purposes we have found the thermophilic organism much more satisfactory. One is not likely to encounter this type of organism as a laboratory contaminant in the sterility test, thereby causing a false positive reaction. This would necessitate the retesting of the load and delay production.

A very important consideration in the selection of the chemical indicator was to make sure that it did not inhibit the growth of the control organisms. Bacteriostatic-type tests were made using diluted spore suspensions with the paper strips, both before and after exposure to the sterilizing gas. No inhibition was noted and controls from small inocula grew quite luxuriantly in the test media.

The spores used to inoculate the indicator strips were prepared by suspending washed spores of *B. stearothermophilus* in distilled water containing 1% sorbitol. The suspension was standardized to approximately 5 million spores per ml. Spore counts were determined by appropriate dilution of the suspension and plating on trypticase soy agar. Plates were incubated at 60° for 24 hr. Paper strips were inoculated with 0.02 ml. of the suspension or approximately 100,000 spores. After sterilization, strips were transferred to fluid thioglycollate medium and incubated at 60° for 7 days to determine survival of spores. Control tubes containing indicator strips, both before and after exposure to ethylene oxide and inoculated with diluted spore suspension, showed no inhibition of the control organism.

APPLICATION

In commercial lots, dye-spore strips with 100,000 spores are prepared in a size to fit the item being sterilized. They have been prepared in sizes similar to the commercially available spore strips[2] for use in the index finger and in the cuff of rubber gloves, since it is felt that these are the most difficult places for the gas to reach in this item. These controls in the index finger change color, and the organisms are killed even if a knot is tied in the wrist part of the glove, indicating adequate penetration. In the sterilization of commercial lots of gloves,[3] the use of these indicators throughout the sterilizer load give assurance that the entire lot has been sterilized.

REFERENCES

(1) Rubbo, S. D., and Gardner, J. F., "A Review of Sterilization and Disinfection," Lloyd-Luke, London, England, 1965, p. 116.
(2) Epstein, J., Rosenthal, R. W., and Ess, R. J., *Anal. Chem.*, **27**, 1435(1955).
(3) Sawicki, E., *et al.*, *ibid.*, **35**, 1479(1963).
(4) Friedman, O. M., and Boger, E., *ibid.*, **33**, 906(1961).

[2] Kilit spore strips, Baltimore Biological Laboratory, Baltimore, Md.
[3] Wilson Rubber Co., Canton, Ohio.

Pharmacokinetic Model for Nalidixic Acid in Man I

Kinetic Pathways for Hydroxynalidixic Acid

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

A model which includes the parameters of absorption, metabolism, and excretion of hydroxynalidixic acid (HNA) is presented. Individual rate constants for the absorption and disappearance of HNA in 5 subjects were determined. Theoretical plasma-level curves based upon these constants were calculated, and good agreement with the experimental data was obtained. Rate constants for glucuronide formation, oxidation, and excretion of HNA were calculated.

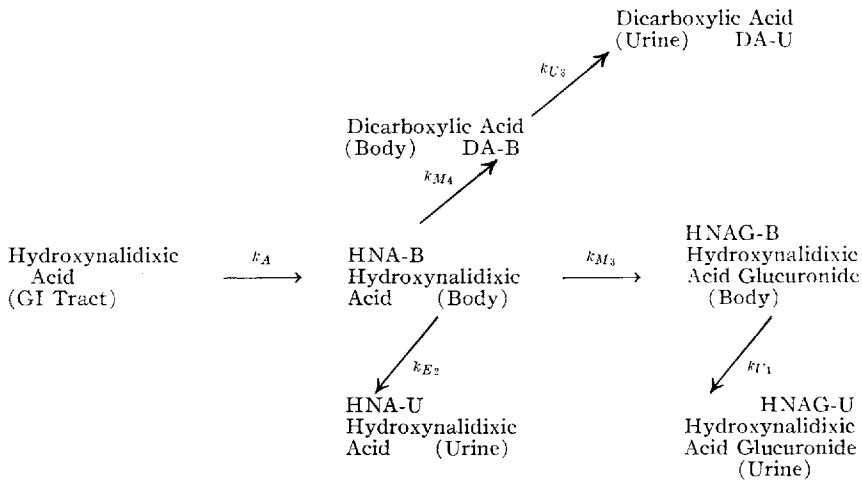
HYDROXYNALIDIXIC acid (1-ethyl-1, 4-dihydro-7-hydroxymethyl-4-oxo-1, 8-naphthyridine-3-carboxylic acid) is 1 of 4 metabolites of nalidixic acid[1] formed in man. It has been isolated and shown to have an *in vitro* antibacterial spectrum similar to that of nalidixic acid (1). The other 3 metabolites, all of which have not shown biological activity, are nalidixic acid glucuronide, hydroxynalidixic acid glucuronide, and the 3,7-dicarboxylic acid.

A previous article by the authors (2) described a simplified working model which permitted calculation of the apparent kinetics of the biologically active and inactive forms of nalidixic acid as separate groups.

Because nalidixic acid has been found to be clinically effective in the treatment of Gram-negative infections (3, 4), more definitive studies of its pharmacokinetics have been continued. These experiments would also illustrate the mechanisms which enabled the simplified model to describe absorption and elimination parameters.

In order to describe the complete pharmacokinetic profile of nalidixic acid, it is necessary to quantitate all the rate processes, including those occurring after its oxidation to the 7-hydroxy derivative. This article deals with a study of the absorption, metabolism, and excretion of hy-

Received September 30, 1965, from the Sterling-Winthrop Research Institute, Rensselaer, N. Y.
Accepted for publication October 27, 1965.
[1] Marketed as NegGram by Winthrop Laboratories, New York, N. Y.



Kinetic pathways of hydroxynalidixic acid in man; k_A = availability rate constant; k_{M_i} = metabolic rate constants; k_{E_2} and k_{U_i} = excretion rate constants. Designations for the rate constants subnumber are those used in the larger model for nalidixic acid (7).

Scheme I

droxynalidixic acid, gives basic information about the compound, and [as will be illustrated (7)] permits a detailed description of the pharmacokinetics of nalidixic acid.

THEORETICAL

The work of McChesney and co-workers (1) leads to the model depicted in Scheme I. This represents the present knowledge of the kinetic pathways for hydroxynalidixic acid in man.

Equations describing the change of each component in Scheme I with respect to time were derived assuming total absorption and first-order rate processes. The total urine recovery was found to be 98.3 ± 2.9% (±S.E.), indicating complete absorption of the drug in every case. Those equations that were 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 hydroxynalidixic 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 disappearance rate constant (k_{d_2}) for hydroxynalidixic acid is taken as the sum of $k_{E_2} + k_{M_3} + k_{M_4}$. The availability rate constant (k_A) from the gastrointestinal tract encompasses many factors, including motility, dissolution rate, and the intrinsic absorption rate.

$$[\text{HNA-B}] = \frac{k_A A_0}{(k_A - k_{d_2})}\left(e^{-k_{d_2}t} - e^{-k_A t}\right) \quad \text{(Eq. 1)}$$

$$k_{E_2} = \frac{k_{d_2}\,[\text{HNA-U}]_\infty}{A_0} \quad \text{(Eq. 2)}$$

$$k_{M_4} = \frac{k_{d_2}\,[\text{HNAG-U}]_\infty}{A_0} \quad \text{(Eq. 3)}$$

$$k_{M_4} = \frac{k_{d_2}\,[\text{DA-U}]_\infty}{A_0} \quad \text{(Eq. 4)}$$

EXPERIMENTAL

Medication and Sampling Protocol.—The sodium salt of HNA was given in hard gelatin capsules

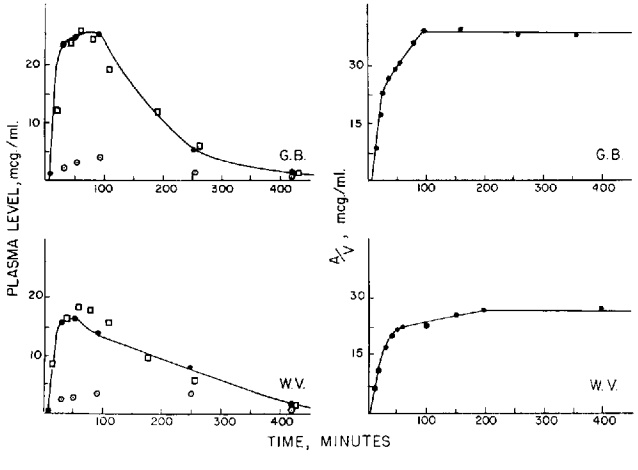


Fig. 1.—Plasma levels and A/V as a function of time. Key: ●, hydroxynalidixic acid; ⊙, hydroxynalidixic acid glucuronide; □, calculated values for hydroxynalidixic acid. Initials identify subjects.

(equivalent to 500 mg. of the acid per capsule) to 5 healthy subjects in a 1-Gm. dose (2 capsules) after overnight fasting. Food, but not water, was withheld until 3 hr. postmedication. Blood samples were taken after 0, 10, 30, 50, 90, 250, and 420 min. after drug administration. Urine collections were made at 0, 8, 24, and 48 hr. after administration of the drug. Plasma and urine concentrations of HNA and HNAG were determined in each sample using a previously published method (1). DA also was determined in the urine samples as previously described (1).

METHODS OF CALCULATION

The rates of availability of HNA to the plasma were determined by the method of Wagner and Nelson (5). Typical plots of A/V values are shown in Fig. 1 and represent the cumulative amount absorbed/apparent volume of distribution as a function of time. Individual disappearance rate constant values (k_{d_2}) were obtained from semilogarithmic plots of plasma hydroxynalidixic acid *versus* time. These rate constants multiplied by the cumulative area under experimental plasma level curves (Fig. 1) at different times, plus the plasma level at each time, gives A/V as defined by

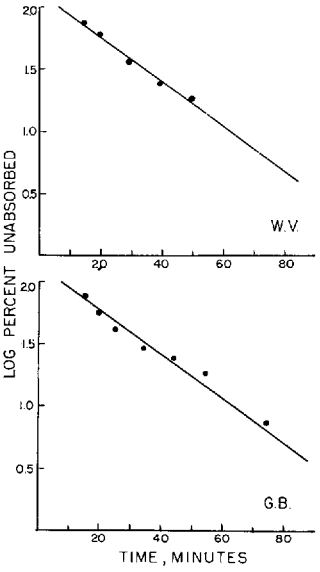


Fig. 2.—Log per cent hydroxynalidixic acid unabsorbed as a function of time. Initials identify subjects.

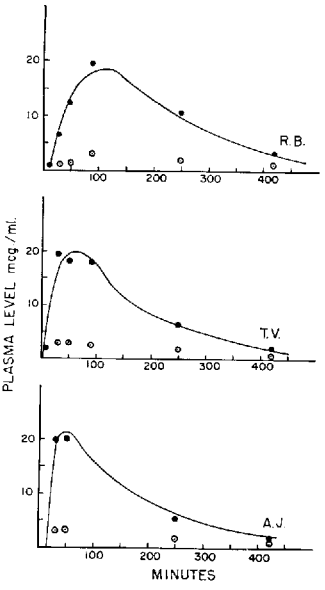


Fig. 3.—Plasma levels as a function of time. Key: ●, experimental hydroxynalidixic acid; ⊙, experimental hydroxynalidixic acid glucuronide; —, calculated curves for hydroxynalidixic acid. Initials identify subjects.

Wagner and Nelson. With these values, the per cent of the drug unabsorbed at different times was calculated. Availability rate and lag time were determined from graphs of log per cent unabsorbed as a function of time as shown in Fig. 2 and as listed in Table I.

Individual values for the excretion rate constant (k_{E_2}) and metabolic rate constants (k_{M_3} and k_{M_4}) were calculated using Eqs. 2, 3, and 4. It was found, as expected, that the 0–48 hr. urinary excretion amounts would represent infinity values for HNA-U, HNAG-U, and DA-U.

From $[A/V]_\infty$ values, the apparent volumes of distribution were calculated and are expressed in Table I as a percentage of body weight.

RESULTS AND DISCUSSION

Theoretical curves of HNA plasma levels *versus* time were calculated using Eq. 1 and the individual parameters. In order to obtain concentration values, HNA-B was divided by the individual's apparent volume of distribution. Excellent agreement was obtained between the theoretical plasma level values based on the model and the experimental values as shown in Figs. 1 and 3. This indicates that small deviations from the first-order rate process (*e.g.*, Fig. 2, G.B.) as measured from the experimental curve are not of great significance.

TABLE I.—KINETIC PARAMETERS FOR SODIUM HYDROXYNALIDIXATE

Subject	% Urinary Recovery	Lag Time, min.	k_A (min.$^{-1}$) × 10^3	k_{d_2} (min.$^{-1}$) × 10^3	k_{E_2} (min.$^{-1}$) × 10^3	k_{M_3} (min.$^{-1}$) × 10^3	k_{M_4} (min.$^{-1}$) × 10^3	Apparent Vol. of Distribution as % of Body Wt.
A.J.	105	13	70	7.37	1.44	4.30	1.99	45.9
G.B.	91	8	41	7.88	2.94	3.50	0.74	40.8
T.V.	97	5	39	7.70	2.39	3.78	1.31	55.5
W.V.	100	6	40	7.00	0.96	4.16	1.44	52.3
R.B.	78[a]	10	17	7.37	...	...	...	41.5
Mean values	98	8	42	7.46	1.93	4.05	1.37	49.0

[a] Incomplete urine collections.

The short lag time of 8 ± 1.5 min. (±S.E.) shows the rapid appearance of the drug in the plasma. That the drug is also rapidly absorbed is apparent from the high values for k_A. Sodium salts of acidic drugs usually have fast dissolution rates (6), and in the case of nalidixic acid (NA), the sodium salt had a shorter lag time and greater availability rate than the acid in capsule form (2). The mean lag time of 8 min. for the sodium salt of HNA (Table I) is very similar to the previously reported mean lag time of 3 min. for the sodium salt of NA (2). Also, the mean availability rate constant for HNA (sodium salt) of 0.042 $min.^{-1}$ is identical to this rate constant for NA (sodium salt) (2). This similarity in lag time and availability rate constant between the sodium salts of NA and HNA is related to their similar pKa, dissolution rate, and chemical structure.

All 5 subjects showed remarkably similar disappearance rate constants (k_{d_2}, range from 7.00–7.88) with a threefold difference in excretion rate constants (k_{E_2}, range from 0.96–2.94). A high excretion rate constant was compensated by a low metabolic rate constant (k_{M_3} and k_{M_4}) and vice versa. This fact could be predicted from the model because of the small variation in disappearance rates.

Glucuronide formation (k_{M_3}) is 2 to 5 times faster than the oxidative formation of the dicarboxylic acid (k_{M_4}). Hydroxynalidixic acid excretion (k_{E_2}) is slower in all cases than the formation of glucuronide (k_{M_3}), and in 2 cases (subjects A. J. and W. V.) slower than the formation of the dicarboxylic acid (k_{M_4}). This high rate of glucuronide formation plus the observed low glucuronide plasma levels (Figs. 1 and 3) indicates that HNAG has a large volume of distribution and/or a rapid excretion rate.

A relatively high apparent volume of distribution is evident for HNA [49.0 ± 3.0% (±S.E.)], indicating that it is distributed in extravascular compartments. The data of McChesney and coworkers (1) of tissue levels in dogs and monkeys showed that NA and HNA have a relatively uniform tissue distribution. This is particularly significant since nalidixic acid is used to treat infections which are localized in tissues.

CONCLUSION

A pharmacokinetic model for hydroxynalidixic acid has been presented and has been shown to be applicable in 5 subjects after oral administration of the sodium salt in capsule form. The good fits of theoretical plasma level curves with the actual data support the definitions of such parameters as lag time, k_A, k_{d_2}, and V_D. Calculated rate constants for excretion and metabolism will be used in a subsequent article in which an extensive model for nalidixic acid will be assumed.

APPENDIX

Expressions relating the amounts of DA-U, HNAG-U, and HNA-U as a function of time are presented. By letting time (t) approach infinity, equations are obtained which enable the calculation of metabolic constants (k_{M_3} and k_{M_4}) and the excretion constant (k_{E_2}) without making any assumptions about the relative values of consecutive rate constants.

These equations were derived from Scheme I 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*.

$$[\text{HNA-U}] = \frac{k_{E_2}k_A A_0}{(k_A - k_{d_2})}\left(\frac{e^{-k_A t}}{k_A} - \frac{e^{-k_{d_2}t}}{k_{d_2}}\right) + \frac{k_{E_2}A_0}{k_{d_2}} \quad \text{(Eq. 1}a\text{)}$$

$$[\text{HNAG-U}] = \frac{k_{U_1}k_{M_3}k_A A_0}{(k_A - k_{d_2})(k_{d_2} - k_{U_1})}\left(\frac{e^{-k_{d_2}t}}{k_{d_2}} - \frac{e^{-k_{U_1}t}}{k_{U_1}}\right) - \frac{k_{U_1}k_{M_3}k_A A_0}{(k_A - k_{d_2})(k_A - k_{U_1})}\left(\frac{e^{-k_A t}}{k_A} - \frac{e^{-k_{U_1}t}}{k_{U_1}}\right) + \frac{k_{M_3}A_0}{k_{d_2}} \quad \text{(Eq. 2}a\text{)}$$

$$[\text{DA-U}] = \frac{k_{U_3}k_{M_4}k_A A_0}{(k_A - k_{d_2})(k_{d_2} - k_{U_3})}\left(\frac{e^{-k_{d_2}t}}{k_{d_2}} - \frac{e^{-k_{U_3}t}}{k_{U_3}}\right) - \frac{k_{U_3}k_{M_4}k_A A_0}{(k_A - k_{d_2})(k_A - k_{U_3})}\left(\frac{e^{-k_A t}}{k_A} - \frac{e^{-k_{U_3}t}}{k_{U_3}}\right) + \frac{k_{M_4}A_0}{k_{d_2}} \quad \text{(Eq. 3}a\text{)}$$

It is apparent that when time (t) is permitted to approach infinity, the above equations reduce to their last terms and give on rearrangement Eqs. 2, 3, and 4 of the text.

REFERENCES

(1) McChesney, E. W., *et al.*, *Toxicol. Appl. Pharmacol.*, **6**, 292(1964).
(2) Moore, W. E., *et al.*, *J. Pharm. Sci.*, **54**, 36(1965).
(3) Carroll, C., *J. Urol.*, **90**, 476(1963).
(4) Barlow, A. M., *Brit. Med. J.*, **2**, 1308(1963).
(5) Wagner, J. G., and Nelson, E., *J. Pharm. Sci.*, **52**, 610(1963).
(6) Levy, G., in "Prescription Pharmacy," Sprowls, J. B., Jr., ed., J. B. Lippincott Co., Philadelphia, Pa., 1963, Chap. 2, p. 69.
(7) Portmann, G. A., *et al.*, *J. Pharm. Sci.*, **55**, 72(1966).