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Androgen radioimmunoassay in the ram : results of direct plasma testosterone and dehydroepiandrosterone measurement and physiological evaluation

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Summary. Different radioimmunoassays of testosterone (T) dehydroepiandrosterone (DHA) and 5 α-dihydrotestosterone (5 α-DHT) were investigated for ram plasma.

Specificity of the antisera, lack of noticeable binding in plasma, very low levels of other androgens allow direct plasma RIA for DHA and T by the double antibody technique. The levels obtained by this simplified method are in agreement with those found after extraction alone, after extraction and celite chromatography and after quantification with a completely different technique such as gas chromatography.

The within assay variabilities for T and DHA were 4.7 p. 100 and 4.6 p. 100 respectively but vary with the level of steroid in plasma. The inter assay variabilities of T were 9.5 p. 100 and 3.2 p. 100 for 1.5 and 11.6 ng/ml of plasma respectively. The antiserum for 5 α-DHT have a specificity such that, even after celite chromatography some androgens (5 β-DHT) may interfere. However determinations of 5 α/5 β-DHT amounts are possible.

The physiological validations of direct plasma T and DHA RIA were studied in various conditions.

The DHA plasma variations are similar to those of T in Ram from birth to puberty, but the levels are lower. DHA plasma levels show a seasonal variation as does testosterone. Variations within 24 hrs of these two androgens were in synchrony.

The direct plasma T and DHA assays are useful and inexpensive tools to characterize ram testicular function.

Introduction.

Many publications have described competitive protein binding assay of androgens in various species. These assays for ovines include or not chromatographic steps, always carried out after extraction (Katongole, 1971 ; Sandford *et al.*, 1974 ; Schanbacher, 1976). However, assay validity has only been determined for testosterone, incompletely for 5 α-DHT, and not at all for DHA.

Only qualitative plasma progesterone assay can be done without extraction in the ewe (Terqui and Thimonier, 1974). This result suggests the possibility of eliminating extraction and measuring steroid directly as done for gonadotrophins.

The aim of this paper is not only to establish the validity of radioimmunoassay of testosterone (T), dehydroepiandrosterone (DHA) and dihydrotestosterone (5 α-DHT) in ram plasma but also to simplify the radioimmunoassay of these steroids without loss of quality.

Material and methods.

Materials.

Animals. — Fourteen Ile-de-France and 4 Prealpes du Sud rams born during the breeding season were used in this study. One jugular blood sample (5 ml) was collected every week from birth to 17 months of age. Plasma was separated by centrifugation and stored at — 20 °C until assay.

Glassware. — All non-graduated glassware was heated at + 300 °C for a period of at least 4 hrs. Conical glass tubes were siliconized with an aqueous suspension of silicone (2 p. 100) and then dried at + 200 °C for 2 hrs.

Solvents. — Analytical grade solvents were used throughout for radioimmunoassay. For gas chromatography, solvents were purified as described by Attal (1970).

Celite. — Celite 535 (Touzart et Matignon) was washed 5 times with 6 N hydrochloric acid and then with redistilled water until neutrality. Final washing was done with redistilled methanol before drying with chloroform. It was heated to + 500 °C overnight before each use.

Steroids. — Recrystallised steroids were used to prepare standard solutions (100 μg/ml) in redistilled methanol or ethanol *. These standard solutions were changed every 4 months. Tritium-labelled steroids from the Radiochemical Centre (Amersham, UK) were diluted to 5 μCi/ml in benzene-ethanol (9 v/1 v) and stored at + 4 °C. The specific activity of testosterone, 5 α-dihydrotestosterone and dehydroepiandrosterone were 83, 120, 15 Ci/mole, respectively.

Antiserum. — Testosterone 3-O-carboxy-methyl-oxime, 5 α-dihydrotestosterone-1 α-CH_2-COOH and dehydroepiandrosterone-15 α-CH_2-COOH prepared by Condom and Emiliozzi (1974) were conjugated to bovine serum albumin, according to Dray *et al.* (1971). Rabbits were immunized according to the technique of Vaitukaitis *et al.* (1971). Antiserum, saturated by BSA, was diluted to 1/100 and stored at — 20 °C.

Specificities of the three antiserums were established by competition with other steroids. The percentage of cross-reaction was defined by the ratio of the amounts of reference steroid to competitor that gave 50 p. 100 displacement of the labelled compound.

Sheep serum anti-rabbit γ-globulins was obtained by the same method. This antiserum was treated with charcoal (10 mg/ml of serum) to strip off the endogenous steroids and then diluted with phosphate buffer.

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* Abbreviations and international nomenclature were presented in table 1.

Methods.

1. *Assay after extraction and chromotography : complete assay* (T, DHA, 5 α-DHT)

The different steps are summarised in figure 1.

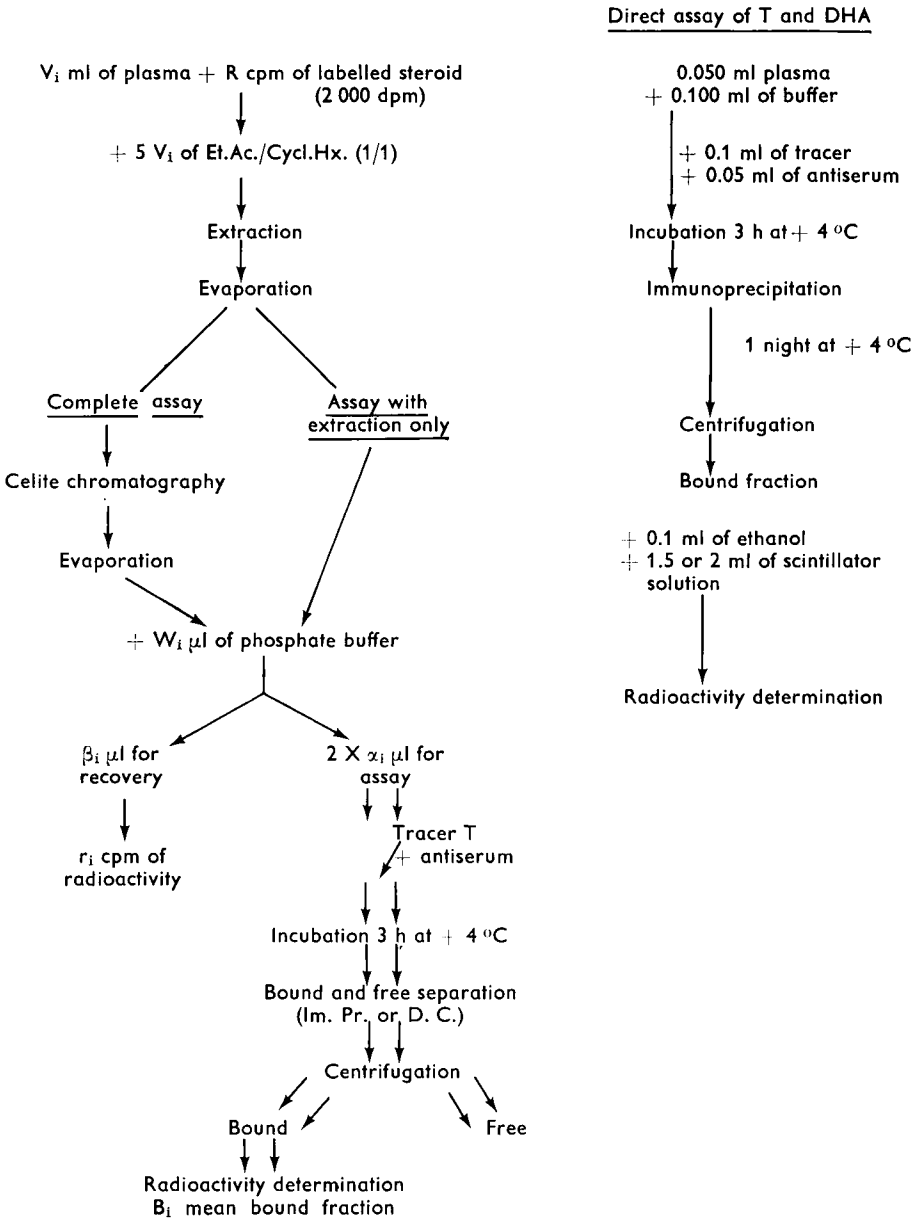

FIG. 1. — Flow sheet for the radioimmunoassays of T, DHT and DHA (Et. Ac : Ethyl Acetate ; Cycl. Hx : Cyclohexane ; Im. Pr. : Immunoprecipitation ; D. C. : Dextran Charcoal).

Extraction. — 0.1 ml of phosphate buffer containing 2 000 d.p.m. of labelled hormones was added to 0.5 to 3 ml of plasma ; these tracers served as the internal standard for recovery estimation. After mixing and equilibration 30 min at room temperature, extraction was carried out once with five volumes of ethyl acetate/cyclohexane mixture (1 v/1 v) by vortex mixing or by horizontal shaking. The lower phase was frozen by dipping tubes in methanol with dry-ice. The organic solvent mixture was poured into siliconized conical glass tubes and evaporated to dryness under filtered air.

Celite chromatography. — Glass columns (diameter 6 mm) were obturated with a glass bead and 0.8 g of mixture (0.5 g of celite 535 and 0.25 ml of formamide) were packed with vacuum. Each column was saturated by approximately 30 ml of heptane.

Dried residue was transferred to celite columns by rinsing twice with 1.75 ml of heptane ; elution was carried out stepwise first using 12 ml of heptane (5 α-DHT and DHA fraction), 2 ml of heptane/benzene (85 v/15 v) which were discarded, and then 10 ml of heptane benzene solution (testosterone fraction).

Radioimmunoassay. — Dried residue of each fraction was dissolved in 0.3 to 1 ml of 0.1 M phosphate buffer saline (pH 7) that contained 1 g/l of gelatine and 1 p. 1 000 of sodium azide. Duplicate aliquots of 0.1 ml were transferred into 10 $\times$ 75 mm polystyrene tubes or into polypropylene tubes (LKB, France) according to bound-free fraction separation techniques. Another aliquot of 0.05 ml or 0.1 ml was used to determine recovery.

The standard curves were constructed using duplicate or triplicate 0.1 ml aliquots of various solutions of standard steroids in buffer (5 to 1 000 pg/0.1 ml).

To each tube were successively added 10 000 d.p.m. of tritiated steroid in 0.1 ml of buffer, and 0.1 ml of the antiserum diluted in buffer containing rabbit γ-globulins. After vortex mixing, tubes were left for 3 hrs at + 4 °C. These conditions were optimal for binding (Guignard, 1972).

Bound and free fractions separation.

— Dextran charcoal method for 5 α-DHT assay : At the end of incubation, 1 ml of dextran (25 mg/100 ml) and charcoal (250 mg/100 ml) in buffer were added to each tube ; 15 min. later, the charcoal was separated by centrifugation for 10 min at 3 000 g. The supernatant was collected into counting vials and mixed with 10 ml scintillation fluid which contained Triton $\times$ 100 according to Patterson and Green (1965) and Terqui, Rombauts and Fèvre (1968).

— Immunoprecipitation for T and DHA assays: At the end of incubation, 0.020 ml of sheep serum antirabbit γ-globulins were added and mixed. Following overnight incubation at 4 °C, 2 ml of buffer were added and the immunoprecipitate was separated by centrifugation at 3 000 g for 40 min at + 4 °C. Supernatants were discarded ; to destroy the binding, 0.1 ml of ethanol or dioxan was added prior to 1.5 to 2 ml of scintillator fluid (toluene 1 l ; PPO 4 g ; dimethyl POPOP 40 mg). After shaking, radioactivity was measured with a Tricarb spectrometer (Packard Instr.).

2. *Assay without chromatography* (T).

As shown in figure 1, the first steps until solvent evaporation were similar to those described before ; the radioimmunoassay was then performed as described above.

3. *Direct assay in plasma* (T, DHA).

As indicated in figure 1, there was no extraction and no recovery measurement. Aliquots of 0.05 or 0.02 ml of plasma and 0.1 ml of buffer were mixed into polypropylene tubes. Standard curves were constructed with 0.050 ml or 0.020 ml of steroid-free plasma and with 0.1 ml aliquots of various standard steroid solutions ranging from 5 pg to 1 000 pg/0.1 ml of buffer. After addition of 0.1 ml of tritiated steroid and 0.05 ml of diluted antiserum, tubes were incubated for 3 hrs at 4 °C. Bound and free fractions were then separated by immunoprecipitation, as described previously.

4. *Testosterone measurement by gas chromatography according to Attal* (1970).

After extraction and purification, testosterone was transformed into 17 β-heptafluorobutyrate. This derivative was purified by thin layer chromatography and the amount was measured by gas liquid chromatography with an electron capture detector.

5. *Computation of unknown samples.*

For direct radioimmunoassay in plasma, the logit transformation of B/B_0 and logarithm of hormone concentration were used to determine DHA and testosterone amounts of unknown samples.

In the case of extraction and extraction + chromatography, radioactivity due to recovery was taken into account. The calculations were done as follows :

V_i	ml plasma volume of the ith sample ;
R cpm	mean amount of radioactivity added to plasma for recovery ;
b_1 cpm	background ;
E pg	amount of steroid equivalent to R cpm ;
w_i µl	total volume of buffer used to redissolve the sample ;
β_i µl	volume used to measure recovery ;
r_i cpm	amount of radioactivity in β_i ;
α_i µl	volume used for the radioimmunoassay ;
T cpm	mean radioactivity used for radioimmunoassay ;
b_2 cpm	non-specific binding radioactivity ;
B_i cpm	mean bound fraction of the ith sample ;

for complementary explanation, see fig. 1.

The standard curve is expressed as : Logit (B/T) = f (ln dose). The curve obtained is formed of two straight lines.

For the ith sample, the total radioactivity is :

$$T_i = T + \frac{\alpha_i}{\beta_i}(r_i - b_1) - b_1 ;$$

To the Logit : $$\ln \left(\frac{(B_i - b_2) \Big/ \left(T + \frac{\alpha_i}{\beta_i}(r_i - b_1) - b_1 \right)}{1 - (B_i - b_2) \Big/ \left(T + \frac{\alpha_i}{\beta_i}(r_i - b_1) - b_1 \right)} \right).$$

corresponds to dose d_i (pg). The level in ng/ml of plasma was :

$$D_i = \frac{1}{10^3} \times \frac{1}{V_i} \left(d_i \times \frac{w_i}{\alpha_i} \times \frac{R - b_1}{(r_i - b_1) \times \frac{w_i}{\beta_i}} - E \right)$$

$$D_i = \frac{1}{10^3} \times \frac{1}{V_i} \left(d_i \times \frac{\beta_i}{\alpha_i} \times \frac{R - b_1}{r_i - b_1} - E \right) .$$

All these computations were run on a programmable calculator (Hewlett Packard 9820).

Results.

A. — *Radioimmunoassay acceptability.*

Testosterone, 5 α-dihydrotestosterone and dehydroepiandrosterone acceptability of the different assays directly in plasma, after extraction and after extraction plus chromatography is investigated. According to Abraham (1974), acceptability depends on specificity, sensitivity, accuracy, precision, and practicability.

TABLE 1

Specificity of androgens antisera : percentage of cross reaction

	Anti-testosterone Final dilution 1/15 000	Anti-5 α-DHT Final dilution 1/45 000	Anti-DHA Final dilution 1/15 000
Testosterone (T)	100	12	< 0.1 *
5 α-dihydrotestosterone (5 α-DHT)	65	100	< 0.1
Dehydroepiandrosterone (DHA)	< 0.1 *	< 0.1 *	100
17 β-sulfooxyandrost-4-ene-3-one	0.9	< 0.1	—
Testosterone propionate	< 0.1	—	—
17 α-hydroxy-androst-4-éne-3-one (epitestosterone)	3.5	< 0.1	—
Androstenedione	0.7	0.5	0.4
17 β-hydroxy-5 α-androstan-3-one (5 β-DHT)	49.5	48	—
3 α-hydroxy-5 α-androstan-17-one	< 0.1	3.9	< 0.1
3 β-hydroxy-5 α-androstan-17-one	—	< 0.1	1.6
3 α-hydroxy-5 β-androstan-17-one	2.8	0.3	< 0.1
5 α-androstan-3 α, 17 β-diol	2	30	< 0.1
5 α-androstan-3 β, 17 β-diol	4	18	< 0.1
5 β-androstan-3 α, 17 β-diol	1.4	5.6	—
3 β-sulfooxyandrost-5-ene-17-one (DHA sulfate)	—	< 0.1	< 0.1
3 β, 17 β-dihydroxy-5-androstene	0.5	0.7	4
11 β, 17 β-dihydroxy-androst-4-ene-3-one	—	< 0.1	—
11 β-hydroxy-androst-4-ene-3,17-dione	—	< 0.1	—
Androst-4-ene-3, 11, 17-trione	—	< 0.1	—
Cyproterone (Schering)	< 0.1	< 0.1	—
3 β-hydroxy-pregn-5-ene-20-one	—	< 0.1	< 0.1
Progesterone	< 0.1	< 0.1	< 0.1
5 α-pregnan-3,20-dione	—	< 0.1	< 0.1
Cortisol	< 0.1	< 0.1	—
17 α, 21-dihydroxy-pregn-4-ene-3,20-dione	—	< 0.1	—
Oestrone	< 0.1	< 0.1	< 0.1
Oestradiol-17 β	< 0.1	< 0.1	< 0.1

* < 0.1 means that 100 ng of steroid did not displace 50 p. 100 of labelled compound.

Specificity. — In cases of direct plasma RIA or after solvent extraction only, the specificity of the assay depends solely on antiserum specificity. Percentages of cross-reaction for 27 steroids are reported in table 1. Neither C_{21}-steroids nor C_{18} steroids cross-react significantly with the three antisera. The major interfering steroids for testosterone are 5 α-DHT (65 p. 100) and 5 β-DHT (49.5 p. 100). The other androgens show little or no significant cross-reaction ; none of the steroids tested compete with DHA (table 1). DHA sulfate and 3 β, 17 β-dihydroxy-5-androstene have little or no effect on antiserum DHA binding (< 0.1 and 4 p. 100 respectively).

Thus, direct plasma and extraction RIA might be considered as very specific for DHA ; for testosterone assays, the specificity depends on the relative amount of 5 α/β reduced compounds.

5 α-DHT antiserum appears to be less specific than the other two antisera ; indeed, as indicated in table 1, 5 β-DHT, testosterone, 5 α-androstan-3 α, 17 β-diol and 5 α-androstan-3 β, 17 β-diol show significant cross-reactions of 48, 12, 30 and 18 p. 100, respectively. Thus, mainly because of high testosterone levels in the male, direct plasma and extraction RIA, are not specific.

To increase specificity, a chromatographic step can be added ; figure 2 indicates the distribution of different steroids in testosterone and 5 α-DHT fractions after celite chromatography. Testosterone is separated from 5 α- and 5 β-DHT which are the more competitive steroids to serum antitestosterone. The other steroids, still present in the testosterone fraction, do not show any significant cross-reaction with serum anti-testosterone.

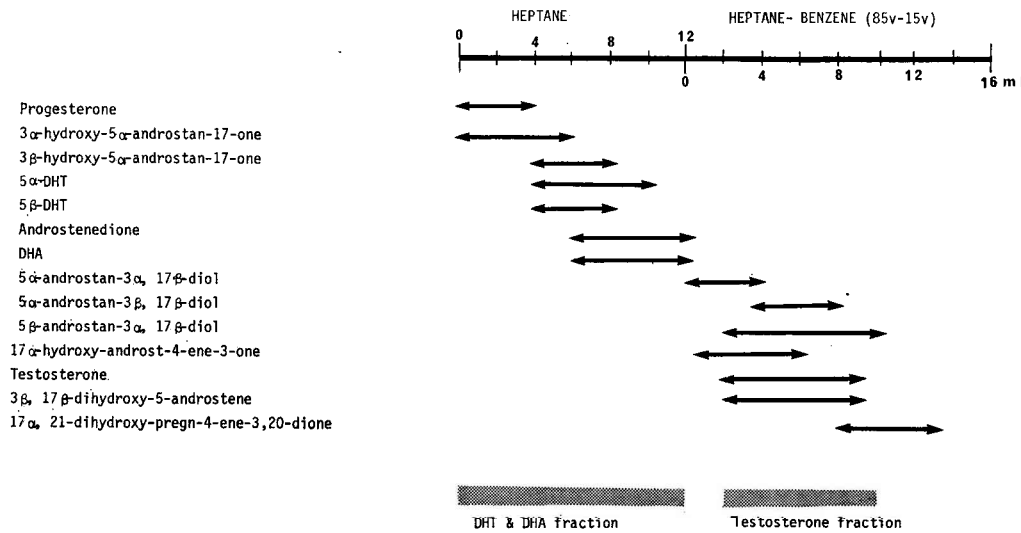

FIG. 2. — *Elution of different steroids after celite chromatography.*

DHA is also separated from 3 β, 17 β-dihydroxy-5-androstene, and the other steroids eluted in the same fraction as DHA are not competitive.

The 5 α-DHT fraction is free from testosterone but 5 β-DHT is also eluted in the same fraction. However, the 5 α-androstanediols are separated from DHT. This assay

TABLE 2

Sensitivity of the radioimmunoassays

Steroids	Assay	Affinity constant of anti-serum M^{-1}	Sensitivity of standard curve	(Logit. = A log dose + B) A	B	r	Recovery in percentage m ± sem	Blank in ng (steroid free plasma)	Sensitivity of the assay ng/ml
Testosterone		4.1×10^9							
	Direct RIA		10 pg	— 0.97	+ 3.96	— 0.991	—	—	0.2 (0.05)
	Extraction		5-10 pg	— 1.1	+ 4.12	— 0.999	90 ± 0.5	0.040 (1 ml)	0.080 (1 ml)
	Extraction + chromato.		5-10 pg	— 1.10	+ 4.12	— 0.999	79 ± 1	0.050 (1 ml)	0.1 (1 ml)
DHA		3×10^9							
	Direct RIA		10-20 pg	— 1.12	+ 4.76	— 0.996	—		0.2 (0.05)
	Extr. + chrom.		10-20 pg	— 0.92	+ 5.1	— 0.997	49 ± 1	0.060 (1 ml)	0.1 (1 ml)
DHT		0.6×10^9							
	Extr. + chrom.		5-10 pg	1.19	5.2	— 0.995	69 ± 1	0.090 (1 ml)	0.150 (1 ml)

( ) Volume of plasma used.

is specific for 5 α-DHT only in cases with relatively low 5 β-DHT levels ; otherwise, it permits estimation of 5 α/5 β-DHT compounds.

Sensitivity. — The sensitivity of the different assays and the factors governing sensitivity such as affinity constant, standard curve sensitivity, recovery percentage, and blank values are presented in table 2, the standard curves are shown in figure 3. Logit-Log. linearisations for the standard curves were relevant since the correlation coefficient and the slope are not different from — 1.0.

TABLE 3

Accuracy of the different assays

Linear regression between amount added to steroid-free plasma and amount recovered ($y = a + bx$)

Steroids	Assays	Range of concentration (ng/ml)	Regression coefficient (b)	Zero value (a)	r	n
Testosterone	Direct	0.2-30	1.003	0.013	0.999	8
	Extract. + chrom.	1-8	1.005	0.086	0.999	8
DHA	Direct	0.2-5	1.050	0.034	0.996	24
	Extract. + chrom.	0.2-5	1.222	0.116	0.992	24
DHT	Extract. + chrom.	0.1-1	1.032	— 0.003	0.994	12

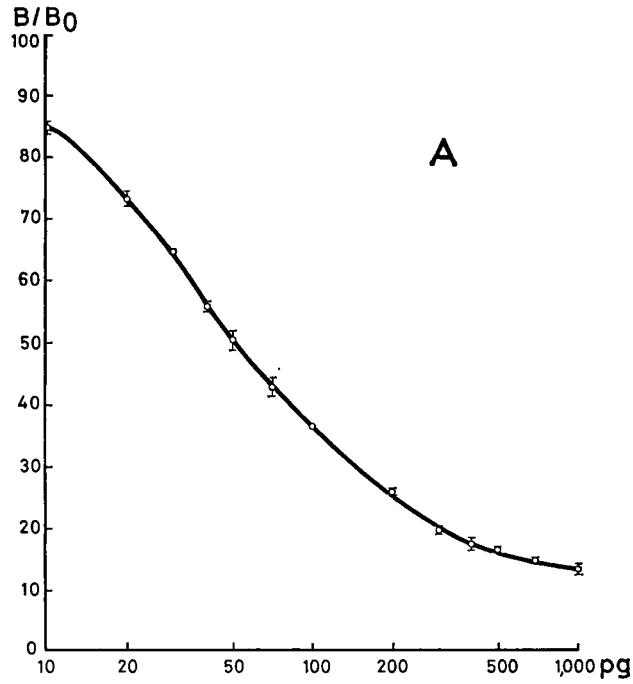

FIG. 3

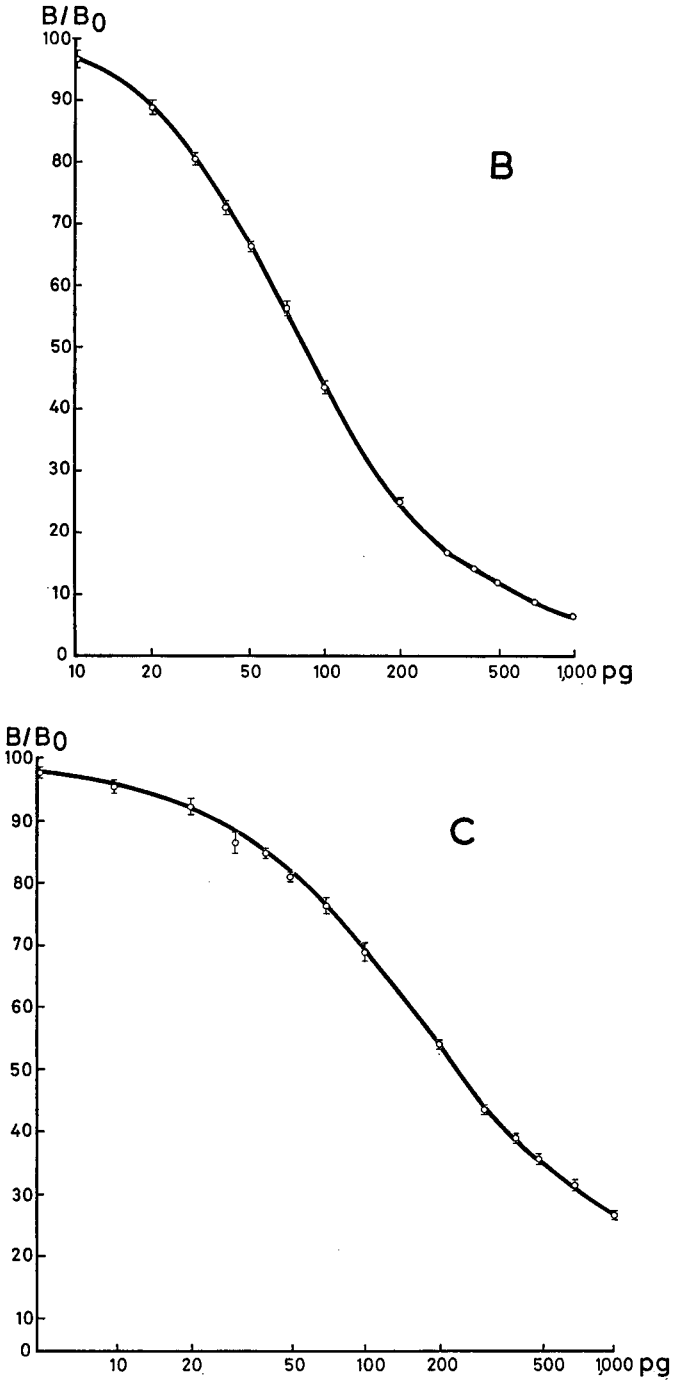

FIG. 3. — *Standard curves for the radioimmunoassays of testosterone (A), of 5 α-DHT (B) and of DHA (C).* T and DHA standard curves are prepared with 0.05 ml of steroid-free ram plasma (m ± sem).

The recovery for direct assay in plasma is assumed to be 100 p. 100. The low recovery after chromatography for 5 α-DHT and DHA are probably due to low solubility in phosphate buffer. Sensitivity of standard curves were around 10 pg/tube and the blanks were of the same order, indicating the absence of noticeable effects due to solvent.

Assay sensitivity for the volume of plasma and buffer commonly used is approximately 0.1 ng/ml of plasma.

Accuracy. — The different steroids were added to steroid-free plasma samples in increasing amounts (table 3). Parameters of the linear regressions presented in table 3 show that the regression coefficients are not different from 1 and that at least 98 p. 100 of the variability is explained by the amount added. The zero values computed from regression line were generally less than 0.1 ng, i. e. inferior to the blank values suggesting no plasma effect.

Accuracy for testosterone was also checked by measurement of unknown ram plasma samples with different techniques. The levels reported in table 4 were similar,

TABLE 4

Accuracy : levels of testosterone (ng/ml) *in RAM plasma samples assayed by three methods*

Sample	Direct RIA	RIA after extraction + chromatography	Gas chromatography
1	1.5 ± 0.08	1.55	1.6
2	11.3 ± 0.4	10.3	—
3	6.5 ± 0.2	—	7.0

TABLE 5

Precision of the different radioimmunoassays

Steroids	Assay	Within-assay variability			Between-assay variability		
		v. c. p. 100	Level ng/ml	nb replicate	v. c. p. 100	Level ng/ml	n × m (assay) (replicate)
Testosterone	Direct RIA	9.9	1.4	6	9.5	1.5	10 × 2
		4.7	11.8	6	3.2	11,6	10 × 2
	Extraction	5.0	2	4		—	—
		3.0	7.4	6		—	—
	Extraction + chromat.	6.1	4.4	9	14	11.4	3 × 2
DHA	Direct RIA	4.6	2.3	4	—	—	—
	Extraction + chromat.	10.8	1.0	9	—	—	—
DHT	Extraction + chromat.	7.1	0.52	6	12.2	1.1	3 × 2

regardless of the technique used : direct plasma RIA, extraction + chromatography with RIA and gas chromatography.

Precision. — The within and among-assay variabilities have been calculated from replicate variance in the same assay and in completely different assays (table 5). The coefficients of variation were between 3 and 11 p. 100 for the within-assay and between 3 to 14 p. 100 for the among-assay. The coefficients of variation differ not only from one steroid to another but also with steroid level (table 5).

B. — *Plasma androgen levels in the ram.*

Peripheral plasma testosterone and DHA levels were investigated with direct plasma assay in different physiological states. The amounts of 5 α/5 β-DHT compounds were also estimated by the complete assay.

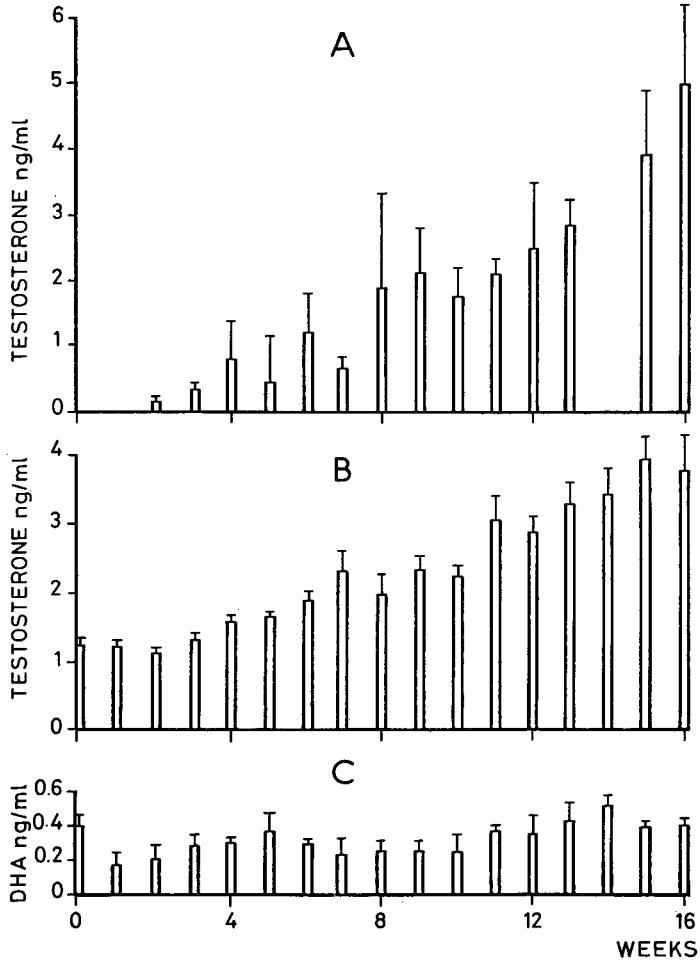

FIG. 4. — *Androgen levels in lamb peripheral plasma from birth to* 16 *weeks of age* (m + sem). A. — Testosterone in Préalpes du Sud breed. B. — Testosterone in Ile-de-France breed. C. — DHA in Ile-de-France breed.

Evolution from birth to puberty (fig. 4). — In the two breeds studied, the mean testosterone levels rise linearly from birth to 16 weeks of age as shown in figure 4. Individual patterns are similar but sometimes high concentrations of testosterone occur during the first week of life, especially in the Ile-de-France breed. At birth, the testosterone levels are lower in the Préalpes du Sud than those of the Ile-de-France breed (0.2 ng/ml *vs* 1 ng/ml). However, at 15 or 16 weeks of age, the testosterone levels seem higher in Préalpes du Sud than in Ile-de-France lambs. The linear regressions between testosterone (T) and age in days (d) for the two breeds were :

— Préalpes du Sud T (ng/ml) = — 0.81 + 0.044 d $r = 0.95$ $P < 0.01$
— Ile-de-France T (ng/ml) = 0.87 + 0.026 d $r = 0.97$ $P < 0.01$.

The slopes of these two linear regressions are different ($P < 0.05$).

In the Ile-de-France breed, peripheral plasma DHA levels are very low after birth (0.3 ng/ml) and reach only 0.5 to 0.6 ng/ml 3 months later.

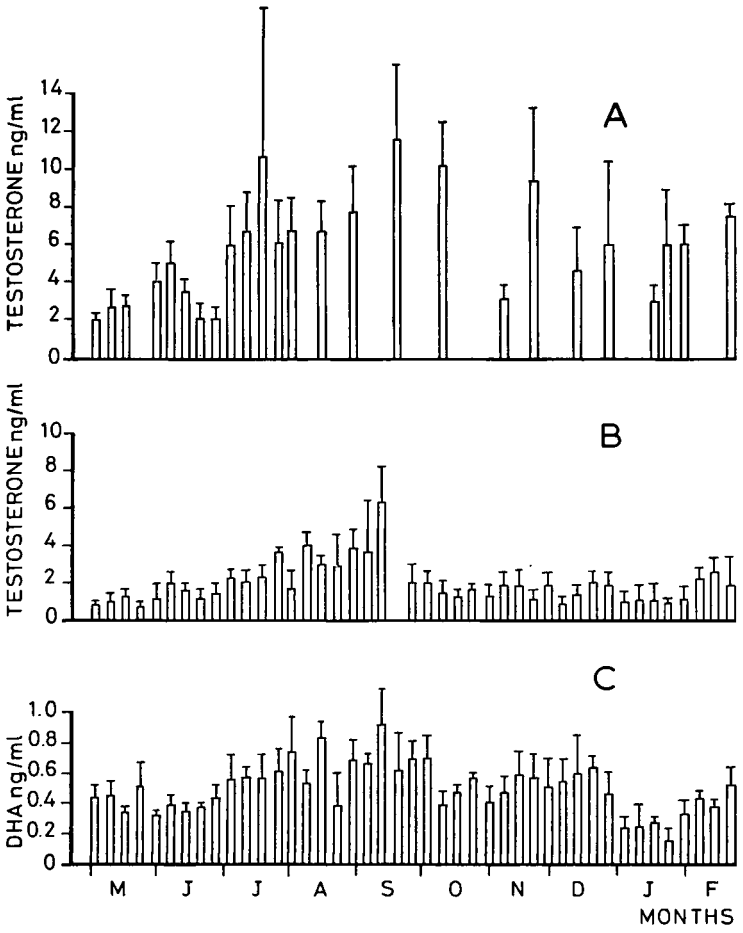

FIG. 5. — *Seasonal variations of androgens in ram* (m + sem). A. — Testosterone in Préalpes du Sud breed. B. — Testosterone in Ile-de-France breed. C. — DHA in Ile-de-France breed.

In young lambs of Ile-de-France breed (15 weeks), the 5 α/5 β-DHT levels appear to be very low and near the sensitivity of the method used : 0.075 ± 0.01 ng/ml.

Seasonal variations in young rams. — Testosterone levels begin to increase in both breeds (fig. 5*a* and *b*) at the end of June and reach the highest values (8 or 12 ng/ml) in September ; they then decrease rapidly in the Ile-de-France breed and more slowly with large fluctuations in the Préalpes du Sud breed.

The peripheral plasma testosterone concentrations are higher in the Préalpes du Sud breed than those observed in the Ile-de-France breed, but perhaps this is true only for this particular first breeding season.

Seasonal variation of plasma DHA in the Ile-de-France breed (fig. 5) was also detected but the magnitude of change was lower than for testosterone.

Plasma DHT levels in Ile-de-France rams were low during the breeding season : 0.13 ± 0.033 ng/ml.

Variations within 24 hours. — Figure 6 depicts individual variations of testosterone and DHA in jugular plasma of two Ile-de-France rams during two 24-hr periods in June and in September. Testosterone secretion appears to be pulsatile, and there is a good coincidence between testosterone and DHA variations. These two samples show that the number of testosterone and DHA peaks are greater in September than in June.

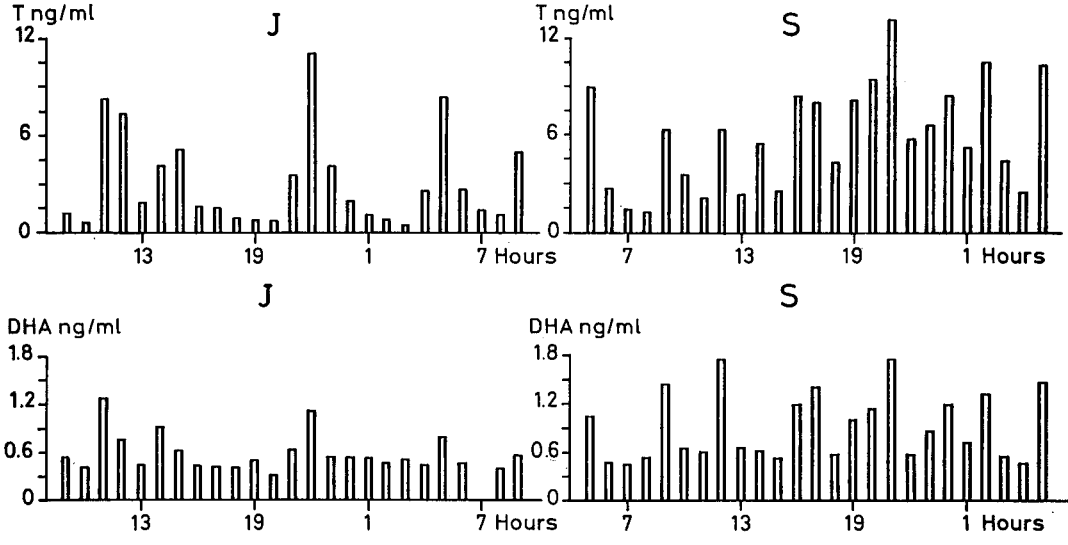

FIG. 6. — *24-hour variations of testosterone and DHA in June* (J) *and in September* (S) *in two rams.*

Discussion.

Validity of assays of three androgens was studied in the peripheral plasma of the ram, and plasma variations of androgens in different physiological states were demonstrated with radioimmunoassays directly in plasma for testosterone and DHA.

In peripheral plasma of the ram the nature and level of androgens determine the specificity of direct and after-extraction assays. Attal (1970) demonstrated that androstenedione and epitestosterone are also present in the unconjugated fraction. Estimates by gas chromatography indicate that these compounds appear in low concentrations ($\leqslant$ 2 ng/ml) in comparison to testosterone (Attal, 1970). From our results 5 α/5 β-DHT compounds appear to be at low levels ($<$ 0.2 ng/ml). Thus, the known androgens in the unconjugated fraction did not interfere with testosterone assays because of the low concentration. This is confirmed by the fact that after celite chromatography, estimates are similar to those obtained after extraction or measured directly in plasma.

The extraction step is necessary only if plasma testosterone binding is extensive and if conjugated steroids bind significantly to the antibody. Dray (1970) and Attal (1970) have shown that testosterone binding by sheep plasma is low. From the specificity of the antiserum used in the present study, neither testosterone sulfate nor dehydroepiandrosterone sulfate shows any significant cross-reaction. Consequently, results obtained by direct RIA in plasma are the same as those given by extraction and chromatography and compare favorably to a completely different technique such as gas chromatography.

The sensitivity of direct plasma assay is perhaps lower than after extraction because a large volume of plasma can be assayed by extraction technique. However, this disadvantage depends on the levels to be measured.

Intra- and inter-assay variability are lower in the direct RIA in plasma than in the other assays described here or published by Abraham (1974), Schanbacher and Ford (1976) and Schanbacher (1976). The lower number of operations with the direct RIA may account for this difference.

For testosterone assay after extraction, or after extraction plus chromatography, the analytical criteria (sensitivity, reproductivity and accuracy) are similar to those obtained by Abraham (1974) for human plasma and by Schanbacher (1976) for sheep plasma.

The existence of DHA in ram plasma was unknown ; from celite chromatography and antiserum specificity, it is possible to conclude that DHA is present in this plasma. Direct RIA in plasma is also possible and valid for this steroid.

5 α-DHT could only determined in ram plasma by the technique described here if the absence of 5 β-DHT or its presence in negligible levels could be shown. This differs from the conclusion of Schanbacher (1976) who claimed to have measured 5 α-DHT but had not studied 5 β-DHT. However, 5 α- and 5 β-DHT can be estimated by this assay which is similar to that used by Bartke and Voglmayr (1977).

Prepuberal, seasonal and 24-hr level variations of peripheral plasma testosterone in ram by direct RIA in plasma are similar to those first described by Attal (1970) and later by Katongole *et al.* (1974), Sandford *et al.* (1974), Purvis *et al.* (1974), Lincoln (1976), Schanbacher (1976), Schanbacher and Ford (1976).

The physiological meanings of these variations were discussed by Cotta *et al.* (1975) for the prepuberal period and by Garnier *et al.* (1977) studying seasonal and 24-hr variations.

The peripheral levels of DHA are very low compared to those of testosterone but plasma DHA variations, especially over 24 hrs, are coincidental to those of testosterone. This observation suggests that the majority of peripheral DHA is also produced

by the testis, as has been shown in boar by Huis In't Veld *et al.* (1964). Direct RIA in plasma for testosterone appears to be as good as other methods. Indeed, the validity of our assay is demonstrated both analytically and physiologically.

Because of its simplicity, validity and lower cost, the direct testosterone assay appears to be more practical for characterizing testicular function in the ram. Such an assay may be applied to other species.

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Résumé. Divers types de dosages radioimmunologiques (RIA) ont été étudiés pour mesurer la testostérone (T), la déhydroépiandrostérone (DHA) et la 5 α-dihydrotestostérone (5 α-DHT) dans le plasma de Bélier.

La spécificité des antisérums, l'absence de liaison de forte affinité avec les protéines plasmatiques, les faibles concentrations des autres androgènes permettent de doser la DHA et la T directement dans le plasma de Bélier. Les concentrations mesurées avec ce dosage direct sont identiques à celles qui ont été obtenues après extraction, après extraction et chromatographie sur célite, et après une technique complètement différente comme la chromatographie en phase gazeuse. La sensibilité de ces dosages est comprise entre 0,1 à 0,2 ng/ml de plasma. La variabilité à l'intérieur d'un même dosage est faible : de 4,7 et de 4,6 p. 100 respectivement pour T et DHA, mais elle dépend de la quantité de stéroïde mesurée. La variabilité entre dosages de T est de 9,5 p. 100 pour 1,5 ng/ml et de 3,2 p. 100 pour 11,6 ng/ml.

Malgré une chromatographie sur colonne de célite, il n'est pas possible de mesurer spécifiquement la 5 α-DHT ; seules, les quantités de 5 α/5 β-DHT peuvent être estimées, mais après extraction et chromatographie. Ainsi, chez l'agneau et chez le Bélier, les concentrations plasmatiques de ces composés sont très faibles (0,07 à 0,2 ng/ml).

La validité physiologique des RIA de T et de DHA a été évaluée dans différentes situations chez le Bélier.

Ainsi, de la naissance à la puberté, ces deux androgènes augmentent progressivement, toutefois les concentrations plasmatiques de DHA sont plus faibles que celles de T. La DHA plasmatique présente, comme la testostérone, une variation saisonnière. Au cours des 24 heures, les concentrations de ces androgènes varient de façon « pulsée » et les « pulses » de DHA sont synchrones de ceux de T.

Les dosages directement dans le plasma de T et DHA sont donc validés et constituen un moyen très pratique et peu onéreux pour caractériser la fonction testiculaire du Bélier.

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