

without any expensive antisera, radioactive steroids or equipment. (Supported by the Ford Foundation, New York.)

25. Progesterone receptor assays in human endometrium

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Precipitation of progesterone receptor (PR) by polyethylene glycol (PEG) provides a simple assay. Soluble tissue preparations and nuclear extracts, equilibrated with labelled steroid, are treated with PEG (final concentration 15%) after addition of γ -globulin. "Blank" precipitation of free steroid was $10.0 \pm 1.6\%$ (mean \pm S.D., $n = 40$). The cytoplasmic and nuclear PR from human endometrium, as determined by this method, were saturable, heat labile (40°C, 30 min) and not dissociated from progesterone (P) during PEG precipitation. For a given tissue, the proportion of labelled P bound to a nonsaturable, heat stable ('non-specific') component decreased as the concentration of labelled steroid was increased from 0.29 to 23 nM. Cortisol was found to increase or decrease non-receptor binding of P, the effect varying between tissues and with P and cortisol concentrations. Glycerol (10%), present during equilibration only, did not increase the affinity of the PR for P. This method is capable of high precision, obviates the use of synthetic P analogues and may find application in studying the properties of PR in relation to its natural hormone.

26. A simple method for separation of viable testicular cells

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The role of steroids on spermatogenesis has been a problem for several decades. This is due to the fact that testicular steroid estimation in each cell type could not be performed due to non-availability of a method for isolating the testicular cells suitable for biochemical study. A technique has been described here in which biochemical estimation of steroids could be performed by which the steroid contents and their role in testis in different experimental conditions could be studied. In this method, testis is minced in a buffer medium. After vigorous shaking the supernatant containing spermatogenic cells is removed and treated with hypotonic KCl, first with 0.075 M to isolate spermatocytes and then with 0.06 M to separate and isolate spermatogonia and spermatids. Leydig and peritubular cells from pellet are separated one after another by treatment with collagenase and pancreatin, respectively. The residue is then passed through a syringe needle to break the tubular fragments. The cell suspension is shaken and layered over a 4% sucrose gradient at 4°C to separate Sertoli cells from other cells (spermatogonia and spermatocytes).

Approximately 5–10% contamination is found in different fractions. All the cells are found to be 90% viable by their ability to exclude trypan blue.

27. Patterns of labelling in [3 H]-steroids by tritium nuclear magnetic resonance spectroscopy

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Tritiated hormonal steroids are widely used in biological research. Tritium nuclear magnetic resonance spectroscopy provides a convenient, reliable and non-destructive method of analysis, giving a direct quantitative measurement of both the positions and stereochemistry of tritium labelling in [3 H]-steroids. Conventional methods using chemical and biochemical procedures to obtain such data are usually very time consuming and tedious. Such methods are also often prone to errors due to non-specific hydrogen-tritium isotope exchange and from the lack of specificity of the reactions used. Examples are given which illustrate the importance of knowing the patterns of labelling in tritiated androgens, oestrogens and corticoids for biological research (aromatase, dehydrogenase and protein (receptor) binding studies).

28. Determination of sex hormone binding capacity using a rapid twin-gel micro-column

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A new method has been developed to study steroid-protein interactions in human plasma. In this instance the method is applied to the determination of 5 α -dihydrotestosterone binding non-specifically to human serum albumin and specifically to sex hormone binding globulin (SHBG). The method presented uses a twin-gel micro-column incorporating an upper layer of Cibacron Blue F3G-A-Sepharose 4B for the affinity removal of HSA and the steroid bound to it, above Sephadex LH-20 to retard the free steroid. Thus after washing of incubate aliquots through the column the emergent fluid contains the specifically bound steroid. The method is evaluated and compared with equilibrium dialysis, ammonium sulphate and charcoal separation procedures, and SHBG binding capacity data is presented for—(a) ovulatory and non-ovulatory cycles in normal female volunteers, (b) diurnal variations in male and female volunteers, (c) post-menopause women before and during oestrogen therapy regimes, and correlated with testosterone and androstenedione levels determined by radioimmunoassay.

3. BIOSYNTHESIS

29. Androgen production *in vitro* by hamster granulosa cells and theca

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Comparative ability of granulosa cells and theca from hamster follicles to produce androstenedione (A), testosterone (T) and dihydrotestosterone (DHT) from endogenous

and exogenous substrate was assessed in 2 h incubations. From endogenous substrate, theca accumulated A(0.41 ng), T(0.11 ng) with DHT non-detectable. LH increased thecal A(1.7 ng), T(0.75 ng) and DHT(0.63 ng). Granulosa cells, \pm LH or FSH, did not accumulate androgens. Only theca converted added progesterone to A, T and DHT, with LH increasing conversion. Similarly, only theca converted added 17 α -OH progesterone to A, T and DHT, with a slight LH effect. With A as added substrate, granulosa cells accumulated 10 \times more T than did theca. FSH increased