

Radioimmunoassay and related Techniques

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Shahid Kamal (Atomic Energy Medical Centre, Jinnah Postgraduate Medical Centre, Karachi.)

Radioimmunoassay systems have been developed to quantitate virtually every hormone and enzyme in the body. Utilizing the potent tools of radioactivity and immunology, these exquisitely sensitive techniques have revolutionized the fields of endocrine physiology and clinical endocrinology. RIA, as the name implies, achieves sensitivity through the use of radionuclides and specificity that is uniquely associated with immunochemical reactions¹. Development of radioimmunoassay initiated with the publication by Greenwood and Hunter²⁻⁴, demonstrating I-131 labelling of proteins using Chloramine-T while fully retaining immunological activity. Yalow and Berson published their RIA for insulin⁵⁻⁸ and Roger Ekins came up with determination of serum thyroxine using a competitive binding assay^{9,10}. The radioimmunological determination of thyroid stimulating hormone was first published by Odell¹¹ and for thyroid hormones by Murphy et al¹². The discovery that happens coupled to carrier proteins could also invoke an antibody production accelerated the development of radioimmunoassays for steroids and low molecular weight substances¹³⁻²⁰. The importance of this method lies in permitting the quantitative measurement of extremely small fractions, in the order of nanogram (10 g) and picogram (10 g), while the measurement is highly specific due to the immunological character of the reaction. The application of this technique to the measurement of protein and peptide hormones was an important advancement in the biochemistry of hormones and endocrinology. Before the advent of radioimmunoassays, the assay of polypeptide hormones was mostly considered a research topic, and their assessment was made indirectly. The earlier method of measuring peptide hormone in plasma was based on bioassay. This was performed by comparing the hormonal effect produced by an unknown in a laboratory animal with that produced by a known standardized preparation of the hormone. Several bioassays have been developed. However, they generally lacked sensitivity or specificity. To improve both sensitivity and specificity, it became necessary to concentrate the hormone in question from a large volume of animal plasma. This animal preparation, as well as the processing of the material for use, was time consuming and tedious. Although these methods yielded much physiological information, the technical requirements and poor reproducibility restricted the clinical use of bioassays to relatively few laboratories²¹. The direct chemical assay of peptide hormones was likewise fraught with problems. The chemical assay of thyroidal and steroidal hormones is possible because they possess distinctive chemical groupings. In addition, their concentration in plasma are three to five orders of magnitude higher than those of the peptide hormones. Unlike thyroidal and steroidal hormones, peptide hormones have evaded accurate assay because they are very few uniquely reacting groups that can be used to identify the hormone in the presence of other peptides. Even when specific reaction sites do exist on the hormone in question, they are not readily distinguishable from the remainder of the plasma proteins, which are present at a million-fold to billion-fold higher concentration. Radioimmunoassay methods possess the unique advantage of being sensitive, specific, and precise and therefore have gained the widest acceptance. The pace of new developments in RIA makes it impossible to create a "current" list of RIA applications²⁰. The applications in cardiology, rheumatology, gastroenterology and gynaecology soon followed rapidly²². Drugs that are used in generally low concentration are difficult to measure chemically and are increasingly being measured by RIA. Most drugs are relatively small molecules with molecular weights less than 1000, are non-immunogenic. Landsteiner²³ showed that many small molecules (called haptens) could be covalently linked to larger carrier molecules such as proteins, poly-peptides and poly-saccharides. Antibodies

formed to the larger substituted carrier molecules could react specifically with the smaller molecule alone. Butler and Chen²⁴ used this technique to produce specific antibodies to digoxin, a technique adapted by Smith et al²⁵ to determine therapeutic and toxic levels of serum digoxin, greatly helping the management of patients in congestive failure where the serum levels of digoxin often fluctuate widely and pose a serious threat of toxicity. The search for specific tumour antibodies which would detect preclinical cancers and provide a means of following therapy and detecting recurrence is a challenging and worthy goal. Carcinoembryonic antigen, a glycoprotein was first identified by Gold and Freedman²⁶. This antigen is detectable in a high number of patients with cancers²⁷. CEA assay has been useful in following the course of therapy and in detecting recurrence. Following total resection of gastrointestinal tumours, the serum levels usually return to normal^{28,29}. Another tumour substance, alpha fetoprotein, which is normally present in foetal tissues, has also been found to be present in patients with hepatomas³⁰, and is also useful to follow the therapy of tumour patients. The detection of microbial antigens and antibodies has developed through a host of techniques including immune diffusion, immune electrophoresis, hemagglutination and bacterial agglutination. RIA lends added ease and sensitivity to these assays, as exemplified in the detection of hepatitis-B antigen^{18,31}. With such rapid development of radioimmunological assay methods in the last twenty years, it is difficult to clearly define the future of this modality. Many experts in the field of radioimmunoassays see an increasing introduction of enzymeimmunoassays because of the ease with which the waste can be disposed of, while others are looking for new and more sensitive immunoassay systems. It is without question that immunoassays in one form or other will play an important role during the next decade as a method of analysis. Radioimmunoassays will remain one of the most important analytical methods in endocrinology even in the future, as very low concentrations of the hormones must be measured. The radio-immunological determination of enzyme concentrations is a research area in which increasing interest is being shown. Radioimmunoassay will continue to be useful tool over the next years in bacteriology and virology especially in the field of fighting infectious diseases. Fields in which radioimmunoassays will increasingly be replaced by either enzyme immunoassays or fluorescence-immunoassay are those where μg or mg amounts are found in serum e.g., controlling drug levels. Commercial enzyme-immunoassays as well as fluorescenceimmunoassays are already available for many drugs and several serum proteins.

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