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SHORT COMMUNICATION

INCREASED CONCENTRATION OF ANDROGENS IN CRYPTORCHID STALLION TESTES

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SUMMARY

The concentrations of both 17β-hydroxyandrogens (total androgens) and testosterone (T) itself in the abdominal testes of five unilaterally cryptorchid stallions were compared with those of their contralateral scrotal testes. Total androgens were quantitated by a competitive protein-binding assay while testosterone was quantitated by a radio-immunoassay following Sephadex LH-20 column chromatography. Abdominally retained testes contained significantly increased concentrations of both total androgens and testosterone when compared to those in the descended testes.

In most adult mammals the testes are carried in the scrotum. Cryptorchidism (failure of normal descent of the testes) in domestic animals occurs most frequently in boars and stallions. In those species which normally have scrotal testes, bilaterally cryptorchid individuals are sterile because spermatogenesis does not occur at body temperature [1]. Although the Leydig cells in retained testes show no regressive changes, it is not known if they function normally [2] but mature cryptorchid stallions behave in much the same way as do stallions with scrotal testes [3]. Here we report having found, in each of 5 unilaterally cryptorchid stallions, a significant increase in the concentration of both 17β-OH androgens as well as testosterone in the retained testes over that of its scrotal companion.

Both testes from each horse were removed at the same time and immediately stored at −20°C. A wedge of tunic-free parenchyma was cut from each of the frozen organs and allowed to thaw on filter paper. From each wedge, one gram of tissue was minced and homogenized in 10 ml of saline in an ice bath. The homogenate was centrifuged at 17,000 *g* for 30 min at 4°C and the supernatant was used for androgen determinations.

17 β-OH androgens were assayed, using a competitive protein-binding technique [4] which is based upon the fact that the Sex Hormone-binding Globulin (SHBG) essentially will bind only 17β-OH androgens and 17 β-estradiol [5, 6]. To the supernatants from testicular tissue homogenates, 0·1 volume of 0·1 N sodium hydroxide was added and the samples were extracted with five volumes of diethyl ether. Alkalinization of the ethereal extract efficiently eliminated 17β-estradiol from the 17 β-OH androgens since the former is a phenolic steroid. Testosterone in each sample was individually fractionated by Sephadex LH-20 column chromatography [4] and quantitated by radio-immunoassay using the Rivanol-treated, highly specific testosterone antiserum [7]. When one gram of equine liver tissue ($n = 9$) was homogenized and carried through the same procedures, the tissue "blank values" obtained were (mean ± S.E.M.), 0·50 ± 0·12 and 0·29 ± 0·08 ng for competitive protein–binding assay and radio immunoassay, respectively.

Histological examination of 4 μm hemotoxylin and eosin stained sections of testes revealed no significant differences in the number of Leydig cells per unit area between the retained and scrotal testes. However, this may be due to the fact that the extensive connective tissue of the cryptorchid testes disrupted the Leydig cell nests thereby rendering quantitative data difficult to obtain.

Testosterone and 17 β-OH androgen concentrations were 2·1 and 1·7 times higher ($0{\cdot}005 > P > 0{\cdot}001$), respectively, in the retained than in the descended testes. Testosterone comprised 64% of the total 17 β-OH androgens in the retained testes as compared to 53% in the descended testes (Table 1).

We believe this is the first demonstration of an increased concentration of 17 β-OH androgens as well as testosterone in naturally occurring cryptorchid testes. However, it is important to note that our methods pro-

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Table 1. Concentration of total 17β -OH androgens (T.A.) and testosterone (T) ng/g, in descended and retained stallion testes

	Descended Testis			Retained Testis		
Stallion*	T.A.†	T†	T/T.A.	T.A.†	T†	T/T.A.
1	499	243	0·49	852	600	0·70
2	283	165	0·58	449	320	0·71
3	470	250	0·53	717	380	0·53
4	185	104	0·56	483	225	0·47
5	521	276	0·53	905	652	0·72
Mean	392	208	0·53	681‡	435‡	0·64
± S.E.	67	32	0·02	93	82	0·05

* All horses were between 18 and 24 months of age.
† Mean of quadruplicate determinations; each mean had a coefficient of variation of between 3 and 5%.
‡ Significantly increased ($0·005 > P > 0·001$).

vide only an index of the levels of these compounds and not an absolute value for their total concentrations, since the differences between the cryptorchid and scrotal testes are sufficiently large and important. The relationship, if any, between the arrest in spermatogenesis characteristic of testes which fail to descend and their increased concentrations of androgens needs further investigation. We are presently engaged in identifying the individual androgenic profile of the retained testes by Sephadex LH-20 column chromatography and high resolution mass spectrometry as previously described for human plasma [8, 9].

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REFERENCES

1. Cummins J. M. and Glover T. D.: *J. Reprod. Fert.* **23** (1970) 423–433.
2. VanDemark N. L. and Free M. J.: In *The Testis* (Edited by A. D. Johnson, W. R. Gomes and N. L. VanDemark). Academic Press, New York, **3** (1970) pp. 233–312.
3. Bishop M. W. H., David J. S. E. and Messervey A.: *Proc. Roy. Soc. Med.* **8** (1966) 769–770.
4. Ganjam V. K. and Amann R. P.: *Acta endocr., Copenh.* **74** (1973) 186–200.
5. Murphy B. E. P.: *Rec. Progr. Horm. Res.* **25** (1963) 563–610.
6. Vermeulen A. and Verdonck L.: In *Karolinska Symposia on Research Methods in Reproduction Endocrinology*: 2nd Symposium, Bogtykkereit Forum, Copenhagen (1970) pp. 239–256.
7. Resko J. A., Malley A., Begley D. and Hess D. L.: *Endocrinology* **93** (1973) 156–161.
8. Ganjam V. K., Murphy B. E. P., Chan T. H. and Currie P. A.: *J. steroid Biochem.* **2** (1971) 155–156.
9. Ganjam V. K., Murphy B. E. P., Chan T. H. and Currie P. A.: *J. steroid Biochem.* **4** (1973) 443–450.