phate fractions was dehydroepiandrosterone, occurring in much greater amounts in the conjugated fraction. The TBDMS ethers showed distinct advantages for the detection of trace steroids, e.g. testosterone using mass chromatography and mass fragmentography. The results are in agreement with those previously obtained by others using radioimmunoassays.

21. Enzymic determination of plasma and urine oestrogens

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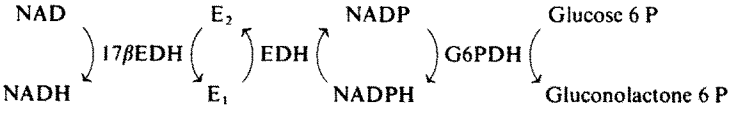
The method uses the transhydrogenase function of the 17β oestradiol dehydrogenase (EDH). We have determined the conditions to obtain a direct relationship between transhydrogenase activity and oestrogen concentrations. The NADPH is produced from a small amount of NADP by the system glucose 6 phosphate + glucose 6 phosphate dehydrogenase (G6 PDH). Hydrogen of NADPH is transfered to an excess of NAD by the oestradiol dehydrogenase free of endogenous oestrogens.



This method is specific for oestrone (E_1) and oestradiol (E_2) which can be determined together or separately if oestrone is previously reacted with hydrazine. In the plasma, this method allows the determination of 10 pg of oestrogen per tube. It seems to be more advantageous than radioimmunologic and immunoenzymatic methods. In urine the determination is performed without extraction on 10 to 25 μl of hydrolyzed urine. This specific and handy method presents advantages on the conventional technics of urinary oestrogens determination.

22. The influence of plasma-extract on the separation of antibody bound and unbound oestrogens by dextran coated charcoal (DCC)

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In radioimmunoassay, the adsorption of the unbound ligand to dextran coated charcoal (DCC) depends on the incubation time in the presence of DCC and on the amount of plasma-extract. Indeed, the latter decreases the effectiveness of DCC to adsorb the unbound ligand. As a result, the presence of excess radioactivity in the supernatant involves an overestimation of the apparent antibody-bound fraction of the tracer and an important underestimation of the amount of oestrogens in the extract. The fact is evidenced by a non-linear relationship between the plasma volumes extracted and the estrogen values. Also, the recovery of added steroid decreases significantly with increasing amounts of plasma-extract. Increasing the concentration of DCC reduces the underestimation of steroid present in the extract; one thus obtains a linear relationship between plasma volumes and amount of oestrogen measured, comparable to the results observed with the more elaborate and time consuming chromatographic method. However, the incubation time with DCC is important and the dissociation velocity of the steroid antibody complex becomes critical. Several examples of the above mentioned aspects will be shown. The nature of the antibody is important, and in routine analysis, the use of different "specific" kits implies the necessity of individual adaptation in so far as DCC concentration and incubation time are concerned.

23. A direct magnetic solid-phase radioimmunoassay for plasma aldosterone

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A simple and direct radioimmunoassay for plasma aldosterone which can be easily automated is described. The assay uses a highly specific aldosterone antiserum coupled covalently to a magnetic cellulose solid-phase and ^{125}I-labelled aldosterone ligands. Aldosterone antisera were produced in sheep. The magnetic cellulose solid-phase antibodies and various ^{125}I-labelled aldosterone ligands were prepared using modifications of previously described methods (aldosterone-3-mono-oxime) [^{125}I]-iodohistamine, aldosterone-3-(*p*-hydroxybenzoyl)hydrazone-[^{125}I], and aldosterone-3-(*p*-hydroxyphenylpropionyl) hydrazone-[^{125}I]). The assay was carried out by adding a 100 μl aliquot of plasma or aldosterone standard to a 100 μl of solid-phase antibody and 10,000 c.p.m. of [^{125}I]-aldosterone ligand in 100 μl phosphate buffer: the tubes were mixed and incubated at room temperature for 4h, placed on a permanent magnet to separate the antibody-bound from free fraction and the supernatant aspirated. The bound fraction was counted. The solid-phase assay was slightly less sensitive but had greater specificity than the liquid-phase system. The sensitivity of the assay was 10 pg/ml with zero blank values. The direct solid-phase radioimmunoassay was evaluated by comparing results with those obtained by a previously validated direct assay using liquid-phase antiserum. This radioimmunoassay for plasma aldosterone is easy to perform, rapid, cheap and uses magnetic solid-phase antiserum which has the major advantage over the liquid form of ease of separation of antibody-bound from free steroid. The use of magnetic solid-phase particles obviates the need for centrifugation. The assay described here and the reagents produced now form the basis of a fully automated plasma aldosterone radioimmunoassay.

24. New analytical methods for steroids, including some comparisons of methods with regard to specificity

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With the aim of carrying out large-scale clinical metabolic studies on estrogens, radioimmunoassay (RIA) methods for urinary estrone, estradiol, estriol, estriol-16α-glucuronide, estriol-3-glucuronide and a mass fragmentographic procedure for a number of estrogens in urine were developed. In addition the first analyses of estrogens in faeces of men, and non-pregnant women during the menstrual cycle have been carried out. With these methods it has been possible to study the influence of diet and drugs on estrogen metabolism and the physiology of the menstrual cycle in detail. Further work on enzymatic fluorometric procedures has resulted in the first method for a synthetic steroid, medroxyprogesterone acetate (MPA). The method can detect 3×10^{-13} mol of standard. Comparisons with a "specific" RIA of MPA revealed that the new method gives almost 50% lower values, which were in the same range as those obtained by mass fragmentography. Thus the use of specific steroid enzymes (in this case 3α,20β-hydroxysteroid dehydrogenase) combined with adequate purification procedures can yield highly specific and sensitive methods

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 ± 1.6% (mean ± 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 spermatogenia 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 5α-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, ± 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 17α-OH progesterone to A, T and DHT, with a slight LH effect. With A as added substrate, granulosa cells accumulated 10× more T than did theca. FSH increased