occurring in significant amounts in urine of patients with hypertension and/or hypokalaemia. The suggested systematic name of the steroid is 11β,20α,21-trihydroxy-3-oxo-4-pregnen-18-al (20-reduced aldosterone).

NEWER TECHNIQUES—II. IMMUNOENZYMOLOGY

Immunoenzymology: enzyme-immunoassay for steroids, EDWARD DAWSON and ANNEKE BOSCH, Biochemical R. & D. Laboratories, Organon, Scientific Development Group, Oss, The Netherlands

Enzyme-immunoassay (EIA) is an assay technique in which an enzyme bound to an immunochemically reactive compound is used as a reagent in the detection of haptens, antigens or immunoglobulins. The choice of enzyme is of great importance since, among others, it influences the detection limit of the assay. In steroid assays the enzyme is covalently linked to a steroid molecule via a bridge. For this conjugation a number of methods are available. Thorough purification of the conjugate is often necessary. The site of attachment, the nature of the bridge as well as the steroid to be labelled, must be carefully selected in conjunction with the antiserum, since the combination of antiserum and steroid conjugate may influence both sensitivity and specificity of the assay. Special attention must be paid to the assay design in general as well as to the way of assessment of the specificity. Since the inception of EIA in 1971, a number of steroid assays based on this technique have been developed. Because sensitivities of EIA's for steroids are comparable to those of already existing radio-immunoassays (RIA) and EIA has some practical advantages, this technique must be regarded as an attractive alternative to RIA.

NEWER TECHNIQUES—III. AFFINITY CHROMATOGRAPHY AND VARIAN STERIODS

New biospecific adsorbents for the purification of estradiol receptor, HÉLÈNE RICHARD-FOY et GÉRARD REDEUILH, Unité 33, INSERM, Hôspital Bicêtre, 78 Rue du Général Leclere, 94 270 Bicêtre, France

The properties of several new biospecific adsorbents obtained by coupling estradiol to agarose and acrylamide were compared for the purification of estradiol receptor from calf uterus. A new method was developed allowing the calculation of the maximal binding capacity of an adsorbent from a linear plot. The binding capacities of the different adsorbents obtained by coupling estradiol 7α to agarose were compared by using this method. It was observed that: (1) The adsorbents are stable in water at 0°C for at least 1 year and in presence of cytosol. After a wash by bovine serum albumin and guanidine chloride their binding properties are not modified. (2) A good biospecific elution recovery was only possible with biospecific adsorbents containing "low amounts" of coupled steroid (<0.03 mg derivative/ml gel). (3) The geometry of the column or the dynamics of the loading have no influence on the binding capacity of the absorbents. (4) The biospecific adsorbents described here are ion exchanger resins. The measurements of the amount of proteins eluted during a low ionic strength wash at 30°C, prior to the biospecific elution, can be used as an index of the ion exchange capacity. (5) The different molecular forms of the receptor do not bind to biospecific adsorbents to the same extent. The binding capacities for the different molecular forms of the receptor were: "4*S*-trypsin" > "4*S*–5*S*-KCl" > "8*S*". The presence of aggregated receptor close to the "4*S*-trypsin" receptor form decreased the receptor binding capacity of the biospecific adsorbents.

The use of affinity chromatography after conventional purification steps (purification = 25 fold) led to a 27,000 fold purification of the receptor, corresponding to 60% pure protein on the basis of 1 estradiol binding site per 60,000 Dalton unit.

Purification of the native form of the estradiol receptor by affinity chromatography, GIOVANNI ALFREDO PUCA, Istituto di Patologia generale, I Facoltà di Medicina e Chirurgia, Università degli Studi di Napoli, Via S. Andrea delle Dame, 2, 80138 Napoli, Italy

The property of the "native" estradiol receptor to form large and irreversible aggregates has until now impeded all attempts at its purification. We describe a property which is peculiar to the "native" form of the estradiol receptor, its ability to interact specifically to heparin bound covalently to Sepharose. This property permits the easy purification of this form of receptor and it may also reflect important, previously unrecognized physiological function. The "native" estradiol-receptor complex is purified to homogenity after chromatography on columns of heparin-agarose, Sephadex G-200 and DEAE-cellulose, followed by two more Sephadex G-200 columns. The purified molecule is a single polypeptide of 69,000 molecular weight by polyacrylamide gel electrophoresis in sodium dodecyl sulphate, it has a sedimentation coefficient of 4.3 S, a Stokes radius of 36.5 Å, a frictional ratio of 1.3 and an isoelectric point of 6.4. The purified estradiol-receptor complex exchanges the radioactive hormone only with estrogenic compounds but not with testosterone, 5α-dihydrosterone and progesterone.

Control of ovarian steroid secretion, D. T. BAIRD, Department of Obstetrics and Gynaecology, University of Edinburgh, Scotland

Ovarian function is controlled by a well recognized feedback system involving the hypothalamus, anterior pituitary and the ovary. In the mature female, depending on the dose, oestradiol either inhibits (negative feedback) or stimulates (positive feedback) the secretion of pituitary gonadotrophins. In virtually all mammals studied so far, oestradiol is the main oestrogen secreted by the Graafian follicle, and it can be assumed that the ovulatory signal is induced by the rising levels of this hormone. Androgens or gestogens of ovarian and adrenal origin may influence ovarian function either indirectly by modulating the feedback effect of oestradiol, or directly by altering the response of the ovarian cells to gonadotrophins. For example, androstenedione will increase the sensitivity of the hypothalamic–pituitary system to the feedback effect of oestradiol. In this way, prolactin, receptors for which occur in high concentration in the adrenal, may influence ovarian function via an effect in adrenal androgen secretion.

During the luteal phase of the cycle, progesterone secreted by the corpus luteum, enhances the negative feedback but abolishes the positive feedback effect of oestrogen. In those species in which the corpus luteum is the major source of oestrogen, e.g. man, follicular development is inhibited during the luteal phase. In contrast, follicular development proceeds continuously in the sheep and the cow in which the corpus luteum does not secrete oestradiol. Thus the apparent differences in the follicular phase of the cycle between different species may reflect differences in the source of oestrogen during the luteal phase.