10

Drug Disposition—Distribution

We have known for a long time that people respond differently to drugs and now know that these differences are the result of both pharmacokinetic and pharmacodynamic variability in the patient population. Pharmacokinetic variability means that the same dose of drug results in different blood levels in different people. The association of drug concentration in blood or plasma with pharmacologic effect, described in Chapter 9, suggests that pharmacokinetic variability is an important factor in how people respond to drugs.

Pharmacokinetic variability is the result of interindividual differences in the absorption and disposition of drugs. Chapters 2 through 6 deal with various aspects of drug absorption. This chapter and the one that follows concern the basic principles of drug distribution and elimination.

The term *disposition* refers to the fate of a drug after absorption. On reaching the bloodstream, drugs are simultaneously distributed throughout the body and eliminated. Distribution usually occurs much more rapidly than elimination. The rate of distribution to the tissues of each organ is determined by the blood flow perfusing the organ and the ease with which the drug molecules cross the capillary wall and penetrate the cells of the particular tissue.

DRUG DISTRIBUTION

Distribution in Blood and Other Fluids

Drug molecules are distributed throughout the body by means of the circulation of blood. The entire blood volume (about 6 L) is pumped through the heart each minute; within minutes after a drug enters the bloodstream it is diluted into the total blood volume. A drug that is restricted to the vascular space and can freely penetrate erythrocytes has a volume of distribution of 6 L. If the drug cannot permeate the red blood cells (RBCs), the available space is reduced to about 3 L (plasma volume).

Nearly all drugs easily cross capillaries and are rapidly diluted to a much larger volume, the extracellular space. Capillaries, except those in the brain, are more like filters than lipid membranes, in terms of permeability. Drugs with molecular weights of up to 500 or 600 daltons quickly diffuse out of the vascular system and reach the interstitial fluid bathing the cells.

Certain body fluids may be relatively inaccessible to drugs in the bloodstream; these include cerebrospinal fluid (CSF), bronchial secretions, pericardial fluid, and middle ear fluid. The degree of access of antibiotics to these fluids may be a limiting factor in treating infections. Inflammation, however, often secondary to infection, increases drug penetration.

Drug concentration in body fluids also depends on the degree of drug binding in the fluid. Drug concentration in CSF and saliva, which are usually protein-free, is usually equal to free (unbound) drug concentration in plasma. Drug concentration in extracellular fluid (ECF) is frequently less than that in plasma, because the ECF has a lower albumin concentration than plasma. Drug concentration in synovial fluid varies with the degree of inflammation because albumin concentration fluctuates with the severity of the disease process.

Cellular Distribution

The penetration of drugs into cells depends on many of the factors that govern gastrointestinal absorption of drugs in solution. Small, water-soluble molecules and ions diffuse through aqueous channels or pores in the cell membrane. Lipid-soluble

molecules penetrate the membrane itself. Water-soluble molecules and ions of moderate size (molecular weights of 50 daltons or more) cannot enter cells easily, except by special transport mechanisms. The penetration of weak acids or bases into cells depends on the pH of the ECF. Unlike the gastrointestinal tract, however, blood and ECF maintain a remarkably uniform pH.

Studies in dogs have shown that carbon dioxide-induced acidosis or sodium bicarbonate-induced alkalosis markedly alters the distribution of phenobarbital, a weak acid.[1] When plasma pH is lowered by carbon dioxide inhalation, there is a decrease in the plasma levels of phenobarbital. Under these conditions, a greater fraction of the drug in plasma is in the un-ionized form and a larger amount of the drug moves into the cells. Sodium bicarbonate produces an elevation of phenobarbital concentration in the plasma because of a shift of drug from the cellular space to the extracellular space. These shifts occur in all tissues, including the brain. Acidosis deepens phenobarbital anesthesia whereas alkalosis lightens it.

Sodium bicarbonate is sometimes used in the treatment of barbiturate intoxication to induce a mild systemic alkalosis to reduce the central nervous system (CNS) burden of drug. This treatment also produces urinary alkalosis, which promotes the urinary excretion of weakly acidic drugs.

Drug Penetration to Central Nervous System

The capillaries in the brain are different in their permeability characteristics from those found in the rest of the body. They possess a cellular sheath that makes them much less permeable to water-soluble substances. This sheath constitutes the so-called blood-brain barrier. The penetration rate of a drug into the brain depends on its degree of ionization in the plasma and its lipid solubility.

Highly lipid-soluble drugs, like thiopental, reach the brain almost immediately after administration. More polar compounds, like barbital, penetrate the CNS at a slower rate.

Failure of antibiotics to effectively penetrate the CNS is a long-recognized concern in the treatment of meningitis.[2] In most cases, this failure can be explained by the physicochemical properties of the drug. For example, penicillin G is a water-soluble, weak organic acid (pKa = 2.6) that is essentially ionized at the pH of plasma. The slow diffusion of penicillins into the CNS reflects the poor lipid solubility of the ionized form of the drug.

Interestingly, the permeability of the blood-brain barrier is increased in meningeal infections because of the abnormal state of the membranes. Variable but significant levels of ampicillin, penicillin G, lincomycin, and cephalothin have been found in the CSF of patients with viral, bacterial, or other meningeal inflammatory states.[3,4] Little or no detectable antibiotic activity is found in the CSF of patients with normal meninges who received these drugs. The steady-state CSF concentration of ampicillin was found to be only 2% of serum concentration in normal rabbits, but increased to 13% of serum concentration in rabbits with meningitis.[5]

Parkinsonism is associated with a depletion of dopamine in the brain. Replacement therapy is ineffective because of the inability of dopamine to cross the blood-brain barrier. On the other hand, levodopa, a precursor of dopamine, is an important drug in the treatment of this disease. Levodopa can penetrate the CNS where it is subsequently metabolized to dopamine.

A serious problem in the long-term management of parkinsonian patients treated with levodopa is the deterioration of control and the development of random fluctuations in motor performance, called the on-off phenomenon. A group of investigators in Oregon sought to determine whether the oscillating clinical response to levodopa reflected fluctuations in brain levels of the drug related to variation in gastrointestinal absorption and transport to the central nervous system.[6]

The role of absorption was evaluated by determining whether it was affected by meals and by studying the clinical response to a stable concentration of levodopa in plasma achieved by intravenous infusion. The role of transport of levodopa from plasma to brain was examined by studying the clinical response to high-protein meals and to certain amino acids given during drug infusion. The investigators hypothesized that certain amino acids might compete with levodopa for transport across the blood-brain barrier.

Patients with Parkinson's disease for at least 8 years, characterized by unpredictable and marked swings in their response to levodopa, were studied. When levodopa was given 3 times a day before meals, the typical profile of plasma levodopa concentration showed peaks about 1 hr after the dose and troughs immediately before the next dose; roughly corresponding oscillations were noted in

mobility and dyskinesia. There were, however, incongruities; sometimes, a peak level was not seen after a dose, and at other times the plasma peak was not accompanied by clinical improvement even though equivalent levels at other periods of the day were effective.

When levodopa was given to patients after an overnight fast, absorption was rapid and reproducible. On the other hand, when the drug was taken after a meal, rate and extent of absorption were decreased and in some cases no peak concentration was evident. A constant rate intravenous infusion virtually eliminated fluctuations in levodopa levels in plasma and produced a stable clinical response lasting from 12 to 36 hr, except when perturbed by a meal or an amino acid challenge.

The administration of a high-protein meal during iv infusion of levodopa had no effect on drug concentration in plasma, but it did cause a deterioration of clinical response. These meals also about doubled the plasma concentration of large neutral amino acids that have been found in animal studies to affect the transport of levodopa across the blood-brain barrier.

To further characterize this loss of efficacy, patients were challenged with individual amino acids during levodopa infusion. Loss of motor control was observed when phenylalanine, leucine, and isoleucine were given but not when glycine or lysine was administered. The investigators concluded that interference with absorption of levodopa by food resulting in variable blood levels and with transport of levodopa across the blood-brain barrier by large neutral amino acids contained in the diet may be responsible, in part, for the on-off phenomenon in Parkinson's disease. The observation that constant infusion markedly decreased fluctuations offers the hope that improved methods of delivering levodopa may eliminate or at least reduce this problem.

The rapid penetration of lipid-soluble molecules in the CNS is of great importance for anticonvulsant or psychotropic drugs, which must act on the brain, but facile penetration may produce unwanted side effects with drugs intended to affect other systems, such as antiarrhythmic drugs. The CSF concentrations of lipid-soluble drugs usually reflect free drug concentrations in plasma. The CSF-plasma concentration ratio in epileptic patients undergoing temporal lobectomy was 0.12 for phenytoin and 0.46 for phenobarbital; the free to total concentration ratio in plasma was 0.15 for phenytoin and 0.50 for phenobarbital.[7] The CSF-plasma concentration ratio for chlordiazepoxide in patients receiving spinal anesthesia was found to be 0.043; the free-to-total concentration ratio of the drug in plasma was 0.04.[8]

Drug levels are usually higher in brain tissue than in the CSF. Phenytoin concentrations in epileptic patients are about 6 times higher in the temporal lobe than in the CSF.[7] Propranolol concentrations are more than 250 times higher in the human brain than in the CSF.[9] This is a result of drug binding to constituents in the brain.

Adequate distribution to the central nervous system is of particular concern in the use of antineoplastic drugs. Certain parenchymal cancers are quite responsive to systemic antitumor agents, but metastases of these tumors to the CNS have been virtually unresponsive to the same chemotherapy, probably because the drugs were unable to cross the blood-brain barrier.

Several approaches are under investigation to enhance drug delivery to the CNS. Spontaneous disruption of the blood-brain barrier during the course of certain diseases (e.g., meningitis) increases the penetration of drugs to the CNS. There is now evidence that certain treatments may permit controlled transient disruption of the barrier.

For example, dimethyl sulfoxide (DMSO) seems to open the blood-brain barrier in mice to the enzyme horseradish peroxidase.[10] Uniform distribution of the enzyme was observed throughout most of the forebrain, brainstem, and cerebellum when it was given with DMSO. In the absence of this penetration enhancer, the enzyme failed to enter brain parenchyma. The effects of DMSO were no longer evident within 2 hr of administration, suggesting that disruption was transient. How DMSO opens the blood-brain barrier is unclear and whether it may be used safely in humans remains to be determined.

A more developed technique, called osmotic blood-brain barrier disruption, utilizes hyperosmolar solutions of mannitol. Osmotic disruption of the blood-brain barrier immediately before administration of methotrexate in a dog model, by infusion of 25% mannitol into the internal carotid artery, resulted in therapeutic levels of drug in the ipsilateral cerebral hemisphere but not in the contralateral hemisphere.[11] The permeability of the barrier appeared to return to normal within 1 hr after treatment.

In preliminary clinical studies, methotrexate was

administered after blood-brain barrier disruption to 6 patients with brain tumors.[12] No permanent complications were seen, and serial enhanced CT scans indicated that disruption increased drug delivery to the tumor and the surrounding brain. More recently, using this technique, Neuwelt et al.[13] documented tumor regression in patients with microglioma, medulloblastoma, and glioblastoma.

Warnke et al.[14] studied the effect of hyperosmotic blood-brain barrier disruption on transport of a water-soluble amino acid, amino-isobutyric acid, in rats with ethylnitrosourea-induced gliomas. Hyperosmotic disruption resulted in a modest, statistically insignificant, increase in tumor uptake. In contrast, a large and significant increase in the uptake of amino-isobutyric acid was found in the tumor-free brain tissue. The investigators concluded that the effects of hyperosmotic blood-barrier disruption are different in normal brain tissue and in brain tumors, and that the benefits of disruption with respect to the rate of drug delivery to brain tumors appear to be marginal. Transient disruption, however, may still be useful to increase drug delivery to brain tissue surrounding the tumor.

Boder and Farag have described a prodrug approach to enhance delivery to the CNS.[15] A biologically active compound covalently linked to a lipid-soluble dihydropyridine carrier easily penetrates the blood-brain barrier. Oxidation of the pyridine part of the drug-carrier prodrug to the ionic pyridinium salt prevents its elimination from the CNS but enhances its elimination from the general circulation. Subsequent cleavage of the prodrug in the CNS results in sustained delivery of the drug to the brain and facile elimination of the carrier.

Bodor and Farag[16] prepared the N-methyl-1,4-dihydronicotinate ester of testosterone via quaternization of testosterone nicotinate with methyl iodide followed by reduction of the quaternary salt. They postulated that the ester, by virtue of its lipid solubility, would cross the blood-brain barrier more easily than testosterone. It was also anticipated that biological oxidation to the corresponding quaternary derivative would follow, thereby capturing the corresponding ionic, hydrophilic product in the central nervous system. Oxidation in peripheral tissue not involving a permeability barrier would favor rapid clearance from the general circulation because the quaternary derivative is excreted more rapidly than the unoxidized form. Oxidation would favor both the accumulation of quaternary derivative in the brain and minimal systemic exposure outside the brain. A subsequent slow hydrolysis to free testosterone in the brain would provide a site-specific, prolonged exposure to testosterone.

After administration of the testosterone ester to female rats, no reduced material could be detected in blood or brain. However, high and persistent levels of the quaternary form of the prodrug were found in the brain, suggesting rapid oxidation. Blood levels of the charged form were initially high but fell quickly. Testosterone was found to be released from the quaternary ester very slowly giving rise to low but persistent levels in the brain ($t_{1/2}$ = 20 hours). Administration of testosterone itself results in high brain levels followed by rapid clearance ($t_{1/2}$ = 15 minutes).

Prodrugs promising brain-selective delivery have also been described for certain progestins, including ethisterone, norethindrone, and norgestrel.[17] Administration of the reduced form of a norethindrone prodrug to rats resulted in relatively high and persistent levels of the oxidized quaternary form of the prodrug in the brain. The "locked-in" quaternary salt hydrolyzed slowly to produce the pharmacologically active parent drug norethindrone. This hydrolysis produced substantially higher and more persistent levels of norethindrone in the brain than those found after the administration of norethindrone itself.

This novel prodrug approach to drug delivery and sequestration in the brain has also been applied to estradiol.[18] The maximum effect of equimolar doses of estradiol prodrug and estradiol itself in suppressing luteinizing hormone secretion in orchidectomized rats was about the same, but the effect of the prodrug persisted for a much longer time. The investigators suggested that this chemical delivery system for estradiol may be useful clinically in the treatment of vasomotor instability associated with ovariectomy or the menopause, particularly in women for whom peripheral estrogen is contraindicated. The prodrug may also be useful in the chronic reduction of gonadotropin secretion for fertility regulation or for the treatment of gonadal-steroid-dependent diseases, such as endometriosis and prostatic hypertrophy.

Placental Transfer of Drugs

Since the thalidomide tragedy, there has been keen interest in the passage of drugs across the placenta.[19–21] The membranes separating fetal capillary blood from maternal blood resemble cell

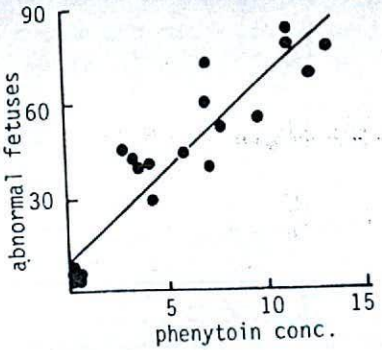

Fig. 10–1. Relationship between phenytoin concentration in maternal plasma (μg/ml) and the frequency (%) of mouse fetuses with malformations. (Data from Finnell, R.H.[24])

membranes elsewhere in the body. Many drugs of moderate to high lipid solubility, including sulfonamides, barbiturates, anticonvulsants, narcotic analgesics, antibiotics, and steroids, can be detected in appreciable concentrations in fetal blood or tissues shortly after administration to the mother.

Although its development has been greatly hampered by experimental and ethical difficulties, the theory of maternal-fetal equilibrium rates is considerable.[22,23] The shortest time possible for a drug to equilibrate between maternal blood and fetal tissue has been estimated to be about 40 min. Drugs like tubocurarine, whose passage across the placenta is impeded by low lipid solubility, large molecular size, ionization, and protein binding, probably require hours for equilibration and may not be detected in the fetus after a single dose to the mother. Fetal exposure to drugs that are rapidly eliminated by the mother is also likely to be small.

Chronic medication presents the greatest concerns. The higher the blood level of drug in pregnant patients on chronic medication, the greater is the risk to the fetus. The occurrence of malformations associated with the fetal hydantoin syndrome in the mouse are strongly correlated with maternal serum concentrations of the anticonvulsant drug phenytoin (Fig. 10–1).[24]

In man, there is some evidence that both the metabolites of phenytoin and a genetic defect in metabolic detoxification contribute to susceptibility to phenytoin-induced birth defects.[25] Lymphocytes from 24 children exposed to phenytoin throughout gestation, as well as from family members, were challenged with phenytoin metabolites generated by isolated mouse liver. Lymphocytes from 14 children were positive, manifesting an increase in cell death on exposure to phenytoin metabolites. Each child with a positive result had at least one parent whose cells also were positive. A positive in vitro challenge was highly correlated with major birth defects, including congenital heart disease, cleft lip and/or palate, microcephaly, and major genitourinary, eye, and limb defects.

In 1982, isotretinoin, a highly effective drug for the treatment of severe chronic cystic acne, was marketed in the United States. In short order, it became the most widely criticized drug of the decade because of its potential to cause severe deformities in children born to women who had taken isotretinoin during the early weeks of pregnancy. The syndrome has been called retinoic acid embryopathy.[26]

Isotretinoin is a retinoic acid, a class of compounds related to vitamin A. Because retinoic acid as well as large doses of vitamin A were known to be teratogenic in laboratory animals, isotretinoin was labeled upon marketing as Category X, indicating that the drug was contraindicated for use during pregnancy. Despite this initial warning and subsequent stronger warnings, the use of isotretinoin during pregnancy has been associated with more than 60 documented reports of adverse reproductive outcomes since the drug went on the market. Some epidemiologists suggest that the number is actually as high as 600.

Since its marketing, the labeling of isotretinoin has become more and more restrictive. In 1984, the manufacturer added to the label a recommendation that a pregnancy test should be performed within 2 weeks of initiating treatment with isotretinoin, and that an effective form of contraception be used for at least 1 month prior to starting therapy, during therapy, and 1 month after therapy is discontinued. Furthermore, in a most unusual move, the Food and Drug Administration advised all blood banks that donations from anyone taking isotretinoin should be deferred for at least 1 month after taking the last dose. The agency, considering the potency of isotretinoin as a teratogen and the possibility that it may be present in the blood for weeks after discontinuance, suggested that there may be a risk to the developing fetus if blood from an isotretinoin-treated donor is transfused into a patient who either is or soon becomes pregnant.

Several other countries, which approved the use of isotretinoin somewhat later than the FDA, con-

trol the distribution of the drug more closely than does the United States. In the United Kingdom, only 200 dermatologists are certified to prescribe the drug. Isotretinoin is available from any licensed physician in the U.S. Sweden has elected not to approve isotretinoin for general marketing, requiring physicians to submit a special request to the government to prescribe the drug.

Since some degree of fetal exposure is likely to occur with virtually all drugs, and since the consequences of such exposure is usually unknown, many advocate that drug administration during pregnancy be severely restricted.

Some investigators are taking advantage of the ease with which drugs cross the placenta to treat the fetus by giving medication to the mother.[27] A deficiency in the enzyme 21-hydroxylase results in congenital adrenal hyperplasia, leading to masculinization of the external genitalia of affected females. This problem might be avoided if fetal adrenal gland function were suppressed.

A woman with mild 21-hydroxylase deficiency, whose previous female child was born with adrenal hyperplasia and masculinization, was given dexamethasone beginning at the tenth week of gestation of another female child. After a normal delivery at 39 weeks, the child was found to have normal external genitalia. Postnatal tests indicated that the infant, like the mother and sibling, was 21-hydroxylase-deficient. The investigators concluded that "this study demonstrates prolonged suppression of the fetal adrenal gland with dexamethasone and suggests it might prevent abnormal masculinization in fetuses with severe congenital adrenal hyperplasia."[27]

Blood Flow

Blood flow is the rate-limiting step in the distribution of most drugs. Accordingly, rapid equilibration of lipid-soluble drugs is observed between the blood and lungs, kidney, liver, heart, and brain, all of which are highly perfused with blood. Less rapid equilibration is found in skeletal muscle, bone, and adipose tissue, which receive a considerably smaller volume of blood per unit mass per minute. Perfusion of relatively large, solid tumors is also low. Changes in blood flow to the liver or kidneys, as a result of disease or other factors, may alter the elimination rate of a drug.

Blood flow rates may also influence drug uptake at receptor sites. Figure 10–2 shows concentrations of procainamide after a short intravenous infusion in nonischemic and progressively ischemic regions of the dog myocardium.[28] Procainamide accumulated more slowly and peak concentrations were lower in the ischemic regions than in the nonischemic region; concentrations were lowest in the most severely ischemic region.

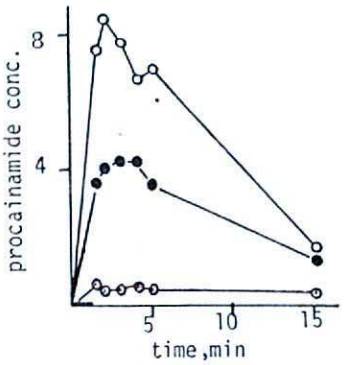

Fig. 10–2. Procainamide concentrations in the myocardium (μg/g) during and after a 1-min infusion in the dog. Key: (○) nonischemic region; (●) moderate ischemia; (⊙) severe ischemia. (Data from Wenger, T.L., et al.[28])

Redistribution of a drug into less well-perfused tissues, rather than metabolism or excretion, may limit the duration of effect of certain drugs at highly perfused sites. For example, thiopental produces anesthesia within seconds after administration because of rapid equilibration between blood and brain. The concentration of drug in the brain rapidly declines, however, and the duration of effect is short-lived despite the fact that thiopental is only slowly metabolized. The rapid decline of drug concentrations in the brain is the result of redistribution into other tissues, particularly skeletal muscle and fat. Inhibition or induction of drug-metabolizing enzymes and subsequent reduction or enhancement of the metabolism rate of thiopental is likely to have no effect on the duration of drug action because redistribution rate is the controlling factor.

Investigators in Sweden studied the distribution and elimination of the narcotic analgesic meperidine in patients recovering from surgery.[29] Five hr after the procedure, the drug was infused intravenously at a rate predicted to produce a steady-state concentration of about 500 ng/ml. Blood samples were collected simultaneously from mixed central venous blood (pulmonary artery) and peripheral arterial blood (radial artery) and assayed for meperidine.

Drug uptake by an organ is expressed as the equilibrium distribution ratio between tissue and blood (K_P). Drug clearance by an organ (CL) is expressed as the product of the organ blood flow (Q) and the extraction ratio (E).

$$CL = Q \times E \qquad (10\text{–}1)$$

The pulmonary extraction ratio (E) is calculated as follows:

$$E = (C_V - C_A)/C_V \qquad (10\text{–}1A)$$

where C_V and C_A are the venous and arterial plasma concentrations, respectively. Blood flow to the lungs is about 5,000 ml/min, the highest flow rate to any organ in the body. Accordingly (see Eq. 10–1), even a modest extraction ratio could result in a large percentage of the dose of a drug being eliminated in a single pass across the lungs.

The investigators found that the venous concentration of meperidine at steady state was 540 ng/ml, whereas the arterial concentration was 523 ng/ml. These values were not significantly different. From these results, one can calculate an extraction ratio of 0.03 which is not significantly different from zero. These findings indicate that the lungs make little or no contribution to the overall clearance of meperidine, estimated at about 800 ml/min.

On the other hand, during the infusion of meperidine before steady state, venous plasma concentrations were higher than arterial levels, suggesting an uptake of meperidine in the lungs. Equilibrium ratios of lung tissue to venous blood were estimated to be quite high, in the order of 25 to 30, suggesting that 10 to 15% of the total amount of meperidine in the body will be found in the lungs.

People exposed to nicotine develop tolerance to many of its effects. When heart rate is measured over time during and after a short constant-rate intravenous infusion of nicotine, a greater increase in heart rate is seen for a given nicotine concentration during infusion (the rising phase of concentrations) than after infusion (the declining phase). This could be the result of acute tolerance. On the other hand, similar differences in blood concentration-effect relationships in rising and falling portions of the blood concentration-time curve may be observed if drug concentrations at the effect site (brain) equilibrate more rapidly with arterial concentrations than drug levels at the (venous) blood sampling site.

To distinguish between these possibilities, rabbits were given nicotine intravenously over 1 min. Blood samples taken from the internal jugular vein (reflecting brain concentration), the femoral vein, and the femoral artery revealed that brain levels peaked before femoral venous concentrations. The results indicated that for nicotine in the rabbit, brain equilibrates with arterial blood about 3 times faster than do peripheral tissues.[30] Using typical human tissue volumes and blood flows, the investigators estimated that in humans, nicotine equilibrates with the brain about 14 times faster than with peripheral tissue.

Nicotine was also given to healthy human subjects by intravenous infusion and peripheral venous blood concentrations and cardiovascular responses were measured. Heart rate peaked before venous concentrations, suggesting more rapid distribution of drug to the heart than to venous blood. The investigators concluded that the apparent development of acute tolerance to the increase in heart rate during nicotine infusion may be due partly, if not completely, to the distribution kinetics of the drug rather than to the rapid development of functional tolerance.

Drug distribution rates are usually determined by simultaneously measuring drug concentrations in fluid or tissue (C_T) and in blood (C), as a function of time after intravenous bolus or infusion. Shortly after drug administration, the ratio of drug concentrations (C_T/C) is small. With time, the ratio increases until a constant value is achieved. This reflects equilibration of drug between the fluid or tissue and the blood.

A semilogarithmic plot of the difference between the equilibrium ratio and the ratio at a given time, as a function of time, will usually be linear, so that one may estimate a distribution half-life for a particular fluid or tissue. At least three paired samples (tissue and blood) must be obtained for this estimate. The need for multiple punctures or multiple biopsies at several times after drug administration severely limits the study of drug distribution. The alternative approach, serial sacrifice of animals at different times after administration, is limited to small species and introduces error due to interindividual variability.

A clever approach to overcome this problem and to obtain a considerable amount of information from a single sample of fluid or tissue has been described.[31] It is based on the administration of a series of different isotopes of a drug at different times before the sample is taken. The investigators suggested that this approach provides the same information about a drug's distribution to a particular

site as that obtained by infusing the drug and carrying out serial collections of fluid or tissue. They applied the method to study the distribution of phenobarbital into the cerebrospinal fluid (CSF) of a dog.

Three forms of phenobarbital with different molecular weights, achieved by stable isotope labeling, were infused intravenously at different times. The heaviest form (+5 PB) was infused first, at −25 min. At −10 min, the next heaviest form (+3 PB) was given; at time 0, unlabeled phenobarbital (+0 PB) was infused. Blood and CSF samples were then collected at 5, 15, 30, 60, and 90 min and the concentrations of each form of phenobarbital were determined in each fluid.

Each paired sample of blood and CSF provides three data points from which a distribution half-life may be estimated. For example, the 15 min sample permits calculation of a CSF/serum ratio 15 min after drug administration (+0 PB), 25 min after drug administration (+3 PB), and 40 min after drug administration (+5 PB). Staggered isotope administration data from each paired sample taken at 5, 15, 30, or 60 min provided estimates of distribution half-life ranging from 22 to 24 min. These estimates were in good agreement with the distribution half-life value determined by infusing unlabeled phenobarbital and taking multiple samples of blood and CSF over time.

Distribution Volumes

There is usually a considerable difference between the apparent volume of distribution of a drug and the actual volume in which it distributes. The apparent volume of distribution is simply a proportionality constant relating the plasma concentration to the total amount of drug in the body. Depending on the degree of binding to plasma proteins and tissues, the apparent volume of distribution of a drug may vary in man from 0.04 L/kg (plasma volume) to 20 L/kg or more.

The actual distribution volume of a drug is related to body water; it can never exceed total body water (TBW), that is, about 60% of body weight or 42 L in a normal 70-kg man. Body water may be divided into 3 compartments: vascular fluid, extracellular fluid (ECF), and intracellular fluid. In man, extracellular water is about one third of the total (i.e., about 19% of body weight or 15 L). This volume includes plasma water, which is about 4% of body weight or 3 L. The whole blood (vascular) volume, including the intracellular water of the erythrocytes, is about twice plasma volume, or about 6 L.

Certain dyes, such as Evans blue, are essentially confined to the circulating plasma and can be used to determine plasma volume (and blood volume, if the hematocrit is known). Certain substances such as chloride and bromide ions distribute rapidly throughout the ECF, but do not cross cell membranes, so they may be used to estimate extracellular water. The volume of TBW may be approximated by determining the distribution of heavy water (D_2O) or certain lipid-soluble but poorly bound substances, such as antipyrine.

The apparent volume of distribution of these tracers approximates their true volume of distribution. This, however, occurs with few substances, only those that are negligibly bound to plasma proteins and tissues. If a drug is preferentially bound to plasma proteins, the apparent volume of distribution is smaller than the real volume of distribution. On the other hand, preferential binding of drugs at extravascular sites results in an apparent volume of distribution larger than the true volume of distribution.

The following equation describes the relationship between apparent volume of distribution, drug binding, and anatomic volumes:[32]

$$V = V_B + (f_B V_T/f_T) \qquad (10\text{–}2)$$

where V is the apparent volume of distribution, V_B is blood volume, V_T is extravascular volume, and f_B and f_T are the free fractions of drug in the blood and extravascular space (tissues), respectively. If a drug is 92% bound to plasma proteins and other elements of blood, then $f_B = 0.08$. For lipid-soluble drugs, V_T is the difference between TBW and blood volume (V_B). For polar drugs, V_T is the difference between ECF volume and blood volume; this applies to most antibiotics.

Equation 10–2 indicates that the apparent volume of distribution increases with increases in anatomic volumes or tissue binding and with decreases in plasma protein or blood binding. The volume of distribution of a drug that is unbound in the body is equal to either TBW (e.g., antipyrine) or ECF volume (e.g., bromide ion), since $f_B = f_T = 1$. Disease factors and concomitant drug therapy that reduce drug binding to plasma proteins result in an increase in apparent volume of distribution.

A more physiologically correct expression for apparent volume of distribution,[33] which takes into

account that plasma proteins are not limited to the blood but are distributed throughout the ECF is:

$$V = V_P (1 + R_{E/I}) + f_P V_P [(V_E/V_P) - R_{E/I}] + V_R f_P/f_T \quad (10\text{–}3)$$

where V_P is plasma volume (3 L in a normal 70-kg man), V_E is the extracellular space minus plasma volume (15 L minus 3 L, or 12 L), V_R is the physical volume into which the drug distributes (i.e., TBW, 42 L, or extracellular space, 15 L) minus extracellular space, f_P and f_T are the free fractions of drug in plasma and tissue, and $R_{E/I}$ is the ratio of the amount of binding protein in the ECF outside the plasma to that in the plasma. Usually, 55 to 60% of the total extracellular albumin is found outside the plasma. Changes in this ratio occur with prolonged bed rest and in severe burns. Assuming that all drug binding proteins are distributed like albumin, then $R_{E/I} \simeq 1.4$.

Substituting these values into Equation 10–3 yields:

$$V = 7.2 + 7.8\, f_P + V_R\, f_P/f_T \quad (10\text{–}4)$$

If a drug distributes only to the ECF and does not enter cells (i.e., $V_R = 0$), the apparent volume of distribution is given by:

$$V = 7.2 + 7.8\, f_P \quad (10\text{–}4A)$$

When the apparent volume of distribution is large (i.e., $V > TBW$), the first two terms in Equation 10–4 are usually negligible and the equation reduces to:

$$V = V_R\, f_P/f_T \quad (10\text{–}5)$$

Changes in apparent volume of distribution are found as a function of body weight, age, and disease because of differences in anatomic volumes or drug binding. The apparent volume of distribution of some drugs may differ in men and women. Studies with chlordiazepoxide show that $V = 34$ L for both sexes. When the data are normalized for differences in total body weight, a significant sex difference is found; $V = 0.58$ L/kg for women and 0.45 L/kg for men.[34]

Investigators in Japan studied factors that affected the apparent volume of distribution of cefazolin, a cephalosporin antibiotic, in newborn human infants.[35] Body weight-normalized volumes of distribution (V/BW) determined in 11 newborns ranged from 0.21 to 0.37 L/kg. The unbound fraction of cefazolin in plasma varied widely, from about 0.2 to 0.8. A strong correlation was observed when V/kg was plotted against unbound fraction ($r = 0.94$). In turn, the unbound fraction was related to bilirubin levels and albumin concentrations in plasma. A statistically significant correlation ($r = 0.81$) between free fraction and the unconjugated bilirubin:albumin molar ratio was observed.

Cefazolin and other β-lactam antibiotics are localized in the extracellular water space and are bound to albumin in both intravascular and interstitial fluids. Predictions of the weight-adjusted apparent volume of distribution of cefazolin as a function of free fraction, based on Equation 10–3, were in good agreement with the values calculated in newborn infants.

DRUG BINDING IN BLOOD

Binding to plasma proteins affects drug distribution and elimination, as well as the pharmacologic effect of a drug. The high molecular weight of plasma proteins restricts passage across capillaries; their low lipid solubility prevents passage across cell membranes. Drug bound to plasma protein is similarly restricted. Only that fraction of the drug concentration that is freely circulating or unbound in extracellular water can penetrate cell membranes and is subject to glomerular filtration. Hepatic metabolism of most drugs is also limited by the free fraction of drug in the blood. The interaction of drugs with plasma proteins is a rapidly reversible process, however, and one may think of drug bound to plasma proteins as being in temporary storage, subject to instant recall.

Drug binding to plasma proteins may involve ionic, Van der Waals, hydrogen, and/or hydrophobic bonds. The most important contribution to drug binding in the plasma is made by albumin which comprises about one half of the total plasma proteins. In healthy individuals, albumin concentration in the plasma is about 4 g/100 ml. Lower levels are found during pregnancy (about 3.5 g/100 ml during the last trimester) and in certain diseases. Albumin binds a wide variety of drug molecules, but plays a particularly important role in the binding of weak acids and neutral drugs.

α_1-Acid glycoprotein (orosomucoid) is also an important binding protein, with an affinity for basic drugs, including imipramine, lidocaine, propranolol, and quinidine. α_1-Acid glycoprotein is a low molecular weight protein (approximately 40,000 daltons). It is an acute phase reactant, and its concentration in plasma rises in inflammation, malig-

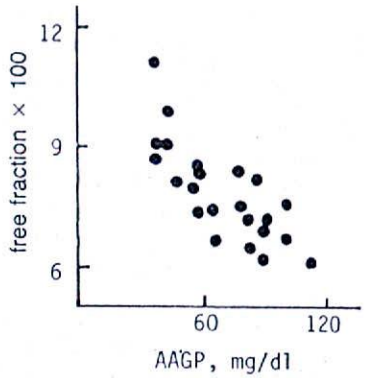

Fig. 10–3. Correlation between the levels of α_1-acid glycoprotein (AAGP) and free fraction of imipramine in healthy human subjects. (Data from Piafsky, K.M., and Borgå, O.[36])

nant disease, and stress and falls in hepatic disease and nephrotic syndrome. The average concentration of α_1-acid glycoprotein in plasma is about 40 to 100 mg/100 ml. The relationship between imipramine binding and α_1-acid glycoprotein concentration in plasma in healthy men and women is shown in Figure 10–3.[36] Other considerations concerning the plasma protein binding of basic drugs have been discussed by Routledge.[37]

The other proteins in plasma play a limited role in drug binding. There is a highly specific interaction between certain steroids, such as prednisolone, and corticosteroid-binding globulin, also known as CBG or transcortin.[38] Transcortin also binds thyroxine and vitamin B_{12}. Gamma globulins react specifically with antigens, but negligibly with most drugs.

The drug-protein interaction may be described by the law of mass action:

$$D_F + \text{Free sites} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} D_B$$

where D_F and D_B are the free and bound drug, respectively, and k_1 and k_2 are the rate constants for association and dissociation, respectively. Thus,

$$K = \frac{k_1}{k_2} = \frac{[D_B]}{[D_F]\,[\text{Free sites}]} = \frac{[D_B]}{[D_F]\,(n[P] - [D_B])} \qquad (10\text{–}6)$$

where K is the equilibrium association constant, n is the number of binding sites per mole of protein, and $[D_F]$, $[D_B]$, and $[P]$ are the molar concentrations of free and bound drug and protein, respectively.

The binding rate constants, k_1 and k_2, appear to be large since equilibrium is established almost immediately. The equilibrium constant, K, varies from about zero, where essentially no drug is bound, to about 10^6, where almost all the drug is bound to the protein.

The fraction of drug in the plasma that is free or unbound, f_P, is given by:

$$f_P = [D_F]\,([D_F] + [D_B]) = [D_F]\,[D_T] \qquad (10\text{–}7)$$

where $[D_T]$ is the total concentration of drug in the plasma. In most cases, for a given amount of drug in the body, the greater the binding of drug to plasma proteins, the larger is the total drug concentration in plasma. Changes in binding usually affect blood levels of total drug and play a role in pharmacokinetic variability.

The fraction of drug free in the plasma depends on the magnitude of K, the total drug concentration, and the protein concentration. In principle, there are a limited number of binding sites on the protein. As the drug concentration in plasma increases, the number of free sites decreases; therefore, the fraction of free drug increases. In practice, however, the fraction of unbound drug in plasma for most drugs administered in therapeutic doses is essentially constant over the entire drug concentration range.

Concentration-dependent changes in the fraction of free drug in the plasma are most likely to occur with drugs having a high association constant (i.e., 10^5 to 10^6), and that are given in large doses (e.g., certain sulfonamides and phenylbutazone). The fraction of disopyramide unbound to plasma proteins varies from about 0.19 to 0.46 over the therapeutic range of total drug concentration in plasma (2 to 8 μg/ml).[39] A total disopyramide concentration of 2 μg/ml provides a free drug level of about 0.4 μg/ml; a 4-fold increase in total drug concentration results in a 10-fold increase in free drug concentration.

Ceftriaxone, a third generation cephalosporin, is unusual in that its plasma protein binding is concentration-dependent.[40] The percentage of free ceftriaxone in plasma increases from 4 to 17% when the total ceftriaxone concentration in plasma is increased from 0.5 μg/ml to 300 μg/ml. Concentra-

tion-dependence in plasma protein binding is also found following usual doses of valproic acid, and aspirin in the treatment of rheumatoid arthritis.

The relationship between bioavailability and area under the drug concentration-time curve (AUC) is nonlinear and absorption-rate dependent when the plasma protein binding of a drug is concentration-dependent.[41] Two products from which a drug is equally well absorbed produce different values for AUC if there is a difference in absorption rate. As a rule, AUC comparisons will overestimate the bioavailability (extent of absorption) of the more slowly absorbed product.

A large degree of intersubject variability in binding is seen with certain drugs. Studies in 26 patients who had been taking phenytoin regularly for more than 2 weeks showed that the free (unbound) phenytoin level in plasma varied from 5.8 to 12.6% of the total drug concentration.[42] A study of 31 patients with cardiovascular disease who were taking warfarin regularly has shown that the free warfarin level in plasma ranges from 0.4 to 1.9% of the total drug concentration.[43] A study concerned with the binding of benzodiazepines in human plasma found that free fraction varied 2-fold for lorazepam, 4-fold for diazepam and chlordiazepoxide, and 20-fold for oxazepam.[44] The free fraction of imipramine in plasma of depressed patients was found to vary 4-fold (5.4 to 21%). This variability may contribute to the difficulty in correlating the plasma levels of antidepressant drugs and clinical outcome.[45] Diurnal variations in plasma protein binding are responsible for variations in the total blood levels of diazepam throughout the day.[46] Normal changes in plasma lipids may account for part of the inter- and intrasubject variability in plasma protein binding found in healthy human subjects, because of potential competition between lipids and drugs for binding sites on plasma proteins.

The classic methods of studying plasma protein binding of drugs are equilibrium dialysis and ultrafiltration. Measurements are made more quickly using ultrafiltration but may not be as accurate as those based on equilibrium dialysis. The plasma protein binding of phenytoin was measured in patients with normal renal function and impaired renal function using a new, simplified ultrafiltration method as well as the traditional equilibrium dialysis method. As expected, unbound fraction was about twice as large in uremic patients as in patients with normal renal function, but in both groups of patients the values for each method were in good agreement. The investigators concluded that the new ultrafiltration device "has a great potential for measurements of unbound concentrations of phenytoin because of its rapidity and reliability."[47]

Plasma Protein Binding and Drug Distribution

Since the protein concentration in extravascular fluid is less than in plasma, the total drug concentration in plasma is usually higher than in lymph, cerebrospinal fluid (CSF), synovial fluid, and other fluids of the extravascular space.

Normal CSF contains so little protein that it is often viewed as an ultrafiltrate of the plasma. The plasma protein binding of nortriptyline in man is about 94%. This value agrees with the finding that the steady-state concentration of nortriptyline in CSF is only 3 to 11% of the plasma level.[48] The CSF concentrations of carbamazepine and its epoxide metabolite in patients being treated for epilepsy are closely related to free drug and free metabolite concentrations in serum.[49]

Normal synovial fluid contains only 1-g albumin/100 ml; however, albumin levels may be elevated in synovial fluid from patients with arthritis or other degenerative joint diseases. The penetration of ampicillin and cloxacillin into synovial fluid has been measured after oral administration of these penicillins to patients with osteoarthritis or rheumatoid arthritis.[50] The results show that both drugs diffused rapidly into synovial fluid but that they differed appreciably with respect to total concentration relative to total concentration in plasma.

For ampicillin, which is not highly bound to plasma proteins (only 10 to 15%), the total drug levels in synovial fluid were similar to the total plasma levels. With cloxacillin, which is highly bound to plasma proteins (95%), the total levels in synovial fluid were considerably lower than those in plasma. However, the unbound levels of cloxacillin in synovial fluid and plasma were similar (Fig. 10–4).

Free and total ibuprofen levels in serum and synovial fluid were determined in patients with arthritis.[51] Albumin concentrations were found to be 3.7 g/100 ml in serum and 2.1 g/100 ml in synovial fluid. Total ibuprofen concentration in joint fluid was about 40% that in serum. The drug was bound to the extent of 99% in serum and 97.5% in synovial fluid. The ratio of total ibuprofen in synovial fluid to that in serum correlated with the albumin concentration ratio ($r = 0.89$). Free ibuprofen concentration in joint fluid (0.19 μg/ml) was similar

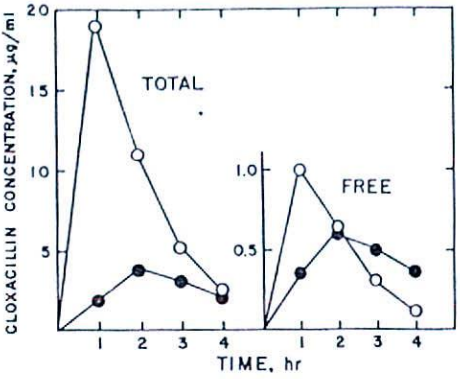

Fig. 10–4. Total and free cloxacillin concentrations in serum (○) and synovial fluid (●) after a single 500-mg oral dose to patients with arthritis. (Data from Howell, A., Sutherland, R., and Rolinson, G.N.[50])

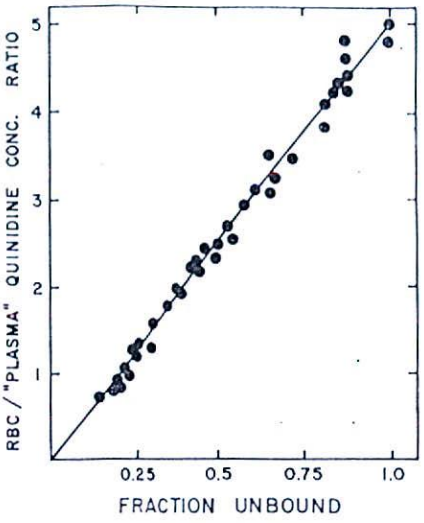

Fig. 10–5. Relationship between the red blood cell (RBC)/plasma (or diluted plasma) quinidine concentration ratio and the fraction of unbound quinidine in the plasma. (Data from Hughes, I.E., Tett, K.F., and Jellett, L.B.[54])

to free serum levels (0.25 µg/ml), indicating that synovial fluid is easily accessible to unbound drug.

Drug Binding to Erythrocytes. Several studies have demonstrated that drug uptake by erythrocytes is a function of plasma protein binding. Linear correlations have been reported between the blood or red blood cell (RBC)/plasma concentration ratio and the percent of unbound drug in the plasma for propranolol,[52] phenytoin,[53] quinidine,[54] and haloperidol.[55] The quinidine data are shown in Figure 10–5.

Determination of the RBC-plasma concentration ratio has been suggested as a simple and rapid technique for the large-scale screening of abnormal plasma binding in routine clinical blood samples. Estimates of free rather than total drug concentration may be more useful for individualization of drug therapy.

Drugs that bind avidly to RBCs may show concentration-dependent uptake from plasma. Erythrocyte accumulation of acetazolamide appears to be a composite of two processes: a nonlinear, saturable process and a linear process (Fig. 10–6).[56] The partitioning of the diuretic, chlorthalidone between RBC and plasma is also concentration-dependent.[57] When the concentration of chlorthalidone in blood is less than 15 to 20 µg/ml, 98% of the drug is bound to the red cells. Increasing the blood concentration results in an abrupt and substantial decrease in the partition ratio, in favor of the plasma, indicating saturable binding sites of chlorthalidone on RBCs. The binding of cyclosporine, an immunosuppressant widely used to prevent rejection in transplant patients, to erythrocytes is also concentration-dependent.[58] Blood/plasma ratio decreases from about 1.5 at low plasma levels of cyclosporine to 1.0 at very high levels of the drug. Whole-blood cyclosporine measurements will be difficult to interpret when hematocrit varies considerably.

Although drug binding to plasma proteins, erythrocytes, and other tissues is usually rapidly reversible, there are examples of apparently irre

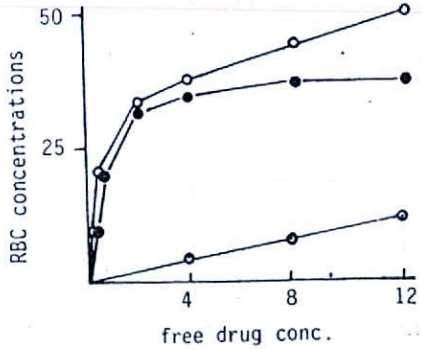

Fig. 10–6. Acetazolamide concentrations (µg/ml) in red blood cells as a function of free drug concentration in plasma. Key: (○) total concentration; (●) bound concentration; (⊙) free concentration. (Data from Wallace, S.M., and Riegelman, S.[56])

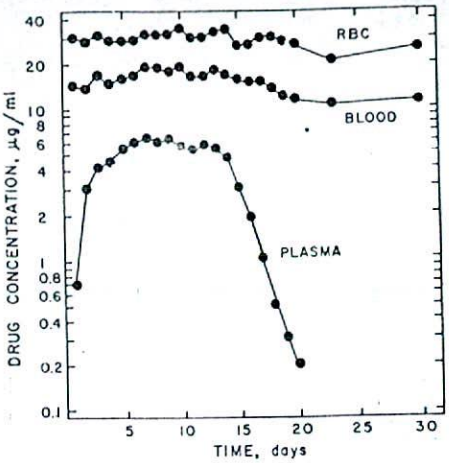

Fig. 10–7. Concentrations of an investigational carbonic anhydrase inhibitor in plasma, blood, and RBCs during and after repetitive oral dosing with 100 mg twice daily for 14 days. (Data from Lund, J., et al.[59])

versible binding. A particularly dramatic example has been reported with an investigational carbonic anhydrase inhibitor in man.[59] Drug concentrations in plasma, blood, and erythrocytes during and after repetitive dosing to human subjects are shown in Figure 10–7. The drug is found in the RBCs in measurable amounts for more than a year after the last dose.

Irreversible binding of drugs to serum albumin has also been demonstrated. Zomepirac is a nonsteroidal anti-inflammatory drug withdrawn from the market because of an unexplained high incidence of immunologic reactions. It is metabolized in humans to a reactive, unstable acyl glucuronide. Because of the similarities of zomepirac glucuronide to bilirubin glucuronide, in structure and stability, and the documented irreversible binding of bilirubin to albumin through its acyl glucuronide, Smith et al.[60] studied the reaction of zomepirac acyl glucuronide with albumin.

Irreversible binding of zomepirac to protein was demonstrated both in vitro and in vivo. Binding correlated with overall exposure to zomepirac glucuronide. When probenecid, which decreases the plasma clearance of zomepirac glucuronide and increases blood levels of the unstable metabolite, was given concurrently with zomepirac, irreversible binding was increased. The investigators concluded that the formation of irreversible protein-bound zomepirac occurs via the acyl glucuronide and the reaction may be general for other drugs metabolized to acyl glucuronides, such as tolmetin.[61]

Plasma Protein Binding and Drug Effects

There is considerable theory but limited experimental evidence to suggest that the concentration of free drug in the plasma is the critical determinant of drug effect. To test this hypothesis, investigators studied the relationship between anticoagulant effect and the concentrations of free and total warfarin in rat plasma.[62] The concentration of total warfarin required to elicit a defined anticoagulant effect varied widely among animals (coefficient of variation of 85%), whereas the required concentration of free warfarin showed much less variation (coefficient of variation of 29%). These studies suggest that the anticoagulant effect of warfarin is more nearly a function of its free than of its total concentration in plasma.

In another study, rats were given phenytoin and its potency against maximal electroshock seizures was determined. The effect of phenylbutazone (a drug that competitively decreases the plasma protein binding of phenytoin and decreases its total plasma concentration) on the potency and on the total and unbound plasma concentrations of phenytoin was then measured. Phenylbutazone treatment increased the potency of phenytoin in terms of dose and total drug concentration (i.e., a lower plasma phenytoin concentration and a lower dose were required to protect against shock-induced seizure) but did not affect the potency of unbound phenytoin.[63] Thus, the anticonvulsant action of phenytoin appears to depend on the concentration of unbound drug in plasma rather than on the total plasma concentration or the dose.

The effects of plasma protein binding on the relationship between propranolol concentration and the antagonism of isoproterenol-induced tachycardia was investigated in healthy subjects and hypertensive patients.[64] A poor correlation was found between effect and total propranolol concentration in plasma ($r = 0.46$), whereas there was an excellent correlation between efficacy and free drug concentration ($r = 0.89$). The effect of propranolol on heart rate is a predictable function of free drug concentration in man; the contribution of individual variation in receptor sensitivity to differences in oral dosage requirement is minor compared to that of variations in systemic availability.

Plasma Protein Binding and Drug Elimination

Free rather than total drug concentration is often the driving force for drug elimination. The glomerular capillaries in the kidneys contain pores that, like those in most other capillaries, permit the passage of most drugs but restrict the passage of plasma proteins. Accordingly, in healthy individuals the glomerular filtrate is an ultrafiltrate of plasma; only free (unbound) drug is filtered. In the absence of active tubular secretion and reabsorption, drug concentration in urine is equal to free drug concentration in plasma.

If a drug or chemical is neither secreted nor reabsorbed by the tubules and is not protein bound, its renal clearance is a measure of glomerular filtration rate (GFR); inulin and creatinine have these characteristics and are often used to estimate GFR. If, on the other hand, the drug is protein bound, the renal clearance of total drug in the plasma is less than GFR but the clearance of free drug is equal to GFR. Studies have shown that the rate of renal excretion of several tetracyclines is inversely related to their extent of plasma protein binding.[65] More recently, studies in an isolated perfused rat kidney, using different amounts of serum albumin in the perfusate, showed that the renal clearance of digitoxin is linearly related to the unbound fraction of drug in perfusate.[66]

The rate of metabolism of certain drugs is also related to the degree of binding in the plasma. For example, a highly significant inverse rank-order correlation has been reported between the rate of metabolism of 11 sulfonamides in man and their degree of protein binding.[67] A strong correlation ($r = 0.95$) has been observed between total clearance of warfarin from plasma and the free fraction of drug in the serum of individual rats.[68] These data are shown in Figure 10–8. The results show that the pronounced intersubject variability (about 10-fold) in the elimination of warfarin in the rat is strongly related to intersubject differences in plasma protein binding of the drug. A statistically significant correlation between the total plasma clearance of warfarin and the free fraction of this drug in serum has also been found in patients with cardiovascular disease who were taking warfarin regularly.[43]

The plasma protein binding and metabolism of diflunisal are both capacity limited in the rat. A plot of clearance of total diflunisal from plasma vs drug concentration described a U-shaped curve. At low diflunisal concentrations (<100 μg/ml) clearance decreased with increasing drug levels, whereas at higher concentrations (>200 μg/ml) clearance increased with increasing drug levels. On the other hand, the clearance of unbound diflunisal from plasma consistently decreased with increasing drug levels. The pattern described when the clearance of total diflunisal was plotted against drug concentration is a consequence of capacity-limited metabolism (low concentration effect) as well as capacity-limited binding (high concentration effect). The results obtained when the clearance of unbound diflunisal was studied simply reflect capacity-limited metabolism.[69]

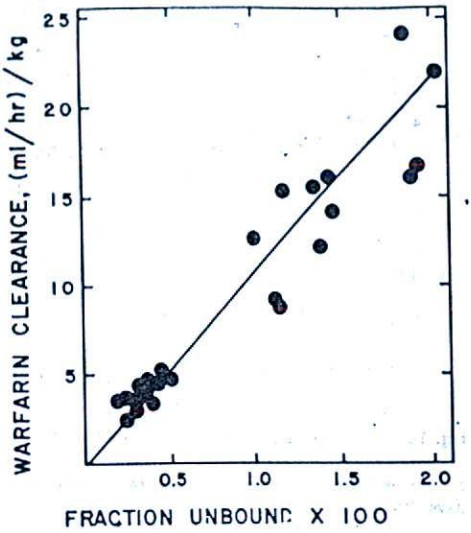

Fig. 10–8. Relationship between the clearance of warfarin from plasma and free (unbound) fraction in the serum of individual rats. (Data from Yacobi, A., and Levy, G.[68])

The clearance of many drugs from the blood is directly proportional to free fraction in the plasma (f_p); the steady-state concentration of these drugs is inversely proportional to f_p. On the other hand, the clearance of some drugs is largely independent of plasma protein binding; drugs in this category include those subject to rapid and extensive hepatic metabolism, such as lidocaine or verapamil, and those undergoing extensive tubular secretion, such as the penicillins.

The effect of plasma protein binding on the half-life of a drug depends on the apparent volume of distribution (V) of the drug.[70] The half-life of drugs with a relatively small V (e.g., <0.25 L/kg) is

sensitive to changes in f_p; a decrease in plasma protein binding results in a shorter half-life. Conversely, the half-life of drugs with larger values of V (e.g., >0.5 L/kg) is essentially independent of plasma protein binding.

DRUG BINDING IN TISSUES

Tissue binding plays an important part in drug distribution, at least in the sense of drug storage. In many cases, more than 90% of the drug in the body is bound in the extravascular or tissue space. Drug binding to tissues is poorly understood. Few studies have been directed to this interaction; almost all have used tissues from experimental animals.[71-73] Tissue binding has no effect on drug clearance or on average steady-state concentrations of drug in blood or plasma. Its principal influence is on the time course of drug in the body. The half-life of drugs with apparent volumes of distribution exceeding 0.5 L/kg is determined by clearance and tissue binding.[70,74] An increase in clearance or a decrease in tissue binding decreases the half-life of a drug. The role of tissue binding in the pharmacologic effects of drugs is unknown.

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