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/