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Ovarian Hormone: Lack of Effect on Reproductive Structures of Female Asian Musk Shrews

Abstract. *Uterine and vaginal weights and histologies are not altered by ovariectomy or estrogen treatment in the Asian musk shrew (*Suncus murinus*). In addition, ovariectomized shrews mate. Thus, the role of ovarian hormones in the control of the reproductive status of this species does not conform to the accepted mammalian pattern.*

It is generally assumed that ovarian estrogens are required for the development and support of the sex accessories of female mammals. This generalization has been derived from observations of a limited number of species. However, the importance of estrogen in the reproduction of female elephants and Asian musk shrews has been questioned. In elephants, Plotka *et al.* (*1*) failed to demonstrate a distinct relation between the reproductive state (immature, mature, and pregnant) and the very low concentrations of estradiol in the plasma. Similarly, the concentration of plasma estradiol in adult musk shrews is below detection by radioimmunoassay (*2*). These observations suggest that the target tissue receptor mechanisms are extremely sensitive to low estradiol titers or that the sex accessories such as the uterus and vagina in these forms are not dependent on estradiol.

Since insectivores are generally considered the most primitive living eutherians (*3*), and the effects of sex steroids have not been studied in them, we have examined the sex accessories in tropical Asian shrews (*Suncus murinus*) subjected to gonadectomy and hormone replacement therapy. The results of this study indicate that the uterus and vagina in this species are apparently not dependent on ovarian hormones.

The history and maintenance of the *Suncus* colony have been described (*4*). Healthy mature animals not previously used for experimental purposes were used throughout (*5*). The effects of ovariectomy on uterine and vaginal histologies are shown in Table 1. Microscopic examination revealed no significant differences between the sex accessories from intact and ovariectomized shrews. Uterine and vaginal weights were also not dependent on ovarian hormones (Table 2). Wet and dry weights of these organs did not decline following ovariectomy and multiple injections of estradiol (*6*) failed to increase these weights in gonadectomized shrews. Changes in vaginal cytology of rodents are commonly employed in the bioassay of estrogenic hormones. Table 2 shows the effects of ovariectomy and estrogen treatment on the vaginal smear in *Suncus*. Nucleated epithelial cells predominated in the vaginal smear of intact shrews (*7*), and this smear was not altered by long-term ovariectomy or injection of massive amounts of the potent synthetic estrogen, diethylstilbestrol.

Since female shrews showed no anatomic dependence on ovarian hormones (Tables 1 and 2) it was of interest to examine the effects of orchidectomy and androgen treatment (*8*) in male shrews (Table 2). Long-term castration resulted in significant reductions in the weights of the prostate, ampulla, and epididymis. In addition, the weights of these organs were increased by testosterone treatment. It has also been shown that the plasma testosterone level in male musk shrews is similar to that observed in other mammalian species (*9*). Thus, maintenance of male sex accessories in *Suncus murinus* conforms to the pattern described for other mammals.

To evaluate the possibility that the apparent lack of ability of the shrew uterus and vagina to respond to estrogenic hormones (Table 2) is due to a corresponding absence of estrogen receptors, various tissues from females were incubated with [^{3}H]estradiol (*10*). After incubation, the amount of [^{3}H]estradiol bound to the nuclear fraction was determined (Fig. 1). The nuclear uptake of [^{3}H]estradiol by corresponding tissues of rats is shown in Fig. 1 for comparison [see also (*11*)]. The uterus and vagina of the rat incorporate more [^{3}H]estradiol than does muscle or kidney. This preferential accumulation is due to the presence of estrogen receptors which are confined to estrogen target tissues (*12*). The shrew uterus and vagina also retain more [^{3}H]estradiol than muscle or kidney, an indication that these reproductive tissues also have specific estrogen receptors.

Uterine and vaginal atrophy following ovariectomy during the reproductive period has been observed in most mammalian orders and has generally been assumed to be a universal phenomenon in mammals. Involution is characterized by

Table 1. Histology of the uterus and vagina in normal and ovariectomized shrews. Shrews were ovariectomized for a period of 1 month. Results are expressed as means ± standard error of the mean of three determinations.

Treatment	Diameter of endometrial gland (μ)	Height of uterine epithelial cells (μ)	Thickness of vaginal epithelium (μ)
Nonovariectomized	55.0 ± 4.5	44.3 ± 4.9	28.7 ± 2.3
Ovariectomized	52.7 ± 2.7	47.2 ± 2.8	30.7 ± 2.5

Table 2. Effects of gonadectomy and sex steroid treatment on the sex accessories of the Asian musk shrew. Tissues were examined 3 months after gonadectomy. Ovariectomized shrews were injected with estradiol (25 μg/day) for 5 days before the uterine and vaginal weights were determined. For determination of the vaginal smears, ovariectomized females received diethylstibestrol (1 mg/day) for 5 days. Vaginal smears in all three groups contained greater than 90 percent nucleated epithelial cells. Orchidectomized males received testosterone (2.5 mg) for 7 days. Results are expressed as means ± the standard error of the mean of five to nine determinations.

Treatment	Female					Male		
	Uterine weight (mg)		Vaginal weight (mg)		Vaginal smear (nucleated epithelial cells)	Prostate (mg)	Ampullae (mg)	Epididymides (mg)
	Wet	Dry	Wet	Dry				
None	8.6 ± 2.0	2.5 ± 0.4	38 ± 4	8.5 ± 0.9	6/6	44 ± 8	47 ± 7	23 ± 2
Gonadectomized	8.3 ± 1.4	2.3 ± 0.3	40 ± 5	9.6 ± 1.2	7/7	11 ± 1	8 ± 1	7 ± 1
Gonadectomized plus sex steroid treatment	10.1 ± 0.7	2.4 ± 0.4	41 ± 5	9.7 ± 1.4	5/5	42 ± 7	33 ± 7	19 ± 4

marked reductions in the weight and histological appearance of the organs. The data in Tables 1 and 2 indicate that ovariectomy is without effect on these parameters in captive Asian musk shrews, and that ovarian hormones are not required for the maintenance of these sex accessories in adult nonpregnant shrews. We cannot exclude the possibility that ovariectomy results in subtle cytological or biochemical changes that were undetected in this work. It is also possible that ovarian hormones are necessary for sexual receptivity or development of the genital tract before puberty and during pregnancy. However, in most mammals the genital tissues become extensively differentiated in response to ovarian and placental hormones during pregnancy, whereas there are no gross histological differences in the uterus and vagina of nonpregnant as opposed to pregnant musk shrews (*13*). In addition, sexually inexperienced shrews mate after they have been ovariectomized (*14*). Thus, in several respects the role of ovarian hormones in the maintenance of reproductive status of this species does not conform to the accepted mammalian pattern.

We cannot exclude the possibility that steroids of adrenal origin may be requisite for reproductive function in *Suncus*. Plasma and adrenal progesterone levels are approximately 20 percent higher in sexually receptive as compared to nonreceptive shrews (*2*).

Correlations have been observed between the concentration of estrogen receptors in the rat uterus and the ability of estradiol to elicit uterine responses; in addition, correlations between receptor content and hormone responses have been observed for nonsteroidal hormones (*15*). The apparent lack of correlation between the presence of estrogen receptors (Fig. 1) and the absence of estrogen-induced responses (Tables 1 and 2) in the uterus and vagina of the musk shrew may be explained by: (i) reproductive tissues may actually respond to estradiol, but the responses produced were not detected; (ii) nuclear-bound estradiol is not bound to the estrogen receptor but to another estrogen-binding component; (iii) the uterus and vagina may lack a component other than the estrogen receptor which is required for the action of estradiol. Which of these factors is responsible for our results remains to be determined.

In mammals that exhibit estrous cycles, the uterus and vagina must be highly responsive to and dependent on gonadal steroids for growth and function. Similarly, in seasonal breeders the female sex accessories are maintained by gonadal steroids during the reproductive season. In musk shrews there is no discernible estrous cycle; ovulation is induced by mating, and Graafian follicles form only after copulation (*13*). In addition, there is no seasonal reproductive quiescence, either under natural conditions (*12, 16*) or in captivity (*13*). These considerations may explain why ovarian hormones are not requisite for the maintenance of reproductive structures of nonpregnant Asian musk shrews.

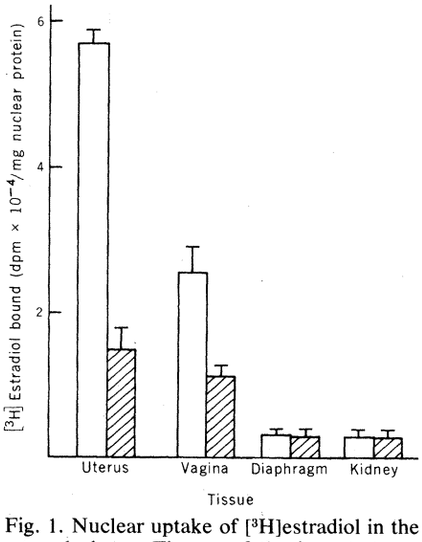

Fig. 1. Nuclear uptake of [^{3}H]estradiol in the rat and shrew. Tissues of the immature rat (open bars) and ovariectomized shrew (crosshatched) were incubated in vitro with [^{3}H]estradiol. After incubation the concentrations of [^{3}H]estradiol bound to the nuclear fractions were determined.

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6. The amount of estradiol employed (about 1 mg per kilogram of body weight per day) is approximately 10^3 greater than the dose required to elicit maximal uterine hypertrophy in the rat [J. N. Anderson, J. H. Clark, E. J. Peck, Jr., *Biochem. Biophys. Res. Commun.* **48**, 1460 (1972); J. C. Eldridge, J. C. McPherson, V. B. Mahesh, *Endocrinology* **94**, 1536 (1974)].
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8. Adults (3 to 6 months old) weighing 36 to 48 g were used. They were bilaterally castrated under ether anesthesia. After treatment they were decapitated and the wet weights of their paired prostates, ampullae (vasal glands positionally similar to rodent seminal vesicles), and epididymides were obtained.
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11. The amounts of [^{3}H]estradiol bound by the tissues of the adult ovariectomized rat, in terms of disintegrations per minute per milligram of nuclear protein, were: uterus, $2.0 \pm 0.2 \times 10^4$; vagina, $1.4 \pm 0.3 \times 10^4$; diaphragm, $0.3 \pm 0.02 \times 10^4$; and kidney, $0.3 \pm 0.05 \times 10^4$. These values are similar to those shown in Fig. 1 for the corresponding tissues of the adult ovariectomized shrew.
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Loss of Y-Cells in the Lateral Geniculate Nucleus of Monocularly Deprived Tree Shrews

Abstract. *In tree shrews* (Tupaia glis) *reared with one eye closed, Y-cells were almost entirely absent in the binocular segment of the lateral geniculate laminae receiving input from the deprived eye. Y-cells were found in the monocular segment of these laminae, and in the binocular segment of the laminae with input from the normal eye. X-cells were present in both the deprived and normal laminae and appeared unaffected by the deprivation. A number of abnormal cells were also found, and these were located primarily in the binocular segment where Y-cells were absent.*

Tree shrews share with primates a well-developed geniculostriate visual pathway and may bear strong resemblance to the common ancestor of the primate line (*1*). They differ from the cat, on which so much of our knowledge of visual functioning is based, not only in evolutionary history, but in a number of features of their visual system. For example, compared with cats, tree shrews have more retinal cones, a larger, more differentiated superior colliculus, and a lateral geniculate nucleus which projects solely to the striate cortex (*1–3*). Despite these and other important differences, we previously found that tree shrews have X- and Y-cells in their lateral geniculate nucleus (*4*). Our suggestion that other more disparate species might also possess X- and Y-cells has recently been confirmed (*5*). In this report, we extend the analogy between the X- and Y-cells of cat and tree shrew to include the effects of visual deprivation on the postnatal development of these cells.

Previous work has shown that in cats reared with sutured eyelids there is a selective reduction in the population of lateral geniculate Y-cells sampled with microelectrode techniques. In cats reared with one eye closed this inability to record from Y-cells is limited to the binocular segment of the lateral geniculate laminae receiving afferents from the deprived eye (that is, the deprived laminae); Y-cells are apparently unaffected in the deprived monocular segment, as are X-cells throughout the nucleus (*6*). We have found that the same pattern of Y-cell "loss" occurs in the lateral geniculate nucleus of monocularly deprived tree shrews, while X-cells seem to be unaffected.

In seven tree shrews (*Tupaia glis*) we sutured the lids of one eye at about 7 days after birth, well before the normal eye opening which occurs at about 20 days of age. The animals were hand-reared until weaning, and then cage-reared normally until 4 to 24 months of age, when they were anesthetized and prepared for single-unit recording. All experimental techniques, recording procedures, and criteria for classification of X-and Y-cells were identical to those we used previously in normal tree shrews and cats (*4, 6–8*).

In each animal we placed our recording electrode in the lateral geniculate nucleus contralateral to the deprived eye. This allowed sampling of neurons both in the binocular and monocular portions of the visual field in the four deprived laminae (*3*). The two laminae which receive ipsilateral nondeprived eye input provided control data from the binocular portion of the visual field.

Our primary finding is that we obtained recordings from very few Y-cells in the binocular portion of the visual field in the deprived laminae, while we found many Y-cells in the monocular portion. Figure 1 shows the distribution in the visual field of the cells sampled in the deprived laminae. In the binocular segment, only two of the 46 relay cells sampled were Y-cells, a significantly smaller proportion than were present in the normal laminae in these same animals (six Y-cells out of 18) or in the comparable laminae of normal tree shrews (15 Y-cells out of 33) (*4, 9*). Ten of the 20 cells sampled in the monocular segment of the deprived laminae were Y-cells, and this ratio is normal (*4*). The receptive-field center diameters, conduction latencies from optic chiasm and from cortex, and responses to visual stimuli of the Y-cells we sampled in these deprived laminae were not significantly different from the values established in our earlier experiment on normal tree shrews (*4, 7, 10*). The X-cells, and a few cells with mixed X- and Y-properties (that is, "mixed" cells) (*4*), were also not significantly different from the cells in normal animals in their response properties, and these cells were present in normal proportions in the deprived and nondeprived laminae of the lid-sutured tree shrews. Our data thus indicate an inability to record Y-cells, and suggest that this "loss" is restricted to the binocular segment of the deprived laminae. Furthermore, we found no evidence for an effect of the deprivation on X-cells (*11*).

In addition to the selective effect on Y-cells in the deprived tree shrews, we found 17 cells with properties that we never observed in normal animals. Sixteen of these 17 abnormal cells were located in the deprived laminae, 11 in the binocular, and five in the monocular segment. These cells were so sluggish in their response to visual stimuli that we could not determine the receptive-field