levels relatively considerably lower in the same extracts. The same steroids have also been assayed in tissue from males with gynaecomastia and with carcinoma of the breast. The endogenous steroid levels in the tumours have been related to other biochemical parameters such as "oestradiol-17β receptor" concentration.

31. Assay of hormonal steroids by gas chromatography–mass spectrometry using stable isotope internal standards

VESTERGAARD, P. and SAYEGH, J. F., Research Center, Rockland State Hospital, Orangeburg, N.Y. 10962, U.S.A.

The mass spectrometer with a multiple ion detector can be used as a very sensitive detector in the gas chromatographic estimation of hormonal steroids. The intermittent focussing in the instrument on selected ions gives high specificity to the assay. The ideal internal standards for this type of assay are hormonal steroids labelled with stable isotopes. They allow correction for any losses in the initial procedures preceding the gas chromatography. Gas chromatography/mass spectrometry with stable isotope labelled internal standards produced by deuteration of appropriate steroids has been applied to the assay of hormonal steroids in body fluids. Practical assays for testosterone and estrogens in body fluids will be compared with radioimmunoassay methods for the same compounds.

2B. Steroid radioimmunoassay—I

32. Synthesis of 11α-C_{19}-steroid–protein conjugates for new RIA antisera

HALLER, W. S., BESCH, P. K. and BUTTRAM, V. C., Reproductive Research Laboratory, Baylor College of Medicine, Houston, Texas 77025 U.S.A.

The synthesis of specific steroid antigens for use in the development of radioimmunoassay (RIA) systems has been the subject of much recent investigation by several authors. Many different sites of conjugation of the steroid to protein have been tried, including the 3, 17, 6, 7 and 11 positions. The most specific steroid derivatives yet found utilize an 11α-hydroxyl group for conjugation to protein. Only in the case of progesterone is the 11α-hydroxy compound readily available. We have succeeded in synthesizing 11α-hydroxy-androstenedione from adrenosterone which is commercially available at low cost. The simple two step method may be run in any laboratory and gives a good yield of the 11α-hydroxy compound without contamination by the 11β-hydroxy isomer. Additionally, routes will be presented to give rise to other 11α-hydroxy androgens, such as, testosterone, dihydrotestosterone and androstanediol for use in developing new RIA systems. (Supported in part by The Robert A. Welch Foundation, Q-560 and St. Luke's Episcopal Hospital).

33. Antisera specific to corticosterone

VETTER, W., VETTER, H., DIETHELM, U., ARMBRUSTER, H. and SIEGENTHALER, W., Department of Internal Medicine, University of Zürich, Switzerland

Five rabbits were immunized with a corticosterone-3-oxime bovine serum albumin (BSA) conjugate carrying 6 steroid molecules. Antibody sensitivity and specificity were tested 10–14 weeks after first immunization. Antibody dilutions that bound 50% of ^{3}H-corticosterone were between 500 and 1000. All antisera showed similar high sensitivity as indicated by the slopes of the standard curves. Three of the 5 sera showed highest cross-reaction with cortisol (70, 43, 26%) and low cross-reaction with deoxycorticosterone (DOC) (7, 5, 5%) and progesterone (3, 4, 5%). With these sera alterations in the chemical and steric configuration at C-11 resulted in a marked decrease in cross-reaction as indicated by displacement experiments with 11-deoxycortisol (0·9, 0·8, 0·7%), cortisone (5, 0·1, 0·06%) and 11-epi-cortisol (0·03, 0·02, 0·02%). Two of the 5 sera showed highest cross-reaction with progesterone (94, 67%) and DOC (82, 88%) and low cross-reaction with cortisol (6, 8%). With these sera, changes in the chemical configuration at C-17 resulted in a decrease in cross-reactivity as indicated by displacement experiments with 17α-OH-progesterone (8,2%). Our results indicate that with corticosterone-3-oxime BSA two different antibody populations can be raised in rabbits: (a) one predominantly directed against β-C-11, the other predominantly against α-C-17. Coupling through the double bond at C-3 preventing rotation of the hapten might have favoured these findings.

34. Specific antisera for estriol-16α-glucosiduronate

WRIGHT, K., COLLINS, D. C. and PREEDY, J. R. K., Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, 30303, U.S.A.

Estrogen conjugates in plasma and urine have usually been determined indirectly after hydrolysis, followed by assay of the free estrogen. Such methods often do not distinguish between individual conjugates. We have developed an antiserum to estriol-16α-glucosiduronate for use in a direct radioimmunoassay of this conjugate. Estriol-16α-glucosiduronate-bovine serum albumin was prepared by coupling the carboxylic acid group of the glucosiduronate to ε-amino groups of lyside residues in bovine serum albumin by the mixed anhydride procedure. The protein conjugate was injected intradermally into rabbits with Freund's adjuvant. At a dilution of 1:2000 the antiserum gave a useful standard curve over the range of 10 pg to 200 pg. The antiserum cross-reacted 4% with estriol-17β-glucosiduronate, < 1% with estriol-3-glucosiduronate, 2% with estradiol-17β-17-glucosiduronate, and 1% with estrone glucosiduronate. As expected, the antiserum showed significant cross reactions with certain free estrogens: 16-epiestriol (12%), estriol (22%), estrone (32%), and estradiol-17β (20%). Thus preliminary routine separation of free estrogens is necessary before radioimmunoassay.

35. Highly specific antisera for solid-phase radioimmunoassay of 11β-hydroxy-4-androstene-3,17-dione

RAO, P. N. and MOORE, P. H., JR., Southwest Foundation for Research and Education, San Antonio, Texas 78284 U.S.A.

The contribution of the adrenal cortex to the androgenic-anabolic interrelationship in the body has not been fully explored. The secretion of androgens by the adrenal, their change in response to environmental stress, and their effect on other organs like the testes are significant features of such concerns. From what is known of the production of C_{19}-steroids by the adrenal and from the analysis of the urinary metabolites of these compounds, it is evident that they are principally 11β-hydroxy secretory products. With a conversion of over 90% of preformed 4-androstene-3,17-dione to its 11β-hydroxy derivative within the adrenal, a measurement of the plasma levels of the latter compound should serve as a rational index of the biosynthesis of