

## PLASMA LEVELS OF TESTOSTERONE IN MALE RABBITS FOLLOWING COPULATION

GARY C. HALTMEYER AND KRISTEN B. EIK-NES

*Department of Physiology, Division of Physiology and Biochemistry of Reproduction, University of Southern California, Los Angeles, California*

(Received 12th June 1968)

**Summary.** Normal, male rabbits were either allowed to copulate with receptive females, or were injected with human chorionic gonadotrophin (HCG), or with adrenocorticotrophic hormone (ACTH). Copulation and HCG injection produced significant increases in plasma testosterone while ACTH administration failed to do so. Rabbits treated with either chlorpromazine or fluoxymestrone before copulation did not increase plasma levels of testosterone following coitus.

### INTRODUCTION

It is well established that the rabbit ovulates in response to mating and a marked ovarian release of  $20\alpha$ -hydroxypregnen-4-en-3-one will occur shortly after coitus in this species (Hilliard, Archibald & Sawyer, 1963). This change in progesterin secretion following mating corresponds with the increase of gonadotrophin in peripheral blood (Hilliard, Hayward & Sawyer, 1964), and the release of interstitial cell stimulating hormone (ICSH) and prolactin from the pituitary gland (Desjardins, Kirton & Hafs, 1967). Moreover, Taleisnik, Caligaris & Astrada (1966) noted a decrease in pituitary content of ICSH and an increase in plasma ICSH in female rats following copulation. The level of ascorbic acid in the ovary of these animals was lower than in control rats.

The hormonal response of the male gonad to copulation is less overt and has received little attention. Copulation will increase the blood levels of ICSH and decrease the pituitary concentration of the hormone in the male rat (Taleisnik *et al.*, 1966). The secretion of androgenic steroids into the spermatic venous blood of anaesthetized rabbits appears to increase shortly after coitus (Endröczy, 1962) and this increase is not found in male rabbits with hypothalamic lesions.

Since testosterone is the major androgen secreted by the male gonad, it was deemed important to study fluctuations of this hormone in the peripheral blood of the rabbit following coitus.

### METHODS

Adult male rabbits of the New Zealand strain were used. The animals were maintained in individual wire cages, given unlimited food and water and housed outdoors. Experiments were conducted only during the winter season.

Twenty millilitres of blood were collected by heart puncture from each of the animals before experimentation to establish their resting levels of plasma testosterone. Ten animals were assigned to each of the following experimental conditions:

- (a) no treatment (resting level control);
- (b) copulation with a receptive female rabbit;
- (c) injection of 500 i.u. of HCG intravenously;
- (d) injection of 5 i.u./kg of ACTH intravenously;
- (e) copulation with a receptive female following subcutaneous injections of 15  $\mu\text{g}/\text{kg}$  of fluoxymestrone (9 $\alpha$ -fluoro-11 $\beta$ -hydroxy-17 $\alpha$ -methyl testosterone) at 18, 12 and 6 hr before collection of blood for measurement of resting level of testosterone;
- (f) copulation with a receptive female following intramuscular injection of 3.5 mg/kg of chlorpromazine (CPZ) 1 hr before collection of blood for estimation of resting level of testosterone.

A sample of 20 ml of blood was collected by heart puncture from all animals 45 min after copulation or treatment with HCG or ACTH. Fluoxymestrone and CPZ were given in an attempt to block the changes of testicular activity induced by copulation. In order to determine if these drugs had inhibited the ability of the testis to produce testosterone, the fluoxymestrone and CPZ animals were given a subsequent intravenous injection of 500 i.u. HCG and blood was taken (by heart puncture) for testosterone estimation 45 min later.

The blood samples were collected in heparinized vacutainers, immediately placed on ice, centrifuged, and the plasma removed and assayed for free testosterone using the method published by our laboratory (Brownie, van der Molen, Nishizawa & Eik-Nes, 1964). The specificity, accuracy and precision of this method have been demonstrated (Brownie *et al.*, 1964; Resko & Eik-Nes, 1966).

## RESULTS

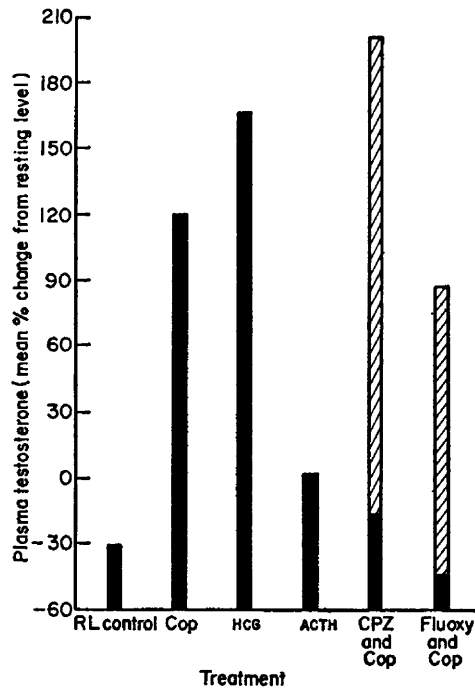
Since the resting level of plasma testosterone was determined in each animal before the experiment began, each rabbit served as its own control and a statistical design of within subject analysis of variance was employed. Use of this type of control experiment was necessary because of a rather large seasonal and individual variation in plasma testosterone which exists in the rabbit. Since both the main effects and their interactions were significantly different from the controls (Table 1), an analysis of simple effects was computed. This analysis indicated no differences between the resting levels of plasma testosterone in each of the experimental groups, the mean level being 37.6 ng/10 ml of plasma. Both copulation ( $F = 6.88$ ) and HCG administration ( $F = 41.43$ ) produced significant increases in plasma testosterone (mean levels of 77.3 and 165.4 ng testosterone/10 ml plasma, respectively). Administration of ACTH (Text-fig. 1) failed to produce an increase in circulating testosterone and no stimulatory effect of copulation could be found in animals treated either with fluoxymestrone or with CPZ before coitus (Text-fig. 1). Since these animals showed an increase in plasma testosterone following the administration of

HCG (Text-fig. 1), the lack of elevation of plasma testosterone following coitus is probably not due to inactivation of testicular enzymes producing this steroid hormone.

TABLE 1  
ANALYSIS OF VARIANCE FOR PLASMA TESTOSTERONE IN MALE RABBITS FOLLOWING COPULATION

Sources of variation	SS	df	MS	F
Between subjects	217,436.7	59		
B (treatment)	119,740.9	5	23,948.2	13.24*
Subjects within group	97,695.8	54	1,809.2	
Within subject	135,042.3	60		
A (time)	10,524.0	1	10,524.0	8.11*
AB	54,436.1	5	10,887.2	8.39*
AX subject within group	70,082.1	54	1,297.8	

\*  $P < 0.01$ .



TEXT-FIG. 1. Mean % change from resting level of plasma testosterone in male rabbits. RL control: resting level 45 min after initial collection of blood. Cop: 45 min after copulation. HCG: 45 min after intravenous HCG. ACTH: 45 min after intravenous ACTH. CPZ and Cop: rabbits treated with chlorpromazine and then permitted to copulate (shaded area indicates response to subsequent injection of HCG). Fluoxy and Cop: rabbits treated with fluoxymestronone and then permitted to copulate (shaded area indicates subsequent response to intravenous HCG).

DISCUSSION

The male rabbit shows a marked increase in plasma testosterone following copulation with a receptive female. These results are comparable to those

obtained by Endröczy (1962) who determined androgens in spermatic venous blood of anaesthetized rabbits and to recent data by Saginor & Horton (1968). The response of the male rabbit to sexual stimulation appears, therefore, to be similar to that of the female, i.e. spontaneous release of pituitary ICSH with subsequent stimulation of secretion of gonadal steroids (Hilliard *et al.*, 1963).

Administration of CPZ or fluoxymestrone before copulation blocked the increase of plasma testosterone associated with this physiological event. The primary effect of CPZ is thought to be on the central nervous system with no appreciable direct effect upon a tissue like the testis. This is supported by our observation that animals treated with this drug still show a normal plasma testosterone response following administration of HCG. Fluoxymestrone would be expected to affect the hypothalamic-pituitary feedback system. Prior treatment with fluoxymestrone blocked the response to copulation and tended to decrease the subsequent effect of HCG on circulating testosterone (Text-fig. 1). Fluoxymestrone may thus have some direct effect upon the testis as well as its expected effect on the hypothalamic-pituitary feedback mechanism. It should, however, be remembered that fluoxymestrone was administered every 6th hr during the day before HCG injection. Treatment with fluoxymestrone for this period of time could have resulted in a gradual decrease of pituitary ICSH secretion and, as a consequence of this change in pituitary activity, the capacity of the testis to produce and secrete testosterone following administration of exogenous gonadotrophin may have also decreased (Eik-Nes, 1964). Whatever the solution to these problems, our data indicate that the central nervous system is involved in augmenting the secretion of testosterone in the male rabbit following copulation.

Removal of two 20-ml samples of blood within 45 min, in an animal weighing about 3 kg, must be classified as a 'stressful' condition. Still this treatment did not lead to augmented levels of plasma testosterone (Text-fig. 1). If anything, these levels tended to decrease slightly. This observation is in keeping with previous findings that the level of testosterone in spermatic vein blood of the dog (Eik-Nes, 1962) and of the rat (Bardin & Peterson, 1967) will decrease during stressful conditions. Such conditions are known to increase pituitary secretion of ACTH. This hormone appears, however, to have no measurable effect on the cells of the testis secreting testosterone (Text-fig. 1). In spite of the fact that ICSH and ACTH show many similarities with regard to mechanism of action (Eik-Nes, 1964), they are most specific in selecting the proper target organ (Eik-Nes, 1962).

At present there is no explanation for the association of copulation with an increased secretion of testosterone in the male rabbit. This animal is known to copulate very rapidly when placed with a receptive female and also to copulate repeatedly. Some workers consider testosterone to be the hormone influencing the sex drive. The initial ejaculation is probably completed before the rise in plasma testosterone is established, though time experiments with regard to this increase must still be done. Testosterone can affect sperm maturation and is present in the epididymal tissue of the rabbit (Frankel & Eik-Nes, 1968). The post-coital increase in testosterone secretion could thus serve the purpose of sperm development in the testis and/or the epididymis following ejaculation.

Macmillan & Hafs (1967) found that sexual preparation before ejaculation greatly increased the sperm count in the first and second ejaculate through its effect upon the epididymides. Testosterone secretion in response to sexual stimulation could also involve mechanisms associated with sperm capacitation. Finally, the observed 'reflex secretion' of testosterone following coitus could reflect a similarity in the design of the hypothalamic-pituitary axis of the male and the female rabbit, and the augmentation in testosterone secretion could serve no known biological function. If so, this reflex mechanism may be present in animals other than the rabbit and the rat (Taleisnik *et al.*, 1966) since preliminary data from our laboratory indicate that the level of testosterone may increase in the plasma of normal men following coitus.

## ACKNOWLEDGMENTS

This work was supported in part by research grants AM-06651 and T01 CA 5000 from the U.S. Public Health Service, Bethesda, Maryland.

## REFERENCES

- BARDIN, C. W. & PETERSON, R. E. (1967) Studies of androgen production by the rat: testosterone and androstenedione content of blood. *Endocrinology*, **80**, 38.
- BROWNE, A. C., VAN DER MOLEN, H. J., NISHIZAWA, E. E. & EIK-NES, K. B. (1964) Determination of testosterone in human peripheral plasma using gas-liquid chromatography with electron capture detection. *J. clin. Endocr.* **24**, 1091.
- DESJARDINS, C., KIRTON, K. T. & HAFS, H. D. (1967) Pituitary levels of LH, FSH and prolactin after coitus in rabbits. (Abstract). *Fedn Proc. Fedn Am. Socs exp. Biol.* **26**, 534.
- EIK-NES, K. B. (1962) Secretion of testosterone in anesthetized dogs. *Endocrinology*, **71**, 101.
- EIK-NES, K. B. (1964) Effects of gonadotrophins on secretion of steroids by the ovary and the testis. *Physiol. Rev.* **44**, 609.
- ENDRÖCZI, E. (1962) Copulation induced testicular secretion in the male rabbit. *Excerpta Med. Internat. Congress Series No.* **47**, 620.
- FRANKEL, A. I. & EIK-NES, K. B. (1968) Steroidogenesis *in vitro* of the epididymis of the rabbit. (Abstract). *Fedn Proc. Fedn Am. Socs exp. Biol.* **27**, 624.
- HILLIARD, J., ARCHIBALD, D. & SAWYER, C. H. (1963) Gonadotropic activation of preovulatory synthesis and release of progesterin in the rabbit. *Endocrinology*, **72**, 59.
- HILLIARD, J., HAYWARD, J. N. & SAWYER, C. H. (1964) Postcoital patterns of secretion of pituitary gonadotropin and ovarian progesterin in the rabbit. *Endocrinology*, **75**, 957.
- MACMILLAN, K. L. & HAFS, H. D. (1967) Semen output of rabbits ejaculated after varying sexual preparation. *Proc. Soc. exp. Biol. Med.* **125**, 1278.
- RESKO, J. A. & EIK-NES, K. B. (1966) Diurnal testosterone levels in peripheral plasma of human male subjects. *J. clin. Endocr.* **26**, 573.
- SAGINOR, M. & HORTON, R. (1968) Reflex release of gonadotropin and increased plasma testosterone concentration in male rabbits during copulation. *Endocrinology*, **82**, 627.
- TALEISNIK, S., CALIGARIS, L. & ASTRADA, J. J. (1966) Effect of copulation on the release of pituitary gonadotrophins in male and female rats. *Endocrinology*, **79**, 49.