the strains isolated from the 2 different areas were of the same subtype. The possibility must be considered that the infected mice could serve as a source of infection for humans and other animals.

Although the question was raised that the serum inhibition of type 3 reovirus may be due to non-specific inhibitors, the type 2 serologic results would appear to be specific, since the occurrence of antibody correlated well with the presence of mice infected with the virus. The possibility that some of the type 2 reactions may represent cross reactions from infection with other reovirus types must be considered; however, no correlation has been observed between the presence of type 3 inhibitor and inhibition of type 2 in HI tests of individual mouse sera, and tests with sera of immunized mice indicate that the HI and neutralization procedures give only minimal cross reaction.

The finding of reovirus type 3 virus in 5 of 7 transplanted mouse leukemias suggests that this virus may be a relatively frequent contaminant of mouse tumors. Also, we have identified the oncolytic virus isolated by Nelson(12) from a transplantable ascites tumor as a strain of reovirus type 3.

Summary. Using virus isolation and serologic survey procedures, the occurrence of natural reovirus infection in mice has been detected. Four isolates of reovirus type 2 were recovered from urban and rural wild mice; 7 strains of reovirus type 3 were isolated from normal laboratory mice and transplant-induced mouse leukemias. Antibody to type 2 was found focally distributed in wild mice, but was not encountered in laboratory mice. A low titer serum inhibitor of type 3 HA and infectivity, possibly representing specific antibody, was prevalent in laboratory mice.

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Effects of Gonadotrophins and Androgens on the Sex Organs of the Estrogen-Treated Male Rat. (26949)

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In an effort to use estrogen-treated immature male rats for LH assays it became apparent that the ventral prostate response to crude and purified preparations was quite different. Data obtained in examining these differences are here presented.

Methods. In the first series of experiments, 17-18 day old male rats (Charles River Lab.) were given a single subcutaneous injection of 5 μg of estradiol dipropionate in peanut oil. In the second series, 20-21-day-old Sprague Dawley rats were injected with 10 μg of estradiol dipropionate in peanut oil. The animals were fed *ad libitum* with Purina Lab Chow.

Subcutaneous injections of the materials

to be tested were begun 3 days after administration of the estrogen. All materials were given for 4 successive days, except testosterone propionate which was given as a single injection. The volume of material injected was 0.25 ml. The following preparations were used:

1. Estradiol dipropionate, Ciba, in sesame oil, diluted with peanut oil to desired concentration,

2. Testosterone propionate, Ayerst, diluted to volume with peanut oil,

3. Luteinizing Hormone (LH) in 15% gelatin—0.2% phenol,*

   a. Equine, Armour Co., Lot #377-279
   b. Ovine, Armour Co., Lot #227-80
   c. Ovine, DEAE—LH (A neutral 75% ethanol precipitate of an ammoniacal-alcohol extract of acetone dried glands was put on a diethylaminoethyl cellulose column in 0.005 M ammonium hydroxide-ammonium acetate buffer at pH 9.8. The first fraction obtained after initiating gradient elution with 0.1 M ammonium acetate was dialyzed, lyophilized and used in these experiments.

4. Acetone-dried ovine pituitaries, ground to 60 mesh, and crude extracts of ovine pituitaries, were suspended or dissolved in 15% gelatin—0.2% phenol,

5. Testosterone (free alcohol), Δ 4-androstene-3,17 dione, progesterone, 17 α hydroxyprogesterone, dehydroepiandrosterone and pregnenolone (G. D. Searle and Co.) were dissolved in peanut oil.

All animals were sacrificed 7 days following administration of estrogen. Body weight was measured. The right testis, ventral prostate, and seminal vesicles were dissected free, blotted on paper, and weighed.

Results. Series I. The 25-26-day-old rats (Charles River Lab.) had ventral prostates that weighed 50.2 ($\pm$ 2.1) mg/100 g rat (n-20). When injected subcutaneously with 5 μg of estradiol dipropionate at 17-18 days of age and autopsied 7 days later, rats of this strain had ventral prostates that weighed 28.5 ($\pm$ 1.7) mg/100 g rat (n = 39).

Data illustrated in Fig. 1 demonstrate the effect of testosterone propionate (TP) on the ventral prostate of the estrogen-treated immature rat. A linear relationship between the logarithm of dose of TP and ventral prostate size (mg/100 g rat) was found to exist, such that a 10-fold increase in TP dose resulted in an average increase in ventral prostate size of 41.5 mg/100 g rat. The minimum dose of TP required to significantly enlarge the ventral prostate of these animals varied from 10 to 20 μg. Ventral prostates heavier than 100 mg/100 g rat were obtained with large doses of TP.

Data indicated that the responses of the ventral prostate to Armour equine LH 377-279 and crude sheep pituitary extracts were similar (Fig. 1). The increase in ventral prostate size following a 10-fold increase in dose of these pituitary extracts ranged from 25.9 to 38.2 mg/100 g rat. Ventral prostate weights between 70 and 115 mg/100 g rat were repeatedly observed following administration of relatively large doses of sheep pituitary powder and crude extracts thereof.

In contrast to TP and crude pituitary extracts, rats given increasing doses of ovine DEAE-LH showed relatively small increases in ventral prostate weight (average slope

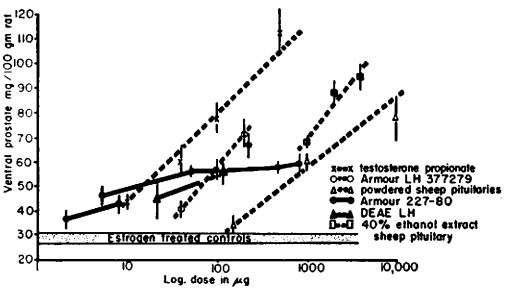

FIG. 1. *Series I*: Effect of testosterone propionate and pituitary extracts on ventral prostate weight of estrogen-treated immature rat.

* Armour LH 377-279 was reported to have 5 times the potency of Armour LH 227-80 when assayed on the basis of response of the ventral prostate of hypophysectomized rats rested 7 days following operation. LH 377-279 was, however, only 15-20% as potent as LH 227-80 when assayed by Dr. Albert Parlow using ovarian ascorbic acid depletion in pseudopregnant rats as the end point. DEAE-LH was approximately 30% as potent as LH 227-80 when assayed in hypophysectomized male rats rested 2 days following surgery, and when assayed by the ovarian ascorbic depletion technique.

TABLE I. *Series II.* Effects of LH, Pituitary Powder, and Various Steroids on Weight of Reproductive Organs of Estrogen-Treated, Immature Male Rats.

Material	Total dose, μg	n	Body wt, g ± S.E.	Seminal vesicle mg/100 g ± S.E.	Ventral prostate mg/100 g ± S.E.	R. testis mg/100 g ± S.E.	
Controls	0	8	70.0 ± 3.4	29.5 ± 1.4	19.2 ± .8	207 ± 15	
Armour LH, Lot 227-80	5	10	73.8 ± 2.8	23.8 ± 1.7	40.5 ± 3.1	300 ± 10	**
	10	5	74.2 ± 1.1	27.4 ± 1.3	47.7 ± 7.3	312 ± 14	**
	20	11	75.8 ± 2.0	30.3 ± .9	57.9 ± 3.3	293 ± 7	**
	40	11	70.1 ± 1.9	28.8 ± 5.3	55.0 ± 5.0	287 ± 18	*
	800	6	70.0 ± 2.8	40.7 ± 5.3	76.7 ± 2.3	349 ± 15	**
Sheep pituitary powder	200	6	71.3 ± 2.7	27.3 ± 1.7	28.2 ± 1.7	246 ± 12	NS
	300	6	72.8 ± 3.3	35.9 ± 3.9	40.9 ± 4.6	285 ± 14	*
	1200	6	77.0 ± 2.6	34.7 ± 3.5	61.0 ± 8.3	358 ± 27	**
	2000	5	74.0 ± 1.6	31.6 ± 2.8	56.3 ± 6.9	329 ± 19	**
	20000	6	70.8 ± 1.5	46.1 ± 3.6	84.4 ± 7.4	370 ± 14	**
	32000	8	75.9 ± 2.0	55.0 ± 3.7	90.5 ± 6.2	371 ± 17	**
Testosterone	100	12	75.6 ± 1.3	34.4 ± 2.3	37.3 ± 2.9	206 ± 6	NS
	400	6	73.7 ± 2.6	40.5 ± 1.5	52.7 ± 7.1	213 ± 9	NS
	800	6	72.7 ± 4.1	63.4 ± 9.7	63.2 ± 2.8	230 ± 10	NS
	1600	9	72.0 ± 1.6	77.0 ± 5.5	82.6 ± 3.4	272 ± 23	NS
	10000	7	76.1 ± 1.8	146.1 ± 3.5	107.4 ± 3.1	284 ± 6	*
Δ_4-Androstene-3,17 dione	100	6	73.0 ± 1.3	33.6 ± 9.1	45.9 ± 6.1	219 ± 17	NS
	400	6	75.5 ± 1.7	26.7 ± .9	54.7 ± 5.2	262 ± 33	NS
	800	6	74.3 ± 1.3	33.9 ± 1.9	68.7 ± 6.3	222 ± 7	NS
	1600	6	73.3 ± 2.0	54.2 ± 4.9	87.8 ± 4.6	245 ± 13	NS
	10000	6	73.1 ± 1.9	107.0 ± 4.6	115.2 ±11.7	297 ± 10	*
17α hydroxyprogesterone	2000	6	77.2 ± 1.0	24.2 ± 1.3	32.9 ± 5.5	231 ± 34	NS
	10000	6	74.3 ± 1.3	32.1 ± 1.9	67.4 ± 4.2	282 ± 15	*
Progesterone	2000	5	74.8 ± 2.5	26.8 ± 2.0	41.9 ± 2.4	223 ± 5	NS
	5000	6	74.7 ± 1.0	27.3 ± 2.2	53.3 ± 7.6	231 ± 14	NS
	20000	6	71.8 ± 2.0	26.3 ± 2.3	66.6 ± 6.4	245 ± 18	NS
Pregnenolone	1000	8	72.9 ± 1.4	30.3 ± 1.7	35.0 ± 2.4	258 ± 8	*
	3000	6	71.5 ± 3.9	27.7 ± 2.0	43.8 ± 3.9	297 ± 17	*
	6000	7	76.4 ± 2.0	32.3 ± 1.4	52.1 ± 3.3	277 ± 6	*
	15000	10	72.5 ± 1.3	26.0 ± 1.6	51.4 ± 3.2	281 ± 8	**
Dehydroepiandrosterone	800	6	76.8 ± 3.5	28.2 ± 1.5	29.4 ± 4.7	248 ± 3	NS
	1600	6	77.7 ± 2.8	30.0 ± .7	35.8 ± 2.8	251 ± 11	NS
	2100	6	73.0 ± 2.8	25.7 ± 2.8	45.2 ± 8.7	279 ± 8	NS
	8400	6	76.8 ± 3.7	47.7 ± 3.9	70.5 ± 5.4	306 ± 11	*

Statistical significance: * $p < .05$; ** $p < .01$; *** $p < .001$; NS—not significant.

9.2). A similar result was obtained with Armour Ovine LH 227-80 (slope 13.4) (Fig. 1). Furthermore, approximately 50 μg of Armour LH 227-80 produced ventral prostate weights of 55-65 mg/100 g rat and increasing the dose to 1.0 mg did not induce further enlargement.

Series II. Data in Table I again demonstrated that increasing doses of Armour Ovine LH 227-80 induced smaller increments in ventral prostate weight (slope 15.7) than did powdered sheep pituitaries (slope 26.7), testosterone (slope 36.0), androstenedione (slope 36.9), progesterone, 17 α hydroxyprogesterone, or dehydroepiandrosterone. Pregnenolone caused ventral prostate enlargement with dose-response characteristics similar to those of Armour LH 227-80 (slope 14.6). Statistical comparisons of these slopes are presented in Table II.

For each material assayed, the weight of the ventral prostate (mg/100 g rat) was plotted against the logarithm of the dose and extrapolated to obtain the minimum effective dose (amount of material required to produce a ventral prostate weighing 21.6 mg/100 g rat). The data were then replotted (Fig. 2A) as mg ventral prostate/100 g rat against the logarithms of multiples of the minimum effective dose (actual doses divided by minimum

TABLE II. *Series II.* A Comparison of Dose-Ventral Prostate Response Slopes Following Administration of Pituitary Extracts and Androgenic Steroids Utilizing the Chi-Square Test for Parallelism.

	Slope (b)	Variance (σ^2b)	χ^2	P	Parallelism
Testosterone *vs*	36.02	5.861			
Armour 227-80	15.65	9.036	27.85	<.001	Not parallel
Sheep pit. powder	26.70	11.237	5.08	<.05 >.02	" "
Androstenedione	36.92	20.902	.03	<.99 >.98	Parallel
Pregnenolone	14.62	12.580	24.83	<.001	Not parallel
Armour 227-80 *vs*					
Sheep pit. powder			6.02	<.02 >.01	Not parallel
Pregnenolone			.05	<.99 >.98	Parallel
Sheep pit. powder *vs*					
Pregnenolone			6.13	<.02 >.01	Not parallel

effective dose). Similarly, where significant enlargement occurred, weights of seminal vesicles (Fig. 2B) and testes (Fig. 2C), were plotted against the logarithms of multiples of the minimum effective dose as calculated from the ventral prostate data. Thus the abscissa of the plot was made the same for all materials assayed and for all organs.

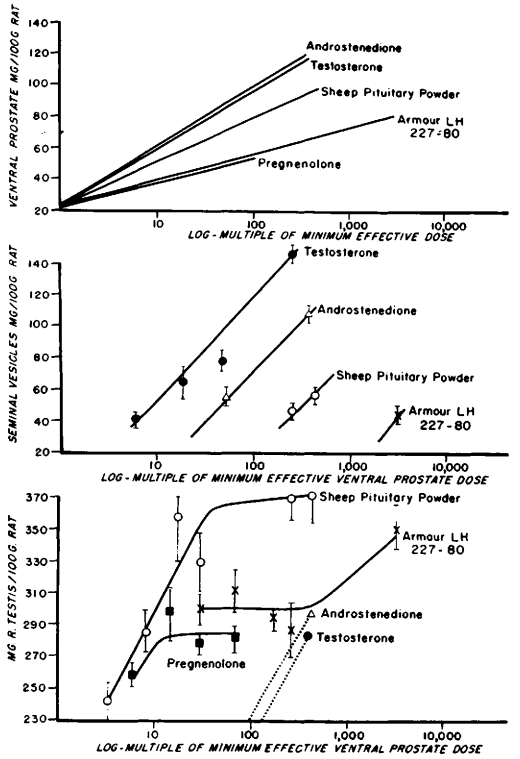

FIG. 2. *Series II*: Effects of graded doses of pituitary extracts and androgenic steroids on sex organs of estrogen-primed rat. (See text for explanation of abscissa.)

The dose of testosterone capable of inducing minimal but definite growth of the seminal vesicles was only 2 to 3 times that necessary to produce minimal growth of the ventral prostate (Fig. 2B). Ten to 20 times as much androstenedione was required to stimulate the seminal vesicles as was needed to stimulate the ventral prostate, while for sheep pituitary powder 100-110 times, and for Armour LH 227-80, 1000-2000 times as much was required. Progesterone, 17 *a* hydroxyprogesterone, pregnenolone, and dehydroepiandrosterone either did not cause enlargement of the seminal vesicles or did so only at high dose levels.

The threshold dose for enlargement of the testes was similar to the threshold dose for enlargement of the ventral prostate when sheep pituitary powder, Armour LH 227-80 or pregnenolone were administered, but was considerably greater when the other androgenic steroids were given (Fig. 2C).

Discussion. Several patterns of response were observed when increasing doses of pituitary extracts or steroids were administered to immature, male rats previously treated with estradiol dipropionate. One response, seen after administration of Armour LH 227-80 or pregnenolone, was characterized by small increments in ventral prostate weight, no increase in seminal vesicle weight except with large doses, and significant enlargement

of the testes with doses barely affecting the size of the ventral prostate.

Another response, occurring with administration of whole pituitary powder, was typified by relatively large increments in ventral prostate size and no increase in seminal vesicle weight until large doses were administered. Similar although not identical effects were noted with Δ 4-androstene-3,17 dione, 17 α hydroxyprogesterone, and dehydroepiandrosterone. These steroids did not increase testes weight except with large doses. Whole pituitary powder, probably because of its FSH and LH content, induced enlargement of the testes even at doses that caused only minimal increase in ventral prostate weight.

A third pattern of response occurred with testosterone which caused nearly coincident stimulation of the seminal vesicles and ventral prostate with relatively great dose-response slopes, and no increase in testes weight except with large doses. The synergistic action of testosterone and estrogens in affecting growth of the seminal vesicles has been reported(1). It would appear that the other androgenic steroids administered in this study, and the endogenous androgens elaborated by the rat when stimulated by ovine gonadotrophins, did not synergize with the administered estradiol dipropionate.

Purified equine and ovine LH preparations produced different ventral prostate dose-response relationships, the equine material having a greater slope than the ovine LH (Series I). The same phenomenon has been found in the hypophysectomized rat and has been explained by the longer biological half-life of the equine LH(2). A similar mechanism, slow absorption of crude pituitary extracts and rapid absorption of purified LH preparations, may account for the differences in dose-response slopes of these two materials. However, use of a gelatin vehicle should have minimized any potential differences in rate of absorption. Moreover, attempts to further delay absorption of purified LH preparations with an oily vehicle did not enhance their action(3).

The finding that a highly purified LH preparation caused only a doubling of ventral prostate weight in hypophysectomized rats tends to substantiate the data presented here(4). The hypothesis that LH is responsible for production of a weakly androgenic substance such as pregnenolone, while crude pituitary extracts lead to formation of a more potent androgen such as androstenedione, is appealing and compatible with the data presented, but is not substantiated by it. Corroboration of the data presented here, and thus circumstantial support for this hypothesis, can be found among previously reported observations. LH has been shown to maintain spermatogenesis and testicular weight in hypophysectomized rats with doses barely affecting seminal vesicle or ventral prostate size(5), while testosterone propionate did so only at dose levels that were distinctly androgenic(6). Similar observations have been made on the hypophysectomized mouse(7). Pregnenolone has been reported to be more potent in maintaining spermatogenesis and testicular weight in hypophysectomized or estrogen-treated rats than either testosterone, androstenedione, or progesterone(8,9).

The factor (or factors) present in crude pituitary extracts making them more effective than purified LH in stimulating growth of the ventral prostate is unknown. Theoretically, this postulated factor could directly enlarge the ventral prostate, augment the response of the ventral prostate to androgen, or facilitate androgen production by the testes. There are conflicting data in the literature concerning these possibilities. No effect on weight of the ventral prostate, nor augmentation of LH or testosterone effects on weight of the ventral prostate weight, has been noted with FSH(10,11,12) or ACTH(11,13). Growth hormone has been reported to enlarge(11) and to have no effect(14) on the ventral prostate of the hypophysectomized-castrated rat. No effect of growth hormone on the ventral prostate of the castrated rat has been noted (13). Growth hormone has been reported to have little(13) or no(14) effect on the response of the ventral prostate to testosterone. Growth hormone has been reported to augment LH effects on the ventral prostate in one strain of hypophysectomized rats but not another(12).

The reported effects of prolactin on the

ventral prostate have also been variable. Prolactin had no effect on the ventral prostate of the hypophysectomized-castrated rat(11,14) and only minor effect on the ventral prostate of the castrated rat(13). Prolactin had been noted to augment the effect of testosterone on the ventral prostate of the hypophysectomized rat(15), but only questionable augmentation has been noted in the hypophysectomized-castrated animal(14). Little(13) or no(16) augmentation was noted in castrated rats. A synergistic effect of LH and prolactin on the ventral prostate has been noted in one strain of hypophysectomized rats(12,17) but not in another(12). It could not be shown that prolactin augmented the ventral prostate response to urinary gonadotrophins (16). No consistent augmentation of LH effects in the estrogen-treated rat could be shown with ACTH, TSH, FSH, or various combinations of growth hormone and prolactin(3).

Summary. The effects of various pituitary extracts and androgenic steroids on the sex organs of the estrogen-treated immature male rat were studied. Testosterone induced growth of the seminal vesicles and ventral prostate with similar threshold-doses and dose-response slopes for both organs, but did not cause testicular enlargement except at high dose levels. Purified ovine LH and pregnenolone induced growth of the ventral prostate but the dose-response slope was considerably less than that obtained with testosterone, androstenedione, 17 *a* hydroxyprogesterone, progesterone, dehydroepiandrosterone or crude pituitary extracts. Unlike testosterone, neither purified LH nor pregnenolone induced growth of the seminal vesicles except at very high dose levels, but both caused enlargement of the testes at doses that barely effected prostate size.

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