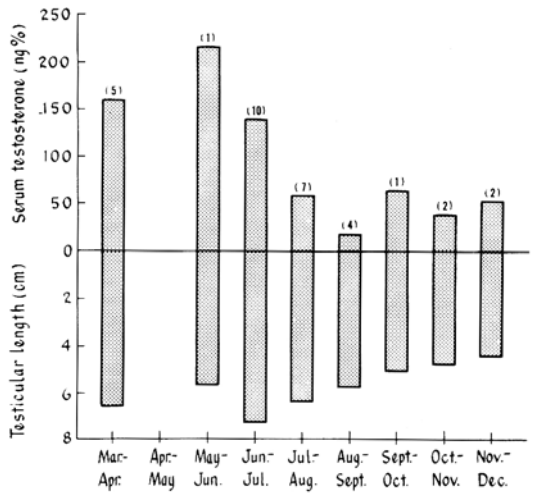

FIG. 1. Mean serum testosterone and testicular length in wild black bears by month. The number in parentheses indicates the sample size.

Despite the significant differences in serum testosterone and testicular lengths, there were no differences in mean age (5.5 years vs. 4.2 years, $P>.1$) or mean baculum length (16.6 cm vs. 16.0 cm, $P>0.1$) in animals captured during the two time periods. This excludes the possibility that a sampling age bias was responsible for the variation found.

To exclude the possibility that the method of capture accounted for the seasonal variation in testosterone found, results for each method were considered separately (Table 2). A significant seasonal variation in serum testosterone was found with each method. Finally, in animals in which serial measurements were obtained, the same trend of greater serum testosterone and testicular measurements during March through mid-July was noted as for the

TABLE 1. Mean age, testicular length, baculum length and serum testosterone concentration in wild black bears sampled during the periods of March through mid-July and mid-July through November.

Date	Age (yrs)	Testicle length (cm)	Baculum length (cm)	Testosterone (ng %)
March — mid-July	5.5 ± .8	6.9 ± .2	16.6 ± .6	153 ± 14
Mid-July – November	4.2 ± .5	5.8 ± .3	16.0 ± .5	47 ± 7
Significance P	N.S.	<.005	N.S.	<.001

All values mean ± standard error of the mean.

population. This provides further evidence that the seasonal variation observed is not a sampling artifact.

Four animals were captured in the fall shortly after entering their dens and five animals were captured in the following spring before they had left their dens. In the intervening period they lost an average of 19 percent of their body weight. Despite this weight loss and despite the fact that the animals spend the winter isolated and without social contact, the mean serum testosterone in the spring was 161 ng percent. This is significantly greater ($P<0.05$) than the mean serum testosterone found in the same animals in the fall, or ($P<0.005$) when compared to all animals sampled from mid-July through November.

DISCUSSION

This study confirms the presence of a seasonal variation in serum testosterone concentration in the black bear (*Ursus americanus*) which parallels the previously reported seasonal variation in testicular size (Erickson et al., 1964). The fact that the serum testosterone concentration was found to rise in the spring before the bears left their dens also provides some insight into the factors responsible for this annual variation in serum testosterone.

A number of environmental factors have been shown to influence serum testosterone in male mammals. In the rhesus monkey, social dominance is associated with higher testosterone levels and forced acceptance of a sub-dominant role produces a marked fall in plasma testosterone (Rose et al., 1975). Since serum testosterone levels were found to be elevated in the bear while the animals were isolated in their dens, it is unlikely that social factors are responsible for the annual variation in serum testosterone observed.

Exposure to receptive females has been shown to elevate circulating testosterone in the rat (Purvis and Haynes, 1974), rabbit (Haltmeyer and Eik-Nes, 1969) and rhesus monkey (Rose et al., 1972). Gordon and Bernstein (1973) demonstrated that visual exposure to female rhesus monkeys was necessary to elicit seasonal sexual behavior in the male rhesus. These authors presented the hypothesis that seasonal reproductive rhythms were controlled by the female and that the male seasonal reproductive cycle was simply a response to the female. Since serum testosterone and testicular size were found to increase in bears isolated in dens, it is unlikely that the seasonal reproductive cycle of male bears is controlled by the female cycle.

Nutrition also may affect serum testosterone

TABLE 2. Seasonal variation in serum testosterone concentration of wild black bears found employing three different capture methods (see text).

Date	Testosterone (ng %)		
	Barrel trap	Foot snare	Den
March — mid-July	137 ± 23	166 ± 22	161 ± 27
Mid-July — November	38 ± 16	38 ± 6	61 ± 7
Significance P	<.025	<.005	<.05

All values mean ± standard error of the mean.

concentration (Grewal et al., 1971). Again, since serum testosterone concentration was found to be elevated before the bears left their dens and began feeding, spring refeeding can be excluded as a cause or trigger for the annual cycle observed. In addition, bears have the greatest body weights during the fall predenning period, a time of maximal testicular regression and low serum testosterone.

Stress has been shown to depress circulating plasma testosterone in the rhesus monkey (Mason et al., 1968) and man (Kreuz et al., 1972). Strenuous muscular exercise has also been shown to elevate plasma testosterone in man (Sutton et al., 1973). In this study the stress of capture or the muscular exercise exerted by the animals trying to escape capture could have influenced serum testosterone. Tranquilization could also have influenced serum testosterone. However, all animals were tranquilized in the same manner and a seasonal variation in serum testosterone was found in animals captured by three different techniques. Thus, stress, muscular exercise or tranquilizing cannot explain the seasonal variation in serum testosterone observed.

In addition to the environmental factors of social hierarchy, exposure to receptive females, nutrition, stress and muscular exercise, there are endogenous events which alter serum testosterone. Diurnal variations in serum testosterone have been reported in a number of species including man (Judd and Parker, 1974) and the rhesus monkey (Michael et al., 1974). Large spontaneous variations in plasma testosterone have also been observed when plasma testosterone samples are collected at frequent intervals (Murray and Corker, 1973; Eaton and Resko, 1974). It is not known whether the bear has a diurnal testosterone rhythm or frequent spontaneous variations in plasma testosterone. In this study neither of these endogenous events were controlled and yet a seasonal variation was demonstrated. From this we conclude that if these events do occur in this species, the variations occur around a seasonally changing mean. It is possible that if serial samples were obtained at the same time of day, much of the variability in samples obtained at approximately the same time of year would be obviated and a sharper seasonal rhythm observed.

The finding of a seasonal elevation of serum testosterone in the male black bear which is coincident with the breeding season yet independent of nutrition and exposure to females raises the question of what does control the annual reproductive cycle in both the male and female black bear? There is ample evidence that day length (photoperiod) is the major environmental factor controlling the seasonal reproductive rhythms of a variety of species (see review by Rieter [1974]). Although the effect of varying day length on the annual reproductive cycle of the black bear has not been studied, this would appear to be the most likely environmental factor synchronizing reproduction in this species. Alternatively, some other physical change such as temperature could be responsible.

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