Biopharmaceutical Investigation of Nalidixic Acid in Man

By W. E. MOORE, G. A. PORTMANN, H. STANDER, and E. W. McCHESNEY

A complex model to illustrate the pharmacokinetic parameters relating to the absorption, metabolism, and elimination of nalidixic acid in man is presented. This model was reduced to a practical working model by combining the two active forms of the drug and by combining the two conjugated forms of the drug. Employing equations derived from the working model, the plasma and urinary excretion curves from eight normal subjects were analyzed by both graphical and computer approaches following the oral administration of four dosage forms given according to a Latin square crossover design. Satisfactory fits of the curves were obtained for three of the four dosage forms. Estimates of lag time, the apparent kinetic rate constants, and the volumes of distribution were compared among the three dosage forms. Significant differences were shown among the formulations with respect to lag time and availability rates.

OVER THE LAST few years, there has been an increasing number of blood level and urinary excretion studies of drugs having various pharmacokinetic pathways ranging in complexity from an intravenous injection–urinary excretion system to one following oral absorption with numerous metabolic and elimination pathways (1–3).

Many studies are not analyzed by kinetic models because either (*a*) the analytical methods required to describe the model sufficiently are not available or (*b*) the costs relative to number of samples, specificity of assay, and computational time are prohibitive or (*c*) both.

One solution which permits a kinetic approach to the study of a complex pharmacokinetic model is to reduce the model to a form where the estimates of the simplified model parameters provide useful information concerning the properties of the drug being studied.

This approach was applied in the present study to a comparison of the kinetics of four nalidixic acid[1] dosage forms that vary in their physical-chemical properties.

THEORETICAL

Complex Model.—A previous work (4) on the metabolism of nalidixic acid led to the development of a complex model. This model (Fig. 1) conveys our current understanding of the absorption, metabolism, and elimination of nalidixic acid in man. One appreciates that this seemingly complex model is in reality an oversimplification of a very complex biological process.

Working Model.—By combining the two microbiologically active forms (nalidixic acid and hydroxylated nalidixic acid) and by combining the two conjugated forms of the active forms, the number of rate processes were thereby reduced to yield the working model described in Fig. 2. The analysis based upon two forms of nalidixic acid, instead of upon only original nalidixic acid, gives rise to apparent first-order rate constant estimations. The authors feel this approximation is justified because the hydroxylated nalidixic acid is equally active microbiologically (4), and any analysis accounting for only original unchanged nalidixic acid would be misleading from a therapeutic potential point of view.

In the working model, lag time is defined as the time interval between ingestion of the dosage form and the appearance of naphthyridine acid in the plasma. The apparent rate constants were considered as all being first order, formally defined as:

k_A = The availability rate from the intestinal tract, including the factors of gastrointestinal motility, dissolution rate, etc., and the absorption rate itself.

k_E = The urinary excretion rate of the microbiologically active drug. This rate function includes the metabolic rate process of conversion of nalidixic acid to its hydroxylated metabolite.

k_M = The metabolic rate constant for the conversion of active drug to its glucuronide. This process also includes the metabolic conversion rate to hydroxylated active drug.

k_U = The excretion rate constant for conjugated drug. This rate function also includes the metabolic conversion rate to hydroxylated drug.

The k_{M_4} pathway was ignored since other studies showed that the dicarboxylic acid metabolite accounted for only a small percentage (less than 5%) of the dose administered.

The following set of equations was derived to describe the changes of nalidixic acid and its derivatives in the compartments of the model.

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[1] Nalidixic acid is 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid. Marketed as NegGram by Winthrop Laboratories, Rensselaer, N. Y.

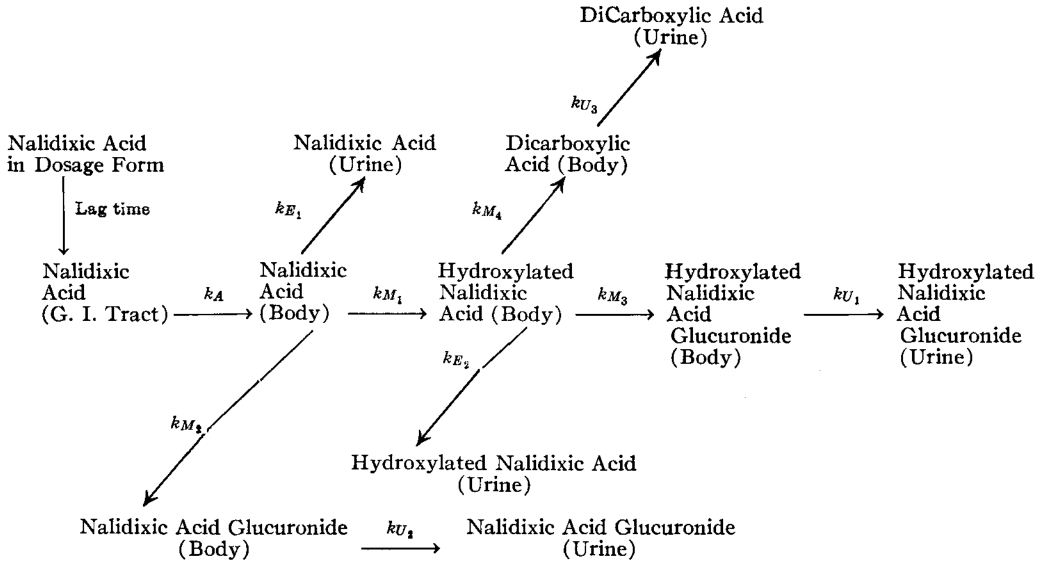

Fig. 1.—Kinetic pathways of nalidixic in man. k_A = Availability rate constant; k_{M_i} = metabolic rate constants; k_{E_i} and k_{U_i} = excretion rate constants.

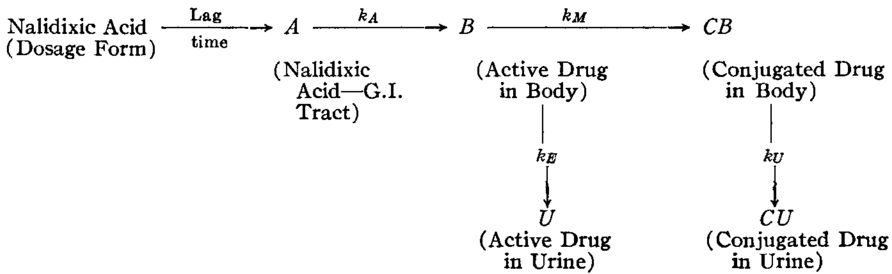

Fig. 2.—Kinetic pathways of nalidixic acid in man, working model.

Nalidixic Acid in G.I. Tract[2] = $A = A_O e^{-k_A t}$ (Eq. 1)

Active Drug in Body =

$$B = \frac{k_A A_O}{k_A - k_d}(e^{-k_d t} - e^{-k_A t}) \quad \text{(Eq. 2)}$$

Conjugated Drug in Body =

$$CB = \frac{k_m k_A A_O}{(k_A - k_d)(k_d - k_U)}(e^{-k_U t} - e^{-k_d t}) - \frac{k_M k_A A_O}{(k_A - k_d)(k_A - k_U)}(e^{-k_U t} - e^{-k_A t}) \quad \text{(Eq. 3)}$$

Active Drug in Urine =

$$U = \frac{k_A k_E A_O}{k_d(k_A - k_d)}(1 - e^{-k_d t}) - \frac{k_E A_O}{k_A - k_d}(1 - e^{-k_A t}) \quad \text{(Eq. 4)}$$

Conjugated Drug in Urine =

$$C_U = \frac{k_A k_M A_O}{(k_A - k_d)(k_d - k_U)}(1 - e^{-k_U t}) - \frac{k_A k_M k_U A_O}{k_d(k_A - k_d)(k_d - k_U)}(1 - e^{-k_d t}) - \frac{k_A k_M A_O}{(k_A - k_d)(k_A - k_U)}(1 - e^{-k_U t}) + \frac{k_M k_U A_O}{(k_A - k_d)(k_A - k_U)}(1 - e^{-k_A t}) \quad \text{(Eq. 5)}$$

[2] Fraction of the dose absorbed is 1 because absorption is essentially complete.

It was convenient to collapse the working model further to obtain estimates of k_A by combining compartments B and CB and defining an additional apparent disappearance rate constant, k_d, as being the sum of k_E and k_M. The combination of B and CB permitted an analysis of the data utilizing the values for total drug in the body.

EXPERIMENTAL

Administration of Dosage Forms.—Eight subjects were given each of four formulations over a 3–4-week period according to a Latin square design. Medication equivalent to 1 Gm. of nalidixic acid was given with water after overnight fasting. To maintain uniformity of absorption food, but not water, was withheld for 3 hours postmedication. The medications were given on four successive Mondays.

Dosage Forms.—Three capsule and one tablet formulations were evaluated. The three capsule formulations contained nalidixic acid in the form of micropulverized crystals (4–12 μ), 60–80-mesh crystals, and the sodium salt, whereas the tablet formulation (NegGram Caplets, 250 mg./Caplet) contained micropulverized crystals (4–12 μ). The capsules contained only pure drug.

Sample Collections.—Seven blood samples were taken after administration of each dosage form at

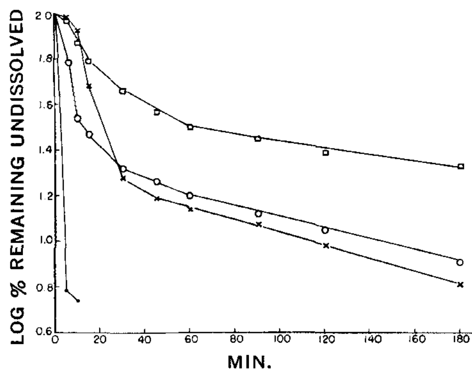

Fig. 3.—Dissolution test results. Key, ●, sodium nalidixate capsules; ×, fine nalidixic acid crystals capsules; ○, fine nalidixic acid crystals Caplet; □, coarse nalidixic acid crystals capsules.

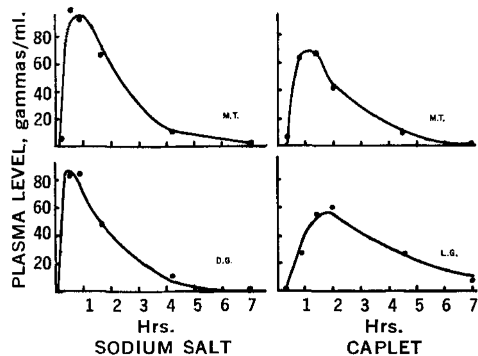

Fig. 4.—Total naphthyridine in plasma; fit of experimental data (●) to model (—). Initials identify subjects.

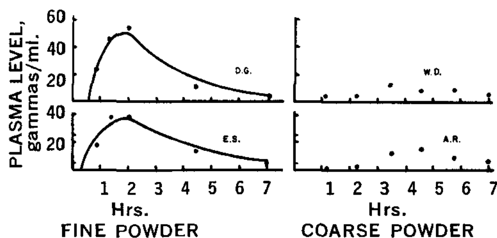

Fig. 5.—Total naphthyridine in plasma; fit of experimental data (●) to model (—). Initials identify subjects. For the coarse powder only experimental points are shown.

appropriate intervals from 0 to 420 minutes. Urine samples were collected at 0, 2, 4, 6, 6 to 24, and 24 to 48 hours after drug administration to assure total collection of urinary excretory products.

In Vitro Dissolution Rates.—The dissolution rates of the four dosage forms at pH 7.5 were strongly dependent upon the stirring rate. At slow stirring rates, the coarse powder dissolved more rapidly than the fine powder because the fine powder did not disperse or wet well in the dissolution medium. A stirring rate was empirically estimated so that the relative rank order in dissolution rate would agree with the order suggested by Levy (6) as the most probable rank order in availability: sodium salt in a capsule > fine powder in a capsule ≥ fine powder in a tablet > coarse powder in a capsule. The method consisted of using a phosphate buffer at pH 7.5 in a volume of 400 ml./500 mg. of nalidixic acid at room temperature. The stirring rate was 400 r.p.m. from a Heller stirrer fitted with a three-bladed propeller. The data are presented in Fig. 3.

Intrinsic dissolution rate data were provided by Dr. Gerhard Levy,[3] who determined that the dissolution rate of nalidixic acid from a rotating disk increases markedly above pH 6.5. At pH 6.5, the compound dissolves three times as rapidly as at a pH of 1.5.

Analytical Results.—Total naphthyridine concentrations were determined by fluorometric assays for all 4 weeks. The active naphthyridine assays were fluorometric (4) for weeks 1 and 4 and microbiological (5) for weeks 2–4. (See Table II.) The agreement between these independent methods is shown under *Results and Discussion.*

Data for curve fitting of conjugated naphthyridine in urine were obtained by difference between total naphthyridine (chemical) and active naphthyridine (microbiological) for weeks 2–4.

RESULTS AND DISCUSSION

Plots of total naphthyridine plasma concentration *versus* time were constructed from the experimental data on an individual basis. From these graphs, lag times were estimated by extrapolating the absorptive phase of the curves to zero plasma level assuming absorption to be a first-order process. Initial estimates of k_A were obtained by assuming the time differences between peak plasma level and lag time as representative of two half-life periods for absorption. Rate constants for disappearance (k_d) were estimated graphically from semilogarithmic plots of the plasma decay using the sodium salt data. Volumes of distribution (V_D) were assumed to be 20% of the subjects body weight. All of these initial estimates (lag time, k_A, k_d, and V_D) were approximately equivalent to the final curve-fitted values.

With the aid of a digital computer, final estimates were obtained by substituting the initial estimates in Eq. 2 (division by V_D is necessary to obtain concentration) for each subject and testing for the goodness of fit of successive estimates by least-squares iterative methods.[4] Examples of these fits are shown in Figs. 4 and 5 for the four dosage forms studied.

Plasma levels are, of course, dependent on the individual's volume of distribution; therefore, comparison of curve height or area must be on an individual basis. It was found consistently that for heavier individuals the plasma levels were lower compared to lighter individuals for the same preparation. Volumes of distribution were found to be proportional to body weights which varied from 113 to 208 lb.

Data from the coarse crystals capsule clearly illustrate why no curve fitting was possible with the proposed model. Very slow absorption occurred, evidenced by low plasma levels and significant excretion during the period 24 to 48 hours postmedication (36 to 202 mg.). In contrast, the other formulations gave high initial blood levels and complete excretion (>95%) in 24 hours.

[3] Private communication, November 1962.
[4] V_D's for nalidixic acid and metabolites were assumed the same.

TABLE I.—ANALYSIS OF KINETIC PARAMETERS ESTIMATED FROM TOTAL NAPHTHYRIDINE IN PLASMA

Formulation	Mean[a] Lag Time, Min.	Mean[a] Availability Rate Constant, k_A, Min.$^{-1}$	Mean[a] Disappearance Rate Constant, kd, Min.$^{-1}$	Mean[a] Apparent Vol. of Distribution, V_D, as % of Body Wt.
A. Micropulverized powder—Caplet	14	0.020	0.011	17
B. Micropulverized powder—capsule	24	0.017	0.011	19
C. Sodium salt—capsule	3	0.042	0.011	16
Statistical Comparisons Between Means				
Formulation *A vs. B*	Sig. p-0.05	N.S.D.[b]	...	N.S.D.[b]
Formulation *B vs. C*	Sig. p-0.01	Sig. p-0.01	...	N.S.D.[b]
Formulation *A vs. C*	Sig. p-0.05	Sig. p-0.01	...	N.S.D.[b]

Analysis of Variance

Source of Variation	d.f.	Lag Time Mean Sq.	k_A, Mean Sq. × 10^6	V_D (% of Body Wt.) Mean Sq.
Latin squares	1	37.5	39.3	26.0
Rows within squares eliminating columns	6	26.0	240	208
Columns within squares ignoring rows	6	81.7	86.8	166
Treatments	2	872[d]	466[d]	18.2
Residual	8[c]	57.5	39.3	7.42

[a] Mean of eight observations. [b] N.S.D., no significant difference, $p > 0.05$. [c] Reduced to 7 d.f. for k_A analysis to compensate for single rejected estimate. [d] Significant, $p = 0.01$.

TABLE II.—COMPARISON OF CHEMICAL AND BIOLOGICAL ASSAYS[a]—URINE SAMPLES

		mg. Active Naphthyridine Excreted in the Time Intervals									
		0–2 Hr.		2–4 Hr.		4–6 Hr.		6–24 Hr.		24–48 Hr.	
Subject	Dosage Form	Chem.	Bio.	Chem.	Bio.	Chem.	Bio.	Chem.	Bio.	Chem.	Bio.
A	Caplet	17.8	17.0	94.4	77.6	43.2	34.0	61.6	46	7.6	<16.5
B	Caplet	7.7	14.6	29.1	30.3	76.2	55.2	37.6	36.6[b]	4.5	<10.9
C	Na salt	22.1	23.0	29.9	32.2	11.8	11.5	35.7	27.0[b]	4.8	<13.4
D	Na salt	172	134.0	...	...	121[c]	94.2	63.2	41.0	2.3	<19.6
E	Fine powder	51.5	43.0	64.0	50.0	42.2	36.0	57.9	51.0	1.5	<19.2
F	Fine powder	17.6	14.0	73.3	60.0	84.2	70.0	128.0	79.0	8.2	<15.6
G	Coarse powder	1.4	2.4[b]	12.0	13.0	15.5	13.0	62.2	69.0	4.7	<9.3
H	Coarse powder	0.4	<1.1	1.2	1.76[b]	39.3	37.0	89.7	67.0	7.0	<43.0

[a] Chemical assay (4); biological assay (5). [b] Value estimate from a one point response. [c] Two to six-hour collection.

The rapidly soluble sodium salt of nalidixic acid was significantly more readily available for absorption with a lag time significantly shorter than the micropulverized powder in either a Caplet or capsule form, as shown in Table I. There was excellent rank-order correlation between the initial dissolution rate of the dosage forms (Fig. 3) and their respective lag times (Table I).

As expected, the disappearance rate constant (k_d), estimated from the experimental data obtained with the sodium salt formulation, did not change with a formulation change.

Satisfactory fits of calculated values with experimental data were also obtained in the case of the plasma active naphthyridine results, examples of which are illustrated in Figs. 6 and 7. Except for the volume of distribution (V_D), the kinetic parameters for total naphthyridine were the same as for active naphthyridine. Because the metabolic rate is the controlling factor, k_d is essentially the same.[5]

The over-all mean of the apparent V_D's, expressed as per cent of body weight for the Caplet, micropulverized powder-capsule, and sodium salt-capsule, was 26% with a range of 20 to 29% in the means (nine observations).

Equation 5 was used for calculation of CU, employing K_A and k_d rate constants estimated from the plasma curves. Representative examples of the fits obtained are illustrated in Fig. 8. The fits of 18

[5] Further studies have shown that kd is also the same for nalidixic acid and will be reported in a future publication.

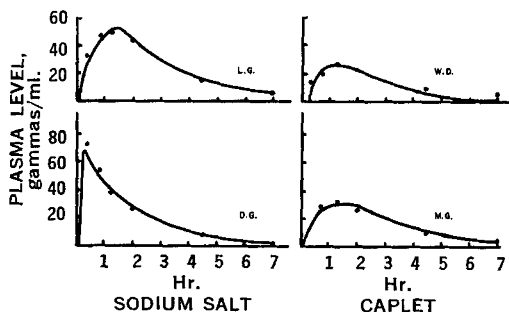

Fig. 6.—Active naphthyridine in plasma, fit of experimental data (●) to model (—). Initials identify subjects.

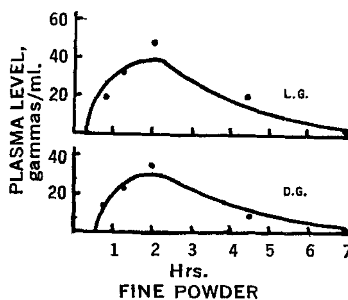

Fig. 7.—Active naphthyridine in plasma, fit of experimental data (●) to model (—).

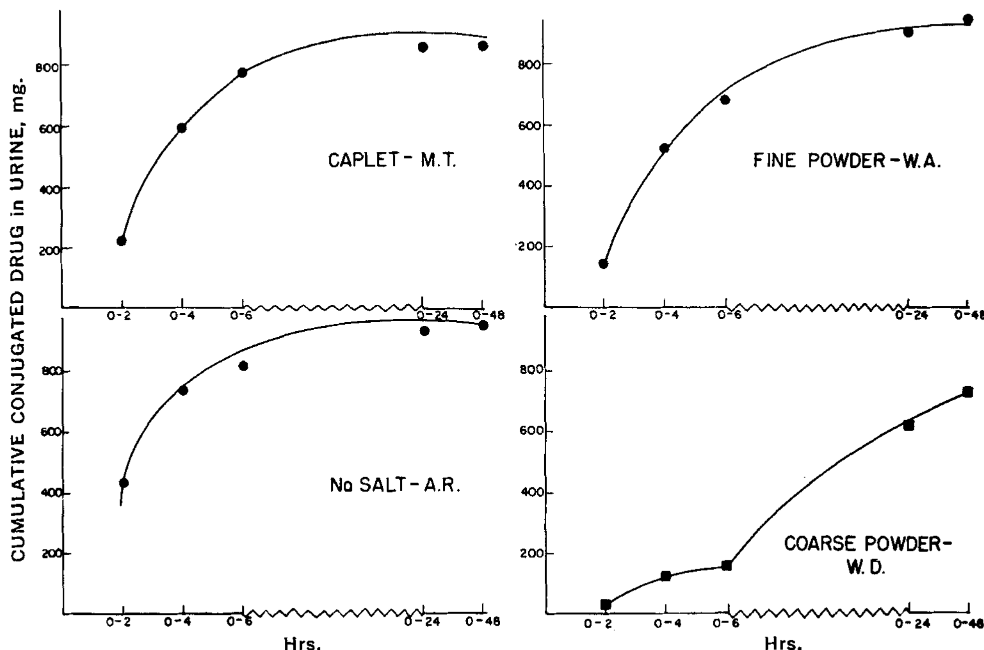

Fig. 8.—Cumulative excretion of conjugated naphthyridine, fit of experimental data (●) to model (—). For the coarse powder capsules, the experimental values were joined (■). Time scale was broken to condense the figures.

TABLE III.—ANALYSIS OF KINETIC PARAMETERS ESTIMATED FROM ACTIVE AND CONJUGATED NAPHTHYRIDINE IN URINE

Formulation	Mean[a] Excretion Rate Constant for Active Naphthyridine k_E Min.$^{-1}$ (Range)	Mean[a] Metabolic Rate Constant, k_M Min.$^{-1}$ (Range)	Mean[a] Excretion Rate Constant for Conjugated Naphthyridine k_U, Min.$^{-1}$ (Range)
Micropulverized powder—Caplet	0.0014 (0.0007–0.00019)	0.0090 (0.00070–0.011)	0.015 (0.0094–0.026)
Micropulverized powder—capsule	0.0015 (0.0007–0.0022)	0.0096 (0.0064–0.013)	0.0099 (0.0044–0.014)
Sodium salt—capsule	0.0014 (0.0005–0.0035)	0.0094 (0.0080–0.011)	0.015 (0.0080–0.028)

[a] Mean of six observations.

TABLE IV.—CALCULATED MEAN PEAK PLASMA TIMES AND CONCENTRATIONS

Formulation	$t_{max.}$	Min. Lag Time	Min. Peak Time	γ/ml. at Peak Time Naphthyridine Total	γ/ml. at Peak Time Naphthyridine Active
Sodium salt capsule	45	3	46	58	47
Micropulverized powder—Caplet	67	14	81	42	36
Micropulverized powder—capsule	73	24	97	36	24

experimental values (Table III) support the definition of k_A and k_d in the collapsed model.

The apparent kinetic rate constants, k_E and k_M, were estimated from active and conjugated naphthyridine in urine (0 to 48 hours) which accounted for over 95% of the dose. The following equations used for these calculations were derived by solving for CU and U in Eqs. 4 and 5 allowing time to go to infinity.

$$k_M = k_d \frac{[CU]_\infty}{A_O} \qquad \text{(Eq. 6)}$$

$$k_E = k_d \frac{[U]_\infty}{A_O} \qquad \text{(Eq. 7)}$$

Table III summarizes apparent excretion and metabolic rate constants obtained from active and conjugated naphthyridine determinations in urine. The k_U results (Table III) show that the glucuronide is rapidly excreted; the rate constant is about 10 times that of k_E and equal to or greater than k_M.

The mean availability and disappearance rate constants can be related to peak plasma level time according to Eq. 8. This value plus the mean lag time for each formulation then should represent the mean observed peak time.

$$t_{max} = \frac{2.3}{(k_A - k_d)} \log \frac{k_A}{k_d} \qquad \text{(Eq. 8)}$$

The calculated mean peak times and mean peak concentrations are listed in Table IV.

CONCLUSION

A useful, simplified kinetic description of nalidixic acid's fate in man has been presented for three of four dosage forms. These results clearly indicate that the pharmacokinetic analysis of a drug must include provision for the biopharmaceutic factors of the dosage form of the drug. When correction is allowed for these modifying factors, the parameters of elimination kinetics become meaningful, as for example, the constancy of k_d.

Lag times and availability rates varied significantly between the dosage forms as expected. However, the differences in behavior between the micropulverized powder in a capsule and in a Caplet are not extreme; they show the same k_A but different lag times. This effect is not due to a capsule as such (the short lag time of the sodium salt shows that a capsule can release its contents quite rapidly), but it is most likely due to the fact that the Caplet contains adjuvants that bring about rapid dispersion of the drug.

The model failed completely to describe the pharmacokinetics of nalidixic acid when administered orally as a coarse powder. A model to describe this slowly absorbed dosage form is the subject of continuing research.

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Structure–Chromogenic Activity Relationship of Phenolic Compounds with Ehrlich Reagent

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Over 100 phenolic compounds have been hitherto tested for their chromogenic ability with a modified Ehrlich reagent. A simple paper spotting technique has been utilized for this study. Mono-, di-, and trihydroxyphenols have been tested, and the possible relationship of color and structure has been investigated. Color tests were observed in the case of many compounds, with resorcinol, phloroglucinol, and several of their derivatives yielding relatively sensitive chromogenic reactions. The chromogenic effect of the alkyl substituent, etherification, and esterification of phenolic hydroxyl group(s) of resorcinols has been studied. The color reactions of three types of carbonyl-substituted resorcinols previously reported were investigated further by examining the effect of electron-donating groups in mitigating the adverse effect of the carbonyl group. Several naturally occurring phenolic compounds were also tested by the same technique. The modified Ehrlich reagent is a useful phenolic chromogen and may be applicable as a differentiating reagent for some of these compounds.

EHRLICH REAGENT (1), consisting of an acidic solution of *p*-dimethylaminobenzaldehyde (PDAB), is generally used in chromogenic analysis for the detection (2–5) of indole derivatives in plant and animal extracts. Spraying this reagent on paper chromatograms containing indole compounds produces purple, blue, and red spots. According to Feigl (2), even pyrrole and its derivatives that have intact CH-group in the α- and β-position relative to the cyclic NH-group will react with PDAB to yield colored products. It was indicated further that aliphatic amines condense with this aldehyde and produce various colored condensates—usually yellow, orange-red, and brown.

Morton (6) found that the Ehrlich reagent was applicable for the chromogenic analysis of phloroglucinol, pyrogallol, orcinol, and resorcinol. The colors were similar to those produced by indoles and pyrroles under conditions of the Ehrlich test. Since then the effect of this reagent on a number of phenolic compounds has been reported (8–12). The studies of these workers revealed that generally most of the phenols did not appear to react on the paper chromatograms with the reagent. Excluding some cinnamic acid derivatives, all that did so were resorcinol or phloroglucinol derivatives. Phloroglucinol-derived compounds reacted instantaneously, whereas the resorcinol-derived compounds developed colors after a while.

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