11. Gel entrapped antibodies: Advantages in determination of steroid hormones, H. A. THOMA, L. HEIDER, M. SPERL, S. HOFMANN, F. ZEHMISCH and E. E. KUSS, I. Frauenklinik der Universität München, Germany

Entrapment of antibodies in various polyacrylamide matrices can solve some problems in assaying steroid hormones:

1. Determination of steroids in the presence of crossreacting substances

Copolymers of acrylamide with acidic and hydrophobic residues are excellently suited for immobilisation of the anti-steroid-antibodies because they are able to differentiate the ligands. It can be shown that the affinities of the anti-estriol antibody to estriol, estriol-3-glucuronoside and estriol-3-sulphate can be varied by using a solid-phase system with different acrylic copolymerisates. The cross-reactivity of estriol-3-glucuronoside and estriol-3-sulphate amounting to 61% and 47% respectively in homogeneous aqueous medium can be diminished to 4% and 5% in the solid-phase assay with an optimal composition of the matrix consisting of acrylamide and different acids.

This way of assaying may circumvent the time-consuming solvent extraction, usually unavoidable in steroid assays, to separate the cross-reacting substances and introduces a system of assaying the steroid hormones directly in serum.

2. Determination of the free, diffusible steroids in the presence of a binding protein

In the determination of steroid hormones in serum more or less bound to specific carrier proteins, the unbound fraction represents the most interesting clinical parameter. The variability of the pore sizes enables an assay of the free diffusible fraction of a protein-bound hapten.

The present report describes the assay of the diffusible cortisol and testosterone in the presence of the protein-bound hormones. The whole assay consists of a sequential procedure of the entrapped antibodies with serum, elution, incubation with tracer and final elution. A formal sensitivity of 20 pg/ml (testosterone) can be achieved with this assay principle. The following concentration ranges (μg/100 ml) of diffusible cortisol were found: normal (1.5–2.5), stimulation (4.5–10.8), suppression (0.15–1.0), pregnancy (3.0–7.8). No significant differences were found by comparison with conventional methods (equilibrium dialysis etc.).

It should be emphasized that this assay principle can be adopted to other substances bound to proteins and that absolute value of the diffusible hapten is obtained directly. In contrast, conventional methods of measurement of free (non-protein-bound) hormones have relied on two unrelated determinations: those of total serum hormone concentration and the diffusible hormone fraction in dialysate.

The data available suggest that it may be advantageous in determination of steroids and other substances to combine the properties of the solid-phase technique—simplicity in operation—with the improved specificity given by optimal matrix composition.

12. Enzyme immunoassay of steroid using a heterologous system, CL. GROS and F. DRAY, Unité de Radioimmunologie Analytique, Institut Pasteur 28, rue du Dr. Roux, 75724-Paris, France

We have recently proposed an enzyme immunoassay for steroids using β-galactosidase linked to haptens as tracer and separation of free from bound fractions by insolubilized anti-gamma globulin serum. In this assay we used a homologous system where the hapten derivative (hemisuccinate) used for the preparation of immunogens and production of antibodies was the same as that used for the coupling reaction with enzyme. We present here a new heterologous system using derivatives of hapten which are different for antibody and enzyme–hapten conjugate formation. In this way the sensitivity of the system is increased; this could be explained by the poorer affinity between hapten–enzyme conjugate and antibodies than that between free hapten and antibodies. The specificity of the system is not altered. The derivative used for immunogen preparation is a hemisuccinate of the hapten and for linking to the enzyme we select a hemimaleate of the hapten. Preparation of hemimaleate is presented and its coupling to β-galactosidase is performed by the intermediate formation of a *N*-hydroxysuccinimide ester of hemimaleate of hapten. This method has been used for progesterone, cortisol and oestrone assays and the good correlation between the results obtained by enzyme and radioimmunoassay in biological fluids is presented.

CENTRAL NERVOUS SYSTEM—I. ANIMAL MODELS

13. The peculiarities of the guinea pig hypothalamus-hypophysis estradiol–receptor system, R. N. SCHEDRINA, L. S. MININA, E. A. NOVIKOV and S. V. STURCHAK, All-Union Research Institute of Obstetrics and Gynecology, Moscow, U.S.S.R.

Some kinetic and thermodynamic parameters of estradiol–receptor (E_2–R) interaction in adenohypophysis and anterior hypothalamus of immature guinea pig females weighing 250–300 g were investigated.

The cytosol was prepared by centrifuging homogenates at 105,000 *g* for one hour. Separation of free and bound hormones was done by charcoal. The experiments were carried out at 30°C. The values of dissociation rate constants (k_{-1}) were determined by Mester and Robertson's method $(0.130 \pm 0.007) \times 10^{-4}\,s^{-1}$ for adenohypophysis and $(0.128 \pm 0.002) \times 10^{-4}\,s^{-1}$ for anterior hypothalamus at 30°C. Half-life time ($T_{1/2}$) of E_2–R-complexes −103 and 91 min, respectively. Equilibrium association constants (K_{ass}) values (Scatchard analysis)—$(0.510 \pm 0.035) \times 10^{10}\,M^{-1}$ and $(0.136 \pm 0.024) \times 10^{10}\,M^{-1}$. The calculated values of association rate constants (k_{+1})—$0.682 \times 10^{6}\,s^{-1}\,M^{-1}$ and $0.158 \times 10^{6}\,s^{-1}\,M^{-1}$.

At zero-temperature k_{-1} value for adenohypophysis E_2–R-complex—$(0.57 \pm 0.04) \times 10^{-4}\,s^{-1}$ and for anterior hypothalamus—$(0.69 \pm 0.09) \times 10^{-4}\,s^{-1}$. The values of $T_{1/2}$ were 202 and 167 min respectively. Under these conditions k_{+1} values 0.31×10^{6} and $0.51 \times 10^{5}\,s^{-1}\,M^{-1}$.

The data obtained indicate the presence of specific E_2–R-systems, which differ in the formation rate and firmness of E_2–R-complexes in guinea pig adenohypophysis and anterior hypothalamus.

Regardless of the temperature (0°C and 30°C), the E_2–R-complexes are formed mainly via hydrogen bonds (−56 and −53 Kdj/M at 30°C and −46 and −41 Kdj/M at 0°C).

Nevertheless, these E_2–R-complexes are nonidentical as can be seen from the absence of coincidence of Kase values, measured experimentally and calculated from data, obtained at another temperature.

Besides, E_2–R-complexes formed at 0°C, dissociated completely during 5 min at 30°C conditions.

The lack of the coincidence in the characteristics of E_2–R-complexes emphasize the necessity of a careful approach to comparison of the data obtained at different temperatures of incubation.