MECHANISMS OF DRUG ABSORPTION AND EXCRETION[1,2]

PASSAGE OF DRUGS OUT OF AND INTO THE GASTROINTESTINAL TRACT

By Ruth R. Levine and Edward W. Pelikan

Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston University Medical Center, Boston, Massachusetts

When one considers the importance of the oral route for administration of drugs it is, indeed, surprising that so little attention has been directed toward systematic investigation of the mechanisms by which drugs leave and enter the gastrointestinal tract. The paucity of new material,[1] ordinarily, would preclude the need for a review at this time were it not for the fact that recent reviews of mechanisms of absorption (1 to 4) were written from a single point of view. In this review, therefore, the authors will re-examine some of the older literature and some of the newer literature from different points of view. The present authors make no claim to being completely free of a bias which may stem from their own special interests. It is hoped that the presentation of differing and different viewpoints may stimulate investigations in areas that appear to have been unduly neglected.

The interpretation of much of the recent work on the passage of drugs across biological barriers is based on the assumption that the biological barrier is relatively static and inert. This has led to the concept that the factors of molecular size, shape, electric charge, and polarity of the drug molecule alone could serve to account for the movement of most drugs (1 to 4). However, the properties of the membrane[3] are dependent not only on the properties of its constituents but also on the manner in which these constituents are organized to form the membrane. The properties of the membrane become the physical-chemical properties of its constituents only to the extent that the breakdown in the required energy supply permits disorganization of the membrane (5). The living membrane is in dynamic equilibrium with its envi-

[1] The survey of the literature pertaining to this review was concluded in June, 1963. Numerous reports have appeared which describe the simple fact or degree of intestinal absorption of single compounds or groups of compounds in various species. Since these reports are not concerned with mechanism of absorption they will not be included in this review.

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[3] The authors will use the words "biological barrier" or "membrane" interchangeably to describe the separation between two phases. This is not to imply that *in vivo* phase separations are either single or homogenous. Biological barriers, such as the intestinal barrier, may consist of several "layers."

ronment, the dead membrane in static equilibrium (5). Concepts that invoke both dynamic and static characteristics of the barrier as well as the physical-chemical properties of the drugs are necessary for a more complete understanding of the mechanisms by which drugs traverse biological barriers.

With respect to any given material, the membrane can play any of several roles during the process of transfer of the solute. Since transfer is the passage of a solute through a barrier interposed between two phases, the solute appearing in the same state in both of the separated phases (6), any reaction of membrane and solute which produces a change in the final state of the solute in either or both phases is not pertinent to this discussion. When we can describe the manner of transfer of a solute across a membrane in terms of the physical-chemical properties of the solute and those of the constituents of the membrane, then the membrane plays a passive role. Here we are dealing with so-called passive diffusion and the force moving the solute across the membrane is the concentration gradient of the solute. The rate of movement is proportional to the gradient, within the limits of viability of the membrane, and the rate is determined by the physical-chemical properties of both the solute and the membrane. During passive diffusion cellular energy is utilized only to maintain membrane structure.

When we can describe the transfer of a solute across a membrane only by implicating a chemical structure or a site on the membrane, the structure or group is said to mediate the transfer. The term "carrier" has been applied to the structure or site in or on the cellular membrane which mediates the transfer by temporary combination with the solute (7), and the term "facilitated diffusion" has been introduced to describe the process (8). If the transfer is unable to proceed against a concentrated gradient (i.e., "uphill"), then the moving force is still the concentration gradient. This is still diffusion, but the role of the membrane is no longer dependent on only the physical properties of its constituents but depends as well on the manner in which the constituents are organized within or on the structure. Transfer mediated by carrier (facilitated diffusion), in contrast to passive diffusion, is characterized by a decrease in the rate of transfer at concentrations of solute which saturate the carrier but do not affect the viability of the cell. The rate of transfer in this case is determined by the properties of the solute and carrier, and a rate-limiting step, the availability of carrier, is introduced. Energy is utilized only to maintain cellular organization.

When a solute, transfer of which is mediated by carrier, is moved from a phase of lower to a phase of higher electro-chemical potential, the process is called "active transport" or a "biological pump." In this case, the cell or cellular membrane participates in the transfer by providing cellular organization and carrier for temporary combination with the solute, and by supplying chemical energy for the work to be done. In active transport there is also a limitation on the rate of transfer, but the rate-limiting step may be determined by either availability of carrier or the supply of energy for osmotic work.

The discussion above permits the establishment of several criteria for adequate characterization of mode of transfer according to the role of the barrier in the transfer process:

(*a*) The description of a dose-effect curve for the transfer phenomena is a *sine qua non* to characterizing the phenomena. The relationship of amount transferred to amount to be transferred must be described as linear or alinear, continuous or discontinuous, limited or unlimited.

(*b*) Similar to the dose-effect curve, a time-effect curve is *sine qua non* to characterizing the transfer phenomena.

(*c*) The capacity of the transfer system to transfer both with and against a concentration gradient (regardless of the direction of transfer under physiological conditions) must be determined.

(*d*) Experimental conditions (of pH, ionic strength, kind and quantity of adventitious molecules, etc.) that depart from the state under which transfer normally occurs must be assumed, until proven otherwise, to have modified the nature of the barrier, as well as that of the transferable solute, and to have altered the mechanism of transfer from that which normally obtains. This is not to say that a single molecular species must be transferred by only one mechanism even under a given set of experimental conditions.

(*e*) Viability or biological integrity of the barrier, under the conditions of the study of transfer, must be verified by independent measures of functional capacity of the barrier.

We shall not discuss the fine points of difference between facilitated diffusion and carrier transport (7, 9) or try to set up yet one more definition of active transport. We shall proceed to apply the above criteria to analysis of published data.

Passive diffusion: It would follow from consideration of the porosity of the cellular membrane and its fundamentally lipoid character that water and small molecules could pass through the pores, and that substances which were soluble in lipids could enter into and pass through the membranes via the lipoid phase (10 to 14). It would be expected that the capacity of the chemicals to traverse the membrane via the lipoid phase would be a function of their degree of lipoid solubility. Using oil-to-water or organic solvent-to-water partition coefficients as a measure of lipoid solubility, it has been possible to show positive correlations between degree of lipoid solubility and rates of absorption of various substances from the intestine (1, 4, 15). While better correlations have been obtained for homologous series of compounds (15, 16), significant correlations can be shown to exist too, for groups of compounds of widely differing structures (17, 18). For example, using the data for a homologous series of substituted barbiturates (16) to calculate the correlation between per cent intestinal absorption and partition ratios, a rank difference correlation coefficient (19) of ± 0.995 is obtained. This indicates a highly significant relationship. It follows, then, that for other compounds in this homologous series, on the basis of the partition coefficients obtained using the same solvent system, one probably could predict quite

accurately the degree to which the compounds would be absorbed.[4] Similar calculations can be made for a group of heterogeneous compounds, such as those studied by Schanker et al. (17, 18). Hogben (18) presented data for partition coefficients in chloroform/water and heptane/water for compounds whose degree of intestinal absorption had been determined previously (17). The rank difference correlation coefficient between per cent absorption and partition coefficients in heptane/water was $+0.607$ ($P < 0.01$) and in chloroform/water was $+0.506$ ($P < 0.05$). Thus, the relationship between absorption rate and partition coefficients can be considered to be significant and unlikely to have occurred on the basis of chance alone. However, for the heptane system only 37 per cent, and for the chloroform system only 25 per cent, of the joint variability can be accounted for by properties common to absorption and to solubility in these organic solvents.[4] Therefore, the relationship between distribution in the solvents and absorption accounts for a *minority* rather than a *majority* of the factors common to both. Ascribing a causal relationship between solubility in organic solvents and mechanism of absorption is to leave out more of the factors in absorption than have been taken into account. To the extent that one can generalize from the results of a limited series of compounds, the ability to make predictions concerning degree of absorption of an individual compound among a group of heterogeneous compounds on the basis of its organic solvent partition ratio is relatively low.

Inferences concerning transfer of drugs have been made on the basis of data obtained in model systems, including systems which measure lipoid solubility. The biological barrier contains lipoid material. What criteria should be used to judge the appropriateness and validity of a system used as a model of the role of the lipid in the barrier? As Hogben (18) has pointed out, agreement between degree of intestinal absorption and partition coefficients in a few selected systems must be considered potentially spurious until external evidence is obtained for the correspondence of the solvent system to *in vivo* systems. In the past a single experimental attack has been used for two purposes: first, to make inferences about the nature of the barrier in terms of the agents which can traverse it (10, 11) and secondly, to infer mechanisms of drug transfer across the barrier (1). This is circular reasoning. What is required to resolve the question are studies of the properties of the barrier independent of, and unconfounded by, the act of drug transfer. Such studies obviously present great difficulty of experimental design and execution.

If the capacity of chemicals to traverse a biological barrier is a function of only their degree of lipoid solubility, the barrier should be almost impermeable to strongly acidic and basic drugs. Conflicting experimental evidence has

[4] The square of the correlation coefficient $\times 100$ determines the per cent of variability in both arrays of data which can be accounted for on the basis of properties common to the two variants.

been presented concerning the absorption of such compounds from the rat intestine (17 to 24). One group of investigators used an *in vivo* perfusion technique and reported that, beginning thirty minutes after perfusion was started, negligible absorption of quaternary ammonium cations and strongly acidic compounds occurred (17). While the insignificant absorption of strongly ionized drugs upon perfusion was interpreted to mean that the intestinal mucosa is impermeable to these ions (1, 17), the results were reconciled with the use of orally administered quaternary ammonium ions as therapeutic agents by the conjecture that the length of time (seven minutes) that the recirculating perfusate was in contact with the intestine during a single cycle was too short to allow for detectable absorption (2). A second group of investigators used an *in vivo* intestinal loop technique and found significant absorption (10–30 per cent) of various quaternary ammonium compounds (20 to 24) and 7–10 per cent absorption of phenol red (25). In these studies of absorption of quaternary ammonium compounds as a function of time it was found that they were absorbed quite rapidly from the *in vivo* intestinal loop (5–15 per cent within 15 minutes), but that only about 20 per cent of the dose was absorbed even after four hours because the initial, rapid, rate was not maintained (20 to 22). Appearance of the quaternary ammonium agents in urine within fifteen minutes after oral administration corroborated the findings of rapid initial absorption (26). Perhaps these differences in results could be reconciled if both groups of workers had designed the experiments to take cognizance of the fifth criterion set forth above, as well as of possible changes in rates of absorption that might occur with the passage of time.

Demonstrating conditions and mechanisms by which absorption can occur is not, by itself, evidence that these are the mechanisms by which it normally does occur. In practice, identification of the normal state requires application of criteria (*d*) and (*e*) above. Specifically, experiments must be designed to measure those conditions and their interactions which cause a departure from the physiological state. Levine and co-workers (24, 25) in studying the influence of some experimental manipulations on the absorption of quaternary ammonium compounds found that: (*a*) fasting of animals and perfusing of the intestinal lumen with small quantities of water prior to the beginning of the experiments increased absorption; (*b*) the relative position in the gut of the absorbing surface influenced absorption, being greatest in the segments closest to the pylorus, and (*c*) administration of drugs in solutions of about pH 3 did not influence rate of absorption of the quaternary ammonium compounds. A later but far more rigorously controlled and carefully analyzed study of the influence of experimental manipulations on intestinal absorption is that of Ragozzino & Malone (27). These investigators first carried out their studies in intact normal rats and found that thiourea significantly increased the rate of intestinal absorption of quinine. Then, they carried out experiments designed to examine the effects of thiourea, two derivatives of thiourea, ionic strength of perfusion solutions, fasting, and some of the interac-

tions of these factors on the absorption of quinine and quinidine, employing an *in vivo* intestinal perfusion procedure similar to that used by Schanker et al. (17). The detail of their elegant factorially designed experiments is indicated by the fact that each of 32 means involved a total of 24 separate determinations, representing four points in time for each of six animals. The rates of absorption for each of the four intervals of time in a single rat were relatively constant. Their pertinent conclusions, based on careful statistical analysis of the data, were as follows: (*a*) results could be replicated in separate samples of tissue regardless of the positions of the samples in the perfusion apparatus; (*b*) fasting routinely decreased the ability of the animals to absorb quinine and quinidine in the *absence* of any additives; (*c*) the rate of absorption of both alkaloids was decreased in nonfasted animals, but was increased in fasted animals in the *presence* of 1-phenyl-2-thiourea; (*d*) differences in ionic strength of the perfusion medium had no significant effect on absorption of the alkaloids. While it may not be always convenient to use such an elaborate design for studies of absorption of drugs, the work of Ragozzino and Malone certainly points up the value of carefully controlled experiments and thoughtful analysis of the results.

The early studies of Travell, recently reviewed (28), and the more recent studies of Schanker et al. (29), and Hogben et al. (18) have demonstrated clearly that changes in the pH of the drug solutions can produce changes in the rates of absorption of weak electrolytes from various parts of the gastrointestinal tract. The result of these studies led to the concept that the mucosa of the gastrointestinal tract, regardless of the site, was selectivity permeable to the unionized form of drugs, since the absorption of acidic drugs increased and that of basic drugs decreased with increasing acidity of the solutions, while opposite changes in absorption were observed with increasing alkalinity of the solution. As is invariably true in the analysis of such complex problems, assumptions had necessarily to be made as a point of departure. Two assumptions made by Hogben et al. (18) were that: (*a*) "the mucosal surface is completely impermeable to the ionized species," and (*b*) implicitly, changes in the pH of the medium to which a membrane is exposed produce no changes in the state of the membrane, per se. Certain observations, such as the rapid absorption of salicylic acid and the absorption of quaternary ammonium ions from the small intestine seemed inconsistent with the concept of an intestinal barrier completely impermeable to the ionized form of the drug (17, 18). Hogben and co-workers (18) postulated that the pH at the site of absorption was lower than the pH within the intestinal lumen and that this would explain the observed inconsistencies. After studies of the steady-state distribution of drugs between the intestinal lumen and plasma, they concluded that a slightly acidic environment did exist at the intestinal-blood barrier (18). However, as Hogben et al. (18) point out, even a relatively negligible permeability to the ionized species would modify the steady-state distribution ratio significantly. In more recent studies, Nogami & Matsuzawa (30, 31) showed

that the application of chemical kinetic analysis permitted inferring that drugs might penetrate through the intestinal barrier, *in vitro*, in both the undissociated and dissociated forms. Theoretical equations were derived from the assumption that the intestinal barrier was measurably permeable to *both* forms of the drug; the permeability coefficients for the undissociated and dissociated forms were determined experimentally. Confirmatory experiments were carried out using solutions at the calculated equilibrium state concentrations in order to test the validity of the equations used and the values for permeability coefficients determined from them. The ratio of the permeability coefficients for the undissociated to the dissociated forms was found to be about six for salicylic acid (30) and about eleven for aminopyrine (31). Procedures employing constant rate perfusion through rat small intestine were used by both groups of investigators, Hogben and co-workers (18) using an *in vivo*, and Nogami & Matsuzawa (30, 31) an *in vitro* preparation. The experimental procedures differed also in the choice of buffers used by the two groups to achieve desired acidic pH's, Hogben and co-workers (18) adding aspartate or acetate, and Nogami & Matsuzawa (30, 31) added citrate or phosphate to bicarbonate buffers. However, until it can be demonstrated experimentallythat differences in methods influenced the outcomeof the experiments,[5] we must question whether the discordant conclusions were not the result of the different theoretical formulations of the problem used by the two groups of investigators. In any event, the validity of the assumption that the intestine is impermeable to the ionized species of a weak electrolyte has yet to be demonstrated.

To the reviewers' knowledge, no adequate studies have been carried out to determine the validity of the second assumption; namely, that changes in the pH of the medium produce no changes in the state of the membrane, or that such possible changes in the membrane have no bearing on the absorption of weak electrolytes. Since the membrane is not a static structure (32), due consideration should be given to the possibility that changes in the state of the membrane, influencing its ability to form complexes with solute molecules, can occur when the surface pH is changed (33, 34).

Indeed, changes in rates of absorption have been shown to occur when the state of the membrane may be assumed to have been changed. This is indicated in studies of the effect of ethylenediaminetetraacetic acid (EDTA) on absorption. Strongly acidic compounds such as phenolsulfonphthalein (35) and a heparinoid (36, 37) were found to be significantly better absorbed in the presence of EDTA than in its absence, but the calcium or magnesium salt of EDTA did not produce an increased absorption of heparinoid (36). Schanker & Johnson (38) found that EDTA enhanced the absorption of

[5] The presence of citrate and phosphate ions, even in a Krebs-Ringer solution, might have an effect on the calcium concentration of the solutions or the tissue with possible subsequent effects on drug transfer.

strong bases (such as decamethonium) and neutral compounds (such as inulin) as well as strong acids. Levine (39) found that the disodium salt of EDTA increased the rate of absorption of the monoquaternary ammonium agent, benzomethamine, but only during the first half- to one hour after administration of both agents. This enhancement of the initial rate of absorption of benzomethamine by the disodium salt of EDTA was reversed by the addition of an equimolar concentration of calcium or magnesium chloride (39). The effect of EDTA to increase absorption appears to be nonspecific in the sense that the degree or kind of ionization of the solute molecule does not determine whether its absorption can be increased by EDTA. The evidence suggests that the influence of EDTA on absorption is indirect and mediated by effective removal of calcium from the membrane. Numerous observations on tissues (40) have led to the concept that the effect of calcium on biological membranes is to increase the proximity of adjacent molecules, thereby decreasing permeability by "solidification" of the membrane (40, 41). Removal of calcium, in contrast, produces an increase in permeability. The bulk of the evidence concerning the nonspecific effect of EDTA on absorption is consistent with the idea that EDTA produces its effect primarily by altering the state of the membrane, since EDTA would not be expected to produce uniform effects on the state or inherent absorbability of the different solutes. However, it must be remembered that EDTA chelates other ions besides calcium. Few of the absorption studies with EDTA have taken into account the possibility that some of the effect of EDTA might be ascribable to its interactions with other ions, such as magnesium.

The conclusion that most drugs are absorbed from the intestinal tract by a process of passive diffusion of the unionized moiety across a lipoid barrier has been reached, primarily, on the basis of a demonstration of a relationship between lipoid solubility, ionization constant, and degree of absorption (1, 2, 16 to 18). However, the existence of this relationship is insufficient, in itself, to justify conclusions as to the mechanism(s) involved in the transfer. These studies have not satisfied, for the most part, the two most important criteria listed above, namely, the description of dose-effect and time-effect curves. Dose-effect relationships, based on two to four doses over a wide range, were obtained for a small percentage of the total number of compounds studied (16, 17). The results of these studies, e.g., that the absolute amount of each drug absorbed was directly proportional to its initial concentration, and that, in the few cases studied, one drug did not alter the rate of absorption of another drug, certainly are consistent with the concept that the agents were transferred by simple diffusion. However, the data are not sufficient to prove or disprove the conclusion concerning mechanism of transfer for all the drugs studied, or for even those drugs studied at more than one dose. Even when the concentration gradient is the moving force, more detailed analysis is necessary before the mechanism of absorption of these drugs can be termed only simple diffusion without additional facilitated diffusion or carrier transport.

Facilitated diffusion: Discrimination between passive diffusion and facilitated diffusion as the mechanism by which a chemical is absorbed is of particular importance when the chemical is used as a drug. Only changes in the biological barrier can influence the rate of absorption of a particular molecular species of a drug which is transferred by passive diffusion. However, the rate of absorption of a species of a drug molecule which is transferred by facilitated diffusion may be increased or decreased by changes in, or on, the membrane, or by the presence of certain solutes. Consideration of the latter may have important consequences in the therapeutic use of a drug or combinations of drugs administered orally.

To the reviewers' knowledge, there have been no studies which have firmly demonstrated that facilitated diffusion is the mechanism by which any therapeutic agent, with the possible exception of iron and cyanocobalamin (42, 43), is transferred out of, or into, the gastrointestinal tract.

An erroneous conclusion might have been made that iron, injected intraperitoneally, could be transferred through the serosa *into* the intestine via mechanisms intrinsic to the intestinal tissue, had the investigators not used histological methods in their work (44). The histological studies indicated that ferritin was transferred into the intestine by diapedesis of iron-laden phagocytes. Obviously, in this case, the membrane across which the critical transfer of iron occurred was that of the leucocyte rather than that of the barrier separating the vascular system from the intestinal lumen. The authors, Thirayothin & Crosby (44), suggested that the small intestine might function as an excretory organ for debris-laden phagocytes. The implications of this speculation are many. For example, potential drugs of limited solubility are frequently tested by intraperitoneal injection in particulate form. To the extent that these can be phagocytized, and the speculations of Thirayothin and Crosby can be extended, one may question the degree to which drugs so injected can be assumed a priori to reach their sites of action in predicted concentrations.

Active transport: It is not probable that evidence already exists that would permit demonstrating that a large number of compounds, some of which may be useful therapeutic agents, are absorbed by an active transport mechanism (or, for that matter, by facilitated diffusion). Unfortunately, much of this information is not readily accessible because of limitations in titling scientific articles and indexing them in the usual bibliographic texts. Williams makes this point in the analogous case of metabolism of drugs by "normal" enzymes (45). For example, in the course of investigations concerned mainly with the mechanism of absorption (or metabolism) of *normal* body constituents or foodstuffs, many *foreign* compounds have been shown to be transported against a concentration gradient (6) (or metabolized by "normal" enzyme). Schanker & Jeffrey (46) have presented evidence that 5-fluorouracil may be transported across the intestinal epithelium against a concentration gradient by a process which transports the natural pyrimidines, uracil, and thymine. Evered & Randall (47), using a similar technique, that of the

everted rat small intestine *in vitro*, found that certain serine and threonine derivatives of nitrogen mustard were actively transported against a concentration gradient. The rates of transfer and the final concentration ratios were comparable both for these derivatives and for the parent amino acids (47). The availability of evidence for absorption by a mechanism of active transport being limited to compounds used in cancer chemotherapy is probably the result of a philosophy governing the design of such drugs. In the case of carcinoclastic agents, the search for active agents involved compounds which, because of structural similarities to normal body constituents, might be antagonistic to abnormal cell development. Such compounds would be expected to enter into a number of chemical reactions appropriate to the normal metabolite they mimic, including any reactions involved in active transport. Since the mode of absorption of even normally occurring body constituents is barely understood, it can hardly be expected that the significance of observations concerning other materials can be viewed in proper perspective.

Strictly speaking, there have been no demonstrations of foreign compounds being actively transported into the intestine through the intestinal epithelium. Active transport of material from the blood into the bile has been amply documented (4, 48). By and large, such materials gain access to the intestinal lumen (from which they may be reabsorbed) via the biliary conduits. Schanker & Solomon (48) have presented evidence for the active transport of a quaternary ammonium compound, procaine amide ethobromide, into bile. This transport process could be markedly depressed by some other quaternary ammonium compounds, including benzomethamine and oxyphenonium (48). It is of interest that the evidence obtained to date suggests that the two latter compounds may be excreted into the intestine by an active transport process (21, 26, 48), and also may pass out of the intestine by some process other than passive diffusion (20, 21, 24, 39). Schanker & Solomon (48), appropriately, point out the similarities in the specialized secretory processes of the liver, kidney, and choroid plexus for the transport of certain organic cations and anions; and that, in all three tissues "transport appears to be oriented in such a way that the ions tend to be removed from the body" although the transport from the cerebrospinal fluid is initially a process of absorption into the blood. In these tissues the direction of transport appears to be determined "teleologically" rather than by embryological orientation of the barriers with respect to the true body surface.

Stewart & Harrison (49), in a quantitative study, have presented evidence for the active secretion into bile of the microbiologically active form of a broad spectrum penicillin, D(−)-6-(α-amino-α-phenylacetamido) penicillanic acid, and have presented evidence for its reabsorption and re-excretion in the bile. The enterohepatic system appears to play a major role also in the physiological disposition of indomethacin (50), but in this case the pharmacologically inactive metabolite, a glucuronide, was excreted via the bile. Indomethacin was largely reabsorbed as the active compound but only in

those species (guinea pig, monkey) in which deconjugation reactions occurred within the intestine.

Augmented absorption: Detailed kinetic studies by two groups of investigators (20, 21, 24, 39, 51 to 55) have shown that the pattern of intestinal absorption of several therapeutic agents is not entirely consistent with a concept of transfer by *only* passive diffusion. Neither group of investigators has yet reported identification of the other mechanism(s) that may be involved in the absorption of the drugs which they have been studying. Both groups of workers used similar methods of data analysis and the same procedure for obtaining the data, that is, determining dose-effect relationships using *in vivo* intestinal loops in rats. Such similarities of methodology may have helped to account for the fact that the two groups of workers drew a similar conclusion about the respective compounds which they studied, i.e., that passive diffusion alone could not explain the total phenomena.

In the case of benzomethamine, a dose-absorption curve determined for many points distributed over a wide dose range indicated that the curve was monotonic but discontinuous, and was composed of three straight line segments as illustrated by the lower line in Figure 1 (24). The dose-effect curve, per se, indicated only that more than one transfer mechanism was potentially available to benzomethamine, and that dose determined the mechanism utilized preferentially. However, since the rate of transfer was maximal only with the intermediate doses, the mechanism subserving this maximum rate was limited and could not be only passive diffusion. Other than this, the dose-effect curve, per se, gave no indication of the nature of the transfer mechanism. Consideration of the time-effect curves (20, 39), as illustrated by the lower and upper lines in Figure 2, indicated that the rate of absorption decreased with time more rapidly than did the calculated concentration gradient. This result, also incompatible with transfer by only passive diffusion, was obtained with doses in the lowest and highest segments of the dose-effect curve. Therefore, only consideration of *both* dose-effect and time-effect curves permits the inference that benzomethamine is not transferred by only passive diffusion in any part of the dose range studied.

Further studies with benzomethamine carried out with simultaneous administration of a phosphatido-peptide fraction of intestine, which augmented total absorption, yielded a dose-effect curve which was essentially linear but which also demonstrated a saturation-like phenomenon as illustrated by the upper curve in Figure 1 (39). In the presence of the phosphatido-peptide fraction it was not possible to demonstrate from the dose-effect curve alone that more than one mechanism of transfer was available to benzomethamine even though the saturation phenomenon indicated that the mechanism available to benzomethamine in the presence of the phosphatido-peptide fraction was probably not just passive diffusion. Since the rate of absorption remained constant although the calculated concentration gradient declined (middle curve of Figure 2), the time-effect curve confirmed the inference that

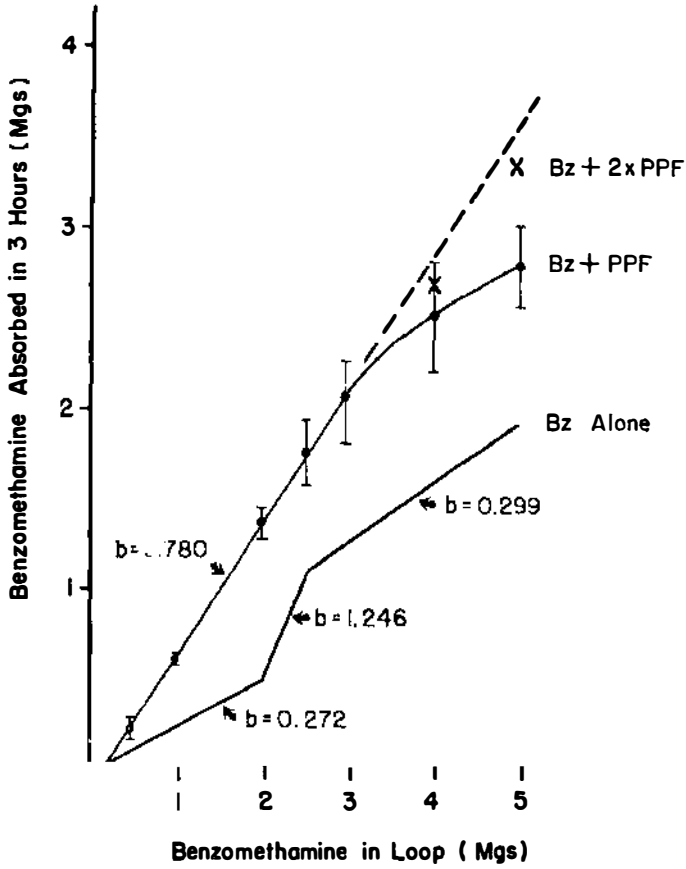

FIG. 1. Dose-effect curves for absorption of benzomethamine from *in vivo* intestinal loops in rats. Bars through each point indicate standard deviation; —— ——: Benzomethamine (Bz) in water. Data from Levine & Pelikan (24); ● ●: Benzomethamine (Bz) in water solution containing 1.5 mg/loop of phosphatido-peptide fraction (PPF). Data from Levine (39); ×: Benzomethamine (Bz) in water solution containing 3.0 mg/loop of phosphatido-peptide fraction (2×PPF); – – – – –: Linear regression line for Bz+PPF with doses of Bz of 3.0 mg or less.

the absorption of benzomethamine in the presence of phosphatido-peptide fraction was not by passive diffusion alone.

Augmentation of absorption by adjuvants, involving a transfer mechanism other than only passive diffusion, has also been demonstrated for some non-quaternary drugs (27). In the case of the quaternary ammonium drug, benzomethamine, enhancement of absorption by an additive (39) obscured the multiplicity of the mechanisms of absorption available to benzomethamine

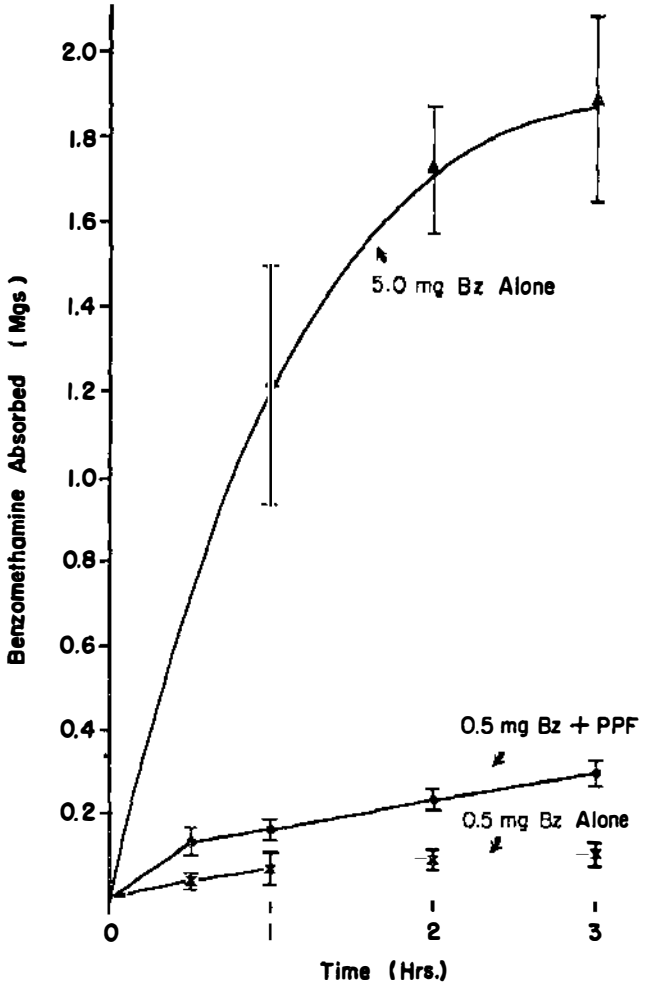

FIG. 2. Time-effect curves for absorption of benzomethamine from *in vivo* intestinal loops in rats. Bars through each point indicate standard deviation; × ×: Benzomethamine (Bz), 0.5 mg/loop, in water; Data from Levine et al. (20); ● ●: Benzomethamine (Bz), 0.5 mg/loop, in water solution containing 1.5 mg/loop of phosphatido-peptide fraction (PPF). Data from Levine (39). ▲ ▲: Benzomethamine (Bz), 5.0 mg/loop, in water.

alone (20, 24). In contrast, in the studies of Ragozzino & Malone (55), the addition of adjuvants was necessary to demonstrate that more than one mechanism of absorption of quinine and quinidine was available under some conditions. Ragozzino & Malone (27) found that the addition of thiourea or

some thiourea derivatives increased the degree of absorption of quinine or quinidine. In order to study the dynamics of this augmentation, they (55) carried out multiple intestinal loop experiments, based on a Latin square design, in unanesthetized rats. The absorption of the alkaloids in the presence of adjuvants was studied as a function of both dose and concentration at a number of levels. The dose-absorption curves for quinine, for quinidine, and for each alkaloid in the presence of each of three adjuvants were found to be linear and practically indistinguishable from each other at the lower dose range. This suggested that a common mechanism of transfer for the alkaloids, consistent with the idea of passive transfer, was operative in this dose range. However, the dose-absorption curves for quinine, but not for quinidine, in the presence of thiourea, and the curves for both quinine and quinidine in the presence of N,N′-diethylthiourea were inflected at the same level (278 μg of alkaloid/intestinal loop). Ragozzino & Malone concluded from their data, as had Levine & Pelikan from similar data obtained with a quaternary ammonium drug (24), that some transfer process in addition to passive diffusion was needed to explain the total phenomena of absorption in all dose ranges.

The studies of enhanced absorption have provided data intrinsically interesting of and by themselves. In the studies of Ragozzino & Malone (27, 55), the significance of the results is heightened when it is recalled that quinidine and quinine differ from each other only by being diasterioisomers. The physical-chemical properties, such as pKa, of the two isomers are virtually identical, and they differ only quantitatively in their biological properties. The uniform effect of N, N′-diethylthiourea to enhance the absorption of both isomers suggests that some part (or parts) of the transfer process is equally available to both. However, the differential effect of thiourea in enhancing absorption of quinine but not quinidine (27, 55) indicates that some step in the absorption process is different for each of the two isomers, and is dependent on the internal structure of the solute and not on the physical-chemical properties of the molecule as a whole.

The effect of the phosphatido-peptide fraction of intestinal tissue has been shown to augment the absorption of other quaternary ammonium compounds besides benzomethamine, such as oxyphenonium, 10-(α-dimethylaminopropionyl)-phenothiazine methobromide (Secergan®), hexamethonium and tubocurarine (51 to 54). The degree of augmentation of absorption was a function of the dose of the phosphatido-peptide fraction and as little as one milligram of fraction was effective (51 to 54). Biological tests were used to confirm the enhancement of absorption of tubocurarine and to demonstrate that the biological properties of the solute absorbed had not been changed by the process of absorption or by the experimental manipulations (54).

Concluding remarks: It is striking that particularly for therapeutic agents in wide and general use there is so little documented information available concerning mechanisms of absorption. The fact that passive diffusion must play a role in the absorption of *all* drugs cannot be denied in view of the properties of membranes and of solutions. The vexing question remains as to the

degree to which facilitated diffusion and active transport, and the environmental conditions in which absorption occurs, determines the rate and amount of absorption of any *specific* agent. The paucity of detailed information on the mechanisms of absorption of many individual drugs makes premature the formulation of generalizations about the mode of absorption of all drugs or of groups of drugs, no matter how desirable it is to make such generalizations.

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