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Effects of Gonadectomy and Sex Hormone Administration on Accessory Reproductive Organs of Protein-Deficient Rats.* (30448)

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It has been reported(1) that hypophysectomy of protein-depleted rats produced a significant decrease in weights of testis and ventral prostate, already markedly reduced as a result of protein deprivation. This finding favored the supposition that small amounts of gonadotrophins were circulating in the blood stream of intact male protein-deficient rats, especially since adrenalectomy—and loss of adrenal androgens—had no such effects on reproductive organs of these animals. The present study describes the effects of gonadectomy on accessory reproductive organs of protein-deficient rats of both sexes. The response of accessory organs to low doses of sex hormones was also investigated in order to test the sensitivity of these organs to exogenous hormones in the absence of dietary protein.

Materials and methods. Female and male Long-Evans rats were gonadectomized and placed for approximately 1 month on diets containing 0% or 20% protein.† They received distilled drinking water and were kept, singly, in cages with wire screen floors. Female rats were nulliparous, 80-85 days of age, and weighed an average of 208 g; male rats were 40-42 days old and averaged 152 g at onset. Groups of female rats, anestrous as judged by vaginal smears, received estradiol benzoate (Nutritional Biochemicals Co.) for 3 days preceding autopsy. Daily amounts ranged from 0.03 μg to 0.30 μg in 0.3 ml sesame oil and were administered subcutaneously in 3 equally divided doses. Male animals were injected subcutaneously with testosterone propionate (Matheson Coleman & Bell) for 10 days preceding sacrifice. The hormone was given in single daily doses ranging from 10 μg to 250 μg in 0.1 ml sesame oil. Vehicle-injected animals and uninjected intact rats fed purified diets served

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† Composition of purified diets was reported earlier (2). The protein-deficient diet is known to reduce food intake; the effects of food restriction and protein deprivation on reproductive function in rats have been compared by paired feeding(3). In both sexes, the consequences of such protein deprivation are much more serious than those following limited consumption of a complete diet. For this reason, and because it is known that underfed rats are still responsive to low levels of sex hormones(6,8), paired feeding was not employed in the present studies.

TABLE I. Effects of Ovariectomy and Estradiol Benzoate on Rats Fed Protein-Free or Complete Diets.

Daily dose, μg	No. of rats	Final wt, g	Wt change, g	Uterus wt, mg	Vaginal smear at autopsy
		Ovariectomized rats fed protein-free diet			
Sesame oil	9	129	−79	103 ± 7*	anestrous
.09	7	133	−76	138 ± 14	"
.15	6	135	−76	263 ± 18	cornified (4/6)
.30	6	129	−77	238 ± 14	" (6/6)
		Ovariectomized rats fed complete diet			
Sesame oil	10	289	+81	116 ± 10	anestrous
.09	7	295	+88	122 ± 5	"
.15	7	288	+76	265 ± 22	cornified (5/7)
.30	6	268	+67	268 ± 22	" (6/6)
		Intact rats fed protein-free diet			
—	8	131	−75	96 ± 9	anestrous
		Intact rats fed complete diet			
—	8	243	+30	389 ± 41	cycling

* Mean ± standard error of mean. Uteri were drained of fluid, if necessary, before weighing.

as controls. At autopsy, accessory reproductive organs were removed, weighed, fixed in Bouin's fluid, and prepared for microscopic examination. Uteri were stained with H & E, seminal vesicles and ventral prostate with Mallory-Azan.

Results. Oil-injected ovariectomized rats fed protein-free or complete diets did not differ from intact protein-depleted animals when judged on the basis of uterine weights or vaginal smears (Table I). The response of ovariectomized rats to increasing doses of estradiol was not affected by diet; daily doses of 0.15 μg doubled uterine weight, stimulated endometrial growth, and induced vaginal cornification in about 70% of animals of either group. The highest dose tested, 0.30 μg, was ineffective in restoring normal uterus weight but caused vaginal cornification in all rats.

Ventral prostate glands were significantly heavier in intact than in vehicle-injected gonadectomized rats fed the protein-free diet (Table II). Low doses of testosterone produced similar gravimetric and histologic changes in sex accessories of gonadectomized protein-deficient and control rats. Absence of dietary protein did significantly reduce, however, the response of these organs to a daily dose of 250 μg. The hormone evoked the well-known gain in body weight of gonadectomized controls(4); it apparently also slowed the body weight loss of castrated protein-deficient rats.

Discussion. These results substantiate the view that small amounts of androgens are circulating in intact male rats fed 0% protein and that the testes are the source of the hormone, for gonadectomy abolished the partial maintenance of accessory sex organs seen in animals of this group. In accord with this interpretation also is the finding that typical castration changes occurred in anterior pituitary glands of rats subjected to gonadectomy and protein deprivation(5). Uteri of untreated ovariectomized and intact protein-deficient rats could not be distinguished by weight or microscopic appearance, evidence that circulating estrogen levels are exceedingly low in the absence of dietary protein. This observation is in line with the findings that gonadotrophic deficiencies are similar in protein depleted and hypophysectomized female rats(1).

Doses of sex hormones which produced significant minimal gravimetric and histologic changes in accessory reproductive organs of gonadectomized controls were equally effective in stimulating initial growth of sex accessories of protein-deficient ovariectomized or castrated rats. Thus, absence of dietary protein does not appear to impair the sensitivity of these organs to the injected hormone. There are few other studies in which graded

TABLE II. Effects of Gonadectomy and Testosterone Propionate on Rats Fed Protein-Free and Complete Diets.

Daily dose, μg	No. of rats	Final wt, g	Wt change, g	Seminal vesicles, mg	Ventral prostate, mg
		Gonadectomized rats fed protein-free diet			
Sesame oil	15	90	−63	17 ± 1*	12 ± 1
10	8	92	−61	32 ± 4	17 ± 1
30	8	91	−61	111 ± 9	51 ± 6
50	8	95	−58	156 ± 17	85 ± 19
100	4	94	−55	236 ± 13	98 ± 3
250	4	95	−54	324 ± 44	126 ± 23
		Gonadectomized rats fed complete diet			
Sesame oil	14	305	+151	19 ± 1	15 ± 1
10	8	306	+155	29 ± 2	24 ± 3
30	8	319	+166	119 ± 12	58 ± 6
50	8	318	+167	176 ± 19	83 ± 15
100	4	326	+175	393 ± 42	99 ± 8
250	4	327	+175	686 ± 23	240 ± 29
		Intact rats fed protein-free diet			
—	3	94	−52	20 ± 4	23 ± 2
		Intact rats fed complete diet			
—	3	356	+208	1072 ± 143	301 ± 40

* Mean ± standard error of mean. Seminal vesicles with fluid, if any, and coagulating glands.

amounts of sex steroids were administered to gonadectomized rats deprived of general or specific dietary factors. Neither caloric restriction nor feeding of vitamin-free casein or glucose influenced the response of the ventral prostate to low doses of testosterone (6), and tryptophane deficiency was reported to increase uterine sensitivity to small amounts of α-estradiol(7). Lerner and Turkheimer(8) recently found that uterine responsiveness to estradiol benzoate was decreased in immature ovariectomized mice fed a protein-free diet. No reduction in the uterotrophic effect of the hormone was evident, however, when the uterine growth response to 0.03 μg, the lowest dose tested, was expressed in relation to body weight.

In the gonadectomized rats of this study the response of the ventral prostate to a high dose of testosterone was significantly less when protein was deleted from the diet. Grayhack and Scott(6) observed a similar relationship if dietary intake was limited, and Leathem(9,10), who noted that the response of seminal vesicles to 250 μg of the hormone was reduced in the absence of dietary protein, suggested that subnormal responsiveness followed depletion of body protein stores.

The influence of protein nutrition on hormone action was again emphasized recently by Oslapas and Leathem(11), who found a reduced uterine response to 3 μg of estradiol benzoate in immature ovariectomized rats receiving a 0% casein diet. Leathem's studies (*loc. cit.*) have also used biochemical and histochemical endpoints as measures of accessory reproductive organ stimulation by sex hormones. The histologic and gravimetric data being reported in the present study do not appear to be at variance with those findings.

Failure of accessory organ weights to advance normally with increasing dosage of testosterone may also stem from the disruptive effect of the diet on pituitary production of growth hormone and, possibly, prolactin. Both of these hormones are known to enhance the androgenic action of testosterone in hypophysectomized-castrate rats(12), and evidence has been presented that the growth-promoting potency of rat plasma is significantly depressed following protein deprivation(2,13). While the effects of protein-free diets on pituitary prolactin function are as yet unknown, inanition has been found to reduce pituitary prolactin content in female rats(14).

Summary. Involution of accessory reproductive organs of male rats fed a 0% protein

diet was accentuated by gonadectomy, attesting to residual testis function in intact protein-depleted rats. Sex accessories of gonadectomized protein-deficient rats responded to the same low levels of estradiol benzoate or testosterone propionate effective in gonadectomized control animals, indicating that sensitivity of these organs to exogenous hormone was unaffected by the diet. A subnormal response of accessory organs to high doses of testosterone was noted, however, in male protein-deficient rats.

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Effect of Allogeneic Bone Marrow on Lethally Irradiated Thymectomized Mice.* (30449)

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Allogeneic adult bone marrow has long been shown capable of inducing graft-*versus*-host reactions in irradiated mice(1,2). Recently, Miller and others(3,4,5,6) have shown that the complete recovery of immunologic competence by lethally irradiated mice seeded with syngeneic marrow depends on the presence of the adult thymus. This implies that the action of the host thymus is necessary for development of immunologic competence in the donor marrow. The absence of the thymus might then be expected to preclude development of a graft-*versus*-host reaction in allogeneic radiation chimeras. The present studies were designed to test this hypothesis.

Materials and methods. The experimental design is outlined in Fig. 1. CBA/JAX‡ male mice, 7-8 weeks old, were divided into 5 groups of 18-22 animals each. The animals of 2 of the groups were thymectomized and of 2 others, sham-thymectomized. The fifth group served as an unoperated control. One week after operation, all the animals were lethally irradiated with a dose of 850 R at 50 R/min. The X-ray facilities consisted of a cross-fire arrangement of 2 tubes run at 184 kvp, 30 ma; filtration 0.28 mm Cu and 0.50 mm Al; target distance 72 cm from each tube. The animals were irradiated in

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‡ All mice were obtained from Jackson Memorial Laboratories, Bar Harbor, Maine.