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EVALUATION OF A RADIOIMMUNOASSAY FOR TESTOSTERONE ESTIMATION

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SUMMARY

A radioimmunoassay technique, which is essentially a modification of the method described by Furuyama *et al.*[1], has been evaluated for the determination of testosterone in human peripheral plasma and rat testis tissue.

The antiserum used was raised against testosterone-3-(0-carboxymethyl)-oxime-bovine serum albumin in female rabbits. It had an association constant of $5{\cdot}5 \times 10^9$ 1/mol, 4°C, at a dilution of 1 in 20,000. The procedure involved addition of [^{3}H]-testosterone internal standard, extraction and chromatography of the plasma extracts on alumina micro-columns prior to assay. Testis tissue extracts were not chromatographed. Known amounts of standard testosterone were subjected to the same procedures.

After incubation with antiserum for 16 h at 4°C total recovery from the extraction, chromatography (when used) and incubation procedures were measured in order to correct for losses. Either toluene scintillation fluid, dextran-coated charcoal or polyethylene glycol were used to separate free and bound testosterone. For human plasma as well as for testis tissue a good correlation was observed between results obtained with radioimmunoassay and a gas chromatographic method using electron capture detection of testosterone chloroacetate[12].

INTRODUCTION

SEVERAL reports in the last few years have described the measurement of testosterone in human peripheral plasma by radioimmunoassay[1–6]. The main advantages of this technique are its high sensitivity and simplicity of assay. Although this method is potentially highly specific, in practice the antibodies to testosterone used so far cross react with other steroids. Therefore, a purification step has to be used to remove cross reacting steroids. In order to verify the specificity and accuracy of the developed testosterone radioimmunoassays several reports have been published describing comparison with other methods. For example, Furuyama *et al.*[1] and Dessypris *et al.*[7, 8] found a good agreement between results of radioimmunoassay and competitive protein binding. Dufau *et al.*[5] compared results of radioimmunoassay with results of both competitive protein binding and isotope derivative assay and observed a satisfactory correlation. Collins *et al.*[6] reported a reasonable correlation between radioimmunoassay and gas–liquid chromatography (g.l.c.) for plasma testosterone. The present report describes an extensive evaluation of a method, which is essentially a modification of the method described by Furuyama *et al.*[1]. Different methods of separating free and bound testosterone have been investigated. The reliability of the method was evaluated through a comparison of results obtained by radioimmunoassay and g.l.c. of testosterone concentrations in peripheral human plasma and rat testis tissue. In addition the inter- and intra-assay variability was evaluated.

EXPERIMENTAL

Standards. [1,2,6,7-^{3}H-]-Testosterone, S.A. 100 Ci/mmol, was purchased from the Radiochemical Centre, Amersham, England; its purity was checked every two months by paper chromatography and t.l.c.. A stock solution in toluene-ethanol (9:1, v/v) containing ± 20,000 d.p.m./50 μl was used.

Non-radioactive steroids were obtained from Steraloids, Pawling, New York, and were used without further purification.

Solvents and reagents. The 0·05 M borate buffer, pH 8·0, was made up fresh every week and stored at 4°C. Krebs–Ringer bicarbonate buffer, pH 7·4, was made up fresh for every estimation. Water was glass distilled before use.

Methanol (Merck), ethanol (Merck), diethylether (peroxide free) (Union Chimique Belge, Brussels, Belgium), n-hexane (U.C.B.), dichloromethane (U.C.B.) and acetone (U.C.B.) were of analytical grade and were redistilled once before use. In later experiments the solvents were used without further purification (see Discussion section). Polyethylene glycol (U.C.B.) was used without purification. Alumina, standardized for the chromatographic adsorption analysis (Merck), was washed with warm 6 N hydrochloric acid, distilled water, methanol and dichloromethane and then heated at 500°C in a furnace for 8 h. The alumina was deactivated with distilled water resulting in activity grade II-III according to Brockmann [18].

Glassware. Assays were carried out in conical glass tubes (10 cm, i.d. 1·4 cm) siliconized with a 5% solution of dimethyldichlorosilane (Merck) in toluene (Hicol, Rotterdam, The Netherlands). Tubes and other glassware were washed by soaking overnight in Extran detergent solution (Merck) and were rinsed with distilled water.

Antiserum. The testosterone-3-(0-carboxymethyl)-oxime-bovine serum albumin was prepared by the method of Erlanger *et al.* [9]. One mg of the conjugate was dissolved in 2 ml sterile saline-complete Freund's adjuvant (Difco) (1:3, v/v), and this emulsion was administered *via* multiple intradermal injections (approximately 30 sites) into female New Zealand white rabbits (weight 3 kg). This procedure was repeated weekly for a month, then two times fortnightly and monthly thereafter. Three months after the initial immunization an antiserum was obtained, which was deemed suitable for use in the radioimmunoassay. A 1:20 (v/v) dilution of the antiserum was preabsorbed on 0·5% bovine serum albumin in 0·05 M borate buffer, pH 8·0, and centrifuged for 15 min, with 3,000 rev/min at 4°C. The supernatant was stored at −20°C. Just before use this 1:20 antiserum was diluted to 1:20,000 (v/v) with 0·05 M borate buffer, pH 8·0, containing 0·06% bovine serum albumin and 0·05% human γ-globulin.

Dextran-coated charcoal. 25 mg Dextran T 250 (Pharmacia, Uppsala, Sweden) was dissolved in 100 ml 0·05 M borate buffer, pH 8·0, and added to 250 mg of Norit GSX charcoal (Clydesdale Co. Ltd., Glasgow).

Radioactivity measurements. Samples were counted in a scintillation fluid prepared by dissolving 80 g naphthalene (U.C.B.), 4 g PPO (Merck) and 40 mg POPOP (Merck) in 1000 ml toluene (Hicol)-methoxyethanol (U.C.B.) (3:2, v/v). When toluene scintillation fluid was used for separating free and bound testosterone all samples were counted in toluene scintillation fluid prepared by dissolving 4 g PPO and 40 mg POPOP in 1000 ml toluene. Radioactivity measurements were performed using an automatic liquid scintillation system (Nuclear Chicago model Isocap/300). Samples were counted to an accuracy of 2%.

METHODS

Extraction procedures

Testis tissue. Samples of rat testis tissue for testosterone estimation were prepared essentially as described previously [20]. After incubation of rat testis tissue samples (approximately 100 mg wet weight per 0·5 ml Krebs–Ringer bicarbonate buffer), the vessels were cooled in ice, immediately followed by addition of the internal standard [1,2,6,7-^{3}H]-testosterone (approximately 20,000 d.p.m.). The samples were sonicated (20 KHz, amplitude 5 μm) at 0°C for 30 sec and then extracted with acetone (2 × 2 ml). Acetone was evaporated under N_2 at 45°C and the remaining water phase was extracted with ether (3 × 1 ml). Testosterone was assayed in aliquots of the combined ether phases. Samples for the standard curve were prepared by addition of internal standard [1,2,6,7-^{3}H]-testosterone (approximately 20,000 d.p.m.) to 0,5,10,25,50 and 100 ng testosterone in duplicate in 0·5 ml Krebs–Ringer bicarbonate buffer. After addition of acetone the standards were treated in the same way as the unknown samples.

Plasma. Human peripheral plasma samples were assayed in duplicate with different amounts of plasma (male: 25 and 50 μl, female: 100 and 200 μl). Following addition of internal standard [1,2,6,7-^{3}H]-testosterone (approximately 40,000 d.p.m.) the plasma samples were extracted with 1·5 ml n-hexane–ether (4:1, v/v) for 60 sec, using a Vortex mixer, and then centrifuged. In order to prepare the standard curve [1,2,6,7-^{3}H]-testosterone and known amounts of testosterone (0, 50, 100, 150, 250 and 500 pg) in duplicate were dissolved in 1·5 ml n-hexane-ether (4:1, v/v) by mixing on a Vortex mixer and then treated in the same way as the unknown samples.

Micro-column chromatography

For chromatography, alumina columns (3 cm), packed in a glasswool-plugged disposable Pasteur pipette (15 cm, i.d. 0·6 cm), were used. Just before use the columns were washed with 3 × 1·6 ml ethanol, 3 × 1·6 ml methanol, 3 × 1·6 ml dichloromethane-methanol (1:1, v/v) and 3 × 1·6 ml dichloromethane. Total n-hexane-ether (4:1, v/v) extracts of plasma samples and standards were transferred to the micro-columns and the columns were then eluted with 1 × 1·6 ml n-hexane-ether (4:1, v/v), 6 × 1·6 ml 0·45% ethanol in n-hexane and 2 × 1·6 ml 0·95% ethanol in n-hexane. The last fraction contained the testosterone (recovery of [1,2,6,7-^{3}H]-testosterone was 67 ± 8 (S.D.)% (n = 7). Testis tissue extracts were not chromatographed.

Incubation with antiserum

Suitable aliquots of the purified extracts and standards were added to siliconized tubes and dried under nitrogen (for tissue extracts and standards, 10–100 μl were added plus a second internal standard [1,2,6,7-^{3}H]-testosterone (approximately 20,000 d.p.m.); for plasma extracts and standards 0·5 and 1·0 ml of the 0·95% ethanol in n-hexane column eluate fraction were used). 250 μl of 1 in 20,000 diluted antiserum was added to each tube. The contents of the tubes were mixed on a Vortex mixer. After standing for 16 h at 4°C, 50 μl was removed into a counting vial in order to estimate the total recovery. Four tubes containing buffer and internal standard but no antiserum were assayed to determine non-specific binding.

Separation of bound and free testosterone

To separate free and bound testosterone in the remaining 200 μl incubation mixture either one of three methods was used:

(a) *Toluene scintillation fluid extraction* [10]. Unbound testosterone was extracted from the incubation mixture at 4°C with 2·5 ml toluene scintillation fluid by mixing on a Vortex mixer for 20 sec. After centrifugation the toluene layer was removed into a counting vial with a Pasteur pipette. The extraction was repeated with the same volume and mixing for 5 sec. Extraction recovery of free [1,2,6,7-^{3}H]-testosterone was 99 ± 1% after an incubation with 0·05 M borate buffer, pH 8·0, containing 0·06% bovine serum albumin and 0·05% human γ-globulin without antiserum.

(b) *Dextran-coated charcoal adsorption.* 500 μl of a dextran-coated charcoal suspension was added at 4°C to the incubation mixture with an Eppendorf pipette. After mixing on a Vortex and standing for 5 min at 4°C tubes were centrifuged 10 min at 4°C (3,000 rev/min). The supernatant (500 μl) containing antibody bound testosterone was removed into a counting vial. Non-specific binding in the absence of antiserum was 1–2%.

(c) *Polyethylene glycol precipitation* [11]. Polyethylene glycol was added to give a final concentration of 12·5%. After centrifugation the free testosterone was estimated by counting 100 μl of the supernatant.

Calculations

A computer programme was used for calculation of results. Corrections for quenching, mass of internal standard, recovery, and non-specific binding were made. The standard curves were linearized by transformation of percentage bound and amount of testosterone in pg to a logit-log scale. Data obtained by the programme included amount of testosterone in sample, average % standard deviation and correlation coefficient of the standard curve. The computer programme used was written by Mr. W. F. Clotscher and was based on programmes published by Rodbard and Lewald [13].

RESULTS

Standard curves

Standard curves over the range 15–170 pg with and without column chromatography are shown in Fig. 1. The precision of the points in the standard curve over an extended range, assuming that the recoveries were the same, is given in Table 1. The effect of adding the antiserum at 4°C and at room temperature before subsequent incubation at 4°C is given in Table 2. The longer the samples were kept at room temperature before being cooled to 4°C, the more the initial binding decreases. This may cause an error in the assay if a large series of samples are being dealt with. Therefore addition of antiserum, incubation and separation of free and bound testosterone were performed at 4°C.

Comparison of three methods for separation of free and bound testosterone

Under the same conditions an overall higher percentage initial binding was obtained with dextran-coated charcoal adsorption, compared with toluene scintillation fluid extraction.

In the absence of antiserum 1–2% of the free testosterone was not adsorbed by

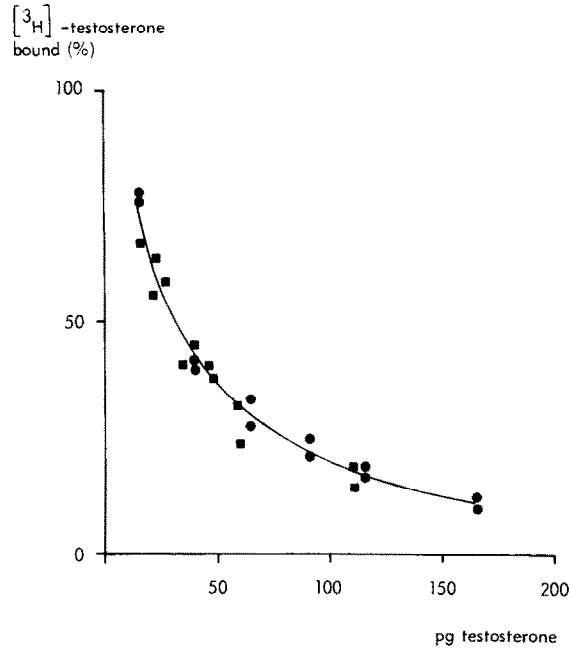

Fig. 1. Standard curves for testosterone with antiserum dilution 1:20,000, with and without alumina micro-column chromatography. (■ with alumina chromatography; ● without alumina chromatography)

Table 1. Precision of standard curve without extraction and chromatography

Amount of pg testosterone added to internal standard [^{3}H]-testosterone*	[^{3}H]-testosterone bound in % (S.D., coefficient of variation† (n = 6)	pg testosterone‡ (S.D., coefficient of variation) as printed out by com puter programme (logit-log transformation)	pg testosterone‡ (S.D., coefficient of variation) as calculated from a plot of % bound versus mass of testosterone
0	60·2 ± 0·6 (1·0%)	2·4 ± 1·5 (62·5%)	1·9 ± 1·5 (78·9%)
50	27·6 ± 1·7 (6·2%)	47·3 ± 4·7 (9·9%)	47·2 ± 4·1 (8·7%)
100	14·0 ± 2·1 (15·0%)	112·8 ± 12·4 (11·0%)	122·2 ± 14·4 (11·8%)
200	8·0 ± 1·2 (15·0%)	184·0 ± 22·8 (12·4%)	196·2 ± 34·6 (17·6%)
250	5·9 ± 1·3 (22·0%)	258·8 ± 52·4 (20·2%)	274·8 ± 56·2 (20·4%)
500	2·4 ± 0·9 (37·5%)	530·0 ± 153·0 (28·8%)	—
1000	0·9 ± 0·5 (55·5%)	976·0 ± 372·0 (38·1%)	—

*Mass of internal standard [^{3}H]-testosterone is 22·7 pg.
†Coefficient of variation (%) = (S.D./mean) × 100.
‡Mass of internal standard[^{3}H]-testosterone subtracted.

the charcoal and in the case of toluene scintillator extraction 1–2% free testosterone was not extracted. Sensitivity and reproducibility of both methods were essentially similar. Polyethylene glycol precipitates antibody bound testosterone. The effect of varying the final concentration of polyethylene glycol for precipitation is shown in Fig. 2. A 12·5% solution in water resulted in a good standard curve. However, a high non-specific precipitation of free testosterone (14%) oc-

Table 2. Effect of adding the antiserum at 4 and 22°C before subsequent incubation at 4°C, using charcoal adsorption for separating free and bound testosterone (n = 6)

Conditions*	(Mean ± S.D.), % bound
1. Antiserum added at 4°C	58·8 ± 1·7
2. Antiserum added at 22°C, then samples immediately cooled to 4°C	56·1 ± 4·0
3. Antiserum added at 22°C, samples left at 22°C for 30 min before being cooled to 4°C	53·2 ± 1·1
4. Same as 3 except left at 22°C for 90 min	52·6 ± 1·6

*Samples contained internal standard only.

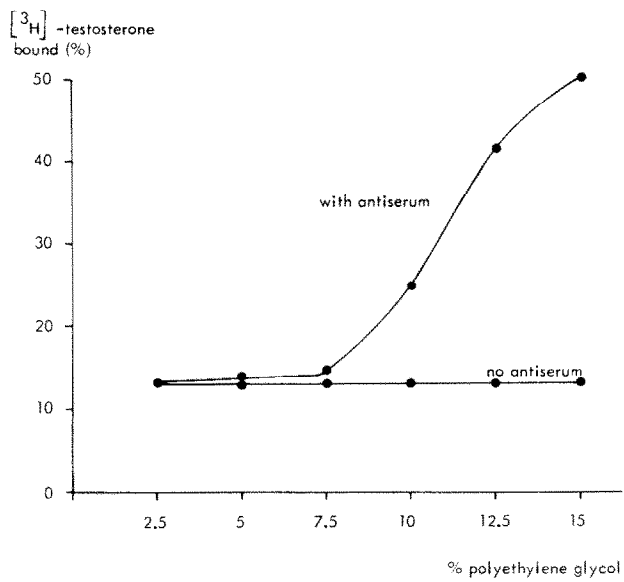

Fig. 2. Effect of varying concentration of polyethylene glycol for separating free and bound testosterone.

curred in the absence of antiserum. This non-specific precipitation could be reduced to 8–10% by washing the precipitates with ethanol. This method as well as the toluene scintillator extraction is, however, time consuming, and therefore the dextran-coated charcoal method to separate free and bound testosterone was found to be the most practical. This method was used throughout the rest of this study.

Accuracy

For plasma samples, recovery of [^{3}H]-testosterone after extraction and micro-column chromatography was 67 ± 8%. Recovery after extraction of rat testis tis-

sue samples was 92 ± 2%. Recovery of [^{3}H]-testosterone after incubation with antiserum was 90 ± 5%.

Accuracy was assessed by replicate assays including chromatography of known amounts of pure testosterone added to distilled water. Mean values and coefficients of variation are given in Table 3. In general, a good correlation exists between amounts of testosterone added and amounts found.

Table 3. Accuracy and precision of testosterone radioimmunoassay

pg testosterone added to water	n	pg testosterone found (mean)	S.D. (in pg)	Coefficient* of variation	% recovery
0	5	3	1	33·0	
50	5	47	4	8·5	94
100	5	96	8	8·3	96
150	5	152	8	5·3	101
200	5	206	35	17·0	103
300	5	260	35	13·5	87

*Coefficient of variation (%) = (S.D./mean) × 100

Precision

Table 4 gives results of multiple estimations of testosterone in plasma from six human males, performed on four different days. The coefficients of variation of the mean estimate on each individual day, which reflects the intra-assay precision, varied between 5 and 28%. The mean value and the standard deviation calculated from the mean values obtained on the different days may give an impression about possible inter-assay variations. In four samples this coefficient of variation was about 6% and it was 14 and 17% in the other two samples.

Table 5 gives results of multiple estimations in plasma from ten human females, performed on different days. The coefficients of variation in each assay are in general higher than with human male plasma, and vary between 0 and 75%. For duplicate determinations of testosterone in rat testis tissue within the range of 1000–3000 pg the standard deviation was 230 pg (n = 45)[21] as calculated according to De Jonge[19].

Specificity

Specificity of the radioimmunoassay depends on the specificity of the antiserum and the specificity of extraction and chromatography. The specificity of the antiserum was tested by comparing the ability of various steroids to displace [^{3}H]-testosterone from the antibody (see Fig. 3). Addition of 100 pg 5α-dihydrotestosterone, 1500 pg 4-androstene-3,17-dione or 650 pg 4-androstene-3β,17β-diol to [^{3}H]-testosterone labelled antiserum appeared to give the same displacement (50%) of radioactivity from initial binding as 50 pg testosterone, using toluene scintillator extraction for separation of free and bound steroid. After alumina micro-column chromatography, the percentage of 5α-dihydrotestosterone remaining in the testosterone fraction was only 3 ± 2 (S.D.)% (n = 7). Another factor influencing the specificity was the blank. The mean value of the blank was very low, varying from zero to four pg. Sometimes very high blanks were found due to unidentified impurities in some batches of ethanol and

Table 4. Variability of R.I.A. of testosterone in human male plasma and comparison with g.l.c. values

Plasma	Assay number	Number of replicates in each assay	Testosterone (μg/100 ml) Inter-assay mean (± S.D., coefficient of variation*)	Testosterone (μg/100 ml) Inter-assay mean (± S.D., coefficient of variation*)	g.l.c. value
M 1	1	6	0·50 ± 0·12 (24%)	0·49 ± 0·03 (6%)	0·48
	2	7	0·45 ± 0·02 (5%)		
	3	8	0·51 ± 0·06 (13%)		
	4	6	0·48 ± 0·07 (14%)		
M 2	1	7	0·75 ± 0·09 (12%)	0·73 ± 0·05 (7%)	1·19
	2	7	0·78 ± 0·10 (13%)		
	3	7	0·72 ± 0·09 (12%)		
	4	6	0·66 ± 0·05 (7%)		
M 3	1	7	0·72 ± 0·08 (10%)	0·69 ± 0·04 (6%)	0·78
	2	8	0·71 ± 0·09 (13%)		
	3	8	0·66 ± 0·07 (10%)		
	4	8	0·65 ± 0·05 (7%)		
M 4	1	6	0·44 ± 0·03 (7%)	0·44 ± 0·03 (7%)	0·50
	2	7	0·43 ± 0·06 (14%)		
	3	8	0·41 ± 0·05 (13%)		
	4	7	0·47 ± 0·04 (8%)		
M 5	1	7	0·58 ± 0·05 (9%)	0·68 ± 0·12 (17%)	0·59
	2	7	0·80 ± 0·10 (13%)		
	3	8	0·57 ± 0·06 (10%)		
	4	7	0·75 ± 0·03 (5%)		
M 6	1	3	0·49	0·41 ± 0·06 (15%)	0·35
	2	8	0·36 ± 0·10 (28%)		
	3	8	0·37 ± 0·07 (19%)		
	4	8	0·42 ± 0·06 (15%)		

*Coefficient of variation (%) = (S.D./mean) × 100.

ether. Even after repeated redistillation of those batches blanks remained high. In practice, specificity was evaluated by comparison of the plasma and tissue testosterone concentrations measured by the radioimmunoassay technique with results obtained by g.l.c. of testosterone using ^{63}Ni electron capture detection of testosterone chloroacetate[12]. For plasma and rat testis tissue samples there was a good correlation between radioimmunoassay and g.l.c. (see Figs. 4 and 5). For male plasmas a correlation coefficient of 0·87 (n = 92) was found, for female plasma it was 0·80 (n = 36). Rat testis tissue samples assayed by both radioimmunoassay and g.l.c. reflected also a good correlation 0·95 (n = 54.)

DISCUSSION

A method for the determination of testosterone, essentially similar to that of Furuyama *et al.*[1], has been evaluated. The reliability of this method was evaluated by the estimation of testosterone levels in peripheral human plasma and

Table 5. Variability of R.I.A. of testosterone in human female plasma and comparison with g.l.c. values

Plasma	Assay number	Number of replicates in each assay	Testosterone (μg/100 ml) Intra-assay mean (± S.D., ± coefficient of variation*)	g.l.c. value
F 1	1	6	0·12 ± 0·01 (8%)	0·13
	2	3	0·07	
	3	5	0·12 ± 0·05 (42%)	
F 2	1	5	0·06 ± 0·02 (33%)	0·05
	2	5	0·03 ± 0·004 (13%)	
F 3	1	6	0·08 ± 0·03 (38%)	0·03
	2	5	0·03 ± 0·01 (33%)	
	3	5	0·07 ± 0·02 (29%)	
F 4	1	5	0·08 ± 0·03 (38%)	0·06
	2	4	0·04 ± 0·03 (75%)	
F 5	1	5	0·10 ± 0·02 (20%)	0·16
	2	5	0·05 ± 0·02 (40%)	
	3	5	0·08 ± 0·01 (13%)	
F 6	1	4	0·03 ± 0·005 (17%)	0·02
	2	5	0·02 ± 0·004 (20%)	
F 7	1	5	0·03 ± 0·01 (33%)	0·03
	2	4	0·02 ± 0·005 (25%)	
F 8	1	5	0·04 ± 0·00	0·03
	2	4	0·03 ± 0·01 (33%)	
F 9	1	5	0·04 ± 0·015 (38%)	0·01
	2	6	0·03 ± 0·019 (63%)	
F 10	1	5	0·04 ± 0·01 (25%)	0·03
	2	4	0·03 ± 0·02 (67%)	

*Coefficient of variation (%) = (S.D./mean) × 100.

in rat testis tissue and by a comparison of results of radioimmunoassay and g.l.c. estimations. The method as applied to plasma and tissue in which duplicate samples were taken and each duplicate was in turn assayed at two levels, had a reasonable reliability.

Accuracy and precision of standard curves and samples were satisfactory. Normally blank values were low and were not subtracted. The high association constant of the antiserum ($5{\cdot}5 \times 10^9$ 1/mol, 4°C) provided a high sensitivity of detection. The reproducibility of the assay was 10–15%.

Non-specific interference from solvents, buffer solutions, chromatography and glassware have from time to time given serious problems with this method. Solvents were the main source of high blanks. Surprisingly the normal distillation plus chemical purification procedures have occasionally introduced high blank

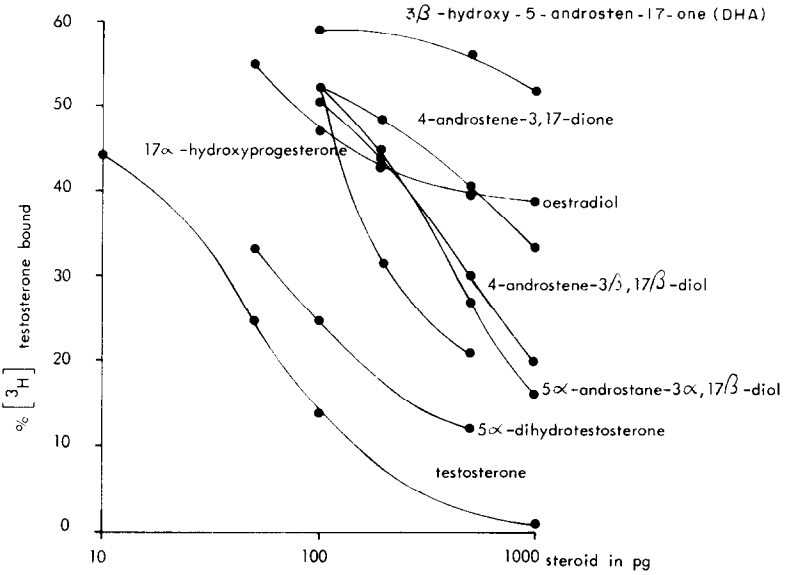

Fig. 3. Displacement of [^{3}H]-testosterone from antiserum (1:20,000) by various steroids, using toluene scintillator extraction to separate free and bound steroid.

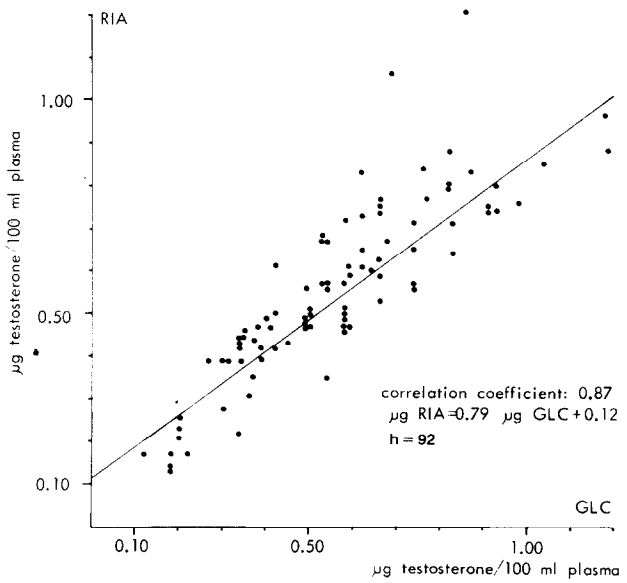

Fig. 4. Testosterone concentrations in human male plasma assayed by gas–liquid chromatography (g.l.c.) and radioimmunoassay (R.I.A.).

values, whereas relatively impure solvents (as judged by g.l.c.) direct from the manufacturer generally gave low blank values. The contribution of each solvent to the blank was therefore routinely determined before use and purity was considered satisfactory when blank values were below 5 pg using 500 μl of evaporated solvent. As a precaution the standards were subjected to the same extraction and

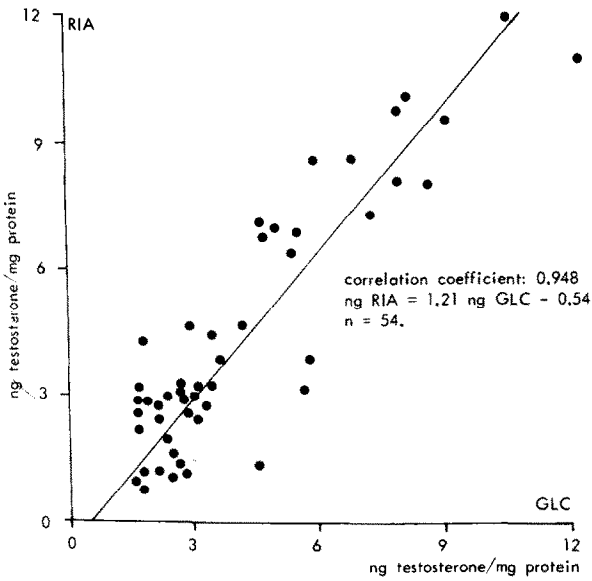

Fig. 5. Testosterone concentrations in testis tissue samples assayed by gas–liquid chromatography (g.l.c.) and radioimmunoassay (R.I.A.).

chromatography procedures as the unknown samples. The total recovery of [^{3}H]-testosterone after incubation with antiserum was measured because of variable recoveries from the incubation (85 to 95%). One technician can determine routinely fifteen plasma samples in duplicate or thirty tissue samples in duplicate in two days.

A good correlation between results of radioimmunoassay and g.l.c. was found for testosterone in both plasma and rat testis tissue samples. The latter samples were not chromatographed, this apparently was unnecessary. Dufau *et al.* [14] have obtained accurate results using a similar radioimmunoassay except that no organic extraction procedure was used. One of the main possible cross reacting steroids which is present in both plasma and testis tissue is 5α-dihydrotestosterone. The mean concentrations of this steroid in male and female plasma and in testis tissue are about 10% [15, 17], 45% [15, 17] and 13% [16] respectively of the mean concentrations of testosterone. Collins *et al.* [6] showed that chromatography on Sephadex LH20 gave lower testosterone values than without chromatography. Using the alumina micro-column chromatography procedure described, it is possible to get an almost complete separation of testosterone and 5α-dihydrotestosterone.

The comparison of results for testosterone concentrations obtained with radioimmunoassay and gas–liquid chromatography for the same samples was only meant to evaluate the reliability of *one* technique relative to the other. The sensitivity and practicability of radioimmunoassay offer definite advantages. In terms of accuracy and precision the two techniques are comparable. Specificity of the gas-chromatographic method, particularly with respect to the separation from 5α-dihydrotestosterone, is better than that of radioimmunoassay without chromatography.

The levels of testosterone estimated with the radioimmunoassay technique in

human male and female plasma are in agreement with those obtained with several other techniques[5, 22]. Although there is a good agreement between radioimmunoassay and g.l.c. results, the absolute values of the concentrations of testosterone found in rat testis tissue are difficult to compare with those found by others[14, 20, 21, 23], because tissue levels may vary depending upon the physiological state and method of isolation of the testis tissue.

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