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Perspective

Prodrugs and Site-Specific Drug Delivery

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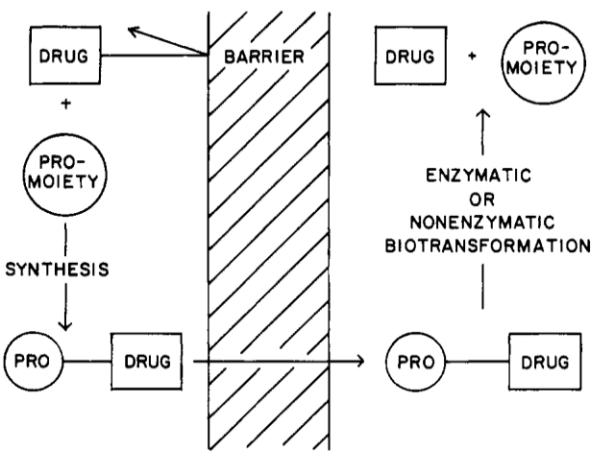
The term prodrug is used to describe an agent which must undergo chemical or enzymatic transformation to the active or parent drug after administration, so that the metabolic product or parent drug can subsequently exhibit the desired pharmacological response. The purpose of this paper is to address, in a critical and quantitative manner, whether prodrugs can provide site-specific delivery or targeting of parent, active drugs to their site of action. The first point that will be made is that prodrugs of *most* currently useful therapeutic agents *cannot* achieve further site-specific delivery. However, site-specific delivery is possible when drugs have certain physicochemical properties. Thus, the thesis presented in this paper is that the physicochemical properties of the parent drug and the properties of the site are both critical in predicting whether a prodrug can succeed in site-specific delivery of the parent drug to that site. Drug design that is guided by such an analysis may be more successful in the development of targeted drug systems utilizing prodrugs.

Although prodrugs have received renewed interest of late,[2,3] the approach is not new. Albert[4] was the first to use the term "pro-drug" or "pro-agent" and suggested that the technique could be used to temporarily alter and so optimize the physicochemical properties and, thus, the pharmacological and toxicological time profiles of an agent. Even before Albert, terms such as drug latentiation[5,6] and bioreversible derivatives were used by various investigators to describe such derivatives.

Scheme I



As the area of biopharmaceutics and pharmacokinetics grew in the late 1960's and early 1970's, knowledge and expertise was at last available that allowed deficiencies such as poor bioavailability to be identified in existing drug products and provided the basis for the better design of new products. Thus, the renewed interest in prodrugs was perhaps due to the growth of these disciplines, an increased understanding of metabolic processes in the body, and the perceived need to approach drug therapy and drug design more rationally.

To date, much of the published work on prodrugs has focused upon what might be called "reclamation" projects. That is, the less than ideal behavior of a currently used therapeutic agent was traced to a particular physico-

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(2) (a) T. Higuchi and V. Stella, in "Abstracts of Papers", 168th National Meeting of the American Chemical Society, Atlantic City, N.J., Sept. 10, 1974, American Chemical Society, Washington, D.C., 1974, Abstr MEDI. (b) T. Higuchi and V. Stella, *ACS Symp Ser.*, **No. 14** (1975).

(3) (a) "Design of Biopharmaceutical Properties through Prodrugs and Analogs". A symposium sponsored by the Medicinal Chemistry Section of the American Pharmaceutical Association, Academy of Pharmaceutical Sciences, 21st National Meeting, Orlando, FL, Nov 16–17, 1976. (b) "Design of Biopharmaceutical Properties through Prodrugs and Analogs", E. B. Roche, Ed., American Pharmaceutical Association, Washington, D.C., 1977.

(4) A. Albert, "Selective Toxicity", Chapman and Hall, London, 1951.

(5) N. J. Harper, *J. Med. Pharm. Chem.*, 1, 467 (1959).

(6) N. J. Harper, *Prog. Drug Res.*, 4, 221 (1962).

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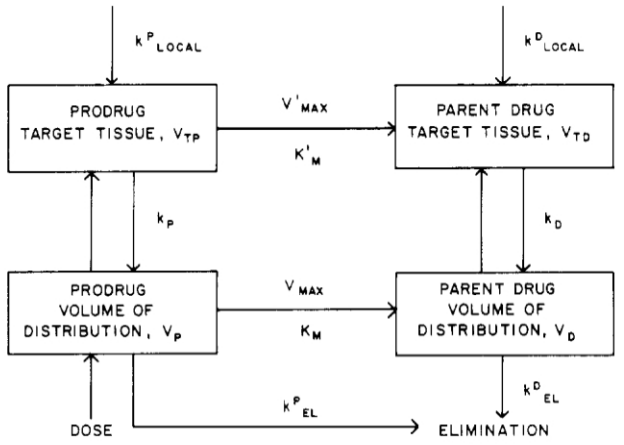
Scheme II



chemical property of that agent. To overcome this limitation, prodrug forms of the agent or other techniques were considered to correct the problem.

Prodrugs Defined on the Basis of Their Problem-Solving Potential. The prodrug approach to problem solving is illustrated in Scheme I. When the parent or active drug is not fully utilized because of some identifiable barrier or problem,[7,8] the physicochemical properties of the drug can be altered by attachment of a pro-moiety. This allows the prodrug to bypass the barrier and, once past the barrier, to revert to the parent compound by a postbarrier enzyme or nonenzymatic process. An alternative to cleavage as a method for obtaining activation is enzyme-mediated synthetic processes such as phosphorylation.

Other literature reviews provide many examples of where prodrugs have been used to solve various problems.[2-16] These reviews should be consulted for a more extensive coverage of the subject. What will be presented in this *perspective* will be some thoughts on one particular direction for future research with prodrugs, i.e., use of prodrugs for site-specific delivery or targeting of receptor-active chemical entities.

Site-Specific Delivery. To achieve truly site-specific delivery, the time profile of drug at the target organ must be optimized, and the burden of drug to other tissues must be minimized. One way to visualize how to use prodrugs for optimizing drug delivery to a particular site would be to develop a hybrid classical/physiologically based pharmacokinetic model in which the various hypothesized optimization prodrug techniques are tested. A simple hypothetical model for a prodrug capable of permeating and releasing drug in a target organ is presented in Scheme II.

The model assumes that the prodrug is introduced into the body as a dose, D, and distributes throughout a volume of distribution, V_P, and into the target organ of volume V_{TP}, with a clearance k_P in mL/min. The prodrug is converted to the parent drug in the target organ or in the rest of the body via a saturable process described by a Michaelis–Menten form defined with K_m and V_{max} values. The prodrug may be cleared via urinary excretion or nonproductive metabolism, k^P_{el}. The parent drug has a volume of distribution, V_D, and a target organ of volume V_{TD} (equal to V_{TP}). The transfer between the target organ and the rest of the body is defined by a clearance term, k_D, while elimination from the body is defined by a clearance term, k^D_{el}. The two input or transport terms, k^P_{local} and k^D_{local}, represent the possibility of direct or local delivery of prodrug or drug to the target site, respectively. To further simplify matters in the initial discussion, the assumptions that the prodrug will be administered systemically ($k^P_{local} = k^D_{local} = 0$ mL/min) and that the prodrug quantitatively regenerates the parent drug ($k^P_{el} = 0$) will be made.

An explanation of the term clearance (k_D, k_P, etc.) is in order. In classical pharmacokinetics, transport in and out of an organ has normally been expressed in terms of forward and reverse first-order rate constants. In this paper, clearance terms have been used instead of forward and reverse rate constants because of their physiological relevance. The rate at which a molecule can be transported to an organ is a function of two terms: the blood flow to the organ and the extraction coefficient of the organ, i.e.,

$$k_P = Q \times E$$

where k_P is the clearance in milliliters/minute, Q is the blood flow to the target organ in milliliters/minute, and E is the extraction coefficient or fraction extracted having the limits of 0 to 1. Poor transport to an organ can come from two sources. First, the physicochemical properties of the drug molecule in question may cause the molecule to be poorly permeable to some rate-limiting membrane, e.g., the blood–brain barrier. If this is the case, then E will be small and k_P might be largely determined by the extractability of the drug. On the other hand, if the drug readily permeates the organ ($E \sim 1$), then blood flow rate may become a limitation. Drug treatment of tumors may provide a good example of this dilemma,[17] because tumors have poor vascularization.[18,19] Thus, simply trying to further increase membrane permeabilities of a drug for which E is approximately unity will have no effect on the ability of the drug to reach the target site, since the rate-determining step is blood flow, not extractability. Therefore, using the model and clearance concepts described above, it is possible to predict the maximum values for k_P for particular organs if blood-flow rate to the organ is known. Lower values than Q might be predicted for k_P if the permeability of a rate-limiting membrane for the organ in question is known.

By setting up mass balance equations for each substance in each tissue it is possible to generate a series of differential equations which can then be solved numerically.[20] A typical mass balance equation for the parent drug in the target tissue is shown in eq 1 and 2, where the various C

(7) E. J. Ariens and A. M. Simonis, in "Pharmacology and Pharmacokinetics", T. Toerell, R. L. Dedrick, and P. G. Condliffe, Eds., Plenum Press, New York, 1974, p 163.
(8) V. J. Stella, T. J. Mikkelson, and J. D. Pipkin, in "Drug Delivery Systems: Characteristics and Biomedical Applications", R. L. Juliano, Ed., Oxford University Press, New York, 1980, Chapter 4.
(9) A. Albert, "Selective Toxicity", 5th ed, Chapman and Hall, London, 1973, pp 21–62.
(10) E. J. Ariens, *Prog. Drug Res.*, **10**, 629 (1966).
(11) E. J. Ariens in "Drug Design", Vol. 2, E. J. Ariens, Ed., Academic Press, New York, 1971, pp 2–127.
(12) G. A. Digenis and J. V. Swintosky, *Handb. Exp. Pharmacol.*, **28**(Part 3), 86 (1975).
(13) A. A. Sinkula and S. H. Yalkowsky, *J. Pharm. Sci.*, **64**, 181 (1975).
(14) A. A. Sinkula, *Annu. Rep. Med. Chem.*, **10**, 306 (1975).
(15) V. J. Stella, *Aust. J. Pharm. Sci.*, **NS2**, 57 (1973).
(16) V. J. Stella in "Formulation and Preparation of Dosage Forms", J. Polderman, Ed., Elsevier/North-Holland, Amsterdam, 1977, pp 91–111.
(17) P. Workman and J. A. Double, *Biomedicine*, **28**, 255 (1978).
(18) L. H. Gray, A. D. Conger, M. Ebert, S. Hornsey, and O. C. A. Scott, *Br. J. Radiol.*, **26**, 638 (1953).
(19) R. H. Thomlinson and L. H. Gray, *Br. J. Cancer*, **9**, 539 (1955).
(20) By use of a modified Hamming's predictor-corrector method.

rate of change of parent drug into the target organ = net rate in (by transport) + rate in (by metabolism of the prodrug) (1)

$$V_{TD}\frac{dC_{TD}}{dt} = k_D(C_D - C_{TD}) + \frac{V_{max}'C_{TP}}{K_m' + C_{TP}} \quad (2)$$

terms represent concentrations of drug as defined by the subscripts. The V_{max}' term in eq 2 has the units of mass of prodrug metabolized per unit time. To convert this to the relative enzymatic activity based on a per unit volume of tissue it must be divided by V_{TP}. Equal enzymatic activity on a per volume basis in the target tissue relative to the rest of the body occurs when $V_{max}'/V_{TP} = V_{max}/V_P$. Values can then be given to each of the parameters in these equations and concentration–time profiles of prodrug and drug in the two tissues generated. By fixing all parameters except for the one to be probed, the effect of parameter change on the time profile of each species can be examined. In the simulations (see Figures 1–5 later), the relative availability of a drug to a particular organ can be determined by the area under the target organ concentration vs. time profile (AUC) of the formed parent drug. Concentration of drug at one particular time point might be deceiving in its interpretation in that it may not be representative of the differences over the entire time course of target organ concentration (see Figure 3 later).

Why many of the prodrug approaches to solving drug site-specific delivery problems have in the past met with limited success can be examined by the use of this model.

Membrane Permeability Alterations and Site-Specific Conversion. Consider the idea that permeability of a membrane is the rate-limiting step to a drug's ability to reach the active site. Creveling et al.[21] and Daly et al.[22] have demonstrated increased permeability of norepinephrine derivatives to the brain (3,4,β-triacetyl and 3,4,β-trimethylsilyl derivatives). These proposed prodrugs enter the brain much more readily than does the polar parent drug, norepinephrine. However, the derivatives survive in the brain largely as noncatechol entities. The norepinephrine prodrugs are able to reach the site, but their inability to convert to the parent drug in the target tissue simply cause the prodrug to drain from the target site (i.e., $k_P \gg k_D$ but $V_{max}'/V_{TP} \lll V_{max}/V_P$). Therefore, using increased permeability as the *only* basis for judging improvement in drug delivery via prodrugs may be an unacceptable or limited criterion for specificity.

An alternative criterion for specificity can be based upon the target organ containing a high level of a particular enzyme which is capable of selectively cleaving the promoiety–drug linkage at that site ($V_{max}'/V_{TP} \gg V_{max}/V_P$). This argument appears promising, but it also suffers from narrow thinking. It has been proposed that the higher concentration of phosphatases and amidases in tumor cells could be used to site specific deliver cytotoxic agents to tumors. In fact, diethylstilbesterol diphosphate has been promoted as a human prostatic tumor-selective agent,[25] as have other phosphate ester derivatives.[26] Again, apart

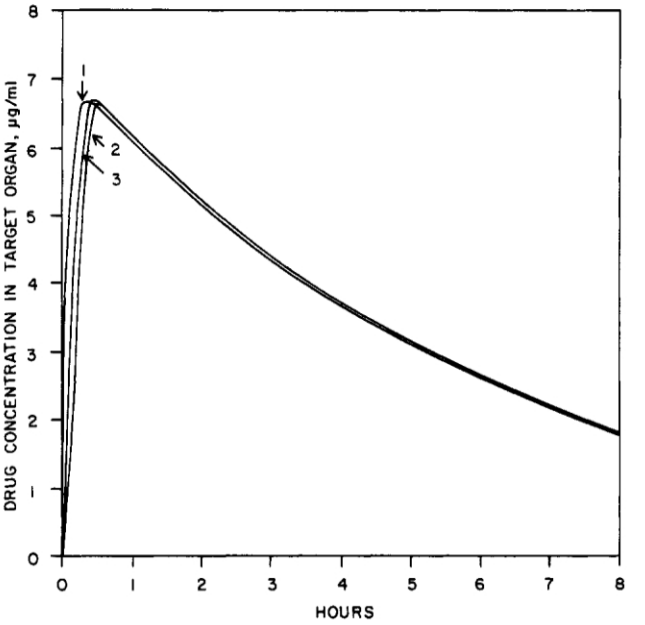
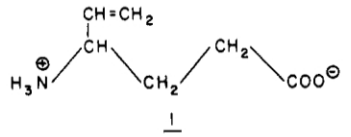


Figure 1. Plots of the effect of varying k_P values, as defined by a hypothetical prodrug model (Scheme II), on drug concentration in the target organ vs. time profile for a drug having a k_D value of 20 mL/min and prodrugs where $V_{max}'/V_{TP} = 10(V_{max}/V_P)$. Line 1 is where drug input is, as the parent drug, placed in volume V_D. Lines 2 and 3 are where drug input is, as prodrugs, placed in volume V_P and having a k_P value of 0.02 and 10 mL/min, respectively.

from other considerations that will be discussed later, these phosphate esters were only partially successful because the prodrug that was designed to convert to the parent compound in a specific tissue was not able to reach that tissue. The ubiquitous distribution of phosphatases in other tissues more highly perfused and accessible to the prodrug, such as bone marrow, small intestines, and liver,[24] are probably able to compete more effectively for the cleavage of the prodrug. Referring to Scheme II, $V_{max}'/V_{TP} \gg V_{max}/V_P$, but $k_D \gg k_P$. This is probably the reason why many attempts using peptidases, glycosidases, sulfatases, and phosphatase enzymes to promote tumor selectivity of cytotoxic agents have failed in the past.[24] Prodrugs attempting to use these enzymes are too polar, the relative enzymatic selectivity is insufficient, and tumor cell perfusion is too poor to achieve the desired goal.

Another example of poor prodrug transport is γ-vinyl-γAbu (1), a γ-aminobutyric acid (γAbu) transaminase k_{cat}



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inhibitor.[25-27] In the case of γ-vinyl-γAbu, its purpose is to selectively inhibit the γAbu transaminase enzyme to raise synaptasomal γAbu levels, which should then lead to anticonvulsant action. Gale and Iadarola[28] have recently shown that intraperitoneal (ip) injection of γ-vinyl-γAbu at 1600 mg/kg to rats does act as an anticonvulsant. However, the high dose needed to elicit the response suggests that very little γ-vinyl-γAbu penetrates the blood–brain barrier. The need for improved delivery of γAbu/glutamate altering agents has been recognized, and

(21) C. R. Creveling, J. W. Daly, T. Tokuyama, and B. Witkop, *Experientia*, **25**, 26 (1969).
(22) J. W. Daly, C. R. Creveling, and B. Witkop, *J. Med. Chem.*, **9**, 273 (1966).
(23) H. Druckrey and S. Raabe, *Klin. Wochenschr.*, **30**, 882 (1952).
(24) For an excellent discussion of this point and drug latentiation in cancer chemotherapy in general, please refer to ref 17 and the references therein.
(25) P. Bey, M. Jung, and B. Metcalf, *Med. Chem., Proc. Int. Symp., 5th, 1976*, 115 (1977).
(26) P. Bey, *Sci. Tech. Pharm.*, **7**, 171 (1978).
(27) M. Jung and B. Metcalf, *Biochem. Biophys. Res. Commun.*, **67**, 301 (1975).
(28) K. Gale and M. J. Iadarola, *Science*, **208**, 288 (1980).

prodrugs of such k_{cat} inhibitors (which themselves can be considered prodrugs[25,26]) and other γAbu/glutamate altering agents having better blood–brain barrier permeability have been proposed.[29]

Illustration of the above cases is possible using the model presented in Scheme II (Figure 1). It is assumed for all of the simulations (Figures 1–5) that $V_{TP} = V_{TD} = 100$ mL, $V_P = V_D = 14000$ mL, dose $= D = 100$ mg, $V_{max} = 10$ mg/min, $K_m = 1.2$ μg/mL, and $k^D_{el} = 40$ mL/min. For this simulation, k_D is fixed at 20 mL/min, which represents a clearance value similar to that of a small tumor where the drug readily permeates the tissue. These conditions are then used to input the parent drug (equivalent to an iv dose of the parent drug into V_D) to generate a target organ drug concentration as a function of time. This profile is then used as a base line for comparison with the profile generated from the input of a prodrug. The conditions $K_m' = K_m$, $k_P = 0.02$, 10 mL/min and $V_{max}' = 0.714$ mg/min (giving a specific activity on a per volume basis ten times higher in the target organ than in the rest of the body, i.e., $V_{max}'/V_{TP} = 10\ V_{max}/V_P$), for the prodrug describes a prodrug that is rapidly metabolized by the target organ but has lower permeability to the target organ than the parent drug. Figure 1 illustrates that the superior target organ metabolism cannot compensate for the decreased availability to the site. In fact, input of the parent drug (line 1) gives more rapid drug input to the target organ than did the prodrug (lines 2 and 3).

To summarize the discussion so far, altered permeability and selective enzymatic cleavage of prodrugs, although important in achieving targeting, cannot be treated as mutually independent factors. As will be demonstrated later, it is possible to trade one factor against another. However the degree of success of such a trade off depends upon another consideration which has not, until now, been fully recognized or discussed.

The Properties of the Parent Compound. If the parent drug molecule reasonably permeates the target organ (note: specificity is not implied by this statement, it simply states that the time profile of drug in the target organ approximately mimics the plasma level time profile, $k_D \geq k^D_{el}$), increased relative permeability by the prodrug and its specific conversion in the target tissue may do little to promote specificity. Figures 2 and 3 are simulations of two drugs where V_{TP}, V_{TD}, V_P, V_D, k^D_{el}, V_{max}, and K_m are the same as in Figure 1. In Figure 2, $k_D = 200$ mL/min, $V_{max}'/V_{TP} = 10(V_{max}/V_P)$, and $k_P = k_D$; in Figure 3, $k_D =$ 20 mL/min, $V_{max}'/V_{TP} = 10(V_{max}/V_P)$, and $k_P = k_D$ and $k_P = 10k_D$. The values for k_D of 200 and 20 mL/min represent cases of very rapid and moderate accessibility and retention of the parent drug to the target tissue, respectively.

With the k_D values of 200 mL/min, no real advantage via prodrug input is seen even with good permeability by the prodrug and a tenfold selectivity in target site conversion vs. body conversion. In fact, drug input via the prodrug delays the appearance of parent drug in the target tissue. With the k_D value of 20 mL/min (Figure 3) some increase in the early drug concentration time points is seen in the target organ, but the overall advantage of prodrug vs. parent drug input is minor. The only real advantage seen is that parent drug input via prodrug delivery provides an alternative target organ input mode for the parent drug. The lack of specificity illustrated in Figures 2 and 3 is due to rapid "leakage" of the parent drug from the target organ, even though a large fraction of the parent drug is formed from the prodrug by metabolism specifically in the target organ.

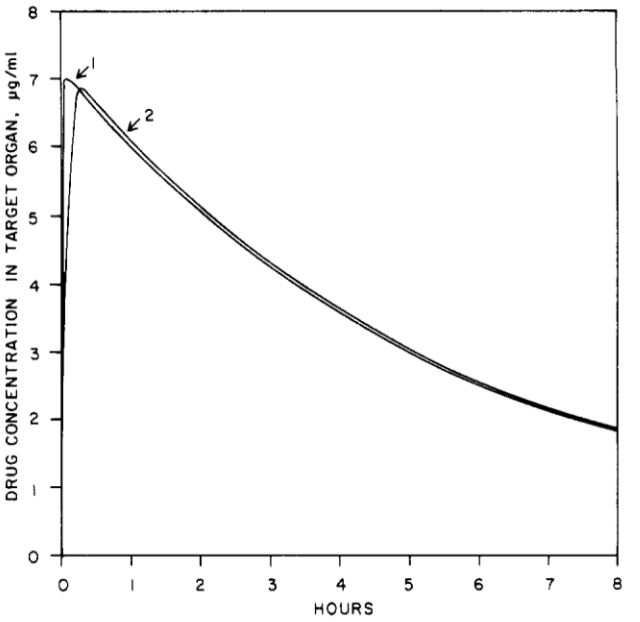


Figure 2. Plots of the effect of varying k_P values, as defined by a hypothetical prodrug model (Scheme II), on drug concentration in the target organ vs. time profile for a drug having a k_D value of 200 mL/min and prodrugs where $V_{max}'/V_{TP} = 10(V_{max}/V_P)$. Line 1 is where drug input is, as the parent drug, placed in volume V_D and line 2 is where drug input is, as a prodrug, placed in volume V_P and having a k_P value of 200 mL/min.

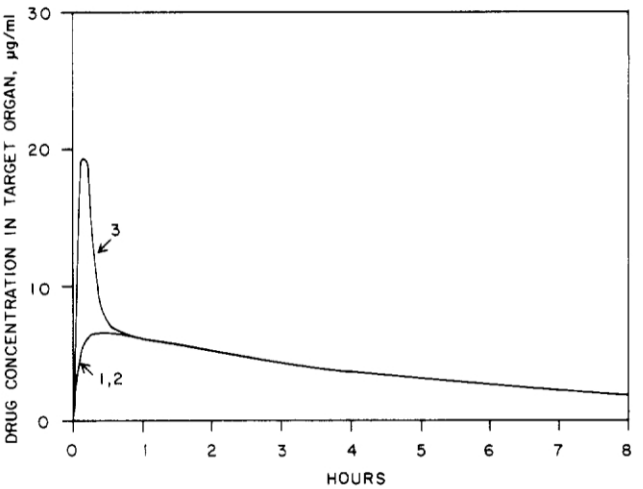
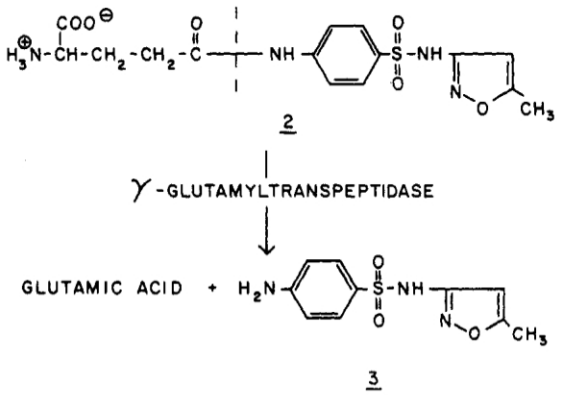


Figure 3. Plots of the effect of varying k_P values, as defined by a hypothetical prodrug model (Scheme II), on drug concentration in the target organ vs. time profile for a drug having a kD value of 20 mL/min and prodrugs where $V_{max}'/V_{TP} = 10(V_{max}/V_P)$. Line 1 is where drug input is, as the parent drug, placed in volume V_D. Lines 2 and 3 are where drug input is, as prodrugs, placed in volume V_P and having k_P values of 20 and 200 mL/min, respectively.

One example of the problem of rapid "leakage" of the formed drug from the target tissue may be γ-glutamylsulfamethazole (**2**), a proposed kidney selection prodrug of sulfamethazole (**3**).[30] This attempted delivery of sulfamethazole is based on earlier studies showing the relatively high kidney activity of the γ-glutamyltranspeptidase

(29) L. Brehm, P. Krogsgaard-Larsen, and P. Jacobsen, *Alfred Benzoin Symp.*, **12**, 247 (1979).

(30) M. Orlowski, H. Mizoguchi, and S. Wilk, *J. Pharmacol. Exp. Ther.*, **212**, 167 (1980).



enzyme. Wilk et al. had previously used this kidney selectivity to deliver L-Dopa (L-3,4-dihydroxyphenylalanine) and, subsequently, dopamine as γ-glutamyl-L-Dopa to the kidney.[31] The high kidney γ-glutamyltranspeptidase activity relative to activity in other tissues serves as the basis for the potential selective renal delivery of sulfamethazole via γ-glutamylsulfamethazole.

The relative sulfamethazole concentrations in mouse kidneys and other tissues 20 min after ip injection of equimolar doses of sulfamethazole and γ-glutamylsulfamethazole were measured by Orlowski et al.[30] Their data suggest that sulfamethazole concentration in tissues other than the kidney and pancreas are slightly diminished, but no great selectivity is seen in the kidney and pancreas. Other derivatives such as *N*-acetyl-γ-glutamylsulfamethazole and *N*-(chloroacetyl)-γ-glutamylsulfamethazole are more encouraging not because they give high levels of sulfamethazole in the kidney but because they do appear to give significantly diminished levels of sulfamethazole in other tissues.[30] Orlowski et al.[30] have addressed some of the possible explanations for the poor behavior of γ-glutamylsulfamethazole. However, another possible explanation not addressed by those authors is that the rate of cleavage of γ-glutamylsulfamethazole to sulfamethazole at the specific site of cleavage allows the sulfamethazole to "leak" from the metabolism site. This leakage and redistribution may occur because sulfamethazole is a relatively nonpolar uncharged species at physiological pH. The other derivatives may cleave at sites where trapping of the sulfamethazole is possible.

A leakage theory gains credibility and validity when the behavior of γ-glutamylsulfamethazole is compared to that of γ-glutamyldopamine (**4**) and γ-glutamyl-L-Dopa (**5**). Wilk et al.[31] and others[32-36] have shown that the γ-glutamyltranspeptidase activity in the kidney can be used to selectively deliver dopamine (**6**) as γ-glutamyldopamine (**4**) or γ-glutamyl-L-Dopa (**5**). The γ-glutamyl-L-Dopa releases L-Dopa (**7**), which then decarboxylates to dopamine.

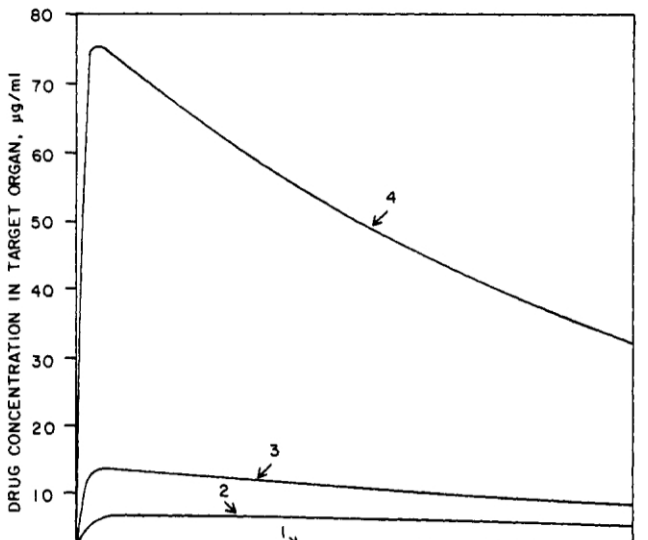
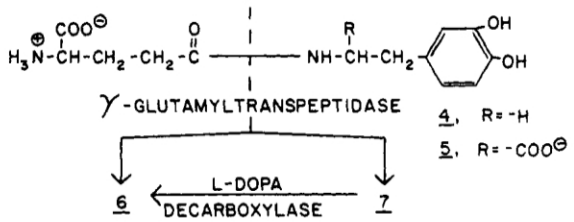


Figure 4. Plots of the effect of varying k_P values, as defined by a hypothetical prodrug model (Scheme II), on drug concentration in the target organ vs. time profile for a drug having a k_D value of 0.2 mL/min and prodrugs where $V_{max}'/V_{TP} = 100(V_{max}/V_P)$. Line 1 is where drug input is, as the parent drug, placed in volume V_D. Lines 2, 3, and 4 are where drug input is, as prodrugs, placed in volume V_P and having k_P values of 0.2, 20, and 200 mL/min, respectively.



Both γ-glutamyldopamine and γ-gultamyl-L-Dopa are as polar as γ-glutamylsulfamethazole, but the released parent drugs, dopamine and L-Dopa, are very polar and charged at physiological pH. Both γ-glutamyldopamine and γ-glutamyl-L-Dopa have been found to be superior kidney delivery forms of dopamine relative to dopamine and L-Dopa themselves, as measured by kidney and other tissue level time profiles and pharmacological activity measurements.[31-36]

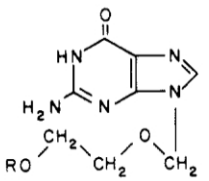
Figure 4 presents a simulation of the importance of parent drug retention. The parameters V_{TP}, V_{TD}, V_P, V_D, k^D_{el}, V_{max}, and K_m are the same as in Figure 1. The transport constant k_D has been given a low value of 0.2 mL/min to represent a drug that has poor transport characteristics into and out of the target organ. This characteristic has been combined with selective cleavage of the prodrug in the target organ [$V_{max}'/V_{TP} = 100(V_{max}/V_P)$] and varying degrees of transport of the prodrug to the target organ (k_P = 0.2 to 200 mL/min). Note that k_P values greater than k_D values can only occur when the limitation is organ extractability, not blood perfusion. If the low value of k_D actually represents a blood-flow limitation (E for the parent drug is approximately unity), then k_P cannot have values greater than k_D. As seen in Figure 4, a substantial improvement in selectivity can be achieved

(31) S. Wilk, H. Mizoguchi, and M. Orlowski, *J. Pharmacol. Exp. Ther.*, **206**, 227 (1978).
(32) J. J. Kyncl, F. N. Minard, and P. H. Jones, *Adv. Biosci.*, **20**, 369 (1979).
(33) J. Kyncl, R. Hollinger, R. Warner, C. W. Ours, F. N. Minard, P. H. Jones, and J. H. Biel, *Kidney Int.*, **10**, 589 (1976).
(34) J. Kyncl, R. Hollinger, C. W. Ours, F. N. Minard, P. H. Jones, and J. H. Biel, in "Abstracts of Papers", 172nd National Meeting of the American Chemical Society, San Francisco, Calif., 1976, American Chemical Society, Washington, D.C., 1976, Abstr MEDI 19.
(35) P. H. Jones, C. W. Ours, J. H. Biel, F. N. Minard, J. Kyncl, and Y. C. Martin, in "Abstracts of Papers", 172nd National Meeting of the American Chemical Society, San Francisco, Calif., 1976, American Chemical Society, Washington, D.C., 1976, Abstr MEDI 17.
(36) F. N. Minard, J. C. Cain, D. S. Grant, C. W. Ours, J. Kyncl, P. H. Jones, and J. H. Biel, in "Abstracts of Papers", 172nd National Meeting of the American Chemical Society, San Francisco, Calif., 1976, American Chemical Society, Washington, D.C., 1976, Abstr MEDI 18.

when all the conditions of transport, selective cleavage, and retention are operative. It should be noted that, while the enzymatic specificity used here is high, similar results are achieved with lower values of V_{max}'.

L-Dopa itself is an example of a prodrug of dopamine. L-Dopa is able to deliver dopamine to the brain, because it is transported to the brain via the active-transport mechanism for L-amino acids.[37] Once in the brain, it is subsequently decarboxylated to dopamine. As a prodrug of dopamine, L-Dopa is not without problems. Peripheral decarboxylation of L-Dopa to dopamine leads to various side effects that are directly attributed to peripheral dopamine and its further metabolites. Selectivity for brain delivery is partially achieved by use of the peripheral L-Dopa decarboxylase inhibitors. That is, the combination of L-Dopa and a peripheral Dopa decarboxylase inhibitor help build selectivity into the delivery of dopamine to the brain.

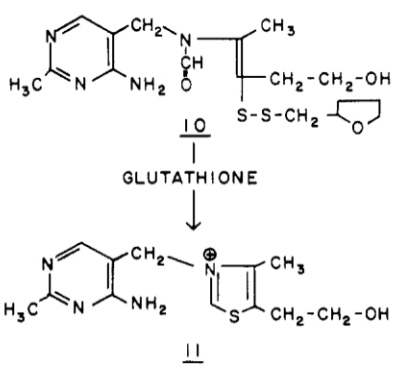
Another possible example of the importance of site permeability and retention is the antiviral agent acyclovir (8), which is selectively activated to its phosphate deriv-



8, R = -H
9, R = $-PO_3^=$

ative (9) in viruses. Acyclovir is an analogue of a vital nucleotide precursor; it is not phosphorylated by mammalian cells but is phosphorylated by the viral enzyme. The active agent 9 is then incorporated into viral DNA, disrupting the virus's replication cycle.[38] The selectivity in this case may come not only from the activation process (phosphorylation of 8 → 9 in viruses) but also from 8 being able to penetrate the virus and from 9 probably being retained by the virus. If 9 is partially released from the virus, it probably would have difficulty being taken up by mammalian cells because of its high polarity. To date, 8 has shown low toxicity in man in phase I studies, and its possible use in the treatment of herpes infections can be a major breakthrough.[39]

An additional example of a site-specific delivery stressing the importance of the physicochemical properties of the parent drug is thiamine tetrahydrofurfuryl disulfide or TTFD (10), a lipid-soluble prodrug of thiamine (11).[8,16,40]



10 → 11

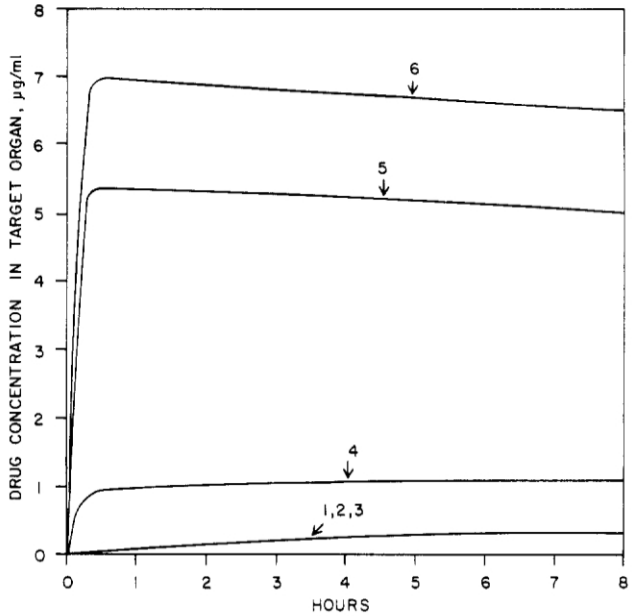


Figure 5. Plots of the effect of varying k_P values, as defined by a hypothetical prodrug model (Scheme II), on drug concentration in the target organ vs. time profile for a drug having a k_D value of 0.02 mL/min and prodrugs where $V_{max}'/V_{TP} = V_{max}/V_P$. Line 1 is where drug input is, as the parent drug, placed in volume V_P. Lines 2, 3, 4, 5, and 6 are where drug input is, as prodrugs, placed in volume V_P and having k_P values of 0.02, 0.2, 2, 20, and 200 mL/min, respectively.

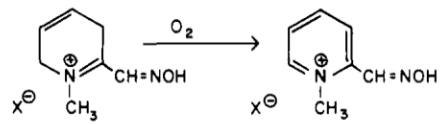
After intravenous (iv) administration of thiamine to rats, thiamine is rapidly cleared primarily via urinary excretion (90% dose) from whole blood with a half-life of 35 min. The thiamine in this case is the plasma fraction of the whole blood. After iv administration of TTFD, whole-blood thiamine half-life is 200 min (all the thiamine is in the red blood cell component of the whole blood) and 76% of the administered dose can be accounted for in the red blood cell fraction within minutes after administration of the TTFD.[40] The long half-life of thiamine from whole blood results from TTFD rapidly and passively permeating the red blood cell membranes and reacting instantaneously with red blood glutathione thus releasing thiamine. The release of the trapped thiamine from the red blood cells is slow at these levels,[40,41] and the long whole-blood half-life actually represents the slow passive permeation of the quaternary thiamine through the red blood cell membrane.[40] Although the initial goal had not been the delivery of thiamine to the red blood cells, this work did show that a considerable fraction of a drug can be delivered to an individual tissue if the right conditions are met.

The cases discussed above all assume enzymatic specificity of the target organ over the rest of the body. However, improved drug delivery may be possible to a specific site even without specificity of the enzymatic process when the parent drug in question has difficulty reaching the desired site. Figure 5 illustrates this case. Again, V_{TP}, V_{TD}, V_P, V_D, k_{el}, V_{max}, and k_m are the same as in Figure 1. The value for k_D is fixed at 0.02 mL/min, and the available enzymatic activity per unit volume in the target tissue is equivalent to the average activity per unit volume in the rest of the body. Assuming these fixed values, k_P is then varied. As can be seen in this figure, delivery of the drug improves significantly to the target

(37) H. Shindo, T. Komai, and K. Kawai, *Chem. Pharm. Bull.*, **25**, 1417 (1977).
(38) J. A. Fyfe, P. M. Keller, P. A. Furman, R. L. Miller, and G. B. Elion, *J. Biol. Chem.*, **253**, 8721 (1978).
(39) G. B. Elion, *Chem. Eng. News*, **58**(15), 24 (1980).
(40) J. D. Pipkin and V. J. Stella, unpublished results.
(41) T. Komai and H. Shindo, *J. Nutr. Sci. Vitaminol.*, **20**, 189 (1974).

tissue even though enzymatic selectivity is not present if k_P is large enough and parent drug retention is possible.

There are some interesting examples of such cases in the literature. Bodor et al.[42,43] and Shek et al.[44,45] have shown that the dihydro derivative (enamine salt, *N*-methyl-3,6-dihydropyridine 2-carbaldoxime hydrochloride, Pro-2-PAM, **12**) of the quaternary ammonium compound *N*-

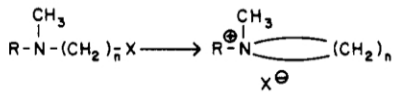


12 13

methylpyridinium 2-aldoxime chloride (2-PAM chloride, **13**) rapidly oxidizes to 2-PAM in vivo. When administered iv, the prodrug shows a 13-fold increase in the amount of 2-PAM delivered to mice brains when compared to 2-PAM.

In the case of Pro-2-PAM, the delivery of 2-PAM is successful not only because the prodrug passes through the blood–brain barrier (the enamine exists as a neutral species at physiological pH), but also because the brain tissue is capable of converting Pro-2-PAM to 2-PAM. Brain conversion is at least competitive with the nonbrain conversion in order for substantial levels of 2-PAM to appear in the brain before the prodrug leaks out of the brain.

Quaternary compounds, because of their polarity, do not readily penetrate nonpolar membranes (see previous examples of thiamine and 2-PAM). In studies similar to those of Bodor et al., Ross et al.[46-50] and others[51,52] have investigated the use of haloalkylamines (**14**) as prodrugs



14 15

of various quaternary compounds (**15**). By manipulating the number of methylene groups, *n*, and the halide group, X, various quantities of **15** via **14** are delivered into nerve and brain tissue that would otherwise not have been accessible if delivered as **15**. The conversion of **14** to **15** takes place via a facile intramolecular reaction and was not enzyme mediated. Another non-enzyme-mediated selective delivery example is methenamine delivery of formaldehyde. Acidification of the urine of patients taking methenamine promotes decomposition of the methenamine to the nonspecific antibacterial agent formaldehyde in the urine.[53] Therefore, methenamine can be used successfully as a prodrug for site-specific delivery of its active agent by utilizing the pH differences between urine (acidic pH) and other body tissues (physiological pH of 7.4).

Other Considerations. Scheme II is an oversimplification of what really occurs in an intact animal. In the simulations presented here, similar volumes of tissues and target organs have been assumed and the effect of V_D and V_P changes have not been considered. Also not specifically considered so far is binding of drug or prodrug in tissues and target organ, or selectivity in uptake using a carrier mechanism (except for L-Dopa). Some preliminary conclusions of the effects of some of these variables upon the time profiles of drug concentration within target organs are presented below.

Changes in the volume of distribution of the prodrug, V_P, relative to V_D can have beneficial or deleterious effects. When V_P is much smaller than V_D, greater driving force for prodrug transport to the target organ occurs, whereas greater V_P dilutes the prodrug, decreases transport to the target organ, and promotes conversion in the body relative to the target organ.

Specificity can be obtained when a prodrug can utilize a specific active-transport process to the target organ, providing enzymatic processes are then available to convert the prodrug to the parent drug in the target organ and leakage of the parent drug is slow.

If the body's ability to utilize a drug depends on a slow input rate of the drug into the body, prodrugs may be used to obtain a depot or prolonged release effect. This can be done by controlling the prodrug release from its depot, altering the prodrug distribution characteristics, or by manipulating the metabolic conversion rate of prodrug to parent drug.[54,55] Specificity may be effected in this case by a more efficient parent drug uptake into the target tissue.

One should not be overoptimistic in cases where a prodrug achieves high target organ concentration due to protein binding, since it is likely that only the free fraction of prodrug will be available for conversion. High prodrug delivery may be possible, but parent drug delivery will be defined by many other variables. Also, except in the case of specific tissue antibodies, prediction of tissue binding characteristics of a chemical entity based upon this chemical's structure is still an evolving science.

Another note of caution is in order. Even though a prodrug may exhibit excellent physicochemical properties for the delivery of parent drug to a tissue, it may also exhibit improved transport to another tissue, thus increasing the incidence of side effects because of the selectivity for the other tissue. An example of this might be bone-marrow toxicity of a prodrug designed for tumor delivery.

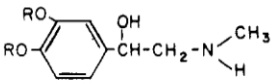
The hypothetical model shown in Scheme II has another limitation. It has been assumed that the target tissue represents a homogeneous system. In fact, models such as those presented in Scheme II may be used to represent compartments within the target organ. For example, just because a particular prodrug may deliver a drug to the brain does not mean that the drug reaches that site within the brain where it can exert its activity. Similarly, this model can also represent at the cellular level the organelles within a cell. Thus, the model presented in Scheme II is

(42) N. Bodor, E. Shek, and T. Higuchi, *Science*, **190**, 155 (1975).
(43) N. Bodor, E. Shek, and T. Higuchi, *J. Med. Chem.*, **19**, 102 (1976).
(44) E. Shek, T. Higuchi, and N. Bodor, *J. Med. Chem.*, **19**, 108 (1976).
(45) E. Shek, T. Higuchi, and N. Bodor, *J. Med. Chem.*, **19**, 113 (1976).
(46) S. B. Ross, R. Sandberg, B. A. Akerman, K. E. Domeii, G. Stening, and S. Svensson, *J. Med. Chem.*, **16**, 787 (1973).
(47) S. B. Ross and O. Froden, *Eur. J. Pharmacol.*, **13**, 46 (1970).
(48) S. B. Ross, J. G. Johansson, B. Lindborg, and R. Dahlbom, *Acta Pharm. Suec.*, **10**, 29 (1973).
(49) S. B. Ross and S. B. A. Akerman, *J. Pharmacol. Exp. Ther.*, **182**, 351 (1972).
(50) S. B. Ross, *J. Pharm. Pharmacol.*, **27**, 322 (1975).
(51) J. G. Johansson, B. Lindborg, R. Dahlbom, S. B. Ross, and S. B. A. Akerman, *Acta Pharm. Suec.*, **10**, 199 (1973).
(52) B. Lindborg, J. G. Johansson, R. Dahlbom, and S. B. Ross, *Acta Pharm. Suec.* 11, 401 (1974).
(53) R. E. Notari, *J. Pharm. Sci.*, **62**, 865 (1973).
(54) P. R. Byron, R. E. Notari, and M.-Y. Huang, *Int. J. Pharm.*, 1, 219 (1978).
(55) R. E. Notari, M.-Y. Huang, and P. R. Byron, *Int. J. Pharm.*, 1, 233 (1978).

recognized as a gross, but useful, oversimplification of what occurs in the intact animal.

Site-Specific Delivery Using Local Administration Routes. Prodrug concepts using local drug-delivery routes specifically to the skin and eye have been reviewed by Stella et al.[8] Qualitatively, the arguments for improved delivery to local tissues, such as the skin or the eye (via corneal drug administration), with prodrugs are very similar to those just discussed for systemic drug delivery.

Dipivaloylepinephrine (**16**) as a prodrug of epinephrine



16, R = -COC(CH$_3$)$_3$

17, R = -H

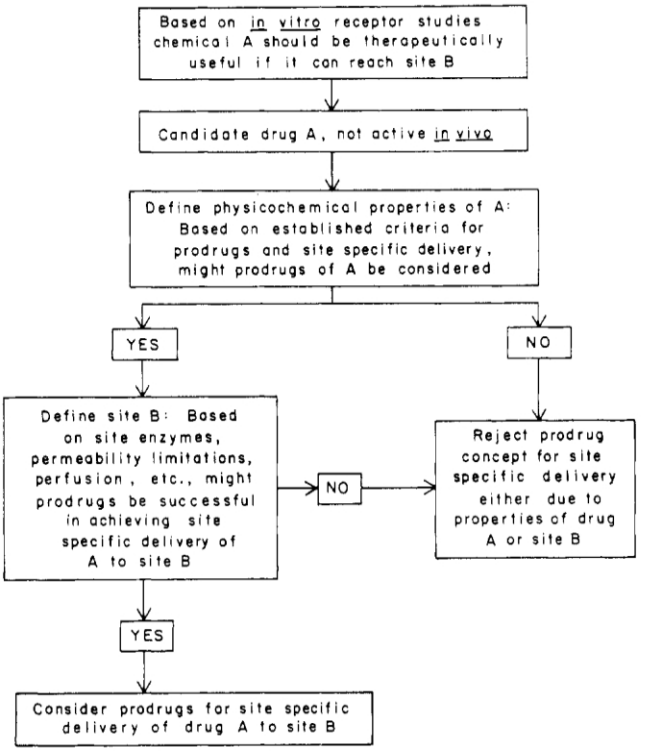
(**17**) has proven to be successful as an antiglaucoma agent[56] not only because it is better able to penetrate the cornea than epinephrine but also because the cornea and aqueous humor have significant esterase activity capable of releasing epinephrine.[57]

Improved dermal delivery of drugs, such as steroids as prodrugs, and antiviral agents, such as *ara*-A as its 5′-valerate ester,[58,59] and the modeling of these systems as examples of diffusion with simultaneous chemical reactions is an area of great interest.[58-60] It is beyond the scope of this *perspective* to go into the details of these studies. However, this is another area in which great strides in optimization of drug delivery via prodrugs and analogues are expected in the near future.

The Drug Design Stage: The Point for Critical Consideration of Prodrugs. Many examples of prodrugs actually represent "reclamation" projects. That is, when a therapeutic agent is less than ideal, attempts are made to improve it via prodrugs. This perspective has pointed out that true site-specific delivery via prodrugs is only possible when the active drug can be somewhat retained by the target organ or site. After making this observation in the simulations, it has been concluded that this is one major reason why many prior attempts at site-specific delivery or targeting via prodrugs proved unsuccessful. By the very fact that most currently used therapeutic agents have to be able to reach their site of action to be therapeutically effective means that the chance of success in promoting their specificity further via prodrugs is diminished.

The observation that parent drug site retention is an important parameter suggests that prodrugs can be used in conjunction with basic drug design concepts to promote targeting. Consider the example of γ-vinyl-γAbu (**1**) discussed earlier. Using a biochemical basis, this is one of many agents designed specifically to affect an enzyme receptor, the γAbu transaminase system. Its in vitro activity suggests that it has possible use as an anticonvulsant.

Scheme III



It has limited in vivo activity probably because those properties that made it a good in vitro candidate limit its in vivo activity. Here is where a prodrug approach, along with analogue development, and other techniques[61,62] might be used to overcome this problem.

Scheme III is an attempt at a flow chart outlining steps to be taken when considering whether prodrugs could be useful for delivery of a chemical A to a site B. It suggests that consideration of prodrugs should occur at the drug design stage of development, since prodrugs are one effective tool for getting receptor-active agents to their site of action with some degree of specificity.

Conclusions

Hopefully this perspective gives a realistic appreciation of the possible utilization of prodrugs to achieve site-specific delivery or targeting of drug molecules. Consideration of prodrugs and other techniques at the drug design level of development should, in the future, mean that fewer reclamation projects will be necessary and the prodrugs, as well as other optimization techniques, will become an integral part of basic drug design. To be successful, prodrug design requires a multidisciplinary approach that draws upon the expertise of biochemists, pharmacologists, toxicologists, synthetic organic and medicinal chemists, pharmaceutical chemists, as well as adequate feedback from clinicians. Hopefully, this perspective will stimulate discussion of its shortcomings, as well as its assumptions, thereby encouraging appropriate research in the targeting of drugs via prodrugs.

(56) D. A. McClure, *ACS Symp. Ser.*, **no. 14**, pp 224–235 (1975).
(57) A. I. Mandel, F. Stentz, and A. E. Kitabchi, *Ophthalmology (Rochester, Minn.)* **85**, 268 (1978).
(58) C. D. Yu, J. L. Fox, N. F. H. Ho, and W. I. Higuchi, *J. Pharm. Sci.*, 68, 1341 (1979).
(59) C. D. Yu, J. L. Fox, N. F. H. Ho, and W. I. Higuchi, *J. Pharm. Sci.*, 68, 1347 (1979).
(60) J. L. Fox, C.-D. Yu, W. I. Higuchi, and N. F. H. Ho, *Int. J. Pharmacol.*, **2**, 41 (1979).
(61) G. Gregoriadis, Ed., "Drug Carriers in Biology and Medicine", Academic Press, New York, 1979.
(62) R. L. Juliano, Ed., "Drug Delivery Systems: Characteristics and Biomedical Applications", Oxford University Press, New York, 1980.