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.

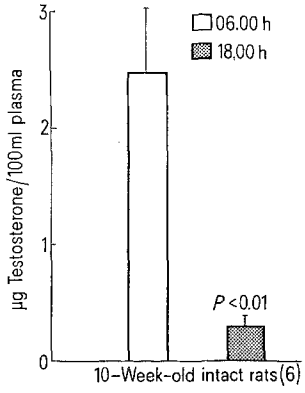

Fig. 1. Diurnal variation in plasma testosterone of intact rats (values are the mean showing the standard error).

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Group	No. of rats	Body weight (g)	Testis weight (mg/100 g body weight)	Accessory sex gland weight (mg/100 g body weight)	Pituitary weight (mg/100 g body weight)	Testicular vein testosterone (μg/100 ml plasma)
Control (sham-operated)	7	546 ± 26	325 ± 13	575 ± 41	2.53 ± 0.09	11.91 ± 1.95
Blinded (significance)	6 P	502 ± 13 n.s.	356 ± 16 n.s.	479 ± 28 0.05 — 0.1	2.41 ± 0.07 n.s.	5.48 ± 1.05 <0.02
Pinealectomized (significance)	6 P	527 ± 14 n.s.	334 ± 12 n.s.	687 ± 28 <0.05	2.42 ± 0.06 n.s.	10.82 ± 2.56 n.s.

Values are the mean ± S.E.; n.s. indicates not significant by *t*-test. Accessory sex gland weight is that of the seminal vesicles and prostate together.

Testicular vein blood was collected as previously described[8] for testosterone determination on the day after the 12-week diurnal sampling and the contralateral testis was removed for the histological evaluation[9] of spermatogenesis.

The diurnal variation of plasma testosterone in normal rats is expressed in Figure 1 which illustrates the significantly higher levels of the hormone at 06.00 h. Sham-operated control animals exhibited similar changes at all 3 periods of investigation although absolute differences between mean testosterone levels at 06.00 h and 18.00 h were not as great (Figure 2) as in the case of the intact rats. 4 weeks after surgery, the diurnal variation in plasma testosterone was somewhat reduced in both blinded and pinealectomized rats and differences were no longer statistically significant. At 8 weeks, the difference between hormone levels at 06.00 h and 18.00 h were also reduced and not significant in the blinded and pinealectomized groups, and this appeared to be largely the result of elevation in testosterone concentration at 18.00 h. Blinding almost completely abolished diurnal variation in testosterone by 12 weeks whereas with pinealectomized rats the plasma levels of testosterone at 06.00 h were elevated and significant diurnal variation has been re-established.

Neither blinding nor pinealectomy caused significant alteration in testis weight when examined 12 weeks later (see Table) and histochemical specimens were identical to those from control animals indicating lack of effect on spermatogenesis. Testicular vein testosterone in blinded rats at 12 weeks was markedly lower than control values and the mean weight of the accessory sex glands was marginally reduced. Further indication that the diurnal responses to pinealectomy might be of a transient nature is supported by the lack of significant change in testicular testosterone. Previous studies[8] have shown that testicular vein testosterone was elevated 4 weeks after pinealectomy in immature rats. In the present investigation, a hypertrophic response of the accessory sex organs at 12 weeks suggests that stimulation of endocrine function of the testis had resulted from pinealectomy. This is further substantiated by the higher than control levels of peripheral testosterone both at 06.00 h ($P < 0.05$) and 18.00 h ($P < 0.02$) in pinealectomized rats at 8 weeks.

The data suggests that photic information, as perceived by the eyes, and also the pineal gland are involved in regulatory mechanisms which cause diurnal variation in circulating testosterone in the laboratory rat. The eyes would seem to be more important to this rhythm than is the pineal gland which may mean that the latter is only partly responsible for mediating environmental lighting influences on testicular endocrine function. These experiments, however, provide no information concerning possible phase shifts in the testosterone rhythm and this merits further investigation.

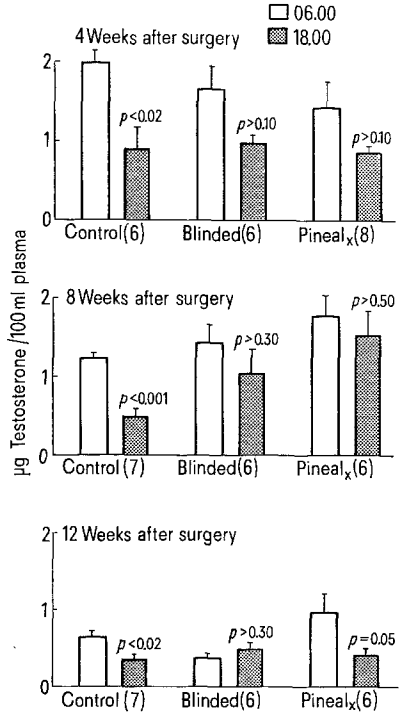

Fig. 2. Effects of blinding and pinealectomy on diurnal variation of testosterone (values are the mean showing the standard error).

Résumé. L'enlèvement des yeux et de la glande pinéale dans les rats adultes a perturbé les variations cliniques des testostérones de la circulation. L'aveuglement fit diminuer les différences diurnales des niveaux de testostérones qui furent abolis en 12 semaines. Toutefois, les effets de la pinéalectomie furent transitoires puisqu'une fluctuation diurnale significative fut parfois rétablie dans les testostérones. Ceci suggère que la glande pinéale n'est pas totalement responsable des effects d'information photique.

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