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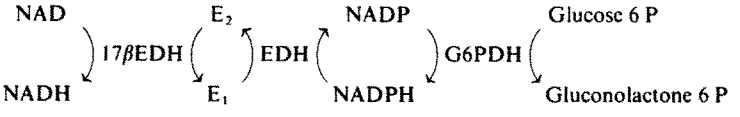
The method uses the transhydrogenase function of the 17β oestradiol dehydrogenase (EDH). We have determined the conditions to obtain a direct relationship between transhydrogenase activity and oestrogen concentrations. The NADPH is produced from a small amount of NADP by the system glucose 6 phosphate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