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Studies on the Prostate Glands of Adult Inbred LSH Hamsters (38838)

MARGARET BUTLER, W. K. SAWYER, T. GIANNINA AND B. G. STEINETZ

Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Ardsley, New York 10502

Homburger and Nixon (1) reported the occurrence of cystic prostatic hypertrophy in two inbred lines of Syrian hamsters (*Mesocricetus auratus*), one of which was Line 2.4 (LSH). This strain appeared to offer a rodent model for studying the development and possible therapy of benign prostatic hypertrophy. We, therefore, have investigated several parameters of prostate function in young and old LSH hamsters. These include prostate weight, acid phosphatase (AcP) activity, and uptake of testosterone-6,7-^{3}H as influenced by castration and testosterone treatment. Surprisingly, we have found that prostates of LSH hamsters undergo a profound atrophy with advancing age in contrast to those of an outbred strain such as Lakeview Lak:LVG(SYR).

Materials and Methods. Thirty male inbred LSH (strain 2.4) hamsters were purchased from TELACO, Bar Harbor, ME at 6 wk (immature) and 6 mo (adult) of age. An additional 180 inbred LSH/SsLak hamsters were subsequently bought from the Lakeview Hamster Colony, a subsidiary of Charles River Breeding Laboratories. Five normal adult Lakeview [Lak:LVG(SYR)] outbred hamsters were likewise obtained from the latter supplier.

The first experiment was performed on the TELACO (strain 2.4) hamsters. The adult and immature hamsters were divided into three groups each; intact, castrated and castrated plus testosterone, 200 μg sc in 0.2 ml sesame oil daily for 7 days starting 1 wk after castration. On the eighth day the animals were killed with ether and the testes (intact hamsters), ventral prostates, and seminal vesicles were removed and weighed. Prostates were immediately homogenized in ice-cold deionized water and AcP was determined by the method of Manning *et al.* (2) using α-naphthyl acid phosphate as substrate. A group of five adult intact Lakeview outbred hamsters (5–7 mo old) was killed at the same time and organ weights and prostatic AcP determined as above.

The second experiment was essentially a replicate of the first except that LSH/SsLak hamsters were used. In this instance immature (40–50 g), young adult (81–90 g) and older (>6 mo; > 100 g) animals were divided into groups of 16 each; intact, castrated, or castrated plus testosterone 200 μg sc in 0.2 ml sesame oil daily for 7 days. On the 7th day 20 μCi [6,7-^{3}H]testosterone were injected iv (jugular vein) into 10 animals from each group 2 hr after the last dose of unlabeled steroids. The animals were killed 2 hr later and the prostates and seminal vesicles of the [^{3}H]testosterone-treated hamsters were removed, weighed, and digested in 0.5 ml Protosol (Packard). Radioactivity was determined in 10 ml Instagel (New England Nuclear) on a Tri-Carb liquid scintillation spectrometer. DPM were determined from quench curves obtained with tissue digested in Protosol to which had been added known amounts of radioactivity. The prostates of the remaining hamsters were homogenized in ice-cold deionized water and AcP determined as previously described.

Results were expressed as means $\pm$ SEM and significance of differences were determined according to Fisher's t tables.

Results. LSH (strain 2.4, Telaco) hamsters. In the immature LSH (2.4) hamsters, neither castration nor treatment with testosterone (T) altered seminal vesicle weight (Table I). Ventral prostate weight was significantly reduced 8 days after castration and essentially restored to the intact level by androgen treatment (Table I). Prostatic AcP concentration was increased by castration and decreased by T. In adult LSH (2.4) hamsters, seminal vesicle and prostate weights were not influenced by castration, but were significantly ($P < 0.05$) increased by T treatment of castrated animals (Table I). Prostatic AcP concentration was similar in intact and castrated adult LSH hamsters and much like that observed in castrated immature animals (Table I). T treatment significantly reduced the enzyme concentration in the older ani-

TABLE I. ORGAN WEIGHTS AND PROSTATIC ACID PHOSPHATASE OF LINE 2.4 LSH HAMSTERS (TELACO).

Group	No. ham.	Final body wt g	Testes (mg)	Sem. ves. (mg)	Ventral prostate		
					mg	Acid phosphatase[a] Units/gland	Units/mg
Immature (6 wks)							
Intact	5	87	499 ± 174	81 ± 12	24 ± 4	42 ± 12	1.6 ± 0.2
Castrate	5	91	—	81 ± 9	14 ± 1	43 ± 4	3.0 ± 0.2
Castrate + test.[b]	5	80	—	83 ± 22	21 ± 3	29 ± 7	1.5 ± 0.3
Adult (6 mo.)							
Intact	5	116	261 ± 22	153 ± 9	22 ± 2	78 ± 10	3.6 ± 0.3
Castrate	5	107	—	177 ± 17	20 ± 1	65 ± 11	3.2 ± 0.5
Castrate + test.[b]	5	107	—	244 ± 25	48 ± 9	67 ± 9	1.4 ± 0.1
Intact Lakeview Hamsters (LaK:LVG)	5	148	3,090 ± 190	288 ± 18	128 ± 13	5 ± 1	0.02 ± 0.002

[a] One unit of acid phosphatase activity liberates 1 μg of α-naphthol from sodium α-naphthyl acid phosphate in 30 min at pH 5.2 and 37° under standard conditions.

[b] Testosterone 200 μg sc daily for 7 days.

mals (Table I). Testis weight of the 6-mo-old hamsters was significantly lower than the mean testis weight of the younger animals (261 ± 22 mg vs 499 mg) although there was an overlapping range in the latter group. Also, prostate weight of intact young and old LSH hamsters did not differ significantly.

Adult Lakeview outbred [Lak:LBG-(SYR)] hamsters had testes weighing about 11 times more than those of the LSH (2.4) adults (Table I). Furthermore, the seminal vesicles and ventral prostates of the Lakeview outbred hamsters were significantly ($P < 0.01$) heavier and their prostatic AcP concentrations were much lower ($P < 0.01$) than those of the LSH (2.4 line) animals.

LSH/SsLak hamsters. Seminal vesicle and prostate weights were reduced significantly ($P < 0.01$) by castration in immature, young adult, and older LSH/SsLak hamsters (Table II). T injections increased seminal vesicle and prostate weight above castration levels in each age group (Table II). However, the organ weight seldom approached those of intact hamsters (Table II). Prostate weight (but not seminal vesicle or testis weight) of the older intact adult LSH/SsLak hamsters was significantly lower than that of the younger intact adult animals (Table II). AcP concentration was increased by castration, whereas the total enzyme per gland remained about the same in young and old adult LSH/SsLak hamsters (Table II).

The total uptake of tritium as well as the concentration of radioactivity in the seminal vesicles was increased by castration of the immature, young, and old adult LSH/SsLak hamsters (Table II). T treatment further increased the total amount of tritiated steroid taken up by the seminal vesicles in each age group without much influencing the concentration of radioactivity in these organs (Table II). The concentration of radioactivity in the prostate 2 hr after injection of [6,7-^{3}H]testosterone was increased ($P < 0.01$) by castration in immature or adult animals (Table II). In young and old adult castrates T injected for 1 wk increased the concentration as well as the total amount of radioactivity in the prostate above the level of untreated animals. In immature castrated hamsters, T treatment was associated with a decreased concentration of tritium in the prostate although total radioactivity per gland was significantly increased ($P < 0.02$).

Discussion. Our findings are contrary to those previously reported (1) that spontaneous prostatic hypertrophy occurs with advancing age in LSH (2.4 line) hamsters. Two inbred strains of LSH hamsters were presently studied, one of which (line 2.4 LSH) was obtained from the source used in the original report (1). Contrary to the original findings, senescent changes were observed in the 2.4 LSH hamsters. The prostates of 6-mo-old animals were no larger than those observed in 6-wk-old animals and about 1/5 as large as those of adult outbred hamsters (Lak:LVG). We interpret the immature size of the 2.4 LSH prostates to be related to the profound testicular atrophy observed in

TABLE II. ACID PHOSPHATASE CONTENT AND TRITIATED TESTOSTERONE UPTAKE OF SECONDARY SEX ORGANS OF LSH/Ss LaK HAMSTERS.

Group	No. of hamsters[b]	Final body wt. (g)	Testes (g)	Sem. ves. (mg.)	Ventral prostate			Uptake of [^{3}H]testosterone			
								Seminal vesicles		Ventral prostate	
					mg	Acid phosphatase[a]		dpm/gland ($\times 10^3$)	dpm/mg ($\times 10^3$)	dpm/gland ($\times 10^3$)	dpm/mg ($\times 10^3$)
						Units/gland	Units/mg				
Immature (40–50 g) Intact	15 (6/9)	79 ± 2	1.84 ± 0.06	125 ± 8	33 ± 3	112 ± 21	2.9 ± 0.6	18 ± 6	0.1 ± 0.04	9 ± 5	0.2 ± 0.1
Castrate	14 (6/8)	79 ± 2	—	27 ± 2	5 ± 1	43 ± 8	3.2 ± 0.5	61 ± 7	2.8 ± 0.4	40 ± 1	26 ± 3
Castrate + test[c]	16 (6/10)	77 ± 2	—	56 ± 5	9 ± 1	38 ± 3	2.8 ± 0.1	125 ± 26	1.2 ± 0.4	68 ± 9	11 ± 2
Young adult (81–90 g)											
Intact	16 (6/10)	95 ± 2	2.53 ± 0.04	273 ± 15	85 ± 4	81 ± 9	1.0 ± 0.2	14 ± 2	0.05 ± 0.005	4 ± 0.7	0.05 ± 0.01
Castrate	14 (5/9)	102 ± 2	—	85 ± 6	8 ± 1	74 ± 15	4.1 ± 0.2	81 ± 15	1.0 ± 0.1	13.9 ± 4.6	1.6 ± 0.7
Castrate + test[c]	16 (6/10)	95 ± 2	—	217 ± 13	19 ± 3	80 ± 5	2.6 ± 0.3	276 ± 46	1.3 ± 0.1	40 ± 4	3.8 ± 0.4
Adult (> 100 g)											
Intact	16 (6/10)	117 ± 4	2.39 ± 0.18	318 ± 28	49 ± 6	132 ± 19	2.4 ± 0.5	23 ± 0.5	0.3 ± 0.1	11 ± 5	0.3 ± 0.1
Castrate	13 (6/7)	110 ± 5	—	169 ± 19	14 ± 3	117 ± 11	4.9 ± 0.2	89 ± 29	0.6 ± 0.2	4 ± 1	1.2 ± 0.2
Castrate + test[c]	16 (6/10)	115 ± 3	—	290 ± 10	29 ± 6	203 ± 34	3.7 ± 0.7	289 ± 23	1.0 ± 0.1	35 ± 2	3.0 ± 0.5

[a] See Table I for acid phosphatase units.

[b] Number of hamsters for average organ weight is first number. In parentheses are shown, respectively, numbers for acid phosphatase and [^{3}H]-testosterone uptake.

[c] Testosterone 200 μg sc 7 days.

these animals. Their testes weighed less than 10% of those of adult outbred hamsters.

Similar but less dramatic senescent changes were observed in LSH/SsLak hamsters. Thus, prostates of older animals were not much larger than those of immatures and were significantly smaller than those of young adults. Testis weight in the older LSH/SsLak hamsters was about the same as that of young adults and significantly lower than that of the outbred Lak:LVG strain.

The reason for the conflicting data obtained by Nixon and Homburger (1) and ourselves is by no means clear. However, a genetic change would appear to be the likeliest explanation. Granados and Dam (3) have reported occurrence of testicular hypoplasia in inbred Syrian hamsters (originally derived from a Jerusalem colony). This defect was either uni- or bilateral and in the latter case resulted in sterility. It seems possible that a similar genetic change may have occurred in the 2.4 LSH hamster strain during the 4-yr interval between the first study (1) and the present one. The main difference in the defects is that the one described by Granados and Dam (3) is developmental, whereas the one observed in the present study is degenerative. It is obvious that hypofunctional testes would likely preclude the development of prostatic hypertrophy and hyperplasia. Neither the life span nor the reproductive performance of LSH (2.4 or SsLak) hamsters appear to deviate much from those of outbred animals. Thus, 2.4 strain LSH hamsters have a median life span of 530 days and the males are used for breeding up to 6 mo of age (F. Homburger, personal communication). LSH/SsLak hamsters have a median life span of 2 yr and male breeders are normally retired at 12 mo (G. Slater, personal communication). Outbred Lak:LVG(SYR) hamsters have similar reproductive and viability characteristics.

Observations on the effects of castration and testosterone on organ weights, AcP and uptake of [^{3}H]testosterone revealed other peculiarities of the LSH hamsters. In immature or adult 2.4 LSH hamsters, castration had little or no effect on weights of the prostates or seminal vesicles. T injection in the castrates effected a modest increase in prostate size of young and old 2.4 LSH animals, but an androgenic response of the seminal vesicles was seen only in the adults. A possible role of adrenal androgens cannot be excluded. Responses of the sex accessory organs to castration and androgen injection were not as aberrant in LSH/SsLak hamsters. In immature, young adult, and old adult animals, castration was followed by atrophy of prostates and seminal vesicles. T increased the weights of these structures in each age group, but appeared to be more effective in the older than in the younger animals.

Total prostatic AcP was unchanged by castration or testosterone treatment of 2.4 LSH hamsters, but the concentration of enzyme appeared to be inversely proportional to circulating androgen levels (endogenous or exogenous). Thus, castration of immature animals increased AcP concentration and this was prevented by T treatment. These findings were unexpected and contrary to the general thesis that prostatic acid phosphatase activity depends upon T levels in rodents (4, 5) and man (e.g. 6). In the intact senescent adult 2.4 LSH hamsters, prostatic AcP was elevated and remained so after castration. T injection depressed AcP concentration. These observations support the thesis that testicular function is decreased in old 2.4 LSH males. The lowest levels of prostatic AcP were found in intact adult outbred Lak/LVG hamsters, in agreement with the normal appearance of their testes and secondary sex organs. AcP concentration in prostates of intact LSH/SsLak hamsters was similar to that observed in 2.4 LSH hamsters and much higher than in outbred Lak/LVG animals. AcP concentration was increased by castration and decreased by testosterone in young and old LSH/SsLak adults.

Relative uptake (dpm/mg) of [^{3}H]testosterone by the prostate and seminal vesicles was enhanced by castration in all age groups of LSH/SsLak hamsters. Absolute uptake of the radioactive steroid (dpm/gland) by prostates and seminal vesicles was also increased by castration in immature and young adult animals. Testosterone pretreatment of castrated animals further increased the total uptake of [^{3}H]testosterone by the prostate

and seminal vesicles in each age group. The T pretreatment was also associated with a decreased uptake of tritiated steroid per mg of tissue by prostates of immature animals and an increased uptake in the young and old adults. There was no correlation between [^{3}H]testosterone uptake and acid phosphatase.

The amount of labeled steroid taken up by an organ depends upon many factors, the most important of which are the affinity of the binding sites for the steroid and the number of binding sites available. The latter in turn depends upon the synthesis and/or degradation of binding macromolecules and the number of sites occupied by endogenously derived hormone molecules. We interpret our data as follows: after castration, endogenous testosterone levels rapidly decline in immature and young adult LSH/SsLak hamsters resulting in an increased number of available binding sites in the prostate and seminal vesicles. These would account for the increased uptake of tritiated testosterone. T treatment of castrates increases the size of the accessory organs and may stimulate the synthesis of steroid-binding macromolecules within these structures. This, in turn, would result in an increased accumulation of radioactivity after administration of [^{3}H]testosterone in accord with what was actually observed. Larger doses of T (e.g., those which would restore prostate weight to the noncastrated level) might be expected to result in a reduced [^{3}H]testosterone uptake by saturating available binding sites with nonradioactive steroid.

The fact that prostates and seminal vesicles of castrated old adult LSH/SsLak hamsters responded to testosterone injections similarly to younger animals suggests that the senescent changes observed are due primarily to decreased testicular function rather than to reduced responsiveness of the target tissues.

Instead of serving as a model for benign prostatic hypertrophy, the LSH hamster would appear to be a suitable model for the study of sensecent changes in the male reproductive system.

Summary. We have studied several parameters of prostate function in two inbred lines (2.4 and SsLak) of young and old LSH hamsters. These included weight, acid phosphatase, and [^{3}H]testosterone uptake as influenced by age, castration, and androgen treatment. In the hamster, prostatic acid phosphatase concentration was found to vary inversely with androgen levels, contrary to the usual assumption that this enzyme is androgen dependent. Prostatic uptake of tritiated testosterone was enhanced by castration and by treatment of castrates with doses of androgen which induced a moderate increase in gland size. With advancing age, the prostates of LSH hamsters (both strains) became atrophic rather than hyperplastic, in contrast with a previous report (1). This atrophy appeared to be a consequence of decreased testicular function. The LSH hamsters appear to be a suitable model for the study of senescent changes in the male reproductive system.

Addendum. Dr. F. Homburger, whom we informed of our findings, has recently autopsied 9 old male 2.4 LSH hamsters at the Bio-Research Institute, Cambridge, Mass. Testes averaged 3.25 ± 0.06 grams and prostates 90 ± 10 mg. Thirteen old 2.4 LSH males were also sacrificed at Telaco: testes weighed 2.98 ± 0.03 grams and prostates 119 ± 80 mg. These data support their previous findings. We plan to compare and exchange 2.4 LSH animals raised at the Bio-Research Institute and at CIBA-GEIGY in an effort to resolve this delemma.

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