The Effect of ACTH, Group Caging, and Adrenalectomy in *Peromyscus leucopus* with Emphasis on Suppression of Reproductive Function[1] (36267)

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Adrenocorticotropin (ACTH) inhibits sexual maturation in immature female house mice and reproductive function in mature female house mice of five strains tested, but has little comparable effect on the reproductive system of male house mice (6). ACTH (natural porcine) also inhibits reproductive function in adrenalectomized females maintained on corticoids, although the effect is not as marked as in intact females (4, 6). Synthetic β^{1-24}-corticotropin (Ciba) has similar effects on the reproductive system of female mice (7). Therefore, the adrenal glands are not essential for this action of ACTH, although they have an augmentative role.

The action of ACTH on reproductive function in female mice apparently is at the level of the pituitary or higher, since it is overcome by injection of crude pituitary extract (6).

Preliminary experiments indicated that reproductive function in both male and female *Peromyscus* is susceptible to inhibition by ACTH (6). This paper describes experiments designed to explore further the actions of ACTH on the reproductive systems of the northern white-footed mouse *Peromyscus leucopus noveboracensis*.

Material and Methods. Animals. The *Peromyscus* used in these experiments were from outbred colonies maintained in our laboratory and were descendants of individuals trapped alive on the Letterkenny Army Depot, Franklin Co., PA, in 1959 *et seq*. They were maintained on 15 hr of light daily, at a temperature of 78°F, caged in heterosexual pairs, and fed D and G (Price Wilhoite) mouse pellets.

All experimental mice were weaned at 21 days and assigned to experimental groups as indicated in Table I. They were caged either in groups of 6 to 8 per cage (group caged) or one per cage (singly caged). Control mice usually were litter-mates of the same sex and weight as the experimental mice.

All injections were made subcutaneously between 8 and 10 a.m. daily. Control animals were injected with either 0.1 ml of the gelatin vehicle for the ACTH (Armour) or with 0.1 ml of 0.8% saline. Daily dosages of ACTH (Porcine: Armour, Organon) and duration of injections are given in Table I.

Adrenalectomy and sham-adrenalectomy were done at 26–28 days of age. Each adrenalectomized mouse was maintained on daily subcutaneous injections of 50 μg of cortisol acetate.

Mice were killed 24 hr after the last injection, the GI tract was removed; and the remainder of the animal, with slices through the larger organs, was put in 10% neutral buffered formalin. Organ weights were obtained after fixation and histological sections prepared as described elsewhere (2).

Areas of the adrenal cortex and its zones were determined by projecting transverse sections through the middle of the glands at a constant magnification, outlining the cortex and its zones, and subsequently planimetering these outlines. Analysis of zonal rela-

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TABLE I. Data as Means and Their Standard Errors for Body Weight and Organ Weights of Adrenalectomized and Intact *Peromyscus*.

Expt. 1	No.	Treatment	Age (days)	ACTH (units)	Days of inj.	Final body wt (g)	Wt (mg)			
							Adrenal	Kidney	Ovary/testis	Uterus/sem. ves.
Female	8	Intact	30	0	10	13.7 ± 0.6	7.5 ± 0.5	175 ± 11	10.9 ± 1.0	12.7 ± 2.4
P. leucopus	8	Adrenex[a]	30	0	10	13.1 ± 0.5		197 ± 7	13.0 ± 1.1	12.0 ± 0.5
	8	Adrenex + ACTH	30	4	10	12.6 ± 0.6		243 ± 3	4.4 ± 0.6	4.6 ± 0.6
	8	Sham + ACTH	30	4	10	12.6 ± 0.8	13.2 ± 0.6	213 ± 7	6.3 ± 0.9	7.0 ± 1.4
Male	8	Intact	30	0	10	14.9 ± 0.5	6.6 ± 0.7	188 ± 11	111 ± 11	14.7 ± 2.5
P. leucopus	8	Adrenex	30	0	10	14.3 ± 0.4		202 ± 8	88 ± 9	11.2 ± 1.4
	8	Adrenex + ACTH	30	4	10	14.3 ± 0.8		229 ± 9	61 ± 14	7.0 ± 1.5
	8	Sham + ACTH	30	4	10	15.3 ± 0.5	10.7 ± 1.3	213 ± 9	64 ± 11	4.0 ± 0.8

[a] Adrenalectomized.

tionships were not considered necessary for every experiment once the relationships between weight and zonal changes were established.

Statistical evaluations were by analyses of variance and *t* tests where appropriate.

Results. The results from all experiments and the means and standard errors of the means of the parameters (Table I and II) are considered in the sequence of their presentation in Table I.

Expt. 1. The role of the adrenals in inhibition of reproductive function by ACTH in intact *Peromyscus* was unknown. Therefore the effects of adrenalectomy on reproductive organs with, and without, ACTH were studied in this experiment.

The results (Table I) indicate that body weight in *P. leucopus* of both sexes was unaffected by adrenalectomy and treatment with ACTH.

ACTH significantly increased adrenal weight of male and female *P. leucopus* ($p < .01$) (Table I). The cells of the adrenal cortex, particularly of the *zona fasciculata* and *zona reticularis*, of *Peromyscus* normally exhibit a remarkable degree of variation in size of the cells and nuclei, and some cells and nuclei are strikingly large. Similar variability in cell size also is seen in mice with, or without, ACTH treatment in the laboratory and in animals trapped in the wild from a variety of localities. Costa (9) described this variability in adrenocortical cells in *Peromyscus leucopus* and ascribed it to a 2c, 4c, and 8c polyploid series although its significance is not clear.

ACTH causes hyperplasia and hypertrophy of the cells of the fasiculata and reticularis and exaggerates the anisocytosis and variability in nuclear size. Stimulation with ACTH also leads to the formation of large, granular, eosinophilic inclusions in some nuclei. There was no inflammation accompanying these changes, nor were the ACTH-treated animals unhealthy. No evidence of infectious disease was found at autopsy or subsequent histological examination of the various organs. Testosterone also causes a marked hypertrophy of the adrenal cortex in *Peromyscus* (5). Its effects on the histology

of the adrenals in males or females closely resemble those of ACTH, although the increase in cortical mass is less than that following ACTH treatment.

ACTH significantly decreased ovarian ($p < .01$) and uterine ($p < .01$) weight in adrenalectomized and sham-adrenalectomized females (Table I). Ovarian and uterine weight was not significantly affected by adrenalectomy. On histological examination, the ovaries of ACTH-treated adrenalectomized and sham-operated mice were found to have atretic tertiary follicles and no corpora lutea. Control and adrenalectomized mouse ovaries contained either corpora lutea or large tertiary follicles. Thus, lower ovarian weight of intact and adrenalectomized ACTH-treated mice was associated with the absence of corpora lutea and viable tertiary follicles. Uteri of ACTH-treated mice were essentially immature with dense endometria and low, nonsecretory mucosal epithelium. In uteri of control and untreated adrenalectomized mice, growth of glandular, stromal, and muscula elements had begun although full maturation had not been reached.

Testicular weight decreased significantly $p < .01$) in ACTH-treated intact and adrenalectomized male *P. leucopus* (Table I). Histologically, the testes of these mice were infantile with tubules containing no lumens or mature germinal elements. Seminal vesicular weight was significantly decreased in adrenalectomized ($p < .05$) and sham-operated ($p < .01$) *P. leucopus* treated with ACTH (Table I).

The kidneys of adrenalectomized *P. leucopus* of both sexes receiving ACTH were significantly enlarged ($p < .01$) compared to control mice (Table I). Microscopically, the kidneys in the four groups of mice of both sexes appeared to be similar. ACTH apparently had no effect on renal glomerular morphology resembling that observed in house mice (6). It is possible ovarian androgens may have had a renotropic effect in adrenalectomized females, since adrenal androgens must be ruled out.

In this experiment, 4 units of ACTH significantly depressed reproductive organ weights in intact and adrenalectomized *P.*

TABLE II. Mean Weights of Adrenal Glands and Reproductive Organs and Their Standard Errors of *P. leucopus* of Both Sexes After Daily Treatment with 5 Units of ACTH.

All mice were intact.

Expt. 2	No.	Method of caging	Age at 1st inj. (days)	ACTH (units)	Days of inj.	Final body wt (g)	Wt (mg)					
							Adrenal	Kidney	Testis/ovary	Sem. ves./ uterus	Thymus	Spleen
Male												
P. leucopus	8	Singly	80–100	5	10	12.3 ± 1.0	17.6 ± 1.9	219 ± 17	179 ± 26	77 ± 12	0	19 ± 3
	7	Grouped	80–100	5	10	15.4 ± 0.7	14.4 ± 1.5	237 ± 10	116 ± 26	30 ± 10	1 ± 0.9	16 ± 1
	8	Singly	80–100	0	10	21.0 ± 1.5	9.0 ± 0.7	278 ± 10	406 ± 29	168 ± 21	22 ± 4	22 ± 1
	7	Grouped	80–100	0	10	20.3 ± 0.5	7.4 ± 0.5	252 ± 15	206 ± 42	76 ± 30	20 ± 4	21 ± 4
Female												
P. leucopus	8	Singly	80–100	5	10	13.2 ± 0.5	22.4 ± 2.0	247 ± 10	8.2 ± 0.4	21.9 ± 3.1	0	15 ± 2
	7	Grouped	80–100	5	10	15.2 ± 0.7	18.9 ± 2.8	247 ± 11	6.8 ± 0.9	20.2 ± 8.9	1 ± 0.8	14 ± 1
	8	Singly	80–100	0	10	19.7 ± 1.4	10.4 ± 0.7	290 ± 18	12.7 ± 2.3	62.8 ±17.6	20 ± 2	21 ± 1
	7	Grouped	80–100	0	10	18.4 ± 0.9	8.7 ± 0.2	251 ± 8	12.8 ± 1.0	29.2 ± 5.7	35 ± 7	24 ± 2

leucopus of both sexes. Although testicular and seminal vesicle weights of corticoid maintained male *P. leucopus* were below control values (Table I), they were not significantly different.

Expt. 2. The preceding experiment was conducted on immature *Peromyscus*; thus the question arose of whether or not ACTH would inhibit reproductive function in mature animals, as well as suppress maturation in immature animals. Also the effect of caging, *i.e.*, caged singly or in groups, needed to be determined for *P. leucopus* in this system. This experiment was designed to answer these questions for mature male and female *P. leucopus* (Table II). A daily dose of 5 IU of ACTH was selected because of the relatively poor response of the adrenals in this species to lower doses within 10 days.

The results are summarized in Table II. Adrenal weight was significantly increased by 5 units of ACTH daily in both sexes, whether grouped or singly caged ($p < .00001$). Adrenals of grouped mice weighted 17% less than those of singly caged mice ($p < .01$) and those of females averaged 28% heavier than those of males ($p < .01$). There were no readily detectable histological differences to explain the lower weights in grouped mice or the higher ones in females, although an increase in cortical area with ACTH was obvious. Growth in terms of gains in body weight, thymic weights and splenic weights were commensurate with increases in adrenocortical function as indicated by increases in adrenal weight (Table II).

An average decline of 48% in testicular weight followed treatment with 5 units of ACTH daily for 10 days ($p < .001$). The main effect, as in younger animals, was damage to, and loss of, the more mature germinal elements and particularly of those in later stages of spermatogenesis. Spermatogonia and, to a lesser degree primary spermatocytes, appeared to be unaffected. Grouping also caused an average decrease in testicular weight of 43% ($p < .001$), and its effect on the germinal elements was similar to that of ACTH, with the more mature stages being affected primarily. Grouping untreated male control mice was followed by as great a decline in testicular weight as was caused by 5 IU of ACTH daily (Table II). Androgen production by the testes, as indicated by weights of the seminal vesicles, also was seriously impaired by ACTH and by grouping. The decreases in weight of the seminal vesicles were significant ($p < .02$ for ACTH and for grouping).

Ovarian weight was 41% lower after 5 IU of ACTH daily for 10 days ($p < .001$), but grouping had no significant effect on the ovaries. The ovaries of every mouse had one or more generations of corpora lutea. Uterine weight reflected ovarian changes: ACTH produced a significant decrease ($p < .02$), but not grouping. Degeneration of the corpora lutea of *Peromyscus* occurs with formation and accumulation of pigments which remain after disappearance of the corpora lutea and therefore serve as markers of past corpora. Similar granules of pigment in the luteal cells designate degeneration. Most corpora lutea in ACTH-treated mice definitely were degenerating and most uteri were dense and inactive. Vaginae of ACTH-treated females were anestrous and mucified.

In this experiment, it is evident that grouping exhibits gonadal function in male *P. leucopus* as much as do 5 units of ACTH daily, for 10 days. In females the effects of 5 units daily of ACTH profoundly depressed ovarian and uterine weights and function, but the effect of grouping for 10 days was not as marked.

Discussion. In these experiments ACTH produced significant enlargement of the adrenals of *P. leucopus*. The doses used in these experiments apparently were within the natural physiological range as far as effect on the adrenal cortex is concerned, as wild specimens of *P. leucopus* from populations of relative high density often have adrenals weighing more than 20 mg and occasionally over 30 mg (8).

Reproductive function in both sexes of *P. leucopus* is inhibited by ACTH in either immature or mature animals. Reproductive organs were essentially the same histologically as in similarly treated house mice (3).

In contrast to male house mice, spermatogenesis, and presumably testicular secretion of androgens, are readily inhibited by ACTH in *Peromyscus*. The effect of ACTH is main-

ly on spermatids in mature mice; whereas it causes spermatogenic arrest in immature mice (6).

ACTH in corticoid-maintained adrenalectomized mice appeared to increase reproductive inhibition in *P. leucopus* of both sexes. Thus, adrenal presence is not mandatory for ACTH-induced reproductive inhibition in *Peromyscus*.

Greater depression of ovarian weight by ACTH in adrenalectomized *P. leucopus* than in intact mice contrasts with the opposite effect seen in female mice (6). The inhibitory action of ACTH on immature female *P. leucopus* ovaries appears to be largely nonadrenal mediated indicating a direct ovarian effect of ACTH in *P. leucopus*.

Groupng, in contrast to caging singly, appears to lower adrenal weight in mature *P. leucopus* of both sexes. The reason for the lower adrenal weight in grouped *P. leucopus* is unknown, but the reduction in males may be due to suppression of testicular androgen secretion preventing an androgen-induced increase in adrenal weight (5). Southwick (10) has reported increases in adrenal weight following grouping of "incompatible" *P. leucopus*, but not in "compatible" animals. On the other hand, Bronson and Eleftheriou (1) found no increase in adrenocortical size or function in grouped male *P. m. bairdii*, although the same strain showed marked adrenal responses to both ACTH and exposure to trained fighters.

In contrast to its uncertain effects on the adrenals of *P. leucopus*, grouping suppresses reproductive function strikingly in both males and females of this species.

In toto, the results of grouping or of ACTH on the reproductive organs of intact and adrenalectomized mice are remarkably similar. Possibly ACTH in *P. leucopus* affects CNS centers to inhibit reproductive function and maturation as postulated in female house mice (6). Perhaps these same centers can be directly affected by grouping to curtail reproduction without the mediation of adrenals. In any event, it is clear that ACTH and grouping profoundly inhibit reproductive function in *Peromyscus*. These effects require further exploration, especially with regard to their possible individual or combined roles in population processes. Also studies relating adrenocortical morphology to function in *Peromyscus* are needed, particularly with regard to grouping, ACTH treatment, and the effects of testosterone. But even without such studies it is clearly evident that reproductive function in *Peromyscus* is more susceptible to inhibition, particularly in males, by ACTH, and grouping than it is in house mice (*Mus*), suggesting the possibility of quite different mechanisms in the regulation and control of population growth.

Summary. Sham-adrenalectomized and adrenalectomized maturing white-footed mice (*Peromyscus leucopus noveboracensis*) of both sexes were injected subcutaneously with 4 units of ACTH daily for 10 days. Mice were adrenalectomized when 26–28 days old and each mouse was maintained thereafter on subcutaneous injections of 50 μg of cortisol acetate/day.

In a second experiment, the effects of 5 units of ACTH daily for 10 days and of group caging (6–8 mice/cage) for 10 days were determined in intact mature *P. leucopus* 80–100 days old at the start of treatment.

ACTH inhibited reproductive function in intact mature and maturing *P. leucopus* of both sexes, and in adrenalectomized maturing mice of both sexes maintained on cortisol. Group caging inhibited reproductive function in both sexes. In contrast to its effects on house mice, grouping reduced adrenal weight in both sexes. Grouping and ACTH inhibit reproductive function in male *P. leucopus* to a much greater extent than in house mice.

ACTH produced no renal lesions in *P. leucopus*.

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