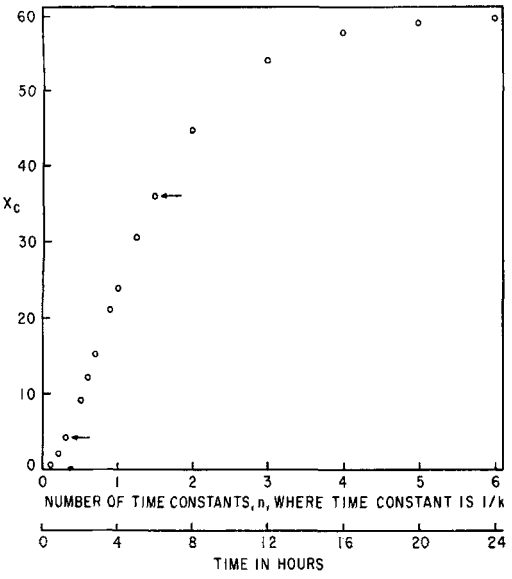

Fig. 9—A plot of the cumulative amount of unchanged drug in the urine, X_C versus time for the model shown as Scheme IV and the constants given in the text.

REFERENCES

(1) Wagner, J. G., and Nelson, E., *J. Pharm. Sci.*, **53**, 1392(1964).
(2) Levy, G., and Hollister, L. E., *ibid.*, **53**, 1447(1964).
(3) Krüger-Thiemer, E., and Bünger, P., *Chemotherapia*, **10**, 61(1965–1966).
(4) Moore, W. E., Portmann, G. A., Stander, H., and McChesney, E. W., *J. Pharm. Sci.*, **54**, 36(1965).
(5) Levy, G., Leonards, J. R., and Procknall, J. A., *ibid.*, **54**, 1719(1965).
(6) Wagner, J. G., "Design and Data Analysis of Biopharmaceutical Studies in Man," presented to the Industrial Pharmacy Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.
(7) Carpenter, O. S., Northam, J. I., and Wagner, J. G., "Antibiotics—Advances in Research, Production and Clinical Use," Herold, M., and Gabriel, Z., eds., Butterworth & Co., Ltd., London, England, 1966, pp. 165–172.
(8) Elliott, H. C., *Proc. Soc. Exptl. Biol. Med.*, **121**, 861(1966).
(9) Nelson, E., and O'Reilly, I., *J. Pharmacol. Exptl. Therap.*, **129**, 368(1960).
(10) Nelson, E., *Antibiot. Chemotherapia*, **12**, 29(1964).
(11) Levy, G., *J. Pharm. Sci.*, **54**, 959(1965).
(12) Wagner, J. G., and Nelson, E., *ibid.*, **52**, 610(1963).
(13) Cummings, A. J., and Martin, B. K., *Biochem. Pharmacol.*, **13**, 767(1964).
(14) Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.*, **56**, 658(1934).
(15) Levy, G., *J. Pharm. Sci.*, **54**, 496(1965).
(16) Nelson, E., Hanano, M., and Levy, G., *J. Pharmacol. Exptl. Therap.*, **153**, 159(1966).
(17) Michaelis, L., and Menton, M. L., *Biochem. Z.*, **49**, 333(1913).
(18) Bartholomay, A. F., "Stochastic Models in Medicine and Biology," Proceedings of a Symposium, Mathematics Research Center, U. S. Army, University of Wisconsin, Madison, Wis., June 1963.
(19) Hollister, L., and Levy, G., *J. Pharm. Sci.*, **54**, 1126 (1965).
(20) Cummings, A. J., Martin, B. K., and Renton, R., *Brit. J. Pharmacol.*, **26**, 461(1966).
(21) Nelson, E., *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 54 (1960).
(22) Bray, H. G., Thorpe, W. V., and White, K., *Biochem. J.*, **48**, 88(1951).
(23) Schachter, D., and Manis, J. G., *J. Clin. Invest.*, **37**, 800(1958).
(24) Cummings, A. J., and Martin, B. K., *Nature*, **200**, 1296(1963).
(25) Butler, T. C., *Federation Proc.*, **17**, 1158(1958).
(26) Cummings, A. J., Martin, B. K., and Park, G. S., *Nature*, **202**, 779(1964).

Pharmacokinetic Model for Nalidixic Acid in Man III

Effect of Repeated Oral Dosage

By E. W. MCCHESNEY, G. A. PORTMANN, and R. F. KOSS

In studies which involved the administration of 1-Gm. doses of nalidixic acid to human volunteers four times daily for a period of 10 days, it has been shown that the resulting plasma levels of nalidixic and of hydroxynalidixic acid could be predicted reasonably well from a model derived from single-dose studies. More satisfactory blood levels are obtained if the doses are taken at least 1 hr. before meals. Repeated dosage results in no important change in the absorption-excretion-metabolism patterns of the drug.

IN PREVIOUS communications (1, 2) a model describing the absorption and elimination of nalidixic acid[1] (NA) in man was presented. This model was derived from observations in seven subjects following the ingestion of single 1-Gm. doses of NA in several physical forms. There remained, however, the question of how well such a model would describe the situation in which the drug is given as a standard therapeutic course, *i.e.*, 1 Gm. four times daily. In particular there was the problem of whether such a dosage regimen would result in a significant carry-over of the drug and its metabolites from one day to another [it was already known (3) that following single 1-Gm. doses the excretion is usually not quite complete within 24 hr.], and whether the repeated administration of 4 Gm. per day would result in significant changes in the way the body handles

Received October 21, 1966, from the Sterling-Winthrop Research Institute, Rensselaer, NY 12144
Accepted for publication January 24, 1967.
The authors thank Dr. D. A. Berberian and Ruth Graham, R.N., for supervision of the medications and collection of the biological materials.
Previous paper: Portmann, G. A., McChesney, E. W., Stander, H., and Moore, W. E., *J. Pharm. Sci.*, **55**, 72(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.

the drug. To answer these questions three separate studies have been conducted as outlined in studies *A*, *B*, and *C*.

Study A—The results of giving 1-Gm. doses four times daily for 3 consecutive days have been determined.

Study B—A comparison of the results obtained when the drug (single 1-Gm. dose) is taken on a full *versus* an empty stomach has been made.

Study C—The results of giving 1-Gm. doses four times daily for 10 consecutive days have been determined, at intervals during the course of this dosage regimen. (*Note:* the daily time schedules for studies *A* and *C* were not identical; see under *Experimental*.)

METHODS OF ANALYSIS

The methods used in the present work have been described (2, 3) and no further details are given here except as noted below.[2]

THEORETICAL

The equation used for calculating the theoretical NA plasma levels following the first 3 doses (in studies *A* and *C*) was that derived by Dost (4) for the case where equal doses are taken at equal time intervals. The average rate constants and apparent volumes of distribution which had been determined previously for another set of seven volunteers (2) were used in making the present calculations. For hydroxynalidixic acid (HNA) the general equation given below was derived by methods similar to those of Dost and of Wiegand *et al.* (5). The model, the basic equations, and the applicable constants have been presented (2). The urinary outputs of NA and of its several metabolic products were calculated in the same manner as the plasma levels.

The elimination rate constants for NA and HNA are designated as k_d and k_{d_2}, respectively. The availability rate constant for NA is designated as k_A, and the rate constant for the oxidation of NA to HNA as k_{M_1}. A_0 represents the total amount absorbed (in this case A_0 = dose administered) and n the number of doses given. The time interval between doses is designated as T, and the time elapsed after the administration of each dose as t. Division of the term HNA-B (that is, HNA in the body) by the apparent volume of distribution gives the plasma concentration:

$$\text{HNA-B} = \frac{k_{M_1}k_A A_0}{k_A - k_d} \times \left[\frac{(k_A - k_d)\, e^{-k_{d_2}t}}{(k_d - k_{d_2})(k_A - k_{d_2})}\left(\frac{1 - e^{-nk_{d_2}T}}{1 - e^{-k_{d_2}T}}\right) + \frac{e^{-k_A t}}{(k_A - k_{d_2})}\left(\frac{1 - e^{-nk_A T}}{1 - e^{-k_A T}}\right) - \frac{e^{-k_d t}}{(k_d - k_{d_2})}\left(\frac{1 - e^{-nk_d T}}{1 - e^{-k_d T}}\right)\right]$$

The average values for the various constants are as follows: k_d = 0.0112^{-1} min.; k_{M_1} = 0.00453 min.$^{-1}$; k_{d_2} = 0.00746 min.$^{-1}$; k_A = 0.0180 min.$^{-1}$; V_d for NA = 34% of body weight; V_d for HNA = 49% of body weight; mean body weight for study *A* = 74 Kg., for study *C* = 65.5 Kg.; lag time = 18 min. There is an almost perfect correlation between the values for V_d and per cent protein-bound: per cent protein-bound NA/HNA = 93/63 = 1.48 (2) V_d(HNA)/V_d(NA) = 49/34 = 1.45.

EXPERIMENTAL

Study A—Four male human volunteers took 1-Gm. doses of NA four times daily for 3 consecutive days, without control of the timing of the meals, or of the amounts eaten. The doses were taken, as 0.5-Gm. tablets, at 8:15 a.m., 1:15 p.m., 6:15 p.m., and at bedtime (about 11:15 p.m.). Oxalated plasma samples were obtained at 8:15 a.m. on each of the 3 days, at 2-hr. intervals thereafter until 4:15 p.m., and at 8:15 a.m. on the fourth day. Urine samples were collected for the interval 8:15 a.m.–4:15 p.m. for the first 4 days, and for the 4:15 p.m.–8:15 a.m. interval of the first 3 nights, (*i.e.*, for 80 hr. following the first dose). The compounds determined in these samples were NA and HNA in both free and ester glucuronide forms.

Study B—Three human volunteers took 1-Gm. doses of NA on an empty stomach, and were provided breakfast 75 min. later, while three other volunteers took 1-Gm. doses immediately after a full breakfast. Three days later the groups of subjects were reversed, to complete the experimental cross-over design. Oxalated plasma samples were obtained from each subject just prior to medication and at intervals of 0.5, 1, 2, and 3 hr. thereafter. These samples were analyzed for free NA and HNA.

Study C—Six human volunteers (four males, two females) took 1-Gm. doses of NA four times daily for 10 consecutive days. On days 1, 7, and 10 a strict schedule of dosage and meals was followed, with the drug being taken at 7:30 and 11:30 a.m., 3:30 and 9:00 p.m., and the meals being eaten at 8:30 a.m., 12:30 p.m., and 6–7 p.m. On the other 7 days the subjects were permitted a wider latitude of choice in the timing of their meals and of the medications, but record cards which they were required to fill out showed that the greatest deviation from the dosage schedule in any case was 1 hr. On days 1, 7, and 10 of this regimen oxalated plasma samples were obtained at 7:30, 9:00, and 11:30 a.m., and at 1:00, 3:30, and 5:00 p.m. Quantitative urine collections were made under toluene for the intervals 7:30 a.m.–4:30 p.m. and 4:30 p.m.–7:30 a.m. of each of these days. The plasma samples were analyzed for free, (*i.e.*, extractable) NA and HNA; the urine samples were analyzed for both free and conjugated NA and HNA, and for the dicarboxylic acid metabolite (3). (Analysis for the last-named is somewhat nonspecific, and is subject to some interference from other drugs which the volunteers may take; *e.g.*, salicylates.)

RESULTS AND DISCUSSION

The data obtained in study *A* are presented in Tables I and II. The plasma levels observed for NA and HNA and their glucuronides are presented in Table I, as are the values for NA and HNA which were calculated from the model (2). Table II pre-

[2] In *Reference 2*, p. 73, the volume ratio of buffer solution (pH 5.63) to toluene was inadvertently not stated. The ratio used is 45/30.

TABLE I—PLASMA LEVELS OF NA AND HNA, FREE AND CONJUGATED, IN FOUR HUMANS TAKING 1 GM. OF NA 4 TIMES DAILY FOR 3 CONSECUTIVE DAYS[a]

Substance Determined[b]	Day of Expt.	hr. After Morning Dose[c] 0	2	4	6	8
NA	1	...	15.6 ± 7.2 (17.3)	13.7 ± 3.4 (6.6)	8.1 ± 5.1 (17.2)	9.6 ± 2.7 (12.0)
HNA	1	...	3.8 ± 1.5 (3.6)	5.0 ± 0.6 (4.7)	3.6 ± 0.7 (3.6)	4.4 ± 2.1 (5.9)
NA-G	1	...	26.5 ± 10.5	9.0 ± 1.3	6.5 ± 2.6	9.6 ± 3.3
HNA-G	1	...	5.9 ± 2.6	2.3 ± 0.8	1.0 ± 0.5	1.2 ± 0.6
NA	2	1.2 ± 0.7[d] (0)	14.5 ± 6.3 (17.3)	11.8 ± 3.4 (6.6)	9.6 ± 4.5 (17.2)	13.2 ± 7.7 (12.0)
HNA	2	1.5 ± 0.5[d] (1.0)	4.9 ± 1.0 (3.9)	6.5 ± 2.3 (4.7)	7.1 ± 4.4 (3.6)	6.4 ± 2.5 (5.9)
NA-G	2	2.1 ± 0.8[d]	10.0 ± 3.2	9.4 ± 0.6	12.4 ± 4.1	9.2 ± 2.4
HNA-G	2	2.1 ± 0.8[d]	2.3 ± 1.7	3.1 ± 1.6	4.7 ± 2.5	1.9 ± 0.8
NA	3	1.5 ± 0.5 (0)	17.8 ± 6.5 (17.3)	9.5 ± 1.8 (6.6)	11.5 ± 6.6 (17.2)	6.2 ± 1.9 (12.0)
HNA	3	2.8 ± 0.8 (1.0)	6.4 ± 1.7 (3.9)	5.8 ± 0.9 (4.7)	8.1 ± 4.3 (3.6)	5.8 ± 2.8 (5.9)
NA-G	3	2.0 ± 0.9	15.8 ± 5.2	9.4 ± 1.3	9.1 ± 3.4	9.4 ± 2.7
HNA-G	3	1.3 ± 0.5	2.3 ± 0.5	1.5 ± 0.6	1.2 ± 0.8	1.2 ± 0.7
NA	4	1.9 ± 1.0 (0)	...	...	...	...
HNA	4	3.4 ± 0.8 (1.0)	...	...	...	...
NA-G	4	4.2 ± 1.8	...	...	...	...
HNA-G	4	1.1 ± 0.2	...	...	...	...

[a] Doses given at 0, 5, 10, and 15 hr. of each day; initial dose given at 8:15 a.m. [b] NA, nalidixic acid; HNA, hydroxynalidixic acid; NA-G and HNA-G, corresponding glucuronides. [c] Values given as mcg./ml. ± S.E.; the calculated values are given in parentheses and were derived from observations on a panel of seven subjects which had one subject common to the present panel (2). [d] Subject who omitted fourth dose on day 1 not included in the means.

TABLE II—URINARY EXCRETION OF NA AND THREE METABOLIC PRODUCTS IN FOUR HUMANS TAKING 1-GM. DOSES OF NA 4 TIMES DAILY FOR 3 CONSECUTIVE DAYS[a]

Substance Determined[b]	Day of Expt.	Interval After Morning Dose[c] 0–8 hr.	8–24 hr.	0–24 hr.
NA	1	−2 ± 7 (13)	−5 ± 8 (22)	−7 ± 14 (35)
HNA		82 ± 51 (130)	255 ± 29 (302)	337 ± 65 (432)
NA-G		485 ± 130	1500 ± 230	1985 ± 310 (2142)
HNA-G		189 ± 80	918 ± 100	1107 ± 35 (911)
Total		754 ± 230	2668 ± 173	3422 ± 130 (3520)
NA	2	1 ± 3 (13)	−5 ± 3 (22)	−4 ± 6 (35)
HNA		66 ± 20 (139)	210 ± 63 (302)	276 ± 49 (441)
NA-G		595 ± 79	1140 ± 229	1735 ± 288 (2142)
HNA-G		267 ± 63	735 ± 194	1002 ± 257 (931)
Total		929 ± 110	2080 ± 215	3009 ± 315 (3549)
NA	3	5 ± 2 (13)	5 ± 3 (22)	10 ± 2 (35)
HNA		70 ± 48 (139)	146 ± 28 (302)	216 ± 42 (441)
NA-G		571 ± 103	1474 ± 217	2045 ± 240 (2142)
HNA-G		330 ± 105	680 ± 97	1010 ± 171 (931)
Total		976 ± 152	2305 ± 139	3281 ± 310 (3549)
NA	4	7 ± 7	—	8 ± 8[d] (105)[d]
HNA		22 ± 5	—	839 ± 135[d] (1314)[d]
NA-G		105 ± 28	—	5941 ± 208[d] (6426)[d]
HNA-G		109 ± 38	—	3191 ± 136[d] (2773)[d]
Total		243 ± 37	—	9979 ± 125[d] (10618)[d]

[a] Doses given at 0, 5, 10, and 15 hr. of each day; initial dose at 8:15 a.m. [b] For definition of terms see Table I. [c] Values given as mg. ± S.E., and in terms of NA equivalents. Figures in parentheses are the calculated values derived from the model. [d] Total for 80 hr.

sents the corresponding urinary excretion data for NA, HNA, and their glucuronides, along with the values for each which were calculated from the model. The results obtained in study *B* are presented in Table III, while the results obtained in study *C* are presented in Tables IV and V. The latter tables are quite comparable to Tables I and II, the principal difference being that Table V includes data on the actual and calculated excretion of the dicarboxylic acid metabolite. The following comments may be made on the data.

There are numerous time schedules which might be followed in taking 1-Gm. doses of any drug four times daily. In the case of a drug which has a half-life of about 60 min., it is especially desirable to use a regimen which will maintain the maximum possible plasma levels over the longest period of time, within the limits of safety. [In this connection it should be noted that the antibacterial spectrum of HNA both *in vitro* and *in vivo* is very similar to that of NA (3, 6, 7); therefore, the biologically effective plasma level is a function of the sum of the concentrations of free NA plus HNA.] As a practical matter, however, there are essentially only two schedules of dosage which are likely to be prescribed: (*a*) 1 Gm. after meals and at bedtime, and (*b*) 1 Gm. between meals and at bedtime. Study *A* was based on the former schedule and the data obtained suggested that, as a result of the absence of any control of the amount of food in the stomach when the doses were taken, some variation in the rate (and possibly completeness) of absorption had occurred. Nevertheless, certain general conclusions could be drawn from the study, as follows.

(*a*) The plasma levels of free NA were generally about equal to those of conjugated NA (Table I). For the 15 separate intervals studied the ratio of free to conjugated NA averaged 1.0 ± 0.3 (S.D.),

TABLE III—PLASMA LEVELS OF NA AND HNA[a] IN MAN AT 4 INTERVALS AFTER INGESTION OF 1-GM. DOSES OF NA

Subject	State of Stomach[b]	Time After Medication, hr. 0.5 NA	0.5 HNA	1.0 NA	1.0 HNA	2.0 NA	2.0 HNA	3.0 NA	3.0 HNA
1	Full	1.5	0.8	4.0	2.3	4.4	3.9	4.4	5.7
2	Full	2.2	0.1	32.5	3.2	16.2	7.8	7.1	6.6
3	Full	0.6	0.2	5.7	0.7	10.1	4.4	17.2	5.4
4	Full	1.1	0.3	7.8	2.1	12.1	3.4	26.7	10.4
5	Full	33.9	1.9	17.6	4.4	11.9	4.4	4.8	4.6
6	Full	0.1	0.1	0	1.1	5.5	0.5	10.4	2.3
Mean	Full	6.6	0.6	11.3	2.3	10.0	4.1	11.8	5.8
S.E.		5.5	0.3	4.9	0.6	1.8	1.0	3.6	1.1
NA/HNA		11.0		4.9		2.4		2.0	
1	Empty	19.5	11.4	13.4	16.4	7.8	15.4	1.7	10.2
2	Empty	9.4	1.6	14.3	3.6	20.8	9.4	9.8	7.0
3	Empty	18.8	4.2	17.8	3.5	15.4	8.8	8.8	7.4
4	Empty	18.6	4.2	24.8	7.6	11.0	8.2	7.0	6.3
5	Empty	18.8	3.4	20.4	5.3	14.8	5.8	5.4	4.1
6	Empty	0.6	0.3	7.6	1.7	11.4	3.5	5.0	3.8
Mean	Empty	14.3	4.2	16.3	6.4	13.5	8.5	6.3	6.5
S.E.		3.2	1.6	2.5	2.1	1.8	1.6	1.2	1.0
NA/HNA		3.4		2.5		1.6		0.97	

[a] Values given as mcg./ml.; zero-hr. blank values (0.1–0.2 mcg./ml.) subtracted. [b] When dose was ingested. The term "full stomach" means that the subjects were allowed as big a breakfast as they wished. The dose was taken immediately after the meal was finished.

TABLE IV—PLASMA LEVELS OF NA AND HNA IN SIX HUMANS TAKING 1 GM. OF NA 4 TIMES DAILY FOR 10 CONSECUTIVE DAYS[a]

Substance Determined[b]	Day of Expt.	hr. After Morning Dose[c] 0	1.5	4	5.5	8	9.5
NA	1	...	12.1 ± 2.6 (21.8)	4.3 ± 0.8 (7.7)	13.5 ± 3.1 (24.5)	10.9 ± 2.3 (8.1)	16.4 ± 5.8 (25.1)
HNA	1	...	5.0 ± 1.5 (2.9)	2.8 ± 0.4 (4.7)	3.7 ± 0.5 (6.8)	7.3 ± 1.2 (6.2)	9.5 ± 1.7 (7.6)
NA	7	1.1 ± 0.7 (0)	21.7 ± 2.8 (21.8)	4.3 ± 0.8 (7.7)	21.0 ± 7.1 (24.5)	7.9 ± 1.2 (8.1)	32.2 ± 6.1 (25.1)
HNA	7	4.5 ± 0.6 (0.9)	12.2 ± 1.6 (3.2)	8.2 ± 1.3 (4.7)	8.4 ± 1.8 (6.8)	8.5 ± 1.3 (6.2)	17.0 ± 1.9 (7.6)
NA	10	1.1 ± 0.2 (0)	17.5 ± 2.9 (21.8)	3.6 ± 1.0 (7.7)	32.2 ± 3.2 (24.5)	6.6 ± 2.0 (8.1)	24.2 ± 4.1 (25.1)
HNA	10	2.9 ± 1.0 (0.9)	11.9 ± 4.1 (3.2)	5.8 ± 1.1 (4.7)	12.6 ± 3.6 (6.8)	10.8 ± 1.9 (6.2)	14.6 ± 2.4 (7.6)

[a] Doses taken at zero (7:30 a.m.), 4, 8, and 13.5 hr. each day. The subjects had breakfast at 1 hr., lunch at 5 hr., and dinner at approximately 11 hr. of each day, based on this time schedule. [b] NA, nalidixic acid; HNA, hydroxynalidixic acid. [c] Values given as mcg./ml. ± S.E.; those values given in parentheses were calculated on the basis of rate constants derived from a panel which included one of the same subjects (2).

and no individual subject showed any consistent change in these ratios as related to time of day or the number of days on medication.

(*b*) There was a small but definite carry-over of naphthyridine in the plasma from one day to another, although the equations derived from the earlier single-dose studies (see under *Theoretical*) suggested that the only carry-over should be a small amount of HNA. The mean carry-over at 24 hr. in all forms determined, for example, was 6.9 mcg./ml.; at 48 hr. it was 7.6 mcg./ml. and at 72 hr. it was 10.6 mcg./ml. Free naphthyridine represented about half of the carry-over and in this category HNA predominated over NA in a ratio of about 3/2 (Table I).

(*c*) The urinary output of free naphthyridine (NA + HNA) per hour was greatest in the 8–24-hr. period of the first day, but otherwise fluctuated according to no obvious pattern. The per hour outputs of this item for the seven successive periods of observation were: 10.0, 15.6, 8.5, 12.8, 9.5, 9.4, and 3.7 mg. The 8–24-hr. period of the first day was also characterized by the largest output of total naphthyridine, for any of the periods studied. The ratio of free naphthyridine to total naphthyridine also varied from one period to another, from a low of 0.065 to a high of 0.119, but according to no consistent pattern.

(*d*) With allowance for the excretion of 4% of the dose in the feces (3) and 8% as the dicarboxylic acid[3] (2), the daily urinary output of total naphthyridine should have averaged 3540 mg. This rate of excretion was not achieved for any one of the 3 days studied, reflecting the fact that in two of the subjects the over-all recoveries were only 84 and 93% of the expected amounts. The subject who gave the lowest total recovery of naphthyridine in the urine also generally gave the lowest plasma levels. (NA + HNA = 29.4 mcg./ml. at 4 hr. and 27.2 mcg./ml. at 26 hr.; otherwise there was no level above 18. The expected peak level for a subject of his body weight, following a single 1-Gm. dose of NA would be about 32.) This subject, considered reliable, was very positive that he had taken all of the doses.

The reasons for the somewhat erratic results obtained in study *A* were revealed in part in study *B*,

[3] In terms of NA equivalents an output of 8 mg. as dicarboxylic acid would be reduced to 7 mg.

TABLE V—URINARY EXCRETION OF NALIDIXIC ACID AND FOUR METABOLIC PRODUCTS IN SIX HUMANS TAKING 1-Gm. DOSES OF NA 4 TIMES DAILY FOR 10 CONSECUTIVE DAYS[a]

Substance Determined[b]	Day of Expt.	Interval After Morning Dose[c] 0–9 hr.	9–24 hr.	0–24 hr.
NA	1	9 ± 7 (17)	18 ± 4 (20)	27 ± 6 (35)
HNA		180 ± 58 (156)	249 ± 48 (280)	429 ± 53 (432)
NA-G		716 ± 65	1145 ± 68	1861 ± 112 (2142)
HNA-G		393 ± 108	862 ± 89	1255 ± 176 (911)
DCA		78 ± 15	165 ± 43	244 ± 51 (320)
Total		1376 ± 117	2439 ± 160	3816 ± 148 (3840)
NA	7	16 ± 5 (17)	15 ± 5 (20)	31 ± 8 (35)
HNA		229 ± 42 (166)	253 ± 46 (280)	482 ± 94 (442)
NA-G		1152 ± 191	1176 ± 91	2328 ± 251 (2142)
HNA-G		528 ± 122	708 ± 158	1236 ± 151 (933)
DCA		84 ± 25	148 ± 39	232 ± 36 (328)
Total		2009 ± 200	2300 ± 91	4309 ± 249 (3880)
NA	10	0 ± 4 (17)	9 ± 3 (20)	9 ± 7 (35)
HNA		189 ± 79 (166)	248 ± 74 (280)	437 ± 71 (442)
NA-G		789 ± 85	1122 ± 156	1911 ± 132 (2142)
HNA-G		471 ± 83	769 ± 98	1240 ± 107 (933)
DCA		104 ± 25	166 ± 70	270 ± 63 (328)
Total		1553 ± 178	2314 ± 218	3867 ± 90 (3880)

[a] Doses taken at 0, 4, 8, and 13.5 hr. of each day (zero hr. = 7:30 a.m.). The subjects had breakfast at 8:30 a.m., lunch at 12:30 p.m., and dinner at approximately 6:30 p.m. each day on which samples were to be taken. One subject failed to take the fourth dose on the first day and is not included in the means for that day. [b] For definition of terms see Table I; DCA, dicarboxylic acid. [c] Values given as mg., in terms of NA equivalents.

which showed definitely that the presence of food in the stomach delays the absorption of NA. In the "full stomach" part of the experiment, one subject gave a peak plasma level at 0.5 hr., and another subject at 1 hr., but in half of the subjects the 3-hr. level was the highest observed (Table III). In contrast, when in the postprandial state the mean plasma level for the same six subjects at 0.5 hr. postmedication exceeded any which was attained when they ingested the dose immediately after a full breakfast, and in this state only one of the subjects reached his peak level as late as 2 hr. postmedication.

In study *C* the expected urinary excretion rate of about 3860 mg./day (including the dicarboxylic acid) was fully realized, as is evident in Table V. The mean outputs of the items determined, as per cent of the total amount excreted, were approximately as follows, for the 3 days studied: conjugated NA, 51; conjugated HNA, 31; free HNA, 11; dicarboxylic acid, 6.5; free NA, 0.5 (all values given in terms of equivalents of NA). On the basis of the single-dose studies (2) the expected partition of these excretory products, listed in the same order, was: 56, 24, 11, 8, 1. Thus, the only important difference between the present and the earlier set of values would be the shift of about 6% of the output from the conjugated NA to the conjugated HNA fraction. This shift was also reflected in the smaller than expected output of free NA. No consistent change in the fraction of the total output represented by any of these compounds becomes evident in a comparison of the results for days 1, 7, and 10. For example, free HNA remained constant throughout at 11.2% of the total, while conjugated NA increased from 48.7 to 54% and then decreased to 49.4% on day 10, and conjugated HNA decreased from 32.9 to 28.6% on day 7 and then increased again to 32.1% on day 10. Meanwhile the per cent of the output represented by DCA varied within the narrow limits of 5.2 and 7%.

With regard to the plasma level curves for the 3 days which were studied, the following generalizations may be made.

(*a*) On the first day the levels for NA deviated considerably from the expected course. Generally the observed values were lower than the calculated, one possible reason being that as a result of the very rapid absorption-elimination kinetics of the drug, the true peak level for each individual could only have been established by multiple blood sampling at times around the expected peak. Practical considerations rendered it impossible to do this. Other contributing factors might be the small number of subjects used relative to the complexity of the pharmacokinetic model, and the fact that only one of the experimental subjects was included in the panel from which the model was derived. The last-named option was deliberate, in the hope that the results obtained on the panel would be sufficiently representative to permit a prediction of what would be observed in the general population. This hope was only partially realized in the present experiments, but in kinetic studies such as those of Engberg-Pedersen (8) and of Krüger-Thiemer and Bunger (9) the possibility of predicting plasma levels of a chemotherapeutic agent from a kinetic model has been well established.

(*b*) Also on the first day, the observed values for HNA never coincided exactly with the calculated values, although the means of the two sets of figures were about equal. In general, therefore, the expected peaks and valleys developed, but these peaks and valleys did not invariably occur at the predicted times, for reasons set forth above.

(*c*) On the seventh and tenth days of the experiment the observed values for NA followed the calculated path quite closely.

(*d*) On the seventh and tenth days of the experiment the observed values for HNA exceeded the calculated values by rather large amounts (exception: day 10, 4 hr.). This discrepancy was due in part to the fact that the daily lows for HNA were considerably higher than the calculated 0.9 mcg./ml. However, in each of the six subjects the carryover of HNA from day 9 was actually less than that from day 6, indicating that there was not a progres-

sive accumulation of the drug in the plasma. For days 7 and 10 the overnight lows for NA in the six subjects ranged from 0.1 to 1.7 mcg./ml., and those for HNA ranged from 1.6 to 12.1 mcg./ml.[4]

(*e*) HNA represented a more constant constituent of the plasma than NA. Evidence for this is the fact that at practically every low point in the NA curves, the level of HNA was higher than that of NA, and *vice versa*. Thus, following each dose of NA the ratio NA/HNA increased to a peak of about 3/1, and then gradually decreased, so that by the time the next dose was taken the ratio was less than 1. (*Note:* at the equilibrium high the calculated plasma levels for subjects of the average weight used in this study would be: NA = 25 mcg./ml.; HNA = 7.5 mcg./ml.)

SUMMARY

The absorption and elimination of nalidixic acid have been studied in subjects receiving the drug four times daily on two different dosage schedules: "after meals" and "between meals." More satisfactory and predictable results were obtained when the doses were given at least 1 hr. before meals, rather than at variable times after meals. Plasma levels and excretion patterns of nalidixic and hydroxynalidixic acids resulting from the administration of 1 Gm. of the former four times daily for 10 days followed quite closely a model derived from single-dose studies. This dosage regimen did not result in any important change in the characteristics of the absorption, excretion, or metabolism of the drug, as based on a comparison of the data for the first, seventh, and tenth days of medication. The multiple dosage regimen resulted in some carry-over of naphthyridine in the plasma from one day to another, but the carry-over did not appear invariably to increase or decrease with the number of days on medication. Sensitivity of the plasma levels to small changes in the rate constants has been shown. The inverse relationship of plasma levels and body weights suggests that dosage should be adjusted to individual body weights, with the initial adult dosage regimen being approximately 15–16 mg./Kg. four times daily.

[4] Since the number of metabolic and excretion processes involved in the over-all picture is considerable, relatively small changes in the rates of several of these could grossly alter the overnight values. For example, a 30% decrease in the mean elimination rate constant for HNA (k_{H2}) would increase the overnight low from 0.8 to 1.9 mcg./ml., and would increase the 9.5-hr. value from 7.6 to 10 mcg./ml. A 1.5-fold increase in the average rate constant for NA → HNA (k_{M1}) without a corresponding change in k_d would increase HNA 1.5 times. Such differences in rate constants have been observed (2).

REFERENCES

(1) Moore, W. E., Portmann, G. A., Stander, H., and McChesney, E. W., *J. Pharm. Sci.*, **54**, 36(1965).
(2) Portmann, G. A., McChesney, E. W., Stander, H., and Moore, W. E., *ibid.*, **55**, 72(1966).
(3) McChesney, E. W., Froelich, E. J., Lesher, G. Y., Crain, A. V. R., and Rosi, D., *Toxicol. Appl. Pharmacol.*, **6**, 292(1964).
(4) Dost, F. H., "Der Blutspiegle," Arbeitsgemeinschaft Medizinische Verlag, G. M. B. A., Leipzig, Germany, 1953, pp. 41, 254.
(5) Wiegand, R. G., Buddenhagen, J. D., and Endicott, C. J., *J. Pharm. Sci.*, **52**, 268(1963).
(6) Lesher, G. Y., Proceedings of the 3rd International Congress of Chemotherapy, Stuttgart (July 23–27, 1963), G. Thieme Verlag, Stuttgart, Germany, 1964.
(7) Goss, W. A., and Deitz, W. H., *Bacteriol. Proc.*, **1963**, 93.
(8) Engberg-Pedersen, H., Morch, P., and Tybring, L., *Brit. J. Pharmacol.*, **23**, 1(1964).
(9) Krüger-Thiemer, E., and Bunger, P., *Chemotherapia*, **10**, 129(1965).

Effects of the Ratio of Calcium to Potassium in the Nutrient Medium on the Growth and Alkaloid Production of *Atropa belladonna*

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The increase of calcium/potassium ratios in nutrient solutions reduces growth of belladonna plants. This is evident in the reduced elongation of all stems, particularly the sympodial flowering branches, and in the fresh and dry weights of all plant parts. There appears to be a concurrent increase in the proportion of leaf to total plant at the expense of stem and root. The increase in calcium/potassium ratio results in higher yields of total nitrogen and alkaloids.

THE IMPORTANCE of calcium and potassium in the nutrition of plants is well established (1–4). The authors' interest was directed to the effect of varying ratios of calcium/potassium concentration on the metabolic reactions in the alkaloid-producing plant *Atropa belladonna* L. The influence of the concentration of one of these cations on the other has been expressed in connection with: (*a*) their being bound on soil particles (5–7), (*b*) their absorption from the soil solution by roots (7), (*c*) their translocation through the plant (8), and (*d*) the rate of a number of metabolic reactions within the plant (9, 10).

Brown (11) reported that interactions involving nutrient elements may directly or indirectly affect all of the major metabolic pathways in plants and animals. The ratio of iron/phosphorus may drastically alter the ability of cells to grow by controlling oxidative mechanisms.

Received August 15, 1966, from the Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois, Chicago, IL 60680
Accepted for publication February 16, 1967.