QUANTITATIVE STUDIES OF PROSTATIC SECRETION*

I. CHARACTERISTICS OF THE NORMAL SECRETION; THE INFLUENCE OF THYROID, SUPRARENAL, AND TESTIS EXTIRPATION AND ANDROGEN SUBSTITUTION ON THE PROSTATIC OUTPUT

BY CHARLES HUGGINS, M.D., M. H. MASINA, B. CHIR., LILLIAN EICHELBERGER, PH.D., AND JAMES D. WHARTON

(*From the Department of Surgery and the Lasker Foundation for Medical Research, the Department of Medicine, of the University of Chicago, Chicago*)

A simple surgical procedure was devised enabling frequent collection of prostatic secretion over long periods of time in dogs. The serial observations of secretion so obtained permitted assay of the physiological work of the prostate, since this gland has no known endocrine function. Reactions of the canine prostate are of special interest because the dog is the only laboratory animal which develops spontaneous prostatic hypertrophy similar to that in man (1).

It is well known that the mature prostate gland is not a self-contained functional unit but directly depends for its maintenance on testis secretion, which in turn is greatly influenced by other endocrine products. While interest was centered in the amounts and quality of secretion as indices of prostatic activity, these values reflected in turn the more general factors influencing the genital complex as a whole and the activity levels of this functional system were accordingly repeatedly determined.

In this paper quantitative data are presented on the prostatic secretion of normal dogs; on the rate of atrophy of the prostate after castration, and the restoration of secretion by injections of male sex hormones in castrate dogs; on the influence of hyperthyroidism and thyroid and suprarenal removal on the prostate; and on the chemical composition of prostatic fluid in these various states. The influence of confinement on the canine testis is also reported.

Studies of prostatic secretion recorded previously have been limited to brief experiments. Eckhard (2) devised a method, which has been used subsequently by several groups (3–5), wherein the neck of the bladder was ligated to prevent the passage of urine

into the urethra and a cannula was placed in the urethra to deliver the prostatic secretion. Farrell (6) made an ingenious modification of Eckhard's technique, although no long-time observations were presented. At the first of two operations, the neck of the bladder was separated from the prostate, both cut ends infolded, and an anastomosis created between the fundus of the bladder and the prepuce; 1 week later a fistula of the urethra was formed in the perineum. We have found the procedure of Farrell to be technically unsound for several reasons: There was a high mortality rate; the skin constantly bathed in urine became macerated and ulcerated; the perineal fistula had a tendency to close, and quantitative collections through it were difficult. The secretion of the prostate has been obtained by electrical stimulation of the hypogastric nerves, or nervus erigens (2–4) or of the gland surface (5), and by the use of pharmacodynamic agents such as pilocarpine (3–8).

In the guinea pig semen can be obtained at frequent intervals by the electric ejaculation test of Battelli (9) as developed by Moore and Gallagher (10), but this animal has large accessory sex glands in addition to the prostate so that the secretion is a mixture of the products of a number of glands, constituting a disadvantage for the purposes of the present experiment. The dog has no seminal vesicles or bulbo-urethral glands (2).

The advantages of the operative technique to be described are that an artificial fistula is not required for the collection of prostatic fluid and that urine excretion is so disposed of that the dogs remain in good health.

Methods

All operations were performed on dogs, under ether anesthesia. The abdomen was opened through a lower left mid-rectus incision and the bladder was delivered. The fat pad and peritoneum covering the prostate gland were incised. The neck of the bladder was transected as close to the prostate as possible and both of the cut ends were closed with a continuous silk suture. The suture line at the base of the bladder was inverted with a second silk suture, care being taken not to compromise the ureters. A large brass cannula—No. 18 in gauge, 9 cm. long and 1 mm. in thickness with flanged feet—was inserted in the dome of the bladder and the incision closed. An important step was to produce ventral fixation of the bladder with an interrupted suture on either side of the cannula penetrating the whole thickness of the abdominal wall (Text-fig. 1). An incision was then made along the entire ventral preputial length and the cut surfaces of mucosa and skin whipped over with a silk suture. An extension of rubber tubing on the cannula drained the urine away from the skin. The cannula was cleaned of salt incrustations frequently to prevent occlusion.

Experimentation was begun 1 week following the operation. Pilocarpine hydrochloride, in 6 mg. doses, was used to induce prostatic secretion. Solutions of the alkaloid in physiological saline were prepared freshly before each experiment. The prostatic secretion was collected by placing the penis in a 250 cc. Erlenmeyer flask. A standard period of 1 hour was adopted for the collections, which were usually done every other day.

Certain dogs subjected to thyro-parathyroidectomy were given calcium lactate as a dietary supplement according to Luckhardt and Goldberg (11). Bilateral suprarenalectomy was carried out in 2 stages in 5 dogs and a preliminary gastrostomy, using a

heavy flanged cannula made of pyrex glass and equipped with a rubber stopper, was found useful for feeding purposes. Following the removal of the second suprarenal gland the dogs each day were fed 6 gm. each of sodium chloride and sodium citrate through the gastric cannula, and suprarenal cortex extract, 1 cc., was injected subcutaneously. Some of the castrate dogs were injected with testosterone propionate, in doses of 2.5 to 25 mg. daily, in sesame oil.[1]

The dogs were maintained in good condition for more than 10 months after the prostatic isolation operation. The diet consisted of a canned meat, vegetable, and cod liver oil mixture, supplemented daily with 8 cc. of a vitamin supplement which was said to supply not less than 4400 units of vitamin A, 630 units of vitamin D, 30 units of vitamin B, 20 units of vitamin G, 6.24 mg. of nicotinic acid, and 13 drops of wheat germ
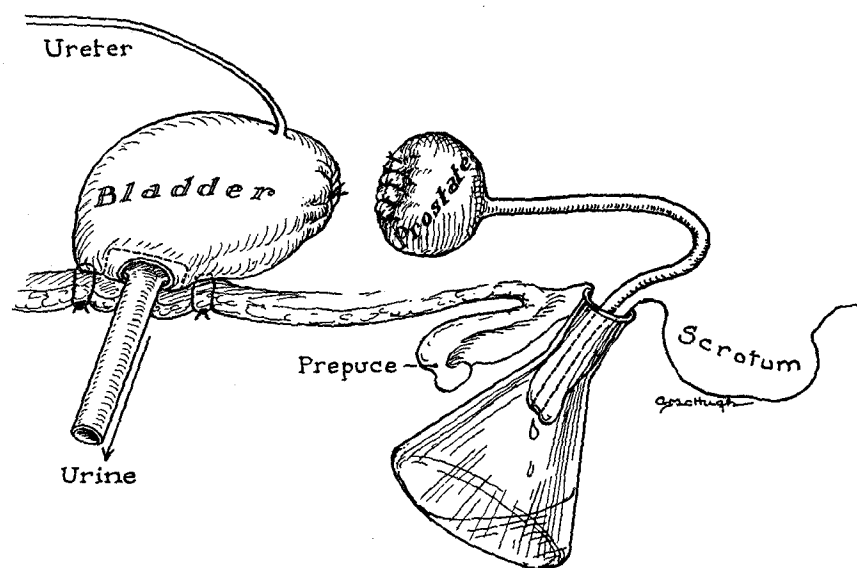
TEXT-FIG. 1

oil. The dogs lived a sedentary life in a room 12 × 16 feet and had little access to direct sunlight.

Counts of spermatozoa were made on the prostatic fluids using a standard blood cell counting chamber and adding a tiny drop of formalin as an immobilizing agent.

Chemical Methods.—The following determinations were made on all fluids: water, sodium, potassium, calcium, chloride, inorganic phosphorus, glucose, and proteins. In some experiments the fluids were also analyzed for pH and total CO_2. All analyses were made in duplicate. The fluids were collected under oil for pH and CO_2 determinations. For all other determinations the fluid was collected in tubes and centrifuged. pH was determined with the glass electrode, and the total CO_2 by the method of Van Slyke and Neill (12). Water determinations were made by drying known weights of the fluid to

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[1] We are indebted to Dr. Erwin Schwenk and the Schering Corporation for generous donations of this substance.

constant weight at 105°.   Sodium was determined by the method of Butler and Tuthill
(13); potassium, by the method of Shohl and Bennett (14); and calcium, by the method
of Kramer and Tisdall (15) as modified by Clark and Collip (16).   Chloride analyses
were carried out by the Wilson and Ball modification (17) of the method of Van
Slyke (18).   Inorganic phosphorus was determined by the method of Fiske and Sub-
barow (19); glucose, by the method of Miller and Van Slyke (20); and total proteins were
determined by the micro Kjeldahl method of Campbell and Hanna (21).   The proteins
were estimated by multiplying by 6.25 the total nitrogen, corrected for non-protein
nitrogen.

RESULTS

The Volume of Secretion of Prostatic Fluid in Normal Dogs.—Following the
stimulation with pilocarpine the prostate gland of normal dogs secreted at
times as much as 4 times its weight of fluid in 1 hour.
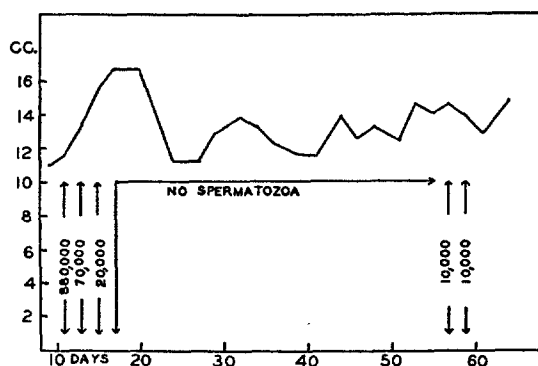
TEXT-FIG. 2. Dog 3-31, excretion of prostatic fluid in a normal dog.   The number of
spermatozoa for each cc. of prostatic fluid is indicated in the lower portion of the chart.

In this and subsequent charts, the ordinates represent prostatic secretion in cc.
collected during 1 hour following an intravenous injection of pilocarpine (6 mg.) and
the abscissae represent time in days.

The curves of the amounts of prostatic secretion obtained over a long
period of time assumed a regularity of appearance with minor peaks and
valleys (Text-fig. 2).   Certain recurrent effects were seen in 4 dogs studied
from 5 to 7 months, and in 10 others observed from 1 to 3 months.   Dog
1-92 in 157 days gave maximum and minimum secretions of 5.8 and 2.0 cc.
respectively and was regarded as in a steady state.   Dog 4-92 secreting 20
cc. daily had a steady decline between the 17th and 44th day and secreted
3 cc. ±1 for 45 days thereafter; a steady state at a level lower than the
original one (Text-fig. 3).   Dog 3-68 over a period of 114 days secreted 6
cc. ±1.14, but during this time had 5 high peaks with secretion of 14 to
34.1 cc.

In 6 dogs there was a decline from the original levels to complete cessation of prostatic secretion. The condition of 3 of these dogs was poor because of pulmonary or renal infection, and they subsequently died. There were 3 dogs apparently in good health that developed total suppression of prostatic output; the secretion, however, returned at 77, 78 and 80 days and was maintained for 3 months thereafter but at a level lower than the initial one.

Effect of Confinement of Normal Dogs on the Production of Spermatozoa.— Spermatozoa disappeared from the ejaculate of most normal dogs soon after their confinement in the laboratory (Text-figs. 2, 3, 4, and 6). Loss of sperm motility was first noted. The spermatozoa then gradually disappeared from the ejaculate and it was many weeks before motile spermatozoa again
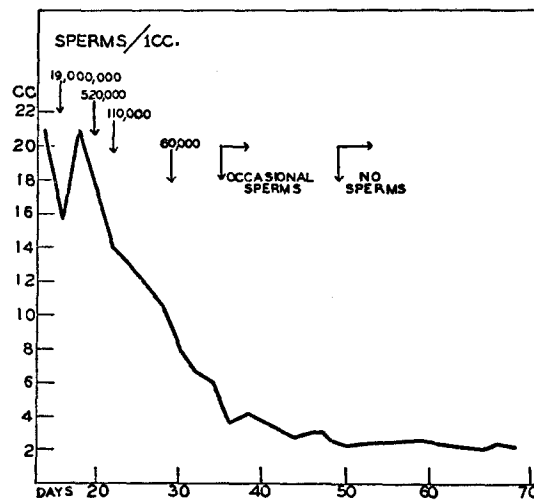
TEXT-FIG. 3. Dog 4-92, excretion of prostatic fluid in a normal dog. The number of spermatozoa per cc. of prostatic fluid is shown in the upper portion of the chart.

appeared. Histological study of the testis showed that the loss of spermatozoa was due to atrophy of the germ cells of the testis. This atrophy was reversible, since the testis recovered its gametogenetic activity. Since there was no decrease in the output of prostatic fluid concomitant with the germinal cell hypoplasia, it was concluded that little disturbance had occurred in the amount of male hormone produced.

The earliest changes in the cells of the testis consisted of swelling of sperm heads, giant cell formation, dissolution and desquamation of the orderly maturation line of the germ cells, so that later the germinal epithelium was reduced to a single layer of basal cells in the tubules resembling the cryptorchid testis. Recovery occurred in areas of tubules, some show-

ing repair while others showed atrophy. No changes were detected in interstitial cell cytology.

The testes of 80 adult dogs were studied by cytological methods using paraffin sections, and were classified in 3 groups: (a) normal; (b) an intermediate state of dissolution; (c) atrophy (Table I). All testes studied at the beginning of confinement were normal. After 8 to 60 days severe disturbances were found. After further confinement the testes usually returned to normal so that dogs maintained for 4 months or more had normal testes.

This atrophy was not due to pilocarpine injections since it occurred in 12 normal dogs in confinement that were not subjected to experimental procedures. 6 control dogs after entering the laboratory were injected with testosterone propionate, 10 mg. daily, for 17 to 20 days without preventing atrophy of the testis.

TABLE I

The Influence of the Length of Cage Captivity on the Testis

Duration of caging	Number of dogs	Normal testis	Intermediate stages of dissolution	Severe testis atrophy
days				
0–10	38	31	7	0
11–20	12	4	8	0
21–60	37	5	11	21
61–365	10	9	0	1

The germ cells following recovery from cage atrophy were hardy, for 2 dogs surviving 36 and 40 days following removal of both suprarenal glands had normal testes, despite having been in severe suprarenal insufficiency several times.

In addition, in this series 4 testes in 3 dogs had malignant epithelial neoplasms.

The Influence of Testis Removal and Androgen Supplements on Prostatic Secretions.—The rate of development of prostatic atrophy as manifested by cessation of secretion following castration was determined in 10 dogs. The shortest time period was 7 days in dog 2-46 whose secretion prior to testis removal was 5 cc. in 1 hour; and the longest time period was 23 days in dog 5-83 (previous secretion 17.5 cc.). In dog 4-89 (Text-fig. 4) whose original secretion was 24 cc., and in dog 5-61, secreting 23.2 cc., fluid ceased at 9 and 15 days respectively.

The ester testosterone propionate was injected in 13 castrate dogs. Subcutaneous and intramuscular injections were equally effective but intra-

venous injections were totally ineffective. In 3 immature dogs, weighing
14 to 18 kilos, with infantile prostate glands, measurable secretion was
obtained after 3 to 7 daily injections of 10 mg. In these dogs the curve of
fluid output rose to a plateau with small undulations (Text-fig. 5). In
dogs with very active prostate glands, as indicated by large secretion,
injections of the ester following the atrophy of castration failed to restore
the fluid to its previous high level. Thus dog 4-89, weighing 28 kilos and
normally secreting at a level of 20 cc. ±4 for 32 days, after the atrophy of
castration was injected with daily doses of 25 mg. for 37 days and reached a
plateau of 13 cc. (Text-fig. 4). However, in dogs with relatively inactive
prostate glands, yielding small secretion, the ester injections following
castration markedly increased the original small output (Text-fig. 6). The
wavy plateau which had been reached following long periods of androgen
injections was broken by high prolonged peaks in 3 dogs, presumably as a
result of better absorption or utilization of the androgenic material.

The effect of the dosage of testosterone on the quantity of prostatic
secretion was determined in 5 castrate dogs, which were maintained for
periods of 21 to 48 days on daily amounts of 2.5, 5.0, 10, and 25 mg. It was
found that there was a maximum effective dose beyond which further
increases did not produce secretion increments. Thus dog 4-2, weighing
14 kilos, which had been maintained for 8 months on a daily ester dosage
of 10 mg., did not have a decrease in secretion during 48 days when main-
tained on 5 mg. amounts, but a significant decrease occurred 22 days follow-
ing reduction to 2.5 mg. daily.

Prostatic secretion ceased following the termination of testosterone in-
jections in 3 castrate dogs in 18, 20, and 21 days, a delay as compared with
the original atrophy following castration of these dogs of 4, 7, and 8 days
respectively.

The Chemical Composition of Prostatic Fluid.—A summary of the data
obtained from the analyses of the fluids from all dogs is presented in Table
II. The points of interest in the data are as follows:

1. The distribution of electrolytes in this fluid is different from the distri-
bution in transudates or blood serum. Serum and transudates constitute
a heterogeneous system in approximate thermodynamic equilibrium, while
the fluid from the prostate is a secreted fluid. The concentration of
osmotically active substances was 335 mM per kilo of water, of which the
total base concentration consisted of 162 mM of sodium and 5.2 mM of
potassium. Calcium was present only in very small amounts (0.3 mM).
It will be noted that the chloride concentration was 163 mM per kilo of
water, thus accounting for most of the acid ions. Inorganic phosphate
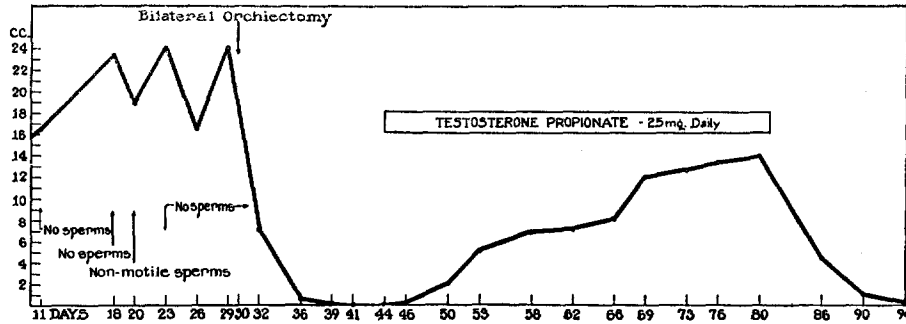
Text-Fig. 4. The rates of (a) cessation of prostatic secretion following bilateral orchiectomy; (b) restoration induced by injections of testosterone propionate (25 mg. daily); and (c) atrophy following termination of androgen injections.
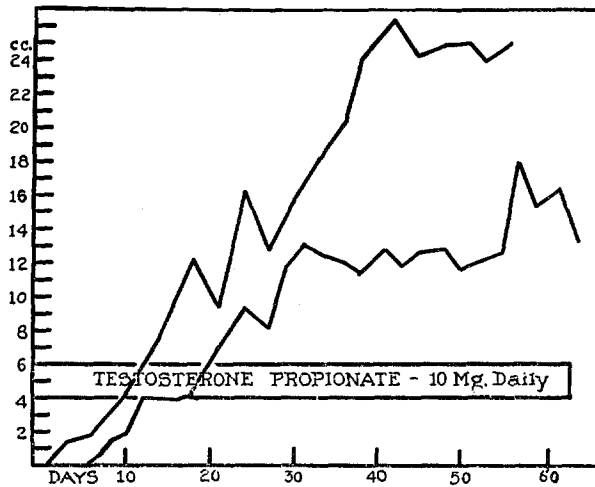
Text-Fig. 5. The onset and rate of development of prostatic secretion in 2 immature dogs receiving subcutaneous injections daily of testosterone propionate (10 mg.).   Before the injections the prostate glands were infantile and did not secrete fluid.   The upper curve shows the findings in dog 4-2, and the lower, in dog 5-10.
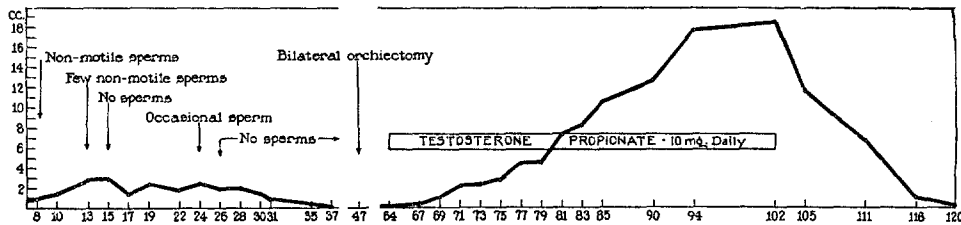
Text-Fig. 6. Dog 4-39, spontaneous cessation of prostatic secretion and restoration with subcutaneous injections of testosterone propionate (10 mg.) following removal of the testes.

was present only in traces, and the total CO_2 in 3 samples of the fluid ranged from 0.8 to 0.9 mM per liter of fluid, thus demonstrating the absence of bicarbonate ion.   The pH in 8 samples varied between 5.29 and 6.16.

The high content of chloride and water in prostatic fluid has been previously reported by Farrell (6).

2. The protein concentration in these fluids was less than 1 per cent.   In normal fluids the concentration was 0.82 gm. per cent, while in the fluids from castrated animals injected with testosterone the mean protein concentration was 0.61 gm. per cent.   This indicates that a small amount of protein may be partially inflammatory in origin and may be partially gained from the testicular cells the fluids bathe so intimately.

3. The electrolyte concentrations per kilo of water in the fluids were not changed by the physiological procedures carried out in this study.

TABLE II

*The Composition of Prostatic Fluid in the Dog*

Mean values are expressed per liter of fluid, with standard deviations.

|	Number of fluids	H_2O	Cl	Na	K	Ca	NPN	Total protein	Glucose
		gm.	*mM*	*mM*	*mM*	*mM*	*gm.*	*gm.*	*mg.*
Normal prostatic fluid	17	981± 3	160±2.7	159±2.6	5.1±0.2	0.3	0.22	8.25	0–30
Castrate, injected with testosterone	17	984± 3	162±3.6	160±3.3	5.2±0.2	0.3	0.31	6.14	0–75
One suprarenal removed	6	981± 2	162±2.2	157±2.1	5.5±0.3		0.24	8.11	27
Two suprarenals removed	3	960±11	153±3.1	154±1.0	4.8±0.4		0.15	5.77	
Thyroid feeding	5	977± 2.5	160±4.0	156±1.7	6.0±0.7		0.16	12.70	40

The Effect of Removal of Thyroids and Suprarenals in Castrate Dogs Whose Prostate Was Maintained with Daily Androgen Injections.—The removal of 1 suprarenal gland did not change the rate of prostatic secretion.   Following extirpation of both suprarenals the dogs were not injected with pilocarpine until the wounds had healed and the animals were in good condition. 2 dogs without suprarenal glands were so injected and a slight decrease in prostatic output occurred which was considered insignificant (Text-fig. 7). The standard dose of pilocarpine, 6 mg., was found to be fatal to suprarenalectomized dogs otherwise in good condition, undoubtedly due to the diversion of large amounts of fluid into the alimentary tract, but these dogs tolerated doses of 3 mg.

Removal of the thyroid and parathyroid glands did not modify the secretion of the prostate in 3 dogs.   Hyperthyroidism produced by feeding

desiccated thyroid powder in large amounts (4 gm. daily) to 3 dogs for 14, 28, and 38 days respectively caused a decrease in prostatic secretion (Text-fig. 8). During these periods in 2 dogs, the curves of secretion showed marked depression; at times, however, there was a return to the expected
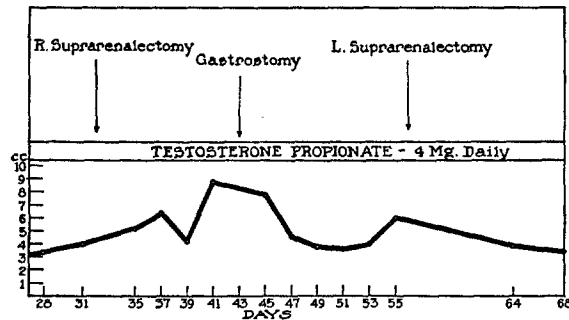
TEXT-FIG. 7. Dog 3-12, a castrated animal whose prostate was maintained by daily injections of testosterone propionate (4 mg.). A slight depression of prostatic output followed removal of the second suprarenal.
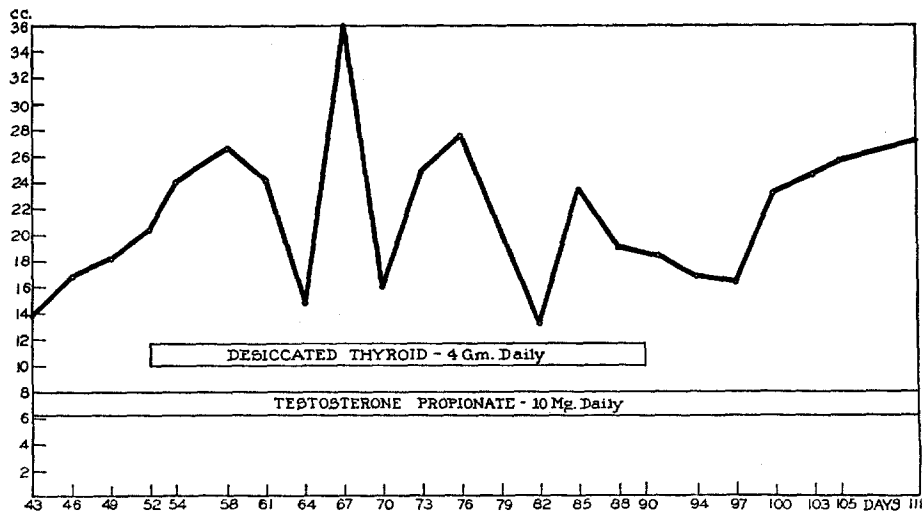
TEXT-FIG. 8. Dog 4-2, a castrated dog whose prostate was maintained by daily injections of testosterone propionate (10 mg.). Hyperthyroidism induced depression of prostatic secretion interrupted by returns to a level normal for this dog.

normal level, suggesting that the decrease was not fundamentally due to an effect on the prostate but to a general depression of the nervous system or body fluids.

Pilocarpine Dosage. Prostatic Fatigue Effect.—Some relationship was

indicated between the amount of pilocarpine injected and the duration and intensity of prostatic secretion. Prostatic fluid was delivered from the urethra between 1 and 12 minutes following an injection of 6 mg. and continued for 50 to 90 minutes; it was found that 95 per cent or more of the secretion occurred in the first hour so that 1 hour was adopted as the test period. Doubling or trebling the dosage of pilocarpine always increased the secretion but not according to a simple linear relationship. Thus dog 1-36, in a steady state, when tested at intervals of 2 days with 6, 12, and 18 mg. of pilocarpine yielded 1.0, 8.1, and 26.1 cc. of fluid respectively.

It was observed when similar doses of pilocarpine were injected twice

TABLE III

Prostatic Secretion Resulting from Two Injections of Pilocarpine Hydrochloride with an Intervening 6 Hour Interval

Dog No.	First injection: Dosage of pilocarpine	First secretion	Fluids injected intravenously in interval	Second injection: Dosage of pilocarpine	Second secretion	Difference
	mg.	*cc.*		*mg.*	*cc.*	*cc.*
1-36	6	1.1	—	6	0.9	−0.2
	6	1.0	—	12	10.7	+9.7
	6	2.5	1000 cc. NaCl, 0.9%	6	2.2	−0.3
1-92	6	5.9	—	6	1.4	−4.5
	6	3.8	—	12	5.8	+2.0
	6	3.9	1000 cc. NaCl, 0.9%	6	1.7	−2.2
5-08	6	8.3	—	6	4.0	−4.3
	6	10.0	—	6	4.9	−5.1
	6	6.8	1000 cc. glucose, 5%	6	13.4	+6.6
	6	10.0	1000 cc. NaCl, 0.9%	6	14.0	+9.0

with an intervening short interval that the volume of secretion obtained from the second injection was always less than from the first; a series of experiments was designed to study this decrement occurring with a 6 hour interval between injections. To overcome dehydration the dogs were given unlimited food and water immediately after the preliminary injection and collection; moreover the water content of the blood serum was determined before each test in 3 dogs and showed no significant change. In 4 dogs, 1 liter amounts of 5 per cent glucose or 0.9 per cent sodium chloride were injected and the final collection begun 30 minutes later. In 3 dogs (Table III) the injection of fluid did not overcome the short interval decrement but in dog 5-08 increased amounts of fluid resulted. It was found that injection

of 12 mg. of the alkaloid always produced more fluid than was secreted with 6 mg. 6 hours previously. In experiments with a short interval it was concluded that the decrement was due to a local disturbance analogous to fatigue since dehydration was not a factor. Also the gland was not absolutely refractory since the decrement could be overcome with increased dosage of the stimulating agent.

The fatigue effect was overcome in dogs within 1 day, since in dogs with constant amounts of secretion no differences were observed with 1, 2, or 4 days intervening between stimulations.

Relative Amounts of Secretion from the Prostate and Urethral Glands.—In acute experiments in 2 dogs, a cannula was ligated in the posterior urethra so that all of the prostatic fluid passed through it. Following recovery from the anesthesia pilocarpine was injected; prostatic fluid was collected through the cannula, and the secretion of the urethral glands from the urethral meatus. In these dogs the volume of the urethral secretion was less than 2 per cent of the volume of prostatic fluid. Thus dog 5-23 secreted 12.9 cc. of prostatic fluid while 4 beads of clear urethral fluid were collected measuring 0.2 cc. or 1.5 per cent of the prostatic secretion.

DISCUSSION

In normal dogs the prostatic output varied little from week to week with occasional sustained rises or depressions lasting over periods of days. The regularity of the secretion curves, with the absence of isolated spikes or sharp dips, indicated that the technique was free from systematic errors and random effects.

In the past the quantitative method of estimating sex gland activity most frequently used has been the determination of weights of the sex organs. It was found in these experiments that the weight of the mature prostate gland could not be correlated with the secretion volume in dogs of the same weight.

The finding of atrophy of the testis occurring in dogs in the laboratory is in agreement with previous observations in other animals. Darwin (22) noted that animals removed from their natural conditions often became infertile. Ceni (23) found in dogs which had received trauma to the brain that the testis underwent severe involution which was maximal 32 days following the incident but control dogs were not studied. Stieve (24) showed that caging temporarily produced gonadal atrophy in caged hens and that the ovary became normal some weeks later. Hartman (25) observed that the female opossum frequently developed ovarian atrophy when confined to a cage; that some animals spontaneously recovered

from the atrophy; and that diet and vitamin A modifications were without effect while exercise was beneficial in aiding recovery. An important feature of the changes in our dogs was the differential involvement of internal secretion and germ cells, since prostatic secretion was supported when the germinal maturation cells were completely atrophied. It is significant that recovery of the germ cells took place without modification of diet or surroundings.

SUMMARY AND CONCLUSIONS

A simple isolation of the prostate enabled quantitative collection of prostatic secretion in dogs over periods of months. The secretory stimulant was pilocarpine and 2 similar amounts injected with a 6 hour interval gave smaller amounts at the second testing, suggesting a fatigue effect. The prostate was not absolutely refractory since doubling the amount of alkaloid injected at the second test increased the volume to equal or exceed the preliminary secretion. The depression effect had disappeared at 24 hours.

In normal dogs the secretory curves were essentially regular, with occasional prolonged rises or depressions. The amount of secretion did not bear a direct relationship to the weight of the gland in adult dogs. The germinal epithelium of the testis underwent atrophy during the first few weeks of cage life while the prostatic secretion was maintained, showing that the atrophy was differential and did not involve the cells producing the androgenic hormone. The atrophy was reversible and all dogs kept for more than 4 months showed restoration of the germ cells. A few dogs developed atrophy of the germinal cells with cessation of prostatic secretion for many weeks but with final recovery. Removal of the suprarenal glands with suprarenal insufficiency did not produce sterility.

The distribution of electrolytes in the prostatic secretion differed from that in the serum-transudate system, although the concentration of osmotically active substances was the same, being made up almost entirely of sodium and chloride. The distribution was not affected by the different physiological procedures used in this study. Protein concentrations were less than 1 per cent.

The rate of prostatic atrophy following castration was determined, and cessation of secretion occurred in 7 to 23 days. The restoration of prostatic fluid in castrate dogs following daily injections of testosterone propionate followed a smooth curve to form a plateau which was interrupted occasionally by prolonged elevations with return to the established level. The prostate having been reconstructed, the dosage of androgenic material injected could be greatly reduced without causing a decrease in secretion.

Ablation of the thyroid and parathyroid glands had no significant influence on prostatic secretion. Hyperthyroidism caused a secretory depression interrupted with returns to normal levels.

BIBLIOGRAPHY

1. Zuckerman, S., and Groome, J. R., *J. Path. and Bact.*, 1937, **44**, 113.
2. Eckhard, C., *Beitr. Anat. u. Physiol.*, 1863, **3**, 155.
3. Mislawsky, N., and Bormann, W., *Centr. Physiol.*, 1899, **12**, 181.
4. Sergijewsky, M. W., and Bachromejew, J. R., *Z. ges. exp. Med.*, 1930, **71**, 303.
5. Winkler, F., *Dermat. Woch.*, 1931, **93**, 1626.
6. Farrell, J. I., *Tr. Am. Assn. Genito-Urin. Surg.*, 1931, **24**, 221.
7. Farrell, J. I., and Lyman, Y., *Am. J. Physiol.*, 1937, **118**, 64.
8. Farrell, J. I., *J. Urol.*, 1938, **39**, 171.
9. Battelli, F., *Compt. rend. Soc. phys. et hist. nat. Genève*, 1922, **39**, 73.
10. Moore, C. R., and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 39.
11. Luckhardt, A. B., and Goldberg, B., *J. Am. Med. Assn.*, 1923, **80**, 79.
12. Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.
13. Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.
14. Shohl, A. T., and Bennett, H. B., *J. Biol. Chem.*, 1928, **78**, 643.
15. Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **48**, 223.
16. Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.
17. Wilson, D. W., and Ball, E. G., *J. Biol. Chem.*, 1928, **79**, 221.
18. Van Slyke, D. D., *J. Biol. Chem.*, 1924, **58**, 523.
19. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.
20. Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.*, 1936, **114**, 583.
21. Campbell, W. R., and Hanna, M. I., *J. Biol. Chem.*, 1937, **119**, 1.
22. Darwin, C., The variation of animals and plants under domestication, New York, D. Appleton and Co., 1894, **2**, 127.
23. Ceni, C., *Arch. Entwcklngsmechn. Organ.*, 1918, **44**, 530.
24. Stieve, H., *Arch. Entwcklngsmechn. Organ.*, 1913, **38**, 8.
25. Hartman, C., *Am. J. Physiol.*, 1924, **68**, 97.