JOURNAL OF Pharmaceutical Sciences

September 1962 volume 51, number 9

Review Article

Prediction of Stability of Drugs and Pharmaceutical Preparations

By EDWARD R. GARRETT

TO PREDICT is "to tell or declare beforehand, foretell or prophesy" (190). In the realms of science, prediction is based on a knowledge of the relations among experimental values under observed experimental conditions. Prediction of the stability of drugs and pharmaceutical preparations depends on quantitative mathematical expressions that permit the calculation of rates of degradation by the substitution of the appropriate values for temperature, concentrations, pressure, time, pH, oxygen content, centrifugal or gravitational stress, light intensities and wavelengths, etc.

These quantitative relations can be obtained from the classical literature of chemistry and physics (83, 169). Judicious experimental design can permit the evaluation of the vital parameters with the minimum expenditure of time and labor. Thus, stability can be predicted for practical circumstances where study, *per se*, is experimentally or economically impractical or unsuitable.

The literature contains many articles on drug incompatibilities or instabilities for which references are cited (18, 134, 158, 192).

However, kinetic and predictive studies of drugs in the pharmaceutical literature are of relatively recent vintage. In fact, *ca.* 1950 can be considered the start of the modern era in the quantitative comprehension and prediction of drug stability.

Received from the College of Pharmacy, University of Florida, Gainesville.

This review will be concerned with those studies on drugs and pharmaceutical formulations where quantitative expressions were obtained to permit prediction of stability. It will consider the basic concepts and the rationales which can be applied to permit prognosis. This review will be practically limited to studies conducted in liquid media involving solvolytic processes and leave the aerobic, bacterial, enzymic, or photolytic degradations to another time or another reviewer. The prediction of stability of the complex systems characterized by colloidal suspensions, emulsions, ointments, and solid formulations will, in general, also be ignored.

The basic concepts of kinetics and their applications to the understanding of the mechanisms of reactions of many simple systems and reactive groups are given in many fine books on these subjects (2, 9, 14, 29, 43, 71, 72, 86, 103, 104, 123, 138). Their reading is recommended.

Predictive Use of the Apparent First-Order Rate Constant.—The chemical action of a solvent and other solutes on the drug in solution is of prime importance. A systematic approach to its understanding and quantification is to consider the rate of degradation of such a drug as proportional to the concentrations of the reactants in the rate-determining step, or proportional to the concentrations of the reactants in the equilibria which precede such a rate-determining step. The rate of drug transformation is given in concentration, c, change per unit of time; (time-unit^{-1}).

In most solvolytic reactions with constant conditions of temperature, pH, buffer kind and concentration, and ionic strength, the rate of change per unit of time is proportional to the first power of the concentration of the drug being transformed

$$dc/dt(M/\text{L./sec.}) = kc^1(\text{sec.}^{-1} \times \text{moles/L.}) \quad \text{(Eq. 1)}$$

The rate is considered as apparent first order since it varies as the first power of the concentration, c, of the substrate. On integration of Eq. 1, the logarithm of the concentration, c, when plotted against time is a straight line of slope $k/2.303$

$$\log c = -kt/2.303 + \log c_0 \quad \text{(Eq. 2)}$$

or the logarithm of the fraction of substrate untransformed when plotted against time is a straight line of the same slope

$$\log c/c_0 = -kt/2.303 \quad \text{(Eq. 3)}$$

where c_0 is the original concentration and k is in reciprocal time units (29, 71). It has been recommended that all rate constants be given in units of seconds for ease of literature comparison (161). Some typical semilogarithmic plots for the degradation of thiamine hydrochloride (47) are given in Fig. 1.

The rate constants, k, are frequently used to compare and estimate rates. An alternative constant to use is the half-life, $t_{1/2}$; the time when a concentration is reduced by half its initial value. For all apparent first-order reactions the $t_{1/2}$ is independent of the magnitude of the initial concentration of the substrate and is related to the first-order rate constant, k, by

$$t_{1/2}(\text{sec.}) = -2.303 \log 0.5/k = 0.693/k \quad \text{(Eq. 4)}$$

from the rearrangement of Eq. 3 where $c/c_0 = {}^1/_2$, $t = t_{1/2}$ and k is in sec.$^{-1}$.

Variations on this theme can be chosen to alculate the time of 5 or 10% loss of substrate, *i.e.*, $t_{0.95}$ or $t_{0.90}$, respectively (117). The half-life is only independent of initial substrate concentrations for first-order reactions. It has been used frequently to describe the stability of a drug and the rate dependencies (35, 95, 98, 130, 132, 193, 198). Nelson (143) provides a convenient table to determine the fractional loss of substrate for n half-lives. However for the purpose of a systematic presentation, the concept of the rate constant will be used in this paper as descriptive of the fraction of substrate remaining on exponential decay

$$c/c_0 = e^{-kt} \quad \text{(Eq. 5)}$$

It is apparent that if the apparent first-order rate constant, k, can be determined as a function of the physical conditions of the solution, the rate of degradation at a given concentration can be determined by substitution of that concentration, c, into Eq. 1; that the half-life under those conditions can be determined by substitution into Eq. 4; that the fraction of substrate remaining at any time, t, can be determined by use of Eq. 5.

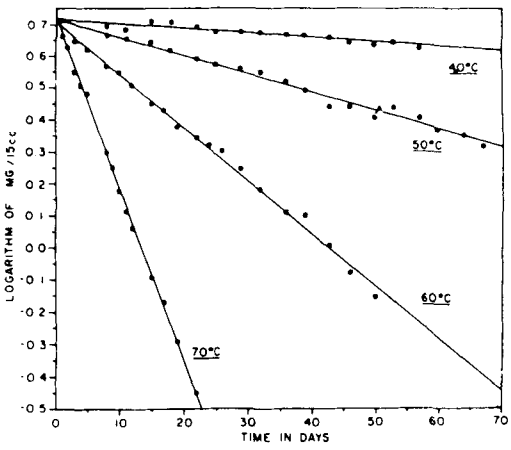


Fig. 1.—First-order plots of the thermal degradation of thiamine hydrochloride in a liquid multivitamin; logarithms of concentration (mg./15 ml.) against time in days. [Figure 1 of Garrett, E. R., This Journal, **45**, 470(1956) (47).]

To transform the half-life or first-order rate constants into other time units such as hours, days, years, etc., the conversions are

$$t_{1/2}(\text{time units}) = t_{1/2}(\text{in sec.})/(\text{seconds/time unit}) \quad \text{(Eq. 6)}$$

$$k(\text{time unit})^{-1} = k(\text{sec.}^{-1}) \times \text{sec./time unit} \quad \text{(Eq. 7)}$$

An appropriate procedure for studies that may be ambiguous is to verify the fact experimentally that the apparent first-order rate constant, k, is independent of substrate concentration. This has been done in many such cases (52, 55, 57, 66, 91, 114, 140, 154, 165, 193). Alternative methods of estimating apparent first-order rate constants are from the slopes of plots of concentration changes against time at the initial concentrations of substrate or by the Guggenheim method (81). Such procedures have been used with steroid hemiester hydrolysis and compared to the k values derived from classical plots (59, 61, 68).

Specific Acid-Base Catalyzed Solvolysis.—The simplest catalysts for solvolysis are hydrogen ions and hydroxyl ions so that the apparent first-order reaction rate constant k would be determined by

$$k = k_1[H^+] + k_6[OH^-] \quad \text{(Eq. 8)}$$

where catalysis by $[H^+]$ and $[OH^-]$ and the respective specific rate constants k_1 and k_6(L./*M*/sec.) are considered specific acid-base catalysis. In general, hydroxyl ion catalysis is negligible when a hydrogen ion catalyzed transformation is operative, and vice versa, so that one method of determining k_1 is to plot the observed rate constant, k, obtained from the slope of the plot of data according to Eq. 2, against the maintained hydrogen ion concentration.

As per Eq. 8 where the contribution of $k_6[OH^-]$ is negligible, the slope of such a plot estimates k_1 in L./*M*/sec. Obviously, k_6 may be similarly obtained by plotting k against $[OH^-]$ when the contribution of k_1 $[H^+]$ is negligible and k_6 in L./*M*/sec. can be estimated. A typical plot in this manner is given in Fig. 2 for the acid hydrolysis of psicofuranine (56). The negative intercept of the plot is due to the fact that the true molarity of hydrochloric acid is the apparent molarity lessened by the amount necessary to neutralize the psicofuranine.

Definition of Hydrogen and Hydroxyl Ion Concentrations and Activities.—The specific bimolecular rate constants are defined in terms of the ratio of the apparent first-order rate constant and the concentration or activity of the catalytic species. The specific rate constants of Eq. 8

$$k_1 = k/[H^+] \text{ when } k_6[OH^-] \sim 0 \quad \text{(Eq.8a)}$$

or

$$k_6 = k/[OH^-] \text{ when } k_1[H^+] \sim 0 \quad \text{(Eq. 8b)}$$

can be defined in terms of the stoichiometric strong acid or strong alkali concentration, *e.g.*, $[H^+]$ = [HCl] or $[OH^-]$ = [NaOH] on the postulate that the hydrogen or hydroxyl ion catalytic contributions are equivalent to the molarity of the strong acid or base. This is not necessarily true, however, as the effective hydrogen (or hydroxyl) ion concentration (activity) of a strong acid (or base) decreases from expectation with increased concentrations of the acid (or base) (89). The high activity of perchloric acid makes it one of the most ideal acids for the study of specific hydrogen ion catalysis (97, 98, 130–132). Ester hydrolysis is most generally catalyzed only by hydrogen and hydroxyl ions (specific acid-base catalysis) (9, 123) but consumes hydroxyl ions, so that if $k_6 = k/[OH^-]$ is to be determined, then the molar concentration of strong alkali should exceed the concentration substrate by at least tenfold, *e.g.*, $[OH^-] > 10c$ when $[OH^-]$ is taken as equivalent to [NaOH]. A specific example is the determination of $k/[OH^-]$ in the hydrolysis of acetylsalicylates (50). When the molar concentration of strong alkali approaches that of the substrate, bimolecular rate expressions must be used to calculate k_6 (9, 43, 104, 123). It is a similar situation for amides when hydrogen ions are taken up by the liberated amines the concentration of strong acid must greatly exceed the substrate concentration for pseudo first-order techniques to be used in the calculation of the apparent bimolecular rate constant k_1 (70).

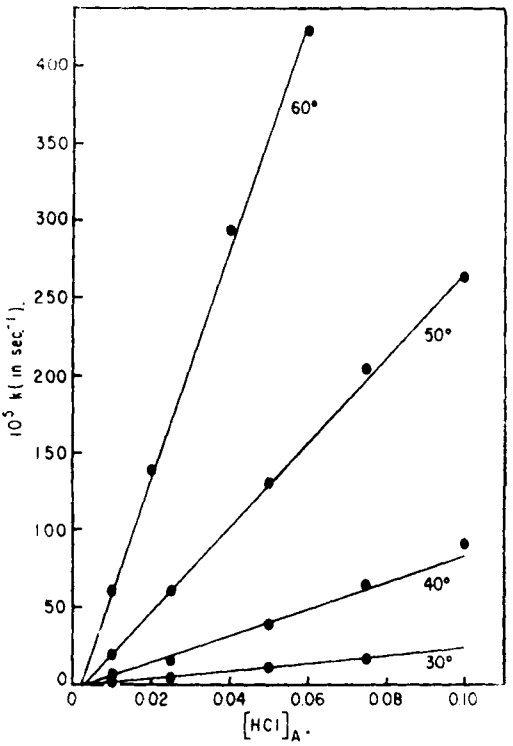


Fig. 2.—Rate constants, k in sec.$^{-1}$, for the apparent first-order degradation of psicofuranine as a function of apparent hydrochloric acid molarity. [Figure 3 of Garrett, E. R., *J. Am. Chem. Soc.*, **82**, 827(1960)(56).]

An alternative method is frequently used to calculate k_1 and k_6 where these values are defined in terms of the activities of hydrogen ions and hydroxyl ions, respectively.

The pH can be experimentally determined and, when k_6 $[OH] \sim 0$, the logarithmic transformation of Eq. 8 yields

$$\log k = \log k_1' - \text{pH} \quad \text{(Eq. 9)}$$

so that the slope of the logarithm of the apparent first-order rate constant against the experimentally determined pH is negative and equal to unity, and the antilog of the intercept yields the bimolecular rate constant k_1'. This k_1' value is related to the specific bimolecular rate constant k_1 by

$$k_1' = f_{H^+} k_1 \quad \text{(Eq. 10)}$$

where f_{H^+} is the activity coefficient (2, 89, 149) for the hydrogen ion and

$$k = k_1' \ 10^{-pH} \text{ when } k_6[OH^-] \sim 0 \quad \text{(Eq. 11)}$$

where the pH is the experimentally determined ($-\log a_{H^+}$), where a_{H^+} is the hydrogen ion activity.

A similar development for k_6' yields

$$\log k = \log k_6' - pOH = \log k_6 - pKw + pH \quad \text{(Eq. 12)}$$

The pK_w for a given temperature and solvent can be obtained from the literature (89, 149) so that k_6 can be calculated from the intercept of the plot of the logarithm of the apparent first-order rate constant against the experimentally determined pH. Thus, Eq. 8 is redefined in terms of pH as

$$k = k_1' \ 10^{-pH} + k_6' \ 10^{-(pKw - pH)} \quad \text{(Eq. 13)}$$

where the k_1' and k_6' values of Eq. 12 will frequently differ slightly from the k_1 and k_6 of Eq. 8 and yet be the desired values for prediction of solvolysis rates in intermediate pH ranges where pH is experimentally determined.

Comparisons of k_1 determined from stoichiometric catalytic concentrations and from pH measurements (*i.e.*, k_1') have been made in the cases of the solvolysis of the antibiotic streptovaricin (53) and the solvolysis of the antibiotic psicofuranine (56). An interesting comparison of measured pH and the pH calculated from kinetic rate constants is given for the solvolysis of acetylsalicylates (50).

It is frequently difficult to determine pH experimentally at high concentrations of hydrogen or hydroxyl ions so as to deduce the bimolecular rate constants k_1 and k_6 as from plots of Eqs. 9 and 12. However, stoichiometric acid concentrations can be converted to pH values corresponding to hydrogen ion and hydroxyl ion activities from the data on electrolytes in the literature (2, 89, 149).

For example, in concentrated hydrochloric acid solutions

$$pH = -\log f_{HCl}[HCl] \quad \text{(Eq. 14)}$$

where the mean activity coefficient of hydrochloric acid, f_{HCl}, is given at various temperatures, solvent mixtures, and HCl concentrations (89, 149). This definition of pH has been used in the study of the acid catalyzed solvolysis of hydrocortisone hemiester in alcohol-water mixtures (59) and of N-acetyl-*p*-aminophenol (114).

In the study of the acid catalyzed solvolysis of the antibiotic streptozotocin (57), the pH calculated from the activity coefficients (89) and the hydrochloric acid concentrations can be compared to the observed pH values of the solutions. Reasonable agreement is obtained above a pH of 1.2 and within 0.15 pH units below that value.

Similarly

$$pH = pKw - pOH = pKw + \log f_{NaOH}[NaOH] \quad \text{(Eq. 15)}$$

where f_{NaOH}, the mean activity coefficient of sodium hydroxide, and pKw are given in the literature for many solvent mixtures, temperatures, and solutes (89, 149). This definition of pOH has been used in the study of the alkaline hydrolysis of the antibiotics psicofuranine (56) and actinospectacin (69), where the k_6 values determined from the calculated hydroxyl ion activities and the stoichiometric [NaOH] have been compared.

Frequently, data exist in the literature to permit prediction of pH of buffer solutions under the conditions of preparation. An example of this procedure is in the study of barbital degradation in an ammonia buffer system (80) where the dissociation constants for the ammonia buffer system (8) and barbital (128) are given in the literature as a function of temperature. By the use of available activity coefficients (89, 115) and discreet approximations (7), the pH at various temperatures was calculated from the equation for the thermodynamic equilibrium constant for the buffer used. It is interesting to compare this approach (80) in the determination of the pH for the dependency of barbital kinetics to that of phenobarbital kinetics where the pH was determined experimentally (88).

Another method of characterizing hydrogen or hydroxyl ion concentrations is by the spectrophotometric determination of the degree of dissociation of a weakly acidic or basic indicator of known pKa where the spectrophotometric absorptivities of the charged and uncharged forms differ (73). The $[OH^-]$ calculated thusly has been used to determine the kinetic dependencies of the solvolysis of homatropine and atropine methyl-bromides (151).

Specific methods of calculating hydrogen and hydroxyl ion concentrations or activities for the prediction of the apparent first-order rate constants for the degradation of various pharmaceuticals are indicated in Table I. Methyl *dl*-α-phenyl-2-piperidylacetate (170) has exhibited specific acid-base catalyzed solvolysis where the log k *vs.* pH profile is given in Fig. 3 and the pH of minimum degradation is *ca.* 3. Specific acid-base catalyzed solvolysis has also been exhibited by the alkyl *dl*-α-(2-piperidyl)-phenyl-

acetates with pH *ca.* 3 of maximum stability (155) and N-acetyl-*p*-aminophenol with a pH 5–6 of maximum stability. The minimum of the catenary as exemplified in Fig. 3 and the pH of minimum overall rate at the isocatalytic point can be calculated from the specific acid-base catalytic constants as demonstrated in the literature (35, 113, 170). Many other examples of such catenaries are given in books on kinetics and mechanisms of reactions (2, 9, 43, 104, 123).

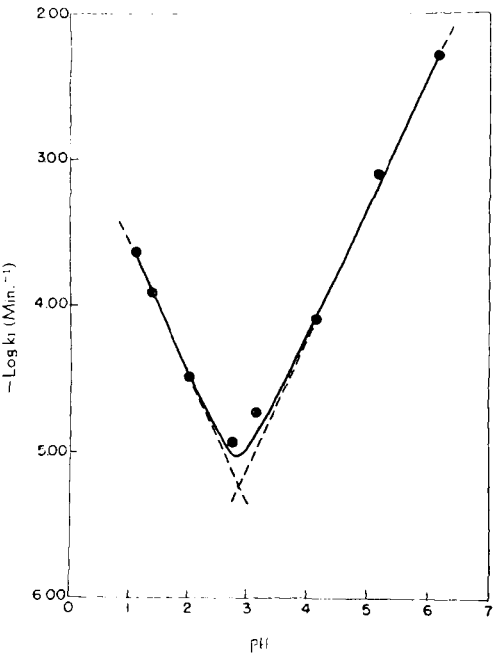


Fig. 3.—pH Dependency of the hydrolysis of methyl DL-α-phenyl-2-piperidylacetate at 80° [Figure 5 of Siegel, S., Lachman, L., and Malspeis, L., THIS JOURNAL, **49**, 431(1959) (170).]

Catalysis by Solvent.—If the apparent first-order rate constants calculated from Eqs. 8 or 13 do not agree with the observed values for intermediate pH ranges, then catalysis by solvent or solutes, intramolecular catalysis, or ionic strength must be considered as contributing to the overall rate.

The simplest modification of Eq. 8 is

$$k = k_1[H^+] + k_6[OH^-] + k_2 \quad \text{(Eq. 16)}$$

When the k plotted against $[H^+]$ has an intercept which cannot be attributed to hydroxyl ion attack, a solvent catalytic effect k_2 is implicated. This is further confirmed when k plotted against $[OH^-]$ has the same intercept or when

$$k = k_2, k_1[H^+] \sim 0 \sim k_6[OH^-] \quad \text{(Eq. 17)}$$

Such solvent attack on a degradable species is frequently apparent from the log k-pH profiles as with thiamine (193), pyridine-2-aldoxime methiodide (35), methyl pyrrolidylacetylsalicylate hydrochloride (51), and streptovaricin (53), which is given in Fig. 4.

General Acid-Base Catalysis.—Not only may hydrogen and hydroxyl ions and solvent alone catalyze the decomposition of drugs in solution, but charged species contributed from excipients or buffers may do so also. A general rule is that if solvent does catalyze, other catalytic species exist which can act as acids or bases with catalytic activity, and

TABLE I.—PREDICTIVE EQUATIONS FOR KINETICS OF SOLVOLYSIS OF DRUGS

Compound	Dependency of Apparent First-Order Rate Constant, k, in pH Region Studied[a]	Remarks	References
	Antibiotics		
Psicofuranine	$k_1 10^{-pH} f_{SH^+} + (k_4 10^{-pH} + k_6 10^{-pOH}) f_S + k_9 10^{-pOH'} f_{S^-}$	*b, d, g, h, i, j, k, l, q, s*	(56, 66)
Actinospectacin	$k_3 10^{-(pKw - pH)} f_{HS} + k_6 10^{-pOH} f_{S^-} + \Sigma_i k_i A_i^-$	*b, d, i, j, k*	(69)
Streptozotocin	$k_1 10^{-pH'} f_{SH^+} + (k_4 10^{-pH} + k_5 + k_6 10^{-(pKw - pH)}) f_S$	*b, d, g, h, i, l, m, s*	(57)
Streptovaricin	$k_1[H^+]^{(or\ k_1 10^{-pH})} + k_2$	*c, e, g, h, i, l, m, n*	(53)
Chloramphenicol	$k_2 + k_3 10^{-(pKw - pH)}$ (chloride solvolysis)	*b, d, i, s*	(91)
	$k_1[H^+]' + k_2 + k_3 10^{-(pKw - pH)} + \Sigma_i k_i \times HA_i + \Sigma_i B_i$ (amide solvolysis)	*b, d, g, h, i*	(97, 98)
	$k_1[H^+]' + k_2$ where $k_1 = k_1' 10^{m/D}[H_2O]$	*b, e, g* *t*	(132)
	Salicylates		
Acetylsalicylic acid	$k_1 10^{-pH} f_{HS} + (k_4 10^{-pH} + k_5 + k_6[OH^-]) f_{S^-}$ where $k_5 = k_5' + k_5''[C_2H_5OH]/[HOH]$	*d, e, f, g, h, i, j, k, l, m, q, t*	(34, 50, 58)
β-Cyclopentylpropionylsalicylic acid			
Trimethylacetylsalicylic acid		*t*	(50, 58)
Diethylacetylsalicylic acid			

TABLE I.—(*continued*)

Compound	Dependency of Apparent First-Order Rate Constant k, in pH Region Studied[a]	Remarks	References
Methyl pyrrolidylacetylsalicylate hydrochloride	$(k_2 + k_A^-[A^-] + k_3 10^{-(pKw - pH)}) f_{SH^+}$	*c, d, g, h*	(51)
	where $k_A^- = k_A' - 10^{(Z_A Z_B A \sqrt{\mu} + C\mu)}$	*u, v, x*	
	where $k_2 = k_2' 10^{Z_A Z_B A \sqrt{\mu}}$	*u, v*	
Diethylaminoethyl acetylsalicylate hydrochloride	$(k_1 10^{-pH} + k_2 + k_3 10^{-(pKw - pH)} + k_A^-[A^-] + k_{HA}[HA]) f_{SH^+}$ (overall rate)	*c, d, g, h*	
	where $k_A^- = k'_A{}^- \times 10^{(Z_A Z_B A \sqrt{\mu} + C\mu)}$	*u, v, x*	(52)
	$(k_1 10^{-pH} + k_2 + k_A^-[A^-] + k_{HA}[HA]) f_{SH^+}$ (to diethylaminoethyl salicylate hydrochloride)	*c, d, g, h*	
	$(k_3 10^{-(pKw - pH)} + k_A^- \times [A^-]) f^s{}_{H^+}$ (to acetylsalicylic acid)	*c, g, h*	
Diethylaminoethyl salicylate hydrochloride	$k_3 10^{-(pKw - pH)}$	*c, d, g, h, l, m, s*	(52)
Alkyl salicylates	$k_3[OH^-] f_{HS} + k_6[OH^-] f_S^-$	*b, d, e, f, k, q, s, t*	(154)
	where $k_3 = k_3' {}^{Z_A Z_B A \sqrt{\mu}}$	*u, v*	
Alkyl *m*- and *p*-hydroxybenzoates	$k_6[OH^-]$	*b, d, e, f, k, t*	(154)
	where $k_6 = k_6' 10^{Z_A Z_B A \sqrt{\mu}}$	*u, w*	
Acetylsalicylic acid anhydride	$k_2 + k_3 10^{-(pKw - pH)} + k_A^-[A^-] - k_{HA}[HA]$	*b, d, f, g, k, j, m, s*	(55)
	where $k_2 = k_2' 10^{(mID + b)}$	*t*	
	Steroids		
21-Hydrocortisone esters of hemisuccinate, hemiadipate, hemipimelate, hemisuberate, acetate, acrylate, hemi-α,α'-diethylglutarate, hemi-β,β'-dimethylglutarate, hemi-α,α'-diethylsuccinate	$k_6 10^{-(pKw - pH)}$	*c, e,*	(59)
Prednisolone hemi-β,β'-dimethylglutarate, 6α-methylprednisolone hemi-β,β'-dimethylglutarate	$k_6 10^{-(pKw - pH)}$	*b, d,*	(61, 68)
21-Hydrocortisone hemisuccinate	$k_1 10^{-pH'}$	*c, e, g*	(59)
	$k_6 10^{-(pKw - pH)}$	*c, e, j*	
	$k_1 10^{-pH} f_{HS} + (k_5 + k_6 10^{-(pKw - pH)}) f_S^-$	*c, e, h, i, j, l*	
Prednisolone	$k_3[OH^-]$	*c, d, k, p*	(84)
21-Hydrocortisone phosphate	$k_4 10^{-npH}$	*b, d, i*	(129)
	Alkaloids		
Atropine	$(k_1[H^+]' + k_3 10^{-(pKw - pH)}) \times f_{SH^+} + k_6 10^{-(pKw - pH)} f_S$	*b, d, g, i, j, k*	(113, 198)
Homatropine	$k_3 10^{-(pKw - pH)} f_{SH^+} + k_6 10^{-(pKw - pH)} f_S$	*b, d, i, j, k*	(150)
Homatropine methyl bromide, Atropine methyl bromide, Scopolamine, Acetylscopolamine, Aposcopolamine, Acetyltropate, Trimethylacetylscopolamine, Scopolamine methyl bromide, Trimethylacetyl scopolamine methyl bromide	$k_6[OH^-]'$	*b, d, i, k*	(151)
Acetylscopolamine methyl bromide, Aposcopolamine methyl bromide, Noratropine, Tropine phenylacetate, Tropine phenoxyacetate, Tropine *p*-nitrobenzoate	$k_6[OH^-]$	*b, d, k*	(49,67)
	$k_3 10^{-(pKw - pH)} f_{SH^+} + k_6 10^{-(pKw - pH)} f_S$	*b, d, i, j, k*	

TABLE I.—(*continued*)

Compound	Dependency of Apparent First-Order Rate Constant k, in pH Region Studied[a]	Remarks	References
Atropine ethylbromide Atropine benzyl chloride	$k_6[OH^-]'$	*b, d, j, k*	(152)
	Others		
Procaine	$(k_1[H^+]' + k_3 10^{-(pKw - pH)}) \times f_{SH^+} + k_6 10^{-(pKw - pH)} f_S$	*b, d, g, h, i, j, l, m, p, r*	(95, 130, 178)
Benzocaine	$k_1[H^+]'$	*b, d, g*	(130)
Procainamide	$k_1[H^+]'$	*b, d, g, p,*	(131)
Epinephrine	$k_1 10^{-npH}$ (racemization)	*b, d, g, p, s*	(165)
N-Acetyl-*p*-aminophenol	$k_1 10^{-pH'} + k_6 10^{-(pKw - pH)}$	*b, d, g, h, i, j, m, s*	(114)
2-(1-Naphthylmethyl)-2-imidazoline	$k_3 10^{-(pKw - pH)} f_{SH^+} + k_6 10^{-(pKw - pH)} f_S$	*b, d, i, j, k, q*	(171)
Alkyl *dl*-α-(2-piperidyl)-phenylacetates	$(k_1 10^{-pH} + k_3 10^{-(pKw - pH)}) \times f_{SH^+}$	*b, d, g, h, i, o*	(155, 170)
	where $k_1 = k_1' 10^{Z_A Z_B A \sqrt{\mu}}$	*g, u, w*	
	where $k_3 = k_3' 10^{Z_A Z_B A \sqrt{\mu}}$	*i, u, v*	
Pyridine 2-aldoxime methiodide	$(k_1 10^{-pH} + k_3 10^{-(pKw - pH)}) \times f_{SH^+}$	*b, d, g, h, i, j, k*	(35)
Chlorobutanol	$k_2 + k_3 10^{-(pKw - pH)}$	*b, d, g, h, i, l, m, r, s*	(140)
Barbital Phenobarbital	$(k_3 10^{-(pKw - pH)} + \Sigma_i k_i B_i) \times f_{HS} + (\Sigma_i k_i B_i + k_6 10^{-(pKw - pH)}) f_{S^-}$	*b, d, g, i, j, q*	(80, 88, 181)
p-Chlorobenzaldoxime	$k_1[H^+] - k_{-1}$ [Products]	*c, e, g*	(60)
N-Butylformamide	$k_1[H^+]$	*b, d, g*	(70)
Thiamine	$(k_1 10^{-pH''} + k_2 + k_3 10^{-(pKw - pH)} + \Sigma_i k_i A_i) f_{SH^{++}} + k_8 f_S$	*b, d, g, h, i, j, k, p, q, s*	(193)
	where $k_2 = k_2' 10^{Z_A Z_B A \sqrt{\mu}}$	*u, v*	
	where $k_3 = k_3' 10^{Z_A Z_B A \sqrt{\mu}}$	*u, v*	
	where $k_1 = k_1$, $k_8 = k_8$	*m*	
	where $k_8 = k_8$	*r*	

[a] SH^+ represents a protonated substrate in equilibrium with an uncharged substrate, S, with a dissociation constant K_a. The uncharged substrate, S, may lose a proton and may equilibrate with a negatively charged substrate, S^-. The symbol HS represents an acidic, uncharged substrate of a dissociation constant Ka, which may equilibrate with an anionic substrate, S^-. The symbol pH is for the experimentally determined value, $pH' = -\log f[HCl]$ and $pOH = -\log f[MOH]$ where f is the mean activity coefficient (2). The pH'' are values determined or calculated from the literature for the buffers used. Other symbols are $[H^+] = [HCl]$, $[H^+]' = [HClO_4]$, $[OH^-] = [NaOH]$. The $[OH^-]'$ is the hydroxyl ion concentration determined by an indicator method. The hydrogen or hydroxyl ion activity is frequently expressed in the form $a_{H^+} = 10^{-pH}$ or $a_{OH} = 10^{-pOH} = 10^{-(pKw - pH)}$. The general form of the kinetic expressions given is $(k_1[H^+] + k_2 + k_3[OH^-])f_{HS\ (or\ SH^+)} + (k_4[H^+] + k_5 + k_6[OH^-])f_{S^-\ (or\ S)} + (k_8 + k_9)[OH^-]f_{S^=\ (or\ S^-)}$ where $f_{HS\ (or\ SH^+)} = 1/(1 + Ka/[H^+])$ is the fraction of substrate as the acid form (uncharged or positively charged), where $f_{S^=\ (or\ S)} = 1/(1 + [H^+]/Ka_1)$ is the fraction of substrate as the base form (negatively charged or uncharged) and if another proton is removed, then $f_{S^-\ (or\ S^-)} = 1/(1 + Ka_2/[H^+])$ is the fraction of substrate further dissociated as a doubly or singly charged anion. The $[H^+]$ in these definitions of fractions of substrate is usually defined in terms of the measured hydrogen ion activity, *i.e.*, 10^{-pH}. The A_i^- and B_i represent charged and uncharged general bases respectively, whereas the HA_i and H^+B_i represent uncharged and charged general acids, respectively, so that $\Sigma_i k_i A_i^-$, $\Sigma_i k_i B_i$, $\Sigma_i k_i Ha_i$, and $\Sigma_i k_i H^+ B_i$ represent the contribution of solutes to general acid-base catalyzed solvolysis of the substrate. [b] Values at all operating temperatures can be calculated from $k_i = Ae^{-\Delta Ha/RT}$ or $\log k_i = \log A - \Delta Ha/2.303\ RT$ where A and ΔH_a can be derived from values given in the references. [c] Studied at one or two temperatures only. [d] Aqueous solutions. [e] Aqueous alcohols (methanol, ethanol, propylene glycol). [f] Aqueous dioxane or aqueous acetone. [g] Strong acid region. [h] Acetate buffer region. [i] Phosphate buffer region. [j] Mildly alkaline region. [k] Strong alkali region. [l] General acid-base catalysis checked and not observed with acetic acid-acetate buffers. [m] Ionic strength effects checked and not observed in region studied. [n] Oxidative degradation checked and not observed in region studied. [o] General acid-base catalysis checked and not observed with phthalic acid-phthalate buffers. [p] Oxidatively degraded when oxygen present. [q] Dissociation constants, Ka, at all operating temperatures can be calculated from Arrhenius expressions or other data. [r] General acid-base catalysis checked and not observed. [s] Checked at different substrate concentrations for first order with respect to substrate. [t] Rates studied as functions of dielectric constant where D is dielectric constant and m and b are constants. [u] $Z_A$and Z_B are the charges on the reacting ions and $\mu = \frac{1}{2}\Sigma c_i Z_i^2$ is the ionic strength. [v] $Z_A Z_B A$ is negative. [w] $Z_A Z_B A$ is positive. [x] The constant C is positive.

conversely. An example of this is the assignment of intramolecular catalysis to the pH-independent hydrolysis of the aspirin anion (50) rather than general acid-base catalysis by water since variations in acetic acid-acetate buffer concentrations had negligible effect on the hydrolysis (34).

Thus, Eq. 8 which has been extended to become Eq. (16) can be further modified

$$k = k_1[H^+] + k_6[OH^-] + k_2 + \Sigma_i k_i[HA_i] + \Sigma_i k_i[A_i^-] + \Sigma_i k_i[B_i] + \Sigma_i k_i[B_i H^+] \quad \text{(Eq. 18)}$$

where B is a general base and BH^+ its conjugate acid, where HA is a general acid and A^- is its conjugate base according to the treatment of Frost and Pearson (43).

An appropriate method to evaluate general acid-base effects is to determine the apparent first-order rate constant in the buffered vehicle and subtract those rate contributions which can be studied separately, such as k_1 $[H^+]$, k_6 $[OH^-]$, and k_2. The excess in degradation rate can be assigned to general acid-base catalysis provided

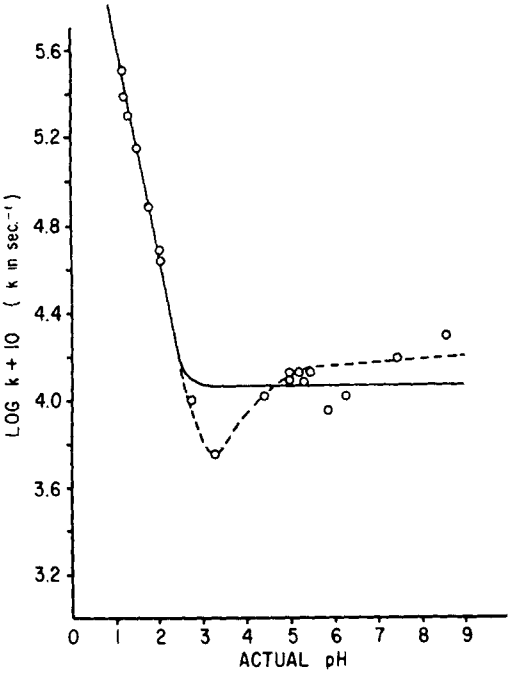


Fig. 4.—The pH profile of the degradation rates of streptovaricin C based on the 432 mμ absorbance disappearance in 25% ethanol at 30.3°. [Figure 3 of Garrett, E. R., THIS JOURNAL, **48**, 169(1959) (53).]

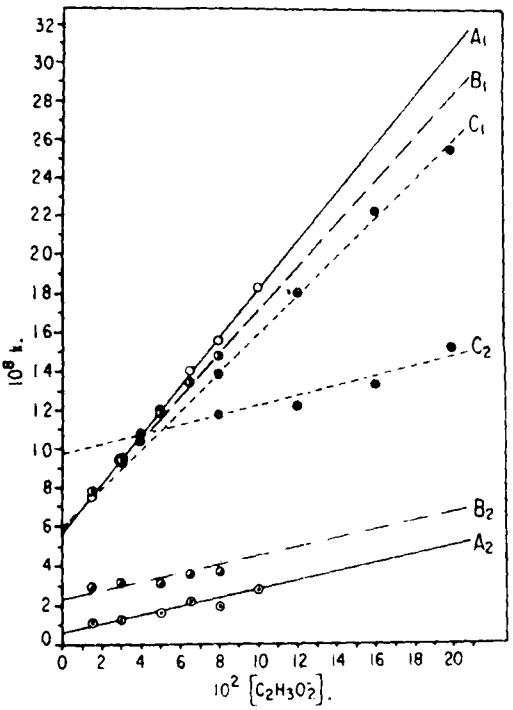
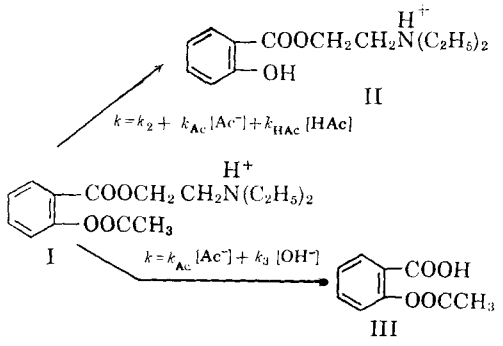


Fig. 5.—Effect of varying acetate ion concentration at constant ionic strength, μ, at several pH values on the simultaneous first-order rate constants of the hydrolyses to diethylaminoethyl salicylate hydrochloride, II, (curves A_1, B_1, C_1) and to aspirin, III, (curves A_2, B_2, C_2) of 10×10^{-4} *M* diethylaminoethyl acetylsalicylate hydrochloride, I, at 30.3°. ○, A, pH 4.00, 0.100 μ., ◑, B, pH 4.62, 0.100 μ., ●, C, pH 5.21, 0.200. [Figure 8 of Garrett, E. R., *J. Am. Chem. Soc.*, **80**, 4049 (1958) (52).]

that ionic strength effects have been controlled by varying buffer concentrations at constant pH and constant ionic strength. The use of a neutral salt such as sodium or potassium chloride is recommended.

A graphic example of a complex general acid-base catalyzed hydrolysis is given in Fig. 5 for the hydrolysis of diethylaminoethyl acetylsalicylate hydrochloride, I (52).



The apparent first-order rate constant, k, is plotted against the acetate ion concentration, $[Ac^-]$, at constant pH and constant ionic strength, μ. The curves A_1, B_1, and C_1, each at a different pH value, have the same intercept, k_2, and thus determine the solvent effect on the hydrolysis of I to diethylaminoethyl salicylic acid hydrochloride, II. The varying slopes, k_{Ac}', of lines A_1, B_1, and C_1 represent the rate constants for acetate ion and acetic acid catalyzed solvolysis of I to II. This k_{Ac}' can be dissected into the component contributions of base (k_{Ac}) and acid (k_{HAc}) catalytic rate constants since

$$k_{Ac}'[Ac^-] = \{k_{Ac} + k_{HAc}/K_a'[H^+]\}[Ac^-] \quad \text{(Eq. 19a)}$$

$$= k_{Ac}[Ac^-] + k_{HAc}[H^+][Ac^-]/Ka' \quad \text{(Eq. 19b)}$$

$$= k_{Ac}[Ac^-] + k_{HAc}[HAc] \quad \text{(Eq. 19c)}$$

where Ka′ is the dissociation constant of acetic acid.

Thus, from the slopes, k_{Ac}', of lines A_1 and A_2 in Fig. 5, the known $[H^+]$ of each curve and the relation

$$k_{Ac}' = k_{Ac} + (k_{HAc}/Ka')[H^+] \quad \text{(Eq. 20)}$$

the acid-base catalytic constants k_{Ac} and k_{HAc} were calculated (52).

The curves A_2, B_2, and C_2 for the hydrolysis of I to aspirin, III, have the same slope within experimental error, and thus only general base

catalysis is concluded. The intercepts of these lines vary and are consistent with hydroxyl ion catalysis.

Since most pH studies of pharmaceutical degradation rates are conducted in acetic acid-acetate, phosphate, borate, or similar buffers, the potential catalytic action of these species must be evaluated. Such effects have also been observed in the hydrolysis of phenylacetates (13, 23, 162) and in the solvolysis of aspirin anhydride (55). In the latter case, undissociated acetic acid had an inhibitory effect. General base catalysis has been shown for thiamine at pH values less than 7 (193), for the antibiotic streptozotocin by the monohydrogen phosphate anion (57), for methyl pyrrolidylacetylsalicylate hydrochloride by acetate ions (51), for barbital and phenobarbital by ammonia (80, 88, 181), for the antibiotic actinospectacin (69) by phosphate, carbonate, and borate buffers. General acid-base catalysis of chloramphenicol (97, 98) has been determined for monohydrogen phosphate ion, undissociated acetic acid, and both monohydrogen and the dihydrogen citrate ions. For the general case where general acid-base catalysis has been found and may be expected, the reader is referred to texts (9, 43, 123). General acid-base effects were tested for but not observed with acetate buffers for psicofuranine (56), for aspirin (34), the antibiotics streptovaricin (53) and streptozotocin (57), diethylaminoethyl salicylate hydrochloride (52), procaine (95, 130), and chlorobutanol (140). They were not observed with phosphate buffers for chlorobutanol (140), for phthalate buffers for alkyl *dl*-α-(2-piperidyl)-phenylacetates (155, 170), and in the high pH region for thiamine (193).

In actuality many of the mechanisms which follow the basic equations for general base catalysis actually go through a nucleophilic participation in the rate-determining step. Examples are given in Bender's fine review (12). Nucleophilic catalysis is involved in the solvolysis of anhydrides (32, 74–77, 79, 108–110) and also aspirin anhydride (55), in the imidazole (13), N-methylimidazole (24), and 4-methylpyridine (108) catalysis of *p*-nitrophenylacetate hydrolysis (24), and in the acetate ion catalyzed solvolysis of methyl pyrrolidylacetylsalicylate hydrochloride (51).

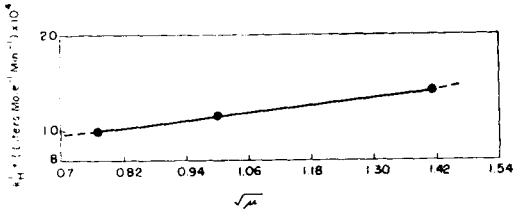


Fig. 6.—Influence of ionic strength on the velocity of a hydronium ion catalyzed solvolysis of a protonated substrate. [Figure 8 of Siegel, S., Lachman, L., and Malspeis, L., THIS JOURNAL, 48, 431(1959)(170).]

Ionic Strength and Dielectric Constant Effects.—The general expression that relates an observed bimolecular rate constant, k_i, with ionic strength, dielectric constant, and other thermodynamic properties of the solution of reactants for ion-ion reactions (1, 2) is

$$\log k_i = \log k_i' + z_A z_B A \sqrt{\mu}/(1 + \beta\sqrt{\mu}) \quad \text{(Eq. 21)}$$

so that

$$k_i = k_i' \, 10^{z_A z_B A \sqrt{\mu}/(1 + \beta\sqrt{\mu})} \quad \text{(Eq. 22)}$$

which to a first approximation may be given as

$$k_i = k_i' \, 10^{(z_A z_B A \sqrt{\mu} + C\mu)} \quad \text{(Eq. 23)}$$

or as

$$\log k_i = \log k_i' + z_A z_B A \sqrt{\mu} \quad \text{(Eq. 24)}$$

so that

$$k_i = k_i' \, 10^{z_A z_B A \sqrt{\mu}} \quad \text{(Eq. 25)}$$

where $\mu = {}^1/_2 \Sigma C_i z_i^2$, the ionic strength, z_A and z_B are the charges on the reacting species, and A and β are functions of the properties of the solution, *e.g.*, dielectric constants, temperature, etc., and thermodynamic constants and can be obtained from the literature (2, 89, 149).

Thus the dependence of log k_i *vs.* $\sqrt{\mu}$ should be a straight line of positive slope for the reactions of ions of like charge sign and negative slope for ions of unlike charge sign. An example of the application of Eq. 24 is given in Fig. 6 for the reaction of the positively charged hydronium ion with the positively charged ester, methyl *dl*-α-phenyl-2-piperidylacetate (170), where the plot of the logarithm of the specific hydrogen ion catalytic rate constant against the square root of the ionic strength is positive. When the positively charged ester is reacted with the negative hydroxyl ion, the salt effect is negative, as evidenced from Fig. 7 where such a plot has a negative slope (170). Such effects have been discussed in detail with many examples by Bell (9) and Amis (2). They have been observed in the acid and base catalyzed hydrolysis of carboxymethyltriethylammonium halide (10, 11, 145).

The salt effect is negative, *i.e.*, $z_A z_B A$, for the hydroxyl ion and general base, A^-, catalyzed solvolysis of the positively charged methyl pyrrolidylacetylsalicylate hydrochloride (51) and of the positively charged thiamine (193); for the

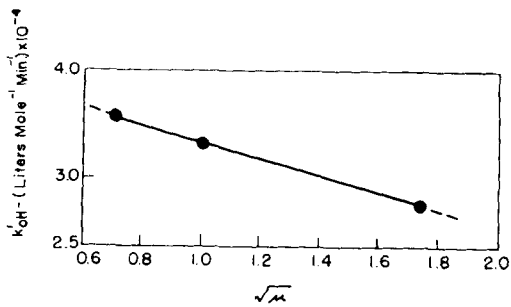


Fig. 7.—Influence of ionic strength on the velocity of a hydroxyl ion catalyzed solvolysis of a protonated substrate. [Figure 9 of Siegel, S., Lachman, L., and Malspeis, L., THIS JOURNAL, **48**, 431 (1959) (170).]

general base, A^-, catalyzed solvolysis of the protonated diethylaminoethylacetylsalicylate hydrochloride (52). The salt effect is positive, *i.e.*, $z_A z_B A$ for the hydroxyl ion catalyzed solvolysis of the negatively charged *m*- and *p*-hydroxybenzoates (154). In one study, salt effects were observed for the solvolysis of barbital (80) but were not quantified.

Salt effects were checked for but not observed in the pH regions studied for the acid and base catalyzed solvolysis of protonated procaine (95, 130), the solvolysis of N-acetyl-*p*-aminophenol (114), the acid solvolysis of chlorobutanol (140), the acid catalyzed solvolysis of doubly positively charged thiamine (193), the solvent catalyzed solvolysis of streptozotocin (57) and streptovaricin (53), the solvolysis of chloramphenicol in phosphate buffer (98), the hydroxyl ion catalyzed solvolysis of diethylaminoethyl salicylate hydrochloride (52), and the solvolysis of acetylsalicylic acid anhydride (55).

Also a plot of log k_i *vs.* the reciprocal of the dielectric constant at $\mu = 0$ should yield a straight line of negative slope for ions of like charge sign and of positive slope for ions of unlike charge sign (1, 2).

It follows that the use of miscible water-organic solvents where the dielectric constant decreases with more of the organic solvent should not increase the stability of a drug if the rate-determining step involves reactants of unlike charge. It can be predicted that the use of alcohol-water, propylene glycol-water, dimethylacetamide-water, sugar-water, or glycerol-water solutions will tend to increase the instability of protonated drugs subject to general or specific base catalysis in the presence of buffer anions if this is the major catalytic species. Examples of such predictions would be the hydrolysis of the scopolamine methylbromides (49, 67), atropine ethyl and benzyl bromides (152), the pH 3–7 solvolysis of methyl *dl*-α-phenyl-2-piperidylacetate (170), and alkyl *dl*-α-(2-piperidyl)-phenylacetates (155), where the major hydrolysis has been assigned to hydroxyl ion attack on a positively charged molecule. Certainly, in the case of the reaction of similarly charged species, such as in the hydroxyl ion catalyzed solvolysis of an anion (59, 61, 68) or the hydrogen ion catalyzed solvolysis of a cation (35, 95, 113, 130, 152, 155, 170, 198), the stability should be increased by decreasing the dielectric constant. The example of the decreased rate of fading of bromphenol blue (negatively charged quinoid dye ion) with hydroxyl ion when the alcohol content of water-ethanol and water-methanol solvents is increased (2, 3) is discussed in detail by Amis (1).

In the limiting case of the attack of an ion on a dipolar molecule (1, 2)

$$\log k' = \log k'_D + K \frac{z\mu}{D} \qquad \text{(Eq. 26)}$$

Thus, with an increase in dielectric constant, D, the rate of the reaction decreases for a positive ionic reactant and increases for a negative ion reactant. If the dielectric constant is decreased the rate should increase for positive ions and decrease for negative ions.

This equation and its indicated interdependencies has been applied with reasonable success to both the hydroxyl and hydrogen ion catalyzed hydrolysis of esters in water and organic solvent-water mixtures.

Examples are the water-ethyl alcohol and water-acetone alkaline solvolysis of ethylacetate (4, 156) and methyl propionate (157); the water-alcohol acid hydrolysis of aspirin (50), the water-acetone acid hydrolysis of methyl propionate (105), ethylacetate (141), ethyl formate (168), and ethyl dichloroacetate (142). In the case of the latter reactions of specific hydronium ion catalyzed ester hydrolysis where a preliminary equilibrium with water prior to the rate determining step may occur, the k' of Eq. 26 may be made specific with respect to water by dividing by the concentration of water. The relation of Eq. 26 is then demonstrated more validly (1, 105, 132, 141). A plot of data consistent to the requirements of this equation for the reaction between a cation and a dipolar molecule is given in Fig. 8 for chloramphenicol hydrolysis (132).

These relationships of rate with dielectric constant can also be used as methods to determine the nature of the reaction. In the hydrolysis of acylsalicylates (50, 58), the increase of rates of solvolysis of the aspirin anion with increasing ethanolic content along with the relative

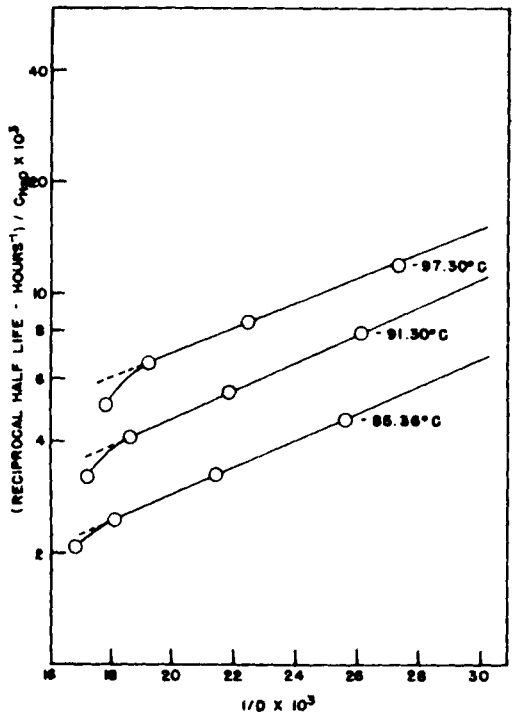


Fig. 8.—Influence of dielectric constant on reaction rates for the apparent reaction between the protonated chloramphenicol and the dipolar molecule water. [Figure 7 of Marcus, A. D., and Taraszka, A. J., THIS JOURNAL, **48**, 77(1959) (132).]

constancy of rate with increasing dioxane content, both concomitant with decreasing dielectric constant, did not implicate direct water attack on the aspirin anion. It led to kinetic evidence for intramolecular catalysis (50) and subsequently to the deduction of a possible rate-determining step in the attack of water or ethanol on an uncharged cyclic intermediate (58). Marcus (132) states that the rates for acid catalyzed solvolysis of protonated chloramphenicol in propylene glycol-water mixture does not fit the requirements for like charged ions and can be explained by the fact that the rate-determining step is an ion-dipolar molecule reaction between hydrogen ions and the hydrated amide. The log $k_1/[H_2O]$ values, when plotted against the reciprocal of the dielectric constant (84) as per Eq. 26, is linear and of positive slope. A point of Marcus (132) is well taken in that it is unwise to make any blanket assumption that lowering the dielectric constant (in general, by increasing the nonaqueous content of a solvent) will increase the stability of a drug. Predictions of stability as functions of the dielectric constant are valid on the basis of the operative mechanisms.

The limiting case for a dipolar molecule-dipolar molecule reaction is usually independent of ionic strength but dependent on dielectric constant (1, 2)

$$\log k_i = \log k_{(D=\infty)} - K(1/D) \quad \text{(Eq. 27)}$$

where a decrease in the dielectric tends to decrease the rate, and conversely.

There are many ways to relate the observed rate constant, k_i, to some function of the dielectric constant, D, for the interaction of dipolar molecules. A general expression to relate linearly the logarithm of the specific rate constant and some function of the dielectric ϕ_i (D), where m and b are constants, is

$$\log k_i = m_i\phi_i(D) + b_i \quad \text{(Eq. 28)}$$

Within small ranges of high dielectric constant, this relation is usually linear for practically any ϕ_i (D), *i.e.*, ϕ_1 (D) = nonaqueous solvent, ϕ_2 $(D) = D$, ϕ_3 $(D) = 1/D$, or ϕ_4 $(D) = (D - 1)/(2D + 1)$ (2, 72) as was shown in the case of the hydrolysis of aspirin anhydride (55). The latter expressions for ϕ_3 (*i.e.*, Eq. 27) and ϕ_4 can be extended to greater ranges of dielectric constant. Examples and further discussion can be found in the literature for the effect of solvent and dielectric constant on rates (38, 125, 153).

A plot of log k *vs.* $(D - 1)/(2D + 1)$ has been made for *t*-butyl chloride (124) in aqueous ethanol and in aqueous dioxane, acetone, ethanol, and methanol (38), for acetic anhydride (74, 78), benzoyl chloride (5, 78), benzyl (111), and substituted benzylsulfonates (112) in aqueous acetone, for ethylene bromo and iodohydrin (28) in aqueous ethanol, for *p*-nitrobenzylbromide (85) in aqueous dioxane. In most cases since m $(1/D) + b$ is the first part of the numerical expansion of $(D - 1)/(2D + 1)$, the fit of log k *vs.* $1/D$ is just as valid. Log k *vs.* log D appears to give the best possible linearity (38).

Solvolysis Rates, pH, and the Ionic Nature of the Substrate.—In many cases the hydrogen and hydroxyl ion catalyzed degradation rates of drugs in solution vary with the kind and amount of charge on the substrate molecule. The complex kinetic expressions that quantify these phenomena have been developed excellently by Higuchi (95) and Edwards (34). The approach of Edwards can be applied to the general case of the charged or uncharged acid substrate. The apparent dissociation constant, K_a, can be defined as

$$K_a = [S^-][H^+]/[HS] = [S][H^+]/[SH^+] \quad \text{(Eq. 28)}$$

In the solvolytic reactions of an uncharged acid the ionic form of the reacting substrate, the catalytic species and the specific rate constant can be given, respectively, as: HS, H^+, k_1;

HS, (H_2O), k_2; HS, OH^-, k_3; S^-, H^+, k_4; S^-, (H_2O), k_5; S^-, OH^-, k_6. With respect to k_2 and k_5 these values may or may not be dependent on the bimolecular attack of water or solvent on substrate and for this purpose are defined as first-order rate constants with respect to substrate rather than the alternatives, $k_2' = k_2/[H_2O]$, or $k_5' = k_5/[H_2O]$. Equivalent definitions and symbolisms and arguments can be constructed for the charged acid, SH^+.

It is reasonable to expect *a priori* that the specific bimolecular rate constant k_1 for the attack of a hydrogen ion on the undissociated uncharged acid, HS, would be less than the k_4 specific rate constant for such an attack on the oppositely charged anion, S^-. This does not necessarily imply that $k_1[H^+][HS] < k_4[H^+][S^-]$ so that the decomposition in acid solution is less than the decomposition in neutral solution since, to achieve a significant concentration of $[S^-]$, the concentration of $[H^+]$ must decrease below the dissociation constant, Ka. Similarly, although it is reasonable to expect *a priori* that the specific bimolecular rate constant k_3 for the attack of a hydroxyl ion on the undissociated uncharged acid, HS, would exceed the specific bimolecular rate constant for the attack of a hydroxyl ion on the anion S^-, it does not necessarily imply that decomposition in solutions of pH less than pKa exceeds that of solutions of pH greater than pKa. In the case of acetylsalicylates, the conditions defined by the dissociation constant of the acids where HS exists at very low concentrations of hydroxyl ion $[OH^-]$, indicate that $k_3[OH^-][HS]$ does not contribute significantly to the overall hydrolysis rate to products (34, 50).

The rate of decrease of the total concentration of substrate, $[HS]_T$, by solvolytic processes is equivalent to the sum of the rates of decrease of the undissociated acid [HS] and the anion $[S^-]$, which in turn can be defined by the specific rate constants

$$-d[HS]_T/dt = k[HS]_T \text{ (at constant pH)} = k([HS] + [S^-]) \quad \text{(Eq. 29)}$$

and

$$-d([HS] + [S^-])/dt = k_1[H^+][HS] + k_2[HS] + k_3[OH^-][HS] + k_4[H^+][S^-] + k_5[S^-] + k_6[OH^-][S^-] \quad \text{(Eq. 30)}$$

It has been shown (34, 95) that

$$k = (k_1[H^+] + k_2 + k_3[OH^-])/(1 + Ka/[H^+]) + (k_4[H^+] + k_5 + k_6[OH^-])/(1 + [H^+]/Ka) \quad \text{(Eq. 31)}$$

where

$$f_{HS} = 1/(1 + Ka/[H^+]) = [HS]/[HS]_T \quad \text{(Eq. 32)}$$

is the fraction of $[HS]_T$ as the undissociated acid [HS] and where

$$f_{S^-} = 1/(1 + H^+/[Ka]) = [S^-]/[HS]_T \quad \text{(Eq. 33)}$$

is the fraction of $[HS]_T$ as the dissociated acid $[S^-]$.

Analogous expressions could be derived for a singly charged (SH^+) or doubly charged substrate (SH^{++}).

If the substrate has two or more functionalities that vary the kind and amount of charge on the reacting molecule with pH, *i.e.*, pKa_1, pKa_2, etc., then a suitable variation of Eq. 31 could be developed on the same principles.

Frequently, kinetic ambiguity may exist. For example, a rate contribution may be assigned to $k_4[H^+][S^-]$ or to $k_2[HS] = k_4K_a[HS]$, as in the case of the hydrolysis of acetylsalicylate (50), and still be consistent with the kinetic data. Similarly, a rate contribution may be assigned to either $k_5[S^-]$ or $k_3[OH^-][HS] = k_5K_a[OH^-][HS]/Kw$, as in the case of the hydrolysis of hydrocortisone hemisuccinate (59).

Another alternative kinetic equivalence is $k_6'[OH^-]^2 f_{HS^{++}} = k_5 f_S$ at high pH values for the solvolysis of thiamine (193) where the mechanism of "spontaneous" decomposition was chosen on the basis of the fact that primary salt and general base catalytic effects could not be observed. Also, for thiamine solvolysis, where $k_3[OH^-]f_{HS^{++}} = k_5 f_{S^+}$, the interaction of hydroxyl ion and double protonated thiamine was chosen over the "spontaneous" decomposition of singly protonated thiamine since a negative dependence on ionic strength pointed to the reaction of a negative and positively charged ion (193). The hydroxyl ion catalyzed solvolysis of the uncharged alkylsalicylate, $k_3[OH^-]f_{HS}$, was chosen over its kinetic equivalent, the "spontaneous" hydrolysis of the ester ion, $k_5 f_{S^-}$, on the basis of the variation of rate with dielectric constant (154). Alternate choices for the solvolysis of pyridine 2-aldoxime methiodide on kinetic grounds are the specific base hydrolysis of the protonated substrate, $k_3[OH^-]f_{SH^+}$, or the spontaneous decomposition of the neutral substrate, $k_5 f_S$ (35).

Predictive equations for the kinetics of solvolysis of drugs are given in Table I in accordance with Eq. 31 for the dependency of the apparent first-order rate constant, k. Where the specific rate constants can be further defined in terms of salt effects (ionic strength) and dielectric constant, this has been done. The log k *vs.* pH profiles given in Figs. 3, 4, and 9–14 show interesting variations when the various specific bimolecular

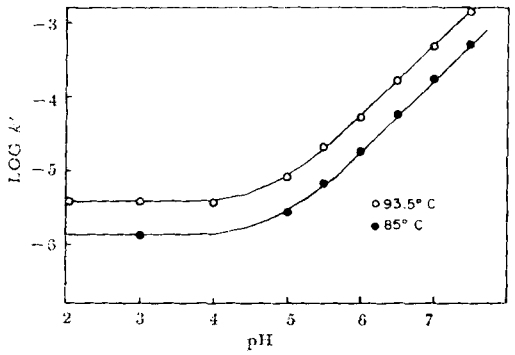


Fig. 9.—The log k-pH profile for the solvolysis of chlorbutanol. [Figure 9 of Nair, A. D., and Lach, J. L., THIS JOURNAL, **48**, 390(1959) (140).]

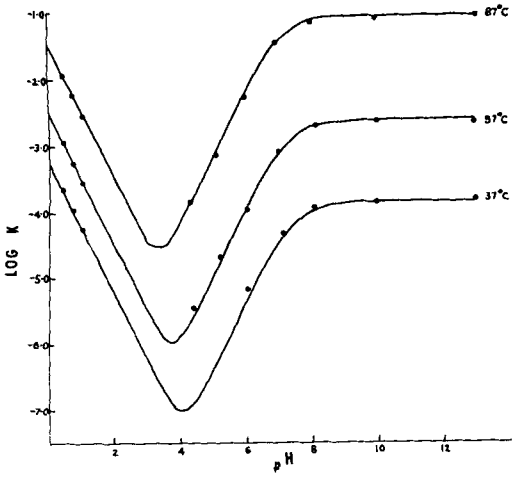


Fig. 10.—The log k-pH profile for the solvolysis of pyridine-2-aldoxime methiodide. [Figure 5 of Ellin, R. I., Carlese, J. S., and Kondritzer, A. A., THIS JOURNAL, **51**, 141(1962) (35).]

rate constants, k_i, $i = 1, 2 \ldots 6$ are observed as having significant contributions to the overall rate. Figure 3 for the protonated methyl *dl*-α-phenyl-2-piperidylacetate (170) exhibits the classic catenary when only specific acid-base catalysis is observed, as does the alkyl *dl*-α-(2-piperidyl)-phenylacetates (155) which exhibits a maximum stability at pH *ca.* 3. A similar profile for N-acetyl-*p*-aminophenol (114) has minimum at pH *ca.* 5–6. The profile of streptovaricin (53) Fig. 4, has a probable minimum plateau at pH values 3–4 due to a probable pH-independent solvolysis when the hydrogen ion concentration is lessened. Chlorobutanol (140), Fig. 9, exhibits no acid catalyzed solvolysis so that its maximum stability is a plateau at pH values less than 5. Pyridine 2-aldoxime methiodide (35), Fig. 10, exhibits minimal instability at pH *ca.* 3–4 and an interesting pH-independent plateau in the alkaline region due to a high sta-

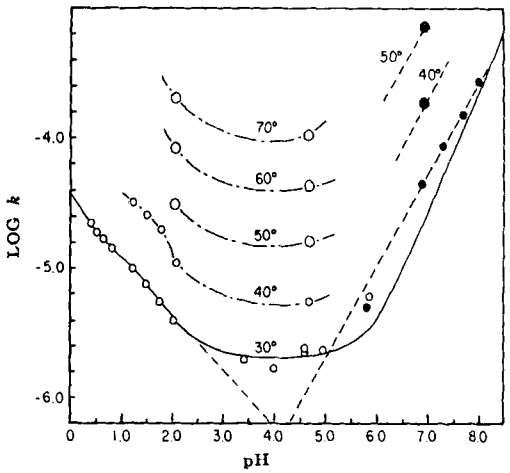


Fig. 11.—The log k-pH profile for the solvolysis of the antibiotic streptozotocin at 30°. [Figure 8 of Garrett E. R., THIS JOURNAL, **49**, 767(1960) (57).]

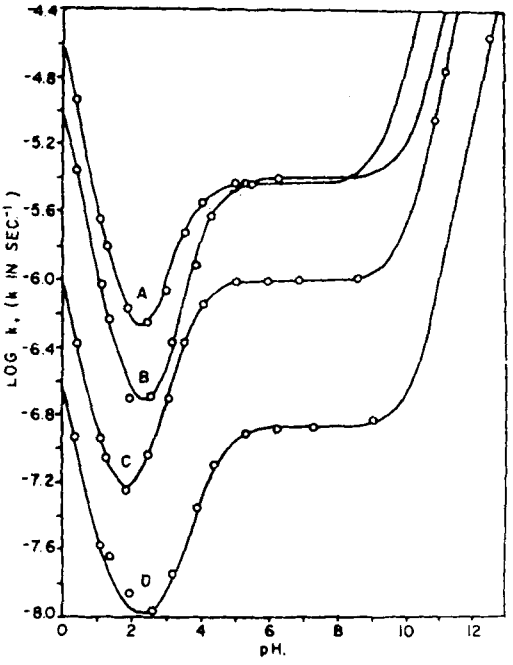


Fig. 12.—The log k-pH profile for the solvolyses of acylsalicylic acids, A, acetyl-; B,β-cyclopentyl-propionyl-; C, trimethylacetyl-; D, diethylacetyl-, at 25°. [Figure 1 of Garrett, E. R., *J. Am. Chem. Soc.*, **79**, 3401(1957) (50).]

bility of the oximate to hydroxyl ion attack. Streptozotocin (57) exhibits two deviations from the expected catenary in its log k-pH profile, Fig. 11. In the alkaline branch, rates in phosphate buffer exceed the specific base catalysis expected due to a general base catalysis by phosphate anions. On the other side of the arte minimum at pH *ca.* 4 in the acid branch, the log k *vs.* pH plots deviates from a slope of unity. Such kinetic data permit the calculation of a dis-

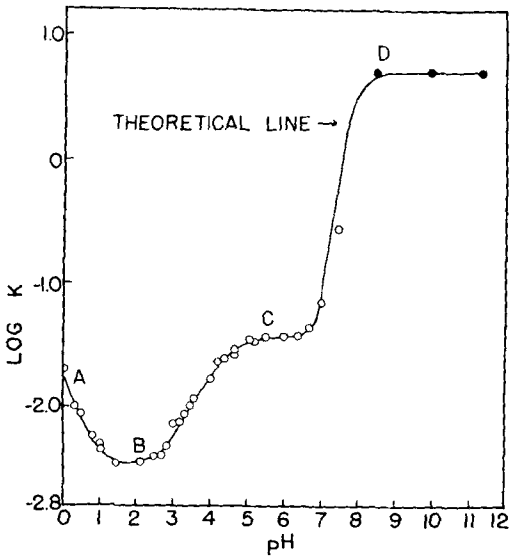


Fig. 13.—The log k-pH profile for thiamine at 96.4°. [Figure 11 of Windheuser, J. J., and Higuchi, T., This Journal, **51**, 354(1962) (193).]

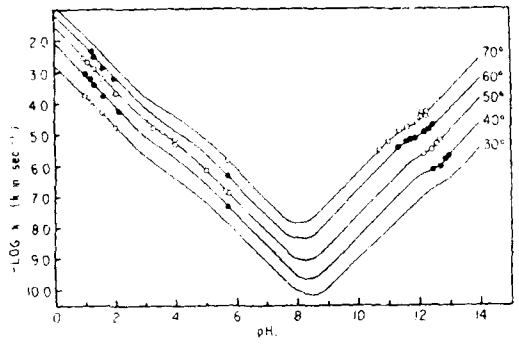


Fig. 14.—The log k-pH profile for psicofuranine. [Figure 4 of Garrett, E. R., *J. Am. Chem. Soc.*, **82**, 827(1960) (56).]

sociation constant difficultly obtainable by potentiometry, and the method described in the original paper (57) may be of interest. The hydrolyses of acetylsalicylic acids (34, 50) have an interesting log k-pH profile, Fig. 12, due to a pH-independent rate of anion solvolysis before hydroxyl ion catalyzed hydrolysis of the anion becomes significant. The pH of maximal stability of these compounds is *ca.* 3. Thiamine (193) also has an interesting profile, Fig. 13, and exhibits the extraordinary phenomenon of a large rate elevation within a short pH range that maintains constancy with large increases in pH. This phenomenon can be assigned to the change in the ionic nature of the substrate with pH where the new solution species of thiamine has a high but constant rate of solvolysis. Psicofuranine's (56) log k-pH profile, Fig. 14, exhibits the classical case of a substrate that can exist in three ionic forms in which the cationic form shows hydrogen ion catalyzed solvolysis, the anionic form hydroxyl ion catalyzed solvolysis, the neutral form is subject to both catalyses, and the pH of minimum hydrolysis is *ca.* 8–8.5.

The importance of a rate-pH profile cannot be underestimated for the prediction of the pH of minimum hydrolysis, the optimum for pharmaceutical stabilities. It can readily be seen that relationships among the magnitudes of the determined k_i values determine the pH of this minimum. Other pH minimum values so obtained are *ca.* pH 1–3 for thiamine (193), *ca.* pH 3 for hydrocortisone hemisuccinate (59) [which rate-pH profile resembles acetylsalicylic acid (34, 50) except for the lack of a significant k_4], and pH < 3 for aspirin anhydride (55) which has no specific acid catalyzed solvolysis similar to chlorobutanol (140). Actinospectacin (69) also showed no pharmaceutically significant acid instability and the barbitals (80, 88, 181) were not attacked by acid.

The isocatalytic point has been discussed for other solvolyses (107), ethylaminoacetate (19), and acetylcholine (25).

Approximative Methods for Log k-pH Relations.—As has been discussed, there are many instances where the ionic strength, general acid-base catalysis, or changes in the ionic nature of the substrate due to pKa properties, known or unknown, will give apparent nonlinear relations between log k and pH. In many cases the relation can be mathematically characterized, however, by an equation of the form

$$\log k = -n\text{pH} + \log k_i \qquad \text{(Eq. 34)}$$

or

$$k = k_i\, 10^{-n\text{pH}} \qquad \text{(Eq. 35)}$$

Similarly

$$k = k_i\, 10^{n\text{pOH}}$$

where the pH or pOH range of effectiveness must be defined. Such relations have been used to characterize the solvolysis of hydrocortisone phosphate in the neutral pH range (132), the racemization of epinephrine (165), the basic solvolytic degradation of actinospectacin (69), and the solvolysis of streptozotocin in phosphate buffers (57) where general base catalysis perturbed the alkaline branch of the specific acid-base catalytic catenary.

Frequently, such perturbations can also be accounted for by defining a rate constant for a particular hydrogen ion or hydroxyl ion range as

$$k = k'[OH^-] + k''; \; a < [OH^-] < b \quad \text{(Eq. 36)}$$

or

$$k = k'[H^+] + k''; \; a' < [H^+] < b' \quad \text{(Eq. 37)}$$

Examples and comparisons of the above two sets (Eqs. 34–37) of approximations with the values given by a true mechanistic dependency are given for actinospectacin (69) and psicofuranine (56, 66).

Prediction of Solvolytic Rates at Various Temperatures.—The quantitative relation of specific reaction rates and temperature is the Arrhenius expression (2, 9, 14, 29, 43, 71, 72, 86, 103, 104, 123, 138)

$$k_i = Ae^{-\Delta H_a/RT} \quad \text{(Eq. 38)}$$

where k is the specific rate of degradation or rate constant, T is the absolute temperature (°C. + 273), R is the gas constant (1.987 calories degree^{-1}mole^{-1}), A is a constant associated with the entropy of the reaction and/or collision factors, and ΔH_a is defined as the heat of activation.

The logarithmic form of Eq. 38

$$\log k_i = -(\Delta H_a/2.303R\,(1/T) + \log A \quad \text{(Eq. 39a)}$$

$$= -S/T + P \quad \text{(Eq. 39b)}$$

shows that the slope, $-S$, and intercept, P, of the plot log k *vs.* $1/T$ and, thus, ΔH_a and A, can be evaluated (Fig. 15) from several studies at various temperatures. Thus, the specific rate constants for any temperature can be calculated from a knowledge of these constants and applied to the prediction of the overall apparent first-order rate constant as previously discussed. It is also apparent that a rate constant at any temperature can be calculated from the knowledge of the heat of activation, ΔH_a and one rate constant at a known temperature (193). The footnotes of Table I specify if A and ΔH_a are available for the various solvolytic degradations of those pharmaceutically important compounds and a list of some of them is tabulated elsewhere (170). Additional sources of such values are in the texts previously cited and may be found for many others, as well as the kinetic dependencies of rates, in two fine compilations published by the National Bureau of Standards (173).

The effect of ΔH_a of the per cent of the organic component in mixed aqueous-organic solvents in the hydrolysis of esters is given by Tommila, *et al.* (154, 182–184). Graphs of this dependency generally pass through a minimum for alkaline solvolysis.

Higuchi and Busse (92) have pointed out that knowledge of the heats of activation for the

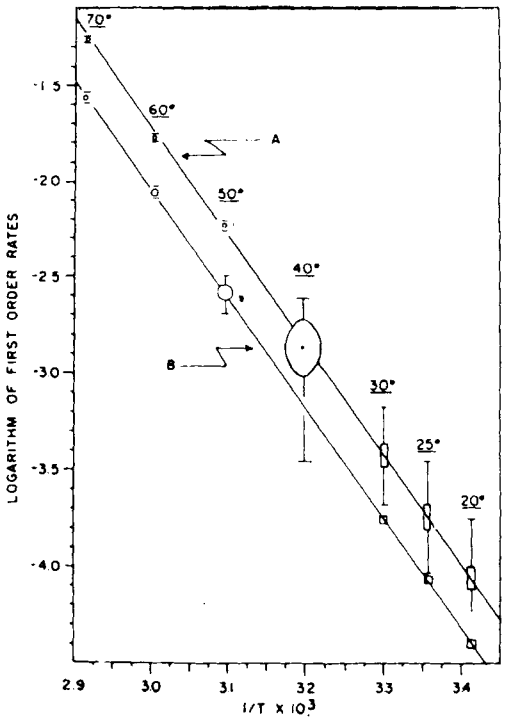


Fig. 15.—Arrhenius plots for thiamine hydrochloride in liquid multivitamin preparations. The circles represent the standard deviation of an experimental log rate in days and the rectangles the standard deviation of a predicted log rate. The horizontal lines represent the 95% confidence limits of experimental and predicted log rates. [Figure 3 of Garrett, E. R., THIS JOURNAL, **45**, 470(1956) (47).]

stability of drugs and the sterilization of microorganisms and their relative magnitudes can permit the proper choice of sterilizing times and temperatures for minimum loss of the drug. For procaine solutions, autoclaving at 120° is preferable to prolonged sterilization at 100°.

Comprehension of the significance of the knowledge of heats of activation can permit the prediction of the feasibility and optimum temperatures and time for the selective degradation of components in a mixture of drugs for the salvaging of lots contaminated by an unwanted material difficult to remove by other methods. This technique has been applied to a mixture of penicillins (65).

A special point must be made of the care that must be taken in estimating the heats of activation which are frequently deduced as "apparent" heats of activation (95, 140, 198).

If rate is dependent on hydroxyl ion concentration, $k_6[OH^-]$, and is determined at constant pH for several temperatures then

$$k_6[OH^-] = k_6Kw/[H^+] = k_6'Kw = Ae^{-\Delta Ha/RT} = A_{k_6'}e^{-\Delta H_{k_6'}/RT}\,A_{Kw}e^{-\Delta H_{Kw}/RT} = Ae^{-(\Delta H_{k_6'}+\Delta H_{Kw})/RT} \quad \text{(Eq. 40)}$$

and the heat of ionization of water, ΔH_{Kw}, must be subtracted to obtain the heat of activation of the specific hydroxyl ion catalyzed reaction $\Delta H_{k_0'}$.

Prediction of Drug Solvolysis in Heterogeneous Systems.—Since rate is a function of the concentration of the substrate, an apparent first-order reaction will become zero order with respect to substrate in saturated solutions as

$$\text{amount/volume/time} = kc_s = k_0c^0 = k_0 \quad \text{(Eq. 41)}$$

since c_s is the concentration of the substrate in the saturated solution.

It is thus possible to decrease the overall rate of hydrolysis of a substrate by solvolytic mechanisms by decreasing its solubility in the pharmaceutical vehicle. Examples are penicillin G procaine suspensions (172) and saturated solutions of acetylsalicylic acids (15, 48). Although the stability of acetylsalicylic acids in homogeneous solution (50) was in the decreasing order; diethylacetyl-, trimethylacetyl-, β-cyclopentylpropionyl-, acetyl-, in saturated solution, β-cyclopentylpropionyl-salicylic acid had the greatest stability due to its lowest solubility. When the kinetic dependencies of the apparent first-order rate constant, k, are known and the solubility, c_s, is also known as a function of pH and temperature, the stability of saturated solutions can be predicted.

The expression (48, 94, 133)

$$\text{pKa}' = \text{pH} + \log c_i/(c_s - c_i) \quad \text{(Eq. 42)}$$

may be used to predict the solubility, c_s, of a material of pKa′ at any given pH where c_i is the intrinsic solubility, *i.e.*, the solubility of the uncharged species which is presumed to be the determining factor of total solubility. The equation has been used to determine the solubility of sulfadiazine sodium (106) and secobarbital (185) in several solvent systems. For example, the stability of saturated solutions of barbitals could be calculated for any pH from the data on solubility (94) and the data on homogeneous kinetics (80, 88, 181) given in the literature.

In general, solubility also follows the Arrhenius law of Eq. 38 where ΔH_a represents the heat of solution, so that with the parameters of this equation, solubility c_s can be predicted for any temperature (54, 185). The complex system of saturated solutions of aspirin anhydride degrading to aspirin to salicylic acid can be quantitatively predicted for any temperature or pH (54). It was also shown, analogous to Eq. 40, that the apparent heat of activation for the degradation of saturated solutions of aspirin anhydride is actually the sum of the heats of solution and of activation of the homogeneous solution hydrolysis for aspirin anhydride (54).

Riegelman (159) has stated that in the heterogeneous system produced by surfactants with benzocaine that hydrolysis rates are more dependent on surfactant concentrations than the chain length of the polymeric surfactant. Anionic and cationic surfactants appear to stabilize the drug to base catalysis.

Bonus Values from Predictive Techniques.—Not only is information obtained from these studies that would permit the prediction of stability with varied substrate concentrations, pH values, salt concentrations, solvent changes, temperature variations, or saturated solutions, but additional information can be gleaned from judiciously designed experiments. The antibiotic psicofuranine is solvolytically degraded to adenine which is uniquely antagonistic to the antibacterial properties of its precursor (66). This was determined from concomitant biological and chemical assays of degrading solutions and the fact that a plot of biological assay *vs.* chemical assay was linear, but of negative intercept. Further investigation proved that the product adenine lessened the biological response. A decision was made that the excellent anticholinergic properties of acetylscopolamine methyl bromide were unique to this compound and not due to its ready gastrointestinal hydrolysis to the already established anticholinergic scopolamine methyl bromide, and that an expensive clinical trial of the former compound was justified (49). Comparison and understanding of the kinetics of solvolysis and solubilities of aspirin anhydride (54, 55) and aspirin (34, 48, 50), plus a comprehension of the rationales for aspirin absorption, led to the clinically confirmed prediction that aspirin anhydride would give higher blood levels of aspirin and less levels of salicylic acid than aspirin on oral administration to fasting subjects (54). The recommended conditions for autoclaving procaine solutions for minimum instability has already been cited (92). Stability studies were consistent with the fact that oral inefficacy of *p*-chlorobenzaldoxime (60) could be ascribed to gastric solvolysis but excluded this as an explanation for the oral inefficacy of N-butylformamide (70). The knowledge of the log k-pH profile of aspirin solvolysis (34, 50) suggested that a protonated amine in the molecule would permit a soluble, stable salicylic acid derivative to be formulated in an acid aqueous media which might readily hydrolyze to aspirin at the pH of the intestine.

TABLE II.—REPRESENTATIVE LOG k *vs.* pH PROFILES FOR VARIOUS KINETIC DEPENDENCIES OF THE APPARENT FIRST-ORDER RATE CONSTANT, k

Figure	k[a]	Substrate HS or S	Reference
3	$k_1[H^+] + k_3[OH^-]$	Methyl *dl*-α-phenyl-2-piperidylacetate	(170)
4	$k_1[H^+] + k_2$	Streptovaricin	(53)
9	$k_2 + k_3[OH^-]$	Chlorobutanol	(140)
10	$(k_1[H^+] + k_3[OH^-])f_{SH^+}$	Pyridine 2-aldoxime methiodide	(35)
11	$k_1[H^+]f_{SH^+} + (k_4[H^+] + k_6[OH^-])f_S$	Streptozotocin	(57)
12	$k_1[H^+]f_{HS} + (k_4[H^+] + k_5 + k_6[OH^-])f_{S^-}$	Acylsalicylic acids	(50)
13	$(k_1[H^+] + k_2 + k_3[OH^-])f_{SH^{++}} + k_8f_S$	Thiamine	(193)
14	$k_1[H^+]f_{SH^+} + (k_4[H^+] + k_6[OH^-])f_S + k_9[OH^-]f_{S^-}$	Psicofuranine	(56)

[a] f_S, f_{SH^+}, f_{HS}, or f_{S^-} represent the fraction of substrate, S or HS, in charged or uncharged form.

One of two such compounds did so readily hydrolyze to aspirin in slightly alkaline media, but both compounds unfortunately exhibited a high degree of general base catalyzed hydrolysis even by water alone in acidic solutions (51, 52). This did lead to a fundamental contribution since general base catalyzed hydrolysis of esters had been presumed to be nonexistent (9, 123) prior to the time of submission of the first paper for publication (51).

An intriguing stabilization technique has been introduced by Higuchi, Lachman, and co-workers (20, 96, 120–122). This technique was based on their comprehension of the fact that a complexed substrate might have negligible degradation rates in contrast to the uncomplexed substrate. Thus, knowledge of the equilibrium constants for the formation of such complexes and kinetic studies should permit the prediction of stability of complex-stabilized substrates. Examples are the stabilization of benzocaine (96, 116), procaine (122), tetracaine (121), and *p*-hydroxybenzoic acid (20) by caffeine, homologs (20, 120), and other agents (116). In many of these instances, the complexed substrate did not hydrolyze at all and the appearance of degradation products could be ascribed solely to the free substrate in equilibrium with the complex. A more recent paper (160) substantiates this principle by demonstrating that boric acid chelation of the catechol nucleus of epinephrine stabilizes against degradation by the attack of sulfite and bisulfite ions on the optically active side chain (100, 102, 118). It has also been shown that lithium chelates with alkylsalicylates and inhibits hydroxyl ion attack (154). This thesis has led workers to consciously seek to find if complexation exists and inhibits degradation rates. Unfortunately, success has not met all such attempts (155, 193).

A more detailed review of the literature on complexation of pharmaceuticals and its potential applications, including the stabilization of these pharmaceuticals, has been recently published by Walker (186, 187). The role of chelation in drug stabilization and other phenomena is covered in a review by Foye (42).

Frequently it is felt that modification of a pharmaceutical compound by appropriate substituents, if therapeutic efficacy was not decreased, could increase the stability of the pharmaceutical. It is apparent that steric blocking was effective in the case where the size and complexity of the acid groups in acetylsalicylic acids (50) and 21-steroid hemiesters (59, 68) varied. The Newman "rule of six" (127, 144) has great predictive power in the estimation of steric hindrance of ester solvolysis.

Another powerful predictive tool is the Hammett linear free energy relation of sigma-rho (43, 86) where

$$\log k = \log k_0 + \sigma\rho \qquad \text{(Eq. 43)}$$

where the substitutent constant σ is determined by the nature of the substituent and independent of the reaction; where the reaction constant ρ is a constant for all substituents. By definition, $\sigma = 0$ when $k = k_0$, the rate constant of the unsubstituted compounds. Hammett determined σ values for various substituents of benzoic acid as the logarithm of the ratio of the dissociation constants of the *m*- and *p*-substituted benzoic to unsubstituted benzoic acids. Astonishing linearity of log k *vs.* σ plots exists for many reactions. Taft (174–176) has extended this concept to the reactions of aliphatic compounds and *ortho*-substituted benzene derivatives. The contributions of Hammond (87) and Grunwald and Winstein (82) should be noted. Recently, Portoghese and Malspeis (155) have shown that when the log k/k_0 for the log ratio of specific base catalyzed hydrolysis rate constant of some alkyl esters of *dl*-α-(2-piperidyl)-phenylacetic acids to that constant for the methyl ester is plotted against a σ obtained from a log ratio of specific base catalyzed hydrolysis of alkyl acetates, astonishing linearity is obtained (Fig. 16). These

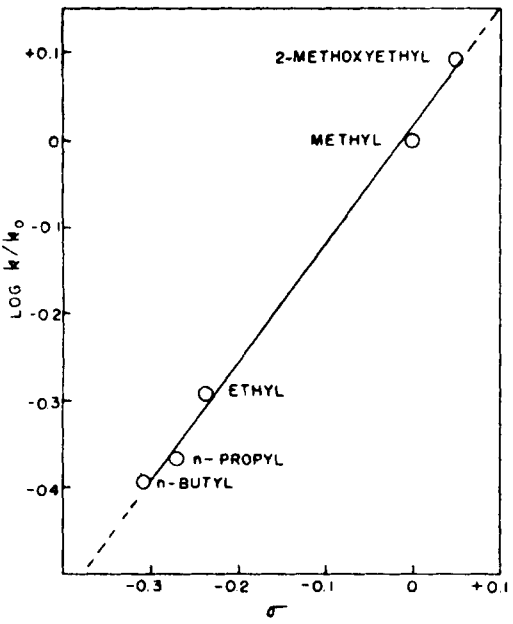


Fig. 16.—Correlation of log k/k_0 values for the specific base-catalyzed hydrolysis of alkyl esters of *dl*-α-(2-piperidyl)-phenylacetic acid at 80°, pH 5.98 with σ values of alkylacetates. [Figure 5 of Portoghese, P., and Malspeis, L., THIS JOURNAL, **50**, 494(1961)(155).]

facts imply that the σ and ρ constants available in the literature may permit the prediction of appropriate substituents to obtain the desired stability of many compounds, or that with a minimum of study on some substituent groups, the effects of many on stability can be quantitatively predicted.

It has also been shown that vitamin A esters of acids in anhydrous solvents have increased stability to proton attack with increasing Ka of such acids and yet in the absence of mineral acids, the instability of the vitamin A ester increases with the Ka of the ester acid portion (41).

Prediction of Stability of Drugs of Unknown Structure.—There is a need in pharmacy to study the changes in structures of molecules of unknown structure to predict stability. The stability of materials must be known for the establishment of treatment of assay standards and for the preparation of samples to be submitted for clinical evaluation.

The application, efficacy, and toxicity of an antibiotic or antibiotic complex is frequently well known before the components can be separated, purified, individually characterized, and structures assigned.

The stability of materials must be known for the establishment of treatment of assay standards and for the preparation of samples to be submitted for clinical evaluation.

Prediction of stability and design of formulations may be based on studies of the rates of change of some physicochemical characteristic. Correlation of changes in the physicochemical properties with the changes in the bioassays may serve as a valid argument for the use of the former. An obvious disadvantage is that variation in the latter may not necessarily reflect in the former although the converse is not probable.

Obvious advantages are that such physicochemical studies will be easy to conduct, less expensive, can lead to prediction of the conditions that affect degradation, and provide information on the properties of the intermediates and products.

Examples of the application of such a philosophy were the stability studies on the antibiotic fumagillin (33, 45, 64), an amebacide, which showed the necessity of protecting the material from light and oxygen and gave the rates of degradation in solution and crystal, as well as Arrhenius' expressions for predicting temperature effects. The ultraviolet absorptivities were correlated with biological activites (64). The prediction of the stabilities of the antitubercular streptovaricins (53) and the antibiotics, streptozotocin (57) and actinospectacin (69), have been previously discussed. The studies on the streptovaricins (53) also provided a correlation of biological activities with spectrophotometric absorptivities, the fact of increased stability in ethanol, physicochemical properties of the degradation product which would abet purification, and a differential kinetic method to assay for individual streptovaricins in the antibotic complex.

The stability studies on streptozotocin (57) correlated the polarographic and color assay due to an N-nitrosomethyl urea function with biological activity, kinetically observed a low pKa, and predicted that the high instability at the pH values of the blood would not permit significant blood levels of the antibotic to be observed. This was found to be true (188).

The stability on actinospectacin (69) correlated a copper chelate assay with biological activity, pointed out that the bioassay reliability was a function of buffer concentration, and kinetically observed a pKa *ca.* 12.

Other Studies.—Other kinetic studies on the stability of drugs that have been reported are the alkaline decomposition of glutethimide (195), the specific acid catalyzed decomposition of chlorothiazine (194) and diphenhydramine hydrochloride (146). The log *k*-pH profile has been given for procaine and tetracaine

(178) hydrolysis, substituted salicylic acid decarboxylation (191) as *p*-aminosalicylic acid (177, 179), and the solvolytic degradation of penicillin (21). The kinetics of formation of anhydrovitamin A for the alcohol and acetate of vitamin A have been investigated (99).

Thermal degradation of glucose solutions exhibited general acid-base catalysis with a rate dependence on substrate concentration (189). The heat of activation was determined from rate constants estimated by the initial slopes of the rate plots. The kinetics of glucose degradation in acid solution were also followed by the initial rate of formation of 5-hydroxymethylfurfural (90). The logarithm of the apparent rate constant varied linearly with the reciprocal of the dielectric constant, as predicted for a reaction between a positive ion and a dipole, as indicated by Eq. 26.

A catenary has been drawn for the pH dependency of the decomposition rate of oxophenarsine hydrochloride and Arrhenius parameters can be calculated from the data given (6).

The kinetics of bisulfite addition to compounds have been studied by Higuchi and Schroeter (100–102, 164, 166). They clearly show that not only can a pharmaceutical degrade in a solution and lose therapeutic potency but that a synthesis to a biologically inactive compound can also occur, as with epinephrine (166) and other benzyl derivatives (100). The probable reaction in these instances is to a benzyl sulfonic acid by reaction with a hydroxyl group (100–102). The bimolecular kinetics of epinephrine with sulfite ion were established, the heat of activation determined, and a quantitative expression derived for the given log *k*-pH profile (102). Bimolecular kinetics of salicyl alcohol reaction with bisulfite and/or sulfite ions were shown to be pH-dependent, with an appropriate predictive expression to explain the log *k*-pH profile (164). The Arrhenius parameters of the reaction were determined and ionic strength effects checked. This work has been recently considered by Schroeter in his discussion on sulfurous acid salts as pharmaceutical antioxidants (163).

The rates of hydrolysis of carbamate and carbonate esters (39, 40, 93) are of great importance in predicting the stability of carbamate esters of pharmaceutical utility (36). Nogami and coworkers have studied the hydrolysis of various quaternary nitrogen compounds (147, 148) and have observed specific acid-base and solvent catalysis, constructed the log *k*-pH profiles, and determined the Arrhenius parameters.

Prediction of Stability in Pharmaceutical Preparations.—A pharmaceutical preparation is frequently complex. Prior discussion in this paper on the prediction of stability has considered those circumstances where most of the variables are known and their specific effects on degradation can be quantified. This is not possible with the many formulations where prediction of market or shelf-life is needed. Typical examples of such a complex formulation are the multivitamin preparations which have been criticized for age on the market (26) and inadequate control, stability, and potency (27).

The legal, moral, economic, competitive, and public health reasons for the need of valid methods for the prediction and verification of drug stability in the marketed formulation have been outlined (26, 27, 44, 62, 63, 117, 136, 137).

Criticism has been leveled at rule-of-thumb methods of predicting shelf-life from accelerated testing (17, 44, 62, 63, 117, 126, 136) since, too frequently, such correlations have been intuitive, based on insufficient numbers of elevated temperatures or on empirical relations found in supposedly similar preparations (16, 63).

The Arrhenius relations of rate with absolute temperature of Eq. 38 and 39 show that any value proportional to the specific rate, k, would permit evaluation of the slope of the plot of log k *vs.* $1/T$ and, thus, prediction of stability at shelf or storage temperatures should be practical.

It is not necessary to determine the mechanisms of degradation. In many cases, the procedure is to follow some property of the degradation as a function of time and to linearize this function. The slopes of such linear plots may be used as estimates of the specific rates (k). The reproducibility of such a degradation rate function at the various elevated temperatures would validate the use of such functions in the prediction of degradation rates at lower temperatures (63).

The order of the degradation rate of a component in a complex preparation is defined as the power, n, of the concentration proportional to the rate of degradation, as is Eq. 1. In most studies on the thermal degradation of complex liquid preparations (46, 47, 63, 135) $n = 0$ or $n = 1$ has served satisfactorily to characterize the loss of initial assay with time within the errors of assay. Analogous procedures can be developed, however, for the other possible values of n. Autocatalytic degradation, however, may need special treatment and the thermal degradation of crystalline fumagillin in air gave apparent second-order kinetics (45).

The decrease in color of a multisulfa preparation was proportional to time (*i.e.*, zero order) at the various elevated temperatures studied (63). The logarithm of the slopes of such plots was linear with the reciprocal of the absolute temperatures of the studies and the values at the extrapolated room temperatures permitted prediction of the color stability at those temperatures. The thermal degradation of many vitamins in liquid multivitamin preparations has also been studied (46, 47, 135). Zero- and first-order plots (a typical example for thiamine hydrochloride is given in Fig. 1) were applicable. The Arrhenius plots, as in Fig. 15 for thiamine hydrochloride, permitted prediction of stability at the lower temperatures.

Although the equations of such plots can be estimated graphically (135), the employment of statistical procedures (22, 30, 31) determines the degree of confidence to be held in the predicted stability. These procedures have been outlined and applied by Garrett (46, 47), and were subsequently given stepwise (135). In general, the rate data are fitted by the method of least squares, as in Fig. 1, to give the best estimated rate constant and estimates of its error as well as estimates of error in the assay methods.

Similarly, the error in a predicted rate constant at a marketing temperature can be estimated (Fig. 15). The final verification of these predictive procedures has been given in detail in the literature (46, 135). An example is the solid line in Fig. 17 which predicted the thermal degradation of thiamine hydrochloride at 30° where the dashed lines encompass the standard error in the predicted thermal degradation and the 95% confidence limits of a single assay. The data were obtained from two separately prepared formulations maintained at 30° and assayed over a year. Another example is given in Fig. 18 and verifies the predicted thermal degradation of folic acid in a liquid multivitamin preparation at 25°. The upper curve was predicted for a modified preparation with an increased concentration of folic acid and was experimentally confirmed. This indicates that it may be used practicably in many cases to predict stability on the basis of one elevated temperature and the known Arrhenius slope.

This type of prediction may serve as an efficient control procedure as, when a batch of one component is introduced from a new source is increased in amount, or a manufacturing process is modified. This procedure is not applicable to two different components; Arrhenius slopes of such will differ even in the same preparation.

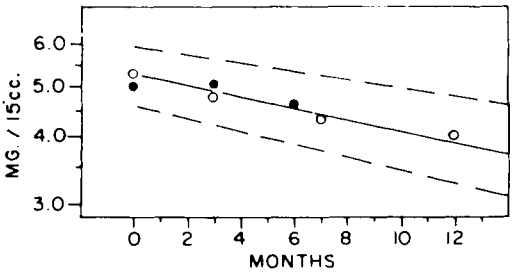


Fig. 17.—Verification of prediction of thiamine hydrochloride stability at room temperature. The solid line is the predicted thermal degradation rate of thiamine hydrochloride in a liquid multivitamin preparation A at 30°. The dashed lines encompass standard error in predicted values and the standard error of a single assay. O, Data from preparation A; ●, data from a similar preparation A′. [Figure 4 of Garrett, E. R., This Journal, **45**, 470(1956) (47).]

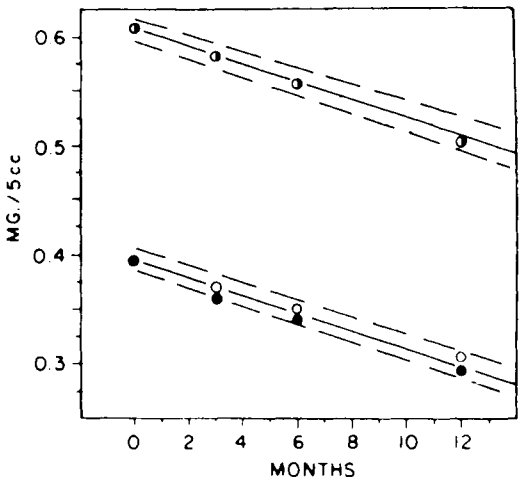


Fig. 18.—Verification of prediction of folic acid stability at room temperature. The solid lines are predicted thermal degradation rates of folic acid in liquid multivitamin preparation A at 25°. The dashed lines encompass standard error in predicted values and 95% confidence of a single assay. O, Data from preparation A; ●, data from a similar preparation B; ◑, data from a modified preparation C with increased initial concentration of folic acid and other vitamin components. [Figure 9 of Garrett, E. R., This Journal, **45**, 171(1956) (46)].

However, when the vehicle or formulation is entirely new, the rate of change of degradation rate of a component with temperature is not the same in both vehicles. Valid prediction is not possible from comparison at only one elevated temperature. The slopes of the Arrhenius equation must be determined anew.

The use of these physicochemical procedures for prediction of stability must be considered in the light of the controlling mechanisms of degradation (47). In the previous discussion components were considered that most probably degrade by solvolytic processes; *i.e.*, reactions

in solution and their heats of activation, as per Arrhenius slope, are generally in the range 10–30 Kcal./mole. Thus, advantage may be taken of significant increases in rate with temperature. However, if diffusion or photolysis are the rate-determining steps, the heat of activation is only of the magnitude 2–3 Kcal./mole and little advantage is gained by accelerated temperature studies in prediction, since the temperature effect on rate is small. In some cases, such rates may be accelerated by increases in pressure or light intensities.

Frequently the thermal degradation of polyhydroxylic materials and the subsequent effect on other components are of interest. However, it must be realized that the heats of activation for such pyrolysis are frequently of the magnitude of 50–70 Kcal./mole. Thus, rates of degradation which may be great at elevated temperatures may not be of any practical significance at the temperature of marketing and storage of the preparation.

Further discussions on design and practice of such predictive methods are given in the literature (44, 117) and the very models of ideal comprehensive stability testing laboratories for application of such techniques are also given (118, 119).

Other examples of the application of these predictive techniques in pharmaceutical formulations are for ascorbic acid in a liquid multivitamin emulsion containing fluoride (180), the kinetics of degradation of amyl nitrite ampuls (196, 197), and the stability of ergonovine maleate (139). Publications of interest in this regard are studies on the influence of the effect of heating and cooling time on prediction of shelf-life by kinetic principles and the Arrhenius equation at exaggerated temperatures (37). It has been shown, however, that for a series of samples subjected to elevated temperatures and when each sample is treated in the same manner (*i.e.*, heated and cooled), the calculated rates of degradation are identical to those measured under isothermal conditions (167).

CONCLUSIONS

The prediction of solvolytic stability of drugs in solution and in liquid pharmaceutical preparations is based on good science. The known quantitative relations between degradative rate and the extant variables (concentration, pH, solvent kind and dielectric constant, ionic strength, substrate pKa's, substrate reactive groups, temperature, solubility, nucleophiles, acids, bases, addend, etc.) have been shown to permit such prediction from a minimum of experiment and data and with a maximum of understanding and confidence. The procedures outlined provide additional pharmaceutical bonuses in the physicochemical characterization of substrates and products of kinetic transformations. They permit the correlation of biological activities and assays, assurance of valid assay methods, and estimations of their error, and the prediction of molecular, solvent, and addend changes to increase stability or increase physiological availability.

The future looms brightly for the prediction of stability of the complicated phenomena involved in photolytic and oxidative transformations. The complex stability problems of suspensions, emulsions, and ointments are leaving the backwoods of empirical correlations and rules-of-thumb and will shortly join the civilized communities of quantified prediction.

REFERENCES

(1) Amis, E. S., *Anal. Chem.*, **27**, 1672(1955).
(2) Amis, E. S., "Kinetics of Chemical Change in Solution," The MacMillan Co., New York, N. Y., 1949.
(3) Amis, E. S., and LaMer, V. K., *J. Am. Chem. Soc.*, **61**, 905(1939).
(4) Amis, E. S., and Siegal, S., *ibid.*, **72**, 674(1950).
(5) Archer, B. L., and Hudson, R. F., *J. Chem. Soc.*, **1950**, 3259.
(6) Banks, C. K., THIS JOURNAL, **38**, 503(1949).
(7) Bates, R. G., "Electrometric pH Determinations," John Wiley & Sons, Inc., New York, N. Y., 1954, p. 39.
(8) Bates, R. G., and Pinching, G. D., *J. Research Natl. Bur. Standards*, **42**, 419(1949).
(9) Bell, R. P., "Acid-Base Catalysis," Oxford University Press, London, 1941.
(10) Bell, R. P., and Lindars, F. J., *J. Chem. Soc.*, **1954**, 4601.
(11) Bell, R. P., and Waind, G. M., *ibid.*, **1950**, 1979.
(12) Bender, M. L., *Chem. Revs.*, **60**, 87(1960).
(13) Bender, M. L., and Turnquest, B. W., *J. Am. Chem. Soc.*, **79**, 1652, 1656(1957).
(14) Benson, S. W., "The Foundations of Chemical Kinetics," McGraw-Hill, New York, N. Y., 1960.
(15) Blaug, S. M., and Wesolowski, J. W., THIS JOURNAL, **48**, 691(1959).
(16) Blythe, R. H., "Tests on Shelf Life and Stability," presented to the Scientific Section, American Pharmaceutical Manufacturers Assoc., New York, N. Y., February 2, 1954.
(17) Blythe, R. H., *Glass Packer*, **25**, August 1954.
(18) Blythe, R. H., and Zirkle, M. S., "Selected References on the Stability of Pharmaceutical Ingredients," presented to the American Chemical Society, New York, N. Y., September 16, 1954.
(19) Bolen, I., *Z. anorg. Chem.*, **143**, 201(1925).
(20) Bolton, S., Guttman, D., and Higuchi, T., THIS JOURNAL, **46**, 38(1957).
(21) Broderson, R., "Inactivation of Penicillin in Aqueous Solution," Einar Munksgaard, Copenhagen, 1949.
(22) Brownlee, K. A., "Industrial Experimentation," Chemical Publishing Co., Inc., New York, N. Y., 1953.
(23) Bruice, T. C., and Schmir, G. L., *J. Am. Chem. Soc.*, **79**, 1663(1957).
(24) Bruice, T. C., and Schmir, G. L., *ibid.*, **80**, 148 (1959).
(25) Butterworth, J., Eley, D., and Stove, G. S., *Biochem. J.*, **53**, 30(1953).
(26) Campbell, J. A., THIS JOURNAL, **44**, 598(1955).
(27) Campbell, J. A., and McLeod, H. A., *ibid.*, **44**, 263 (1955).
(28) Cowan, H. D., McCabe, C. L., and Warner, J. C., *J. Am. Chem. Soc.*, **72**, 1194(1950).
(29) Daniels, F., and Alberty, R. A., "Physical Chemistry," John Wiley & Sons, Inc., New York, N. Y., 1955.
(30) Davies, O. L., "Statistical Methods in Research and Production," 2nd ed., Oliver & Boyd, London, 1949.
(31) Deming, W. E., "Statistical Adjustment of Data," John Wiley & Sons, Inc., New York, N. Y., 1943.
(32) Denny, D. B., and Greenbaum, M. A., *J. Am. Chem. Soc.*, **79**, 370(1957).
(33) Eble, T. E., and Garrett, E. R., THIS JOURNAL, **43**, 536(1954).
(34) Edwards, L. J., *Trans. Faraday Soc.*, **46**, 723 (1950).
(35) Ellin, R. I., Carlese, J. S., and Kondritzer, A. A., THIS JOURNAL, **51**, 141(1962).

(36) Ellis, S., Plachte, F. L., and Straus, O. H., *J. Pharmacol. Exptl. Therap.*, **79,** 295(1943).
(37) Eriksen, S. P., Irwin, G. M., and Swintosky, J. V., THIS JOURNAL, **49,** 632(1960); Eriksen, S. P., Pauls, J. F., and Swintosky, J. V., *ibid.*, **47,** 697(1958).
(38) Fainberg, A. H., and Winstein, S., *J. Am. Chem. Soc.*, **78,** 2770(1956).
(39) Faurholt, C., *J. chim. phys.*, **22,** 1(1925).
(40) Faurholt, C., *Z. physik. Chem.*, **126,** 211(1927).
(41) Forlano, A. J., and Harris, L. E., THIS JOURNAL, **49,** 458(1960).
(42) Foye, W. O., *ibid.*, **50,** 93(1961).
(43) Frost, A. A., and Pearson, R. G., "Kinetics and Mechanism," 2nd ed., John Wiley & Sons, Inc., New York, N. Y., 1961.
(44) Garrett, E. R., *Am. Perfumer Aromat.*, **74,** 23(1959).
(45) Garrett, E. R., THIS JOURNAL, **43,** 539(1954).
(46) Garrett, E. R., *ibid.*, **45,** 171(1956).
(47) Garrett, E. R., *ibid.*, **45,** 470(1956).
(48) Garrett, E. R., *ibid.*, **46,** 584(1957).
(49) Garrett, E. R., *J. Am. Chem. Soc.*, **79,** 1071(1957).
(50) Garrett, E. R., *ibid.*, **79,** 3401(1957).
(51) Garrett, E. R., *ibid.*, **79,** 5206(1957).
(52) Garrett, E. R., *ibid.*, **80,** 4049(1958).
(53) Garrett, E. R., THIS JOURNAL, **48,** 169(1959).
(54) Garrett, E. R., *ibid.*, **48,** 676(1959).
(55) Garrett, E. R., *J. Am. Chem. Soc.*, **82,** 711(1960).
(56) Garrett, E. R., *ibid.*, **82,** 827(1960).
(57) Garrett, E. R., THIS JOURNAL, **49,** 767(1960).
(58) Garrett, E. R., *J. Org. Chem.*, **26,** 3660(1961).
(59) Garrett, E. R., *J. Med. Pharm. Chem.*, **5,** 112(1962).
(60) Garrett, E. R., THIS JOURNAL, **51,** 410(1962).
(61) Garrett, E. R., *ibid.*, **51,** 445(1962).
(62) Garrett, E. R., "Third Stevens Symposium on Statistical Methods in Chemical Industry," January 24, 1959, American Graphic, Irvington, N. J., 1959.
(63) Garrett, E. R., and Carper, R. F., THIS JOURNAL, **44,** 515(1955).
(64) Garrett, E. R., and Eble, T. E., *ibid.*, **43,** 385(1954).
(65) Garrett, E. R., and Ford, J. H., personal communication.
(66) Garrett, E. R., and Hanka, L. J., THIS JOURNAL, **49,** 526(1960).
(67) Garrett, E. R., and Moffett, R. B., *J. Am. Chem. Soc.*, **77,** 1245(1955).
(68) Garrett, E. R., and Royer, M. E., THIS JOURNAL, **51,** 451(1962).
(69) Garrett, E. R., and Umbreit, G. R., *ibid.*, **51,** 436 (1962).
(70) Garrett, E. R., and Weber, D. J., *ibid.*, **51,** 387 (1962).
(71) Glasstone, S., "Textbook of Physical Chemistry," 2nd ed., D. Van Nostrand Co., Inc., New York, N. Y., 1946.
(72) Glasstone, S., Laidler, K. J., and Eyring, A., "The Theory of Rate Processes," McGraw-Hill, New York, N. Y., 1941.
(73) Goddu, R. F., and Hume, D. N., *Anal. Chem.*, **26,** 1679(1954).
(74) Gold, V., *Trans. Faraday Soc.*, **44,** 506(1948).
(75) Gold, V., and Butler, A. R., *Proc. Chem. Soc.*, **1960,** 15.
(76) Gold, V., and Butler, A. R., *J. Chem. Soc.*, **1961,** 2305.
(77) Gold, V., and Butler, A. R., *ibid.*, **1961,** 4362.
(78) Gold, V., Hilton, J., and Jefferson, E. G., *ibid.*, **1954,** 2756.
(79) Gold, V., and Jefferson, E. G., *ibid.*, **1953,** 1409.
(80) Goyan, J. E., Shaikh, Z. I., and Autian, J., THIS JOURNAL, **49,** 627(1960).
(81) Guggenheim, F. A., *Phil. Mag.*, **2,** 538(1936).
(82) Grunwald, E., and Winstein, S., *J. Am. Chem. Soc.*, **70,** 846(1948).
(83) Guillot, M., *Am. J. Hospital Pharm.*, **17,** 540(1960).
(84) Guttman, D. E., and Meister, P. D., THIS JOURNAL, **47,** 773(1958).
(85) Hackett, J. W., and Thomas, H. C., *J. Am. Chem. Soc.*, **72,** 4962(1950).
(86) Hammett, L. P., "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1940.
(87) Hammond, G. S., *J. Am. Chem. Soc.*, **77,** 334(1955).
(88) Hasegawa, J., Ikeda, K., and Matsuzewa, T., *Pharm. Bull. Tokyo*, **6,** 36(1958).
(89) Harned, H. S., and Owen, B. B., "The Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold Publishing Co., New York, N. Y., 1958.
(90) Heimlich, K. R., and Martin, A. N., THIS JOURNAL, **49,** 592(1960).
(91) Higuchi, T., and Bias, C. T., *ibid.*, **42,** 707(1953).
(92) Higuchi, T., and Busse, L. W., *ibid.*, **39,** 411(1950).
(93) Higuchi, T., and Dittert, L. W., preprint D-III, symposia papers, Scientific Section, A.PH.A., 1962.
(94) Higuchi, T., Gupta, M., and Busse, L. W., THIS JOURNAL, **42,** 157(1953).
(95) Higuchi, T., Havinga, A., and Busse, L. W., *ibid.* **39,** 405(1950).
(96) Higuchi, T., and Lachman, L., *ibid.*, **44,** 521(1955).
(97) Higuchi, T., and Marcus, A. D., *ibid.*, **43,** 530(1954).
(98) Higuchi, T., Marcus, A. D., and Bias, C. T., *ibid.*, **43,** 129(1954).
(99) Higuchi, T., and Reinstein, J., *ibid.*, **48,** 155(1959).
(100) Higuchi, T., and Schroeter, L. C., *ibid.*, **48,** 535 (1959).
(101) Higuchi, T., and Schroeter, L. C., *ibid.*, **49,** 331(1960).
(102) Higuchi, T., and Schroeter, L. C., *J. Am. Chem. Soc.*, **82,** 1904(1960).
(103) Hine, J., "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1956.
(104) Hinshelwood, C. N., "The Kinetics of Chemical Change," Oxford University Press, London, 1940.
(105) Hockersmith, J. L., and Amis, E. S., *Anal. Chim. Acta*, **9,** 101(1953).
(106) Hom, F. S., and Autian, J., THIS JOURNAL, **45,** 608 (1956).
(107) Ingold, C. K., *J. Chem. Soc.*, **1930,** 1032.
(108) Johnson, S. L., *J. Am. Chem. Soc.*, **84,** 1729(1962).
(109) Kilpatrick, M., *ibid.*, **50,** 2891(1928).
(110) Kilpatrick, M., *ibid.*, **52,** 1410, 1418(1930).
(111) Kochi, J. K., and Hammond, G. S., *ibid.*, **75,** 3445 (1953).
(112) Kochi, J. K., and Hammond, G. S., *ibid.*, **75,** 3452 (1953).
(113) Kondritzer, A. A., and Zvirblis, P., THIS JOURNAL, **46,** 531(1957).
(114) Koshy, K. T., and Lach, J. L., *ibid.*, **50,** 113(1961).
(115) Krahl, M. E., *J. Phys. Chem.*, **44,** 449(1940).
(116) Lach, J. L., and Pauli, W. A., *Drug. Standards*, **27,** 104(1959).
(117) Lachman, L., *Bull. Parenteral Drug. Assoc.*, **13,** 8 (1959).
(118) Lachman, L., and Cooper, J., THIS JOURNAL, **48,** 226(1959).
(119) Lachman, L., and Cooper, J., *ibid.*, **48,** 234(1959).
(120) Lachman, L., Guttman, D., and Higuchi, T., *ibid.*, **46,** 36(1957).
(121) Lachman, L., and Higuchi, T., *ibid.*, **46,** 32(1957).
(122) Lachman, L., Ravin, L. J., and Higuchi, T., *ibid.*, **45,** 290(1956).
(123) Laidler, K. J., "Chemical Kinetics," McGraw-Hill Book Co., New York, N. Y., 1950.
(124) Laidler, K. J., and Eyring, H., *Ann. N. Y. Acad. Sci.*, **39,** 303(1940).
(125) Laidler, K. J., and Landskroener, P. A., *Trans. Faraday Soc.*, **52,** 200(1956).
(126) Levy, G. B., *Drug & Cosmetic Ind.*, **76,** 472(1955).
(127) Loening, K. L., Garrett, A. B., and Newman, M. S., *J. Am. Chem. Soc.*, **74,** 3929(1952).
(128) Manov, G. G., Schuette, K. E., and Kirk, F. S., *J. Research Natl. Bur. Standards*, **48,** 84(1952).
(129) Marcus, A. D., THIS JOURNAL, **49,** 383(1960).
(130) Marcus, A. D., and Baron, S., *ibid.*, **48,** 85(1959).
(131) Marcus, A. D., and Taraszka, A. J., *ibid.*, **46,** 28 (1957).
(132) Marcus, A. D., and Taraszka, A. J., *ibid.*, **48,** 77 (1959).
(133) Martin, A. N., "Physical Pharmacy," Lea and Febiger, Philadelphia, Pa., 1960.
(134) Martin, E. W., "Husa's Pharmaceutical Dispensing," 5th ed., Mack Publishing Co., Easton, Pa., 1959.
(135) McLeod, H. A., Pelletier, O., and Campbell, J. A., *Can. Pharm. J.*, **March 1958,** 55.
(136) Meyers, E. L., "Drug Stability, Data Requirement Under New Drug Regulations," Pharmacy Congress, St. John's University, Jamaica, N. Y., March 17, 1959.
(137) Meyers, E. L., *Drug Standards*, **27,** 84(1959).
(138) Moelwyn-Hughes, E. A., "Kinetics of Reactions in Solution," Oxford University Press, Oxford, 1946.
(139) Moore, W. E., *Drug Standards*, **27,** 187(1959).
(140) Nair, A. D., and Lach, J. L., THIS JOURNAL, **48,** 390(1959).
(141) Nair, P. M., and Amis, E. S., *Anal. Chim. Acta*, **9,** 111(1953).
(142) Nair, P. M., and Amis, E. S., *J. Am. Chem. Soc.*, **77,** 3452(1955).
(143) Nelson, E., in Martin, E. W., "Husa's Pharmaceutical Dispensing," 5th ed., Mack Publishing Co., Easton, Pa., 1959.
(144) Newman, M. S., *J. Am. Chem. Soc.*, **72,** 4783(1950).
(145) Nielson, R. F., *ibid.*, **58,** 206(1936).
(146) Nogami, H., and Masayoshi, H., *Chem. Pharm. Bull. Tokyo*, **8,** 510(1960).
(147) Nogami H., Masayoshi, H., Awazu, S., and Yamada, H., *ibid.*, **6,** 277(1958); through Chem. Abstr., **53,** 5589 (1959).
(148) Nogami, H., and Nakajima, N., *ibid.*, **6,** 283(1958); through *Chem. Abstr.*, **53,** 5589(1959).
(149) Parsons, R., "Handbook of Electrochemical Constants," Butterworth's Scientific Publishers, London, 1959.
(150) Patel, J. L., and Lemberger, A. P., THIS JOURNAL, **47,** 878(1958).
(151) Patel, J. L., and Lemberger, A. P., *ibid.*, **48,** 106 (1959).
(152) Patel, J. L., and Lemberger, A. P., Preprint D-V symposia papers, Scientific Section, A.PH.A., 1962.
(153) Pearson, R. G., *J. Chem. Physics.*, **20,** 1478(1952).
(154) Pekkarinen, L., and Tommila, E., *Acta Chem. Scand.*, **13,** 1019(1959).
(155) Portoghese, P. S., and Malspeis, L., THIS JOURNAL, **50,** 494(1961).
(156) Potts, J. E., Jr., and Amis, E. S., *J. Am. Chem. Soc.*, **71,** 2112(1949).
(157) Quinlan, J. E., and Amis, E. S., *ibid.*, **77,** 4187 (1955).
(158) Martin, E. W., "Remington's Practice of Pharmacy," 12th ed., Mack Publishing Co., Easton, Pa., 1961.
(159) Riegelman, S., THIS JOURNAL, **49,** 339(1960).

(160) Riegelman, S., and Fischer, E. Z., *ibid.*, **51**, 206 (1962).
(161) Rollefson, G. K., Bartlett, P. D., Hammett, L. P., and Long, F. A., *J. Am. Chem. Soc.*, **77**, 2666a(1959).
(162) Schmir, G. L., and Bruice, T. C., *ibid.*, **80**, 1173 (1958).
(163) Schroeter, L. C., THIS JOURNAL, **50**, 891(1961).
(164) Schroeter, L. C., *ibid.*, **51**, 258(1962).
(165) Schroeter, L. C., and Higuchi, T., *ibid.*, **47**, 426 (1958).
(166) Schroeter, L. C., Higuchi, T., and Schuler, E. E., *ibid.*, **47**, 723(1958).
(167) Scott, M. W., and Lachman, L., *ibid.*, **51**, 125(1962).
(168) Shah, N. P., and Amis, E. S., *Anal. Chim. Acta*, **11**, 401(1954).
(169) Shou, S. A., *Pharm. Acta Helv.*, **34**, 398(1959).
(170) Siegel, S., Lachman, L., and Malspeis, L., THIS JOURNAL, **48**, 431(1959).
(171) Stern, M. J., King, L. D., and Marcus, A. D., *ibid.*, **48**, 641(1959).
(172) Swintosky, J. V., Rosen, E., Robinson, T. J., Chamberlain, R. E., and Guarini, J. R., *ibid.*, **45**, 37(1956).
(173) "Tables of Chemical Kinetics: Homogenous Reactions," National Bur. Standards, Circular 510, U. S. Dept. of Commerce, Washington, D. C., 1951, and Suppl. 1, 1956.
(174) Taft, R. W., Jr., in Newman, M. S., "Steric Effects in Organic Chemistry," John Wiley & Sons, New York, N. Y., Chap. 13, 1956.
(175) Taft, R. W., Jr., *J. Am. Chem. Soc.*, **74**, 589, 599, 2729, 3120(1952).
(176) Taft, R. W., Jr., *ibid.*, **75**, 4321(1953).
(177) Tanaka, N., *J. Pharm. Soc. Japan*, **81**, 604(1961).
(178) Tanaka, N., Harada, K., and Sugawara, S., *ibid.*, **81**, 903(1961).
(179) Tanaka, N., and Nakagaki, M., *ibid.*, **81**, 591, 597, 600(1961).
(180) Tingstad, J. E., Macdonald, L. H., and Meister, P. D., Preprint D-I symposia papers, Scientific Section A.PH.A., 1962 meeting.
(181) Tishler, F., Sinsheimer, J. E., and Goyan, J. E., THIS JOURNAL, **51**, 214(1962).
(182) Tommila, E., and Hietala, S., *Acta Chem. Scand.*, **8**, 257(1954).
(183) Tommila, E., Koivisto, A., Lyrra, J. P., Antell, K., and Heimo, S., *Ann. Acad. Sci. Fennicae Ser.* A II, **47**, 3(1952).
(184) Tommila, E., and Maltamo, S., *Suomen Kemistilehti B*, **28**, 73, 118(1955).
(185) Udani, J. H., and Autian, J., THIS JOURNAL, **49**, 376(1960).
(186) Walker, G. C., *Bull. Ontario Coll. Pharm.*, **10**, 95 (1961).
(187) Walker, G. C., *ibid.*, **11**, 3(1962).
(188) Wallach, D. P., private communication.
(189) Webb, N. E., Jr., Sperandio, G. J., and Martin, A. N., THIS JOURNAL, **47**, 101(1958).
(190) "Webster's New Collegiate Dictionary," 2nd ed., G. and C. Merriam Co., Springfield, Mass., 1953.
(191) Willi, A. V., *Trans. Faraday Soc.*, **55**, 433(1959).
(192) Whittet, T. D., "Decomposition of Medicaments. . ." presented at Symposium on Drug Stability at 19th International Conference of Pharmaceutical Sciences, Zurich, Switzerland, September 1959.
(193) Windheuser, J. J., and Higuchi, T., THIS JOURNAL, **51**, 354(1962).
(194) Yamana, T., Koike, H., and Tamura, Y., *J. Pharm. Soc. Japan*, **81**, 1528(1961).
(195) Yamana, T., and Mizukami, Y., *ibid.*, **81**, 1381(1961).
(196) Yunker, M. H., and Higuchi, T., THIS JOURNAL, **47**, 621(1958).
(197) Yunker, M. H., Szulczewski, D., and Higuchi, T., *ibid.*, **47**, 613(1958).
(198) Zvirblis, P., Socholitsky, I., and Kondritzer, A. A., *ibid.*, **45**, 450(1956).

Research Articles

Biliary and Urinary Excretion Patterns of Chlorpromazine in the Dog

By T. L. FLANAGAN, L. W. REYNOLDS†, W. J. NOVICK, T. H. LIN, I. M. RONDISH, and E. J. VAN LOON

Chlorpromazine instilled intraduodenally in dogs was excreted in bile and urine as "free" and "bound" chlorpromazine and chlorpromazine sulfoxide. Based upon chromatographic studies and ultraviolet analysis, chlorpromazine and its sulfoxide undergo two types of binding. Strong alkaline treatment hydrolyzed one type while treatment with β-glucuronidase hydrolyzed both types. In urine, the concentration of the "bound" phenothiazines liberated by alkaline hydrolysis was 2–3 times as great as the "free" material. In bile, the concentration of this type of "bound" chlorpromazine and chlorpromazine sulfoxide was 10 to 15 times greater than the "free" forms of these materials.

VARIOUS investigators have reported on the urinary excretion of chlorpromazine and/or its metabolic products in man and experimental animals (1–9). Ross, Young, and Maass (10), Walkenstein and Seifter (11), and Fishman and Goldenberg (12) reported that the side chain which is attached to the phenothiazine nucleus is demethylated. Nadeau and Sobolewski (9) reported that β-glucuronidase treatment increases the amount of phenothiazine-like extractable material from human urine, and Lin, Reynolds, Rondish, and Van Loon (13) reported on the isolation and characterization of glucuronic acid conjugates of chlorpromazine in human urine. Fyodorov (14) administered chlorpromazine-S^{35} to dogs and found that the bile was distinguished by a high degree of radioactivity, especially 3–6 hours after administration.

The purposes of the present studies were (*a*) to obtain an insight into the distribution and

Received October 19, 1961, from the Research and Development Division, Smith Kline and French Laboratories, Philadelphia 1, Pa.
Accepted for publication December 14, 1961.
† Present address: General Electric, Space Science Division, Philadelphia, Pa.