Effects of ACTH on Voles (*Microtus pennsylvanicus*) Related to Reproductive Function and Renal Disease[1] (35558)

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Both natural and synthetic corticotropins have been found to inhibit reproductive function in intact female house mice (2, 6). Suppression of reproductive function by ACTH has also been produced in adrenalectomized female *Mus* and in intact female *Peromyscus leucopus* and *Peromyscus maniculatus* (3, 4). ACTH induced only a slight decrease in seminal vesicle weight in male house mice while testicular weight and spermatogenesis were unaffected (4). Sexual maturation, spermatogenesis, and reproductive function, however, are greatly inhibited by ACTH in both mature and immature male *Peromyscus* (4). The inhibitory action of ACTH on reproductive function is thought to be due to inhibition of secretion of pituitary gonadotropins by acting at the level of the pituitary or higher since ACTH inhibits reproductive function in adrenalectomized mice (1).

ACTH has also been found to induce renal glomerular disease in house mice and woodchucks (4). It is not known how ACTH produces this disease. The glomerular lesions are characterized by deposition of PAS-positive intercapillary material. Interstitial and tubular involvement are secondary events appearing, if ever, late in the disease. The severity of the disease was found to be dose dependent. ACTH also produces glomerular disease in adrenalectomized *Mus*, although the lesions are less severe than those in intact mice (7). Doses of ACTH that produced renal disease in house mice failed to do so in *Peromyscus*; and daily doses of ACTH of up to 40 units produced no demonstrable morphological changes in the renal glomeruli of laboratory rats (3, 4, 10).

Because of these species differences and the importance of voles in population research, we wished to see what effects ACTH might have on the reproductive organs and kidneys of male and female meadow voles (*Microtus pennsylvanicus*).

Methods. The *M. pennsylvanicus* were from a colony in this laboratory originally derived from individuals live-trapped on the Letterkenny Army Depot in south central Pennsylvania. The voles were maintained at 75°F, with 15 hr of light/day and were fed corn, sunflower seeds, and D and G (Price Wilhoite) mouse pellets. The females were kept 8/cage and males 12/cage. Experimental and control *Microtus* were the same age (30 days), weight, and sex.

Each vole was injected subcutaneously with 4 or 8 units of ACTH in gelatin (Organon) daily for 10 days or 4 units daily for 21 days. All injections were made between 9 and 10 a.m. (Table I). Noninjected animals served as controls. The voles were killed 24 hr after the last injection, weighed, the gastrointestinal tract was removed, muscle samples were taken, and the remainder of the animal was put in 10% neutral buffered formalin. Muscle tissue was taken from the right thigh of each animal, dried to a powder, and fat was removed by ether extraction. The resulting powder was digested with nitric acid and electrolytes were determined in duplicate by flame photometry according to a method by Fuhrman and Crismon (8). Following fixation, organ weights were obtained and histological sections were prepared. Sections of reproductive organs, kidneys, and

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adrenals were stained with hematoxylin–eosin and with Lillie's PAS–allochrome procedure (9).

Results. Body weight. Weight gain over 10 days was significantly reduced ($p<0.01$) in males receiving 8 units of ACTH. Weight gains of other ACTH-treated voles of both sexes at 10 days tended to be reduced, although not significantly (Table I). After 4 units of ACTH for 21 days, weight gain in female voles was 94% less ($p<0.01$) than control values (Table I) while weight gain in treated males was not significantly affected.

Adrenals. Adrenal weights of male and female *Microtus* were significantly increased ($p<0.05$) by ACTH at 10 and 21 days compared with controls (Table I). Adrenal enlargement was related to dose in males, but in female *Microtus* the degree of adrenal enlargement was similar after 4 or 8 units of ACTH for 10 days (Table I). The fasciculata and reticular zones of the adrenals of treated animals were increased in width relative to those of untreated voles. The degree of involution of the X-zone of females increased as ACTH dosage increased.

Reproductive organs. Weights of ovaries and uteri were significantly decreased relative to control values by 4 and 8 units of ACTH ($p<0.05$) after 10 days and by 4 units after 21 days treatment ($p<0.01$) (Table I). Microscopic examination of the organs revealed that ovaries of ACTH-treated voles contained no corpora lutea and that the number of primary and secondary follicles appeared to be increased as in the case of house mice although counts were not made. The tertiary follicles present were atretic. Uteri in ACTH-treated females were infantile with dense endometria and no glandular development.

Testicular weight was significantly depressed ($p<0.05$) by 8 units of ACTH for 10 days or 4 units for 21 days (Table I). Seminal vesicular weight was significantly decreased ($p<0.05$) at 10 and 21 days with doses used (Table I). In contrast to testes from controls of the same age (Fig. 1), testes that were significantly affected by ACTH had small seminiferous tubules in which spermatogonia and primary spermatocytes predominated and with no mature germinal elements present (Fig. 2).

TABLE I. Weight Gain and Organ Weights of *Microtus* Receiving 0, 4, and 8 Units of ACTH for 10 and 21 Days.

Data as means and their standard errors.

Microtus	No. of animals	Injection (days)	ACTH (units)	Wt change (g)	Weight (mg)			
					Adrenal	Kidney	Testis/ovary	Sem. ves./uterus
Male	12	10	0	+5.1 ± 1.1	6.9 ± 1.1	137.7 ± 4.4	533.1 ± 105	5.8 ± 1.1
	12	10	4	+2.8 ± 1.2	11.1 ± 0.7	140.7 ± 5.2	321.7 ± 160	3.4 ± 0.9
	12	10	8	+0.8 ± 0.6	25.1 ± 3.6	272.7 ± 13.4	288.2 ± 42	1.2 ± 0.2
	5	21	0	+0.9 ± 0.8	7.5 ± 1.4	—	839.8 ± 44	74.0 ± 21.0
	4	21	4	+0.8 ± 0.8	19.96 ± 1.4	—	318.0 ± 67	9.5 ± 3.3
Female	8	10	0	+2.7 ± 0.8	20.2 ± 1.1	220.3 ± 11.8	10.7 ± 0.9	36.7 ± 4.7
	8	10	4	+3.3 ± 1.2	24.3 ± 1.8	284.7 ± 13.6	6.6 ± 0.9	25.3 ± 3.6
	8	10	8	+1.5 ± 0.7	24.6 ± 2.2	269.1 ± 5.6	5.3 ± 1.0	12.2 ± 2.0
	5	21	0	+5.4 ± 2.0	26.4 ± 3.0	—	6.3 ± 1.9	108.3 ± 41.1
	4	21	4	−5.1 ± 1.2	44.4 ± 5.7	—	3.5 ± 0.7	64.6 ± 14.5

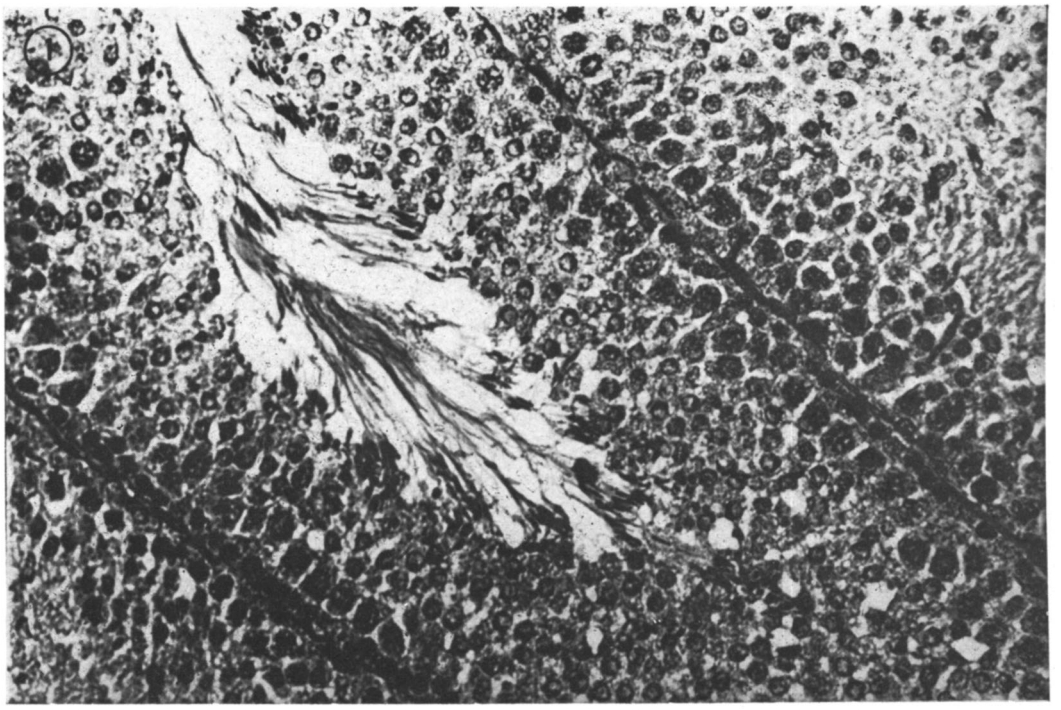

FIG. 1. Section of *Microtus* testis from untreated control: Note size of seminiferous tubules and that spermatogenesis has proceeded to completion; ×390.

Kidneys and electrolytes. Kidney weight in male *Microtus* was significantly increased ($p<0.05$) with 8 units of ACTH (Table I). Kidney weight in females was significantly increased ($p<0.05$) by both 4 and 8 units of ACTH although the degree of kidney enlargement was similar for both doses of ACTH. Renal glomerular morphology was unaffected by treatment with ACTH. However, treatment with ACTH resulted in vacuolation of the epithelial cells of the distal tubules and collecting ducts of the kidney. In addition, there were PAS-positive granules in the cytoplasm of the epithelial cells of the collecting ducts resembling those associated with potassium deficiency (12). Electrolyte determinations indicated muscle sodium and potassium were significantly decreased ($p<0.01$) by 4 and 8 units of ACTH in *Microtus* of both sexes (Table II). In males the depression of potassium appeared to be dose dependent. There were no significant differences in concentrations of sodium and potassium between sexes.

TABLE II. Sodium and Potassium Content of *Microtus* Muscle After Treatment with ACTH for 10 Days.

Data are given as means and their standard errors; FFDS = fat free dry solids.

Microtus	ACTH (units)	Na (meq/100 g of FFDS)	K (meq/100 g of FFDS)
Male	0	15.5 ± 1.0	54.4 ± 1.8
	4	12.6 ± 1.2	46.3 ± 1.9
	8	11.5 ± 1.7	34.8 ± 4.3
Female	0	18.6 ± 2.7	57.8 ± 4.8
	4	11.8 ± 1.7	33.3 ± 2.4
	8	13.7 ± 1.6	36.2 ± 2.9

Discussion. Doses of 4 and 8 units of ACTH for 10 days and 4 units for 21 days resulted in essentially infantile ovaries and uteri in female voles and produced inhibition of spermatogenesis in males. Thus, in both sexes of *M. pennsylvanicus* ACTH inhibited reproductive function and sexual maturation much as it does in both sexes of *Peromyscus,* and in female house mice (4). Direct and/or indirect ACTH-induced gonadotropin inhibition may be involved here as postulated for

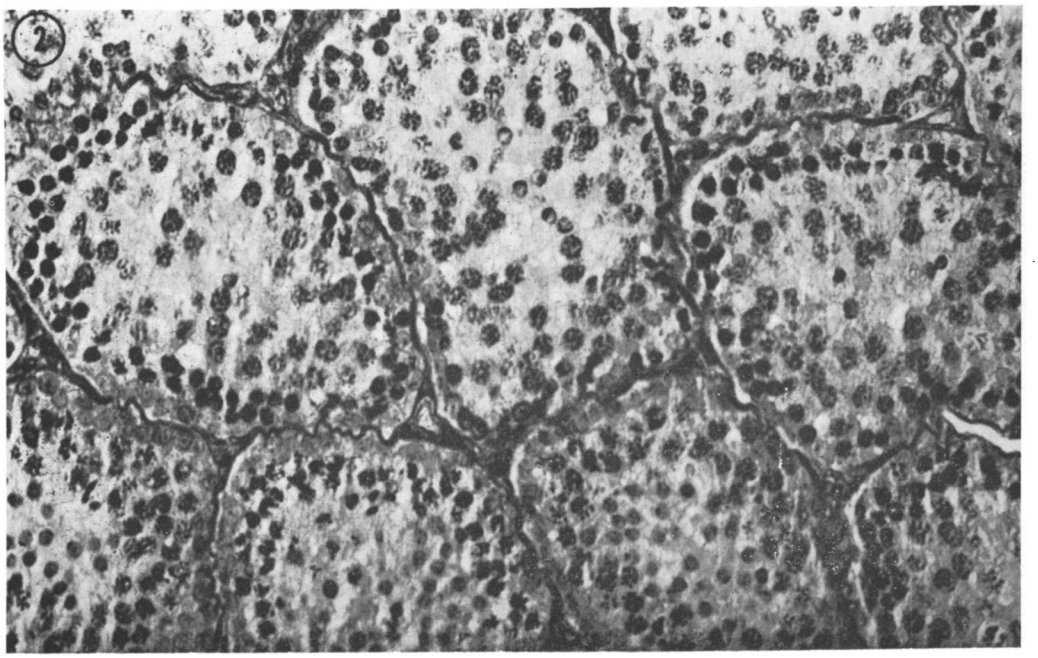

FIG. 2. Section of *Microtus* testis treated with 4 units of ACTH for 21 days: Note absence of mature forms; compare with testis of control (Fig. 1); ×390.

Mus and *Peromyscus* (4).

Contrary to the effects of ACTH in house mice but like those in *Peromyscus*, doses up to 8 units a day produced no observable effect on renal glomerular morphology in *Microtus*. There is no explanation for these differences in species responses to ACTH. ACTH treatment resulted in potassium decrease, presumably mediated by the adrenals, as indicated by characteristic changes in the epithelial cells in the renal papilla and by muscle electrolyte determinations. The decrease in muscle sodium in ACTH-treated *Microtus* was unexpected, but may reflect a decrease in aldosterone output which can occur after continued ACTH administration (11).

The reason for ACTH producing a dose-dependent adrenal enlargement after 10 days treatment in males but not in females is unknown. It may be that further adrenal enlargement was impossible in females of this age. Another explanation may be that ACTH-induced involution of the X-zone offset the increase in the fasiculata-reticularis zones in females. The fact that adrenal weight normally is three- to fourfold greater in female than in male *Microtus* in the controls of these experiments as well as under natural conditions (5) may have a bearing on these results.

Summary. Reproductive function in *Microtus pennsylvanicus* appears to be quite sensitive to inhibition by ACTH while renal glomeruli of voles are remarkably resistant to ACTH. The effects of ACTH on *Microtus* reproductive function are similar to what has been found in female house mice and both sexes of *Peromyscus* (4). ACTH had no apparent effect on the renal glomeruli of *Microtus*.

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