Structural Changes of the Pineals of Blinded Rats Fed Long Term

It has generally been accepted that the pineal function is activated by keeping the animals in darkness, and contrarily decreased by keeping them in continuous light. However, uterine weight was not decreased by blinding alone[1], and the activity of N-acetyltransferase of the pineal was significantly decreased in about one-half of the blinded and the blinded-anosmic rats[2]. In addition, it was noticed on the long-term feeding of blinded hamsters that the gonads returned to the normal state[3,4]. OTA and OBARA[5] reported also no increase of urinary gonadotrophin-inhibiting substance in the blinded rat. In man, menarch occurs at an early age in blind girls as compared with those with normal eyesight[6].

In the present experiment of blindness, the regressive or hypofunctional changes of the pineals were observed, contrary to the results of experiments in darkness published up-to-date.

Material and methods. 9 male rats of the Wistar strain, aged 8 weeks, were used. 5 of them were bilaterally enucleated. 30 weeks after enucleation, the pineals were removed. 4 other normal, intact rats were used as controls. They were fed in the usual day-night condition, i.e. overhead fluorescent lamps were kept on from 05.30 h to 19.30 h daily.

The pineal was removed under anesthesia with ether. The removed pineals were immersed in the glutaraldehyde solution, and followed by postfixation in the osmium solution. After dehydration, they were embedded in Epon. Ultra-thin sections were doubly stained with uranylacetate and lead nitrate. 1 μm thick sections were also obtained for light microscopy from the Epon blocks. They were stained with methylene blue.

Results and discussion. The pinealocytes are light- and electron microscopically divided into 2 types, clear and dark cells[7–10]. The clear cells are considered the activated or functional cells[7–10], while the dark ones are the resting or reserve cells[9,10]. In the controls, the clear cells were more numerous than the dark cells.

In the blinded rats, the pinealocytes of both types were generally shrunken. The dark cells were increased both in number and cytoplasmic stainability. The clear cells showed increased transparency of the cytoplasm. The cytoplasm was occasionally water-clear in the clear cells. The nucleoli were not found in more than one-half of the clear cells.

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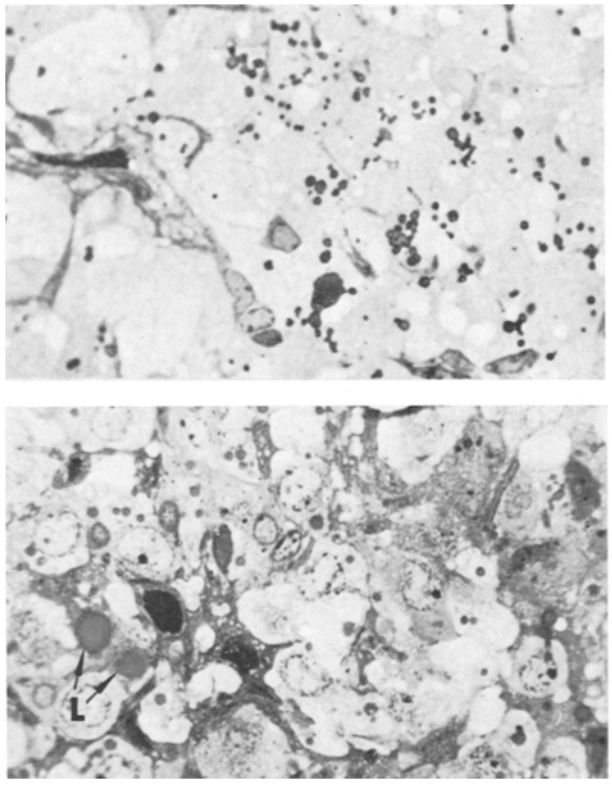

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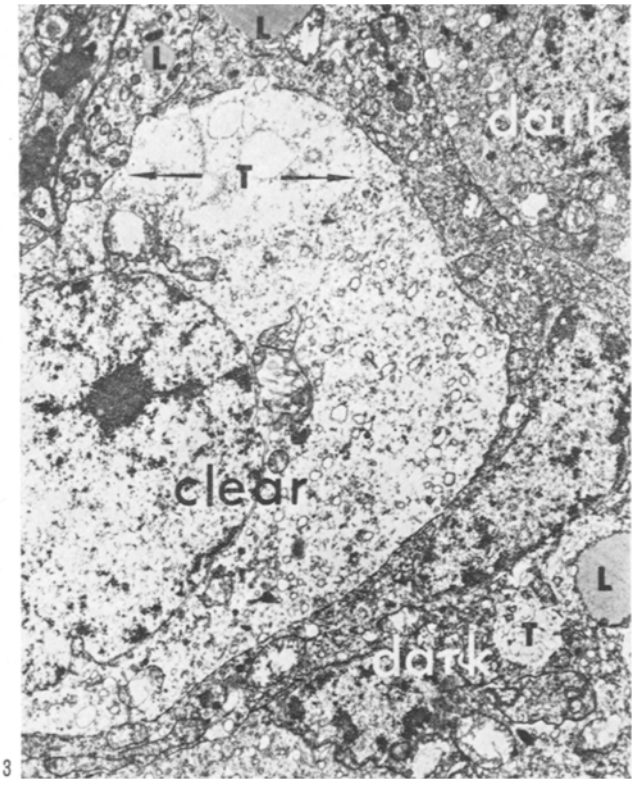

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Fig. 1 and 2. Light micrographs of the pineals. 1. Control with almost clear cells and a number of strongly stained lipid granules. 2. Blinded rat with increased number of dark cells and decreased number of small lipid granules, and with decreased stainability. 2 Large lipid granules are indicated with arrows and other small granules are also seen. The pinealocytes of both types are a little shrunk in a blinded rat as compared with the control. × 520.

Fig. 3. Electron micrograph of the pineal of a blinded rat. Clear and dark cells are found. Organelles and ribosomes are sparse, especially in the clear cell. A transparent area (T) in the clear cell and in the dark one is present with remnants of organelles. Destruction of the remaining mitochondria is obvious in the pinealocytes of both types. Lipid granules (L) are also faintly stained in the electron micrograph. × 5,600.

The number of the lipid granules was significantly decreased. It was mainly caused by diminution of small lipid granules. The remaining lipid granules stained faintly greenish color with methylene blue.

Electron-microscopically, the cytoplasmic organelles of the clear cells were more sparse in the blinded rats than in the controls. The small transparent areas containing a very small number of the cytoplasmic organelle remnants were found in the pinealocytes of both types, especially often in the clear cells.

The mitochondria were decreased in number in both types of cells. The decrease was more apparent in the clear cells than in the dark ones. The mitochondria, especially in the clear cells, were swollen and showed destroyed inner structure. The vesicles were also decreased in number, but the vacuoles, which might be the enlarged vesicles, appeared in small number. The small particles showing high electron density diminished remarkably. Decrease of ribosomes was also very noticeable in the clear cells. In the pericapillary space, the nerve endings were decreased in number and became atrophic.

The histological and cytological changes of the pineals obtained from the blinded rats which have been described here, are of regression or degeneration. The clear cells, which are usually regarded as the activated or functional cells, may not be in the functional state in the pineal of the blinded rats, but in exhaustion or in degeneration.

Some authors[7,11,12] postulated that the variation of the lipid granules might be related to secretory activity of the pinealocytes. PERRELET et al.[12] have noted that the electron density of the lipid granules is lessened in the pinealocytes if the rat is treated with p-chlorophenylalanine which inhibits the tryptophan hydroxylase and serotonin synthesis.

KAPPERS[13] has concluded from the presence of nerve fibre degeneration in the pineal after extirpation of the superior cervical ganglion that the pineal receives innervation from this ganglion. BOSTELMANN[8] also noticed regression of the pineals of the rat 1 month after bilateral superior cervical gangliectomy. Their nerve endings also disappeared 3 months after gangliectomy. The changes of the nerve endings after blinding in the present experiment were a little similar to those by gangliectomy[14].

Zusammenfassung. Elektronenoptische Untersuchungen der Pinealzellen von Ratten, die 30 Wochen nach beidseitiger, okularer Enukleation gehalten wurden, zeigten eine Vermehrung der dunklen Pinealzellen. Überdies konnten nur wenig intrazelluläre Organellen und Lipidgranula in hellen und dunklen Zellen sowie schwache Elektronendensität der Granula beobachtet werden.

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Effects of Blinding and Pinealectomy on Diurnal Variations in Plasma Testosterone

Environmental lighting and the pineal gland are undoubtedly associated in the regulation of male reproductive functions[1-3] although the physiological nature of such relations are not clearly defined. Endocrine function of the rat testis and circulating levels of testosterone are subject to both seasonal[4] and diurnal[5] variation. The present series of experiments were therefore carried out to determine whether or not the eyes and the pineal gland are responsible for, or involved in, diurnal fluctuations of testosterone in mature rats.

Rats were Sprague-Dawley derived and were housed individually under controlled ambient conditions. The daily light cycle was 12 h light: 12 h dark (lights on 06.00–18.00 h) and room temperature was 21 ± 1 °C with relative humidity 45–65%. Samples of peripheral blood were taken from the tail vein of the animals subdued by ether anaesthesia, at 06.00 and 18.00 h (± 20 min). An equal volume (0.5–0.8 ml) of warm isotonic saline was injected i.p. following the collection of each blood sample in an effort to restore circulating fluid volumes. Heparinized blood samples were immediately centrifuged at 900 × *g* for 20 min and 0.05 ml aliquots of the plasma was used for the estimation of testosterone by radio-immuno assay[6].

Rats were received at 8 weeks of age and blood samples were first taken from intact animals 2 weeks later. At 12 weeks of age, surgery was undertaken and 3 groups of animals were prepared, i.e. sham-pinealectomized control, blinded and pinealectomized groups. Blinding was achieved by orbital enucleation and pinealectomy was performed by the technique of BRUINVELS et al.[7]. Blood samples taken at 06.00 and 18.00 h were collected from these rats at 4, 8 and 12 week intervals following the surgery.

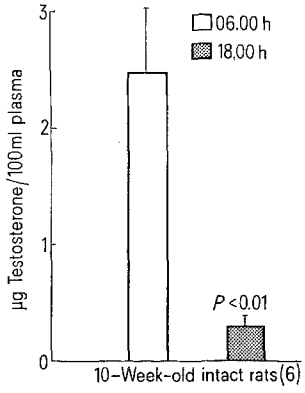

Fig. 1. Diurnal variation in plasma testosterone of intact rats (values are the mean showing the standard error).

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