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Seasonal variations in thyroxine and testosterone levels in relation to the moult in the adult male mink (*Mustela vison* Peale and Beauvois)

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Plasma thyroxine (T_4) and testosterone concentrations were measured in adult male mink maintained outdoors under natural light and fed *ad libitum* the whole year round. Plasma T_4 concentrations presented a biphasic seasonal change, the highest values occurring in the spring and autumn months and the lowest values in the winter months. The plasma testosterone cycle showed an annual maximum in January–February. The possibility of testis–thyroid interactions is discussed. The changes observed are correlated with environmental temperature, photoperiod, and molting cycle.

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Les concentrations plasmatiques en thyroxine (T_4) et testostérone sont mesurées chez le vison mâle adulte élevé dans des conditions naturelles de lumière et nourri de façon constante tout au long de l'année. La concentration plasmatique en T_4 offre un profil biphasique, les valeurs les plus fortes sont observées au printemps et en automne, les valeurs les plus faibles pendant les mois d'hiver. Le cycle de la testostérone présente un maximum annuel en janvier–février. La possibilité d'interactions testo-thyroïdiennes est envisagée. Les variations observées sont discutées en fonction de la température ambiante, de la photopériode et du cycle du pelage.

Introduction

Several authors have already shown the existence of a sexually active period clearly defined but of short duration, in the mink (Hemmingsen 1967; Onstad 1967; Tiba *et al.* 1968; Kostron and Kukla 1969, 1970, 1971; Venge 1959, 1973; Nieschlag and Bieniek 1975; Pilbeam *et al.* 1979; Boissin-Agasse and Boissin 1979). The endocrine testicular activity and the gametogenesis begin in December and are followed by a period of sexual inactivity starting in early spring.

Since Rowan's first observations on birds (1925) and Bissonnette's work on mammals (1933), it is generally agreed that changes in day length play an important part in the reproductive cycle. Recent studies have shown that in the mink, photoperiodism is involved, both in the male and the female, in two very different mechanisms: the increase and the decrease of the gonads during the year and also the secretion of progesterone by the corpus luteum and the initiation of the implantation of the blastocyst (Boissin-Agasse and Martinet 1980; Allais and Martinet 1978). Furthermore, other studies have shown that in birds and mammals with a seasonal reproductive cycle, there exists an internal multiendocrine regulation in which testis–thyroid interactions play an important part (Jallageas and Assenmacher 1979). It is on the basis of these considerations that the present work has been carried out on the mink. Fluctuations in the plasma thyroxine and testosterone concentrations throughout the year are discussed in relation to environmental temperature, photoperiod, and annual molting cycle.

Materials and methods

These experiments were carried out in Chizé Forest located in western France (latitude: 46°19′ N; longitude: 00°24′ W).

Eight standard dark adult male minks were used in this study. There were no female minks in the vicinity. The minks were housed outdoors in roofed open sheds and exposed to natural weather and daylight conditions. Each animal was in an individual wire cage with a small nest at one end. Food (Pelsifood) and water were provided *ad libitum*. All animals were sampled once between 0900 and 1100 hours during the last week of each month from December 1976 to September 1978. They were lightly anesthetized with fluothane (Halothane, Imperial Chemical Industries).

Body weights were recorded and 5 mL of blood was taken from the jugular vein. The heparinized blood was immediately centrifuged and the plasma was stored frozen at −25°C until the hormonal levels were determined 6–12 months later. Fur development was rated according to the descriptions by Bassett and Llewellyn (1949).

Total plasma thyroxine was determined according to the competitive protein-binding method described by Vigouroux (1972) and modified by Astier *et al.* (1978). Blood plasma samples were extracted with ethanol and after centrifugation aliquots were taken in duplicate for assay. The sensitivity of the assay was 50 pg/tube. The intra- and inter-assay coefficients of variation were 3–5% and 8–10% respectively. T_4

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appears to be stable for at least one year in plasma stored frozen at −25°C (our unpublished observations).

Testosterone levels were measured using radioimmunoassay already described and validated (Boissin-Agasse and Boissin 1979). Testosterone assays were performed with BioMérieux's antiserum which shows a cross-reaction with 5-α-dihydrotestosterone (DHT). Since the plasma samples were not chromatographed, the results reported are the sum of testosterone and DHT concentrations. It did not appear necessary to isolate the testosterone, because in the mink 5-α-DHT is produced in very small amounts, so the error due to this cross-reactivity will account always for less than 5%.

All data are presented as the mean ± SEM; statistical significance was done using an analysis of variance performed on Digital P.D.P. 11.40.

Results

Seasonal variations of the plasma thyroxine (Fig. 1)

The plasma concentration in T_4 exhibited biphasic variations with a remarkable difference between maximum and minimum values. The lowest values were observed between December and April (mean level from December 1976 to April 1977: 8.38 ± 0.17 ng/mL; from December 1977 to April 1978: 8.73 ± 0.40 ng/mL).

There was a sharp increase in the thyroxine level during spring. Plasma thyroxine concentrations observed in May 1977 were significantly higher ($P < 0.05$) than those obtained in March 1977. Similarly, between March 1978 and May 1978 the increase in T_4 level was highly significant ($P < 0.01$).

The level of thyroid hormone remained high from spring to midsummer (mean level from May to July 1977: 13.55 ± 0.13 ng/mL; from May to July 1978: 14.96 ± 0.52 ng/mL), then decreased at the end of summer (July 1977 versus September 1977 $P < 0.05$; July 1978 versus October 1978 $P < 0.05$).

A second important increase took place in autumn (October–November 1977: 13.38 ± 0.30 ng/mL), then fell to a low level at the beginning of winter.

Seasonal variations of the plasma testosterone (Fig. 1)

The endocrine testicular activity presented a clearly defined annual cycle. From early spring to late autumn, the plasma concentration in testosterone was extremely low (mean level from March 1977 to November 1977: 1.30 ± 0.24 ng/mL; from April 1978 to December 1978: 1.07 ± 0.15 ng/mL).

In one month from December 1976 to January 1977 and from November 1977 to December 1977, the hormone level showed a threefold increase (December 1976 versus January 1977 $P < 0.05$; November 1977 versus December 1977 $P < 0.05$).

In January, the plasma concentration of testosterone was at its highest (January 1977: 7.97 ± 2.14 ng/mL; January 1978: 10.96 ± 2.45 ng/mL).

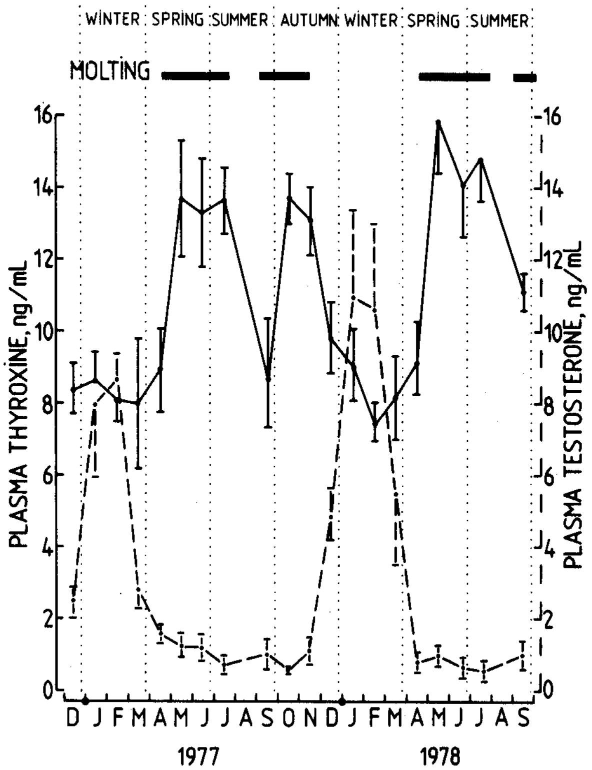

FIG. 1. Annual variations of the plasma thyroxine (solid line) and testosterone levels (broken line) in the male mink (nanograms per millilitre, mean ± SEM).

The breeding period was, however, a short one. In March, we observed a statistically significant decrease.

Thus, there exist inverse phase relationships between seasonal variations in the plasma concentration of testosterone and thyroxine. In fact, the thyroid hormone level increases in early spring when the testosterone level is at its lowest; inversely, in late autumn the endocrine testicular function resumes its activity only after a sharp decrease in the thyroxine level.

We tried to determine the existence and the nature of correlations between plasma T_4 and testosterone levels for the following periods: from December 1976 to December 1977 and from December 1977 to September 1978. Table 1 shows that between the two endocrine parameters, there always exist negative correlations which are highly significant during the two periods.

Discussion

The results which have just been analyzed show that in the mink the plasma thyroxine concentration is low during winter. On the other hand, high values are

TABLE 1. Correlations between plasma T_4 and testosterone levels in the adult male mink

Sampling period	No. of values (n)	Correlation coefficient (r)	Statistical significance (P)	Regression linear curve $y = ax + b$
December 1976 to December 1977	96	−0.43	$P < 0.01$	$y = -0.42x + 7.41$
December 1977 to September 1978	72	−0.42	$P < 0.01$	$y = -0.66x + 12.43$

observed in spring, in early summer, and again in autumn.

These results are contradictory to some data mainly provided by experiments on deep cold effects on laboratory mammals. It is generally accepted that among environmental factors likely to influence the thyroid gland function, outer temperature plays a preponderant part. More particularly, experiments carried out on the rat show that exposure to cold is shortly followed by stimulation of the thyroid function (Astier, 1975*a*, 1975*b*).

In wild mammals, many authors have shown a winter hypothyroidism (Hoffman and Robinson 1966: white-tailed deer; Delost 1966: grey squirrel; Rigaudière 1969: various microtines; Aleksiuk and Cowan 1969: Canadian beaver; Aleksiuk and Frohlinger 1971: muskrat; Maurel and Joffre 1975; Maurel *et al.* 1977; Maurel and Boissin 1979: badger; Cadariu *et al.* 1976: roe deer; Maurel and Boissin 1981: red fox). The same findings have been noted in birds (Jallageas, Tamisier *et al.* 1978: Pekin ducks and teal; Garbutt *et al.* 1979: ruffed grouse).

In wild animals, low temperatures during the cold season do not seem, consequently, to have a stimulating effect on thyroid activity. The hypothesis according to which the decrease in the plasma thyroxine concentration would be a result of the increased utilisation of the thyroid hormone during the winter months could be taken into consideration, but the results obtained for another mustelid (the badger) have shown that winter depletion lowers all the parameters considered for characterising the thyroid activity: [^{131}I] thyroid uptake, secretion of thyroid hormones, plasma thyroxine concentration, and biological half-life (Maurel and Boissin 1979).

Thus, in wild animals whose fur ensures efficient thermal protection and which are acclimatized to outside life, the correlations between annual variations of thermoperiod and thyroid function do not show the synchronizing effect of that physical factor on the endocrine cycle studied.

Considering the seasonal variations of thyroid activity in relation to the annual cycle of reproduction, several authors have suggested the possibility of internal regulation due to the existence of negative testis–thyroid interactions in birds (Jallageas 1975; Jallageas, Astier *et al.* 1978) and also in mammals (Maurel *et al.* 1977; Saboureau and Boissin 1978). Given the results obtained in the mink, showing strict phasic opposition between the thyroid and testicular cycles, possible interrelations between the two endocrine functions can be envisaged for this species. However, it is evident that such an observation does not allow us, at least for the time being, to establish a causal relationship between the two cyclic phenomena.

Besides, it is possible to establish correlations between the pelage cycle and the annual evolution of the two endocrine functions. Spring molting (from April 15th to July 21st) starts after the fall in testosterone, and autumn molting (from September 1st to November 15th) is over before the beginning of testicular activity. During the two molting periods there is an important increase in the thyroxine rate. Between the two phases of pelage replacement the level of thyroid hormone decreases.

The inverse phase relationships between the reproductive cycle and the molting cycle observed in the mink confirm, finally, that pelage replacement cannot take place unless the level of sexual hormone is low. The results of the present study, which lead us to draw the same conclusions as those of Duby and Travis (1972), suggest that there exists a precise correlation between the inverse evolution of the plasma concentration in testosterone and thyroxine during the two molting periods.

Since the studies by Bissonnette and Wilson (1939), Hammond (1951, 1954), and Stout *et al.* (1969), it is generally agreed that furring cycles in the mink are under photoperiodic control, in which case each molting would be controlled by inverse variations in day length. A decrease in daylight duration initiates a change to a heavy, dense pelage and an increase in day length leads to a sleek, less dense, summer coat. The neuroendocrine and endocrine regulations of this biological cycle and the metabolism changes implied are as yet unknown (Rust *et al.* 1965). Recently, Allain and Rougeot (1980) proceeding on the basis of Rust and Meyer's studies on the weasel (1969) have shown in the mink that implantation of melatonin pellets in summer immediately brings about molting, which in turn leads to winter pelage. If we take into account the results described above, it seems difficult to exclude the participation of thyroid hormones. A similar conclusion has been drawn by Reineke *et al.* (1962): if thyroxine does not play a decisive part in starting the molting process, it is probable that an increased synthesis of thyroid hormone is necessary for pelage growth.

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