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EFFICACY OF TESTOSTERONE-FILLED POLYDIMETHYLSILOXANE IMPLANTS IN MAINTAINING PLASMA TESTOSTERONE IN RABBITS

L. G. STRATTON,* L. L. EWING† AND C. DESJARDINS‡

Department of Physiological Sciences, Oklahoma State University, Stillwater, Oklahoma 74074, U.S.A.

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Summary. Experiments were designed to examine the release of testosterone through polydimethylsiloxane (PDS) capsules *in vitro* and *in situ* and to test the efficacy of PDS capsules in maintaining testosterone in the peripheral circulation of castrate male rabbits for several months. The results demonstrate that (1) tritiated testosterone passed through PDS capsules suspended in water, (2) the tritiated material released into water had the same chromatographic mobility as authentic testosterone when subjected to thin-layer chromatography, (3) the rate of release of testosterone into water was dependent upon capsule surface area, (4) the release of testosterone from implants *in situ* was dependent upon surface area but independent of implantation site (i.e. subcutaneous versus intraperitoneal implants), and (5) the release of testosterone from PDS capsules *in situ* remained relatively constant over a span of 3 months. Castration abolished libido and caused accessory sex organ atrophy in male rabbits, but subcutaneous placement of testosterone-filled PDS implants (430 mm^2, 0·245 mm thick) maintained these androgen-dependent characteristics at normal levels. The concentrations of seminal fructose, citric acid and plasma testosterone noted in castrate rabbits receiving testosterone implants *in situ* were virtually identical to those noted in intact rabbits receiving similar implants containing cholesterol for 3 months.

INTRODUCTION

Enclosure of a number of steroids such as oestradiol, progesterone, androstenedione, testosterone and cortisol in polydimethylsiloxane (PDS) membranes permits them to diffuse at a uniform and predictable rate into distilled water and plasma *in vitro* (Dziuk & Cook, 1966; Kincl, Benagiano & Angee, 1968). Placement of PDS testosterone implants in castrate male rats has been shown to

* Present address: Department of Animal Science, University of Arkansas, Fayetteville, Arkansas 72701, U.S.A.

† Present address: Division of Reproductive Biology, Department of Population Dynamics, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205, U.S.A.

‡ Present address: Department of Zoology, The University of Texas, Austin, Texas 78712, U.S.A.

maintain accessory sex gland weight for as long as 16 weeks (Moon & Bunge, 1968). Chang & Kincl (1968) compared the biological activity of 6-methyl-17α-acetoxypregna-4,6-diene-3,20-dione using three different routes of administration in female rats: subcutaneous injection, gavage and PDS implants. Administration of this steroid in a PDS implant required 6 to 25 times less steroid than the other routes of administration to produce comparable biological effects. These encouraging results suggest that PDS implants may be used in experimental or clinical situations where it is necessary to maintain specific concentrations of plasma testosterone in males over protracted time periods. Before this can be accomplished, however, experiments must be conducted to determine the efficacy of testosterone PDS implants in maintaining specific plasma testosterone concentrations in animals over protracted time periods. Experiments were therefore carried out to confirm that testosterone passes through PDS capsules and that the rate of release is directly proportional to capsule surface area. We also showed that subcutaneous placement of testosterone implants for 3 months maintained libido, accessory sex gland weights and secretory activity and plasma testosterone concentrations in orchidectomized rabbits at levels identical to those observed in intact rabbits receiving PDS cholesterol implants.

MATERIALS AND METHODS

General

Mature male rabbits were supplied by Redwood Game Farm, Salt Lake City, Utah. Rabbits were housed individually in an air-conditioned room on a 14-hr light/10-hr dark schedule and were allowed free access to Purina Rabbit Chow and water.

Polydimethylsiloxane tubing and Silastic Medical Adhesive Silicone Type A cement were obtained from Dow-Corning Corporation, Midland, Michigan. Polydimethylsiloxane capsules were prepared according to Kincl *et al.* (1968) by selecting silastic tubing of the desired length, sealing one end with silicone cement, filling the capsule with appropriate material and sealing the open end. Each seal was allowed approximately 12 hr for cross-linking and hardening. Each capsule was implanted with an 11-gauge trocar under aseptic conditions. No evidence of adhesions or infections was detected after placement of implants *in situ* for as long as 3 months.

Assessment of libido and accessory sex gland function

Libido was monitored by quantifying the latency required for mounting and ejaculation after exposure to a teaser female. The latency between the exposure of the male to the teaser female and mounting and ejaculation into an artificial vagina was recorded. Males not responding to the female test stimulus within 180 sec were presented with a new teaser female and afforded another 180 sec to respond. Failure to mount and ejaculate was recorded as 360 sec in both categories. These criteria were measured every other day for 20 days after subcutaneous placement of PDS testosterone implants for 70

days. This method is discussed in full by Macmillan, Desjardins, Kirton & Hafs (1969).

Each semen sample obtained after sexual activity tests was immediately cooled to 5° C. Seminal volume was recorded before and after removal of gel mass. The gel mass was discarded and 2·0 ml of 20% trichloroacetic acid were added to each ejaculate. Following centrifugation, the fructose content (Davis & Gander, 1967) and the citric acid content (Saffran & Denstedt, 1948) of the supernatant were determined.

Determination of testosterone-tritium release

The release of testosterone-tritium from PDS capsules *in vitro* (Exp. 1) was determined by measuring the radioactivity present in 1-ml aliquots of the water bathing the capsule. Aliquots were placed in scintillation vials with 14 ml Aquasol (New England Nuclear, Boston, Massachusetts), mixed and cooled in the dark at 5° C for 4 hr before determining their radioactivity.

In Exps 2 and 3, 1-ml aliquots of plasma obtained from rabbits receiving subcutaneous testosterone-tritium PDS capsules were placed in a scintillation vial with 14 ml Aquasol, mixed by vigorous shaking, and allowed to equilibrate in the dark at 5° C for 48 hr before measuring radioactivity.

Determination of plasma testosterone

Testosterone present in blood plasma was extracted, isolated and quantified by means of gas–liquid chromatographic (GLC) procedures with electron capture detection as originally described by Brownie, van der Molen, Nishizawa & Eik-Nes (1964) and modified by Kirschner & Coffman (1968). Testosterone loss before GLC was determined and corrected to 100% recovery by measuring the loss of [1,2-^{3}H]testosterone added to each plasma sample before extraction. After radioactive samples were placed in scintillation vials, they were evaporated to dryness under nitrogen. Subsequently, 12 ml scintillation fluid (15·15 g 2,5-diphenyloxazole and 151·4 mg 1,4-bis-2,5-phenyloxazole per 3·7 litres of spectro quality toluene) were added to each vial and the contents were counted for radioactivity.

Measurement of radioactivity

Radioactivity was measured in a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3365) equipped with automatic external standardization. In all cases, sufficient radioactivity was allowed to accumulate to give a probable error of 3% or less. All radioactivity values were corrected to 100% efficiency (i.e. d/min) by counting each sample against a ^{137}Cs-external standard and comparing the value obtained to a standard correlation curve (count rate of external standard versus the percentage efficiency of samples of known radioactivity containing varying amounts of a quenching additive) for tritium in the scintillator solutions.

Experiment 1

The release *in vitro* of [1,2-^{3}H]testosterone from PDS capsules was examined by measuring the rate of appearance of tritium from 225-mm^{2}, 450-mm^{2} and

900-mm^2 capsules (0·65-mm wall thickness). Six capsules of each size were filled with a mixture of recrystallized testosterone and [1,2-^{3}H]testosterone (specific activity of mixture = 1900 ct/min/μg) and were incubated individually at 22 to 25° C in 100 ml of distilled water. Aliquots (1 ml) were obtained for tritium determination at 6, 12 and 24 hr after incubation.

Experiment 2

The relationship of capsule surface area to the level of tritium in the peripheral plasma of intact male rabbits was determined after implantation of testosterone-tritium (specific activity = 1900 ct/min/μg) PDS implants. Capsules were prepared with a wall thickness of 0·65 mm and external surface areas of 225, 450 or 900 mm^2 and implanted subcutaneously into a total of twenty-one rabbits (seven replicates per dimension). Tritium concentrations in samples of peripheral blood plasma were determined at weekly intervals for 1 to 7 weeks for each animal.

Experiment 3

The effect of implantation site on the concentration of tritium in the peripheral blood plasma of intact male rabbits was measured by preparing capsules (containing testosterone-tritium; specific activity = 1900 ct/min/μg) with external surface areas of 450 and 900 mm^2 (wall thickness, 0·65 mm) and implanting them either subcutaneously or intraperitoneally. Five animals were used for each capsule dimension at both implantation sites. The concentration of tritium in samples of peripheral blood plasma was determined at weekly intervals for 1 to 6 weeks for each animal.

Experiment 4

Twenty-eight rabbits were trained to mount a female and ejaculate into an artificial vagina. Twenty-one animals were anaesthetized and bilateral castration was performed. Seven castrated rabbits were allocated to each of three experimental treatment groups. These treatment groups received subcutaneous PDS implants as follows: (1) cholesterol-filled 430-mm^2 capsule, (2) testosterone-filled 215-mm^2 capsule, or (3) testosterone-filled 430-mm^2 capsule. The remaining seven intact rabbits (control group) received cholesterol-filled 430-mm^2 capsules. The wall thickness of all implants was 0·245 mm. Sex behaviour measurements were taken 70 to 90 days after implantation. The animals were exsanguinated 90 days after implantation and the accessory sex organs were removed and peripheral blood samples were obtained. Accessory sex glands were dissected free of extraneous tissue, separated according to the method described by Bern & Krichesky (1943), blotted to remove excess fluid and then weighed.

Statistical analysis

The results of Exps 1 to 4 were subjected to individual factorial analyses of variance (Snedecor & Cochran, 1967). When the analyses of variance revealed significant variations, the differences in treatment means were detected by Duncan's Multiple Range Test (Steel & Torrie, 1960).

RESULTS

Experiment 1

Release *in vitro* of [1,2-^{3}H]testosterone from capsules into distilled water increased with respect to time for each capsule size (Table 1). The release rate with respect to capsule surface area, although variable, showed a direct relationship between rate of release and capsule surface area. Statistical analysis showed a significant variation ($P<0{\cdot}01$) due to time of incubation and capsule surface area. Tritium extracted from the water bathing PDS capsules had the same chromatographic mobility on thin-layer chromatography (benzene: methanol, 87·5:12·5, v/v) as authentic testosterone.

Table 1. Release of testosterone from polydimethylsiloxane capsules into distilled water at 22 to 25° C

Capsule surface area (mm^2)	*Time (hr)*		
	6	12	24
225	20 ± 1·6	33 ± 2·3	62 ± 4·4
450	32 ± 4·1	53 ± 6·6	96 ± 7·8
900	96 ± 6·8	138 ± 9·7	212 ± 9·4

Each value is expressed as μg testosterone/100 ml water and represents the mean ± S.E. of six replicates.

Table 2. Tritium concentration in the peripheral plasma of intact male rabbits containing subcutaneously placed PDS [1,2-^{3}H]testosterone capsules

Capsule surface area (mm^2)	*Time (weeks)*						
	1	2	3	4	5	6	7
225	269 ± 12	256 ± 56	196 ± 20	152 ± 27	162 ± 20	181 ± 31	182 ± 24
450	331 ± 29	350 ± 22	307 ± 26	250 ± 30	252 ± 31	347 ± 26	277 ± 27
900	797 ± 198	741 ± 89	690 ± 74	746 ± 81	723 ± 117	687 ± 93	661 ± 158

Each value represents the mean ± S.E. of seven rabbits and is expressed as tritium d/min/ml blood plasma.

Experiment 2

A simple method of monitoring testosterone release from capsules *in situ* is to quantify the levels of tritium derived from [1,2-^{3}H]testosterone-filled PDS implants in peripheral plasma. The results in Table 2 show a direct relationship between capsule surface area and the amount of tritium detectable in peripheral blood plasma over a 7-week period. Increasing the surface area from 225 mm^2 to 900 mm^2 caused a significant increase ($P<0{\cdot}05$) in the tritium concentration in peripheral plasma, but no significant differences were noted between the 225- and 450-mm^2 or the 450- and 900-mm^2 capsules. Moreover, there was no significant difference in plasma tritium levels due to time ($P>0{\cdot}25$).

Although it was possible to demonstate the release of [1,2-^{3}H]testosterone from PDS implants *in situ*, the results in Table 3 show that only 1 to 2% of the total plasma tritium present in rabbits receiving subcutaneous [1,2-^{3}H]-testosterone PDS implants was actually associated with tritiated testosterone.

Experiment 3

Capsules (450 and 900 mm^2) of PDS containing [1,2-^{3}H]testosterone were placed subcutaneously and intraperitoneally into mature male rabbits. The

Table 3. Distribution of tritium in the peripheral plasma of intact male rabbits containing subcutaneous PDS [1,2-^{3}H]testosterone implants*

Capsule surface area (mm^2)	*Total tritium in plasma sample (d/min/sample)*	*Total tritium in dichloromethane extract*		*Testosterone tritium*	
		d/min/ sample	*% of total tritium*	*d/min/ sample*	*% of total tritium*
225	5733	108	2	109	1·9
450	13514	275	2	160	1·2
900	14384	750	5·2	187	1·3

* Plasma obtained during the 5th week of blood collection reported in Table 2 was pooled and a 1-ml aliquot was removed from pooled plasma and used for radioactivity determination in Aquasol. The remainder of the plasma pool was extracted three times with 2·5 vols of dichloromethane. An aliquot was removed and counted in Aquasol. The remainder was concentrated under nitrogen, developed in the thin-layer chromatography system, benzene:ethylacetate (110:100), and the area corresponding chromatographically to testosterone was eluted and counted in Aquasol. All counts were corrected for quenching, for loss through the respective purification procedures and for aliquot losses.

Table 4. Tritium concentration in the peripheral plasma of intact male rabbits containing subcutaneous or intraperitoneal PDS [1,2-^{3}H]testosterone implants

Capsule surface area (mm^2)	*Site*	*Time (weeks)*					
		1	2	3	4	5	6
450	s.c.	293 ± 25	304 ± 22	293 ± 30	305 ± 15	277 ± 37	256 ± 28
	i.p.	343 ± 65	375 ± 24	326 ± 30	366 ± 46	264 ± 28	348 ± 30
900	s.c.	727 ± 96	327 ± 13	708 ± 60	578 ± 24	600 ± 13	658 ± 64
	i.p.	786 ± 94	709 ± 64	581 ± 92	523 ± 115	726 ± 117	846 ± 108

Each value represents the mean ± S.E. of seven rabbits and is expressed as tritium d/min/ml blood plasma. s.c. = Subcutaneous; i.p. = intraperitoneal.

capsules used in this experiment were the same ones that were used in Exp. 2. The results in Table 4 show that there was no significant difference ($P > 0{\cdot}25$) in tritium concentration due to time or to site of implantation.

The release of testosterone-tritium *in situ* was constant over a 13-week period since the same capsules were used as in Exps 2 and 3. The [1,2-^{3}H]testosterone release *in vitro* and *in situ* was directly related to surface area of the Silastic capsules.

Experiment 4

Goodwin (1972) showed that the rate of production of testosterone by mature male rabbits was approximately 400 μg/24 hr, and results obtained in Exps 1 to 3 suggested that a testosterone capsule with a wall thickness of 0·245 mm providing a surface area of 430 mm^2 should release approximately 400 μg/24 hr. An experiment was therefore designed to compare the effect of 215- and 430-mm^2 testosterone PDS implants upon androgen characteristics and plasma testosterone concentrations in castrate male rabbits.

Sexual behaviour was undetected in rabbits castrated for 70 to 90 days, but subcutaneous placement of a 215-mm^2 testosterone PDS implant significantly ($P<0·05$) increased the sexual behaviour score to 6·0. Animals receiving 430-mm^2 testosterone PDS implants showed a further increase to 9·8. The sex behaviour scores of intact (8·4) and castrate (9·8) rabbits receiving a 430-mm^2 testosterone PDS implant were not significantly different from each other ($P>0·25$).

Table 5. Effect of PDS testosterone implants on the weights of accessory sex organs of castrate male rabbits

Contents and size of implants	*Testes*	*Organ weights*			
		Seminal vesicles	*Prostate gland*	*Vesicular gland*	*Bulbo-urethral gland*
Cholesterol 430 mm^2	–	135 ± 10	113 ± 20	129 ± 29	216 ± 50
Cholesterol 430 mm^2	+	508 ± 70	693 ± 60	807 ± 70	622 ± 90
Testosterone 215 mm^2	–	297 ± 40	415 ± 50	539 ± 60	424 ± 30
Testosterone 430 mm^2	–	476 ± 90	572 ± 40	778 ± 90	616 ± 60

Each value represents the mean ± S.E. of seven rabbits following subcutaneous placement for 90 days.

In parallel with these observations, the results in Table 5 show that castration for 90 days significantly ($P<0·01$) decreased seminal vesicle, prostate, vesicular and bulbo-urethral gland weights below those of control rabbits receiving PDS cholesterol-filled implants. Testosterone PDS implants resulted in increased accessory sex organ weights which were proportional to the surface area of the PDS implant. The weight of the accessory sex organs of rabbits receiving 430-mm^2 testosterone implants was not significantly different ($P>0·25$) from intact rabbits receiving PDS cholesterol implants.

The data in Table 6 show that secretory function as well as accessory sex organ weight was maintained by subcutaneous PDS testosterone implants. Castration for 70 to 90 days reduced the seminal volume, citric acid and fructose concentrations to zero. Castrate male rabbits receiving 215-mm^2 PDS testosterone implants produced seminal plasma with significantly ($P<0·01$) lower citric acid and fructose concentrations than castrate males receiving 430-mm^2 PDS testosterone implants. There was no significant difference ($P>0·25$) in

the seminal volume, citric acid or fructose concentrations of castrate males receiving 430-mm^2 PDS testosterone implants or of intact males receiving cholesterol PDS implants.

Differences observed in libido, accessory sex organ weight and secretory activity (Tables 5 and 6) paralleled the plasma testosterone concentrations.

Table 6. Effect of PDS testosterone implants on secretory function of accessory sex organs of castrate male rabbits

Contents and size of implants	*Testes*	*Seminal plasma*		
		Volume of ejaculates (ml)	*Citric acid (mg/ml)*	*Fructose (mg/ml)*
Cholesterol 430 mm^2	−	0·0	0·0	0·0
Cholesterol 430 mm^2	+	0·43 ± 0·08	1·73 ± 0·30	2·30 ± 0·34
Testosterone 215 mm^2	−	0·39 ± 0·15	0·62 ± 0·23	0·93 ± 0·14
Testosterone 430 mm^2	−	0·59 ± 0·05	2·11 ± 0·23	2·46 ± 0·52

Each value represents the mean ± S.E. of ten determinations made on seven rabbits following subcutaneous placement of the implants for 70 to 90 days.

This was shown by the finding that testosterone was undetectable in the blood of castrate males containing PDS cholesterol implants but averaged 2·1 ± 0·6 ng/ml plasma in intact animals and 2·09 ± 0·72 and 2·3 ± 0·76 ng/ml plasma 90 days after subcutaneous placement of 215- and 430-mm^2 testosterone implants.

DISCUSSION

The present results demonstrating that [1,2-^{3}H]testosterone passed through PDS capsules *in vitro* and *in situ* agree with the findings of previous investigators (Dziuk & Cook, 1966; Kincl *et al.*, 1968). Tritium extracted from distilled water bathing PDS capsules had the same chromatographic mobility as authentic testosterone, supporting the contention that testosterone passed through PDS membranes unchanged. These results imply that different dosages of testosterone may be administered parenterally by varying the surface area of PDS implants. Further exploitation of such a possibility necessitates answers to two questions: does a direct relationship exist between capsule surface area and testosterone release *in situ*, and is it possible to obtain a sustained release of testosterone from PDS capsules *in situ*?

The results of Exps 2 and 3 demonstrate that testosterone release from PDS capsules was continuous for 13 weeks, and was dependent upon surface area and independent of site of implantation. Comparison of release rates versus site of implantation was not intended to provide evidence to select the most efficacious site for PDS testosterone implants, but to demonstrate that the factor limiting [1,2-^{3}H]testosterone release from subcutaneous PDS implants

was the surface area of the capsule. The subcutaneous site was chosen because it limits the amount of tissue available for absorption while the peritoneal cavity offers a comparatively larger absorbing surface from which drugs enter the circulation rapidly (Fingl & Woodbury, 1970). The fact that [1,2-^{3}H]-testosterone release was independent of the site of implantation supports the conclusion that the rate of release is influenced primarily by the external surface area of PDS capsules. However, the observation that 98 to 99% of the [1,2-^{3}H]-testosterone released was metabolized to another moiety suggests that the surface area-dependent release rate from PDS capsules may not be a primary factor limiting the practical application of this technique to experimental and clinical conditions. An aspect previously overlooked in experiments studying this mode of administering steroidal hormones may be the homeostatic mechanisms regulating the metabolism and excretion of these natural products.

The finding that less than 2% of the tritium in the peripheral circulation was associated with testosterone *per se* supports the argument that the only meaningful assessment of the efficiency of testosterone PDS implants to release testosterone is to determine their ability to sustain specific concentrations of biologically active testosterone in the peripheral circulation. The fact that plasma testosterone levels, sexual behaviour, accessory sex organ size and secretory activity were maintained in castrate male rabbits for 3 months by the 215-mm^2 and 430-mm^2 PDS testosterone capsules attests to the efficacy of these implants. The fact that the androgen-dependent characteristics of the castrate rabbits receiving the 430-mm^2 implant were similar to those of the intact controls suggests that PDS implants of this dimension mimic endogenous testosterone secretion from the testes.

It seems worthwhile to speculate about possible practical applications of this mode of testosterone administration. Testosterone PDS implants should facilitate investigations pertaining to the effects of specific plasma testosterone concentrations upon the hypothalamic-hypophysial neuroendocrine system governing gonadotrophin secretion in castrate males. This technique should also allow investigation of the concept that specific testosterone therapy by means of PDS implants might create azoospermia in experimental animals and man without altering accessory sex organ size or secretory activity. Provided testosterone PDS implants are more efficacious in maintaining plasma testosterone concentration than injections or testosterone-filled cholesterol pellets, it might be possible to elucidate the testosterone requirement for spermatogenesis in intact and hypophysectomized males. The possibilities noted above merely serve to typify some important applications of testosterone-filled PDS capsules and it seems reasonable to suggest, on the basis of the present results, that PDS testosterone capsules may provide an effective means of investigating the complete spectrum of testosterone-dependent actions in both animals and man.

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