Journal of Pharmaceutical Sciences

FEBRUARY 1975
VOLUME 64 NUMBER 2

REVIEW ARTICLE

Rationale for Design of Biologically Reversible Drug Derivatives: Prodrugs

A. A. SINKULA [x] **and S. H. YALKOWSKY**

Keyphrases □ Drug derivatives, biologically reversible—rationale for design of prodrugs, physicochemical and biological considerations, applications, review □ Prodrugs—rationale for design, physicochemical and biological considerations, applications, modifications affecting absorption, site direction, depot, taste and odor, and irritation, review □ Reversible drug derivatives—rationale for design for prodrugs, physicochemical and biological considerations, applications, review □ Chemical modification—prodrugs, physicochemical and biological considerations, applications, review □ Latentiated drugs—design of prodrugs, review

CONTENTS

Nearly all therapeutic agents possess various physicochemical and biological properties, some desirable and others undesirable. In general, the pharmaceutical world is concerned with minimizing the number and magnitude of undesirable properties of a drug while retaining the desirable therapeutic activity.

Improvement of drug efficacy can be accomplished by biological, physical, or chemical means. The biological approach entails varying the route of administration. Examples include the injectable route to optimize onset of action, maximize bioavailability (enhanced blood levels), and eliminate gastric irritation and acid-catalyzed drug degradation. Versatility is severely limited when utilizing the biological approach, because alternative routes of administration are frequently unavailable and are always less convenient than oral administration.

A greater degree of flexibility of drug modification is offered by the physical approach, commonly referred to as dosage form design. The elements and philosophy of this approach were discussed by Schroeter (1) and others (2–8). The highest degree of flexibility in altering drug efficacy, however, is offered by the chemical approach.

Drug derivatization has been long recognized as an important means of producing better pharmaceuti-

cals. Bayer, as far back as 1899, synthesized the drug aspirin in an attempt to improve the therapeutic activity of salicylic acid. Since that time, literally thousands of drug derivatives have been synthesized and tested. These drug derivatives can be broadly classified into two categories: irreversible or reversible. Irreversible derivatives or analogs are usually synthesized for the purpose of finding a similar, new, biologically active entity possessing increased potency, a broader spectrum of activity, or some other desirable property not possessed by the parent compound. A reversible drug derivative utilizes a chemical moiety of proven biological activity (the parent molecule) and seeks to deliver it to the site of action while overcoming some inherent drawback to the use of the parent compound.

In the case of the analog, precautions must be taken as to what functional group can be modifed since indiscriminate modification may destroy all bioactivity. The reversible derivative can be modified at any functionality without undue concern for its involvement at the receptor level since it is reversible by definition. These facts eliminate the need to determine the bioactive center(s) in the molecule and offer the chemist a greater number of chemical sites at which to modify.

In general, three approaches are followed in the search for new drug agents: *(a)* the general screening approach in which chemical substances from any source are tested for their effect against a predetermined disease or disease state, *(b)* the chemical modification of existing drug substances whose biological effects are known, and *(c)* mimicking nature by biochemical design, where a compound is made to exert an action in a manner similar to a known biochemical substance (9). Any lead compounds obtained from these three approaches are usually further modified chemically to gain the biologically most potent representatives of the series.

This review will focus on approach *(b)*, the chemical modification of existing drug substances whose biological effects are known. It will be limited solely to biologically reversible derivatives, *i.e.*, those compounds that, upon introduction to the appropriate biological system, revert back to the parent molecule by virtue of enzymatic and/or chemical lability. To provide an interpretive review of the area, references from the journal and patent literature will be selected to illustrate the various principles discussed. The discussion will of necessity not be comprehensive but will nonetheless cover the significant aspects of the discipline.

Reversible derivatives (10–14) have also been termed prodrugs (15–22) and latentiated drugs (23–25) and have been designed to eliminate a variety of undesirable properties such as bitterness, odor, gastric upset, and poor absorption. Many comprehensive reviews of both reversible and irreversible drug derivatives (23–32) have appeared recently.

Harper (25) must be credited with coining the term "drug latentiation," and he defined it as "the chemical modification of a biologically active compound to form a new compound, which upon *in vivo* enzymatic attack will liberate the parent compound." Kupchan *et al.* (33) extended this definition operationally by including nonenzymatic processes as well for regeneration of the parent compound. By inference, latentiation implies a time lag element or time component involved in regenerating the bioactive parent molecule *in vivo*. Since most latentiated drug substances *per se* are biologically inactive, this concept is important for those drugs that are metabolized or excreted too rapidly to provide adequate clinical efficacy.

Albert (34, 35), in discussing the selective toxicity of drug molecules, elucidated the proagent or prodrug concept. The term prodrug is general in that it includes latentiated drug derivatives as well as substances that are converted after administration to the actual substance that combines with receptors. These actual substances may be active metabolites of the parent molecule. Harper (24), on the other hand, discussed the concept of structural formulation and defined it as "the modification of a biologically active compound at a point not essential for binding to an active site in the biological receptor, so that although the desired biological effect is retained, the resulting changes in physicochemical properties cause alteration in the absorption, distribution or metabolism of the drug; the parent compound, however, is not liberated in the body." Structural formulation differs from irreversible derivative formation in that the former retains bioactivity *in vivo* whereas the latter may or may not do so. The distinction is subtle but may have profound ramifications in the rational approach to the synthesis of bioactive agents.

Any number of inherent disadvantages may preclude the use of the parent drug molecule in clinical practice. Among those properties considered disadvantageous in a drug molecule are bitterness or tartness, offensive odor, gastric or intestinal upset and irritation, pain on injection, lack of absorption, slow or rapid metabolism, and lack of stability in the bulk state, the dosage form, or *in vivo* (*i.e.*, gastric instability).

In many cases, undesirable properties in a drug molecule cannot be overcome by conventional pharmaceutical formulation or route of administration changes, so the method of choice becomes reversible derivative formation. In the intelligent design of reversible drug derivatives, it is necessary to consider two questions:

1. What structural modification(s) of the parent molecule are necessary to reduce or eliminate the particular undesirable effect?
2. What conditions are available *in vivo* (enzymes, pH, *etc.*) to regenerate the parent molecule from the derivative?

The first question requires an extensive knowledge of structure–activity relationships as they apply to elimination of these undesirable properties. The second question is dependent on a rather sophisticated knowledge of biology. Complete answers to these two questions are obviously not yet available to the medicinal chemist. A limited body of knowledge is available, however, and this knowledge, if used judiciously, can form a basis for the rational design of revers-

ible drug derivatives. The remainder of this review will consider specific undesirable drug properties and possible means of eliminating these properties by using reversible drug derivatives. Examples of drugs whose properties have been successfully modified are provided in the tables.

GENERAL CONSIDERATIONS

Physicochemical—*Absorption*—Probably the most fruitful area of reversible derivatization is the improvement of passive drug absorption through epithelial tissue. Many studies involving *in vivo, in situ,* and *in vitro* systems have been conducted to elucidate the role of chemical structure in drug absorption. The similarities and differences among some more widely accepted theories also will be discussed here.

Nearly all of the empiricisms and theories in current use agree that the addition of a hydrophobic group to a compound usually increases its absorption. It is also agreed that this increase in absorption is a direct consequence of the increase in the biological lipid–water partition coefficient resulting from the added hydrophobic moiety. Although there is a lack of agreement as to what, if any, *in vitro* partitioning system best mimics the biological situation, octanol–water apparently is the most useful for correlative purposes.

From both the physicochemical and the biological point of view, octanol–water is the most extensively studied system. Leo *et al.* (36) tabulated and critically evaluated thousands of octanol–water partition coefficients and developed a system of rules for estimating values for compounds that have not yet been studied.

It is important to realize that the theoretical and empirical relationships to be discussed are equally applicable to reversible derivatives as well as nonreversible derivatives (analogs), because all of these relationships rely upon partitioning and because the presence of a reversible linkage does not normally alter the relative partition coefficients of the members of a series. For example, in the octanol–water system, the ratio of the partition coefficients of ethyl and butyl benzoates is the same as the ratio for *p*-ethyl- and *p*-butylbenzoic acids, even though the esters are reversible while the acids are not. Another consequence of the dependence of absorbability on partitioning is the fact that the unionized form of a dissociable molecule is absorbed more efficiently than its ionic species. The quantitative relationships between pH, pK, and partition coefficient are well known (37, 38) and will not be discussed here.

To get a clear picture of the role of hydrophobicity in drug transport, it is necessary to cover a wide range of partition coefficients. The most convenient and economical way of accomplishing this task is to construct a homologous series. The partition coefficient of the *n*th number of any homologous series, PC_n, in any solvent system can be described by:

$$\log(PC_n) = \log(PC_0) + \pi^s n \qquad \text{(Eq. 1)}$$

Table I[a]—Values of π_{CH_2} for Some Common Solvents and for Red Blood Cell Ghosts

Solvent	π_{CH_2}
Ether	0.573
Ether	0.612
Octanol	0.500
Chloroform	0.609
Olive oil	0.525
Castor oil	0.545
Red blood cell ghosts	0.526

[a] Adapted, with permission, from Ref. 46.

where PC_0 is a constant dependent on both the series and the solvent system, and π^s is a constant dependent only on the solvent system. According to the notation of Leo *et al.* (36), π can be an incremental constant for any substituent; but when dealing primarily with homologous series, π will designate π_{CH_2} unless otherwise specified.

The values of π (π_{CH_2}) for several organic solvents and red blood cell ghosts against water are listed in Table I. From the octanol–water π value of 0.5, it can be seen that only seven consecutive homologs are needed to cover a 1000-fold range of partition coefficients in half-log increments. Spanning this broad range of values minimizes the effects of random error and normal biological variation. Homologous series are particularly well suited for studying transport because the alkyl group usually does not interfere with the interaction of the active portion of the molecule and the receptor site. Therefore, it is frequently possible to measure the relative biological activities of homologs and then equate these values to relative transport rates.

The earliest structure–activity or structure–transport workers all recognized the parallelism between the logarithms of the biological response *(BR)* and the lipid–water partition coefficient *(PC)*. Equations of the form:

$$\log(BR) = a + b\log(PC) \qquad \text{(Eq. 2)}$$

were proposed (39–44). For the simple case of a homologous series, Eqs. 1 and 2 are combined giving:

$$\log(BR) = b\pi^s + b\log(PC_0) + a \qquad \text{(Eq. 3)}$$

which describes the so-called linear structure–activity relationship.

The value of a in Eq. 2 or 3 is a measure of the degree of similarity of the *in vitro* and the true *in vivo* partitioning systems. If a is equal to unity, the systems are equivalent in their relative affinities for a methylene group. (It is possible for π_{CH_2} to be the same for a pair of systems and, at the same time, for other substituent constants such as π_{OH} to be quite different. For specific examples, see Ref. 36.) When considering a homologous series, it is not necessary to use any reference *in vitro* partitioning system. Flynn and Yalkowsky (45) showed that $b\pi^s$ is equal to π^B, the π value in the biological system. Thus, Eq. 3 becomes:

$$\log(BR) = A + \pi^B n \qquad \text{(Eq. 4)}$$

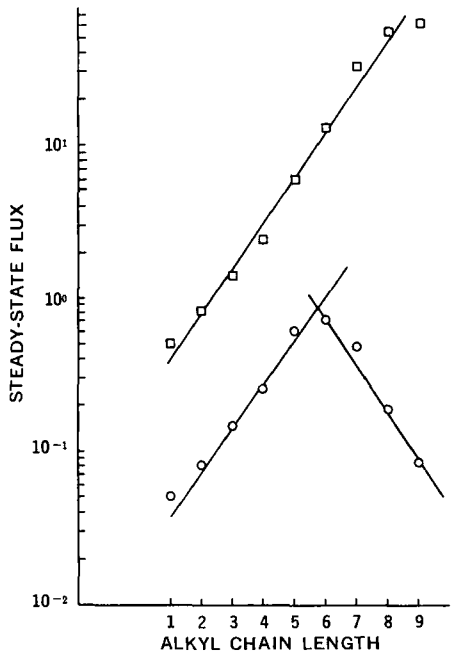


Figure 1—*Data of Scheuplein and Blank (48) for permeation of normal aliphatic alcohols across human stratum corneum* in vitro. *Key:* □, *concentration-normalized flux; and* ○, *flux from 0.10* M *solution (last four points represent saturated solutions). (Adapted, with permission, from Ref. 46.)*

where A is equal to the constant portion of Eq. 3 and is, therefore, dependent upon both the biological system and the homologous series.

Yalkowsky and Flynn (46) recently evaluated a large number of linear chain-length–activity relationships which are dependent upon passive transport. They observed, as expected, that π^B for a particular biological system is essentially independent of the series under study. They found that the value of π^B for most simple organisms (bacteria, fungus, erythrocytes, *etc.*) is 0.46 ± 0.03; *i.e.*, on the average, there is a 2.9-fold increase in activity for each additional methylene group. It was also observed that the value of π^B for epithelial and GI tissue of higher animals is around 0.25, which corresponds to an incremental constant for each methylene group of about 1.8 for the change in activity (or transport) with chain length. This low value is in agreement with the fact that these tissues contain a significant fraction of polar components.

The linear increase in the logarithm of the transport rate with chain length is expected from basic permeability theory. The flux, F, or transport rate of a substrate across a rate-determining lipid phase or membrane separating two aqueous phases at a concentration differential of ΔC is:

$$F = \frac{PC}{R}\Delta C \qquad \text{(Eq. 5)}$$

where PC is the lipid–water partition coefficient of the substrate, and R is the resistance of the membrane to its diffusion. Collander (47), using chara cells, was among the first to show the relationship between permeability and partitioning in a biological system. More recently, excellent correlation was shown (48) between the permeability of nine aliphatic alcohols across excised human stratum corneum and their stratum corneum–water partition coefficients. From these data (Fig. 1), it can be seen that there is a great increase in permeability between methanol and nonanol.

The exponential increase in transport rate and thus activity with extention of the alkyl group cannot go on indefinitely. If experiments are carried to high enough chain lengths, there is a leveling off or plateauing of the curve and ultimately a decrease in activity with increasing hydrophobicity. While there is reasonably good agreement in the literature about the ascending portion of the structure–activity curve, there is a great deal of controversy about the reason for and even the shape of the apical or descending portions of the curve.

Hansch (49) described the overall shape of the structure–activity curve by a parabolic equation of the form:

$$\log(BR) = a + b\log(PC) + c\log^2(PC) \qquad \text{(Eq. 6)}$$

He showed that Eq. 6 gives better statistical fit to many sets of data than the linear Eq. 2. The improved correlation is undoubtedly at least partially due to the greater degree of flexibility produced by the additional variable c; but since c is always negative, it cannot be regarded as simply another adjustable parameter. One difficulty that arises with the parabolic equation is that the values of the coefficients, a and b in Eqs. 2 and 6, have no relationship to one another and parabolic equations having the same ascending slopes can appear quite different.

Since Hansch's parabolic relationship is based upon a countercurrent distribution-type model (49), the curve is nowhere linear and is symmetrical about an optimum value of log (PC). The nonlinearity of the parabola makes it difficult to reconcile with the linear case described by Eq. 2. Furthermore, the symmetry is not consistent with the many reasons given by Hansch and Clayton (50) to explain the decrease in activity with chain length. Nevertheless, in spite of the theoretical shortcomings, the Hansch parabolic relationship can be extremely useful in the empirical analysis of structure–activity data and in the prediction of optimum partition coefficients for biological activity. From a practical standpoint, it is frequently more useful than the more theoretically valid relationships that will be discussed.

Wagner and Sedman (51) recently analyzed much of Hansch's data and found that a statistically better fit is obtained with an equation of the form:

$$(BR) = \frac{1}{a + (b/PC)} \qquad \text{(Eq. 7)}$$

Others also showed good fit of biological activity data and transport data to equations similar to Eq. 7 (46, 52, 53). One of the earliest uses of this equation was by Zwolinski *et al.* (54) who studied the permeability of various plant cells to the members of several homologous series. They also showed that double-recip-

rocal plots [$(BR)^{-1}$ *versus* $(PC)^{-1}$] are linear and can be used to evaluate the constants a and b conveniently. Another important feature of Eq. 7 is that it is applicable to both linear and nonlinear data.

The mathematical form of Eq. 7 can be obtained directly from Eq. 5 by the incorporation of an additional resistance in series with that of the membrane. Equation 5 then becomes:

$$BR \propto F = \frac{\Delta C}{(R_m/PC) + R_{aq}} \qquad \text{(Eq. 8)}$$

This added resistance, R_{aq}, results from the unstirred aqueous layers adjacent to the membrane which must be traversed by any solute passing through the membrane. For a more complete description of the derivation of Eq. 8 and the importance of unstirred layers (or diffusion layers as they are often called), the reader is referred to Refs. 45 and 46. For an alternative treatment based on extraction theory leading to another equation of the same form as Eq. 7, see Ref. 51.

For the study of a homologous series, it is convenient to combine Eq. 1 with the logarithmic form of Eq. 8 (or 7) to get:

$$\log(BR) = \log(\Delta C) - \log(PC_0) - \pi n - \log(R_m + R_{aq}PC_0 10^{\pi n}) \qquad \text{(Eq. 9)}$$

which is shown schematically in Fig. 2. It can be seen that, according to Eq. 9, there is a linear increase in log BR with chain length but that at some point [when $R_{aq} \geq (R_m/PC)$] the curve levels off and approaches a limiting value. These equations can satisfactorily describe most structure–activity data, but they do not explain the descending portion of the curve.

Because of the wide variety of reasons for a decline in activity with increasing chain length, no single equation can explain all available data. Hansch and Clayton (50) and Yalkowsky and Flynn (46) listed about a dozen possible reasons for this decline. These reasons can be broadly classified into those that are dependent upon a biological parameter (*e.g.*, enzyme specificity, conformational distortion of the active site, metabolism, and poisoning of enzymes) and those that are related to some physical property (*e.g.*, solubility, complex formation, micelle formation, partitioning into inert phases, and binding to inert surfaces). The former are the most difficult to correlate by simple theories but can be handled quite satisfactorily by equations such as Eq. 6. The latter all have one important feature in common; they can generally be described mathematically by:

$$\log(P_n) = \log(P_0) + \alpha n \qquad \text{(Eq. 10)}$$

where P_n is the value of any property of the nth homolog of the series, P_0 is a reference value, and α is a well-defined constant[1].

[1] Occasionally, it is necessary to use a higher order polynomial of n to describe P_n. This would alter the subsequent mathematical treatment slightly but not the general conclusions (see Ref. 52).

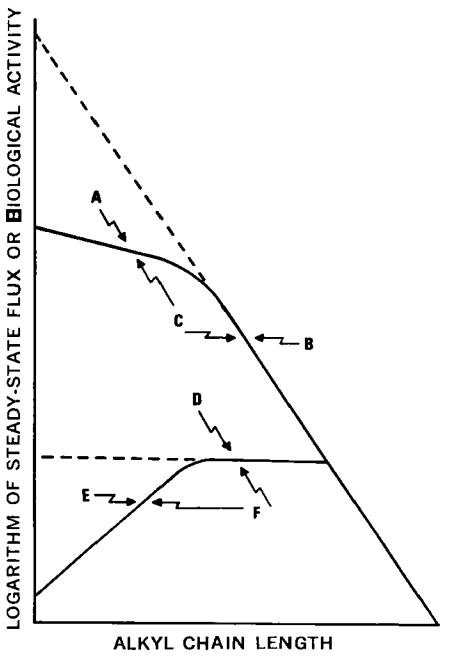


Figure 2—*Hypothetical chain-length activity relationships. (Adapted, with permission, from Ref. 52.)*

Yalkowsky *et al.* (46) theoretically characterized the effects of behavior on the transport rate by using solubility as an example. Based on literature data for nearly 20 homologous series in water, they found[1]:

$$\log(S_n) = \log(S_0) - \delta n \qquad \text{(Eq. 11)}$$

where S_n is the solubility of the nth member of the series, and S_0 is a constant.

The effect of solubility on transport rate is to limit the attainable concentration differential so that the maximum value of Eq. 8 becomes:

$$F = \frac{S}{(R_m/PC) + R_{aq}} = \frac{(S_0 10^{-\delta n}/R_m)}{PC_0 10^{\pi n} + R_{aq}} \qquad \text{(Eq. 12)}$$

which, in logarithmic form, is:

$$\log(F) = \log(S_0 PC_0) + (\pi - \delta)n - \log(R_m + R_{aq}PC_0 10^{\pi n}) \qquad \text{(Eq. 13)}$$

These equations can now describe a "parabolic" structure–activity curve on the basis of transport-limited activity and basic physical–chemical relationships. Figure 2 shows the expected dependence of transport across a biological barrier (and activity dependent thereupon) for the members of a homologous series predicted by Eqs. 9 and 13. The scales are arbitrary but show that the break occurs at the same chain length for the equimolar and saturated cases. Figure 3 shows an experimental verification of Eqs. 9 and 13. These data were obtained from turnover time experiments with goldfish (52). The agreement between experimental and theoretical data, while not necessarily proving the theory, gives a positive indication of its utility.

Solubility—The primary role of solubility in determining drug absorption is obvious since only the

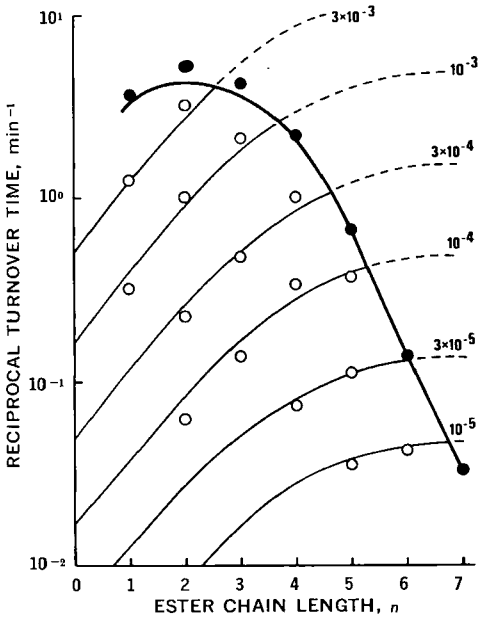


Figure 3—*Turnover times produced by various concentrations and saturated solutions of* n-*alkyl* p-*aminobenzoates. Points are experimental:* ○, *unsaturated; and* ●, *saturated. Lines are theoretical:* ——, *unsaturated; and* ——, *saturated. (Reprinted, with permission, from Ref. 52.)*

drug that is in solution is available for absorption. Since virtually any structural modification that alters solubility will also alter ionization and partitioning, it is somewhat difficult to provide clearcut examples of the relationship between solubility and absorption. Some effects of structure on aqueous solubility will be discussed later. The importance of solubility was apparent in the previous discussion of homologous series. It frequently is the factor responsible for the parabolic shape of many structure–activity curves.

For homologous series, solubility in aqueous media generally decreases by a factor of 4.0 for each methylene unit. Branched alkyl moieties decrease aqueous solubility to a lesser extent than linear chains. It was shown that this results because the former have a lower surface area than the latter and because the solubility of over 70 aliphatic alcohols (linear, cyclic, and branched) is directly proportional to the hydrocarbon surface area (55). An earlier study (56) showed a similar correlation for hydrocarbons. Many empirical correlations have appeared which are useful for estimating the aqueous solubility of organic liquids (57–59). These correlations, while far from complete, can be of value in evaluating the effect of a structural modification on solubility.

Since many drugs are either weak acids or weak bases or their salts, dissociation must be regarded as an important factor in determining absorbability. It is generally known (37, 38) that the unionized form of a drug is absorbed far more efficiently than the ionic species, even though the latter is more soluble. The explanation for this observation lies in the fact that the increase in the partition coefficient in going from a salt to free acid (or free base) usually exceeds the corresponding decrease in solubility by several orders of magnitude. This is analogous to π being greater than δ in Eq. 13. The greater absorbability of the unionized species over the salt is further amplified by the fact that it is rarely practical to give saturated solutions of the salt.

Many workers (37, 38) regard the absorption of ionic species as nonexistent and treat the undissociated species in a manner similar to the treatment of nonelectrolytes discussed. Others (60, 61) have shown that certain ionic drugs are absorbed in their undissociated state, either directly or by ion-pair or complex formation. While these mechanisms are certainly operative in specific instances, they are not of sufficient importance to be of concern here. Consequently, the term ΔC in Eqs. 5, 8, and 9 must refer only to the concentration differential of the undissociated form of the drug. In each aqueous phase, the concentration of unionized species, C_u, can be related to the total concentration, C_t, the pH, and the pK of the weak acidic drug by:

$$C_u = \frac{C_t}{1 + 10^{(\mathrm{pH}-\mathrm{pK})}} \qquad \text{(Eq. 14)}$$

A similar equation can be written for basic drugs. The combination of Eqs. 14 and 5 is known as pH–partition theory. The direct application of this theory to gastric and intestinal absorption has been only partially successful. The data seem to indicate that the intestinal pH is closer to 5.5 than it is to the accepted value of 7.4. This observation led to the postulation of a region of the intestinal lumen adjacent to its surface which has a pH of 5.5 and which is in equilibrium with the bulk of the lumen (37). This virtual pH hypothesis has been criticized (62) because its existence would have no effect on the amount of unionized drug at the luminal surface.

Dissociation behavior, however, is important in designing reversible derivatives when the linkage involves the ionizable group. Thus, a pH-sensitive drug such as 15-methylprostaglandin $F_{2\alpha}$ becomes unionizable when converted to its methyl ester.

If absorption is to be increased by adjusting hydrophobicity, a linkage must be selected that will remain intact until absorption is complete, with subsequent release of the parent molecule into the bloodstream or at some specified tissue. To choose such a linkage rationally, an awareness of what linkage-cleaving enzymes are present in the GI tract, the liver, the blood, and the various body tissues is necessary.

Biological—*Enzymes*—In a broad sense, the basis for the rational design of biologically reversible drug derivatives is predicated on the ability of the host tissue or organism to regenerate the drug derivative to the bioactive parent molecular species. The manner in which this is frequently accomplished is through the mediation of an enzyme or enzyme system within the host. In this respect, Bender (63) and Jencks (64) reviewed the mechanisms of enzyme catalysis. The enzyme(s) may be widely distributed throughout the host tissue (*e.g.*, esterase) or localized in site-specific tissue (*e.g.*, amidase). Enzymes *per se* have been characterized chemically, but their efficiency and

Table II—Reversible Derivative and Prodrug Linkages and Enzymes Responsible for Their *In Vivo* Hydrolysis

Linkage	Hydrolyzing Enzyme	Tissue	Reference
Ester			
Short–medium chain aliphatic	Cholinesterase	Liver, kidney, gut	149–160, 161, 162
	Ester hydrolase	Blood, intestinal mucosa	
	Lipase		
	Cholesterol esterase		
	Acetylcholinesterase		
	Acetyl esterase		163
	Aldehyde oxidase	General distribution	
	Lipase	Liver	
	Carboxypeptidase	Gut	105, 107
Long chain aliphatic	Pancreatic lipase		103, 116
Carbonate	Pancreatin	Intestine	
	Lipase		164
	Aliesterase		165–168
	Carboxypeptidase		
	Cholinesterase	Blood	
Hemiester	Esterases	Blood	169
Phosphate, organic	Acid phosphatase	Blood	170–173
	Alkaline phosphatase	Blood	
	Acid phosphatase III	Blood	174, 175
Pyrophosphate	Pyrophosphatase I	Liver, gut, blood	176
Sulfate, organic	Steroid sulfatase	Liver, blood	177
	Arylsulfohydrolase A and B (phenolsulfatase, arylsulfatase)	Liver	178, 179
	Arylsulfohydrolase C	Liver	180
	Estrogen sulfohydrolase	Liver, gut	181, 182
	Steroid-3β-sulfohydrolase	Liver	183–185
	Steroid-21-sulfatase	Placenta	186
Amide	Amidase	Liver	156, 187–193
		Neoplastic tissue	
		Walker carcinosarcoma 256	194
		Dunning rat leukemia	195
		Neoplastic tissue	191
Amino acid	Proteolytic enzymes	Gut	105, 107, 196
		Neoplastic tissue	110
	Chymotrypsins A and B		106
	Trypsin		105, 107
	Carboxypeptidase A		105, 107
	Carboxypeptidase B		108, 109, 197, 198
Azo	Azoreductase	Liver	199
		Walker rat carcinoma	200
		Sarcoma 180	
		Adenocarcinoma 755	201
		Lymphoid leukemia L-1210	
		Liver	202–205[a]
		Gut microflora	66, 68
Carbamate	Carbamidase	Liver	206
		Walker carcinosarcoma 256 (rats)	115
		Adenocarcinoma 755 (mice)	115
		Liver	207
Phosphamide	Phosphoramidases	Neoplastic tissue (liver)	208–216
		Liver	
Glucosiduronate (β-glucuronide)	β-Glucuronidase	Liver	217–220
		Gut	221
		Blood	
		Gut microflora	66, 68
N-Acetylglucosaminide	α-*N*-Acetylglucosaminidase	Liver, gut, blood	222, 223
	β-*N*-Acetylglucosaminidase		224
β-Glucoside	β-Glucosidase	Gut, liver	176
		Gut microflora	66, 68

[a] This excellent review covers a wide variety of bioreversible nitrogen mustard drug derivatives.

specificity have generally not been quantitatively determined *in vivo*.

Quantitative predictions of the effect of drug derivative enzymatic catalysis *in vivo* are difficult due to the variety and complexity of the enzyme systems involved. The liver, for example, considered the most important organ for drug degradation, contains a complex, nonspecific variety of enzyme systems. The reactivity of these systems varies widely in individuals and largely depends on genetic and hormonal factors, sex, age, *etc.*, making absolute predictions impossible. Furthermore, *in vitro* enzyme hydrolysis studies can be misleading if not conducted with several animal species. For example, a species specificity was demonstrated when hydrolysis rates of the reversible derivative 4-acetamidophenyl-2,2,2-trichloroethyl carbonate were compared using human and rat plasma (65). Hydrolysis proceeded about four times faster in rat plasma than in human plasma, probably due to a greater abundance of specific and/or nonspecific esterases present in rat plasma. This same phenomenon has been demonstrated for a se-

ries of lincomycin 2,7-dialkylcarbonate esters (12).

A number of chemical linkages covalently bonded to a drug substrate that are known to be hydrolyzed by certain common enzymes are listed in Table II. The host tissues in which these enzymatically catalyzed reactions occur are also shown. Although qualitative in nature, this outline provides the medicinal chemist with a powerful tool for designing bioreversible drug derivatives tailored to meet a specific therapeutic need, route of administration, and dosage regimen.

Enzymes considered important to orally administered reversible derivatives are found in the gut, gut wall, blood, and liver. The gut of several animal species, including humans, contains a variety of GI microflora whose enzyme systems are capable of hydrolyzing a number of reversible drug linkages. Thus, the naturally occurring glycosides amygdalin and cycasin are hydrolyzed by gut microfloral enzymes to their aglycones methylazoxymethanol and mandelonitrile, respectively (66). β-Glucuronidases of similar microflora are capable of hydrolyzing stilbestrol and indomethacin glucuronides excreted in the bile. The parent drug is then reabsorbed from the intestine. This hydrolytic activity thus serves as an important event in the enterohepatic circulation of these drug entities (66, 67). Azo linkages are hydrolytically reduced by azoreductase enzymes present in gut microflora. The reversible drug derivative sulfachrysoidine[2] is converted to the parent bioactive species sulfanilamide by this mechanism (66, 67).

Experiments in humans and germ-free rats have shown that sulfasalazine (salicylazosulfapyridine), a drug used in the treatment of ulcerative colitis, is enzymatically reduced to 5-aminosalicylate and sulfapyridine in the GI tract. The former has been implicated for its direct effect on the colon (68, 69). Other chemical linkages known to be hydrolyzed by GI microorganisms are carboxylic esters, sulfate and nitrate esters, amides, and sulfamates (70).

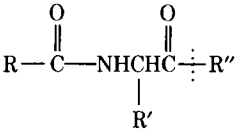
Esterases—The distribution of esterases is ubiquitous and includes a wide assortment of vertebrate tissues and blood serum (71–75). Nonspecific esterases, as the name implies, hydrolyze a variety of ester types including aliphatic, aromatic, and thiol esters (76, 77) and amino acid esters (78, 79), as well as a number of aromatic amides (76) such as acetanilid, phenacetin, and lidocaine. Specific esterases include acetylcholinesterase (80), cholesterol esterase (81), and possibly vitamin A esterase (82). For a comprehensive review of carboxylic ester hydrolases, see Ref. 76. Many bioreversible drug derivatives have been designed to take advantage of the catalytic activity of these esterases.

Lipases, another subclass of hydrolases, can be considered as carboxylesterases with one difference—they are unable to hydrolyze substrate ester derivatives that are fully dispersed (soluble) in water. There appears to be a minimum degree of molecular aggregation necessary for their hydrolytic activity (83). Lipases have recently been shown to hydrolyze molecules in a micelle (84, 85). Two classes of lipases are considered important for hydrolysis of reversible drug derivatives: tissue lipases and lipases found in the digestive tract due to discharge by specialized organs. The latter type is exemplified by pancreatic lipase. Good correlation was established between the hydrolysis rate of a micellar solution of tripropionin and lipase esterolytic activity on these solutions containing approximately 13 monomers per micelle (84).

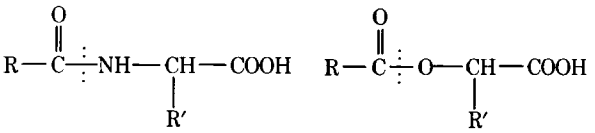
The rapid hydrolysis of clindamycin palmitate given orally may be due to such hydrolytic activity since this drug exists as a micellar solution (86). A similar lipase is thought to hydrolyze micellar solutions of cholesterol and vitamin A esters (87–90). The fact that short chain fatty acid esters are not hydrolyzed as readily as longer chain (C_{10}–C_{18}) esters may be due to the fact that short chain esters are, in general, water soluble whereas the longer chain esters tend to form micellar solutions.

Other lipases that may affect bioreversible derivatives have been found in the microsomal fraction of rat intestinal mucosa (91) and postheparin plasma (92). They hydrolyze short chain monoglycerides and simple esters but not cholesterol esters or long chain di- and triglycerides. A gastric lipase has been found in the microsomal fraction of gastric mucosa (93, 94) which hydrolyzes a number of variable chain-length triglycerides (95). Another lipase of interest is the so-called lipoprotein lipase. It can be detected in plasma within 20 sec after an injection of heparin, suggesting that it may be attached to blood capillary walls (96, 97). The liver contains at least three known lipases: one acidic lipase (98–100) and two alkaline lipases (99, 101). These and other lipases have been reviewed (102, 103).

Another class of hydrolases that may be important in the hydrolysis of bioreversible drug derivatives is the proteolytic enzymes (peptidases) found in the duodenum and intestinal epithelium. The amino acid derivatives hydrolyzed by the endopeptidases can be represented by the general formula:



Those hydrolyzed by the carboxypeptidases can be represented by the general formulas:



peptide substrates ester substrates

The dotted lines represent the point of hydrolysis by the peptidase. Studies with glycylglycine indicate that hydrolysis occurs within the intestinal epithelial cell rather than at the microvilli surface (104). Some of the better known endopeptidases include the chy-

[2] Prontosil.

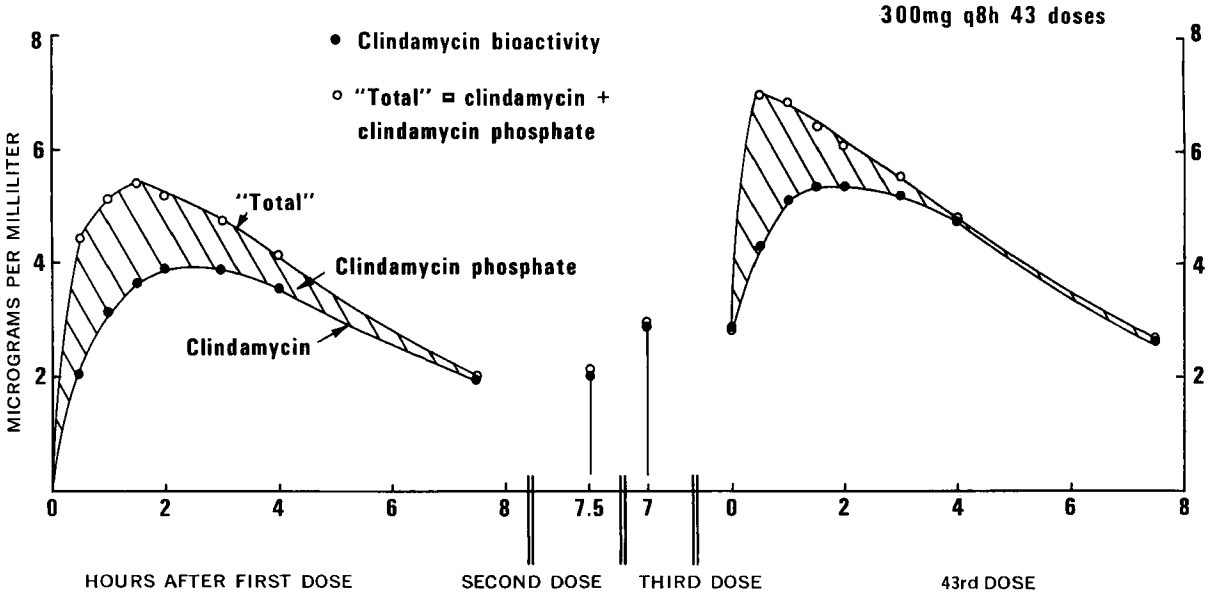


Figure 4—*Mean serum concentrations (micrograms per milliliter) of clindamycin and clindamycin phosphate after intramuscular injections of clindamycin phosphate. (Adapted, with permission, from Ref. 173.)*

motrypsins. These enzymes catalyze the hydrolysis of amide substrates containing the aromatic amino acids phenylalanine, tryptophan, and tyrosine. Moreover, esters of these derivatives are cleaved more rapidly than the amides (105). Chymotrypsin C differs from chymotrypsins A and B in its ability to hydrolyze more rapidly the ester, amide, and peptide bonds involving leucine (106). Trypsin, another peptidase found in the intestine, acts exclusively on peptide, amide, and ester bonds formed with arginine and lysine (105, 107).

Carboxypeptidase A and B, exopeptidases found in the intestine, catalyze the hydrolysis of ester and peptide bonds of derivatives containing aliphatic side chains such as alanine (105), leucine (105), isoleucine (105), and hippuryl-L-lysine (108, 109).

The abundance of proteolytic enzymes found in cancer cells led to the synthesis of glycine bis(2-chloroethyl)amide as a bioreversible form of nitrogen mustard which might selectively be hydrolyzed in such cancer cells (110).

Other investigators have reported on the effect of amidases on the hydrolysis of aminoacylanilides (111–113) and penicillin derivatives (114).

Carbamidases present in Walker carcinosarcoma 256 were deemed responsible for hydrolysis of various bis(1-aziridinyl)phosphinyl carbamates to the bioactive alkylating bis(1-aziridinyl)phosphinyl radical (115).

Keller (116) and Rhodes (117) have reviewed the properties and functions of a wide variety of proteolytic enzymes, and other comprehensive reviews have appeared elsewhere (118).

Alkaline Phosphatase—Alkaline phosphatases (orthophosphoric monoester phosphohydrolases) catalyze the hydrolysis of many alkyl and aryl monophosphate esters. This family of enzymes is localized mainly in kidney and intestinal mucosa (119). Ossifying cartilage is also a rich source of such phosphatase (120) and has an optimum activity at pH 8.4–9.4 (121), similar to that found in blood plasma (122). Isotope labeling studies support the belief that alkaline phosphatase acts *via* a transferase mechanism whereby a phosphoryl moiety is directly transferred from the phosphate ester to an acceptor alcohol (123). It was also shown that Zn^{+2} (2–3 g-atoms/mole of enzyme) was essential for catalytic activity (124, 125). These enzymes catalyze the hydrolysis of a wide variety of ester substrates, including phosphate monoesters, diesters, and phosphoroamidate (126–128).

Acid Phosphatase—Like alkaline phosphatase, the acid phosphatases or orthoester phosphohydrolases are widely distributed throughout nature. Their distribution in mammalian tissue differs somewhat from alkaline phosphatase, being found predominantly in red blood cells (129, 130), plasma (131), prostatic tissue (132–134), spleen (135, 136), and liver (137).

The availability of acid phosphatases in red blood cells and plasma may account for the rapid hydrolysis of clindamycin phosphate to clindamycin when clindamycin phosphate is administered intramuscularly (Fig. 4). Peak serum concentrations of clindamycin appear in less than 0.5 hr, reflecting rapid hydrolysis of the phosphate ester in the systemic circulation. Intramuscular administration of this derivative results in delayed absorption of clindamycin, due in part to rate-limiting diffusion from the injection site as the phosphate ester, with subsequent serum hydrolysis.

The biologically reversible antineoplastic drug, diethylstilbestrol diphosphate, was designed to take advantage of the high phosphatase activity in prostatic carcinoma tissue. Given as an intramuscular injection, the derivative was thought to diffuse from the injection site as the ester and circulate to the prostatic tissue where hydrolysis occurred, thus accu-

Table III—Reversible Drug Derivatives Utilized as Modifiers of Absorption (Oral, Percutaneous, and Parenteral)

Parent Molecule	Reversible Modification or Linkage	Route of Administration	Property Modified	Reference
Convallatoxin	Ketal	Oral	Resorption	228
Hydantoin	Alkyl ester	Oral	Absorption	229
	Amino ester			
Chlorphenesin	Glycine ester	Oral	Absorption	230
	Alanine ester			
Acetaminophen	Caffeine complex	Oral	Absorption	231
Acetylsalicylic acid (aspirin)	THAM salt	Oral	Absorption	232
Acetylsalicylic acid (aspirin)	Acetamidophenyl ester	Oral	Absorption	233
N-Allylnoroxymorphone (naloxone)	Acetate ester	Oral	Absorption	148
	Sulfate ester			
Nicotinic acid	Mesoinositol penta-nicotinate	Oral	Absorption	234
15-Methylprostaglandin $F_{2\alpha}$	Methyl ester	Oral	Absorption	227
Procaine	Polyethylene glycol (carbamate)	Percutaneous Conjunctival	Absorption	235
α-Amino-*p*-toluenesulfonamide (4-homosulfanilamide)	Acetate salt	Percutaneous	Absorption	30, 236
Hexachlorophene	Ester	Percutaneous	Absorption	21
	Ether			
	Hemiester			
Oleandomycin	Acetate ester	Oral	Absorption	237
Erythromycin	Ester, alkyl	Oral	Absorption	14, 238, 239
Clindamycin	Ester, alkyl	Oral	Absorption	13, 240
	Ester, phosphate	Intramuscular		241
α-Carboxybenzylpenicillin	Ester, mono- and dialkyl	Oral	Absorption	242
α-Aryl-β-aminoethyl penicillin	Alkoxymethyl esters	Oral	Absorption	243
Penicillin, general structure	Diethylaminoethyl ester, alkoxymethyl esters, ether	Oral	Absorption	244
α-Aminobenzylpenicillin (ampicillin)	Azide	Intravenous, oral	Absorption	245, 246
6-(D-α-Sulfoaminophenylacetamido)penicillin	Pivaloyloxymethyl ester	Oral	Absorption	247
Carbenicillin	Indanyl ester	Oral	Absorption	248, 249
α-Aminobenzylpenicillin (ampicillin)	Acyloxymethyl ester	Oral	Absorption	225, 226, 250–253
Penicillin G	Amide with 1,2-benziso-thiazol-3(2*H*)-one 1,1-dioxide	Intramuscular	Absorption	254
Penicillin V				
α-Aminobenzylpenicillin (ampicillin)	*N*,*N*-Isopropylidene adduct	Oral	Absorption	255, 256
Hetacillin	Pivaloyloxymethyl ester	Oral	Absorption	257
Doxycycline	Polymetaphosphate complex	Oral	Absorption	258
Colistin	Lower alkyl esters	Oral	Absorption	259
Tetracycline	Betaine salt	Oral	Gastric absorption	260
7-Acylaminocephalosporins	Ring-substituted acyloxybenzyl esters	Oral	Absorption	261
	Acyloxyalkyl esters	Oral	Absorption	262
α-Amino (or ureido) cyclohexadienylalkyl penicillins and cephalosporins	Acyloxymethyl esters	Oral	Absorption	263
7-Acylaminocephalosporins	Imino ether	Oral	Absorption	264
Nandrolone	Phenylpropionate, decanoate esters	Intramuscular	Absorption	266
9α-Fluorohydrocortisone (fludrocortisone)	Acetate ester	Subcutaneous	Absorption	266
Estradiol	Enol ether	Oral, parenteral	Absorption	267
	Acetal			
Oxymetholone	Ethoxycarbonate ester	Oral, subcutaneous	Absorption	268
Methylprednisolone	Acetate ester	Intramuscular	Absorption	269
Testosterone	*N*-Acetylglucosaminide	Oral	Absorption	270, 271
	Glucosiduronate (trimethylsilyl) ether	Oral		
19-Nortestosterone	17-β-Adamantoate	Intramuscular	Absorption	272
9-(β-D-Arabinofuranosyl) adenine	Acetate, formate, propionate, esters	Oral	Absorption	273
Cortisol, prednisolone, dexamethasone	21-Phosphate ester	Intramuscular, intravenous	Absorption	274
	21-Hemisuccinate ester	Subcutaneous		
Prostaglandin	Alkylsilyl ether	Percutaneous, oral, intrauterine	Absorption	275
Salicylic acid	Carbonate ester	Oral	Absorption	276, 277

mulating as diethylstilbestrol in this localized site (23). Good antitumor activity was similarly demonstrated in mouse plasma cell tumor ADJ/PC6A with a series of phosphate esters of *p*-hydroxy-*N,N*-bis(2-chloroethyl)aniline. The activity was attributed to hydrolysis of the phosphate esters by acid and alkaline phosphatases present in the tumor (138, 139).

Comprehensive reviews on these important classes of enzymes have been written (140, 141), and Schapira reviewed the properties of various enzymes present in neoplastic tissue (142).

Sulfatases—The sulfate ester-hydrolyzing enzymes are not necessarily limited to the catalytic hydrolysis of sulfate ester anions but exert their effects on sulfatophosphates (sulfatophosphate sulfohydrolases) and sulfamates (sulfamatases). The sulfatases can be subdivided into aryl, alkyl, and steroid sulfatases. The function of the aryl and alkyl sulfatases is obscure, although they are found in liver (143, 144) and brain (145, 146) tissue. The steroid sulfatases, however, catalyze the hydrolysis of several types of steroid sulfate esters including estrone sulfate, androstenolone sulfate, etiocholanolone sulfate, and cortisone-21-sulfate.

A series of estrogen sulfates was synthesized (147), but no biological testing of these esters was reported. Linder and Fishman (148) found low narcotic antagonist activity upon intravenous administration of an isomeric monosulfate and a disulfate ester of naloxone.

Although virtually no drug derivatives containing a sulfate ester moiety have been explored, their use could be rationalized in therapeutic indications analogous to those of drug phosphate esters.

For obvious reasons, drugs possessing low aqueous solubility would be excluded from the intravenous route of administration. Reversible derivatives designed to enhance aqueous solubility of such drugs *in vivo* include hemiesters, phosphate, and, possibly, sulfate esters.

APPLICATIONS

Absorption—Table III lists some presently marketed drugs that contain reversible linkages designed to enhance absorption of the parent molecule. This list of examples is designed to highlight the variety and scope of chemical linkages utilized to modify a wide selection of parent drug molecules. Parent molecules include representatives from many diverse classes of drugs, and chemical linkages include complexes and salts as well as covalently bound moieties. Since absorption is a general phenomenon, several routes of administration are included to illustrate the versatility of this form of drug modification.

One fine example illustrating the importance of reversible derivative formation and its effect on absorption is found in studies with ampicillin (D-α-aminobenzylpenicillin) (225, 226). This drug is relatively stable in an acidic medium but poorly absorbed orally. A series of acyloxymethyl esters (I*a*–I*h*) was synthesized to provide a hydrophobic form of ampicillin that would be rapidly hydrolyzed *in vivo* after absorption to enhance serum levels of this antibiotic.

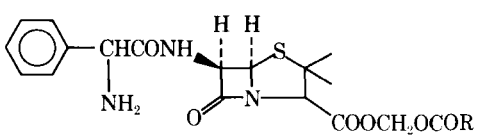
Table IV[a]—Hydrolysis of Acyloxymethyl Esters of Ampicillin at pH 7.4 and 37° in the Presence of 10% Human Serum[b]

Ester	Percent Hydrolyzed[c] after 30 min
Acetoxymethyl (I*a*)	89
Propionyloxymethyl (I*b*)	80
κ-Butyryloxymethyl (I*c*)	85
Isobutyryloxymethyl (I*d*)	89
Pivaloyloxymethyl (I*f*)	22
α-Ethyl-κ-butyryloxymethyl (I*g*)	23
Benzoyloxymethyl (I*h*)	67

[a] Adapted, with permission, from Ref. 225. [b] The starting concentration of the esters was 1/35 mmole ~ 10 μg/ml of free ampicillin. [c] The figures do not indicate the exact degree of hydrolysis since, under the applied conditions, ampicillin as well as its esters undergoes transformations (probably polymerization) resulting in loss of antibacterial activity.

In vitro hydrolysis studies were carried out on several ampicillin esters (Table IV). It was apparent that the straight chain esters were more rapidly hydrolyzed than those with branched chains. This *in vitro* study, while indicative of the relative degree of enzymatic *in vitro* lability of these esters, was not conclusive in determining which ester might suitably be used orally *in vivo*. It would appear that the pivaloyloxymethyl ester (I*f*) is hydrolyzed too slowly to be of use clinically. In point of fact, absorption studies in humans (226) indicated that this ester was absorbed almost quantitatively and enzymatically hydrolyzed rapidly after absorption (Fig. 5). This ester hydrochloride of ampicillin represents the right degree of hydrophobicity for absorption coupled with the required amount of enzymatic lability necessary for hydrolysis to produce extremely high plasma levels of ampicillin. Peak serum levels of ampicillin (administered as pivampicillin) were more than three times greater than ampicillin administered above. This ratio of peak serum levels was invariant in both fasted and fed subjects. Since this study, acyloxymethyl esters of several antibiotics have been made in an attempt to duplicate the success achieved with ampicillin (Table III).

15-Methylprostaglandin $F_{2\alpha}$ and its methyl ester provide yet another example of how a bioreversible derivative can enhance absorption of a parent drug molecule. Magee *et al.* (227) determined the rate of absorption of 15-methylprostaglandin $F_{2\alpha}$ and its methyl ester in rats by using an *in situ* method of sequential sampling of the duodenal contents. They found that absorption of the ester was biphasic with



I*a*: R = CH_3
I*b*: R = CH_2CH_3
I*c*: R = $(CH_2)_2CH_3$
I*d*: R = $CH(CH_3)_2$
I*f*: R = $C(CH_3)_3$
I*g*: R = $CH(CH_2CH_3)_2$
I*h*: R = C_6H_5

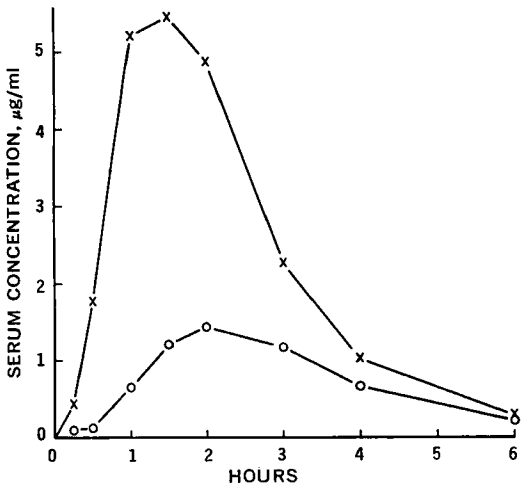


Figure 5—*Mean serum levels of ampicillin in normal volunteers following oral administration of 250 mg of amoicillin (O) and 358 mg of pivaloyloxymethylampicillin hydrochloride (≈250 mg of ampicillin) (X) immediately after breakfast. (Adapted, with permission, from Ref. 225.)*

initial rapid uptake followed by slow continuous absorption (Fig. 6).

The rapid phase of absorption declined as the ester was cleaved to the parent free acid. Higher doses of ester resulted in more drug being absorbed, with no ester being detectable in the serum (Fig. 7). These researchers concluded that the methyl ester was absorbed very rapidly, with hydrolysis by esterases occurring in the intestinal lumen and serum. Serum

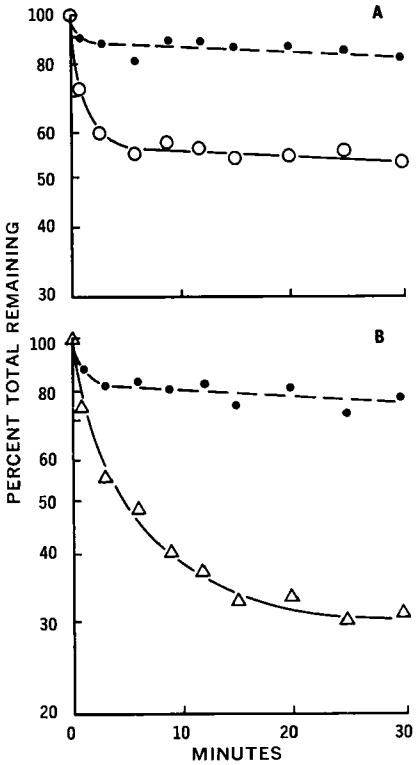


Figure 6—*Absorption of a low dose (A) (0.05 mg/ml) and of a high dose (B) (0.5 mg/ml) of 15-methyl-^{14}C-prostaglandin $F_{2\alpha}$ methyl ester. The dashed lines show changes in fluid volume. (Adapted, with permission, from Ref. 227.)*

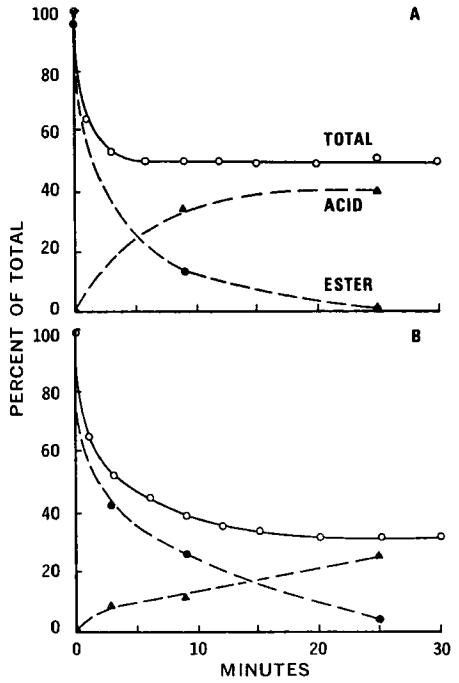


Figure 7—*Hydrolysis of 15-methyl-^{14}C-prostaglandin $F_{2\alpha}$ methyl ester to the free acid in the lumen of the small intestine. Same experiment as shown in Fig. 6. Key: A, low dose; B, high dose; ▲, 15-methyl-^{14}C-prostaglandin $F_{2\alpha}$; ●, 15-methyl-^{14}C-prostaglandin $F_{2\alpha}$ methyl ester; and ○, ● + ▲. (Adapted, with permission, from Ref. 227.)*

levels of parent prostaglandin 10–20 times greater were observed when the methyl ester was administered.

Site Direction—Historically, research in the area of site-directed, reversible derivative chemotherapy has been generated mainly by empirical data and by intuitive notions and correlations. The fault lies not with the chemist and biologist but rather with the paucity of definitive data that might be used for planning and execution of meaningful studies in this area.

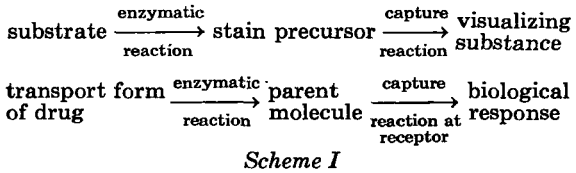
The concepts of reversible drug derivative chemotherapy to specific tissue can be generally divided into two classes:

Site directed, in which the reversible derivative is transported and distributed preferentially to site-specific tissue according to the physicochemical properties of the derivative (*e.g.*, hydrophobicity–hydrophilicity relationships, pKa, molecular size and geometry, and solubility). This subject was discussed in the *Absorption* section.

Site activated, which relates to the regeneration of the parent molecule either by enzymatic or chemical hydrolysis in the target organ or site-specific tissue.

The rationale for the synthesis of site-directed drugs evolved primarily in the area of cancer chemotherapy but can be summarized by the following basic premises that apply to chemotherapy of any localized disease state:

1. Certain disease conditions are localized in specific tissue.
2. The drugs utilized in these disease states affect



Scheme I

normal as well as diseased tissue and may be metabolized and excreted prior to reaching the diseased tissue.

3. Diseased tissue possesses enzymes or enzyme systems differing in their nature and abundance from normal tissue.

4. These enzymes are capable of regenerating the bioactive species of a reversible derivative.

Several excellent papers and reviews are based on these principles (160, 163, 278–283).

Harper (23), in discussing drug localization in specific tissue, drew analogies to enzyme histochemistry in which the histochemical staining process was likened to site-specific drug localization (Scheme I). By drawing inferences from Scheme I and observing the relationship of the low solubility of certain dyes and their high association by hydrogen bonding, substituent effects and steric properties, and subsequent binding to protein, it was concluded that optimal conditions for drug transport to site-specific tissue involved steric interactions (fit) of the drug with the receptor and also the influence of distribution, transport, excretion, and duration of action to these drug–protein interactions.

The ideal reversible drug derivative might possess the following characteristics:

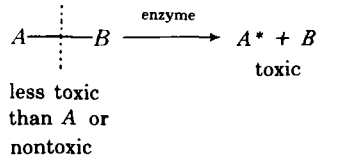
1. Exclusive and complete transport to the diseased tissue or target organ, including affinity and interaction with these cell systems and tissues.

2. Absence of binding of the derivative to protein or tissue not specifically diseased without degradation (hydrolysis) or metabolism of the derivative prior to contact with the diseased bioenvironment; lack of toxicity for normal tissue within the diseased environment and other normal tissue in the body.

3. Complete elimination from the body once the disease state is eliminated or once complete remission is effected.

The ideal derivative consists of a bioactive parent molecule containing a suitable transport group sufficiently stable to deliver the molecule to the site of activity (site direction) containing the necessary enzyme systems and/or chemical bioenvironment sufficient to regenerate the parent molecule at the diseased site (site activation). While the ideal derivative is yet to be made, several approaches have been utilized in a variety of attempts at providing optimal therapeutic response and site specificity. They can be categorized as: *(a)* utilization of the enhanced enzymatic activity present in diseased or dividing cells, especially neoplastic cells, but not present in normal, nondividing cells; *(b)* utilization of the lower pH of neoplastic tissue relative to that of most normal cells; and *(c)* utilization of lipophilic and/or hydrophilic derivatives to enhance transport and subsequent bioactivity to site-specific tissue.

Following the first approach, Tsou *et al.* (191) synthesized a series of nitrogen mustard reversible amide derivatives designed to regenerate the parent molecule in neoplastic tissue by taking advantage of the difference in the level of enzymatic amidase activity between normal and neoplastic cells. Assuming amidase activity to be localized in the tumor cell, they schematically represented what might occur at the site (Scheme II):



Scheme II

where *A—B* represents a nontoxic bioreversible derivative of *A* (toxic to neoplastic tissue), and the dotted line represents the site of hydrolysis of the derivative by the enzyme.

Of several derivatives synthesized, *N,N*-diallyl-β-aziridinopropionamide (DAAP) was found to be active intraperitoneally against the Dunning leukemia system in both ascites and solid form, and data were obtained at doses between 20 and 40 mg/kg. Leucopenia, a common manifestation of toxicity with other bisfunctional alkylating agents, was not apparent with *N,N*-diallyl-β-aziridinopropionamide, indicating that the derivative was selective in its action against dividing (neoplastic) tissue.

Other examples of this approach can be found in Table V.

Another property, namely lower relative pH of certain neoplastic cells (284, 285), has been exploited in the design of bioreversible cancer chemotherapy drug derivatives. The fact that pH is lower in tumor cells may be due to the high rate of aerobic and anaerobic glycolysis, the end result being the production of lactic acid. This acid may accumulate in tumor cells if its production is greater than an offsetting neutralization due to diffusion of buffering substances into the cells from the arterial circulation and by acid diffusing from the cell into venous blood (286, 287). If the preceding elimination mechanisms were not operative, the pH of the tumor cells would probably decrease to a limiting value of ~6 (288).

Papanastassiou *et al.* (287) investigated the *in vivo* acidic lability of a series of alkylaryldiazoamino compounds known to undergo facile acid hydrolysis *in vitro* (Scheme III). Of those compounds tested, *p*-[*N,N*-bis(2-methanesulfonoxyethyl)amino]benzenediazonium fluoroborate (II), while somewhat toxic, prolonged the lifespan of L-1210 leukemia-implanted mice to 157% of controls at a dose of 1.25 mg/kg. It produced cures in five out of six mice with ascites Dunning leukemia. Several other compounds tested were inactive when R = CH_2CH_2F and Y = NO_2 or

$$YArN{=}NNR_2 \xrightarrow{H_3O^+} YArN_2^+ + HNR_2$$

Scheme III

$COOC_2H_5$. The choice of R and Y substituents appears to be critical to activity.

Table V—Reversible Drug Derivatives Utilized as Modifiers and Enhancers of Drug Transport to Site-Specific Tissue

Parent Molecule	Reversible Modification or Linkage	Route of Administration	Target Tissue or Organ	Reference
Isocyanate, R—N=C=O	Haloalkyl nitrosourea	Intraperitoneal Intravenous Intracerebral Subcutaneous Oral	L-1210 leukemia	301–303
Testosterone	Propionate ester	Intramuscular	Breast carcinoma	304
Dromostanolone	Propionate ester	Intramuscular	Breast carcinoma	305
Urea	Hydroxyl		Myelogenous leukemia	306
Normeperidine	Amide	Subcutaneous	CNS	307
Morphine, phenazocine	Ether	Subcutaneous	CNS	308
Tetrahydrocannabinol	Ester	Oral, intramuscular	CNS	152
Diethylstilbestrol	4,4′-Diphosphate ester	Intramuscular	Prostatic carcinoma	23
	4,4′-Dicarbamate	Intramuscular	Prostatic carcinoma	309
Methotrexate, 3′,5′-dichloromethotrexate	Dialkyl esters		L-1210 leukemia	154
Phenethylamine, *dl*- and *d*-amphetamine, *l*-ephedrine, *dl*-*p*-hydroxyamphetamine	Carbonate	Intraperitoneal	Brain	207, 310
Dopamine, 3,4-dihydroxyphenethylamine	Diacyl esters		Brain	311
Norepinephrine	Silyl ether Acetate ester	Intravenous	Brain Heart	312
Cytosine arabinoside (cytarabine)	5′-Acylate 5′-Sulfonate	Intraperitoneal Subcutaneous	L-1210 leukemia	151
Hydroxyurea	Alkyl ether	Intravaginal	Vaginal mucosa	206
Dichloroisocyanatophosphine oxide	Carbamate		Walker carcinosarcoma 256	115
N,*N*-Bis(2-chloroethyl)-phosphorodiamidic acid	Cyclic phosphoramide	Intraperitoneal Intravenous	Malignant lymphomas Neuroblastoma Ovarian adenocarcinoma Breast carcinoma Wilms tumor	210, 214 215 297, 299
Nitrogen mustard (2,2′-dichloro-*N*-methyldiethylamine)	Phosphorodiamidic acid	Intraperitoneal	Dunning ascites tumor L-1210 leukemia	216
Nitrogen mustard	Aminomethyl tetracycline	Intraperitoneal	Adenocarcinoma 755 L-1210 leukemia	192
Allylamine	Amide	Intravenous	Adenocarcinoma 755	191
Nitrogen mustard	Cholesterol, estradiol, dehydroepiandrosterone esters	Oral	Mammary tumor Leukemia	155, 313
Nitrogen mustard	Peptide (glycine, phenylalanine)	Intraperitoneal	Hepatoma Walker carcinosarcoma 256	314 315, 316
5-Diazoimidazole-4-carboxamide	Dialkyl triazene	Intraperitoneal	Sarcoma 180 Adenocarcinoma 755 L-1210 lymphoid leukemia	201
Nitrogen mustard	Azobenzene	Intraperitoneal	Liver	200, 205, 317
Dichlorodiamine, dichlorodialkylamine	Platinum coordination complex	Intraperitoneal Intravenous	Solid sarcoma 180 Walker 256 carcinosarcoma Dunning leukemia Primary Lewis lung carcinoma	318, 319

In other studies utilizing the neoplastic cell pH hypothesis, Shealy *et al.* (201) found that the lifespan of L-1210-implanted mice increased 57% over controls when dosed with 5(or 4)-(dimethyltriazeno)imidazole-4(or 5)-carboxamide (III). These compounds are acid labile (300) and were thought to regenerate the parent bioactive 5-diazoimidazole-4-carboxamide *in vivo* as the internal salt (IV). A pH difference between neoplastic and normal tissue and its effect on bioactivity of antineoplastic agents were also studied (289).

A third approach, currently receiving increased attention, concerns the correlation of structure–activity relationships with lipophilic character of the drug derivative. A study (290) of some quantitative structure–activity relationships of a series of nitrosoureas and triazinoimidazoles found that for each series the lipophilic character (a crude measure of absorption and transport) of each type of drug was parabolically related to its activity against L-1210 leukemia in mice. A parabolic relationship was found for 23 nitrosoureas when log P was plotted against log $(1/c)$ (Fig. 8).

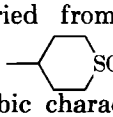
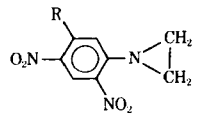
Log P was taken as a measure of hydrophobic character of the derivative molecule where P was its octanol–water partition coefficient. Log $1/c$ is a measure of the bioactivity of the drug. When X = Cl or F and R substituents varied from 4-*tert*-butylcyclohexyl (log $P = 4.51$) to  (log $P = -0.41$), the optimum hydrophobic character occurred at log P from about -0.4 to -0.6. Steric and electronic effects on bioactivity were not considered in this theoretical study (290). Based primarily on hydrophobic consid-

Table VI[a]—Solubility, Partition Coefficients, Substituent Constants, and Biological Activity of *N*-Substituted 1-Aziridino-2,4-dinitrobenzamides[b]



Compound	R	*S*, μmoles/ml	P^E	P^B	Log P^E	π	π'	ED_{90}, μmoles/kg	LD_{50}, μmoles/kg
1	H	1.01	30	>100	1.48	—	—	100	1100
	CONHR′								
16	R′ = H	5.04	0.50	0.14	−0.28	−1.76	—	1.6	112
41	CH_3	1.41	0.87	1	−0.06	−1.54	0.22	<9	113
42	C_2H_5	0.75	2	3.1	0.30	−1.18	0.58	3.6	100
43	*n*-Propyl	0.13	5.4	12	0.73	−0.75	1.01	34	95
44	Isopropyl	0.32	4.5	9	0.65	−0.83	0.93	5.1	54
45	Cyclopropyl	0.41	1.7	3.3	0.23	−1.25	0.51	3.4	110
46	Isobutyl	0.14	14	30	1.15	−0.33	1.43	29	146
47	*tert*-Butyl	0.68	30	55	1.48	0	1.76	65	227
	$CONHR_2$								
52	$R_2 = (CH_3)_2$	3.3	1.1	15	0.04	−1.44	0.32	−ve	65
53	$(C_2H_5)_2$	2.6	7.5	>100	0.86	−0.62	1.14	−ve	81
54	$—(CH_2)_5—$	0.68	9.2	>100	0.95	−0.53	1.23	109	281
55	$—(CH_2)_2O(CH_2)_2—$	1.3	1.2	15	0.08	−1.40	0.36	186	560

[a] Adapted, with permission, from Ref. 289. [b] *S* = solubility in water containing 2% dimethyl sulfoxide at 25°, P^E = ether–water partition coefficient, P^B = benzene–water partition coefficient, $\pi = \log P^x - \log P^H$ (for ether–water coefficients), and $\pi' = \log P^x - \log P^{CONH_2}$ (for ether–water coefficients).

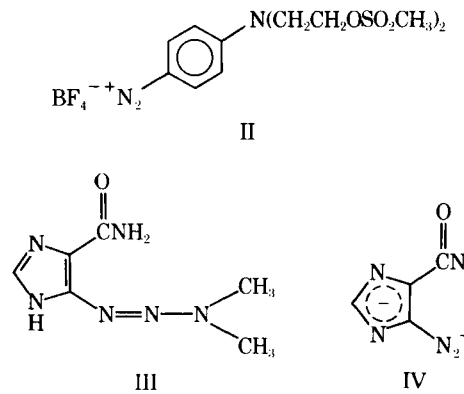
erations and the parabolic relationship, however, it was suggested that nitrosourea derivatives containing more hydrophilic substituents might yield more potent and less toxic drugs, although there are not enough data in Fig. 8 to substantiate the maximum value accurately. The trend, however, is apparent.

It was also shown that an approximate measure of the derivatives' LD_{10} (dose required to kill 10% of test animals) was related parabolically to log *P*. Maximum toxicity was found at log $P_0 = 0.4$. Log P_0 for toxicity was determined by taking the derivative *d* log 1/*c*/*d* log *P* and was found to be approximately 1 log unit higher than for potency, implying that derivatives containing more hydrophilic substituents, while increasing potency, simultaneously decrease toxicity. Wheeler *et al.* (291) confirmed these observations.

In contrast to the finding of Hansch *et al.* (290) of a parabolic relationship correlating log *P* with activity in a series of nitrosourea derivatives, Khan and Ross (289) found a linear relationship between log *P* and activity in a series of *N*-substituted 1-aziridino-2,4-dinitrobenzamides. Thus, they demonstrated a correlation between the water–lipid partition coefficients of a number of substituted amides of 5-aziridino-2,4-dinitrobenzamide (CB 1954) and the expected amide linkage sensitivity toward enzymatic hydrolysis in one instance and the chemotherapeutic index in the Walker tumor assay on the other. Not surprisingly, the highest antitumor activity was demonstrated by the more hydrophilic and easily hydrolyzed substituted amide derivatives. Table VI summarizes the results of solubility, partition coefficient, substituent constants, and bioactivity data for this series of amide derivatives.

The derivatives were tested as inhibitors of growth in transplanted Walker rat carcinoma 256; the LD_{50}, or median lethal dose for tumor-bearing animals, and the ED_{90}, or the dose that produced a 90% inhibition of tumor growth, were reported. The LD_{50}/ED_{90} ratio was taken as the chemotherapeutic index (C.I.). A plot of log $1/ED_{90}$ or log $1/LD_{50}$ *versus* π results in a linear relationship for a series of monosubstituted (but not disubstituted) amide derivatives (Fig. 9).

It can thus be seen that the presence of a monosubstituted amide group in 5-aziridino-2,4-dinitrobenzamide confers favorable water–lipid partitioning prop-



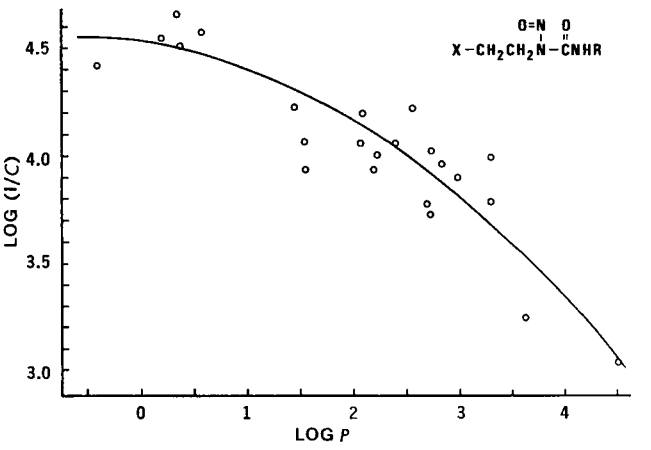


Figure 8—*Experimental data (log 1/C) for 23 nitrosourea derivatives plotted against log P. (Adapted, with permission, from Ref. 290.)*

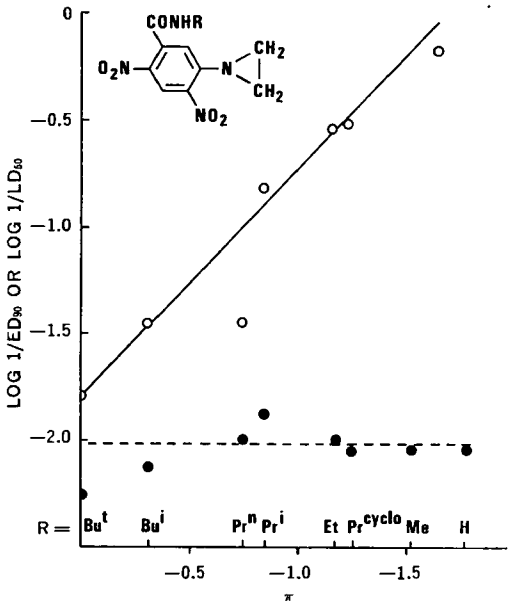


Figure 9—*Plot of substrate constant, π, against log 1/ED_{90} (○) and log 1/LD_{50} (●) for 5-aziridino-2,4-dinitrobenzamides. (Adapted, with permission, from Ref. 289.)*

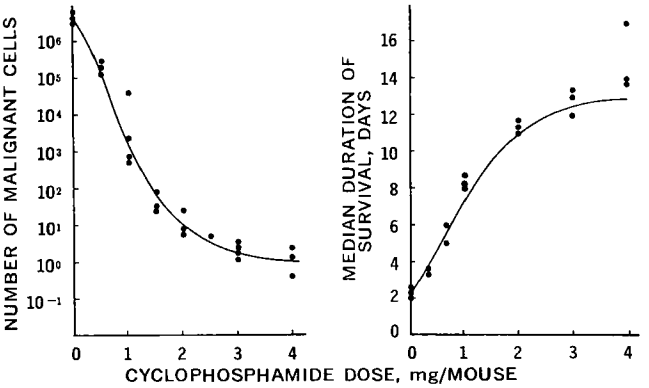
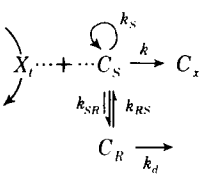


Figure 10—*Dose–cell survival and dose–animal survival curves for the effect of cyclophosphamide on lymphoma cells in the mouse femur. The cell survival line was obtained by least-squares fit of the data to the model of Scheme IV. The theoretical duration of animal survival curve was generated with the values* $C_T^0 = 2.2 \times 10^7$ *cells,* $k_s = 0.0603\ hr^{-1}$, *and cell number causing animal death* $= 8.6 \times 10^8$ *cells. (Adapted, with permission, from Ref. 293.)*

erties yet maintains sufficient *in vivo* stability *via* the amide linkage such that hydrolysis is selective within sensitive tumor cells. These two factors, coupled with the fact that host toxicity of the derivatives is not highly dependent on π or ease of amide hydrolysis, suggest that membrane permeabilities of Walker tumor cells differ from normal cells. Possibly the tumor intercellular enzymatic activity is sufficient to hydrolyze the amide to a nondiffusing anion, thus destroying toxicity as well as bioactivity.

Similar studies were performed on busulfan[3] (1,4-dimethanesulfonoxybutane, 1,4-butanediol dimethanesulfonate) and related sulfonic acid esters (292). Good correlations between water–lipid ratios and bioactivity (neutrophil-depressing activity) were found.

In a study aimed at providing a rational approach to the development of improved site-specific chemotherapeutic agents, Jusko (293) developed a pharmacodynamic model utilizing dose–time–cell survival curves generated by the administration of cell cycle specific agents. The model assumes an irreversible, bimolecular mechanism of drug–receptor interaction that serves as the interface between the pharmacokinetic parameters of the drug and the cell cycle–cell proliferation kinetics of both normal and neoplastic cells. Dose–time–cell survival curves were analyzed using the model for several drugs including the reversible derivative cyclophosphamide. The effects of this derivative on hematopoietic and lymphoma cells were examined, and it was found that the primary *in vivo* determinant of selective chemotherapy was operative during the proliferative state of the cells.

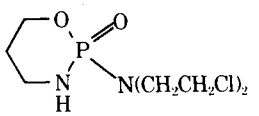


Scheme IV

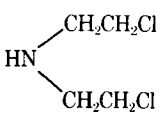
Scheme IV illustrates the model utilized by Jusko to characterize the effects of these chemotherapeutic agents, where C_S represents the concentration of cells (proliferative) sensitive to the drug, C_R is the number of insensitive (resting) cells, k_{RS} and k_{SR} are interconversion rate constants between the two cell populations, X_t represents the transient amount of drug to which the cells are exposed, and k_S and k_d are rate constants acting on sensitive *(C_S)* and insensitive *(C_R)* cells, respectively. The concentration of sterilized or killed cells is represented by C_x, and k is the association rate constant for bimolecular, irreversible drug–receptor interaction.

Perhaps the most important parameter of the model is k_S, the "chemotherapy constant," which is determined by the assumptions that: *(a)* k_{RS} and k_{SR} are relatively constant, and *(b)* the rate constant for cell kill $(k \times X_t)$ can be related to the dose (D_0) and the number of doses *(N)* of drug by $k \int_0^\infty X_t \times dt = Nk_SD_0$.

This parameter implicitly includes the distribution, elimination, and drug–receptor association rate constants. Figure 10 illustrates dose–cell survival and dose–animal survival curves for cyclophosphamide on lymphoma cells.

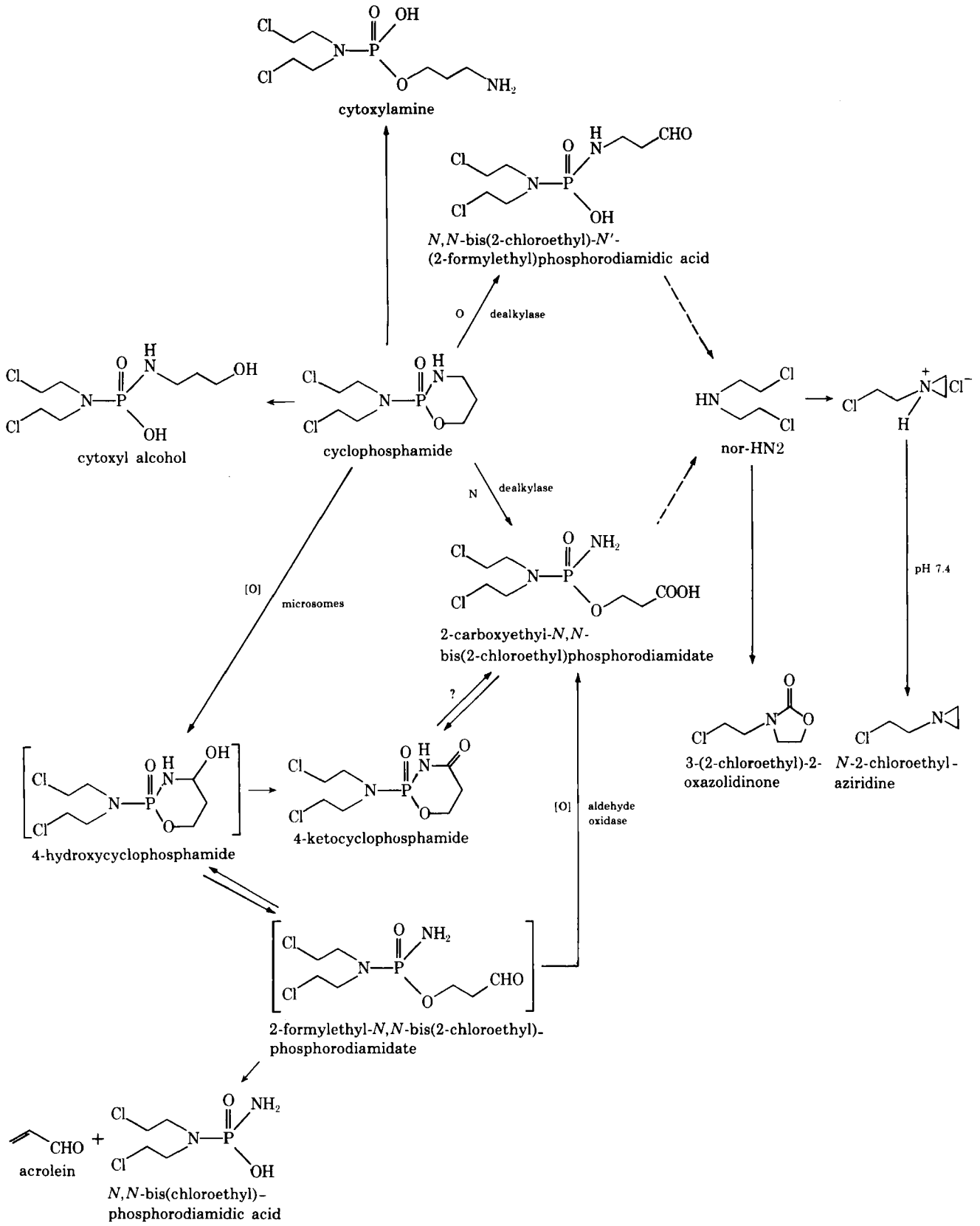


V



VI

[3] Myleran.



Scheme V

Pharmacokinetic studies such as this allow estimates of biological effects (cell kill) to be made at the receptor level as a function of both dose and time. Obviously, absorption and distribution of derivative to site-specific tissue must also be considered as important variables (see discussion on absorption). This type of modeling allows the medicinal chemist to evaluate grossly the effects of a parent molecule at

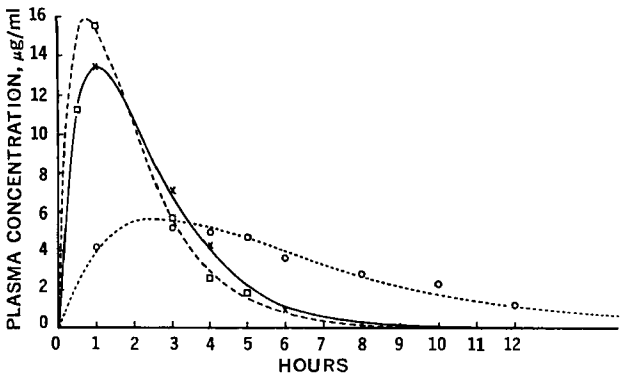


Figure 11—*Concentration of dihydrostreptomycin in the plasma of dogs as a function of time after intramuscular administration of 4 mg/kg of dihydrostreptomycin (as sulfate) in water (□) and in cottonseed oil (×) and of 4 mg/kg of dihydrostreptomycin (as pamoate) in cottonseed oil (○). Dotted lines give one-compartment open model fits. (Adapted, with permission, from Ref. 321.)*

the receptor level and to provide an approximation of the magnitude of the effect of the reversible linkage on transport and distribution to such site-specific tissue.

The interesting bioreversible cytotoxic derivative whose *in vivo* biodegradation has remained obscure for many years is cyclophosphamide [2-[bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide (V)]. This agent differs from other alkylating agents in that it shows a significantly greater selectivity against a variety of tumor cells (211, 294, 295) as judged by its relatively high therapeutic index *in vivo* (215). The greater safety margin of this drug is due to its reduced general and organotropic toxicity. Furthermore, leucotoxicity is less severe and more readily reversible.

Cyclophosphamide was originally synthesized as a reversible derivative of 2,2′-dichlorodiethylamine (nor-HN2, VI). Hindsight proved this assumption to be false based on subsequent work on this derivative.

On administration of cyclophosphamide to animals containing a variety of tumor systems, however, the cytotoxicity was significantly greater than with nor-HN2 (211, 296). A concerted effort (209, 210, 214, 215, 297–299) was undertaken to characterize the bioreversibility and metabolism pattern of this drug (Scheme V). Several of the products and metabolites possess cytotoxic activity comparable to the activity of administered cyclophosphamide. Recent studies with ^{14}C-labeled cyclophosphamide in mouse liver microsomal systems showed *N,N*-bis(2-chloroethyl)-phosphorodiamidic acid to be an active cytotoxic agent generated in this system (297, 298). The detection of acrolein as a product of microsomal oxidation suggested that metabolism of V proceeded *via* an open chain aldehyde to *N,N*-bis(2-chloroethyl)phosphorodiamidic acid (Scheme V). Montgomery *et al.* (160) treated in detail the metabolism of this drug and its various forms.

The example of cyclophosphamide and the implications apparent from a reversible derivative standpoint are profound. The relatively simple modification of a drug molecule (nor-HN2?) has gradually developed into an exceedingly complex and as yet unresolved problem in the determination of the mechanism of action of this important drug.

Table V illustrates selected examples of several classes of drugs that have been reversibly modified for utilization as site-directed and site-activated chemotherapeutic agents.

Depot—Both the physical and chemical approaches have proved useful in imparting depot activity to a drug. Ritschel (320) recently published a thorough review of the various synthetic and mechanical techniques for producing depot activity in parenterals.

The basic premise for depot activity is the localization of the drug derivative in some biological depot or site within the organism, with subsequent slow release from that site at a sufficient rate to provide the active form of the drug in therapeutically efficacious quantities. The duration of activity of such a drug depends upon its physical and chemical properties.

Two important considerations in rational design are: *(a)* the solubility of the derivative in the vehicle (*e.g.*, oleaginous) and the bulk aqueous phase (extracellular water at the site of administration) where the release rate is solubility controlled, and *(b)* diffusion of drug derivative from the vehicle where release is diffusion controlled.

Once the derivative is released (solubilized) at the site of administration, two factors become important: *(a)* regeneration of parent molecule at or near the site by slow, nonenzymatic hydrolysis on contact with body or tissue fluids, *e.g.*, repository sulfones; and *(b)* regeneration of the parent bioactive substance by enzymatic hydrolysis either at the site or in a central compartment (*e.g.*, blood). This sequence of events can be described by a consecutive first-order model (Scheme VI):

$$A \xrightarrow{k_a} B \xrightarrow{k_r} C \xrightarrow{k_e} D$$

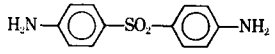
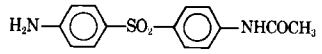
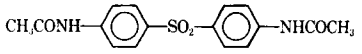
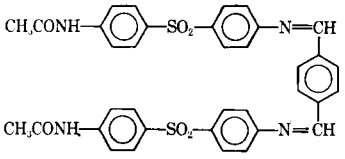
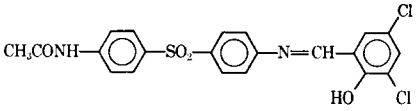
Scheme VI

where A represents the amount of drug at the injection site, B is the amount of derivative in the central body compartment (blood and rapidly equilibrating tissue), C is the amount of regenerated active species, and D is the amount of drug eliminated from the central compartment at any time. The terms k_a, the absorption rate constant, k_r, the derivative's regeneration rate constant, and k_e, the elimination rate constant, govern the drug transfer rate from the depot and central compartment.

Caldwell *et al.* (321) utilized this model in studying the depot properties of dihydrostreptomycin pamoate and in estimating k_a (absorption rate constant) for this salt. The plasma concentration–time plots for the sulfate ($t_{1/2}$ = 0.33 and 0.52 hr) and pamoate ($t_{1/2}$ = 3.46 hr) salts of dihydrostreptomycin are shown in Fig. 11.

The bioavailability of the parent molecule depends on the type and rate of hydrolysis of the reversible derivative. Elslager and coworkers (322–325) synthesized several bioreversible dapsone (diaminodiphenyl sulfone, 4,4′-sulfonyldianiline, DDS) derivatives designed to hydrolyze either enzymatically or nonenzy-

Table VII[a]—Comparative Antimalarial, Antileprotic, and Metabolic Data on Dapsone and Derivatives

Compound	Structure	Weeks Mice Protected		Rats[d]			
				Urinary Excretion			
		Plasmodium berghei[b]	*Mycobacterium leprae*[c]	% Excreted in 30 days	Estimated Half-Life, days	Peak Blood Level, μg/ml	Peak Methemoglobin Level, g/100 ml
Dapsone (DDS)		<1	2	57	9	13.8	3.9
4′-Sulfanilylacetamide (MADDS)		3.5	—	50	32	1.3	1.2
Acedapsone (DADDS)		12	>8	7	>200	0.2	0
4′,4‴-[*p*-Phenylene-bis(methylidyneimino-*p*-phenylenesulfonyl)]-bisacetanilid (PSBA)		5–7	>8	40	55	0.4	0.2
4′-[*N*-(3,5-Dichloro-salicylidene)sulfanilyl]-acetanilide (DSA)[e]		9	>8	31	35	0.7	1.0

[a] Adapted, with permission, from Ref. 324. [b] Estimated number of weeks 50% of mice were protected following a single subcutaneous 400-mg/kg dose of drug suspended in benzyl benzoate–castor oil (40:60). [c] First drug injection was 400 mg/kg given 58 days after infection with *M. leprae;* subsequent injections were 200 mg/kg at intervals of 0.5, 1, or 2 months. [d] Drugs given as single subcutaneous doses of 400 mg/kg in a volume of 5 ml/kg of 1.5% pectin and 0.1% Tween 60 in distilled water. [e] Reviewers' abbreviation.

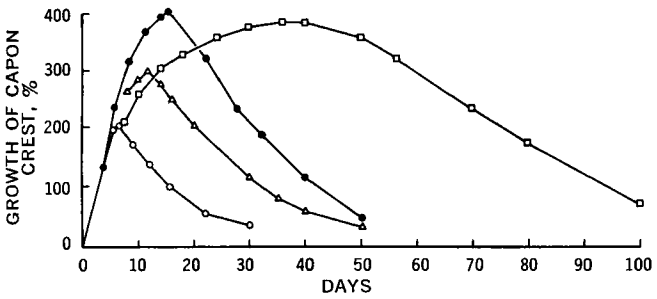


Figure 12—*Influence of ester type of testosterone on growth of capon crest. Key: ○, propionate ester; △, isobutyrate ester; ●, n-valerate ester; and □, undecylenate ester. (Adapted, with permission, from Ref. 327.)*

matically *in vivo*. A series of 4′-(*N*-acylsulfanilyl)anilides (322) was made to take advantage of their antimalarial and antileprotic activities in a repository form. The acyl group varied from hydrogen to palmityl, with a range of solubilities in pH 7 buffer solutions from 0.013 (hydrogen) to 0.0002 (palmityl) mg/ml. Optimum repository activity occurred with acedapsone (4′,4‴-sulfonylbisacetanilide, DADDS) at a solubility of 0.003 mg/ml. This derivative was slowly hydrolyzed by deacetylases to regenerate dapsone *in vivo*.

Other derivatives were designed to take advantage of nonenzymatic lability in body tissue and fluid and included a variety of 4′,4‴-[bis(amino-*p*-phenylenesulfonyl)]bisanilides (323) and 4′-[(*N*-aralkylidene-, benzylidene-, and naphthylidene)sulfanilyl]anilides (324). Of these latter compounds, 4′,4‴ - [*p* - phenylenebis (methylidyneimino-*p*-phenylenesulfonyl)] bisacetanilide (PSBA) and 4′-[*N*-(3,5-dichlorosalicylidene)sulfanilyl]acetanilide (DSA) proved noteworthy. Antimalarial and antileprotic activity data as well as approximate pharmacokinetic data for these derivatives are summarized in Table VII.

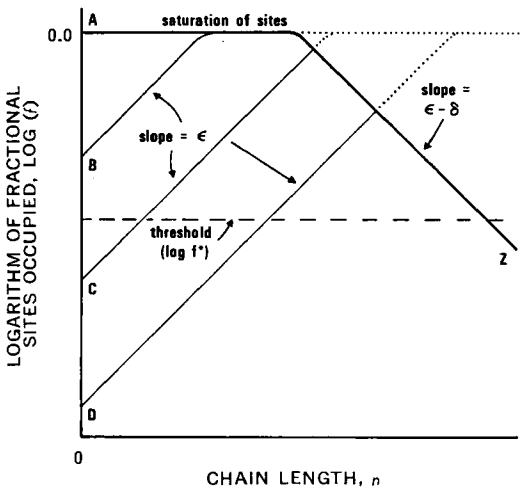


Figure 13—*Theoretical curves for the fractional occupancy of a perfect Langmuirian receptor phase as a function of alkyl chain length under the condition that* $S_0(k_1/k_{-1})_0 \gg 1$. *The heavy line, line A–Z, is the expected trend for saturated solutions. Profiles initiating at points B, C, and D are idealized profiles obtained using fixed concentrations* C_1, C_2, *and* C_3, *respectively, where* $S_0 > C_1 > C_2 > C_3$. *In such cases, log (f) increases linearly with chain length until the saturation limitation is obtained. Thereafter, the profiles are superimposed on the saturation profile. The dotted lines represent the continuing trends expected, neglecting the solubility restriction. "Parabolic" curves can be generated at a fixed concentration. The apex of the "parabola," the chain length of maximal activity, can be flat or broad, depending on concentration. Its position on the chain-length axis is also concentration dependent. (Reprinted, with permission, from Ref. 367.)*

Pinder (326) recently reviewed antimalarial drugs possessing depot and repository properties.

Steroids have been the subject of intense study as clinically acceptable forms of depot drugs. Steroidal bioactivity has been prolonged by forming esters of various lengths and various degrees of steric hindrance. Figure 12 illustrates the effect of chain length on duration of activity (the bioactivity of the parent steroid manifested as capon crest growth) for a series of testosterone esters (327). Winter and Porter (266) similarly correlated rates of absorption of several esters of hydrocortisone and 9α-fluorocortisone as a function of duration of activity.

Miescher *et al.* (328), in a classic study, demonstrated extended duration and bioavailability in a series of testosterone aliphatic esters. With the long chain esters (palmitate and stearate), the increased duration was probably due to delayed absorption (solubility rate controlled) and steric hindrance (slowed hydrolysis of ester). Rapala *et al.* (272) demonstrated a prolonged activity due to steric hindrance with substituted and nonsubstituted adamantoate esters of testosterone.

Various drug classes designed specifically as agents for depot therapeutic efficacy by use of the reversible derivative are listed in Table VIII.

Organoleptic Properties—*Taste*—Taste is a complex combination of sensations including gustation, olfaction, tactility, and responses to heat and cold. In humans, the sensation of taste is confined primarily to the dorsal surface of the tongue, the soft palate, the epiglottis, and parts of the gullet. In children, however, the taste receptors are distributed over larger areas of the mouth. Basically, there are four so-called primary tastes: sour, bitter, salty, and sweet. Others include astringent, metallic, and alkaline (soapy). Pfaffman (362) estimated that a human has 10^4 taste buds including taste nerves and other sensory nerves stimulated by foods. Each taste bud is innervated by from two to 10 nerve fibers with another 10^4 sensory fibers that serve as monitors for pain, temperature, tactility, and other stimuli.

Mechanism of Taste Receptor Stimulation—Electrophysiological measurements have been made by inserting microelectrodes into single taste cells and measuring the electric potential change between the inside and outside of the cell after stimulation with a chemical substance (363, 364). It was found that the molecular geography of the receptor surface changes after such stimulation, thus initiating a chain of events that ultimately manifests itself as a taste response.

Potassium ions, present in abundance within the cell, are released due to increased cell permeability near the associated nerve axon, and this phenomenon accounts for the electric potential change. This change initiates electrical nerve impulses of a few

Table VIII—Reversible Drug Derivatives Utilized as Modifiers of Duration of Activity (Prolonged, Depot Action)

Parent Molecule	Reversible Modification or Linkage	Route of Administration	Reference
Nicotinic acid	Dextran and carboxymethyldextran esters	Oral	329
Streptomycin	Alginic acid salt	Intramuscular	330
Dihydrostreptomycin	Pamoate salt	Intramuscular	321
Cytarabine (ara-C)	5′-Adamantoate ester	Intraperitoneal	331
Ara-adenosine (ara-A)	5′-Palmitate and 5′-benzoate esters	Intraperitoneal	332
Deoxyribonucleosides, ribonucleosides	5′-Adamantoate ester	Intraperitoneal	333
3-*o*-Toloxy-1,2-propanediol	Phenoxyacetate ester	Intraperitoneal	334
	Hemisuccinate ester	Intraperitoneal	335
Ampicillin	Dibenzylethylenediamine salt	Intramuscular	336
Substituted acetamidopenicillins	Carboxamido ester	Intramuscular	337
Amphotericin B	Methyl ester	Intraperitoneal	338
Neomycin	Dextran sulfate salt	Intramuscular	339
Fluphenazine	Heptanoate and decanoate esters	Intramuscular	340
Pipothiazine	Palmitate ester	Intramuscular	341–343
	Undecylenate ester	Oral	
Nandrolone	Phenylpropionate and decanoate esters	Intramuscular	265
Hydrocortisone	21-Alkyl esters	Local (topical)	266
Testosterone	17-β-Alkyl esters	Intramuscular	33, 272, 328, 344
6α-9α-Difluorocorticosteroid	Alkyl ortho esters	Intramuscular	345
Estradiol	17-Cycloalkenyl esters	Oral	346
	Oligomeric esters	Subcutaneous	347
Estradiol	Enol ethers and acetals	Oral, parenteral	267
Estradiol	Formate ester	Intramuscular	348
Nortestosterone	Terpenoate ester	Subcutaneous	349
Aldotestosterone, 1-dehydro-aldosterone	21-Benzoate ester	Oral, parenteral	350
Estrogen	3-Cyclopentyl ether	Parenteral	351
Betamethasone	21-Adamantoate ester	Oral, rectal, parenteral	352
Phendimetrazine	Pamoate salt	Oral	353[a]
Imipramine	Pamoate salt	Oral	354
Oleandomycin	Acetate, propionate ester	Oral	355
Aspirin (acetylsalicylic acid)	Alkyl carbonate ester	Oral	356
2-Phenylacetamido-, 2-amino-2-phenylacetamido-, phenylimidazolidinylpenicillins	*N*-Acyl-6-acylamino amide	Oral	337
Prostaglandin	2-(4-Imidazolyl)ethylamine salt	Oral	357
Dopamine	Amino acid amide	Oral	193
Chloramphenicol	Mono- and bis(trimethylsilyl) ethers	Parenteral	358
Dapsone (diaminodiphenylsulfone)	Acetamide	Intramuscular	359
	Formamide	Oral	360
Cycloguanil	Pamoate salt	Intramuscular	361

[a] This article tabulates a variety of pamoate salts for several classes of drugs.

milliseconds duration, with 100–200 impulses/sec. The intensity of the signal to the brain is a function of the number of nerve fibers activated and the impulse frequency.

Beidler (365) found that the magnitude of a taste response increases at a declining rate with concentration until further concentration increases fail to elicit a response. The relationship between concentration and the magnitude of a steady-state response is:

$$R = \frac{CKR_m}{CK + 1} \qquad \text{(Eq. 15)}$$

where C is the concentration of the stimulant, R is the electrical response magnitude, R_m is the maximal response magnitude at saturation, and K is the reaction equilibrium constant of stimulus–receptor. Plotting R *versus* C affords a sigmoidal curve, the middle portion of which can be utilized for comparisons between concentration and response. The utility of the comparison is invalid at low and high concentrations. Other studies provided evidence of weak substrate adsorption to the taste receptor surface (366).

When suspensions are considered, simply replace C by S for drug solubility. The effects of a homologous series on adsorption onto receptors was studied recently (367). In an analysis similar to that presented for absorption (see section on absorption), it was shown that for subsaturated solutions the response increases with alkyl chain length until R reaches R_m. It was also shown that for suspensions there can be an increased response with chain length for the lower homologs. This results from the fact that the value of K, which is essentially a Langmuirian adsorption constant, increases as a homologous series is ascended. However, as the series becomes highly hydrophobic, the decrease in solubility that accompanies chain extention overshadows the increase in the adsorption constant and the response begins to diminish with chain length until it drops below the threshold value. These relationships are summarized in Fig. 13.

Duncan (368) postulated that taste stimulation may be due to enzymatic mediation. This contention was disputed by Beidler (369) who cited lack of experimental evidence for this thesis plus the fact that such interactions are highly pH dependent whereas a taste response elicited by sodium chloride, for in-

Table IX—Reversible Drug Derivatives Utilized as Modifiers of Taste and Odor Properties

Parent Molecule	Reversible Modification or Linkage	Property Modified	Reference
Pivampicillin	Probenecid salt	Bitterness	378
Tetracycline	3,4,5-Trimethoxybenzoate salt	Bitterness	379
Propoxyphene	2-Naphthalenesulfonic acid salt	Bitterness	380
	4-Chloro-*m*-toluenesulfonic acid salt and 3,4-dichlorobenzenesulfonic acid salt	Bitterness	381
Oleandomycin	Acyl ester/*N*-oxide	Bitterness	355
Chloramphenicol	Palmitate ester	Bitterness	221
	α-Haloalkyl ester, phenyl ester	Bitterness	382
	Phosphite ester	Bitterness	383
	(Trimethylsilyl) ether		358
Lincomycin	Alkyl ester	Bitterness	10–12
	Phosphate ester	Bitterness	175
Clindamycin	Alkyl ester	Bitterness	13
Erythromycin	Alkyl ester/salt	Bitterness	238
	Alkyl ester	Bitterness	14, 384, 385
Ethyl mercaptan	Phthalate ester	Odor	386, 387
Trichloroethanol	Carbonate ester	Odor	16
N-Arylanthranilic acid	Glyceryl ester	Bitterness	388

stance, can occur between pH 3 and 11. He felt that the initial response was electrochemical in nature and due to creation of a disturbance in the surface cell geography with subsequent depolarization of the receptor cell.

Molecular configuration of the drug molecule plays an important role in adsorption efficiency at the receptor level. Saccharin has a taste threshold approximately $\frac{1}{500}$ that of sugar, whereas *N*-methyl-, ethyl-, or bromosaccharin is tasteless (369). Analogously, the magnitude of a bitter taste response may be modified by changing the molecular configuration of the parent molecule. Such is the case for clindamycin (parent molecule) and a series of clindamycin 2-esters (reversible derivative) (13). Solubility, as already mentioned, may play an important role in decreasing the intensity of a bitter response.

A combination of factors is perhaps operative in the demonstration of a taste response. Bitterness of a molecule, for example, may be due to the efficiency of the taste receptor–substrate adsorption reaction, which is related to the molecular geometry of the substrate. If alteration of the parent molecule occurs by derivative formation, the geometry is altered, affecting the adsorption constant. This effect, in turn, acts in concert with the aqueous solubility (or lack of such) of the derivative to eliminate the bitter taste response. Beidler (369) and Moncrieff (370) published reviews on taste theory.

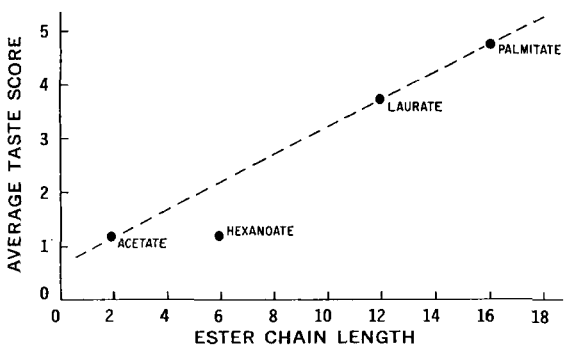


Figure 14—*Effect of clindamycin ester chain length on taste of ester in syrup. (Reprinted, with permission, from Ref. 13.)*

Odor—Odor, another organoleptic property amenable to reversible derivatization, plays an important role in the quality and intensity of taste perception. The magnitude of an olfactory response is related to concentration (371) and can be measured by recording the electrical nerve impulses generated by the olfactory receptors when they are stimulated by an odor (372). Olfactory receptor sensitivity is well known and probably depends on the adsorption of the stimulus molecule to the surface of the receptor. Certain insects can respond to concentrations of less than 10^{-17} *M* (373).

The nature of the functional group and the shape of the molecule, in conjunction with volatility, determine the intensity and duration of such adsorption. Studies (365) have been successfully applied to olfaction using the amyl acetate olfactory response (372). Several reviews and papers have been written correlating odor and molecular constitution (374–376), stereochemistry (377), and physical variables (372).

Taste Studies—The extremely bitter antibiotics have been the focus of much work in reversible drug modification. A study of clindamycin was undertaken to provide derivatives that could suitably be utilized as a tasteless pediatric form of this antibiotic (13). A series of 2- and 3-monoesters and several 2,3-biscarbonate esters was made. Four clindamycin 2-acyl esters of varying chain length (palmitate, laurate, hexanoate, and acetate) were dissolved in 30% sucrose solutions. The clindamycin ester solutions were coded A, B, C, and D, and samples of each were given to a taste panel of 26 people, with 1 hr allowed between each sample. The taste of each was rated on a scale of 1–9 (1 = poor, 9 = excellent). The raw score data were analyzed by computer to show any significant differences between samples.

The average scores (Fig. 14) indicate a linear trend of taste improvement with increasing chain length. The palmitate ester was significantly better than the laurate at the 5% level of confidence. The laurate, in turn, was significantly better than both the hexanoate and the acetate. The latter two compounds

Table X—Reversible Derivatives Utilized as Modifiers of Irritation on Injection

Parent Molecule	Reversible Modification or Linkage	Route of Administration	Reference
Chloramphenicol	1-Hemisuccinate sodium salt	Intramuscular, intravenous	169, 400
	1,3-Bishemisuccinate disodium salt or piperazine salt	Intramuscular	
Oleandomycin	Acetate, propionate esters, *N*-oxide	Intravenous, intramuscular	355
Clindamycin	Phosphate ester	Intramuscular	391, 398, 399

were ranked equally low, being very bitter tasting.

Clindamycin 2-palmitate was found to be devoid of the characteristic bitter taste of clindamycin.

Table IX illustrates several types of drug molecules that have been reversibly modified to eliminate offensive taste and odor properties inherent in the parent drug molecule.

Irritation—*Intramuscular or Intravenous Injection*—Perhaps the most effective yet intimidating mode of drug administration is *via* the parenteral route. It is efficacious in the sense that it provides an immediate therapeutic dose of medicament to those patients not able to self-administer medication such as infants and hospitalized and comatose patients. Furthermore, the frequency of dosing may be reduced by utilizing an injectable depot or repository form of the drug (see *Depot* discussion).

Patient reluctance develops, however, when an initially painful intravenous or intramuscular injection must be administered in a multiple-dose regimen. Such pain is usually accompanied by hemorrhage, edema, inflammation, and tissue necrosis (389). The intensity of pain due to injectable substances has been evaluated by use of tenderness profiles (390) and elevation of enzyme levels such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and creatine phosphokinase (CPK)(390–392). Ballard (393) tabulated a variety of local reactions caused by the injection of several drug solutions and suspensions.

Several factors apparently influence the occurrence, intensity, and duration of pain generated by an injection. Among these, the most important are considered to be the solubility of drug in an aqueous medium, viscosity, pH, tonicity of the injected drug solution, volume injected, site of injection, pain tolerance of the patient, and technique of administration (394). Other factors include precipitation of drug at the injection site (391, 393, 395–397) and localized cell lysis (391).

The approach usually taken by the product formulation specialist is to add a local anesthetic to the product to reduce pain by blocking the sensory nerves around the injection site. A more rational approach involves the reversible modification of certain physicochemical properties of the offending parent drug to eliminate such pain and irritation. A number of drug molecules have been reversibly modified in such a manner to alleviate these frequently bothersome side effects (Table X).

A good example of this type of modification involves clindamycin hydrochloride, a semisynthetic antibiotic used in the treatment of infections due to sensitive Gram-positive organisms. This drug is irritating when administered by intramuscular or intravenous injection. It was postulated that this irritation might result from precipitation of the clindamycin free base at the injection site or from rapid partitioning of drug into tissue cells surrounding the injection site, resulting in cell lysis (391). Both mechanisms may be important since the aqueous solubility of the hydrochloride salt is more than 100 mg/ml at pH 6 but is only 3 mg/ml at physiological pH (~7.4), thus supporting the precipitation hypothesis.

Additionally, clindamycin free base has a partition coefficient of 1.85, which should favor passage of this species through a lipoidal barrier or membrane. At physiological pH, the free base form (pKa = 7.6) would be present at about one-half of the injected dose, lending credibility to the cell partitioning hypothesis.

The synthesis (398) and subsequent intramuscular testing (399) of clindamycin phosphate indicated that this reversible derivative of clindamycin was virtually devoid of the irritation exhibited by the parent antibiotic. There were no signs of local intolerance, and intravenous infusions of the ester produced no superficial thrombophlebitis or irritation. The phosphate ester is intrinsically inactive against bacteria but is rapidly hydrolyzed to clindamycin *in vivo*. Other studies designed to characterize the intramuscular absorption, distribution, and excretion of this derivative indicated excellent bioavailability of the derivative from the injection site as the parent antibiotic (173).

Figure 4 illustrates the findings of a multiple-dose study utilizing clindamycin and the 2-phosphate ester. The lower curve represents clindamycin, and the upper curve represents both clindamycin and clindamycin 2-phosphate. It can be seen that ester

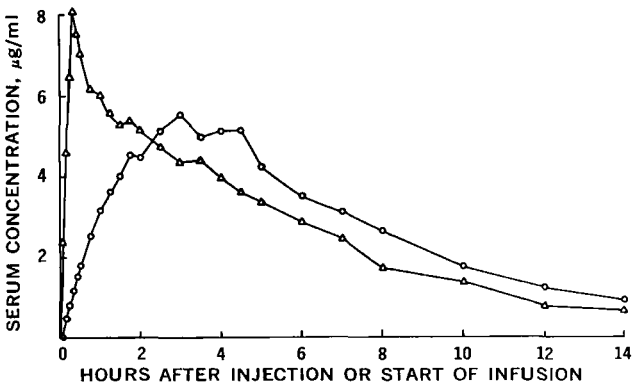


Figure 15—*Serum concentration (micrograms per milliliter) of clindamycin after 600 mg iv (△) and 600 mg im (○) clindamycin phosphate in the same subject. (Adapted, with permission, from Ref. 173.)*

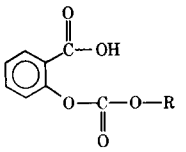


Table XI[a]—Aspirin and Carbonate Esters of Salicylic Acid: Solubilities, Partition Coefficients, and Hydrolysis Rates

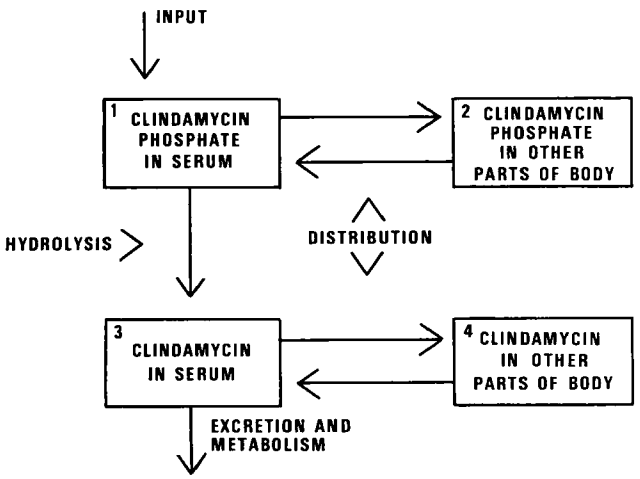
R	Solubilities, mg/ml, 37°		Cyclohexane–0.1 *N* HCl Partition Coefficient, 25° [b]	Half-Lives for Hydrolysis, 37°				
				Phosphate Buffer (0.1 *M*)		Enzyme in Phosphate Buffer (pH 7.4, 0.1 *M*)[c]		
	0.1 *N* HCl[b]	Cyclohexane		pH 7.4, hr	pH 12, min	2% Human Plasma, hr	0.05% Human Pseudocholinesterase, hr	0.05% α-Chymotrypsin, hr
Ethyl	6.7	1.1	0.17	41	3.8	13.3 (3.1)	7.4 (5.5)	53 (—)
n-Butyl	2.8	2.7	1.0	29	4.5	11.7 (2.5)	1.23 (24)	1.38 (21)
n-Hexyl	0.28	5.6	20	15	4.8	8.2 (1.8)	1.33 (11)	0.25 (58)
Trichloroethyl	1.1	0.24	0.22	0.53	1.1	0.35 (1.5)	—	—
Aspirin	5.3	0.06	0.05	10	1.5	21 (—)	3.3 (3)	6.3 (1.6)

[a] Adapted, with permission, from Ref. 276. [b] The 0.1 *N* HCl was used in these experiments to assure that the compounds were completely unionized in the aqueous phases and to reduce the degree of hydrolysis in the aqueous phase during equilibration. [c] The numbers in parentheses are $t_{1/2}$ buffer (pH 7.4)/$t_{1/2}$ enzyme ratios, which represent the degree of enzymatic catalysis.

hydrolysis was virtually complete at the 4-hr sampling time.

A more precise characterization of serum concentration–time profiles of the ester was determined by dosing intramuscularly and intravenously (Fig. 15). Ester hydrolysis occurred more rapidly with intravenous administration, and intramuscular administration showed both slower absorption and decreased peak concentration of the parent antibiotic. The implications concerning the route of administration for such a reversible derivative are important in life-threatening infections, the intravenous route being indicated.

Few studies are available in which pharmacokinetic principles have been applied to reversible drug derivatives designed for injectable purposes. Again, the studies on clindamycin phosphate are illustrative (173). A four-compartment model was used to explain much of the data generated in the study (Scheme VII). The data show that clindamycin phosphate is rapidly hydrolyzed in serum and that the parent drug is widely distributed after both intravenous and intramuscular administration. Equilibrium is reached after the third dose in a multiple-dose regimen, and serum levels of clindamycin are readily predictible.



Scheme VII—Four-compartment pharmacokinetic model for clindamycin phosphate. (Adapted, with permission, from Ref. 173.)

GI Disturbances—It has long been known that a variety of drugs can cause a wide spectrum of GI disturbances, ranging from mild gastritis to severe ulceration (401–404). How these phenomena occur is still a matter of speculation, but several modes of action have been postulated:

1. Injury to gastric mucosa through direct contact of drug to produce a localized irritating necrotizing effect.
2. Damage due to circulating drug that stimulates increased gastric secretion. [It has been shown, however, that this mechanism is nonoperative for aspirin in human subjects and animals and, in fact, may lower gastric secretory activity (405, 406), but it may be operative for other gastroirritant drugs.]
3. Interference with the mechanism(s) responsible for maintaining the integrity of the GI mucous or "mucous barrier."

This barrier, consisting of glycoprotein, is relatively resistant to proteolytic enzyme activity and acts as a protective boundary to underlying epithelial tissue.

Table XII[a]—Toxicity, Gastric Irritability, and Potency of Salicylic Acid Hexylcarbonate and Acetylsalicylic Acid (Aspirin)

	Salicylic Acid Hexylcarbonate	Acetylsalicylic Acid
LD_{50} in mice[b]	10.1	7.0
LD_{50} in rats[b]	13.0	7.6
Incidence of gastric erosions in rats with equimolar doses, %	0	90
Incidence of gastric irritation in dogs with equimolar doses, %	0	100
Antipyretic activity	Equal potency	
Analgesic activity	Equal potency	
Anti-inflammatory activity	Equal potency	

[a] Data from Ref. 277. [b] Average acute oral toxicity in moles/kg × 10^{-3}.

Table XIII—Reversible Drug Derivatives Utilized as Modifiers of GI Irritability

Parent Molecule	Reversible Modification or Linkage	Property Modified	Reference
Salicylic acid	Carbonate ester	Gastric irritation and hemorrhage	276, 277
Aspirin (acetylsalicylic acid)	4-Acetamidophenyl ester	GI blood loss	233
Aspirin (acetylsalicylic acid)	Alkyl ester	GI irritation	417
	Cyano ester		
Aspirin (acetylsalicylic acid)	Carbonate anhydride ester	GI bleeding	356
Nicotinic acid	Hydrazide	GI irritation	409
	Hydroxamic acid		
N-Arylanthranilic acid	Glyceryl ester	GI ulceration	388
21-Hydroxysteroid	Phosphate and sulfate ester	Gastric hemorrhage	418
Oleandrin	Acetate ester	GI irritation	415, 416
Gitoxin	Pentaacetate ester	GI irritation	414
Prostaglandin	Ethyl ester	Diarrhea	413
4,4′-Dihydroxydiphenyl-(2-pyridyl)methane	Alkyl ester	Gastric irritation	411, 412
	Sulfate ester		
Trichloroethanol	Carbonate ester	Stomach irritation, nausea, vomiting	16
	Phosphate ester		410
	4-Acetamidophenyl ester		17

The systemic administration of cortisone and aspirin alters the composition of this barrier in a way that decreases its protective ability (407, 408). This may be due to cellular injury resulting in decreased mucous production and increased ionic flux.

A study of the absorptive properties of a series of organic acids, including acetylsalicylic acid (aspirin), through the mucosal barrier found increased fluxes of hydrogen, chloride, sodium, and potassium ions after administration of these acids; these increases persisted after removal of the compounds (402). Evidence of bleeding was also apparent. Diffusion of acetic acid, which is unionized at gastric pH, through the gastric barrier proceeded more rapidly than diffusion of hydrochloric acid (ionized), implying that unionized hydrophobic molecules are more readily and efficiently absorbed.

In an effort to eliminate gastric irritation produced by salicylic acid, a series of hydrophobic carbonate esters of this important analgesic was prepared (276). The rationale for utilizing carbonate esters of this drug lies in the fact that they are readily hydrolyzed *in vivo* and might possess hydrophobic properties adequate to allow absorption and distribution over a greater area of the GI tract, thus reducing localized irritation. Several esters were synthesized and characterized according to their physicochemical properties (Table XI).

Based on partitioning studies and relative ease of hydrolysis (half-life) in pH 7.4 buffer and enzyme media, the hexylcarbonate ester of salicylic acid was chosen for further study. Blood level studies in dogs indicated that this ester was absorbed more slowly than acetylsalicylic acid and the other carbonate esters. After 2 hr, however, the plasma concentration–time profiles did not differ for any of the esters. A comparison of the LD_{50} values, gastric irritability, and bioactivity of salicylic acid hexylcarbonate *versus* acetylsalicylic acid (aspirin) in animals indicated significant differences in toxicity between these drug derivatives (277) (Table XII).

This study (276) stressed to the medicinal chemist the importance of performing selected physicochemical studies (*e.g.*, solubility and partitioning) and *in vitro* tests (half-lives in a variety of buffers and enzyme-rich milieu) to evaluate initially a series of reversible derivatives based on a preliminary rationale for making such derivatives. It is obvious that when such studies are performed, the choice of primary and backup candidates for further testing becomes less difficult.

Other experiments have been designed and carried out with the foregoing rationale as their basis, and the results of several of these studies are summarized in Table XIII.

REFERENCES

(1) L. C. Schroeter, "Ingredient X," Pergamon Press, Elmsford, N.Y., 1969.

(2) S. A. Kaplan, *Drug Metab. Rev.*, **1**, 15(1972).

(3) S. S. Davis, *Brit. Med. J.*, **1**, 102(1972).

(4) J. W. Poole, *Semin. Drug Treat.*, **1**, 148(1971).

(5) K. Munzel, *Pharm. Acta Helv.*, **46**, 513(1971).

(6) J. Sjögren, *Acta Pharmacol. Toxicol.*, **29**, 68(1971).

(7) E. R. Garrett, *ibid.*, **29**, 1(1971).

(8) L. Z. Benet, in "Drug Design," vol. 4, E. J. Ariens, Ed., Academic, New York, N.Y., 1973, p. 1.

(9) A. Cammarata, *Annu. Rep. Med. Chem.*, **1970**, 256(1971).

(10) A. A. Sinkula, W. Morozowich, C. Lewis, and F. A. MacKellar, *J. Pharm. Sci.*, **58**, 1389(1969).

(11) W. Morozowich, A. A. Sinkula, F. A. MacKellar, and C. Lewis, *ibid.*, **62**, 1102(1973).

(12) A. A. Sinkula and C. Lewis, *ibid.*, **62**, 1757(1973).

(13) A. A. Sinkula, W. Morozowich, and E. L. Rowe, *ibid.*, **62**, 1106(1973).

(14) A. A. Sinkula, *ibid.*, **63**, 842(1974).

(15) J. V. Swintosky, H. J. Adams, H. C. Caldwell, L. W. Dittert, T. Ellison, and D. E. Rivard, *ibid.*, **55**, 992(1966).

(16) H. C. Caldwell, H. J. Adams, D. E. Rivard, and J. V. Swintosky, *ibid.*, **56**, 920(1967).

(17) J. V. Swintosky, H. C. Caldwell, C. W. Chong, G. M. Irwin, and L. W. Dittert, *ibid.*, **57**, 752(1968).

(18) L. W. Dittert, H. C. Caldwell, H. J. Adams, G. M. Irwin, and J. V. Swintosky, *ibid.*, **57**, 774(1968).

(19) L. W. Dittert, G. M. Irwin, C. W. Chong, and J. V. Swintosky, *ibid.*, **57**, 780(1968).

(20) E. S. Rattie, E. G. Shami, L. W. Dittert, and J. V. Swintosky, *ibid.*, **59**, 1738(1970).

(21) H. Takechi, T. Higuchi, and I. H. Pitman, "Abstracts of Papers," APhA Academy of Pharmaceutical Sciences, Houston, Tex. meeting, Apr. 1972, p. 64.

(22) V. Stella and T. Higuchi, *ibid.*, p. 76.

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

(24) N. J. Harper, *Progr. Drug Res.*, **4**, 221(1962).

(25) N. J. Harper, in "Absorption and Distribution of Drugs," T. B. Binns, Ed., Williams & Wilkins, Baltimore, Md., 1964, p. 103.
(26) E. J. Ariens, *Progr. Drug Res.*, **10,** 429(1966).
(27) E. J. Ariens, *Farmaco, Ed. Sci.*, **24,** 3(1969).
(28) E. J. Ariens, "Drug Design," Academic, New York, N.Y., 1971, p. 1.
(29) R. E. Notari, *J. Pharm. Sci.*, **62,** 865(1973). See p. 875 for treatment of reversible derivatives.
(30) V. J. Stella, *Aust. J. Pharm. Sci.*, **NS2,** 57(1973).
(31) H. Bundgaard, *Dan. Tidsskr. Farm.*, **45,** 73(1971).
(32) C. Runti, *Farmaco, Ed. Prat.*, **28,** 451(1973).
(33) S. M. Kupchan, A. F. Casy, and J. V. Swintosky, *J. Pharm. Sci.*, **54,** 514(1965).
(34) A. Albert, "Selective Toxicity," 2nd ed., Wiley, New York, N.Y., 1960, p. 30.
(35) A. Albert, "Selective Toxicity," 3rd ed., Wiley, New York, N.Y., 1964, p. 57.
(36) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71,** 525(1971).
(37) C. A. M. Hogben, D. J. Tocco, B. B. Brodie, and L. S. Schanker, *J. Pharmacol. Exp. Ther.*, **125,** 275(1959).
(38) L. S. Schanker, in "Drugs and Membranes," vol. V, C. A. M. Hogben, Ed., Macmillan, New York, N.Y., 1963, p. 121.
(39) C. Hansch, A. R. Steward, J. Iwasa, and E. N. Deutsch, *Mol. Pharmacol.*, **1,** 205(1965).
(40) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86,** 5175(1964).
(41) J. Ferguson and H. Pirie, *Ann. Appl. Biol.*, **35,** 532(1948).
(42) R. Zahradnik, *Arch. Int. Pharmacodyn. Ther.*, **135,** 311(1962).
(43) K. H. Meyer and H. Hemmi, *Biochem. Z.*, **277,** 39(1935).
(44) J. C. McGowen, *J. Appl. Chem.*, **1,** 1205(1951).
(45) G. L. Flynn and S. H. Yalkowsky, *J. Pharm. Sci.*, **61,** 838(1972).
(46) S. H. Yalkowsky and G. L. Flynn, *ibid.*, **62,** 210(1973).
(47) R. Collander, *Acta Chem. Scand.*, **3,** 717(1949).
(48) R. J. Scheuplein and J. H. Blank, *Physiol. Rev.*, **51,** 702(1971).
(49) C. Hansch, *Accounts Chem. Res.*, **2,** 232(1969).
(50) C. Hansch and J. M. Clayton, *J. Pharm. Sci.*, **62,** 1(1973).
(51) J. G. Wagner and A. J. Sedman, *J. Pharmacokinet. Biopharm.*, **1,** 23(1973).
(52) S. H. Yalkowsky, T. G. Slunick, and G. L. Flynn, *J. Pharm. Sci.*, **63,** 691(1974).
(53) K. R. M. Vora, W. I. Higuchi, and N. F. H. Ho, *ibid.*, **61,** 1785(1972).
(54) B. J. Zwolinski, H. Eyring, and C. E. Reese, *J. Phys. Chem.*, **53,** 1426(1949).
(55) G. L. Amidon, S. H. Yalkowsky, and S. Leung, *J. Pharm. Sci.*, **63,** 1858(1974).
(56) R. B. Hermann, *J. Phys. Chem.*, **76,** 2754(1972).
(57) C. Hansch, J. E. Quinlan, and G. L. Lawrence, *J. Org. Chem.*, **33,** 347(1968).
(58) F. Irmann, *Chem. Ing. Tech.*, **37,** 789(1965).
(59) G. J. Pierotti, C. H. Deal, and E. L. Derr, *Ind. Eng. Chem.*, **51,** 95(1959).
(60) H. Rosen, A. Leaf, and W. B. Schwartz, *J. Gen. Physiol.*, **48,** 379(1964).
(61) W. L. Hayton and G. Levy, *J. Pharm. Sci.*, **61,** 362(1972).
(62) R. J. C. Barry, M. J. Jackson, and D. H. Smyth, *J. Physiol. (London)*, **182,** 150(1966).
(63) M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins," Wiley-Interscience, New York, N.Y., 1971.
(64) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N.Y., 1969.
(65) R. Siegert and W. Vomel, *Arzneim.-Forsch.*, **6,** 714(1956).
(66) R. R. Scheline, *Proc. Eur. Soc. Study Drug Toxicity*, **13,** 35(1972), and references cited therein.
(67) R. T. Williams, *Toxicol. Appl. Pharmacol.*, **23,** 769(1972).
(68) P. Goldman, *N. Engl. J. Med.*, **289,** 623(1973).
(69) H. Schroeder, R. M. Lewkonia, and D. A. Price Evans, *Clin. Pharmacol. Ther.*, **14,** 802(1973).
(70) R. R. Scheline, *Pharmacol. Rev.*, **25,** 451(1973).
(71) W. N. Aldridge, *Biochem. J.*, **53,** 110(1953).
(72) K. B. Augustinsson, *Ann. N.Y. Acad. Sci.*, **94,** 844(1961).
(73) F. Margolis and P. Feigelson, *J. Biol. Chem.*, **238,** 2620(1963).
(74) W. Pilz, E. Stelzell, and I. Johann, *Enzymol. Biol. Clin.*, **9,** 97(1968).
(75) I. Dawson and J. Pryse-Davies, *Gastroenterology*, **44,** 745(1963).
(76) K. Krisch, in "The Enzymes," 3rd ed., vol. 5, P. D. Boyer, Ed., Academic, New York, N.Y., 1971, p. 43.
(77) W. Franz and K. Krisch, *Z. Physiol. Chem.*, **349,** 575(1968).
(78) T. A. Krenitsky and J. S. Fruton, *J. Biol. Chem.*, **241,** 3347(1966).
(79) J. K. Stoops, D. J. Horgan, M. T. C. Runnegar, J. de Jersey, E. C. Webb, and B. Zerner, *Biochemistry*, **8,** 2026(1969).
(80) G. A. Alles and R. C. Hawes, *J. Biol. Chem.*, **133,** 375(1940).
(81) J. Hyun, H. Kothari, E. Herm, J. Mortenson, C. R. Treadwell, and G. V. Vahouny, *ibid.*, **244,** 1937(1969).
(82) J. A. Olson, *Pharmacol. Rev.*, **19,** 559(1967).
(83) L. Sarda and P. Desnuelle, *Biochim. Biophys. Acta*, **30,** 513(1958).
(84) B. Entressangles and P. Desnuelle, *ibid.*, **159,** 285(1968).
(85) M. Semeriva and C. Dufour, *ibid.*, **260,** 393(1972).
(86) E. L. Rowe, "Abstracts of Papers," APhA Academy of Pharmaceutical Sciences, San Francisco, Calif., meeting, Mar. 1971, p. 62.
(87) F. H. Mattson and R. A. Volpenhiem, *J. Lipid Res.*, **7,** 536(1966).
(88) R. G. H. Morgan, J. Barrowman, M. Filipek-Wender, and B. Borgstrom, *Biochim. Biophys. Acta*, **146,** 314(1967).
(89) R. G. H. Morgan, J. Barrowman, and B. Borgstrom, *ibid.*, **175,** 65(1969).
(90) C. Erlanson and B. Borgstrom, *ibid.*, **167,** 629(1968).
(91) J. R. Senior and K. J. Isselbacher, *Biochem. Biophys. Res. Commun.*, **6,** 274(1961).
(92) H. Greten, R. I. Levy, and D. S. Fredrickson, *Circulation*, **38,** 8(1968).
(93) M. Cohen, R. G. H. Morgan, and A. F. Hoffman, *Fed. Proc.*, **27,** 574(1968).
(94) S. B. Clark and P. R. Holt, *ibid.*, **27,** 331(1968).
(95) F. Schoenheyder and K. Volgvartz, *Acta Physiol. Scand.*, **11,** 349(1946).
(96) D. S. Robinson and J. E. French, *Quart. J. Exp. Physiol.*, **42,** 151(1957).
(97) R. J. Havel, *Amer. J. Clin. Nutr.*, **6,** 662(1958).
(98) S. Mahadevan and A. L. Tappel, *J. Biol. Chem.*, **243,** 2849(1968).
(99) W. Guder, L. Weiss, and O. Wieland, *Biochim. Biophys. Acta*, **187,** 173(1969).
(100) K. Hayase and A. L. Tappel, *J. Biol. Chem.*, **245,** 169(1970).
(101) Y. Biale, E. Gorin, and E. Shafrir, *Biochim. Biophys. Acta*, **152,** 28(1968).
(102) P. Desnuelle, in "The Enzymes," 3rd ed., vol. 7, P. D. Boyer, Ed., Academic, New York, N.Y., 1972, p. 575.
(103) P. Desnuelle, in "Handbook of Physiology," vol. 5, sect. 6, C. F. Code, Ed., Williams & Wilkins, Baltimore, Md., 1968, p. 2629.
(104) H. Newey and D. H. Smyth, *J. Physiol. (London)*, **152,** 367(1960).
(105) N. M. Green and H. Neurath, in "The Proteins," vol. 2, H. Neurath and K. Bailey, Eds., Academic, New York, N.Y., 1954, p. 1057.
(106) J. E. Folk and E. W. Schirmer, *J. Biol. Chem.*, **240,** 181(1965).
(107) H. Neurath and G. W. Schwert, *Chem. Rev.*, **46,** 69(1950).
(108) J. E. Folk, *J. Amer. Chem. Soc.*, **78,** 3541(1956).
(109) J. E. Folk and J. A. Gladner, *J. Biol. Chem.*, **231,** 379(1958).
(110) S. Bien and O. M. Friedman, *Chem. Ind. (London)*, **1962,** 2062.
(111) I. C. Geddes, *Acta Anaesthesiol. Scand. Suppl. XVI*, **1965,** 37.
(112) B. Akerman, A. Astrom, S. Ross, and A. Telc, *Acta Pharmacol. Toxicol.*, **24,** 389(1966).
(113) S. B. A. Akerman, S. B. Ross, and A. Telc, *ibid.*, **32,** 88(1973).
(114) E. Brandl, *Sci. Pharm.*, **40,** 1(1972).

(115) Z. B. Papanastassiou and T. J. Bardos, *J. Med. Pharm. Chem.*, **5**, 1000(1962), and references cited therein.
(116) P. J. Keller, in "Handbook of Physiology," vol. 5, sect. 6, C. F. Code, Ed., Williams & Wilkins, Baltimore, Md., 1968, p. 2605.
(117) J. B. Rhodes, in *ibid.*, p. 2589.
(118) "The Enzymes," 3rd ed., vol. 3, P. D. Boyer, Ed., Academic, New York, N.Y., 1971.
(119) P. Grosser and J. Husler, *Biochem. Z.*, **39**, 1(1912).
(120) R. Robison, *Biochem. J.*, **17**, 286(1923).
(121) R. Robison and K. M. Soames, *ibid.*, **18**, 740(1924).
(122) M. Martland and R. Robison, *ibid.*, **20**, 847(1926).
(123) O. Meyerhof and H. Green, *J. Biol. Chem.*, **183**, 377(1950).
(124) D. L. Engstrom, *Biochim. Biophys. Acta*, **52**, 36(1961).
(125) D. R. Harkness, *Arch. Biochem. Biophys.*, **126**, 503(1968).
(126) A. Asakawa, *J. Biochem. (Tokyo)*, **10**, 157(1928).
(127) *Ibid.*, **11**, 143(1929).
(128) M. Ichihara, *J. Biochem. (Tokyo)*, **18**, 87(1933).
(129) M. Martland and R. Robison, *Biochem. J.*, **18**, 765(1924).
(130) M. A. M. Abul-Fadl and E. J. King, *ibid.*, **45**, 51(1949).
(131) W. H. Fishman and H. M. Davidson, *Methods Biochem. Anal.*, **4**, 257(1957).
(132) W. Kutscher and H. Wolberg, *Z. Physiol. Chem.*, **236**, 237(1935).
(133) W. Kutscher and J. Pany, *ibid.*, **255**, 169(1928).
(134) G. Siebert, G. Jung, and K. Lang, *Biochem. Z.*, **326**, 464(1955).
(135) E. Bamann and K. Diederichs, *Chem. Ber.*, **67**, 2019(1934).
(136) A. Chersi, A. Bernardi, and G. Bernardi, *Biochim. Biophys. Acta*, **129**, 12(1966).
(137) E. Bamann and K. Diederichs, *Chem. Ber.*, **68**, 6(1935).
(138) M. A. Bukhari, J. L. Everett, and W. C. J. Ross, *Biochem. Pharmacol.*, **21**, 963(1972).
(139) C. R. Ball, J. A. Double, and J. Goodban, *Brit. J. Cancer*, **78**, 81(1973).
(140) H. N. Fernley, in "The Enzymes," 3rd ed., vol. 4, P. D. Boyer, Ed., Academic, New York, N.Y., 1971, p. 417.
(141) V. P. Hollander, in *ibid.*, p. 499.
(142) F. Schapira, *Advan. Cancer Res.*, **18**, 77(1973).
(143) H. Baum, K. S. Dodgson, and B. Spencer, *Biochem. J.*, **69**, 567(1958).
(144) H. Baum and K. S. Dodgson, *ibid.*, **69**, 573(1958).
(145) W. Bleszynski and A. Leznicki, *Enzymologia*, **33**, 373(1967).
(146) W. Bleszynski, A. Leznicki, and J. Lewosz, *ibid.*, **37**, 314(1969).
(147) H. Fex, K. E. Lundvall, and A. Olsson, *Acta Chem. Scand.*, **22**, 254(1968).
(148) C. Linder and J. Fishman, *J. Med. Chem.*, **16**, 553(1973).
(149) R. B. Billiar and K. B. Eik-Nes, *Anal. Biochem.*, **13**, 11(1965).
(150) Chinoin GVTG, West German pat. 2,242,792 (Apr. 12, 1973).
(151) G. D. Gray, F. R. Nichol, M. M. Mickelson, C. W. Camiener, D. T. Gish, R. C. Kelly, W. J. Wechter, T. E. Moxley, and G. L. Neil, *Biochem. Pharmacol.*, **21**, 465(1972), and references cited therein.
(152) A. D. Little Inc., U.S. pat. 3,728,360 (Apr. 17, 1973).
(153) J. A. Durden, A. A. Sousa, and J. F. Stephen, "Abstract of Papers," American Chemical Society, 165th meeting, PES 31, 1973.
(154) D. J. Johns, D. Farquhar, M. K. Wolpert, B. A. Chabner, and T. L. Loo, *Drug Metab. Dispos.*, **1**, 580(1973).
(155) U. Schaeppi, I. A. Heyman, R. W. Fleischman, H. Rosenkrantz, V. Ilievski, R. Phelan, D. A. Cooney, and R. D. Davis, *Cancer Chemother. Rep.*, **4**, Part 3, 85(1973).
(156) K. C. Tsou, K. Hoegerle, and H. C. F. Su, *J. Med. Chem.*, **6**, 435(1963).
(157) M. M. Nachlas and A. M. Seligman, *J. Biol. Chem.*, **181**, 343(1949).
(158) A. K. Balls and M. B. Matlack, *ibid.*, **123**, 679(1938).
(159) A. M. Seligman and M. M. Nachlas, *J. Clin. Invest.*, **29**, 31(1950).
(160) J. A. Montgomery, T. P. Johnston, and Y. F. Shealy, in "Medicinal Chemistry," 3rd ed., A. Burger, Ed., Wiley-Interscience, New York, N.Y., 1970, p. 690.
(161) K. B. Augustinsson and D. Nachmansohn, *Science*, **110**, 98(1949).
(162) A. M. Morgan and E. B. Truitt, *J. Pharm. Sci.*, **54**, 1640(1965).
(163) A. M. Seligman, M. M. Nachlas, L. H. Manheimer, O. M. Friedman, and G. Wolf, *Ann. Surg.*, **130**, 333(1949).
(164) H. L. Herzog, L. Weber, and E. L. Shapiro, U. S. pat. 3,740,316 (June 19, 1973).
(165) K. C. Tsou and A. Seligman, *J. Amer. Chem. Soc.*, **76**, 3704(1954).
(166) H. A. Ravin, K. C. Tsou, and A. M. Seligman, *J. Biol. Chem.*, **191**, 843(1951).
(167) G. Wolf and A. M. Seligman, *J. Amer. Chem. Soc.*, **73**, 2080(1951).
(168) H. A. Ravin and A. M. Seligman, *J. Biol. Chem.*, **190**, 391(1951).
(169) A. J. Glazko, H. E. Carnes, A. Kazenko, L. M. Wolf, and T. F. Reutner, *Antibiot. Annu.*, **1957–1958**, 792.
(170) V. Botte and S. S. Koide, *Biochim. Biophys. Acta*, **152**, 396(1968).
(171) V. Botte and S. S. Koide, *Endocrinology*, **82**, 1062(1968).
(172) E. M. Sellers, M. L. Lang, S. D. Cooper, and J. Koch-Weser, *Clin. Pharmacol. Ther.*, **14**, 147(1973).
(173) R. M. DeHaan, C. M. Metzler, D. Schellenberg, and W. D. Vandenbosch, *J. Clin. Pharmacol.*, **13**, 190(1973).
(174) D. L. DiPietro, *J. Biol. Chem.*, **243**, 1303(1968).
(175) W. Morozowich, D. J. Lamb, H. A. Karnes, F. A. MacKellar, C. Lewis, K. F. Stern, and E. L. Rowe, *J. Pharm. Sci.*, **58**, 1485(1969).
(176) "Biology Data Book," P. A. Altman and D. S. Dittmer, Eds., Federation of American Societies for Experimental Biology, Washington, D.C., 1964, p. 279.
(177) J. C. Warren and A. P. French, *J. Clin. Endocrinol. Metab.*, **25**, 278(1965).
(178) E. Mehl and H. Jatzkewitz, *Biochim. Biophys. Acta*, **151**, 619(1968), and references cited therein.
(179) K. S. Dodgson and C. H. Wynn, *Biochem. J.*, **68**, 387(1958).
(180) K. S. Dodgson and B. Spencer, *ibid.*, **53**, 444(1953).
(181) M. O. Pulkinnen and I. Paunio, *Ann. Med. Exp. Biol. Fenn.*, **41**, 283(1963); through "Metabolic Conjugation and Metabolic Hydrolysis," vol. I, W. H. Fishman, Ed., Academic, New York, N.Y., p. 267.
(182) M. Levitz and J. Katz, *J. Clin. Endocrinol. Metab.*, **28**, 862(1968).
(183) K. D. Roberts, R. L. Vande Wiele, and S. Lieberman, *J. Biol. Chem.*, **236**, 2213(1961).
(184) M. O. Pulkinnen, *Acta Physiol. Scand. Suppl.*, **180**, 9(1961).
(185) J. C. Warren and C. E. Timberlake, *J. Clin. Endocrinol. Metab.*, **22**, 1148(1962).
(186) J. R. Pasqualini, L. Cedard, B. L. Nguyen, and E. Alsatt, *Biochim. Biophys. Acta*, **139**, 177(1967).
(187) J. P. Greenstein, "Biochemistry of Cancer," Academic, New York, N.Y., 1954, p. 379.
(188) J. A. Goldbarg and A. M. Rutenburg, *Cancer*, **11**, 283(1958).
(189) M. S. Burstone, *J. Nat. Cancer Inst.*, **16**, 1149(1956).
(190) S. Hosoda, S. Takase, K. Yoshida, and J. Tohoku, *J. Exp. Med.*, **73**, 86(1960).
(191) K. C. Tsou, S. B. Damle, R. W. Crichlow, R. G. Ravdin, and H. W. Blunt, *J. Pharm. Sci.*, **56**, 484(1967).
(192) M. A. Kaplan, W. T. Bradner, F. H. Buckwalter, and M. H. Pindell, *Nature (London)*, **205**, 399(1965).
(193) P. H. Jones, J. H. Biel, C. W. Ours, L. Klundt, and R. L. Lenga, "Abstracts of Papers," American Chemical Society, 165th Meeting, MEDI 9, 1973.
(194) W. C. J. Ross and J. G. Wilson, *J. Chem. Soc.*, **1956**, 3616.
(195) R. Jones, Jr., W. B. Kessler, H. E. Lessner, and L. Rane, *Cancer Chemother. Rep.*, **10**, 99(1960).
(196) O. Warburg, *Chem. Ber.*, **38**, 187(1905).
(197) J. E. Folk and J. A. Gladner, *Biochim. Biophys. Acta*, **33**, 570(1959).
(198) E. Wintersberger, D. J. Cox, and H. Neurath, *Biochemistry*, **1**, 1069(1962).
(199) A. Bukhari, *J. Nat. Cancer Inst.*, **50**, 243(1973).
(200) W. C. J. Ross and G. P. Warwick, *J. Chem. Soc.*, **1956**,

1364.
(201) Y. F. Shealy, J. A. Montgomery, and W. R. Laster, Jr., *Biochem. Pharmacol.*, **11**, 674(1962).
(202) A. T. Fuller, *Lancet*, **1**, 194(1937).
(203) V. Hamon, *Ann. Chim. (Paris)*, **2**, 233(1947).
(204) J. L. Everett and W. C. J. Ross, *J. Chem. Soc.*, **1972**, 1949.
(205) W. C. J. Ross, "Biological Alkylating Agents," Butterworth, London, England, 1962.
(206) R. J. Yu and E. J. vanScott, *Proc. Amer. Ass. Cancer Res.*, **14**, 46(1973).
(207) A. J. Verbiscar, U.S. pat. 3,742,022 (June 26, 1973).
(208) O. M. Friedman, E. Boger, V. Grubliauskas, and H. Sommer, *J. Med. Chem.*, **6**, 50(1963).
(209) C. L. Maddock, A. H. Handler, O. M. Friedman, G. E. Foley, and S. Farber, *Cancer Chemother. Rep.*, **50**, 629(1966).
(210) O. M. Friedman, *ibid.*, **51**, 327(1967).
(211) K. Sugiura, F. Schmid, and M. Schmid, *Proc. Amer. Ass. Cancer Res.*, **3**, 271(1961).
(212) N. Brock, *Cancer Chemother. Rep.*, **51**, 315(1967).
(213) M. Lane, *ibid.*, **51**, 359(1967).
(214) G. E. Foley, O. M. Friedman, and B. P. Drolet, *Cancer Res.*, **21**, 57(1961).
(215) N. Brock and H.-J. Hohorst, *Cancer (Philadelphia)*, **20**, 900(1967).
(216) O. M. Friedman, *Cancer Chemother. Rep.*, **51**, 347(1967).
(217) J. T. Hill and C. C. Irving, *Biochemistry*, **6**, 3816(1967).
(218) J. T. Hill, Ph.D. thesis, University of Tennessee, Memphis, Tenn., 1968.
(219) H. Ide, S. Green, K. Kato, and W. H. Fishman, *Biochem. J.*, **106**, 431(1968).
(220) G. A. Levvy and J. Conchie, in "Glucuronic Acid: Free and Combined. Chemistry, Biochemistry, Pharmacology and Medicine," G. J. Dutton, Ed., Academic, New York, N.Y., pp. 301–364.
(221) A. J. Glazko, W. H. Edgerton, W. A. Dill, and W. R. Lenz, *Antibiot. Chemother.*, **2**, 234(1952).
(222) S. Roseman and A. Dorfman, *J. Biol. Chem.*, **191**, 607(1951).
(223) B. Weissman, G. Rowin, J. Marshall, and D. Friederici, *Biochemistry*, **6**, 207(1967).
(224) J. Conchie, J. Findlay, and G. A. Levvy, *Biochem. J.*, **71**, 318(1959).
(225) W. von Daehne, E. Frederiksen, E. Gunderson, F. Lund, P. Mørch, H. J. Peterson, K. Roholt, L. Tybring, and W. O. Godtfredsen, *J. Med. Chem.*, **13**, 607(1970).
(226) W. von Daehne, W. O. Godtfredsen, K. Roholt, and L. Tybring, *Antimicrob. Ag. Chemother.*, **1970**, 431.
(227) W. E. Magee, S. B. Armour, and O. V. Miller, *Biochim. Biophys. Acta*, **306**, 270(1973).
(228) Godecke AG, West German pat. 2,042,646 (Mar. 2, 1972).
(229) V. Stella and T. Higuchi, *J. Pharm. Sci.*, **62**, 962(1973).
(230) Eisai Co. Ltd., West German pat. 2,242,781 (Mar. 8, 1973).
(231) Arakawa Chotaro Gomei Kaisha, Japanese pat. 7,303,363 (Jan. 30, 1973).
(232) J. Klosa, West German pat. 2,134,672 (Jan. 25, 1973).
(233) D. N. Croft, J. H. P. Cuddigan, and C. Sweetland, *Brit. Med. J.*, **3**, 545(1972).
(234) G. Linari, *Arzneim.-Forsch.*, **22**, 1419(1972).
(235) B.-Z. Weiner and A. Zilkha, *J. Med. Chem.*, **16**, 573(1973).
(236) M. G. White and M. J. Asch, *N. Engl. J. Med.*, **284**, 1281(1971).
(237) W. D. Celmer, *Antibiot. Annu.*, **1958–1959**, 277.
(238) V. C. Stephens, J. W. Conine, and H. W. Murphy, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 620(1959), and references cited therein.
(239) S. M. Bell, *Med. J. Aust.*, **2**, 1280(1971).
(240) A. A. Forist, R. M. DeHaan, and C. M. Metzler, *J. Pharmacokinet. Biopharm.*, **1**, 89(1973).
(241) R. E. Kauffman, D. W. Shoeman, and S. H. Wan, *Clin. Pharmacol. Ther.*, **13**, 704(1972).
(242) K. Butler and E. S. Hamanaka, U.S. pat. 3,681,342 (Aug. 1, 1972).
(243) E. K. Frederiksen and W. O. Godtfredsen, U.S. pat. 3,719,668 (Mar. 6, 1973).
(244) A. B. A. Jensen and T. J. Russell, *J. Chem. Soc.*, **1965**, 2127.
(245) E. Hansson, L. Magni, and S. Wahlqvist, *Antimicrob. Ag. Chemother.*, **1967**, 568.
(246) C. H. Ramsey, N. O. Bodin, and E. Hansson, *Arzneim.-Forsch.*, **22**, 1962(1972).
(247) J. M. Essery and J. R. West, U.S. pat. 3,654,265 (Apr. 4, 1972).
(248) A. R. English, J. A. Retsema, V. A. Ray, and J. E. Lynch, *Antimicrob. Ag. Chemother.*, **1**, 185(1971).
(249) D. C. Hobbs, *ibid.*, **2**, 272(1972).
(250) E. L. Foltz, J. W. West, I. H. Breslow, and H. Wallick, *ibid.*, **1970**, 442.
(251) M. C. Jordan, J. B. deMaine, and W. M. M. Kirby, *ibid.*, **1970**, 438.
(252) E. R. Hultberg and B. Backelin, *Scand. J. Infect. Dis.*, **4**, 149(1972).
(253) E. K. Frederiksen and W. O. Godtfredsen, U.S. pat. 3,697,507 (Oct. 10, 1972).
(254) J. A. Yurchenco, M. W. Hopper, T. D. Vince, and G. H. Warren, *Chemotherapy*, **17**, 405(1972), and references cited therein.
(255) W. J. Jusko and G. P. Lewis, *J. Pharm. Sci.*, **62**, 69(1973).
(256) W. J. Jusko, G. P. Lewis, and G. W. Schmitt, *Clin. Pharmacol. Ther.*, **14**, 90(1973).
(257) J. M. Essery, U.S. pat. 3,679,663 (May 25, 1972).
(258) I. Villax, Belgian pat. 774,717 (Feb. 14, 1972).
(259) Kayaku Antibiotics Research Co. Ltd., Japanese pat. 7,211,961 (Apr. 14, 1972).
(260) Koniklijke Nederlandsche Gist en Spiritusfabriek, French pat. 2,126,443 (Oct. 6, 1972).
(261) Yamanouchi Pharmaceutical Co. Ltd., Japanese pat. 4,720,187 (Sept. 27, 1972).
(262) Yamanouchi Pharmaceutical Co. Ltd., Belgian pat. 781,659 (July 31, 1972).
(263) E. R. Squibb and Sons Inc., West German pat. 2,152,745 (Apr. 27, 1972).
(264) Glaxo Labs Ltd., West German pat. 2,223,375 (Nov. 23, 1972).
(265) J. van der Vies, *Acta Endocrinol.*, **49**, 271(1965).
(266) C. A. Winter and C. C. Porter, *J. Amer. Pharm. Ass., Sci. Ed.*, **46**, 515(1957).
(267) R. Gardi, R. Vitali, G. Falconi, and A. Ercoli, *J. Med. Chem.*, **16**, 123(1973).
(268) D. D. Evans and P. J. Palmer, *Steroids*, **5**, 441(1965).
(269) E. Novak, S. Stubbs, and D. J. Chodos, *Clin. Pharmacol. Ther.*, **13**, 148(1972).
(270) D. K. Fukushima, M. Matsui, H. L. Bradlow, and L. Hellman, *Steroids*, **19**, 385(1972).
(271) E. Chang and V. K. Jain, *J. Med. Chem.*, **9**, 433(1966).
(272) R. T. Rapala, R. J. Kraay, and K. Gerzon, *ibid.*, **8**, 580(1965), and references cited therein.
(273) T. H. Haskell and S. Hanessian, U.S. pat. 3,651,045 (Mar. 21, 1972).
(274) J. C. Melby and M. St. Cyr, *Metabolism*, **10**, 75(1961).
(275) Alza Corp., U.S. pat. 3,764,673 (Oct. 9, 1973).
(276) L. W. Dittert, H. C. Caldwell, T. Ellison, G. M. Irwin, D. E. Rivard, and J. V. Swintosky, *J. Pharm. Sci.*, **57**, 828(1968).
(277) A. Misher, H. J. Adams, J. J. Fishler, and R. G. Jones, *ibid.*, **57**, 1128(1968).
(278) W. C. J. Ross, in "Biological Alkylating Agents," Butterworth, London, England, 1962, p. 148.
(279) J. F. Danielli, *Nature*, **170**, 863(1952).
(280) V. T. Oliverio and C. G. Zubrod, *Annu. Rev. Pharmacol.*, **5**, 335(1965).
(281) C. Hansch, *Cancer Chemother. Rep.*, **56**, 433(1972).
(282) W. J. Waddell, *Annu. Rev. Pharmacol.*, **13**, 153(1973).
(283) W. H. Fishman and J. R. Baker, *J. Histochem. Cytochem.*, **4**, 570(1956).
(284) W. C. J. Ross, *Biochem. Pharmacol.*, **8**, 235(1961).
(285) T. A. Connors, B. C. V. Mitchley, V. M. Rosenoer, and W. C. J. Ross, *ibid.*, **13**, 395(1964).
(286) S. Weinhouse, *Advan. Cancer Res.*, **3**, 321(1955).
(287) Z. B. Papanastassiou, R. J. Bruni, E. White, V, and P. L. Levins, *J. Med. Chem.*, **9**, 725(1966).
(288) O. Warburg, K. Posener, and E. Negelein, *Biochem. Z.*, **152**, 309(1924).
(289) A. H. Khan and W. C. J. Ross, *Chem.-Biol. Interactions*, **1**, 27(1969–70).
(290) C. Hansch, N. Smith, R. Engle, and H. Wood, *Cancer Chemother. Rep.*, **56**, 443(1972).

(291) G. P. Wheeler, B. J. Bowdon, J. A. Grimsley, and H. H. Lloyd, *Cancer Res.*, **34**, 194(1974).
(292) R. F. Hudson, G. M. Timmis, and R. D. Marshall, *Biochem. Pharmacol.*, **1**, 48(1958).
(293) W. J. Jusko, *J. Pharmacokinet. Biopharm.*, **1**, 175(1973).
(294) H. Arnold, F. Bourseaux, and N. Brock, *Naturwissenschaften*, **45**, 64(1958).
(295) N. Brock, *Arzneim.-Forsch.*, **8**, 1(1958).
(296) H. E. Skipper and L. H. Schmidt, *Cancer Chemother. Rep.*, **17**, 1(1962).
(297) M. Colvin, C. A. Padgett, and C. Fenselau, *Cancer Res.*, **33**, 915(1973).
(298) M. Colvin and O. M. Friedman, *Proc. Amer. Ass. Cancer Res.*, **14**, 119(1973).
(299) A. N. Milner and M. P. Sullivan, *Cancer Chemother. Rep.*, **51**, 343(1967).
(300) K. H. Saunders, "The Aromatic Diazo Compounds and Their Technical Applications," Edward Arnold, London, England, 1949.
(301) V. T. DeVita, P. P. Carbone, A. H. Owens, Jr., G. L. Gold, M. J. Krant, and J. Edmonson, *Cancer Res.*, **25**, 1876(1965), and references cited therein.
(302) J. A. Montgomery, R. James, G. S. McCaleb, and T. P. Johnston, *J. Med. Chem.*, **10**, 668(1967), and references cited therein.
(303) T. P. Johnston and P. S. Opliger, *ibid.*, **10**, 675(1967).
(304) E. Fels, *J. Clin. Endocrinol.*, **4**, 121(1944).
(305) D. G. Seay, J. D. Bradshaw, and N. T. Nicol, *Cancer Chemother. Rep.*, **56**, 89(1972).
(306) W. N. Fishbein, P. P. Carbone, E. J. Freireich, D. Misra, and E. Frei, *Clin. Pharmacol. Ther.*, **5**, 574(1964).
(307) S. M. Kupchan and A. C. Isenberg, *J. Med. Chem.*, **10**, 960(1967).
(308) S. M. Kupchan and A. F. Casy, *ibid.*, **10**, 959(1967).
(309) Fabrica de Medicamente Biofarm., West German pat. 2,231,486 (Feb. 15, 1973).
(310) A. J. Verbiscar and L. G. Abood, *J. Med. Chem.*, **13**, 1176(1970).
(311) C. Casagrande and G. Ferrari, *Farmaco, Ed. Sci.*, **28**, 143(1972).
(312) C. R. Creveling, J. W. Daly, T. Tokuyama, and B. Witkop, *Experientia*, **25**, 26(1969).
(313) M. E. Wall, G. S. Abernethy, Jr., F. I. Carroll, and D. J. Taylor, *J. Med. Chem.*, **12**, 810(1969).
(314) O. M. Friedman and R. Chatterji, *J. Amer. Chem. Soc.*, **81**, 3750(1959), and references cited therein.
(315) F. Bergel and J. A. Stock, *J. Chem. Soc.*, **1954**, 2409.
(316) L. F. Larionov, A. S. Khokhlov, E. N. Shkodinskaja, O. S. Vasina, V. I. Troosheikina, and M. A. Novikova, *Lancet*, **269**, 169(1955).
(317) A. Bukhari, T. A. Connors, A. M. Gilsenan, W. C. J. Ross, M. J. Tisdale, G. P. Warwick, and D. E. V. Wilman, *J. Nat. Cancer Inst.*, **50**, 243(1973).
(318) B. Rosenberg, *Naturwissenshaften*, **60**, 399(1973).
(319) M. J. Cleare, *Chem. Ind. (London)*, **1973**, 921.
(320) W. A. Ritschel, in "Drug Design," vol. 4, E. J. Ariens, Ed., Academic, New York, N.Y., 1973, pp. 37, 75.
(321) H. C. Caldwell, A. B. Rednick, G. C. Scott, G. J. Yakatan, and D. Ziv, *J. Pharm. Sci.*, **59**, 1689(1970).
(322) E. F. Elslager, Z. B. Gavrilis, A. A. Phillips, and D. F. Worth, *J. Med. Chem.*, **12**, 357(1969).
(323) E. F. Elslager, A. A. Phillips, and D. F. Worth, *ibid.*, **12**, 363(1969).
(324) D. F. Worth, E. F. Elslager, and A. A. Phillips, *ibid.*, **12**, 591(1969).
(325) E. F. Elslager, D. B. Capps, and D. F. Worth, *ibid.*, **12**, 597(1969).
(326) R. M. Pinder, *Progr. Med. Chem.*, **8**, 258(1971).
(327) R. Meier and E. Tschopp, *Naunyn-Schmeidebergs Arch. Exp. Pathol. Pharmakol.*, **226**, 532(1955); through W. A. Ritschel, in "Drug Design," vol. 4, E. J. Ariens, Ed., Academic, New York, N.Y., 1973, p. 75.
(328) K. Miescher, A. Wettstein, and E. Tschopp, *Biochem. J.*, **30**, 1977(1936).
(329) Kutnowskie Zakady Farmaceutyzine Polfa, French pat. 1,604,123 (Aug. 20, 1971).
(330) H. A. M. El Shibini, M. Abdel Nasser, and M. M. Motawi, *Pharmazie*, **26**, 630(1971).
(331) G. L. Neil, H. H. Buskirk, T. E. Moxley, R. C. Manak, S. L. Kuentzel, and B. K. Bhuyan, *Biochem. Pharmacol.*, **20**, 295(1971).
(332) H. E. Renis, D. T. Gish, B. A. Court, E. E. Edison, and W. J. Wechter, *J. Med. Chem.*, **16**, 754(1973).
(333) K. Gerzon and D. Kau, *ibid.*, **10**, 189(1967).
(334) F. Bottari, M. F. Saettone, and M. F. Serafini, *ibid.*, **11**, 904(1968).
(335) F. M. Berger and R. F. Riley, *J. Pharmacol. Exp. Ther.*, **96**, 269(1949).
(336) Andreu SA, West German pat. 2,237,267 (Feb. 1, 1973).
(337) J. H. Sellstedt and M. Wolf, U.S. pat. 3,692,774 (Sept. 19, 1972).
(338) D. P. Bonner, W. Mechlinski, and C. P. Schaffner, *J. Antibiot.*, **25**, 261(1972).
(339) P. Malek, J. Kolc, M. Herold, and J. Hoffman, *Antibiot. Annu.*, **1957–1958**, 546.
(340) J. Denham and L. Adamson, *Acta Psychiat. Scand.*, **47**, 420(1971).
(341) A. Villeneuve, A. Pires, A. Jus, R. Lachance, and A. Drolet, *Curr. Ther. Res., Clin. Exp.*, **14**, 696(1972).
(342) J. B. Thomsen and D. Birkerod, *Acta Psychiat. Scand.*, **49**, 119(1973).
(343) L. Julou, G. Bourat, R. Ducrot, J. Fournel, and C. Garret, *Acta Psychiat. Scand., Suppl.*, **241**, 9(1973).
(344) D. Gould, L. Finckenor, E. B. Hershberg, J. Cassidy, and P. L. Perlman, *J. Amer. Chem. Soc.*, **79**, 4472(1957).
(345) R. Gardi, R. Vitali, G. Falconi, and A. Ercoli, *J. Med. Chem.*, **15**, 556(1972).
(346) G. Falconi, F. Galletti, G. Celasco, and R. Gardi, *Steroids*, **20**, 627(1972).
(347) H. Kuhl and H. D. Taubert, *ibid.*, **22**, 73(1973).
(348) American Home Products Corp., U.S. pat. 3,647,784 (Mar. 7, 1972).
(349) G. Pala, S. Casadio, A. Mantegani, G. Bonardi, and G. Coppi, *J. Med. Chem.*, **15**, 995(1972).
(350) Warner Lambert Co., U.S. pat. 3,649,620 (Mar. 14, 1972).
(351) American Home Products Corp., U.S. pat. 3,649,621 (Mar. 14, 1972).
(352) Glaxo Labs Ltd., Netherlands pat. 7,209,345 (Jan. 9, 1973).
(353) F. Saias, A. Jondet, and J. Philippe, *Ann. Pharm. Fr.*, **27**, 557(1969).
(354) W. C. Miller, D. B. Marcotte, and L. McCurdy, *Curr. Ther. Res., Clin. Exp.*, **15**, 700(1973).
(355) W. D. Celmer, Canadian pat. 779,315 (Feb. 27, 1968).
(356) A. G. Kallianos, J. D. Mold, and M. I. Simpson, U.S. pat. 3,646,201 (Feb. 29, 1972).
(357) A. Zaffaroni, U.S. pat. 3,708,492 (Jan. 2, 1973).
(358) R. L. Houtman, U.S. pat. 3,442,926 (May 6, 1969).
(359) A. B. G. Laing, G. Pringle, and F. C. T. Lane, *Amer. J. Trop. Med. Hyg.*, **15**, 838(1966).
(360) D. F. Clyde, *Mil. Med.*, **135**, 527(1970).
(361) G. R. Coatney, P. G. Contacos, and J. S. Lunn, *Amer. J. Trop. Med. Hyg.*, **13**, 383(1964).
(362) C. Pfaffman, *Amer. Sci.*, **52**, 187(1964).
(363) K. Kimura and L. M. Beidler, *J. Cell. Comp. Physiol.*, **58**, 131(1961).
(364) H. Tateda and L. M. Beidler, *J. Gen. Physiol.*, **47**, 479(1964).
(365) L. M. Beidler, *ibid.*, **38**, 133(1954).
(366) L. M. Beidler, in "Progress in Biophysics and Biophysical Chemistry," Pergamon Press, London, England, 1961, p. 107.
(367) G. L. Flynn, S. H. Yalkowsky, and N. D. Weiner, *J. Pharm. Sci.*, **63**, 300(1974).
(368) C. Duncan, *J. Theoret. Biol.*, **5**, 114(1963).
(369) L. M. Beidler, *Advan. Chem. Ser.*, **56**, 1(1966).
(370) R. W. Moncrieff, "The Chemical Senses," Leonard Hill Books, London, England, 1967, p. 245.
(371) D. G. Moulton and D. Tucker, *Ann. N.Y. Acad. Sci.*, **116**, 380(1964).
(372) D. Tucker, *J. Gen. Physiol.*, **46**, 453(1963), and references cited therein.
(373) D. Schneider, in "Olfaction and Taste," Y. Zotterman, Ed., Pergamon Press, London, England, 1963, p. 85.
(374) M. G. J. Beets, *Amer. Perfum. Aromat.*, **76**, 54(1961).

(375) J. E. Amoore, *J. Soc. Cosmet. Chem.*, **21**, 99(1970).
(376) M. G. J. Beets, *Soc. Chem. Ind., (London), Monogr. No. 1*, **1957**, 54.
(377) Y.-R. Naves, *ibid.*, **1957**, 38.
(378) Merck and Co., Inc., West German pat. 2,256,538 (May 24, 1973).
(379) Officina Terapeutica Italiana SRL; French pat. 2,099,449 (Mar. 17, 1972).
(380) C. M. Gruber, Jr., V. C. Stephens, and P. M. Terrill, *Toxicol. Appl. Pharmacol.*, **19**, 423(1971).
(381) V. C. Stephens, U.S. pat. 3,728,379 (Apr. 17, 1973).
(382) Sumitomo Chemical Co. Ltd., West German pat. 2,244,179 (Mar. 29, 1973).
(383) E. P. Taylor, *J. Pharm. Pharmacol.*, **5**, 254(1953).
(384) R. K. Clark, Jr., and E. L. Varner, *Antibiot. Chemother.*, **7**, 487(1957).
(385) R. K. Clark, Jr., U.S. pat. 2,967,129 (Jan. 3, 1961).
(386) G. E. Davies and G. W. Driver, *Brit. J. Pharmacol.*, **12**, 434(1957).
(387) G. E. Davies and G. W. Driver, *Nature*, **182**, 664(1958).
(388) Schering Corp., U.S. pat. 3,767,811 (Oct. 23, 1973).
(389) J. E. Gray, *Arch. Pathol.*, **84**, 522(1967).
(390) R. M. DeHaan, D. Schellenberg, and J. T. Sobota, *J. Clin. Pharmacol.*, in press.
(391) E. Novak, J. G. Wagner, and D. J. Lamb, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **3**, 201(1970).
(392) J. E. Gray, R. Weaver, M. Prestrud, and R. DeHaan, *Toxicol. Appl. Pharmacol.*, **17**, 297(1970).
(393) B. E. Ballard, *J. Pharm. Sci.*, **57**, 357(1968).
(394) L. Joubert, J. P. deMenezes, and C. A. Fernandez, *J. Int. Med. Res.*, **1**, 61(1972).
(395) J. Baldwin and A. B. Amerson, *Amer. J. Hosp. Pharm.*, **30**, 837(1973).
(396) E. M. Ditlefsen, *Acta Med. Scand.*, **159**, 105(1957).
(397) W. Wallis, H. Kutt, and F. McDowell, *Neurology*, **18**, 513(1968).
(398) W. Morozowich, D. J. Lamb, R. M. DeHaan, and J. E. Gray, "Abstracts of Papers," APhA Academy of Pharmaceutical Sciences, Washington, D.C., meeting, Apr. 1970, p. 63.
(399) H. T. Edmondson, *Ann. Surg.*, **178**, 637(1973).
(400) S. Ross, J. R. Puig, and E. A. Zaremba, *Antibiot. Annu.*, **1957–1958**, 803.
(401) J. T. Scott, I. H. Porter, S. M. Lewis, and A. S. Dixon, *Quart. J. Med.*, **30**, 167(1961).
(402) H. W. Davenport, *Gastroenterology*, **46**, 245(1964).
(403) H. W. Davenport, B. J. Cohen, M. Bree, and V. D. Davenport, *ibid.*, **49**, 189(1965).
(404) K. J. Ivery, *ibid.*, **61**, 247(1971), and references cited therein.
(405) A. Lynch, H. Shaw, and G. W. Milton, *Gut*, **5**, 230(1964).
(406) E. I. Winkelman and W. H. J. Summerskill, *Gastroenterology*, **40**, 56(1961).
(407) R. Menguy and Y. F. Masters, *Surg. Gynecol. Obstet.*, **120**, 92(1965).
(408) R. Menguy and Y. F. Masters, *Surgery*, **54**, 19(1963).
(409) D. M. Bailey, D. Wood, R. E. Johnson, J. P. McAuliff, J. C. Bradford, and A. Arnold, *J. Med. Chem.*, **15**, 344(1972).
(410) B. A. Hems, R. M. Atkinson, M. Early, and E. G. Tomich, *Brit. Med. J.*, **1**, 1834(1962).
(411) M. H. Hubacher and S. Doernberg, *J. Pharm. Sci.*, **53**, 1067(1964).
(412) T. Bruzzese, A. Goi, and M. Riva, *Farmaco, Ed. Sci.*, **28**, 121(1972).
(413) N. H. Anderson and N. M. Weinshenker, U.S. pat. 3,723,423 (Mar. 27, 1973).
(414) K. R. H. Repke and R. Megges, *Therapiewoche*, **23**, 2314, 2317(1973).
(415) Beiersdorf AG, West German pat. 2,212,830 (Sept. 27, 1973).
(416) Beiersdorf AG, West German pat. 2,212,831 (Sept. 27, 1973).
(417) Synthelabo, Belgian pat. 798,557 (Aug. 16, 1973).
(418) I. Villax, U.S. pat. 3,773,803 (Nov. 20, 1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received from *The Research Laboratories, The Upjohn Company, Kalamazoo, MI 49001*

Adapted in part from an oral presentation by A. A. Sinkula to the 14th Annual National Industrial Pharmaceutical Research Conference, Land O' Lakes, Wis., June 1972.

ˣ To whom inquiries should be directed.