CONTINUOUS DARKNESS INDUCES STRUCTURAL CHANGES IN THYROID GLAND OF ADULT MALE RATS

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ABSTRACT

Background

The thyroid glands function and structure are influenced by several psychomotor stimulants like: temperature, drugs, chemicals and light. The purpose of this study was to investigate the effect of continuous darkness on the thyroid glands of male rats. Despite so many previous works to explore the effect of exogenous or endogenous melatonin on thyroid, there are few studies dealing with the outcome of progressively increasing phases of darkness on thyroid architecture, according to the available literatures.

Objectives

This work is intended to study the effect of rising period of continuous darkness on thyroid tissues in adult male rats.

Methods

Adult Wister albino rats were kept in total 24 hours darkness for successive 4 phases. These rats were divided into 8 groups. Group II, III, IV and V were cited in continuous darkness for 2, 4, 6 and 8 weeks in that order. Group I[a], Group I[b], Group I[c], and Group I[d] were control groups of group II, III, IV and V likewise. After the last day of the dark phase dedicated for each group, the animals were sacrificed under effect of anesthesia. The thyroid gland was separated, weighed and right lobe was practiced to study its structural changes.

Results

The results illustrated no key structural effect by short and medium phases of darkness, while on long phases; there was detrimental effect on thyroid tissues.

Conclusion

The continuous darkness for a long time has undesirable histological and anatomical changes on the thyroid tissues of the adult male rats in a manner correlated with the length of publicity.

Keywords: *Thyroid, Melatonin, Darkness, Endocrine.*

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INTRODUCTION

The main pineal gland hormone named melatonin is secreted in all mammals during the dark phase of the circadian day cycle. The role of melatonin in controlling sexual maturity, sexual cycling, stress, mood, body weight, cancer, immune response and its location as a regulator of aging and senescence makes the pineal gland likely to be a factor in welfare of all the physical organs [1-4].

The thyroid gland consists of right and left lobes connected by a narrow isthmus. It is a vascular organ surrounded by a sheath derived from the pretracheal layer of deep fascia. The sheath attaches the gland to the larynx and the trachea.

Each lobe is pear shaped, with its apex being directed upward as far as the oblique line on the lamina of the thyroid cartilage; its base lies below at the level of the fourth or fifth tracheal ring.

The isthmus extends across the midline in front of the second, third, and fourth tracheal rings. A pyramidal lobe is often present, and it projects upward from the isthmus, usually to the left of the midline [(2)].

Its hormones act on nearly all cells in the body. They operate to augment the basal metabolic rate, change protein synthesis, aid regulate the long bone growth in synergy with growth hormone and neuronal maturation. They also increase body's sensitivity to catecholamines. Thyroid hormones are essential to appropriate development and differentiation of every cell of the human body. Also they regulate protein, carbohydrate and fat metabolism, affecting the use of energetic composites. They also stimulate the vitamins metabolism [(2, ,5 6& 7)].

Thyroid hormones production is illegitimated by the pituitary gland. If there is an inadequate amount of thyroid hormone circulating in the blood, the release of TSH is amplified by the pituitary to stimulate more thyroid hormone making. In contrast, when there is too much of circulating thyroid hormone, TSH level goes down as the pituitary attempts to diminish the production of thyroid hormone [(8, 9, 10 &11)].

MATERIALS AND METHODS

Fit adult male Wister albino rats, they were aged eight weeks and having an average weight of 416.63±72.9 (gm), forty in no. were employed in this work. They were alienated into 8 groups, each contained 5 rats. Each animal was positioned aside individually in a stainless wire meshed steel cage. The room temperature was set 22 ± 2 C º, fed freely ordinary experimental diet and the tap water was provided for drinking *ad libitum.*

Group I^{a}, Group I^{b}, Group I^{c}, and Group I^{d} were reserved in 12:12 light – dark cycle and they were regarded the control groups of group II, III, IV, and V sequentially. Animals of group II, III, IV, and V were put in continuous darkness for phases of 2, 4, 6 and 8 weeks in that order. At the last day of 1st couple of weeks of darkness, the animals of group II with its own control (Group I^{a}) were sacrificed under the upshot of the diethyl ether anesthesia, the whole intact thyroid was weighed after the removal of nearby tissues with the aid of a dissecting microscope, next fixed in Boun's solution right away and arranged for histological inspection by means of light microscopy, via serial paraffin sections of 5 µm thickness stained by routine haematoxyline and eosin staining [(12 & 13)].

In the same manner, the animals of group III were managed at the end of the planed 4th weeks of darkness with its own control group (Group I^{b}). The rats of group IV with its own control group (Group I^{c}) were handled at equivalent way at end of the planed 6th week of darkness, and rats belonging to group V with its control group (Group I^{d}) were handled at end of the planed 8th week of darkness. Histological as well as anatomical examinations were achieved. Histological assessment was done both as morphometric and explanatory.

The morphometric analysis was projected by way of Zeiss Integrating Micrometer – disk Turret as I of 25 points - system, put on a light microscope: which determined roughly the virtual surface area by counting up the points superimposed via a disk put on the microscope eye piece, the number of the estimated points actually allied to the relative capacity of the surface set [(14)], the summation of points overlaid the surface area of thyroid follicle's wall, as well as on the lumen, were considered. For each section 5 fields were taken at haphazard examined at 150X magnification field. Number of thyroid acini was calculated per field; by counting these acini seen in random microscopic 5 fields from each slide and using 5 slides from each thyroid sample. All the values were taken as mean ± SD of 5 rats. The significance for the difference between each of darkness-groups and its own control group was estimated by means of student – t – test [16].

RESULTS

Morphometry as well as explanatory studies for all groups were prepared, as follows:

Body weight: It was regarded as anatomical data. The body weight was pretentious in all groups, taking in regards the natural gaining in weight that must occur and the rising in animal growing with time (Table 1). Thyroid weight was not affected preciously in group II and III, whereas it was significantly unnatural in group IV and V (table 2).

Results of morphometry:

1 -The numeral of points that superimposed via the Micrometer – disk Turret overlying the epithelial wall of the acini (follicles), was almost as anticipated normally in group II and III, at the same time it was significantly perverted in group IV and V (table 3).

2- The numeral of the points placed over on the lumen of the acini, showed a reverse fashion to that of the points sited over the wall (Table3).

3- The number of acini per field was changed according to the size of the acinus (Table3).

The explanatory histological outcomes:

In both group II and group III: The cells of the epithelial wall of thyroid acini, were roughly remarkably analogous to those of the control groups; so every acinus was bounded by a single layer of definite thyroid epithelium which rest on a insubstantial basement membrane delimited a lumen filled with thyroid colloid that seen as a pink homogenous material. In the same microscopic field; some acini contained squamous epithelium, further acini were limited by cuboidal or low columnar epithelium (Fig.1& 2).

In group IV: Epithelial cells of the acini were almost cuboidal or low columnar epithelium lined the acini which were packed by a pink colloid with very few vacuoles at the peripheries of their lumens present between the colloid and epithelial cells (Fig.3).

In group V: The acini encompassed thickened basement membrane. There was abundance of thyroid epithelial cells and they were auxiliary elevated in longitudinal dimension (high columnar). The acini were appeared smaller in size with filled with colloid and the edges of the colloid material were scalloped, nevertheless, these acini were numerous and they increased in number (Fig.4).

Table 1. The effect of continuous darkness on body weight (in grams) of 10 weeks old male rats.

Time of keeping rats in continuous darkness	Body wt of rats at 1st day of experiment	Body wt of rats at last day of experiment	Difference in body wt
Control (Group I[a])	411.17±62.9	422.95±74.9	Added 11.78±0.9
2 wk continuous darkness	414.84±77.1	428.54±75.2NS	Added 13.17±1.6NS
Control (Group I[b])	410.97±65.1	431.42±51.1	Added 20.45±0.8
4 wk continuous darkness	422.03±82.9	432.11±80.2*	Added 10.08±0.9*
Control (Group I[c])	423.23±44.0	454.56±79.0	Added 31.33±1.2
6 wk continuous darkness	416.00±72.8	408.09±90.1*	lost 7.91±0.9*
Control (Group I [d])	418.02±74.6	457.19±75.2	Added 39.17±2.0
8 wk continuous darkness	419.25±64.1	402.25±87.7**	Lost 17.00±1.4**

-Results were expressed in mean ± SD of 5 rats.
--The difference of each group was statistically significant (*P<0.03, **P<0.01) when compared with its control, NS=not significant (P>0.05).
- The differences in wt of body among the 4 control groups are due to the difference in age.

Table 2. The effect of continuous darkness on thyroid weight (in grams) of adult male rats.

Time of keeping rats in continuous darkness	Thyroid weight at autopsy (mg)
Control (Group I[a])	18.98±2.4
2 wk continuous darkness	19.11±3.3 NS
Control (Group I[b])	19.02±2.6
4 wk continuous darkness	20.19±1.7 *
Control (Group I[c])	19.23±3.0
6 wk continuous darkness	21.22±3.1*
Control (Group I [d])	19.76±2.7
8 wk continuous darkness	23.12±2.1**

-Results were stated in mean ± SD of 5 rats.
--The difference of each group when compared with its own control was statistically significant as follows:*P<0.04, **P<0.01, NS=not significant P>0.05.
- The differences in wt. of the thyroid among the 4 control groups are due to the difference in age.

Table 3. Number of points (seen via the Micrometer– disk Turret) overlying the thyroid acini wall and lumen of adult male rats placed on continuous darkness (in unit area of 0.0025mm²) and number of acini per field.

Time of keeping rats in continuous darkness	Points on thyroid acini wall	Points on thyroid acini lumen	Number of acini per field
Control (Group I[a])	14.81±1.2	7.91±1.3	18.94±1.4
2 wk continuous darkness	14.22±1.7 NS	8.12±1.7 NS	19.12±2.7 NS
Control (Group I[b])	14.52±1.5	7.84±1.8	19.16±2.1
4 wk continuous darkness	14.04±1.8 NS	8.61±1.9 NS	18.25±1.9 NS
Control (Group I[c])	14.81±1.6	7.96±1.9	18.89±2.4
6 wk continuous darkness	15.27±1.2*	9.23±1.3*	19.68±1.5*
Control (Group I [d])	14.93±1.4	8.02±1.8	18.97±2.3
8 wk continuous darkness	18.11±1.1**	6.12±0.9**	20.14±1.6*

-Data were stated as mean ± SD of 5 rats.
-When every group was compared with its control, the statistical significance in differences was as follows: * P<0.03; ** P<0.01; NS= non-significant =P>0.5.

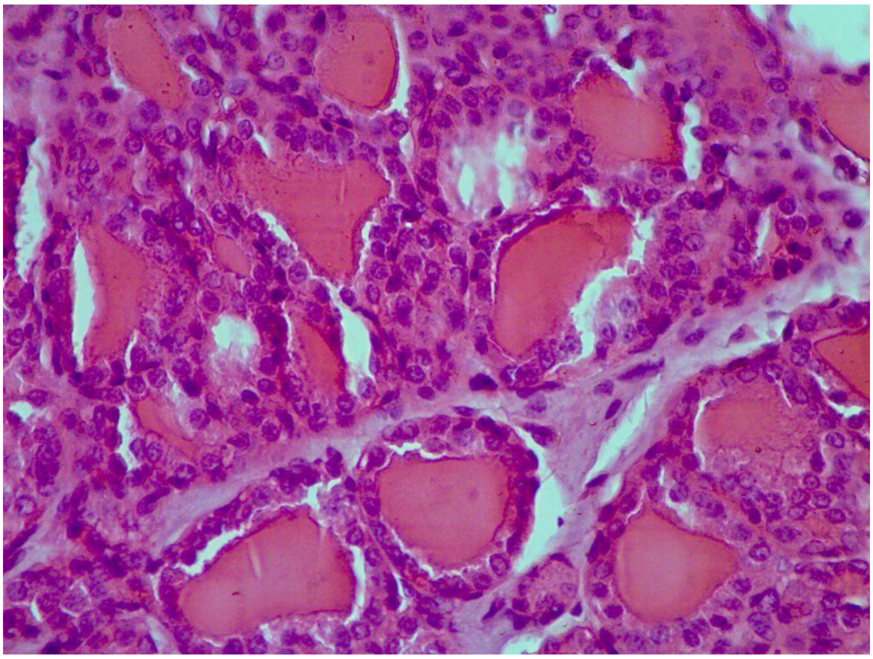

Figure 1. Control group: Thyroid tissues of adult male rat, H&E stain X250.

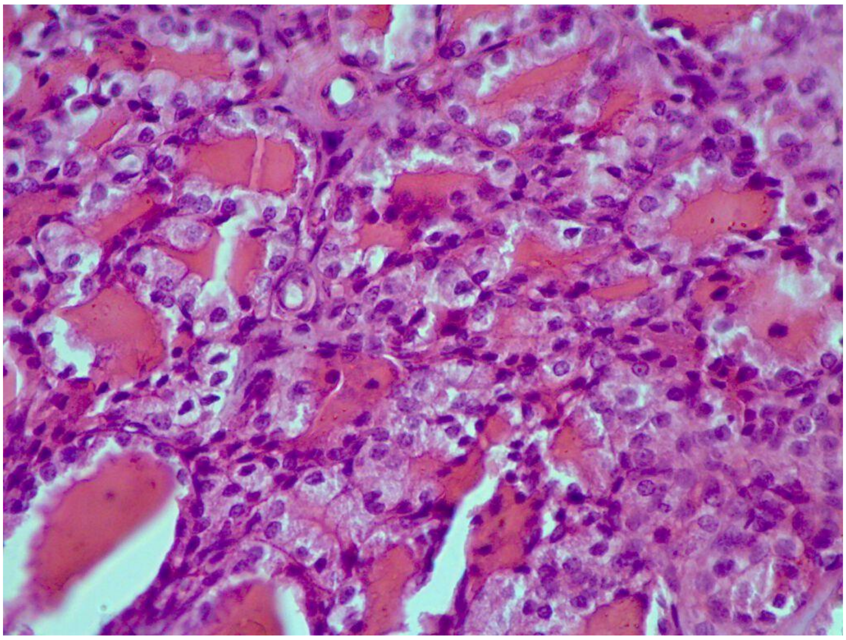

Figure 2. Thyroid tissues in group II of adult male rat, H&E stain X250.

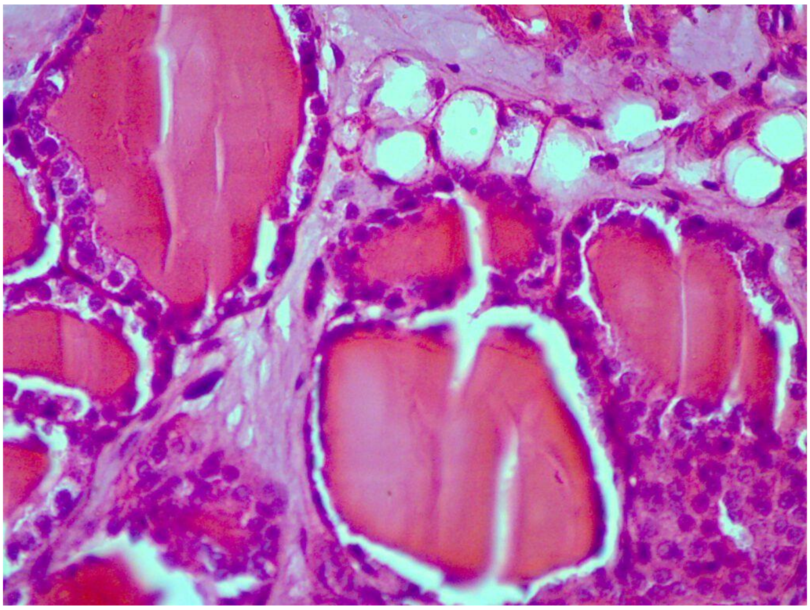

Figure 3: Thyroid acinus in adult male rats of group IV. The epithelium of acinar cells are roughly low columnar, H&E stain X250.

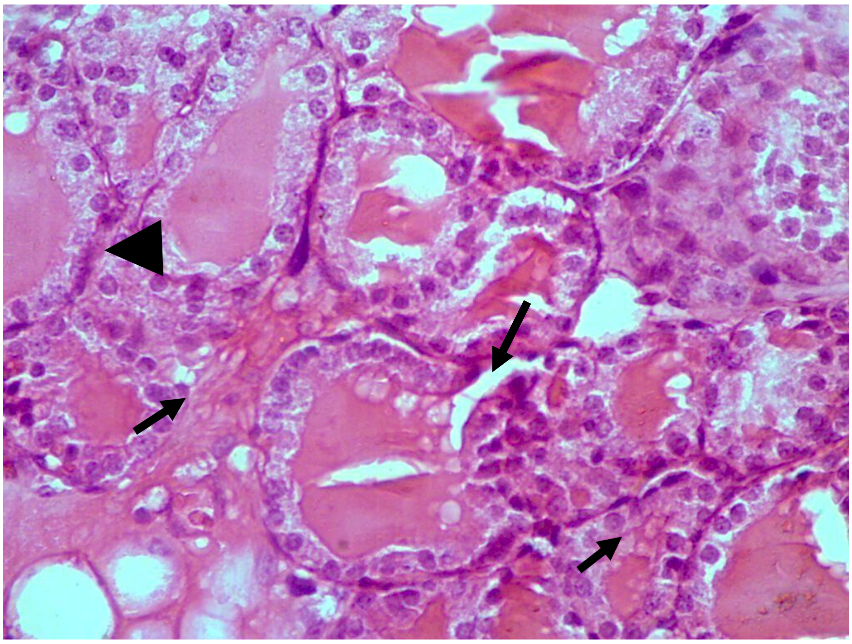

Figure 4: Thyroid tissues in adult male rats of group V: edges of colloid are scalloped (arrows), the columnar epithelium rest on thick basement membrane (arrow head), H&E stain X250.

DISCUSSION

Melatonin hormone is produced barely at night. It operates as the endocrine transducer of the photoperiodic messages [17, 18].There is a high-quality correlation between usual level of endogenous melatonin and thyroid hormones role in various bodily vital natural processes such as reproduction, so the endocrine means of animal seasonal reproduction and the confirmation that long day light and thyroid hormones, are mandatory to the appropriate timing of reproduction. Recent substantiation for a circadian-based molecular mechanism of the pituitary, which is induced by the short duration melatonin indicator reflected by long day, provokes the hypothalamic augment of T3 via a thyroid-stimulating hormone [19].

There were clear increases in the body weight of rats in all groups, nevertheless not all variations were statistically significant, this might be enlightened by the veracity that the darkness and its hormone termed melatonin [1, 3] may decreases the bodily metabolism and/or increases the food intake in rats, hence, affects the entire body mass in rats [20-22].

Table 2 gave an idea about the thyroid weight which was not affected significantly in group II and III, whereas it was significantly affected in group IV and V. The effect of melatonin on thyroid tissues could be simplified by the fact that endogenous melatonin proceeds through specific receptors in the almost entire bodily tissues [23-25].

When melatonin reaches the various tissues; it puts forth its achievement instantaneously, and since it is well verified to have a dose –dependent physiologic action [26-28]. This is why in the relatively small phase of continuous darkness (credible relatively low melatonin level); no major outcome was perceived, while with long dark phases (credible relatively elevated melatonin level); there was an evident histological shift which is a warning of a physiological disorder, because the function of thyroid acini, is indomitable from its size, height of the facing epithelium and the amount of its colloid [29-30].

Those products could be explained by the verity that endogenous melatonin has undesirable effects if it is formed in a large quantity [26-28]. The increase in the thickness of the basement membrane seen in the long phases of darkness; could be the corollary of the augment in production of fibrocollagenous tissues, in view of the fact that melatonin hormone has exceptional stimulating effect on fibroblasts [31, 32], which are the dynamic collagen – secreting cells and the crucial connective tissues forming cells [2].

Group IV: The histological check revealed the following verdicts: there were big acini lined by a layer of almost cuboidal glandular cells, together with large quantity of colloid which gave the histolopathological features of mild degree of hyperthyroidism [29, 30]. The cause for that hyperthyroidism might be the enormous stimulation of thyroid gland by the elongated exposure to continuous darkness leading to manufacture of high level of melatonin hormone which encourages the hypothalamic- pituitary-thyroid axis, thence a large quantity of thyroid hormone in particular; thyroxin was secreted and accumulated inside the thyroid acini that tendered interpretations rather comparable to early stages of thyroid hyper-function category [33-35].

In group V and VI (table 2): in those both groups the gland weight was enhanced and more or less similar histological picture, which could be enlightened by the fact that whichever thyroid disorder presents, it may induces enlargement of the gland whether there is hypothyroidism or hyperthyroidism [29, 34]. The little drop in the amount of thyroid colloid in the last group, that might be conferred by the fact that melatonin in its huge amount may excite the anterior pituitary to turn out thyroid stimulating hormone (TSH) which leads to massive stimulation of thyroid gland to ooze its own hormones, mainly T3 and T4, so this would present a histological appearance extremely analogous to the histological appearance of thyroid tissue of that group [29, 30]. The acini were virtually smaller in size with not as much of quantity of colloid and the peripheries of the colloid were scalloped, but they had a thicker wall and a taller columnar lining and they were more in number, hence the end weight result is larger than the control one (Fig.4). That histological appearance was quite similar to that of the hyperthyroidism, and the scalloped edges of the colloid indicated an energetic reuptake of the thyroid secretion [29, 30, 34]. The unhelpful and damaging effects could be due to sustained time of continuous darkness, because the effect of melatonin may diverge with long duration of the production time [35].

From this on hand study; it may be concluded that the time period of continuous darkness for no more than one month had more or less no apparent risky effect on thyroid gland tissues whilst longer dark periods brought on detrimental outcomes on the thyroid gland architectures of adult male Wister albino rats.

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