Practical Endocrine Diagnosis

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THIRD EDITION

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PREFACE

Endocrinology is perhaps the most exacting discipline in medicine at present, with the precision of the laboratory allowing quantitation of subtle abnormalities that defy detection on clinical grounds alone. Because of physiologic variations and complex hormone interactions, the conditions of diagnostic testing for endocrine diseases are sometimes more important than the precision of the laboratory assay. To effectively use the clinical laboratory in endocrine diagnosis, the physician must be familiar with the limitations of each assay, and the clinical conditions under which meaningful values can be obtained.

The purpose of this manual is to provide a consensus in areas of diagnostic endocrine tests, with up-to-date methods brought together from medical journals and textbooks and proven in clinical application. The emphasis is on hormone measurement; no attempt has been made to be complete in subjects treated, and nonhormonal aspects of disease or diagnosis will be mentioned primarily where their importance is related to the understanding of the hormone abnormality.

Since endocrine diseases must be defined in terms of abnormalities of hormone secretion, the key to accurate endocrine diagnosis is the knowledge of the dynamics of a given hormone system and, in most instances, the ability to modify the dynamics of a system in a measurable and reproducible fashion. A single measurement is rarely enough to give one confidence in endocrine diagnosis, no matter how accurate the measurement. Awareness of modifying factors that are clinically important and the opportunity to make sequential measurements under controlled conditions of stimulation and/or suppression allow one to clearly define most abnormalities. One must be careful to adhere to well-studied protocols so as to be able to apply accepted values to the results, and to rely on referral of more complex cases to specialists in clinical and/or laboratory medicine.

The need for this type of presentation appeared when we encountered a great deal of difficulty in extracting the specific information we needed from various medical writings. Many test descriptions were so general (". . . give some of this medication and measure the response sometime later") that the studies could not be used in specific instances. In other situations, classic diagnostic approaches had been superseded by more accurate and simpler procedures, yet these had not been widely accepted because of lack of exposure.

This manual began as the result of a series of workshops for the American Society of Clinical Pathologists entitled MODERN SOLUTIONS TO COMMON ENDOCRINOLOGIC PROBLEMS, and has evolved from the

interaction between the physician involved in patient care and the physician in charge of the clinical laboratory. This is the third edition; the previous two were well received by all levels of physicians, medical students, house staff, generalists, as well as specialists in pathology and endocrinology. We feel that the interaction between the physician involved in patient care and the physician in charge of the clinical laboratory must continue to encourage the most sensitive and specific, yet cost effective, approach to diagnosis.

This manual should add to the ease and accuracy with which endocrine diagnoses are made by making available in one volume criteria for selection of tests, protocols for testing, and laboratory methods, with the advantages and drawbacks of each, from the initial contact with the patient, to the performance of the test in the patient, the final result of the laboratory procedure, and back to the patient. Reading from cover to cover will be informative, but not necessary. We hope the reader can extract the material pertinent to his patient or the problem at hand with a minimum of distraction.

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Chapter 1

Basic Concepts

FEEDBACK SYSTEMS

Control of interaction, secretion, and release of various hormones must be understood in terms of "feedback" control. Particularly important to understand is the concept of negative feedback, or feedback inhibition, where the rising concentration of a hormone inhibits the system which causes the release of that particular hormone. Most systems of feedback are "closed loop," where the output (such as thyroid hormone) affects the response of the system to input (inhibition of release of thyroid stimulating hormone from the pituitary). At the cellular level of hormone action, systems are often "open loop," where the input affects the output but the converse is not true.

Many of the subjects under discussion involve multiple steps which are subject to modification by outside influences. In addition to the effect that one hormone may have on the release or action of another, biologic rhythms and other nonhormonal stimuli may alter a system at various points. With any endocrine disorder, an understanding of these factors is the key to proper interpretation of laboratory and clinical findings. These factors are fairly well worked out for most systems, and are particularly clear in the case of target glands which are influenced by specific pituitary trophic hormones—the gonads, thyroid, and adrenal glands; the pituitary trophic hormones in turn are controlled by corresponding hormones from the hypothalamus. The hormone(s) produced by the target gland can exert feedback effects at the level of both the hypothalamus and pituitary.

HORMONE TYPES

There are three main classes of hormones in man: steroid, polypeptide, and amine. Their characteristics vary with regard to

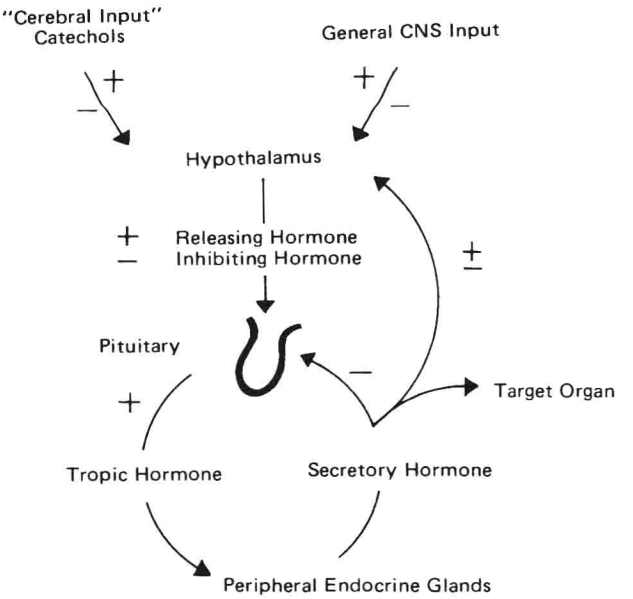

Fig. 1–1. General feedback scheme.

storage, release, transport, degradation, and mechanism of action. These chemical differences are important clinically both in understanding the effects of these hormones in health and disease and in interpreting laboratory results.

The steroid ring is the basic framework for steroid hormones, with specific activity of each hormone conferred by specific side chains. Steroid hormones are usually found in blood bound reversibly to specific carrier proteins. The bound fraction is biologically inactive, with the free, or unbound, fraction being the pool with biologic activity. The steroid hormones include adrenal glucocorticoids, mineralocorticoids, gonadal hormones, and vitamin D.

Polypeptide hormones are made up of amino acids linked together. Often, a small number of amino acids in the sequence provide full biologic activity, and various sized fragments of the same hormone may be circulating simultaneously, due to an inactive portion of the molecule which may protect from degradation or enhance specific binding. This heterogeneity, and variation in biological and chemically assayable activity, may present difficulty in the clinical interpretation of laboratory results. Hypothalamic hormones, pituitary hormones, parathyroid hormone, insulin and other gastrointestinal hormones are examples of polypeptide hormones.

The amine hormones include the neurotransmitter hormones (acetylcholine and catecholamines) and thyroid hormones.

The term "prohormone" is used to describe a substance of low specific activity which is converted to a more active configuration. The conversion from prohormone to active hormone may take place prior to release from the cell of origin (e.g., proinsulin to insulin), in the circulation (angiotensin I to angiotensin II), or at the level of cellular activity (testosterone to dihydrotestosterone, thyroxine to triiodothyronine). The same polypeptide hormone may exist in varying sizes in the circulation due to the presence of active and inactive fragments. Steroid hormone activity may be altered by modifying the side chain on the steroid ring. If a precursor to a prohormone is discovered, it is called a "pre-prohormone." This may lead to some confusion in terminology as well as in assay results.

PHYSICAL CHEMISTRY

Although far behind most of us in our academic experience, physical chemistry is vital in our daily experience with patients. There are two main aspects for consideration, in vivo and in vitro.

In vivo, hormone localization in target gland tissue is a reversible reaction. This can be expressed mathematically as a binding constant (K). It is fascinating to observe that minute quantities of hormone have such selective yet dramatic influences. This can be explained by the avidity of the cellular membrane or intracellular receptors, that is, a great tendency for binding and a minimal tendency to release the hormone. By contrast, the carrier proteins (for example, transcortin) have a lesser binding affinity than the cellular receptor, so they will readily release their cargo at the appropriate site. This concept is crucial when one measures serum levels of hormones. Specifically, a total thyroxine level tells us little in the borderline range, since the binding removes about 99.9% of thyroxine from physiologic activity. Furthermore, levels of thyroxine-binding globulin vary immensely, and not always predictably, among the population. This is certainly of daily importance.

In vitro, the basic concept is equally significant. Taking advantage of the reversibility of the reaction, we can introduce isotopically labeled hormone to displace endogenous hormone. The subsequent separation of *bound* isotope from *free* permits the establishment of a ratio that is fundamental to saturation analysis assays (radioreceptor assay). From this, it should become apparent that the binding protein should have a high affinity for the substance to be measured, and little or none for functionally or structurally similar substances. Consequently, antibodies meeting these criteria continue to be developed, replacing older carrier protein assays.

CELLULAR MECHANISMS OF HORMONE ACTION

At the cellular level, hormones increase the activity of certain enzymes selectively, thereby predictably modifying cellular function.

Cyclic AMP. Most polypeptide hormones affect cellular metabolism through the adenyl cyclase system. The circulating hormone binds rapidly and reversibly to the hormone-specific receptor on the surface of the cell membrane. This binding results in activation of the enzyme adenyl cyclase; this enzyme converts ATP to cyclic AMP (3′, 5′ adenosine monophosphate). Phosphodiesterase rapidly converts cyclic AMP to inactive AMP. Before its inactivation, cyclic AMP activates an enzyme kinase system which modifies cellular function. The hormone dissociates from its receptor site as the hormone concentration outside the cell falls. The specific hormone as "first messenger" and cyclic AMP as a "second messenger," as outlined above, may be similar for hormones such as insulin and growth hormone which have not been shown to activate adenyl cyclase, but may work through as yet unidentified "second messengers."

Measurement of cyclic AMP generated by the effect of parathyroid hormone on the kidney has helped us to understand the pathophysiology of hyperparathyroidism and pseudohypoparathyroidism.

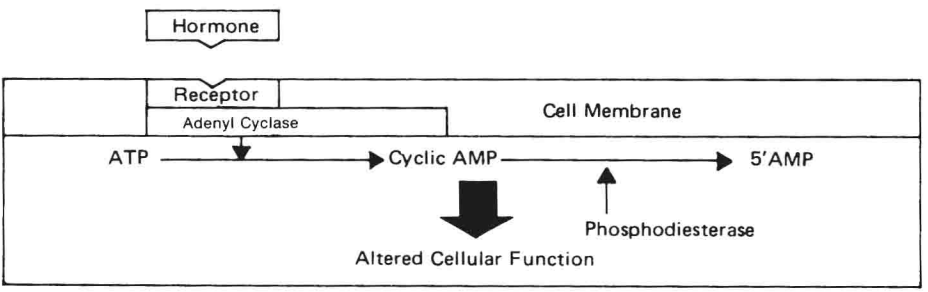

Fig. 1–2. Adenyl cyclase activity. (From Liddle GW, Hardman JC: Cyclic adenosine monophosphate as a mediator of hormone action. N Engl J Med *285*:560, 1971.)

Steroid hormone action. A different concept has been advanced to explain the mechanism of steroid hormone action in cells. Entering the cytoplasm readily, steroids associate with specific cytoplasmic receptor proteins and this complex is transported into the nucleus. The hormone-receptor complex is "activated" at this point, and binds to nuclear protein. This interaction induces the formation of messenger RNA, which enters the cytoplasm and increases the synthesis of specific cellular proteins.

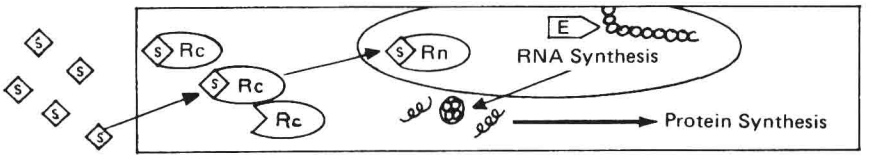

Fig. 1–3. Steroid hormone action. (From O'Malley BW: Mechanisms of action of steroid hormones. N Engl J Med *284*:370, 1971.)

Measurement of receptor levels for estrogen and progesterone in breast cancer tissue has added considerably to our understanding of the effects of hormones on this tumor, and this measurement is both practical and available through many reference laboratories.

Thyroid hormone action. Thyroid hormones (mainly triiodothyronine) easily pass through cell membranes. They may be reversibly bound to sites in the cytoplasm. Hormone action begins when the hormone binds to receptors in the nuclear chromatin (these receptors,

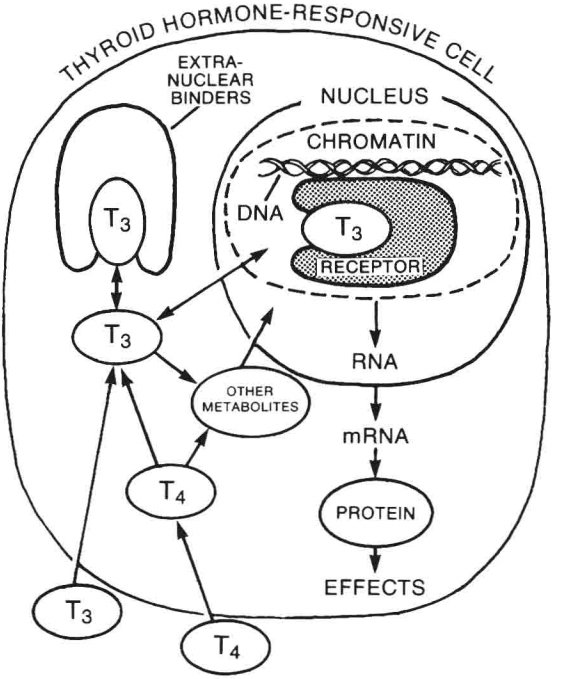

Fig. 1–4. Thyroid hormone action. (Reprinted by permission from Baxter JD, Funder JW: Hormone receptors. N Engl J Med *301*:1149, 1979.)

in contrast to steroid hormone receptors, are present in the nucleus whether the hormone is present or not). The hormone-receptor combination activates messenger RNA, which in turn affects the synthesis of cellular proteins.

Clinical significance of hormone receptors. Changes in receptors may cause disease or be the result of disease. A primary deficiency in cell receptors accounts for testicular feminization, pseudohypoparathyroidism, and other uncommon conditions. A reduction in insulin receptor number secondary to elevated insulin levels may present as non-insulin-dependent diabetes mellitus. Antibodies to receptors may cause disease (myasthenia gravis, hyperthyroidism). Receptor content of tissues may determine their response to drugs (estrogen and progesterone receptors in breast cancer).

ECTOPIC HORMONE PRODUCTION

It appears that all tissues have the capacity to produce hormones. Frequently, cancers are associated with syndromes of hormone excess. Recently, the use of sensitive and specific assays has shown a high frequency of production of hormones or the precursors of hormones in neoplastic disease. Since the hormones produced may vary in biologic activity (e.g., proACTH associated with lung cancer will be measured with radioimmunoassay but not bioassay, parathyroid hormone produced by neoplastic tissue may be indistinguishable from that produced by normal parathyroids), it is important to be aware of this, particularly since cancer in some patients with a "paraneoplastic" syndrome may be occult at the time of presentation. Also, it is important to remember that patients with cancer do not necessarily have all their problems explained by their malignancy (patients with cancer and hypercalcemia may well have primary hyperparathyroidism).

ASSAY TECHNIQUES

When one is seeking quantitative biochemical data for application to a clinical situation, the assay is the means to an end, not an end in itself. Consequently, an ideal assay is accurate (it measures what is stated and is close to the absolute value) and precise (reproducibility within a narrow range). Deviation from this ideal may be significant enough to exclude the use of an assay in one disease, yet be of little significance in another disease. TSH measurement, for example, is not sensitive enough to be useful in hyperthyroidism, but is excellent for hypothyroidism. It is not necessary, for the latter condition, to make costly or time-consuming modifications in the assay. The moral: clinical

laboratories exist to make reliable and useful data available, rather than entertain themselves over the niceties of chemical refinements. Other desirable qualities in an assay are low cost, simplicity (the ability to be performed by persons with a great variety of skills), and rapidity. Rarely are all these qualities found, yet they are rapidly being approached. A few words will suffice to outline currently used assay methodologies:

Colorimetry. Classic application of Beer's observation that color is proportional to the concentration of the colored substance remains the most widely used principle in clinical chemistry. Calcium continues to be measured optimally by use of this principle (SMA-12). By contrast, because of the interference in the "visible" range of the light spectrum by numerous medications as well as naturally occurring substances, colorimetry generally is not desirable. The extension of this method to the ultraviolet range of the spectrum has been a major advance over the years, primarily through the use of highly specific enzyme reagents with automatic blanking of the background color-absorbing substances. Consequently, it is now appropriate to routinely measure glucose with an enzyme assay rather than by means of a nonspecific reducing method with a colorimetric end point. The influx of enzyme assays relies on Beer's law for measurement of the end point. Interference with the enzyme indicator must be assessed.

Fluorometry. Certain substances, notably those with complex organic ring structures, will fluoresce when concentrated and placed in an appropriate chemical environment. The basic principle involves absorption of light energy at one wavelength (exciting wavelength) and emission of a characteristic spectrum (emitting wavelength). Fluorometric cortisol assays of blood and urine formed the foundation for the classic work in the field of adrenal cortical disease. Metanephrine and catecholamine assays continue to be satisfactorily performed by this approach. More specific and far more sensitive than colorimetry, fluorometry continues to be useful. Its greatest value, as a combined tool with immunoassay, is currently being realized.

Immunoassays. The explosion in knowledge of endocrine physiology could never have proceeded so rapidly without the development of immunoassays. This term is chosen specifically to avoid equating the concept with "radioimmunoassay." The emphasis is intended to point out that the specificity of the assay is due to the use of an antibody as a reagent. This is a remarkable testimony to the myriad capabilities of the plasma cell. Similarly, although sensitivity depends to a great extent on the antibody and its binding affinity for small concentrations of the substance being measured, to a greater extent the ability to measure small concentrations is dependent upon the end point chosen. Many

variations on the theme have been developed. The isotope has inherent qualities that have enhanced the immunoassay, now dignified as the "radioimmunoassay" (RIA). The enzyme end point has become increasingly successful and offers advantages in the application of the immunoassay to standard enzyme instrumentation and, in some cases, lower cost. On the other hand, the inherent stability and predictability of the isotope end point, free of any chemical effects or extraneous interference, ensure a continuing role for the radioimmunoassay. Fluoroimmunoassay can be expected to replace the bulk of the applications involving the radioisotope because of the longer shelf life, inherent economic advantages, and the avoidance of regulatory controls, particularly the expense of radioisotope disposal.

One should continually assess the major variables among various assays. These are:

1. The number reported as a level of hormone is only as meaningful as the specificity of the antibody used as a reagent in the procedure. For example, can the antibody used discriminate between cortisol, 11-deoxycortisol, and corticosterone? If not, then the answer is no better than one obtained with a competitive protein binding and reflects total blood corticoids rather than true cortisol. The antibody need not be free of cross reactions with steroids not likely to be encountered physiologically. However, it is apparent that one would not be able to distinguish between adrenal hyperplasias with a nonspecific antibody.

2. Separation of the antibody-bound hormone from free hormone in the reaction is an entire discipline in itself. Various approaches show more or less favorable results in individual assay systems. The increasing use of solid-phase bound reagents is justified by improving technology. This permits prompt completion of an assay within the framework essential for clinical utility.

3. Three forms of antigen compete for binding with the antibody. They must have similar binding affinity. These are: (a) the actual substance in the patient's serum or plasma, which we want to measure; (b) the "pure" standard, which we assay to establish a curve; and (c) the radioisotopically labeled tracer material which always differs structurally and chemically if the isotope label is extraneous to the inherent molecule. Such has been the case with assays of iodinated steroids.

A corollary to these points is that all kits are not created equal; comparability of data from laboratory to laboratory (and reference literature) is often quite disparate, and one must be extremely knowledgeable to attempt development of a total assay system including one's own antibody- and radiolabeled material. Evaluation of the significance of these hormone measurements (specificity and sensitivity) often necessarily relies heavily on close coordination of the laboratory worker with the clinician and evaluation of corroborative data (for example, low T_4 with high TSH).

In considering whether to incorporate a new procedure, each hospital laboratory should evaluate two basic points:

1. How valuable is the assay to patient care, specifically in a decision-making time-related way?
2. Can it be accurately performed, providing reliable information rather than misinformation?

A laboratory that can and should perform a needed assay, but does not, is just as open to criticism as one that should not and yet, incompetently, does perform the assay.

Receptor assays. Target organs of a given hormone contain receptor sites, either on the membrane of the target cell or within the cytoplasm (cytosol). The characteristics of these are an extremely high affinity for the hormone being measured and a remarkable degree of specificity. The most widely useful clinical application of this at the present time is in measurement of estrogen and progesterone receptors in breast carcinoma. Efforts have been made to use these receptor proteins as reagent systems in the assay of hormones. Most notably, assay of human chorionic gonadotropin by the use of stabilized membrane receptors of bovine corpus luteum has been demonstrated. Widespread application of these principles to the clinical laboratory is not appropriate at this time because of the inherent instability of these receptors, which are highly subject to denaturing influences.

Enzyme immunoassay. In place of an isotope end point, one can use an enzyme that is either activated or inactivated by the dynamics of antigen-antibody combination in an assay system. Two alternate methodologies are enzyme-multiplied immunoassay technique (EMIT) and enzyme-linked immuno-sorbent assay (ELISA). Each system makes use of the saturation-assay principle, and of enzyme activity as an indicator of the combination of reagent antibody or antigen with the antibody or antigen being measured. The EMIT system is of great interest because it does not require separation of bound and free. The entire assay occurs in a single test tube. ELISA requires this separation, but avoids the requirement for radioisotope counting equipment. Commercially available reagents for the EMIT system are widely available in the United States. This has been a particular boon to the clinical laboratory in the measurement of drugs. Numerous commercial sources of enzyme and fluoroimmunoassay reagents are now available.

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