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Can Androgens Alone Fully Restore Seminal Vesicle Epithelium?[1] (41131)

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Abstract. The purpose of this work was to determine if injected testosterone alone could restore the involuted seminal vesicle epithelium of hypophysectomized guinea pigs. Adult male guinea pigs were castrated, hypophysectomized, or hypophysectomized and castrated. Seminal vesicle epithelium was then isolated, weighed, analyzed for cytoplasmic protein content, and studied for its ability to synthesize four soluble, cell-specific, secretory proteins *in vitro* using immunoprecipitation techniques. Hypophysectomy, like castration, caused profound involution of this androgen-dependent tissue including loss of its protein-synthesizing capability. Testosterone replacement therapy restored wet weight, general cytoplasmic protein content, and *in vitro* protein synthesis to values found in castrated guinea pigs given testosterone. Thus, with regard to the parameters studied, the tissue responded to testosterone alone without apparent need for a pituitary hormone. Under the experimental conditions the administration of ovine prolactin simultaneously with testosterone did not enhance the action of testosterone in hypophysectomized–castrated guinea pigs.

In this study we asked if injected testosterone alone can stimulate the growth and development of seminal vesicle epithelium from its involuted state in adult hypophysectomized guinea pigs. It has been postulated off and on for many years that pituitary secretions might be required for the full expression of androgen action. Prolactin, for example, has been said to contribute to the support of male sex accessory tissue (1, 2). This study is germane to several aspects of androgen research. For example, what hypophyseal polypeptide(s), if any, might need to be included in a culture medium to maintain the tissue in a differentiated and functional state? Few critical attempts have been made to answer this question.

Guinea pig seminal vesicle epithelium is very sensitive to androgen depletion and repletion. One can isolate the epithelium easily as a sheet of cells which will synthesize and secrete four intrinsic and unique proteins *in vitro* (3–6). Because the preparation comprises androgen-sensitive parenchymal cells without stromal elements and because there are specific biochemical markers to be measured, we consider the isolated epithelium to be an ideal biological preparation to investigate the effects of testosterone in the absence of pituitary hormones.

Materials and Methods. All guinea pigs were from the Mayo inbred colony; adults, 700–800 g, were used. The isolation of seminal vesicle epithelium, the incubation conditions with L-[U-^{14}C]lysine and [U-^{14}C]-glycine, and the immunoprecipitation methods for the study of cell-specific protein synthesis *in vitro* have been described (4–6). Protein synthesis was quantitated in terms of the DPM of ^{14}C from labeled amino acids incorporated into newly formed protein (4–6). Testosterone propionate (TP) USP (100 mg/ml) for injection was obtained from Spencer Mead Inc. (Valley Stream, N.Y.). This was diluted with sesame oil to 20 mg/ml and injected intramuscularly (2.6 mg/kg) every other day. Ovine prolactin, NIH-P-S12, was obtained from the National Pituitary Agency. The potency was 35 IU/mg. In this hormone preparation there was less than 0.020 NIH-FSH-S1 units/mg and less than 0.0050 NIH-LH-S1 units/mg. For injection the prolactin powder was dissolved in 0.050 *M* Na(H)PO_4 buffer of pH 7.5 to give a final concentration of 2.0 mg/ml. Injections (267 μg/kg) were given intramuscularly once daily at approximately 9 AM; guinea pigs were sacrificed 24 hr following the last injection.

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For the development of a hypophysectomy procedure, the anatomic relationship between the sella turcica and various bony landmarks was established by gross dissection and by multiple X-ray film exposures of the head and neck. We established that the pituitary sits 4–5 mm superior to a midline bony prominence of the sphenoid bone. Adult animals were anesthetized by intraperitoneal injection of 32 mg of sodium pentobarbital and local injection of 1.0 ml of 1% Xylocaine (Lidocaine) (Astra Pharmaceutical Products). Under as sterile conditions as possible, a midline incision was made from sternum to mandible and tracheostomy performed. At the median raphe of the sphenoid bone which was reached by blunt dissection of submandibular muscles, the bony prominence was exposed and drilled to a depth of 4 mm and a diameter of 4–5 mm. A high-speed electric drill (Dremal) with a 2-mm round cutting drill bit was used. The pituitary was quickly removed either intact or in two pieces by gentle suction. The gland was visualized in a trap bottle placed in the suction line. Gel foam was placed in the hole for prompt control of bleeding; careful wound closure was then carried out. Garamycin (1 mg) (Schering Pharmaceutical Corp.) was injected on the day of surgery. Cortisone acetate (2 mg, Upjohn) was injected on the day of and after surgery and every other day thereafter until sacrifice. Sodium levothyroxine (10 μg, Flint Labs) was injected on the fourth day after hypophysectomy to maintain the euythyroid state. A solution of tap water containing 1.5% (w/v) NaCl was provided together with unsalted tap water. Mortality was about 20% but completeness of hypophysectomy was the rule. Hypophysectomy which caused profound changes in appearance, weight, skin, and hair was verified by postmortem inspection of every animal. In control hypophysectomized animals, i.e., those not given androgen replacement therapy, a rapid and extreme involution of seminal vesicle epithelium occurred. Data are presented as mean ± SD.

Results. Hypophysectomy caused a profound involution of the epithelium (Table I, groups 1 and 2). The data should be compared with those from the intact controls (group 6) as well as those from the 5-day castrated (group 8). The effects of

TABLE I. SEMINAL VESICLE EPITHELIUM OF ADULT GUINEA PIGS AFTER HYPOPHYSECTOMY

Group	TP injections	Wet wt (mg)	Cytoplasmic protein (mg)	*In vitro* protein synthesis (dpm × 10^{-3})	No. of seminal vesicles
Hypox before sacrifice					
1 5 days	—	93 ± 34	2.0 ± 1.1	148 ± 76	10
2 9 days	—	90	1.5	115	4[a]
3 [b]	3	270 ± 52	6.9 ± 1.4	547 ± 129	6
4 [b]	4	340 ± 29	7.8 ± 1.3	687 ± 81	8
5 [b]	6	332 ± 45	7.4 ± 1.1	726 ± 98	12
6 Intact	—	427 ± 134	11.1 ± 2.4	926 ± 237	18
7 Intact – injected with prolacitin[c]	—	369 ± 83	8.3 ± 2.0	875 ± 122	16
8 Castrated 5 days	—	173 ± 30	3.8 ± 0.6	236 ± 52	7
9 Castrated 5 days	6	343 ± 77	9.9 ± 1.6	760 ± 81	8

Note. The treated hypophysectomized animals were given a TP (2.6 mg/kg) injection im every other day. Four days afer a sham hypophysectomy mean values for epithelium from two seminal vesicles were 270 mg wet wt, 7.2 mg cytoplasmic protein,and 570,000 dpm *in vitro* protein synthesis.

[a] Only half the seminal vesicles had sufficient epithelium to isolate.

[b] Guinea pigs were hypophysectomized 5–8 days before start of TP replacement therapy and sacrificed 24 hr after the last injection.

[c] Injected (267 μg/kg) once daily for 10 days.

hypophysectomy on the epithelium were progressive during each of the first 4 days following surgery (not shown). After the fifth day postsurgery, it was seldom possible to obtain significant quantities of epithelium. In the hypophysectomized guinea pigs even three injections of testosterone caused significant positive changes (group 3). Six injections restored wet weight, cytoplasmic protein and protein synthesis to the same extent that six injections restored them in the castrated guinea pigs (compare groups 5 and 9). Injected prolactin did not result in data significantly different from those of the untreated controls (compare groups 6 and 7).

In the hypophysectomized and castrated guinea pigs (Table II), testosterone replacement alone was very effective: wet weight, cytoplasmic protein, and *in vitro* protein synthesis were the same as for the testosterone-treated castrated group (Table I). The addition of prolactin to the replacement program caused no greater changes in the parameters studied.

Discussion. Numerous publications have suggested a direct role for the pituitary in the full expression of androgen action in male sex accessory tissue (1, 2, 7–13). When an effect of prolactin was sought, tissue weight or tissue content of fructose or citrate was frequently measured. In contrast, Thomas and colleagues measured nucleic acids and proteins as well (1, 12), and we measured the rate of synthesis of cell-specific proteins *in vitro*. Without doubt testosterone injection alone as used was very effective in restoring not only wet weight and cytoplasmic protein content but also protein synthesizing capabilities of tissue from hypophysectomized and castrated guinea pigs. One may conclude that for the major cell function, i.e., synthesis of secretory proteins, testosterone was sufficient and pituitary secretions are not absolutely required. In our experience with the seminal vesicle epithelium preparation, only a structurally well-developed and differentiated cell can secrete abundant secretory proteins. Thus, testosterone alone supported cell growth and development from the involuted state (Tables I and II).

The evidence for a prolactin effect on prostate tissue is convincing (1, 12). However, prostate is cellularly heterogeneous and in that regard, a more complicated tissue than seminal vesicle epithelium. Furthermore, prostate has an embryologic origin different from that of seminal vesicle which derives from Wolffian duct tissue. Such differences may account for our failure to demonstrate a need for prolactin. Other explanations include the possibility that prolactin effects become demonstrable only with smaller doses of testosterone. Another possibility is that prolactin may influence developmental aspects of seminal vesicle epithelium in the preadolescent

TABLE II. SEMINAL VESICLE EPITHELIUM RESPONSE IN HYPOPHYSECTOMIZED AND CASTRATED ADULT GUINEA PIGS

Injections		Isolated epithelium			
TP	Prolactin	Wet wt (mg)	Cytoplasmic protein (mg)	*In vitro* protein synthesis (dpm × 10^{-3})	No. of seminal vesicles
—	—	60 ± 14	1.2 ± 0.5	57 ± 12	8[a]
6	—	370 ± 63	8.5 ± 0.9	808 ± 37	12
6	+	390 ± 33	9.3 ± 1.4	748 ± 124	12

[a] Four of the eight seminal vesicles had insufficient epithelium to dissect from the stroma.

Note. Five days post hypophysectomy and castration, guinea pigs were injected with TP or TP and prolactin. TP was injected every other day over a 12-day period. Prolactin was injected daily for 11 days. At sacrifice, untreated animals had been hypophysectomized and castrated for at least 10 days. All animals received cortisone and levothyroxine replacement therapy.

animal or functional parameters in the adult animal other than those which we have studied.

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