Seminal Composition and Sexual Activity after Castration and Testosterone Replacement in Rabbits* (33950)

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Mann and Parsons (1) were the first to demonstrate that fructose nearly disappears from semen within 2 weeks after castration of rabbits and that normal seminal fructose levels may be restored by testosterone injections. In fact, growth and secretory activity of male rat accessory sex organs can be maintained even when they are transplanted into females, provided that the females are treated with testosterone (2). These and analogous results from several species illustrate the dramatic influence of testosterone on male accessory organs (3). But the influence of testosterone on sexual activity of males is less marked. For example, differences in sexual behavior of male guinea pigs before castration were unaltered by administration of testosterone after castration (4), and rats and rabbits possessed ability to ejaculate for a long as 1 month after castration (1, 5) in the absence of any steroid hormone treatment.

The following experiment was designed to compare the demise of secretory function in male accessory organs with the demise of sexual activity after castration of rabbits. Another objective was to compare the restoration of the normal secretory and behavioral responses with various levels of injected testosterone in the same castrated rabbits.

Materials and Methods. Eight sexually mature rabbits of mixed breeding were trained to mount a single female and ejaculate into an artificial vagina. Each rabbit was given an opportunity to ejaculate every second day throughout a 10-day preliminary period and an 86-day postcastrational period. Sexual reaction of each rabbit at each opportunity to ejaculate was scored from greatest sexual reaction (10) to none (0), according to the following objective system: 10) Rabbit ejaculated within 45 sec with routine female; 9) Rabbit failed 10, but ejaculated between 45 and 180 sec with routine female; 8) Rabbit failed 9, but ejaculated within 45 sec with new female; 7) Rabbit failed 8, but ejaculated between 45 and 180 sec with new female; 6) Rabbit failed 7, mounted and thrust vigorously with both females, but did not ejaculate; 5) Rabbit failed 6, but mounted and thrust vigorously only with new female; 4) Rabbit failed 5, but mounted routine female within 30 sec and failed to thrust vigorously; 3) Rabbit failed 4, but mounted routine female between 30 and 180 sec and failed to thrust vigorously; 2) Rabbit failed 3, but mounted new female within 30 sec and failed to thrust vigorously; 1) Rabbit failed 2, but mounted new female between 30 and 180 sec and failed to thrust vigorously; and 0) Rabbit failed to mount either female. Thus each rabbit was allowed up to 360 sec to ejaculate every second day. The same routine female was always the mount during the first 180 sec, but one of several new females was substituted for the routine female during the final 180 sec. The female was always brought to the male's cage.

Immediately following the fifth control (preliminary period) ejaculation, the rabbits were castrated via a midventral incision. Except for routine postoperative care, the rabbits were untreated for 26 days while each was given an opportunity to ejaculate every second day. Beginning on postoperative day

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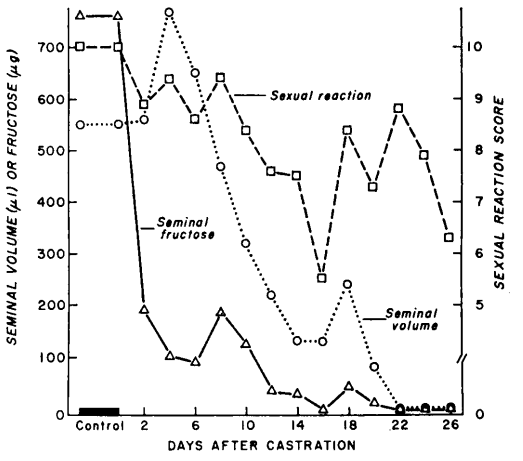

FIG. 1. Loss of libido, seminal volume, and seminal fructose after castration.

27, each rabbit was given 0.5 mg testosterone propionate (TP) subcutaneously in 0.5 ml of corn oil every second day for 10 days. This was followed by five similar injections of 1.0, 2.0, 4.0, 8.0 and 12 mg of TP which began on days 37, 47, 57, 67 and 77, respectively. Injections were always given on days alternate to ejaculations.

Each semen sample was frozen immediately after ejaculation. After thawing, seminal volume was recorded before and after removal of any gel mass. The gel mass was discarded, and 2.0 ml of 20% trichloroacetic acid was added to each ejaculum. Following centrifugation, fructose was determined (6) in 0.5 ml of the supernatant fluid. A value of zero was recorded when a rabbit exhibited orgasm with no perceptible seminal volume. But no data were recorded for a rabbit which had no orgasm. Consequently, analyses of variance of these data were performed with unequal subclass numbers.

Two statistical analyses were performed on the sexual reaction scores. One considered the data from the 26-day postoperative period and included orthogonal polynomial partitioning of the 12 degrees of freedom for days after castration. The last five ejaculation days of the same period were taken as representative of zero TP and compared to averages of the five ejaculation days on each of the other six levels of TP in a Duncan's multiple range test.

Results. The decline in average sexual reaction score (Fig. 1) with increasing duration of castration showed a significant linear component ($p<0.01$) (but most rabbits continued to mount and thrust vigorously throughout this 26-day period. Seminal volume declined gradually for the first 3 weeks of castration, but there was a precipitous decline in the average seminal fructose content during the first 2 days of castration.

The average sexual reaction score (Table I and Fig. 2) increased significantly ($p<0.01$) with 0.5 mg relative to no TP, and 1.0 mg of TP caused another significant increase in sexual reaction ($p<0.01$). In fact, 1.0 mg of TP resulted in orgasm in each rabbit on each ejaculation day. Increments in reaction score with additional TP above 1.0 mg were more gradual and reaction scores with 8.0 or 12.0 mg of TP did not differ significantly from precastrational control values.

Injections of 0.5 mg of TP had no measurable infuence on seminal volume (Table I, Fig. 2). Both seminal volume and fructose were markedly increased at 2.0 mg TP, and the responses of these two seminal characteristics to increasing levels of TP were generally parallel. Further, both attained values at 8.0 mg of TP which did not differ significantly from precastrational control levels. And at

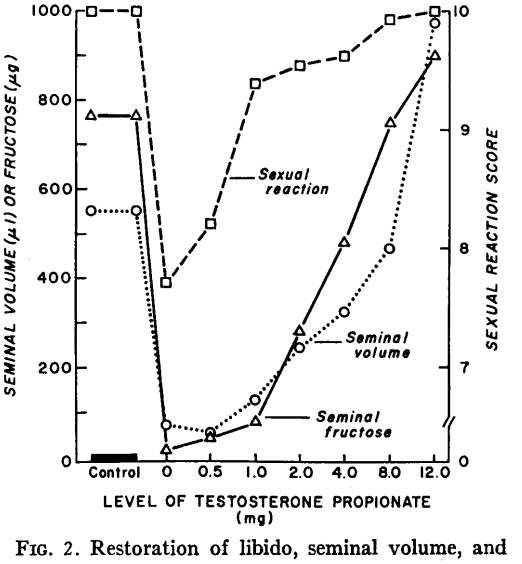

FIG. 2. Restoration of libido, seminal volume, and seminal fructose by testosterone propionate.

TABLE I. Restoration of Libido and Seminal Characteristics of Castrate Rabbits with Testosterone Propionate (TP).

Rabbits	Level of TP[a] (mg)	Sexual reaction score[b]	Seminal characteristics[c]				
			No. of ejacula	Volume (ml)	Fructose (μg)	No. of gel mass	Volume of gel mass (ml)
Intact	0.0	10.00	40	0.55	760	11	0.84
Castrate	0.0[d]	7.70	25	0.06	14	0	—
	0.5	8.20	31	0.05	39	0	—
	1.0	9.40	40	0.12	75	0	—
	2.0	9.55	40	0.27	286	0	—
	4.0	9.60	39	0.32	480	0	—
	8.0	9.95	40	0.46	750	7	0.52
	12.0	10.00	40	0.97	900	12	1.12

[a] Testosterone propionate injected in corn oil every second day.

[b] Scored from normal libido = 10 to none = 0 (see text).

[c] An opportunity to ejaculate was provided for each rabbit every second day, on days alternate to TP injections. Seminal values are averages per ejaculum.

[d] Values for 0.0 TP are averages for 18 to 26 days after castration.

12.0 mg of TP, both were increased significantly above control levels. No gel mass was detected in any ejaculum after castration until rabbits were injected with 8.0 or 12.0 mg of TP.

Discussion. In agreement with previous observations (1) one of the more dramatic seminal changes after castraction in rabbits is the precipitous decline in fructose during the first 48 hr. The present experiment also demonstrates complete absence of gel mass from rabbit semen within 48 hr after castration. Thus, fructose synthesis and gel mass formation are among the most sensitive indicators of testicular androgen. Seminal volume is less sensitive because it declined rather gradually and largely linearly for 20 days. Measurable seminal volumes were obtained from only two rabbits at 20 days and from none at 22–26 days after castration.

We assume that, once accessory glands cease secretion, they probably atrophy. Any seminal volume response to exogenous androgen under these conditions would be gradual and require prior accessory gland growth. Consequently, we suggest that seminal volume responses to injected TP may be more rapid in experiments like that described above, where TP is administered soon after seminal volume disappears, than in experiments where androgen injections are begun at longer intervals after castration (e.g., 7).

Postcastrational decline in sexual reaction described for the rabbits in the present experiment has been observed in most animals (8). But, our rabbits had average reaction scores less than 7.0 on only two ejaculations days. We suggest that maintenance of comparatively high libido in these castrate rabbits may have been associated with the relatively frequent ejaculation (every second day). Nevertheless, administration of as little as 0.5 mg of TP resulted in increased reaction scores within 24 hr. The average reaction scores for the five ejaculation days during injections of 0.5 mg TP were 7.0, 8.0, 8.6, 8.6 and 8.8. Other than this nonsignificant trend for increased libido with increasing duration of a level of TP, there was no indication for increased libido with increasing time at any level of TP. Rather, increments in libido occurred within 24 hr after the first injection of the next higher level of TP. Although there was a marked increase in sexual reaction at 1.0 mg of TP, (av = 9.40), the reactions of these rabbits were quite variable because 25 of these 40 observations were scores of 10. Precastrational reaction scores were reestablished only upon injections of 8.0 or 12.0 mg of TP.

At the final injection of 8.0 mg of TP, each rabbit had received a total of 77.5 mg — considerably less than the 150 mg which De Fremery and Tausk (7) indicated may be required to reestablish normal libido. The greater level of TP reported by them may have reflected a longer interval from castration to beginning of TP treatment. Others (4, 9) reported that normal libido of hamsters and guinea pigs was reestablished only gradually with injections of a constant level of TP, suggesting an initial period of refractoriness to TP.

Cheng and Casida (10) reported that daily injections into intact rabbits of 1 or 3 mg of TP reduced the time required to obtain one ejaculate on every fourth day from 25.5 to 11.4 sec for the lower dose and from 42.7 to 11.5 sec for the higher dose. The scoring procedure used in the present experiment did not distinguish between normal and increased sexual activity, as the maximum score was 10 and lower scores reflected reduced libido. However, the averages for the sexual reaction scores at levels of TP (2.0 and 4.0 mg of TP every 48 hr) comparable to those used by Cheng and Casida (10) were significantly less than 10.0, suggesting that these dose levels did not maintain normal libido in castrate rabbits, much less intensify it. This disparity may be due to our injections of TP every 48 hr instead of every 24 hr. Alternatively, the influence of TP injections on libido may be additive with that of endogenous androgen in intact rabbits.

In contrast to the response of libido, seminal volume and fructose increased very little with 0.5 or 1.0 mg of TP. But in agreement with the restoration of libido, seminal volume and fructose were restored to control levels with 8.0 mg of TP. This difference between behavioral and seminal effects of TP may be associated with some atrophy of the accessory reproductive organs before beginning TP injections. The 8.0-mg level of TP required (every second day) to restore normal libido and normal seminal characteristics is greater than the daily injections of 2.5 mg of TP reported by Bern (11). But Bern measured histological criteria of accessory organs and maintenance of normal micromorphology may not reflect accurately the secretory activity of accessory reproductive organs (12).

From the time of castration through the time of injections of 0.5 through 4.0 mg of TP, no seminal gel mass was observed. Then three ejacula contained gel mass within 24 hr after the first injection of 8.0 mg of TP. This suggests that formation of a gel mass requires at least 8.0 mg of TP, similar to the other criteria measured in this experiment. Cheng and Casida reported that 1.0 mg of TP increased the volume of gel mass from intact rabbits, just as 12.0 mg of TP increased gel mass volume in the present castrated rabbits. Thus the unpredictable and erratic occurence of gel mass among normal rabbits may be a function of marginal androgen levels. Levels of androgen greater than those normally available in intact rabbits and levels of TP in castrate rabbits greater than 8 mg may materially increase the incidence of ejacula with gel mass.

Summary. Losses of libido and seminal volume, fructose and gel were quantified in eight rabbits ejaculated every second day for 26 days after castration. Androgen requirement for restoration of normal libido and seminal characteristics was established in the same rabbits by injections of testosterone proprionate (TP) at levels of from 0.5 to 12.0 mg every second day. Gel mass disappeared from ejacula immediately after castration and seminal fructose declined from 760 μg before castration to 192 μg/ejaculum within 48 hr. But seminal volume and libido declined only gradually and linearly for 3 weeks after castration. Even after 26 days, nearly all rabbits mounted and thrust vigorously although unable to ejaculate. Although libido of castrate rabbits was increased significantly by 0.5 or 1.0 mg of TP, seminal characteristics were not. Control levels of both were reestablished at 8.0 mg of TP.

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Enhancement of Lysozyme Activity by Anodal Tear Protein* (33951)

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The anodal protein of human tears is a major constituent of the lacrimal secretion. This protein migrates as a "prealbumin" in starch or acrylamide gel electrophoresis and has an s_{w20} value of 2.0 in the analytic ultracentrifuge (1). Human anodal tear protein is antigenic (2) and specific antiserum has been prepared in rabbits. This serum shows no reactivity with any of the protein components of saliva, spinal fluid, urine, or serum when tested by agar diffusion techniques (1). Because lysozyme, a basic protein, is also present in tears, it was postulated that the anodal tear protein is important in either the secretion or action of lysozyme. The enhancement of the action of lysozyme in the lysis of *Micrococcus lysodeikticus* in dilute salt solution by the anodal tear protein is here described.

Methods. Rabbit lacrimal secretions were obtained by placing sterile Schirmer tear test strips in the rabbit conjunctival sac. The papers were permitted to become saturated with conjunctival fluid over a 5-min period. The lacimal protein from two strips was eluted with 0.25 ml of an 0.9% NaCl solution buffered at pH 7.2 with 0.05 *M* phosphate buffer and 100 to 200 such eluates were pooled. After 10-fold concentration by dialysis against polyvinylpyrrolidone, the tear eluates were subjected to Sephadex G200 gel filtration in phosphate buffered saline, pH 7.2. The final peak of the gel filtrate contained lysozyme and the anodal protein, and was concentrated again and dialyzed against phosphate buffer, 0.01 *M* pH 6.7. This protein was passed through a carboxymethyl cellulose column equilibrated with the same buffer. The "fall-through" peak was collected, dialyzed against distilled water and lyophilized. The protein thus obtained was homogeneous by acrylamide gel electrophoresis and had migratory properties in acrylamide gel and immunoelectrophoresis similar to those of human anodal tear protein. Equilibrium ultracentrifugation of rabbit anodal tear protein indicated a molecular weight of 23,000. Assays of lysozyme activity were carried out in a Cary spectrophotometer by the continuous recording of the decrease in optical density of a 0.3 mg/ml suspension of *M. lysodeikticus* (3). The suspension was prepared in 0.01 or 0.1 *M* phosphate buffer, pH 7.0. To a 2.9-ml aliquot of the bacterial suspension was added either 0.1 ml of a solution of rabbit anodal tear protein in distilled water at a concentration of 5 mg/ml or 0.1 ml of distilled water. After a base line reading to

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