EFFECTS OF MELATONIN AND THYROID HORMONES ON REPRODUCTIVE STATUS IN THE FEMALE HAMSTER

BY

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Abstract

The objective of this study was to ascertain a possible relationship between melatonin and thyroid status in regulating the hormones of reproduction in the female hamster. The effects of daily melatonin injections on the reproductive system were studied in control female hamsters, in female hamsters made hypothyroid with thiourea in the drinking water, and in hypothyroid female hamsters receiving thyroxin replacement. Daily melatonin injections disrupted estrous cyclicity, reduced the number of developing and mature follicles and corpora lutea, and resulted in atrophy of the uterus and vagina. Melatonin injections also resulted in elevated serum LH and FSH levels; these changes coincided with depressed serum and pituitary PRL and depressed circulating levels of estradiol, T4 and T3. Papanicolaou stained vaginal smears showed that melatonin injections prevented the proliferation of the vaginal epithelium; the estrous cycles of all animals receiving melatonin injections were halted at the diestrous 2 stage.

Ovaries of hypothyroid hamsters displayed a reduced number of developing and mature follicles and corpora lutea; pronounced follicular atresia was also noted. Thiourea administration lengthened the diestrous 2 stage of the cycle, but, unlike melatonin, did not block estrous cyclicity.

Thyroxin replacement reversed some of the effects of hypothyroidism on the ovary; it prevented the decrease in number of developing and mature follicles and corpora lutea, and reduced the extent of follicular atresia. Thyroxin replacement did not prevent the effects of melatonin

on the ovary and on estrous cycles, nor on the circulating levels of serum estradiol, PRL and LH; neither was the melatonin induced inhibition of pituitary PRL prevented by T4 replacement. Among the three groups of animals treated with melatonin, pituitary LH and FSH were significantly elevated in animals receiving thiourea compared to animals receiving melatonin alone or compared to animals receiving melatonin and thiourea plus T4 replacement.

The effects of melatonin on uterine and pituitary weight, and on pituitary and serum prolactin were prevented by estradiol at doses which inhibited pituitary gonadotropins.

These results suggest that:

1. melatonin disrupts the feedback relationship between estradiol and gonadotropins.
2. thyroxin is necessary for luteinization; this effect of thyroxin can be blocked by melatonin.
3. gonadotropin secretion is influenced by a CNS interaction of melatonin and thyroxin.

dedicated to
my mother Antonina and
my brother Henryk
whose constant support, encouragement
and confidence have shown me
nothing is impossible
if we work for it

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History of pineal

Early Greek and Roman writers referred to the pineal as the cervical body (bonos "cone"; soma "body") and the pineal body (pineale "pine cone"; corpus "body") respectively. The pineal has also been termed epiphysis cerebri, derived from the Greek words epi,"upon"; and phyesthai, "to grow" (1).

Modern research on the pineal gland evolved from the isolation and identification of melatonin (N-acetyl-5-methoxytryptamine) from bovine pineal glands by Lerner and colleagues in 1958/1959 (2,3). The substance isolated by Lerner was termed melatonin because of its skin blanching effects on tadpole skin. The discovery by Quay (4) and Wurtman (5) that environmental lighting influenced the synthesis of melatonin resulted in Wurtman and Axelrod (6) to postulate that the mammalian hormone melatonin had antigonadal action and the synthesis of this hormone was influenced by environmental lighting. Once this hypothesis was established, the bulk of initial studies concentrated on the influence of the pineal gland in reproduction (7).

Anatomy-anatomical relationships

The mammalian pineal gland exhibits variability in form, size and location (1). Vollrath has classified the mammalian pineal gland according to form, size and location; his system also covers the relationship of the pineal to the third ventricle and diencephalon (8).

A relationship exists between geographic location and size of the pineal (9). Animals resident at high latitudes including the walrus, seal and sea lion have large pineals (10,11) whereas animals residing at low latitudes, example; anteater, armadillo and sloth have smaller pineals (9,12). The rhinoceros reportedly has no pineal gland (9,12).

In primates, the pineal is located underneath the splenium of the corpus callosum. Rodents however, like the Syrian hamster, have a superficial pineal, embedded in the dura of the confluens of sinuses. The pineal stalk attached ventrally to the posterior commissure and dorsally to the habenular commissure, connects the pineal to the brain. The pineal recess of the third ventricle is the cavity or space between the ventral and dorsal roots of the pineal stalk.

Arterial supply and venous drainage

The arterial supply to the pineal is supplied by the branches of the posterior choroidal arteries, derived from the posterior cerebral arteries (13,14). Compared to other organs and endocrine glands, the pineal gland has a blood flow (4 ml/min/gram of tissue) which is surpassed only by the kidney (15,16). The blood supply to the pineal is greater during the night than during the day; perhaps associated with the nocturnal rise in indole metabolism (1,17,18). Blood supply within the pineal is somewhat unequal; the cortical tissue receives 1.2 - 2.0 times greater flow than the medullary tissue (17,19).

Venous drainage of the pineal is by venules joining the internal cerebral veins and great cerebral vein of Galen which drains into the superior saggital sinus (15).

Innervation

The pathway of light information reaching the pineal has been partially described. In the retina, light information is converted into neural information and travels through the optic chiasm via the optic nerve. After decussation, fibers involved in the photoregulation of melatonin leave the neural pathway involved in vision. Via the retinohypothalamic pathway, impulses are transmitted to the suprachiasmatic nuclei (SCN) (20-22). The SCN projects caudally into the hypothalamus, via one or more neurons reaches the lateral hypothalamus and synapses with central sympathetic fibers running through the medial forebrain bundle (20,21). These fibers project to the intermediolateral cell column of the thoracic cord, the source of preganglionic fibers to the superior cervical ganglia. The preganglionic neurons travel up the sympathetic trunk, synapsing with postganglionic fibers in the superior cervical ganglia. Via the tentorium cerebelli, the postganglionic fibers reach the pineal and enter as the nervi conarii (15).

Ultrastructure

The pineal is a solid parenchymal structure surrounded by a

capsule of pia mater and connective tissue. The main cell population, the chief cells or pinealocytes, thought to derive from ependymal cells (23), contain one or more cytoplasmic processes terminating between other pinealocytes or pericapillary spaces (24,25). Like other cells, these cells contain most of the usual array of organelles: mitochondria, ribosomes, and endoplasmic reticulum. A second population of cells are supporting cells, of neuroglial derivation.

The synaptic ribbon, an organelle found in pinealocytes in all mammalian pineal glands, consists of an electron dense rod surrounded by vesicles (26). It has been suggested that this structure is involved in cell-to-cell communication between adjacent pinealocytes (27). The precise function of these ribbons is not known.

Dense core vesicles in the pinealocyte process and perikarya have been reported in the hamster (28). Romijn and Gelsema (29) provided in vitro evidence that these vesicles contain a secretory product. The chemical nature of these dense core vesicles has not yet been determined.

There is little ultrastructural evidence to support the view that the pineal stores its hormonal product. Melatonin, the secretory product, appears to be released immediately after it is synthesized.

Scattered sporadically throughout the pineal gland are basophilic concretions, acervuli (corpora arenacea) or "brain sand". These concretions found in human pineal glands, contain calcium phosphate and calcium carbonate and arise after puberty (30). The significance of these structures is not well understood.

Biochemistry

Synthesis and regulation of melatonin secretion

Melatonin is synthesized in the pineal gland from the precursor serotonin in two steps: 1) serotonin is acetylated by an acetyl donor, acetyl coenzyme A, and serotonin N-acetyltransferase (SNAT) (31); 2) a methyl group from S-adenosylmethionine is transferred to N-acetyl serotonin via hydroxyindole-O-methyltransferase (32,33).

Serotonin is synthesized in the pinealocytes from tryptophan; circulating tryptophan is hydroxylated by tryptophan hydroxylase to produce 5-hydroxytryptophan which is converted to serotonin by 5-HTP decarboxylase (34,35).

Sympathetic input via the nervi conarii (15,36) stimulates the synthesis of melatonin from serotonin. Melatonin levels in the pineal and plasma follow a distinct circadian pattern and are cued to daily light cycles (37); the enzyme SNAT and melatonin are increased during the dark phase of the light cycle. The night-time rise in melatonin and SNAT is decreased if lights are turned on during the dark phase of the daily light-dark cycle (38,39).

In organ culture, the secretion of SNAT and melatonin is stimulated by norepinephrine (40,41). Melatonin secretion is under noradrenergic control since the administration of a beta-adrenergic blocker like propranolol in humans, prevents or inhibits melatonin secretion (42,43,44). The beta-adrenergic stimulation of melatonin and SNAT is regulated by cyclic AMP (45,46,47).

A mechanism exists in mammalian pineal glands to prevent circulating levels of norepinephrine and other catecholamines from stimulating melatonin secretion. It has been reported in the rat that during the day, circulating norepinephrine is taken up by sympathetic nerve terminals (48). This may be a mechanism to prevent adrenal mediated stress from having any great influence on melatonin secretion.

Distribution and metabolism of melatonin

Studies with tritiated melatonin have shown that melatonin binds to plasma proteins (49). The disappearance curve from plasma is biphasic; the initial phase following administration of tritiated melatonin into rats is distribution and binding followed by elimination (50). Thus the half-life of melatonin disappearance depends on what part of the disappearance curve is examined. The half-time of the distribution phase is 2 minutes and the half-time of the elimination phase is 20-40 minutes (50,51)

Melatonin is metabolized in the liver to 6-hydroxymelatonin, which is conjugated primarily with sulphate and excreted in the urine (and to a lesser extent in faeces) (51-53). It has been reported that the mammalian brain may convert melatonin to one or more 5-methoxykynurenamines (54).

Photoperiod, Pineal and Reproduction

Effect on gonads and reproductive hormones

The variation of photoperiod has a dominant role in regulating reproductive function in the female Syrian hamster (Mesocricetus auratus). In a stimulatory photoperiod (>12.5 hours light per day) females exhibit normal 4-day ovulatory cycles (55). Light deprivation via orbital enucleation or exposure to short photoperiods (<12.5 hours of light per day) disrupts estrous cyclicity and renders the females anestrous and anovulatory (55-61). This is accompanied by morphological changes in the reproductive organs: the uteri undergo atrophy, the number of endometrial glands in the uteri are reduced, and the uteri epithelium is reduced in height. The ovaries of such animals contain few growing and mature follicles, rare corpora lutea, and increase in weight due to hypertrophic ovarian interstitium (56-59). The ovarian interstitium is apparently active in the synthesis of progesterone (62).

The sympathetic nervous system and the pineal gland mediate the photosensitivity of the hamster gonads (57,61,63). Since pinealectomy or superior cervical ganglionectomy reversed the effects of short photoperiod, these effects were considered the result of an activated pineal gland (7,57). Hamsters blinded and pinealectomized had regular estrous cycles and reproductive organs that were indistinguishable from females maintained in stimulatory light conditions (56,57,60). Neither pinealectomy nor superior cervical ganglionectomy influenced the estrous cycles or reproductive organs of hamsters in a long photoperiod (57).

Regardless how gonadal atrophy was induced (by prolonged exposure to reduced lighting conditions or orbital enucleation) the

gonads begin to regenerate between 20-27 weeks (55,56). Hamsters remain refractory to light restriction until they are exposed to a long photoperiod for at least 10 weeks (64,65). In hamsters with intact eyes and optic nerve the refractory state is broken during this period and the animals can again respond to reduced lighting conditions.

Stetson and Tay (66) found that there are two periods of melatonin sensitivity during the day. The first one, one hour prior to lights on and the other a longer period extending throughout late afternoon and early evening. This extended the work of Tamarkin (67), who inititally showed that the effectiveness of melatonin depended upon the time of administration. Tamarkin reported that daily subcutaneous injections of 25 g melatonin late in the afternoon duplicated the effects of short photoperiod or light deprivation (67-69). Hamsters receiving the same melatonin treatment early in the morning, 3 hours after lights on, maintained reproductive competency (67). Melatonin injected during the middle of the dark period had little or no influence on the hamster reproductive capacity (70). Tamarkin suggested that melatonin administered to pineal intact hamsters prior to onset of darkness and onset of light, were temporally adding to endogenous melatonin from the pineal gland to induce gonadal involution (70). Reiter (71) also regarded melatonin as the hormone which mediated gonadal regression following exposure of female hamsters to inhibitory photoperiods. This view is supported by the similarities in the results of daily melatonin injections and reduced lighting conditions; both resulted

in acyclicity in 3-6 weeks.

Continuously available melatonin in silastic capsules or beeswax pellets implanted subcutaneously counteracted the reproductive effects following light deprivation, short photoperiod or daily afternoon melatonin injections (72,73). This paradoxical counterantigonadal effect is similar to that observed in males (74). A mechanism postulated to explain this counterantigonadal effect is that continously available melatonin in large amounts may down-regulate melatonin receptors (75). This hypothesis is rather difficult to test since melatonin receptors are not readily demonstratable.

The changes in serum gonadotropin levels during the 4-day cycle of the female hamster are characterized by a gonadotropin surge on the day of proestrus (76,77). Exposure to short photoperiods or light deprivation results in a daily surge of gonadotropin secretion, rather than a 4-day ovarian cycle (55,68,69,73,78,79), with increased concentrations of LH and FSH and decreased concentrations of prolactin within the anterior pituitary (58,73,80). Chronic daily afternoon injections of melatonin into female hamsters also resulted in an afternoon surge of LH and FSH every day (67).

The ovarian feedback system is already functional in hamsters between 20 and 26 days of age (81). In the absence of ovarian feedback in ovariectomized hamsters in a stimulatory light environment, both the pituitary content and concentrations of LH and FSH are elevated and prolactin levels are depressed (82-84). Plasma levels of LH and FSH are increased whereas plasma levels of prolactin are decreased (55,83,84). Following ovariectomy, hamsters

exhibited daily LH and FSH surges (55,62,78,79). Ovariectomy failed to eliminate the daily surge of gonadotropin secretion in short photoperiod induced acyclic hamsters (55,78).

The results of experiments with female hamsters are consistent with the view that the pineal gland via melatonin acts upon the brain. It has been suggested that the neural mechanism utililized in triggering the daily surges of gonadotropin in light deprived hamsters is similar to the mechanism utilized in triggering the preovulatory gonadotropin release in hamsters undergoing normal cycles (77). The magnitude and time of occurrence of the gonadotropin surges in hamsters under the two conditions coincides (77).

Very little work has been done on the role of the pineal in reproduction in human females. Wetterberg, however has reported a variation in serum melatonin during the menstrual cycles of Swedish women (85). He suggested that low melatonin secretion by the pineal gland at midcycle was permissive for ovulation.

Effects of light deprivation and melatonin on thyroid hormones

Female hamsters deprived of light, either by exposure to short photoperiods or by blinding, had reduced circulating plasma levels of T4 and TSH (86,87). Pinealectomy reversed the reduction in circulating T4 (86). Vriend and collaborators (87,88) suggested the secretion of endogenous melatonin is modified by changing the photoperiod; altered secretion of melatonin was regarded as influencing T4 levels in their experiments. Blinding also resulted in an increase in TRH

content of the hypothalamus, which was also prevented by pinealectomy (89). Vriend (89) suggested that the pineal gland has an inhibitory influence on TRH release in hamsters.

Melatonin injections in female hamsters have also been reported to inhibit circulating levels of T4, T3 and TSH, but this was dependent on the dose and mode of administration (87,90). Subcutaneous administration of 50-100 g melatonin per day inhibited thyroid secretion rate (91). Daily injections of 25 g melatonin inhibited plasma T4, T3 and TSH (87,90). Pinealectomy, or superior cervical ganglionectomy, prevented the melatonin induced reduction in plasma T4 (92,93). Melatonin injections given in the evening prior to lights out were more effective in inhibiting plasma T4 levels than if administered in the morning after lights on (87,92).

High blood levels of melatonin continuously maintained by pharmacological doses of melatonin (2.5 mg daily), by implants of melatonin or by placing melatonin in the drinking water, had no inhibitory effect on plasma T3, T4 or TSH levels (87,94). These protocols prevented the inhibitory effects of daily injections of 25 g melatonin and the inhibitory effects of short photoperiod. Gonadectomy did not prevent the melatonin induced inhibition of circulating T4 (87).

The potential interaction of T4 in prolactin regulation has received little attention. Since TRH releases both prolactin and TSH (95), a major factor regulating TRH induced prolactin release is the number of TRH receptors in the pituitary. Thyroid hormones have been demonstrated to regulate TRH receptors both in vitro and in vivo (96-98). Changes in sensitivity of TSH secretion to T4

feedback as a result of melatonin can be predicted but such changes have not yet been demonstrated (99). Vriend and Ralcewicz (99) suggested that changes in sensitivity to T4 feedback would result in changes in TSH secretion as well as prolactin secretion. Although prolactin secretion is modified in hamsters injected with melatonin or exposed to short photoperiods, the relationship of these changes to T4 and TRH has not yet been established (99).

Similarities between antigonadal and antithyroid actions of the pineal

The inhibitory effect of light restriction and melatonin injections on the gonadal and thyroid axes occurs simultaneously (67,88). Pinealectomy or superior cervical ganglionectomy prevents both the antithyroid and antigonadal effects of light restriction and melatonin injections (58,100,101). Melatonin can have counter-inhibitory effects on both the pituitary-gonadal and pituitary-thyroid axis (75,87,88), if administered on a continuous basis. Since the same neural pathways are used and the antithyroid and antigonadal effects of an activated pineal gland occur simultaneously, they appear to be different aspects of a syndrome produced by melatonin at a single CNS site (88).

Current investigation

In the present study the effects of melatonin on serum and pituitary levels of gonadotropin and prolactin were investigated

in female hamsters made hypothyroid with thiourea in the drinking water; effects of melatonin were studied in hypothyroid female hamsters receiving thyroxin replacement. Melatonin induced changes in reproductive histology and in serum and pituitary hormones were compared to changes that depend on thyroid status.

Melatonin combined with various doses of estradiol were used to determine if the changes in reproductive histology and pituitary and serum hormones in melatonin induced female hamsters were due to changes in serum estradiol levels.

Melatonin induced hamsters exhibited depressed pituitary and serum PRL levels. L-dopa is known to inhibit PRL release. Various doses of L-dopa were used to determine if L-dopa could produce effects similar to melatonin.

Materials and Methods

One hundred and ten female Syrian hamsters, age 10 weeks, strain LAK:LVG (Charles River), were used for this study. They were kept under controlled lighting and temperature conditions (22± 2°C). Food and water were provided ad libitum.

Experimental protocols

Experiment 1

Thirty hamsters were acclimatized for 1 week in a long photoperiod (14L:10D, lights on 0400-1800). Thirty hamsters were acclimatized for 1 week in a short photoperiod (2L:22D, lights on 1400-1600). The animals were randomly assigned to one of 6 groups, (n=5): 1) control hamsters receiving daily afternoon injections of ethanolic saline; 2) hamsters receiving daily afternoon injections of 25 µg melatonin in ethanolic saline; 3) hamsters receiving daily afternoon injections of 25 µg melatonin in ethanolic saline plus 0.4% thiourea in their drinking water; 4) hamsters receiving daily afternoon injections of melatonin, plus thiourea in their drinking water and an injection of 5 µg thyroxin during the last 2 weeks of the experiment; 5) hamsters receiving thiourea in the drinking water plus the T4 replacement injections during the last 2 weeks of the experiment; 6) hamsters receiving only thiourea in the drinking water. Ethanol was used to dissolve the melatonin. All injections were given between 1545-1555 h.

Vaginal smears were taken daily after 6 weeks of treatment. The smears were fixed in 50:50 ether-ethanol for 30 minutes and stained with Papanicolaou's stain.

The hamsters were sacrificed by decapitation after 10 weeks. Serum and pituitaries were collected and stored for later hormonal assay of gonadotropins, prolactin, TSH, T3 and thyroxin. Serum estradiol levels were also determined in hamsters under LD 14:10.

Experiment 2

Thirty hamsters were acclimatized for 1 week in a long photoperiod (14L:10D, lights on 0400-1800) and randomly assigned to one of 6 groups, (n=5): 1) control hamsters receiving daily afternoon injections of ethanolic saline; 2) hamsters receiving daily afternoon injections of 25 μg melatonin in ethanolic saline; 3) control hamsters receiving daily afternoon injections of peanut oil; 4) hamsters receiving daily afternoon injections of 25 μg melatonin in ethanolic saline and an injection of 1 μg estradiol in peanut oil; 5) hamsters receiving daily afternoon injections of 25 μg melatonin in ethanolic saline and an injection of 5 μg estradiol in oil; 6) hamsters receiving daily afternoon injections of 25 μg melatonin in ethanolic saline and an injection of 10 μg estradiol in oil. Peanut oil was used to suspend the estradiol. All injections were given between 1545-1555 h. Vaginal smears were taken daily after 6 weeks of treatment, fixed and stained as previously described. The hamsters were sacrificed by decapitation after 10 weeks. Serum and pituitaries were collected for later assay of gonadotropins,

prolactin and TSH. Serum estradiol levels were also determined.

Experiment 3

Twenty hamsters acclimatized for 1 week in a long photoperiod (14L:10D, lights on 0400-1800) were randomly assigned to one of 4 groups, (n=5): 1) control hamsters receiving daily afternoon injections of 1% ascorbic acid in saline; 2) hamsters receiving daily afternoon injections of 1 μg L-dopa in 1% ascorbic acid; 3) hamsters receiving daily afternoon injections of 25 μg L-dopa in 1% ascorbic acid; 4) hamsters receiving daily afternoon injections of 100 μg L-dopa in 1% ascorbic acid. The L-dopa was dissolved in 1% ascorbic acid. All injections were given between 1545-1555 h. Daily vaginal smears were taken. Hamsters were sacrificed after 10 weeks by decapitation. Serum and pituitaries were collected for hormonal assay of gonadotropins, prolactin and TSH.

All hamsters were sacrificed by decapitation between 1100-1600 h. Prior to sacrifice body weights were recorded. Pituitaries were collected, their weights recorded, stored frozen and subsequently sonicated in phosphate buffered saline. The homogenates were diluted for the hormone assays mentioned. Ovary and uteri weights were recorded. Ovaries, uteri and vagina (in experiment 2 and 3) were removed at the time of sacrifice and prepared for histology. Sections obtained were fixed in Bouin's, dehydrated, embedded in paraffin, sectioned 5 μm and stained with Harris hematoxylin and eosin.

Radioimmunoassays

Serum T4 was determined by the TETRA TAB RIA assay of Nuclear Medical Laboratories (Dallas, Texas). Human hyperthyroid, euthyroid and hypothyroid control sera were run with the assay. Standards from 1.0 to 17.9 μg/dl were used for the T4 standard curve. Assay for T3 was performed using Beckman RIA materials and protocol. Standards from 50.4 to 820 ng/dl were used to prepare the standard curve for T3. Serum and pituitary LH, FSH and TSH were determined by radioimmunoassay using NIAMDD reagents and protocol. Pituitary and serum prolactin were determined using the homologous radioimmunoassay of Soares et al. (102). Serum levels of estradiol were determined by RIA materials and protocol of Travenol Canada Inc. The estradiol standard curve was prepared from standards made up in stripped hamster serum. All samples were assayed in duplicate. The tubes were counted in a LKB gamma counter and hormone concentrations calculated from a spline fit of the standard curve data.

Statistical analysis

The data were analyzed by analysis of variance followed by Duncan's multiple range test. Log transformations were performed on pituitary and serum LH and serum estradiol prior to an analysis of variance.

Results

Experiment 1

Compared to ovaries of saline controls in hamsters in LD 14:10 (Figs. 1 and 2a), the ovaries of hamsters injected daily with 25 μg melatonin (alone or combined with thiourea, or thiourea and T4) contained few mature and growing follicles, rare corpora lutea and a predominance of interstitial tissue (Figs. 2b,2d,&2f). Ovaries of hamsters treated with thiourea alone were also nearly devoid of corpora lutea and also contained fewer developing and mature follicles than controls (Figs. 2e and 3b). It was noted that many of the mature follicles that were found in the ovaries of this group were atretic. In contrast, hypothyroid hamsters receiving thyroxin replacement for the last two weeks of the experiment had ovaries which revealed an increase in the number and size of developing and mature follicles compared to the ovaries of hypothyroid hamsters receiving no T4 replacement. Reduced atresia of mature follicles was observed in hypothyroid hamsters receiving T4 replacement. Several corpora lutea were observed in each ovary of this group (Figs. 2c and 3a).

Histologically, the ovaries of all hamsters in LD 2:22 were similar to ovaries of melatonin injected hamsters in LD 14:10.

Papanicolaou smears of control hamsters displayed regular 4-day estrous cycles (Fig. 4). All hamsters receiving daily melatonin injections had ceased cycling by 6 weeks of treatment. Estrous cycles were halted at diestrus 2, a stage characterized by over 90%

Figure 1. Histological analysis of ovaries from control hamsters in LD 14:10 illustrating the progressive stages of the 4-day estrous cycle. Sections (5 µm) were stained with H & E. A, Proestrus (magnification x5.5). Growing follicles under the influence of FSH develop into mature follicles. B, Estrus (magnification x6.0). The large mature follicle is characterized by a large follicular antrum filled with follicular fluid. C, Diestrus 1 (magnification x7.0). Oocyte is released. Remaining granulosa cells transformed into lutein cells forming a young corpora lutea. D, Diestrus 2 (magnification x5.8). Blood vessels infiltrate the corpora lutea and the corpora lutea degenerates. p, primary follicle; g, growing follicle; a, atretic follicle; cl, corpora lutea; m, mature follicle.

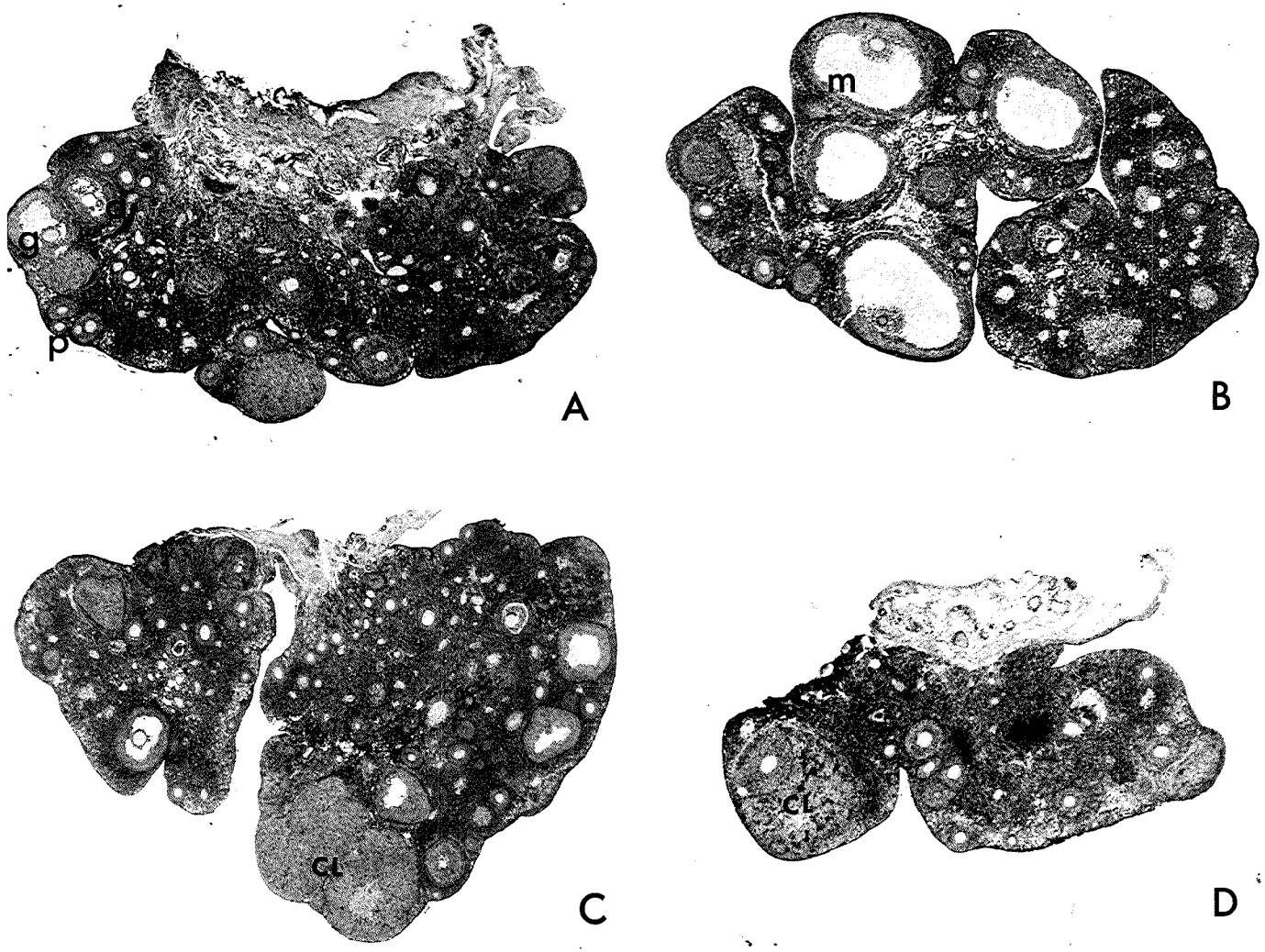
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Figure 2. Histological analysis of ovaries from hamsters in LD 14:10 treated with melatonin, thiourea and T4. Sections (5 μm) were stained with H & E. A, Control (magnification x4.5). Note follicles in various stages of development. B, 25 μg melatonin daily (magnification x3.0); C, 0.4% thiourea in drinking water and 5 μg thyroxin daily for 2 weeks (magnification x5.2). Note presence of numerous corpora lutea. D, 25 μg melatonin daily, 0.4% thiourea in drinking water and 5 μg thyroxin daily for 2 weeks (magnification x3.6); E, 0.4% thiourea in drinking water (magnification x6.0). Note numerous atretic follicles and few corpora lutea. F, 25 μg melatonin daily and 0.4% thiourea in drinking water (magnification x5.0). Note in B,D,& F the absence of growing and mature follicles, (the few growing follicles present are atretic), devoidance of corpora lutea and predominance of ovarian interstitium.

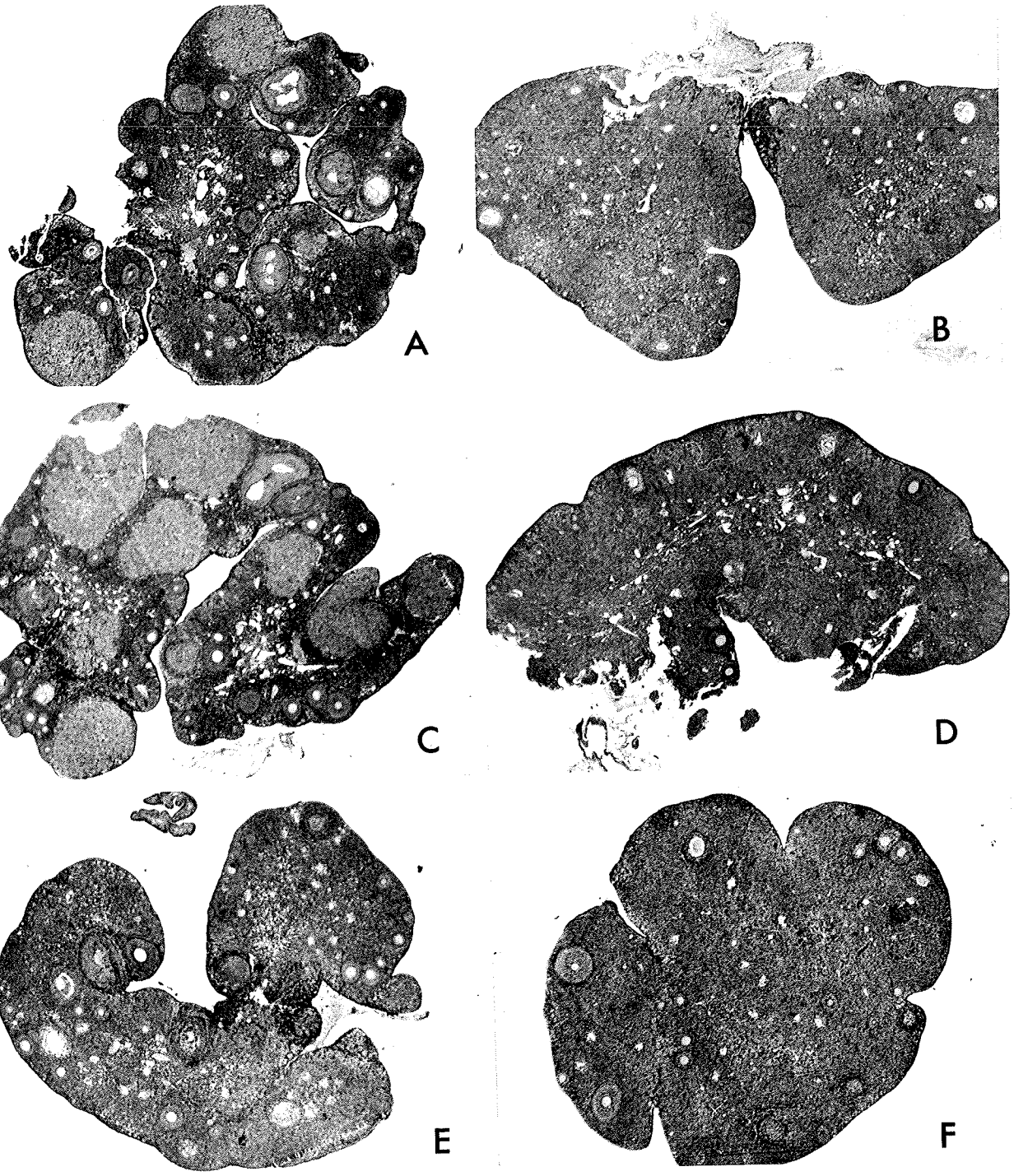
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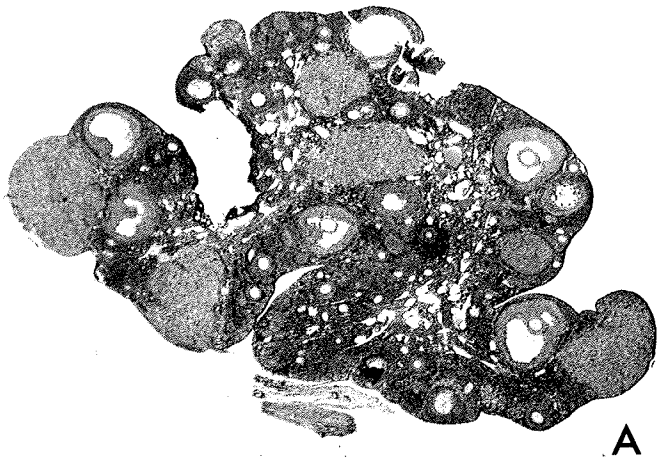
Figure 3. Histological analysis of ovaries from hypothyroid hamsters and ones receiving thyroxin replacement in LD 14:10. Sections (5 μm) were stained with H & E. A, hypothyroid (0.4% thiourea in drinking water) and T4 replacement for 2 weeks (5 μg thyroxin daily) (magnification x4.8). Note presence of several non-atretic follicles in various stages of development and presence of numerous corpora lutea. B, hypothyroid (0.4% thiourea in drinking water) (magnification x5.1). Note the presence of few corpora lutea and that the developing and mature follicles are atretic.



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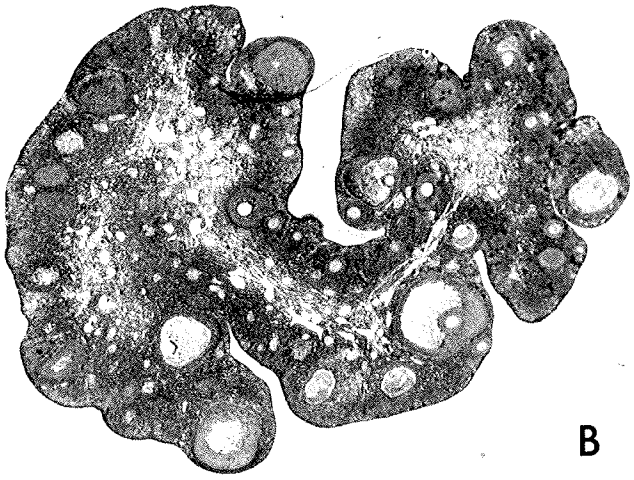
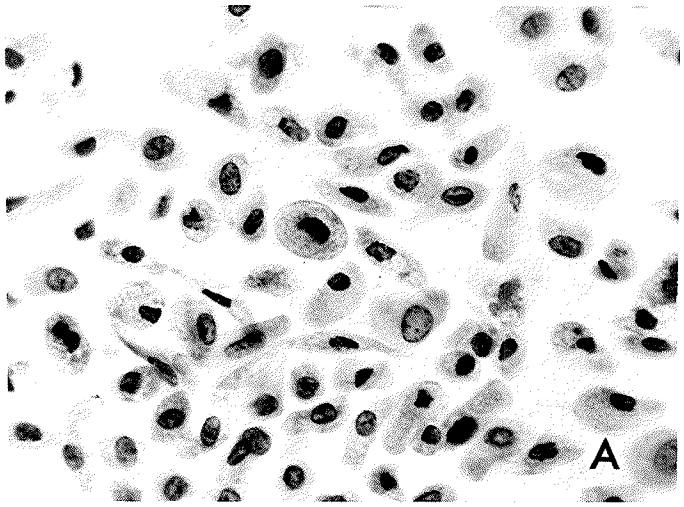
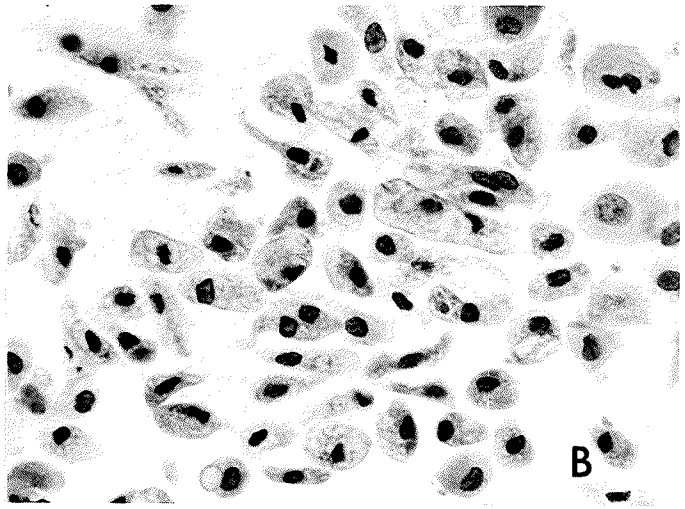


B

Figure 4. Cytological vaginal smears from control hamsters in LD 14:10 illustrating the progressive stages of the 4-day estrous cycle. Smears were stained with Papanicolaou. A, Proestrus; B, Estrus. In A & B the cytological changes are due to increase in estrogen secretion. In both A & B the predominant cell type is superficial acidophils with vesicular nuclei. Basophils with vesicular nuclei are also present. When peak of estrus is reached, superficial acidophils (cornified cells) predominate. Nuclei of both cell types becomes pyknotic and cells become elongated and spindle shaped. C, Diestrus 1. This is the post-ovulatory stage. Cellular desquamation is characterized by the presence of superficial acidophils and basophils. In normal smears, the presence of intermediate cells is less than 10% and the presence of parabasal cells is less than 5%. Neutrophils also appear and increase in number towards the end of this stage. D, Diestrus 2. This stage is characterized by a predominance of neutrophils (over 90%) and few superficial cells. (magnification x120).


A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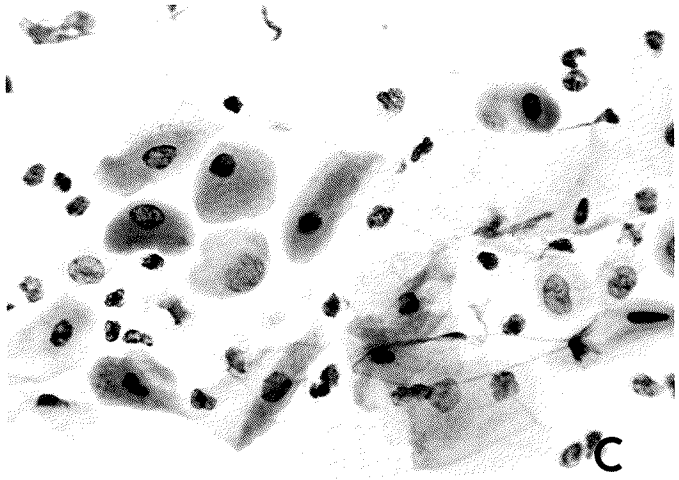

C


D

neutrophils, sparse epithelial cells and presence of intermediate and parabasal cells (Figs. 5b,5d,&5f). Hypothyroid hamsters, with or without thyroxin replacement displayed irregular estrous cycles. Smears of hypothyroid hamsters exhibited a high proportion of intermediate and parabasal cells (Fig. 5c) compared to hypothyroid hamsters receiving T4 replacement, that revealed a reduction in both of these cell types (Fig. 5e).

Pituitary TSH and serum TSH, T3 and T4 levels of hamsters in this experiment verified that hamsters receiving thiourea were hypothyroid. As shown in Table 1, thiourea administration increased pituitary TSH 12-fold, increased serum TSH 2-fold, thyroxin concentrations were reduced to less than 20% of controls and T3 concentrations to less than 45% of controls in both photoperiods. Thyroxin replacement resulted in serum T3 levels approximately twice the control values ($p < .01$). Melatonin injections resulted in a reduction of circulating T4 levels to 47% of controls ($p < .01$). T3 levels in melatonin injected hamsters were reduced to 66% of controls ($p < .05$).

Students t-test revealed a significant increase in pituitary content of LH (181%) in the group treated with melatonin and thiourea compared to controls (Fig. 6a) ($p < .01$); T4 replacement restored the increase to levels not different from hamsters receiving melatonin alone. Serum LH was increased approximately 10-fold (9-14 fold) of controls ($p < .01$) in all groups of hamsters receiving melatonin injections (Fig. 6b). Although serum LH values of hypothyroid hamsters were higher compared to controls, the increase was not statistically

Figure 5. Cytological vaginal smears from hamsters in LD 14:10 treated with melatonin, thiourea and thyroxin. Smears were stained with Papanicolaou. A, Control. The stage is prior to ovulation. B, 25 µg melatonin daily; C, 0.4% thiourea in drinking water. The stage is Diestrus 1. Note the presence of numerous intermediate cells (boat-like shape) and parabasal cells. Superficial acidophils and basophils as well as neutrophils are present. D, 25 µg melatonin daily, 0.4% thiourea in drinking water and 5 µg thyroxin daily for 2 weeks; E, 0.4% thiourea in drinking water and 5 µg thyroxin daily for 2 weeks. The stage is Diestrus 2. Note the reduction in intermediate and parabasal cells. F, 25 µg melatonin daily and 0.4% thiourea in drinking water. Note in B,D,& F the predominance of neutrophils and few superficial acidophils and basophils. (magnification x120).

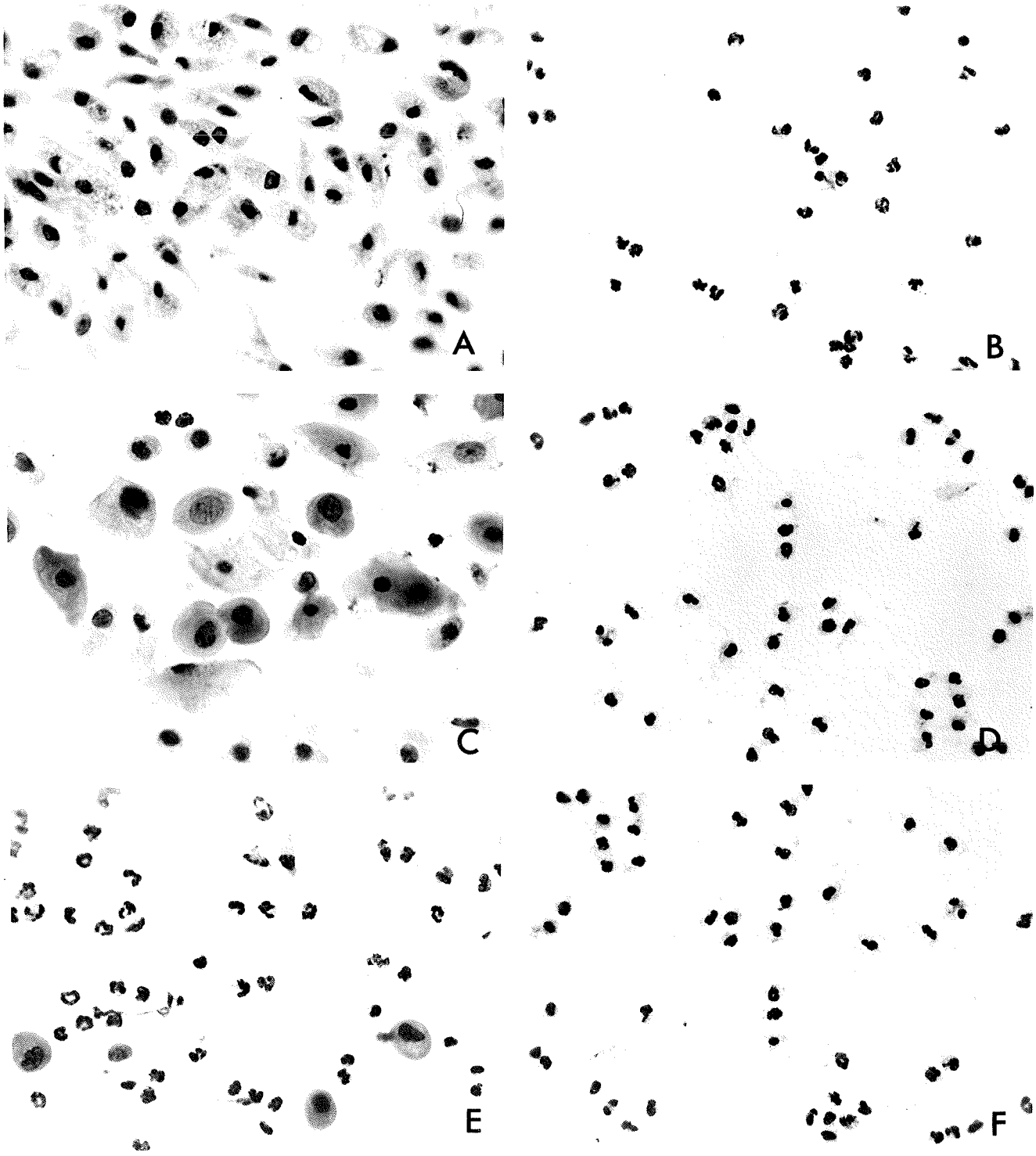
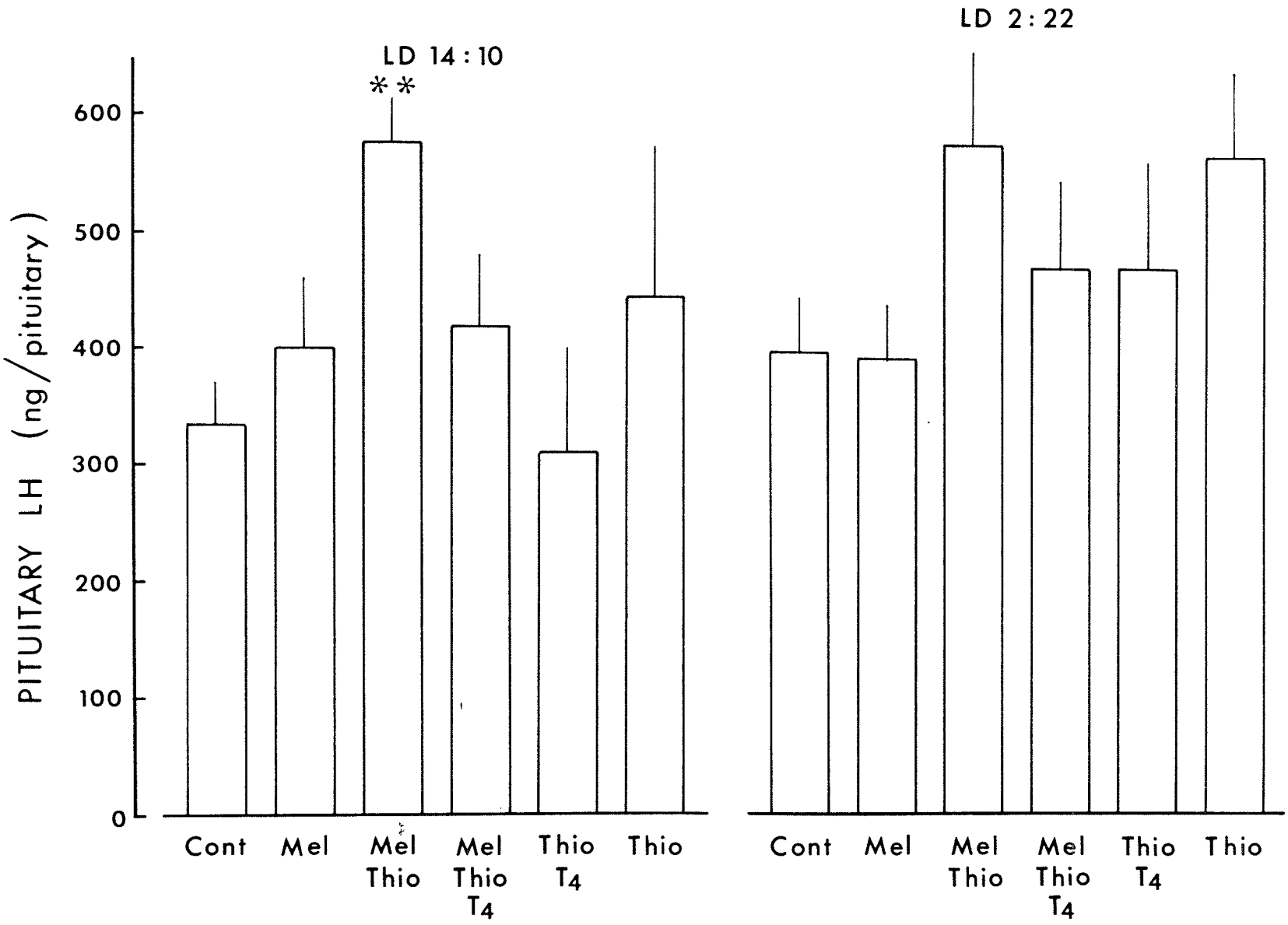

A
B
C
D
E
F

Table 1. Effects of melatonin and thyroid status on TSH and thyroid hormones in female hamsters exposed to LD 14:10 and LD 2:22.

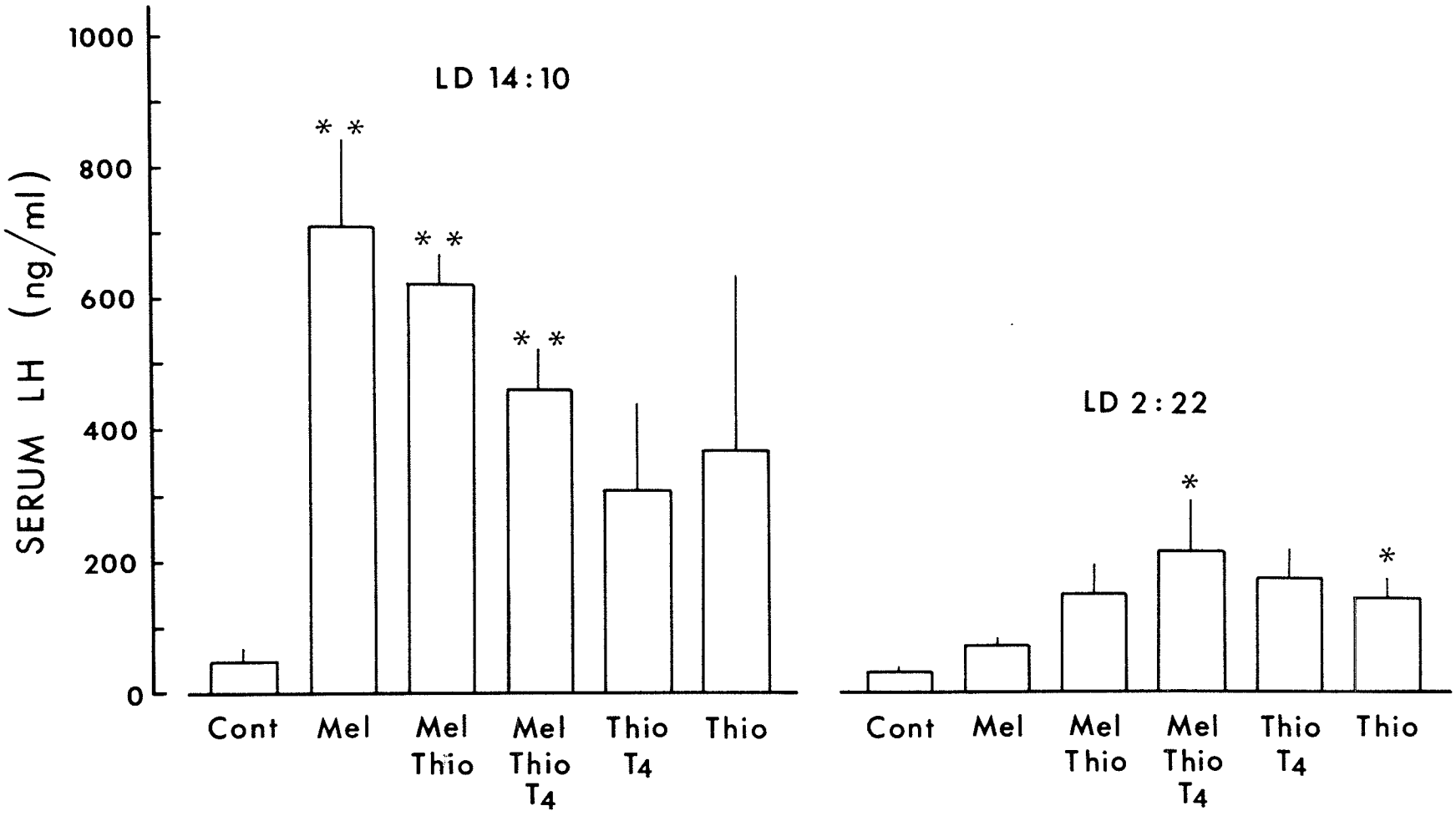
Treatment		TSH ng/pit	TSH ng/ml	T4 ug/dl	T3 ng/dl
Cont	14L	87±6	48±5	6.4±.2	100±1
	2L	86±5	43±5	5.1±.6	93±9
Mel	14L	76±4	45±4	3.0±.4**	67±6 *
	2L	88±9	47±4	3.0±.3**	72±5
Mel+Thio	14L	1076±90 **	109±10**	1.1±.1**	35±4 **
	2L	1193±156**	104±6 **	1.1± 0**	44±6 **
Mel+Thio+T4	14L	98±14	40±3	- - - -	201±16**
	2L	104±11	35±2	- - - -	201±20**
Thio+T4	14L	124±17	38±4	- - - -	202±13**
	2L	81±12	35±5	- - - -	234±9 **
Thio	14L	1456±201**	81±16**	2.7± 1**	43±12**
	2L	1042±82 **	109±3 **	0.9±.1**	30±2 **

Data expressed as means ± SEM.
Asterisks indicate significant difference from controls:
* $p < .05$; ** $p < .01$.
Cont, Control; Mel, Melatonin; Thio, Thiourea.

Figure 6. Effects of melatonin, thiourea and thyroxin replacement on pituitary and serum LH concentration in female hamsters maintained in LD 14:10 and LD 2:22. Mean and standard error displayed. Analysis of variance was performed on log transformed data. Significant difference from controls: * $p < .05$; ** $p < .01$.


LD 14 : 10
**
LD 2 : 22
PITUITARY LH (ng/pituitary)
0
100
200
300
400
500
600
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio

A


SERUM LH (ng/ml)
1000
800
600
400
200
0
LD 14:10
* *
* *
* *
LD 2:22
*
*
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio

B

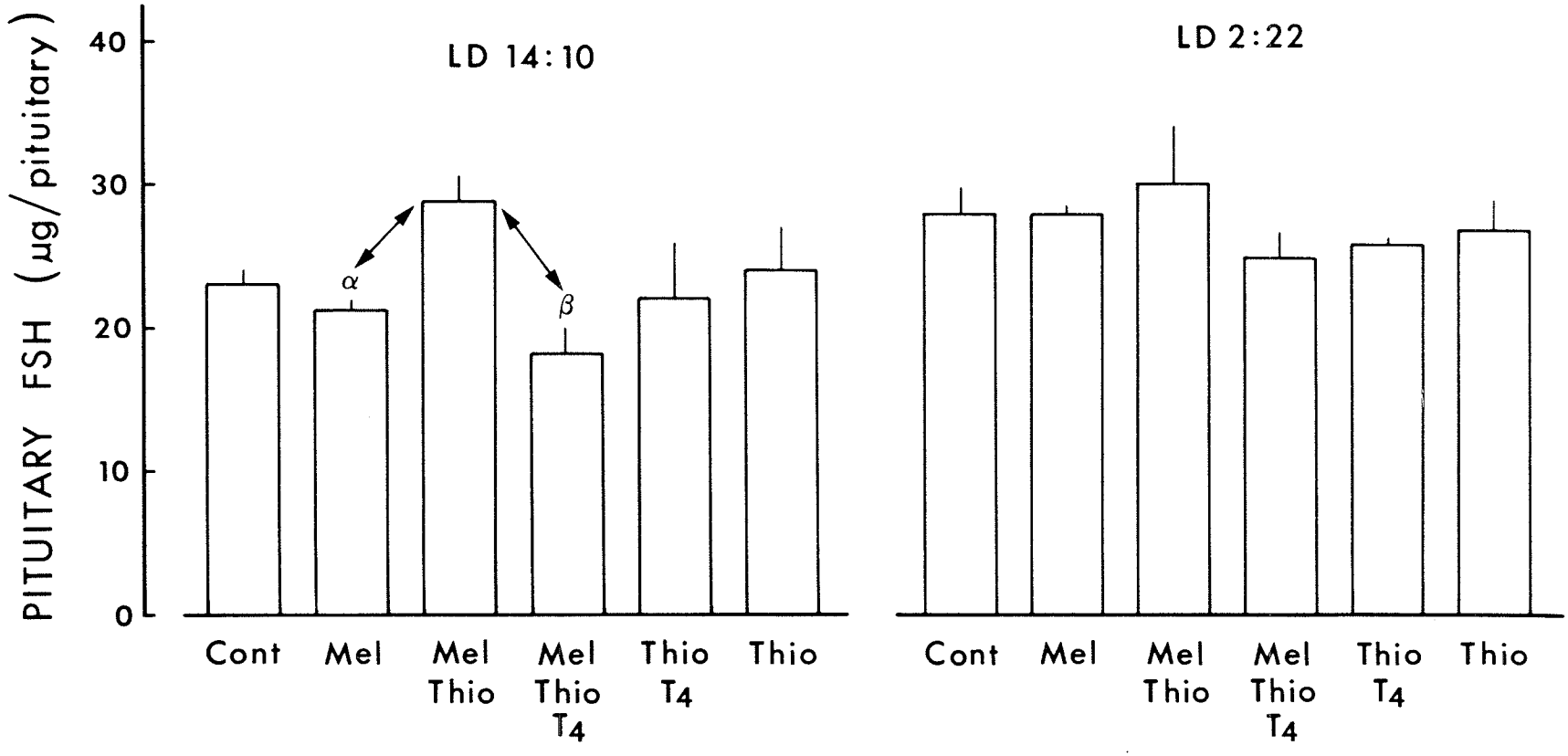
significant. Some caution is required in interpreting results in which both mean and variance are influenced by treatment.

As noted for pituitary content of LH, melatonin injections combined with thiourea also increased FSH content (Fig. 7a) (to 127% of levels observed in animals receiving melatonin alone) ($p < .05$), whereas T4 replacement restored the increase to levels not significantly different from animals receiving melatonin alone ($p < .01$). Neither melatonin injections alone, nor thiourea treatment alone, had a significant effect on pituitary content of FSH. Serum FSH (Fig. 7b) was increased to 148% of controls by melatonin injections ($p < .01$). This was not observed in animals receiving thiourea and T4 in addition to melatonin. Among the 4 groups which received thiourea in the drinking water, only those receiving melatonin had significantly raised levels of circulating FSH ($p < .05$).

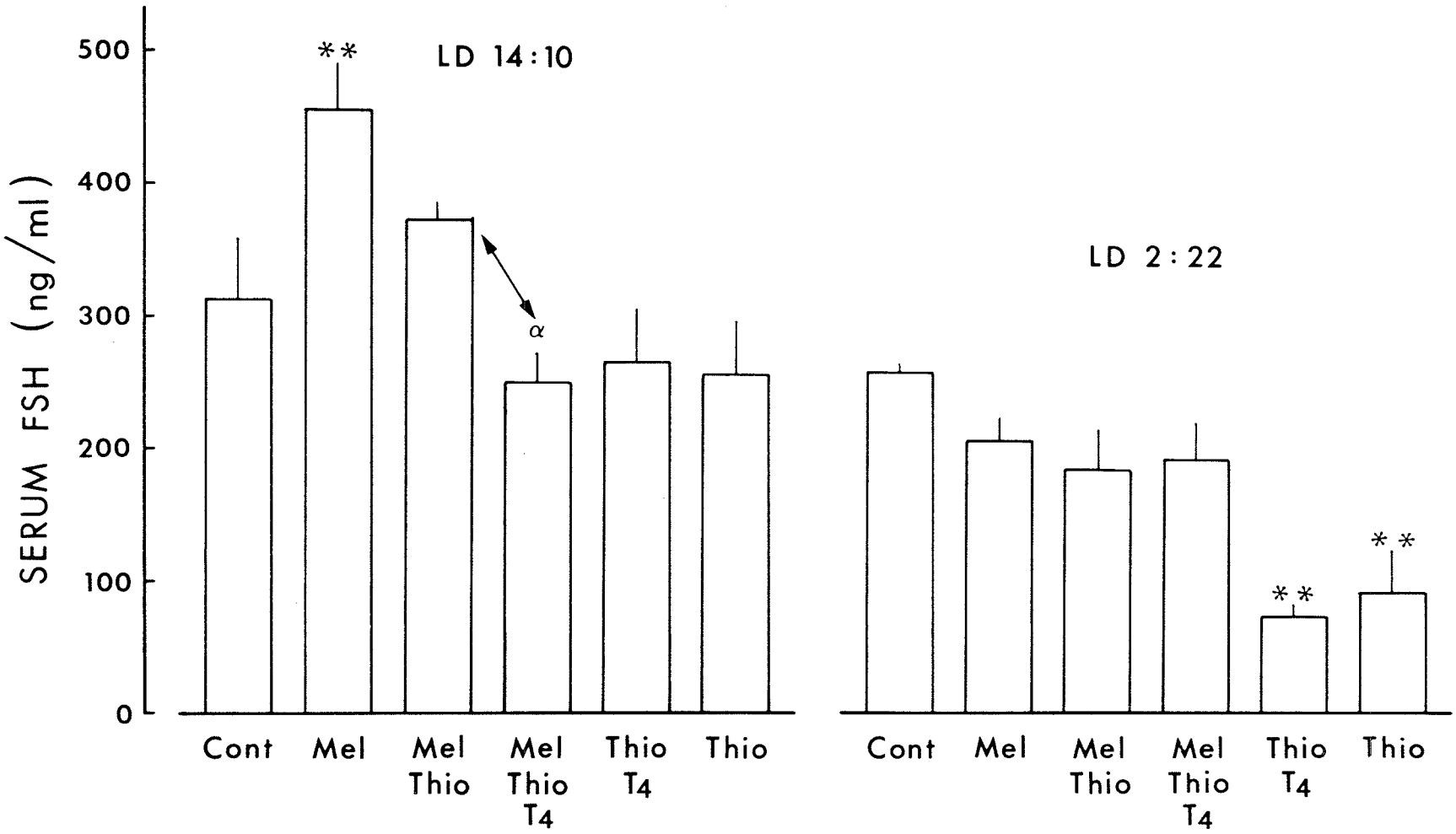
Pituitary content of PRL (Fig. 8a) was reduced to less than 12% of controls by melatonin injections ($p < .01$). Thiourea administration reduced pituitary PRL to 49% of controls ($p < .01$). T4 replacement in animals receiving thiourea did not result in any significant difference in PRL compared to levels observed in animals receiving thiourea alone. Serum PRL (Fig. 8b) was reduced to less than 15% of controls by melatonin injections ($p < .01$). A significant reduction in serum PRL, (to 45% of controls), was also observed in hamsters receiving thiourea ($p < .05$); no significant reduction was observed in similar hamsters receiving T4 replacement.

Serum estradiol (Fig. 9) in LD 14:10 was reduced to less than 25% of controls by melatonin injections ($p < .01$). In 4 out of the 5

Figure 7. Effects of melatonin, thiourea and thyroxin replacement on pituitary and serum FSH levels in female hamsters in LD 14:10 and LD 2:22. Mean and standard error shown. Significant difference from controls:
* $p < .05$; ** $p < .01$. α $p < .05$; β $p < .01$.

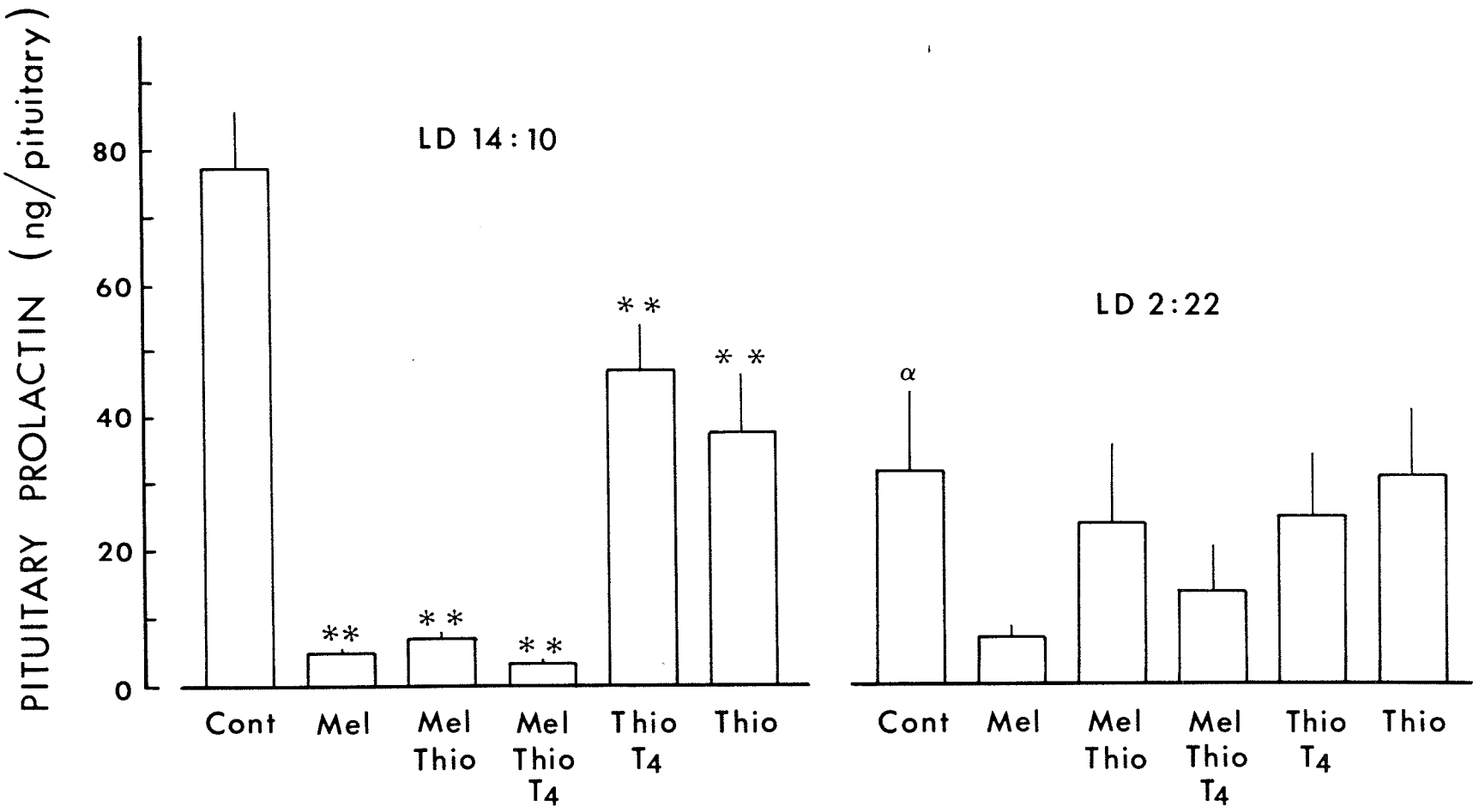

PITUITARY FSH (µg/pituitary)
40
30
20
10
0
LD 14:10
LD 2:22
α
β
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio

A

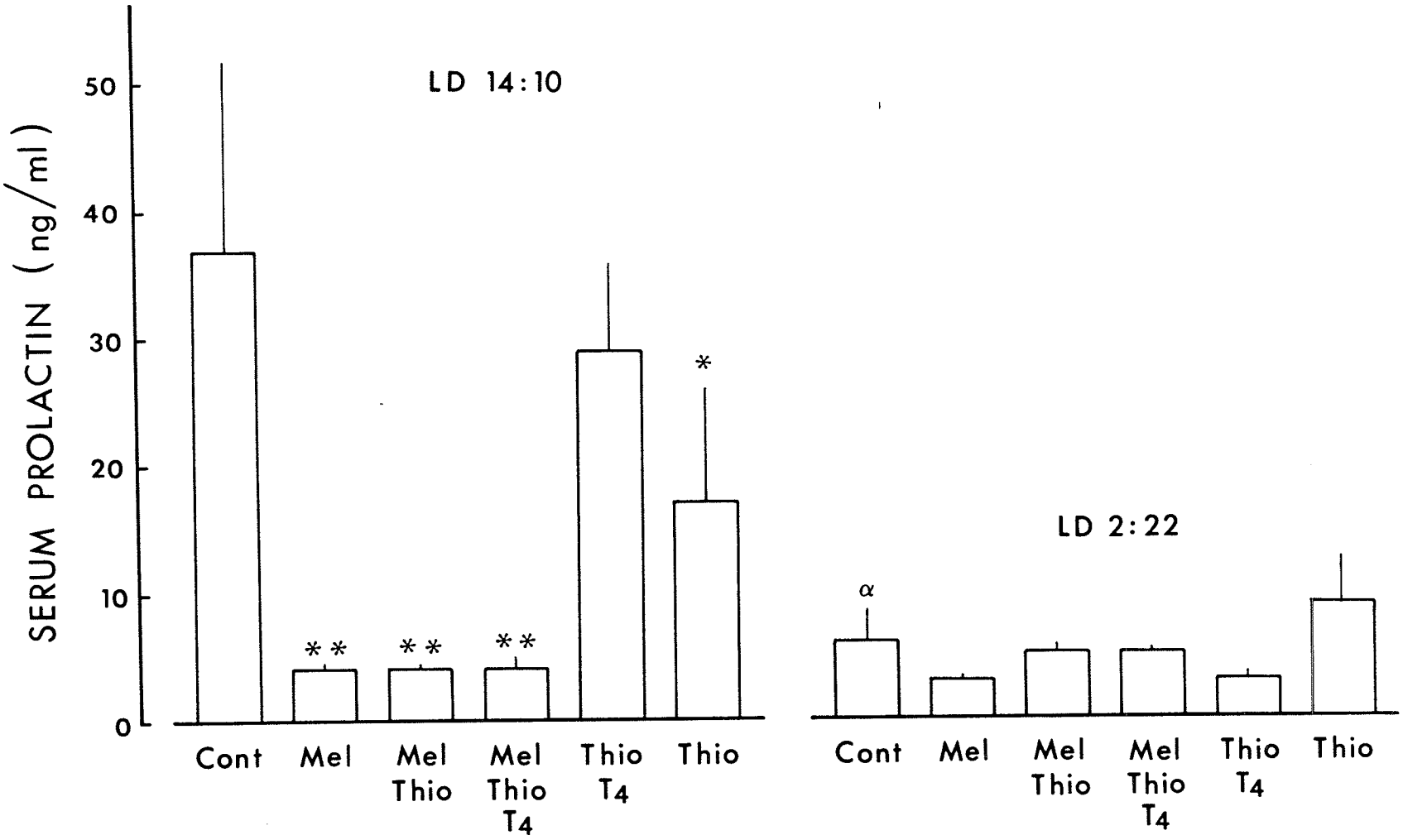

SERUM FSH (ng/ml)
500
400
300
200
100
0
**
LD 14:10
α
LD 2:22
**
**
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio

B

Figure 8. Effects of melatonin, thiourea and thyroxin replacement on pituitary and serum prolactin levels in female hamsters under LD 14:10 and LD 2:22. Mean and standard error displayed. Asterisks indicate significant difference from controls: * $p < .05$; ** $p < .01$. α indicates significant difference from LD 14:10 controls; $p < .01$.


PITUITARY PROLACTIN (ng/pituitary)
0
20
40
60
80
LD 14:10
LD 2:22
**
**
**
**
**
α
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio

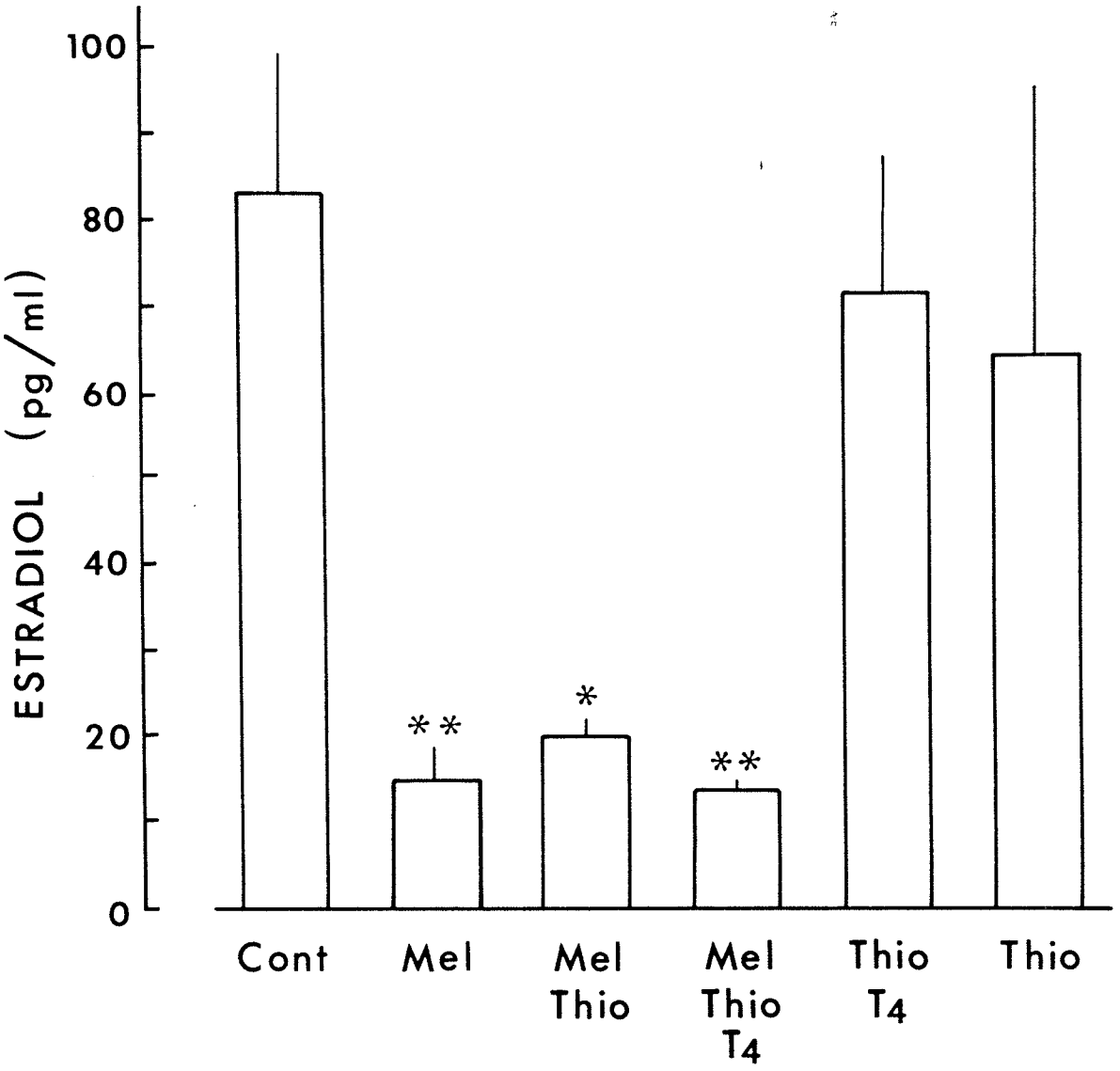
A


SERUM PROLACTIN (ng/ml)
50
40
30
20
10
0
LD 14:10
**
**
**
*
LD 2:22
α
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio

B

Figure 9. Effects of melatonin, thiourea and thyroxin replacement on serum estradiol levels in female hamsters under LD 14:10. Mean and standard error displayed. Anova was perfomed on log transformed data.

* $p < .05$; ** $p < .01$ compared to controls.


ESTRADIOL (pg/ml)
100
80
60
40
20
0
**
*
**
Cont
Mel
Mel Thio
Mel Thio T4
Thio T4
Thio

hypothyroid hamsters, serum estradiol was reduced compared to controls. Serum estradiol in hypothyroid hamsters receiving T4 replacement did not differ significantly from controls.

Ovary weights (Table 2) were slightly increased by melatonin ($p > .05$), and significantly by short photoperiod ($p < .01$). Thiourea administration reduced ovary weights to less than 75% of controls in animals under both photoperiods, irrespective of other treatments.

Uterus weights (Table 2) were reduced to 43% of controls by melatonin injections ($p < .01$). Short photoperiod resulted in a reduction to 64% of controls ($p < .01$). Thiourea administration reduced uterus weights to 56% of controls ($p < .01$), an inhibition partially reversed by T4 replacement in animals under LD 14:10.

Pituitary weights of hamsters under LD 14:10 were reduced to 67% of controls by melatonin administration ($p < .01$). This reduction was partially reversed by thiourea administration ($p < .05$) and restored by T4 replacement ($p < .05$). Short photoperiod also significantly reduced pituitary weights ($p < .05$). In animals under LD 14:10, thiourea administration alone resulted in a small decrease in pituitary weights ($p < .05$).

Body weights (Table 2) were significantly increased by short photoperiod ($p < .05$) and significantly reduced by thiourea administration in hamsters under both photoperiods ($p < .01$).

Experiment 2

In experiment 2, treatment of female hamsters with melatonin

Table 2. Effects of melatonin and thyroid status on body (g) and organ weights (mg) in female hamsters in LD 14:10 and LD 2:22.

Treatment	Uteri	Ovary	Pituitary	Body
14:10;Cont	486±33	47±2	6.1±.4	195±7
14:10;Mel	210±21**	54±3	4.1±.3**	197±7
14:10;Mel+Thio	188±26**	33±6**	5.1±.3*	144±5 **
14:10;Mel+Thio+T4	176±5 **	30±3**	4.1±.3**	141±7 **
14:10;Thio+T4	368±22*	32±2**	5.0±.3*	132±3 **
14:10;Thio	274±60**	34±2**	5.3±.1*	123±5 **
2:22;Cont	310±36	66±7	5.2±.2	226±4
2:22;Mel	252±24	63±3	4.8±.2	208±10*
2:22;Mel+Thio	252±36	33±1**	5.4±.4	150±3 **
2:22;Mel+Thio+T4	214±21	33±2**	4.8±.1	154±4 **
2:22;Thio+T4	232±32	34±1**	4.4±.2	150±7 **
2:22;Thio	244±38	32±3**	5.7±.3	156±8 **

Mean and standard errors shown.
Asterisks indicate significant difference from controls:
* p <.05; ** p <.01.
LD:Light/Dark; Cont, Control; Mel, Melatonin; Thio, Thiourea
α difference between the 2 control photoperiods p < .01
β difference between the 2 control photoperiods p < .05
Cont, Control; Mel, Melatonin; Thio, Thiourea.

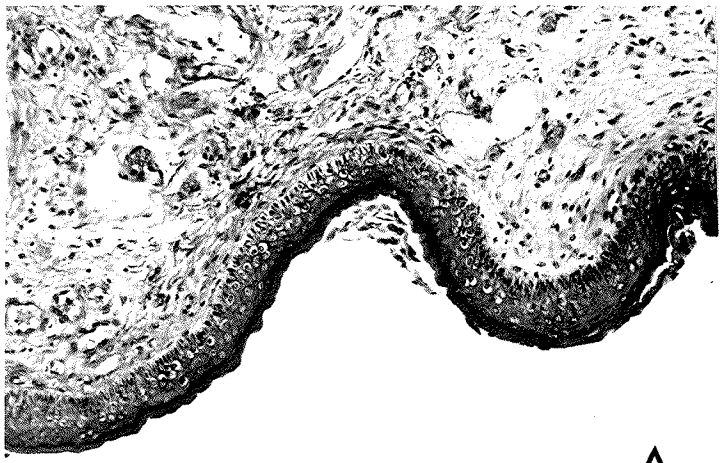
resulted in atrophy of the vagina (Fig. 11b); compared to controls (Figs. 10 and 11a), there was a reduction in proliferation of vaginal epithelium, the stratified squamous epithelium was replaced by columnar epithelium and no mitotic figures were noted in vaginal sections of hamters treated with melatonin.

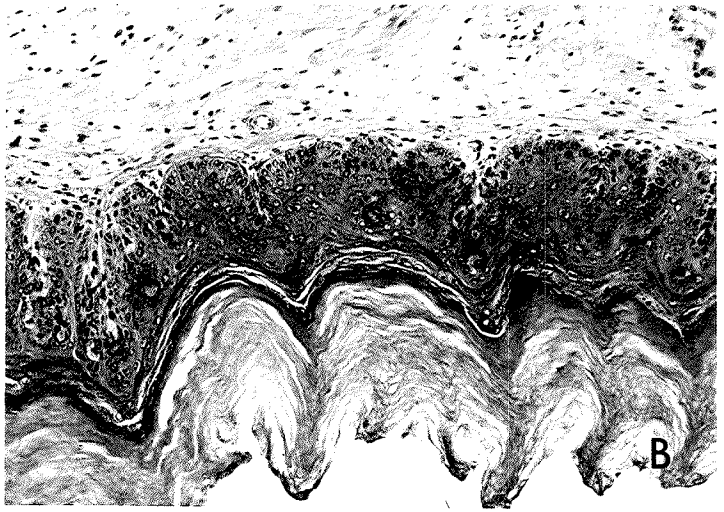
Estradiol injections increased the proliferation of vaginal epithelium; the vaginal epithelium was stratified squamous and mitotic figures were observed in vaginal sections of hamsters treated with estradiol and melatonin. With the higher doses of estradiol, the proliferating vaginal epithelium appeared to be breaking down by exfoliation (Fig. 11d).

As in experiment 1, melatonin injections in hamsters under LD 14:10 resulted in atrophy of the uterus; the dormant uterus had a reduced number of endometrial glands and a reduction in the endometrial epithelium (Fig. 12b). The reduction in weight resulting from melatonin injections was reversed by estradiol (Table 5). Estradiol injections restored the dormant uterus of melatonin treated hamsters at a dose of 1 μg; at this dose, however, cyst formation was present in the endometrium (Fig. 12c). With increasing doses of estradiol, the cystic hyperplasia increased and reduced the endometrial epithelium (Figs. 12d & 12e).

Compared to controls, the ovaries of hamsters in LD 14:10 treated with melatonin alone contained few growing and mature follicles, rare corpora lutea and a predominance of interstitial tissue (Fig. 13b). Estradiol injections resulted in an increase in the number of growing follicles in animals receiving melatonin; these growing follicles, however, were atretic (Fig. 13c). No mature follicles or corpora lutea

Figure 10. Histological analysis of vagina from control hamsters in LD 14:10 demonstrating the progressive stages of the 4-day estrous cycle. Sections (5 μm) were stained with H & E. A, Proestrus. The vagina is under estrogen activity. The vagina is characterized by a thin red superficial cornified epithelial layer. B, Estrus. The superficial cornified layer of the vagina increases in thickness. C, Diestrus 1. Post-ovulatory stage characterized by cellular desquamation of the superficial cornified layer. D, Diestrus 2. The vagina epithelium undergoes regeneration. (magnification x30).


A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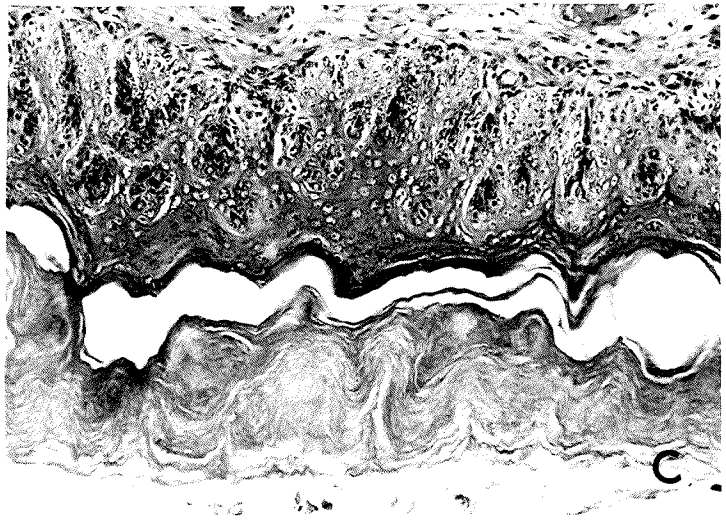
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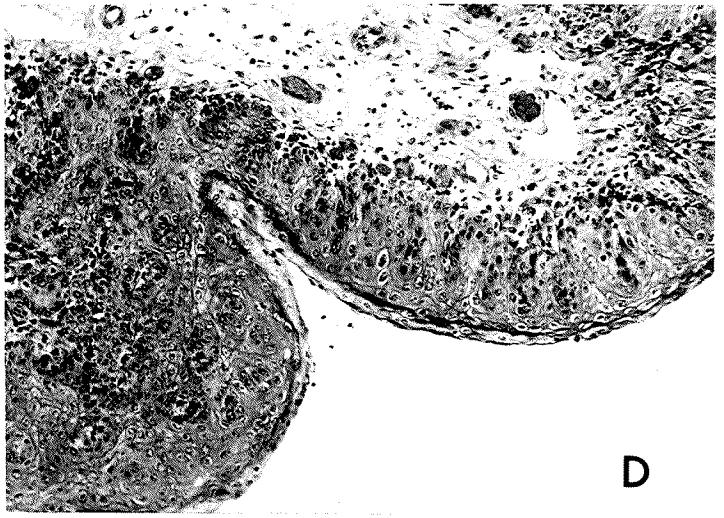

D

Figure 11. Histological analysis of vagina from hamsters in LD 14:10 treated with melatonin and various doses of estradiol. Sections (5 µm) were stained with H & E. A, Control. The vagina is in Diestrus 2. B, 25 µg melatonin daily. Note the atrophy of the vagina epithelium. The stratified squamous epithelium is replaced by columnar epithelium. C, 25 µg and 5 µg estradiol daily. Note the vagina epithelium is stratified squamous and no longer atrophic. D, 25 µg melatonin and 10 µg estradiol daily. Note the breakdown of the superficial stratified squamous epithelium into the lumen. (magnification x30).

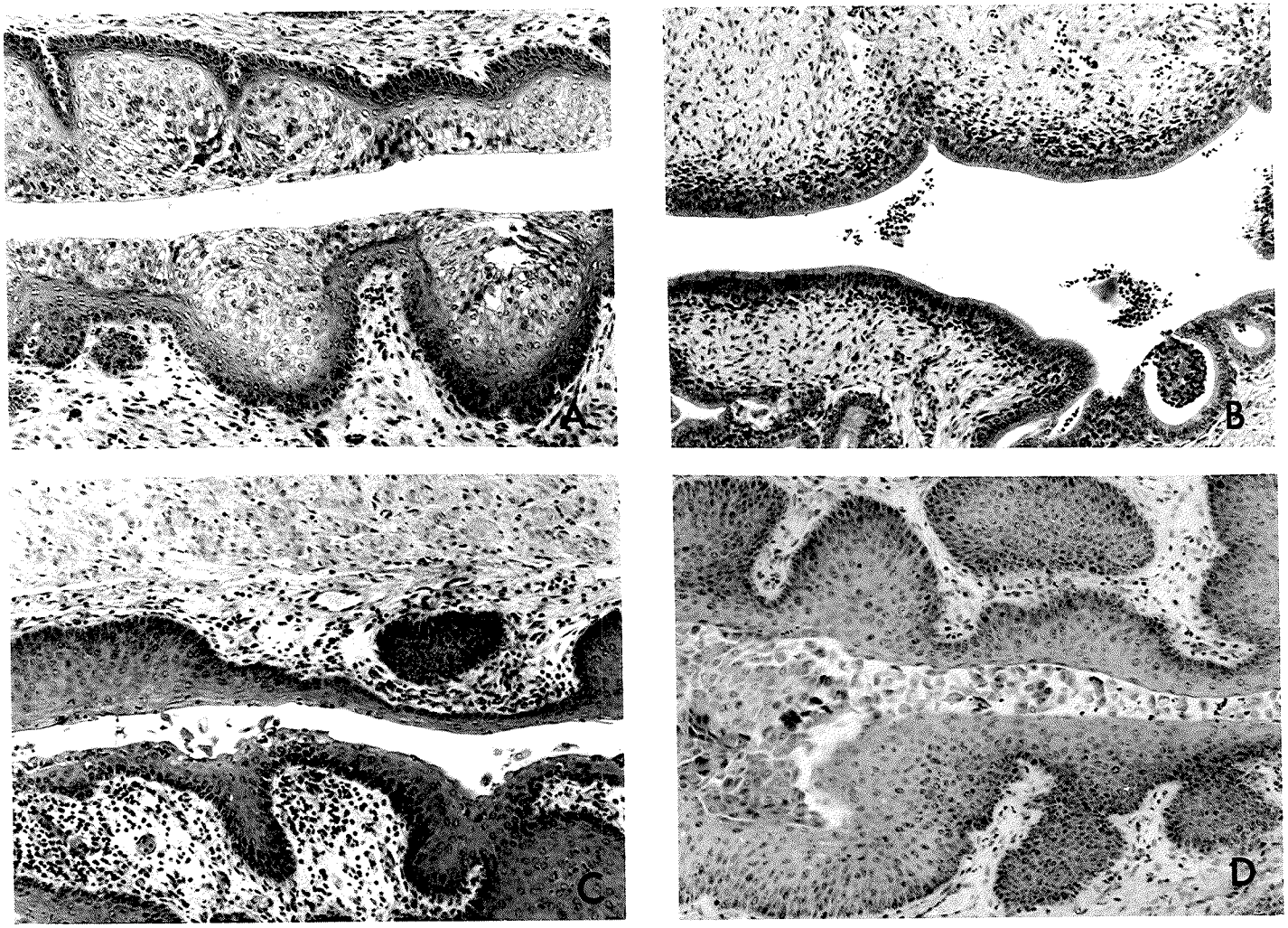

A
B
C
D

Figure 12. Histological analysis of uterus from hamsters in LD 14:10 treated with melatonin and various doses of estradiol. A, Control (magnification x4.2); B, 25 μg melatonin daily (magnification x8.0). Note the dormant uterus; the atrophied uterus has fewer endometrial glands, reduced vasculature and an increase in connective tissue. C, 25 μg melatonin and 1 μg estradiol daily (magnification x4.5). Note the increase in vascularization and the number of endometrial glands in the endometrium. D, 25 μg melatonin and 5 μg estradiol daily (magnification x5.0); E, 25 μg melatonin and 10 μg estradiol daily (magnification x4.5). Note in D & E the endometrium contains cysts surrounded by secretory endometrial epithelium. e, endometrium; m, myometrium; p, perimetrium; arrows, endometrial glands.

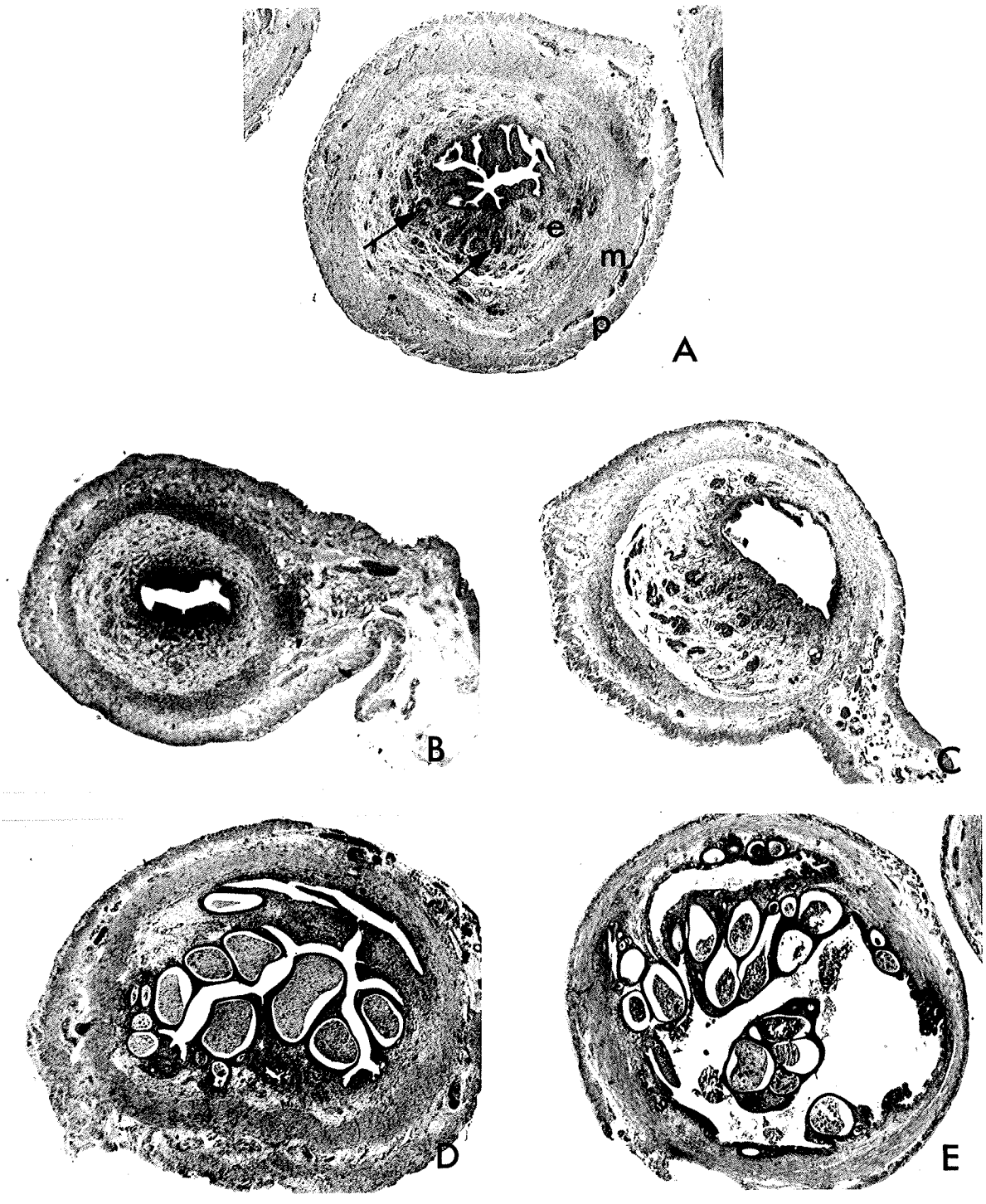

e
m
p
A
B
C
D
E

Table 5. Effects of melatonin and various doses of estradiol on body (g) and organ weights (mg) in female hamsters exposed to LD 14:10.

Treatment	Uteri	Ovary	Pituitary	Body
Saline control	390±45	104±13	6.1±.4	184±7
Melatonin	138±10**	70±6 *	3.8±.1**	189±17
Oil control	522±40	99±2	6.3±.7	169±9
Mel+Est (1 μg)	367±39*	80±4	4.9±.1	185±10
Mel+Est (5 μg)	660±24*	92±7	5.5±.4	165±12
Mel+Est (10 μg)	968±54**	97±7	6.0±.2	164±8

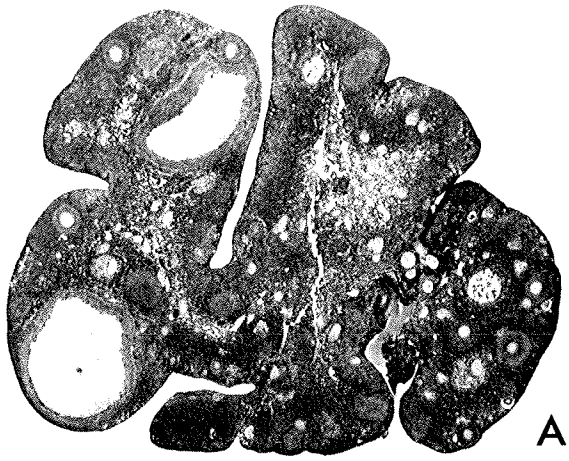
Mean and standard errors displayed.

Asterisks indicate significant difference from controls:

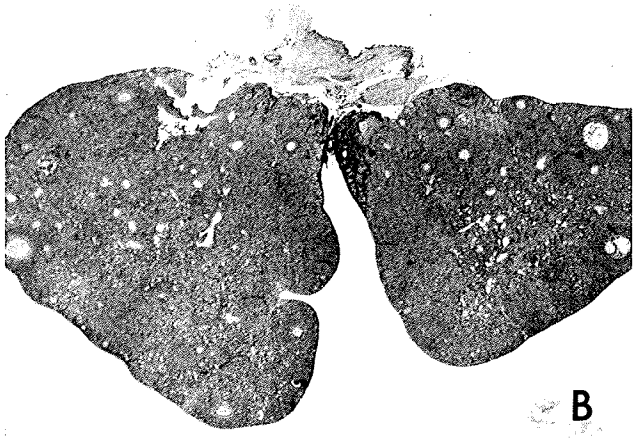
* $p < .05$; ** $p < .01$.

Mel, Melatonin; Est, Estradiol.

Figure 13. Histological analysis of ovaries from hamsters in LD 14:10 treated with melatonin and various doses of estradiol. Sections (5 μm) were stained with H & E. A, Control (magnification x5.8); B, 25 μg melatonin daily (magnification x3.0). Note the absence of growing and mature follicles, devoidance of corpora lutea and abundance of ovarian interstitium. C, 25 μg melatonin and 1 μg estradiol daily (magnification x5.5). Note that the growing follicles are atretic. D, 25 μg melatonin and 5 μg estradiol daily (magnification x5.0); E, 25 μg melatonin and 10 μg estradiol daily (magnification x4.5). Note in D & E that the number of growing, but atretic follicles are reduced with increasing doses of estradiol.



A



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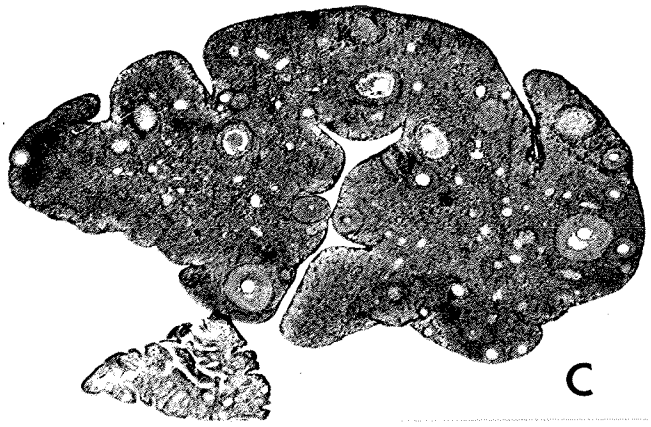
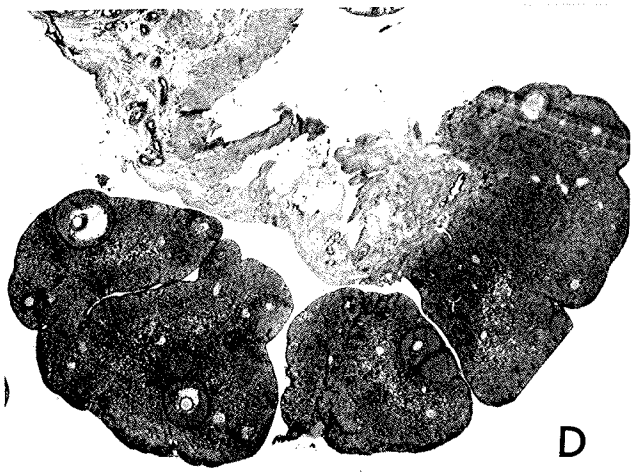
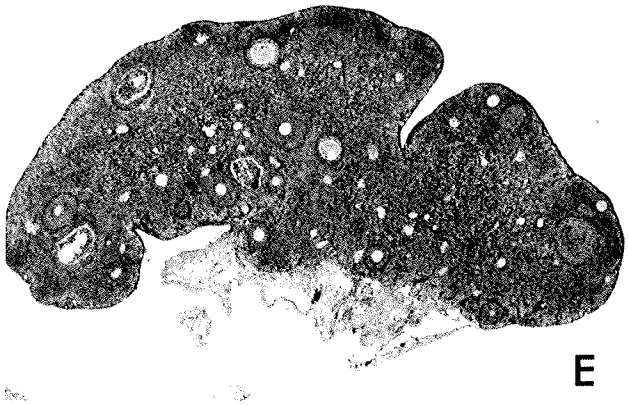


C



D



E

were observed in hamsters receiving melatonin plus estradiol (Fig. 13d). With increasing doses of estradiol, the number of growing, but atretic, follicles were reduced (Fig 13e).

Pituitary content of LH (Table 3) was reduced to less than 20% by estradiol in animals receiving melatonin ($p < .01$). Serum LH was not significantly influenced by melatonin in Experiment 2. In combination with 1 μg daily estradiol injections serum LH was increased to 30% of controls ($p < .05$). With the higher doses of estradiol serum LH levels were restored to control levels.

Pituitary content of FSH (Table 3) was increased to 146% of controls by melatonin alone ($p < .05$). In melatonin treated hamsters pituitary FSH was significantly reduced by estradiol injections in a dose dependent fashion. Serum FSH was not significantly influenced by melatonin in Experiment 2.

Pituitary content of PRL (Table 3) was reduced to 8% of controls ($p < .01$) by melatonin alone. This decrease was reversed (to 87% of saline controls) by estradiol injections. Serum PRL was also increased, in hamsters receiving melatonin, by estradiol, in a dose dependent fashion.

No significant effect of melatonin was noted on pituitary or serum TSH in this experiment.

Serum estradiol was reduced by melatonin alone (Table 4). Estradiol injections increased serum estradiol to values at least 7 times that of oil carrier controls.

Experiment 3

Table 3. Effects of melatonin and various doses of estradiol on TSH, PRL, LH and FSH in female hamsters in LD 14:10.

	LH		FSH		PRL		TSH	
Treatment	ng/pit	ng/ml	ng/pit	ng/ml	ng/pit	ng/ml	ng/pit	ng/ml
Saline Cont	679±83	33±6	239±15	251±125	72±7	7±2	105±19	9±1
Mel	867±94	25±5	348±29*	167±28	6±2**	2±1	93±15	10±1
Oil Cont	958±122	29±6	244±45	128±38	83±7	47±17	87±6	13±1
Mel+Est (1μg)	170±10**	87±40*	222±34	289±71	47±5**	5±0	73±12	9±2
Mel+Est (5μg)	115±15**	55±12	133±29*	352±24*	63±8*	100±37	77±10	11±1
Mel+Est (10μg)	128±9 **	33±3	90±15*	238±15	61±3*	145±24**	89±17	8±18*

Mean and standard error displayed.
Asterisks indicate significant difference from controls:
* $p < .05$; ** $p < .01$.
Cont, Control; Mel, Melatonin; Est, Estradiol.

Table 4. Effects of melatonin and various doses of estradiol on serum estradiol levels in female hamsters under LD 14:10.

Treatment	pg/ml
Saline control	54 ±20
Melatonin	21±0
Oil control	175±57
Melatonin+Estradiol (1 μg)	1343±225**
Melatonin+Estradiol (5 μg)	1363±97 **
Melatonin+Estradiol (10 μg)	1971±151**

Mean and standard error displayed.

Asterisks indicate significant difference from controls:

* $p < .05$; ** $p < .01$.

No significant difference was noted in the histology of the uterus, ovary and vagina in hamsters treated with various doses of L-dopa compared to controls.

No significant difference was noted in pituitary and serum LH, FSH, PRL and TSH among the experimental groups treated with various doses of L-dopa (Table 6).

Uterus, ovary and pituitary weights (Table 7) did not differ significantly from controls. Body weights (Table 7) were increased in a dose dependent fashion to 121% of controls by 25 μg and 100 μg L-dopa ($p < .01$).

Table 6. Effects of various doses of levodopa on TSH, PRL and gonadotropins in female hamsters maintained in LD 14:10.

	LH		FSH		PRL		TSH	
Treatment	ng/pit	ng/ml	ng/pit	ng/ml	ng/pit	ng/ml	ng/pit	ng/ml
Saline Cont	763±93	22±3	280±16	244±93	66±7	16±3	95±12	10±1
L-dopa (1μg)	846±58	31±8	293±33	210±105	76±4	31±12	92±8	8±1
L-dopa (25μg)	1028±140	31±4	274±25	44±22	76±7	53±22	88±13	9±1
L-dopa (100μg)	872±124	62±64	242±22	220±93	75±8	41±22	93±8	7±3

Mean and standard error displayed.
Cont, Control.

Table 7. Effects of various doses of levodopa on body (g) and organ weights (mg) in female hamsters maintained in LD 14:10.

Treatment	Uteri	Ovary	Pituitary	Body
Saline control	398±17	85±7	5.7±.3	169±7
L-dopa (1 µg)	508±53	85±6	6.5±.3	187±5
L-dopa (25 µg)	552±57	93±6	6.4±.5	205±4**
L-dopa (100 µg)	423±59	95±3	6.3±.2	204±9**

Mean and standard error displayed.

Asterisks indicate significant difference from controls:

** $p < .01$.

Discussion

The main objective of the study was to ascertain a possible relationship between melatonin and thyroid hormones in influencing the reproductive system in the female hamster.

There are several findings in the present study concerning the effects of melatonin and its relation to thyroid status. In Experiment 1, daily melatonin injections rendered female hamsters acyclic after 6 weeks of treatment, an observation first reported by Tamarkin et al. (67). Histologically, the ovaries of melatonin-injected hamsters were characterized by few developing and mature follicles, rare corpora lutea and a predominance of interstitial tissue. These observations are consistent with an earlier report by Vaughan et al. (68).

In Experiment 1, serum levels of LH and FSH were significantly elevated by melatonin injections given near the end of the daily light period of the light-dark cycle; these results are in agreement with those of Tamarkin et al. (67). In some studies, the increase in serum LH and FSH with daily melatonin injections was not consistently observed (67,68). The elevated serum LH in the present study provides further evidence that the growth of ovarian interstitium is dependent upon LH (56,103).

In Experiment 1, data obtained from a homologous PRL radioimmunoassay of Soares et al. (102) showed that daily injections of 25 μg melatonin reduced pituitary PRL to 12% and serum PRL to 15% compared to control levels. Melatonin induced depression of pituitary and serum PRL was previously reported in studies using a heterlogous radioimmuno-

assay for PRL (68,72), although a reduction in plasma PRL was not a consistent finding. This could be related to the labile nature of serum prolactin levels (104).

Depressed levels of circulating thyroxin are consistently found in experiments with melatonin injections in hamsters (87,92); in Experiment 1, circulating levels of thyroxin were reduced to 47% of controls, indeed a substantial decrease. Circulating levels of TSH have been reported as depressed by melatonin injections in one study (87), but in the present study, daily melatonin injections had no significant effect on circulating levels of TSH.

The present study (Experiment 1) is the first to report that daily melatonin injections resulted in reduced circulating levels of estradiol.

The effects of melatonin could be explained by an action on one or more of the hypothalamic releasing factors. Although there are no reports of the effects of melatonin on LHRH release, short photoperiod (which presumably acts via endogenous melatonin) has been reported to interfere with LHRH release (105,106). Melatonin could interfere with both LHRH and PRF (prolactin releasing factor) release, altering gonadotropin and PRL secretion. Alternatively, the effects of melatonin could be explained as a result of a change in TRH release. Melatonin has been shown to alter TRH content of the medial basal hypothalamus (89). A decrease in TRH release would be expected to reduce pituitary release of TSH and PRL. Thyroid hormones, on the other hand, have been demonstrated to regulate TRH receptors in the pituitary (96-98), providing a mechanism by which thyroid hormones may under some circumstances influence

PRL secretion. Estradiol also influences TRH binding in the pituitary (96). Factors influencing the number of TRH receptors in the piutuitary do not seem to be a major factor in the effects of melatonin in the present study. Changes in PRL secretion would be expected to decrease gonadotropin receptors on the ovary (107-110). A decline in gonadotropin receptors could account for the decrease in estradiol production observed in melatonin treated hamsters. Reduced circulating levels of estradiol would result in elevated serum levels of LH and FSH due to the loss of the negative feedback effects of estradiol. Thus although estradiol may influence PRL levels, the melatonin induced decrease in PRL is interpreted as initially the result of melatonin influencing PRF or PIF (prolactin inhibiting factor) release.

Similar to melatonin injections, short photoperiod, LD 2:22, also resulted in a cessation of estrous cycles. Histologically, the ovaries were similar to those of melatonin-treated hamsters maintained under LD 14:10.

It was reported that female hamsters kept under a short photoperiod have a daily surge in circulating gonadotropin levels (55,62); this was interpreted as a result of low or absent estrogen (111). In normal cycling hamsters stimulatory effects of estrogen on LH surges (which occur every 4 days) were reported (82,112). It has been suggested that following the proestrous surge of LH, high levels of estradiol prevent the repetitive daily LH surges (113) which could otherwise occur. Low levels of estradiol have also been reported to account for the low serum PRL in short photoperiod induced anestrous in female hamsters (114). Estrogen has been known to stimulate PRL release at the pituitary level

(115) in women, hamster and rat (114,116,117). In the present study, low estradiol could contribute to maintaining low PRL levels in melatonin-injected hamsters.

The results of Experiment 1 are consistent with the view that the changes in circulating gonadotropins and PRL observed in melatonin-injected hamsters under LD 14:10 are also a result of low or absent estrogen levels. In Experiment 2, estradiol injections reversed the inhibitory effects of melatonin on several parameters at doses that significantly influenced the release of pituitary gonadotropins. The reduction in pituitary weight, the reduction in uterine weight, the atrophy of the vagina, and the depression in pituitary PRL and serum PRL, were all reversed by estradiol injections. These results suggest that many of the effects of melatonin are caused by changes in the secretion of estradiol.

Changes in prolactin secretion appear to play an important role in the melatonin induced inhibition of estrous cycles. Early investigations have established prolactin as a luteotropic hormone in maintaining the corpora lutea during the normal cycle in both the rat (118) and hamster (103). Differentiation of granulosa cells into luteal cells involves a reduction in LH, FSH and estradiol receptor content and an increase in PRL receptors (119-121) which coincides with increased responsiveness of the luteal cells to prolactin (107).

Thiourea induced hypothyroidism influenced several aspects of the reproductive system of the hamster. Hypothyroidism had no significant effect on pituitary LH or FSH content. Both pituitary and serum

prolactin were significantly reduced by thiourea; the reduction in serum PRL was partially reversed by T4 replacement.

In four of the five animals in LD 14:10 receiving thiourea alone, serum estradiol was reduced. However, one hamster displayed high serum estradiol levels which increased the variance for that group. Animals receiving thiourea plus T4 replacement had estradiol levels that were not significantly different from controls.

Hamsters made hypothyroid with thiourea in the drinking water displayed irregular estrous cycles. Papanicolaou smears showed numerous intermediate and parabasal cells compared to smears of hamsters receiving thiourea plus T4 replacement. It was noted that the parabasal cells were aglycogenic, suggesting estrogen deficiency. Since the maturation of squamous epithelia is estrogen dependent, this implies that the moderate estrogen deficiency in 4 of the 5 hypothyroid hamsters, would explain the presence of intermediate and parabasal cells in Papanicolaou smears. Hamsters receiving T4 replacement had higher levels of estrogen than hypothyroid hamsters, therefore explaining the reduction in intermediate and parabasal cells. Papanicolaou smears of melatonin treated hamsters showed intermediate and parabasal cells also suggesting estrogen deficiency.

The ovaries of hamsters receiving thiourea were nearly devoid of corpora lutea and contained fewer developing and mature follicles than controls. The effects of thiourea on the ovary were noted previously by Reiter et al. (122). Thyroxin replacement resulted in ovaries that contained many developing and mature follicles and several corpora lutea, comparable to control ovaries. One possible explanation for the

effects of thyroid hormones on the ovary is that thyroid status regulates PRL binding sites on the ovary. Hyperthyroidism was reported to increase and hypothyroidism to decrease the number of PRL receptors in kidney membranes (123). Both hypophysectomy and thyroidectomy in male rats reduced PRL binding whereas a single thyroxin injection rapidly restored binding to intact controls (123). Therefore, reduced thyroxin levels would be expected to reduce the concentration of PRL receptors on the ovary. A reduction in thyroxin would also influence PRL release. Low serum PRL levels would reduce the concentration of gonadotropin receptors (PRL has been shown to induce LH receptors on the ovary), ultimately reducing the estradiol production, as evident in 4 of the 5 animals treated with thiourea alone. Low serum estradiol would further contribute to maintaining low serum PRL, since estrogen has been shown to stimulate PRL release at the pituitary level (114-117). Reduced serum PRL levels and reduced concentration of PRL receptors on the ovary would prevent the differentiation of granulosa cells into luteal cells, resulting in atretic follicles and fewer corpora lutea.

Thiourea significantly reduced uterus weights in animals under LD 14:10, (the uterus is estrogen dependent); this effect of thiourea was partially reversed by T4 replacement.

A comparison can be made between the effects of thiourea administration and melatonin administration. Both interfered with estrous cycles and influenced the levels of reproductive hormones. Melatonin, however, was much more effective in blocking estrous cyclicity and in interfering with the hormones of reproduction. This may be because melatonin has an effect on a CNS site whereas thiourea has an effect

on a local site, the thyroid gland. A direct effect of thyroxin on the ovary appears to be required for normal luteinization. Experiment 3 showed that similar effects could not be obtained with L-dopa.

Experiment 1 was designed to study not only the independent effects of melatonin and hypothyroidism, but also the interaction between the two treatments. Results showed that ovarian histology and vaginal smears of hamsters receiving melatonin were similar irrespective of thyroid status. Pituitary LH and FSH content, although not significantly changed by melatonin alone, were significantly increased by melatonin in thiourea treated hamsters; these effects were reversed by T4 replacement. Reduction in T4 could result in a change in the synthesis of neurotransmitters (norepinephrine, dopamine or serotonin), altering LHRH release which changes the secretion of LH and FSH. These results suggest that the effect of melatonin on gonadotropin regulation depends on thyroid status. Hypothyroidism, with or without T4 replacement, on the other hand, did not significantly influence melatonin induced inhibition of pituitary or serum prolactin. Similarly, the effect of melatonin on circulating estradiol levels was not significantly influenced by thyroid status.

The site of action of melatonin is not well understood. The present results suggest that thyroid hormones and estradiol do play a role in the sequence of events which lead to atrophy of the reproductive tract in female hamsters. Although thyroid status did not influence the effect of melatonin on pituitary and plasma levels of PRL in Experiment 1, thyroxin has been reported to influence prolactin's action in liver and mammary gland of mammals (124,125) and in lower vertebrates (126).

Although many of the effects of melatonin observed in this study can be interpreted as results of low estrogen levels, the observations are also consistent with the view that melatonin acts via one or more of the hypothalamic releasing factors.

In conclusion, these results suggest that 1) melatonin blocks changes in the reproductive tract and the hormones of reproduction associated with the estrous cycle by disrupting the feedback relationship between estradiol and gonadotropins 2) although similar changes occurred in hypothyroid hamsters, melatonin was more effective 3) T4 replacement reversed the effects of hypothyroidism on the ovary; this T4 replacement effect was prevented by melatonin 4) melatonin induced changes in the uterus and vagina were prevented by estradiol at doses which inhibited pituitary gonadotropins 5) gonadotropin secretion is influenced by a CNS interaction of melatonin and thyroid hormones.

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