(VIII*a*) was also similarly tested in desoxycorticosterone–saline rats (intraperitoneally) (10).

Acute toxicities were determined in male mice weighing 20 ±2 g. A dose was given orally to each of five animals and mortalities were recorded 1 week later. The LD_{50} was calculated according to a previous procedure (11).

RESULTS AND DISCUSSION

Table I summarizes the results of the pharmacological assays. All the compounds studied showed appreciable activity in the spontaneously hypertensive rat assay. In decreasing order of activity they are: VIII*a* > VIII*d* > VIII*e* > VIII*b* > V*c* > V*a* > V*b* > V*e* > IX*e* > IX*b* > IX*d* > IX*a*.

The hydrazino group is not essential for activity although it does considerably increase antihypertensive activity and toxicity. Group VIII compounds are thus the most active and the most toxic. Furthermore, the antihypertensive activity of V compounds may be related to that of other non hydrazine antihypertensive compounds (12, 13). It seems that this is the first report of antihypertensive activity in the oxo and triazole metabolites of pyridazino–hydrazine antihypertensives.

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Simple Methods for Estimating Percent Disintegrated–Time Data

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Abstract □ Two techniques are described for the treatment of dissolution rates to estimate the percent disintegrated–time data for tablets and capsules. The first is an extension of an equation derived previously with the assumption of first-order disintegration and dissolution processes; whereas, the second involves the determination of the rate constant from the terminal segment of the curve and the use of numerical derivatives according to a disintegration kinetics-independent approach. The dissolution data of six commercial tablet and capsule formulations were treated according to the described techniques. Good agreement was found between the percent disintegrated–time data estimated by the second approach for an acetaminophen tablet and those obtained by a well-established model where a Weibull function was employed.

Keyphrases □ Dissolution—tablets, simple methods for estimating percent disintegrated–time data □ Disintegration—tablets, simple methods for estimating percent disintegrated–time data, dissolution

There are few methods available for utilizing dissolution rate data to estimate the fraction disintegrated as a function of time for tablets (1, 2). These techniques, however, lack simplicity and require computer programs which may not be available.

A simple method was described recently for the disintegration–dissolution analysis of percent dissolved–time data (3). The technique is based on a biexponential equation with the assumption of first-order disintegration and dissolution according to a simple dissolution model.

The present report describes two approachs that can be used to estimate the percent disintegrated–time data for tablets and capsules. The first is an extension to the above report (3), whereas the other is based on disintegration kinetics—independent approach. Both techniques are simple and rapid and allow computations to be carried out manually.

THEORETICAL

Estimation of Percent Disintegrated–Time Data (Approach I) —Assuming first-order disintegration and dissolution processes, if A, A_p, and A_s represent the amounts of drug in dosage form, small particles, and solution, respectively, at any given time, and it is assumed that the disintegration and dissolution are first-order processes whose apparent rate constants are k_d and k_s, respectively, or:

$$A \xrightarrow[\text{first-order disintegration}]{k_d} A_p \xrightarrow[\text{first-order dissolution}]{k_s} A_s$$

Scheme I

the following equation can be derived (3):

$$100 - f_s = \frac{100\,k_d}{k_d - k_s} e^{-k_s t} - \frac{100\,k_s}{k_d - k_s} e^{-k_d t} \qquad \text{(Eq. 1)}$$

where f_s is the cumulative percent dissolved at time t.

Based on Scheme I, the following also can be derived:

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Table I—Percent Disintegrated–Time Data for the Formulations Used According to *Approaches I* and *II*

Formulation Code	Midinterval Time, min																	
	1		3		5		7		9		12.5		17.5		22.5		27.5	
	I[a]	II[b]	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
A	27.5	10.4	61.8	31.3	79.9	63.3	89.4	80.6	94.4	85.6	98.2	96.4	99.6	94.8	100	100	100	100
B	36.9	14.6	74.9	37.3	90.0	79.3	96.0	87.3	98.4	97.6	99.7	106.0	100	100	100	100	100	100
C	36.6	35.0	74.5	64.6	89.8	77.8	95.9	85.0	98.3	89.0	99.7	94.2	100	102	100	104	100	100
	Midinterval Time, min																	
	1.25		3.75		6.25		8.75		12.5		17.5		22.5		27.5			
	I[a]	II[b]	I	II	I	II	I	II	I	II	I	II	I	II	I	II		
D	36.6	36.6	74.6	75.7	89.8	93.6	95.9	96.2	99.0	98.7	99.8	99.5	100	99.9	100	100		
E	40.3	33.4	78.7	72.3	92.4	90.4	97.3	92.2	99.4	99.5	99.9	101	100	99.1	100	99.1		
F	22.4	0	53.3	22.4	71.9	41.0	83.1	71.2	97.1	75.3	97.1	90.7	99.0	104	99.6	110		

[a] Estimated according to *Approach I*. [b] Estimated according to *Approach II*.

$$A_d = A_0 (1 - e^{-k_d t}) \quad \text{(Eq. 2)}$$

where A_d is the amount disintegrated at time t and equal to the amount of drug in dosage form at time 0 (A_0) minus the amount of drug in dosage form at time t (A). Multiplying both sides of Eq. 2 by $100/A_0$ yields:

$$f_d = 100 (1 - e^{-k_d t}) \quad \text{(Eq. 3)}$$

where $f_d = 100A_d/A_0$ and is the cumulative percent disintegrated at time t.

The disintegration profile can be obtained by substituting for k_d in Eq. 3 the values determined graphically according to Eq. 1 where k_s can also be determined (3).

Disintegration Kinetics Independent Approach (Approach II)—If the dissolution process is assumed to follow first-order kinetics while disintegration is taken as a kinetics-independent process, the following can be derived using a simple kinetic approach:

$$f_{d(t)} = \frac{(df_s/dt)_t}{k_s} + f_{s(t)} \quad \text{(Eq. 4)}$$

where $(df_s/dt)_t$ is the dissolution rate at time t, and $f_{d(t)}$, $f_{s(t)}$, and k_s have the same meaning as described earlier. Equation 4 can also be derived using a convolution technique (2). Employing Eq. 4, a direct calculation of the cumulative percent disintegrated–time data can be achieved once $(df_s/dt)_t$ is calculated and k_s determined. The apparent first-order rate constant for dissolution (k_s) can be determined from the terminal straight line segment of $\ln(100 - f_s)$ *versus* time plot.

RESULTS AND DISCUSSION

The dissolution data obtained for six commercial tablets and capsules containing isoniazid, theophylline, ampicillin, tetracycline hydrochloride, and tetracycline hydrochloride–phosphate complex (3) were employed to examine the use of the described techniques to estimate the percent disintegrated–time data.

The apparent first-order disintegration and dissolution rate constants (k_d and k_s, respectively) were determined graphically according to Eq. 1. The values of k_d were substituted in Eq. 3, and the percent disintegrated at various time values were calculated and are presented in Table I.

When *Approach II* was employed for the determination of the percent disintegrated–time data, the dissolution rate $(df_s/dt)_t$ was calculated for each time interval, and the midinterval time was taken as t. The cumulative percent dissolved at midinterval time (t) was estimated by the arithmetic mean of the percent dissolved at the beginning and the end of the interval. Table I presents the percent disintegrated–time data determined by this approach.

As demonstrated in Table I, the data obtained by *Approach I* were generally comparable to those obtained by *Approach II*. Indeed, for formulations C, D, and E, the data produced by both techniques were very close. The disagreement in the results, when existent, generally occurred in the initial data points. This may indicate less than perfect fit of these data points to Eq. 1. More initial data points might have led to better estimates of k_d; however, this would require the automation of the dissolution test. A good fit of the dissolution data to Eq. 1 is essential if the percent disintegrated–time data were to be estimated accurately according to Eq. 3.

To examine the reliability of the described techniques, the dissolution rate data of an acetaminophen tablet were subjected to the described analysis. These data were reported and employed (2) to demonstrate the application of a model to estimate the fraction disintegrated–time data. The data were well represented by a Weibull function (2), and a good estimate of k_d according to Eq. 1 could not be obtained. However, the curve produced by plotting $\ln(100 - f_s)$ *versus* time had a linear terminal segment whose slope was equal to 1.389 min^{-1}. This value was used as k_s in Eq. 4, and the percent disintegrated–time data were determined after the dissolution rate $(df_s/dt)_t$ was calculated for each interval. The percent dissolved at midinterval time $(f_s)_t$ was reported.

In a continuous function approach using convolution techniques, an equation was derived (2) which was employed to analyze these data. The equation was obtained by substituting a Weibull function for f_s in Eq. 3. The two parameters for this Weibull function were determined by a nonlinear regression of the Weibull function to the above tablet data. The first-order dissolution parameter was determined (2) from separate dissolution tests of acetaminophen powder and plotting the data semilogarithmically against time.

As can be seen in Table II, the percent disintegrated–time data by *Approach II* were similar to those obtained previously (2). Therefore, while the existent methods (1, 2) involve either a system of linear equations which require computer programs to solve or continuous functions with three parameters to be determined, the estimation of the percent disintegrated–time data by *Approach II* is simple and requires no separate dissolution testing of drug powder. The estimation of the first-order dissolution rate constant can be performed easily by plotting the tablet dissolution data semilogarithmically against time. The terminal data points most frequently behave in a first-order fashion. This approach for determination of k_s is valid here, because the acetaminophen tablet dissolution was less rapid than disintegration (1, 2), and k_s was smaller than k_d for the other formulations used (Table I).

While the usefulness of Eq. 3 to estimate the percent disintegrated–

Table II—Percent Disintegrated–Time Data for an Acetaminophen Tablet

Midinterval Time, min	$(df_s/dt)_t$, %/min	$f_{s(t)}$, %	$f^a_{d(t)}$, %	$f^b_{d(t)}$, %
0.17	2.73	0.4	2.4	1.9
0.50	8.53	1.7	7.8	7.0
0.83	15.2	6.5	17.4	13.7
1.17	21.5	12.8	28.3	21.7
1.50	22.9	19.8	36.3	30.3
1.83	22.4	26.9	43.0	39.2
2.17	24.8	35.6	53.5	48.3
2.5	20.3	43.1	57.7	56.8
2.83	23.6	50.1	67.1	64.6
3.17	21.2	57.6	72.9	71.9
3.50	20.9	64.4	79.4	77.9
3.83	19.7	71.1	85.3	83.1
4.17	19.4	77.7	91.7	87.4
4.50	18.2	84.7	97.8	90.8
4.83	11.2	89.1	97.2	93.4
5.17	10.9	92.9	100.8	95.4
5.50	7.3	95.5	100.8	96.9
6.00	2.6	98.2	100.8	98.3

[a] Estimated according to *Approach II*. [b] Reported previously (2) and labeled as continuous function.

time is limited to the cases where the dissolution data are well described by Eq. 1, *Approach II* provides a simple, rapid, and reliable tool for prediction of disintegration profiles. The computation involved is simple and can be carried out manually without the use of computer programs as required by other techniques.

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Lysine and Polylysine: Correlation of their Effects on Polyphosphoinositides *In Vitro* with Ototoxic Action *In Vivo*

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Received November 19, 1981, from the **Kresge Hearing Research Institute and* ‡*College of Pharmacy, The University of Michigan, Ann Arbor, MI 48109.* Accepted for publication February 3, 1982.

Abstract □ Low concentrations of poly-L-lysine, a polycationic hydrophilic molecule, caused a large expansion of polyphosphoinositide monolayers and produced a significant loss of cochlear microphonic potentials in perilymphatic perfusions in the guinea pig. In contrast, the monomeric L-lysine had only slight effects on polyphosphoinositide monolayers and did not affect cochlear microphonic potentials even at concentrations as high as 10 m*M*. These data substantiate the hypothesis that the expansion of polyphosphoinositide monolayers by a drug is an indicator of its ototoxicity.

Keyphrases □ Polyphosphoinositides—correlation of effects of lysine and polylysine *in vitro* with ototoxic action *in vivo* □ Lysine—correlation of effects on polyphosphoinositides *in vitro* with ototoxic action *in vivo*, polylysine □ Polylysine—correlation of effects on polyphosphoinositides *in vitro* with ototoxic action *in vivo*, lysine

Evidence has previously been provided in *in vivo* and *in vitro* studies for an involvement of polyphosphoinositides in the toxic actions of aminoglycoside antibiotics (1). Monolayer studies have shown that polyphosphoinositides are unique among various anionic phospholipids with respect to both the type and magnitude of the interactions with neomycin and Ca^{2+} (2). The observed increase in surface pressure was indicative of a very strong preference of the polyphosphoinositide film for neomycin over Ca^{2+} and other cations. It was further shown that the degree of interaction of eight aminoglycoside antibiotics and fragments with polyphosphoinositides correlated well with their ototoxicity in the guinea pig (3).

In order to test further the hypothesis that the degree of expansion of polyphosphoinositide monolayers is correlated with ototoxicity, monolayer studies, and ototoxicity tests for two compounds unrelated to aminoglycosides, poly-L-lysine and L-lysine, are reported here. Poly-L-lysine represents a polycationic hydrophilic compound, capable of occupying an area-determining position in lipid films (4), and L-lysine represents the corresponding singly-charged hydrophilic monomer.

EXPERIMENTAL

Materials and Methods—Poly-L-lysine had a molecular weight of 3400 (polymerization grade, 16)[1]. Polyphosphoinositides were purified from ox brain by chromatography on immobilized neomycin (5).

A polytef beaker (8-cm diameter) held 100 ml of subphase containing 50 m*M* sodium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate, pH 7.0, 1 m*M* $CaCl_2$, and sufficient sodium chloride to adjust the ionic strength to 0.2. A stationary syringe, with the needle immersed in the subphase throughout the experiment, delivered the additions of lysine and polylysine. The solutions were stirred with a magnetic bar at slow speed without the film being disturbed. The polyphosphoinositide mixtures (phosphatidylinositol phosphate–phosphatidylinositol bisphosphate, 1:2 molar ratio) were spread as a solution in *n*-hexane–ethanol–chloroform (80:5:15, v/v/v). Approximately 0.2 μg of lipid/cm^2 was spread to obtain the desired surface pressure (14–15 dyne/cm) which was read with a balance[2] after the film had stabilized for 10 min. Polylysine or L-lysine was then injected into the subphase for final concentrations of 10^{-7}–10^{-4} *M*. After each addition, the subphase was mixed for 15 min before measurements were taken. Experiments were performed at 25±2° in duplicate, and surface tension readings always agreed within 0.1 dyne/cm.

Perilymphatic Perfusions—Perilymphatic perfusions were carried out in male albino guinea pigs (200–300 g). An animal with a positive Preyer hearing reflex was anesthetized with pentobarbital (20 mg/kg of body weight ip), atropine sulfate (0.05 mg/kg sc), and 0.5 ml/kg of body weight im of a solution containing 0.4 mg of fentanyl and 20.0 mg of droperidol/ml (6). The animal's body temperature was maintained at 37 ±1° with a heating pad and artificial respiration was provided through a tracheal cannula. Surgical and perfusion techniques were similar to that described previously (3).

The perilymphatic spaces of the cochlea were perfused at a rate of ~30 μl/min. Cochlear microphonic potentials were measured in response to a sound stimulus of white noise, 20–4000 Hz, delivered through an earphone. The sound intensity level was adjusted to give an initial microphonic potential of 200–400 μV. After the potential had stabilized for 30 min (control period), ototoxicity was determined as its loss after 30 min of a subsequent perfusion with added drug.

[1] Poly-L-lysine and L-lysine were obtained from Sigma Chemical Co., St. Louis, Mo.
[2] Wilhelmy balance.

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