a two-step protonation. As a check of the method, the pKa of *o*-iodobenzoic acid was determined concurrently, yielding the value of −7.4 which is in good agreement with previously published data (12).

A first protonation of compound I could lead to structures I*b*, I*d*, I*c*, or I*f*; loss of water from I*b* will yield I*c*. (Scheme I.)

Of these structures for a single protonation, I*b* and I*f* are considered least likely. Protonation of a conjugated carbonyl function, such as that of benzophenone, occurs at pKa = −6.41 (16), and protonation of a lactone occurs at the carbonyl rather than the ether oxygen; an electronic shift would convert "structure" I*f* into I*e*. Examination of Fig. 2 reveals that protonation of compound I gives rise to a new peak at 256 mμ, while the doublet at 276–284 coalesces to a single peak at 288 mμ. By comparison, *o*-iodobenzoic acid shows a peak at 285 mμ which, in strong acid, shifts to 328 mμ. Thus, the 288 mμ peak of mono-protonated 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole could be indicative of the presence of a carboxylic acid function (I*e*). However, not enough is known about the spectral characteristics of iodonium compounds to rule out the possible contribution of structure I*d*.

The possibility of a sulfonation reaction appears unlikely, as dilution of the above concentrated sulfuric acid solutions resulted in an absorption spectra identical to that obtained by mild acidification of an aqueous solution.

The second protonation (H_0 = −5.75) may be associated with that of a carbonyl group, leading to structures such as I*g*, I*h*, I*i*, I*j*, or I*l*; loss of water could lead to I*k*, or I*h*. The shift of the absorption band from 288 mμ to 296 mμ is quite small. Structures I*j* and I*l* would contain a protonated lactone oxygen, and are therefore considered far less likely than structure I*i*, which represents a classically protonated carboxylic acid and is resonance stabilized. Although the pKa observed is significantly less than those recorded for other *o*-substituted benzoic acids (−6.78 to −7.78), the effect of an *o*-iodonium function must be taken into consideration. Thus, no conclusion can be made at present on the possible significance of these data on deciding between structures I*i* and I*h* for the doubly protonated species, or between I*e* and I*d* for the singly protonated molecule.

The ionization properties of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole and homologs thus support the heterocyclic nature of these compounds.

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Pharmacokinetic Model for Nalidixic Acid in Man II

Parameters for Absorption, Metabolism, and Elimination

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

The absorption, metabolism, and excretion of nalidixic acid in man is illustrated by a model, and appropriate equations are derived. A total of 7 rate constants are calculated: 4 metabolic constants, 2 excretory constants, and 1 availability constant. All 5 components of the urine compartment and 2 components of the plasma compartment are measured. Comparisons between experimental and calculated values are good.

THE CONSTRUCTION of pharmacokinetic models, their utility in dosage form design, and the mathematical description of the fate of a drug in the body has been adequately treated by Wagner (1, 2), Nelson (3), Levy (4), and others (5).

A complex model illustrating the pharmacokinetic parameters relating to the absorption, metabolism, and elimination of nalidixic acid[1] in man has been presented previously (6). A practical model was developed which enabled calculation of the biologically active and inactive forms as separate groups. Another article by the authors describes a pharmacokinetic

Received September 30, 1965, from the Sterling-Winthrop Research Institute, Rensselaer, N. Y.
Accepted for publication October 27, 1965.
Previous paper: Portmann, G. A., McChesney, E. W., Stander, H., and Moore, W. E., *J. Pharm. Sci.*, **55**, 59 (1966).

[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, New York, N. Y.

model for hydroxynalidixic acid (9) which is an active metabolite.

The present article gives further experimental results which permit the complete pharmacokinetic description of nalidixic acid in man. This model has been applied to blood levels and urinary excretion data obtained from two different tablet formulations.

THEORETICAL

Equations describing the change of each component in Scheme I with respect to time were derived, assuming total absorption and all rate processes to be first order. The recovery of the drug from the urine in terms of all forms detected analytically was found to be 95 ± 2% (± S. E.) indicating essentially total absorption in every case.

The equations used in the present study are listed below. Lag time is defined as the time interval between ingestion of the dosage form and the appearance of nalidixic acid in the plasma. A_0 is equal to the administered dose since complete absorption occurs. Time (t) is measured after the lag time interval. The nalidixic acid (NA) disappearance rate constant (k_d) is equal to the sum of $k_{E_1} + k_{M_1} + k_{M_2}$. The disappearance rate constant (k_{d_2}) for hydroxynalidixic acid (HNA) is the sum of $k_{E_2} + k_{M_3} + k_{M_4}$. The availability rate from the intestinal tract (k_A) is a resultant of such factors as gastrointestinal motility, dissolution rate, etc., and the absorption rate itself.

$$A = A_0 e^{-k_A t} \qquad \text{(Eq. 1)}$$

$$\text{NA-B} = \frac{k_A A_0}{(k_A - k_d)} (e^{-k_d t} - e^{-k_A t}) \qquad \text{(Eq. 2)}$$

$$\text{HNA-B} = \frac{k_{M_1} k_A A_0}{(k_A - k_d)(k_A - k_{d_2})} (e^{-k_{d_2} t} - e^{-k_d t}) - \frac{k_{M_1} k_A A_0}{(k_A - k_d)(k_A - k_{d_2})} (e^{-k_{d_2} t} - e^{-k_A t}) \qquad \text{(Eq. 3)}$$

$$\text{NA-U} = \frac{k_{E_1} A_0}{(k_d - k_A)} (1 - e^{-k_A t}) + \frac{k_{E_1} k_A A_0}{(k_d - k_A) k_d} (e^{-k_d t} - 1) \qquad \text{(Eq. 4)}$$

$$k_{E_1} = \frac{k_d [\text{NA-U}]_\infty}{A_0} \qquad \text{(Eq. 5)}$$

$$\text{HNA-U} = \frac{k_{E_2} k_{M_1} k_A A_0}{(k_A - k_d)(k_d - k_{d_2})} \left(\frac{e^{-k_d t}}{k_d} - \frac{e^{-k_{d_2} t}}{k_{d_2}} \right) - \frac{k_{E_2} k_{M_1} k_A A_0}{(k_A - k_d)(k_A - k_{d_2})} \left(\frac{e^{-k_A t}}{k_A} - \frac{e^{-k_{d_2} t}}{k_{d_2}} \right) + \frac{k_{E_2} k_{M_1} A_0}{k_{d_2} k_d} \qquad \text{(Eq. 6)}$$

$$[\text{HNA-U}]_\infty = \frac{k_{E_2} k_{M_1} A_0}{k_d k_{d_2}} \qquad \text{(Eq. 7)}$$

$$k_{M_2} = \frac{k_d [\text{NAG-U}]_\infty}{A_0} \qquad \text{(Eq. 8)}$$

$$[\text{HNAG-U}]_\infty = \frac{k_{M_3} k_{M_1} A_0}{k_d k_{d_2}} \qquad \text{(Eq. 9)}$$

$$[\text{DA-U}]_\infty = \frac{k_{M_4} k_{M_1} A_0}{k_d k_{d_2}} \qquad \text{(Eq. 10)}$$

$$k_{M_1} = \frac{k_d [\text{HNA} - \text{U} + \text{HNAG} - \text{U} + \text{DA} - \text{U}]^\infty}{A_0} \qquad \text{(Eq. 11)}$$

EXPERIMENTAL

Protocol.—Two tablet formulations,[1] both containing micropulverized nalidixic acid crystals, but with different tablet excipients, were given orally as a 1-Gm. dose (2 Caplets) to 8 subjects according to a crossover design, with 1 week between experiments. The drug was administered with water after overnight fasting. To maintain uniformity of absorption, food, but not water, was withheld until 3 hr. postmedication.

Blood samples were taken at 0, 20, 50, 80, 120, 270, and 420 min. postmedication. A 0- and 24-hr. urine sample was also collected. Previous experiments have shown essentially complete recovery in 24 hr.

Analytical Methods.—Nalidixic and hydroxynalidixic acids may be extracted from biological materials by means of toluene at pH 1–2, as previously described (7). Prior hydrolysis for 1 hr. at this pH releases both compounds from their glucuronide conjugates. It is possible to distinguish between NA and HNA on the basis of their differing extractabilities as a function of pH, as described below.

(*a*) Free (or total) naphthyridine is determined by extracting with toluene at pH 1.2 (0.1 *N* HCl), transfer from toluene to 0.5 *M* borate pH 9, and reading the fluorescence at 325/375 mμ after acidification as described previously (7). The results are calculated on the basis of NA standards carried through the same procedures; the recovery of NA, therefore, is 100%, while that of HNA is 134%. The latter figure is a result of 2 factors: the extractability of HNA under the conditions used in 77%, but, since its fluorescence on a weight basis is 172% of that of NA, a net value of 134% will be obtained for HNA read against NA standards.

(*b*) A second aliquot of the material to be analyzed (1–2 ml.) is diluted as necessary with an aqueous buffer of pH 5.63. (Composition of buffer per liter: *sodium chloride*, 10 Gm.; *potassium acid phosphate*, 12.25 Gm.; basic potassium phosphate, 1.75 Gm.) The solution is extracted with 30 ml. of toluene, of which 25 ml. is extracted with the 0.5 *M* borate, which is assayed in the usual way. Under these conditions the extractability of NA is 5.8 times that of HNA, but the relative fluorescence factor of 1.72 reduces the advantage in favor of NA to 3.4. When read against NA standards carried through the same steps, this method will recover 100% of the NA present, and 33% of the HNA. The following relationship, therefore, exists:

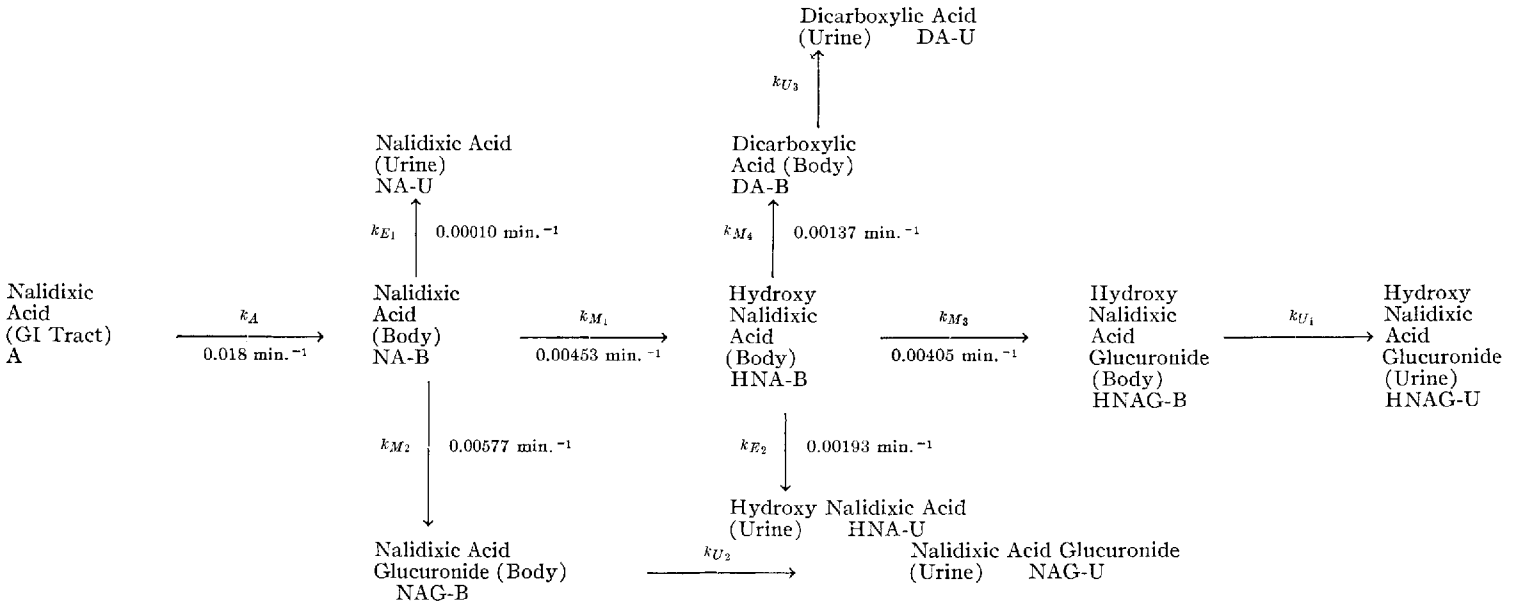
step *1* = 100% NA + 134% HNA

step *2* = 100% NA + 33% HNA

step *1* − step *2* = 101% HNA

For practical purposes, then, step *1* − step *2* is taken simply as equal to the amount of HNA present, and the amount of NA is readily calculated from either *1* or *2*. When applied to the analysis of mixtures of the 2 compounds ranging from 5–40 mcg. of NA and 60–5 mcg. of HNA, the recovery

[1] Negram Caplets, 500 mg./Caplet.



Kinetic pathways of nalidixic acid in man; k_A = availability rate constant; k_{M1} = metabolic rate constants; k_{E1} and k_{U1} = excretion rate constants.

Scheme I

TABLE I.—PARAMETERS FOR NALIDIXIC ACID IN FORMULATIONS A AND B

Subject	% Urinary Recovery A	% Urinary Recovery B	Lag Time, min. A	Lag Time, min. B	k_A (min.$^{-1}$) × 10^3 A	k_A (min.$^{-1}$) × 10^3 B	kd (min.$^{-1}$) × 10^3 A	kd (min.$^{-1}$) × 10^3 B	k_{M1} (min.$^{-1}$) × 10^3 A	k_{M1} (min.$^{-1}$) × 10^3 B	k_E (min.$^{-1}$) × 10^3 A	k_E (min.$^{-1}$) × 10^3 B	k_{M2} (min.$^{-1}$) × 10^3 A	k_{M2} (min.$^{-1}$) × 10^3 B	V_D as % of Body Wt. A	V_D as % of Body Wt. B
A. J.	96	97	35	12	40.5	15.1	12.8	12.8	5.64	4.16	0.35	0.13	6.25	8.15	29.7	45.1
G. B.	[a]	93	[a]	18	[a]	18.2	[a]	11.6	[a]	6.98	[a]	0.04	[a]	3.74	[a]	25.6
D. F.	103	94	47	28	18.3	15.3	13.6	12.8	5.40	4.59	0.15	0.33	8.60	6.81	38.2	40.0
J. R.	96	97	8	10	8.50	9.77	10.5	10.0	2.33	3.42	0.02	0.05	7.70	6.21	26.6	30.8
W. H.	90	97	9	15	25.6	15.3	7.7	6.1	2.20	1.72	0.02	0.03	4.71	4.18	39.2	43.5
R. B.	[b]	[b]	0	36	15.6	0.99%/min.[c]	11.6	12.8	...	...	...	...	...	...	...	...
J. D.	95	83	6	14	0.70%/min.[c]	0.59%/min.[c]	10.0	12.8	5.53	7.53	[d]	[d]	3.98	3.14	29.8	25.8

[a] Incomplete crossover. [b] Incomplete urine collection. [c] Zero order. [d] No detectable NA.

of HNA was 96.5 ± 5% of the amount added, and the recovery of NA was 103 ± 9%. If total NA + HNA is determined following acid hydrolysis in the same way, and the values for free NA and HNA are subtracted, the amounts present as conjugated NA and conjugated HNA may be estimated, NA and HNA being stable to this hydrolysis.

In addition, urine levels of NAG, HNAG, and DA were determined with previously described methods (7).

RESULTS AND DISCUSSION

The Wagner and Nelson method (8) of calculating availability rates of drug to plasma was used, and results (k_A) are shown in Table I. Typical plasma level curves and A/V values are shown in Figs. 1 and 2 for 2 individuals and 2 dosage forms. For these same subjects, the logarithm of the per cent unabsorbed at various times was calculated and is shown in Fig. 3. Lag time is read as the time when 100% is unabsorbed by extrapolating the line to log 2.

Three of 13 observations for availability rates are apparently of the zero-order type. The mean of the 10 first-order availability rates is 0.018 min.$^{-1}$ which is very similar to the mean rate of 0.020 min.$^{-1}$ reported for nalidixic acid in caplet form for 8 subjects (6). The use of total plasma naphthyridine for determining absorption rates (6) results in a small error because practically all of the naphthyridine is in the form of nalidixic acid.

With the exception of W. H., subjects showed little variation in disappearance rates, either among themselves or between dosage forms. Using Eq. 2, with division by V_D to obtain concentration, the theoretical values of NA were calculated for the 2 subjects shown in Figs. 1 and 2. Excellent agreement was obtained between the calculated values and those from the actual plasma level curve.

Because of low HNA plasma levels (Figs. 1 and 2), it is apparent that very little difference would be seen between the disappearance rates of NA and NA + HNA. In fact, in our previous study (6), where active naphthyridine (NA + HNA) was measured in 8 subjects, their apparent average disappearance rate was 0.011 min.$^{-1}$, which is identical to the average rate for NA (Table I).

Individual values for excretion (k_{E_1}) and metabolic (k_{M_1}, k_{M_2}) rate constants were calculated using Eqs. 5, 8, and 11 with the 0–24-hr. urinary data as the infinity values (Table I). Data previously obtained (6) indicated that a 24-hr. urine analysis would represent complete excretion. Rate constants for the formation of NAG (k_{M_2}) are the

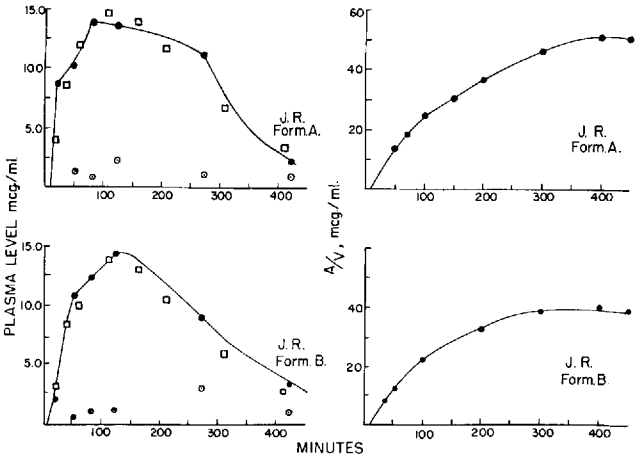


Fig. 1.—Plasma levels and A/V as a function of time. Key: ●, NA; ⊙, HNA; □, calculated values for NA. Initials identify subjects.

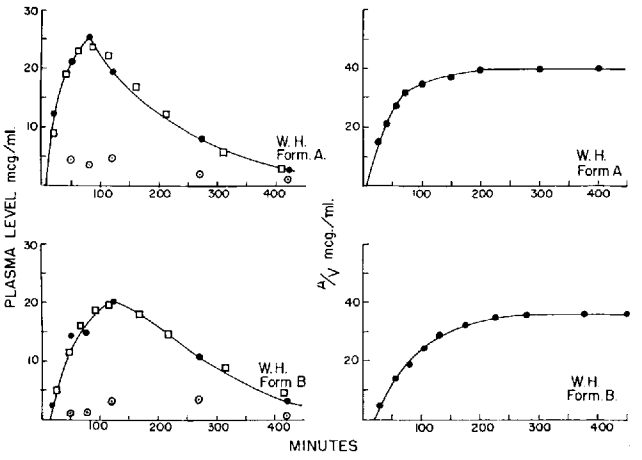


Fig. 2.—Plasma levels and A/V as a function of time. Key: ●, NA; ⊙, HNA; □, calculated values for NA. Initials identify subjects.

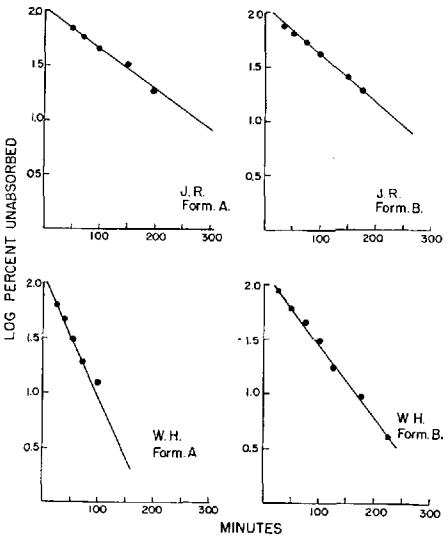


Fig. 3.—Log per cent nalidixic acid unabsorbed as a function of time. Initials identify subjects.

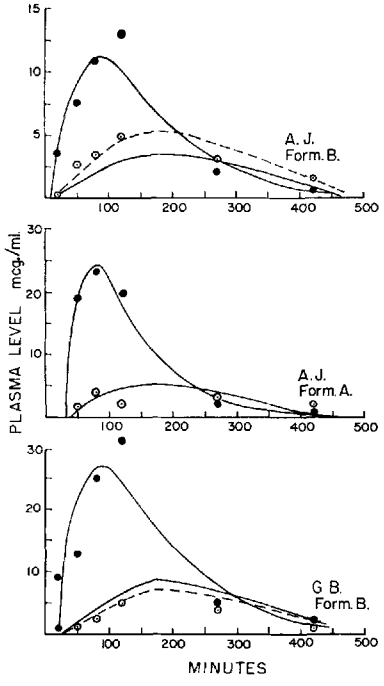


Fig. 4.—Plasma levels as a function of time. Key: experimental ●, NA; experimental ⊙, HNA; —, calculated curve for HNA and NA. (20 min. experimental value for A. J., form A was 0.)

highest of the various constants for disappearance in 4 of 6 subjects. The variation in metabolic rates with each individual between forms A and B is slight compared to the variation among individuals.

Because of the low excretion rate of nalidixic acid and its high metabolic rates, the over-all effect is an extremely small quantity of NA appearing in the urine. In fact, if the active drug is measured in the urine (NA + HNA), the observed apparent excretion rate constant should be essentially that of HNA. The apparent mean excretion rate constant of the active drug (NA + HNA) in the urine has been reported as 0.0015 min.$^{-1}$ ± 0.0003 (18 observations) (6) compared to an average excretion rate constant for HNA of 0.0019 min.$^{-1}$ ± 0.0004 (4 observations) (9).

The excretion of acidic and basic organic compound is sometimes modified greatly as a result of changes in urinary pH usually induced by large doses of sodium bicarbonate or ammonium chloride. McChesney and co-workers (7) have shown that the urinary excretion of active naphthyridine (NA + HNA) is increased with $NaHCO_3$ and decreased with NH_4Cl given simultaneously with nalidixic acid. No attempt was made to control urinary pH in the present study since normal variations in excretion rates were sought.

The apparent volumes of distribution for NA and HNA are inversely proportional to the per cent of plasma protein binding at pH 7.4 in therapeutic concentration ranges. These are 93% for NA and 63% for HNA using equilibrium dialysis techniques.

In order to evaluate the processes beyond the formation of HNA, the results of an experiment in which HNA is given orally (9) are needed. For 2 subjects (A. J. and G. B.) common to both these experiments, their individual disappearance rate constants (k_{d_2}) and apparent volumes of distribution for HNA (9) plus their k_A, k_{M_1}, k_d, and V_D values for NA (Table I) were used in Eqs. 2 and 3 to calculate plasma levels. These results which are shown in Fig. 4 indicate a reasonable agreement with the experimental data for NA.

For HNA, the agreement with experimental data for A. J. (form A) is reasonable, however, for G. B. and A. J. (form B) the general shape of the theoretical curve is good indicating that the rate constants are sufficiently good estimates, but the heights of the curves are too low or too high. The complexity of the HNA system is of such a magnitude that good fits should not be expected with the use of apparent distributive volumes and disappearance rate constants from separate experiments. Good fits can be obtained by only changing the volume of distribution. By increasing the V_D for G. B. from 40.8 to 50.0% of body weight, the height of the plasma level curve is lowered (dashed curve, Fig. 4), producing a good correlation with experimental values. For A. J. (form B), decreasing the V_D from 45.9 to 31% of body weight also gives a good fit with experimental values (dashed curve, Fig. 4).

Assuming average rate constants and using Eqs. 5, 7, 8, 9, and 10, the average urinary excretion values at infinity were calculated. A comparison between these values and the average experimental values is given in Table II. Again a reasonable agreement is apparent.

The plasma level comparison (NA and HNA) plus the good agreement with urinary excretion data indicate that these average rate constants (Table II) will give a good picture of the metabolism and excretion of nalidixic acid and its metabolite (HNA). Such a picture is presented in Figs. 5 and 6 where the changes occurring in plasma and urine

TABLE II.—COMPARISON OF CALCULATED AND EXPERIMENTAL VALUES FOR NALIDIXIC ACID AND METABOLITES

Compd.	Calcd.[a] Urinary Excretion, mg.	Exptl. Urinary Excretion (mg.) Av. ± S.E.
NA-U	9	8 ± 3
NAG-U	517	537 ± 49
HNA-U	105	129 ± 8
HNAG-U	221	229 ± 32
DA-U	74	43 ± 6

[a] Average rate constants are: k_d, 0.01116 min.$^{-1}$; k_{M_1}, 0.00453 min.$^{-1}$; k_{E_1}, 0.00010 min.$^{-1}$; k_{M_2}, 0.00577 min.$^{-1}$; k_{d_2}, 0.00746 min.$^{-1}$; k_{E_2}, 0.00193 min.$^{-1}$; k_{M_3}, 0.00405 min.$^{-1}$; k_{M_4}, 0.00137 min.$^{-1}$.

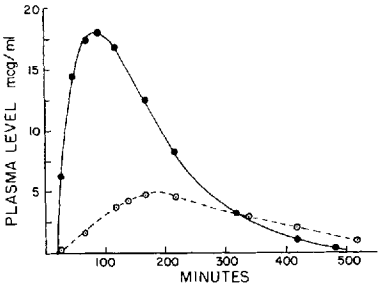


Fig. 5.—Calculated average plasma levels as a function of time. Key: ●, NA; ⊙, HNA.

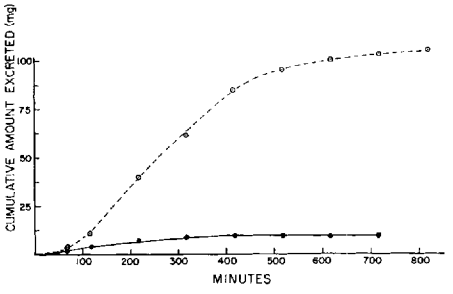


Fig. 6.—Calculated average cumulative urinary excretion as a function of time. Key: ●, NA; ⊙, HNA.

with respect to time for the active components of the model are presented. Average rate constants and V_D's were used in Eqs. 2, 3, 4, and 6 to generate these curves for a 1-Gm. dose of nalidixic acid.

SUMMARY

A differential extraction procedure has been developed which enabled the separate determination of NA and HNA in plasma and urine.

The solution of a pharmacokinetic model for nalidixic acid which involves 5 components in the body and urine compartments has been mathematically described in terms of rate equations. A total of 7 rate constants have been determined: 2 for glucuronide formation, 2 for oxidation, 2 for excretion of NA and HNA, and 1 for the availability of NA. The reasonable agreement between calculated and experimental curves has been illustrated.

APPENDIX

Equations relating the quantities of NAG-U, HNAG-U, and DA-U as a function of time are presented. By letting time (t) approach infinity, equations are obtained which enable the calculation of metabolic and excretion constants without making any assumptions about the relative values of consecutive rate constants.

These equations were derived from the model (Fig. 1) where the rate constants are assumed to be first order and the compartments are defined as the: (a) GI tract, (b) body or apparent volume of distribution, and (c) urine. Definitions of terms are stated under *Theoretical*.

NAG-U =

$$\frac{k_{U_2}k_{M_2}k_A A_0}{(k_A - k_d)(k_d - k_{U_2})}\left(\frac{e^{-k_d t}}{k_d} - \frac{e^{-k_{U_2}t}}{k_{U_2}}\right) - \frac{k_{U_2}k_{M_2}k_A A_0}{(k_A - k_d)(k_A - k_{U_2})}\left(\frac{e^{-k_A t}}{k_A} - \frac{e^{-k_{U_2}t}}{k_{U_2}}\right) + \frac{k_{M_2}A_0}{k_d} \quad \text{(Eq. 4a)}$$

HNAG-U =

$$\frac{k_{M_3}k_{M_1}k_A k_{U_1}A_0}{(k_A - k_d)(k_d - k_{d_2})(k_{U_1} - k_{d_2})}\left(\frac{e^{-k_{U_1}t}}{k_{U_1}} - \frac{e^{-k_{d_2}t}}{k_{d_2}}\right) - \frac{k_{M_3}k_{M_1}k_A k_{U_1}A_0}{(k_A - k_d)(k_d - k_{d_2})(k_{U_1} - k_d)}\left(\frac{e^{-k_{U_1}t}}{k_{U_1}} - \frac{e^{-k_d t}}{k_d}\right) - \frac{k_{M_3}k_{M_1}k_A k_{U_1}A_0}{(k_A - k_d)(k_A - k_{d_2})(k_{U_1} - k_{d_2})}\left(\frac{e^{-k_{U_1}t}}{k_{U_1}} - \frac{e^{-k_{d_2}t}}{k_{d_2}}\right) + \frac{k_{M_3}k_{M_1}k_A k_{U_1}A_0}{(k_A - k_d)(k_A - k_{d_2})(k_{U_1} - k_A)}\left(\frac{e^{-k_{U_1}t}}{k_{U_1}} - \frac{e^{-k_A t}}{k_A}\right) + \frac{k_{M_3}k_{M_1}A_0}{k_d k_{d_2}} \quad \text{(Eq. 5a)}$$

DA-U =

$$\frac{k_{M_4}k_{M_1}k_A k_{U_3}A_0}{(k_A - k_d)(k_d - k_{d_2})(k_{U_3} - k_{d_2})}\left(\frac{e^{-k_{U_3}t}}{k_{U_3}} - \frac{e^{-k_{d_2}t}}{k_{d_2}}\right) - \frac{k_{M_4}k_{M_1}k_A k_{U_3}A_0}{(k_A - k_d)(k_d - k_{d_2})(k_{U_3} - k_d)}\left(\frac{e^{-k_{U_3}t}}{k_{U_3}} - \frac{e^{-k_d t}}{k_d}\right) - \frac{k_{M_4}k_{M_1}k_A k_{U_3}A_0}{(k_A - k_d)(k_A - k_{d_2})(k_{U_3} - k_{d_2})}\left(\frac{e^{-k_{U_3}t}}{k_{U_3}} - \frac{e^{-k_{d_2}t}}{k_{d_2}}\right) + \frac{k_{M_4}k_{M_1}k_A k_{U_3}A_0}{(k_A - k_d)(k_A - k_{d_2})(k_{U_3} - k_A)}\left(\frac{e^{-k_{U_3}t}}{k_{U_3}} - \frac{e^{-k_A t}}{k_A}\right) + \frac{k_{M_4}k_{M_1}A_0}{k_d k_{d_2}} \quad \text{(Eq. 6a)}$$

It is apparent that when time (t) is permitted to approach infinity, the above equations reduce to their last term and give Eqs. 8, 9, and 10 of the text.

Equation 11 of the text is derived from the sum of the expressions for DA-U, HNAG-U, and HNA-U at $t = \infty$.

$$[\text{DA-U}]_\infty + [\text{HNAG-U}]_\infty + [\text{HNA-U}]_\infty = \frac{k_{M_4}k_{M_1}A_0}{k_d k_{d_2}} + \frac{k_{M_3}k_{M_1}A_0}{k_d k_{d_2}} + \frac{k_{E_2}k_{M_1}A_0}{k_d k_{d_2}} \quad \text{(Eq. 7a)}$$

which reduces to

$$[\text{DA-U}]_\infty + [\text{HNAG-U}]_\infty + [\text{HNA-U}]_\infty = \frac{k_{M_1}A_0}{k_d k_{d_2}}(k_{M_4} + k_{M_3} + k_{E_2}) \quad \text{(Eq. 8a)}$$

By definition $k_{d_2} = k_{M_4} + k_{M_3} + k_{E_2}$.

Therefore, Eq. 8a, upon substituting for k_{d_2}

and solving for k_{M_1} reduces to Eq. 11 of the text. This equation permits the calculation of the metabolic rate constant (k_{M_1}) for the oxidation of nalidixic acid to hydroxynalidixic acid.

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Drug Standards

Determination of Free Salicylic Acid in Aspirin and Aspirin Products

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In a previously described method for the determination of free salicylic acid in aspirin, the salicylic acid is isolated on a partition chromatographic column as its purple ferric–phenol complex, using ferric chloride solution as immobile phase. With the addition of a high concentration of urea to the ferric chloride, the method is significantly improved. The separation is more sharply defined, making feasible the analysis of larger samples of aspirin and permitting the use of a more easily prepared chromatographic column.

A PROCEDURE has been described (1) for the isolation and determination of small amounts of salicylic acid which occur in aspirin and aspirin products. The salicylic acid is retained on a Celite:2% ferric chloride partition chromatographic column as its ferric complex while the nonphenolic aspirin is eluted with chloroform. The ferric complex is then dissociated with acetic acid and the free salicylic acid eluted with chloroform.

Several investigators have encountered difficulty with the published method (2). During the elution of the aspirin, the salicylic acid migrates slowly down the column (as evidenced by the position of the purple complex) and spreads out into a diffuse band, which sometimes becomes difficult to discern. Unless the chromatographic column is packed with great uniformity, channeling may occur during both the elution of aspirin and the recovery of salicylic acid.

A radical improvement in the chromatographic separation is achieved with a modified ferric chloride reagent, which contains a high concentration of urea. The band of the ferric–salicylate complex obtained with this reagent is much more deeply colored than that obtained with the simple ferric chloride reagent. The dense, sharply delineated band migrates only very slightly during the elution of aspirin. The use of shorter columns, which do not require extraordinary care in packing, is therefore feasible. Columns prepared with the modified reagent will also accommodate much larger samples of aspirin than those prepared with ferric chloride alone.

Addition of urea to the ferric chloride reagent was suggested from the report of a urea–salicylic acid complex by Bolton (3). It is apparent that the formation of this binary complex does not account for the trapping of salicylic acid by the ferric chloride–urea reagent, however, since urea solutions alone, at any concentration, are completely ineffective in removing salicylic acid from chloroform solution.

Optimum results are obtained with an immobile phase containing 5% ferric chloride and which is 10 *M* with respect to urea. It must be maintained at a pH between 3.1 and 3.3. At lower pH levels the salicylic acid band becomes diffuse and more loosely retained, while at higher levels recovery of salicylic acid from the column may be incomplete using the specified volume of eluant.

The concentration of urea in the reagent must

Received May 19, 1965, from the General Methods Branch, Division of Pharmaceutical Chemistry, Bureau of Scientific Research, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.
Accepted for publication November 4, 1965.