PASSAGE OF DRUGS ACROSS BODY MEMBRANES

LEWIS S. SCHANKER

Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Bethesda, Maryland

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I. INTRODUCTION

The access of drugs to the vertebrate organism is hindered by a succession of membranes. First there are membranes such as the skin, the gastrointestinal

epithelium, and the lining of the respiratory tract which delay the entrance of drugs into the body. Then there are membranes enclosing the blood and other body fluids, membranes around individual tissue cells, and membranes around intracellular structures further to restrict drug penetration. An understanding of how drugs cross these boundaries would not only furnish a useful guideline for predicting the extent of absorption, distribution, and excretion of a new drug, but would also help to define the nature of living membranes.

II. NATURE OF BODY MEMBRANES

Body membranes are of three main types: those made up of several layers of cells, for example the skin; those consisting of a single layer of cells, like the intestinal epithelium; and those consisting of a boundary less than one cell in thickness, for example the membrane of a single cell, mitochondrion, or nucleus. Thus, except for the boundaries around intracellular structures, all body membranes are composed of a fundamental structure, the cell membrane—or, as it is often called, the plasma membrane.

The question of the nature of cell membranes has always fascinated the biological scientist, and has stimulated a vast amount of research. The problem has been approached in several ways: 1) attempts to deduce the physical nature of membranes from their permeability characteristics; 2) attempts to determine directly the chemical composition and physical properties of membranes; and 3) attempts to describe membranes from their microscopic appearance. Although none of these approaches has led to a solution of the problem, each has revealed a number of pertinent facts from which there has evolved a useful working hypothesis of the nature of the cell membrane.

One of the first clues to the character of living membranes was supplied in 1902 by Overton's study of the osmotic behavior of muscle in solutions of various substances (88). A frog sartorius muscle was soaked in Ringer's solution containing a dissolved organic compound; if the substance entered, the weight of the muscle remained virtually unchanged, whereas loss of weight indicated an osmotic withdrawal of fluid and hence impermeability to the solute. Noting that the tissue was in general more readily penetrated by lipid-soluble substances than by lipid-insoluble substances, Overton concluded that the cell membrane is lipoid in character.

As the study of cell permeability progressed, it soon became evident that the concept of a simple lipoid membrane was inadequate, for it did not explain the rapid cellular penetration of small, lipid-insoluble molecules such as urea, formamide, and water. Taking this shortcoming into account, Collander and Bärlund (23) proposed that the lipoid film is not continuous; rather, it is interrupted frequently by small water-filled channels or pores, and is best described as a lipoid-sieve membrane. With this model, lipid-soluble molecules penetrate readily by diffusing through the lipoid regions, while lipid-insoluble molecules penetrate only if they are small enough to pass through the pores.

In the meantime, evidence in support of the lipoid membrane thesis had begun

to arise from other lines of investigation. Using a novel approach to the problem, Gorter and Grendel (47) extracted the lipids of the red cell and determined that their area, when spread in a monolayer, was twice the surface area of the cell. This led them to suggest that the cell membrane is a bimolecular layer of lipoid molecules; the molecules are oriented perpendicularly to the cell surface with their polar groups located at the two membrane-water interfaces.

An apparently serious objection to the view of Gorter and Grendel arose several years later when measurements of the interfacial tension of cells in aqueous media (21, 52) revealed a tension much lower than that to be expected for a simple lipoid-aqueous interface. However, the contradiction was resolved when Danielli and Harvey (33) showed that the interfacial tension of oil droplets surrounded by protoplasm is much lower than that of oil droplets surrounded by a protein-free solution; evidence was presented that the lipoid boundary of cells, like the oil droplet in protoplasm, is covered with a layer of adsorbed protein which lowers markedly the cell surface-water interfacial tension. Subsequently, Danielli and Davson (32, 37) proposed that the cell membrane consists of a bimolecular layer of oriented lipoid molecules with an adsorbed monolayer of protein on both sides.

The lipo-protein nature of the cell membrane has also been suggested by the results of chemical analyses of the ghosts (stromata) of red cells (93, 94). The ghosts, which contain nearly all of the lipids of intact red cells (172), have a lipid-to-protein ratio of approximately 1:1.7 by weight; this amounts to about 70 lipid molecules for each molecule of protein.

In recent years, electron microscopic pictures and x-ray diffraction patterns of a variety of cells have revealed that the cell membrane, as well as the mitochondrial and nuclear membranes, have a property in common: when suitably fixed and stained, they appear as two dark lines separated by a lighter band (9, 31, 42, 51, 113, 137). This pattern is usually interpreted as representing a bimolecular lipoid sheet bounded on both sides by protein. The thickness of the membranes is of the order of 100 Å, a value previously predicted from the bimolecular lipoid model.

Thus over the past 60 years, considerable evidence has accumulated in support of Overton's thesis that the cell membrane is lipoidal in character. However, much remains to be learned before there can be a good understanding of the relation between membrane structure and membrane permeability. For example, there is the question of the nature of membrane pores; their presence in the lipoid layer seems necessary to explain the hydrodynamic flow of water and the ready diffusion of small, lipid-insoluble molecules and ions across cell membranes (2, 7, 89, 139). Then there is the perplexing question of how certain inorganic ions and certain large, lipid-insoluble molecules like glucose and amino acids rapidly cross the cell boundary; studies of these processes suggest that membrane components ("carriers") actively participate in accomplishing the transfers. And finally, in the case of the passage of solutes across multicellular membranes, there is the question of whether the intercellular spaces provide aqueous passageways for the transfer of small lipid-insoluble molecules and ions.

III. PROCESSES BY WHICH SUBSTANCES CROSS MEMBRANES

The diverse ways in which solutes move across membranes may be grouped into two general categories: passive transfer processes, and specialized transport processes. The term *passive transfer* implies that the membrane behaves as an inert solvent layer or system of aqueous channels through which the solute passes. The term *specialized transport* implies that the membrane displays an active character, transporting the solute in a manner that cannot be explained by the structure or physical properties of the membrane.

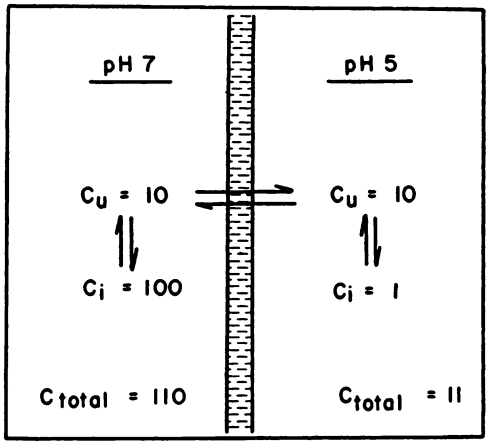


FIG. 1. Theoretical distribution of a weak acid, pKa 6, between aqueous solutions of different hydrogen-ion concentration

The solutions are separated by a membrane permeable only to the unionized form of the compound. C_u is the concentration of unionized compound, and C_i that of ionized compound.

A. Passive transfer

1. Simple diffusion. Many substances move across membranes by simple diffusion; that is, their rate of transfer is directly proportional to their concentration gradient across the membrane. Some of the substances penetrate the membrane as though it were a layer of lipoid material, the speed of penetration being determined by the lipid-to-water partition coefficient of the substance. In contrast, a number of lipid-insoluble substances cross the membrane as though it were a fine sieve, the smaller molecules and ions crossing faster than the larger ones. With ions, however, the speed of transfer may be determined more by the charge than by the size; for instance, in the red cell anions penetrate rapidly, while cations penetrate very slowly (37, 63, 127, 139).

When an unionized substance attains a steady-state distribution across a membrane, its concentration is the same on both sides. In contrast, a partly ionized substance may distribute itself unequally, either because of a Donnan type of ionic distribution or because of a difference in hydrogen-ion concentration on the two sides of the membrane.

A Donnan distribution occurs whenever the solution on one side contains some ions that cannot cross to the other side. In the red cell, for example, the presence of hemoglobin anions affects the distribution of a diffusible ion like chloride so that its cell-to-plasma concentration ratio is about 0.7 instead of 1.0 (55, 159).

A difference in pH on the two sides of a membrane affects the distribution of a partly ionized substance because of the preferential permeability of membranes to the lipid-soluble, unionized forms of compounds. The process may be portrayed as in Figure 1, which shows the distribution of a weak acid (pKa = 6) between solutions of pH 7 and pH 5; the solutions are separated by a membrane permeable only to the unionized form of the compound. At the steady state, the concentration of the unionized solute is the same in both solutions; 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 the solute (ionized plus unionized) on both sides of the membrane is a function of the pH of the two fluids and the dissociation constant of the solute. This relationship is expressed in the following equations (64):

for a weak acid,

$$\frac{C_1}{C_2} = \frac{1 + 10^{(pH_1 - pKa)}}{1 + 10^{(pH_2 - pKa)}}$$

and for a weak base,

$$\frac{C_1}{C_2} = \frac{1 + 10^{(pKa - pH_1)}}{1 + 10^{(pKa - pH_2)}}$$

where C_1 and C_2 are the concentrations of solute in the two fluids, and pKa is the negative logarithm of the acidic dissociation constant of the weak acid or base.

Some of the above-described characteristics of simple diffusion processes are difficult to demonstrate with body membranes except in a very approximate fashion. For instance, it may be impossible to determine the steady-state distribution of a compound between a cell and its environment, because the compound is rapidly metabolized within the cell. Moreover, there is the uncertainty of whether a compound is bound to nondiffusible materials inside the cell, and the problem of measuring the intracellular concentration of the unbound form of the compound (127).

A major obstacle in attempting to correlate membrane penetration with the molecular size of solutes stems from the fact that molecules have different shapes as well as different sizes; it is difficult to judge which of two molecules is the larger, when one is a long-chain aliphatic compound and the other a round-shaped aromatic one. An additional complication arises if solutes become adsorbed to materials lining the membrane channels. Thus, it is easy to understand why the best correlations have been obtained with compounds of closely related chemical structure such as the members of a homologous series (56).

With lipid-soluble substances, comparisons between the oil-to-water partition

coefficients and rates of membrane penetration have usually revealed only rough correlations (22, 56, 120). The main reason for the shortcoming is the failure of various oils and lipid solvents to mirror accurately the solvent properties of a lipoid membrane. Moreover, peculiar interactions between solutes and membranes, as well as between solutes and lipid solvents, introduce variables that cannot readily be taken into account. Accordingly, the best correlations have been obtained with compounds belonging to a homologous series.

2. Filtration. When water flows, in bulk, across a porous membrane, any solute that is small enough to pass through the pores flows with it. For example, the water that filters across the glomerular membrane is accompanied by all of the solutes of plasma except the large protein molecules. Since filtration (hydrodynamic flow) occurs as a result of a hydrostatic or osmotic pressure difference across a membrane, the flow of the water and its solutes is passive in nature.

B. Specialized transport

1. Carrier transport. The concept of membrane carriers has arisen as a tentative explanation for the peculiar permeability of cell membranes to certain lipid-insoluble solutes. Carriers are pictured as membrane components capable of forming a complex with the solute (substrate) at one surface of the membrane; the complex moves across the membrane, the substrate is released, and the carrier then returns to the original surface. The subject of carrier transport has been reviewed recently in this journal (171).

At least three types of carrier transport are recognized: *active transport; facilitated diffusion* (30); and *exchange diffusion* (157). These headings are useful for the present discussion; however, they are superficial and will no doubt be replaced by more specific terms, or at least supplemented with descriptive subheadings, as more is learned about the nature of the processes.

a. Active transport. This term is used to designate processes having the following characteristics. 1) The solute moves across the membrane against a concentration gradient, that is, from the solution of lower concentration to the one of higher concentration; or if the solute is an ion, it moves against an electrochemical potential gradient. 2) The transport mechanism becomes saturated when the concentration of solute is raised high enough. 3) The process shows specificity for a particular type of chemical structure. 4) If two substances are transported by the same mechanism, one will competitively inhibit the transport of the other. 5) The transport mechanism is inhibited noncompetitively by substances which interfere with cell metabolism.

Transport processes with these characteristics have been demonstrated most convincingly *in vitro* using cellular membranes like frog skin or intestinal wall bathed on both sides by simple aqueous solutions. With preparations of this type, it is easy to establish whether transport occurs against an electrochemical potential gradient, and it is a simple matter to control the substrate concentration, add various inhibitors, and so forth (26, 131, 158, 177).

A more difficult task is demonstrating active transport in the intact animal. Since the bloodstream and interstitial fluid usually represent the solution on one

side of a body membrane, the substrate concentration is difficult to control because of the variables of metabolism, renal excretion, and binding to blood and tissue proteins. Moreover, if intracellular fluid represents the solution on the other side of the membrane, the difficulties are even greater because of the problem of distinguishing freely diffusible substrate from bound substrate inside a cell. In addition, misleading results might be obtained when the transport rates are measured at high concentrations of the substrate or in the presence of inhibitors; the substrate or inhibitor might appear to affect the transport system, when in reality it is acting indirectly through an effect on blood flow, respiration, hormone release, and so forth.

b. Facilitated diffusion. This term is generally used to designate carrier transport processes in which the substrate does not move against a concentration gradient. As an example, glucose readily penetrates the human red cell, attaining the same concentration inside the cell as that on the outside, by a process that shows specificity, saturability, and a sensitivity to certain metabolic inhibitors (70).

c. Exchange diffusion. In this process, a carrier is thought to transport the substrate from one surface of the membrane to the opposite surface, where it releases the substrate, picks up another molecule of substrate, and transports it to the original surface (157). Thus, if a different isotope of the substrate is placed on either side of the membrane, there will be a rapid exchange of the isotopes without a net transfer of substrate. Exchange diffusion may occur in any carrier transport system that is near saturation (171).

2. Facilitated diffusion not associated with membrane carriers. Processes other than carrier transport have been proposed to explain the accelerated diffusion of certain lipid-insoluble substances across membranes. For example, there is the idea of Stein and Danielli (144) that penetration might occur through hydrogen-bonding structures ("polar pores") extending through the thickness of the lipoid membrane. Polar substances would penetrate rapidly if their stereochemical characteristics corresponded closely to those of the hydrogen-bonding pore. In addition, there would be competition for transfer through the pores, and the process would be saturable.

Another possible mechanism of facilitated diffusion has been suggested by Stein (143) from his studies of the passage of glycerol into human red cells. Evidence was obtained that glycerol molecules are bound in pairs to specific sites on the membrane surface; it was proposed that the bound molecules form dimers which penetrate the lipoid membrane with ease because of their relatively low polarity.

3. Pinocytosis. Cells growing in tissue culture take up small droplets of the external medium by an engulfing or sucking-in process known as pinocytosis (76, 77). The same phenomenon is seen in the amoeba, and there is electron-microscopic evidence suggesting that it occurs in certain mammalian cells (61). Pinocytosis can be induced in amoebae by adding certain proteins or salts to the external medium, and there are indications that adsorption of these charged solutes to the cell surface initiates the process (6, 10, 61).

Very little is known about the physiological significance of pinocytosis. Although the process appears to operate too slowly to account for the rapid cellular uptake of natural substrates such as the amino acids (19a), it could account for the uptake of small amounts of protein and other macromolecules.

IV. METHODS OF STUDY

A widely used method of assessing the rate at which a drug crosses a membrane involves measuring the concentration of drug on both sides of the membrane from time to time until a steady-state distribution is achieved. If the concentration of drug on one side is kept constant throughout the experiment, the data can be analyzed by simple application of the diffusion equation (35); furthermore there will be little error in estimating the distribution ratio of drug at the steady state. If, on the other hand, the drug level on one side is allowed to decline rapidly (as a result of metabolism, excretion, etc.), analysis of the data will be complicated, and in addition, a true steady-state distribution might not be attained because the continuous redistribution of drug across the membrane may not keep pace with the rapidly falling level. Accordingly, in studying the passage of a drug from blood into some other fluid or tissue, the drug is administered by continuous intravenous infusion at a rate that will keep the blood level from changing. Or if transfer is measured *in vitro*, for instance from an aqueous solution into a group of single cells, the volume of the outside solution is made so large relative to the volume of the cells that the outside concentration of drug remains essentially constant. Under these conditions, the results may be expressed as a concentration ratio, that is:

$$\frac{\text{concentration of diffusible (unbound) drug in the inside solution}}{\text{concentration of diffusible (unbound) drug in the outside solution or plasma}},$$

and plotted graphically against time (or against the logarithm of time) as shown in Figures 2 and 3. In the figures, C_1 represents the drug concentration in the fluid being entered, and C_2 that in the plasma or outside solution; the curves represent the concentration ratios of three drugs, A, B, and C, which cross the membrane by simple diffusion. Drugs B and C attain a steady-state concentration ratio of 1.0, as would be expected for neutral molecules; in contrast, drug A attains a ratio of 2.0. Drug A might be a partly ionized substance which distributes itself unequally, either because of a pH differential across the membrane, or because of a Donnan effect.

The problem is to determine the relative speeds with which the drugs cross the membrane. One way of doing this involves use of the curves in Figures 2 or 3 to estimate the time required for each drug to reach a concentration ratio that is one-half of the steady-state ratio. Thus, drugs A and B have a half-time of 0.68 hour, and drug C a half-time twice this value, 1.36 hours (Fig. 3). This means that the membrane is equally permeable to A and B, and only half as permeable to C.

Another way of determining the relative rates of transfer involves expression

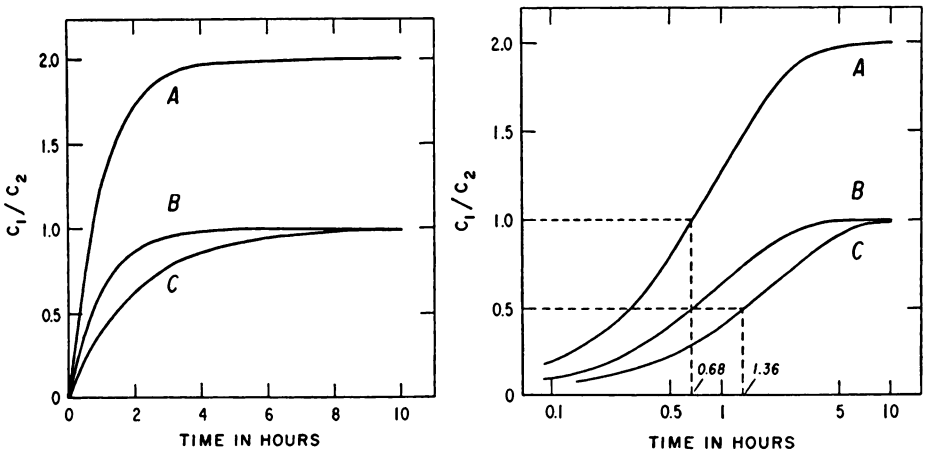
of the results in terms of Fick's law:

$$\frac{d(C_2 - C_1)}{dt} = -P(C_2 - C_1)$$

where t is time, and P is a permeability coefficient which is a measure of the relative ease with which the drug crosses the membrane. When C_2 is made constant (*e.g.*, a constant plasma level), the equation may be integrated to give:

$$Pt = -\ln\left(1 - \frac{C_1}{C_2 R}\right),$$

where R is the ratio C_1/C_2 at the steady state (38). On substituting in the equa-



FIGS. 2 and 3. For explanation see text

tion the observed concentration ratios, and plotting the values of the right-hand side of the equation against time, a straight line is obtained (Fig. 4). The permeability coefficient P is given by the slope of the line, $-\ln(1 - C_1/C_2R)/t$. Thus, drugs A and B have the same coefficient, 1.0, while drug C has a coefficient half as great, 0.5.

Although the general method described above has wide application in studies of the passage of drugs from the bloodstream into other body compartments, it is not useful in a situation in which the drug passes in the opposite direction. For example, in studies of intestinal absorption, the blood level of drugs cannot be used as a measure of relative absorption rates because of the variables of drug distribution, metabolism, and excretion. Accordingly, if the drugs are neither destroyed nor precipitated within the intestinal lumen, their relative speeds of absorption are assessed from the rates at which they disappear from the intestine. In practice, an experimental time is selected that allows a partial disappearance (*e.g.*, 20 to 80%) of the compounds, and the results are expressed as the per cent or fraction of total compound absorbed in that time.

Another situation in which measurement of drug concentration on both sides of a membrane fails to give a direct estimate of drug transfer is encountered in the renal excretion of drugs. The concentration of a drug in urine is dependent not only on the permeability of the tubular epithelium, but also on variables such as glomerular filtration rate, urine pH, and the degree of tubular reabsorption of water. Furthermore, the disappearance of drug from plasma does not reflect the rate of urinary excretion because of the variables of drug distribution, metabolism, excretion in bile, and so forth. In this complex system, the principle of *plasma clearance* has been extremely useful. Clearance is defined as the volume

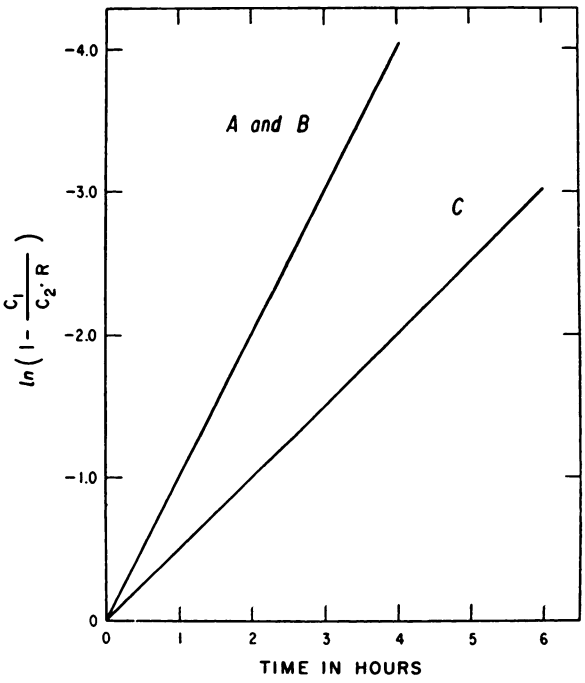


FIG. 4. For explanation see text

(ml) of plasma that is completely cleared of drug in one minute; it is calculated by dividing the amount of drug excreted in the urine in one minute by the plasma concentration of drug. In a clearance experiment, the plasma concentration of drug is kept constant by continuous intravenous infusion, and correction is made for the degree of plasma protein binding. By comparing the clearance values of various drugs with that of a substance like inulin, which is filtered across the glomerulus but does not penetrate the tubular epithelium, it is possible to estimate the relative speeds with which the drugs pass from the tubular fluid into the bloodstream, and to demonstrate the active tubular secretion of drugs. Use of the clearance principle is by no means limited to investigations on the kidney; it has been applied, for example, in studies of hepatic excretion (24, 141) and gastrointestinal absorption and secretion (57, 59, 128).

The general procedures outlined above are sometimes modified to overcome certain technical difficulties, eliminate certain biological variables, or obtain special information about a transfer process. Detailed descriptions of these procedures as applied to specific body membranes can be obtained from the following references: kidney (85, 95, 138, 141); stomach, intestine, and colon (26, 27, 56, 59, 65, 71, 119, 128, 132, 177); liver (24, 122, 123, 141); central nervous system (13, 34, 35, 91, 103); placenta (29); mammary gland (106); blood capillary (90, 92, 134); skin (45, 78, 156, 158, 173); eye (34, 35, 38); mesentery (8); isolated cells and tissue slices (28, 37, 39, 62, 95, 127). Although most of these references deal with studies of passive transfer processes, several concern active transport. The kinetic analysis of active transport and other specialized transport processes has been reviewed recently in this journal by Wilbrandt and Rosenberg (171).

V. PASSAGE OF DRUGS ACROSS VARIOUS BODY MEMBRANES

The passage of drugs across many of the body membranes has been discussed rather comprehensively in a number of recent reviews. Accordingly, these topics will be presented in a general way, pointing out similarities and differences between the various membranes. More detailed discussions will be given for topics not adequately reviewed in recent years.

A. Blood capillary

Numerous studies of capillary permeability, well reviewed by Pappenheimer and Renkin (90, 111), have suggested that solutes traverse the capillary wall by a combination of two processes, diffusion and filtration. Diffusion is the predominant mode of transfer for lipid-soluble molecules as well as for small, lipid-insoluble molecules and ions. Filtration (hydrodynamic flow), on the other hand, predominates for large, lipid-insoluble molecules whose rates of diffusion across the capillary endothelium are relatively slow.

The capillary wall is a good example of a membrane that behaves as a lipoid-pore layer; thus, lipid-soluble substances penetrate readily at rates roughly parallel to their oil-to-water partition coefficients, and lipid-insoluble substances penetrate less readily at rates inversely related to their molecular sizes. While the latter substances appear to pass through aqueous pores whose total cross-sectional area comprises less than 0.2% of the capillary surface, lipid-soluble substances appear to penetrate through the entire surface (90, 92, 109, 110, 111).

But despite the faster rates of transfer of lipid-soluble substances, it is important to point out that all substances, lipid-soluble or not, cross the capillary wall at rates which are extraordinarily rapid in comparison with their rates of passage across other body membranes. In fact, the supply of most drugs to the various tissues is limited by the rate of blood flow rather than by the restraint imposed by the capillary wall. A notable exception is encountered in the central nervous system where the cerebral capillaries appear to impede markedly the passage of many solutes into the extracellular fluid of the brain. However, it is possibly not the capillary wall itself, but rather the surrounding layer of tightly packed glial cells which forms the so-called blood-brain barrier.

B. Central nervous system

The exchange of drugs between the brain, cerebrospinal fluid, and blood has received a great deal of study in the past few years, and has been the subject of a number of recent reviews (12, 36, 105, 115, 121). Much progress has been made toward explaining why some drugs penetrate the central nervous system with ease, while others penetrate with considerable difficulty. In addition, significant advances have been made toward understanding the fate of substances injected directly into the cerebrospinal fluid.

1. Passage of drugs from the bloodstream into brain and cerebrospinal fluid. The view that the blood-brain and blood-cerebrospinal fluid barriers behave as lipoid membranes toward foreign organic compounds (12, 15, 35, 67, 121) has received strong support from a number of recent studies. For example, Mayer, Maickel and Brodie (83) have shown that a variety of drugs penetrate the brain and cerebrospinal fluid (CSF) of rabbits at rates roughly parallel to the lipid-to-water partition coefficients of the drugs at pH 7.4. It was noted for several compounds that the rates of entry into brain and CSF are very similar, and that the transfer process is best described as simple diffusion.

Similarly, Mark and co-workers (79, 80) have demonstrated a relation between the rate of passage into brain and the oil-to-water partition ratio at pH 7.4 of a number of oxy- and thio-barbiturates. Thiopental, with its very high lipid-solubility, enters the brain of dogs so rapidly that the rate appears to be limited only by the rate of cerebral blood flow.

An investigation by Rall, Stabenau and Zubrod (103) 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 unionized forms of weak organic acids and bases. Antipyrine, *p*-aminobenzoic acid, and several sulfonamides were shown to attain steady-state CSF-to-plasma concentration ratios which approximated those calculated for a lipoid membrane separating solutions of the pH of blood (7.4) and CSF (7.3). Furthermore, when the pH gradient between the two fluids was altered by changing the pH of plasma, compounds whose degree of ionization was significantly affected attained new CSF-to-plasma ratios which approached the predicted ratios; drugs like antipyrine and sulfanilamide, which remained essentially unionized at the various pH values, showed no change from their normal distribution ratio of 1.0. The distributions of ammonia (142) and barbital (13) in the dog, and of sulfadiazine (100) in man have also been shown to be a function of the pH of the two fluids and the ionization constants of the compounds.

Additional 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 such as quaternary ammonium compounds and sulfonic acids. These substances penetrate the brain and CSF much more slowly than do most nonionized drug molecules (13, 49, 50, 74, 82, 104).

To evaluate the factors of lipid-solubility and degree of ionization in the passage of drugs into CSF, Brodie, Kurz and Schanker (13) have investigated in the dog a large number of compounds of diverse structures and physical proper-

ties. The compounds were found to diffuse passively from plasma into CSF at widely different rates. Lipid-solubility was shown to be the rate-limiting factor with drugs that are mainly unionized in plasma; these compounds penetrated the blood-CSF boundary at rates roughly related to the lipid-to-water partition coefficient of the unionized 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 unionized 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 more likely the dominant characteristic, since the relevance of the degree of ionization is probably a consequence of the poor lipid-solubility of organic ions.

Although the blood-CSF and blood-brain barriers behave very much alike with regard to their permeability to drugs and other foreign organic compounds, anatomically they are quite different. For example, the blood-CSF barrier appears to consist mainly of the epithelium of the choroid plexuses, whereas the blood-brain barrier seems to be either the brain capillary wall or its surrounding layer of glial cells. But despite this anatomical difference, the barriers are, in a pharmacological sense, not completely divorced from one another. For instance, a highly lipid-soluble drug, having sped across the blood-brain barrier, can diffuse across the ependyma and pia mater into regions of the CSF that are so remote from the choroid plexuses that they have not yet attained diffusion equilibrium with the blood (34, 35).

After a drug has crossed the blood-brain barrier and gained access to the extracellular fluid, it may penetrate various regions of the brain tissue at different rates. For example, Roth and co-workers (41, 46, 115), using autoradiographic techniques with the cat brain, have shown that phenobarbital and urea enter the white matter more slowly than the gray matter. Apparently the thick layer of lipoid membranes which forms the myelin sheath of individual nerve fibers in white matter constitutes a greater hindrance to drug penetration than the single lipoid membrane that surrounds the nonmyelinated fibers of gray matter (115).

2. Passage of drugs from cerebrospinal fluid into the bloodstream. Despite the marked parallelism between the lipid-to-water partition ratios of drugs and their speeds of entry into CSF, the passage of drugs in the reverse direction, that is from CSF to blood, is only partly dependent on lipid-solubility. For example, Mayer, Maickel and Brodie (84) have shown that after intracisternal injection in rabbits, compounds with a low lipid-solubility leave the CSF almost as rapidly as those with a high lipid-solubility. Moreover, Prockop, Schanker and Brodie (97, 98) have reported that mannitol, sucrose, inulin, and dextran, lipid-insoluble substances which enter CSF at extremely slow rates when administered intravenously, all pass readily at similar rates from CSF to blood after injection into a lateral cerebral ventricle of rabbits. Similarly, Rothman *et al.* (116, 117), in a study of the exchange of inulin, dextran, and serum albumin between CSF and blood in the dog, have found that the three substances readily leave the CSF at about the same rate.

One way in which lipid-insoluble compounds might leave the CSF would be by filtration across the arachnoid villi as CSF flows, in bulk, from the subarachnoid space into the dural venous sinuses (35, 36). The villi, which *in vitro* are permeable to particles as large as yeast cells and erythrocytes (170), would provide the extremely porous boundary needed to explain why large saccharide molecules (mol. wt. 50,000) escape from the CSF just as rapidly as do small ones (mol. wt. 182) (97, 98). Evidence that the molecules are indeed swept across the arachnoid villi as CSF drains into the dural blood sinuses has been supplied by a recent study comparing the rate of exit of inulin with the rate of drainage of CSF (96). When the rate of CSF drainage in rabbits was lowered to various degrees by reducing the hydrostatic pressure difference between CSF and dural sinus blood, the exit rate of intraventricularly administered inulin was lowered proportionately; and when CSF drainage was prevented by reversing the normal pressure gradient across the villi, the outflux of inulin was virtually halted.

An additional process by which a compound may leave the CSF is by active transport. For example, Pappenheimer, Heisey and Jordan (91) have demonstrated that phenol red and iodopyracet (Diodrast) are actively transferred from CSF to blood in the vicinity of the fourth ventricle of the goat, possibly *via* the choroid plexus. The compounds are transferred against a concentration gradient, they compete with one another for transfer, and the process is saturable. A similar transport of phenol red appears to occur in the rabbit (98) and the dogfish (102).

Thus, in considering the fate of drugs injected into the CSF, it is apparent that several routes of exit are available. All drugs, regardless of molecular size or lipid-solubility, would be expected to leave the CSF by a nonspecific process of filtration across the arachnoid villi. If a drug is lipid-soluble, it can also leave the CSF by diffusing across lipoid portions of the blood-CSF boundary, and by diffusing into the brain and thence across the blood-brain barrier. In addition, certain drugs may escape from the CSF by specialized, active transport processes.

3. Passage of drugs from cerebrospinal fluid into brain. A recent investigation by Rall, Oppelt and Patlak (101), concerning the passage of lipid-insoluble substances from the perfused lateral ventricle into the brain substance of dogs, has suggested that the ependymal lining of the ventricles offers little resistance to the transfer of solutes. For example, inulin, dextran, and sulfate ion were reported to diffuse from the ventricular CSF into the extracellular fluid of brain at rates approaching the coefficients of free diffusion of the substances. It should be emphasized, however, that because the movement of solutes in an unmixed fluid is a very slow process, the substances did not spread readily throughout the entire extracellular space; rather, some 5 hours were required for tissue at a depth of 3 mm to attain a concentration half of what it would contain at the steady state. From the concentrations of the compounds in brain tissue adjacent to the ventricular surface, the authors calculated that the extracellular space of brain is between 7 and 14 % of the wet tissue weight.

As additional evidence of the highly permeable nature of the ependyma, organic ions like bromphenol blue (43) and N^1-methylnicotinamide (99) have been shown to pass readily from ventricular CSF into the brain.

C. Eye

1. Ocular fluid. The detailed investigations of Davson (34, 35, 36, 38), Friedenwald (44a), and Kinsey (66a), concerning the transfer of solutes between the aqueous humor and blood, indicate that there are two main routes by which organic compounds penetrate into the ocular fluid: 1) through the epithelium of the ciliary body; and 2) across the capillary walls and surrounding connective tissue of the iris.

Drug entrance by way of the ciliary body is thought to involve both diffusion and secretion (35, 36). Thus, drugs diffuse from the blood capillaries into the epithelial cells of the ciliary body; since these cells secrete fluid and solutes (the primary aqueous humor) into the posterior chamber, the more rapidly a solute diffuses into the cells, the greater will be its concentration in the secretion. Of course the rate of fluid secretion becomes a rate-limiting factor with solutes that diffuse into the cells very rapidly.

Drug entrance by way of the iris is primarily a diffusion process. Solutes diffuse out of the iris capillaries, pass either through or between the connective tissue cells of the iris, and enter the fluid of the anterior chamber. The intercellular route is probably unimportant except for lipid-insoluble solutes, which cannot readily penetrate through the cells (35, 36).

Whatever the precise nature of the above routes of transfer, it is apparent from the relation between the lipid-solubility of drugs and their rate of passage into the ocular fluid that diffusion across cell membranes is an important part of the transfer process. For example, Ross (114) and Davson and Matchett (34, 38) have shown that a number of organic compounds, including ethanol, glycerol, creatinine, sucrose, sulfonamides, and thiourea derivatives, enter the aqueous humor of rabbits at rates roughly parallel to their lipid-to-water partition coefficients. Moreover Dayton *et al.* (40), in studies with dogs, have shown that thiopental, with its high lipid-solubility, penetrates the ocular fluid much more rapidly than does the moderately lipid-soluble barbital.

Drugs escape from the ocular fluid in several different ways. All solutes can leave *via* the drainage route of the ocular fluid, passing through the spaces of Fontana and the canal of Schlemm into the bloodstream (35, 36). If a solute is lipid-soluble, it can also leave the eye by diffusion across the lipoidal boundary separating ocular fluid from blood. A third mechanism of exit, recently demonstrated in the rabbit by Becker and Forbes (5, 44), is active transport. In this process Diodrast, a lipid-insoluble anion, is transferred from ocular fluid to blood against a concentration gradient. The process is saturable and is inhibited by phenol red, penicillin, and *p*-aminohippurate, anions which presumably compete for the transport mechanism. Evidence that the ciliary body is the site of transport has been provided by studies *in vitro* which have shown that this structure accumulates Diodrast by a process exhibiting all of the characteristics of active transfer (5).

It is interesting to compare the ocular fluid and the cerebrospinal fluid with regard to the entrance and exit of organic substances. Both fluids are separated from blood by similar boundaries, which allow the ready passage of lipid-soluble

compounds and impede the passage of lipid-insoluble compounds. In addition, the drainage structures for both fluids are permeable to virtually all solutes, lipid-soluble or not, ocular fluid and its solutes flowing through the spaces of Fontana and canal of Schlemm into the bloodstream, and cerebrospinal fluid flowing across the arachnoid villi to reach the bloodstream. Finally there are specialized processes in both systems which actively transport certain organic anions into the blood.

2. Cornea. Many drugs traverse the cornea at rates related to their degree of ionization and lipid-solubility. For example, Cogan and Hirsch (20), in applying solutions of drugs of different hydrogen-ion concentration to the excised cornea of rabbits, have observed a rapid rate of passage for substances present as unionized molecules, and a very slow rate of passage for substances present as ions. Thus, an organic acid like salicylic acid penetrated most readily from solutions of low pH value, while organic bases like aniline, atropine, ephedrine, and pilocarpine penetrated most readily from solutions of high pH value. The results were confirmed in the living animal by showing that aniline passes from the corneal surface into the aqueous humor at rates directly related to the proportion of drug present as unionized molecules.

The importance of lipid-solubility in determining the speed of penetration of drugs into the cornea has been demonstrated by Swan and White (145) in experiments with a number of naphthalene derivatives. These compounds penetrated the corneal tissue of living rabbits at rates roughly parallel to their relative oil-to-water solubility ratios. Evidence that the lipoidal barrier of the cornea is located within the anterior epithelial layer was supplied by the almost identical rates at which lipid-soluble and lipid-insoluble compounds entered corneas from which the epithelium had been removed. Similarly, Cogan and Hirsch (20), in experiments with excised corneas devoid of their epithelium, have shown that the lipid-insoluble, ionized form of drugs crosses the tissue just as readily as does the lipid-soluble, unionized form.

D. Gastrointestinal tract

The transfer of drugs and other foreign compounds across the gastrointestinal epithelium, discussed at length in recent reviews by the author (120, 121), is for the most part explainable in terms of simple diffusion across a lipoid-pore membrane. The transfer of certain inorganic ions, sugars, amino acids, pyrimidines, and other natural substrates, on the other hand, seems to involve specialized transport processes (25, 27, 58, 121, 130, 131, 176).

1. Stomach. Studies of the gastric absorption of drugs in the rat (128) and the human (59) have shown that the epithelial lining of the stomach is permeable to the lipid-soluble, unionized form of drugs, and relatively impermeable to the ionized form. For example, weak acids such as the salicylates and barbiturates, which are mainly unionized in the acid gastric contents, are readily absorbed; conversely, weak bases such as aminopyrine, quinine, ephedrine, and tolazoline, which are highly ionized, are hardly absorbed at all. Moreover, completely ionized sulfonic acids like phenol red, and completely ionized quaternary amines like tetraethylammonium, are not appreciably absorbed.

Additional evidence that it is mainly the nonionized form of a drug which crosses the gastric epithelium is supplied by the reversal of the above-described pattern of absorption that results when the gastric contents are made alkaline (pH 8) with sodium bicarbonate. Many basic compounds become unionized in the alkaline medium and show an increased rate of absorption; conversely, acidic compounds become more ionized and show a decreased rate of absorption (128, 154).

The preferential permeability of the gastric mucosa to the nonionized form of weak electrolytes is further emphasized by the predictable manner in which drugs become distributed between gastric juice and plasma. For instance, when various drugs are administered intravenously to dogs, they are distributed between gastric juice (pH 1) and plasma (pH 7.4) according to their pKa value: basic drugs like antipyrine, aminopyrine, mecamylamine, and levorphan become more concentrated in gastric juice than in plasma, the higher the pKa value, the greater the gastric juice-to-plasma concentration ratio; acidic drugs like salicylate, phenylbutazone, thiopental, and barbital become more concentrated in plasma, the lower the pKa value, the smaller the gastric juice-to-plasma ratio (135, 136, 178).

An indication that lipid-solubility is the chief physical property which governs the passage of unionized molecules across the gastric epithelium is provided by the rough parallelism between the rates of absorption of barbiturates and the lipid-to-water partition coefficients of their unionized forms. For example, thiopental is absorbed very rapidly, secobarbital less rapidly, and barbital relatively slowly (128).

2. Small intestine. Investigations of drug absorption in the rat have revealed that the epithelium of the small intestine, like that of the stomach, allows the ready penetration of lipid-soluble, unionized molecules and impedes the penetration of ionized moieties. Thus, there is a relation between the degree of ionization and the rate of absorption of drugs: many weak acids and bases, including salicylates, barbiturates, phenols, xanthines, antipyrine, aminopyrine, various plant alkaloids, and a number of aniline derivatives, are readily absorbed; stronger, more highly ionized acids and bases such as *o*-nitrobenzoic acid, tolazoline, and mecamylamine are less readily absorbed; and completely ionized compounds like phenol red, sulfosalicylic acid, tetraethylammonium, and edrophonium (Tensilon) are very slowly absorbed (132).

Another indication that weak acids and bases cross the intestinal boundary mainly in their unionized form is provided by the change in the rate of absorption which results from a change in pH of the intestinal contents. For example, raising the intestinal pH increases the absorption of bases such as quinine and aminopyrine, and decreases the absorption of acids such as benzoate and salicylate (60).

Since the rates of intestinal absorption of numerous drugs are dependent on the proportion of lipid-soluble, nonionized drug molecules, and not on the molecular weight of the compounds, it appears that the main pathway of absorption is through lipoid areas of the intestinal boundary rather than through small aqueous pores. In support of this view, many lipid-soluble compounds of high molecular

weight are absorbed more rapidly than small, lipid-insoluble molecules like D_2O and urea (132). That the latter two compounds do pass through pores in the intestinal epithelium is apparent from their rates of absorption, which are much faster than those of larger, lipid-insoluble molecules such as inulin and mannitol (60, 132). With lipid-insoluble ions like sulfonic acids and quaternary amines, there is no indication of whether their slow absorption results from diffusion (through pores or lipoid regions of the boundary) or from some more complicated process (75, 120, 121).

Evidence that lipid-solubility is the physical property that dictates the rate of passage of unionized molecules across the intestinal epithelium has been furnished by studies showing a rough parallelism between the rates of absorption of various weak electrolytes and the lipid-to-water partition ratios of their unionized forms (60, 120). Furthermore, a recent investigation of the intestinal absorption of a large number of steroid compounds in the rat has shown that the compounds are absorbed at rates roughly parallel to their lipid-solubilities (133).

Calculations based on the steady-state distribution of drugs between plasma and the intestinal lumen suggest that the effective pH of the rat small intestine is considerably lower than that of the intestinal contents (57, 60, 120). For example, although the intestinal contents may have a measured pH of 6.6, and the plasma a pH of 7.4, drugs are distributed between the two fluids as though the pH values were 5.3 and 7.4, respectively. A zone with a pH of 5.3, possibly located at the surface of the intestinal epithelial boundary, may thus determine the degree of ionization of drugs as they approach the boundary to be absorbed.

Although most drugs and other foreign organic compounds appear to cross the intestinal boundary by a process of simple diffusion, there is evidence that a drug can be absorbed by a specialized active transport process if its chemical structure is similar enough to that of the substrate transported naturally. For example the foreign pyrimidines, 5-fluorouracil and 5-bromouracil, are actively transported across the intestinal epithelium by the process which transports the natural pyrimidines, uracil and thymine (124). Moreover, several foreign sugars, structurally similar to glucose, are actively absorbed by the monosaccharide transport process of the intestine (175).

3. Colon. The pattern of drug absorption in the rat colon is very similar to that in the small intestine (119). Thus, weak acids and bases are in general readily absorbed, and stronger, more highly ionized acids and bases are more slowly absorbed. Furthermore, the absorption of drugs is favored by changes in the colonic pH which increase the proportion of drug in the unionized form.

The rough proportionality between the rate of absorption of a number of barbiturates and the lipid-to-water partition coefficient of their unionized forms emphasizes the importance of lipid-solubility in determining the rate of passage of drugs across the colonic epithelium (119).

E. Oral mucosa

The absorption of drugs from the oral cavity has not been studied in a systematic, quantitative manner. Nevertheless the results of semiquantitative investiga-

tions such as those of Walton (164, 165, 167) strongly suggest that the mucosal lining of the mouth, like that of the rest of the alimentary canal, behaves as a lipoidal barrier to the passage of drugs. Walton's procedure was to measure the pharmacologic response to a drug after subcutaneous administration, and then to determine the sublingual dosage required to elicit a response of similar magnitude; the sublingual/subcutaneous dosage ratio provided a rough measure of the extent of oral absorption. Using this method in dogs, he showed that the relative degrees of oral absorption of cocaine, strychnine, atropine, and several opium alkaloids parallel roughly the oil-to-water partition ratios of the compounds (165, 166).

F. Skin

There is no general agreement among investigators as to the main pathway by which drugs traverse mammalian skin. Some authors have stressed the importance of the epidermal route, while others have contended that the appendageal route—through hair follicles, sweat glands, and sebaceous glands—is the predominant one (19, 45, 78, 118, 174). The problem has remained unsolved mainly because of the difficulty of devising suitable quantitative experiments to evaluate drug transfer by the various pathways. Recently, however, Tregear (155) has developed a technique whereby drug penetration can be assessed using small areas of skin which contain either a desired number of hair follicles or none at all. Studying in this way the absorption of tri-*n*-butyl phosphate from the skin of living pigs, he has shown that the hair follicle is no more penetrable than an equivalent area of epidermis; in fact, regions of the skin devoid of hair follicles were penetrated slightly more rapidly than regions containing these structures.

Whatever the relative importance of the various skin structures as routes of absorption, the results of numerous studies make it clear that drugs penetrate the skin predominantly by passing through a lipid-like barrier. This conclusion is based on many isolated observations that lipid-soluble molecules are absorbed much more readily than lipid-insoluble molecules and ions (19, 45, 78, 118, 174), and on Treherne's (156) comprehensive study of the passage of nonelectrolytes across the excised rabbit skin. The latter investigator has shown that various alcohols and urea derivatives diffuse across whole skin at rates roughly proportional to the ether-to-water partition coefficients of the compounds. He concluded that the lipoid 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.

G. Salivary glands

The molecular size, lipid-solubility, and degree of ionization of organic compounds are important determinants of their passage into saliva. The relevance of the size and lipid-solubility of molecules is apparent from results obtained by Amberson and Höber (1) in a study of the salivary excretion of nonelectrolytes. Using the isolated, perfused submaxillary gland of the cat, they showed that small lipid-insoluble molecules such as acetamide, propionamide, urea, and di-

hydroxyacetone pass into saliva much more readily than do larger molecules like glucose, galactose, fructose, and disaccharides (1, 56). The importance of lipid-solubility became evident on comparing the extents of salivary excretion of butyramide, dimethylurea, and malonamide, compounds with nearly identical molecular sizes; the first two substances, which have appreciable lipid-solubilities, entered the saliva considerably faster than did malonamide, which has a very low lipid-solubility (1, 56).

A similar pattern of salivary excretion was obtained by Burgen (14) in a study of the permeability of the parotid gland. The parotid duct of dogs was cannulated, various organic nonelectrolytes were administered intravenously by continuous infusion, and the secretion of saliva was enhanced by stimulation of the auriculotemporal nerve. Lipid-insoluble compounds such as urea, glycerol, creatinine, and mannitol passed from plasma to saliva at rates roughly related to their molecular weight, the smaller the molecule, the more rapid the rate. In contrast, a lipid-soluble substance like chloramphenicol entered the saliva very rapidly despite its relatively large molecular weight of 325. Except at very low rates of salivary secretion, the compounds were less concentrated in saliva than in plasma, mannitol having the lowest concentration, 2 to 4% of the plasma level, and most of the other compounds having concentrations ranging from 10 to about 90% of the plasma level. In sharp contrast, the parotid tissue levels of all the compounds were about the same as those in plasma—a particularly interesting observation in the case of mannitol, since most cells (except those of the liver) are almost impermeable to the compound. Thus, the side of the parotid epithelium that faces the plasma behaves as a highly porous boundary.

Evidence that the parotid gland epithelium is almost impermeable to the ionized form of drugs has been provided by an investigation by Killmann and Thaysen (66) of the salivary excretion of acidic compounds. The compounds were administered orally or by continuous intravenous infusion to human subjects, saliva was collected from the parotid duct, and plasma samples were obtained at various times. An almost completely ionized compound like *p*-aminohippuric acid appeared in saliva in very low concentration, the saliva-to-plasma concentration ratio being 0.015. In contrast, a number of sulfonamide compounds, partly to completely unionized in plasma, appeared in saliva in relatively high concentrations; the saliva-to-plasma ratios, which ranged from 0.31 to 0.94, paralleled roughly the pKa values of the drugs so that the more unionized the compound, the more of it was found in the saliva.

H. Sweat gland

The sweat gland epithelium appears to be another example of a membrane that is preferentially permeable to the unionized form of drugs. Evidence for this view has been provided by a study by Thaysen and Schwartz (151) of the distribution of drugs between sweat and plasma. Various acidic compounds, administered parenterally or orally to human subjects, attained sweat-to-plasma concentration ratios dependent on the pKa value of the compound, the lower the pKa, the lower the ratio. For example, the ratio for sulfanilamide (pKa 10.4)

was about 0.7; sulfapyridine (pKa 8.4), 0.6; sulfathiazole (pKa 7.1), 0.13; and *p*-aminohippuric acid (pKa 3.8), 0.02. The data conform roughly to the pattern that would be expected for the distribution of weak acids across a lipid-like membrane separating plasma of pH 7.4 from a fluid of somewhat lower pH value.

Like many other body membranes, the sweat gland epithelium is readily penetrated by urea but not by inulin (151).

I. Placenta

Although the exchange of drugs between the mother and fetus has been the subject of innumerable reports as well as a number of recent reviews (3, 4, 48, 81), almost nothing is known about the nature of the process. Much confusion about placental permeability has arisen from an abundance of conflicting data obtained in poorly designed experiments. For example, the failure to detect significant amounts of a drug in the fetus after administration to the mother has often been interpreted in terms of placental impermeability without due regard for the possibility that the drug might have been metabolized, localized, or excreted by the mother. Moreover, results with various drugs have been obtained using so many different experimental procedures, in different animal species, and at different stages of the gestation period that it is difficult to make valid comparisons.

With the paucity of information concerning the comparative transfer rates of substances, there is no clear-cut indication of the extent to which the lipoid membrane thesis applies to the placental boundary. However, a hint that the lipid-solubility of drugs might be a factor in determining their speed of passage across the placenta is obtained when drugs are divided into two groups: those which pass from mother to fetus to a significant extent, and those which do not. For example, compounds that have been detected in significant concentrations in fetal blood or tissues shortly after administration to the mother include the following: all the anesthetic gases and vapors; many barbiturates, sulfonamides, and salicylates; a number of alkaloids such as quinine, methadone, and meperidine; and numerous other drugs of moderate to high lipid-solubility (3, 4, 81). In contrast, substances which scarcely reach the fetus in a short period of time include inulin, dextran, and a number of quaternary ammonium ions (3, 4, 48, 49, 50, 81)—compounds almost insoluble in lipids.

An additional suggestion of a lipoid character of the boundary has been supplied by the measurements of Dancis and co-workers (29) of the relative rates at which some estrogens and their glucuronides cross the guinea pig placenta. They showed that estriol and the lipid-soluble metabolites of estradiol readily traverse the placenta in either direction, while the lipid-insoluble glucuronides of these compounds cross hardly at all.

J. Mammary gland

Evidence that the mammary gland epithelium is permeable to the unionized form of drugs and almost impermeable to the ionized form has been supplied by Rasmussen's studies of the distribution of drugs between milk and plasma (106, 107, 108). In experiments with lactating cows and goats, drugs were admin-

istered by continuous intravenous infusion, samples of milk and plasma were obtained periodically, and the concentration of drug was measured in ultrafiltrates of the two fluids. Basic compounds appeared in milk in a concentration greater than that in plasma. For example, erythromycin (pKa 8.8) had a milk-to-plasma concentration ratio of about 7.0. In contrast, acidic drugs appeared in milk in a concentration less than that in plasma, the lower the pKa value, the lower the milk-to-plasma ratio. For instance, benzylpenicillin (pKa 2.7) had a ratio of about 0.2, sulfathiazole (pKa 7.1) a ratio of 0.35, and sulfadimidine (pKa 7.4) a ratio of about 0.6. The ratios were in fairly close agreement with those calculated for a lipoid membrane separating solutions of pH 6.6 and 7.5, the usual pH values obtained for milk and plasma, respectively. Moreover in a few experiments in which the pH of milk varied between 6.5 and 7.7 without an appreciable change in plasma pH, the milk-to-plasma ratios of drugs varied in a manner predictable from the pH gradient and the pKa of the drug.

As would be expected from these results, unionized substances like ethanol, urea, and antipyrine had a milk-to-plasma ratio of 1.0, and this was independent of the pH of the milk.

K. Erythrocyte membrane and other cell membranes

1. Erythrocyte. In an investigation of the permeability of the human erythrocyte to a variety of organic bases, Schanker, Nafpliotis and Johnson (127) have shown that the compounds penetrate the cell at rates related to their lipid-to-water partition coefficients at pH 7.4. For instance drugs of high lipid-solubility like antipyrine, bufotenine, aniline, and procaine amide entered very rapidly; drugs of relatively low lipid-solubility like epinephrine, norepinephrine, and serotonin entered decidedly more slowly; and drugs of very low lipid-solubility like tetramethylammonium and edrophonium entered extremely slowly. Moreover, the cell membrane was shown to be preferentially permeable to the lipid-soluble, unionized form of the compounds; for example when the pH of the extracellular fluid was varied, serotonin entered the cells at rates directly related to the proportion of compound present as the unionized moiety.

Organic acids have also been shown to penetrate the red cell at rates roughly parallel to their lipid-solubilities; however, highly ionized acids of very low lipid-solubility such as phenol red, sulfanilic acid, and hippuric acid diffuse into the cell much more rapidly than do highly ionized basic compounds of a similar low lipid-solubility (125, 126). Thus, the entry of organic anions resembles that of inorganic anions, which also penetrate the erythrocyte at rates greatly exceeding those of cations.

To explain the unusual permeability of the erythrocyte to anions, it has been suggested that the cell is bounded by a lipid-like membrane which is perforated with positively-charged aqueous channels of various diameters (63, 93, 127, 139, 153). According to this idea, small lipid-soluble molecules penetrate both by dissolving in the lipoid phase and by diffusing through the aqueous channels; larger lipid-soluble molecules enter mainly through the lipoid phase. Lipid-insoluble molecules as well as anions enter the cell if they are small enough to

pass through the channels, whereas cations are largely excluded because of their lipid-insolubility and their inability to pass through the positively-charged channels.

2. Various tissue cells. In a study of the distribution of phenobarbital in dogs, Waddell and Butler (161) noted that the partition of the drug between plasma and tissues could be altered by varying the plasma pH. When the pH was lowered, the plasma level of drug decreased, and the tissue levels (brain, fat, muscle, liver) increased; conversely, on raising the plasma pH, the plasma level increased, and the tissue levels decreased. The results were consistent with the view that the cell membranes are preferentially permeable to the unionized form of drugs, and that drugs are distributed between the intracellular and extracellular fluids according to the difference in pH of the fluids. Moreover the same authors (163) have 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 (18) based on the distribution of carbon dioxide.

Robbins (112) has reported that the dye 2,8-diaminoacridine (proflavin) penetrates human conjunctival cells in tissue culture at rates related to the pH of the extracellular fluid. The results suggested that it is the unionized form of the dye which penetrates the cell membrane.

L. Mitochondria

Studies by Tedeschi and Harris (147, 148, 149, 150) of the osmotic swelling of mitochondria in solutions of organic compounds suggest that the boundary of these structures is lipoid in character. For example, rat liver mitochondria, suspended in solutions of nonelectrolytes such as polyhydric alcohols, aliphatic acid amides, and urea derivatives, were shown to swell at rates paralleling roughly the oil-to-water partition coefficients of the compounds. When the data were expressed in terms of penetration rates, and permeability constants calculated for the different compounds, the resulting values were similar to those that have been reported for the passage of these substances into *Chara* (23) and other cells.

M. Kidney

Studies of the renal excretion of drugs, so well reviewed in a number of recent articles (9a, 85, 87, 95, 141, 146), have indicated that the tubular epithelium has a dual character with regard to the transfer of foreign organic compounds: it behaves as a lipoid boundary allowing the ready passage of unionized, lipid-soluble molecules, and it has specialized processes for transporting many organic ions from plasma to urine.

Drugs of high lipid-solubility do not appear in the urine in large proportions, because most of the drug molecules filtered at the glomerulus return to the bloodstream by diffusing across the lipid-like boundary of the tubular cells (12, 16). Conversely, compounds of low lipid-solubility are readily excreted in the urine, because they are only partly reabsorbed in the tubule.

When the pH of tubular fluid is altered, the changes in the rates of urinary excretion of weak acids and bases are consistent with the view that the tubular epithelium is selectively permeable to the lipid-soluble, unionized form of drugs. For example when the tubular urine is made more alkaline than plasma, weak bases become less concentrated in urine than in plasma, and as a result are excreted more slowly; when the urine is made acidic, the bases become concentrated in the urine and are excreted more rapidly (85, 86). Conversely, weak acids are excreted more readily in an alkaline urine, and more slowly in an acidic urine (85, 161, 162).

The tubular epithelium possesses at least two specialized processes for transporting substances from plasma to urine: one for organic anions such as phenol red, Diodrast, and *p*-aminohippurate, and one for organic cations such as tetraethylammonium, mepiperphenidol (Darstine), and N^1-methylnicotinamide, to mention only a few of the compounds (9a, 95, 141, 146). Numerous studies have shown that these substances are transported against large concentration gradients, that there is competition for transport among various anionic compounds, and similarly competition for transport among various cationic compounds. Such observations have led to the conclusion that many strong organic acids and bases are secreted into the urine by active transport processes of rather low structural specificity. Moreover it has become apparent in recent years that certain weak acids and bases are also excreted in part by these transport processes. For example, the ionized form of salicylic acid is secreted into urine by the mechanism that secretes *p*-aminohippurate, and the ionized form of quinine is secreted by the mechanism that secretes N^1-methylnicotinamide (87, 95, 152, 160, 168, 169). Thus, the renal excretion of certain weak electrolytes appears to involve three processes: glomerular filtration, active tubular secretion of the ionized moiety, and passive tubular reabsorption of the unionized moiety.

N. Liver

One of the most widely studied aspects of hepatic permeability is the specialized process by which certain organic acids are concentratively transferred from blood into bile. The transported acids are highly ionized ones—phenol red, bilirubin, the bile acids, bromsulphalein, fluorescein, and penicillin—and the transfer process thus resembles in some respects that which transports organic anions across the kidney tubule. Studies showing that these compounds are transferred into bile against sizable concentration gradients, that the transfer mechanism is saturable, and that various acids compete for transfer have been thoroughly reviewed by Sperber (141) and Brauer (11).

Recent studies of the hepatic excretion of organic bases have suggested a further analogy with the kidney. Thus, Schanker and Solomon (122, 140) have shown that the quaternary ammonium compound, procaine amide ethobromide (PAEB), is transferred from the blood to the bile of rats by a process exhibiting the characteristics of an active transport process. For example, when the plasma level of drug is kept constant, the drug concentration in bile is at least 80 times that in plasma. Furthermore, the transport mechanism is saturable as evidenced

by the failure of a rise in the plasma level to result in an increase in the amount of drug excreted. Moreover certain organic cations appear to compete with PAEB for transport. For instance, quaternary amines like Darstine, oxyphenonium, glycopyrrolate, benzomethamine, and N^1-methylnicotinamide, some of which are known to be excreted in bile in significant proportions (72, 73, 122, 129), depress markedly the biliary excretion of PAEB. An indication that this transport process is different from the one which secretes organic anions is supplied by the failure of high doses of bromsulphalein to inhibit the hepatic transport of PAEB.

In addition to the above-mentioned acids and bases, many polar metabolites of drugs including glucuronides and other conjugates appear in bile in high concentrations, suggesting that specific transport processes might be involved. An alternative explanation is that these substances, formed within the liver cells, are "dragged" into the bile capillaries by a nonspecific process of filtration (hydrodynamic flow) resulting from the osmotic effect of actively secreted bile acids and inorganic ions (123).

The passage into bile of large, lipid-insoluble molecules like dextran, inulin, sucrose, and mannitol (24, 53, 54, 123, 129) indicates that the boundary between bile and hepatic sinusoidal blood is highly porous in nature. The above-mentioned saccharides appear in the bile of rats in concentrations inversely related to their molecular size, mannitol having the same concentration in bile as in plasma water (123, 129). Moreover, mannitol (17, 123) and sorbitol (17) penetrate into the intracellular water of liver and rapidly equilibrate with all of the hepatic water, suggesting that large pores exist in the membrane of the hepatic parenchymal cell as well as in the wall of the blood sinusoid.

Although the parenchymal cell has a high degree of permeability to saccharides that are confined to the extracellular fluid of most tissues, it has an even greater permeability to fat-soluble substances. For example, in studies with the isolated, perfused rabbit liver (maintained at a temperature near 0°C to minimize the metabolism of drugs), Kurz (68, 69) has shown that even though drugs of low lipid-solubility diffuse from the circulation into the tissue at relatively rapid rates, compounds of high lipid-solubility enter much faster and at rates roughly parallel to their lipid-to-water partition ratios. Extending his study to include the entrance of drugs into rabbit liver slices (at 0°C), Kurz (68, 69) has observed that compounds of widely different lipid-solubility all penetrate the tissue slices with extreme rapidity and at approximately the same rate. This finding has led him to suggest that the parenchymal cell membrane is more permeable than the endothelium of the blood sinusoid, and that the latter membrane constitutes the main barrier between plasma and the intracellular fluid of the liver. However, this view seems inconsistent with observations of the entrance of saccharides into the liver *in vivo*. For example, inulin (123, 129), dextran (129), and raffinose (17) penetrate the sinusoid wall rather rapidly as evidenced by the readiness with which they equilibrate between plasma and liver; but they must enter the parenchymal cell extremely slowly since they are confined largely to what appears to be the extracellular space of the tissue—a space of some 20 to 25% of the wet tissue weight (17, 51, 123, 129). The discrepancy between the observa-

tions *in vitro* and *in vivo* might be the result of an abnormal increase in permeability of liver cells in the tissue slices.

Although much work is needed to clarify the various aspects of hepatic permeability, the bulk of the experimental evidence now at hand suggests the following picture. The endothelium of the blood sinusoids, like that of blood capillaries in general, behaves as an extremely porous membrane permitting the ready equilibration between plasma and the extracellular fluid of liver of virtually all molecules and ions whose size is less than that of protein molecules. The membrane of the hepatic parenchymal cell acts as a lipoid membrane containing fairly large aqueous pores; the pores are large enough to admit a number of lipid-insoluble substances that do not penetrate many other body cells, but are smaller than the pores of the sinusoidal endothelium. Finally, the bile duct epithelium must have the properties of a lipoid membrane that is relatively impermeable to large, lipid-insoluble molecules and ions; otherwise glucuronides, bile acids, and certain other organic anions and cations would not remain in bile in concentrations far greater than those in plasma.

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