MECHANISMS OF DRUG ABSORPTION AND DISTRIBUTION[1,2]

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During the past three to four years, considerable progress has been made toward defining the mechanisms of drug absorption and distribution. Perhaps one of the reasons for this rapid advance has been the adoption of the view that the mechanisms of absorption and distribution of foreign organic compounds may be considerably simpler than those of the natural cell substrates. This idea has developed from the numerous studies of membrane permeability of the past 60 years. The classical work of Overton (1) and of Collander & Bärlund (2) on the permeability of plant and animal cells to organic compounds led to the view that the cell boundary is essentially a lipid-like layer interspersed with small aqueous channels; nonpolar substances would penetrate the membrane by dissolving in the lipoid phase, and polar molecules would penetrate only if they were small enough to diffuse through the open channels. Later workers recognized that the cell membrane also possessed specialized processes for transporting lipid-insoluble substrates required by the cell (3). For example, certain sugars and amino acids were found to cross the cell boundary by processes which differed from simple diffusion in that they exhibited specificity, saturability, and a requirement for energy. Although the voluminous literature dealing with specialized transport processes tended to relegate to the background the lipoid sieve theory of membrane permeability, recent studies of the absorption and distribution of drugs have shown the great importance of this early concept. The gastrointestinal epithelium, the renal tubular epithelium, the blood-brain and blood-cerebrospinal fluid boundaries, and the boundaries of various tissue cells behave as lipid-like barriers to the passage of many foreign organic compounds. Numerous drugs diffuse across these boundaries as lipid-soluble, nonionized molecules, and the rates of transfer are, in general, related to the relative lipid/water partition coefficients of the molecules. The almost lipid-insoluble, ionized forms of drugs diffuse across these boundaries with relatively great difficulty.

However, the lipoid membrane thesis does not explain completely the passage of drugs across the boundaries of mammalian cells. Specialized transport processes appear to be responsible for the rapid cellular transfer of certain foreign organic ions and polar molecules.

This article reviews a number of investigations of the past few years which attempt to define the mechanisms of drug absorption and distribution.

[1] The survey of literature pertaining to this review was concluded in May, 1960.

[2] Abbreviations used in this chapter include: CSF (cerebrospinal fluid); EDTA (ethylenediamine-tetraacetic acid).

Passage of Drugs Across the Gastrointestinal Epithelium

Distribution of drugs between plasma and gastric juice.—A study by Shore *et al.* (4) of the distribution of drugs between plasma and gastric juice has supplied strong evidence that drugs cross the gastric epithelium in their nonionized form, and that the ionized form penetrates very slowly, if at all. In the experiments, various acidic and basic drugs were administered intravenously to dogs with Heidenhain gastric pouches, and the concentrations of drug in gastric juice and plasma measured. At the steady state, basic drugs appeared in gastric juice in concentrations ranging

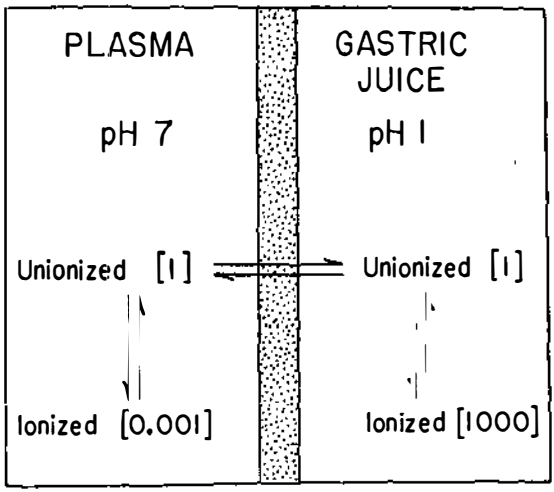

Fig. 1. Theoretical distribution of an organic base, pKa 4, between plasma and gastric juice, assuming that the fluids are separated by a boundary permeable only to the un-ionized drug molecule.

from one to 40 times that of plasma. In contrast, acidic drugs appeared in gastric juice in low concentrations which ranged from zero to six-tenths that of plasma. The results were explained in terms of a model system in which gastric juice is separated from plasma by a barrier permeable only to the nonionized form of a weak electrolyte (Fig. 1). At the steady state, the concentrations of nonionized drug in plasma and gastric juice are the same (correcting for the degree of plasma binding), but the concentrations of the ionized form are unequal because of the difference in hydrogen-ion concentration of the two fluids. Accordingly, the total concentration of drug (ionized plus nonionized) on both sides of the gastric mucosa is a function of the pH of the two fluids and the dissociation constant of the drug. From this relationship, it can be readily calculated that a basic drug will be more concentrated in gastric juice than in plasma, and an acidic drug will be more concentrated in plasma than in gastric juice. The gastric juice/plasma concentration ratios observed in this study were generally in close agreement with the calculated ratios; however the maximum ratio of 40, observed

for a number of basic compounds, was considerably less than the values calculated for these compounds. The apparent inconsistency was resolved when it was shown that the ratio of 40 was a limiting value imposed by the rate of gastric mucosal blood flow.

This interpretation of the distribution of drugs across the gastric mucosa is consistent with the observations of a number of earlier workers that basic dyes, administered parenterally, appear in the gastric lumen, whereas acidic dyes do not. The hypothesis is also supported by the recent findings of Zawoiski *et al.* (5), who studied the gastric secretion of mecamylamine in the dog. This organic base readily passes from plasma into the gastric lumen where it becomes concentrated to an extent dependent on the pH of the gastric juice—the lower the gastric pH, the higher the gastric juice/plasma concentration ratio.

The establishment of concentration gradients of weak electrolytes across the gastric epithelium is, as shown later in this article, but one example of a type of drug distribution observed with many biologic membranes. All that is required for such a distribution is that the membrane have a preferential permeability to the nonionized form of the weak electrolyte, and that the fluids bathing either side of the membrane have different hydrogen ion concentrations. The process is physical in nature and does not display the characteristics of a specialized transport system; there is, however, an indirect expenditure of energy by the organism in maintaining the pH differential across the membrane.

Absorption of drugs from the stomach.—Most of the recent studies of the mechanism of gastrointestinal drug absorption, as well as some of the earlier papers on this topic, have been reviewed by the author (6).

Schanker *et al.* (7) have shown that a variety of drugs are absorbed directly from the rat stomach by simple diffusion of the nonionized drug moiety. Of the large number of compounds studied, ready absorption was observed for all of the acidic drugs except the highly ionized sulfonic acids. In contrast, none of the basic compounds were absorbed except those so weakly basic that they are partially nonionized in the gastric contents.

Additional evidence that it is mainly the nonionized form of a drug which is absorbed was obtained by changing the degree of ionization of drugs by raising the pH of the stomach contents. Basic compounds, which become more un-ionized at higher pH values, were more readily absorbed from the alkaline medium. Conversely, acidic compounds, which become more ionized at higher pH values, were less readily absorbed. Evidence of the passive nature of the absorption process was supplied by the observations that various drugs did not interfere with the absorption of other drugs, and that the amount of drug absorbed was directly proportional to the concentration within the stomach.

An indication that lipid solubility is the physical property governing the passage of uncharged molecules across the gastric epithelium was provided by a study of three barbiturates with similar pKa values. These compounds were absorbed at rates roughly proportional to the lipid/water partition co-

efficients of their nonionized forms. For example, thiopental was absorbed very rapidly, secobarbital less rapidly, and barbital relatively slowly.

Hogben *et al.* (8) reported that the pattern of absorption from the stomach of man was the same as in the rat (7). Acidic drugs like salicylic acid, acetylsalicylic acid, thiopental, and secobarbital were readily absorbed. Basic compounds like quinine, ephedrine, and aminopyrine were not absorbed. Of considerable interest was the observation that thiopental and the two salicylates were absorbed more rapidly than ethyl alcohol; heretofore ethanol had often been considered the rather unique example of a drug absorbed from the stomach.

Absorption of drugs from the small intestine.—Schanker *et al.* (9) have found that the epithelial lining of the intestine, like that of the stomach, allows the ready penetration of undissociated drug molecules but impedes the passage of ionized moieties. In experiments with rats, the entire small intestine was perfused with a drug solution, and the extent of absorption estimated from the difference in the concentration entering and leaving the intestine. A relation between the degree of ionization and the rate of absorption of drugs was revealed: the weaker acids and bases were readily absorbed; stronger, highly ionized acids and bases were more slowly absorbed; and the completely ionized quaternary ammonium compounds and sulfonic acids were hardly absorbed at all. It was pointed out that the failure to observe any measureable absorption for some of the very highly ionized compounds was not inconsistent with the slow but definite absorption of these substances known to occur in therapeutics; in the perfusion experiments, the drug solution passed through the intestine in only seven minutes, contrasted with the several hours that a drug remains in the intestine when used therapeutically.

The direct proportionality between the amount of salicylic acid or aniline absorbed and the concentration of drug within the intestine suggested that absorption occurred by simple diffusion rather than by a specialized transport process which would be expected to become saturated at the higher drug concentrations. Additional evidence of the passive nature of the absorption process was the failure of various drugs to alter the rates of absorption of other drugs.

Since the rates of intestinal absorption of drugs were related to the proportion of lipid-soluble, undissociated drug molecules and not to the molecular weight of the compounds, it was suggested that the main pathway of absorption is through the lipoid areas of the intestinal boundary rather than through small aqueous channels. In support of this view, many lipid-soluble drugs of high molecular weight were absorbed more rapidly than small, lipid-insoluble molecules like urea and D_2O.

Hogben *et al.* (10) studied the effect of changes of intestinal pH on the absorption of a number of weak organic acids and bases in the rat. It was shown that the rate of absorption varied with the proportion of drug present as nonionized molecules. For example, raising the intestinal pH increased

the absorption of bases and decreased the absorption of acids; moreover, compounds which remained essentially undissociated at the various hydrogen-ion concentrations tested showed no change in their rate of absorption.

These investigators also measured the distribution of various drugs between plasma and the intestinal lumen. In the experiments, the rat intestine was perfused with a solution of the drug, and the animal also received the drug intravenously. At the steady state, when there was no net passage of drug from intestine to plasma or from plasma to intestine, the gut/plasma concentration ratios approached, but did not equal the ratios calculated for a system in which plasma (pH 7.4) and intestinal contents (pH 6.6) are separated by a lipoid membrane. The results suggested that the effective pH at the site of absorption might be different from the pH of the intestinal contents. On calculating the effective pH of the intestine from the observed gut/plasma concentration ratios, a value of 5.3 was obtained. A zone with a pH of 5.3, possibly located at the surface of the intestinal epithelial boundary, was shown to be quantitatively consistent with the pattern of drug absorption in the rat intestine.[3]

Evidence that lipid solubility is the physical property that determines the rate of passage of uncharged molecules across the intestinal epithelium was provided by the observation that the rates of absorption of a large number of weak acids and bases were roughly parallel to the lipid/water partition ratios of the nonionized drug molecules.

Intestinal absorption of quaternary ammonium ions.—Although the intestinal absorption of weak organic electrolytes can be explained in terms of simple diffusion of uncharged molecules across a lipoid boundary, the question remains how organic ions are absorbed. The rates at which organic anions and cations cross the intestinal epithelium are extremely slow compared with the rates of passage of most uncharged molecules (9, 10). Nevertheless, it is well known that ions like the quaternary ammonium compounds are absorbed to a significant extent when administered therapeutically. For example, it has been estimated that 5 to 10 per cent of an oral dose of hexamethonium is absorbed in man (13, 14, 15).

Levine *et al.* (16, 17) studied the absorption of a number of quaternary ammonium ions by measuring their rates of disappearance from loops of the rat small intestine. It was observed that in general the rate of absorption declined markedly with time. For example, several quaternary ammonium compounds were absorbed to the extent of about 15 per cent in four hours, but most of the absorption took place during the first hour. An exception is penthienate, which was continuously absorbed throughout five hours [Levine & Clark (18)]. It was suggested that the poor absorption of these drugs is attributable to the formation of nonabsorbable complexes with mucin. This view was supported by the observation that adding mucin to the intestinal loop depressed the degree of absorption of benzomethamine (16).

[3] The phenomenon of the difference in pH between a biologic surface and the solution which bathes it has been discussed by Albert (11) and by Caldwell (12).

More recently Levine (19) reported that, although intestinal mucus can form nonabsorbable complexes with quaternary ammonium compounds, removal of the mucus by washing the intestine results in a decrease rather than an increase in the absorption of these ions. It was suggested that the mucus might contain some component capable of forming an absorbable complex with these drugs. However a variety of constituents of normal intestinal contents failed to augment the absorption of benzomethamine. For example, adenylic acid, glucose-1-phosphate, chondroitin sulfate, and hyaluronic acid were found to inhibit absorption; amino acids, saturated fatty acids, bile, bile salts, sorbitol, glucuronic acid, and mucic acid had no effect on the absorption of benzomethamine.

The available information raises a number of questions about the mechanism of absorption of quaternary ammonium ions. Does intestinal mucus promote as well as inhibit the absorption of these cations? Does the binding of quaternary ammonium compounds to mucus have something to do with the process by which these drugs cross the intestinal epithelium, or does it result only in a lowering of the concentration of diffusable drug? If quaternary ammonium ions are absorbed as complexes, what is the nature of the complex, and what happens to it on reaching the bloodstream? Whether these compounds are absorbed in the unchanged form or as complexes, do they cross the intestinal epithelium by simple diffusion or by specialized transport processes analogous to those which transport certain inorganic cations? A clue to the answer of the last question might be obtained from a thorough study of the kinetics of the absorption process.

Intestinal absorption of tetracycline.—Tetracycline is a highly ionized drug which, like the quaternary ammonium compounds, is only partially absorbed from the gastrointestinal tract when administered therapeutically [Maynard *et al.* (20)]. During the past few years, reports from several laboratories have claimed that the absorption of tetracycline in man is significantly enhanced when the antibiotic is administered in capsules containing citric acid, sodium hexametaphosphate, or glucosamine. Numerous comparisons of the effectiveness of the various adjuvants have been made, and conflicting results reported. For example, various investigators found different adjuvants to be superior to the others tested, and some investigators found that the adjuvants had no effect on the absorption of tetracycline.

In reviewing a number of these reports, Finland (21) concluded that several of the claims of enhanced absorption were not supported by the experimental data. He also pointed out that much of the conflicting data was the result of failure to control the amount of calcium salts in the various capsule preparations. Dicalcium phosphate has been shown to depress markedly the absorption of tetracycline [Dearborn *et al.* (22)]; the same salt is commonly used as a filler in capsule preparations on the assumption that it is inert. Finland (21) and Kunin *et al.* (23) have concluded that there is little or no difference in the blood levels of tetracycline achieved in clinical practice whether the antibiotic is administered as the

pure (calcium-free) hydrochloride salt or in combination with various adjuvants.

From a study in rats, in which the blood level of tetracycline was taken as a measure of the degree of absorption, Dearborn *et al.* (22) concluded that both calcium ion and food interfered with the absorption of tetracycline, and that citric acid and sodium metaphosphate partially reversed the effect. The authors suggested that citric acid and sodium metaphosphate facilitate the absorption of tetracycline by complexing with calcium and other multivalent metal ions which are present in the gastrointestinal tract and which form complexes with tetracycline.

Although the results of the above studies are interesting and have important implications in the therapeutic use of tetracycline, they give no indication of the process by which this drug is absorbed. The work of Pindell *et al.* (24) has supplied the only clear information regarding the mechanism of absorption of tetracycline. These workers measured directly the extent and rate of absorption of the drug from loops of the dog small intestine. Only about 3 per cent of an administered dose of tetracycline was absorbed in 1.5 hours, and the rate of absorption was constant throughout this period. The amount of tetracycline absorbed was directly proportional to the concentration over a tenfold range, and it was concluded that absorption occurs by passive diffusion.

Considering that tetracycline exists largely as a zwitterion at neutral pH (25), it would be interesting to know whether this drug is absorbed predominantly as the zwitterion, anion, cation, or neutral molecule.

Intestinal absorption of large molecules.—Loomis (26) has reported that heparin, a polysaccharide ordinarily not absorbed from the gastrointestinal tract, can be absorbed to a small extent when the intestinal contents are made acidic. In the experiments, solutions of heparin were placed in duodenal loops of the dog, and the degree of absorption was estimated from changes in the clotting time of blood as well as from the amount of drug recovered from the intestine after five to seven hours. An anticoagulant effect was noted when the drug was administered in a citrate-phosphate solution of pH 4; in contrast, no absorption occurred from a similar solution of pH 8 or from physiological saline solution. Since the anticoagulant effect lasted only three hours, and only about 10 per cent of the dose was absorbed, it appeared that absorption ceased as the intestinal pH rose from 4 to 6.5. It was suggested that the absorption of heparin from a solution of low pH might be attributed to the lowered degree of ionization of the molecule's carboxyl groups.

Windsor & Cronheim (27) have found that heparin and a synthetic heparinoid, sulfopolyglucin, are absorbed from the gastrointestinal tract of the dog and rat when administered with certain salts of ethylenediaminetetraacetic acid (EDTA). The sodium, potassium, and ammonium salts of EDTA were effective, but the calcium and magnesium salts were not. It was suggested that the alkali salts of EDTA facilitate the absorption of heparin and sulfopolyglucin by forming chelates with calcium and mag-

nesium ions in the intestinal lumen. In support of this view, the oral administration of calcium ion blocked the enhancement of absorption produced by alkali salts of EDTA.

Trace amounts of polypeptides and proteins are known to be absorbed from the gastrointestinal tract. For example, Laskowski *et al.* (28) estimated that in six hours somewhat less than 3 per cent of a dose of insulin was absorbed from a loop of the rat small intestine when the drug was administered together with a pancreatic inhibitor of trypsin and chymotrypsin. When insulin was not protected from the actions of proteolytic enzymes, absorption did not occur. From similar experiments in the rat, Danforth & Moore (29) concluded that insulin was absorbed to the extent of about 0.05 per cent in three hours when administered with diisopropylfluorophosphate, another inhibitor of the actions of proteolytic enzymes.

The toxicity of the ingested exotoxin of *Clostridium botulinum* and the allergic response to ingested proteins of food are familiar examples of the intestinal absorption of protein molecules. Lamanna (30) has recently shown that the diphtheria and tetanus toxins, as well as the botulinus toxin, are absorbed from the gastrointestinal tract of mice. Absorption could be demonstrated only when huge amounts of the toxins were administered, the oral LD_{50} values being hundreds of thousands of times greater than the intraperitoneal LD_{50} values. It was suggested that protein molecules are absorbed by passing through imperfections in the intestinal boundary. Pinocytosis ("cell drinking") has also been suggested as a process by which large molecules may penetrate cell boundaries [Leake & Pomerat (31)].

Absorption of drugs from the colon.—Schanker (32) found that the pattern of drug absorption in the rat colon was very similar to that in the small intestine. Weak acids and bases were in general readily absorbed; stronger, more highly ionized acids and bases were more slowly absorbed; and the completely ionized quaternary ammonium compounds and sulfonic acids were very slowly absorbed. Moreover, the absorption of weak acids and bases was favored by a change in colonic pH which increased the proportion of drug in the nonionized form. The lipid solubility of drugs was shown to be an important physical property in governing the rate of absorption. For example nine barbiturates with similar pKa values were absorbed at rates roughly proportional to the chloroform/water partition ratios of the nonionized drug molecules.

Active transport across the intestinal epithelium.—Although the intestinal absorption of numerous drugs and other foreign organic compounds may be explained in terms of simple diffusion across a lipid-like boundary, it is also clear that certain natural substrates are absorbed by specialized active transport processes. It has long been recognized that the small intestine possesses two independent active transfer processes for organic compounds: one for monosaccharides, and one for amino acids. A third such process, which actively transports the pyrimidines thymine and uracil, has recently been described by Schanker & Tocco (33).

The active absorption mechanisms are poorly understood and can be

described only in terms of the characteristics which distinguish them from simple diffusion. These include: transport of the solute against a concentration gradient; saturation of the transport mechanism when the concentration of solute is raised high enough; specificity of the process for a certain molecular structure; competition between two solutes for the same transfer mechanism; and inhibition of the transport process by substances which interfere with cell metabolism.

There is evidence that a drug can be transported by one of the specialized processes if its chemical structure is similar enough to that of the normal substrate. For example, Wilson & Landau (34) have shown that several foreign sugars, structurally similar to glucose, are actively transferred by the monosaccharide transport mechanism of the small intestine. Further, the foreign compounds, 5-fluorouracil and 5-bromouracil, are actively transported across the intestinal epithelium by the pyrimidine transport system (35).

Absorption of Drugs Through the Skin

The percutaneous absorption of drugs has been the subject of several recent reviews (36 to 39). Although it has long been recognized that in general lipid-soluble molecules penetrate the skin much more readily than do lipid-insoluble molecules and ions, conclusive evidence in support of this view has been obtained only recently. In a study of the permeability of excised rabbit skin to a number of nonelectrolytes, Treherne (40) clearly demonstrated the lipoid character of the epidermal barrier. It was shown that various organic substances diffuse across whole skin at rates roughly proportional to the ether/water partition coefficients of the compounds. It was concluded that the lipid-like barrier of the skin is located within the epidermal layer, since the dermis is freely permeable to many solutes and displays the characteristics of a highly porous membrane.

Ions and lipid-insoluble molecules, which penetrate the skin very slowly, appear to by-pass the epidermal barrier by diffusing through the walls of the hair follicles and sebaceous glands [Malkinson (36)].

Passage of Drugs Across the Renal Tubular Epithelium

It is beyond the scope of this article to present a detailed account of studies of the mechanisms of renal drug excretion. From several comprehensive reviews that have appeared recently, it is possible to make the following generalizations.

The renal tubular epithelium appears to have a dual character regarding the transfer of foreign organic compounds: it behaves as a lipoid boundary which allows the ready passage of nonionized, lipid-soluble molecules, and it has specialized processes for the transport of many organic ions.

Drugs of high lipid solubility like thiopental do not appear in the urine in appreciable amounts, since nearly all of the drug molecules filtered at the glomerulus return to the bloodstream by diffusing across the lipid-like

boundary of the renal tubule (41, 42, 43). Conversely, compounds of relatively low lipid solubility like barbital are more readily excreted in the urine because they are only partially reabsorbed in the tubule.

The changes in the rate of urinary excretion of weak acids and bases that result from changes in the pH of tubular fluid are consistent with the view that the tubular epithelium is selectively permeable to the nonionized, lipid-soluble form of drugs. Thus the distribution of weak acids and bases between tubular urine and plasma can be explained by the same principles outlined previously in this article for the distribution of drugs between gastric juice and plasma. For example, when the tubular urine is more alkaline than plasma, weak bases become less concentrated in urine than in plasma, and, as a result, are slowly excreted; when the urine is acidic, weak bases become concentrated in the urine and are rapidly excreted [Orloff & Berliner (44)]. Moreover, weak acids are readily excreted in an alkaline urine, and more slowly excreted in an acidic urine [Waddell & Butler (45, 46)]. In recent reviews, Milne *et al.* (47) and Peters (48) evaluate the importance of passive tubular transfer of nonionized molecules in the urinary excretion of weak acids and bases.

The renal tubular epithelium appears to possess at least two specialized transport processes: one for the secretion of strong organic acids, and one for the secretion of strong organic bases. Many of the compounds shown to be excreted by these processes are completely ionized at pH 7.4 and would not readily diffuse across a lipoid membrane. Numerous studies have indicated that these substances are transported across the renal tubule against large concentration gradients, that there is competition for transport among various acidic compounds, and that there is competition for transport among various basic compounds. These observations have led to the conclusion that many strong organic acids and bases cross the renal tubule by active transport processes. Peters (48) has recently reviewed the renal excretion of organic bases, and Sperber (49), that of organic acids; Taggart (50) presents an interesting discussion of the renal transport processes.

The mechanisms of urinary excretion of weak acids and bases and of strong acids and bases are often considered to be essentially different, the former involving passive diffusion, and the latter active transport. But surely the kidney tubule cannot distinguish a weak acid from a strong acid, or a weak base from a strong base; more likely, the tubular epithelium can only distinguish an ion from an uncharged molecule. Accordingly, one might expect the ionized form of a weak acid or base to be actively transported, provided that it possessed the structural characteristics required by the transport system. Evidence of this has been supplied by a recent study of the renal excretion of salicylic acid [Weiner *et al.* (51)]. It was shown that the salicylate ion is secreted into the tubular fluid by an active transport process resembling the one which transports *p*-aminohippurate. The tubular reabsorption of salicylic acid appears to occur by passive diffusion of the unionized drug molecule and is governed by the difference in pH between tubular urine and plasma.

A similar three-phase excretion process—glomerular filtration, active tubular secretion, and passive tubular reabsorption—has not yet been demonstrated for weak organic bases, although it was proposed a number of years ago by Jailer *et al.* (52) and is strongly suggested by the recent work of Peters and co-workers (48).

Passage of Drugs into Bile

The mechanisms of biliary excretion of drugs are poorly understood. Many substances appear in bile in a concentration similar to that of plasma. On the other hand, a number of highly ionized organic acids are secreted into bile in very high concentrations, suggesting the presence of an active transport system analogous to the one of the kidney. Studies of the passage of drugs and other substances into bile have been reviewed by Sperber (49) and by Brauer (53).

Schanker & Hogben (54) have recently investigated the biliary excretion of some lipid-insoluble molecules in the rat. The appearance in bile of such large molecules as inulin and sucrose suggests that the boundary between the blood and the bile is a highly porous structure. The observation that sucrose and mannitol invade the intracellular water of liver, mannitol rapidly equilibrating with all of the hepatic water, implies that the pores are located in the membrane of the hepatic parenchymal cell. This cell would thus appear to be unique among animal cells with its unusual permeability to solutes generally confined to the extracellular compartment of body tissues.

Passage of Drugs into the Central Nervous System

The view that the blood-brain and blood-cerebrospinal fluid barriers behave as lipoid membranes toward foreign organic compounds [Davson (55, 56); Brodie & Hogben (41)] has received strong support from several recent studies. Mayer *et al.* (57) showed that a variety of drugs penetrate the brain and cerebrospinal fluid (CSF) of rabbits at rates roughly parallel to the lipid/water partition coefficient of the drugs at pH 7.4. It was noted for several compounds that the rates of entry into brain and CSF were very similar and that the transfer process was best described in terms of simple diffusion.

Mark *et al.* (58, 59) have demonstrated a relation between the rate of passage into brain and the oil/water partition ratio at pH 7.4 of several barbiturates. Thiopental, with its very high lipid solubility, entered the brain of dogs so rapidly that the rate was probably limited only by the rate of cerebral blood flow.

A study by Rall *et al.* (60) of the distribution of drugs between the CSF and plasma of dogs has indicated that the blood-CSF boundary, like other biologic membranes, is preferentially permeable to the nonionized form of weak organic acids and bases. Antipyrine, *p*-aminobenzoic acid, and several sulfonamides were shown to attain steady-state CSF/plasma concentration ratios which approximated those calculated for a lipoid membrane separating solutions of the pH of blood and CSF. Furthermore, when the pH gradient between blood and CSF was altered by changing the pH of plasma,

compounds whose degree of ionization was significantly affected attained new CSF/plasma concentration ratios which approached the predicted ratios; drugs like antipyrine and sulfanilamide, which remained essentially undissociated at the various pH values, showed no change from their normal distribution ratio of 1.0. The distribution between CSF and plasma of ammonia [Stabenau *et al.* (61)] and barbital [Brodie *et al.* (62)] has also been shown to be a function of the pH of the two fluids and the dissociation constant of the compound.

More direct evidence that the blood-brain and blood-CSF barriers are highly resistant to the entry of foreign organic ions has been supplied by studies of completely ionized substances like the quaternary ammonium compounds and sulfonic acids. These cations and anions penetrate the brain and CSF at extremely slow rates [Brodie *et al.* (62); Mayer & Bain (63); Rall & Zubrod (64)].

Brodie *et al.* (62) have evaluated the factors of lipid solubility and the degree of ionization in the passage of drugs into cerebrospinal fluid. Fourteen compounds of diverse structures and physical properties were found to diffuse passively from plasma into the CSF of dogs at widely different rates. Lipid solubility was shown to be the rate-limiting factor with drugs that are mainly undissociated in plasma; these compounds penetrated the blood-CSF barrier at rates roughly related to the lipid/water partition coefficient of the nonionized molecules. The degree of ionization was shown to be the rate-limiting factor with compounds that are highly ionized in plasma; these drugs entered the CSF at rates roughly parallel to the proportion of drug nonionized at pH 7.4. It was suggested that although both lipid solubility and the degree of ionization are important in governing the transfer of drugs into CSF, lipid solubility is probably the dominant characteristic since the relevance of the degree of ionization is probably a consequence of the lipid insolubility of organic ions.

Although the mechanism of entry of drugs into the central nervous system appears to be fairly well understood, the same cannot be said for the mechanism of exit. Mayer *et al.* (65) reported that a number of drugs rapidly disappeared from the CSF of rabbits after intracisternal injection. Although compounds with high lipid solubilities left the CSF somewhat more rapidly than those with low lipid solubilities, the difference in the rates of exit was slight when contrasted with the widely diverse rates at which the same compounds enter the cerebrospinal fluid. It was suggested that the chief mode of exit from the CSF for many drugs may be diffusion from the subarachnoid space through the arachnoid villi into the bloodstream; lipid-soluble drugs might also leave the CSF to some extent by penetrating the blood-CSF and blood-brain barriers in the reverse direction. The observation that drugs may leave the CSF quite rapidly regardless of their rate of entry would explain why a slowly entering compound like sulfanilic acid (60) never attains a CSF/plasma ratio of unity.

A recent study by Pappenheimer *et al.* (66) indicates that certain compounds may leave the CSF by a process other than simple diffusion. For ex-

ample iodopyracet (Diodrast), phenol red, and *p*-aminohippuric acid were shown to be actively transported from CSF to blood in both the cat and goat. The drugs appear to be secreted from a region somewhere between the aqueduct of Sylvius and the cisterna magna; secretion does not occur from the lateral ventricles, the third ventricle, or from the cortical subarachnoid spaces. At low drug concentrations, at which the transport mechanism is not saturated, the rate of active secretion from CSF is about 10 times the rate of passive transfer.

Passage of Drugs into Tissue Cells

Effect of plasma pH on drug distribution.—In a study of the distribution of phenobarbital in dogs, Waddell & Butler (45) found that the partition of the drug between plasma and tissues could be altered reversibly by varying the plasma pH. When the plasma pH was lowered, the plasma drug level decreased and the tissue levels (brain, fat, liver, and muscle) increased; conversely, raising the plasma pH resulted in an increase in the plasma level and a decrease in the tissue levels. The results were consistent with the assumption that cell membranes are preferentially permeable to the nonionized form of phenobarbital and that the drug distributes between the intracellular and extracellular fluids according to the difference in pH of the fluids.

Waddell & Butler (67) have recently used the pH-dependent distribution of a weak acid, 5,5-dimethyl-2,4-oxazolidinedione (DMO) to estimate the intracellular pH of skeletal muscle in the dog. The value of 7.0 obtained with this compound is in close agreement with earlier reported values based on the distribution of carbon dioxide.

Effect of plasma protein binding on drug distribution.—Numerous studies have shown that drugs bound to plasma proteins cannot cross body membranes. For example, it is only the unbound fraction of drug in plasma which penetrates the blood-brain and blood-cerebrospinal fluid boundaries (57, 60, 62), the renal tubular epithelium (48, 49), or the gastrointestinal epithelium (4, 10, 32).

Anton (68) has found that the distribution of a drug between tissues and plasma can be altered by changing the extent to which the drug is bound to plasma proteins. For example, sulfinpyrazone displaces the highly bound sulfonamide, sulfaethylthiadiazole, from plasma albumin; as a result the plasma level of sulfonamide declines, and the tissue levels (brain and muscle) rise. Certain other compounds were found to have a similar effect on the distribution of the sulfonamide, and the magnitude of the effect was proportional to the extent to which the compound displaced the sulfonamide from plasma albumin. These findings might have important implications in therapeutics when two or more drugs are administered simultaneously.

Penetration of drugs into erythrocytes and other cells.—Schanker & Nafpliotis (69) have shown that the rates at which a number of drugs enter human red cells are roughly related to the lipid/water partition co-

efficient of the compounds at pH 7.4. Weak organic bases with high lipid solubilities, like antipyrine and aniline, entered the cells very rapidly; those with low lipid solubilities, like serotonin, epinephrine, and norepinephrine penetrated more slowly. Quaternary ammonium compounds, which have very low lipid solubilities, entered at either very slow rates or not at all. A relation between the lipid/water partition ratio and rate of cell penetration was also observed for acidic compounds; however strong acids appeared to enter much more rapidly than strong bases of similar lipid solubility. It was suggested that the entry of organic anions resembles that of the inorganic anions, which also penetrate the red cell at rates greatly exceeding those of cations.

Robbins (70) has reported that the dye 2,8-diaminoacridine (proflavin) penetrates human conjunctiva cells in tissue culture at rates related to the pH of the extracellular fluid. The results were consistent with the view that it is the nonionized form of the dye which penetrates the cell membrane.

Localization of Drugs in Tissue Cells

Accumulation of drugs in body fat.—It has been known for some time that certain drugs of high lipid solubility localize to a considerable degree in body fat [Brodie & Hogben (41); Richards & Taylor (71)]. The mechanism of the distribution appears to be a simple partitioning of lipid-soluble, nonionized molecules between intracellular lipids and body water (41). This type of distribution has been observed for a number of thiobarbiturates, N-alkyl thiobarbiturates, dibenamine, and dibenzyline. The role of body fat in regulating the duration of pharmacologic action of drugs has been discussed frequently (41, 71 to 74).

Intracellular binding of drugs.—Once a drug has penetrated the boundaries of the various tissue cells, its degree of binding to the cell components becomes the most significant factor in determining its distribution in the body. Despite the great importance of intracellular binding, almost nothing is known about it.

The binding of several barbiturates to homogenates of various tissues [Goldbaum & Smith (75)] suggests that the tissue binding and the plasma protein binding of these compounds may be governed by the same principles. On the other hand, the almost limitless capacity of liver-cell nuclei to bind mepacrine [Brodie & Hogben (41); Tomkins & Brodie (76)] suggests a type of binding different from the various proposed types of plasma protein binding.

Tissue localization of endogenous substrates.—Although endogenous substances like serotonin, the catechol amines, amino acids, purines, pyrimidines, various hormones, and so forth are of considerable interest to the pharmacologist, it is beyond the scope of this review to discuss the numerous studies of their physiological distribution. Whenever one of these natural substrates is found to be highly localized in a particular tissue, it may be presumed that the mechanism involved is either active transport or

some type of binding to cell components, or both. For example, serotonin appears to be localized in blood platelets by an active transport mechanism (77 to 80), whereas histamine appears to be concentrated in mast cells because of intracellular binding (81).

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