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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