4-androstene-3,17-dione by the adrenal. We have therefore developed a method and obtained a highly specific antiserum for use in measuring 11β-hydroxy-4-androstene-3,17-dione by a solid-phase radioimmunoassay procedure. This antiserum was generated in rabbits using the hapten, 6β,11β-dihydroxy-4-androstene-3, 17-dione 6-hemisuccinate coupled to bovine serum albumin. The antiserum showed a titer of 68% binding of 50 pg of 11β-hydroxy-4-androstene-3,17-dione-[1,2,6,7-^{3}H] at a dilution of 1:12500 in the assay. Among the numerous steroids tested for cross-reactivity, only 4-androstene-3,17-dione and 5α-androstene-3, 17-dione showed 5% and 2% cross-reactivity respectively. All other structurally related steroids, including C_{21} compounds, showed no detectable cross-reaction. The linearity of the Scatchard plot indicated that the antibody was essentially homogeneous with respect to its binding of 11β-hydroxy-4-androstene-3,17-dione, with a K_a of $9{\cdot}7 \times 10^8$. The Rivanol-treated antiserum was coupled to Enzacryl AA, a synthetic polymer and the complex so obtained showed 50% binding with the labelled antigen. Determination of cross-reactivities employing this complex proved it retained its high specificity and can readily be adopted for a simple solid-phase RIA.

36. Preparation of antigenic conjugates of 3-oxosteroids by coupling to a macromolecule through position—1

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A general method for rendering 3-oxosteroids antigenic by coupling to a macromolecule through position 1 has been developed as part of a program aimed at generating antibodies able to discriminate between closely related steroid hormones and their metabolites. Nucleophilic attack on the 1,2-dehydro derivatives of ring A saturated 3-oxo or Δ^4-3-oxosteroids by ambidendate reagents gave the corresponding 1α-thioether alkanoic acids. Thus addition products with β-mercaptopropionic acid were obtained from 5α-dihydrotestosterone, testosterone, progesterone and androstenedione. These were covalently attached to either thyroglobulin or bovine serum albumin (BSA). Immunization of rabbits with testosterone-1α-carboxyethyl-thioether-thyroglobulin gave rise to antisera of high affinity to testosterone that showed minimal cross-reaction with 5α-dihydrotestosterone (3%), androstenedione (< 0·1%) and with a variety of 17-oxo-androstane compounds (< 0·1%). Conversely, immunization with 5α-dihydrotestosterone-1α-carboxyethyl-thioether-BSA yielded an antiserum with high affinity for 5α-dihydrotestosterone but little cross-reaction for testosterone (10–15%) and androstenedione (< 0·5%).

37. Some studies of the specificity of antisera to C_{18} and C_{19} steroid–BSA conjugates

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Antisera for use in radioimmunoassay techniques have been developed for five C_{19} and three C_{18} steroids involving exposure of alternative regions of the steroid moiety as immunogenic determinants. The ability of related steroids and other compounds to interfere with steroid-antibody binding has been investigated. Antigens prepared for this study were:—17β-hydroxy-5α-androstan-3-(O-carboxymethyl) oxime–BSA 4-androstene-3,17-dione-11α-hemisuccinate–BSA 4-androstene-3,17-dione-6β-hemisuccinate–BSA 17β-hydroxy-4-androsten-3-one, 11α-hemisuccinate–BSA 3β-hydroxy-5-androsten-17(O-carboxymethyl) oxime–BSA 1,3,5(10)-estratrien-3,16α,17β-triol-6(O-carboxymethyl) oxime–BSA 1,3,5(10)-estratrien-3,17β-diol-6(O-carboxy-methyl)oxime–BSA 1,3,5(10)-estratrien-3-ol, 17-one-6(O-carboxymethyl)oxime–BSA.

38. A high affinity testosterone antibody gives lower female testosterone values

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Antisera to testosterone reported, thus far, have an affinity of $1{-}2 \times 10^9$ L/M. We have generated an antibody to testosterone-3 conjugate which has an affinity of 2×10^{10} L/M using a conjugate containing 38 residues per albumin. This serum is of higher specificity as well (DHT = 25%, 4-Adione = 0·3%, 4-Adiol = 3·2%, F = 0·001%, Prog = 0·06%). Assay of hexane extracts directly or after alumina column chromatography gave the mean ± SD (Range) 28·4 ± 14·5 (5·6–56·2)ng% and 14·6 ± 9·0 (3·1–33·9)ng%, respectively, for 19 normal young women and 401·4 ± 174·7 (192–808)ng% and 378·1 ± 167·9 (181–795)ng%, respectively, for 14 normal men. A comparison of 10^9 L/M antisera with 10^{10} L/M antisera after alumina chromatography gave 33·6 ± 19·5 ng% vs 15·8 ± 6·5 ng% for 6 normal women. Values by the 10^9 antiserum compare with other methods. Therefore, the difference lies in the more specific antiserum rather than the methodology.

39. The use of steroid coupled bacteriophage in the steroid field

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Estradiol-17β and progesterone have been covalently conjugated to bacteriophage T4. The bacteriophages surviving the coupling process were able to form plaques on Petri dishes when mixed with their host *E. coli.* B. These "steroidated" phages could be neutralized at 0° or 37° according to pseudomolecular first order kinetics when incubated in very dilute anti-steroid sera. Preincubation with increasing amounts of free steroids resulted in a decrease of the neutralization which allowed us to construct standard curves for these two steroids and the related compounds. The average equilibrium constants of the antisera against free steroids could be directly calculated from the standard curves. We compared this "viroimmunoassay (VIA) with radioimmunoassay (RIA) for sensitivity in the antisera titer and amount of steroid detected. VIA could detect antisera concentrations 100 times lower than RIA. In addition it could quantify with good reliability 1–2 pg of estradiol or progesterone (RIA could only detect 5–10 pg). Lastly the average equilibrium constants of the antisera against the immunogenic steroids and others have been found to be the same by dialysis equilibrium and VIA. We used this VIA to assay progesterone and estradiol levels directly in diluted plasma of pregnant women. The values found in these conditions were in good agreement with those determined after extraction by RIA.

2B 2. Steroid radioimmunoassay—II

40. One column chromatography and simultaneous radioimmunoassay of testosterone and dihydrotestosterone

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