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Influence of Light Regimen and the Time of Year on Circadian Oscillations of Thyroid Hormones in Rats

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Summary

Male SPF rats (Wistar strain) were adapted in the course of the year to natural light (N) and to a 12 : 12 h (light : dark) artificial light (A) regimen. At approximately the spring and autumn equinox and the summer and winter solstice, rats were killed at 3-h intervals over a 24 h period and their serum thyroxine (T_4), triiodothyronine (T_3) and reverse T_3 levels were determined. The light regimen and time of year significantly influenced the basic characteristics of the oscillations of the hormones. In the N regimen, T_4 levels (T_3 levels less) culminated in all seasons in correlation to sunrise. In the A regimen they culminated irregularly after daybreak. In animals with the N regimen, the oscillations of the hormones were rhythmic in all seasons, but in the A regimen in only some seasons. In the N regimen, the mean daily T_4 concentration value (the mesor) was the highest in the spring and the lowest in the autumn; in the A regimen the mesors were the same, except for a low mesor in the autumn. In both light regimens, the T_3 mesors were the highest in the autumn and low in the winter; the rT_3 mesors were a mirror image of the T_3 mesors. The annual mean of serum T_4 concentrations was lower in the N group than in the A group.

Key words:
Circadian rhythms – Light regimens – Time of year – Rat thyroid hormones

Introduction

Light from a solar – i.e. natural – source has different physical properties and biological effects from artificial light and so a comparison of the effect of the two types of light is still highly topical in chronobiology. As distinct from artificial light, natural light has a broader wavelength spectrum, a changing ratio of the light to the dark part of the day, with transition periods between the two parts, variable intensity during the day and year in correlation to the latitude and greater intensity at night owing to the moon and the stars. In an artificial light regimen, the intensity of the light to which a caged animal is exposed is the same throughout the day and the year.

We were interested in the degree to which qualitatively different light – natural light with a variable photoperiod and artificial light with a stable 12 : 12 h

(light : dark) photoperiod – would influence the circadian oscillations of the thyroid hormones in the serum of laboratory rats in the course of the year.

Material and Methods

Male rats (Wistar strain) of an originally specific pathogen-free colony, exposed from birth to an artificial light regimen (12 h light and 12 h dark – LD 12 : 12) and with an initial weight of 180 g, were divided immediately after delivery from the breeders (Velaz, Prague) into two groups and were adapted five weeks to artificial light (LD 12 : 12) and natural light in each of the four seasons. The temperature in the vivarium was 23 ± 2 °C and relative humidity 60 – 70 %. The animals were kept in cages in groups of four, with a free access to food and water (LD pellets, Velaz, Prague). Cold light of 150 lux intensity from a fluorescent lamp in the cage was switched on automatically at 7 a.m. and off at 7 p.m.. After the adaptation to the natural (N) and artificial (A) light regimen had been completed the rats were killed by rapid decapitation at 3-hour intervals over a period of 24 hours in the last week of March, June, September and December, approximately at the time of the spring and autumn equinox and the summer and winter solstice. The thyroxine (T_4), triiodothyronine (T_3) and reverse triiodothyronine (rT_3) concentrations were determined radioimmunochemically in serum obtained from a mixed blood, using commercial kits (for T_4 and T_3 from Institute of Radioecology and Applied Nuclear Techniques, ČSFR, for rT_3 from Hypolab Biodata, Switzerland) and groups of eight animals each. The experiment was evaluated statistically by cosinor analysis (Halberg *et al.* 1967), with a chosen 24-h rhythm period and an unpaired t-test (the mesors). When studying the effect of different photoperiods on the circadian oscillations of the given parameters, the basic characteristics of the oscillations were evaluated using two acrophases – the computative acrophase determined by the relationship of the peak to 0^{00} h local time, whose values are given in a cosinor analysis table, and the external acrophase, which is related to a given point on the synchronizing external cycle – in our experiment the beginning of the light period (sunrise, switching on the light). The external acrophase allows more exact evaluation of the effect of the photoperiod on the tested parameters.

Results

Thyroxine (T_4). The curves of circadian oscillation of the thyroxine concentration in the serum of rats with the N regimen had two peaks in every season, but they differed in rats with the A regimen. Rhythm with a 24 h period was present in the group N in all seasons, but in the A group was not detected in the summer. The computative acrophase occurred at roughly the same time during the year in both groups of rats, with a 6-h phase shift forward in group A (compared with group N) in the summer. In rats with the N regimen, the localization of the external acrophases was roughly the same – 2 to 3 hours after sunrise – in every season. With the A regimen, the external acrophase was localized 4 h after daybreak in the winter, 6 h after in the summer and shortly after daybreak in the intervening seasons. The mesor (the mean daily concentration of fitted curve of the parameter) for T_4 was the highest in the spring and lowest in the winter in rats with the N regimen, while in animals with the A regimen it was the lowest in the autumn and in the other seasons was approximately the same. The T_4 mesors for rats with the N regimen were significantly lower in the summer and winter than for rats with the A regimen. The mean thyroxine concentration for the whole year was statistically significantly lower in rats with the N regimen ($P < 0.01$) (Fig. 1 – 4 and 6, Tab. 1).

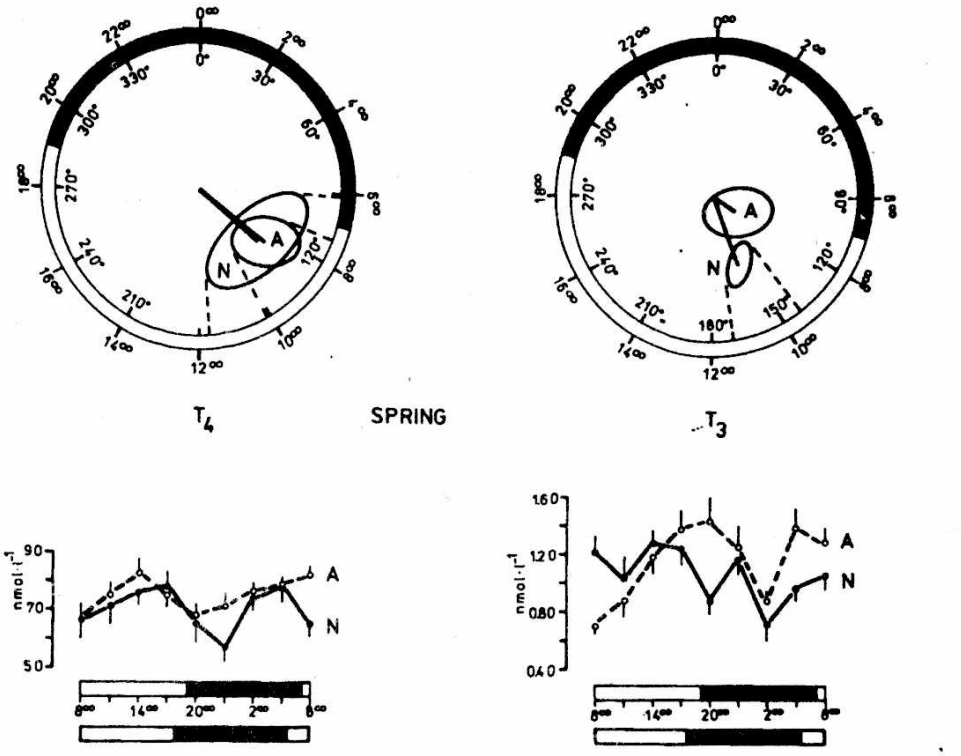

Fig. 1
Circadian oscillations (lower part) and cosinor diagram (upper part) of serum thyroxine (T_4) and triiodothyronine (T_3) concentration in rats kept in natural (N) and artificial (A) lighting conditions during the spring. The oscillations are presented as the arithmetical means ± S.E.M. White part of time data – light, black part – darkness, upper abscissa – A regimen, lower abscissa – N regimen; the time data in the cosinor diagram illustrate the A regimen only. The basal characteristics of the oscillations are illustrated in the cosinor diagrams: the vector originating from the center of the circular system of coordinates represents the amplitude of the oscillations. The acrophase is represented by the angle formed by the vector with hour 0. Rhythm is present if the ellipse of the errors does not overlap the origin of the coordinates; the tangents to the ellipse represent the 95 % confidence interval for the acrophase. The exact time of sunrise and sunset on the day the rats were killed:

March	23: 5.31 a.m., 5.51 p.m.;	June	26: 4.34 a.m., 8.42 p.m.
September	29: 5.31 a.m., 5.21 p.m.;	December	22: 7.25 a.m., 3.43 p.m.

Triiodothyronine (T_3). Circadian oscillations of serum triiodothyronine levels were marked in both groups (A and N) in every season except the winter. Rhythm, which was present in all seasons in the group N, was demonstrated in the spring and summer in the group A. The computative acrophases were localized at approximately the same time in rats with both light regimens. The external acrophases in rats with the N regimen were situated 5 – 6 h after sunrise for the whole of the year except the winter, when they occurred 1 h after sunrise. In the A regimen, T_3 concentrations culminated within 1.5 h after daybreak in the spring, summer and winter and 5.5 h after daybreak in the autumn. The T_3 mesor, in both groups, was the highest in the autumn and was lower in the winter (N) or in the winter and summer (A). In rats with a natural light regimen, the mesors were

distinctly lower (in the autumn and winter) than in rats kept in artificial light. The annual mean of T_3 concentration was lower in rats with the N regimen than in group A, although the difference was not significant (Fig. 1 – 4 and 6, Tab. 1).

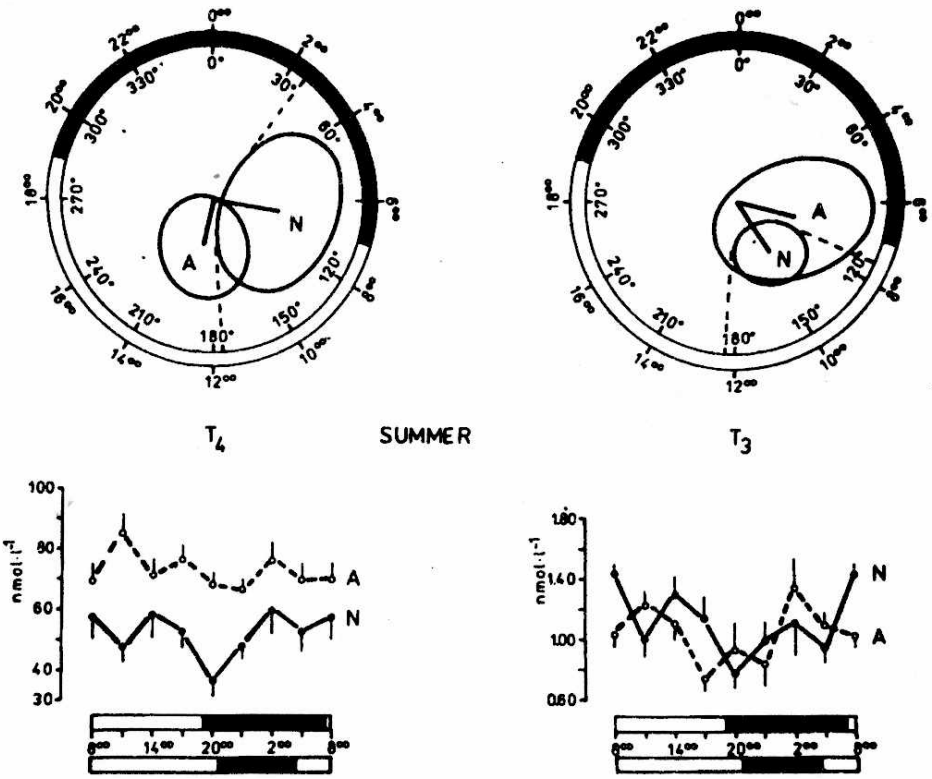

Fig. 2
Circadian oscillations and cosinor diagram of serum T_4 and T_3 concentration during the summer. Other details as in Fig. 1.

Reverse Triiodothyronine (rT_3). The serum rT_3 concentration oscillated rhythmically during the 24 hours in both groups in all seasons. In both groups (A and N), computative acrophases had roughly the same localization. In the N regimen, external acrophases were situated 3.5 – 4.5 h after sunrise from the spring to the autumn and 2 h after sunrise in the winter. In the A regimen, external acrophases were situated in the first hour after daybreak. In both groups (A and N), the mesor was the highest in the winter; in group N it was the lowest in the autumn and in group A it was low in the summer and autumn. In rats with the N regimen, the autumn mesor was significantly lower than in animals with the A regimen. There was no difference between the annual mean rT_3 concentration in the two groups (Fig. 5 and 6, Tab. 1).

Table 1

Characteristics of the cosinor test: the mesor (the mean value of the fitted curve) and amplitude are given in the employed units; the acrophase is given in an angular and a time interpretation.

CI – confidence interval; its limits in the presence of rhythm are given in brackets.
N – natural, A – artificial light regimen.

		Rhythm detection (95% level)	Mesor ± S.E.M.	Amplitude ± CI (95%)	Acrophase ± CI (95%) Degrees	Hours/minutes)
Thyroxine						
Spring	*N*	+	69.52±2.20	16.19(10.20;22.18)	132°(91°;176°)	8^{48}(6^{04};11^{44})
	A	+	74.33±2.40	16.99(11.14;22.84)	129°(110°;151°)	8^{36}(7^{20};10^{04})
Summer	*N*	+	53.09±2.59	8.70(1.20;16.20)	100°(37°;177°)	6^{40}(2^{28};11^{48})
	A	–	71.27±3.49	5.91(– ; –)	192°(– ; –)	12^{48}(– ; –)
Autumn	*N*	+	56.45±2.21	16.26(10.63;21.89)	118°(98°;147°)	7^{52}(6^{32}; 9^{48})
	A	+	59.84±2.65	11.08(6.16;16.00)	110°(88°;148°)	7^{20}(5^{52}; 9^{52})
Winter	*N*	+	40.13±2.25	10.51(3.51;17.51)	150°(62°;235°)	10^{00}(4^{08};15^{40})
	A	+	70.57±2.14	18.92(13.22;24.62)	160°(137°;176°)	10^{40}(9^{08};11^{44})
Triiodothyronine						
Spring	*N*	+	1.07±0.05	0.29(0.23; 0.35)	161°(145°;172°)	10^{44}(9^{40};11^{28})
	A	–	1.14±0.05	0.10(– ; –)	127°(– ; –)	8^{28}(– ; –)
Summer	*N*	+	1.12±0.05	0.17(0.09; 0.25)	147°(113°;185°)	9^{48}(7^{32};12^{20})
	A	–	1.04±0.05	0.16(– ; –)	105°(– ; –)	7^{00}(– ; –)
Autumn	*N*	+	1.48±0.05	0.96(0.73; 1.19)	171°(141°;207°)	11^{24}(9^{24};13^{36})
	A	+	1.82±0.05	1.03(0.62; 1.44)	187°(172°;202°)	12^{28}(11^{28};13^{28})
Winter	*N*	+	0.61±0.04	0.14(0.05; 0.23)	130°(211°: 70°)	8^{40}(14^{04}; 4^{40})
	A	+	1.04±0.04	0.30(0.25; 0.35)	126°(98°;164°)	8^{24}(6^{32};10^{56})
Reverse Triiodothyronine						
Spring	*N*	+	86.12±3.45	21.25(14.17;28.33)	139°(111°;161°)	9^{16}(7^{24};10^{44})
	A	+	94.76±3.81	30.24(16.01;44.47)	122°(55°;189°)	8^{08}(3^{40};12^{36})
Summer	*N*	+	76.76±3.66	17.73(9.55;25.91)	138°(105°;161°)	9^{12}(7^{00};10^{44})
	A	+	80.19±3.52	18.52(7.12;29.92)	120°(94°;176°)	8^{00}(6^{16};11^{44})
Autumn	*N*	+	63.04±2.95	14.16(3.69;24.63)	140°(77°;196°)	9^{20}(5^{08};13^{04})
	A	+	80.54±3.55	15.94(12.27;19.61)	123°(95°;152°)	8^{12}(6^{20};10^{08})
Winter	*N*	+	115.20±4.51	46.61(31.63;61.59)	143°(115°;167°)	9^{32}(7^{40};11^{08})
	A	+	104.14±2.89	20.59(17.43;23.75)	104°(87°;137°)	6^{56}(5^{48}; 9^{08})

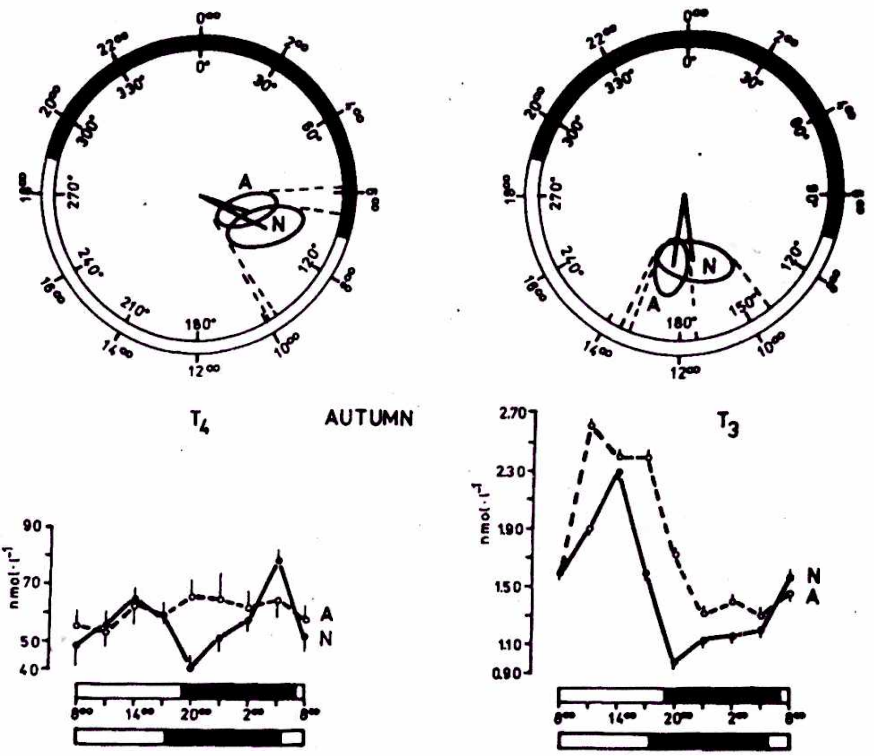

Fig. 3
Circadian oscillations and cosinor diagram of serum T_4 and T_3 concentration during the autumn. Other details as in Fig. 1.

Discussion

As a dominant synchronizer of biological rhythms, light plays an important role in circadian and seasonal (circaannual) variation of a broad spectrum of indicators, including the thyroid hormones. A short photoperiod did not induce involution of the gonads in adult laboratory rats, which were therefore regarded as photoperiodically inactive animals. However, the administration of testosterone, together with the effect of short daylight, caused the gonads of laboratory rats to regress (Wallen *et al.* 1987) and this, together with seasonal variation of various indicators, is indicative of photoperiodicity of certain traits.

In the laboratory rat, the activity of the thyroid gland fluctuates in the course of the day and of the year. Seasonal changes in the oscillations of serum thyrotropic hormone (TSH) levels in rats, with the highest mean circadian concentration in the autumn and the lowest in the winter and spring (Jordan *et al.* 1983) were recorded. Wong *et al.* (1983), in the serum of male rats of different ages and breeds, described the circadian T_4 and T_3 concentration as higher in the summer than in the winter, with a LD of 14 : 10. Independently of these authors, Ahlersová *et al.* (1984), who investigated circadian oscillations of these hormones in the spring and autumn as

well as in the summer and winter and also described the circadian oscillations of reverse T_3, observed similar seasonal changes in the T_4 and T_3 concentrations in the serum of male rats exposed to a LD of 12 : 12.

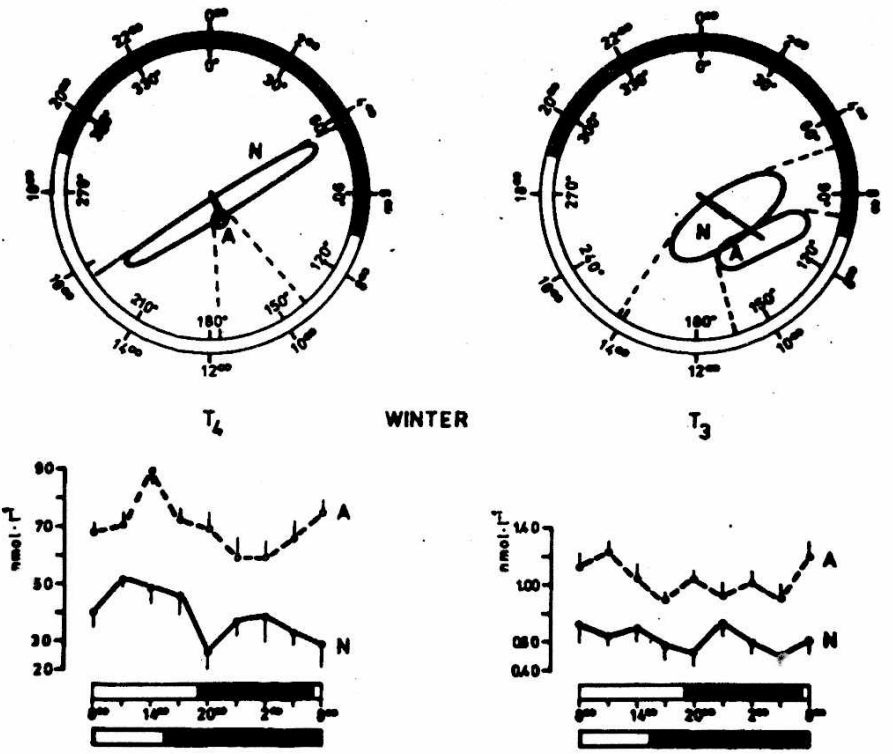

Fig. 4
Circadian oscillations and cosinor diagram of serum T_4 and T_3 concentration during the winter. Other details as in Fig. 1.

In the literature we failed to find any publication on the influence of natural light in the various seasons on circadian oscillations of the thyroid hormones in the serum of laboratory rats, or any study comparing the effect of light from a natural and an artificial source on the same hormones. These are highly topical problems from the aspect of detailed definition of the role of the pineal body in the regulation of thyroid activity and in the modification of circadian and seasonal rhythms of the thyroid hormones in rats. In the mammalian pineal body, a light signal is transformed to a biochemical signal, melatonin, whose rhythm of synthesis is regulated by light of adequate intensity, with a pacemaker in the suprachiasmatic nuclei of the hypothalamus. A high melatonin concentration appears in the pineal body in the dark part of the day in all species of animals, both nocturnal and diurnal. The melatonin level acts as a signal measuring the length of the day in photoperiodic species (for a review see Arendt 1987). Interaction of pineal body and thyroid has been studied in hibernators (chiefly Syrian hamsters) and in rats and mice. Blinding the hamsters, or exposing them to a short photoperiod, raised the

amount of thyrotropin-releasing hormone (TRH) in the hypothalamus and reduced the serum TSH and T_4 concentration. Pinealectomy or removal of the superior cervical ganglia prevented changes in these parameters (Vriend *et al.* 1982, Vriend 1983, Vriend and Wilber 1983). These studies draw attention to the inhibitory effect of the pineal body on TRH secretion in hamsters – a finding also made in other laboratory animals (Niles *et al.* 1979, Relkin 1978, Vriend 1978). Morphological and functional signs of raised thyroid activity were observed in pinealectomized rats and mice (Vriend 1983). Immature rats kept in permanent darkness had markedly lower plasma TSH and protein-bound iodine levels than rats with a LD 12 : 12 regimen (Relkin 1972, 1978). Niles *et al.* (1979) observed a marked increase in plasma TSH levels in pinealectomized rats with a LD 1 : 23 regimen compared with rats with an intact pineal body.

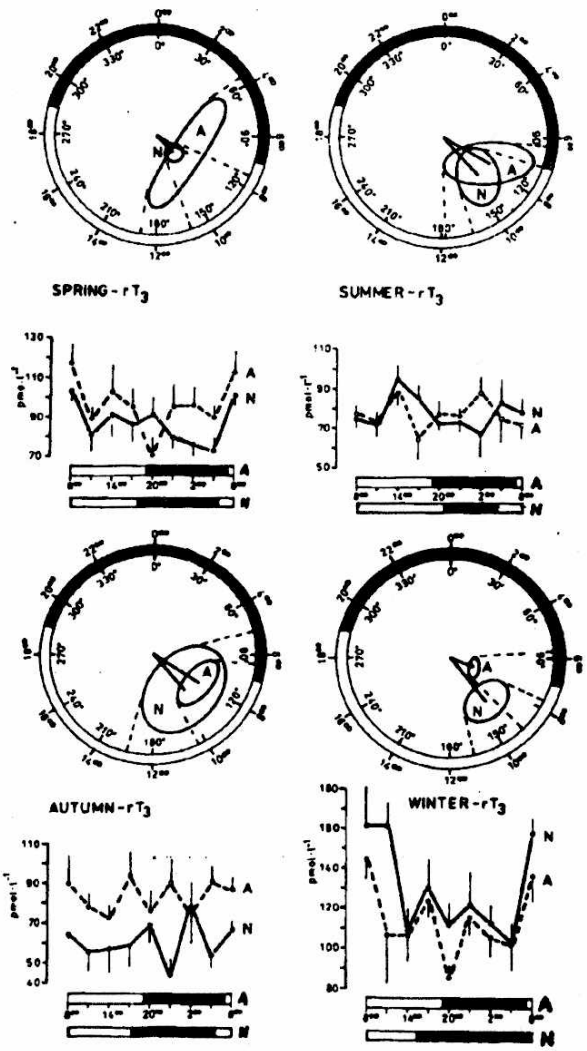

Fig. 5
Circadian oscillations and cosinor diagram of serum rT_3 concentration in the course of the year. Other details as in Fig. 1.

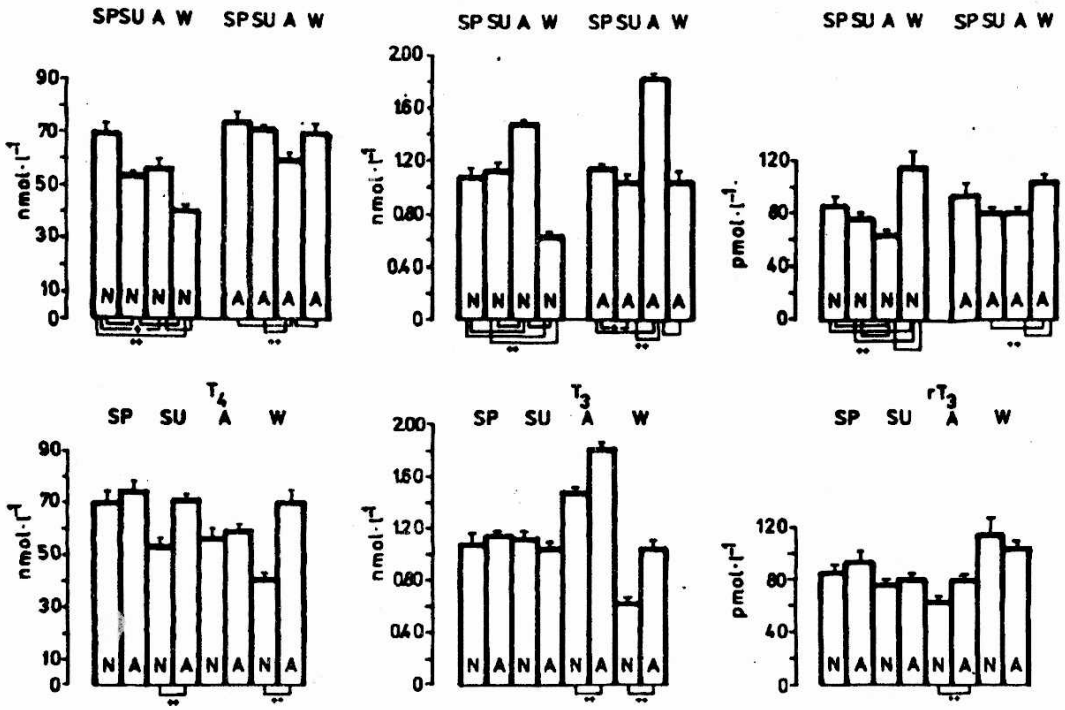

Fig. 6
The columns illustrate the mean values (mean ± S.E.M) of the fitted curves (mesors) of T_4, T_3 and rT_3 serum concentration in the course of the year. SP – spring, SU – summer, A – autumn, W – winter. Significance of differences between seasons and light regimens: ++ for $P<0.01$ and + for $P<0.05$. Other details as in Fig. 1.

We found that changes in circadian serum T_3 and rT_3 oscillations were similar in rats exposed to the two given types of light regimens, but not the same. Culmination of the levels of the two hormones in both groups of rats (N and A), expressed by the external acrophase, were different in relation to the beginning of daylight. Marked seasonal differences in T_3 mesors were observed in animals with the N regimen (the lowest in the winter, the highest in the summer). Similarly, seasonal changes in rT_3 mesors were more pronounced in rats with the N regimen than in animals with the A regimen.

The influence of natural light on circadian oscillations of the thyroid hormones in the various seasons was most pronounced in the case of thyroxine. In rats with the N regimen, the trend of changes in T_4 mesors during the year (the highest in the spring, lower in the summer and autumn, the lowest in the winter) differed from the findings in rats with the A regimen, in which the mesor value did not alter significantly, except in the autumn, when it fell. The T_4 concentration culminated during the year at approximately the same time after sunrise (expressed by means of the external acrophase), indicating that thyroxine oscillations are correlated with the appearance of the light stimulus. No such correlation was observed in rats with the A regimen. Assuming the existence of pineal body-

hypothalamus-thyroid regulatory relationships, these changes are determined by differences in the action of natural and artificial light on this axis, as manifested mainly in the circadian rhythms of thyroxine, the basic thyroid hormone. The response of circadian T_3 and rT_3 oscillations to different light regimens was less marked and seasonal influences dominated. In rats kept in natural light, Laakso *et al.* (1988) recorded a higher rhythm amplitude and content of melatonin in the pineal body at night and lower levels in the daytime compared with rats kept in artificial light (in both regimens LD was 13 : 11). The authors ascribed a great importance to gradual light changes at daybreak and nightfall in the induction and inhibition of melatonin secretion. N-acetyltransferase, the key enzyme in melatonin synthesis in rat pineal body, reacted like melatonin to the gradual onset of light and dark in natural light during the year (Illnerová and Vaněček 1980). These authors found the shortest period of N-acetyltransferase activity in natural light in June and the longest in December; this would explain the low serum T_4 and T_3 values in the winter and higher values in seasons with a longer photoperiod in our experiments. The findings of Illnerová and Vaněček (1980) and Laakso *et al.* (1988) help to explain the lower circaannual thyroxine and triiodothyronine mesors during the year in rats with the N regimen compared with the A regimen in the present study.

From the high T_3 concentration and low rT_3 concentration in the autumn, we conclude that metabolism is intensified during preparation for the winter. In the winter, in laboratory rats, as in animals living freely in open, we see a decrease in the T_4 and T_3 levels, associated with an increase in the rT_3 level. In the spring, the thyroid hormone levels (mainly T_4) rise again. Seasonal changes in the thyroid hormones are a part of developmental adaptations to more or less favourable conditions of existence in various seasons

We demonstrated circadian variation of serum reverse triiodothyronine levels in rats and the influence of the time of year on these oscillations.

The length of the daily photoperiod during the year played a dominant role in the regulation of metabolism. The persistence of seasonal changes in thyroid hormone (mainly thyroxine) concentrations in laboratory rats is a marked photoperiodic trait in an animal regarded as being little photoperiodic.

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References

AHLERSOVÁ E., AHLERS I., MILÁROVÁ R., ĎATELINKA I., TOROPILA M.: Circadian oscillations of thyroid hormones, insulin and glucagon in the blood of laboratory rats in the course of the year. *Physiol. Bohemoslov.* **33**: 309–319, 1984.

ARENDT L.: Chronobiology of melatonin. *ISI Atlas Sci. Pharmacol.* **1**: 257–261, 1987.

HALBERG F., TONG Z.L., JOHNSON E. A.: Circadian system phase – an aspect of temporal morphology: procedures and illustrative examples. In: *The Cellular Aspects of Biorhythms.* H. MAYERSBACH (ed.), Springer, Berlin, 1967, pp. 20–48.

ILLNEROVÁ H., VANĚČEK J.: Pineal rhythm in N-acetyltransferase activity in rats under different artificial photoperiods and in natural daylight in the course of year. *Neuroendocrinology* **31**: 321–326, 1980.

JORDAN D, PERRIN F., MORNEX R.: Circaannual variations of TSH circadian rhythm parameters in the rat. *Neuroendocrinology* **36**: 17–20, 1983.

LAAKSO M. J., PORKKA-HEISKANEN T., ALILA A., PEDER M., JOHANSSON G.: Twenty-four-hour patterns of pineal melatonin and pituitary and plasma prolactin in male rats under "natural" and artificial lighting conditions. *Neuroendocrinology* **48**: 308–313, 1988.

NILES L., BROWN G. M., GROTA L. J.: Role of the pineal in diurnal endocrine secretions and rhythm regulation. *Neuroendocrinology* **29**: 14–21, 1979.

RELKIN R.: Effects of pinealectomy and constant light and darkness on thyrotropin levels in the pituitary and plasma of the rat. *Neuroendocrinology* **10**: 46–52, 1972.

RELKIN R.: Use of melatonin and synthetic TRH to determine the site of pineal inhibition of TSH secretion. *Neuroendocrinology* **25**: 310–318, 1978.

VRIEND J.: Testing the TRH hypothesis of pineal function. *Med. Hypothesis* **4**: 376–387, 1978

VRIEND J.: Pineal-thyroid interactions. *Pineal Res. Rev.* **1**: 183–206, 1983.

VRIEND J., WILBER J. F.: Influence of the pineal gland on hypothalamic content of TRH in Syrian hamsters. *Horm. Res.* **17**: 108–113, 1983.

VRIEND J., RICHARDSON B. A., VAUGHAN M. K., JOHNSON L. Y., REITER R. J.: Effects of melatonin on thyroid physiology of female hamsters. *Neuroendocrinology*, **35**: 79–85, 1982.

WALLEN E. P., DEROSCH M. A., THEBERT A., LOSEE-OLSON S., TUREK F. W.: Photoperiodic response in male laboratory rat. *Biol. Reprod.* **37**: 22–27, 1987.

WONG C., DÖHLER K. D., ATKINSON M. J., GEERLINGS H., HESCH R. D., VON ZUR MÜHLEN A.: Influence of age, strain and season on diurnal periodicity of thyroid stimulating hormone, thyroxine, triiodothyronine and parathyroid hormone in the serum of male laboratory rats. *Acta Endocrinol.* **102**: 377–385, 1983.

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