Biochemical and physiological studies of androgen-binding protein in the reproductive tract of the ram

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Summary. The electrophoretic mobility, effect of pronase, temperature stability, affinity constant and specificity of androgen-binding protein (ABP) were compared in rete testis fluid (RTF), cauda epididymal plasma (CEP) and seminal plasma (SP) of the ram in which the levels of ABP, dihydrotestosterone (DHT), total protein and the number of spermatozoa were also measured. The characteristics of the ABP appeared to be almost identical in all 3 fluids. ABP was highly concentrated in the cauda epididymidis although 50–75% of it was utilized or destroyed during transit through the epididymis. The levels of ABP were higher in the breeding season and positively correlated with DHT in RTF and SP. It is concluded that ABP might be responsible for the increase in DHT in the reproductive tract of the ram during the breeding season and that ABP in the SP might serve as a useful marker of Sertoli cell function in the ram.

Introduction

An androgen-binding protein (ABP) has been found in the testis and the epididymis of several mammals (Hansson, Ritzén, French & Nayfeh, 1975a). In the rat and the rabbit, ABP is produced in the testis and secreted in the epididymis via the efferent ducts (French & Ritzén, 1973; Danzo, Eller & Orgebin-Crist, 1974; Ritzén & French, 1974). A similar binding protein has been found in ram rete testis fluid (Hansson *et al.*, 1975a; Jégou, Dacheux, Terqui & Garnier, 1976), cauda epididymal plasma (Jégou *et al.*, 1976; Voglmayr *et al.*, 1977) and seminal plasma (Jégou, Dacheux & Terqui, 1978a).

The present work was undertaken to compare certain physicochemical properties of ABP in the different parts of the reproductive tract of the ram. As the ram is a seasonal breeder a comparison of the ABP concentrations in its breeding and non-breeding states was also made. In addition, other parameters such as dihydrotestosterone levels, total protein concentrations and numbers of spermatozoa were measured.

Materials and Methods

Animals and samples

Adult Ile-de-France rams were used throughout these investigations. Rete testis fluid (RTF) and cauda epididymal plasma (CEP) were collected from different groups of animals housed in natural lighting. In the group of rams whose RTF was sampled, the breeding season collections were made in September and October (breeding season) or from January to March (non-breeding season).

Seminal plasma samples were collected from two similar groups of rams subjected to opposing 6-monthly reproductive cycles induced artificially by manipulating the photoperiod (Alberio & Colas, 1976). In these rams, the antero-posterior diameter of the testis (testicular diameter less the thickness of the scrotal skin) was measured with precision calipers.

The collection of RTF was performed as described previously (Jégou, Dacheux, Terqui, Garnier & Courrot, 1978b). Cauda epididymal plasma was collected by retroflushing of the contents of the ductus deferens. Seminal plasma was obtained from ejaculates collected in an artificial vagina. The semen was centrifuged (1500 g, 15 min, 4°C) and the sperm concentration was measured with a haemocytometer. All samples were then rapidly stored at -20°C.

Estimation of the flow rate of the cauda epididymal plasma

In rams, epididymal absorption of spermatozoa is negligible (Bedford, 1975), and therefore the total number of spermatozoa entering the epididymis is equal to the number of spermatozoa leaving it, i.e. RTF sperm concentration $\times$ RTF flow rate = CEP sperm concentration $\times$ CEP flow rate. If the concentrations of spermatozoa and the flow rates of the RTF (Table 3) are known, the flow rate of CEP can be calculated from the above equation.

Extraction of endogenous steroids

Endogenous steroids were extracted from all samples by charcoal adsorption (Charcoal Norit A: Nutritional Biochemical Co) at a final concentration of 10 mg/ml for 15 min at 4°C with shaking. Charcoal was removed by 3 centrifugations at 2500 g. Samples treated in this manner are referred to below as 'extracted'.

Analytical procedures

Binding studies. Binding was measured by polyacrylamide gel electrophoresis (PAGE) (Corvol, Chrambach, Rodbard & Bardin, 1971; Ritzén, Nayfeh, French & Dobbins, 1971) and the dextran-coated charcoal method. These binding studies and studies of temperature stability, specificity and the effects of pronase were conducted under the conditions described previously (Jégou *et al.*, 1978b) except for the following modifications for PAGE. After extraction, diluted CEP (1:5 or 1:10) and seminal plasma (1:3 or 1:5) were incubated with 50 nM-[³H]5 α -dihydrotestosterone for 2 h at 4°C. Electrophoresis of fluids was carried out on gels to which 5 nM-[³H]5 α -dihydrotestosterone had been added before polymerization. After migration, the gels were cut into 2.5 mm slices which were placed in counting vials containing 10 ml of an ethanol-toluene scintillator.

Radioimmunoassay. Dihydrotestosterone concentrations in the 3 fluids were measured as described by Garnier, Cotta & Terqui (1978). Briefly, steroids were extracted from fluids with an ethylacetate-cyclohexane mixture, and dihydrotestosterone was then purified and separated from testosterone by celite chromatography. Radioimmunoassay was performed with an antiserum raised against 5 α -dihydrotestosterone-1 α -carboxymethyloxime-BSA. Dextran-coated charcoal was used for bound and free separation. For each fluid, different volumes of a pool were assayed. The slopes of the regression lines between logit Bo/T and ln volume (RTF, -1.11; CEP, -1.05; seminal plasma, -1.15) were not statistically different from those of standard curves (-1.11) and did not differ from each other when analysed by an analysis of variance. The recovery percentage did not differ from one fluid to another and the mean ($\pm$ s.e.m.) was $49.0 \pm 2.37\%$ ($n = 22$). Water gave a blank of 0.057 ng/ml. The within-assay coefficient of variation was 7.1% for 0.52 ng/ml and the between-assay coefficient of variation was 12.2% for 1.1 ng/ml.

Radioactivity was measured in a triton-toluene mixture (1:2) (4 g PPO and 100 mg POPOP per litre toluene) or in an ethanol-toluene scintillator (4 g PPO, 40 mg POPOP and 300 ml ethanol per litre toluene).

Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) as modified by Hartree (1972).

Statistical analysis

The tests used were Mann-Whitney U test for comparisons between groups or Spearman's rank correlation test.

Results

Characteristics of the androgen-binding protein

Electrophoretic mobility. A single major binding peak was present in all 3 fluids (Text-fig. 1). The protein-[^3H]5 α -dihydrotestosterone complex ran in the gel with same relative mobility (R_F) for the RTF (0.56), CEP (0.57) and seminal plasma (0.56). This binding was saturable since a 100-fold excess of unlabelled 5 α -dihydrotestosterone in the incubation medium and gel ensured the total displacement of the peak of radioactivity (Text-fig. 1).

Text-fig. 1. PAGE analysis of (a) rete testis fluid, (b) cauda epididymal plasma (1:10), and (c) seminal plasma (1:3) of the ram in the presence of [^3H]5 α -dihydrotestosterone (—) or [^3H]5 α -dihydrotestosterone plus 100-fold excess of unlabelled hormone (---). The arrow indicates the position of the bromophenol blue marker.

Effects of pronase. The binding activity in the 3 fluids was completely destroyed by incubation with pronase (Table 1), suggesting that [^3H]5 α -dihydrotestosterone is bound by a proteinaceous component.

Table 1. Effect of pronase on binding of ABP in the ram

Fluids	Controls (c.p.m.)	Pronase (0.1%) (c.p.m.)	Background (c.p.m.)
Rete testis fluid	3781	185	185
Cauda epididymal plasma	761	65	59
Seminal plasma	618	65	59

Temperature stability. The binding capacity of the ABP in RTF was unaltered by heating at 21 and 32°C for 30 min but was partly destroyed at 50°C (47% decrease) and completely destroyed at 60°C. The ABP in seminal plasma behaved similarly except that 66% of binding was destroyed at 50°C. Binding in the CEP resisted heating at 21, 32 and 50°C but was destroyed at 60°C. The fact that binding in RTF and seminal plasma was slightly less than in CEP after heating to 50°C was perhaps due to inactivation by different proteolytic enzymes, as suggested for rabbit ABP (Hansson, Ritzén, French, Weddington & Nayfeh, 1975b).

Affinity constant. The affinity constants (K_a at 4°C) of the ABP, measured by Scatchard plots (Scatchard, 1949) and using the dextran-coated charcoal method were high and in the same order of magnitude, being $2.7 \pm 0.1 \times 10^9 \text{ M}^{-1}$ ($n = 7$) for RTF, $3.2 \pm 0.5 \times 10^9 \text{ M}^{-1}$ ($n = 6$) for CEP, and $1.8 \pm 0.4 \times 10^9 \text{ M}^{-1}$ ($n = 4$) for seminal plasma.

Specificity. The ABP in all 3 fluids bound 5 α -dihydrotestosterone very strongly. Binding of testosterone was also elevated. In contrast, binding of oestradiol-17 β , dehydroepiandrosterone and 5 β -hydrotestosterone was low and there was almost no binding of androstenedione and cyproterone. Competition by cyproterone acetate, progesterone and cortisol was negligible in the range of concentrations used (Table 2). The specificity appeared to be identical in the three parts of the genital tract.

Table 2. Data relating to the specificity of the assay for 5 α -dihydrotestosterone (DHT) in rete testis fluid (RTF), cauda epididymal plasma (CEP) and seminal plasma (SP) of the ram

Competing steroids	Relative binding (% of control)		
	RTF	CEP	SP
5 α -Dihydrotestosterone	100	100	100
Testosterone	32	35	35
Oestradiol-17 β	4	4	6
Dehydroepiandrosterone	2	2.5	3
5 β -Dihydrotestosterone	1	1	2
Androstenedione	0.3	0.8	0.8
Cyproterone	0.2	0.4	0.1
Cyproterone acetate	0*	0*	0*
Progesterone	0*	0*	0*
Cortisol	0*	0*	0*

Relative binding was defined by the ratio of the amounts of 5 α -DHT to competitor which gave 50% displacement of the [^3H]5 α -DHT. Bound and free radioactivity was separated by dextran-coated charcoal.

* There was no displacement of [^3H]5 α -DHT bound to ABP observed in the range of concentrations (0–1.2 μM) used.

Measurement of ABP, dihydrotestosterone, total protein and spermatozoa

ABP. The ABP concentration in RTF and seminal plasma was significantly higher (+41% and +43% respectively) in the breeding season than in the non-breeding season. In CEP, the concentration of ABP tended to be higher in the breeding season (+20%), but was in fact more highly concentrated ($\times 10^{-9} \text{ M}$) in the CEP in both seasons than in the other fluids.

However, taking in consideration the flow rates of RTF and CEP (Table 3) the amount of ABP entering the caput epididymidis was 2.9×10^{-12} mol/h in the non-breeding season and 6.2×10^{-2} mol/h in the breeding season whereas the amount of ABP leaving the cauda epididymidis was 0.7×10^{-12} mol/h in the non-breeding season and 3.2×10^{-12} mol/h in the breeding season. Thus, despite its higher molar concentrations in the epididymis, ABP lost about 50–75% of its binding capacity according to the season.

Table 3. Mean $\pm$ s.e.m. concentrations of ABP, DHT, spermatozoa and total protein in the rete testis fluid (RTF: data taken from Jegou *et al.* 1978b), cauda epididymal plasma (CEP) and seminal plasma (SP) of the ram during the breeding and the non-breeding season

	RTF		CEP		SP	
	Breeding season	Non-breeding season	Breeding season	Non-breeding season	Breeding season	Non-breeding season
No. of rams	6	7	5	7	5	5
ABP ($\times 10^{-9}$ M)	*4.4 $\pm$ 0.98†	2.6 $\pm$ 0.62	84 $\pm$ 7.27 (4)	67.4 $\pm$ 8.75	*35.3 $\pm$ 2.78 (4)	20.2 $\pm$ 3.82
DHT (mg/ml)	*1.0 $\pm$ 0.14	0.7 $\pm$ 0.06	**4.4 $\pm$ 0.36	2.5 $\pm$ 0.42	**7.2 $\pm$ 0.98	2.2 $\pm$ 0.44
Spermatozoa ($\times 10^9$ /ml)	***0.177 $\pm$ 0.022	0.049 $\pm$ 0.009	***6.4 $\pm$ 0.3	4.9 $\pm$ 0.4	5.2 $\pm$ 0.2	3.9 $\pm$ 0.6
Total protein (mg/ml)	**1.3 $\pm$ 0.06	0.95 $\pm$ 0.09	13.35 $\pm$ 0.86	12.65 $\pm$ 0.99	47.2 $\pm$ 2.90	36.2 $\pm$ 6.4
Flow rates (ml/h)	*1.4 $\pm$ 0.11	1.1 $\pm$ 0.07	39 $\times 10^{-3}$ §	11 $\times 10^{-3}$		

Values significantly different from those in the non-breeding season: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† Dextran-coated charcoal method.

‡ PAGE method.

§ See calculation in 'Materials and Methods'.

Dihydrotestosterone, total protein and spermatozoa. The concentrations of dihydrotestosterone were significantly higher during the breeding season in all 3 fluids. The concentrations of total protein were significantly higher in the breeding season only in RTF. These total protein concentrations increased from the testis to the seminal plasma, but if one considers the flow rates, the amount of the total protein entering the epididymis (1 mg/h in the non-breeding season; 1.8 mg/h in the breeding season) was 4–5 times higher than that leaving it (0.2 mg/h in the non-breeding season; 0.5 mg/h in the breeding season). The concentrations of spermatozoa were significantly higher in the breeding season than in the non-breeding season in RTF and CEP but the difference for seminal plasma did not reach significance ($P = 0.075$). The two groups of rams selected for the measurement of these parameters in seminal plasma were definitely experiencing different sexual seasons because the mean antero-posterior diameter of the testis of rams in the breeding season group (70 ± 1.7 mm) was significantly greater ($P < 0.01$) than that of rams in the non-breeding season group (47 ± 1.2 mm). Spermatozoa were concentrated in the cauda epididymidis as a consequence of the resorptive capacity of the epididymis. This was shown by the difference between the flow rates of fluids entering and leaving the epididymis.

Table 4. Spearman's rank correlation table between ABP, spermatozoa, DHT and total protein in the seminal plasma and the antero-posterior diameters of the cauda epididymidis and the testis of rams ($N = 9$)

	Spermatozoa	DHT	Total protein	Diam. of cauda epididymidis	Testis diam.
ABP	0.39	0.77**	0.29	0.87***	0.60*
Spermatozoa		0.49	0.33	0.58*	0.66*
DHT			0.33	0.69*	0.62*
Total proteins				0.60*	0.65*
Diam. of cauda epididymidis					0.88***

* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$.

Correlation analysis

No significant correlation was found between the different parameters studied in CEP, but significant correlations were found for several seminal plasma characteristics (see Table 4).

Discussion

These results confirm the presence of an ABP with a high affinity and specificity for 5 α -dihydrotestosterone in RTF, CEP and seminal plasma of the ram. The characteristics of the ABP, such as the R_F in PAGE, the specificity and the K_a , appear to be identical in these three parts of the genital tract. In addition the properties of ram ABP appear to resemble those of rat and rabbit ABP very closely (Hansson *et al.*, 1975a, b).

Whereas ABP was found in small amounts in the fluid of the cauda epididymidis of the rat (Hansson *et al.*, 1974) and mature rabbit (Weddington *et al.*, 1974; Danzo, Cooper & Orgebin-Crist, 1977), in the adult ram, ABP ($\times 10^{-9}$ M) is highly concentrated in the cauda epididymidis, as found by Voglmayr *et al.* (1977). The concentrations of ABP, expressed in terms of the protein present (pmol ABP/mg protein), in the breeding and non-breeding seasons are 3.4 and 2.75 in the RTF, 6.3 and 5.3 in the CEP, and 0.75 and 0.55 in the seminal plasma. Consequently, the ABP gradient across the epididymis in the mature ram is the reverse of that found in the rabbit (Danzo *et al.*, 1977). The molar concentrations of ABP are about 10 times lower in RTF than in seminal plasma (Table 3), in which the high levels reflect the existence of the high concentrations of ABP in CEP although they are lower than those in the CEP because of the dilution which occurs at the moment of ejaculation.

Calculation of the secretion of ABP in the rete testis and in the cauda epididymidis (see above) shows that ABP is partly destroyed, inactivated and/or taken up as it passes through the epididymis. Furthermore, our results show that probably more ABP enters the epididymis, and less of it is lost during its passage through the epididymis, in the breeding season than in the non-breeding season.

The difference in total protein entering and leaving the epididymis of the ram shows that, in spite of a possible contribution of epididymal secretion to the epididymal plasma, as described in other mammals (Amann, Killian & Benton, 1973; Jones, 1974), the amount of protein leaving the epididymis is markedly lower than that entering it. The total proteins entering are either utilized or destroyed during their transit through the epididymis.

The levels of dihydrotestosterone were higher in all 3 fluids studied during the breeding season than in the non-breeding season. Assuming one binding site of dihydrotestosterone per molecule of ABP it is easy to calculate that the concentration of the hormone is equivalent to that of ABP in RTF, but lower than ABP in CEP and seminal plasma. The positive correlation found between ABP and dihydrotestosterone in seminal plasma has been reported previously for efferent duct fluid of the rabbit (Guerrero, Ritzén, Purvis, Hansson & French, 1975), for ram RTF (Jégou *et al.*, 1978b), and for the cytosol of rat epididymis (Purvis & Hansson, 1978).

Unlike the rat, in which the same ABP is present in testis and blood plasma (Gunsalus, Musto & Bardin, 1978), in the ram two different binding proteins for 5 α -dihydrotestosterone have been described which have the same R_F in PAGE and the same K_a (Jégou, 1976): the ABP in the reproductive tract and the sex-steroid binding protein (SBP) in blood plasma. This SBP is still present in the blood of rams 3 years after castration (B. Jégou, unpublished results). As the technique of collection of RTF, CEP and the utilization of seminal plasma for analysis avoid blood contamination, the 5 α -dihydrotestosterone binding component studied in this paper may be taken as evidence for testicular secretion of ABP.

The physiological role of ABP has yet to be elucidated. ABP is not indispensable in all species since it has not been detected in the RTF, CEP or seminal plasma of boars or in the CEP

and seminal plasma of stallions (B. Jégou & J. L. Dacheux, unpublished results; Jégou & Le Gac-Jégou, 1978). When present, ABP may play a role in the transport and concentration of androgen in the testis and epididymis (Hansson *et al.*, 1975a). In the ram, this role of ABP would explain the increase of the levels of androgen in the reproductive tract during the breeding season which is correlated with increasing ABP. ABP may also protect androgens against metabolism, regulate the incorporation of 5 α -dihydrotestosterone by the cellular receptor of the epididymis and/or play a role in sperm storage in the cauda epididymidis. Our results also show that a seasonal increase of the concentrations of ABP in seminal plasma of the ram exists. This increase is similar to that previously demonstrated in ovine RTF (Jégou *et al.*, 1978b). As ABP is produced by the Sertoli cells (Hagenas *et al.*, 1975; Fritz, Rommerts, Louis & Dorrington, 1976), the seasonal variation of ABP possibly reflects a seasonal metabolic activity of Sertoli cells, this activity probably being related to the seasonal variation in the production of RTF (Dacheux, Pisselet, Blanc, Hochereau-de-Reviers & Courot, 1977) and the seasonal increase in the size of nuclei of Sertoli cells (Hochereau-de-Reviers, Loir & Pelletier, 1976). This seasonal variation in the production of ABP may also be related to the seasonal variation in plasma FSH (Davies, Main & Setchell, 1977), plasma LH (Sandford, Winter, Palmer & Howland, 1974; Davies *et al.*, 1977) and plasma testosterone (Attal, 1970; Katongole, Naftolin & Short, 1974).

As a consequence of the similar order of variation of ABP in RTF and seminal plasma we may consider that the measurement of the binding ability of ABP in the semen may accurately reflect testicular activity in the ram. Thus, the presence of ABP in ram semen may be useful for studying the seminiferous tubular compartment without the intervention of surgery and it may be possible to conduct a longitudinal study of this compartment by sampling the seminal plasma once a reliable and repeatable sampling protocol has been established.

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References

- Alberio, R. & Colas, G. (1976) Influence of photoperiodism on the sexual development of the young Ile-de-France ram. *Proc. 8th. Int. Congr. Anim. Reprod. & A. I.*, Cracow, Vol. 3, pp. 26–29.
- Amann, R.P., Killian, G.J. & Benton, A.W. (1973) Differences in the electrophoretic characteristics of bovine rete testis fluid and plasma from the cauda epididymidis. *J. Reprod. Fert.* **35**, 321–330.
- Attal, J. (1970) *Mesure des oestrogènes et des androgènes testiculaires et plasmatiques dans l'espèce ovine des microméthodes de chromatographie en phase gazeuse: influence de l'âge, de la saison et du cycle diurne*. Thèse Doc. ès Sci. Nat., Paris, CNRS, No. AO 6317, 173 pp.
- Bedford, J.M. (1975) In *Handbook of Physiology* Section 7. Endocrinology V., pp. 173–200. Eds. R. O. Greep & D. W. Hamilton. Am. Physiol. Soc., Washington D.C.
- Corvol, P.L., Chrambach, A., Rodbard, D. & Bardin, C.W. (1971) Physical properties and binding capacity of testosterone-oestradiol binding globulin in human plasma, determined by polyacrylamide gel electrophoresis. *J. biol. Chem.* **11**, 3437–3443.
- Dacheux, J.L., Pisselet, C., Blanc, M., Hochereau-de-Reviers, M.T. & Courot, M. (1977) Variations saisonnières des sécrétions exocrines (fluide et spermatozoïdes) chez le bœuf adulte (Ile-de-France). *J. Physiol., Paris*, 99A.
- Danzo, B.J., Eller, B.C. & Orgebin-Crist, M.C. (1974) Studies on the site of origin of the androgen-binding protein present in epididymal cytosol from mature intact rabbits. *Steroids* **24**, 107–122.
- Danzo, B.J., Cooper, T.G. & Orgebin-Crist, M.C. (1977) Androgen binding protein (ABP) in fluids collected from the rete testis and cauda epididymidis of sexually mature and immature rabbits and observations on morphological changes in the epididymis following ligation of the ductuli efferentes. *Biol. Reprod.* **17**, 64–77.
- Davies, R.V., Main, S.T. & Setchell, B.P. (1977) Seasonal changes in plasma follicle-stimulating hormone, luteinizing hormone and testosterone in rams. *J. Endocr.* **72**, 12P.
- French, F.S. & Ritzén, E.M. (1973) Androgen binding protein in efferent duct fluid of rat testis. *J. Reprod. Fert.* **32**, 479–483.

- Fritz, I.B., Rommerts, F.F.G., Louis, B.G. & Dorrington, J.H. (1976) Regulation by FSH and dibutyryl cyclic AMP of the formation of androgen-binding protein in Sertoli cell-enriched cultures. *J. Reprod. Fert.* **46**, 17–24.
- Garnier, D.H., Cotta, J. & Terqui, M. (1978) Assessment of androgen radioimmunoassay in sheep: direct plasma RIA of testosterone and dehydroepiandrosterone and physiological evaluation. *Annls Biol. anim. Biochim. Biophys.* **18**, 265–281.
- Guerrero, R., Ritzén, E.M., Purvis, K., Hansson, V. & French, F. S. (1975) Concentration of steroid hormones and androgen-binding protein (ABP) in rabbit efferent duct fluid. In *Hormonal Regulation of Spermatogenesis*, pp. 213–221. Eds F. S. French, V. Hansson, E. M. Ritzén & S. N. Nayfeh. Plenum Press, New York.
- Gunsalus, G.L., Musto, N.A. & Bardin, W.C. (1978) Immunoassay of androgen-binding protein in blood: a new approach for study of the seminiferous tubule. *Science, N.Y.* **200**, 65–66.
- Hagenas, L., Ritzén, E.M., Ploen, L., Hansson, V., French, F.S. & Nayfeh, S.N. (1975) Sertoli cell origin of testicular and androgen-binding protein (ABP). *Molec. cell. Endocr.* **2**, 339–350.
- Hansson, V., Trygstad, O., French, F.S., McLean, W.S., Smith, A.A., Tindall, D.J., Weddington, S.C., Petrusz, P., Nayfeh, S.N. & Ritzén, E.M. (1974) Androgen transport and receptor mechanisms in testis and epididymis. *Nature, Lond.* **250**, 387–391.
- Hansson, V., Ritzén, E.M., French, F.S. & Nayfeh, S.N. (1975a) Androgen transport and receptor mechanisms in testis and epididymis. In *Handbook of Physiology, Endocrinology*, Vol. V, pp. 173–200. Eds R. O. Greep & D. W. Hamilton. Am. Physiol. Soc., Washington, D.C.
- Hansson, V., Ritzén, E.M., French, F.S., Weddington, S.C. & Nayfeh, S.N. (1975b) Testicular androgen-binding protein (ABP): comparison of ABP in rabbit testis and epididymis with a similar androgen binding protein (TeBG) in rabbit serum. *Molec. cell. Endocr.* **3**, 1–20.
- Hartree, E.F. (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Analyt. Biochem.* **43**, 422–427.
- Hochereau-de-Reviere, M.T., Loir, M. & Pelletier, J. (1976) Seasonal variations in the response of the testis and LH levels to hemicastration of adult rams. *J. Reprod. Fert.* **46**, 203–209.
- Jégou, B. (1976) *Etude des protéines de liaison des Androgènes dans le tractus génital et le plasma sanguin du bélier (Ile-de-France)*. Thèse Doc. 3e cycle, Paris, 56 pp.
- Jégou, B. & Le Gac-Jégou, F. (1978) Androgen-binding protein in the seminal plasma of some mammalian species. *J. Endocr.* **77**, 267–268.
- Jégou, B., Dacheux, J.L., Terqui, M. & Garnier, D.H. (1976) Etude de la protéine de liaison de la 5 α -dihydrotestosterone et de la testostérone dans le tractus génital mâle (bélier). *J. Physiol., Paris* **72**, 44A.
- Jégou, B., Dacheux, J.L. & Terqui, M. (1978a) Mise en évidence d'une protéine de liaison spécifique des androgènes de type ABP (androgen binding protein) dans le plasma séminal du bélier. *C. r. hebdomadaire Séanc. Acad. Sci., Paris D* **286**, 347–350.
- Jégou, B., Dacheux, J.L., Terqui, M., Garnier, D.H., & Courrot, M. (1978b) Studies of the androgen binding protein in the rete testis fluid of the ram and its relation to sexual season. *Molec. cell. Endocr.* **9**, 335–346.
- Jones, R. (1974) Absorption and secretion in the cauda epididymidis of the rabbit and the effects of degenerating spermatozoa on epididymal plasma after castration. *J. Endocr.* **63**, 157–165.
- Katongole, C.B., Naftolin, F. & Short, R.V. (1974) Seasonal variations in blood luteinizing hormone and testosterone levels in rams. *J. Endocr.* **60**, 101–106.
- Lowry, O.H., Rosebrough, N.J., Farr, A. L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- Purvis, K. & Hansson, V. (1978) Androgens and androgen-binding protein in the rat epididymis. *J. Reprod. Fert.* **52**, 59–63.
- Ritzén, E.M. & French, F.S. (1974) Demonstration of an androgen binding protein (ABP) in rabbit testis: secretion in efferent duct fluid and passage into epididymis. *J. Steroid Biochem.* **5**, 151–154.
- Ritzén, E. M., Nayfeh, S.N., French, F.S. & Dobbins, C. (1971) Demonstration of androgen binding protein components in rat epididymis cytosol and comparison with binding components in prostate and other tissues. *Endocrinology* **89**, 143–151.
- Sandford, L.M., Winter, J.S.D., Palmer, W.M. & Howland, B.E. (1974) The profile of LH and testosterone secretion in the ram. *Endocrinology* **95**, 627–631.
- Scatchard, G. (1949) The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51**, 660–672.
- Voglmeier, J.K., Musto, N.A., Saksena, S.K., Brown-Woodman, P.D.C., Marley, P.B. & White, I.G. (1977) Characteristics of semen collected from the cauda epididymidis of conscious rams. *J. Reprod. Fert.* **49**, 245–251.
- Weddington, S.C., McLean, W.S., Nayfeh, S.N., French, F.S., Hansson, V. & Ritzén, E.M. (1974) Androgen binding protein (ABP) in rabbit testis and epididymis changes with age in comparison with androgen binding protein (TeBG) in serum and separation from the intracellular receptor in epididymis. *Steroids* **24**, 123–134.

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