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Seasonal Variations of Thyroid Activity in the Adult Male Badger, (*Meles meles* L.)

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Seasonal variations of thyroid activity (radioactive iodide uptake, plasma thyroxine, and biological half-life of the radioactive-labeled thyroxine) were studied in the male badger. These variations were characterized by a maximum occurring at the end of summer and in autumn, and a winter and late spring minimum. A significant spring activity was observed. Thyroid uptake 24 hr after injection of radioactive iodide exhibited a maximum from September to December. A second peak is also observed in April. In winter and summer this parameter was strongly depressed. The annual cycle of plasma thyroxine concentrations presented a biphasic seasonal change with a maximum in summer and autumn, a minimum in winter and a second maximum in spring. The metabolism of exogenous thyroxine was reduced in winter and moderate rates of inactivation were observed in summer and autumn. However, during this latter period there is a short, important increase of the biological half-life of thyroxine. Thyroxine metabolism is accelerated at the end of winter. Relationships to seasonal changes in body weight, general activity cycle and climatic conditions are considered.

The European badger, *Meles meles* L., is a wild mammal who lives underground in an extensive branched system of burrows (sets). This animal offers a general locomotor activity exclusively nocturnal. Under natural conditions in temperate climates this activity is not constant over the year: The general activity out of the set is less important in the winter time. From a seasonal viewpoint, too, the badger will show a clearly marked body weight cycle, getting fatter during the cold months. Once these characteristics are known, it is important to examine the possible annual variations of thyroid function owing to its importance in the process of metabolic adaptation to climatic conditions.

Because of the wide range of tests used to measure thyroid function, the biology and the life conditions of the species reported on here as related to information given in literature is sometimes contradictory as far as the environmental endocrinology of wild mammals is concerned. One can find classical descriptions of hypothyroidism among hibernating mammals (Kayser and Aron, 1950; Hoffman and Zarrow, 1958; Lachiver and Petrovic, 1960; Lachiver, 1964; Hulbert and Hudson, 1976). However Hudson and Deavers (1976), in the ground squirrels, *Spermophilus* sp. have shown that it is difficult to set up causal relationships between period of hibernation and period of reduced thyroid activity in autumn and winter. They observed an increase of thyroid activity of *S. richardsoni* during the time of the year which corresponds with the middle of the natural hibernation period. A similar result has been described by Lachiver *et al.* (1957) on the garden dormouse, *Eliomys quercinus*, and by Petrovic and Kayser (1958) on the hamster, *Cricetus cricetus*. In the chipmunk, *Tamias striatus*, the thyroxine plasma level is the same for hibernating or normothermic animals (Hudson, 1977). In nonhibernating animals, many authors have shown a thyroid cycle distinguished by a winter hypoactivity

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(Hoffman and Robinson, 1966, white-tailed deer, *Odocoileus virginianus*; Delost, 1966, grey squirrel, *Sciurus vulgaris*; Rigaudière, 1969, various cricetines; Aleksiuk and Cowan, 1969, Canadian beaver, *Castor canadensis*; Aleksiuk and Frohlinger, 1971, muskrat, *Ondatra zibethica*; Cadariu *et al.*, 1976, roe deer, *Capreolus capreolus*) or by a summer hypoactivity and an autumn maximum (Hoffman and Kirkpatrick, 1960, American grey squirrel, *Sciurus carolinensis*) or a winter maximum (Eleftheriou and Zarrow, 1961, *Peromyscus maniculatus*).

Because of the heterogeneity in the functional level of a number of thyroid follicles observed in the badger, we found it essential to start our study of the seasonal function of this endocrine gland by using the conventional exploratory tests with radioactive iodide (^{131}I) release rate together with the assay of plasma thyroxine and an examination of the peripheral metabolism of that hormone.

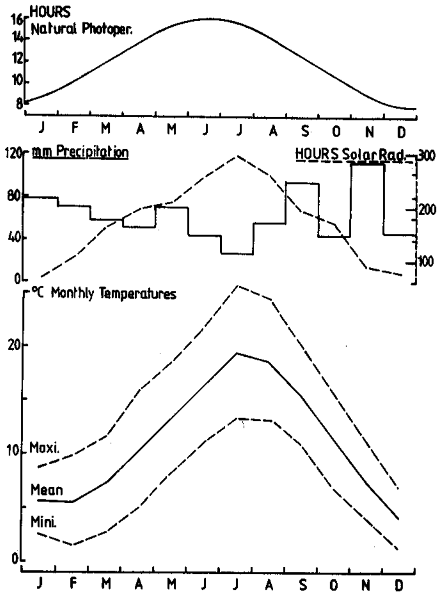

FIG. 1. Climatic conditions of the Chizé Forest in the west of France (7 years of monthly means).

MATERIAL AND METHODS

These experiments were carried out from 1975 to 1977 in Chizé Forest located in Western France (latitude, 46° 19′N; longitude, 00° 24′W). In this region, the winters, while not very cold, are distinct and not very sunny. Summers are not extremely hot but present an important solar radiation with temperatures about 25° (Fig. 1).

The animals used, all of them adult males, were provided by the numerous burrows located in the area of the laboratory. They were captured all the year round by "digging up," thus ensuring a continuous supply of one to three badgers per month. Animals have to get accustomed to captivity conditions for 1 month before joining the experimental group. They are submitted to natural weather conditions and fed daily during time of study with 1-day-old chicks specially hatched in standard farms. Body weight changes, thyroid uptake of radioactive iodide, plasma level of T4, and the peripheral use of radioactive-iodide-labeled thyroxine (^{131}I) have been studied monthly.

Besides, we studied the general activity rhythm of badgers living in their own natural environment in the Chizé Forest through direct watching of five burrows.

Each family lives in a burrow (adult male and female and their litter). We carried out observations with an infrared field glass. We determined thus a frequency index by assessing the number of entrances used or cleared and the importance of the digging at each entrance after deciding upon an arbitrary scale (0 = no entrance used, no noticeable digging; 100 = all entrances used and important digging at main entrances).

In vivo study of the thyroid uptake of radioactive iodide. The animal has been slightly anesthetized with ether and placed in dorsal décubitus. After intraperitoneal injection of 50 μCi of ^{131}I (Ref. S2, C.E.A., F.-Saclay) a time series has been adopted to study the kinetic of thyroid uptake as follows: 3, 6, 12, 24, 48, and 72 hr (sometimes up to 7 days).

Radioactivity of the thyroid gland was measured with a solid scintillation counter (Scintibloc) equipped with a collimator and adjusted to an analyzer (Intertechnique SA 41). Thyroid uptake as a function of time was expressed in percentage of the injected dose (% i.d.) corrected for radioactive physical decay.

Because of the radioactivity of juxtathyroidal tissues, a correction is to be brought to the calculations with regards to the rate between juxtathyroidal radioactivity (measured after cutting off thyroids) and that of the leg according to the method advised by Querière and Lachiver (1957).

On the other hand, we have compared the radioactivity of the neck of the living animal with that of the thyroid after killing the animal and dissecting the gland. *In vivo* and *in vitro* measures were in complete accordance. The evolution of thyroid uptake along the time has been graphically represented, and the calculation, by means of the least square method, of the decreasing phase, 24 to 48 hr after injection of tracer, provides information on the speed of hormone release. According to Pipes *et al.* (1956), we have calculated the true rate constant for release of thyroid hormone ($k4$) with the equation of Brownell (1951): $k4 = k'4 / 1\text{-}U$ where $k'4$ is the observed rate of release of thyroidal ^{131}I, based upon the calculated slope of the regression line (exponential decay) of thyroidal radioactivity after the maximum uptake of ^{131}I and U the theoretical uptake of ^{131}I (% i.d.) obtained by extrapolation to zero time. This $k4$ is, then, expressed as hormonal secretion as percentage injected dose per day: $k4$ (% i.d./day).

Study of the peripheral metabolism of thyroxine. The labeled hormone (^{131}I-L-thyroxine, Amersham, Ref. IB 14; R.A.S.: 30 mCi/mg) diluted in physiological salt solution was injected (30 μCi) into the radial vein. In order to prevent the thyroid uptake of the iodide resulting from deiodination of the labeled hormone, an antithyroidal was given 30 min after injection and then every 12 hr. Potassium perchlorate was chosen because it does not alter the distribution and excretion of injected thyroxine (Sandford and van Middlesworth, 1960; Escobar del Rey and Morreale de Escobar, 1961). It has been used in an aqueous solution (200 mg/ml) and in the quantity of 0.5 ml/kg body wt. Blood samples were taken at various times after injection of ^{131}I-thyroxine (after 6, 12, 24, 36, 48, and 72 hr) from the opposite radial vein. Then, plasma radioactivity was directly measured.

As we could not possibly carry out a chromatographic identification under our working conditions, we cannot certify that we have only measured the radioactivity of the plasma T4, so this is only a crude estimate.

Assay of plasma thyroxine. Total plasma thyroxine was assayed by the isotopic competition technique described by Vigouroux (1972), after all validation tests of the method have been carried out. Blood samples were collected daily between 9:00 and 11:00 AM. Each plasma sample was duplicated.

All data are presented as the mean ± 1 SE; statistical significance was assessed using an analysis of variance performed on the Digital P.D.P. 11-40 of the laboratory.

RESULTS

Seasonal Variations of the Body Weight and of the General Activity Rhythm

The badger exhibits a seasonal cycle in body weight clearly marked (maximum/minimum ratio: 1.8) with a winter maximum (11.8 ± 0.6 kg) and a minimum (6.6 ± 0.3 kg) taking place at the end of the summer and the beginning of autumn ($P \leq 0.01$) (Fig. 2). Between November and December, there is a marked increase in weight. It occurs along with a reduction in the general activity of the animals, as evidenced by the study on the sets used in the forest (weak activity index).

During winter and early spring, greater activity at openings of dens and heavier weights of animals have been observed. During late spring and summer, animals proceed to take longer walks through the forest but den activities are reduced. In autumn there is an important digging activity as the increasing number of entries used and stacking up of earth by the badgers tend to prove.

Changes in the Thyroid Uptake of Radioactive Iodide

General aspect of the graph shown in Fig. 3 of the ^{131}I uptake, expressed in percentage

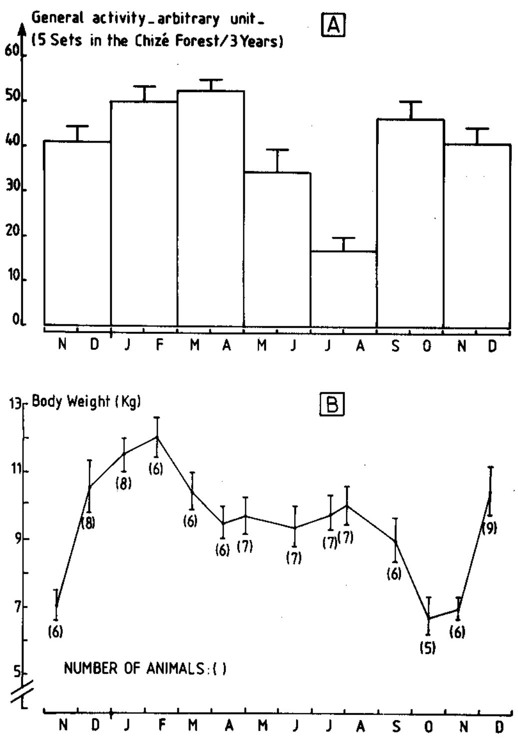

FIG. 2. Seasonal variations of the set activity (A) and the body weight (B) in the male badger (mean ± SEM).

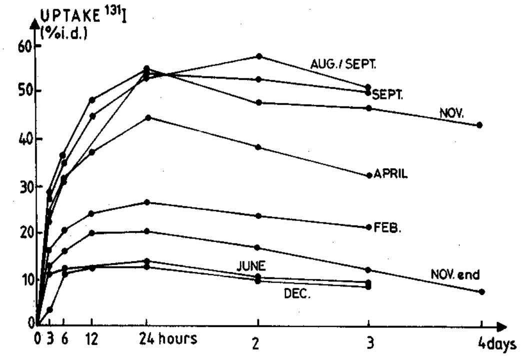

FIG. 3. Kinetic of the ^{131}I thyroid uptake at different times of the year.

of the injected dose (^{131}I % i.d.) gives evidence for a rapid increase, corresponding to the maximum speed of thyroidal uptake, ending 3 hr after injection of the tracer, a maximum fixation at 24 hr, and, then, a decreasing phase, following the excretion of synthetized thyroid hormones.

In order to describe the seasonal evolution of the thyroid function, we shall consider successively the variations in thyroid uptake 24 hr after injection of radioactive iodide and the slope of the decreasing phase: *K4* coefficient (Table 1).

We can thus observe in the badger (Fig. 4) a well-marked annual cycle, with a bimodal aspect, for the capacities in fixing iodide. The maximum activity period occurs in autumn, from September to November (from 54.2 to 55.4%). A second peak, with an amplitude slightly shorter than the first one is also observed in spring, in April (45.0%), September–November vs April: N.S.

In winter, thyroid uptake of iodide is strongly depressed. It varies between 17.1 and 28.6% i.d. (from September to November vs from December to March: $P \leqslant 0.01$; from December to March vs April: $P \leqslant 0.01$). The second sequence of hypoactivity, late spring and summer, from 14.3 to 20.9%, presents an amplitude comparable to the first one (from June to August vs April: $P \leqslant 0.01$; from June to August vs from December to March: N.S.).

Secretion of thyroid hormones, expressed by the variations of *K4* coefficient also presents a well-marked seasonal change (Table 1). Here too, the cycle has a bimodal aspect with an autumn maximum and a progressive fall from October to early winter (August vs November: $P \leqslant 0.01$). Then, *K4* values remain low during colder months. In spring, though, hormonal secre-

TABLE 1

SEASONAL VARIATIONS OF THE *K4* COEFFICIENT EXPRESSED IN PERCENTAGE OF INJECTED DOSE PER DAY (MEAN ± SEM)

Months	No. of animals	Mean ± SEM (% i.d. / day)	Statistical analysis	
End August, beginning September	5	14.6 ± 2.2^{a}		
Mid-October	5	9.3 ± 2.0^{b}	N.S.$^{(a,b)}$	$P \leqslant 0.01^{(a,c)}$
Mid-November	6	6.5 ± 1.3^{c}	N.S.$^{(b,c)}$	
Mid-December	8	1.0 ± 1.2^{d}	$P \leqslant 0.01^{(c,d)}$	
Mid-January	7	2.0 ± 0.4^{e}	N.S.$^{(d,e)}$	
Beginning February	6	2.1 ± 0.6^{f}	N.S.$^{(e,f)}$	
Mid-March	6	2.2 ± 0.5^{g}	N.S.$^{(f,g)}$	
Beginning April	6	19.5 ± 5.5^{h}	$P \leqslant 0.01^{(g,h)}$	
End April, beginning May	6	13.6 ± 5.3^{i}	N.S.$^{(h,i)}$	$P \leqslant 0.05^{(h,j)}$
End May	4	1.2 ± 0.2^{j}	$P \leqslant 0.05^{(i,j)}$	
Mid-June	7	4.0 ± 1.5^{k}	N.S.$^{(j,k)}$	
Mid-July	3	0.9 ± 0.4^{l}	N.S.$^{(k,l)}$	
End July, beginning August	5	0.8 ± 0.2^{m}	N.S.$^{(l,m)}$	
Beginning September	4	16.3 ± 4.4^{n}	$P \leqslant 0.05^{(m,n)}$	

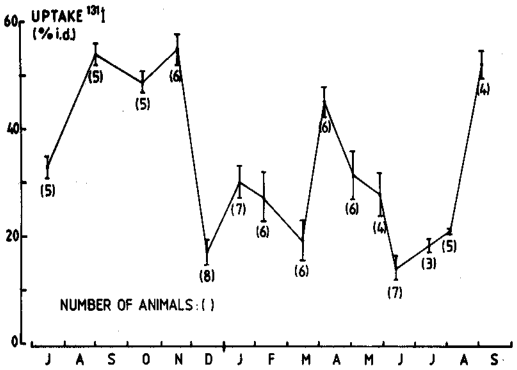

FIG. 4. Seasonal variations of the thyroid uptake (24 hr after the ^{131}I injection) in the male badger (% i.d., mean ± SEM).

tion is important (March vs April: $P \leqslant 0.01$), and this second maximum, taking place in April, is followed by a sequence of hyposecretion (May vs April: $P \leqslant 0.05$) that will last till early September (August vs September: $P \leqslant 0.05$).

Annual Cycle of Plasma Thyroxine Concentrations

Study of the graph shown in Fig. 5 shows biphasic seasonal variation of the plasma concentration of thyroxine. Higher values take place in September (22.6 ± 2.2 ng/ml), but the rate will rapidly fall to a very low level by mid-December (11.7 ± 0.7 ng/ml). From December to February, the plasma thyroxine stays at a low level and we observe a tendency to a rise in March (December vs March: $P \leqslant 0.05$). This persists until mid-May (18.7 ± 1.6 ng/ml). This second maximum is statistically significant (February vs May: $P \leqslant 0.01$).

After a late spring diminution (end of June 12.6 ± 1.6 ng/ml) statistically significant (mid-May vs end of June: $P \leqslant 0.01$) plasma concentration of thyroid hormone increases markedly one more time (end of June vs July: $P \leqslant 0.01$), from July, to reach a maximum in September (29.8 ± 1.0 ng/ml).

Seasonal Variations of Biological Half-Life (t ½) of Thyroxine

Peripheral use of thyroid hormone is not constant during the year (Table 2). At the end of summer or beginning of autumn metabolism of the hormone is low (t ½ = 26.7 ±

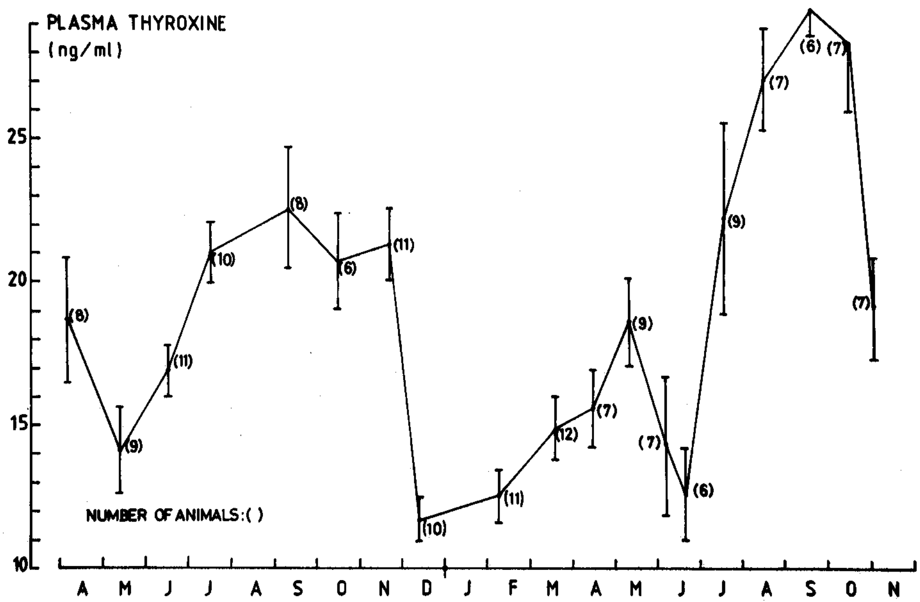

FIG. 5. Annual variations of the plasma thyroxine in the male badger (ng/ml, mean ± SEM).

TABLE 2
SEASONAL EVOLUTION OF THE RADIOACTIVE THYROXINE HALF-LIFE
(t ½, HOURS) IN THE MALE BADGER

	First year		Second year	
Mid-April	19.2 ± 1.4[a]	(5)[b]	17.9 ± 0.8	(7)
Mid-May	16.8 ± 0.5	(9)	11.0 ± 0.8	(18)
End May	24.6 ± 0.9	(9)		
Mid-June	18.7 ± 0.8	(11)		
Mid-July	17.9 ± 0.7	(10)	17.7 ± 1.5	(6)
Beginning September	26.7 ± 0.8	(10)		
Mid-October	19.1 ± 0.7	(10)		
Mid-November	20.5 ± 0.8	(11)	21.2 ± 1.0	(7)
Mid-December	22.8 ± 0.8	(11)		
Beginning February	–		22.3 ± 1.2	(11)
End February	15.6 ± 0.9	(6)		
Mid-March	17.7 ± 0.7	(12)		

[a] Values are shown as means ± SEM.
[b] The number of animals are in parentheses.

0.8 hr). There is a statistically significant stimulation of the metabolism ($P \leq 0.01$) midautumn (t ½ = 19.1 ± 0.7 hr). During the winter, from November to February, peripheral use of thyroid hormone is still going slowly. Hormonal metabolism is then stimulated at the end of winter and in spring December vs end of February: $P \leq 0.01$), but maximum will only be reached in early May (t ½ = 16.8 ± 0.5 hr the first year, 11.0 ± 0.8 hr the second year). This maximum is followed by a minimum occurring at the end of May (t ½ = 24.6 ± 0.9 hr; mid-May vs end May: $P \leq 0.01$). In the summer, the hormonal metabolism exhibits a medium activity (17.7 ± 1.5 hr $\leq t$ ½ $\leq$ 18.7 ± 0.8 hr).

DISCUSSION

Results achieved show that in the male badger, the thyroid gland exhibits seasonal variations marked by a maximum occurring at the end of summer and in autumn. There is a winter and preaestival minimum. A significant spring activity can be observed as well.

Such interpretation can be safely put forth if one considers the perfect concordance of the features characterizing the various graphs. As a matter of fact, a summer and autumn high plasma level of T4 corresponds to an important isotopic thyroid uptake and a high rate of excretion. During the same period, peripheral utilization of thyroxine is high, though this parameter is depressed in September. During early winter, the fall in plasma concentration of hormone accompanies the decrease of thyroid uptake of iodide and the important decrease in hormonal excretion. Hormonal peripheral metabolism remains at a low level.

Spring thyroid activation is marked by a sudden rise in thyroid uptake, rate of thyroxine excretion, and thyroxine plasma level. During the same period, shortening of the half-life of radioactive thyroxine provides evidence for a stimulation of peripheral metabolism. While at the end of spring, changes in all measured parameters enables one assume that thyroid function is strongly depressed. At the end of summer, though, after a sudden stimulation, glandular activity is very important.

If one disregards the winter behavioral adaptation of the badger, who lives underground and exhibits a seasonal rhythm of general locomotor activity characterized by a strong diminution of the time spent outside during the cold months, such results will seem contradictory to some data mainly provided by experiments on deep-cold effects on laboratory animals. It is generally accepted that among environmental factors likely to influence the thyroid gland function outer temperature plays a preponderant part. More particularly, the set of experiments carried out on the rat shows that exposure to cold will be shortly followed by stimulation of thyroid function (important bibliographical survey in Astier, 1975a, b). In the long run (1 month and more of exposure to cold), activation will persist but no longer bears the spectacular feature observed during the first days of exposure to cold, although one may note discrepancies between all various studies, certainly due to differences in experimental protocols or in animals diets. Hyperactivity of the thyroid gland seems to appear through a higher ^{131}I/PBI (Kuroshima *et al.*, 1971), and an increased thyroxine secretion rate (Bauman and Turner, 1967; Bauman *et al.*, 1968) but amplitude of stimulation is weaker if exposure to low temperature lasts a long time.

Besides, one may note that, in spite of differences from the thermal regulatory viewpoint, wild or domestic birds will generally react to chronic cold with a constant thyroid activation, like laboratory mammals. Laboratory experiments on domestic bird (Astier, 1975a,b) confirm observations on seasonal thyroid cycles characterized by an early winter thyroid hyperactivity among wild birds in temperate regions: pigeon (Riddle, 1925), starling (Burger, 1938), English sparrow (Davis and Davis, 1954), *Zonotrichia leucophrys* (Wilson and Farner, 1960; Farner, 1964).

Among wild mammals acclimatized to outside life, with a fur ensuring an efficient thermal protection and with seasonal behavioral rhythms clearly marked and adapted to weather conditions, correlations between annual variations of thermoperiod and thyroid function do not express the synchronizing effect of that physical factor on the endocrine cycle considered, to which some of the data in the literature mentioned above testify.

On the other hand, thyroid function is certainly not subordinate to environmental factors only. One should examine possible gonad–thyroid interrelations as well, if one considers opposed phase relations that take place between the two annual rhythms (Maurel *et al.* 1977). Indeed, to low plasma concentrations in testosterone correspond, at the end of the summer, important rates of thyroxine secretion. While in winter a fresh start in testicular function will occur only at the beginning of the thyroid hypoactivity sequence. Yet one should characterize the nature of these endocrine interactions by a study based on physiological and bioclimatic experiments.

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