not by the second, there is insufficient data to say which is correct. In either case, the peptide bond is broken via acid catalysis by Tyr 248. If an acyl enzyme were formed, the subsequent attack by H_2O would be aided by the proximity of the Tyr 248 conjugate base.

5.4.2. *Leucine aminopeptidase*

Although it is thought of as the 'classic' N-terminal exopeptidase, there is relatively little information available on leucine aminopeptidase (LAP). This is due partly to difficulty in identifying aminopeptidase obtained from different sources and partly to its size. It was originally isolated from porcine kidney [90], but many studies have been done on samples extracted from bovine eye lens [91, 92].

The molecular weight of the enzyme is around 3×10^5 g/mole. The porcine kidney sample is thought to consist of four subunits [93], whereas the bovine eye lens sample consists of 10 subunits [94]. Low-angle X-ray diffraction studies done on the eye lens sample indicate that the enzyme is shaped like a hollow cylinder with inside diameter of about 32 Å [95]. No efforts to investigate the detailed tertiary structure have been made and, since efforts to determine end groups have been unsuccessful, the primary and secondary structures are completely unknown. However, Himmelhoch [96] has found that the enzyme contains approximately one atom of Zn per subunit.

Unlike CPA, the tertiary structure of LAP is stabilized by the Zn ions: once the Zn has been removed, catalytic activity is lost permanently. However, the Zn can be replaced with other ions [97]. LAP is strongly activated by Mn^{2+} and Mg^{2+}, inhibited by Cd^{2+}, Cu^{2+}, Hg^{2+} and Pb^{2+}, and unaffected by Ca^{2+}, Co^{2+} and Ni^{2+}. The Zn (or other metal ion) has been shown to play an essential role in the catalytic process but to be unessential for substrate binding [98].

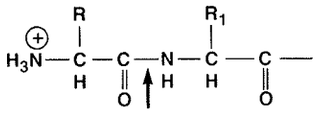


Fig. 12. LAP catalyzes the hydrolysis of the indicated peptide bond.

The primary action of LAP is indicated in Figure 12. It can also function as an esterase but at about 10% of the rate for the analogous peptide [97]. There are two absolute requirements for potential LAP substrates [97]: the N-terminal residue must be of the L configuration and the terminal $-NH_2$ groups must be free (i.e., not acylated). In addition to peptides and esters, amides have long been known to be good substrates for LAP (e.g., L-leucine-amide [99]). Almost any N-terminal L-amino acid (or glycine) will be released at a measurable rate, although substrates with a hydrophobic R-group are best. Generally, substrates wherein the N-terminal residue is Leu, norLeu or norVal are hydrolyzed the fastest, although L-Ala-L-leucinamide is the best substrate known. Compounds having an N-terminal D-amino acid residue are

not hydrolyzed at measureable rates, and having a D-amino acid residue in the penultimate position greatly reduces the reaction rate. Also, compounds of the types Pro-X or X-Pro (Pro = proline) are very poor substrates. Compounds which act as inhibitors fall into three categories: those which interact with the metal ion (e.g., EDTA, citrate), those which are hydrophobic and compete for the binding site (e.g., large aliphatics, alkanols), and those which do both of the above (e.g., L-leucine, L-leucinol).

Kinetic studies on LAP have been reviewed by Smith and Hill [97] and are not particularly illuminating. Some substrates are hydrolyzed according to first-order kinetics, while others follow zero-order kinetics. The enzyme is maximally active between pH 9.0 and 9.5, depending on the substrate and on the metal ion. In general, V_{max} for the Mn^{2+}-activated enzyme is greater than that for the Mg^{2+}-activated enzyme, although the values of K_m are comparable [100].

5.4.3. *Prodrug considerations*

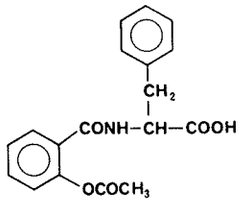
Since carboxy and aminopeptidase are digestive enzymes, prodrugs utilizing these enzymes as reconversion sites will be restricted to oral dosage forms. Carboxypeptidase can be used for the reconversion of prodrugs having a free carboxyl group. Amino acid derivatives of aspirin in which the carboxyl group phenylalamine and phenyllactic acid ethyl esters of aspirin were targeted for sequential reconversion by α-chymotrypsin and carboxypeptidase have been reported [2 – 4]. Choice of these esters led to high shelf life for aspirin and a reasonable enzymatic rate of regeneration in vitro by the combined effect of α-chymotrypsin and carboxypeptidase A. Table 10 shows the

TABLE 10
Kinetic Parameters for Carboxypeptidase Hydrolysis at pH 7.5

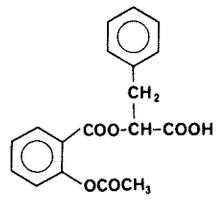
Substrate	K_m (mole/liter)	k_{cat} (sec^{-1})	K_i (mole/liter)	$\frac{k_{cat}}{K_m}$ (moles/second/liter)
Cinnamoyl phenylalanine[a]	6×10^{-4}	2.1×10^{-2}	–	35
Indoleacryloyl-phenylalanine[a]	5.84×10^{-4}	1.4×10^{-3}	–	2.39
Aspirin phenylalanine[b] (I)	1.84×10^{-4}	2.8×10^{-3}	–	15.2
Cinnamoyl phenyllactic acid	1.87×10^{-4}	67	5.78×10^{-5}	3.58×10^5
Furylacryloyl phenyllactic acid	1.32×10^{-4}	47	–	3.56×10^5
Aspirin phenyllactic acid (II)	1×10^{-4}	25	4×10^{-6}	2.5×10^5

a. Taken from Ref. 3.
b. Performed at pH 8.5.

kinetic parameters for carboxypeptidase A hydrolysis of aspirin phenylalamine (I) and aspirin phenyllactic acid (II).



I



II

Isolation and identification of carboxy and aminopeptidase from enterocyte brush borders and cytosol have shown different though somewhat overlapping specificities. It appears that these exopeptidases function at the surface or interior of the absorptive cell to further reduce small oligopeptides released by pancreatic protease action. Their radial distributions appear to be geared to couple small peptide and amino acid intestinal transport. Tetrapeptide activity is associated exclusively with the brush border membrane, tripeptide activity is distributed about equally between membrane and cytosol, while enzymes that cleave dipeptides are more prevalent in the cytosol than brush border [101]. Axial distributions appear to be fairly even over the entire small intestinal length and less subject to species, age, and nutritional input parameters than is alkaline phosphatase. However, this may be attributed to the fact that isoenzymes and specific peptidases have not been well characterized with respect to their association with particular axial regions [102]. Intestinal absorption of L-lysine-*p*-nitroanilide, L-alanine-*p*-nitroanilide and glycine-*p*-nitroanilide was studied in the presence of competitive inhibitors in perfused rat intestine in order to establish the coupled brush border hydrolysis and transport [103]. This study has shown that the peptidase in the brush border region that serves as the hydrolysis site requires a free α-amino group (an aminopeptidase).

6. Alkaline phosphatases

The mammalian alkaline phosphatases are distributed among various tissues of the body. Examples of tissue exhibiting alkaline phosphatase activity are: the intestinal mucosa, placenta, kidney, bone, liver, lung and spleen [104]. Alkaline phosphatases may not be as readily useful as targeted reconversion site for prodrugs as the hydrolytic enzymes of the GI tract. Fishman [105] has investigated the organ-specific behavior of rat alkaline phosphatases toward a variety of compounds.

A typical alkaline phosphatase (e.g., human placental alkaline phosphatase) has a molecular weight around 125,000 g/mole and contains approximately 3 moles of

Zn^{2+} per mole of enzyme [106]. Limited amino acid sequencing data around the active site has been reported [107] and the sequence ASP-'active Ser'-Ala has been observed for the active site.

Alkaline phosphatases catalyze the hydrolysis of most phosphate esters. Harkness [108] has shown that human placental phosphatase hydrolyzes a variety of substrates of comparable rates. Inorganic phosphate [110] and arsenate [108, 109] anions have been found to be potent competitive inhibitors. Cysteine and histidine have been found to act as non-competitive inhibitors by chelating the Zn^{2+} ion essential for catalytic activity [111]. L-Phenylalanine has been shown to be an uncompetitive inhibitor [112, 113]. Kinetic studies have indicated that (1) as the substrate concentration is increased, the optimum pH increases from about pH 8 to about pH 10, and (2) substrate inhibition is significant. The data [114] in Figure 13 illustrate both these observations.

Alkaline phosphatase activity is not distributed evenly in the axial direction of the vertebrate gastrointestinal tract. In many species, though not in all, enzymatic activity begins abruptly at the pylorus and diminishes gradually from the duodenum to the ileum. This is found in the dog, mouse and adult rat. In rats, for example, the ratio of duodenal to jeujunal to ileal alkaline phosphatase (per mg protein) is 16:4:1 [115]. In humans, high ileal activity has been reported [116, 117]. In several species, including mouse and rat, axial activity distribution varies with growth and development [118, 119]. For neonates of these species, alkaline phosphatase activity is distributed more evenly along the small intestinal length. Fasting, fat ingestion, vitamin D deficiency, and dietary zinc have been shown to correlate with increases in intestinal alkaline

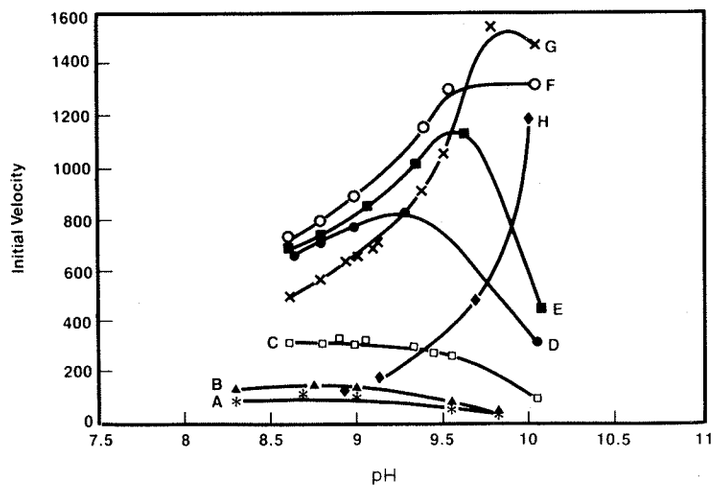


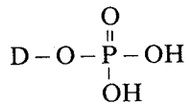
Fig. 13. Hydrolysis of phenyl phosphate by calf intestinal alkaline phosphatase. The curves refer to the following substrate concentrations: A, 25 μM; B, 50 μM; C, 100 μM; D, 500 μM; E, 750 μM; F, 2.5 mM; G, 25 mM; and H, 75 mM. Initial velocities are expressed as μmol product per mg enzyme per minute.

phosphatase activity, while calcium, sucrose and alcohol intake have been correlated with reduced activities [120, 121].

There is also considerable evidence that intestinal alkaline phosphatases exist as several isoenzymes [122]. In this regard, it has been shown that the ileal enzyme is precipitated less easily than duodenal enzyme by mixed anti-phosphatase, L-phenylalanine inhibition is more marked for duodenal than jejunal enzyme, heat stability is greatest for duodenal alkaline phosphatase, and the phenylphosphate to beta-glycerophosphate activity ratio is reversed from duodenum (high ratio) to ileum. It is believed this enzyme functions in the small intestine for absorption of phosphate from dietary phosphates, but much uncertainty remains concerning its physiologic function.

6.1. Prodrug considerations

Since phosphatase activity is distributed widely in mammalian tissues, phosphate monoester prodrugs are not restricted to oral dosage forms. However, for planned and specific targeting using phosphate monoesters, alkaline phosphatase in the intestine is the enzyme of choice. Since, in general, P-N bonds are not hydrolyzed, a free hydroxyl group in the drug moiety would be required. Prodrugs of the form



would be potential phosphatase substrates.

The concept of alkaline phosphatase hydrolysis coupled with facilitated transport has been studied recently [123] using the 21-phosphate ester of hydrocortisone. Bioavailability experiments in beagle dogs were done along with the parent drug, succinate and lysinate esters in equivalent molar doses. The phosphate derivative, which was shown previously to have high rat intestinal wall permeability in the upper intestine, showed high early peak plasma concentration which dropped off rapidly, leading, however, to a somewhat reduced bioavailability for this prodrug. The bioavailability results are shown in Figure 14. The fact that intestinal alkaline phosphatase distribution in dog is predominantly in the upper small intestine and that the ionized prodrug is not well absorbed passively accounts for the data.

These results indicate that prodrugs targeted for intestinal alkaline phosphatase potentially can improve oral drug delivery. For stable phosphate derivatives, axial enzyme distribution in the particular species under consideration will determine the relative success for these prodrugs.

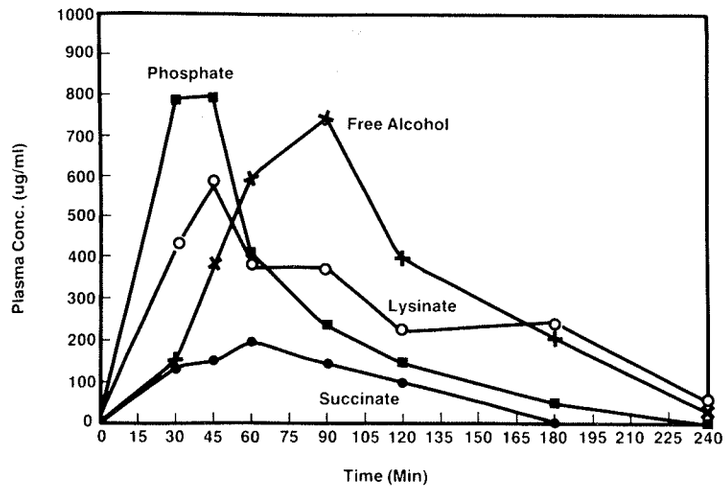


Fig. 14. Pooled dog plasma concentration after a 0.15 mM oral dose of the prodrugs.

7. Carboxyl esterases and lipases

7.1. Carboxyl esterases

Carboxyl esterases are distributed widely in vertebrate tissues and blood serum, the highest mammalian activities being found in the liver, kidney, duodenum and brain [124]. The physiological functions of most carboxyl esterases are not clearly understood. Myers and Mendel [125] were among the earliest group of scientists to suggest that these enzymes do not themselves participate in normal metabolic processes. Krisch [124], observing that carboxyl esterases hydrolyze a wide variety of compounds not normally found in the body, suggested that these enzymes play an important role in the body's detoxification system. Carboxyl esterases have been classified [126] according to their behavior toward organophosphorus compounds such as diethyl-*p*-nitrophenyl phosphate. A-esterases hydrolyze organophosphorus compounds as substrates whereas B-esterases do not hydrolyze and are inhibited by such compounds. A third type, C-esterase, has been found in porcine kidney extracts, which neither hydrolyzes nor is inhibited by organophosphorus compounds.

There is very little information available concerning the structure of the carboxylesterases, which exist in many forms. As many as 13 electrophoretically distinct esterases have been found in the rat liver alone [127]. Studies on esterase isozymes have been reviewed by Latner and Skillen [128]. Carboxylesterases from various sources have been found to have molecular weights around 160,000 g/mol [124]. It has also been shown that the equivalent weight of several esterases is approximately half of the molecular weight [129 – 132]; the dissociation of pig and ox liver esterases into active

'half-molecules' has been observed in several laboratories (see, Refs. 132, 133). Krish [124] has reviewed studies of the amino acid composition of various esterases. The B-esterases, which have received far more attention than the other two classes, are phosphorylated by inhibitors such as diethyl *p*-nitrophenyl phosphate at a serine residue. This fact has enabled the determination of the amino acid sequence in the vicinity of the 'active serine' for a number of different carboxylesterases [124].

Esterases catalyze the hydrolysis of esters according to the reaction:

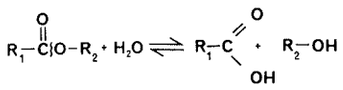


Table 11 lists a few of the many compounds known to be esterase substrates. Although some labile ester substrates are also hydrolyzed by several endopeptidases (e.g., α-chymotrypsin) and other enzymes, these catalytic rates are, typically, 4 to 5 orders of magnitude less than those of 'true' esterases [138, 139, 145]. For example, using *p*-nitrophenyl butyrate as the substrate, Stoops et al. [138] reported that, at pH 8.1, $K_m = 2.3 \times 10^{-4}$ M and $k_{cat} = 3.72 \times 10^{2}$ sec^{-1} for pig liver carboxylesterase. For the same substrate under similar conditions, Cane and Wetlaufer [146] found $k_{cat} = 7.7 \times 10^{-3}$ sec^{-1} for α-chymotrypsin. Also, like α-chymotrypsin, liver and kidney esterases have been shown to catalyze the hydrolysis of several amino acid esters [132, 134, 138] at comparable rates. For the carboxylesterase- and α-chymotrypsin-catalyzed hydrolysis of L-tyrosine ethyl ester, Stoops et al. [138] found k_{cat} values of 71 and 39 sec^{-1}, respectively. However, in contrast with the peptidases, peptides are not hydrolyzed by porcine liver carboxylesterase [134], although some amides have been found to be poor substrates. With $K_m = 2.5 \times 10^{-3}$ M and $k_{cat} = 0.1$ sec^{-1}, phenacetin was typical of the several amide substrates studied by Franz and Krisch [131].

They also noted that procaine was an equally poor ester substrate of porcine kidney carboxylesterase, having K_m and k_{cat} values of 4×10^{-4} and 0.1 sec^{-1}, respectively. Levy and Ocken [135] have distinguished three groups of carboxyl ester substrates: (1) unsubstituted monocarboxylate esters (which are hydrolyzed with the highest relative velocities), (2) substituted monocarboxylate esters, and (3) dicarboxylate diesters and substituted diesters, of which only one ester group is hydrolyzed. Charged compounds are extremely poor substrates [134, 135]. Hofstee [136] has found that the reactivity of fatty acid esters of *m*-hydroxybenzoic acid increases as the acyl chain length is increased. Chain length effects have been discussed in more detail by Dixon and Webb [137], who reported the reactivity and affinity (defined as the inverse of K_m) of 33 esters (in which both the acyl and alkyl chain lengths ranged between C_1 and C_9) with respect to horse liver carboxylesterase. They concluded that the alkyl and acyl chain length effects are largely independent. Both the affinity and reactivity increase as either chain is lengthened to about C_4 to C_6. Fur-

TABLE 11
Examples of Esterase Substrates

1. Carboxyl esters
   (a) Esters of unsubstituted fatty acids
   - Phenyl formate (acetate, propionate, butyrate, valerate)
   - Ethyl formate (acetate, propionate, valerate)
   - *m*-Carboxyphenyl esters of a homologous series of *n*-fatty acids (chain length from C_2 to C_{12})
   - Ethyl acetate
   - Glyceryl triacetate (triacetin)
   - *p*-Nitrophenyl acetate
   - *O*-Nitrophenyl acetate
   - 2,6-Dichlorobenzenone-indophenyl acetate
   - Vitamin A acetate
   - Methyl butyrate
   - Methyl butyrate (3-methylbutyrate, pentanoate, 3- and 4-methylpentanoate, hexanoate, heptanoate)
   - Glyceryl tributyrate (tributyrin)
   - Ethyl butyrate
   - *O*-, *m*-, and *p*-Nitrophenyl butyrate
   - 2,4-Dinitrophenyl butyrate
   - *m*-(*n*-Pentanoyloxy) benzoic acid
   - *m*-(*n*-Heptanoyloxy) benzoic acid

   (b) Esters of other acids
   - Ethyl benzoate (benzenesulfonate, lactate, acetoacetate, diethyl succinate, fumarate, asparate, *p*-hydroxybenzoate, bromomalonate, terephthalate, and other ethyl esters)
   - Procaine (2-diethylaminoethyl *p*-aminobenzoate)
   - L-Tyrosine ethyl ester (and many other amino acid esters)

2. Thioesters
   - 6-*S*- and 8-*S*-acetoacetyl monothioloctanoate
   - 8-*S*-Acetoacetyl, 6-ethyl monothioloctanoate
   - 8-*S*-Acetoacetyl dihydrolipoic acid
   - 6-*S*- and 8-*S*-Acetyl dihydrolipoic acid
   - 6-*S*-Acetoacetyldecanoate
   - *S*-Acetyl- and *S*-acetoacetyl-BAL
   - *p*-Nitrothiophenyl hippurate
   - Thiophenyl acetate

3. Aromatic amides
   - Acetanilide
   - Phenacetin
   - Monoethylglycine 2,6-xylidide
   - Diethylglycine 2,6-xylidide (Xylocaine; lidocaine)
   - *N*-(*n*-Butylamino) acetyl 2-chloro, 6-methylanilide · HCl (hostacaine: butacetoloid)
   - L-Leucyl *p*-nitroanilide
   - L-Leucyl beta-naphthylamide
   - 2-(N^4-*n*-Propylaminoacetyl)-sulfanilamido 4,6-dimethylpyridine HCl

a. Taken from the review by Krisch [124]. See that article for references.

ther increase in the alkyl chain length results in a decrease in both the affinity and reactivity, suggesting that the alkyl binding site is only large enough to accomodate a butyl to hexyl chain. Further increase of the acyl chain length produces the same decline in reactivity but a sharp rise in affinity, suggesting the existence of a secondary acyl binding site which leads to non-optimal orientation at the esteratic site. Similarly, branched-chain substrates exhibit higher affinities but lower reactivities [137]. Esterases are also capable of hydrolyzing thioesters [121, 122] and aromatic amides, including acetanilide [129, 132, 134] and phenacetin, at reduced rates. Lastly, carboxylesterases can also act as transferases as was first observed by Bergmann and Wurzel [140] in 1953.

Kinetic studies on mammalian carboxylesterases consistently report the pH optimum to be in the range of 7.5 – 9.0 [124]. It is generally agreed that, like other serine hydrolases (e.g., α-CT), carboxylesterase catalysis proceeds through an acyl-enzyme intermediate [141]. Deviations from normal Michaelis-Menten kinetics attributable to substrate activation have been observed by several workers (see, e.g., Refs. 138 – 142). The details of the catalytic mechanism are still unknown, although the participation of a histidine residue has been discussed [137, 144, 145] but not verified.

7.2. Lipases

The lipases are a distinct subset of the large family of esterase enzymes. In mammals, lipases are found in the digestive tract, in such tissues as the heart, brain, muscle, arteries, kidney, adipose tissue and serum, and have also been identified in milk [147]. Their primary function is the metabolism of triglycerides.

Most of the lipase found in the GI tract is produced in the pancreas. The lipase isolated from lyophilized aqueous extracts of fresh porcine pancreas was found to be almost entirely in the form of a high-molecular-weight complex of enzyme and lysolecithin-rich micelles [148]. Treatment of this complex with organic solvents produced enzyme molecules weighing approximately 48,000 g/mole [149]. The amino acid compositions of several lipases are known [149, 150], but there is no information on their structure. The observation that the lipase activity of porcine pancreatic extracts is significantly depressed upon chromatography [151] or gel filtration [152] pointed to the existence of a pancreatic colipase, which subsequently was isolated and purified [153]. It was found to be a small protein (molecular weight, about 8,000 g/mole) which binds to the lipase in a 1:1 molar ratio [153] and increases the lipase activity when bile salts are present [154, 155].

Lipases are best distinguished from the other carboxylesterases by the physical state of the substrate. Unlike other esterases, lipases do not catalyze the hydrolysis of dissociated (i.e., totally solvated) substrate molecules. Lipases generally act at hydrophobic interfaces or on emulsified particles. However, some lipases are known to hydrolyze micelles [156, 157] and the minimum extent to which substrate

molecules must be aggregated is still unknown. This lipase-esterase distinction is clearly illustrated in Figure 15, taken from the work of Sarda and Desnuelle [158]. Whereas the esterase activity reached a plateau as the solution became saturated with triacetin, lipase activity on the same substrate was negligible until the solubility limit was passed and emulsified particles were formed. Agents which promote the micellization or emulsification of substrate molecules, e.g., NaCl [154] and bile salts [147], also produced the expected increase in lipase activity. Bile salts also promote in vivo lipolysis by forming mixed micelles with the lipolysis products (i.e., fatty acids, soaps) and removing them from the hydrophobic interface where the lipase acts [147]. Lastly, bile salts are known to shift the optimum pH of lipases from about 8.5 to 6, which is much closer to the pH of the upper intestine [159].

Kinetic studies on lipases are complicated by the requirement that the substrate molecules must be aggregated. Any factor which affects the affinity of the enzyme for the resulting interface or the packing and orientation of the substrate molecules at that interface will also affect the observed lipase activity. Also, the rate of lipolysis is not dependent simply on the concentration of substrate molecules but, rather, on the available surface area of aggregated substrate. Assuming a constant particle-size distribution, Sarda and Desnuelle [158] prepared triglyceride emulsions of various 'concentrations' and, using arbitrary units, demonstrated that lipolysis proceeds according to Michaelis-Menten-like kinetics.

p-Nitrophenyl phosphate incorporated into bile salt micelles causes almost complete inactivation of pancreatic lipase [160]. As with other esterases, the organophosphate has been shown to bind to a serine residue [161] undoubtedly in-

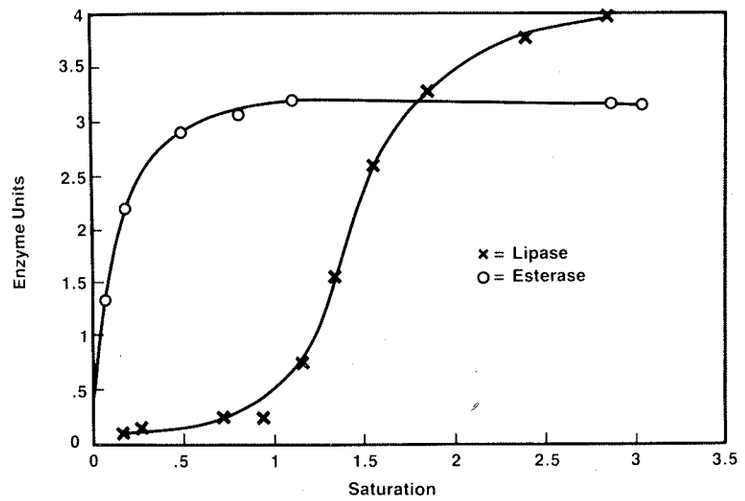


Fig. 15. Hydrolysis of triacetin by a lipase and an esterase. Increasing amounts of triacetin were added to a fixed volume of buffer and the resulting mixtures were incubated with pure lipase (×) or purified horse liver esterase (○). Beyond saturation of 1.0 the solution is oversaturated and, consequently, emulsified particles begin to form.

volved in the catalytic mechanism. There is also considerable evidence for the participation of a histidine residue in the catalytic process [162], but the detailed mechanism is quite unknown.

Lipases can catalyze the hydrolysis of a variety of esters, although, owing to their tendency toward self-association, triglycerides are preferred. Only the 1- and 3-positions of triglycerides are attacked by lipase. However, di- and monoglycerides undergo rapid isomerization (especially at slightly acidic pH) whereby a chain attached at the 2-position migrates to a primary (1- or 3-position) carbon [163]. Thus, 1,3-di- and 1-monoglycerides are found both in vitro and in vivo; in vitro lipolysis of triglycerides sometimes goes to completion. In contrast with the above positional specificity, lipases do not recognize [164, 165] the 'biological asymmetry' ascribed to glycerol derivatives in other instances [166, 167]. The effect of acyl chain length on substrate reactivity has been the subject of many investigations, but it is now known to be less important [147] than the length of the alcohol chain. In a study of over 100 esters in which both the acyl and alcohol chain lengths ranged between C_2 and C_{18}, Mattson and Volpenhein [168] concluded that the rate of hydrolysis is influenced independently by the acyl and alcohol chain lengths. Esters of heptyl alcohol were found to be hydrolyzed faster than esters of other alcohols, and esters of dodecanoic (followed by butyric) acid were hydrolyzed faster than esters of other fatty acids. They ascribed the influence of the acyl chain length to typical enzyme-substrate specificity, whereas the influence of the alcohol chain length was attributed to orientiation of the substrate molecules at the substrate/water interface.

7.3. Prodrug considerations

Since the carboxylesterases and lipases are distributed widely in mammalian tissues, prodrugs designed for reconversion by these enzymes are not restricted to oral dosage forms. In view of the broad specificity and heterogeneity of these enzyme classes, many esters are likely to be at least poor substrates. In general, amides are much poorer substrates than the corresponding esters. Prodrug suggestions would include $C_4 - C_6$ esters of drugs possessing a free hydroxyl group and simple $C_4 - C_6$ esters of drugs with free carboxyl groups. Glyceride esters would be potential lipase substrates, as would esters that are largely hydrophobic and present, predominantly, at the interface of emulsified particles. Suggestions would include dodecanoate and butyrate esters of drugs with free hydroxyl groups and heptyl esters of drugs with free carboxyl groups. The alcoholic, as opposed to the acyl, portion of the ester appears to be the more important in determining lipase activity. However, since proper orientation of the substrate at the interface is also important for lipase activity, the drug portion of the prodrug may significantly affect lipase activity as well as determining whether the lipases or esterases would be the primary reconversion site. Finally, it should be noted that many prodrugs currently available (steroid esters, for example) are probably substrates for the lipases and/or esterases. Clearly, a more detailed biochemical characterization of these enzyme classes is needed before more specific suggestions can be made.

8. Microfloral enzymes in the colon

The glycosidase activity of the colonic microflora offers opportunity of designing a colon-specific drug delivery system. Colon-specific delivery of bioactive compounds is known to occur in man. In the plant kingdom, a great many compounds are found as glycosides. Upon ingestion, many of these glycosides pass through the upper intestine and into the colon. Once there, the glycosidases of the colonic microflora liberate aglycones, which may then be absorbed. Certain sulfa drugs [169] are now known to be activated by the azo-reductase activity of the colonic microflora. A prodrug system based on polymers has been reported [170, 171] in which certain aromatic amines are released by reduction of an azo link between the drug and the polymeric carrier.

Based on this concept, a 'colon-specific' prodrug design has been reported recently [172]. Dexamethasone-21-beta-D-glucoside and prednisolone-21-beta-D-glucoside were synthesized for possible treatment of inflammatory bowel disease. Hydrolysis of the prodrugs by beta-glucosidase and fecal homogenates in vitro caused the release of the free steroids. Nearly 60% of an oral dose of glucoside of dexamethasone was shown to have reached the cecum, whereas less than 15% of glucoside of prednisolone did. When free steroids were administered orally, they were absorbed almost exclusively in the small intestine.

The rat model suffers from the problem of a relatively high bacterial population and subsequently high level of glycosidase activity present in its stomach, upper small intestine and lower small intestine. There are an average of $10^{7.7}$, $10^{6.9}$ and $10^{7.7}$ microorganisms/g wet weight in the rat stomach, upper small intestine and lower small intestine, respectively. In contrast, the bacterial population in man's stomach and small intestine is much lower. There are only an average of 10^0, $10^{2.6}$ and $10^{4.2}$ microorganisms/g wet weight residing in the human stomach, upper small intestine and lower small intestine, respectively [173, 177]. Bacterial population of the large intestines of rat and man are more nearly comparable ($\approx 10^{8.3}$ microorganisms/g wet weight) [173]. ***Bacteroides*** and ***Bifidobacteria*** are the bacterial species comprising the majority of microorganisms in the gasterointestinal system of both the laboratory rat and man. Both species have been shown to produce measurable quantities of beta-glucosidase in vivo [173].

Modification of the enzymatic activity of the gut microflora to improve delivery is a significant feature in this system. It has been shown that certain enzymes produced by gut bacteria are inducible with diet [174, 175]. For instance, bean diets dramatically increase α-galactosidase activity in human subjects while bran diets increase β-glucosidase activity. Manipulation of glycosidase activity by diet may be

very useful in standardizing glycosidase activity and also in raising enzyme activity in patients with a diseased colon, where enzyme levels may be depressed.

Modification of the glycone, aglycone and glycosidic linkage can probably all be varied to improve or alter the rate and location of drug release. Altering the sugar residue by modifying functionalities [176] or using an oligosaccharide carrier might hinder the rate of hydrolysis in vivo. Slow hydrolysis of refractory prodrugs in the colon might prove to be an effective mechanism for sustained release. Changing the aglycone, as was demonstrated in this work, can also alter delivery. Furthermore, the stereochemistry of the glycosidic link might be utilized to vary rates and sites of release. A sustained release system for the small intestine could be based on α-glycosides designed to be cleaved by digestive enzymes. Absorption would then be moderated by the rate of release in the small intestine, as well as the physicochemical properties of the parent drug.

9. Summary

A rationale for the effective design of prodrugs through the consideration of enzyme-substrate specificities has been developed. The diverse properties of the various amino acid residues combined with their low toxicity provide a wide range of application for drug derivatization with amino acids. Considerable knowledge of the enzyme or enzyme system is necessary for effective targeting of the prodrugs.

Prodrug reconversion in the intestinal lumen can be utilized as an oral drug delivery strategy for drugs with poor stability in acidic pH and causing gastric irritation. These prodrugs may be targeted to the pancreatic enzymes in the intestinal lumen – pancreatic amylase, pancreatic lipase, elastase, trypsin, α-chymotrypsin and carboxypeptidase A. Prodrugs designed to increase drug aqueous solubility may also be targeted to the luminal enzymes to improve dissolution from solid oral dosage form. However, luminal reconversion after dissolution regenerates the limitations on aqueous transport of the poorly soluble drug. In such cases, it is advisable to target these prodrugs for enzymes associated with the intestinal brush border membrane. Reconversion at the brush border membrane releases the more membrane permeable parent drug at a point adjacent to enterocyte membrane. A special transport advantage for phosphate and amino acid prodrugs may be gained if enzyme binding and catalytic sites are located so as to permit a favorable positioning of the drug molecule for membrane permeation.

α-Chymotrypsin, trypsin and elastase exhibit a wide range of specificities for substrates of the form $D-X-\overset{\overset{O}{\|}}{C}-R$, where X is O or NH, D the drug moiety and R the specificity site-directing group. Size, aromaticity and hydrophobicity of the R group determines which of the three enzymes becomes the reconversion site. Carboxy and aminopeptidases show specificity for substrates of the form

$D-\overset{\overset{O}{\|}}{C}-NH-\overset{\overset{R}{|}}{CH}-COO^-$ and $D-X-\overset{\overset{O}{\|}}{C}-\overset{\overset{R}{|}}{CH}-NH_3^+$, respectively. These enzymes preferentially hydrolyze prodrugs containing nonpolar R group. The physical properties of drugs with free -OH, $-NH_2$ or -COOH groups may be altered to almost any desired direction by appropriate choice of the R group.

The carboxyl esterases and the lipases exhibit a broad specificity towards esters. The prodrug must be present at a micellar or emulsified particle interface, with proper orientation in order for lipase to be active. Colipase increases the lipase activity in the presence of bile salts. C_4-C_6 esters of drugs possessing -OH or -COOH group may be suggested for using these enzymes as reconversion sites. The phosphatases require prodrugs of the form $D-O-\overset{\overset{O}{\|}}{P}-(OH)_2$ and, consequently, the drug molecule must possess free -OH groups. The phosphatases, esterases and lipases are distributed widely in mammalian tissues and prodrugs targeted to these reconversion sites need not be restricted to oral dosage forms.

The enzymes of the colonic microflora may be utilized for targeting drugs to the colon for treatment of colonic diseases. The predominant microfloral enzyme systems, glycosidase and azo-reductase, require the prodrugs to contain glycoside and azo groups, respectively. Appropriate choice of these groups may prevent significant absorption of the unconverted prodrugs before reaching the colon and complete reconversion in the colon.

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Design of Prodrugs (Bundgaard, H., ed.)

CHAPTER 3

Pharmacokinetic aspects of prodrug design and evaluation

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1. Introduction

From a pharmacokinetic viewpoint, prodrugs may be regarded as drug delivery systems. Following their introduction into a specific environment, they must initiate drug release, which should proceed at an appropriate rate. The mechanism employed to activate this process has been referred to as the 'trigger' [1]. 'Triggers' may include the sudden presence of water or enzymes, or a change in the environmental pH. The ideal site and rate for drug release will depend upon the specific delivery problems which were meant to be overcome by the prodrug design. The earliest site can vary from in vitro within the solution prepared for administration to a specific organ or tissue in vivo. Once at the site, prodrug conversion rate may vary from nearly instantaneous (which is the usual case) to very slow, which is the case when conversion of circulating prodrug is the means for extending drug duration.

All prodrugs may begin conversion following successful completion of their specific goals. With the exception of site-specific conversion and rate-limiting conversion, prodrug conversion to drug must be completed no later than their arrival in the blood. Some examples of prodrug goals and the earliest site wherein conversion may occur are given in Table 1, together with the simplest mechanism or 'trigger' for each case. As suggested in the table, dry powder stability may require only water to hydrolyze chemically the prodrug upon its reconstitution just prior to use. The rate may further be regulated by controlling the pH. However, if a solution is to be stored, the differential between physiological pH and an acceptable formulation pH will not be sufficient to allow both prodrug stability in vitro and complete, rapid prodrug conversion in vivo. It would be necessary to design a chemically stable prodrug which would be metabolically converted on arrival in or during transport to the blood.

TABLE 1
Examples Showing How the Mission of the Prodrug Determines the Earliest Acceptable Site and the Potential Mechanism for Conversion to Drug

Prodrug goal	Earliest site for drug release	'Trigger'
Dry powder stability	Reconstituted solution	Water
Improved taste	Stomach	ΔpH or enzymes
Stomach acid stability	Intestines	ΔpH or enzymes
Gastrointestinal solubility	Blood	Enzymes
Solubility or stability of reconstituted parenterals	Blood	ΔpH or enzymes
Solubility or stability in stored solutions	Blood	Enzymes
Increased permeability for absorption	Blood	Enzymes (oral) Enzymes or ΔpH (topical)
Increased duration by im depot	Blood	Enzymes
Increased duration by iv administration	Slowly in blood or tissues	Enzymes or water
Site enrichment	Specific organ or tissue	Site-specific enzymes

Problems associated with control of prodrug conversion rates, evaluation of data, and development of pharmacokinetic theory have been discussed for more than a decade [2, 3]. Equations describing the time-courses for drugs as a function of the prodrug goals [4], theory for the optimization of intramuscular depot prodrug absorption rates [5, 6] and the application of pharmacokinetic theory to prodrug practice [7] all provide positive evidence for progress toward a rational basis for pharmacokinetic theory. Nevertheless, significant areas remain inadequately defined, and contradictions between theory and observations still exist. In theory, true site specificity cannot be achieved since the drug released in the organ or tissue will then re-equilibrate and assume its normal disposition pattern [8]. This allows only what might be termed transient enrichment, wherein the relative drug distribution pattern favors the site only until normal equilibrium is established. Even this advantage would become insignificant for a prodrug which is administered continuously or repeatedly until the steady-state is achieved. If site specificity is measured using the ratio of organ or tissue concentration to plasma concentration under steady-state conditions, then prodrug and drug should provide equivalent results. Results which are contrary to this indicate that theory, experimental methods or both remain inadequate. Theory and practice are at even greater odds for the case wherein prodrug is converted rapidly in blood, yet tissue to plasma drug ratios reportedly are increased by prodrug administration. When intact prodrug cannot be found in the systemic circulation the prodrug can only behave as any other device which simply presents bioavailable drug. It cannot alter drug disposition any more than a tablet formulation can do so.

One common source of experimental error is conversion during the preparation of samples for assay. The potential for this source of misinterpretation of kinetic data has been reviewed [3]. It has often gone unnoticed because of the fact that both drug and prodrug are expected components of the sample. The degree of error is dependent upon the intact prodrug in the sample, the fraction converted during the procedure and the resultant apparent time-course for drug which reflects the contributions from both species. Control experiments are required since results of assays which are specific for drug and prodrug do not in themselves indicate whether or not there is conversion [1].

The use of in vitro conversion rates to predict in vivo prodrug conversion constant values obtained by compartmental pharmacokinetics has remained largely unsuccessful. Methane sulfonic acid prodrugs of sulfonamides were successfully modeled in rabbits but the observed in vitro rate constant values in blood were assigned as part of the calculated in vivo prodrug elimination constant and not determined independently [9]. Ancitabine, a prodrug which undergoes chemical hydrolysis to cytarabine, was chosen as an ideal candidate to test for a potential in vitro – in vivo correlation [10]. It was concluded that the non-physiological nature of the apparent volume terms in pharmacokinetic compartmental schemes precludes a direct meaningful correlation, in spite of the agreement observed in this study. The difficulties arise in that these parameters are not real volumes, may not be equal for drug and prodrug and, in any case, would not indicate that prodrug and drug were in the same physiological space. The use of an in vitro conversion constant in blood therefore becomes speculative when correlated with a calculated apparent constant in the model.

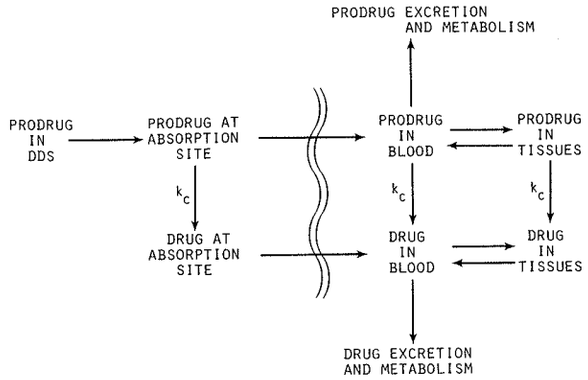
While prediction of in vivo ratios would be a most significant factor in advancing prodrug theory and design, this is not yet feasible. Progress in prodrug development has been largely empirical. Rapid conversion in blood or before arrival in blood has often been the goal. In this case, one can be certain that the in vivo conversion rate will be equal to or greater than the in vitro rate in blood.

The degree of success in attaining a prodrug goal may be assessed by model-independent methods based on assays for drug and prodrug. The specific prodrug goal will dictate the ideal kinetic pattern, which, in turn, defines the necessary data and its interpretation. This chapter provides brief surveys of the theory accompanying each goal, methods for evaluating the success of the prodrugs and the practical limitations as evidenced by examples of prodrugs, their successes and failures.

2. Theory

2.1. General description of prodrug pharmacokinetics

Scheme 1 summarizes the rate processes which may follow the extravascular (ev) ad-



Scheme 1

ministration of a prodrug in a drug delivery system (DDS). As indicated by the conversion rate constants (k_c), drug may be formed at the absorption site, in the blood or within some other distribution site in the body. Figure 1 provides an example of the time-courses for prodrug in the DDS, at the ev site and in the blood, together with drug in the blood for the simple case wherein conversion is limited to blood. Of these four sets of data, only the concentrations of prodrug and drug in blood would be assayed in practice. This example is intended solely to illustrate the kinetic patterns for the key species in Scheme 1. It is not optimized for a specific goal. The ideal profiles will depend upon the prodrug goal. Furthermore. the blood concentration time-courses for drug and prodrug are critical in order to assess the degree of success in achieving the goal.

2.2. Extravascular administration of prodrugs to increase the bioavailable fraction of drug

The bioavailable dose of a poorly absorbed drug may be increased by administration of a suitable prodrug. The kinetics in Scheme 1 should then be optimized to favor

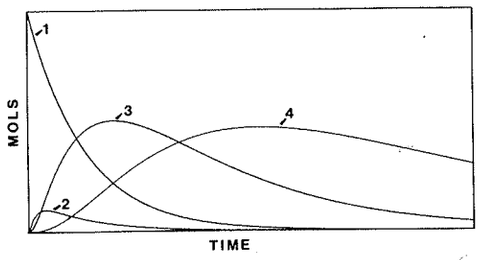
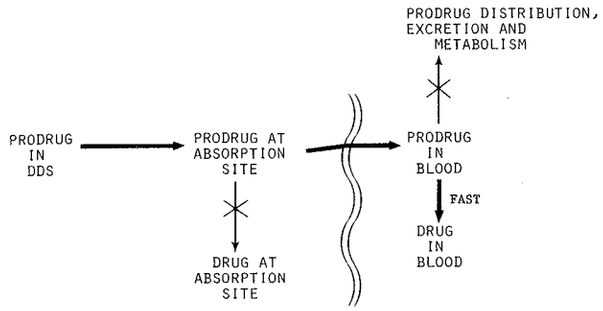


Fig. 1. Time-courses following extravascular administration of prodrug in accordance with Scheme 1, with conversion restricted to the blood. Curves represent: 1, prodrug remaining in the DDS; 2, prodrug at the absorption site; 3, prodrug in the blood; and 4, drug in the blood.

increased drug delivery to the blood. The predominant rate processes should be those which carry the prodrug from the DDS to the blood followed by rapid conversion to the drug, as illustrated in Scheme 2. Conversion prior to arrival in the blood would be counterproductive if this process reverted to the original absorption problem associated with the drug itself. One exception to Scheme 2 is that of a drug having a bioavailability problem due solely to gastric instability. In this case, prodrug conversion may take place in the intestines or may be initiated in the intestines and completed on arrival in the blood.



Scheme 2

The rates of distribution, excretion and metabolism of prodrug should be sufficiently slow, relative to prodrug conversion in blood, to be relatively insignificant. Prodrug excretion and metabolism cause a decrease in drug yield, and thus a reduction in the potential bioavailable dose of drug. Distribution of intact prodrug causes concern for the potential effects of the prodrug on various tissues and for the nonspecific distribution of drug which may be released throughout the body. If the prodrug does not circulate intact, but in fact converts at once to the drug, then the pharmacology, toxicology and pharmacokinetics are all that of the original drug, provided that the disposable moiety is inert.

Figure 2 illustrates the increased delivery of drug by administration of a prodrug which converts rapidly and completely in the blood. The bioavailable fraction from the prodrug is twice that observed following an equimolar dose of the drug itself. The time-course for prodrug concentration in blood is insignificant relative to that for the drug which it delivers. This prodrug time-course could be reduced to undetectable levels by further increasing its conversion rate in the blood. In this example the rate has been limited intentionally to allow visibility of the prodrug time-course in blood. The absorption rate constant for prodrug and drug have been set at equal values. Thus, the prodrug is increasing only the bioavailable dose. In this case the prodrug is acting like a drug delivery system from which drug is absorbed to a greater extent.

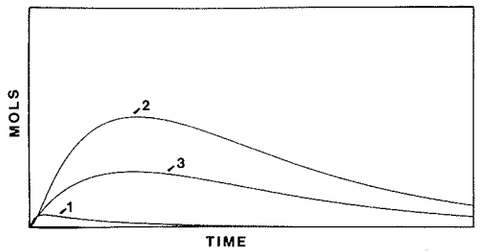


Fig. 2. The absorption rate-constant is unchanged but the prodrug is absorbed twice as much as drug and is converted rapidly in blood. Curves represent the concentration time-courses in blood for: 1, prodrug; 2, drug following extravascular prodrug administration; and 3, drug following an equimolar dose of the drug itself.

2.3. Extravascular Administration of Prodrugs to Increase the Rate of Appearance of Drug in Blood

The bioavailability of a drug is characterized by two features: the extent to which absorption occurs and the rate of absorption. In the previous section only the extent of absorption (i.e., the bioavailable dose) was increased by the prodrug. Some drugs may work best when they present a high, albeit transient, concentration maximum in the blood, C_{max}. For a given dose, the C_{max} will increase in direct proportion to the absorption rate constant but the duration above a selected minimum concentration, C_{min}, may decrease with increased absorption rate. The limiting case of this phenomenon is the duration of an intravenous (iv) dose in comparison to that of an equal bioavailable dose given by an ev route. The iv dose presents the highest C_{max} but the ev dose can persist above an appropriate C_{min} for the longer duration.

Thus, for a drug wherein C_{max} is the overriding factor for effectiveness, an increased rate of presentation of drug from prodrug will represent an improvement. Optimization of the kinetic properties can again be described using Scheme 2 and its accompanying explanation. The difference between this case and the previous one is that the rate of prodrug absorption will exceed that of the original drug, although the extent of absorption of each is similar.

Figure 3 illustrates the drug and prodrug concentration time-courses in blood following equimolar doses of drug and a more rapidly absorbed prodrug that is then converted rapidly and completely in the blood. The extent of absorption is the same for each, but the rapidly absorbed prodrug provides a higher C_{max} value for the drug at a shorter time interval, t_{max}. As in Figure 2, the prodrug time-course is visible due to the value chosen for k_c in the blood. If k_c were increased sufficiently, relative to the absorption rate constant for prodrug, the prodrug time-course in blood could be reduced to near-zero concentrations.

In Figure 2 only the bioavailable dose was increased by prodrug whereas in Figure 3 only the absorption rate constant was increased. In both cases conversion was

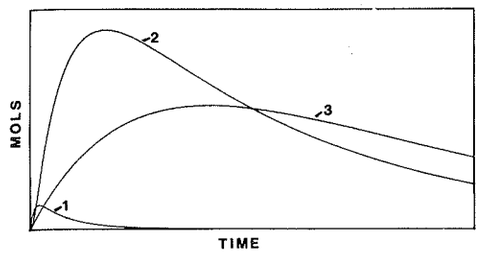


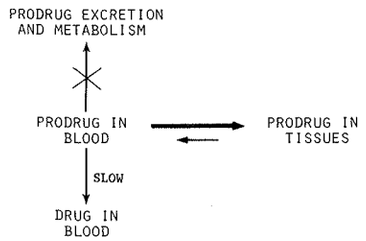
Fig. 3. The prodrug is more rapidly absorbed than the drug and is then converted rapidly and completely in the blood. Concentration time-courses in blood are: 1, prodrug; 2, drug following an extravascular dose of prodrug; and 3, drug following an equimolar dose of drug. The total absorbed from drug and prodrug is equal.

limited to blood where the process was complete. These restrictions were used to simplify the examples under consideration. In practice, a prodrug may alter simultaneously the bioavailable dose and the rate of absorption. These may be increased or decreased relative to drug itself. Conversion will not be complete if the prodrug is lost to non-drug-producing processes. Assessment of the success of the net result of the many opposing rate processes is discussed in the section on evaluation.

2.4. Intravenous Prodrug Administration for Increased Drug Duration

In theory, the time-course for drug concentration in blood may be prolonged by iv administration of a rate-optimized prodrug. This could occur through a slow conversion of prodrug to drug in blood, as shown in Scheme 3. Two alternate mechanisms include either the slow release of tissue-bound prodrug followed by conversion in blood or a tissue to blood prodrug ratio which minimizes prodrug concentration available for conversion in blood, thus limiting its rate of conversion.

Figure 4 compares the time-course for drug concentration in blood following an iv dose of prodrug to that following an iv dose of drug itself. In this example, the conversion rate constant is less than the apparent rate constant for loss of drug. The



Scheme 3

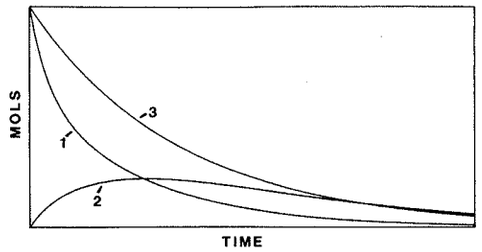


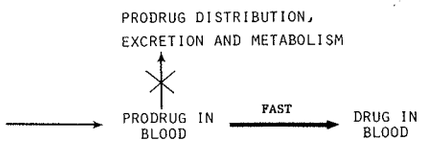
Fig. 4. Example of increased drug duration from rate-limiting conversion of an iv dose of prodrug. Blood concentration time-courses are: 1, drug following an iv dose of drug; 2, drug following an iv dose of prodrug; and 3, prodrug given intravenously.

terminal portion of the drug concentration time-course following the prodrug dose is thus described by the slower conversion rate constant instead of the apparent elimination rate constant for the drug itself according to a phenomenon known as 'flip-flop' [11].

This approach is difficult to achieve, due to several problems. Since circulating prodrug is required as a depot for the supply of drug, it predisposes prodrug to loss by excretion and metabolism. The greater the loss to these processes, the smaller is the yield of drug. Circulating prodrug would also undergo distribution throughout the body. This increases the problems associated with determining prodrug accumulation in tissues and the pharmacology and toxicology associated with prodrug accumulation.

2.5. Prodrugs to increase solubility in parenteral formulations

Increased solubility for parenteral solutions has long been a common prodrug goal with a high degree of feasibility. An ideal kinetic pattern would be the rapid conversion of prodrug to drug in blood with no loss of prodrug to distribution, excretion or metabolism independent of whether the prodrug is given by iv or intramuscular (im) administration, as illustrated in Scheme 4. The resultant concentration time-course for drug should be bioequivalent to an equimolar dose of drug itself. Figure 5 illustrates an iv dose of rapidly converting prodrug and an equimolar dose of the drug itself. The resulting time-courses for drugs are similar, except for a short initial time period. They could be made to appear identical by increasing the rate of pro-



Scheme 4

drug conversion to drug sufficiently to result in a prodrug time course that approaches zero concentration.

If prodrug conversion is not fast relative to excretion and metabolism, then the bioavailable dose of drug will decrease. This is also illustrated in Figure 5, wherein the example shown represents the drug concentration time-course for the case where 66% of the prodrug is excreted or metabolized and 33% is converted to drug. Thus, the bioavailability of drug has been significantly compromised, even though prodrug administration was by the iv route.

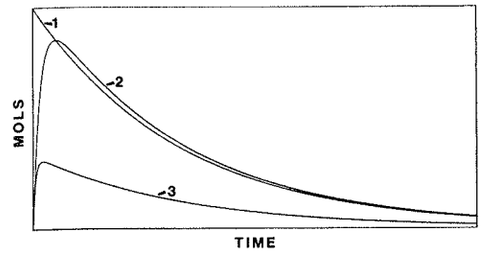
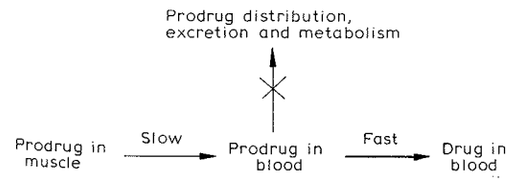


Fig. 5. Drug concentration time-courses in blood following (1) an iv dose of drug as compared to (2) a rapidly and completely converted iv dose of prodrug. In curve 3, 33% of the iv prodrug dose has been converted to drug and 67% has been lost to excretion or metabolism.

2.6. Increased drug duration by extravascular prodrug administration

The most practical means of prolonging the duration of drug in the body has been through an im depot injection of a slowly-releasing prodrug. The ideal kinetic pattern would be the slow release of prodrug from the muscle to the blood followed by rapid conversion to drug, as shown in Scheme 5. Under these conditions the terminal phase of the drug concentration time-course in blood would be controlled by rate-limiting release from the muscle in accordance with the so-called 'flip-flop' theory. An example is shown in Figure 6, where the time-course for drug following the im administration of a slowly-released prodrug is compared to that observed from an equimolar im dose of the drug itself. Conversion is both rapid and complete in the blood so that the prodrug concentrations are relatively small. The prodrug time-course could be decreased to the point where it was no longer visible by increasing the rate of prodrug conversion in blood.



Scheme 5

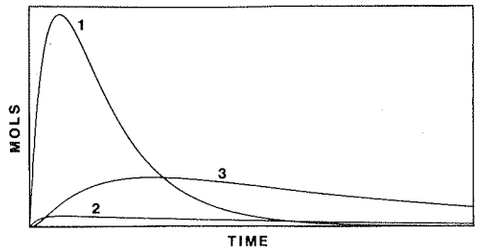


Fig. 6. Increasing the drug duration by im depot injection. Concentration time-courses in blood are: 1, drug following an im dose of the drug itself; 2, prodrug following an equimolar im dose of the prodrug; and 3, drug following the prodrug dose.

3. Evaluation; relating the theory to the goals

Prodrug goals may be broadly classified as pharmaceutical or pharmacokinetic. Pharmaceutical goals include efforts to overcome drug formulation problems or to increase patient acceptability. Pharmacokinetic goals involve the intentional modification of the time-course for drug in the patient. The pharmacokinetic evaluation of the prodrug will depend upon its specific goal.

3.1. Bio-equivalency

Pharmaceutical prodrug goals such as improving taste or odor, decreasing pain on injection, increasing storage stability, decreasing gastric distress or increasing solubility in the formulation may all be regarded as pre-systemic goals. They should not alter significantly the time-course for drug concentration in blood since that is not the stated purpose of the prodrug. Therefore, the pharmacokinetic evaluation should demonstrate that there is no difference in either the extent or rate of drug bioavailability from prodrug as compared to the drug itself. A positive proof would be that the blood concentration time-course following administration of prodrug is not significantly different from that following an equimolar dose of drug by the same route of administration in the same formulation. Since the time-courses are similar, the C_{max}, t_{max} and bioavailable fraction (f) values would also be similar in evidence of equivalence in both the rate and extent of bioavailability.

These data would be required regardless of the route of administration. As discussed in reference to Figure 5, an iv injection of a prodrug can result in decreased bioavailability of drug. Although bio-equivalency is the goal, prodrug may inadvertently alter the bioavailability of the drug. Extravascular prodrug administration may influence the rate and/or the extent of drug bioavailability in either a positive or negative manner. A prodrug which produces a drug concentration time-course which exceeds that from drug itself, but which has a similar t_{max} value, has

increased primarily the extent rather than the rate of bioavailability. The drug time-courses from prodrug and drug can then be made similar by reducing the prodrug dose. A prodrug which alters the rate or both the rate and extent cannot be made to produce a blood concentration time-course similar to that from drug itself. This case may necessitate a re-examination of the dosage regimen and its resultant accumulation pattern during repetitive dosing since the drug time-course in the body will be altered by the prodrug. While the final result may be overall improvement in bioavailability, this is fortuitous since it has no relationship to a starting goal such as improvement in taste.

3.2. Biovailability

A prodrug may be designed to increase the extent or rate of drug bioavailability, or both. An increase in the extent of bioavailability is indicated by an increased bioavailable fraction (f) whereas an increased rate is reflected by an increase in C_{max} and a decrease in t_{max}.

3.2.1. Bioavailable fraction

The time-course for the concentration of drug in blood following a rapid iv injection of drug may often be described by

$$C = \sum_{i=1}^{n} C_i e^{-\lambda_i t} \tag{1}$$

where C is concentration and t is time. A drug is said to be described by linear pharmacokinetics when this form of equation is adequate independent of dose and when the apparent rate constants, λ_i, are unchanged while the preexponential terms, C_i, are directly proportional to the dose.

In practice, it may not be prudent to administer certain drugs by the iv route. The extravascular route of interest may be employed to demonstrate linear pharmacokinetics. In this case, the concentration at any fixed time should be directly proportional to the dose if linear kinetics are in effect.

The bioavailable fraction, f, of drug is a measure of its absolute bioavailability from an ev route. For a drug behaving by linear pharmacokinetics, the absolute bioavailable fraction may be calculated from

$$f = AUC_{ev}/AUC_{iv} \tag{2}$$

where AUC is the area under the drug concentration time-course in blood from time zero to infinity. The AUC value may be calculated from the parameters values in Eqn. 1, using

$$\text{AUC} = \sum_{i=1}^{n} C_i/\lambda_i \tag{3}$$

In theory, a drug described by Eqn. 1 may be described by

$$C = \sum_{i=1}^{n} C_i e^{-\lambda i t} - \left[\sum_{i=1}^{n} C_i\right] e^{-k a t} \tag{4}$$

following an ev dose. In practice, the equation describing ev data may contain hybrids of the various rate constants and C_i terms. As long as the time course can be described as a series of exponential terms, the AUC from an ev dose is given as the sum of the contributions, $\Sigma\,[C_i/\lambda_i]$, wherein one of the terms will provide a negative component. Both iv and ev concentration time-courses may also be treated by the trapezoidal rule to estimate the AUC values. If the final portion of the curve from time t' to ∞ is not available, this can be approximated from

$$\text{AUC} = C_{t'}/\lambda_n \tag{5}$$

provided that $C_{t'}$ is in the terminal phase wherein the only significant contribution to the remaining time-course is

$$C \cong C_n e^{-\lambda n t} \tag{6}$$

The absolute bioavailable fraction of drug from an equimolar dose of prodrug may be calculated from

$$f = \text{AUC}_{\text{pd}}/\text{AUC}_{\text{iv}} \tag{7}$$

where both AUC values refer to the drug concentration time-course and pd indicates administration of prodrug while iv is an intravenous dose of the drug itself. Since these AUC represent only drug itself, the only requirement is that the drug pharmacokinetics are linear. Evaluation using Eqn. 7 should be applied to prodrugs given either ev or iv since conversion following iv administraton may be incomplete. Where it is not possible to administer equimolar doses, the AUC values must be dose-adjusted, which can be done directly from the dose in the case of linear kinetics. If an iv bolus dose cannot be administered, a constant-rate infusion can be substituted and the AUC values may be calculated directly from the concentration time-course. The limits for bioavailable fraction are $0 \leq f \leq 1.0$. It is not possible to exceed the upper limit. This represents complete transfer of drug to the blood, which therefore makes the AUC value equivalent to placing drug directly in the blood by the intravenous route. This concept continues to be violated in the literature when prodrugs are alleged to provide higher AUC values than equal iv

doses of drugs themselves ($f > 1.0$). The most common source of this misconception is that of a non-selective assay which includes a contribution from intact prodrug, which exaggerates the AUC value.

3.2.2. Increased extent of absorption

A prodrug may be designed to increase the extent of drug bioavailability following ev administration. This would be reflected by an increased value for f, as calculated by Eqn. 7, in comparison to that obtained by Eqn. 2. However, iv data for the drug itself may not be available, so that absolute bioavailability may not be known. In this case the relative bioavailability may be used to evaluate the prodrug. Since the total body clearance of drug is defined as

$$CL = f \cdot D/\text{AUC} \tag{8}$$

where D is the dose of drug, the bioavailable fraction from a prodrug dose (f') may be incorporated as

$$CL = f' \cdot D'/\text{AUC}' \tag{9}$$

where D' is the prodrug dose normalized for drug content. Since CL is constant for the drug, the ratio of bioavailable fractions may be written

$$f'/f = (\text{AUC}' \cdot D)/(\text{AUC} \cdot D') \tag{10}$$

If the molecular doses are held constant or the AUC values are dose-adjusted, the ratio, which may be called the relative bioavailability, may be written

$$\text{relative bioavailability} = \text{AUC}'/\text{AUC} \tag{11}$$

where AUC′ is the value for the drug concentration time-course following ev prodrug administration and AUC is that following ev drug administration in the same formulation. The theoretical upper limit on the relative bioavailability value is $1/f$. If f is not known there is no theoretical basis to reject a large increase in bioavailability by an ev dose of prodrug.

Experimental protocols for demonstrating an increase in drug bioavailability from orally administered prodrugs would be similar to those appropriate for testing a tablet or capsule designed for increased bioavailability. A crossover study, using the AUC values for drug and the relative bioavailability from prodrug compared to a reference standard, may be employed [12]. The 75/75 rule for bioequivalency has as its criterion that at least 75% of the subjects show the test dose to have a relative bioavailability greater than 0.75 using each subject as his or her own control. This rule, which is suggested only to show bioequivalency (not superiority), must be ap-

plied with caution as it has known limitations accompanying high inter- and intra-subject variability [13, 14].

3.2.3. *Increased absorption rate*

Increased absorption rate should be achieved without sacrificing total bioavailability. Success would therefore require an increased C_{max} value, a decreased t_{max} value and relative bioavailability value calculated from Eqn. 11 that is equal to or greater than 1. No further advantage can be gained in data treatment by calculating the apparent absorption rates. As shown in Scheme 1, the delivery of drug from prodrug is a complex multiple step process. The meaning of the apparent absorption rate constant for drug following administration of prodrug is even more obscure than that for drug, which in itself is open to question [15]. The evaluation should thus be based directly on the prodrug goal, which is to provide higher C_{max} values through increased absorption rate. A direct comparison such as that shown in Figure 3 with a comparison of the AUC values using Eqn. 11 will serve to evaluate the degree of success in achieving this goal.

3.3. Duration

In theory the duration of drug concentration in blood may be increased by either iv prodrugs or ev prodrugs. Because of the difference in mechanisms, iv prodrug administration will require circulating intact prodrug whereas ev prodrug administration ideally should provide rapid conversion on arrival in the blood. This predisposes the iv prodrug approach to assay specificity problems. There are two factors which determine the potential for error due to post-sampling prodrug conversion: 1, the ratio of prodrug to drug in the original sample; 2, the degree to which conversion occurs during the sample preparation and assay procedures. Since the iv approach requires circulating prodrug to act as a reservoir for drug release, the prodrug/drug ratio is relatively high. Successful ev prodrugs for extending duration have been limited to depot injections given intramuscularly or subcutaneously rather than orally or intravenously.

Because prodrug excretion and metabolism competing with conversion is so great, the iv prodrug for increased duration has not proven to be a practical approach. Evaluation may be conducted by comparison of the drug concentration time-course following iv administration of prodrug to that of drug itself. While the study should include equivalent doses, it may also contain a much larger dose of prodrug in the successful case. If prodrug acts as a slow-release reservoir it may require the equivalent of several drug doses to demonstrate the total duration which may be achieved by the prodrug. The control should either include an equivalent dose of the drug or provide kinetic evidence showing that such a dose would provide an excessively high C_{max} value with an overall duration that is less than that of the prodrug.

An ideal im depot prodrug should extend the duration of action of drug without significant concentrations of intact prodrug in the blood. The increased duration should reflect slow release of prodrug or drug from the depot. A comparison of the drug time-course following prodrug administration to that following an equivalent dose of drug itself in the same formulation may or may not indicate the degree of success. Positive results in the form of an extension of the drug concentration time-course would indicate success. Negative results in the form of a higher C_{max} and shorter duration or an insignificant change in duration would indicate failure. However, the lack of detectable concentrations of drug in blood or the lack of a pharmacological response being used as a test does not in itself mean the prodrug candidate cannot prolong the duration of drug action. If the rate of release from the depot is sufficiently slow relative to prodrug conversion in blood and to drug elimination from the body, the drug will approach a near-zero steady-state concentration profile. This slow release could in fact be ideal but the prodrug would be eliminated by a screening procedure based on comparisons of a normal single dose of drug to an equivalent dose of prodrug. It may be necessary to administer multiple doses as a single unit in order to provide adequate large levels for assay or observable response. Therefore, the control may be difficult or impossible to administer. It may not be feasible, for example, to administer 20 doses of the drug itself, due to the resulting high C_{max} value. The control for drug may have to be simulated using appropriate kinetic studies at lower doses to allow a dependable prediction of the result which would not be observed from the high drug dose.

3.4. Urinary excretion data

Total urinary excretion of drug following prodrug administration is of limited utility in the evaluation of prodrug goals and is not recommended as a substitute for blood-concentration time-course data. There are several problems associated with the use of urinary data. At best, data for total amount of drug excreted can be employed to estimate the bioavailable fraction of drug resulting from prodrug administration. This does not evaluate the bio-equivalency, absorption rate, duration, C_{max} and t_{max} values. Theoretically, the time-courses for cumulative amounts of drug and prodrug excreted in the urine can be employed to assess bio-equivalency and duration. In practice, the estimates are subject to a high degree of variability and are less reliable than those from concentration time-courses in blood.

Even the relatively simple assessment of bioavailable fraction using total urinary excretion of drug presents several problems. It is necessary to collect data for a period of time equal to five times the half-life of the rate-determining step in order to achieve a 97% theoretical recovery following a single, totally available dose. The control experiment normally would be that of iv administration of drug itself together with urinary collection during five times the biological half-life. The percentage recovery should be independent of the drug dose over the range of

urinary excretion data which applies to the final prodrug evaluation. Administration of the prodrug would require urinary collection over a period of time corresponding to five times the half-life corresponding to the terminal phase of a drug concentration time-course following a prodrug dose. This cannot be assumed to be equal to the biological half-life of the drug. When a prodrug is designed to increase the duration, this half-life may reflect the prodrug absorption, conversion or a composite of both. If a successful long-acting prodrug provides a very slow release of drug, then the resulting low urinary concentrations may become difficult to assess at all. When the drug cannot be administered intravenously, a comparison can be carried out based on administration of drug by the same route and formulation. Comparisons must be adjusted for differences in molecular weight.

A multiple-dose steady-state protocol may be employed if the time to achieve steady-state blood levels can be estimated. This still requires a knowledge of the rate-determining half-life, which will correspond to the terminal half-life ($t^*_{½}$) for the time-course of the drug concentration in blood following prodrug administration. Provided that the dosage interval, τ, is sufficiently short to allow accumulation, $\tau \leq 3\ (t^*_{½})$, the steady-state drug levels will provide increased urinary excretion at a more uniform rate than that following a single dose. In addition, it is a condition of the property of the steady-state that a bioavailable dose is excreted during each dosage interval. Urinary collection over this entire interval can provide an estimate for the fraction of prodrug excreted intact and the bioavailable fraction through assay for drug and prodrug in urine. Controls must be used to justify the relationship between bioavailable drug and urinary excretion just as is required for single dose evaluations.

In the simplest of cases, wherein prodrug can be shown to convert instantly to drug in blood, urinary data might provide an acceptable means for evaluating the bioavailability of drug from prodrug. However, when the drug concentration time-course in blood is intimately related to the prodrug goal, the use of urinary data to imply blood level results is usually equivocal. The combination of total urinary recovery of drug and prodrug coupled with comparisons of drug concentration AUC values can provide a means to distinguish between insufficient prodrug absorption and incomplete conversion. Examples of this approach are given in section 4.2.1.

4. Practice; relating the evaluation to the goals

This section is a brief survey of some prodrugs which have been chosen to amplify some of the salient features associated with the prodrug kinetic theory and evaluation sections.

4.1. Pharmaceutical goals

4.1.1. Solubility

Success in increasing drug solubility by prodrug formation predates the popular usage of the term prodrug. In particular, there are many marketed examples of parenteral formulations employing soluble prodrugs, such as hydrocortisone sodium succinate, methyldopate hydrochloride, methylprednisolone sodium succinate and dexamethasone sodium phosphate.

Some prodrugs commonly employed by iv administration are not converted sufficiently rapidly in all patients to provide a bioavailable fraction of unity. In eight adult patients with normal renal and hepatic function, the unhydrolyzed chloramphenicol succinate prodrug, collected in the urine, varied from 13 to 36% of the iv dose [16]. In 12 patients from 2.5 months to 20 years of age the urinary recovery of this same prodrug varied from 8 to 43% following iv administration [17]. These data indicate that conversion of the monosuccinate ester of chloramphenicol is not sufficiently rapid to ensure complete bioavailability in all patients. The palmitate ester of chloramphenicol is a very slightly water-soluble prodrug employed in pediatric suspensions to avoid the bitter taste of chloramphenicol. Hydrolysis is probably effected by intestinal and pancreatic lipases. The bioavailability of orally administered palmitate was found to be superior to that of the intravenously administered succinate in a study using 18 children, aged 2 months to 14 years [18]. Using Eqn. 11, where chloramphenicol plasma concentrations are employed to calculate the dose-normalized mean AUC′ value from the oral palmitate and the mean AUC value from the iv succinate, provides a relative bioavailability estimate of 1.4. This illustrates that it is not a strictly theoretical possibility that an iv prodrug may be less bioavailable than an ev prodrug of the same parent drug.

Metronidazole is not available as a rapid iv injection, due to its limited solubility near neutral pH values ($\approx$10 mg/ml) relative to its large dose requirements. A phosphate ester prodrug was shown to have a solubility equivalent to 500 mg/ml of metronidazole at pH 7 [19]. The relative bioavailability of drug following subcutaneous administration of this prodrug to rats was 0.88 that of the drug itself. However, zero-order conversion in human serum required 8 – 12 hours for complete conversion, thus predisposing this prodrug to bioavailability problems in humans at this dosage level. Eight water-soluble amino acid esters of metronidazole have also been prepared for achieving the same goal [20, 21]. The hydrochloride salt of the *N,N*-dimethylglycine ester is soluble > 50% w/v, has a 9.5 minute half-life for hydrolysis in whole human blood and is sufficiently stable for clinical use at a pH of 4.4, which corresponds to the pH of aqueous solutions of the salt ($t_{0.9}$ = 73 hours at 23°C). Disappearance of plasma ester given intravenously to dogs is first-order, with approximately a 5-minute half-life which appears to be due to quantitative conversion to metronidazole [21].

4.1.2. Stability

Stability may be increased by employing steric hindrance of the degradation mechanism, using electronic effects to decrease the reactivity of the labile moiety or by reversibly derivatizing that part of the drug molecule which is responsible for either intramolecular participation or drug dimerization. Hetacillin is a prodrug which, by derivatizing the nucleophilic primary amino group, prevents dimerization which occurs in concentrated nonacidic ampicillin solutions [22]. Epinephryl borate prevents inactivation of epinephrine by the anti-oxidant bisulfite in ophthalmic solutions [23].

In concentrated solutions, cycloserine undergoes dimerization involving attack by the primary amino group. The prodrug, pentizidone, was formed by condensing acetylacetone and cycloserine to yield an enamine. This prodrug was stable to temperature and humidity conditions which degraded cycloserine [24]. Urinary recovery of cycloserine in mice was higher following oral administration of prodrug than after an equal oral dose of drug. This is similar to the approach used to decrease the dimerization of ampicillin by formation of hetacillin. Conversion of hetacillin in humans is rapid ($t_{1/2} \simeq 10$ minutes) and complete [25].

The nafate ester prodrug of cefamandole has increased dry powder stability relative to the parent drug [26]. While conversion in humans appears to be incomplete, the commercial preparation is buffered to initiate prodrug conversion following reconstitution. The total prodrug conversion in clinical use is then the sum of in vitro and in vivo conversion, which is nearly 90% [27].

Dinoprostone, a prostaglandin, is unstable at room temperature, wherein decomposition following liquefaction occurs during a few months storage. High-melting phenyl esters were prepared to resist liquefaction and increase storage stability. Under conditions wherein dinoprostone degraded 44 – 59% in 12 months, the liquid esters also resulted in decomposition but the crystalline esters showed no decomposition for 23 – 30 months [28]. Although no bioavailability studies were carried out, the prodrugs were not equivalent to dinoprostone in three biological activity testing procedures.

4.1.3. Patient acceptance

Prodrugs have been used to overcome such deterrents to patient compliance as pain on injection or unpleasant taste or odor. Prodrugs which reduce unpleasant taste are chloramphemcol palmitate, piperazine estrone sulfate, phenoxyl methyl penicillin hydrabamine, acetyl sulfisoxazole, triamcinolone diacetate, the tetrahydropyranyl ether of acetaminophen, erythromycin ethylsuccinate and clindamycin palmitate. Evaluation of four clindamycin 2-acyl esters indicated that increasing the chain length improved the taste from bitter (acetate) to complete loss of the bitter taste (palmitate) [29].

Clindamycin causes pain on injection whereas its 2′-phosphate ester prodrug is well tolerated. Although this prodrug is hydrolyzed rapidly in vivo ($t_{1/2} \simeq 10$ minutes), the intact prodrug has been assayed in the blood [30].

4.2. Pharmacokinetic Goals

4.2.1. Bioavailability

Oral bioavailability may be increased by alteration of the partition coefficient of the parent compound, increasing the gastrointestinal stability or reducing first-pass metabolism. The prodrug bond must be sufficiently stable to deliver the drug to the systemic circulation, yet labile enough to prevent excretion of intact prodrug [7].

Salsalate is a salicylester prodrug of salicylic acid which can hydrolyze to form two molecules of salicylic acid. Consequently, a 358-mg dose of salsalate is equivalent in salicylic acid content to 500 mg of acetylsalicylic acid. A study, in six arthritic patients, combined data for salicylic acid plasma concentration time-courses and total urinary recoveries to evaluate both the prodrug bioavailability and its conversion to salicylic acid [31]. Approximately 88% was recovered in the urine as salicylates and gentisates following equivalent oral doses of either acetylsalicylic acid or salsalate. However, the relative bioavailability based on salicylic acid concentrations using Eqn. 11, where AUC′ represents data following salsalate administration, is 0.85. The fact that both drug and prodrug provide equivalent and nearly complete urinary recoveries shows that total absorption was also equal, so that the decrease in plasma salicylate AUC value must be due to incomplete prodrug hydrolysis relative to its metabolism to glucuronide conjugates.

A similar approach has shown that the stearate ester prodrug of etilefrine appears to be less absorbed than is etilefrine itself. In this case, the relative etilefrine bioavailability calculated using Eqn. 11 and that based on a comparison of 24-hour urinary recoveries of drug both indicated that the total prodrug absorbed and converted was only 50% relative to etilefrine [32]. However, the mean urinary recovery of etilefrine following drug administration was nearly complete (93%), while no prodrug could be found in the urine following administration of the stearate ester. In contrast to the salsalate case, these data suggest that this prodrug decreases bioavailability due to poor absorption.

Dipivalyl epinephrine (dipivefrin) appears to penetrate the cornea approximately 17 times greater than epinephrine [33]. This diester has an increased o/w partition coefficient and appears to work as a prodrug, based on evidence for its conversion in aqueous humor following corneal application to eight patients. In contrast, the pivalate monoester of phenylephrine has been reported to have α-adrenergic activity of its own, the extent of which is approximately half that of phenylephrine [34]. In this respect, it would be regarded as a drug which is metabolized to the active metabolite, phenylephrine. Unlike dipivefrin, pharmacological response to phenylephrine pivalate was not prevented by prior administration of the cholinesterase inhibitor, echothiophate iodide.

Penicillin prodrugs which are absorbed well and converted rapidly have been reviewed previously [3]. The relative bioavailability from pivampicillin, talampicillin and bacampicillin is approximately 1.7 that of ampicillin given orally. These

prodrugs are converted on or before arrival in the blood. Their tissue to plasma concentration ratios should be similar to that for ampicillin at similar plasma concentrations. This has been observed [35] and reviewed [7] in at least one study on bacampicillin. However, extended ampicillin time-courses were observed in middle ear effusate, sinus mucosa and sputum. The ratio of bronchial secretion ampicillin concentration to serum concentration was four times higher following 800 mg of oral bacampicillin than that observed following 1 g of ampicillin itself [36]. These observations apparently serve as the basis for a 12-hour dosage interval using bacampicillin. This is one illustration of the inadequacy of either theory, experimental or both. In theory, it is not possible to change the time-course in a specific organ or tissue if the prodrug does not circulate intact, as is the case for all three of these ampicillin prodrugs [37].

Carbenicillin is acid-unstable ($t_{1/2} \cong 18$ minutes at pH 2, 35°C) and has low lipid solubility, thus limiting it to parenteral use. Two prodrug esters, carfecillin and carindacillin, are six times more stable to β-lactam hydrolysis at pH 2, whereas their calculated conversion half-lives at pH 7, 37°C (where carbenicillin is relatively stable, $t_{1/2} \simeq 500$ hours) are 8.5 and 17 hours [38]. Both esters provide carbenicillin plasma concentration time-courses which are adequate for oral use.

4.2.2. Duration

Increased duration of drug action through prodrug formation has been achieved primarily through depot injections. A successful preparation will provide rate-determining drug input from the injection site, resulting in the terminal phase for drug loss being controlled by drug input [6]. Several examples are: desoxycorticosterone pivalate, estradiol cypionate, fludrocortisone acetate, hydrocortisone cypionate, methylprednisolone acetate, testosterone propionate, dexamethasone acetate and triamcinadone hexacetonide. Fluphenazine enanthate (im) has been reported to provide a terminal phase half-life of 3.5 days, while the decanoate provided an 8-day half-life and the half-life following an im injection of drug itself was only 15 hours [39].

4.2.3. Distribution

A prodrug which is converted rapidly to drug on or before its arrival in blood cannot alter the disposition kinetics for the drug itself. It behaves instead as a drug delivery system which may alter only the bioavailable dose and input kinetics. As previously discussed, this represents the case for the prodrugs pivampicillin, talampicillin and bacampicillin, which are well absorbed orally and converted rapidly to ampicillin.

Although the bioavailable fraction of ampicillin is only 0.5, that of its analog, amoxicillin, is approximately 0.9. Sarmoxicillin was prepared to increase amoxicillin distribution. In contrast to the ampicillin prodrugs, sarmoxicillin, the methoxymethyl ester of hetamoxicillin, is absorbed partially as intact prodrug and partially as amoxicillin [40]. The AUC values (μg hour/ml) for the amoxicillin saliva concentration time-courses were increased from 0.21 following a 1000 mg oral dose amoxicillin to 0.67 for an equivalent dose of sarmoxicillin. This increased delivery of amoxicillin to saliva was ascribed to the presence of circulating prodrug. While the increased drug distribution is regarded as advantageous, sarmoxicillin was found to have the disadvantage of decreasing oral bioavailability of amoxicillin. The relative bioavailability values at three dosage levels (250, 500 and 1000 mg) as calculated by Eqn. 11 are 0.76, 0.63 and 0.59 and those based on amoxicillin urinary recovery are 0.75, 0.63 and 0.51. This agreement, together with the fact that sarmoxicillin was not found in the urine, implies incomplete absorption of prodrug rather than failure to convert the prodrug, which has an in vivo $t_{1/2}$ of 21 minutes and an in vitro $t_{1/2}$ of 25 minutes in human serum at 37°C.

N-t-Butylarterenol (tBA) is an active β-adrenoceptor agonist of short duration. Rapid metabolism following oral administration contributes to the limited duration. Bitolterol is the di-*p*-toluate prodrug ester of tBA. Bitolterol is absorbed intact orally and retained primarily in the lungs as the intact prodrug. The observed prolonged bronchodilation is attributed to slow release of the ester from lung, with hydrolysis to tBA. Pharmacological activity is terminated by tBA metabolism via conjugation or 3-*O*-methylation [41]. This successful concentration of a bronchodilator prodrug in the lungs represents an interesting example of site specificity. In contrast, Brazzell and Kostenbauder [42] found that the dipivalate and di-*p*-toluate prodrug esters of isoproterenol increased the first-pass metabolism of parent drug to 3-*O*-methylisoproterenol in the isolated perfused rabbit lung. Although these prodrugs exhibited rapid uptake by lung tissue, their subsequent conversion to parent drug was too rapid to provide any extension of isoproterenol duration in lung tissue. This is similar to the situation wherein rapid prodrug conversion in the liver results in first-pass metabolism of parent drug.

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Design of Prodrugs (Bundgaard, H., ed.)

CHAPTER 4

Sustained drug action accomplished by the prodrug approach

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1. Introduction

The use of the prodrug approach has a long tradition in therapeutics for the development of agents with prolonged duration of activity. This approach is predicated on the premise that, after administration to the host organism, a chemically modified drug molecule will result in slow release of the therapeutically active drug over a relatively defined period of time. Certain precautions must be observed when prodrugs are used to prolong drug action. Each prodrug possesses different physicochemical, pharmacokinetic, biopharmaceutic and toxicologic properties from those of its parent drug molecule, and structure-activity comparisons among prodrugs of a given drug class are virtually meaningless. Each prodrug thus becomes a drug development program in its own right. There is, however, a body of literature that serves as a guide to present and future endeavors in this area of sustained and controlled drug delivery. Many of these studies have been reviewed recently [1, 2].

This brief review will attempt to update and augment the previously cited reviews, with selected special emphasis on recent clinical experience with these drugs. Additionally, a discussion of polymeric prodrug sustained release delivery systems will be included.

2. Steroids

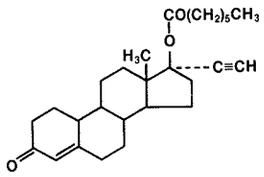
More clinical experience has been gathered with steroid prodrugs than with any other class of sustained release drugs. As with any prodrug, the steroid prodrug must be hydrolyzed prior to manifestation of therapeutic response.

2.1. CONTRACEPTIVE STEROIDS

Hormonal contraceptive prodrugs have been used clinically for about 25 years. During that time, issues relating to the complex nature of sustained release contraceptive therapy have surfaced, and involve epidemiological, sociological and political as well as therapeutic aspects, all of which continue to be discussed and debated [3]. Many advantages accrue from the use of long-acting injectable contraceptive steroids, however, and include (a) high user effectiveness (b) ease of administration, (c) elimination of patient compliance problems, (d) lack of gastrointestinal side effects and (e) ease of use in Third World countries. Two primary groups of agents have received worldwide recognition as effective contraceptives and are classified according to their duration of therapeutic activity. The first group consists of progestagens that have a duration of 3 – 6 months. The other group is the progestagen-estrogens with durations of 2 – 3 months.

2.1.1. Progestagens

2.1.1.1. Norethisterone enanthate. This C_{18} steroid ester is a derivative of 19-nortestosterone and chemically is 17α-ethinyl-17β-hydroxy-4-estrene-3-one-17-yl heptanoate (1).



Structure 1

It is administered as an oily solution and the prolonged duration is only partially due to slow rate of hydrolysis in the plasma and the liver [4]. Human plasma hydrolysis appears to play a minor role in the regeneration of the parent steroid molecule [5]. The long duration of activity of structure 1 (3 months) is postulated to be due primarily to slow release from the injection site and storage in fatty tissue [6].

The administration regimen plays an important role in the efficacy (frequency of occurrence of pregnancies) of structure 1. A study by the World Health Organization indicated that three-fourths of all pregnancies occurred during the first 3 months of norethisterone enanthate use [7], with no pregnancies occurring after week 32 (Fig. 1). Thus, WHO suggested that future studies be carried out with injections at 2-month intervals for the first 6 months, followed by a 3-month interval thereafter.

Pharmacokinetic studies [4, 8, 8a] on 1 revealed an interesting ethnic difference in the return of ovulation among Indian and Swedish women. Non-caucasian women appeared to ovulate more rapidly, perhaps accounting for its shorter

duration of activity in this population compared to other long-acting contraceptive steroids.

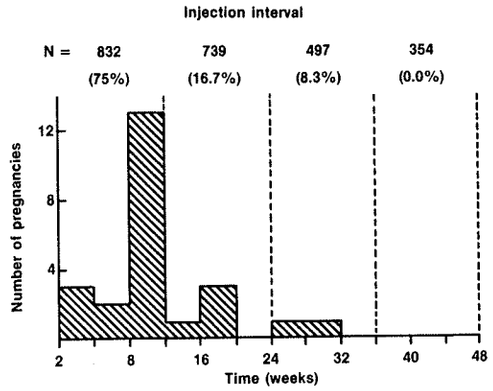
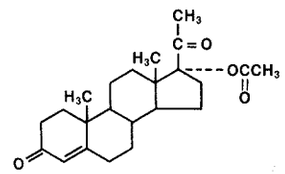


Fig. 1. Incidence of pregnancies in relation to treatment period: norethisterone enanthate [7].

Studies in rats, in which norethisterone was covalently linked to a water-soluble polymer, poly(N^5-hydroxypropyl-L-glutamine), revealed that norethisterone was released over a 270-day period. Rates decreased slowly over time but simulated zero-order kinetics. The polymeric prodrug was implanted subcutaneously and shows promise as a slow-release chemical drug delivery system in clinical medicine.

2.1.1.2. Medroxyprogesterone acetate (MPA). MPA (2) is a synthetic progesterone analog used throughout the world as a safe and effective long-acting contraceptive steroid. A dose of 150 mg given intramuscularly every 12th week inhibits ovulation for approximately 3 months and eliminates mid-cycle surging of lutenizing hormone seen usually at time of ovulation. Chemically, it is 17α-acetoxy-6α-methylpregnene-3,20-dione. The reputed bioactive species is MPA itself and not 17α-hydroxy-6α-methylpregnene-3,20 dione (medroxyprogesterone). Conventional wisdom and experience with this type of drug would indicate that hydrolysis of the 17-acetate ester might occur in vivo, thus producing medroxyprogestrone as the active drug. The fact that no concerted effort has been made to identify the 17α-hydroxy compound after administration of MPA raises fundamental questions concerning not only the bioactive species, but also the reasons for the extended duration



Structure 2

of activity and the actual mechanism of action of MPA as a contraceptive steroid. A radioimmunoassay has been developed for determining serum levels of MPA. The method utilizes goat antibodies produced against a bovine serum albumin conjugate of 3-(*O*-carboxymethyl)oxime medroxyprogesterone acetate [9] Cross-reaction of 17α-hydroxyprogesterone to the antiserum of MPA was low (< 0.25%), indicating that, if 17α-hydroxyprogesterone were present in serum, it would not be detected by this assay [10]. If MPA were indeed the prodrug form of 17α-hydroxyprogesterone, the several-months protection against ovulation by MPA would be due not only to its relative insolubility and slow release from the injection site, but also to the necessity for deacetylation to occur prior to manifestation of bioactivity.

The confusion surrounding this issue of the bioactive species could be clarified by determination of the relative ease of enzymatic hydrolysis in various animal species by sampling plasma, liver, kidney, gut wall and muscle tissue after administration of MPA. Further, an attempt must be made to determine subcellular levels of MPA and its metabolites since this is almost certainly the relevant locus of activity. It no longer suffices to determine only plasma levels of MPA, 17α-hydroxyprogesterone and their metabolic products in clinical studies since this only serves to allay concerns about bioavailability but not about bioactivity.

2.1.2. Progestagen-estrogen combinations

The combination products were designed to overcome the severe bleeding abnormalities of long-acting progestagens when given alone. Most combination preparations are administered on a monthly basis and have not been entirely successful because of the doubtful and difficult quest for an effective long-acting estrogen [11].

2.1.2.1. Dihydroxyprogesterone acetophenide/estradiol enanthate. This contraceptive combination enjoys limited marketing in several European and Latin American countries and contains 150 mg of 16α,17α-dihydroxy-4-pregnene-3, 20-dione-16,17-acetophenide and 10 mg estradiol-17β-heptanoate. It acts by inhibition of the normal functioning of the hypothalamic-pituitary-ovarian axis [12]. There have been no reported pregnancies in more than 32,000 women-months experience with this combination [13]. Injections were repeated on a monthly basis.

2.1.2.2. MPA/estradiol cypionate. Another combination that has been used on a limited basis is medroxyprogesterone acetate and the 17β-cyclopentylpropionate ester of estradiol. The duration of activity of this combination is about 30 days. Studies performed during the mid-1970s indicate that no pregnancies occurred with a total of about 1000 woman-years of use. The WHO is currently sponsoring further trials to increase experience with user-effectiveness of this suspension formulation.

2.2. Treatment of menopause

The symptomatic treatment of menopause (sweating, hot flushes, depression) has been successfully accomplished by the use of 200 mg of dehydroepiandrosterone-3-heptanoate and 4 mg of estradiol-17β-valerate in a suspension of a castor oil/benzyl benzoate vehicle. While the parent non-esterified steroids are metabolized rapidly and exhibit a short duration of action, the prodrug steroid ester combination is efficacious for an average of 5 weeks after administration [14]. Onset of action was delayed for up to 3 days as determined by radioimmunoassay. Plasma levels of parent drug were detectable for an average of 14 days for 17β-estradiol and 18 days for dehydroepiandrosterone. Hydrolysis of the ester occurred rapidly after the prodrug entered the blood stream in soluble form.

2.3. Formulation considerations

Most long-acting steroids are administered as oil solutions or aqueous suspensions and, depending on the degree of lipophilicity and steric hindrance of the prodrug, the judicious selection of a combination of steroid prodrug and formulation enables the clinician to control the duration of action. Two processes predominate when adjustments must be made to alter the duration of drug activity: (a) solubility-controlled release of insoluble drug from the vehicle to the cellular milieu, and (b) diffusion-controlled release of solubilized drug in vehicle to extracellular water. A recent study by Gerrity et al. [15] described a method of using hydrogenated soybean oil (HSO) as the vehicle for releasing testosterone, testosterone propionate (TP) and testosterone enanthate (TE) at varying rates. Results were compared to release of these drugs in a widely used vehicle, namely liquified soybean oil (LSO). Studies were undertaken using castrated male rats. The animals were killed at 1, 3, 5 or 7 days after injection and testosterone (T) parent drug serum levels as well as lutenizing hormone levels were measured using antibody radioimmunoassays. Accessory gland weight (AGW) growth was also determined. A significant difference in functional androgenicity was noted in all three parameters when HSO was used as the vehicle. Not only did accessory gland weights in the castrated animals approach those of intact controls by day 7, but serum levels of testosterone as well remained high throughout the course of the experiment, especially after administration of testosterone proprionate and testosterone enanthate. High serum levels of testosterone, however, do not correlate with potency in suppression of lutenizing hormone levels, the order of potency being TE>T in HSO>TP>T in LSO. Testosterone may thus act on the pituitary gland by way of a different mechanism than either of the testosterone esters.

HSO appears to be a unique vehicle for administering testosterone. Heating to 60°C solubilizes the drug and injection of the solution causes a solid depot to form at body temperature at the injection site. Testosterone and its esters are thus released over a long period of time. A dose of 15 mg of testerone in this vehicle maintains levels for 8 weeks and maintains AGW in orchidectomized rats [16] within intact control range for a similar period. The depot mass does not lose its integrity after

8 weeks and, in fact, can be removed from the injection site, remelted, and reinjected into another animal with continued slow release of drug.

These studies illustrate the complexity of evaluating one parameter, i.e., sustained release, while varying both the vehicle and prodrug. The differing physicochemical properties of the prodrug can have significant effects on solubility, thus affecting pharmacokinetic properties, possibly the mechanism of action, and ultimately bioactivity.

Another study by James and co-workers [17] examined the influence of the solvent on bioavailability of TP from oil-based intramuscular injections in rat gastrocnemius muscle. Release of TP from octanol, isopropylmyristate and light liquid paraffin into muscle mass was rapid, followed by storage in depots elsewhere in the body. Slow release from these naturally occurring biodepots (fatty tissue) may account for the extended biological half-lives of testosterone. These half-lives in muscle can be roughly correlated with distribution coefficients of the drug (Table 1). ^{14}C urinary levels are significantly longer than in muscle and are essentially constant regardless of solvent, implying that release from the injection site is not rate determining.

TABLE 1
Solvent Effects and Testosterone Availability

Solvent	Distribution coefficient[a]	Half-lives (hours)	
		Urine	Muscle
Light liquid paraffin	1.4	33.4	5.4
Isopropylmyristate	4.2	34.3	18.0
1-Octanol	5.3	36.7	22.3

Data from [17].
a. Solvent/water $\times 10^3$.

3. Neuroleptics

Neuroleptics are used in the treatment of a variety of mental disorders, especially psychoses. A depot neuroleptic can be regarded as one which affords therapeutic levels of parent drug for at least 7 days after a single administration. Virtually every long-acting neuroleptic is (a) a prodrug, (b) given by the intramuscular route, and (c) bioactive at the microgram level. Neuroleptic prodrug esters are extremely lipophilic and, when dissolved in an oil vehicle, exhibit a sustained release profile due to their high oil/water partition coefficients and subsequent slow release from the injection site. The prodrug is hydrolyzed rapidly after entry into the bloodstream [18].

Clinically, depot neuroleptics possess several advantages over the short-acting oral forms. Principle among these are (a) ease of administration, (b) reliable therapeutic effect with no increase in tolerance, (c) enhanced patient compliance, (d) reduced and more efficient daily dosage, (e) reduced relapse and rehospitalization rate, and (f) enhanced rate and incidence of 'normal-life' reintegration and resocialization.

A study of 76 patients consisting in part of 38 paranoid schizophrenics and 23 residual psychotics compared treatment of these patient populations with oral versus depot therapy [19]. With the use of the depot form of the drug (fluphenazine decanoate, flupenthixol decanoate), the number of relapses requiring hospital treatment averaged 0.8 per patient, while the average was 2.5 per patient on short-acting orally administered neuroleptics (penfluridol, fluspirilene). Hospital residence time for treatment was reduced from an average of 34 to 8 weeks. There was no readily discernable correlation to sociodemographic data such as social class, academic background, professional achievement, sex, age, or age of onset regarding treatment type (long vs. short, oral vs. intramuscular).

A survey of primary schizophrenic patients indicated that the greatest advantage for the use of depot neuroleptics was the relief afforded these patients without the necessity of hospitalization [20]. Some disadvantages were noted, however, and include (a) the fear of injection, (b) side effects such as drowsiness, tiredness, and extrapyrimidal syndrome, (c) onset of depression, especially with fluphenazine decanoate, and (d) loss of control of course of therapy once administration has occurred.

The question of the value of neuroleptics in prevention of relapse during the lifetime course of schizophrenia has never been answered sufficiently, primarily due to the challenge that such long-term therapy poses to any system of medicosocial care. Issues such as organization and management of information required to ensure the current status of patients living in a community setting, the duration of therapy and the non-traditional clinical doctor/patient situation all tend to complicate the acquisition of meaningful data. In a 12.5-year study, a total of 143 schizophrenics were followed on various regimens of depot neuroleptics [21]. The pre-depot and post-depot experience of the entire patient population indicated that pre-depot treatment hospitalization amounted to 19,510 days whereas post-depot treatment hospitalization totaled 4,376 days. It was demonstrated further that hospital stays did not increase over time, indicating that patient tolerance to depot neuroleptic drugs did not increase. These few studies dramatically illustrate the profound impact of this mode and administration of neuroleptic therapy via the prodrug approach.

3.1. Haloperidol Decanoate

Haloperidol is a potent, orally active central nervous system depressant, sedative

and tranquilizer. Peak plasma levels are seen within 2 – 6 hours after administration. Excretion is slow, with trace amounts detectable in plasma for several weeks. Haloperidol decanoate is the depot form of haloperidol and, when administered as a 50 mg/ml sesame oil solution, results in assayable levels of haloperidol for about 1 month [22].

Multidose administration (4-week intervals) of haloperidol decanoate resulted in peak levels of haloperidol within 3 – 9 days. Steady-state levels were achieved after three to six injections and were related to the dose administered [23]. Good correlation was found between the dose of haloperidol decanoate and free haloperidol plasma levels. No long-term accumulation of the prodrug or parent drug molecule was seen. While a high blood level of haloperidol does not necessarily substantiate high or adequate brain receptor levels of the drug, low blood levels are probably a primary cause for poor or declining clinical response [24].

The determinant for the long duration of activity of haloperidol is due to the slow release of haloperidol decanoate from the injection site. The ester is hydrolyzed rapidly once it enters the circulation. The prodrug thus acts primarily as a lipophilic form of haloperidol that is easily solubilized in the oleaginous vehicle.

3.2. Fluphenazine decanoate and flupenthixol decanoate

Both fluphenazine and flupenthixol are potent neuroleptics and tranquilizers that have found wide acceptance in psychiatry. Both drugs suffer from the usual problems associated with short-duration therapy. The onset of action for fluphenazine is about 1 hour, with a duration of 6 – 8 hours. The range is similar for flupenthixol, which is also accompanied by extrapyramidal side effects. Sedation is also common. The decanoate esters of these drugs illustrate interesting differences between the drugs. The most significant is the elevation of mood, with fewer associated extrapyramidal side effects ascribed to flupenthixol decanoate. A comparative clinical trial in a group of 22 chronic and acute schizophrenic outpatients indicated no difference in measured clinical response between the two prodrugs [25]. Flupenthixol decanoate enhanced work performance to a significant degree, while fluphenazine decanoate significantly increased selected mood elation scores. These clinical and pharmacological indices of clinical well being lasted longer than 4 weeks.

The relative efficacy and side effects of fluphenazine enanthate and decanoate was recently compared in a 7-month double-blind study of 50 schizophrenic outpatients [26]. It has long been assumed that the duration of activity is greater for the decanoate (C_{10}) than the enanthate (C_7) ester of fluphenazine. An injection interval of 4 weeks for the decanoate and 2 weeks for the enanthate was chosen to test this premise. Comparisons were made between the need for long-term antiparkinsonian medication since one of the side effects of fluphenazine therapy is parkinson-like symptoms (akinesia, akathisia, and tremors). Since such side effects have been implicated with fluphenazine administration, the duration and intensity of such effects

might be expected to correlate with duration of therapy. There were no statistically significant differences, however, between the treatment groups as far as the number of patients requiring antiparkinson medication using the Extrapyramidal Symptom Rating Scale. Brief Psychiatric Rating Scale (BPRS) scores, a measure of the patients' mental status, at week 26 of the study indicated that at the midpoint of the fluphenazine decanoate 4-week injection interval no differences were apparent in the ability of the two ester prodrugs to suppress schizophrenic symptoms. At week 28, however, fluphenazine enanthate was superior in suppressing symptoms, indicating that fluphenazine decanoate efficacy does not extend for 4 weeks and should be administered at shorter intervals. This type of study serves to illustrate the complexity of ramifications that are produced by the apparently simple change of ester chain length on the parent drug molecule.

4. Dopamine agonists

4.1. Apomorphine and *N-n*-propylnorapomorphine

Apomorphine (APO) and its analog *N-n*-propylnorapomorphine (NPA) are potent dopamine agonists and act selectively and directly on dopamine (DA) receptors. They have been used for more than 80 years as sedatives as well as for the management of a variety of psychoses, including schizophrenia [27, 28]. Clinical success has been limited due to low oral absorption and short duration of activity. Baldessarini et al. [29] synthesized a series of 10,11-*O,O'*-diester prodrugs of APO designed to enhance its availability to the CNS as well as increase the duration of action. It was found that the decreased hydrolysis rates of these esters (dipropionyl, di-*i*-butyryl, dipivaloyl) prolonged and increased the behavioral activity (stereotypy) in the rat and mouse up to six times the duration of APO itself [30]. The diesters find limited clinical utility, however, because they are not active orally.

An interesting prodrug of NPA that simulates the action of NPA and APO is 10,11-methylenedioxy-*N-n*-propylnorapomorphine (MDO-NPA) [31]. This derivative is equally potent by oral and parenteral administration in stimulating motor activity in rats. Oral activity was attributed to protection of the hydroxyl groups by the cyclic alkylene linkage, thus preventing 'first pass' oxidation, substitution or conjugation. The duration of activity for MDO-NPA is about 60% longer than that of NPA or APO alone (Table 2). Further, duration is dose-dependent. The duration of stereotypy (gnawing, sniffing, startle response) as a function of dose was revealing (Fig. 2). Unlike APO or NPA, MDO-NPA exhibited a depot effect when the dose was increased, and this can be explained by the lipophilic nature of the NPA prodrug. The prodrug is retained in tissue for longer periods of time, and dissolves at a slower rate at physiological pH, with subsequent hydrolysis to the parent drug molecule. No clinical utility has been demonstrated for MDO-NPA at present [32].

TABLE 2
Correlation of Behavior Response, Duration of Effect and Route of Administration

	Stereotypy score[a]			Duration of effect (minutes)		
	Perorally	Subcut-aneously	Intraper-itoneally	Perorally	Subcut-aneously	Intraper-itoneally
MDO-NPA	17.0 ± 1.2	17.5 ± 0.4	16.5 ± 0.8	112 ± 20	106 ± 10	116 ± 12
NPA	0	17.5 ± 0.8	17.5 ± 1.0	0	72 ± 6	70 ± 10
APO	0	17.5 ± 0.4	16.5 ± 2.4	0	70 ± 5	72 ± 12

a. 0 = No stereotypy to 3 = continuous sniffing, mouth movement, low exploratory activity; maximum score = 18/hour. Dose = 1 mg/kg given to six rats per group [31].

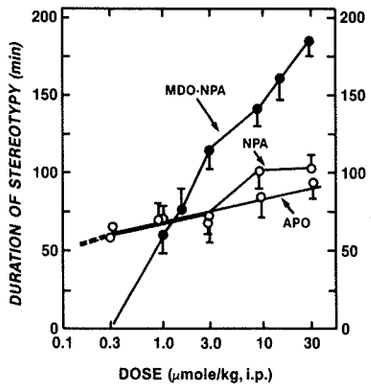


Fig. 2. Relationship of dose to duration of stereotyped behavior for selected aporphines [31].

4.2. ADTN

ADTN is a cyclic analog of dopamine found to cause a strong stimulation of locomotor activity in conscious mice for up to 18 hours when injected intraventricularly [33]. In the urethane-anesthetized guinea pig, ADTN injected intravenously exhibited a hypotensive response similar to dopamine. Lipophilic prodrug esters of 6,7-ADTN (2-amino-6,7-dihydroxytetrahydronaphthalene) were synthesized in an effort to transport the parent drug across the blood-brain barrier [34]. Of the four diesters tested intraperitoneally (diacetyl, di-*i*-butyryl, dipivaloyl and dibenzoyl) the latter two prodrugs produced sustained levels of 6,7-ADTN parent drug for several hours in the rat corpus striatum and cerebellum. Peak levels of ADTN after administration of the dibenzoyl ester of ADTN intraperitoneally were almost 6× greater in rat striatum than those resulting from an equimolar dose of

6,7-ADTN itself. The onset of action was about 3 hours, indicating that hydrolysis of the diester is somewhat slow. Other factors, however, that cause this delay in appearance of peak ADTN levels include solubility in physiological fluid and metabolic inactivation by catechol-*O*-methyltransferase (COMT). The metabolite is devoid of dopaminergic activity [35].

5. Sympathomimetics

5.1. Bronchodilators

5.1.1. Bitolterol mesylate

This sympathomimetic amine prodrug is the *p*-toluene diester of *N*-*t*-butylarterenol. Chemically it is α-[(*tert*-butylamino)methyl]-3,4-dihydroxybenzyl alcohol 3,4-di-*p*-toluate methanesulfonate. It has been shown to concentrate selectively in lung tissue, which is rich in esterases, and slowly hydrolyze to *N*-*t*-butylarterenol, the active β_2-adrenoreceptor agonist [36]. The onset of action is rapid and the bronchodilator effect lasts for more than 6 hours [37]. A good split in activity is seen with bitolterol mesylate. Only 1/13× the chronotropic (β_1-adrenergic cardiac stimulatory) effect of isoproterenol was seen with bitolterol [38]. The primary β_2-adrenergic (bronchodilator) effect was attributable to the greater concentrations of esterase in pulmonary tissue vs. heart, thus concentrating hydrolyzed prodrug or parent drug in the lung.

5.1.2. Bambuterol

The polar bronchodilator molecule terbutaline (α-[(*tert*-butylamino) methyl]-3,5-dihydroxybenzyl alcohol sulfate) has been made highly lipophilic by the formation of its bis-*N*,*N*-dimethylcarbamate prodrug derivative (bambuterol). Single dose oral administration of bambuterol to dogs results in therapeutic levels of terbutaline for up to 20 hours duration [39]. Terbutaline itself exhibits effective levels for 5 – 7 hours. The reason for the extended duration of bambuterol appears to be survival of first-pass metabolism by virtue of the stability inherent in the carbamate linkage. Hydrolysis occurs stepwise to the monocarbamate and finally to terbutaline parent drug. Delayed onset of action is most likely due to this stepwise activation.

Bambuterol is currently undergoing phase I and II clinical evaluation as an orally effective β_2-adrenergic bronchial smooth muscle relaxant and antiasthmatic agent in man [39].

5.1.3. Dipivalyl epinephrine

The routine control of elevated intraocular pressure caused by glaucoma has traditionally been treated with epinephrine. Treatment with this drug suffers many shortcomings, including poor bioavailability, high frequency of administration, and

troublesome side effects. The preparation of the 3′,4′-dipivaloyloxy ester of epinephrine (dipivefrin, DPE) enhanced by oil/water partition coefficient to greater than 600× that of epinephrine [40]. After penetration as the intact prodrug ester the eye, hydrolysis occurs in the corneal tissue. Ocular penetration studies of DPE indicate that concentrations of substrate are about 10× greater than when epinephrine alone is administered [40]. A dose of 0.1% DPE applied topically to the eye twice daily is equivalent to a 2% application of epinephrine administered with the same frequency. Incidence of side effects (blood pressure elevation, conjunctivitis, corneal edema) were significantly reduced with DPE use.

While the use of DPE does not enhance duration in the classical sense, its use does overcome many problems relating to effective treatment not found with epinephrine. DPE 0.1% solution, however, did maintain intraocular pressures below 21 mmHg for a period of 6 months on a b.i.d. regimen [41].

6. Polymeric prodrugs

The chemotherapeutic utility of polymeric prodrugs, in which a drug is attached covalently to a polymer backbone, has been the focus of intense research in the past decade. The range of options in preparing polymeric prodrugs for a specific purpose is limited only by the imagination of the investigator. Thus, a wide variety of synthetic or naturally derived biopolymers possessing varying degrees of solubility that contain functional groups and spacers for attaching all types of drug molecules which can be actively or passively targeted to specific organs, tissues or cells are currently under study. This rational approach, grounded in a fundamental understanding of biochemical, physical chemical, immunologic and toxicologic principles, is perhaps the new frontier in drug delivery research.

Polymeric prodrugs possess unique properties distinct from those of lower-molecular-weight prodrug substances. Because of their molecular size, polymers could be expected to pass with difficulty across biomembranes, thus altering their excretion patterns. Further, solubility can be easily altered by varying the type and number of solubilizing groups on the polymer backbone, and a relatively insoluble polymeric prodrug could be utilized as a sustained release delivery system. Thus, by altering solubility, which in turn affects the rate of absorption and excretion, one can easily fabricate a long-acting prodrug. In general, the pharmacokinetic properties of the polymer can be adjusted to reflect desirable traits in the prodrug. The addition of solubilizing moieties to one area of the polymer, for example, can render the entire macromolecule nontoxic and readily soluble at physiological pH. A second region of the macromolecule can be utilized as the 'drug attachment center' in which the parent drug molecule is covalently linked, with or without spacer groups, to the polymer backbone. Another region of the macromolecule can be chemically programmed to serve as the site-specific transport system for the entire polymer pro-

drug. Technology is currently available via block copolymerization and statistical telomerization techniques to program complex macromolecules to achieve the desired pharmacokinetic properties. These considerations are summarized graphically in Figure 3. Ringsdorf, in two excellent reviews [42, 43], has elaborated further on the notion of bioactive polymer/drug systems.

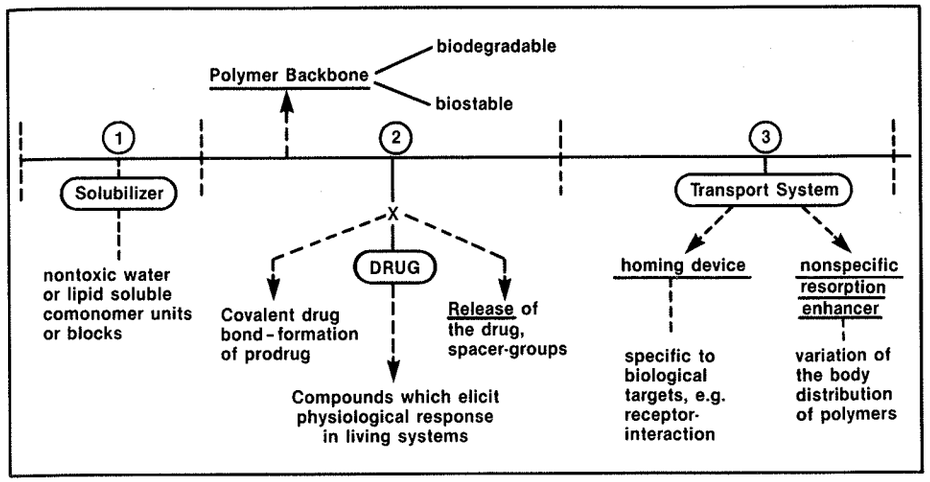
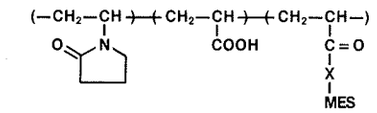


Fig. 3. Alteration of pharmacokinetic properties of polymeric prodrugs – general scheme (adapted from [42]).

Characteristics that are desirable in a polymeric prodrug delivery system include: (a) timely release of drug parent molecule from the drug-polymer conjugate at the appropriate site by biochemical or other means, (b) polymeric backbone must be nontoxic, nonimmunogenic and nonantigenic and (c) the backbone must be either biodegradable or easily removed after elicitation of therapeutic response. When dealing with such complex prodrug delivery systems, certain assumptions must be made. Perhaps a most important consideration is that the polymeric backbone is not intrinsically bioactive but merely acts as the 'delivery system' portion of the drug delivery system. Secondly, in the case of depot drugs, the backbone serves as the primary regulator of those pharmacokinetic properties that enhance the depot response, e.g., solubility and absorption. Third, regeneration of the parent drug is essential since covalent linkage of the pharmacophore renders the drug biologically inactive.

There exist many bioactive polymers, both naturally occurring [44] and synthetically derived [43]. Included among these are enzymes, DNA, interferon and insulin, which occur naturally, and the synthetic plasma substitutes (polyvinylpyrrolidone), antiviral agents (polymethacrylic acid), antisilicosis agents (poly(2-vinylpyridine-1-oxide)) and others.

Attachment of the drug to the polymer backbone has received much attention due to the importance of the availability of a nontoxic, easily bioreversible linkage that can present the parent drug molecule to the appropriate receptor. Many polymerizable active esters and amides are available for selectively attaching the drug to the polymer chain [45] and include imidazole, trichlorophenol, *N*-hydroxysuccinate and *N*-hydroxybenztriazole. In many instances, direct attachment of the drug to the polymeric functional group is not appropriate since hydrolysis of the prodrug cannot occur due to steric problems encountered in promoting a proper drug-receptor interaction. The use of temporary or permanent spacers is necessary in order to distance the drug from the polymer backbone or coil so that hydrolysis and the therapeutic response can occur. Thus, when the psychotomimetic agent mescaline (MES) was attached directly to copolymers of *N*-vinylpyrrolidone, acrylic acid and amides, no release occurred in vivo. Excretion for up to 17 days occurred, however, when the spacer group glycyl leucine was used [46]. Administration of free mescaline provided excretion levels for a period of 20 hours.



X = -; no hydrolysis or excretion
X = -GLY-LEU-: 17 days excretion
Mescaline (MES) only: excretion for 20 hours

One of the most creative approaches combining the use of a soluble, site-directed delivery system containing a covalently bound alkylating agent 'warhead' was undertaken by Rowland et al. [47]. Initial attempts to directly conjugate these agents with rabbit antitumor immunoglobin (Ig) were successful; however, tumor suppression was dependent on the use of large quantities of conjugate. Direct coupling of drug to immunoglobulin resulted in drastic physicochemical changes in antibody activity and solubility which resulted in low bioactivity. To overcome this problem, a polyanionic polymer backbone, polyglutamic acid (PGA) was used to covalently attach the alkylating drug *p*-phenylenediamine mustard (PDM) as well as effect the covalent linkage of the Ig. The resulting chemical drug delivery system is illustrated in Figure 4. The PGA polymer was in the molecular weight range of 35,000. The Ig was derived from a rabbit antiserum against EL4 mouse lymphoma cells made specific by repeated absorption with normal mouse spleen cells [48]. After purification dialysis, the complex contained PDM/PGA/Ig in the ratio of 2:8:10 mg per ml. Efficacy was determined by intraperitoneal innoculation of live EL4 cells (5×10^4) in several groups of C57BL/6 mice. This challenge is about 10,000× the LD_{50} dose. Each mouse received 4× daily injections 24 hours after the challenge dose. The fascinating results are outlined in Table 3.

It is apparent that the conjugated PDM/PGA/Ig copolymer possesses the right combination of solubility, site-specificity and biolability to make PDM an ef-

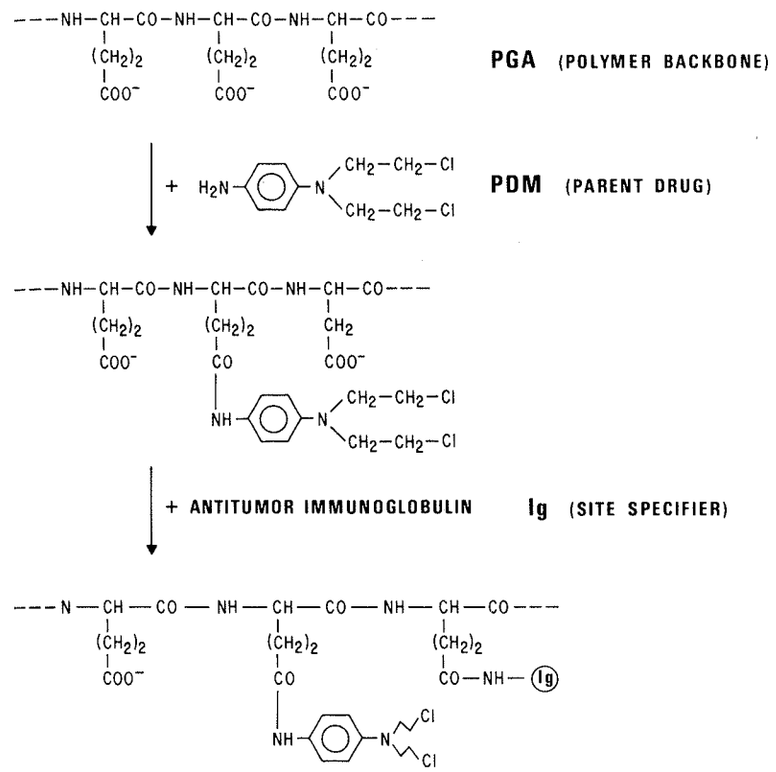


Fig. 4. PDM polymeric prodrug delivery system (adapted from [43]).

TABLE 3
Duration of Efficacy of PDM Polymeric Prodrug

Drug	Median survival time for 50% (days)	Mice alive free of tumor at day 60
NaCl-solution (control)	13	0/5
Ig alone	19	0/5
PGA-PDM-conjugate	25	1/5
PGA-PDM-conjugate + free Ig	38	2/5
PGA-PDM-Ig-conjugate	>100	5/5

Data from [47].

ficacious, long-acting (survival time) cytotoxic agent. Finally, the PDM/PGA/Ig polymeric prodrug enhances the therapeutic ratio of PDM, which results in an agent that is less toxic to normally proliferating cells. The LD_{50} of the prodrug is about 200 while the LD_{50} of PDM = 5. Such enhanced efficacy provides yet another reason for utilizing a polymeric prodrug as an alternate and creative approach to the design of efficient and effective drug delivery systems. Rowland [49] has obtained similar results to those obtained above by using the neutral and water-soluble polymer, dextran, conjugated to PDM and Ig.

Several well-written reviews have appeared on the general topic of polymeric drug delivery systems [42–44, 50–54]. Most treat the subject from the standpoint of site-directed rather than sustained release drug delivery.

6.1. Anticancer agents

6.1.1. Mitomycin C

Mitomycin C (MMC) has been used extensively in the clinic as an antitumor antibiotic against human neoplasms, including chronic myelogenous leukemia and solid tumors of various organs [55]. Shortcomings to its use include toxicity (GI damage and delayed myelosuppression) and short duration of activity. A covalent mitomycin C-dextran conjugate (MMC-D) was synthesized by Kojima et al. [56] and shown to exhibit plasma concentrations of MMC for up to 8 hours after intraperitoneal administration in mice bearing Ehrlich ascites carcinoma or B16 melanoma. A significant reduction in toxicity and increase in survival time was noted when compared to administration of MMC alone. The advantage was apparent only at doses above 5 mg equivalent MMC kg^{-1}. MMC administered as parent drug was active at 1–5 mg kg^{-1}, indicating that the MMC-D conjugate acted as a prodrug and release of MMC from the dextran polymer backbone was rate-limiting. This slower release, however, resulted in a substantial decrease in toxicity. MMC given alone exhibited plasma levels for approximately 2 hours. Urinary excretion rates correlated quite consistently with plasma absorption profiles. Mean survival time against B16 melanoma was 13 days for MMC (2.5 mg kg^{-1}) versus >33 days for MMC-D (8.0 mg MMC equivalent kg^{-1}).

Other high-molecular-weight prodrug polymers of MMC were prepared and their physicochemical properties vis-a-vis antitumor activities examined [57]. Included in this study were conjugates of MMC and bovine serum albumin (MMC-BSA), poly-L-glutamic acid (MMC-PGA) and dextrans (MMC-D) of varying molecular weight. In general, as the molecular weight of the MMC-polymer conjugate increased, the maximum activity against B16 melanoma or P388 leukemia in mice was greater. The mean survival time (days), an indication not only of site-specificity but also of duration, increased in a dose-related manner. Table 4 summarizes the effects of molecular weight, dose and MMC conjugate type on survival time. The results, although somewhat equivocal, illustrate the merit in the use of polymeric prodrugs

TABLE 4
Comparison of Dose, Molecular Weight (MW) and Survival Time for Mitomycin C Prodrugs in Mice with B16 Melanoma

Compound	Dose[a]	MW of polymer backbone	MMC content (w/w%)	Mean survival (days)	T/C%[b]
Control	–	–	–	15.6	100.0
MMC	5.0	–	100	30.3	194.4
MMC-D	5.0	9900	10.8	20.2	129.3
	10.0			25.7	164.5
MMC-D	5.0	64,400	11.8	21.5	137.8
	10.0			27.3	175.2
MMC-D	5.0	487,000	10.1	19.0	121.8
	10.0			>32	>205
MMC-PGA	5.0	14,000	21.2	>39.5	>253
	10.0			30.3	194.4
MMC-BSA	5.0	66,000	5.0	17.7	113.3

Data from [57].
a. mg equivalent MMC/kg.
b. Ratio of mean survival time of treated vs. control group.

for enhancing antitumor efficacy while concurrently diminishing toxicity and extending duration (survival time). The interplay of several factors, such as the ionic charge state and aggregation and folding propensity of the various polymers in a physiological environment, lipid and aqueous solubility of the conjugates and ease and degree of regeneration of MMC from the prodrug polymer, all serve to frustrate a straightforward interpretation of the results. Preliminary results are encouraging enough, however, to continue the search for other polymeric antitumor prodrugs that could enhance depot bioavailability.

Sasaki et al. [58] have examined a series of lipophilic mitomycin C benzyl- and benzoyloxy-1α-*N*-substituted prodrugs for activity against P388 and L1210 leukemia systems in vivo. While certain of these derivatives exhibited activity equal to or better than MMC, no depot effects were apparent.

Amethopterin (methotrexate, MTX) has been linked covalently to bovine serum albumin and shown to exert a dramatic effect in prolonging survival time in BDF_1 mice innoculated with 10^6 L1210 cells [59]. Survival time increased from 8 days (control) to 15 days with use of the MTX-albumin polymer. Hydrolysis proceeds slowly, thereby increasing by almost 2-fold the availability of MTX.

6.2. Antilipolytic agents

6.2.1. Nicotinic acid

Covalently linking nicotinic acid (NA) to the non-toxic polymers starch and dextran

resulted in substantial depot bioavailability of NA over a period of 12 – 13 hours when given orally to the rat [60]. Nicotinic acid exhibits lipolytic activity for a period of 2 hours. The decrease in plasma free fatty acids (FFA), a measure of lipolytic activity, was of lesser magnitude but more constant after administration of the NA conjugates. Further, no rebound in FFA levels was seen at the end of the trial.

7. Summary

The area of chemical sustained release drug delivery systems is emerging as an important approach to the efficient delivery of complex and often toxic molecules to specific sites within the body for prolonged periods. The examples cited in this brief overview are designed to impart the flavor and variety of the approaches that have been successfully employed and that are currently under intense investigation. Especially true of polymeric prodrugs, the future appears to offer interesting possibilities for targeting drugs to specific organ, tissue, and cellular and subcellular systems. The rational use of the principles of cell biology, immunology, biophysics, pharmacology and physical organic chemistry will provide a much needed interdisciplinary approach to the study and solution of some of the most difficult problems in this important drug delivery technology.

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Design of Prodrugs (Bundgaard, H., ed.)

CHAPTER 5

Site-specific drug delivery via prodrugs

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1. Introduction

The goal in most drug development programs is the design of agents which are optimally effective in curing a defined ill while producing minimally harmful or unpleasant side effects. Since early this century, when Ehrlich [1] proposed his 'magic bullet' concept, scientists have been attempting to design drugs and drug delivery systems that 'target' drugs for particular sites, be they a tissue, organ, cell line, enzyme, bacteria or virus, etc., with the hope that such site-directed or site-specific drug delivery would optimize therapy and minimize toxicity.

The purpose of this chapter is to address, in a critical manner, whether prodrugs can provide site-specific delivery or the targeting of parent active drugs to their site of action. The idea that prodrugs could provide for such delivery is not new. Ehrlich's 'magic bullet' concept of a drug-carrier complex which is designed to deliver the drug exclusively to a particular target cell type could be considered the forerunner of such an idea if the drug-carrier degrades, releasing the drug at the site of action. Albert [2], as early as 1958, also described the idea of a 'pro-agent' or 'pro-drug' that could be used as a non-toxic therapeutic agent.

Unfortunately, except for a few isolated exceptions, many of which could be considered a posteriori, scientists have not been very successful at achieving systemic targeting of drugs in vivo via any drug delivery method. This is primarily because of the complexity of systemic site-specific delivery and because many conceptually attractive theories for trying to achieve targeting are partially flawed. Stella and Himmelstein [3, 4] recently critiqued, via modelling experiments, the idea of achieving site-specific delivery via prodrugs. They concluded that such delivery is possible when the physicochemical properties of the parent drug and prodrug are optimized in accord with the properties of the target site. Since there appeared to be important interplay between the properties of the parent drug, site, and prodrug, it was concluded that prodrugs of most currently useful therapeutic agents would have dif-

ficulty achieving further site-specific delivery. This point will be discussed in greater detail later.

In this chapter we will briefly reiterate the basis for and the conclusions of our previous modelling studies and review the literature for examples of site-specific delivery based on site-specific transport, drug release, and the importance of the physicochemical properties of the parent drug to the site-specific delivery of drugs via prodrugs. This chapter will not address extensively the question of local drug delivery since the best examples of this subject are dermal and ophthalmic drug delivery and these are adequately addressed in Ch. 8 and various other chapters. In addition, the chapters on prodrugs and cancer chemotherapy (Ch. 9), and macromolecular prodrugs (Ch. 10) will address aspects of site-specific drug delivery via prodrugs. Therefore, this review will not be comprehensive but will summarize our thoughts on the current and future status of research into low-molecular-weight prodrugs for systemic site-specific delivery.

2. Modelling of prodrugs and site-specific drug delivery

In our earlier papers [3, 4] we proposed a simplified pharmacokinetic model for a prodrug which reflected its distribution to a target organ or site and its metabolic conversion to the parent molecule in the body proper as well as in the target organ. This model is shown in Figure 1. The model assumes that the prodrug is introduced

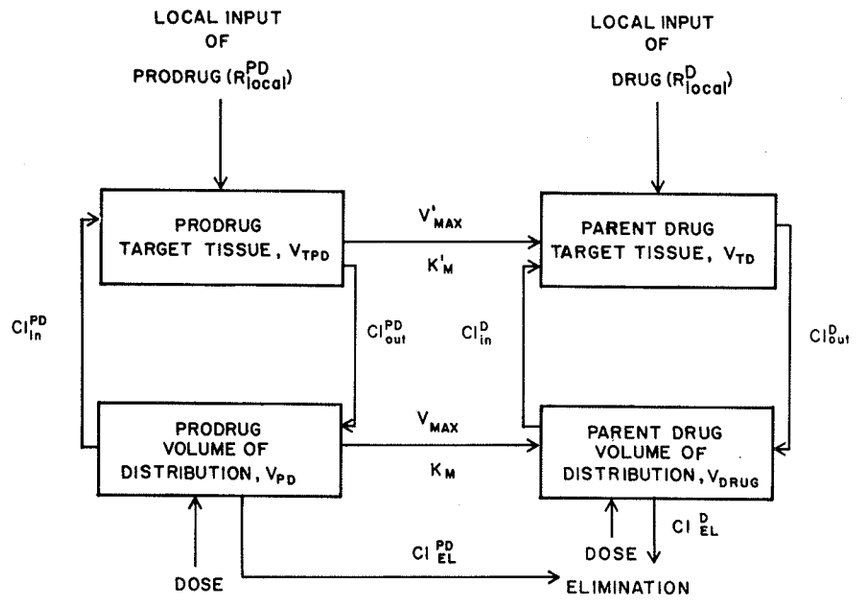


Fig. 1. Pharmacokinetic model used in simulations for site-specific delivery of drugs via prodrugs.

into the body as a single dose with various input functions and distributes through a volume, V_{PD}, and into the target organ of volume V_{TPD}, with a linear clearance or transport rate in, CL^{PD}_{in} (in ml/minute), and a clearance or transport rate out, CL^{PD}_{out} (in ml/minute). The prodrug is converted to the parent drug in the target organ and in the rest of the body via a saturable process described by a Michaelis-Menten form with suitable K_m and V_{max} values. The V_{max} and V'_{max} terms have the units of mass of prodrug metabolized per unit time and are converted to relative enzyme activity on a per unit volume of tissue, by dividing the values of V_{max} and V'_{max} by V_{PD} and V_{TPD}, respectively.

Similarly, transfer clearances and volumes are defined for the parent drug. The prodrug and drug are cleared from the total body via urinary excretion or metabolism (non-productive in the case of the prodrug), CL^{PD}_{el} or CL^{D}_{el}, respectively. The assumption is made that $CL^{PD}_{el} = 0$ ml/minute, and local input routes and rates, reflected by the R^{PD}_{local} and R^{D}_{local} symbols in Figure 1, are not considered here. In all the simulations only systemic drug or prodrug administration via intravenous bolus dosing is considered.

All simulations are performed by setting up the appropriate mass balance equations, e.g., (rate of change of parent drug accumulation in the target organ) = (net rate in by transport) + (rate in by metabolism of the prodrug). The equations are numerically integreated by computer and a modified Hamming's predictor-corrector method.

Simulation curves are generated by fixing various parameters in the model while varying others. As a result of a systematic study involving many simulations, the following general conclusions are found. For site-specific delivery to be achieved via prodrugs:

1. The prodrug must be readily transported to the site of action, and uptake to the site must be rapid, essentially perfusion rate-limited.
2. Once at the site, the prodrug must be selectively cleaved to the active drug relative to its conversion at other sites, i.e., it must be selectively cleaved relative to cleavage in highly perfused tissues such as liver, kidney, etc.
3. The active drug, once selectively generated at the site of action, must be somewhat retained by the tissue.

Until the studies by Stella and Himmelstein [3, 4], the first two points had generally been addressed. The last point, site retention, is not generally recognized as being important, but is probably the basis for many of the failures.

These modelling experiments [3, 4] reinforced the fact that there is a strong interplay between various parameters, such that optimal site-specific delivery will only be truly possible when all aspects are considered. However, it is interesting to consider historically the various factors that previous investigators have considered desirable for achieving site-specific delivery, i.e., site-specific transport and release.

3. Systemic site-specific transport

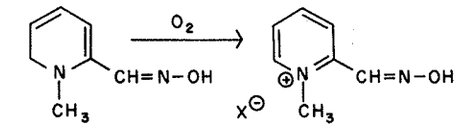
3.1. ALTERED PASSIVE TRANSPORT

If drug availability to a site limits its activity, should not a prodrug capable of reaching the site improve the effectiveness of the drug? Creaveling et al. [5] and Daly et al. [6] have demonstrated increased permeability of norepinephrine derivatives to the brain (3,4-triacetyl and 3,4-trimethylsilyl derivatives). These proposed prodrugs enter the brain 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 causes the prodrug to drain from the target site. A similar observation was made recently by Shashoua et al. [7] when they studied the brain uptake of γ-aminobutyric acid (GABA) in the form of various aliphatic and steroidal esters. All the esters were taken up into the brain substantially faster than GABA, but only the cholesteryl ester of GABA depressed the general motor activity of mice and rats. The authors concluded that the activity of the esters "was dependent on their capacity to release GABA by enzymatic activity and their lipid solubility". The issue of transport and release is critical.

Improved passive permeability to a target site presents other problems. To begin with, a more lipophilic prodrug may be better transported to its target site, but this improved transport is non-specific, resulting in all tissues possibly receiving greater drug (or prodrug) exposure.

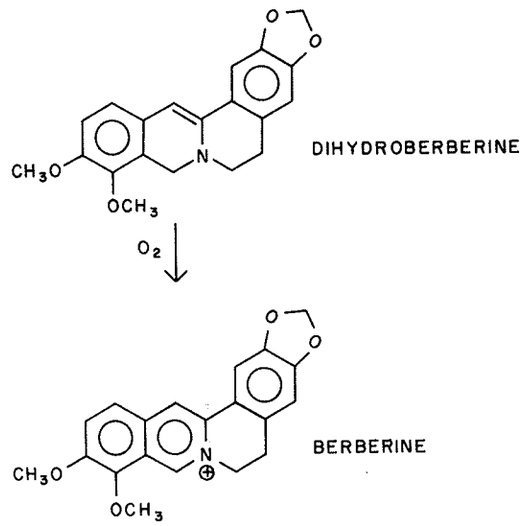
One tissue where improved passive permeability can lead to significant advantages is drug delivery to the brain through the blood-brain barrier (BBB). Entry of drugs into the central nervous system (CNS) through the BBB is made difficult because, unlike systemic capillaries, the endothelial cells of CNS capillaries have very tight junctions and are devoid of intercellular spaces [8 – 10]. The endothelial cells are also relatively void of pinocytotic activity [8,11]. Therefore, drugs which are capable of entry into other systemic tissues have a greater difficulty entering the CNS. For example, the agent, *N*-methylpyridinium-2-carbaldoxime chloride (2-PAM), used in the treatment of organophosphate poisonings by reactivating acetylcholinesterase by the nucleophilic attack of the oxime oxygen on the phosphorous of the phosphorylated enzyme, is not capable of entering the CNS because of its polarity. If a patient has been exposed to an organophosphate poison capable of entering the CNS, CNS deactivated acetylcholinesterase cannot be reactivated by the administration of 2-PAM. A prodrug of 2-PAM, the corresponding dihydropyridine (Pro-2-PAM), was synthesized and evaluated by Bodor et al. [12, 13] and Shek et al. [14, 15] on the suggestion of Dr. R. Borchardt. Pro-2-PAM is relatively unstable and is oxidized rapidly in vivo (including in the CNS) to 2-PAM [12 – 15].

Unlike the studies with the norepinephrine derivatives, Pro-2-PAM was not only capable of penetrating the BBB but was also capable of regenerating 2-PAM in the



PRO-2-PAM 2-PAM

CNS, since after systemic administration of Pro-2-PAM and 2-PAM to mice, 13 times as much 2-PAM was present in mice brains after prodrug treatment compared to 2-PAM treatment. This was confirmed further by the fact that acetylcholinesterase reactivation in mice brain after the mice had been pretreated with diisopropyl flurophosphate was far superior with the prodrug compared to the parent drug [10]. The reader is directed to an extensive review by Bodor and Brewster [10] on the subject of the use of the dihydropyridine approach to improved CNS delivery of pyridinium quaternary ammonium compounds, including the delivery of berberine as its dihydro prodrug. Berberine has an octanol/water partition coefficient of 0.062 at pH 7.4, whereas its dihydro derivative has a partition coefficient of 2.59 [10].



An additional example of altered transport that significantly modified the distribution of an agent due in part to changes in passive prodrug transport is thiamine tetrahydrofurfuryl disulfide (TTFD), a lipid-soluble prodrug of the quaternary ammonium vitamin, thiamine or vitamin B1. After intravenous administration of thiamine to rats, Pipkin and Stella [16] showed that thiamine was cleared rapidly, primarily via urinary excretion (> 90% dose), from whole blood, with a half-life of 35 minutes. The thiamine was found to be essentially in the plasma fraction of the blood (Table 1). After intravenous administration of TTFD, whole blood

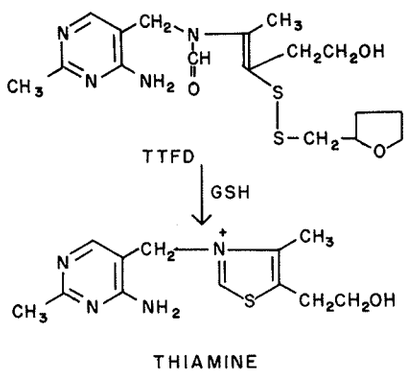


TABLE 1
Ratio of Thiamine in Plasma to Whole Blood as a Function of Time After a 4 mg/kg Intravenous Injection of Thiamine Hydrochloride to Rats

Time post-injection (minute)	Plasma/whole blood thiamine ratio[a]
1	1.63
3	1.83
5	1.90
10	1.64
20	1.42
30	1.80
60	1.63
90	1.43
Mean ± S.D.	1.66 ± 0.06[b]

a. Mean of 1 – 3 determinations at each time point.
b. Expected ratio if hematocrit is 0.45 and if all drug in the plasma fraction is 1.82.

thiamine half-life was 200 minutes and all the thiamine was essentially in the red blood cell fraction of the whole blood and 76% of the total administered dose could be accounted for in the red blood cell fraction of the blood within minutes after administration of the TTFD [17,18]. The long half-line of thiamine from whole blood probably results from TTFD rapidly and passively permeating the red blood cell membranes and reacting instantaneously with red blood cell glutathione to release thiamine. The thiamine is thus trapped in the red blood cells and the longer half-life of thiamine from whole blood simply represents the slow passive efflux of thiamine from the red blood cells. The original goal of the above work was to deliver thiamine more effectively to the CNS, a goal that was somewhat achieved [17], and not thiamine to red blood cells. However, this work did show that a considerable fraction of a drug could be delivered to an individual tissue if the right conditions were

met. In this case all three criteria for significant site specific delivery were present, i.e., access to the tissue by the prodrug, site-specific conversion, and site retention by the tissue of the parent molecule. Of course, depletion of red blood cell glutathione by reaction with TTFD could present a problem. This example of specific red blood cell delivery could serve as a model for the delivery of drugs used for the treatment of red blood cell-related diseases, e.g., sickle cell anemia and malaria.

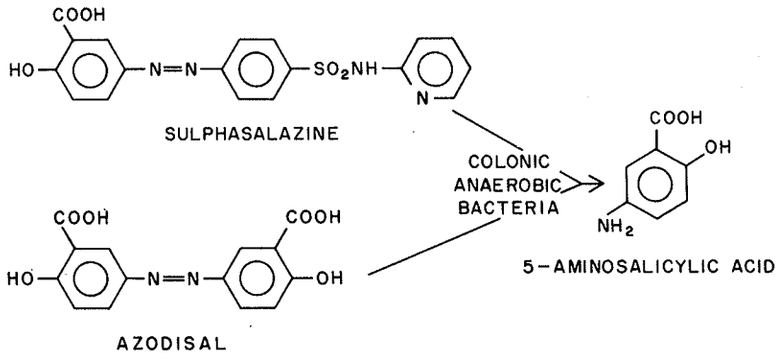
The three examples mentioned above, i.e., prodrugs of 2-PAM, berberine and thiamine, represent cases of altered permeability to a site with a well-defined barrier to polar drugs. A fact not generally appreciated is that permeability through an in vivo lipoidal barrier is not totally limited to the 'lipophilicity' of the molecule. For example, in vivo, once a molecule has sufficient lipophilicity its ability to access a site may become flow or perfusion rate-limited. Fenstermacher et al. [9] recently reviewed various methods for quantifying the transport of drugs across the BBB. They concluded that "for drugs that have octanol:water partition coefficient (PC) values greater than 1.0 or olive oil:water PC values greater than 1.0, their rates of blood-brain transfer will be limited by" the blood perfusion rate of the drug to the brain. "The rates of blood-brain transfer of drugs that have octanol:water PC values less than 0.1 or olive oil:water PC values of less than 0.01 will usually be limited by the permeability of the BBB . . ." while "if the PC value of a drug falls between either of these sets of limits, the transfer of that material from blood to brain will be a function of" permeability and perfusion rate. These conclusions are generally limited to small molecules of molecular weights $< 450-650$ [19], and molecules not capable of utilizing a carrier-mediated mechanism [9].

Uptake of peptides to the brain has been the subject of a number of studies, with variable results [20, 21]. Whether prodrugs of peptides could be used to alter their BBB permeability has not really been well tested, but is an area of intense study in many centers. The conclusions drawn for the delivery of drugs to the CNS would be qualitatively similar for other organs, except that most do not have as well a defined barrier to passive permeability as the BBB and are more capable of intercellular and pinocytotic transport mechanisms.

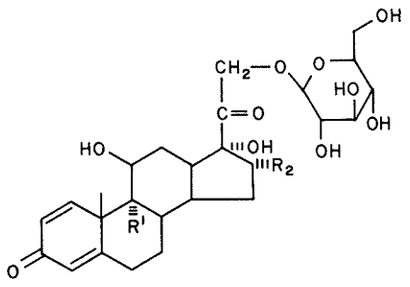
Increasing the polarity of an agent can be used to help direct delivery to a specific site by limiting uptake to sites that require the drug to be non-polar. Two specific examples of this possibility are prodrugs of 5-aminosalicylic acid [22] and some steroidal anti-inflammatory agents [23]. These agents are used in the treatment of inflammatory bowel disease. However, administration of the drugs themselves results in their systemic gastrointestinal absorption such that little of the drug reaches the colon membrane, where they could exert their maximal effect. Also, their systemic absorption exposes the rest of the body to the drugs, with their unwanted side effects.

Two reasonably colon-specific prodrugs of 5-aminosalicylic acid are sulfasalazine and azodisal sodium. Both are azo-linked prodrugs that release the parent molecule

by the action of anaerobic colonic bacteria on the azo linkage. However, this is not the only reason for their colon specificity. The selectivity comes also from the fact that the prodrugs themselves are poorly absorbed from the small intestines because of their polarity. Sulfasalazine suffers from the fact that the released sulfapyridine is absorbed and contributes to the side effects of the prodrug [24]. On the other hand, azodisal, which is a dianion under the pH conditions found in the small and large intestines, is itself very poorly absorbed from the gastrointestinal tract because of this polarity, and releases only 5-aminosalicylic acid [25, 26].



Friend and Chang [23] have proposed recently that drug glycosides of some steroidal anti-inflammatory agents, such as dexamethasone and prednisolone, could be used as colon-specific prodrugs for the parent molecules. They argued that, since a certain degree of non-polarity is necessary for a drug to be absorbed from the GI tract, polar glycosides should be poorly absorbed from the small intestines. The glycosides should then pass into the large intestines and colon, where the action of glycosidases found in colonic bacteria would act on the glycosides to release (specifically) the anti-inflammatory agents. Dexamethasone 21-β-D-glucoside and



$R_1 = F$, $R_2 = CH_3$ (DEXAMETHASONE 21-β-D-GLUCOSIDE)

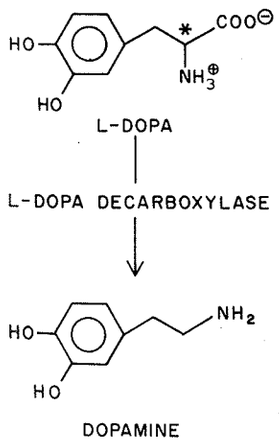
$R_1 = R_2 = H$ (PREDNISOLONE 21-β-D-GLUCOSIDE)

prednisolone 21-β-D-glucoside were synthesized and tested in rats. The delivery of dexamethasone was more specific than that for prednisolone, with nearly 60% of the dexamethasone glucoside reaching the cecum whereas < 15% of the other prodrug reached the cecum. When the two anti-inflammatory agents themselves were administered they were essentially totally absorbed from the small intestines, with less than 1% of the dose reaching the cecum [23].

Again, the apparent success of these examples points out the fact that not only must the prodrug have specificity for the site, in the above cases determined by the high polarity of the prodrugs, but a source of enzyme for the conversion of prodrug to active agent, the colonic bacteria, was necessary for the success of the concept. In this case, site retention of the parent molecule was not quite as critical, as the prodrugs represent almost a case of local rather than systemic drug delivery.

3.2. Altered transport due to pH effects

Differences in pH across membranes have not been used extensively as a means of altering site-specific transport. There are obvious differences in pH across membranes such as those seen along the GI tract. Windows for oral drug absorption are often attributed to the gradual increase in pH seen in passing down the GI tract from stomach to colon. The example of azodisal discussed earlier is one where absorption of the prodrug was limited by the fact that as the dianion (determined by the pH of the GI tract), it was not well absorbed until it released 5-aminosalicylic acid. Urine pH often differs from the physiological pH of 7.4, and it has been proposed that the pH of the interstitial spaces of tumors is considerably more acidic than pH 7.4. As far as transport properties go, pH differences across tissues rarely are used to effect specific transport, but may hold some promise for future research.



3.3. Use of carrier-mediated transport

The lack of transport specificity, imparted in altering passive permeability, may not be a problem if a carrier-mediated transport is utilized. If the uptake-limiting membrane of a tissue or organism has a unique transport mechanism that will allow it to absorb (or exclude) a particular type of molecule, it may be possible to design a prodrug that can take advantage of this differentiation.

Consider the prodrug of dopamine, L-dopa. Dopamine is a very polar catecholamine that on oral dosing is both poorly absorbed and extensively metabolised. L-Dopa, on the other hand, is reasonably well absorbed from the GI tract, although not without problems of its own, and apparently is transported into the CNS by the L-amino acid transport mechanism [27]. The D-isomer of dopa is not transported into the CNS [27]. Once in the brain, L-dopa is decarboxylated to dopamine. Bodor and Brewster [10] have recently suggested that the presence of aromatic amino acid decarboxylase in the BBB, which is used to control or balance neurotransmitter release, metabolism and uptake, may explain the need for giving such large doses of L-dopa in the treatment of CNS dopamine deficiencies.

Other carrier mechanisms have also been demonstrated for transport through the BBB. There appear to be specific carriers for neutral, acidic and basic amino acids, for hexoses, monocarboxylic acids, choline, nucleosides and thyroid hormones [10]. In theory, these could be utilized to help deliver agents to the CNS.

Other cells and tissues may have specific carrier mechanisms that help that tissue, cell line or organelle perform a specific function in the body. If identified, it may be possible to utilize this specific carrier to impart some selectivity to a prodrug. However, as emphasized throughout this chapter, selective transport in and by itself may not be sufficient to guarantee site-specific delivery of the active drug.

A possibly interesting approach to selective transport is the use of peptide and glycopeptide carriers. For example, Firestone et al. [28] showed that amine drugs acylated with a *Z*-Gly-Phenalanylgroup were transported by a pinocytotic mechanism into leukemia L 1210 cells, where presumably the prodrug is cleaved by lysosomal enzymes to release the parent drug. Malignant cells have been reported to have higher pinocytotic or endocytotic activity than non-malignant cells. This higher endocytotic activity has been well demonstrated in cultured cells, but in vivo it is not quite so clear whether malignant cells can compete with other macrophages for uptake of macromolecules. In particular, the high perfusion rate of liver cells along with their high phagocytotic activity makes it hard for other cells with some endocytotic capacity to compete with such highly perfused tissues.

Glycopeptides have been proposed as cell surface-selective receptor agents to promote the transport of drugs to macrophages [29]. The idea is that the specific affinity for a cell surface receptor could be used as a basis for the selectivity. In theory, such cell surface-specific carriers with drug appended will become attached to the cell surface, where internalization of the complex will occur. On lysosomal cleavage

of the carrier complex, the released drug will exert its specific therapeutic effects. This concept is novel and shows some potential.

Some cellular or subcellular barriers may be less selective to side chain specificity and size for the transport of peptides. For example, Steinfeld et al. [30] and others [31] have proposed that the more 'sloppy' peptide-uptake mechanisms in yeast cells versus mammalian cells could be used to deliver selectively anti-yeast entities as part of a peptide prodrug. This approach is not limited to large peptides, as Boehm et al. [32] and others have proposed that drugs attached to small peptide fragments are carried into bacterial as well as fungal cells. Drugs attached to such carriers or determinants may prove useful in future development of chemotherapeutic agents.

The same can be said for drugs attached to monoclonal antibodies, albumin, DNA, as well as synthetic peptides and other macromolecules [33]. In these cases the problems of specificity for a desired tissue, stability in the blood stream, and the mechanism of drug release from the carrier become critical. Sezaki and Hashida [33] recently have critically reviewed the whole area of macromolecular-drug conjugates. Some factors which are often not considered adequately when macromolecular-drug conjugates are considered as potential therapeutic agents are the difficulties of reproducibly synthesizing these macromolecules, their quality control, and whether the agents are acting as prodrugs or analogues. If acting as prodrugs, do the conjugates need to release the drug extracellularly or intracellularly? If intracellular release is necessary, how are the conjugates transported into cells (pinocytosis?) and can this transport compete with uptake of the macromolecules in highly perfused tissues with a significant concentration of cells capable of endocytotic activity?

On the question of the use of peptides as carriers, it should be recognized that the success (or lack thereof) may not be so dependent on whether the concept works as much as on the problems associated with the delivery of peptides to the body, in general. If these peptide conjugates were to be administered orally, peptidases in the GI tract could prematurely release the active drug from the carrier. The researcher should have in mind a statement made by Albert [34] that "although a detailed knowledge of permeability and enzymes can assist a skillful designer in finding pro-agents, he will have in mind an organism's normal reaction to a foreign substance is to burn it up for food".

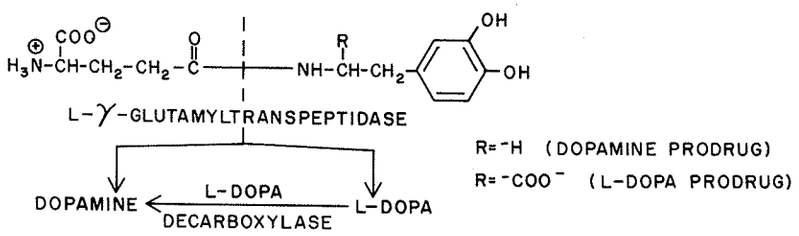
4. Site-specific drug release

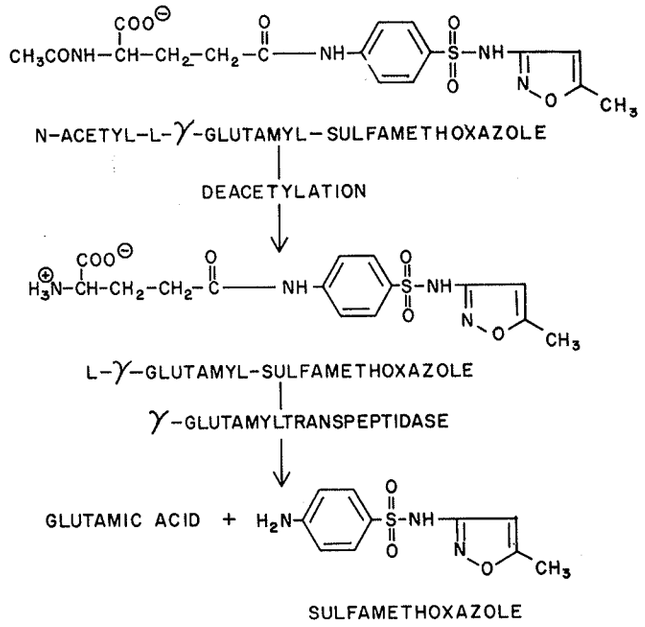
4.1. Enzyme-mediated drug release

It has been argued by many researchers that site-specific prodrug activation should lead to selective delivery of the parent drug to its target site. As mentioned earlier, this is only one important criteria for site-specific delivery. For example, it has been

proposed that the higher concentration of phosphatases and amidases in tumor cells could be used to deliver site-specifically cytotoxic agents to tumors. In fact, diethylstilbestrol diphosphate has been promoted as a prostatic tumor-selective agent [35], as have other phosphate ester derivatives [36]. Again, apart from other considerations, these phosphate esters probably had difficulty permeating the tissue because of the polarity of the prodrug. The ubiquitous distribution of phosphatases in other more highly perfused tissues, such as bone marrow, small intestines and liver, are probably able to compete more effectively for the cleavage of the prodrug [36]. This is probably why many attempts using atypical enzyme levels such as plasminogen activators [37 – 39], peptidases, glycosidases, sulfatases, and phosphatase enzymes to promote tumor selectivity have met with limited success in the past [36]. Prodrugs attempting to use these enzymes are too polar to reach the enzyme site, the relative enzymatic selectivity is insufficient, and the tumor cell perfusion rate is too poor to achieve the desired goal.

There are some good examples of site-specific drug delivery where site-specific drug release did play a very important role in achieving the selectivity. One of the best examples has been the work on L-γ-glutamyl derivatives of various amines and amino acids. L-γ-Glutamyl transpeptidase is an enzyme that is distributed very selectively throughout the body. In particular, it is concentrated in the brush border of the cells of the proximal tubules in the kidney. The enzyme is primarily responsible for the transfer of the L-γ-glutamyl group from the terminal end of a peptide to another peptide or amino acid or water. Therefore, it could be argued that the L-γ-glutamyl derivative of an amino acid or amine will be selectively cleaved in the proximal tubules, releasing a drug selectively in that tissue. Thus, Wilk et al. [40] and others [41] have developed L-γ-glutamyl derivatives of L-dopa and dopamine as kidney-specific prodrugs for the delivery of the renal vasodilator, dopamine. In the case of L-γ-glutamyl-dopamine the prodrug is cleaved only slowly on passage through the kidney. The dopamine is then metabolized rapidly and excreted into the urine without re-entering the systemic circulation, where it would produce adrenergic stimulation of the heart and other side effects. In the case of the L-dopa derivative, Wilk et al. [40] showed that L-γ-glutamyl-L-dopa is selectively accumulated in the kidney and that L-dopa released from the L-γ-glutamyl linkage by L-γ-glutamyl transpeptidase is decarboxylated by dopa decarboxylase, which is also abundant in the kidney [42].





Orlowski et al. [43] attempted to extend this idea to other L-γ-glutamyl derivatives. In particular, they studied whether L-γ-glutamyl and *N*-acyl-L-γ-glutamyl derivatives of sulfamethoxazole could be used as kidney-selective prodrugs of sulfamethoxazole [43]. Their results were not as encouraging as those for dopamine. Reproduced in Table 2 are the relative enzymatic cleavage rates for L-γ-glutamyl-sulfamethoxazole and *N*-acetyl-L-γ-glutamyl-sulfamethoxazole in various

TABLE 2
Rates of Release of Sulfamethoxazole from L-γ-Glutamyl-sulfamethoxazole and *N*-Acetyl-L-γ-glutamyl-sulfamethoxazole by Homogenates of Various Mice Organs [43]

Organ	Sulfamethoxazole release rate (nmol/mg protein/min)	
	L-γ-Glutamyl-sulfamethoxazole	*N*-Acetyl-L-γ-glutamyl-sulfamethoxazole
Kidney	71.5	0.46
Pancreas	10.4	0.001
Small intestines	1.25	0.001
Liver	0.02	0.002
Spleen	0.126	0.001
Lung	0.094	0.001
Heart	0.005	0.001
Brain	0.25	0.05

tissues. The highest activity is seen in the kidney for both potential prodrugs, but higher apparent selectivity is seen with the *N*-acetyl derivative. The levels of sulfamethoxazole in the same tissues after intraperitoneal administration to mice of the two prodrugs compared to sulfamethoxazole itself are shown in Table 3.

TABLE 3
Concentrations of Sulfamethoxazole in Various Mice Tissues 20 Minutes After Intraperitoneal Administration of Sulfamethoxazole, L-γ-Glutamyl-sulfamethoxazole, and *N*-Acetyl-L-γ-glutamyl-sulfamethoxazole [43]

Organ	Study 1		Study 2	
	Sulfamethoxazole (0.25 μmol/g)	L-γ-Glutamyl-sulfamethoxazole	Sulfamethoxazole (0.25 μmol/g)	*N*-Acetyl-L-γ-glutamyl sulfamethoxazole
Kidney	108 ± 9	97 ± 8	62 ± 4	91 ± 9
Pancreas	51 ± 4	66 ± 4	29 ± 3	5 ± 1
Small intestines	54 ± 2	35 ± 2	19 ± 2	4 ± 1
Liver	78 ± 4	55 ± 4	43 ± 5	11 ± 1
Spleen	51 ± 3	32 ± 4	30 ± 1	4 ± 1
Lung	58 ± 3	47 ± 3	30 ± 1	4 ± 1
Heart	57 ± 3	47 ± 4	37 ± 3	5 ± 1
Brain	11 ± 1	5 ± 1	5 ± 2	2 ± 1

Values are μg/g tissue.

Orlowski et al. [43] explained the apparent better performance of the *N*-acetyl derivative as being due to its slower overall cleavage rate, probably due to the fact that deacetylation was necessary before the L-γ-glutamyl transpeptidase could act on the prodrug. The deacetylation step was also faster in the kidney, so a coupling of the two processes appeared to provide the selectivity. Other hypotheses may also help to explain the observations.

As discussed in the site-specific transport section of this chapter, TTFD provided site-specific delivery of thiamine to red blood cells. One of the determinants for this selectivity appeared to be the rather high glutathione levels in the red blood cells. Table 4 gives the relative glutathione levels in various rat tissues. The selective, inadvertent, delivery of thiamine to the red blood cells was in large part due to the exposure of the TTFD to the red blood cell glutathione when it was administered intravenously, where immediate contact with the red blood cells would occur. Whether the same degree of selectivity would be seen after oral delivery of TTFD, where it is exposed to mucosal cell and liver cell glutathione, has not been determined.

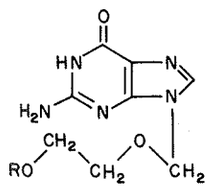
In the earlier discussion of azodisal sodium as a colon-specific prodrug of 5-aminosalicylic acid and the glycosidic prodrugs of dexamethasone and prednisolone, these prodrugs owed their apparent specificity to at least two factors, their polarity, which restricted their ability to be absorbed from the small intestines, and the fact that both required the action of colonic bacteria to release the active drug from the prodrug specifically in the colon. In the case of azodisal this required cleavage of an azo bond and for the glycosides it required the action of glycosidases from the colonic bacteria.

TABLE 4
Distribution of Glutathione in Rat Tissues [44]

Organ	Glutathione (mg/100 g tissue)
Liver	172
Adrenal	109
Brain	102
Spleen	92
Kidney	77
Heart	65
Pancreas	56
Blood	39
Red blood cells	87[a]

a. Calculated from whole blood concentration assuming little or no glutathione in plasma and a hematocrit of 0.45.

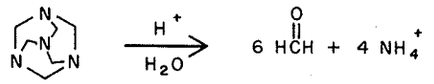
Viruses are less selective in their phosphorylating ability than are mammalian cells. This fact has been utilized in the development of some of the newer antiviral agents, such as acyclovir [45] and others. The suspected prodrugs are analogues of a vital nucleotide precursor; they are not phosphorylated by mammalian cells but are phosphorylated by the viral enzyme [45]. The active agent is actually the phosphorylated prodrug, which is then incorporated into viral DNA, disrupting the virus's replication cycle [45]. The selectivity in this case comes primarily from the activation process, but may also have contributions from the prodrugs being able to penetrate the virus, and possibly from the active drugs being retained by the virus. If the phosphorylated prodrugs are released from the virus they may have some difficulty being taken up by mammalian cells because of their high polarity.



R=-H, ACYCLOVIR
R=-$PO_3^=$, ACYLOVIR PHOSPHATE

4.2. pH-MEDIATED DRUG RELEASE

The pH of most physiological fluids is 7.4. The pH of interstitial fluid around tumor cells and infection sites has been found to be more acidic than physiological pH. These small differences could be exploited to selectively transport or cleave potential prodrugs. However, to date little success has been achieved in this area. In theory, the varying pH in the GI tract could be used to selectively cleave agents at specific sites along the GI tract; however, this idea has not been studied extensively. Urinary pH not only tends to be more acidic than pH 7.4, but it can also be manipulated by the administration of acidifying and alkalinizing agents such as ammonium chloride or sodium bicarbonate, respectively. Methenamine is a prodrug of formaldehyde which is cleaved non-enzymatically to formaldehyde at acidic pHs.



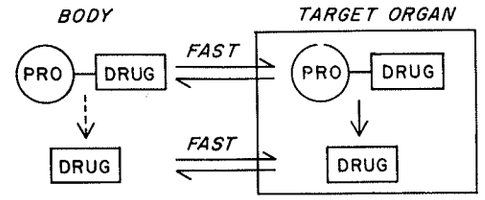
METHENAMINE

Acidification of the urine of patients taking methenamine formulated in an enteric-coated tablet to prevent premature cleaving under gastric pH conditions, promotes decomposition of the methenamine to formaldehyde in the urine, where it acts as a non-specific antibacterial agent [46]. This utilization of pH differences between urine pH and body pH is a rather unique example. For the idea to be successful the breakdown of the prodrug to drug must not only be very pH-sensitive but the cleavage rate must be such that the desired reaction takes place efficiently.

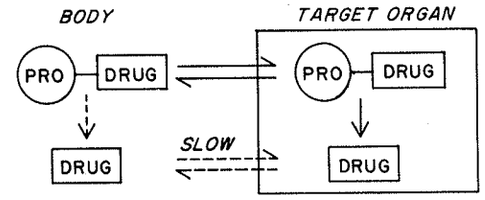
5. Parent molecule properties

Prodrug transport to and parent drug release at the target tissue are generally appreciated as being important factors for site-specific drug delivery. What was not generally well appreciated, until recently, was the role that the properties of the parent drug plays [3,4]. The work by Stella and Pipkin [17] with TTFD, the thiamine prodrug, and the work reviewed by Bodor and Brewster [10] on the delivery of drugs to the CNS, and a study by Repta et al. [47] on a prodrug of 6′-acetylpapaverin, a quaternary ammonium cytotoxic agent, all showed that the retention of polar parent molecules in the target tissue is an important factor. This concept is qualitatively illustrated in Scheme 1. In example 1 the prodrug readily equilibrates with the target organ and the prodrug is converted selectively to drug in the target organ. However, since the drug (parent molecule) rapidly 'leaks' from the target organ, only a momentary burst of drug is seen in the target organ [3]. In example 2, the prodrug more readily accesses the target organ, and when the drug is produced from the prodrug, its 'leakage' out of the target organ is slow. In this

Example 1. Parent drug accessible to target organ



Example 2. Parent drug poorly accessible to target organ



Scheme 1

case true targeting will occur. The importance of site retention of the parent molecule was tested mathematically by Stella and Himmelstein [3, 4] and shown to be very important, if not critical. This is illustrated in Figures 2 and 3, which are plots of target tissue drug concentration as a function of time for two hypothetical drugs, A and B, whose pharmacokinetics are defined by the model illustrated in Figure 1 (these are actual simulations of examples 1 and 2 in Scheme 1).

In these simulations the parameters listed in Table 5 are fixed and only the clearance values to and from the target organ ($Cl^{D}_{in,\,out}$) for the parent compound are varied.

Drug A represents a drug which has good accessibility to the target organ to begin with, i.e., $Cl^{D}_{in} = Cl^{D}_{out} = 10$ ml/minute which represents a transport rate to a 100-ml organ comparable to blood flow to bone marrow, a tumor or muscle, or a clearance to organs like the kidney, liver, heart or brain, where there is approximately a 50–90% diffusional resistance to organ uptake. Drug B represents a drug which has poor accessibility to the target organ, i.e., $Cl^{D}_{in} = Cl^{D}_{out} = 0.1$ ml/minute. Drug A might be a therapeutically useful agent as such, since it can reach the target organ at reasonable levels. Drug B might be a drug that behaves well in an in vitro screen, but because of its poor accessibility to the target organ it may behave poorly in vivo. The parameters listed in Table 5 were chosen so as to represent a drug with a fairly short half-life ($Cl^{D}_{el}/V_{drug} = 5 \times 10^{-3}$ min^{-1}, $t_{1/2} = 139$ minutes) that distributes throughout approximately total body water. The $V'_{max}/V_{TPD} = 357 \times V_{max}/V_{PD}$ represents a case where there is a 357-fold selective rate of metabolism of the prodrug to drug in the target organ over the rest

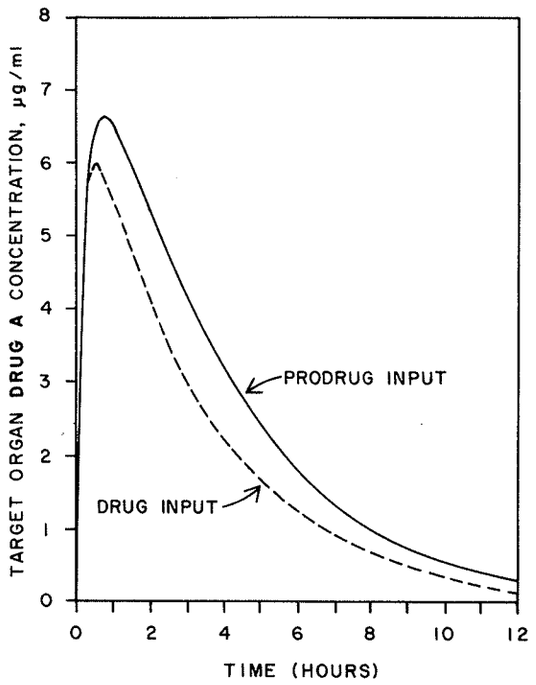


Fig. 2. Plots of target organ drug A concentration versus time for drug A delivery via a prodrug (solid line) having good accessibility to the target organ, and which is cleaved 357-fold more selectively to drug A in the target organ than in the rest of the body, or via drug A input (broken line). Drug A is a drug which has good accessibility to and from the target organ.

TABLE 5
Parameters Used for Simulating the Importance of the Effect of Clearance of the Parent Drug to and from the Target Organ on the Targeting Ability of a Prodrug

Dose (D) = 350 mg	K_M = 10 µg/ml
$R_{INPUT}^{PD,\,D}$ = iv bolus	V'_{MAX} = 7.14 mg/min
V_{drug} = 50 liters	K'_M = 10 µg/ml
V_{TD} = 100 ml	Cl_{in}^{PD} = 10 ml/min
V_{TPD} = 100 ml	Cl_{out}^{PD} = 10 ml/min
V_{PD} = 50 liters	Cl_{el}^{D} = 250 ml/min
V_{MAX} = 10 mg/min	

The parameter that was varied was $Cl_{in,\,out}^{D}$.

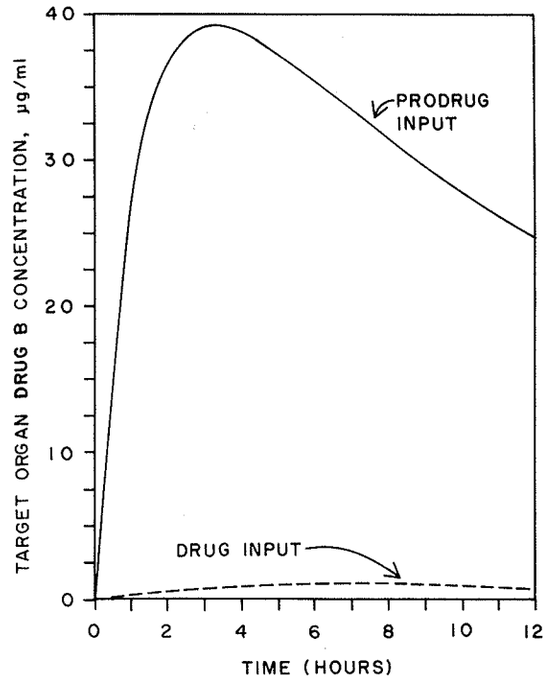


Fig. 3. Plots of target organ drug B concentration versus time for drug B delivery via a prodrug (solid line) having good accessibility to the target organ, and which is cleaved 357-fold more selectively to drug B in the target organ than in the rest of the body, or via drug B input (broken line). Drug B is a drug which has poor accessibility to and from the target organ.

of the body for the prodrug forms of drug A and drug B. What is compared in Figures 2 and 3 is the target organ drug concentration (µg/ml) after intravenous administration of either drug A or B as a prodrug, whose properties are defined above, or drugs A and B themselves.

The delivery of drug B (Fig. 3) as a prodrug with reasonable transport characteristics to the target organ and a 357-fold selective rate of metabolism to drug B in the target organ, relative to the rest of the body, results in a substantial improvement in the target organ drug concentration (solid line) relative to input via the drug itself (broken line, Fig. 3). Only a small improvement in delivery is seen in the case of drug A (Fig. 2). Most of the examples of successful site-specific delivery via prodrugs, e.g., dopamine as L-γ-glutamyl-L-dopa and L-γ-glutamyl-dopamine, thiamine as TTFD, 2-PAM as Pro-2-PAM, berberine as dihydroberberine, acyclovir phosphate as acyclovir, formaldehyde as methenamine, all produce relatively polar parent molecules. The case of L-γ-glutamyl-sulfamethoxazole may fit into the category of a poorly site-retained drug. The recent

work on plasmin-activated prodrugs for cancer chemotherapy [37 – 39] also supports the hypothesis that parent drug retention may be important. In the in vitro cell culture experiments, the prodrugs that were able to be taken up by the cultured cells and generated a rather polar parent compound showed the better selectivity.

6. Conclusion

At what point in drug development should prodrugs be considered for site-specific delivery of a potential therapeutic agent? To date, most attempts at delivery of agents to specific sites via prodrugs have involved trying to deliver already known therapeutically effective agents. As stated earlier, prodrugs of such agents will only be potentially successful if the therapeutic agent itself is poorly deliverable to its site of action to begin with.

There is no doubt that the development of biological and biochemical techniques to isolate receptors and aberrant biochemical pathways will lead to the development of agents capable of specifically interacting with these sites. It is also anticipated that more of these agents are likely to have limited in vivo activity because those physicochemical properties that made it a good in vitro candidate may limit its in vivo activity. Here is where a prodrug approach along with analog development and other optimization techniques might be useful in overcoming this problem.

The following sequence of steps might be envisaged in deciding whether the prodrug approach might be useful in promoting site-specific delivery.

Step 1. Based on receptor or other biochemical studies, a potential drug should be therapeutically useful if it can reach a particular site.

Step 2. The candidate drug is found to be inactive in vivo experiments but active in in vitro screens.

Step 3. Could the lack of activity in vivo be traced to the physicochemical properties and/or susceptibility of the drug to rapid metabolism?

Step 4. If it appears that the candidate drug, based on its physicochemical properties, is unable to reach its site of action then can an identifiable barrier to that transport be isolated?

Step 5. Define the barrier to delivery and attempt to distinguish those characteristics, such as site enzymes, pH differences, etc., unique to this tissue, which could be utilized for differential prodrug delivery.

Step 6. If after asking these questions it is decided that the physicochemical properties of the drug can be chemically altered to a prodrug that would rationally suggest its delivery to, and selective metabolism at the site target, then the prodrug approach may be worth pursuing.

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Design of Prodrugs (Bundgaard, H., ed.)

CHAPTER 6

Decreased toxicity and adverse reactions via prodrugs

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1. Introduction

Prodrugs have been extensively investigated as a means of targeting drugs directly to their site of action, consequently minimising the toxicological effects observed in non-target tissue. The prodrug's therapeutic index has been taken as a measure of the separation of the drug's therapeutic properties, from its clinically limiting toxicological side-effects, and as an indication of its potential clinical efficacy. Over the years the search for compounds with superior therapeutic indices has been intense, and this review summarises such studies. Examples have been taken from amongst steroidal and non-steroidal anti-inflammatory agents, β-stimulants and anticancer agents. These areas select themselves naturally, as the current generation of drugs within these classes, have a proven and often exciting profile of benefits, which are marred and limited by their profile of side-effects. In some of these classes the toxicology encountered was specific for a particular organ.

The problem of increasing therapeutic indices can be broken down into a number of separate stages, which may be considered individually (see Fig. 1). The synthesis of prodrugs (Stage 1), although an integral part of such studies and often troublesome, will not be discussed. The delivery of the drug to the target tissue (Stage 2) remains the major and, in a general sense, an unsolved problem. The simplest, and so far the most efficient, solution implemented involves direct delivery of the prodrug by mechanical means to the target organ. Examples where such an approach has been successful include the topical application of latentiated drugs to the skin, eye or lung and the intra-articular injection of encapsulated masked steroids into joint cavities. The alternative approach of biochemically targeting drugs to specific organs or receptor sites has been most rewarding in cases depending

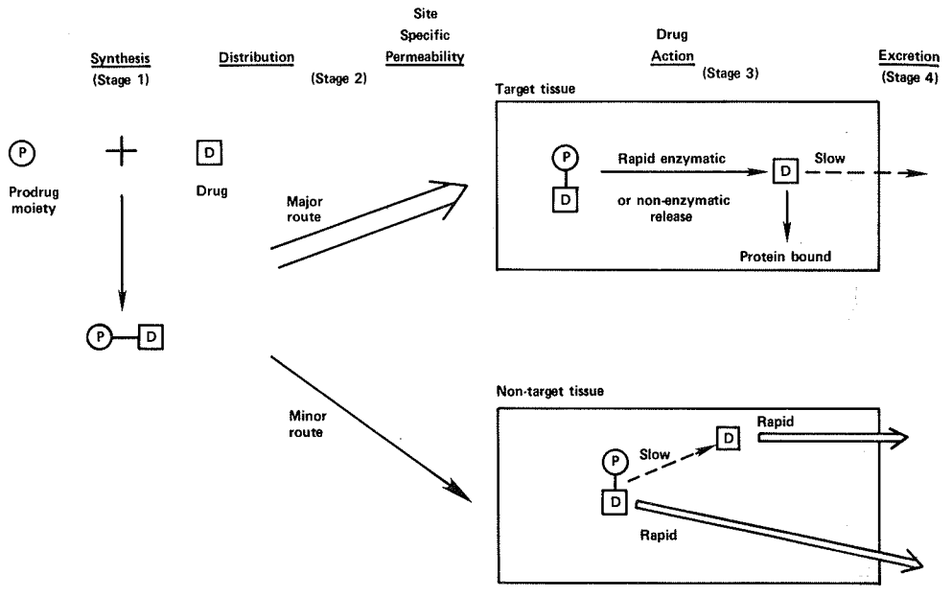


Fig. 1. The distribution and metabolic fate of a prodrug.

on active amino acid transport systems or on organ-specific enzyme systems. In this way 3,4-dihydroxyphenylalanine has been delivered, using the amino acid-transport system, to the brain in a moderately specific manner. Auspicious use has been made of the kidney-specific α-glutamyl-transpeptidase for enhancing the release of sulphamethazole or 3,4-dihydroxyphenylalanine and 3,4-dihydroxyphenylethylamine within this organ. The more demanding problem of specifically delivering cytotoxic agents to malignant cells without damaging other rapidly dividing cellular systems, such as those in bone marrow, remains an enigma which will be discussed. In recent years, the search for prodrugs with higher selectivities for the malignant cell has been widened to include latentiated drugs constructed from macromolecules, such as deoxy nucleic acid, synthetic proteins and natural proteins, and monoclonal antibodies.

The simpler converse problem of minimising drug concentrations in particular toxicologically sensitive organs, whilst maintaining reasonable drug concentrations in other target tissue, seems to fall within the range of what is currently possible using prodrug techniques. The prototype example within this class being the therapy-limiting gastrointestinal irritancy encountered with non-steroidal anti-inflammatory agents, or the analogous cardiotoxicity seen with the anthracyclines.

Having thus considered the delivery of the prodrug to the target tissue, a number of conditions need to be satisfied before the drug can finally accomplish its therapeutic role (Stage 3). The drug must be released at a satisfactory rate from the

latentiated form. The drug having been released must avoid the pitfall of becoming irretrevably bound to large biological molecules, such as albumin, within the active organ. Most target organs, for example the brain, are not homogeneous media and a further degree of localisation even within the target organ may be necessary before the desired biological effect is elicited. Leakage from, or metabolism within the target tissue must be slow compared to the rate of release of drug from prodrug, thus ensuring that the target organ is exposed to significant concentrations of drug for reasonable periods of time.

The design of prodrugs which satisfy all the varied criteria noted above cannot be realised easily in a single molecule. Each of the criteria often place conflicting structural demands on the nature of the latentiated form, leading to the need for compromise in the design process. This review documents the search for the optimum position for compromise.

2. Prodrug analogues of β-stimulants with reduced cardiovascular toxicity

Ester prodrug derivatives of synthetic and natural β-stimulant agonists have been investigated as a means of improving the therapeutic index of the parent drugs. Adrenergic agents such as adrenaline (1a) have been used for many years in the treatment of glaucoma [1, 2], a disease of the eye, characterised by an increase in intra-ocular pressure, leading progressively to damaged retina and a decreased visual field. Adrenaline has a 2-fold effect on the maintenance of intra-ocular pressure. The first occurs by a β-adrenergic mechanism causing a dilation of the canal of Schlemm and a loss of aqueous fluid. In addition, adrenaline produces a vasoconstrictive effect, acting through α-adrenergic receptors, which restricts the production of aqueous humour from the ciliary body. Nonetheless, adrenaline has a number of shortcomings in glaucoma; amongst these are short duration of action, ocular and systemic side-effects, poor bioavailability and inadequate stability. The ocular side-effects observed following topical application of adrenaline include hyperemia, mydriasis, corneal oedema, allergic sensitivity, browache, adrenochrome deposits, tolerance following chronic dosing and maculaopathy. The systemic side-effects observed include cardiovascular side-effects such as arrhythmias, elevated blood pressure and cerebrovascular accidents; these are sometimes accompanied by pallor, dizziness and tremor. Occasionally, central nervous system disturbances such as anxiety and tenseness are encountered.

The pharmacological properties of dipivaloyl adrenaline (1b) [3] were investigated in the anticipation that some of these toxicological problems could be circumvented. The prodrug (1b), on account of the slow release by local esterase enzymes of the pivaloyl moieties, functions as a 'sustained release' adrenaline. When dipivaloyl adrenaline (1b) was compared with adrenaline (1a) for effects on blood pressure and heart rate in dog and cats [4], it was evident that the prodrug had significantly less

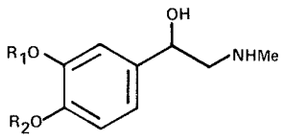
effect than the parent drug on both these parameters. Dipivaloyl adrenaline (1b) in a smaller topical dose gave a greater and more sustained reduction in intra-ocular pressure when applied to rabbit and beagle [5] eyes than a larger dose of adrenaline. A study of the pharmacokinetics and biotransformations of dipivaloyl adrenaline in rabbit eyes revealed that the prodrug penetrated the cornea 17 times more readily than its parent. Once inside the eye, the prodrug was hydrolysed to adrenaline with a half-life for the conversion of 18 minutes [6]. The main metabolite following topical application of either the prodrug or the drug was 3-methoxy adrenaline (1c) [7].

In clinical use dipivaloyl adrenaline has been shown to be nearly as effective as adrenaline at lowering intra-ocular pressure in glaucoma patients [8 – 10]. The prodrug did not cause discolouration of soft contact lenses and had a significantly improved toxicological profile when compared with adrenaline [11].

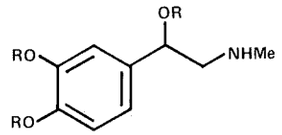
Witkop and co-workers [12], whilst searching for a derivative of the catecholamines able to penetrate the central nervous system, evaluated the 3,4,β-Tris(trimethyl silyl) derivative of noradrenaline (2a) and 3,4,β-triacetyl noradrenaline (2b) as possible prodrugs. Using labelled samples, it was demonstrated that both derivatives (2a,b) entered the central nervous system more readily than the polar parent drug adrenaline following intravenous administration. The distribution and nature of the radioactive species detected in brain, following administration of the prodrug as opposed to noradrenaline, were clearly different.

The preparation of the pivaloyloxy methyl ethers of adrenaline (3a), noradrenaline (3b) and phenylephrine (3c) have been described recently [13]. Dipivaloyloxymethyl adrenaline (3a) was 10 – 30-fold more effective as a topical anti-inflammatory agent than the parent drug in an ear burn rat inflammation model.

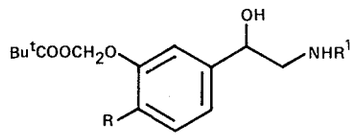
In 1947 Bretschneider [14] prepared the 3,4-diacetate (4a) and 3,4-dipropionate esters of isoprenaline (4b) and other catecholamines, but no pharmacological data were reported. Zolss [15] repeated the preparation of some of these diesters and indicated that their pharmacological properties were qualitatively similar to the parent compounds, often with lower potencies but with prolonged duration of biological effects. More significantly, the esters (4a – d) showed an improved therapeutic ratio over the parent isoprenaline when bronchodilator activity was compared to cardiovascular effects.



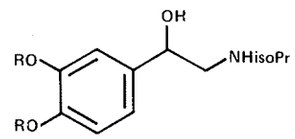
1 (a) $R_1 = R_2 = H$
(b) $R_1 = R_2 = Bu^tCO$
(c) $R_1 = Me$ $R_2 = H$



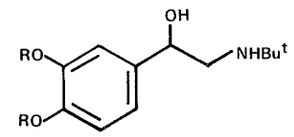
2 (a) $R = SiMe_3$
(b) $R = COCH_3$



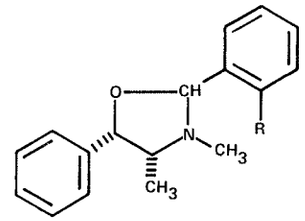
3 (a) $R = OCH_2OCOBu^t$ $R^1 = H$
(b) $R = OCH_2OCOBu^t$ $R^1 = Me$
(c) $R = H$ $R^1 = Me$



4 (a) $R = CH_3CO$
(b) R = EtCO
(c) R = (4Me)PhCO
(d) = Bu^tCO



5 (a) R = H
(b) R = 4MePhCO–



6 (a) R = H
(b) R = OH

The di-*p*-toluoyl (4c) and dipivaloyl isoprenaline (4d) esters were studied recently in the perfused rabbit lung preparation [16]. The prodrugs were investigated because the duration of action of isoprenaline aerosol is short, and the frequent dosing required to maintain the desired bronchial effect can lead to serious cardiac stimulation. Kinetic analysis of the adsorption and metabolism of the prodrugs (4c,d) in rabbit lung revealed absorption of both the diesters intact from the airways into the lung, followed by an equally rapid hydrolysis of the prodrug, first to monoesters and then to active drug. No accumulation of the prodrugs in lung tissue was observed. These results do not support the contention that the prolonged activity observed clinically for β-stimulant prodrugs can be explained by accumulation of the prodrug in the lung and a rate-limiting slow release of the active catechol species from the prodrug depot. Further studies, particularly with respect to tissue distribution of the drug and the latentiated form, are necessary before a satisfactory explanation of this phenomenon can be advanced.

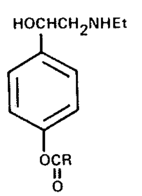
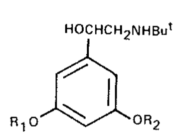
Since *N-t*-butylnoradrenaline (5a) was known to possess improved selectivity over isoprenaline when bronchial effects were compared with cardiovascular effects, a series of 4-monoesters and 3,4-diesters of this compound were synthesised [17] and evaluated biologically. From this series the di-*p*-toluate ester of *N-t*-butylnoradrenaline (5b) was selected for clinical evaluation as a bronchodilator in both oral and aerosol forms. Bitolerol mesylate (5) when administered by aerosol [18] was shown to be an effective bronchodilator drug, without any significant cardiac side-effects. This was attributed to the fact that esterase activity in pulmonary tissue was greater than in the heart.

The kinetics of hydrolysis of oxazolidines derived from (–)-ephedrine and benzaldehyde (6a) or salicyladehyde (6b) were studied [19] in order to to assess their suitability as prodrug forms for β-amino alcohols. The hydrolytic half-lives of these

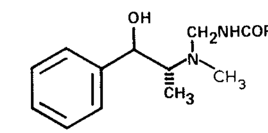
derivatives at pH 7.4 and 37°C were 5 minutes (6a) and 5 seconds (6b), which would severely limit their usefulness as prodrugs. The *N*-acyl Mannich bases of (−)-ephedrine (7) have also been prepared and investigated as potential bioreversible lipophilic forms of the drug. These products were again found to be relatively unstable, even at neutral pH [20].

R-(−)-Phenylephrine (8a), a well-known sympathomimetic amine which has been used extensively topically as a nasal decongestant and as a mydriatic, has recently

TABLE 1
Enzyme Hydrolysis Rates of Ester Prodrugs of β-Stimulants

Compound	R		Relative hydrolysis rates in human blood	Relative hydrolysis rates in rat blood		Reference
	CH_3		57.5	44		22
	CH_3CH_2		47.5	46.5		
	$CH_3(CH_2)_2$		32	25.4		
	$(CH_3)_2CH$		34.5	32		
	$CH_3(CH_2)_3$		30.7	34		
	$(CH_3)_3C$		5.6	4.6		
	$PhCH_2$		6.9	80.5		
	$PhCH(CH_3)$-		3.0	2.4		
	4MePh		9.0	0.2		
	2MePh		0.8	0.3		
	R_1	R_2		Relative hydrolysis rates in cat blood	Relative hydrolysis rates in dog blood	
	CH_3CO	CH_3CO	7.0	2.6		27
	H	CH_3CO	9.8	1.1		
	CH_3CH_2CO	CH_3CH_2CO	7.3	1.9		
	$(CH_3)_2CHCO$	$(CH_3)_2CCO$	1.0	1.0	1.0	
	H	$(CH_3)_2CHCO$	8.8	0.5	0.7	
	CH_3CH_2CHCO- CH_3	CH_3CH_2CHCO CH_3	0.16	0.4		
	$(CH_3)_3CCO$	$(CH_3)_3CCO$	0.02	0.02		
1b			$t_{1/2}$ = 30 min	($t_{1/2}$ = 10 min, rabbit plasma)		2
10b			$t_{1/2}$ = 3 sec			28
11			$t_{1/2}$ = 10 hrs			28
12			$t_{1/2}$ = 1.5 hrs			28
13			$t_{1/2}$ > 10 hrs			28
14			$t_{1/2}$ > 2 hrs			28

been converted to its *R*-(−)-dipivaloyl ester (8c) and its biological activity investigated [21]. The prodrug was found to be 15 times more potent than the parent drug as a mydriatic agent. Unexpectedly also, the enantiomeric prodrug (8c) was found to be three times, rather than twice, as active as the racemic prodrug. A series of 3′-(*O*-acyl) derivatives of etilefrine (9a) [22], the *N*-ethyl analogue of phenylephrine (8a), have been synthesised and evaluated. Correlations between structure and solubility, pK_a value, lipophilicity and esterase-catalysed hydrolysis were demonstrated. The rate of enzymatic hydrolysis (Table 1) of the aliphatic acyl radicals marginally decreased in human blood with increasing chain length (acetyl > *n*-propionyl > *n*-pentanoyl), but was reduced significantly when the acyl radical was highly branched (*n*-butanoyl >> trimethyl acetyl). In most cases, rat blood was slightly less active in hydrolysing the ester functions than was human blood, whereas rat liver homogenate was more efficient, particularly for the aliphatic acyl residues.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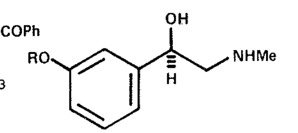
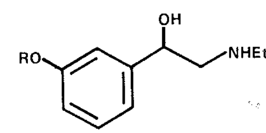
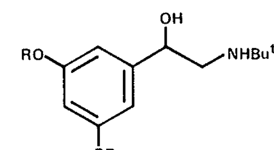

8 (a) R = H
(b) R = Bu^{t}CO
(c) R = Bu^{t}CO (R(−) enantiomer)


9 (a) R = H
(b) R = acyl


10 (a) R = H
(b) R = $(CH_3)_2CHCO$

Wetterlin and Svensson [23] reported in 1968 the preparation of the di-isobutyric acid ester of terbutaline (10b). The pharmacology of the prodrug ibuterol (KWD 2058) (10b) was qualitatively but not quantitatively similar to that of its parent [24]. Ibuterol was demonstrably inactive on isolated cat bronchial preparations, pretreated with the esterase inhibitor eserine in concentrations that relaxed the normal preparations. This indicated that the effect of ibuterol was due to the formation of terbutaline (10a) and not to the unchanged diester. Administered orally ibuterol was six times more potent that terbutaline, reflecting the improved bioavailability of the prodrug. Ibuterol also exhibited a tendency towards less cardiovascular effects in relation to bronchodilator effects and generally was considered to have a superior separation of toxic side-effects when compared with the drug in laboratory tests. Clinical testing [25, 26] of ibuterol confirmed its improved potency as a bronchodilator, 2 mg of ibuterol being equivalent in its bronchodilating effect to 5 mg of terbutaline, both compounds being dosed orally. No significant changes in heart