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/

acetone 92·5:7·5). Six major radioactive spots were separated; the immunoreactive compound isolated in this way was submitted to t.l.c. in systems 1 and 2 (chloroform/ethyl acetate 95:5) where it behaved as a single peak. (Chromatographic controls demonstrated no chemical damage or loss of activity after 30 days.) The new tracer was compared in a RIA with both the tritiated and tyrosine-iodinated (TI) antigen. The standard inhibition curve obtained with the new tracer can almost be superimposed on the curve obtained using ^{3}H-NET. This suggests that the immunoreactive portion of the molecule maintained its characteristics. The sensitivity of the new method is 3 pg; this is approximately 5 times that reached with the tritiated antigen and in the same range of that obtained with the TI antigen.

48. Measurement of anabolic steroids by radioimmunoassay

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The aim of this study was to develop a radioimmunoassay capable of detecting anabolic steroids in blood and urine. Antiserum was raised in rabbits against 17α-methyltestosterone linked *via* the 3-oxo group to human serum albumin. The specificity was in theory thus directed towards the structural features of the steroid D ring (i.e. 17α-methyl, 17β-hydroxy). Many of the anabolic steroids have this common feature (Dianabol, Stanozolol, Oxymetholone etc.). This specificity was found to be such that anabolic steroids with a 17α-methyl group did crossreact, whereas those with a 17α-ethyl group did not. However, testosterone, the natural male hormone, crossreacted to the extent of 5·0% which was unacceptably high. Testosterone acetate did not crossreact at all and this fact was used to eliminate the effect of testosterone in the assay by conversion to the 17-acetate. Organic solvent extracts of blood or urine were therefore acetylated before assay. The 17α-alkylated anabolic steroids are not acetylated and remain unchanged. A coated tube (solid phase) radioimmunoassay was developed using a steroid-^{125}I-histamine conjugate as label. Using this "group specific" antiserum, anabolic steroids with a 17α-methyl group have been readily detected in both blood and urine samples from healthy males after an oral dose of the steroid. (This work was supported by a grant from the British Sports Council).

49. Antisera for radioimmunoassay of mestranol and ethynylestradiol

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Antisera for the synthetic estrogens, mestranol and ethynylestradiol, have been prepared by immunizing rabbits with 6-(0-carboxymethyl) oxime-bovine serum albumin conjugates prepared from 6-oxo-mestranol and 6-oxo-17α-ethynylestradiol, respectively. The antisera for mestranol showed a titer of 50% binding of 50 pg of mestranol-9α, 11ξ-^{3}H) at a dilution of 1:5,000 and the antisera for 17α-ethynylestradiol-(9α, 11ξ-^{3}H) at a dilution of 1:25,000. Individual rabbit sera vary greatly in their spectrum of sensitivity and cross-reactivity between mestranol and EE and other known metabolites of these steroids. These sera are compared to antisera obtained by others which have been coupled at the C-7 position.

50. The use of ^{125}I labelled tracers for the radioimmunoassay of oestradiol-17β and norethindrone

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Previous reports on iodinated tracers for steroid radioimmunoassay have described the conjugation of the steroid to a protein or to a tyrosyl methyl ester followed by radioiodination of the steroid conjugates. In steroids with phenolic ring A the introduction of iodine into the A ring has been shown to destroy immunoreactivity. In the present study this has been avoided by first iodinating tyrosine or histamine which was then conjugated to the steroid. Oestradiol-17β-6-(0-carboxymethyl)oxime was conjugated to ^{125}I-tyramine by the mixed anhydride method. A maximum specific activity of 6·2 mCi/μg oestradiol-17β-6-(0-carboxymethyl)oxime was obtained. The high specific activity enabled a dilution of 1 ml of the antiserum to oestradiol-17β-6-(0-carboxymethyl)oxime-bovine serum albumin of 1:12,000,000 sufficient for 120,000,000 assay units. When using 2,4,6,7-^{3}H oestradiol-17β as tracer this antiserum was used in a dilution of 1:100,000. The high dilution of the antiserum in combination with the iodinated tracer made possible the use of radioimmunoassay systems detecting levels below 1 pg of oestradiol-17β. Norethindrone-3-(0-carboxymethyl)oxime was conjugated to ^{125}I-histamine by the mixed anhydride method. Specific activities of about 1 mCi/μg was obtained. This tracer was used in a radioimmunoassay system with an antiserum to norethindrone-3-(0-carboxymethyl)oxime-bovine serum albumin. The antiserum was diluted 1:50,000 in combination with the iodinated tracer. Five pg of norethindrone was found to give a reduction of 15% of bound counts (O pg = 100%). When using 6,7-^{3}H norethindrone as tracer this antiserum could only be diluted 1:500 and 250 pg of norethindrone could be read off the standard curve as significantly different from zero. ^{125}I- as compared to ^{3}H-tracers resulted in assay systems with increased sensitivity and specificity.