Current Topics in Experimental Endocrinology

volume 1

edited by
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and
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Current Topics in EXPERIMENTAL ENDOCRINOLOGY

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PREFACE

Endocrinology is now one of the most rapidly advancing of the biological sciences, and both novel experimental procedures and the development and exploitation of new concepts have radically altered endocrinological views over the last few years. The ever-extending use of new experimental procedures, such as isotopic methods for tracing molecular events or defining minute hormonal concentrations, has substantially extended our knowledge, and these and other techniques are now making it possible to study endocrine events in considerable detail. The purpose of this series is to provide readers with a continuing and critical review of the field. For this purpose, the editorial board has attempted to select certain aspects of experimental endocrinology which appear particularly dominant or fundamental and which are also of sufficient general interest to justify reviewing in detail. The invited authors have been offered the opportunity to discuss their field critically from a personal standpoint rather than to provide an extensive reference list to the area under discussion. In this way it is hoped that the reader will be able to derive from this survey a closer understanding of contempory problems and advances in these particular areas.

L. MARTINI
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BASIC CONCEPTS OF SATURATION ANALYSIS TECHNIQUES

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I. Introduction

One of the major factors which has led to the advance of endocrinology in the last few years has been the emergence of analytical methods

with sensitivities encompassing the range of concentration at which many hormones exert their effects in biological systems. Many of these methods have relied essentially upon the use of radioactive reagents—a dependence which reflects the delicacy of radioactive measurement as compared with many other physical detection methods. These radioanalytical methods may in turn be broadly subdivided into two classes: labeled derivative techniques, and those termed by the author "saturation assay" methods. This article will deal almost exclusively with the latter group; nevertheless a brief review of the labeled derivative method is not out of place since some of the reagents and techniques employed are common to both assay techniques notwithstanding the fundamental difference in principles on which they each rely.

The derivative method was originally introduced by Keston and his colleagues in 1946 (Keston *et al.*, 1946) and subsequently exploited in the measurements of the steroid hormones by various groups, notably those of Tait and co-workers in England (Avivi *et al.*, 1954) and Peterson (1959) in the United States. The method was likewise adopted for the measurement of the thyroid hormones by Whitehead and Beale (1959).

Fundamentally the technique is akin to "activation analysis" (commonly employed in the measurement of trace elements) in that it depends upon the radioactivation of the compound under test. However, in contrast with conventional activation analysis, activation is achieved by chemical reaction with a labeled reagent rather than by physical methods. The initial amount or concentration of the test compound can be deduced from a measurement of the labeled derivative formed, the latter being quantitated (after its isolation from labeled contaminants) by a measurement of the radioactivity appearing in the purified product. "Indicator" compounds, either the original test compound or its derivative, labeled with a second isotope, are usually added at an appropriate point in the procedure to monitor recovery of the labeled derivative through the necessary extraction and purification stages.

In this simple form, the technique suffers from a major disadvantage: that is, the nonspecificity of the primary chemical reaction on which the method depends. Most labeled reagents used in these procedures (acetic anhydride, thiosemicarbazide, fluorodinitrobenzene, etc.) react with a considerable range of compounds, with the consequence that the specificity of the method depends critically on the efficiency of the procedures whereby the particular reaction product is separated from all other derivatives, from residual unused reagent, and from other radioactive contaminants. Such procedures are frequently time consuming, and they severely limit the number of samples that may be processed.

Moreover, they are never completely successful, and labeled contaminants persisting through successive purification stages almost invariably restrict the ultimate assay detection limits achieved to values far greater than those dictated by the final radioactive measurements (Brodie and Tait, 1969).

Although laborious, the labeled derivative method has formed the basis of major advances in endocrinology in the last decade, particularly in the steroid field. Nevertheless the more recently introduced saturation assay techniques, because of their greater simplicity and, frequently, sensitivity, are tending to displace the original derivative methods.

A recent development, however, has circumvented the basic objection to the derivative technique by imparting a much higher degree of chemical specificity to the initial reaction. This is the immunoradiometric technique, initiated by Miles and Hales (1968), wherein labeled antibody, specific in its reaction with the test compound, is employed as the labeled reagent. This approach has, at the present time, been employed in the assay only of protein hormones. Nevertheless, there seems to be little doubt that its use will be extended to other compounds, such as the thyroid and steroid hormones, against which antisera can be raised by appropriate methods. Ultimately also, it is probable that other specific binding proteins may be isolated in a sufficiently purified form to enable their use as labeled reagents to be contemplated.

II. Saturation Assay

A. Basic Principle

The fundamental principle of the saturation assay method is shown in Fig. 1. (In practice there are many variants of the basic sequence of steps, and those shown in this figure are representative rather than obligatory.) The first step consists of the addition of radioactive P to the biological medium under test. After equilibration of exogenous labeled and endogenous unlabeled compounds, P may, if necessary, be extracted from its biological milieu and purified, extraction recovery being monitored by the radioactivity present in the final extract. Subsequently the extracted compound is mixed with a specific reagent, shown in Fig. 1 as Q, in such relative concentration that part of P reacts with Q (bound, or reacted, P) and part remains in the unreacted form (free P). The distribution of reacted and unreacted P, as revealed by the ratio of radioactivity appearing in the two fractions, is dependent upon the total concentration of P present, so that, provided certain conditions

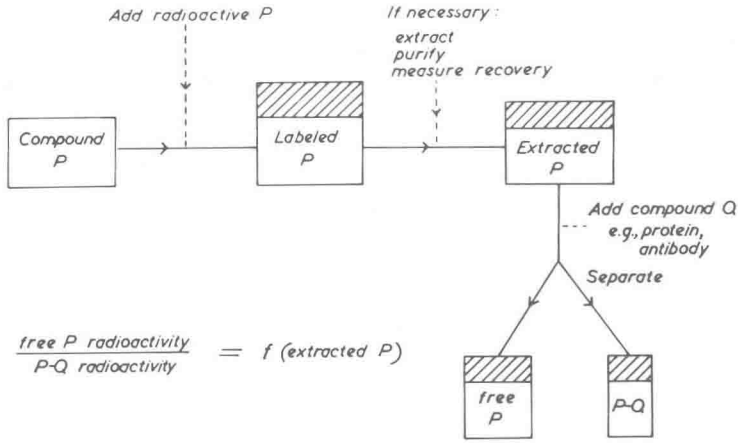

Fig. 1. Fundamental principle of saturation analysis.

are fulfilled, the distribution can be used to deduce the concentration of an unknown amount of P introduced into the system.

To emphasize the generality of this analytical principle, its broad areas of application are set out in Table I. The list is by no means

Table I
Saturation Analysis
(Displacement Analysis, Radiostereoassay, Competitive Radioassay)

Class of reagent	Common name	Compounds to which applied
Specific antibodies	Radioimmunoassay	Polypeptide hormones Proteins Steroid hormones Thyroid hormones Cyclic nucleotides Tumor antigens Viral antigens
Specific serum and tissue proteins, and other binders (e.g., intrinsic factor, milk)	Competitive protein-binding assay	Thyroid hormones Steroid hormones Vitamins Trace elements Cyclic nucleotides Polypeptide hormones
Specific enzymes	Radioenzymatic assay	Folic acid Cyclic nucleotides
Microorganisms	Radiomicrobiological assay	Folic acid
Inorganic reagents	Substoichiometric assay	Metals

comprehensive, and almost any attempt to make it so would be futile by virtue of the rapidity of current developments in the field. Conversely, certain categories of compound have been included under particular headings because their assay using a particular type of specific reagent is *potentially* possible, or desirable, although the experimental procedures may not as yet have been described.

Several terms have been proposed—other than saturation analysis—which encompass the several classes of specific, saturable, reagents which have been exploited in methods of this general type. There are, however, in the author's view, conceptual objections to many of these terms. For example, the adjective "competitive" misrepresents the fundamental principle, since the method does not essentially depend upon competition with or "displacement" of, a labeled competitor compound. Indeed, *any* physicochemical measurement of the distribution of the test compound can serve as the response parameter enabling unknown amounts of that compound to be estimated. Only when measurements are confined to exceedingly low values does the addition of radioactive tracer provide a convenient means of quantitating the distribution. The word "competitive" should perhaps be restricted to the description of those techniques which rely on a genuine competition between two chemically distinct compounds for identical reaction sites.

The single basic requirement common to all forms of exploitation of the principle is that the specific reagent (Q) should be present in the assay system at such concentration that the amount of P that can react is limited, so that the distribution of the latter is a rapidly changing function of the total weight or concentration present. This implies that the reactive sites associated with Q must be saturated or approaching saturation (although it is evident from a consideration of the law of mass action that reaction sites can never be *fully* saturated). Clearly the concentration of Q must be selected so that the distribution of P changes most rapidly in the particular concentration range of interest; hence, for the measurement of high values of P, a larger concentration of Q is appropriate than for measurements falling in the lower ranges. In general, therefore, the concentration of Q must be of the same order of magnitude as the range of values of P that it is desired to assay.

The purpose of this presentation is essentially to enumerate and discuss the fundamental principles contributing to an accurate measurement of P. This necessitates consideration of the theoretical relationships between the concentrations of reagents, and their optimization to yield assay systems displaying maximal precision. Some of the factors affecting specificity (and, hence, accuracy) can likewise be considered from a theoretical point of view. Other factors of a more practical nature, not