General Description about the method of R.IA. and I.R.M.A.

Immunological Methods in the field of hormone research and examination

Introduction into the immunological proof methods:

The discovery of the principle of immunological hormone determination in the early sixties by Salomon Berson and Rosalyn Yalow has made endocrinology what it is today. This is attributed to the fact that only these methods supply the specificity and sensitivity required for hormone determinations in body liquids.

The method is based on the reaction of a hormone to be determined with specific antibodies which can be obtained in sufficient specificity and quantity against any hormone known to date.

The advantage of immunological hormone determination lies in its simplicity and high proof sensitivity. Its disadvantage is that the preliminary stages of hormones or waste products are also bound by antibodies if they only contain the peptide sequence necessary for antibody bonding.
For this reason it is sometimes necessary, in addition to immunological determination, to measure the biological activity of hormones in order to solve certain problems. The comparison methods are the older variations of the immunological proof methods, however, immunological determinations are generally more sensitive.

Comparison Methods:

The comparative immunological hormone methods of determination are based on the comparison of a constant quantity of marked hormones with different quantities of unmarked hormones from a sample around a limited number of bonded points at a constant quantity of a specific antibody.
Therefore the quantity of unmarked hormone determines the portion of the marked hormone bonded to the antibody.
Marking can be made by introducing a radioactive atom (Radio Immuno Assay) or by covalent coupling to a suitable enzyme, e.g. peroxidase (Enzymelogical Immuno Assay, EIA).

These types of hormone determination differ regarding the separation of bonded hormones from free hormones. Electrophoretic separations, salt outs, precipitations with a second antibody directed against the hormone antibody complex and adsorption of the free hormones by activated carbon and ion exchangers are used.
The quantity of marked hormones, bonded or free, can be measured with suitable instruments (in the case of Radio Immunological Testing by Radio Activity Measurement and in the case of Enzymelogical Testing by Enzyme Activity Determination) and the hormone contents of the sample determined by a calibration curve consisting of known quantities of unmarked hormones.

Immunometrical determinations:

The Immunometrical proof methods are all *Fixed Phase Methods* which exclude a separation step.
In addition to this, marked antibodies are mainly used. With the usual immunometrical proof methods (Immuno Enzymatic Assay) the antibodies are bonded to a fixed phase, generally this is the wall of a test tube.
The existing available fixed phase antibody reacts after this with the existing antigen in the sample, which is bonded to the antibody.

The actual proof method is now made by a marked antibody (radioactive or with an enzyme) against additional antigen determinants on the antigen itself.
The defining quantities are, depending on the mode of marking, the remaining freely marked antibodies, resp. the marked antibodies bonded to the tube´s wall.

In a variation of the immunometrical method of testing it is also possible to determine the antibody contents of a tissue sample or body liquid by the reaction with a fixed phase immobilised antigen and proof with a second antibody.

Brief summary:

Proof techniques are selected according to the hormones´s chemical characteristics. Basically, a differentiation is made between hormone proof from body liquids (blood) and the proof in tissue (histochemical).

Radio Immuno Assay (RIA)
In the field of endocrinology it is often the case that, with respect to differential diagnostics, function tests are applied, e.g. simulation tests for thyroid glands and insulin using TRH/TSH or glucose.
It is possible to advance into the trace area of the hormones (up to femto mol) with specific development of special bonding antibodies (AB) - either as antiserum or as monoclonic AB.

The principle:

Suppression of the unmarked hormones (antigens) by the radioactive marked hormones on the antibody bonding points.
The more the determinable unmarked hormone becomes available, the less the marked hormone is able to form radioactive antigen-antibody-complexes with the defined specified antibody.
These are measured after separation in a radioactivity detector (e.g. TRACERLAB Gamma Counter).

The production and execution of an RIA can be made in the following steps:

1. Marking of the hormone to be determined with a radioactive tracer
2. Production of specific bonding antibodies, antiserum or monoclonic antibodies
3. Creation of a dilution curve with known reference concentrations (standards)
4. ASSAY with buffer, protective colloid
5. Separation of the antigen-antibody-complex from the ligands

Measurement and evaluation with suitable hardware and software, logic log, spline function (TRACERLAB Gamma Counter)

Example of an assay, R.I.A., Progesterone