Metabolism of Isosorbide Dinitrate in the Isolated Perfused Rabbit Lung

PHILIP R. MAYER*, WILLIAM C. LUBAWY, PATRICK J. MCNAMARA, and HARRY B. KOSTENBAUDER ˣ

Received February 1, 1982, from the *College of Pharmacy, University of Kentucky, Lexington, KY 40536.* Accepted for publication June 25, 1982. *Present address: School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.

Abstract □ The uptake and metabolism of isosorbide dinitrate was investigated in the recirculating isolated perfused rabbit lung and in lung homogenate 9000×*g* supernatant. Concentration *versus* time profiles from the isolated lung experiments indicate rapid metabolism of isosorbide dinitrate and corresponding increases in the metabolites 5-isosorbide mononitrate, 2-isosorbide mononitrate, and isosorbide. The data suggest that the mononitrates formed in the lung tissue were converted to isosorbide at an extraordinarily high rate. Surprisingly, the rate of appearance of completely denitrated isosorbide was greater when isosorbide dinitrate was administered to the lung than when the mononitrate metabolites of isosorbide dinitrate were administered. The results suggest rapid metabolism of a substantial portion of the mononitrates formed endogenously from isosorbide dinitrate before partitioning of mononitrates into the perfusion medium could occur. The metabolism of isosorbide dinitrate in lung homogenate 9000×*g* supernatant exhibited a metabolic scheme kinetically different from the intact lung studies, as isosorbide was formed slowly from a mononitrate intermediate and not by a near-simultaneous cleavage of both nitrate ester groups. Intravascular multiple-dose studies did not demonstrate any inhibition between isosorbide dinitrate and the mononitrates.

Keyphrases □ Isosorbide dinitrate—metabolism in isolated perfused rabbit lung, kinetic profiles □ Metabolism—of isosorbide dinitrate and metabolites in isolated perfused rabbit lung, kinetic profiles □ Metabolites—of isosorbide dinitrate, kinetic profiles in isolated perfused rabbit lung

The lung has been evaluated previously for many functions other than the physiological exchange of gases. The lung is now known to absorb many drugs, filter the air and blood, and serve in fibrinologic, endocrinologic, and metabolic capacities (1, 2). Uptake of a drug into the lung may be by passive diffusion (3) or carrier-mediated transport (4) and may have reversible, competitive, or saturable components (5). The lung can metabolize efficiently many biologically active substances once they are distributed in the lung (2, 3, 6).

Isosorbide dinitrate is a vasodilator used primarily in the treatment of angina pectoris and congestive heart failure (7, 8). Organic nitrates including isosorbide dinitrate are metabolized extensively by the enzyme glutathione organic nitrate reductase (9, 10), and their oral effectiveness has been questioned due to extensive first-pass liver metabolism (11, 12). The objective of this study was to investigate the uptake and metabolism of isosorbide dinitrate in the isolated perfused rabbit lung, and to evaluate in a preliminary manner this organ as a route of administration.

EXPERIMENTAL

Materials—Randomly labeled [^{14}C]isosorbide dinitrate (168 mCi/mmole)[1] was purified by the chromatographic procedure described in *Analytical Methods* and extracted from the silica gel with methanol. A working solution of >96% purity was obtained and checked at 6-month intervals. Radiolabeled metabolites were recovered in the same manner. Nonlabeled isosorbide dinitrate was obtained as a 25% lactose mixture[2], and the pure compound was recovered by extraction with ethyl acetate and evaporation of the solvent. Nonlabeled 5-isosorbide mononitrate[3], 2-isosorbide mononitrate[4], and isosorbide[5] were used without further purification. All other solvents and reagents were reagent grade.

Isolated Perfused Rabbit Lung Experiments—The isolated lung preparation has been modified slightly from procedures described previously (6, 13). The isolated lung was perfused at 225 ml/min by 100 ml of an artificial medium prepared the day of or day before an experiment. The perfusion medium was composed of Krebs–Henseleit bicarbonate buffer (500 ml) with 4.5% bovine serum albumin (22.5 g)[6] and 5 mmoles of glucose (90 mg)[7]. The pH, adjusted to 7.4, was maintained during the experiment by a constant 0.005-ml/min infusion of a 1.0 meq/ml sodium bicarbonate solution containing 10% glucose and by appropriate adjustments in an air–carbon dioxide ventilating mixture supplied to the lungs.

For intravascular bolus administration, the compounds were presented to the lung by addition to the upper reservoir. Generally ~8 μCi of the compound was added in each study, and supplementary unlabeled compounds were added to achieve the required dose. Doses ranged from 10^{-7} to 10^{-5} mole for isosorbide dinitrate and were 10^{-6} mole for the mononitrates. Each experiment was performed in duplicate. One-milliliter perfusion medium samples were withdrawn from the upper reservoir at appropriate intervals during the 1–2 hr experiment. In the multiple-dose studies, a second (10^{-7}-mole) dose of isosorbide dinitrate was given 60 min after the first (10^{-5}-mole) dose, with subsequent metabolism followed for an additional 60 min.

Lung 9000×*g* Homogenate Experiments—The lungs of New Zealand rabbits were separated from all extraneous tissue, weighed, and

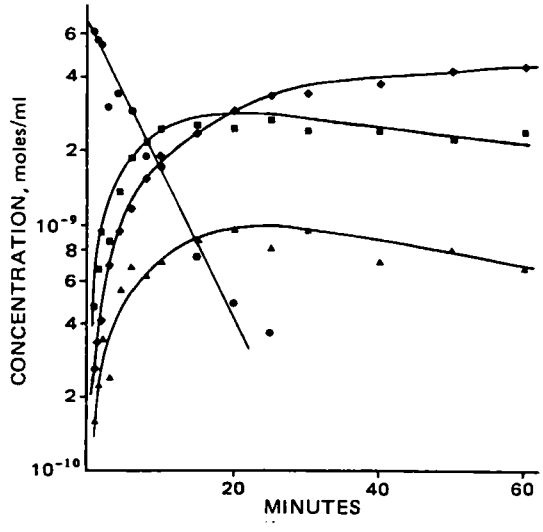
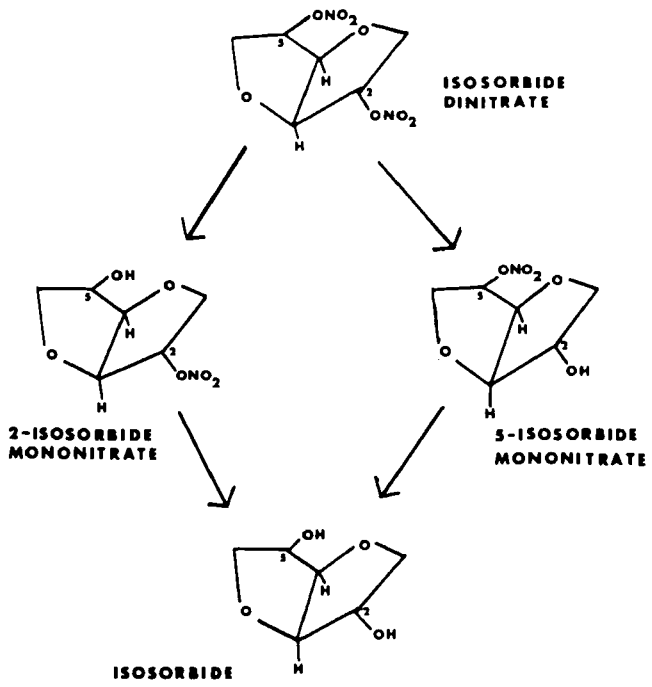


Figure 1—*Semilogarithmic perfusion medium concentration* versus *time profiles following a 10^{-6}-mole iv administration of isosorbide dinitrate. Key: (●) isosorbide dinitrate; (■) 5-isosorbide mononitrate; (▲) 2-isosorbide mononitrate; (◆) isosorbide. Lines are visual aids only.*

[1] New England Nuclear, Boston, Mass.

[2] Lot D-40-177, Wyeth Laboratories, Philadelphia, Pa.
[3] Lot 3369-17-2, Wyeth Laboratories, Philadelphia, Pa.
[4] Lot 82-1, Wyeth Laboratories, Philadelphia, Pa.
[5] Riches-Nelson, Greenwich, Conn.
[6] Rhesus Chemical Co., Phoenix, Ariz.
[7] Eastman Organic Chemicals, Rochester, N.Y.

0022-3549/83/0700-0785$01.00/0



Scheme I—Pathways of isosorbide dinitrate metabolism.

added to a beaker containing two volumes of 0.04 *M* phosphate buffer, pH 7.4. The lungs were homogenized and centrifuged at 9000×*g* for 15 min in a centrifuge kept at 0°. For the incubations, 24 separate culture tubes were arranged containing 10^{-9} mole of isosorbide dinitrate, 5-isosorbide mononitrate, or 2-isosorbide mononitrate to allow a more precise quantitation of each metabolic step. Reduced glutathione[8] and sodium cyanide[9] were added in quantities of 2×10^{-9} moles each, according to the method of Needleman and Krantz (9). The experiment was initiated by the addition of 0.5 ml of the 9000×*g* supernatant. The culture tubes were placed in a shaker incubator kept at 37°, and one culture tube containing each compound was removed at every time interval for analysis. A control experiment was conducted to measure the hydrolysis of isosorbide dinitrate in the presence of glutathione, cyanide, and phosphate buffer. Each experiment was performed in duplicate.

Analytical Methods—The perfusion medium and homogenate samples were extracted with 10 ml of ether, and the organic phase was evaporated to dryness in a water bath not allowed to exceed 40°. Residues were reconstituted in 100 μl of methanol; half of this solution was streaked across 250-μm silica gel TLC plates[10]. Chromatograms were developed 15 cm with dichloromethane–ethyl acetate (4:1). A radiochromatogram scanner[11] was used to locate the radioactive zones on the TLC plates corresponding to isosorbide dinitrate, 5-isosorbide mononitrate, and 2-isosorbide mononitrate, which had R_f values of 0.63, 0.19, and 0.32, respectively. Appropriate bands were scraped into a scintillation vial[12] and dispersed with 15 ml of a scintillation gel prior to counting in a liquid scintillation counter[13]. The gel was composed of 112 g of naphthalene, 14 g of 2,5-diphenyloxazole (PPO), and 0.7 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)-benzene] (dimethyl POPOP) dissolved in 1400 ml of a mixture of xylene (1 part), dioxane (3 parts), and ethylene glycol monoethyl ether (3 parts), followed by the addition of 84 g of a thixotropic gelling agent[14]. All samples were corrected for quenching by an external standardization method.

Isosorbide was measured by suspending 0.1 ml of the perfusion medium remaining after the ether extraction in a toluene–ethoxyoctylphenol[15] scintillation cocktail (6). To confirm that only isosorbide was measured, perfusion medium samples from a lung experiment were streaked on TLC plates and developed with a solvent system of 2-propanol–ammonium hydroxide (4:1) (14). The plates were scanned, scraped, and counted in 15 ml of the silicon dioxide cocktail. Unlabeled, authentic isosorbide was visualized by spraying the plates with a reagent composed of sodium metaperiodate, potassium permanganate, and sodium carbonate (15). Isosorbide concentrations found in the 2-propanol–ammonium hydroxide TLC system corresponded within 15% to those seen by direct measurement of the 0.1 ml postextraction perfusion-medium sample.

[8] Sigma Chemical Co., St. Louis, Mo.
[9] Fisher Scientific Co., Pittsburgh, Pa.
[10] Analtech Inc., Newark, Del.
[11] Model 7201, Packard Instrument Co., Downers Grove, Ill.
[12] Research Products International, Elk Grove Village, Ill.
[13] Model 2425, Packard Instrument Co., Downers Grove, Ill.
[14] Cabosil, Research Products International, Elk Grove Village, Ill.
[15] Triton X-100, Research Products International, Elk Grove Village, Ill.

Table I—Pharmacokinetic Parameters Obtained After Intravascular Administration of Isosorbide Dinitrate to Isolated Perfused Lung

Dose, moles	AUC, moles min/ml	*CL*, ml/min	$k_{\text{Isosorbide Dinitrate}}$, min^{-1}	Half-life, min
10^{-5}	1.1×10^{-6}	8.8	0.061	11.4
10^{-5}	1.2×10^{-6}	8.4	0.054	12.8
10^{-6}	5.2×10^{-8}	19.0	0.118	5.9
10^{-6}	6.0×10^{-8}	16.6	0.116	6.0
10^{-7}	5.0×10^{-9}	20.1	0.128	5.4
10^{-7}	3.6×10^{-9}	27.6	0.149	4.7

RESULTS AND DISCUSSION

The perfusion medium concentrations obtained after a 10^{-6}-mole iv dose of isosorbide dinitrate are shown in Fig. 1. The metabolites (5- and 2-isosorbide mononitrate) show increasing perfusion medium levels which peak after 40 min and have a slight decline when there is virtually no isosorbide dinitrate left to be converted. The 2-isosorbide mononitrate levels are only half those attained by the 5-isosorbide mononitrate, probably due to the stereoselective cleavage of the 2-nitrate group (Scheme I). The isosorbide concentration shows the same rapid increase, but no tendency to decline at the end of the experiment.

Similar concentration *versus* time profiles were obtained for the 10^{-5}- and 10^{-7}-mole doses of isosorbide dinitrate. Pharmacokinetic parameters for isosorbide dinitrate disposition obtained from these studies are given in Table I. Areas under the isosorbide dinitrate perfusion medium concentration *versus* time curve (AUC) were obtained by the trapezoidal rule. Clearances were calculated as the dose divided by the respective AUC. Rate constants and half-lives were obtained from the slope of a line determined from a least-squares analysis of the isosorbide dinitrate concentrations. Linear first-order kinetics appeared to be in effect at the

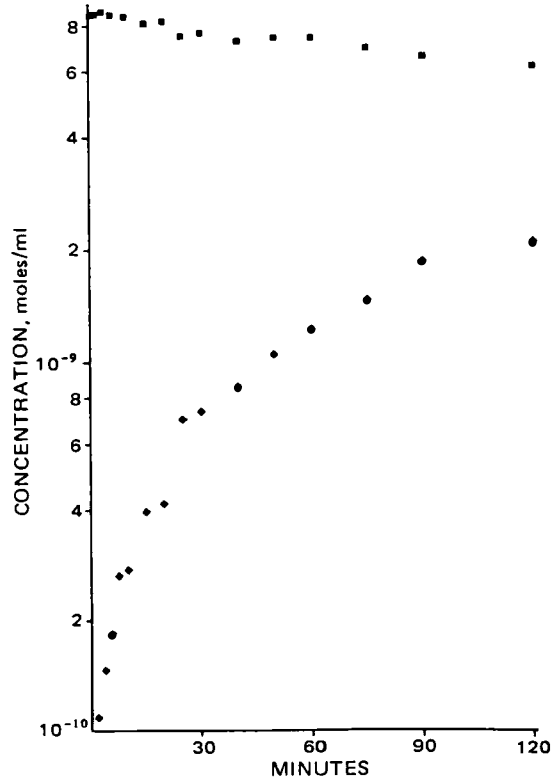


Figure 2—*Semilogarithmic perfusion medium concentration* versus *time profiles following a 10^{-6}-mole iv administration of 5-isosorbide mononitrate. Key: (■) 5-isosorbide mononitrate; (◆) isosorbide.*

Table II—Apparent First-Order Rate Constants for the Formation of 5-Isosorbide Mononitrate, 2-Isosorbide Mononitrate, and Isosorbide after Isosorbide Dinitrate Administration to Isolated Perfused Lung [a]

Dose, moles	$k_{5\text{-Isosorbide Mononitrate}}$ min^{-1}	$k_{2\text{-Isosorbide Mononitrate}}$ min^{-1}	$k_{\text{Isosorbide}}$ min^{-1}
10^{-5}	0.030	0.014	0.018
10^{-5}	0.028	0.015	0.021
10^{-6}	0.056	0.017	0.038
10^{-6}	0.055	0.027	0.041
10^{-7}	0.069	0.024	0.035
10^{-7}	0.084	0.032	0.039

[a] Based on initial rate data.

lower two doses, as demonstrated by the closeness in the clearance values. Lower clearances at high concentrations suggest some saturation of the organic nitrate reductase enzyme at the largest dose.

The role of the lung in the metabolism of isosorbide dinitrate in relationship to the overall disposition of the drug can be elucidated by comparison of the clearance and flow rate of the perfusion medium. The fraction of the dose which passes through the lung vasculature but is not eliminated would be calculated by:

$$F = 1 - \frac{CL}{Q_B}$$

where CL is the perfusion medium clearance (ml/min) and Q_B is the perfusion flow rate (ml/min) (16). For the case of isosorbide dinitrate, nearly 90% of the drug entering the lung would escape first-pass pulmonary metabolism. This relatively slight metabolism suggests that lung metabolism would not be a major factor in the overall elimination of this compound compared with the rapid hepatic denitration demonstrated by isolated, perfused rat liver experiments (17).

Initial rates of formation for the individual metabolites after administration of isosorbide dinitrate were determined from rectilinear plots using a tangent drawn to the respective data points collected in the first 2 min of the experiment. The corresponding apparent first-order rate constants were calculated by dividing these initial rates by the average isosorbide dinitrate concentration in the same time period. Rate constants for the formation of each of the individual metabolites are presented in Table II. In all cases, 5-isosorbide mononitrate was formed more rapidly than isosorbide, which in turn was formed more rapidly than 2-isosorbide mononitrate. The rapid monoexponential decline of the parent drug (Table I) corresponded to the rate of increase in metabolite levels as determined from the sum of the rate constants for the appearance of the three metabolites (Table II). The mean difference of these rate constants was 6.1% ($SD \pm 6.6\%$).

The profiles following administration of 10^{-6}-mole doses of 5- and 2-isosorbide mononitrate are shown in Figs. 2 and 3, respectively. The

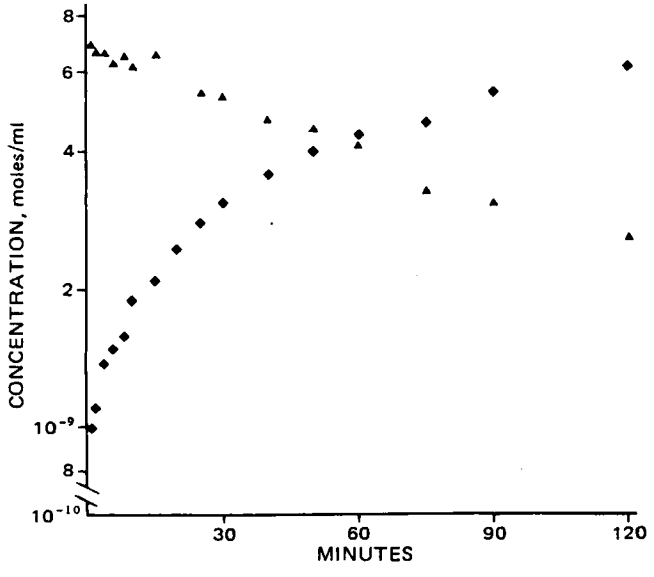


Figure 3—*Semilogarithmic perfusion medium concentration* versus *time profiles following a 10^{-6}-mole iv administration of 2-isosorbide mononitrate. Key: (▲) 2-isosorbide mononitrate; (◆) isosorbide.*

Table III—Apparent First-Order Rate Constants for the Formation of Isosorbide from Isosorbide Dinitrate, 5-Isosorbide Mononitrate, and 2-Isosorbide Mononitrate in Isolated Perfused Lung [a]

Compound	Rate Constant, min^{-1}
Isosorbide dinitrate	0.038 ± 0.003 [b]
5-Isosorbide mononitrate	0.0025 ± 0.0005 [c]
2-Isosorbide mononitrate	0.0073 ± 0.0011 [c]

[a] Mean ± *SD*. [b] $n = 4$. [c] $n = 2$.

2-isosorbide mononitrate shows a more rapid disposition (0.0073 min^{-1}) than the 5-isosorbide mononitrate (0.0025 min^{-1}), probably due to the exo positioning of the 2-nitrate group. In both cases, the rate constants were determined from semilogarithmic rate plots of the ascending isosorbide concentrations. The slight decrease in mononitrate levels appeared also to correlate well with the terminal slopes of the mononitrates in the isosorbide dinitrate experiments.

In Table III, rate constants for the formation of isosorbide from the two mononitrates (as determined from the terminal slopes of the semilogarithmic mononitrate plots) are compared with the apparent first-order rate constant calculated from initial rates of appearance of isosorbide after administration of 10^{-6}- and 10^{-7}-mole doses of isosorbide dinitrate to the lung. These results indicate that the isosorbide was formed much more rapidly when isosorbide dinitrate was given as the dose rather than 5- or 2-isosorbide mononitrate. The rate constants for formation of isosorbide when mononitrates were added to the lung account for <25% of that formed when isosorbide dinitrate was added. These data show that the large amount of isosorbide formed in the isosorbide dinitrate experiments could not have been formed by either mononitrate that had partitioned from the lung into the perfusion medium. The rapid formation of isosorbide from these experiments was not typical of a two-step metabolic process. There is no indication from the mononitrate concentration–time profiles in the perfusion medium that these compounds serve as intermediates to the completely denitrated molecule. Therefore, the rapid formation of isosorbide following isosorbide dinitrate administration appears to be the result of a high intrinsic clearance of the mononitrates formed in the metabolizing organ prior to their diffusion into the perfusion medium.

The differences in the rate of elimination of the mononitrates following

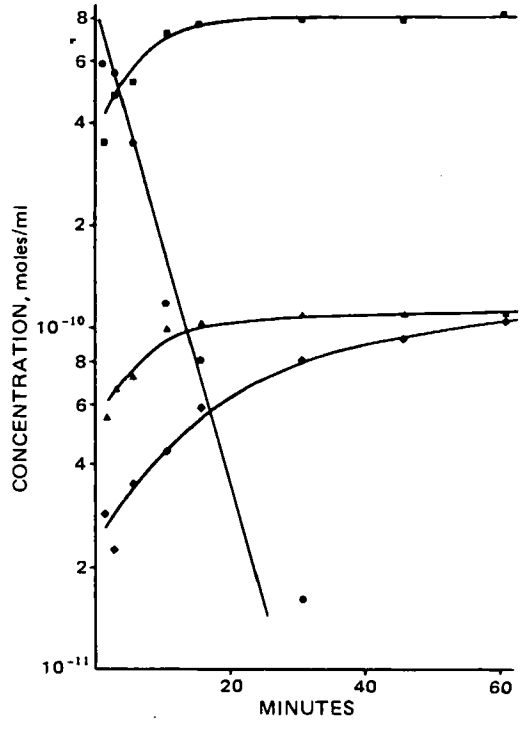


Figure 4—*Semilogarithmic concentration* versus *time profiles showing metabolism of 10^{-9} mole of isosorbide dinitrate in lung homogenate 9000×g supernatant. Key: (●) isosorbide dinitrate; (■) 5-isosorbide mononitrate; (▲) 2-isosorbide mononitrate; (◆) isosorbide. Lines are visual aids only.*

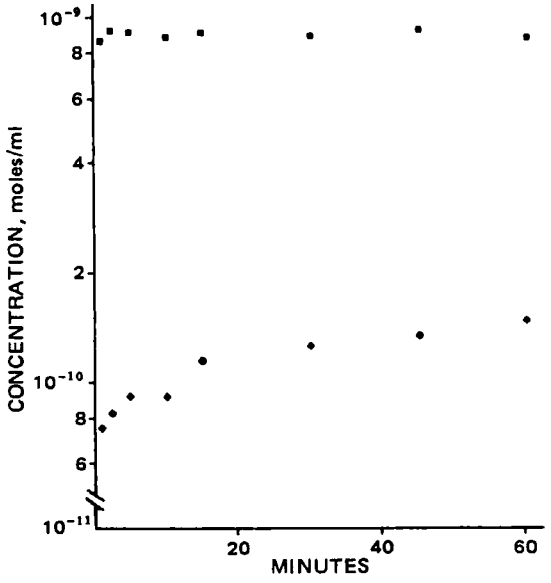


Figure 5—*Semilogarithmic concentration* versus *time profiles showing metabolism of 10^{-9} mole of 5-isosorbide mononitrate in lung homogenate 9000×g supernatant. Key: (■) 5-isosorbide mononitrate; (◆) isosorbide.*

administration of preformed mononitrates to the isolated perfused lung and the rate of production of isosorbide following administration of isosorbide dinitrate may be attributed to the different distribution characteristics of the mononitrates and the dinitrate. As a precursor, isosorbide dinitrate may deliver the 5- and 2-isosorbide mononitrates to sites in the proximity of the metabolizing tissue of the lung, to which the mononitrates themselves have relatively poor access. The second nitrate ester could be quickly denitrated at that point. The mononitrates formed in the lung may also diffuse into the perfusion medium, where their distribution characteristics lead to the slower metabolism seen after an intravascular dose of the mononitrates. Thus, the accessibility to the enzymatic site will directly affect the metabolism of the mononitrates to isosorbide.

There is other evidence that the rate at which a metabolite formed in a metabolizing organ achieves its fate cannot be assumed to be the same as a preformed metabolite presented to that organ. In the studies of Brazzell and Kostenbauder (18), greater metabolism of isoproterenol in the lung was observed when it was formed by the hydrolysis of two diester prodrugs than when it was delivered to the isolated perfused rabbit lung as isoproterenol. The extensive experiments of Pang and Gillette (19) demonstrated the sequential elimination of acetaminophen as it was

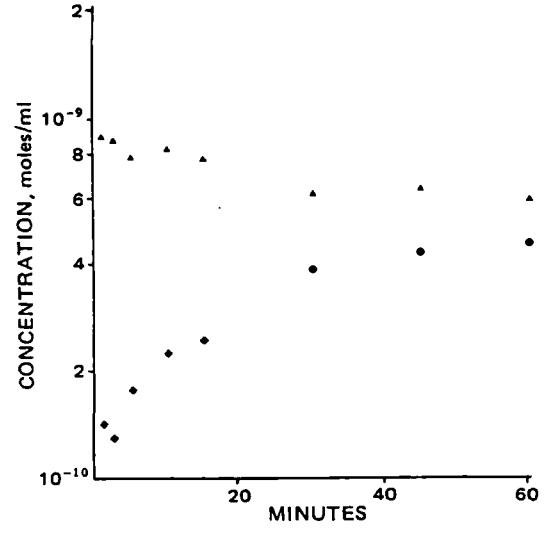


Figure 6—*Semilogarithmic concentration* versus *time profiles showing metabolism of 10^{-9} mole of 2-isosorbide mononitrate in lung homogenate 9000×g supernatant. Key: (▲) 2-isosorbide mononitrate; (◆) isosorbide.*

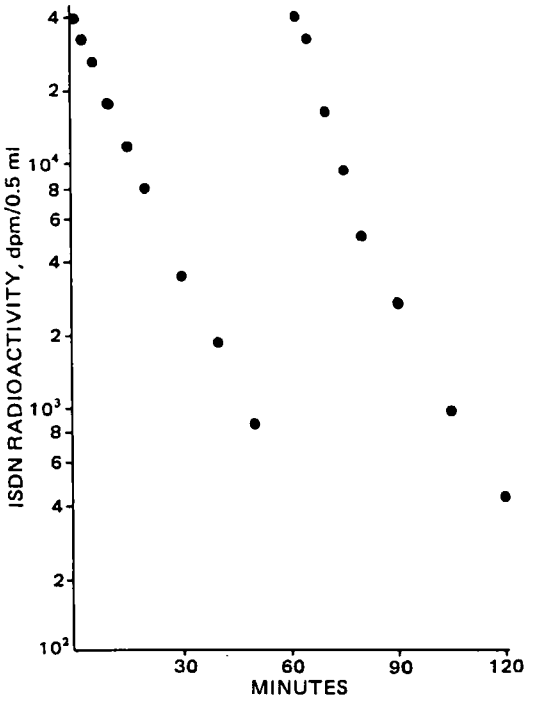


Figure 7—*Semilogarithmic radioactivity* versus *time profile of isosorbide dinitrate following successive doses of 10^{-5} and 10^{-7} mole.*

being formed from phenacetin in a single pass through the isolated perfused liver. Although diffusional barriers were not needed for an explanation of the differences in the metabolism of preformed acetaminophen and acetaminophen formed from phenacetin, these types of barriers to metabolism were hypothesized (20). Rate-limiting diffusional parameters have also been shown to affect metabolism and subsequent elimination in the isolated perfused rat kidney (21). Due to diffusion into renal tubular cells, salicylic acid was cleared more rapidly when it was formed in the kidney from salicyluric acid than when it was presented to the kidney as intact salicylic acid.

The 9000×*g* lung homogenate studies were designed to investigate the rates of metabolism of these organic nitrates independent of any cell wall barriers that might exist. In these experiments, isosorbide dinitrate was rapidly metabolized to the mononitrates in the lung supernatant (Fig. 4). The rate of appearance of isosorbide lagged behind that of both mononitrates and was consistent with sequential first-order dinitrate-to-mononitrate-to-isosorbide metabolism. This concentration *versus* time profile is markedly different from the virtually simultaneous appearance of mononitrate and isosorbide following administration of isosorbide dinitrate to the isolated perfused lung.

Figures 5 and 6 show examples of the slow metabolism of the 5- and 2-isosorbide mononitrates, respectively, in 9000×*g* supernatant. There is only a slight increase in isosorbide levels as these mononitrates are slowly denitrated. Further, the amount of isosorbide formed from the mononitrates in these lung homogenate experiments confirms that the isosorbide formed in the isosorbide dinitrate experiments is a direct product of the metabolism of the mononitrate intermediates alone. Therefore, in the homogenate studies, unlike the intact lung studies, isosorbide formation kinetics are not altered quantitatively by administering isosorbide dinitrate instead of a mononitrate. In a control experiment, isosorbide dinitrate was not hydrolyzed in the presence of reduced glutathione and sodium cyanide, so the metabolism was a direct result of the lung supernatant. The homogenate studies were interesting in the requirement of cyanide for the metabolism of isosorbide dinitrate. In the presence of lung 9000×*g* supernatant and reduced glutathione alone, the drug was not metabolized. The addition of sodium cyanide kept glutathione in its reduced form (9), and isosorbide dinitrate was metabolized quite rapidly.

The multiple-dose experiments were designed to investigate the possibility of product inhibition of the organic nitrate reductase enzyme. A constant infusion of the mononitrates, with coadministration of isosorbide dinitrate, would be inappropriate to demonstrate inhibition, since only small amounts of the mononitrates would reach the enzymatic site. By administering the parent compound, the mononitrates could reach that site by virtue of lung metabolism. Therefore, two separate intra-

vascular doses of isosorbide dinitrate, 10^{-5} and 10^{-7} moles, were given 60 min apart, and the rate of elimination was ascertained for each dose. Plots of isosorbide dinitrate radioactivity for the two doses of the drug in one experiment are shown in Fig. 7. Two parallel lines, representing a half-life of 8 min, indicated that there was no increase in the half-life exhibited with the second dose. There was thus no indication from these studies that the mononitrate metabolites could inhibit denitration of isosorbide dinitrate, whether the mononitrates were preformed or formed *in situ*.

In summary, this paper illustrates how access to selected organs or tissues may alter the expected metabolism of a drug. Release of a drug from a precursor in the proximity of metabolizing tissue may lead to more rapid metabolism than seems likely from studies involving presentation of the preformed drug to that organ or tissue.

REFERENCES

(1) A. P. Fishman and G. G. Pietra, *N. Engl. J. Med.*, **291**, 884 (1974).

(2) M. W. Anderson and T. E. Eling, *Environ. Health Perspect.*, **16**, 77 (1976).

(3) B. W. Blase and T. A. Loomis, *Toxicol. Appl. Pharmacol.*, **37**, 481 (1976).

(4) H. J. Hawkins, A. G. E. Wilson, M. W. Anderson, and T. E. Eling, *Prostaglandins*, **14**, 251 (1977).

(5) M. W. Anderson, T. C. Orton, R. D. Pickett, and T. E. Eling, *J. Pharmacol. Exp. Ther.*, **189**, 456 (1974).

(6) J. P. McGovren, W. C. Lubawy, and H. B. Kostenbauder, *ibid.*, **199**, 198 (1976).

(7) P. Needleman and E. Johnson, in "The Pharmacological Basis of Therapeutics," 6th ed., A. G. Gilman, L. S. Goodman, and A. Gilman, Eds., Macmillan, New York, N.Y., 1980, p. 819.

(8) J. A. Franciosa, L. A. Nordstrorm, and J. N. Cohn, *J. Am. Med. Assoc.*, **240**, 443 (1978).

(9) P. Needleman and J. C. Krantz, *Biochem. Pharmacol.*, **14**, 1225 (1965).

(10) P. Needleman and F. E. Hunter, *Mol. Pharmacol.*, **1**, 77 (1965).

(11) P. Needleman, S. Lang, and E. Johnson, *J. Pharmacol. Exp. Ther.*, **181**, 489 (1972).

(12) J. C. Krantz and C. D. Leake, *Am. J. Cardiol.*, **36**, 407 (1975).

(13) R. W. Neimeier and E. Bingham, *Life Sci.*, **11**, 807 (1972).

(14) S. F. Sisenwine and H. W. Ruelius, *J. Pharmacol. Exp. Ther.*, **176**, 296 (1971).

(15) F. J. DiCarlo, J. M. Hartigan, and G. E. Phillips, *Anal. Chem.*, **36**, 2301 (1964).

(16) M. Rowland, L. Z. Benet, and G. G. Graham, *J. Pharmacokinet. Biopharm.*, **1**, 123 (1973).

(17) E. M. Johnson, A. B. Harkey, D. J. Blehm, and P. Needleman, *J. Pharmacol. Exp. Ther.*, **182**, 56 (1972).

(18) R. K. Brazzell and H. B. Kostenbauder, *J. Pharm. Sci.*, **71**, 1274 (1982).

(19) K. S. Pang and J. R. Gillette, *J. Pharmacol. Exp. Ther.*, **207**, 178 (1978).

(20) K. S. Pang and J. R. Gillette, *J. Pharmacokinet. Biopharm.*, **7**, 275 (1979).

(21) I. Berkersky, L. Fishman, S. A. Kaplan, and W. A. Colburn, *J. Pharmacol. Exp. Ther.*, **212**, 309 (1980).

ACKNOWLEDGMENTS

This work was presented in part at APhA Academy of Pharmaceutical Sciences meeting (the Basic Pharmaceutics Section) in Washington, D.C., April 1980.

Abstracted from a dissertation submitted by P. R. Mayer to the University of Kentucky in partial fulfillment of the Doctor of Philosophy requirements.

Age-Related Pharmacokinetics of *N*-Acetylprocainamide in Rats

AVRAHAM YACOBI ‡×, BURDE L. KAMATH *, HERMAN F. STAMPFLI, ZEE M. LOOK, and CHII-MING LAI

Received April 19, 1982, from the *Department of Pharmaceutical Development, Research and Development Department, American Critical Care, McGaw Park, IL 60085.* Accepted for publication June 18, 1982.

Present Address: *Xavier University, College of Pharmacy, New Orleans, LA 70125. ‡Lederle Laboratories, Pharmacodynamics Dept., Pearl River, N.Y. 10965.

Abstract □ The pharmacokinetics of *N*-acetylprocainamide, administered orally or intravenously, were studied in 3-, 6-, and 12-month-old rats using a two-way crossover study design. At 3, 6, and 12 months of age, the half-life values of *N*-acetylprocainamide were 1.66, 1.82, and 2.29 hr, respectively; the apparent volumes of distribution were 4.75, 3.35, and 1.98 liter/kg, respectively. The elimination rate constant, clearance, and absolute bioavailability of the drug (determined by AUC measurements and the amounts excreted unchanged in the urine) decreased significantly with age. The rate of absorption remained unchanged. The amounts of *N*-acetylprocainamide in the liver and kidneys were significantly higher in the 12-month-old animals. These results clearly demonstrate a significant alteration with age in the bioavailability, distribution, and elimination of *N*-acetylprocainamide in rats. In long-term toxicity studies of this and other drugs that show age-dependent pharmacokinetics, an adjustment in the chronically administered dose is essential.

Keyphrases □ *N*-Acetylprocainamide—oral and intravenous pharmacokinetics in rats, bioavailability, age-related changes □ Pharmacokinetics—*N*-acetylprocainamide in rats after oral and intravenous administrations, age-related changes □ Bioavailability—*N*-acetylprocainamide in rats after oral and intravenous administrations, age-related changes

It has been established that many physiological changes are associated with aging. Lean body mass, plasma albumin, and total body water decrease with age. In humans, the cardiac output decreases 1%/year from ages 19 to 86, and the blood flow to the kidneys is also reduced with age (1). In rats, there are pronounced decreases (>30%) in the cardiac index and in hepatic, renal, and GI tract blood flow at 11–12 months of age (2). These changes can account for significant alterations in the pharmacokinetics and pharmacodynamics of drugs. In toxicity and oncogenicity studies in animals, which are conducted over a long period of time (1 year or more), aging may account for (3, 4): (*a*) diminished absorption of drugs through the GI tract; (*b*) diminished rate of metabolism and renal excretion of drugs; (*c*) accumulation of drugs in blood and receptor sites; (*d*) changes in affinity and sensitivity of the receptor sites to drug molecules; and (*e*) an increased rate of mortality. For these reasons, it was considered prudent to

0022-3549/83/0700-0789$01.00/0