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Virginia A. LiVolsi
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ENDOCRINE PATHOLOGY

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ENDOCRINE PATHOLOGY

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ENDOCRINE PATHOLOGY

This book is dedicated to our teachers, colleagues, and especially our patients, who have all taught us the nature and consequences of endocrine diseases.

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Preface

The field of endocrine pathology has progressed rapidly during the past decade. The specialties of cytopathology, genetics, and molecular biology have been applied to endocrine disorders, and new insights have been obtained. The chapters in this text include classical approaches to the diagnosis of endocrine diseases and information about modern techniques that enhance the diagnostic ability of the pathologist and service to the patient.

This book includes discussions not only of the classical endocrine organs (pituitary, thyroid, parathyroid, and adrenal) but also of those organs and tissues not normally considered "endocrine organs" that can give rise to hormonally active proliferations and tumors (e.g., skin, gastrointestinal tract, lung, and placenta). These discussions maintain the philosophy that endocrine hyperplasias and neoplasias frequently reflect systemic disorders and that the anatomical pathologist must be familiar with and utilize modern techniques to assist in the understanding of these lesions and their clinical importance and relevance.

The authors of the individual chapters are experts in their fields and have been at the forefront of application of this philosophy. We thank each of them and the editorial staff at W.B. Saunders for their efforts in making this project a reality.

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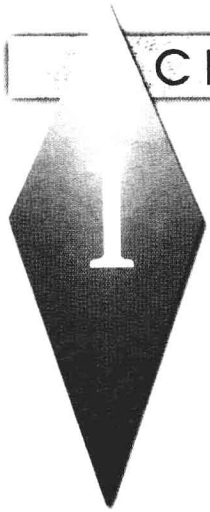

The Contribution of Laboratory Medicine to the Diagnosis and Management of Endocrine Tumors

David B. P. Goodman

The clinical pathology laboratory plays two critical roles in the diagnosis and management of endocrine tumors. First, during the preoperative evaluation of patients, measurement of circulating hormone concentrations provides evidence of loss of feedback loops that are normally operative in controlling hormone secretion. As well, assessment of hormone concentration is important in evaluating ectopic hormone production frequently observed when hormone is produced by tumors outside endocrine glands. Second, measurement of hormone concentration is important postoperatively as a tumor marker to monitor the completeness of initial surgical therapy and the return of disease either at the primary site or as a distant metastasis.

Originally, hormones were categorized into three classes based on their chemical structure: peptide and protein hormones, steroid hormones, and amino acid–related hormones. As new hormones are discovered, this simple categorization does not remain valid: e.g., prostaglandins are produced from fatty acids. Hormones are chemical messengers that send a signal from one point to another in a physiologic system. There are four hormonal communication systems, each with distinct anatomic relationships.

Systematic. In this classic endocrine system, hormone is synthesized and stored in specific cells in a defined endocrine gland. Hormone is released on receipt of an appropriate physiologic signal, a change in some component in the blood (e.g., glucose $[K^+][Ca^{2+}]$), or receipt of a neural signal. Once secreted into the blood stream, the hormone can then travel to a distant cellular target. Frequently, the hormone, particularly if it is a hydrophobic molecule like a steroid hormone, is carried in the blood stream complexed to a specific transport protein (e.g., transcortin for cortisol, vitamin D–binding protein for $1,25(OH)_2D3$). The target cell for a specific hormone is determined by the presence of specific high-affinity receptors for that hormone at the target cell. As a consequence of the presence of hormone at the target cell, a signal transduction process follows, and a specific set of biologic responses is generated. Frequently, some aspects of the biologic response result in a change in the concentration of some blood component such that a feedback signal is sent to the originating endocrine gland to decrease the biosynthesis and secretion of the hormone. In the pancreas, beta cells secrete insulin in response to elevated glucose. Insulin then acts on liver, muscle, and adipose tissue to decrease blood glucose concentration, and the decreased blood glucose feeds back to the pancreatic beta cells to diminish insulin secretion.

Paracrine. In the paracrine system, the distance from the secretion site to the target cell is greatly reduced. Secreted hormone diffuses from the secreting cell to immediately adjacent target cells. For example, testosterone is secreted by the Leydig cells in the testes and diffuses to adjacent seminiferous tubules.

Autocrine. A variation of the system is when the hormone-secreting cell and the target cell are the same. Examples of autocrine hormones are the prostaglandins, thromboxanes, leukotrienes, and lipoxins.

Neurotransmitters. It is now generally accepted that neurotransmitters are hormones, chemical messengers. Thus, a neuron normally innervates a single cell. The electric signal may travel a long distance over the axon. This electric signal is then transformed into a chemical mediator, the neurotransmitter, which is secreted by the axon. The neurotransmitter diffuses locally across the synapse to the adjacent receptor. Thus, neurotransmitters such as acetylcholine and norepinephrine may be considered paracrine hormones.

An understanding of the utility of determining hormone concentration in blood is essential in the management of endocrine tumors. The analytic test procedures are assessed for precision (reproducibility) and accuracy (closeness to correct or true value). A test may be referred to as sensitive (capable of measuring small quantities) and/or specific (free of interference by other substances). These

terms are used in quite a different way when describing the clinical utility of a test (see below).

The distinction between a test result being a true positive (TP) or true negative (TN) is determined with reference to a selective normal range based on 95% confidence limits. Because some normal people fall outside this range (*false positive* [FP]) and some with disease fall within it (*false negative* [FN]), the term reference range (RR) is preferred. For any particular disease, attention is focused at either the upper or lower limit of this range, so we are really considering a reference value (RV). A major problem is to define for each analyte the RV that provides the greatest usefulness in the process of coming to a decision. This is called the decision threshold or decision point.

SENSITIVITY AND SPECIFICITY

To properly utilize hormone concentration analysis, several statistical constructs must be understood.[1, 2] These laboratory test statistics are best illustrated as a 2×2 decision matrix (Fig. 1–1). The *sensitivity* of a test is a measure of its positivity, the percentage of patients with the disease that fall beyond the RV (Fig. 1–2). This is 100% at point A.

$$\text{Sens} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100$$

= % of disease cases that give a positive result with the RV chosen

The *specificity* of a test is a measure of its negativity, the percentage of patients without the disease that fall within the RR. It is 100% at point C in Figure 1–2.

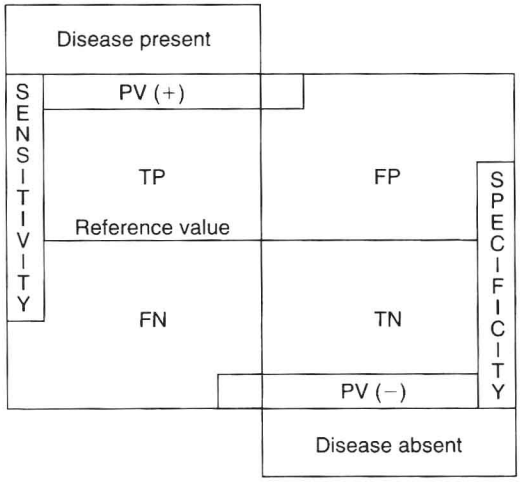

Figure 1–1. A 2×2 decision. The matrix illustrates the meanings of sensitivity, specificity, and predictive value (PV) of a clinical laboratory assay.

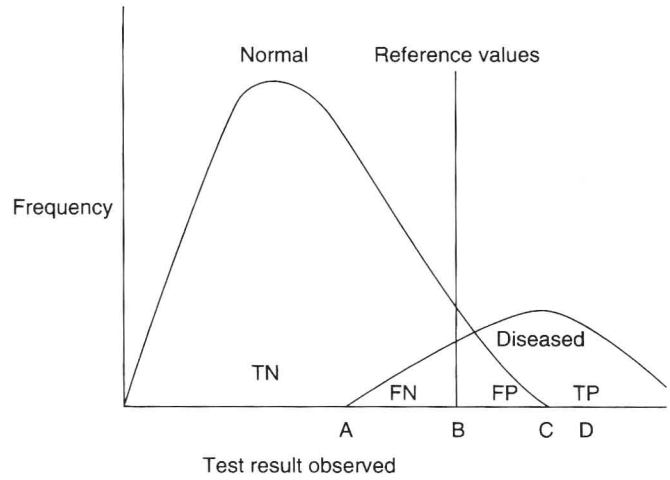

Figure 1–2. Distribution of results for a biochemical analyte in normal and diseased populations. *A, B, C,* and *D* are decision points referred to in the text.

$$\text{SPEC} = \frac{\text{TN}}{\text{TN} + \text{FP}} \times 100$$

= % of nondisease cases that give a negative result with the RV selected

Moving the RV toward the normal range increases sensitivity for ruling disease out. Moving the RV toward the disease range increases specificity for ruling disease in. The particular clinical situation will give the optimal choice of RV. Very few laboratory analyses have such high sensitivity and specificity that they are pathognomonic for a particular disease. Tests currently employed utilizing molecular diagnostic techniques to determine DNA polymorphisms may eventually hold this position.

The essential characteristic of any test is its predictive value (PV). PV depends not only on sensitivity and specificity but also on disease prevalence. The PV is also affected by RV and the TP or TN rates. The PV indicates the information that can be gained by performing the test.

The predictive value of a positive test:

$$\text{PV (+)} = \frac{\text{TP}}{\text{TP} + \text{FP}} \times 100$$

The predictive value of a negative test:

$$\text{PV (−)} = \frac{\text{TN}}{\text{TN} + \text{FN}} \times 100$$

Laboratory data are most useful ruling out rather than ruling in a diagnosis. A test with a high PV^+ works best in a high-prevalence population. In a low-prevalence population, tests with high PV are more useful. As the prevalence increases, the PV of a positive test increases, and the PV of a negative test decreases. As the diagnosis becomes less likely, the PV

of a negative test increases, and the PV of a positive test decreases. Note that 100−PV is the probability that a patient has a particular disease in spite of a negative test result.

The efficiency of a laboratory test is the percentage of all results that are true results, whether TP or TN. Efficiency is expressed as follows:

$$\frac{TP + TN}{\text{Total tests}} \times 100$$

$$\text{This is the same as } \frac{\text{sens} + \text{spec}}{2}$$

The efficiency of flipping a coin is 50%. A perfect test would have 100% efficiency. Generally, a laboratory test is not worthwhile unless its efficiency is greater than 80%.

A receiver operating characteristic (ROC) curve is useful both in assessing the clinical value of different tests and in selecting the best RV for the test chosen. The ROC curve is based on the relation of the true positive to the false positive rate. It is obtained by plotting the TP ratio [TP (TP + FN) = SENS] versus the FP ratio [FP/(FP + TN) = 100−SPEC] for a series of reference values. One can also calculate a *likelihood ratio,* which is the ratio of these two conditional probabilities. The ratio of TP to FP for detecting disease and the ratio of FN to TN for excluding diseases are as follows:

$$\text{For a positive test } \frac{\%\ TP}{\%\ FP} = \frac{SENS}{100 - SPEC}$$

$$\text{For a negative test } \frac{\%\ FN}{\%\ TN} = \frac{100 - SENS}{SPEC}$$

COMPETITIVE-BINDING ASSAYS FOR HORMONE CONCENTRATION MEASUREMENT

Berson and Yalow's[3] observations in the 1950s on the behavior of insulin-binding antibodies gave rise to the technique known as competitive assay. Ligand assay has proliferated because it permits sensitive and accurate quantitation of a variety of compounds (peptides, steroids, vitamins, thyroid hormones, drugs) of biologic importance over a wide range of concentrations.[4] Before development of ligand assay technology, many hormones were measured by crude chemical or in vivo assays using various laboratory animals.

Ligand competition-binding assays have several principles in common. They depend on similar behavior of the standard to be measured and that of the unknown. They depend on the laws of mass action to partition the compound to be measured between the free state and the state of being bound to a binding reagent with a specific but limited capacity to bind the compound. They depend on a means of distinguishing bound compound from free compound. Many different specific binding agents have been used in ligand-binding assays, including antibiotics, naturally occurring proteins, hormone receptors, and enzymes. All of these binding reagents have advantages and disadvantages. The most important differences in the binding agents relate to their affinity (how avidly they bind the compound to be measured) and specificity (how specifically they bind only the compound being measured). The association constant (Ka) of a binding reagent is obtained by measuring how strongly a compound is bound to a binding reagent. Generally, the greater the Ka of a binding reagent, the more stable the association of the binding reagent and specifically bound compound.[5]

Radioimmunoassay

Radioimmunoassay (RIA) is based on the interaction between a radioisotopically-labeled compound and an antibody directed against it that can be inhibited by an unlabeled compound (Fig. 1-3). RIA is commonly used because antibodies can be prepared to a wide variety of compounds including proteins, peptides, steroids, and medications.

Potential drawbacks to RIA include cross-reactivity of antibody with compounds other than the one to be measured; difficulty inducing antibody to the same compounds; multiple antibodies formed to the antigen in each individual immunized animal and the resultant difference in characteristics or immunologic properties of different antibodies in one animal for a specific compound; and antibodies induced to the same specific antigen in different animals of the same species will vary in their properties, making it necessary to characterize the individual antibodies from different animals to the same antigen. Finally, in an RIA immunologic reactivity rather than biologic activity is being measured.

When an animal is immunized with an antigen, that animal may produce more than one antibody to the antigen. Currently, cell lines are more usually developed that produce antibody to a single antigen determinant. These monoclonal, antibody-forming cell lines—hybridomas—are produced by fusing an antibody-forming mouse spleen cell with myeloma cells. These hybridomas are capable of producing large quantities of monoclonal homogeneous antibody.

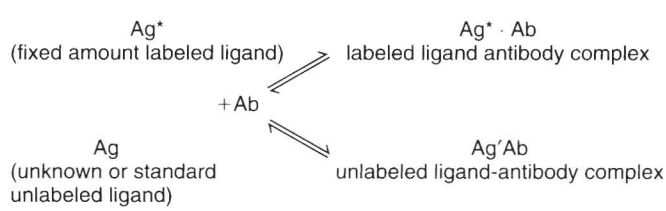

Figure 1-3. Representation of the principle of competitive radioimmunoassay.

This can be contrasted with heterogeneous or polyclonal antibodies that recognize different antigen determinants of the same compound that are formed when an animal is immunized.[6]

Radioassay

Binding proteins that occur in nature may be used as specific binding reagents. Compared with RIA, these binding proteins have a lower binding affinity and are less specific.[7] Serum ordinarily contains proteins that bind cortisol thyroid hormone, testosterone, and vitamin B_{12}. When these proteins are used in an assay, similar serum-binding proteins must be destroyed by either heating the sample or precipitation of interfering binding activity prior to radioassay analysis. The advantage of radioassay using naturally occurring binding proteins relates to their availability and relative inexpensiveness, ease of preparation, storage stability, and consistency from one preparation to another. The disadvantage of these naturally occurring binding proteins is that they have lower affinity (sensitivity) and specificity than RIA. Binding proteins are also available for a limited number of compounds.[4]

Receptor Assays

Receptors for various compounds have been localized on cellular membranes and in both the cytoplasm and the nuclei of cells. These receptors are generally directed toward the biologically active portion of a molecule, which need not be the most immunologically active portion. Disadvantages of receptor assays are that they tend to be unstable; they must be prepared from a source where they are present in small amounts, then concentrated; the techniques used to separate receptors often decrease the number of intact receptors isolated and may adversely alter the binding characteristics of those receptors; receptors may be altered to recognize small changes in compounds produced by radioisotope labeling, making the labeled compound a less than optimal tracer for a particular assay; receptor assays are generally less sensitive than RIAs; and receptors may be occupied by nonspecific binding agents. Advantages of receptor assays include uniformity of characteristics from preparation to preparation and assessment of biologic activity as opposed to immunologic activity.

Ligand Labels for Competitive Binding Assays

Labels in competitive binding assays have included radioactive isotope–labeled molecules, enzyme a tivity, fluorescence, and chemiluminescence. The labeled molecule need not behave exactly as the unlabeled compound, but the two compounds must display similar behavior in the assay system.[5, 8]

Immunoassays can be divided into two types: homogeneous, in which free label can be distinguished from bound label without physical separation of the two, and heterogeneous, in which free and bound label must be separated physically before the assay is completed. It is desirable for the labeled and unlabeled compounds to have similar affinities for the binder. If the affinity of the labeled compound is less than that of the unlabeled compound, the sensitivity of the assay will be decreased. Additionally, if the labeled compound interacts with the binder differently than does the unlabeled compound or has biochemical properties different from those of the unlabeled compound, assay results may not be predictable.[4, 5, 8]

Rad oactive Labels

Radiolabels have been used in immunochemical assays because they offer great flexibility and allow for extremely sensitive assays. Two principal types of label are employed: beta emitters such as tritium (^3H) and gamma emitters such as iodine 125 (^{125}I). Each isotope group has different properties, which form the basis for their being employed in specific assays. ^3H has a long half-life (12.3 years) when compared with ^{125}I (60.2 days), and ^3H is easily incorporated into organic compounds. However, ^3H has several disadvantages: the specific activity of ^3H is much less than for ^{125}I-labeled compounds, and ^3H, a beta emitter, can be assayed using only a liquid scintillation counting system. Gamma-emitting radioisotopes have several advantages over beta-emitting radioisotopes when used in immunoassays. Gamma detection is possible directly from the assay tube. Because of its high specific activity, less gamma-emitting radioisotope is required for reliable determination of the quantity of isotope in a sample, and counting time is reduced.[9]

Labeled Binders— Immunoradiometric Assays

Immunoradiometric assays (IRMAs) differ from RIAs and radioassays in that the compound to be measured binds to a labeled antibody present in the assay in excess. Free and bound antibodies are separated, often by exposing the reaction mixture to antigen coupled to a solid phase. The radioactive-labeled antibody that is not bound to the solid phase–attached antigen reflects the concentration of antigen in the sample (Fig. 1–4). In some IRMAs, two antibodies are reacted to the antigen being measured. One of the antibodies is coupled to a solid phase and the other, which is radiolabeled, is added to the assay after incubation with the solid phase–bound antigen-specific unlabeled antibody. This type of assay is called a two-site IRMA or sandwich IRMA. In a two-site IRMA, the amount of radiolabeled antibody attached to the solid phase reflects the concentration of antigen present.[10]

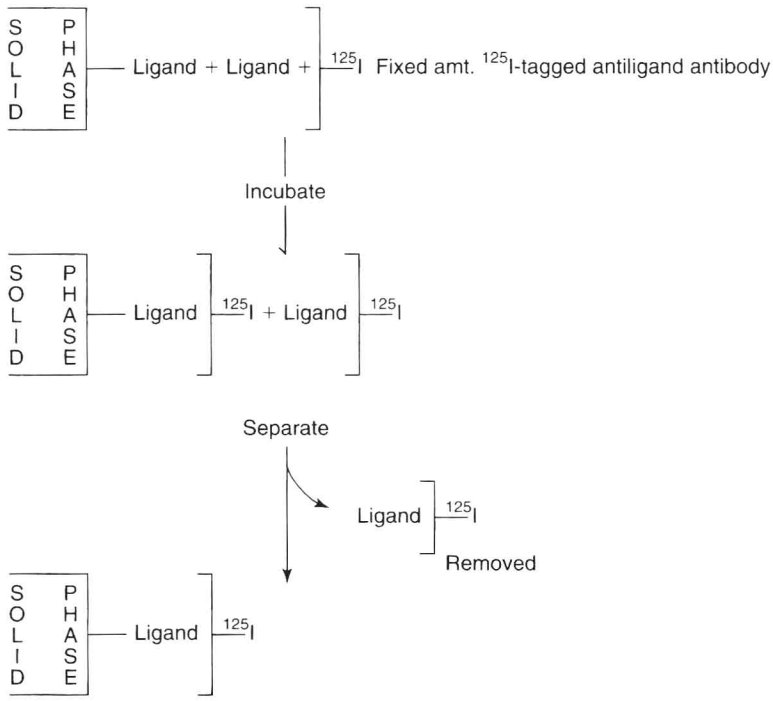

Figure 1-4. Representation of an immunoradiometric assay.

Enzyme Labels, Enzyme-Linked Immunosorbent Assays

Enzymes may be employed in place of radioisotope to label antigens or antibodies. In this approach, analogous to that of RIA, the enzyme is coupled to the antigen to be studied. Enzymatic activity is then determined in either bound or free antigen after separation of the two moieties. Enzymes are convenient to use because they are stable. Urease, peroxidase, and alkaline phosphatase are frequently employed. Enzymes may be bound to antigen-specific antibodies and used in a method analogous to that of an IRMA sandwich assay. In enzyme-labeled systems, in which the antigen is covalently linked to the enzyme, binding of the antigen to antibody may decrease enzyme activity. Thus, by determining enzymatic activity, the amount of antibody-bound and free enzyme–labeled antigen in the sample can be determined. This particular enzyme-linked immunosorbent assay is homogeneous because it is not necessary to separate bound and free antigen before assessing enzyme activity. It is called an enzyme multiplied immunoassay technique. The inhibition of enzyme activity in antibody-bound enzyme-labeled antigen results from either conformational changes occurring in the enzyme as a result of antibody binding to the antigen-enzyme complex or as a result of antibody preventing conformational changes necessary for the enzyme activity. Disadvantages of enzyme-linked immunosorbent assay arise due to enzyme labels hindering antigen-antibody binding, resulting in diminished assay sensitivity. Additionally, enzymatic labeling of antigens and antibodies may be difficult, and naturally occurring substances may inhibit or interfere with quantitation of enzyme activity.[11]

Fluorescent Labels

The advantages of fluorescent labels include their low cost, stability, relative inexpensiveness of instrumentation used to measure fluorescence, and the speed of assay performance. Fluorescent label immunoassays are inherently less sensitive than RIAs. Additionally, the sensitivity of these assays is diminished by background fluorescence. Time-resolved fluorometry minimizes background fluorescence by using a pulse of light to excite the fluorescent label. Fluorescence is then measured during a specific time after excitation. Some fluorescent immunoassays do not require separation of bound and free label to quantitate the analyte being measured. This technology includes the fluorescence polarization method of immunoassay and homogeneous assays using nonfluorescent precursors as enzyme substrate. In fluorescent polarization immunoassay, polarized light excites a fluorescent label. As this label is bound, the degree of polarization of the fluorescent emission from the label increases. The disadvantage of this method is a nonlinear relationship between the concentration of the test compound and the polarization of the fluorescent emission. This type of assay also has a limited concentration range over which the compound being assayed can be accurately measured.[12]

CLINICAL APPLICATION OF LIGAND-BINDING ASSAYS

Discrepancies in Hormone Ligand-Binding Assays

Hormone ligand assays have greatly increased understanding of endocrine physiology and pathophysiology. However, data obtained from ligand-binding assays must be evaluated critically.[13] Examples of discrepancies when clinical observations and bioassay data are compared with results obtained in ligand-binding assays are numerous. Confounding factors may include immunologic or binder-active hormone with diminished biologic activity[14]; biologically active hormones that are immunologically or binder nonreactive in a given assay[15]; immunologically related hormones with different biologic functions[16]; hormone fragments that are immunologically active but biologically inactive[14, 17]; the influence of drugs or other substances that interfere with an assay, such as native antibody, against the ligand being assayed or against the binding agent[18]; and the influence of disease or an unusual circumstances on hormone clearance.[19]

PEPTIDE HORMONE HETEROGENEITY

When a serum sample is treated by various separation techniques and then assayed for ligand binding activity, many polypeptide hormones are found to exist in multiple or heterogeneous forms.[20] Adrenocorticotropic hormone (ACTH) and gastrin-producing tumors are good examples. Neoplasms often produce a very large polypeptide called big ACTH.[21] This molecule has only about 5% of the biologic activity of native ACTH, and it often causes Cushing's syndrome. If native ACTH is assayed following serum fractionation in such cases, the concentration of biologically active ACTH might be underestimated. Conversely, when such a serum is assayed without fractionation, high levels of ACTH might be observed in the absence of clinical illness. This would be due to the presence of an increased concentration of immunologically reactive but biologically inactive big ACTH. In the case of gastrin, normal gastrin (a 34-amino-acid peptide) as well as a 17-amino-acid peptide is produced in response to feeding. Both peptides are biologically active, but the 17-amino-acid peptide is cleared considerably faster. Consequently, a clinical condition such as a gastrinoma could theoretically arise in which the 17-amino-acid peptide was produced and immunologic reactivity for gastrin might be normal in the presence of hypersecretion of gastrin and gastric acid (Zollinger-Ellison syndrome).[20]

LIGAND-BINDING ASSAYS OF THYROID HORMONES

Ligand-binding assay is one of the prime tools employed in assessing thyroid functions. As with steroid hormones, a plasma-binding protein for thyroid hormone was the basis for some early thyroid hormone ligand-binding assays, as is true for cortisol-binding globulin and sex hormone–binding globulin. Therefore, thyroid hormone levels increase in pregnancy, estrogen therapy, and chronic liver disease. However, the fraction of free thyroxine would remain normal. As is the case with other hormones where plasma-binding proteins are present, absolute certainty about hormone status requires measurement of the free fraction of hormone. In assessing thyroid status, currently utilized thyroid-stimulating hormone assays have sufficient sensitivity to accurately assess thyroid status over the entire clinical spectrum.[22]

AUTOANTIBODIES AND LIGAND-BINDING ASSAYS

Assay problems and pathophysiologic states can arise as a result of antibody production to an antigen (hormone or ligand), the immunoglobulin used to assay an antigen, or the antigen's receptor site. The use of monoclonal antibody in immunoassay and the increasing clinical application of mouse monoclonal antibody for targeted imaging and immunotherapy have created a new problem. Treated patients produce heterophilic antibodies against murine antibodies, which interfere with immunoassays. In most cases, the cross-linking of divalent heterophilic antibody to both the primary monoclonal antibody and the enzyme-conjugated secondary monoclonal antibody mimics an antigen, resulting in a falsely elevated value. However, interference with antigen binding by the heterophilic antibodies can decrease the results of an immunoassay.[23] It is known that 15%–40% of individuals may have one or more heterophilic antibodies. Heterophilic antibody interference can be reduced by addition of mouse serum or nonspecific mouse immunoglobulin to the immunoassay, which will complex the heterophilic antibody and make it unavailable for binding to the murine monoclonal employed in the assay.

AUTOMATED LIGAND-BINDING ASSAYS

The pressure to contain costs has necessitated the development of less expensive hormone assay techniques. This cost saving, along with vastly improved assay turnaround times, has been realized recently with the development of automated assays systems for ligand-binding assays. These automated systems vary considerably and generally include automated pipetting, separation steps, assay calculation, and reporting assay data in an appropriate format. The fact that assay pipetting, washing, and quantitation are automated leads to reduced inter- and intra-assay variability.