
METHODS IN RADIOIMMUNOASSAY OF PEPTIDE HORMONES

Reprinted from *Methods in Investigative and Diagnostic Endocrinology*,
Volumes 2A and 2B, edited by Solomon A. Berson (deceased)
and Rosalyn S. Yalow, 1973

Compiled by

ROSALYN S. YALOW

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Preface

The preface to Volume 2 of **METHODS IN INVESTIGATIVE AND DIAGNOSTIC ENDOCRINOLOGY** described the seminal role of radioimmunoassay in our understanding of hormonal physiology. It stated,

"The development of radioimmunoassay and the studies in understanding of hormonal physiology during the late 1950s and the 1960s coincided with a period of great advances in the chemistry of peptide hormones. It was during this time that highly purified preparations of most of the known peptide hormones became available to investigators. That hormones could be obtained which were sufficiently pure for labeling and in adequate supply for immunization was an essential element in the development of radioimmunoassay techniques. In turn, radioimmunoassay provided the sensitivity, specificity and reliability that made possible studies of in vivo hormonal regulation, which would otherwise not have been possible. The synergistic interaction between advances in the biochemistry of peptide hormones and in investigations in physiology, particularly those using radioimmunoassay, has resulted in an information explosion. However, the rate of increase in this field appears to be slowing down and now is an appropriate time to pause and bring together a compendium of the varied methodology and interpretation involved in the interwoven biochemical, physiologic and clinical studies relating to the peptide hormones."

Since the completion of that volume in 1972 there have been relatively few significant changes in radioimmunoassay methodology for the peptide hormones described therein. There has, however, been an enormous increase in the applicability of radioimmunoassay to other peptides and to hundreds of other substances of biologic interest. The problems, practices and pitfalls of radioimmunoassay presented in that volume, both in the general chapters and in those devoted to particular hormones, should prove to be of value not only to the investigators and others employing the described procedures but also to all concerned with developing new assays for any substance and for improving those already in use. It therefore seemed

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worthwhile to extract from the original volume the relevant chapters on radio-immunoassay and to republish them in a format which it is hoped will be helpful and convenient for laboratory use.

Rosalyn S. YALOW
November 1975

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CHAPTER 1

General methodology*

by Solomon A. Berson and Rosalyn S. Yalow

1.1 PRINCIPLE

The basis of the radioimmunoassay method is the competitive inhibition by unlabeled hormone of binding of labeled hormone to its specific antibody (Yalow and Berson 1959) according to the competing reactions shown in fig. 1.

Here, F and B represent concentrations of free and antibody-bound *labeled* hormone, respectively. The *fractions* of the labeled hormone which are bound and free

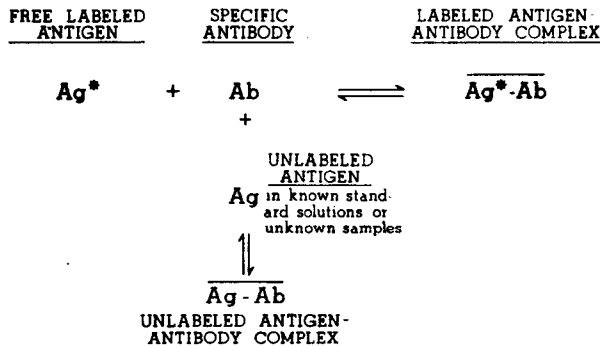


Fig. 1. Competing reactions that form the basis of the radioimmunoassay.

* Standard hormone preparations are available from the Hormone Distribution Officer, Office of the Director, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. 20014 (A list of available preparations is published at least once a year in Endocrinology and in J. Clin. Endocrinol. Metab.) and from the United Kingdom Medical Research Council, Division of Biologic Standards, National Institute for Medical Research, Hampstead Laboratories, Holly Hill, London NW3 6RB, England. When standard reference preparations cannot be obtained, the use of crude glandular extracts or plasma containing a high hormone concentration permits evaluation of relative concentrations of hormone in unknown samples.