and the fluorescent antibody method. Thirty-eight were found to contain poliovirus Types 1, 2 or 3 by the regular procedure, and 34 of these were correctly identified by the fluorescent antibody method. The other 4 of the 38 were negative by fluorescent antibody. The 47 specimens found not to contain poliovirus by the routine method were negative by fluorescent antibody. Eighteen of these contained other enteroviruses. Thus, although the fluorescent antibody procedure was found slightly less sensitive than the routine procedure, it was entirely specific in this study.

1. Liu, C., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 883.

2. Coons, A. H., *Internat. Rev. Cytol.*, 1956, v5, 1.

3. Buckley, S. M., *Arch. f. Virusforsch.*, 1956, v5, 388.

4. Kalter, S. S., Hatch, M. H., Ajello, G. W., *Bact. Proc.*, 1959, 89.

5. Wenner, H. A., Miller, C. A., Kamitsuka, P. A., Wilson, J. C., *Am. J. Hyg.*, 1954, v59, 221.

6. Coons, A. H., Kaplan, M. H., *J. Exp. Med.*, 1950, v91, 1.

7. Riggs, J. L., Seiwald, R. J., Burckhalter, J. H., Downs, C. M., Metcalf, T. G., *Am. J. Path.*, 1958, v34, 1081.

8. Coons, A. H., Leduc, E. H., Connolly, J. M., *J. Exp. Med.*, 1955, v102, 49.

9. Goldman, M., *ibid.*, 1957, v105, 557.

Received December 16, 1960. P.S.E.B.M., 1961. -107.

Evidence for an Hypothalamic Focus of Inhibition of Gonadotropin by Androgen in the Male.* (26517)

JULIAN M. DAVIDSON† AND CHARLES H. SAWYER
Department of Anatomy, University of California Medical Center, Los Angeles

Control of pituitary gonadotropic secretion is generally acknowledged to be partially a function of the level of circulating gonadal hormones. The view has recently become widespread that in the female this "feedback" influence of sex hormones is an indirect one, operating through the hypothalamus, wherein lie receptor structures which respond to the sex steroids by modifying adenohypophysial activity(1,2,3). This report presents evidence that also in the male an area within the hypothalamus responds to the gonadotropin-inhibiting influence of the sex-specific gonadal hormone, testosterone.

Methods. Testosterone propionate was implanted into the brains or hypophyses of 11 mongrel male dogs. The crystalline steroid was heated in a glass tube, and immediately on reaching the melting point some of the hormone was drawn by capillary action into the end of 22 gauge stainless steel tubing. After cooling, the steel tube was cleaned on the outside with solvents, and a minute amount of solid testosterone (of the order of 0.5 mg) retained at the tip. With the aid of the dog stereotaxic instrument(4) tubes so prepared were implanted through the calvaria, each tip being located in the brain or hypophysis as described in the Table.

Semen ejaculates were collected and evaluated as described previously(5). Sperm count and motility were measured on 2 to 4 occasions (6-20 days) before implantation and at intervals after implantation of testosterone. The dogs were autopsied 24-33 days postoperatively. Testes and prostates were removed, weighed, fixed in SUSA solution, sectioned, and stained with hematoxylin and eosin. Brains were perfused and fixed in 10% formalin and sectioned by the freezing method for location of testosterone implantation sites. After removal of the tubes, testosterone was generally still clearly visible at their tips.

Results. Only dogs with pre-implantation ejaculates containing over 100 million highly motile spermatozoa were used in this study. In one group (Table I, Group I) implantation of testosterone into the ventral hypothalamus was followed by failure of ejaculation. In the case of dogs A and G, the first postoperative

* Supported by USPHS grant.

† USPHS Postdoctoral Research Fellow.

TABLE I. Effects of Implanting Testosterone Propionate into Brain or Pituitary Gland of the Male Dog.

Group	Dog	Location of implant	Testicular wt (mg/kg body wt)	Testicular histology	Prostatic histology
I	A	Immediately above post median eminence	1,130	Moderately severe atrophy*	Atrophy
	C	Posterior tuber	711	Complete atrophy†	"
	G	Mid median eminence	653	*Idem*	"
	K	Post median eminence	617	"	"
II	F	Pituitary	1,395	Normal	Normal
	H	"	1,550	"	"
	I	"	1,890	"	"
	L	" ‡	1,080	Some tubular disorganization	Slight degeneration
III	D	Thalamus	1,480	Normal	Normal
	E	"	1,280	"	"
	J	Ventromedial area of hypothalamus	1,320	"	"

* Somewhat less atrophied than the condition in hypophysectomy.
† Similar to condition in hypophysectomy.
‡ Location of implant not verified *post mortem*.

attempt to collect semen (one week after implantation), and all other attempts, were unsuccessful. In dogs C and K there was a gradual decrease to zero in sperm count and motility. Animals with pituitary implants (Group II) showed in 2 cases (dogs F and I) little or no deterioration of ejaculates following implantation, while in the 2 others there was a sharp drop in semen quality with eventual failure of ejaculation in dog H. In dogs of Group III, seminal ejaculates remained normal and essentially unchanged. The location of the testosterone implant in each of the animals is shown diagrammatically in Fig. 1.

A striking difference between the effects of testosterone implantation in the pituitary and in the posterior median eminence area becomes obvious from consideration of the parameters of testicular function in Table I. Testicular weights in the 4 dogs of Group I averaged 0.773 g/kg body weight; in Group II, 1.48 g/kg, and in Group III, 1.36 g/kg. Histologically, all animals of Group I showed marked gonadal atrophy (Fig. 2). In the testes of dogs C, G and K, spermatozoa were absent, spermatids absent or rare, and many seminiferous tubules were lined with Sertoli cells and spermatogonia alone. The lumina of the tubules were closed with amorphous material. In dog A a few scattered spermatozoa remained, but many tubules were disorganized and closed. It is of interest that in this animal the testosterone implant was not quite inside the posterior median eminence, but a little above it (Fig. 1). In all 4 dogs

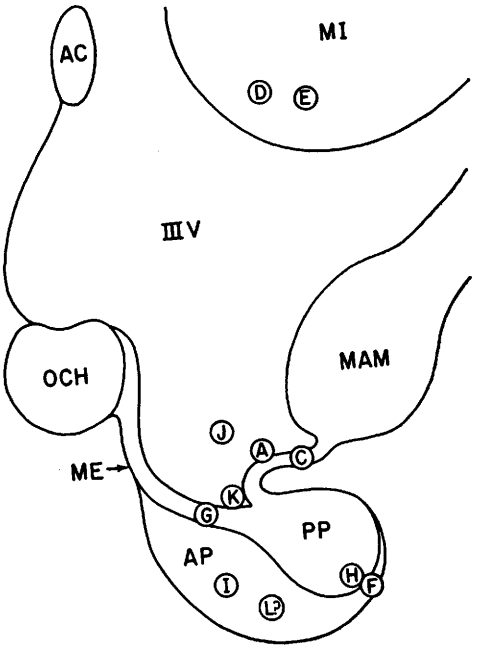

FIG. 1. Location of testosterone implants in dog brain and pituitary. Abbreviations: AC, anterior commissure; AP, anterior pituitary or pars distalis; MAM, mammillary body; ME, median eminence; MI, massa intermedia; OCH, optic chiasm; PP, posterior pituitary or infundibular process; III V, 3rd ventricle.

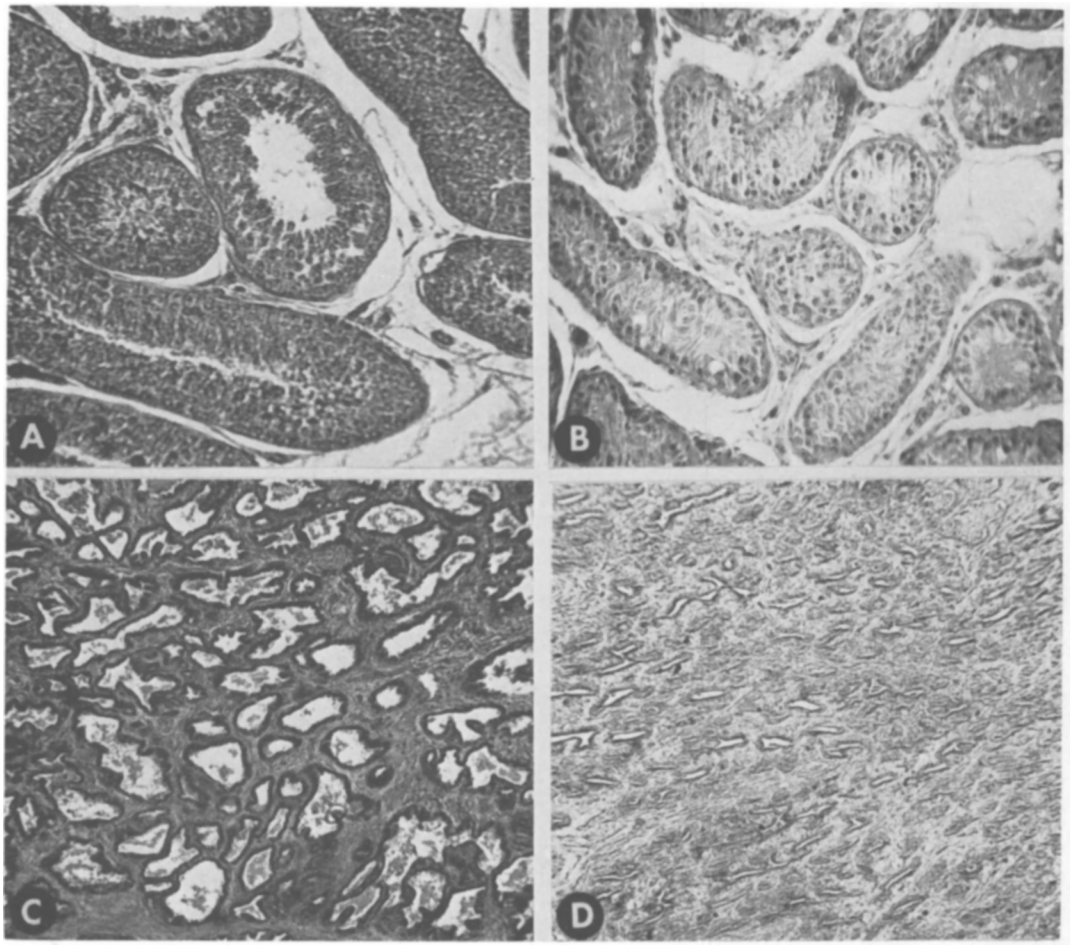

FIG. 2. A, C—Testis (×150) and prostate (×40) of Dog F (testosterone in pituitary gland). B, D—Testis (×150) and prostate (×40) of Dog G (testosterone in mid median eminence).

of Group I the testicular interstitial tissue was characterized by involution of the Leydig cells. Similarly, the prostates in these animals were markedly atrophic, with obliteration of the lumina of the glands and disorganization and flattening of the secretory epithelium (Fig. 2, D).

In Group II (pituitary implants), testicular and prostatic histology did not in general differ from that of normal dogs. Dog L showed the only marked deviation from normal: some disorganized seminiferous tubules and small prostatic gland lumina with, however, a generally columnar epithelium. Both testes and prostate of Dog L had a decidedly more healthy appearance than those of Dog A, the animal with the least degree of atrophy in Group I. Although the location of the implant in Dog L was not confirmed *post mortem*, its position in radiographs appeared to be definitely hypophysial.

Discussion. In male dogs(6,5) as well as in males and females of other species(7-10) destruction of the posterior median eminence region has been shown to lead to gonadal atrophy. This condition is apparently due to inhibition of production and secretion of FSH and ICSH(11). But although the hypothalamus appears to be essential for gonadal maintenance, its role in the "negative feedback" mechanism whereby gonadal hormones influence gonadotropin secretion has not yet been clearly established. Evidence that estrogen acts directly on hypothalamic structures to re-

duce gonadotropin secretion comes from experiments in female animals, involving transplantation of ovarian tissue to the hypothalamus(1) and implantation of solid estrogen therein(2,3).

Hohlweg and Daume(12) injected estrogen in fluid medium into the diencephalon of male birds and rats, with resulting gonadal atrophy. Their work, however, did not demonstrate the localization of the "feedback center," for the estrogen might well have diffused to the pituitary and acted there. The present study indicates that the fixed local implantation of minute amounts of solid androgen into the posterior median eminence area results in aspermia and profound atrophy of testes and prostate similar to that found in hypophysectomized dogs or those with lesions in the posterior median eminence. This effect was not seen with similar implants in the pituitary gland or some other areas of the brain.

It seems clear that the gonadal and prostatic atrophy observed here must be ascribed to the presence of testosterone in the median eminence. The possibility is remote that the results were due to mechanical destruction of the tissue by introduction of steel tubes, for the following reasons: (1) In the case of dogs C and G, the tubes were located just outside the critical area, destruction of which has previously been shown to lead to gonadal atrophy. 2) The pituitary implantations must have entailed at least as much damage to the median eminence as the implantations aimed directly at this structure. The tubes in dogs H and F (group II) passed through exactly the same areas of brain as those of dog C (group I).

It is concluded that this study provides positive evidence for the view that in the male dog gonadotropin secretion is held in check by the action of androgen on the posterior median eminence, and that the latter site is a critical area involved in the "negative feedback" of androgen on pituitary function.

Summary. The chronic implantation of minute amounts of solid testosterone propionate into the posterior median eminence-posterior tuberal region of the hypothalamus in 4 dogs has led to aspermia and profound testicular and prostatic atrophy. Although decreases in quality of semen were noted in 2 of 4 other dogs with similar implants in the pituitary, none of these 4 dogs showed gonadal or prostatic atrophy. No decrease in semen quality nor gonadal or prostatic atrophy was noted in either of 2 dogs with thalamic implants or one with an implant in the ventromedial hypothalamus.

The authors would like to thank Enid Arozena, Cora Rucker and Arlene Koithan for skilled technical assistance.

• 1. Flerko, B., Szentagothai, J., *Acta Endocrin.*, 1957, v26, 121.
• 2. Lisk, R. D., Personal communication, 1960.
• 3. Davidson, J. M., Sawyer, C. H., *Acta Endocrin.*, 1961, In press.
4. Hume, D. M., Ganong, W. F., *EEG clin. Neurophysiol.*, 1956, v8, 136.
• 5. Davidson, J. M., Ganong, W. F., *Endocrinology,* 1960, v66, 480.
• 6. Ganong, W. F., Frederickson, D. S., Hume, D. M., *ibid,* 1955, v57, 355.
• 7. Dey, F. L., *ibid.,* 1943, v33, 75.
• 8. McCann, S. M., *Am. J. Physiol.,* 1953, v175, 13.
• 9. Sawyer, C. H., Robison, B., *J. clin. Endocrinol. & Metab.,* 1956, v16, 914.
• 10. Sawyer, C. H., *J. Exp. Zool.,* 1959, v142, 227.
• 11. Davidson, J. M., Contopoulos, A. N., Ganong, W. F., *Endocrinology,* 1960, v66, 735.
• 12. Hohlweg, W., Daume, E., *Endokrinologie,* 1959, v38, 48.

Received February 27, 1961. P.S.E.B.M., 1961, v107.