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ORIGINAL ARTICLE

Light and electron microscopic examination of pineal gland in rats exposed to constant light and constant darkness

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Abstract

OBJECTIVE: This study was aimed to examine the pineal gland of rats exposed to constant light and darkness at light and electron microscopic level.
DESIGN: For this purpose 18 male Wistar rats were used. Animals were divided into three groups. Rats in group I (Control) were kept under 12 hrs light: 12 hrs dark conditions. Rats in group II were exposed to constant darkness, while rats in group III were exposed to constant light for 6 weeks. At the end of the experiment, all animals were killed by decapitation. The pineal glands of rats were removed, then processed for light and electron microscopy.
RESULTS: In our study, extensive number of pinealocytes was observed in the structure of pineal gland of rats exposed to constant darkness and some of the observed pinealocytes were determined to contain double nucleoli. Furthermore, mitochondria and lipid droplets in the cytoplasm of pinealocytes were increased and rough endoplasmic reticulum sacs were enlarged in this group. Whereas, in rats those exposed to the constant light, a decrease in pinealocyte intensity was associated with increase in the connective tissue between parenchymal cells. Additionally, mitochondria and lipid droplets in the cytoplasm of cells were decreased.
CONCLUSIONS: It was observed that the pinealocyte cell activity of rats exposed to constant darkness was increased but decreased in rats exposed to constant light.

Introduction

The pineal gland which is a neuroendocrine tissue regulates changes exclusively in the functions of the endocrine system as well as the functions of many other systems according to light and dark conditions, and functions like a biological clock along with suprachiasmatic nuclei (SCN) located in the hypothalamus. Biological clocks are cellular structures which help to measure time in an organism. The pineal gland sends time signals to other parts of the body in circadian rhythm through melatonin hormone which the pineal gland secretes in darkness. Thus, it takes part in regulation of the physiological cycles related with different times of year and day. It has an important role, in particular, in control of reproduction functions and in evaluation of seasonal changes in day length [1–4].

Being different from the other endocrine glands, the endocrine activity of the pineal gland depends on neurological innervation. Light and darkness have a special significance in regulation of melatonin secretion from the pineal gland. Generally, light decreases the production of melatonin, whereas darkness increases it [1]. By being converted into electrical impulses in the photoreceptors of retina, light impulses are sent to suprachiasmatic nuclei, which is in hypothalamus, through retinohypothalamic tract. The circadian rhythm of melatonin secretion is regulated by suprachiasmatic nuclei. SCN suppresses the melatonin synthesis according to the amount of light [1–5].

The mammalian pineal gland contains two types of parenchymal cells. The pinealocytes which form the majority of parenchymal cells and responsible for melatonin secretion in the pineal gland. Glial cells serve as supporting cells and they are fewer in number than pinealocytes [1, 6]. The most important transmitter in postganglionic sympathetic nerve ends is norepinephrine. Suprachiasmatic nuclei effectively stops the release of norepinephrine from these nerve ends in light during the day. Norepinephrine release from nerve ends starts in darkness. Norepinephrine is connected to β-adrenergic receptors in pinealocyte membrane. By β-adrenergic receptors being stimulated, firstly adenilat cyclase is activated in the cell and cAMP increases. As a result, melatonin synthesis in the cell increases [1–5, 7].

In the previous studies carried out experimentally, it has been reported that light and darkness given in different periods cause changes in the functions and histological structure of the pineal gland [8–18]. So, in our study it was aimed to examine the pineal gland of rats exposed to constant light and darkness at light and electron microscopic level.

Material and Methods

Adult male Wistar rats (weighing 180–200 g, n = 18) were used in this study. Animals were divided into three groups. Rats in Group I (Control) (n=6) were kept under 12 hrs light and 12 hrs dark conditions. Rats in Group II (n=6) were exposed to constant darkness, whereas rats in Group III (n=6) were exposed to constant light. During the whole experiment the animals were kept at a constant temp (21 ± 1 °C). Food (standard pellet diet) and tap water were supplied *ad libitum*.

At the end of 6-weeks experiment period, all rats were killed by the method of decapitation. By removing the pineal glands of the rats, paraffin and electron microscopic blocks were prepared with routine histological methods. By taking sections, of which thickness is 5 micron, from paraffin blocks, these were stained with haematoxylen-eosine. Those prepared light microscopic preparations were examined on Olympus BH-2 microscope. Thin and semi-thin sections were taken from electron microscopic blocks. Semi-thin sections were stained with Toluidin blue and were used for light microscopic examinations. Thin sections were stained with Lead citrate-Uranil acetate and were examined on Carlzeiss-900 Electron Microscope.

Results

Light microscopic findings

When the pineal gland structure belonging to rats in control group was examined, it was determined to have a normal appearance. Two types of cells in pineal gland parenchyma namely pinealocytes and glial cells were observed. The distinction of these cells were made according to the staining features of their nuclei. Pinealocyte nuclei were euchromatic and were larger than glial cell nuclei. The cytoplasm of pinealocytes were stained with pale color. Due to chromatin density, glial cells nuclei were stained with dark color. The cytoplasm of these cells were seen as dark. Capillaries were observed between pinealocytes and glial cells (Fig. 1, 2).

When the pineal tissue of rats exposed to constant darkness was examined, the gland structure was observed to have an active appearance. In semi-thin sections stained with Toluidin blue, it was observed that extensive number of pinealocytes in the structure of pineal gland of rats exposed to constant darkness. Some of the observed pinealocytes were determined to contain double nucleoli. Furthermore, a considerable increase in lipid droplets was observed in this group (Fig. 3, 4, 5).

When the pineal tissue of rats exposed to constant light was examined, a decrease in the pinealocyte cell intensity and an existence of more glial cells were noticed. Additionally, the connective tissue between parenchymal cells was increased. (Fig. 6, 7).

Electron microscopic findings

While it was observed that pinealocytes belonging to control group had a normal structure (Fig. 8), an increase in mitochondria and lipid droplets of the pinealocyte cell cytoplasm of the rats exposed to constant darkness was observed. Furthermore, rough endoplasmic reticulum sacs were enlarged in the cell cytoplasm in this group (Fig. 9, 10).

A decrease in mitochondria and lipid droplets of the pinealocyte cytoplasm was seen in rats exposed to constant light when compared to the control group. Additionally, rough endoplasmic reticulum sacs that we had observed in pinealocytes of rats exposed to constant darkness was seen less in this group (Fig. 11).

Discussion

By being different from the other glands, the endocrine activity of the pineal gland depends on the neurological innervation. Light and darkness have a special importance in regulation of melatonin secretion by the pineal gland. Light decreases the production of melatonin, whereas darkness increases it [1].

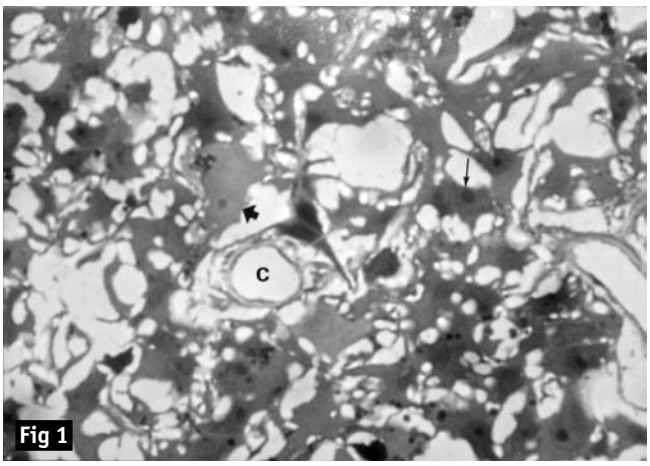
Light impulses are sent to suprachiasmatic nuclei, which is in hypothalamus, through retinohypothalamic tract by being converted into electrical impulses in the photoreceptors of retina. The circadian rhythm of melatonin secretion is regulated by suprachiasmatic nuclei. SCN supresses the melatonin synthesis according to the amount of light. The most important transmitter in postganglionic sympathetic nerve ends is norepinephrine. SCN effectively stops the release of norepinephrine from these nerve ends in light during the day. Norepinephrine release from nerve ends starts in darkness. Norepinephrine is connected to β-adrenergic receptors in pinealocyte membrane. By β-adrenergic receptors being stimulated, firstly adenilat cyclase is activated in the cell and cAMP increases. As a result, melatonin synthesis in the pinealocytes increases [1–5, 7].

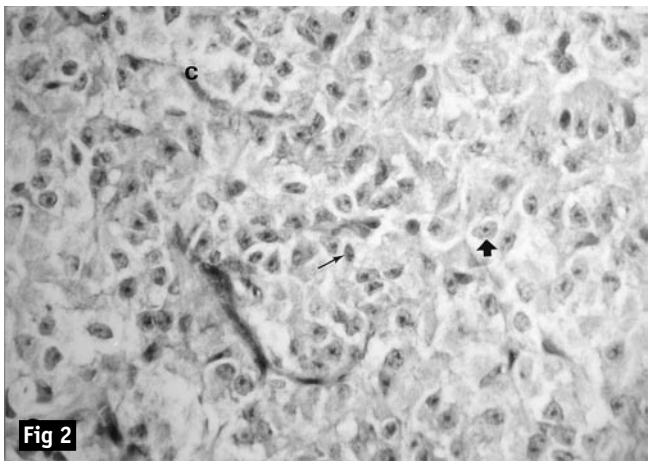
For the melatonin synthesis, firstly tryptophan aminoacid should be taken from the blood into the pinealocytes [1, 2, 4]. A great part of tryptophan taken into cell is utilized for melatonin synthesis and a small part is utilized for protein synthesis. So, an increase in protein synthesis to be observed in pinealocytes naturally demonstrates the increase in melatonin synthesis [2].

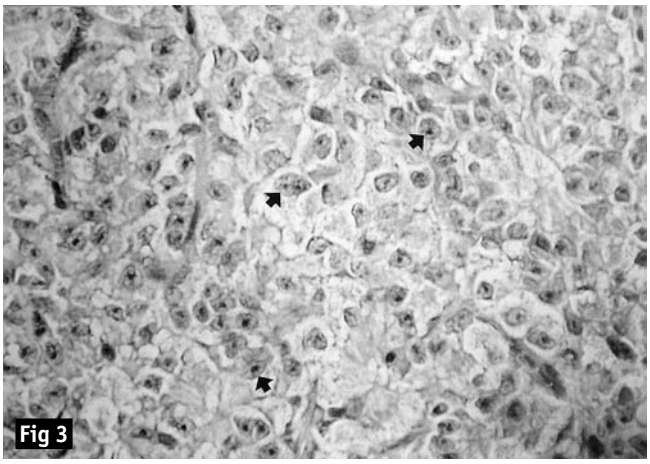
In this study at light and electron microscopic level, microscopic changes which show increase in pinealocyte cell activity were observed in rats exposed to constant darkness. When the pineal gland structure of the rats exposed to constant darkness was examined, it was noticed that extensive number of pinealocytes in the structure of the pineal gland. Some of the observed pinealocytes nuclei were determined to contain double necleoli. Furthermore, a considerable increase in lipid droplets was observed in the gland structure. Along with increases of mitochondria and lipid droplets in the cytoplasm of pinealocytes, enlargements were seen in rough endoplasmic reticulum sacs.

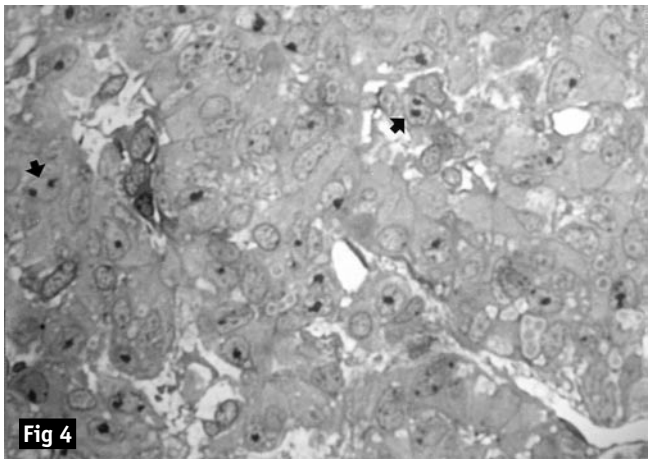
In the previous studies, it was reported that exposure of constant darkness or light restriction caused an activation increase in the pineal gland [12, 16–20]. In the electron microscopic study carried out by Swietoslawski and Karasek [17] on hamsters, they reported that mitochondria, lipid droplets and lisosomes were increased in cell cytoplasm, rough endoplasmic reticulum sacs were enlarged as ultrastructural changes which indicate the increase of pinealocyte activity at night. In the another study carried out by Karasek et al. [12] on rats, light restriction was reported to an increase in pinealocyte cell activation. It was observed on their electron microscopic examinations that an increase in number of mitochondria, ribosomes and cytoplasmic dense bodies in the pinealocyte cytoplasm of the rats exposed to darkness for 16 hrs of the day occured and rough endoplasmic reticulum sacs were enlarged. Krakowski and Cieciura [16] expressed the increase of mitochondria in the pinealocyte cytoplasm of rats exposed to darkness for 23 hrs of the day. Matsushima et al. [18] reported that pinealocyte cell nuclei of hamsters exposed to darkness for 16 hrs of the day were enlarged. In a research performed by Dombrowski and McNulty [19] on hamsters, pinealocytes were examined in electron microscope eight weeks after the optic inoculation made experimentally. It was seen on their ultrastructural examinations that an increase in the volume of pinealocyte cell nuclei and in cytoplasmic dense bodies, and rough endoplasmic reticulum sacs were enlarged. Upson and Benson [20] reported that hypertrophy in the pinealocytes of mice exposed to experimental optic inoculation. Results of our study are in compliance with the findings of the studies mentioned above [12, 16–20].

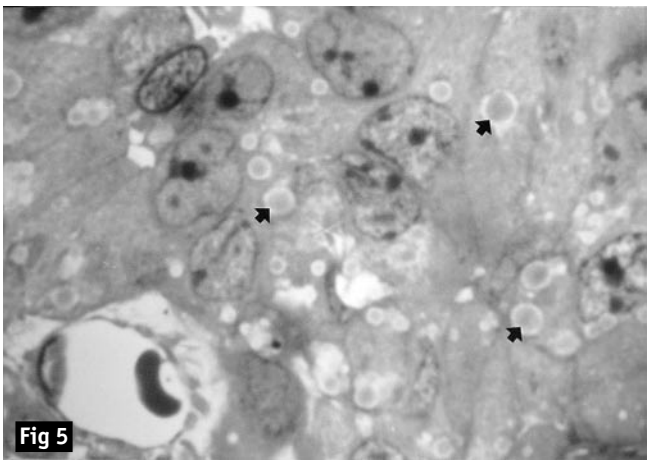
On the other hand, in the previous experimental studies, findings were reported regarding the pinealocyte cell activity decrease in animals exposed to light for a long period of time or exposure to constant light [8–15]. In our study, microscopic changes indicating the decrease in pinealocyte cell activation of the rats exposed to constant light were determined. When the pineal gland structure of these rats were examined, a decrease in number of pinealocytes in the gland structure and existence of more glial cells were observed at light microscope. Furthermore, the connective tissue between parenchymal cells was increased. In electron microscope, a decrease in mitochondria and lipid droplets in pinealocyte cytoplasm was determined. In a similar ultrastructural study carried out by Upson et al. [8], a decrease in lipid droplets of the gland structure and pinealocyte cell volume, and a decrease in amount of mitochondria in cell cytoplasm in the rats exposed to constant light for 70 days were determined. In the studies performed by Karasek et al. [12, 13] a decrease in melatonin synthesis in the pineal gland of animals exposed to constant light was reported. In their microscopic examinations, they expressed that a decrease in number of mitochondria, lysosomes, ribosomes and lipid droplets in the pinealocyte cytoplasm as a result of constant light exposure. Cieciura and Krakowski [14] reported that there was a decrease in number of mitochondria in pinealocyte cytoplasm of rats exposed to constant light. Heredia Chans et al. [15] reported that a decrease in lipid droplets in the pineal gland structure of rats exposed to constant light for 4 weeks. Lewczuk et al. [9] expressed that there was a decrease in mitochondria and lipid droplets in cell cytoplasm with findings regarding the pinealocyte cell activity decrease in lambs exposed to light for 16 hrs of the day

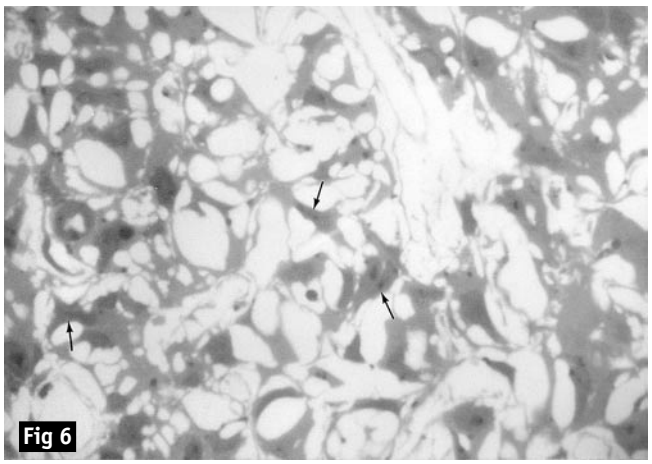












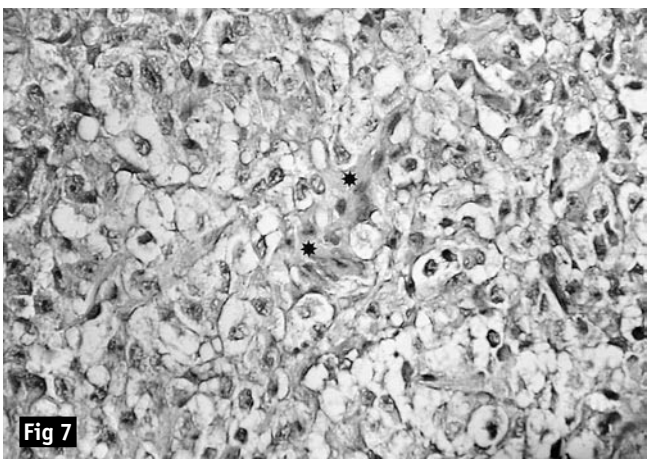
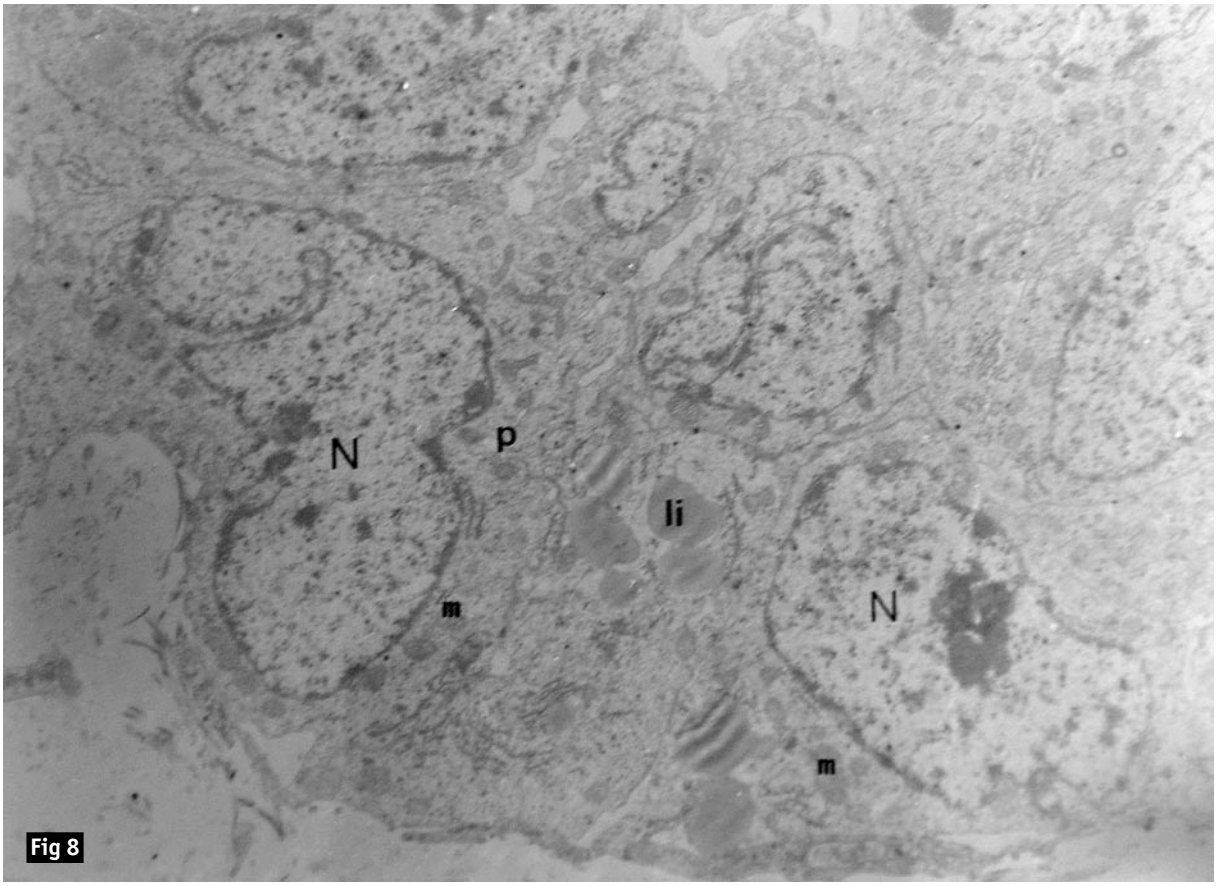


Fig. 1. Semi-thin section stained with toluidine blue of the pineal gland in control group. Large arrow: pinealocytes; small arrow: glial cells; c: capillaries. Magnification, X40.
Fig. 2. Pinealocytes (large arrow), glial cells (small arrow) and capillaries (c) are shown in the control group. H.E. Magnification, X40.
Fig. 3. An increase in pinealocyte cell intensity (arrows) was observed in rats exposed to constant darkness. H.E. Magnification, X40.
Fig. 4. Extensive number of pinealocytes was observed in the structure of pineal gland of rats exposed to constant darkness and some of the observed pinealocytes were determined to contain double nucleoli (arrows). Toluidine blue. Magnification, X40.
Fig. 5. Increased lipid droplets (arrows) in the structure of pineal gland of rats exposed to constant darkness. Toluidine blue. Magnification, X40.
Fig. 6. A decrease in pinealocyte cell intensity and an existence of more glial cells (arrows) were seen in rats exposed to constant light. Toluidine blue. Magnification, X40.
Fig. 7. Increased connective tissue (asterisks) between parenchymal cells in the pineal gland of rats exposed to constant light. H.E. Magnification, X40. *(Publisher's note: Figs. 1–7 85% of original size)*



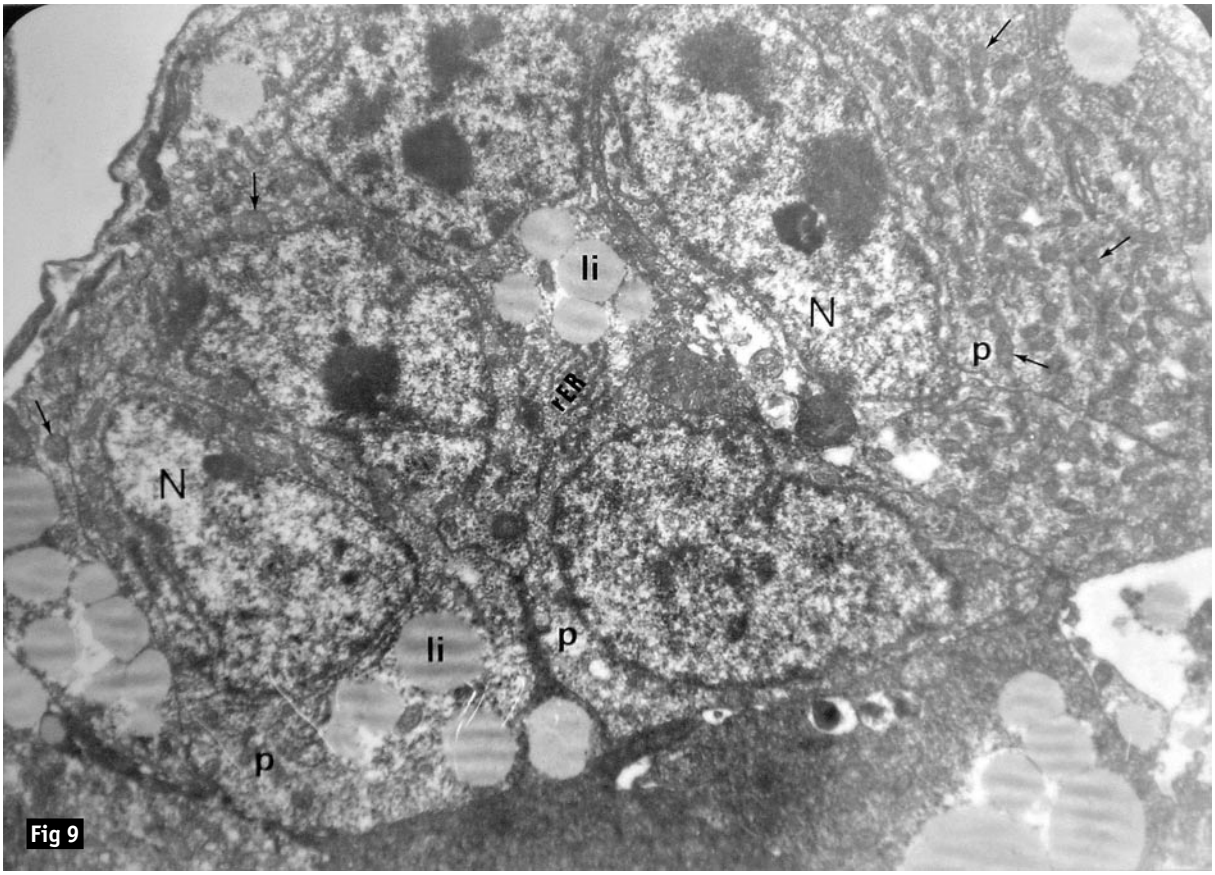
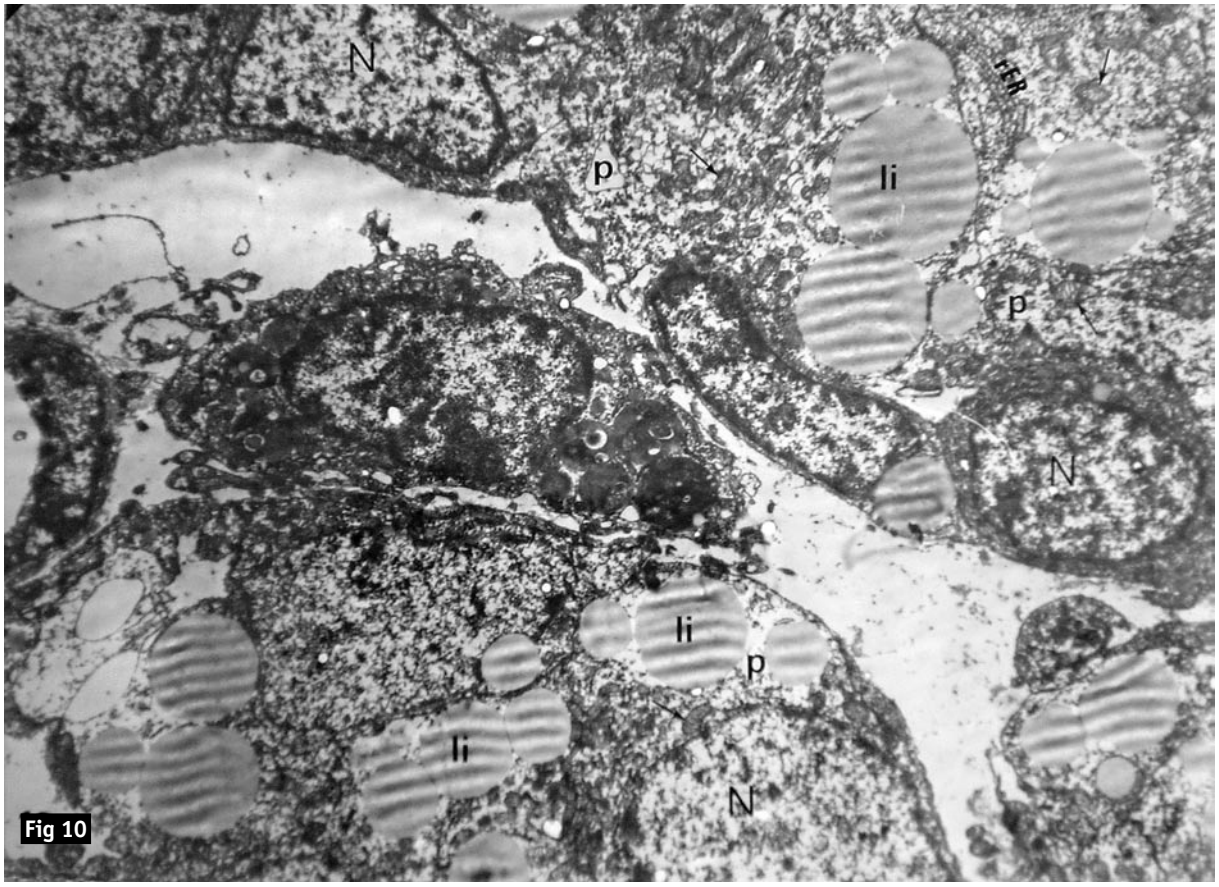


Fig. 8. Electron micrograph of the pineal gland in control rats. P: pinealocyte; N: nucleus; li: lipid droplets; m: mitochondrion. Magnification, X3000.

Fig. 9. Electron micrograph of the pineal gland in rats exposed to constant darkness. Increased number of mitochondria and enlarged rough endoplasmic reticulum sacs were observed in the cytoplasm of pinealocytes. P: pinealocyte; N: nucleus; arrows: mitochondrion; rER: rough endoplasmic reticulum; li: lipid droplets. Magnification, X3000.



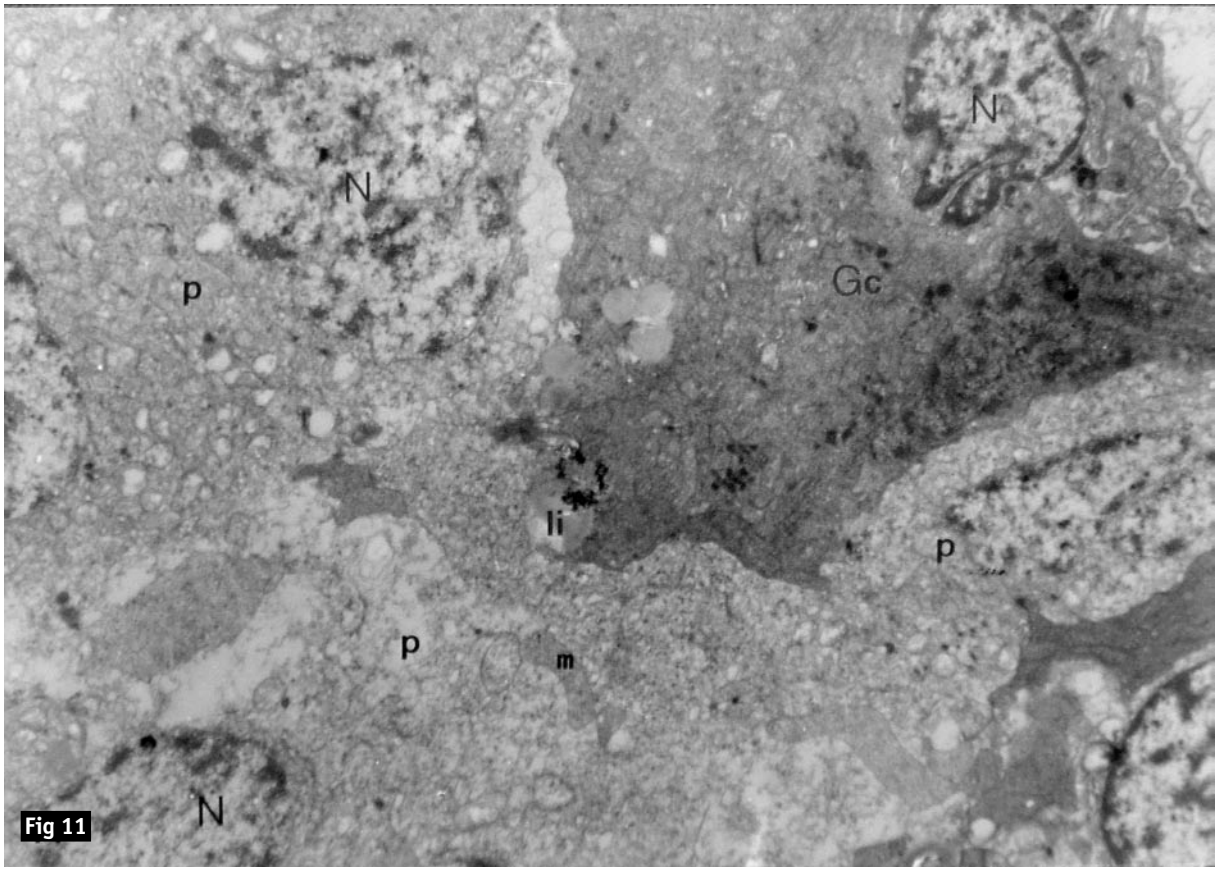


Fig. 10. Increased number of lipid droplets in the ultrastructure of pineal gland of rats exposed to constant darkness. P: pinealocyte; N: nucleus; li: lipid droplets; arrows: mitochondrion; rER: rough endoplasmic reticulum. Magnification, X3000.

Fig. 11. Electron micrograph of the pineal gland in rats exposed to constant light. A decrease in mitochondria and lipid droplets was observed in the cytoplasm of the pinealocytes. P: pinealocyte; Gc: glial cell; N: nucleus; m: mitochondrion; li: lipid droplets. Magnification, X3000.

during 30 days. Ralph [11] stated that constant light exposure caused atrophy in pinealocytes. In terms of microscopic changes which the constant light exposure created in the pineal gland, our study is in agreement with the previous studies [8, 9, 11–15].

As a result of our study at light and electron microscopic level, it was observed that the pinealocyte cell activity of rats exposed to constant darkness was increased whereas it was decreased in the group exposed to constant light.

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