Chapter 7
ORGAN FUNCTION

ORGAN FUNCTION

Organ function is assessed by measuring how well an organ is doing what it is supposed to be doing. For example, the pump function of the heart is studied by measuring the cardiac output and the blood pressure; the cognitive function of the brain is investigated using intelligence tests. The functional status of synthetic organs, organs of cell generation (such as the marrow), organs of elimination, and organs of absorption can also be evaluated. The laboratory studies used to make these measurements may assay the organ's function directly, such as analyzing semen to determine testicular function. More often, though, the studies assess function only indirectly, using the blood or plasma concentration of some secreted, eliminated or absorbed substance as a quantitative marker of the functional status.

SYNTHESIS AND CLEARANCE

The indirect assessment of an organ's synthetic function is based upon the logical notion that the functional status of an organ is reflected in the rate of synthesis of the product secreted by the organ. Given an understanding of the relationship between the plasma concentration of the product and the synthetic rate of the product, a measurement of the product's plasma concentration can be used to infer the organ's functional status. In a similar fashion, the functional status of an organ of elimination is assumed to be revealed by the rate of removal of a substance by the organ. Given the relationship between the plasma concentration of the removed substance and the rate of its removal, the functional status of the organ can be inferred from the plasma concentration of the substance.

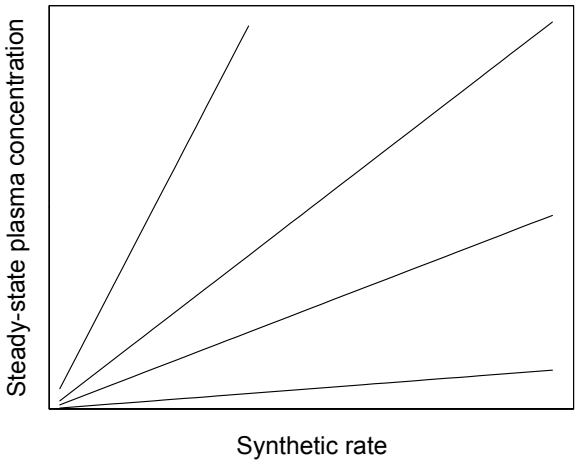

Figure 7.1 Steady-state relationship between substance concentration and synthetic rate for four different clearance rates.

Under steady-state conditions, the relationship between the rate of synthesis of a substance secreted into the plasma and its plasma concentration and the relationship between the removal rate of a substance and its plasma concentration are expressed in a single simple equation (DiStefano 1976),

$$\textit{plasma concentration} = \frac{\textit{synthetic rate}}{\textit{clearance rate}}$$

Synthetic rate quantifies the entry of a substance into the plasma. Its dimensions are amount per unit time (e.g., millimoles per hour). It appears in the numerator because the plasma concentration of a substance is directly related to its rate of entry into the plasma; the more rapid the entry, the greater the concentration. Figure 7.1 shows the direct relationship between plasma concentration and synthetic rate at various clearance rates. Clearance rate quantifies the removal of substance from the plasma. It has the dimensions volume per unit time (e.g., milliliters per minute). Substance concentration is inversely related to its rate of removal, so this term appears in the denominator. Figure 7.2 illustrates the hyperbolic relationship between substance concentration and clearance rate at different synthetic rates.

The volume of distribution is the constant of proportionality between the concentration of a substance and the amount of the substance. The substance concentration in the steady state does not depend upon the volume of distribution of the substance; it is determined solely by the synthetic rate and clearance rate. On the other hand, the time needed to attain the steady state (as a rule of thumb, five half-lives of the substance) depends upon both the clearance rate and the volume of distribution.

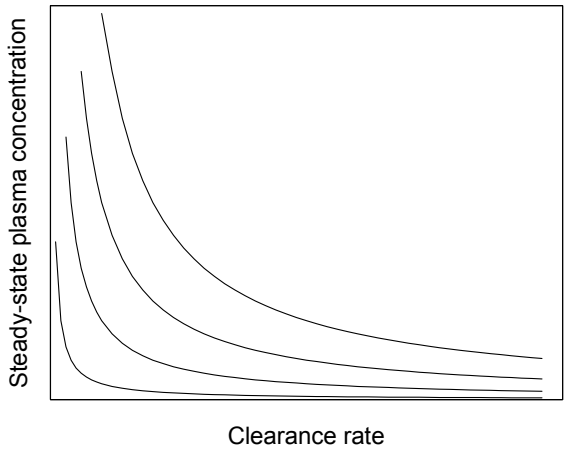

Figure 7.2 Steady-state relationship between substance concentration and clearance rate for four different synthetic rates.

This dependence is revealed in the following form of the formula for half-life:

$$\textit{half-life} = 0.693 \times \frac{\textit{volume of distribution}}{\textit{clearance rate}}$$

Organ synthetic rate

Direct measurement of organ synthetic rate is possible only for a limited number of organs: the stomach, pancreas, and testes. The secretory products of all other organs are released in ways that do not permit their collection. Consequently, the functional status of these organs must be assessed indirectly. The indirect measurement of organ synthetic rate is based upon the steady-state relationship between the plasma concentration of a marker substance and its synthetic rate. Rearrangement of the relationship yields

$$\textit{synthetic rate} = \textit{clearance rate} \times \textit{plasma concentration}$$

If it is assumed that the marker substance is secreted only by the organ of interest and that the clearance rate of the substance is a constant, organ synthetic rate can be estimated from the plasma concentration of the marker substance. Because the relationship is not only linear but directly proportional, the magnitude of a change in synthetic function is exactly reflected in the proportional change in the concentration of the marker. If the clearance rate remains constant, a twofold elevation in the marker substance levels, for instance, indicates an identical twofold increase in synthetic activity; a one-third reduction in the concentration of the marker reflects a one-third decrease in synthetic rate.

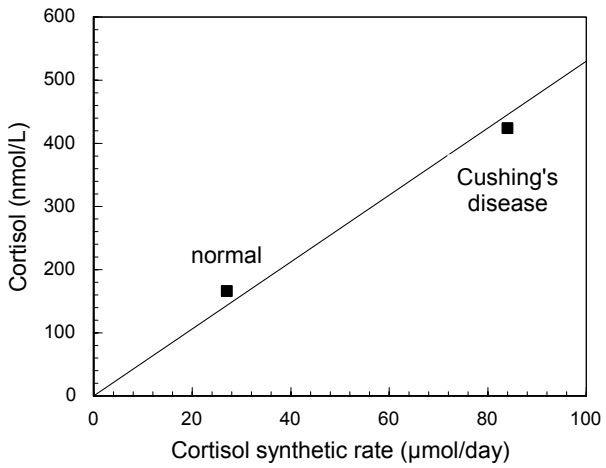

Figure 7.3 Steady-state relationship between plasma cortisol concentration and cortisol synthetic rate. The mean concentration and synthetic rate pairs for normal adults and for patients with Cushing's disease are indicated.

Figure 7.3 illustrates the proportional relationship between the plasma concentration of cortisol and its synthetic rate. The cortisol synthetic rate and daily average plasma concentration in normal adults and in patients with Cushing's disease are also indicated in the figure (Esteban *et al.* 1991). Individuals with Cushing's disease have increased cortisol synthetic rates; the patients represented in the figure have a cortisol synthetic rate 3 times greater than normal and a daily average cortisol concentration which is 2.5 times larger than normal.

Organ clearance rate

Organ clearance rate can be measured directly if the eliminated substance can be collected. The substance being removed is collected over time and its elimination rate is determined. During the collection a blood specimen is taken, and the concentration of substance in the plasma is assayed. The organ clearance rate is then calculated using the formula for clearance rate defined by

$$\textit{clearance rate} = \frac{\textit{elimination rate}}{\textit{plasma concentration}}$$

In practice the collection of eliminated substances is possible only for substances excreted into the urine. Direct measurement of organ clearance rate is thereby limited to the kidney.

Indirect measurement of clearance function is required for such organs as the liver and lungs. The clearance function of the kidney can also be evaluated by indirect measurement, which is particularly useful when the collection of a timed urine specimen

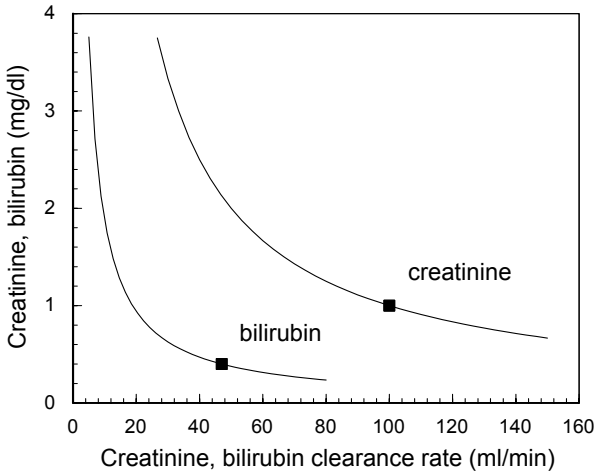

Figure 7.4 Steady-state relationship between plasma concentration and clearance rate for unconjugated bilirubin, creatinine, and carbon dioxide. The mean concentration and clearance rate pairs for normal adults are indicated by squares.

is impractical. Indirect measurements are based upon the steady-state relationship of the plasma concentration of a marker substance and the substance's clearance rate. Rearrangement of the relationship yields

$$\textit{clearance rate} = \frac{\textit{synthetic rate}}{\textit{plasma concentration}}$$

If it is assumed that the marker substance is eliminated solely from the organ of interest and the rate of synthesis of the substance is a constant, organ clearance rate can be estimated from the plasma concentration of the marker substance. Unlike the synthetic rate, however, this relationship is not linear but hyperbolic. Consequently, the magnitude of the change in the plasma concentration of a marker substance following the alteration in organ function will depend not only upon the magnitude of the alteration but also upon the clearance rate present prior to the alteration. Figure 7.4 shows the hyperbolic relationship between clearance rate and plasma concentration for creatinine (Cockcroft and Gault 1976), which is cleared by the kidneys, and unconjugated bilirubin (Berk *et al.* 1969), which is cleared by the liver. Representative normal values for the plasma concentrations and clearance rates are shown as squares. For both these substances, starting from a normal clearance rate, a reduction in the clearance rate will result in only a very modest elevation in the plasma concentration. When organ function is poor, however, and the clearance rate is low, even a slight additional reduction in the clearance rate will cause a marked increase in plasma substance concentration. Thus, marker substances of organ clearance function show increasing sensitivity to changes in organ function as the organ's functional status deteriorates.

Clearance of exogenous substances. Exogenous substances can be used instead of endogenous markers for the evaluation of clearance function. Renal clearance rates can be measured directly, but the clearance of exogenous substances by other organs must be measured indirectly. Of course, renal clearance can be measured indirectly also. As is true for endogenous substances, exogenous marker substances must be eliminated solely from the organ of interest for the indirect measurements to be valid.

If an exogenous substance is administered by continuous infusion its clearance rate can be calculated, once the steady state is achieved, by using the relationship

$$\textit{organ clearance rate} = \frac{\textit{infusion rate}}{\textit{plasma concentration}}$$

It is often impractical to administer a continuous infusion or to wait for steady state to be achieved, however. In such cases the exogenous substance can be given either by bolus injection or by short infusion or it may be given orally. The plasma concentration of the substance is followed over time, and a plasma disposition curve is constructed, as illustrated in Figure 7.5. The clearance rate can then be calculated using the formula

$$\textit{organ clearance rate} = \frac{\textit{amount administered}}{\textit{AUC}}$$

where AUC is the area under the disposition curve.

The number and timing of samples is of obvious practical importance when performing clearance studies. The sampling schedule should be designed to achieve minimum measurement variability in the estimation of the clearance rate while minimizing the discomfort and inconvenience to the patient and the expense of analyzing the specimens. If the substance is administered by continuous infusion, one specimen is adequate but it must be obtained after reaching steady state. If the substance is administered by bolus injection and has a monoexponential plasma clearance curve, two specimens should be taken, the first as soon as practical after the injection of the substance is complete and the second at the anticipated turnover time of the substance in the patient (1.44 times the plasma half-life). This sampling scheme results in the maximum precision in the estimation of the clearance rate (Dossing *et al.*

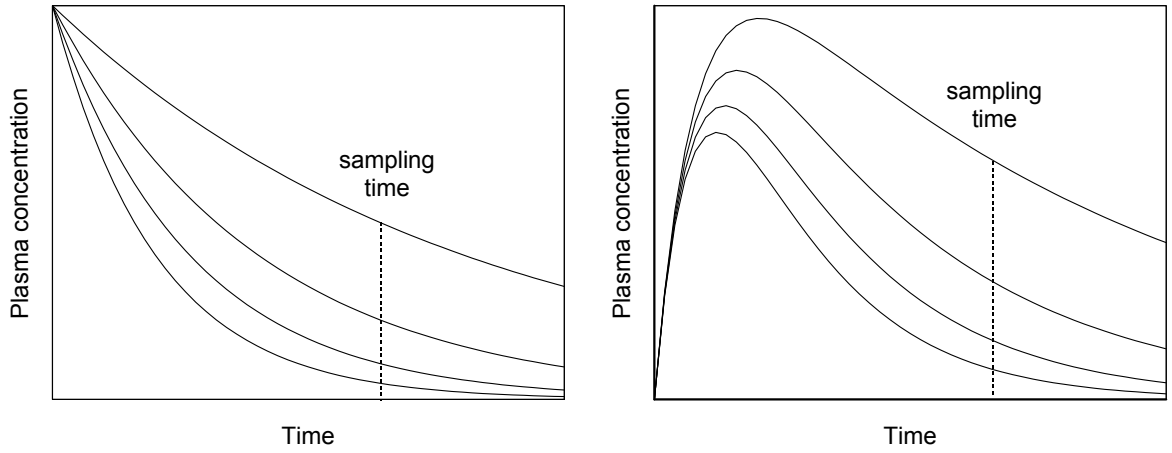

Figure 7.5 Plasma disposition curves for an exogenous substance given by bolus injection (left graph) and by oral administration (right graph). For each mode of administration, the disposition curves for four different clearance rates are shown.

1983). The clearance rate is calculated using the formula,

$$\textit{organ clearance rate} = \frac{\textit{amount administered}}{\frac{C_1}{ln\{C_1/C_2\}}\,\tau}$$

where C_1 is the plasma substance concentration immediately following administration and C_2 is the plasma substance concentration of the second specimen, drawn at time, τ.

If the substance is administered orally, by short intravenous infusion, or if it has a multiexponential plasma clearance curve, multiple specimens will be needed. The number and timing of the samples will vary considerably from substance to substance so the appropriate literature should be consulted as an aid in the design of the sampling schedule and the calculation of the area under the plasma clearance curve.

In clinical practice, clearance studies often rely upon a single concentration determination. In such studies the actual clearance rate is not calculated but, rather, the extent of substance removal, as indicated by the plasma concentration of the substance at a set time following the administration of the substance, is measured (Figure 7.5). This is a less accurate way to conduct a clearance study because of the variability in plasma substance concentrations that results from interindividual variability in the volume of distribution and rate of absorption of the marker substance. Even if the sampling time for a one-sample clearance study can be set at a time that results in little measurement variability for a particular clearance rate, that time cannot be well suited for measuring the clearance of substance in patients with different clearance rates. In particular, for patients who have a clearance rate less than the one for which the study performs well, the specified sampling time will fall earlier in the plasma clearance of the substance when the effects of variability in the volume of distribution or in the rate of substance absorption are larger.

ABSORPTION

Absorption is the uptake of a substance by an organ that conveys that substance to or from the external environment. Of particular concern to the clinician are the absorption of oxygen by the lungs, the absorption of nutrients by the gastrointestinal tract, and the reabsorption by the renal tubules of substances filtered at the glomerulus.

Direct measurement of absorptive function is possible and is frequently employed; the rate or amount of a substance entering an organ of absorption can be determined by sampling the input material and the rate or amount of substance leaving the organ can be ascertained by sampling the output of the organ. For instance, the tubular reabsorption of sodium in the kidney can be quantified by calculating the rate of sodium input into the tubules (computed as the product of the plasma sodium concentration and the glomerular filtration rate) and by measuring the rate of sodium excretion in the urine (computed as the product of the urine sodium concentration and the urine flow rate). Dividing the rate of excretion by the rate of input yields the fraction of sodium excreted. One minus that fraction is the fraction of sodium reabsorbed.

Indirect measurement of absorptive function is also possible. One approach is to use the

steady-state relationship of the blood concentration of an absorbed substance and the substance's clearance rate,

$$\textit{blood concentration} = \frac{\textit{absorption rate}}{\textit{clearance rate}}$$

Rearrangement of this equation yields

$$\textit{absorption rate} = \textit{clearance rate x blood concentration}$$

If it is assumed that the substance is absorbed continuously and that the clearance rate of the substance is a constant, the absorption rate of the substance can be estimated from its concentration in the blood. This is a very convenient way to assess absorptive function but the assumptions that enter into the calculation restrict its applicability. For example, gastrointestinal absorption is episodic, not continuous.

Another approach to the indirect measurement of absorptive function is to administer a marker substance and, by measuring the plasma concentrations of the substance over time, deduce the extent of absorption of the substance. The relationship between the amount of substance administered and the substance's plasma concentrations is

$$AUC = \frac{\textit{fraction absorbed x amount administered}}{\textit{clearance rate}}$$

where AUC is the area under the disposition curve. Rearrangement of this equation gives,

$$\textit{fraction absorbed} = \frac{\textit{clearance rate x AUC}}{\textit{amount administered}}$$

If the clearance rate of the substance is constant, the relationship between the fraction of the substance absorbed and the AUC (normalized to the amount of substance administered) will be linear. Importantly, no assumptions need to be made concerning the kinetics of absorption for the relationship to hold.

Determinants of absorption

When abnormal absorptive function is identified in a patient, it maybe desirable to evaluate the patient further to determine if the abnormality can be localized to the lumen, to the absorptive epithelium, to the vascular bed, or to transport proteins in the blood.

Factors in the lumen that influence absorption include the amount of input and the intraluminal processing that must occur to render a substance absorbable. The first of these, the amount of input, is not a derangement of absorptive function *per se* but it is an important mechanism of abnormal absorptive intake. Decreased input (i.e., dietary deficiency or hypoventilation) as a cause of subnormal absorption of a substance is evaluated by enriching the input with the substance. If absorptive function is normal, the delivery of increased amounts of substance to the absorptive organ will result in an increase in the amount of the substance absorbed. This can be detected as an increase in the concentration of the substance in the plasma or as an increase in a measure of the total body stores of the substance. Disordered intraluminal processing of a nutrient (i.e., maldigestion) is commonly the cause of decreased gastrointestinal absorption. The disorder may be nearly global, such as occurs in pancreatic insufficiency, may affect a class of nutrients, such as the effect of bile salt deficiency upon the absorption of the fat-soluble vitamins, or may be specific for a single nutrient, such as the inability to absorb vitamin B12 due to intrinsic factor deficiency in pernicious anemia. The administration of a preprocessed form of an affected nutrient will allow for normal absorption of the nutrient if maldigestion is the cause of the nutrient's subnormal absorption. This is the basis of the Schilling test for pernicious anemia in which the extent of absorption of vitamin B12 administered already bound to intrinsic factor is compared to that of vitamin B12 administered alone.

The uptake of substances by an absorptive epithelium is accomplished by simple diffusion, by passive transport, or by active transport. Uptake by simple diffusion is impaired by a reduction in the surface area for absorption. This can occur as a result of segmental loss of functioning organ tissue or by localized or generalized remodeling of the architecture of the absorptive surface, such as in the flattening of intestinal villi in celiac disease and the loss of alveolar walls in emphysema. Passive and active transport may also decrease when the absorptive surface area is reduced. However, if homeostatic regulation of an absorbed substance includes regulation of the expression of the epithelial membrane transport proteins or intracellular binding proteins that effect the transport, increased expression of the proteins will partly compensate for decreases in the surface area. Hypoabsorption or hyperabsorption of a regulated substance may occur as a result of a disordered homeostatic system's effect upon the epithelial expression of its transport proteins. The absorptive dysfunction may even

serve as a marker of the disorder. Hyperparathyroidism, for example, leads to decreased renal tubular reabsorption of phosphate. Renal phosphate reabsorptive capacity therefore can, and indeed has, been used as a diagnostic study in this disease.

The distribution of blood flow through the vascular bed is a major determinant of the uptake of oxygen by the lungs. This is because of the alveolar structure of the lungs: capillary blood absorbs oxygen from only one alveolus; if the blood supplies an alveolus that is hypoventilated or if the blood is shunted around the alveolus into the venous circulation, oxygen uptake will be reduced. Normally, autoregulatory mechanisms redistribute blood away from hypoventilated alveoli and the degree of shunting is small. However, in the presence of pulmonary disease, appreciably amounts of pulmonary blood flow may pass through shunts (right-to-left shunting) and the distribution of blood flow may be impaired with overperfusion of hypoventilated alveoli and reciprocal underperfusion of normo- and hyperventilated alveoli (called, ventilation-perfusion mismatch). The contribution of these two mechanisms of oxygen hypoabsorption to a patient's hypoxemia can be assessed by increasing the oxygen content of the air inspired by the patient. This will correct the impaired absorption due to hypoventilation and therefore to ventilation-perfusion mismatch. This maneuver will not reverse the hypoxemia due to shunting. Because normally aerated blood is highly saturated with oxygen, when unshunted pulmonary blood is exposed to alveolar air with an high oxygen content, the increase in oxygen carriage in the unshunted blood is very small. Consequently, the shortfall in the oxygen content in shunted blood cannot be made up for by an increase in the oxygen content of unshunted blood; the mixed pulmonary venous blood will remain underoxygenated.

Finally, if the capacity of the blood to transport a substance from its site of absorption depends upon the substance binding to a protein in the blood, the blood concentration of the transport protein will also affect the absorption of the substance. Measurement of the transport protein concentration is then an important component of the evaluation of absorptive function. For example, the uptake of oxygen in the lungs is determined largely by the concentration of hemoglobin in the blood as the oxygen transport capacity of plasma is very small. So, the clinical evaluation of oxygen transport must include measurement of the blood hemoglobin concentration.

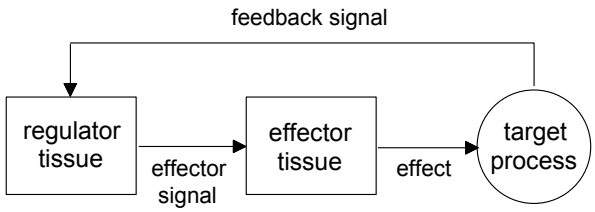

Figure 7.6 A model of active homeostatic regulation.

HOMEOSTATIC SYSTEMS

One of the fundamental activities of physiologic systems is to maintain a constant internal environment despite perturbing stimuli. This is homeostasis (from the Greek *homio*, similar or like, and *sta*, to stand). Homeostasis most often is accomplished actively. Figure 7.6 illustrates the essential components of active homeostatic regulation (Houk 1988). A regular tissue controls the state of the target process through the magnitude of an effector signal. This signal is received by a biologic transducer, most often an effector tissue, the cells of which contain receptors for the effector signal. The regulator tissue maintains a vigilant surveillance of the target process. The magnitude of a feedback signal from the process is monitored by sensors in the regulator tissue. Alterations in the state of the process as reflected in alterations in the feedback signal initiate the corrective changes in the level of the effector signal.

An example of such a homeostatic system is found in the regulation of the plasma calcium concentration. The regulator tissue is the parathyroid glands. The glands monitor the ionized calcium concentration (the feedback signal) through a calcium sensor. They secrete parathyroid hormone (the effector signal) in response to the magnitude of the feedback signal: high calcium concentrations decrease parathyroid hormone secretion and low concentrations increase it. The effector signal, i.e. the hormone, acts through specific membrane receptors in many body tissues (the effector tissues). In the renal tubules, for instance, the binding of parathyroid hormone to its cell-surface receptor activates the cAMP intracellular messenger pathway (the effect) leading to increased tubular reabsorption of calcium (the target process).

The equilibrium state of a regulated process, usually called the homeostatic set-point, is determined by the control response curve of the regulator tissue and the effect response curve of the effector

tissue. The point of intersection of the two curves defines the state of the target process at which the magnitude of the effector and feedback signals are exactly in balance. Panel A of Figure 7.7 shows a pair of control and effect response curves similar to those of the parathyroid hormone-calcium regulatory system. The control response curve shows the magnitude of the effector signal that results from the indicated levels of the feedback signal. When the feedback signal is small, the effector tissue produces a strong signal, when the feedback signal is large, the effector signal is small. The effect response curve relates the state of the target process, as measured by its feedback signal, to the magnitude of the effector signal. At low levels of the effector signal, the target process is suppressed and the feedback signal is small; when the effector signal is large, the target process is stimulated and the feedback signal is large. Only at the point of intersection of the two curves are the magnitudes of the effector and feedback signals in balance. If the target process is in any other state than the one defined by this equilibrium position, the imbalance between the effector signal and the feedback signal will lead to adjustment of the process toward equilibrium.

Abnormal function of a regulator tissue may be due to a disorder of the tissue itself, in which case the dysfunction is referred to as primary, to a disorder of the effector tissue, in which case the dysfunction is called secondary, or to concomitant malfunction of both tissues (Turner *et al*. 1987). In terms of the equilibrium model of homeostasis, primary disorders of homeostasis are those in which there is an alteration in the location of the control response curve. As shown in panel B of Figure 7.7, displacement of the control response curve away from normal will result in the establishment of an abnormal homeostatic set-point. The functional status of both the regulator tissue and the target process will be similarly perturbed, both being either increased (primary hyperfunction) or decreased (primary hypofunction). Secondary disorders of homeostasis are those caused by an alteration in the location of the effect response curve (panel C, Figure 7.7). The abnormal homeostatic set-points in these conditions are characterized by divergence of the functional status of the regulator tissue and the target process. Secondary hyperfunction of the regulator tissue is associated with a decreased level of activity of the target process due to hypofunction of the effector tissue. Secondary hypofunction of the regulator tissue shows an increased level of activity of the target process due to hyperfunction of the effector tissue.

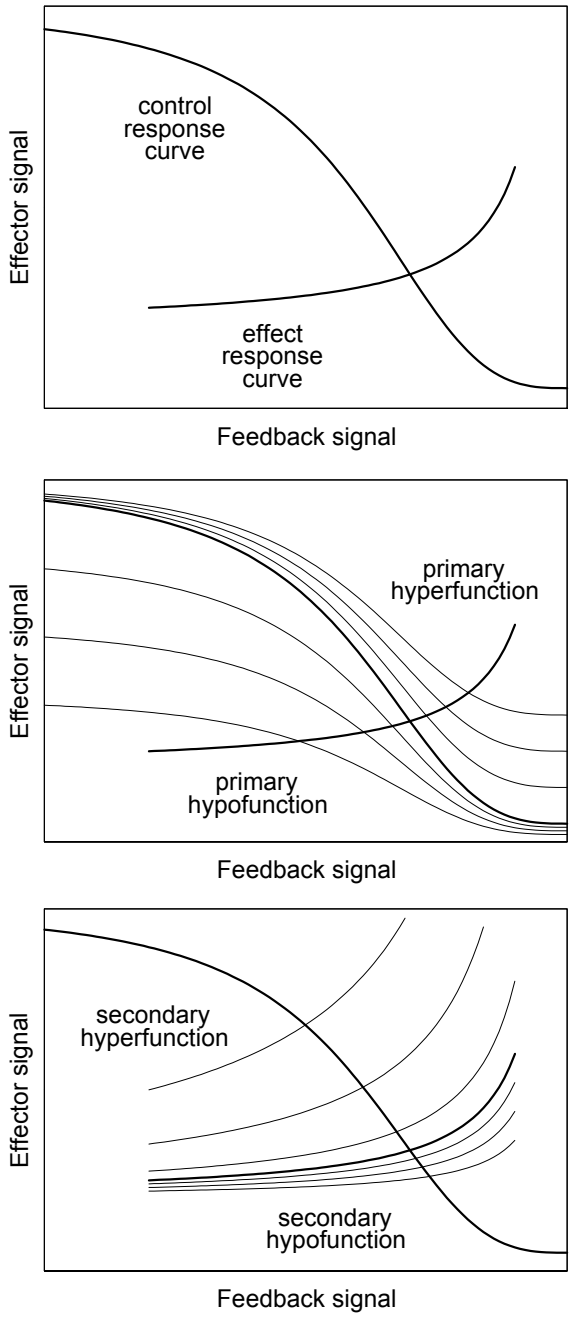

Figure 7.7 The equilibrium model of homeostatic control. The top grpah shows the normal control response curve for the regulator tissue and effect response curve for the associated effector tissue. The middle graph depicts primary dysfunction of the regulator tissue; the control response curve is displaced from its normal location. The bottom graph depicts secondary dysfunction of the regulator tissue; the effect response curve is displaced from its normal location.

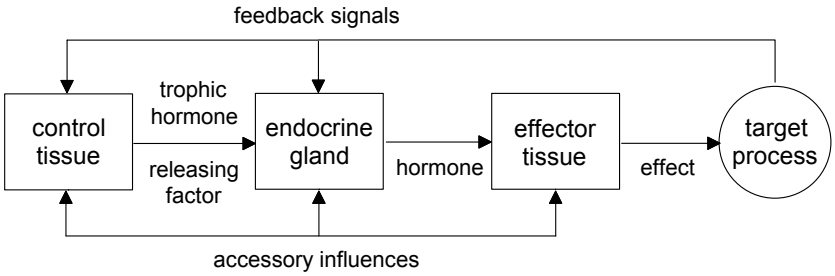

Figure 7.8 A simple model of endocrine homeostasis.

Endocrine systems

In higher animals systemic homeostasis is the responsibility of the endocrine glands. They are the regulators and their hormones are the effector signals. In contrast to the simplicity of the control model shown in Figure 7.6, hormone systems may produce multiple hormones, regulate multiple target processes, and in turn be regulated by other tissues. Additional complexity is found in the interdependent networks of endocrine regulation. Consider, for example, that the pituitary and the adrenal glands produce hormones that act to counterbalance the regulatory effects of insulin on plasma glucose. And the pancreatic islets themselves are the source of the most important counterregulatory hormone, glucagon. Still, the physiology of most endocrine glands is well represented by the comparatively simple model shown in Figure 7.8. The control tissue for many endocrine organs is the pituitary and for the pituitary it is the hypothalamus. In the first case, the control signal is the trophic hormone; in the second, the control signal is a hormone-releasing factor or a neurotransmitter.

Primary endocrine dysfunction resides in the gland itself. Primary hypofunction is usually due to a reduced number of functional cells but may also arise from reduced functional capacity of the cells of an intact organ as in the heritable disorders of hormone synthesis or secretion. Primary hyperfunction usually results from neoplasia of the gland with at least some degree of autonomy of hormone production present in the neoplastic cells. Secondary endocrine dysfunction occurs as a result of either abnormal control tissue function or a disorder in the effector tissue response to hormone.

Primary endocrine dysfunction produces disease in which the clinical picture is consistent with the gland's functional state, but so does secondary endocrine dysfunction due to disordered control tissue. Consequently, the level of the control signal, i.e., the plasma concentration of the trophic hormone, must be measured to separate these two possibilities. In contrast, when secondary endocrine dysfunction is attributable to an abnormal effector tissue, the clinical findings referable to that effector tissue are inconsistent with the endocrine functional state. The diagnosis of this form of secondary dysfunction is thereby readily made. Consistent findings can be expected, however, for other effector tissues that are not abnormal.

Evaluation of endocrine function

The laboratory evaluation of endocrine gland function proceeds in two steps. First, the functional state of the gland, defined as the rate of secretion of its hormone product, is assessed using function markers, the most important of which is the steady-state plasma concentration of the hormone itself. Second, trophic hormones or feedback markers are measured to compare the gland's functional state to the level of physiologic stimulation or endocrine function. Such an analysis makes possible the distinction of primary from secondary dysfunction.

Because hormone clearance rates remain nearly constant in primary endocrine disease, the secretory rate of an endocrine gland, and hence its functional state, is directly proportional to the plasma concentration of the hormone,

synthetic rate =

clearance rate x plasma hormone concentration

where clearance rate is a constant. As will be discussed later in this chapter, for hormones that experience significant plasma protein binding, i.e., steroid and thyroid hormones, the concentration of bioactive hormone must be measured rather than the concentration of total hormone. Similarly, it is the clearance rate of the bioactive hormone that remains constant in endocrine disease, not the clearance rate of total hormone. When circulating hormone

concentrations cannot be measured with adequate reliability, functional status is assessed by the measurement of some other function marker, the quantity of which is predictably related to the plasma concentration of bioactive hormone. There may be cosecreted products that are released from the endocrine gland at rates proportional to the hormone secretion rate. Catabolic products arising from degradation of the hormone within the gland itself and within peripheral tissues can be used. So can effector metabolites, which are either intracellular messengers, e.g., cyclic adenosine monophosphate, or products of intermediary metabolism that are released from effector tissue in proportion to the degree of hormone binding. These function markers may be quantified by measurement of their plasma concentrations or by measurement of their renal elimination rates. The hormone's physiologic effect also may be evaluated, including its effect upon the plasma concentration of its trophic hormone. In diabetes mellitus, insulin secretory capacity is estimated from the clearance rate of a glucose load, insulin's physiologic effect. Insulin production by an insulinoma, on the other hand, is monitored by measurement of plasma C-protein, a cosecreted product.

The measurement of an endocrine function marker rarely permits complete separation of patients according to functional status. More typically, the function categories overlap, sometimes extensively, when even the most reliable function marker available is used. To separate the function categories more completely, an endocrine gland can be stimulated or suppressed. Stimulation of the gland by administration of its trophic hormone or by depressing the feedback signal is especially useful in the separation of primary hypofunction from normal function. When transiently stimulated, a normal gland will, for a period of time, increase the rate of secretion of hormone. A hypofunctional gland will not respond to the stimulation with a comparable increase in secretion rate. Figure 7.9 illustrates why this is so. The control response curve for a hypofunctional gland will be displaced from the normal response curve and will be flattened because the maximal secretory capacity of the gland is reduced (top graph). Consequently, perturbation of the hypofunctional system by an exogenous stimulus (the time course of stimulation is presented in the middle graph) will not result in changes in the magnitude of the function marker equal to those seen with a normally functioning gland (bottom graph). The area between the function marker curve and the basal level of the function marker is proportional to the amount of hormone secreted in excess of the basal output,

$$\text{area between the curves} = \frac{\text{amount of excess hormone secreted}}{\text{clearance rate}}$$

The clearance rate for circulating hormone is usually the same in the setting of endocrine hypofunction as it is during normal function, so the index "area between the curves" is a reliable marker of excess hormone secretion during stimulation. A hypofunctional gland will secrete less excess hormone and therefore will have a measurably smaller "area between the curves." In this way a hypofunctional gland can be distinguished from a normally functioning one. In the figure, the "area between the curves" for the normal gland is 3 times larger than that for the hypofunctional gland even though the basal function marker levels differ by less than 10 percent.

The suppression of endocrine gland hormone secretion by alteration of the feedback signal can be used to improve the diagnostic separation of primary hyperfunction from normal function. During the period of suppression, a normal gland will markedly reduce its rate of hormone secretion. The reduction in the secretion rate will be less is a hyperfunctional gland. Figure 7.10 shows that the reason for this is that hyperfunctional glands also have displaced and flattened control response curves. In a suppression study, the area between the function marker curve and the basal level of the function marker is a reliable marker of the hormone secretion deficit. A hyperfunctional gland will not suppress its secretion very much below its basal state so its secretion deficit will be small compared to normal.

Rather than following the full time course of a function marker during a stimulation or suppression study, a single measurement taken at a specified time, preferably at a time near the extreme point of the function marker curve, can be used to quantify the hormone secretion excess or deficit. The magnitude of the function marker at the specified time will be linearly related to the amount of the excess or deficit. Such a one-sample study technique introduces measurement variability due to interindividual differences in the time to peak function marker change but clinical experience has shown that

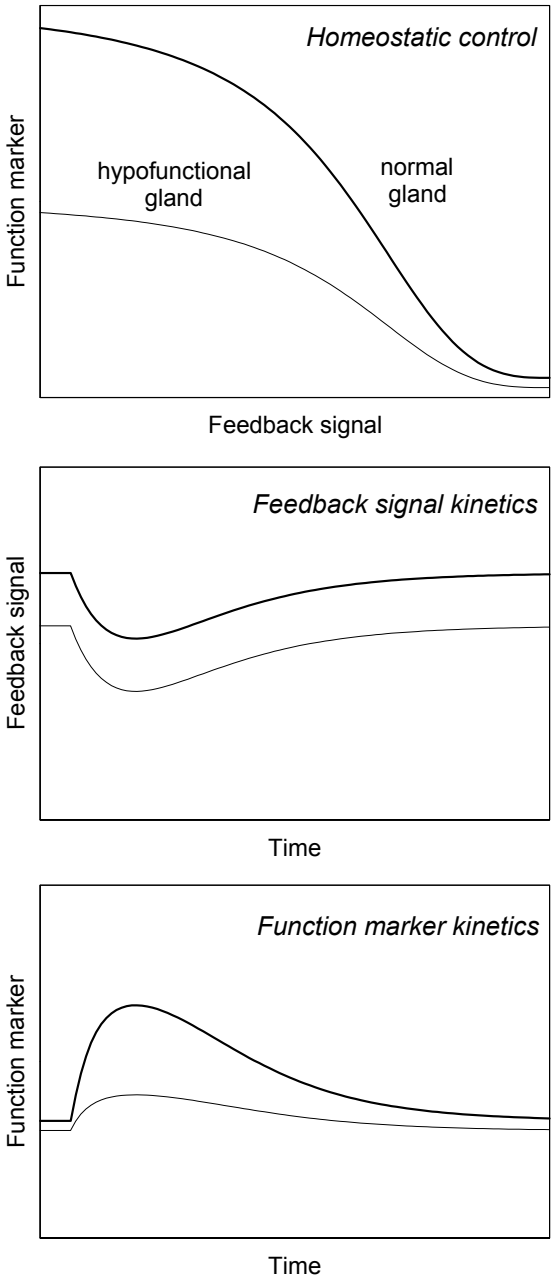

Figure 7.9 The separation of normal function from primary hypofunction in a stimulation study.

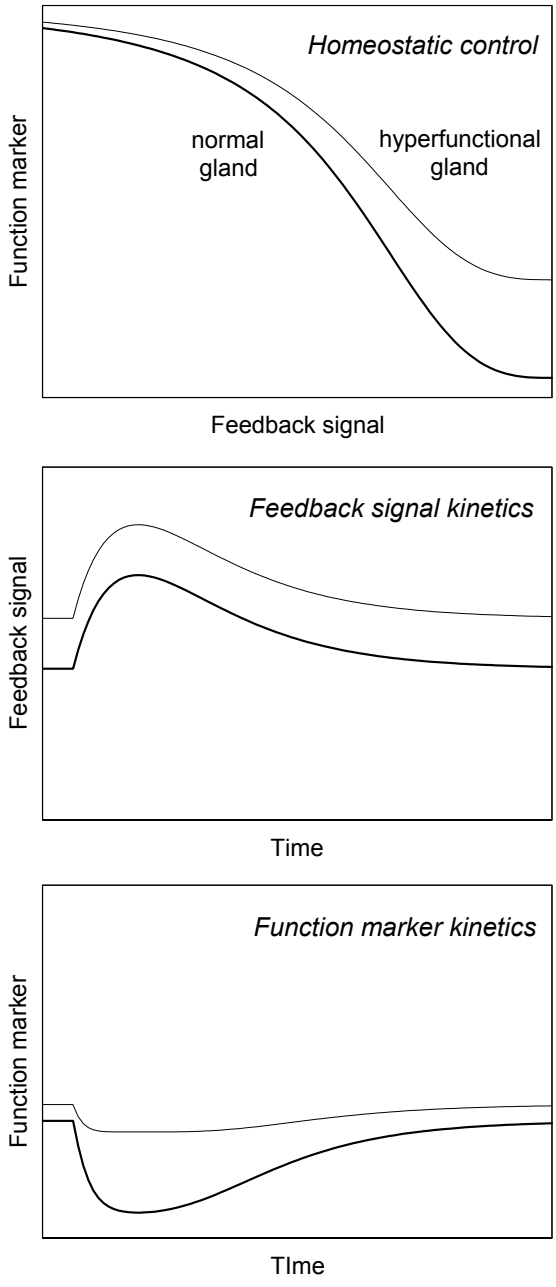

Figure 7.10 The separation of normal function from primary hyperfunction in a suppression study.

satisfactory separation of patients according to the endocrine function categories is still usually possible. The practical advantages of the one-sample techniques over the multiple sample approach is obvious.

Secondary forms of endocrine dysfunction are common. Thyroid, adrenal, ovarian and testicular dysfunction secondary to disorders of the anterior pituitary are evaluated by measurement of the plasma concentration of the appropriate trophic hormone. Secondary disorders of the posterior pituitary, parathyroids, and renin-aldosterone system arising from abnormal effector tissue function or responsiveness are identified by measurement of the appropriate feedback signal and by direct evaluation of effector tissue function.

The most informative manner in which to interpret measurements of feedback signals is to plot the signal value versus the concentration of the function marker for the gland. As shown in Figure 7.7,

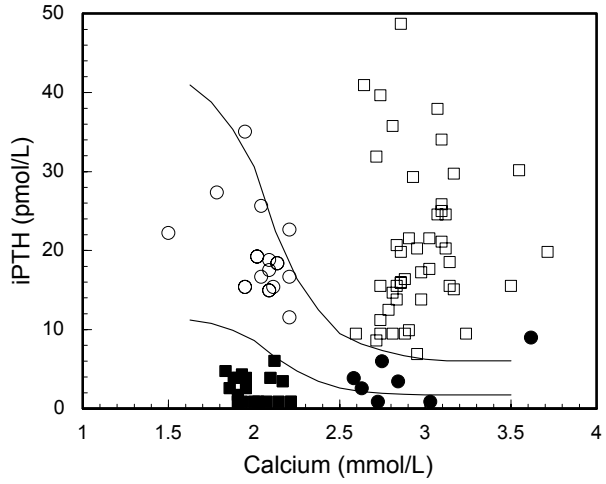

Figure 7.11 Diagnostic plot for the separation of disorders of the parathyroids based on plasma total calcium concentration and plasma immunoreactive parathyrin (iPTH) concentration (redrawn from Figs 3 and 4, LePage *et al.* 1988). The data shown came from hypercalcemic patients with surgically proven primary hyperparathyroidism (open squares) and various forms of secondary hyperparathyroidism (filled circles), and hypocalcemic patients with primary hypoparathyroidism (filled squares), and secondary hyperparathyroidism without renal failure (open circles).

result pairs from patients with secondary disorders of endocrine function will fall along the normal control response curve while the result pairs from patients with primary disorders will be located along the normal effect response curve. Even though there is considerable variability in the location of these curves among individuals, clinical experience shows that there is only a slight overlap of the diagnostic regions. The diagnostic efficacy of this approach is illustrated for parathyroid disease in Figure 7.11. Here, the function marker is immunoreactive parathyrin (iPTH) and the feedback signal is the plasma total calcium concentration, although, of course, the true feedback signal is the ionized calcium concentration. Lepage *et al.* (1988) defined the region characterizing the normal control response curve from a study of 11 normal individuals made hypercalcemic using $CaCl_2$ infusion and hypocalcemic using Na_2EDTA infusion. Individuals with paired calcium and iPTH concentrations in this region have secondary calcium disorders because their homeostatic set-points are consistent with a normal control response curve. Patients with paired calcium and iPTH concentrations outside of this region have homeostatic set-points that could not arise from a normal response curve; they, therefore, have primary calcium disorders. The data from these patients define the region characterizing the normal effect response curve.

Effector tissue function is tested directly by monitoring hormone effect following the administration of the hormone. If physiologic concentrations of the hormone produces a typical response, the effector tissue is functional. If the hormone fails to evoke an adequate response, the function of the effector tissue is impaired. Decreased functional mass of the effector tissue is the most common cause of diminished function. Less commonly, an adequate effector tissue mass is present but it is composed of cells with abnormal or sparse hormone receptors, such as in nephrogenic diabetes insipidus, or post-receptor-binding defects in cellular function, such as in pseudohypothyroidism.

PROTEIN-BOUND TRANSPORT IN BLOOD

Many substances in the blood are transported partly or completely bound to proteins. Oxygen and some carbon dioxide is transported bound to intraerythrocytic hemoglobin and various plasma constituents are bound to plasma binding proteins (Table 7.1).

Blood constituents that have limited solubility in plasma achieve much greater blood concentrations when transported bound to a protein. This is the vehicle function of protein binding. The transport of oxygen is an example of this binding function.

The storage function of protein binding, in which the binding of a blood constituent to a transport protein results in a decreased passage of the substance into the glomerular filtrate and thence the urine, is one mechanism to reduce wasteful renal loss of trace nutrients and thereby lower dietary needs for the nutrients. The maintenance of body stores of iron, for examples, is accomplished not only by the transport of plasma iron as transferrin but also by the protein binding of iron-bearing hemoglobin and heme released into the plasma following intravascular hemolysis. Urinary loss of iron occurs only when the amount of hemoglobin or heme released from a hemolytic episode exceeds the binding capacity of their transport proteins. An equally elaborate scheme for maintaining the body stores of a trace nutrient exists for retinol (vitamin A). Retinol binds with high affinity to the low-molecular-weight protein, retinol-binding protein. The pair then forms a complex with prealbumin, a minor thyroxine-binding globulin. Unlike the

Table 7.1
Transport Functions of Protein Binding

Constituent	Transport Function			Specific Binding Protein
	Vehicle	Storage	Delivery	
oxygen	yes			hemoglobin
bilirubin	yes		yes	albumin
hemoglobin		yes	yes	haptoglobin
heme		yes	yes	hemopexin
iron	yes	yes	yes	transferrin
copper		yes		albumin
90% of plasma copper is contained in ceruloplasmin				
zinc		yes		albumin
30% of plasma zinc is contained in α_2-macroglobulin				
calcium				albumin
magnesium				albumin
retinol	yes		yes	retinol-binding globulin
cobalamin			yes	transcobalamin II
cholecalciferol	yes		possible	vitamin D-binding globulin
thyroxine			possible	thyroxine-binding globulin, prealbumin
triiodothyronine			possible	thyroxine-binding globulin, prealbumin
aldosterone			possible	
cortisol			possible	cortisol-binding globulin
testosterone			possible	sex hormone-binding globulin
estradiol			possible	sex hormone binding globulin

retinol, retinol-binding protein pair, the two-ligand, two-binding protein complex is too large to be filtered at the glomerulus (Blomhoff *et al.* 1990).

Binding proteins which serve a delivery function render the plasma constituents bound to them available for uptake in certain tissues, and unavailable to others. The differential bioavailability of bound constituents is achieved in two ways. First, protein binding may render a plasma constituent available only to tissues with specific receptors for the constituent. An example is (unconjugated) bilirubin which, when unbound, has a high lipophilicity and can readily diffuse across plasma membranes and nonspecifically enter tissue cells. When bound to albumin, bilirubin is excluded from tissue cells except the hepatocytes which appear to have a plasma membrane receptor for bilirubin (Muller-Eberhard and Nikkilä 1989). Second, protein binding may make a plasma constituent available only to tissues with specific receptors for the binding protein. Iron bound to transferrin, for example, is selectively delivered to red cell precursors because they have a high density of transferrin receptors on their surface. Iron is also available to liver and placenta as these tissues also express cell-surface transferrin receptors in large numbers but iron uptake in other tissues, which have much smaller numbers of receptors, is meager (Huebers and Finch 1987).

Another possible but unproven function for plasma protein binding is to "buffer" acute changes in the extracellular concentration of a ligand: if the protein-bound form of the ligand is not bioactive, bound ligand could serve as a ready source of the ligand if the bioactive unbound form were acutely depleted and unoccupied binding sites could serve as a ready sink for the ligand in the event of an acute plasma excess of ligand. This function is usually presented as a mechanism to protect the organism from the detrimental effects that would otherwise attend a sudden change in a substance's extracellular concentration. Calcium and magnesium are likely candidates for such protective buffering. Acute changes in the extracellular concentration of the free form of either cation can have catastrophic effects upon cardiac and nervous system function. Buffering could also be a mechanism to maintain more stable plasma concentrations of substances otherwise

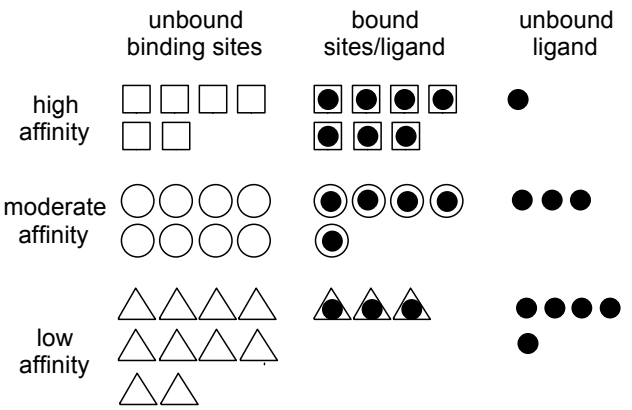

Figure 7.12 The effects of binding protein affinity upon the distribution of a substance between its bound and unbound forms.

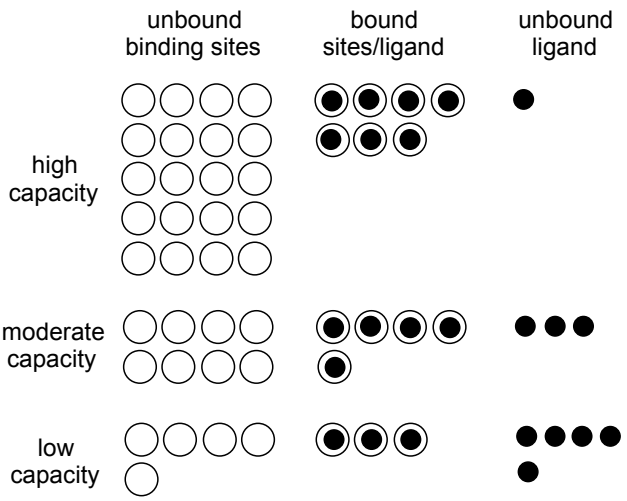

Figure 7.13 The effects of binding protein capacity upon the distribution of a substance between its bound and unbound forms.

subject to swings in concentrations as a result of irregularities in the dietary intake or synthesis of the substance or of a precursor. The protein binding of the thyroid hormones and of cholecalciferol may serve this function. Consider, for example, that a temporary deficiency in iodine intake with consequent impairment of the synthesis of the thyroid hormones could be partially ameliorated by the transfer of TBG-bound hormone to the bioactive fraction. Similarly the effect of seasonal variations in cholecalciferol production upon the synthesis of $1,25\text{-}(OH)_2D_3$ the could be blunted by the reservoir of $25\text{-}(OH)D_3$ maintained bound to DBG.

The bioactive fraction

When referring to the effects of plasma protein binding, the bioactive fraction of a blood constituent is that fraction which participates in physiologic processes when passing through a vascular bed. Its magnitude depends largely upon the distribution of the constituent among its unbound and protein-bound forms. This distribution is determined by the affinity of the binding proteins for the substance and the plasma concentration of the binding sites, i.e., the capacity of the binding proteins. In the case of a single binding protein, the distribution satisfies the simplified equilibrium mass action equation,

$$k_a\,[\textit{free binding sites}] = \frac{[\textit{bound substance}]}{[\textit{unbound substance}]}$$

where k_a is the association constant. (The association constant is equal to the rate constant of association of the substance and the binding protein divided by the rate constant of dissociation of the complex). Inspection of the equation reveals that the ratio of bound to unbound substance is large when the association constant is large (high affinity binding) or if the concentration of binding protein, and hence free binding sites, is large (high capacity binding). Conversely, the ratio is small if there is low affinity or low capacity binding. Figure 7.12 shows the partition of a constant amount of a ligand between protein-bound and unbound forms for three binding proteins of equal capacity but unequal binding affinity. In this example, the association constants are 1.17, 0.21, and 0.06 for the high-, moderate-, and low-affinity binding, respectively. The effect of varying the capacity of a binding protein (association constant, 0.21) is depicted in Figure 7.13.

The relative affinities and capacities of the plasma proteins binding the thyroid and steroid hormones are listed in Table 7.2. Notice that the hormones may bind to a number of proteins and that each binding protein may associate with more than one hormone. Calculating the distribution of these hormones among their protein-bound forms is not at all easy. Using computer-based techniques, the distribution can be calculated by solving a system of general equilibrium mass action equations (Feldman *et al.* 1972); for $i = 1, \ldots, n_2$

$$[\textit{bound substance}_i] = \sum_{j=1}^{n_j} \frac{k_{ij}[\textit{binding site}_j][\textit{unbound substance}_i]}{1 + \sum_{g=1}^{n_2} k_{gj}[\textit{unbound substance}_g]}$$

where n_1 is the number of binding sites, n_2 is the number of ligands, and k_{ij} is the association constant of the ith ligand. Equilibrium binding distributions

Table 7.2
Relative Affinities and Capacities of Plasma Proteins Binding the Thyroid and Steroid Hormones

Affinity	Capacity	
	High	Moderate-to-low
High	TBG triiodothyronine SHBG estradiol prealbumin thyroxine	TBG thyroxine SHBG testosterone CBG cortisol
Intermediate	CBG testosterone aldosterone prealbumin triiodothyronine	
Low	albumin thyroid hormones steroid hormones	

Table 7.3
Common Causes of Altered Plasma Concentrations of Binding Proteins

Protein	Increase	Decrease
all	acute dehydration	malnutrition liver failure nephrosis protein-losing enteropathy
albumin		acute-phase response
TBG	hypothyroidism pregnancy hyperestrogenemia	hyperthyroidism
CBG	pregnancy hyperestrogenemia	
SHBG	pregnancy hyperestrogenemia hyperthyroidism liver failure (males)	hyperandrogenemia hypothyroidism (females)

have been calculated for the steroid hormones (Dunn *et al*. 1981), thyroxine (Oppenheimer 1989), and vitamin D_3 (Dunn 1988).

Laboratory measurement of the bioactive fraction

The plasma concentrations of calcium, magnesium, and the hormones are regulated by homeostatic systems. Because the concentration of the bioactive fraction in the regulator tissue is the entity that is homeostatically controlled, a change in binding protein capacity or affinity will lead to a secondary alteration in the total concentration of the regulated substance. The concentration will change just enough to reestablish the set-point plasma concentration of the bioactive fraction. Significant abnormalities in binding protein affinity are unusual but variability in binding protein capacity are common in clinical practice (Table 7.3). Therefore, the extent of plasma protein binding must be considered when measuring the concentration of these analytes.

Calcium and magnesium. Calcium and magnesium are physiologically active in their ionic (unbound) forms only. The bound forms of these cations are made up of diffusable species (complexed calcium and magnesium) and nondiffusable protein-bound complexes. For calcium, protein binding accounts for about 45 percent of the total calcium and complexed calcium for 10 percent. Total calcium concentrations are altered only very little by small changes in the plasma albumin concentration and small to moderate changes in the concentrations of the calcium-complexing anions (phosphate, carbonate, and citrate). In those cases, the concentration of total calcium adequately reflects the ionic calcium concentration. Moderate changes in plasma albumin concentration and large changes in the concentrations of the calcium-complexing anions may alter the total calcium levels enough to obscure the true status of the homeostatic control of the ionic calcium concentration. In such settings, the concentration of ionic rather than total calcium can be measured using ion-selective electrode potentiometry. Alternatively, the concentration of ionic calcium can be calculated using any of a number of formulas if changes in albumin concentration are the sole binding abnormality (Vanstapel and Lissens 1984).

Albumin-bound magnesium represents only 25 per cent of the total magnesium and complexed forms about 15 per cent. Only large alterations in the plasma concentration of albumin or the magnesium-complexing anions (phosphate, carbonate, and citrate) will change the total magnesium levels enough to disallow the use of the total magnesium concentration as the indicator of the ionic magnesium concentration.

Hormones. In tissues with short mean capillary transit times, i.e., in essentially all tissues but the liver, the bioactive form of a hormone is the

unbound hormone (Mendel 1989, 1992). This means that all hormones with a significant degree of protein binding will have a bioactive fraction much less than 1. For these hormones, the measurement of the total hormone concentration is not a reliable estimate of the concentration of the bioactive hormone; the concentration of unbound hormone must be measured.

Unbound hormone concentration is measured by separation of the protein-bound from the unbound hormone and determination of the hormone concentration in the sample portion containing the unbound form. Methods used to separate bound from unbound hormone include equilibrium dialysis, ultrafiltration, kinetic radioimmunoassay, and chromatography. Equilibrium dialysis remains the reference technique for the measurement of unbound analyte concentrations for all protein bound analytes, but the technique is too tedious and time consuming for routine laboratory use. The separation techniques of ultrafiltration and kinetic radioimmunoassay are practicable and therefore are preferred.

In vivo plasma ultrafiltration can be taken advantage of in the assay of cortisol. The renal glomerular filtration of unbound cortisol results in urine cortisol levels that can be readily and precisely measured. Empirically, the daily renal excretion of unbound cortisol correlates well with the mean daily circulating levels of unbound cortisol. The other hormones cannot be analyzed this way because their greater protein binding and lesser plasma concentrations result in urine hormone concentrations too small for accurate detection. Another *in vivo* separatory process occurs in the formation of saliva. The unbound form of the steroid hormones enter saliva from the salivary gland capillaries but not the bound forms. As a result, salivary concentrations of hormone are very similar to circulating unbound hormone concentrations (Vining and McGinley 1986).

The concentration of unbound hormone can be estimated by calculation of indices proportional to its concentration. One such index is the ratio of the concentration of hormone to the concentration of specific hormone-binding protein. This index is derived from the general equilibrium mass action equation. If there is minimal competition from other hormones for the binding sites on the binding protein and if the fraction of hormone bound to albumin is small compared to that bound to the specific binding protein, the equation simplifies to,

$$[\text{bound hormone}] = \frac{k_{bp}[\text{binding sites}][\text{unbound hormone}]}{1 + k_{bp}[\text{unbound hormone}]}$$

where k_{bp} is the association constant of the hormone for the specific binding protein. Substituting the concentration of total hormone for the concentration of bound hormone, based upon an assumption of extensive protein binding of the hormone, and dividing through by the concentration of specific binding protein yields,

$$\frac{[\text{hormone}]}{[\text{binding sites}]} = \frac{k_{bp}[\text{unbound hormone}]}{1 + k_{bp}[\text{unbound hormone}]}$$

The ratio of the concentration of hormone to the concentration of specific binding protein is, therefore, proportional to the concentration of unbound hormone. Note, however, that the relationship is nonlinear. In addition, it is based upon a number of assumptions which limit its reliability: it is valid only for highly protein-bound hormones that are largely bound to their specific binding protein.

A hormone:hormone-binding protein ratio in clinical use is the testosterone:SHBG ratio (also called the androgen index). Testosterone is almost completely protein bound in the plasma but a protein other than sex-hormone binding globulin contributes significantly to its binding; approximately 50 percent is bound to albumin (Dunn 1981). As a result, the testosterone:SHBG ratio has only an approximate relationship to the unbound testosterone concentration.

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