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The Effects of Constant Darkness and Constant Light on the Pineal Gland and Thymus Morphology in the Rats

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Abstract: It is known that periods of constant darkness and constant light cause stimulation and inhibition of melatonin secretion from the pineal gland. It is also suggested that neuroendocrine responses to environmental stimuli, such as light, can influence immune responses through the pineal gland. For these reasons, in this study the effects of the alterations in the photoperiod rhythm on the pineal gland and thymus morphology were experimentally investigated.

30 Swiss albino rats, aged 4 wk, were divided into 3 groups. Group I rats were kept in a dark room, group II in a room under a bright artificial light and group III (control) animals were exposed to a 12:12 h light: dark cycle. All animals were killed after 5 wk. Overall body weight was not affected by the dark or light periods. In group I, the thymus weight increased by 38%, the increase in the thymus cortex thickness was 34.5%. In group II, the thymus weight decreased by 21% and the decrease in the thymus cortex thickness was 18%. The differences among groups in the thymus weight and the thymus cortex thickness were found statistically significant.

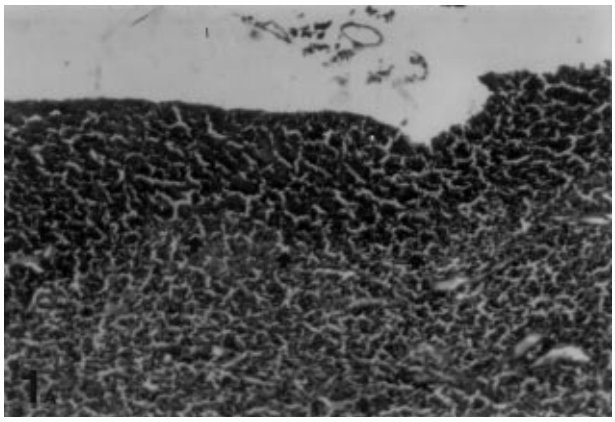
In differences in the diameter of the pineal gland and the pineal cell number of the darkness group and the light group according to the control group was not found statistically significant. However, the differences in he diameter of he pinealocyte nuclei among the groups were statistically significant. These findings point out that the changes in the periods of darkness and light have effects on the pineal gland and immune system.
Key Words: Photoperiodicity, pineal gland, immune system, morphometry, rat.

Introduction

Environmental stimuli, such as light and temperature, are transduced into neuroendocrine signals by the cyclic circadian synthesis and release of melatonin by the pineal gland (1,2). Melatonin is the best-known pineal neurohormone. Its synthesis and secretion are physiologically regulated by photic stimuli (length and intensity of light and duration of darkness)(3). Therefore, pineal and serum concentrations of melatonin show a circadian, as well as a seasonal periodicity in most including man (4). Psychosocial factors and stress also seem to modify the production of melatonin, and alterations of he circadian rhythm of its release have been associated with anxiety states, autoimmune disorders and cancer(5). Furthermore, the pineal gland and melatonin in particular seem to exert an oncostatic role in a variety of experimental and clinical models(6). It is reported that functional and pharmacological inhibition of melatonin synthesis leads to a significant depression of humoral and cell-mediated immune responses in mice(7,8). In this regard, an increasing amount of information has demonstrated the intimate connections existing between the neuroendocrien and the immune systems(8). The thymus gland was considered to represent a target site for immunomodulatory actions of the environmental stimuli and pineal gland. For these reasons, we studied the effect of permanent darkness and permanent light on pineal gland and thymic morphology.

Material and Methods

30 Swiss albino rats (15 female, 15 male), aged 4wk, were used. Because previous research has been also made on rats, we preferred to use rats to be able to compare our results with the literature. The animals were housed 5 female or 5 male percage. They were fed a regular diet and water ad libitum under controlled conditions. The animal rooms were maintained at a temperature of 23°±2°C. The rats were divided into 3 experimental groups, each comprising 5 females and 5 males. The 1st group was kept in a dark room for a period of 5 wk, the 2nd



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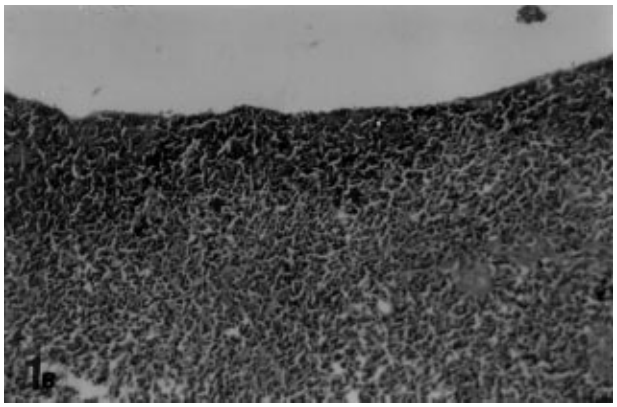

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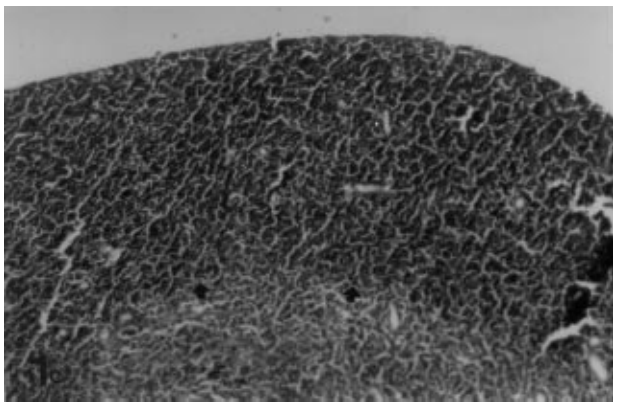

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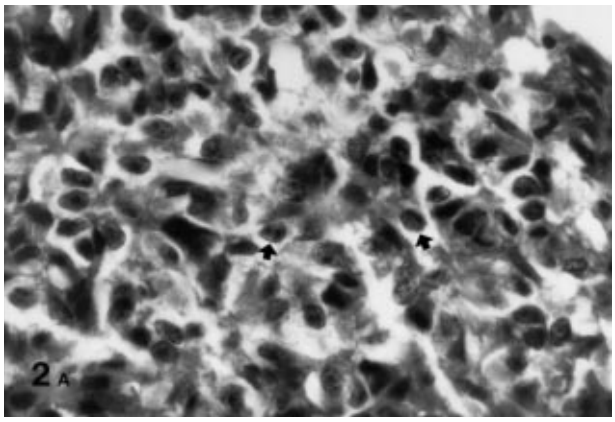
Fig 1. Thymic cortex thickness (H-E x 200). A) Control, B) Constant light, C) Constant darkness (Arrows indicates corcito-medullary line)



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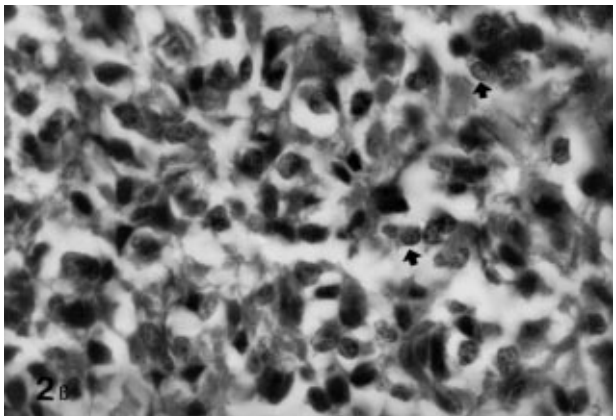

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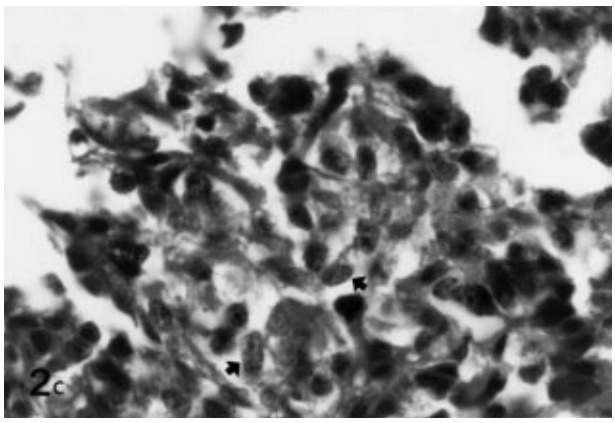

(C)

Fig 2. Pinealocyte nuclei diameter (H-E x 1000). A) Control, B) Constant light, C) Constant darkness

group under a bright artificial light for 5 wk and the 3rd in the animal house where the photoperiod was day light-darkness 12-12 h. In order to observe the effects of the constant darkness and constant light on the thymus and the pineal gland morphology the body weight, thymus weight, thymus cortex thickness, pineal gland diameter, pinealocyte number and pinealocyte nuclei diameter were measured.

Following ether anesthesia, all animals were weighed and were sacrified by cardiac perfusion with 10% formalin solution. The thymus was removed, dissected from adjacent connective tissue and weighed. The entire pineal gland was also removed. After dissection, the thymus and the pineal gland were fixed in 10% buffered formalin solution and for further histological examinations the specimens were embedded in paraffin, 6 mm sections were cut and stained with hematoxylin and eosin. The binocular Zeiss Axiophot photomicroscope was used for the morphological analyses.

In each specimen three different measurements were made (the thickest, medium, the thinnest) for thymus cortex thickness and their means were calculated. For pineal gland diameter estimation sections covering the entire extent of the epiphysis were made and greatest length and greatest width of he pineal gland were measured and their mean values were calculated in each specimen.

Sections containing the pineal gland were projected by a camera to a monitor. The pineal cell count and nuclei mesaurements were made from the monitor's secreen. To determine the pinealocyte number (numeric density), per unit area, in every specimen 5 different areas were counted and means were calculated. For the diameter of pinealocyte nuclei, in each specimen 50 pinealocyte nuclei were measured (totally 500 nuclei for each group). Each group's mean was then calculated.

Data were expressed as the mean ± the standard error of the mean (SEM). Since sample sizes were too small to know their distributions, statistical analysis of data were performed by the Kruskal Wallis variance analysis and Wilcoxon rank sum test.

Results

Overall body weight was not affected by he dark or light periods.

Thymus weight and cortical thickness were markedly increased or decreased at the end of the dark or light periods respectively. Compared to control group, in group I (darkness) the thymus weight increased by 38% and the increase in the thymus cortex thickness was 34.5%, while in group II (light) the thymus weight decreased by 21% and the decrease in thickness affecting the cortex was 18% (Fig. 1, Table). The differences among groups in the thymus weight and thymus cortex thickness were found statistically significant. The mean diameter of he pineal gland in group I (darkness) was 730 µm, in group II (light) 565 µm and in group III (control) 615 µm. While the difference in the diameter of the pineal gland between group I (darkness) and group II (light) was statistically significant, the difference between group I (darkness) and group III (control) and between group II (light) and group III (control) were not statistically significant. The differences in the pineal cell number (between group I and group III, group I and group II, group II and group III) were observed to be insignificant. However, the differences in the pinealocyte nuclei diameter among the groups which were exposed to different lighting conditions were considered statistically significant (Fig. 2, Table). For all parameters there were no significant differences between male and females.

Table. The effects of constant darkness and constant light on body weight, thymus weight and thymic cortex thickness the pineal gland diameter, pineal cell number and pinealocyte nuclei diameter in rats (mean±S.E.M.).

	Controls	Dark	Light
Body weight (gr)	126.0±4.8	131.5±5.0	124.0±6.4
Thymus weight (mg)	296.7±18.0	408.9±19.5*	231.9±9.1*
Thymic cortex thickness(µm)	194.0±7.3*	261.0±15.3*	165.3±6.2*
Pineal gland diameter (µm)	615.0±15.4	730.0±45.0	565.0±39.0
Pineal cell number(per unit area)	45.6±1.6	46.3±1.9	43.8±2.0
Pinealocyte nuclei diametre (µm)	5.90±0.11	6.65±0.19*	5.06±0.06*

* : $p<0.01$ vs control

**: $p<0.05$ vs control

Discussion

It is well established that lighting conditions are directhly responsible for the functional state of the pineal gland in that light inhibits and darkness enhances its activity(3). It is also reported that seasonal variations have affected the sizes of pinealocytes or pineal glands in many wild animals under natural conditions (9,10,11). Pinealocyte volumes or pinealocyte sizes are generally larger in winter or during hibernation(11). By contrast, pinealocytes of the hare are larger in size from summer to fall(9), and maximall diameters of pinealocytes of the bat are obtained in the fall (beginning o hibernation) in males and in early spring (ending of hibernation) in males and in early spring (ending of hibernation) in females(10). In feral animals under field conditions, it is possible that seasonal changes in a variety of environmental factors other than photoperiods may cause changes in size of pinealocytes and pineal glands. Thus, it is difficult to determine to what degree the photoperiods is involved in such seasonal variations. The present study demonstrated clearly the effects of photoperiods on size of pineal gland and pinealocyte nuclei diameter in rats under laboratory conditions.

Pineal gland diameter in the rats which were kept in constant darkness and constant light for 5 weeks did not show significant differences when compared to that in control group, while the difference between the dark and light groups was significant. We could not find difference in the pineal cell number (numeric density). However, the pineealocyte nuclei diameter decreased in light group while it increased in dark group. The difference in the pinealocyte nuclei diameter among groups was found statistically significant. Larger nuclei under constant darkness are believed to be functionally more active than smaller nuclei under constant light(12). Vollrath(13) stated that constant light induced atrophy of pinealocytes. However, Matsushima et al (12) have studied on Chinese hamsters raised under light: dark 16:8 and light: dark 8:16 and stated that the ones raised under long photoperiods (L:D 16:8) have larger pineal glands composed of larger pinealocytes than those raised under short photoperiods (L:D 8:16). They also observed that the pinealocytes in he Chnese hamsters raised under L:D 16:8 were not larger than those in animals raised under photoperiods shorter or longer than 16hr. Thus, L:D 16:8 may be the optimal photoperiod for the development of the pinealocytes in the Chinese hamster (12). However, Dombrowski & McNulty (14,15), who studied on intact and blinded Golden hamsters for 8 weeks, observed that pinealocyte nuclei and cytoplasm volume decreased while the nucleolar size was enhanced following optic enucleation. They stated that these changes in the nucleolar size were the indications of increased synthetic activity in respond to light deprivation (14,15). We observed an increas in the diameter of the pinealocyt nuclei which indicates an increasing functional activity in respond to darkness. Though Quay(10) stated that the maximal nuclear diameter in pinealocytes of the bat are obtained at the beginning of hibernation in males, and at the end of hibernation in females, we did not observe any sex related differences in the pineal diameter, pinealocyte number and the diameter of the pinealocyte nuclei.

There are relatively few studies on a possible connection between lymphopoietic system and pineal gland or enviromental stimuli(16). Mice kept for 3 generations under constant environmental light do not grow normally and show marked atrophy of the thymolymphatic system, and permanent light causes a significant depression of humoral and cell mediated immune responses in this species(7). It has been demonstrated that exogenous melatonin enhanced antibody production through an increase in spleen cellularity, and that when normal nonstressed mice were injected with sheep red blood cells, an injection of melatonin led to increase in antibody production without thymic enlargement(17). In addition, the immun reactivity and circulating lymphocytes were shown to fluctuate according to a circadian rhythm(18,19,20). Although Maestroni and Pierpoli(17) reported that the growth was not normal in mice raised under constant light for 3 generations we did not observe difference in he body weight of the rats raised under constant light or constant dark for 5 weeks. While an experiment of 5 weeeks was not sufficient to effect the body weight, we observed significant changes in the thymus weight and thymus cortex thickness at the end of the same amount of time. In group dark the thymus weight increased by 38%, the increase in the thymus cortex thickness was 34.5% while in group light the thymus weight decreased by 21% and the decrease in the thymus cortex thickness was 18%. Mahmoud et al.,(21), who did similar studies, reported that the thymus weight increased by 315% and the increase in the thypmus cortex volume was 190% in the rats raised constant darkness for 4 weeks and that the thymus weight decreased by 53% and the decrease in the thymus cortex volume was 61% in the rats kept under constant light for 4

weeks. The rate of increase and decrease in our findings was not so high as that in theirs. However, the increase in group dark was higher (almost twice as high as) than the decrease in group light, as was in their findings.

Acute stress and adrenal steroids are known to inhibit the immune response, and involution of the thymus is generally accepted as a reliable sign of the immunosuppressive action of steroid hormones(22,23). Clinical and experimental studies have shown that melatonin has important immunoregulatory functions (4,6). It is reported that the involution of he thymus induced by acute stress could be countered by exogenous melatonin(17,24). Since both melatonin and adrenal steroids do effect immune reactivity in opposite modes, the thymus gland was considered to represent a target site for the immunomodulatory interactions of those hormones(25,26,27) reported that the effect of the melatonin on he thymic morphology was directly dependent on the anti-glucocolrticoidal effect. In the contrary, Familari & Funder(28) pointed out that melatonin was not a glucocorticoid antagonist. Furthermore, the effects of the melatonin were shown to be exerted through opioid peptides, namely B-endorphin, had direct effects on the cell of the immune system(17,24). The findings of Mahmoud et al(21) and ours, in which the increase in the thymus weight and thymus cortex thickness in group dark was higher than the decrease in group light, seem to support the findings of Familari & Funder(28) and Maestroni et al.(17,24). The effects of he environmental conditions to the pineal gland and through the pineal gland to the thymus, direct or indirect, may have important physciological and immunotherapeutic implications.

References

1. Cavallo A. The pineal gland in human beings: Relevance to pediatrics. The Journal of Pediatrics 123: 843-851, 1993.
2. Reiter RJ. Pineal gland interface between the photoperiodic environment and the endocrine system: Trends in Endocrinology and Metabolism 2: 13-19, 1991.
3. Kappers JA. The mammalian pineal gland, a survey, Acta Neurochirurgica 34: 109-149, 1976.
4. Erlich SS, Apuzzo MLJ. The pineal gland: anatomy, physiology and clinical significance. Journal of Neurosurgery 63: 321-341, 1985.
5. Arendt J, Wirz-Juctice A, Bradtke J. Annual rhythm of serum melatonin in man. Neurosci Lett. 7: 327-330, 1977.
6. Blask DE. The pineal: an oncostatic gland? In: RJ Reiter (ed) The pineal gland Raven Press New York 1984, pp: 253-284.
7. Maestroni GJM, Pierpoli J. Pharmacologic control of the hormonally mediated immune response. In: Psychoneuroimmunology (ed. R. Ader). Academic Press, New York, 1981 pp: 405-425.
8. Maestroni GJM, Conti A, Pierpoli W. Role of the pineal gland in immunity. Circation synthesis and release of melatonin modulates the antibody responses and antagonizes the immunosupressive effect of corticosterone. J Neuroimmunol 13: 19-30, 1986.
9. Lincoln GA. Seasonal changes in the pineal gland related to the reproductive cycle in male hare, Lepus europaeus. Journal of Reproduction and Fertility 46: 489-491, 1976.
10. Quay WB. Seasonal cycle and physiological correlates of pinealocyte nuclear and nucleolar diameters in the bats, myotis lucilugus and myotis sadalis. Gen Comp. Endocrinol 29: 369-375, 1976.
11. Mcnulty JA, Dombrowsky TA, Spurrier WA. A seasonal study of pinealocytes in the 13 lined ground squirrel, Spermophilus tridecemlineatus. Reproduction, Nutrition, Development 20: 665-672, 1980.
12. Matsushima S, Sakai Y, Hird Y. Effect of photoperiod an pineal gland volume and pinealocyte size in the Chinese Hamster, Circetulus griseus. Am J Anat 187: 32-38, 1990.
13. Vollrath L. The pineal organ. In: Handbuch der Mikroskopischen Anatomie des menscheu. Vol VI/7. A. Oksche and L. Vollrath, eds. Springer, Berlin 1981, pp: 41-43, 67-71, 242-244.
14. Dombrowsky TA, Mcnulty JA., Morphometric analysis of the pineal complex of the Golden Hamster over a 24 hour light: dark cycle: I. The superficial pineal in untreated and optically enucleated animals. The Am J Anat 171: 359-368, 1984.
15. Dombrowsky TA, Mcnulty JA. Mcnulty JA. Morphometric analysis of the pineal complex of the Golden Hamster over a 24 hour light-dark cycle: II. The deep pineal in untreated and optically enucleated animals. Am J Anat 171: 369-376, 1984.
16. Jankovyc BD, Isakovyc K, Petrovic S. Effect of pinealectomy on immune reactions in the rats. Immunology 18: 1-16, 1970.
17. Maestrony GJM, Conti A, Pierpoli W. Role of the pineal gland in immunity: II. Melatonin enhances the antibody response via a opiatergic mechanism. Clin Exp. Immunol 68: 384-391, 1987.
18. Fernandes G, Halberg F, Yunis ES, Good Ra. Circadian rhythmic plaque forming cells; response of spleens from mice immunized with SRBC. J Immunol 117: 962-966, 1976.
19. Abo T, Kawate T, Itoh K, Kumagai K. Studies on the bioperiodiity of the immune response. I. Circadian rhythms of human T, B and K cell traffic in peripheral blood. J Immunol 126: 1360-1363, 1981.

20. Kawate T, Abo T, Hinuma S, Kumagai K. Studies on the bioperiodicity of the immune response. II. Co-variations of murine T and B cells and a role of corticosteroid. J Immunol 126: 1364-1367, 1981.

21. Mahmoud I, Salman SS, Al-Khateeb A. Continuous darkness and continuos light induce structural changes in the rat thymus. J Anat 185: 143-149, 1994.

22. Munck A, Guyre PM, Holdbrook NJ. Physiological functions of glucocorticoids in stress and their relationship pharmalogical action. Endocr Rev. 5: 25-44, 1984.

23. Buzzetti R, Mcloughlin L, Scaw D, Rees LH. A critical assessment of the interactions between the immune system and the hypothalamo-pituitary-adrenal axis. J Endocrinol 120: 183-187, 1989.

24. Maestroni GJM, Conti A, Pierpoli A. Role of h pineal gland in immunity: III. Melatonin antagonizes the immunosuppressive effect of acute stress via a opiatergic mechanism. Immunology 63: 465-469, 1988.

25. Persengiev S, Patchev V, Velev B. Melatonin effects on thymus steroid reseptors in the course of primary antibody responses: Significance of circulating glucocorticoid levels. International Journal of Biochemistry 23: 1487-1489, 1991.

26. Mori W, Aoyama H, Mori N. Melatonin protects rats from injurious effect of a glucocorticoid, dexamethasone. Japan J Exp. Med. 54: 255-261, 1984.

27. Aoyama H, Mori W, Mori N. Anti-glucocorticoid effects of melatonin in young rats. Hormone and Metabolic Research 36: 423-428, 1986.

28. Familari M, Funder JW. Melatonin and glucocorticoid hormones. Japan J Exp. Med 58: 73-77, 1988.