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A specific RIA for testosterone (T) and dihydrotestosterone (DHT) was developed using a single celite column for separation. Antibody was raised in rabbits using a conjugate of human serum albumin linked to T *via* an oxime at the three position. In an initial dilution of 1:20,000, one antiserum bound 35% of tritiated T(T^{H3}). DHT cross-reacted to the extent of 60%. There was no significant binding by any other steroid tested. With this diluted antiserum a RIA was established using T^{H3} or DHT^{H3} as the labelled hormone to measure T and DHT respectively. After incubation at room temperature for 1 h and 4 °C for 15 min separation of free from bound hormone was accomplished by dextran charcoal suspension and centrifugation. The sensitivity of each RIA was 10 pg and water blanks were consistently below this level. Recovery losses were assessed by adding a small number of counts of both labelled steroids to serum samples. The ether extract from ·25 ml serum for adult males and 1·0 for females was dried, reconstituted with isooctane, and applied to a 5 cc celite column previously washed with isooctane. The DHT fraction was eluted with 7 ml of isooctane, the column rinsed with 3 ml of isooctane and the T fraction then eluted with 5 ml of 10% ethyl acetate in isooctane. The average recovery for both T and DHT was 85%. When known amounts of T and DHT were added to sera and assayed, the mean recovery for DHT was 102% ± 3·5(S.E.M.) of the expected value, and for T 111% ± 5·5. Interassay variation for DHT was 13·8%, and for T 10·2%. Intra-assay variation was 5·7% for DHT and 4·9% for T. Values for 17 young healthy male medical students were 555 ± 131 ng%(mean ± S.D.) for T and 63·1 ± 20·2 ng% for DHT. Reproductive age female levels were 25·7 ± 11·3 ng% for T and 14·1 ± 6·5 ng% for DHT.

41. The simultaneous determination of six C_{19} steroids in human peripheral plasma using a convenient radioimmunoassay technique

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A method is described for the determination of 5α-androstane-3,17-dione(androstanedione);4-androstene-3,17-dione (androstenedione), 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone); 3β-hydroxy-5-androsten-17-one (dehydroepiandrosterone); 17β-hydroxy-4-androsten-3-one (testosterone) and 5-androstene-3β,17β-diol (androstenediol) in peripheral venous plasma using a combination of solvent extraction and thin-layer chromatography after the addition of ^3H labelled internal standards. This is followed by radioimmunoassays developed specifically to measure each of the individual steroids. The concentration of these compounds are given for healthy subjects and patients with endocrine dysfunction. The method attempts to resolve some of the problems associated with clinical assays which only measure the plasma concentration of a single C_{19} steroid.

42. Standardization of specific radioimmunoassays for plasma estrone, estradiol, progesterone and androstenedione

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The wide variety of steroid-RIA's carried out in a clinical laboratory calls for standardization of all steps involved in the procedure. Specific RIA's for estrone (E_1), estradiol (E_2), progesterone (Prog) and androstenedione (A), that require only solvent extraction of plasma and no chromatography, were developed by following identical protocols from immunization schedules to processing of RIA raw data. E_1-6-CMO:BSA, E_2-6-oxo:BSA, Prog-11-0:BSA and A-6-ol hemisuccinate were used as antigens. Antisera of different titers were generated in rabbits 6–12 weeks after the first antigen injection and after one to three booster injections of 100 μg of antigen. Working solutions of antisera were made to achieve 50% binding under standard assay conditions (300 μl reaction volume, incubation 16 h at 4 °C, charcoal separation). Further characterization of the assays included factors such as cross reactivity of antisera, sensitivity, precision and accuracy. Antisera for E_1, E_2 and Prog did not cross react significantly with other steroids at their physiological concentrations. High specificity of the A-assay was obtained with petroleum ether extraction of the plasma samples. The raw data were processed by means of a computer program based on a logit/log transformation of the dose response curve and especially designed for the Hewlett–Packard 9810. Measuring ranges for the assays were as follows: E_1: 10–500 pg, E_2: 10–500 pg, Prog: 20–2000 pg, A: 20–1000 pg. These standardized RIA's have proved to be useful in processing large numbers of samples in studies concerned with ovarian function in normal and abnormal menstrual cycles.

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43. Simultaneous determination of 6-sex-steroids on a 2 ml plasma sample

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A R.I.A. method has been developed for the determination of progesterone (P), estradiol (E_2), dehydroepiandrosterone sulphate (DS), testosterone (T), androstenedione (Δ_4) and dihydrotestosterone (DHT) in a single 2 ml plasma sample. 10 μl of plasma is used for the direct determination of DS, using DS-17HS–BSA antibody (AB). To the rest of the sample, internal ^3H-standards (1000 d.p.m.) of E_2, Δ4, T and DHT are added and the sample is extracted with petrol-ether, yielding the P fraction. The plasma is subsequently extracted with ether, and the extract fractionated through a LH-20 microcolumn, yielding the estradiol fraction and a fraction containing T, Δ4 and DHT. The latter are separated by t.l.c. on silicagel (benzene-methanol 85:15 v/v). Steroids are measured by RIA using specific antibodies.

	Normal values (ng/ml)		*C.V.* (%)	*Recovery* (%)	*Sensitivity*
	Men	Women			
DS (μg/ml)	1·8–2·9	1·0–2·9	13	102 ± 9	10 pg
Progesterone	0·1–0·22	0·2–15	18(M)–10(F)	96 ± 12	10 pg
Estradiol	0·8–2	1·0–20	6	98 ± 7	10 pg
T	2·8–8·5	0·1–0·7	7	96 ± 8	10 pg
Δ4	0·6–2·1	0·6–3·1	8	102 ± 10	10 pg
DHT	0·3–1·4	0·1–0·4	14	108 ± 11	12 pg

The specificity of the methods and physiopathological factors influencing these levels will be discussed.

44. Radioimmunoassay of plasma 16α-hydroxyprogesterone in man

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Although the rate of adrenal production of 16α-hydroxyprogesterone (16-OHP) is augmented by enhanced endogenous ACTH secretion, information on plasma levels of this steroid in man is lacking. A method utilizing radioimmunoassay (RIA) for measuring 16-OHP in plasma has been developed. Antiserum to 16-OHP (anti-16 OHP) was obtained by immunization of rabbits with 16-OHP-3-(O-carboxymethyl)oxime conjugated to BSA. The anti-16-OHP has an association constant of $8{\cdot}9 \times 10^{11}$ L/m, and is highly specific. Steroids with greatest cross-reactivity are 16α-hydroxypregnenolone (4·7%) and progesterone (< 0·5%); others have < 0·1% cross-reactivity. The optimal dilution of anti-16-OHP for the standard curve is 1:10,000 and the logit transformation is linear from 50 to 1000 pg (corr. coefficient > 95% in serial assays). Plasma is extracted with ethyl acetate and 16-OHP is isolated after a single chromatography (t.l.c.). Recovery of labelled 16-OHP added to plasma following t.l.c. is 70·0 ± 17·2% (n = 205). When 500 pg of 16-OHP is added to water the total recovery is 470 ± 10·3 pg (n = 8). At 08·30, 5 normal males have a plasma level of 153 ± 72·3 ng/100 ml in the upright posture. In plasma re-assayed (× 4) 16-OHP is 49 ng/100 ml in an Addisonian, 1,117 ng/100 ml in early normal pregnancy, and 2345 ng/100 ml in cord blood. Such findings have stimulated inquiry into factors regulating blood concentrations of 16-OHP. (Supported by USPHS Grant AM 15809).

45. Radioimmunoassay of 11-deoxycortisol (compound S) in plasma

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Rabbits were immunized with a complex of 11-deoxycortisol-21-hemisuccinate and bovine serum albumin. The antiserum had a titer of 1:6000 and a low cross reactivity with cortisol (1·7%), corticosterone (0·06%) and 11-deoxycorticosterone (1·2%). In CCl_4 extracts of various plasma samples paper chromatographic analysis revealed that the area of tritriated 11-deoxycortisol corresponded to the area occupied by the radioimmune reaction. Therefore 11-deoxycortisol was measured after CCl_4 extraction without chromatographic separation. The sensitivity of the method is about 50 pg; no blank value was detectable. Intra-assay variation was ± 5·3% (n = 42) and interassay variation ± 8·8% (n = 30). The average plasma concentration of 11-deoxycortisol in adults (n = 24) was 0·147 ± 0·044 (SD) μg/100 ml (range 0·091–0·242). After the oral administration of 30 mg/kg metyrapone plasma concentration of 11-deoxycortisol rose to 8·99 ± 2·19 (SD) μg/100 ml (range 5·8–12·9). The reaction to metyrapone was depressed in 9 of 14 patients, from whom a pituitary adenoma had been removed. The method is sufficiently sensitive to measure low plasma concentrations in patients with adrenal insufficiency or ACTH suppression by dexamethasone. A radioimmunoassay with any antiserum against a 3-oxime derivate of 11-deoxycortisol (obtained from UCLA Clinical Laboratory, Los Angeles) gave similar results.

2B 3. Steroid radioimmunoassay—III

46. Steroid radioimmunoassays using micro-liquid scintillation counting

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The increasing volume of steroid radioimmunoassays suggests a role for an efficient and inexpensive ^{3}H-assay of steroids, e.g., in standard curve samples, extraction recovery step, and the free and bound fractions of a steroid. A micro-liquid scintillation counting (MLSC) procedure is described in which the usual 0·1 to 0·5 ml aliquot is counted with only 0·2 to 1·0 ml of 25% Triton X-114 in xylene gel scintillator (0·6% ppo + 0·1% popop w/v), in a small 5 ml glass vial or glass tube (17 × 55 mm), capped with polyethylene stopper and placed in an uncapped standard polyethylene vial used as a holder for MLSC. The scintillator-containing aqueous sample in 9 to 50% H_2O concentration will yield a ^{3}H-efficiency in the range of 33 to 20%, therefore 0·1 to 0·5 ml aliquots of assay buffer with twice its volume of scintillator (33% H_2O conc.) are counted with a high ^{3}H-efficiency of 26%. The background counts are low to 25 c.p.m. Furthermore, by keeping a fixed ratio between sample and scintillator, a constant efficiency can be maintained for a wide range of sample volumes. This gel mixture gives higher efficiency for a broad range of acqueous volumes than the commercial or other gel scintillators. The mixture is prepared for less than $4·0/l. The steroid radioimmunoassays are carried out quite inexpensively through MLSC with the advantages: (1) lower costs in view of rising prices and shortage of organic solvents like toluene or xylene, (2) high efficiency and low background maintained, (3) cost and material economy by the use of small vials and micro-volume of scintillator, and (4) reduced problems of storage, transportation, disposal and environment pollution.

47. A new radioimmunological technique for the assay of synthetic steroid hormones

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Radioimmunoassay (RIA) techniques utilize as tracer either a tritiated steroid or a radioiodinated derivative. Recently, direct radioiodination (RI) of the steroid moiety has been attempted for compounds possessing an aromatic ring. However, by substituting hydrogens with atoms whose molecular weight is approximately half of the entire steroid molecule, the resulting tracer loses most of its immunoreactive characteristic features. The present report outlines a new direct RI technique, that allows the preparation of tracers possessing both a high specific activity and an unaltered immunospecificity and can be applied to steroids possessing unsaturated lateral chains. Conditions for RI were studied in detail using norethisterone (NET). Three nmol of the steroid were labelled with 1 mCi of ^{125}I in the presence of 10 nmol of H_2O_2, using acetic acid as solvent. The reaction was carried out in a final volume of 225 μl during 2 h, using a sealed vial heated at 100°C. Separation of reaction products was obtained by submitting the reaction mixture directly to bidimensional t.l.c. in system I (benzene/