Effect of Biogenic Amines on the Endocrine Axis and Esterase Activity of Hamster Testes[1] (35004)

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Bilateral orbital enucleation (blinding) or exposure of adult hamsters to short photoperiods causes a significant loss of testis weight within 4–6 weeks (1). This loss is prevented by simultaneous pinealectomy; it is transient even without pinealectomy (2). Because the weight is altered, it is pertinent to inquire whether or not blinding interferes with functioning of the hypothalamo–pituitary–gonad axis, and thus, affects the weights of other reproductive organs or endocrine organs.

A pineal complex–gonadal relationship is known for the rat (3). This relationship is interesting because of the discovery of melatonin and serotonin in the bovine pineal complex (4, 5), as well as the different effects produced by exogenous preparations of these biogenic amines. The administration of serotonin decreases adrenal gland weights in the rat (6); similar treatment with melatonin decreases prostate and seminal vesicle weights (7). Melatonin apparently suppressess the secretion of pituitary LH, and not FSH, and thus, does not retard testis weight.

Neither serotonin nor melatonin has been isolated from the pineal complex of the hamster, but the presence of a gonadal inhibitory factor is suggested (8). Evidence for an antigonadal factor is shown by the decreased testis weights of normal hamsters united parabiotically to blinded hamsters (2). The discovery of a doubling in activity of the melatonin-forming enzyme of blinded hamsters indicates that this antigonadal factor may be melatonin (9). There is a need thus to evaluate the effects of exogenous preparations of these biogenic amines on both reproductive and endocrine organs of the hamster.

Information is unavailable whether enzyme levels change in the hamster testis after pinealectomy or blinding. The assay of enzyme activity provides a means of monitoring the observed morphological changes, and it permits some insight into mechanisms involved in these changes. Study of enzymes and their causal involvement in physiological processes, such as germinal differentiation, are of significance in reproductive biology (10). Because of the role of esterases in protein metabolism (11), we decided to quantitate this activity in the testis. This paper reports the results of administering exogenous preparations of melatonin and serotonin to normal, blinded, pinealectomized, and blinded and pinealectomized hamsters; it describes the effects of these biogenic amines on esterase levels of the testis, weights of the testis, seminal vesicle, pituitary and adrenal glands; and correlates some of these changes with alterations in histology and ultrastructure of the testis.

Materials and Methods. Male golden hamsters, *Mesocricetus auratus*, obtained from Lakeview Hamster Co., were kept in small colonies and maintained under an artificial light schedule of 12-hr light: 12-hr darkness. Purina brand pellets and water were given *ad libitum* throughout the course of the investigation. When 4 months of age, the animals were randomly divided into the following four groups: blinded; pinealectomized; blinded and pinealectomized; and controls (no surgical procedure). Animals from each of the above groups were subjected to three

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TABLE I. Comparison of Effect of Blinding, Pinealectomy, and Blinding and Pinealectomy, on Organ Weights and Esterase Activity of Hamster Testis.

Treatment	Weight	Organ weight (mg/100 g body weight)				Esterase activity (U/mg protein × 100)[a]
		Testis	Seminal vesicle	Adrenal	Pituitary	
Blinding (10)[b]	134	505 ± 71[c,d]	359 ± 79[d]	8.88 ± 0.58	3.03 ± 0.14	6.92 ± 1.94[d]
Pinealectomy (6)	116	1300 ± 68	575 ± 53	10.00 ± 0.70	3.32 ± 0.12	4.23 ± 0.67
Blinding + pinealectomy (6)	120	1290 ± 40	577 ± 71	9.55 ± 0.46	3.00 ± 0.14	4.24 ± 1.51
Control (7)	110	1388 ± 25	582 ± 70	9.00 ± 0.54	3.53 ± 0.22	3.52 ± 0.64

[a] One unit of esterase activity is defined as micromoles of β-naphthol liberated/min at 25°.
[b] Number of animals per group is given in parentheses.
[c] Mean ± the standard error of the mean.
[d] Fisher *t* significant to greater than .001.

types of experiments: (1) hamsters receiving no treatment (Table I); (2) hamsters receiving daily ip injections of serotonin (100 μg/0.5 ml saline) for 4 weeks (Table II); and (3) hamsters receiving daily ip injections of melatonin (100 μg/0.5 ml saline) for 4 weeks (Table III).

The hamsters were killed by ether anesthesia, and the body weights were determined. Testes, seminal vesicles, adrenal and pituitary glands were removed and their weights were determined on an analytical balance. The testes were frozen and stored at —20° until assayed. From each animal, 20–40 mg of one testis were homogenized with a glass tissue grinder in 2 ml of 0.2 *M* sodium phosphate buffer, pH 7.4. One-half of the resultant homogenate was diluted to 10 ml with the same buffer, and then both halves were centrifuged at 3500 rpm for 10 min. Duplicate 1-ml samples of the diluted supernatant fluid were used for assay of esterase activity; the remaining sample of undiluted fluid was used for quantitation of total protein.

Assay of total esterase activity was done by modifying a technique described elsewhere (12). Assays were done by mixing 5 ml of buffered β-naphthol acetate (Sigma Chemical Co.) to 1 ml of each supernatant fluid. Each sample was incubated for 30 min at 37° and then cooled under tapwater for 10 min. Controls for each sample contained 5 ml

TABLE II. Effect of Serotonin on Organ Weights and Esterase Activity of the Hamster Testis.

Treatment	Weight	Organ weight (mg/100 g body weight)				Esterase activity (U/mg protein × 100)[a]
		Testis	Seminal vesicle	Adrenal	Pituitary	
Blinding + serotonin (7)[b]	107	702 ± 211[c,d]	374 ± 31[d]	13.1 ± 0.18	3.55 ± 0.36	9.01 ± 0.57[d]
Pinealectomy + serotonin (6)	112	1445 ± 54	643 ±109	14.0 ± 0.65	3.63 ± 0.52	5.90 ± 0.76
Blinding + pinealectomy + serotonin (6)	100	1329 ± 160	451 ± 32	14.7 ± 1.32	3.82 ± 0.38	3.59 ± 0.78
Control + serotonin (7)	108	1290 ± 118	514 ± 46	13.7 ± 0.27	3.71 ± 0.24	4.91 ± 0.13

[a] See footnote *a*, Table I.
[b] See footnote *b*, Table I.
[c] See footnote *c*, Table I.
[d] See footnote *d*, Table I.

Table III. Effect of Melatonin on Organ Weights and Esterase Activity of the Hamster Testis.

Treatment	Weight	Organ weight (mg/100 g body weight)				Esterase activity (U/mg protein × 100)[a]
		Testis	Seminal vesicle	Adrenal	Pituitary	
Blinding + melatonin (6)[b]	109	751 ± 53[c,d]	352 ± 42[d]	13.1 ± 2.09	3.49 ± 0.34	4.94 ± 0.77
Pinealectomy + melatonin (7)	104	1690 ± 29	643 ± 97	10.2 ± 0.91	3.06 ± 0.21	4.92 ± 2.02
Blinding + pinealectomy + melatonin (6)	124	1325 ± 49	526 ± 14	9.7 ± 1.22	3.44 ± 0.33	4.97 ± 0.96
Control + melatonin (6)	108	1408 ± 60	526 ± 46	9.8 ± 0.74	3.25 ± 0.52	5.60 ± 1.37

[a] See footnote *a*, Table I.
[b] See footnote *b*, Table I.
[c] See footnote *c*, Table I.
[d] See footnote *d*, Table I.

of buffered substrate solution, but the 1 ml of enzyme solution was not added until after cooling. One milliliter of fast red TRN was added to each tube to evoke the reaction, and then the samples were read 20 min later against their respective controls. Values were read at 540 mμ using a Bausch and Lomb Spectronic 20 colorimeter kept at 25°. Optical density values were compared with optical density values on a standard curve prepared with β-naphthol. Protein concentrations were determined in duplicate from each undiluted sample by the biuret method (13), using bovine serum albumin as a standard. Values were read at 550 mμ with the same colorimeter.

Tissue for light microscopy was fixed in Bouin's solution; it was dehydrated in a graded series of alcohol, embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin. Tissue for electron microscopy was fixed in 2% buffered osmium tetroxide. After dehydration in a graded series of alcohol, the tissues were embedded in D.E.R. 332. Ultrathin sections were cut on a Porter-Blum MT-II microtome, stained with lead citrate, and examined with an RCA, EMU 3 G electron microscope.

Results. Compared with the multitude of differentiating cells in the seminiferous tubules of control (Fig. 1) and pinealectomized hamsters, primarily spermatogonia occur in the seminiferous tubules of blinded hamsters (Fig. 2). Electron micrographs show that the spermatogonia of control hamsters have the typical array of lysosomal bodies and few osmiophilic droplets (Fig. 3), whereas micrographs of spermatogonia of blinded hamsters exhibit numerous lysosomal bodies and prominent osmiophilic droplets containing lipid (Fig. 4).

The mean testis and mean seminal vesicle weights of blinded hamsters are significantly smaller ($p<.001$) than the mean testis and mean seminal vesicle weights of pinealectomized, blinded and pinealectomized, and control hamsters (Table I). The mean adrenal gland and mean pituitary gland weights fail to differ significantly ($p>.05$) in any of the groups.

Treatment of animals with exogenous preparations of serotonin (Table II) or melatonin (Table III) does not produce an additive effect of reducing reproductive organ weights, or significantly affect endocrine organ weights.

Of the three parameters investigated, that is, blinding, pinealectomy, and blinding and pinealectomy, only blinding increases the mean total esterase activity of the testis (Table I). Elevated levels of esterase activity occur after treatment with serotonin; this in-

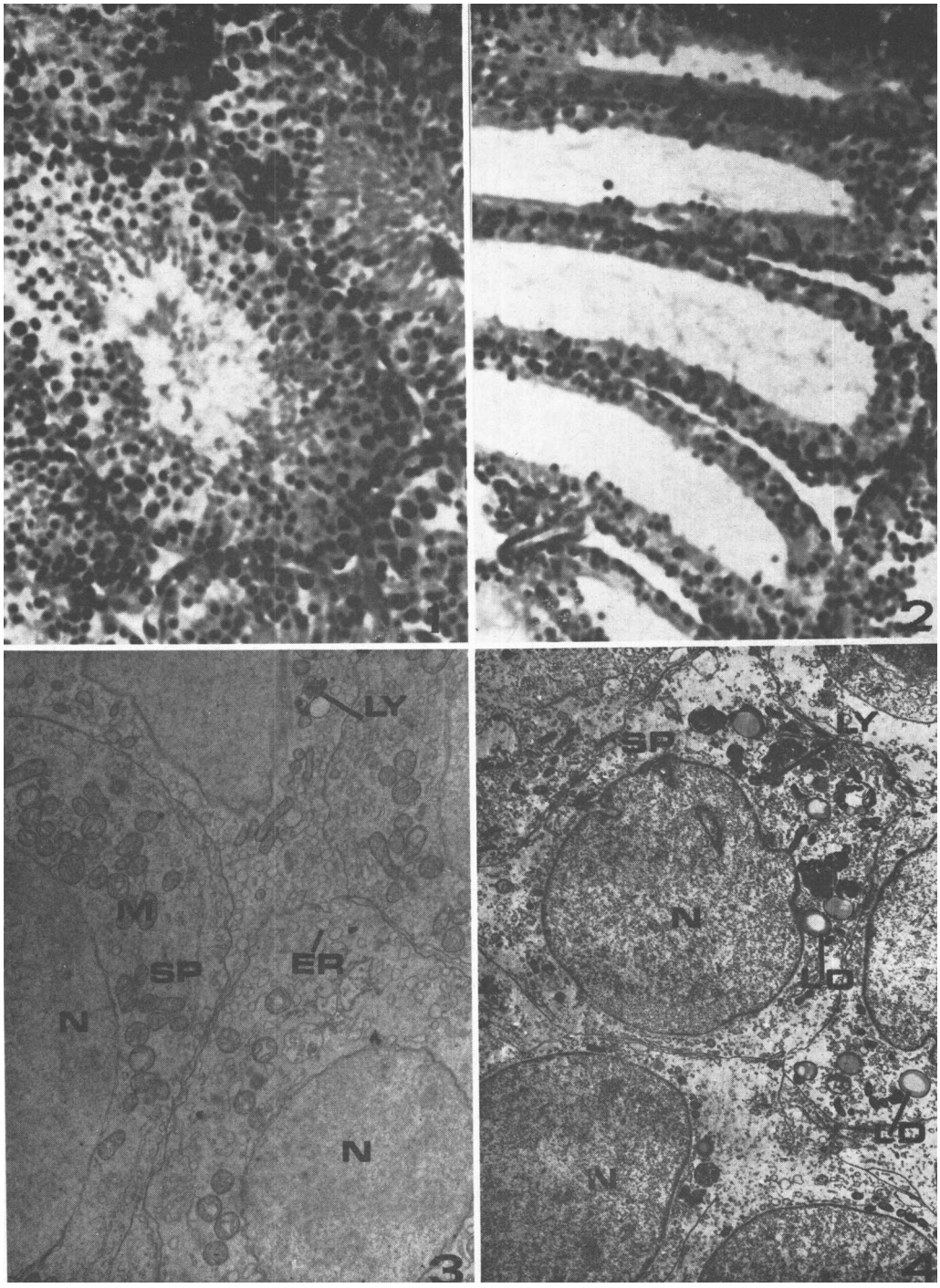

FIG. 1. Control; the testis shows a normal degree of spermatogenic activity. ×200.

FIG. 2. Blinded; the seminiferous tubules are involuted and the germinal epithelium consists primarily of spermatogonia and Sertoli cells. Only rare spermatids are observed. ×200.

FIG. 3. Control; spermatogonium (SP) exhibits a nucleus (N), mitochondrion (M), smooth endoplasmic reticulum (ER), and an occasional lysosomal body (LY). ×6000.

FIG. 4. Blinded; note the accumulation of lipid droplets (LD) and lysosomal bodies (LY) in the cytoplasm of the spermatogonium (SP); nucleus (N). ×4000.

crease is significantly higher for blinded animals (Table II). Injection of melatonin reduces this increase in total esterase activity of blinded hamsters, in spite of the smaller mean testis weights of individuals in this group (Table III).

Discussion. Our study shows that the administration of melatonin does not significantly decrease testis and seminal vesicle weights of blinded hamsters. That treatment with melatonin fails to produce an additive effect is consistent with the lack of an effect of exogenous preparation of melatonin on rat testis weight (7). But our results as well as those of Motta *et al.* (7) disagree with the results of other researchers (14) who obtained an additive effect with a smaller quantity of melatonin than used either by Motta *et al.* (7) or by us. The variable results suggest that melatonin may not be the primary antigonadal substance secreted by either the hamster or the rat pineal complex. The discovery that bovine pineal complex extract (15), and not melatonin, inhibits the stimulatory effect of human gonadotropin on the uterine weights of mice supports our view.

The experiments show that hamsters receiving injections of melatonin have smaller adrenal gland weights than the hamsters receiving treatment with serotonin. These results are in contrast with the findings of Wurtman *et al.* (6) who reported that serotonin, but not melatonin, inhibits adrenal gland weights in the rat. The quantities of biogenic amines used in the study (6) of rats are similar to those used by us. Hamsters and rats also respond differently to these biogenic amines in other respects. Our experiments and those of other researchers (16) show that hamster adrenal gland weights do not appreciably regress after blinding, whereas in the rat a significant retardation occurs after blinding (17). The present study reveals that treatment of hamsters with moderate levels of biogenic amines does not alter pituitary gland weights. The administration of a quantity of melatonin similar to that used by us reduces anterior pituitary gland weights of rats (18).

It is difficult to accept that melatonin operates by suppressing only LH secretion in the hamster. Unlike the studies of the rat (7), both testis and seminal vesicle weights decrease in blinded hamsters, and the effect is not more pronounced after treatment with melatonin. Perhaps the quantity of melatonin given in our study is too low to overcome the action of LH or FSH. In rats kept under continuous light, administration of a moderate quantity of melatonin reduces seminal vesicle weights, while a substantially higher dose is required to reduce testis weights (18).

The histologic studies reveal that blinding temporarily obliterates germinal differentiation in the hamster. The existence of coalesced lipid droplets in spermatogonia of blinded hamsters is similar to the results of prior studies (1) demonstrating the presence of aggregated lipid droplets in spermatogonia and high levels of testicular cholesterol of hamsters kept on short photoperiods and in the cold. The mechanism responsible for this lipid aggregation is not apparent, but aggregation may result from a secondary rather than from a primary effect on the testis. Carvalho (19) reported that rats fed high levels of cholesterol develop severe and irreversible testicular lesions, and that the testes undergo fatty degeneration. Lipid infiltration of blinded hamster spermatogonia could involve a similar pathway, and the mechanism changes soon enough to prevent total fatty degeneration.

Clearly, blinding raises the esterase level of the testis, and the administration of melatonin lessens part of the effect of blinding. This increase in activity of blinded hamsters is associated with a predominance of lysosomal bodies in spermatogonia. During the breakdown of connective tissue there is high peptidase activity (20). These observations suggest that some of the hamster testicular activity is proteolytic in nature. We are currently investigating these possibilities.

Summary. Pinealectomy reverses the loss of testis and seminal vesicle weights of blinded hamsters. Treatment with moderate doses of serotonin and melatonin does not cause further decrease in reproductive organ

weights, or appreciably affect adrenal and pituitary gland weights of blinded hamsters. The seminiferous tubules of blinded hamsters exhibit primarily spermatogonia containing lipid aggregations and numerous lysosomal bodies. Blinded hamsters have significantly higher levels of testicular esterase activity than do control, pinealectomized, and blinded and pinealectomized hamsters. This suggests that the higher esterase activity results from greater lysosomal activity.

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