| | |
|---|---|
| $CL_{12}$ | Clearance from plasma to cell water = $f(T_{max},K_m)$ |
| $CL_{21}$ | Clearance from cell water to plasma |
| **Amounts** | |
| $D_0$ | Dose |
| $X_B^{ss}$ | Amount in body at steady-state |
| **Rates** | |
| $k_0$ | Zero-order infusion rate |
| $T_{max}$ | Maximum cellular transport rate |
| **Ratios** | |
| $K_d$ | Tissue-plasma partition coefficient |
| $K_{pi}$ | Individual tissue-plasma partition coefficients |

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# Pharmacokinetics of Nitroglycerin after Parenteral and Oral Dosing in the Rat


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Received May 17, 1982, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication June 23, 1983. Present addresses: *National Institute of Hygienic Sciences, Tokyo, Japan, ‡Kyoto University Hospital, Kyoto, Japan, and § Schering Corporation, Bloomfield, NJ 07003.



**Abstract** □ The pharmacokinetics of nitroglycerin was characterized in detail using venous plasma after different intravenous bolus doses (0.15-2.48 mg/kg), intra-arterial infusion (8.2 μg/min over 5 h), and oral doses (7-100 mg/kg). Venous plasma clearance was found to be ~650 mL/kg and was independent of the intravenous or intra-arterial dose. This confirmed earlier reports that the venous plasma clearance of nitroglycerin in rats exceeded the value of normal cardiac output. A terminal half-life of ~15 min was observed after high intravenous bolus doses of nitroglycerin. This slow disappearance phase was likely rate limited by redistribution of drug back into the plasma. The bioavailability of oral nitroglycerin ($F$) showed an apparent Michaelis-Menten dependency on dose. $F$ was <5% at doses <20 mg/kg, but increased to a plateau of ~20% from 50-100 mg/kg. First-pass metabolism of nitroglycerin is thus apparently controlled by at least two systems (sites or enzymes). Coadministration of mannitol hexanitrate, a potential competitive inhibitor of first-pass metabolism, did not increase $F$.



**Keyphrases** □ Pharmacokinetics—nitroglycerin, parenteral and oral dosing, rats □ Nitroglycerin—pharmacokinetics, parenteral and oral dosing, rats


Interest in the clinical use of intravenous nitroglycerin has intensified in recent years because of its demonstrated efficacy in treating the resultant manifestations of myocardial infarction, congestive heart failure, and unstable angina. Several reports (1-3) have shown that intravenous infusion of nitroglycerin can appreciably decrease cardiac work load and relieve the increase in left ventricular and diastolic pressure secondary to congestive heart failure or myocardial infarction (2).

Despite the increasing popularity of the use of intravenous nitroglycerin in therapy, very little is known about the pharmacokinetic behavior of this potent drug. There are indications


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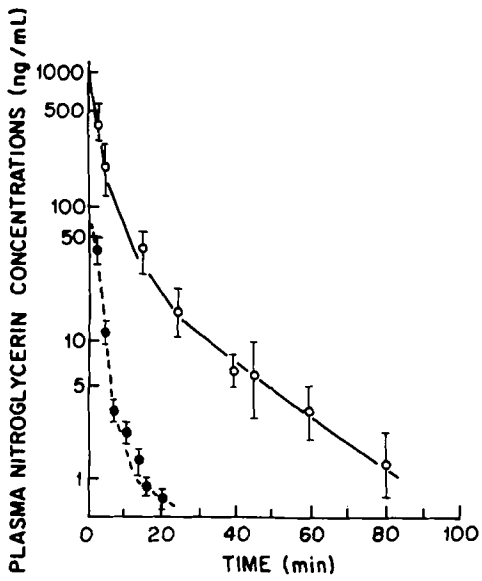


**Figure 1**—*Mean ± SE (bars) of plasma nitroglycerin concentrations after intravenous bolus doses of 0.15 mg/kg (●, n = 4) and 2.48 mg/kg (○, n = 6). The lines are drawn based on the pharmacokinetic parameters shown in Table I for each of the doses.*

(4–6) that circulatory changes in heart rate and blood pressure in humans may parallel those in nitroglycerin plasma concentrations. Several reports have also suggested the observed systemic clearance in humans (7–9) and in rats (10) to be greater than the normal cardiac output in the respective species. However, the underlying mechanism for this observation is still unclear, and many of the basic aspects of nitroglycerin kinetics remain unknown.

Another important issue in nitroglycerin therapy relates to the efficacy of this drug when given orally. Numerous metabolic and pharmacological studies show that nitroglycerin undergoes nearly complete first-pass metabolism when the drug is administered at <3 mg/kg in several animal species (11–14). It was argued (14) that similar first-pass metabolism of oral nitroglycerin also exists in humans, and that oral use of this drug and other organic nitrates in therapy is irrational and ineffective. This viewpoint was strongly contested and refuted by Krantz and Leake (15) based on clinical experience. Convincing clinical data have appeared (16) that also affirm the prophylactic effectiveness of oral nitroglycerin against angina attacks.

There is no literature report on the effect of an oral dose on the extent of first-pass inactivation of nitroglycerin in humans or animals. If the extent of inactivation is capacity limited, oral bioavailability of nitroglycerin may be dose dependent and, thus, extrapolation of absorption data from trace or low doses to infer lack of bioavailability at other doses may be incorrect.

In this report, we describe the effect of intravenous dose on nitroglycerin disposition kinetics and the effect of oral dose on the bioavailability of nitroglycerin in the rat. Since the aqueous solubility of nitroglycerin is limited to ~1 mg/mL (17) and doses of nitroglycerin in this study necessitated the use of an organic solvent mixture of 10% ethanol in propylene glycol, a comparison of the bioavailability of nitroglycerin from this organic vehicle and water was carried out. Finally, the possible interference of nitroglycerin bioavailability by coadministration of another organic nitrate, *viz.*, mannitol hexanitrate, was explored.

## EXPERIMENTAL SECTION

**Materials**—All reagents were of analytical grade unless otherwise stated.

**Preparation of Stock Nitroglycerin Solutions**—Nitroglycerin was isolated from the lactose adsorbate[1]. The powder was dissolved in distilled water and nitroglycerin was extracted with ether in a separatory funnel. The ether was separated and evaporated under a gentle stream of dry nitrogen. Nitroglycerin was recovered as a liquid and a weighed amount was dissolved in the appropriate solvent. The precise concentration of the stock solution was determined either by the USP assay (18) or the kinetic method developed by Fung *et al.* (19).

**Intravenous Bolus Dosing**—Male Sprague-Dawley rats, 250–360 g, underwent right jugular vein cannulation according to the procedure developed by Weeks and Davis (20). The animals were fasted overnight and administered an intravenous bolus dose into the jugular vein cannula, followed by flushing with saline and then with circulating blood. Blood samples were collected into heparinized tubes *via* the jugular vein cannula. The dosing volume was 0.5–0.75 mL in aqueous vehicle after appropriate dilution of the stock solution; the doses administered were 0.15, 0.35, 1.75, and 2.48 mg/kg of nitroglycerin with 4, 7, 5, and 6 animals in each group, respectively. A bolus intravenous dose of 3.5 mg/kg was also attempted but was found to cause severe convulsions in the animals. Pharmacokinetic information was not obtained from this group of animals.

**Intra-Arterial Infusion**—Four male Sprague-Dawley rats, 250–300 g, had jugular vein and carotid artery cannulas surgically implanted. One day after surgery, nitroglycerin was infused into the carotid artery, and blood samples were withdrawn *via* the jugular vein cannula at appropriate intervals. The infusion time was 5 h and the infusion rate was 6.8 μL/min *via* an infusion pump. The infusion solution concentration was 1.2 mg/mL, and the dosing rate was 8.2 μg/min in each animal.

**Crossover Study of Oral Dosing**—Six male Sprague-Dawley rats, weighing ~300 g, underwent jugular vein cannulation and were given an oral dose of either 20 or 100 mg/kg of nitroglycerin in a randomized crossover fashion. The vehicle was 10% alcohol in propylene glycol. All animals were fasted overnight, and the drug was given *via* gastric intubation under light ether anesthesia. The interval between doses was 3 d. Blood was harvested from the jugular vein cannula. The separated plasma was assayed for nitroglycerin as described later.

**Bioavailability of Nitroglycerin at Five Different Oral Doses**—Different male Sprague-Dawley rats were each given an oral dose of nitroglycerin in 10% alcohol in propylene glycol. Each dose, 7, 20, 50, 75, and 100 mg/kg, was adminstered by oral intubation under light ether anesthesia. The oral dose of vehicle was normalized to body weight so that each animal received the same volume on a mL/kg basis (~1 mL per animal). Blood (~0.3 mL) was collected from the tail vein at appropriate intervals, and the plasma was separated immediately for determination of nitroglycerin concentrations. All animals were fasted overnight with free access to water prior to drug administration.

**Organic Nitrate Interaction Study**—Since mannitol hexanitrate has been shown (12) to be a better *in vitro* substrate for organic nitrate reductase (the enzyme responsible for nitroglycerin metabolism), the possibility of enhancement in nitroglycerin bioavailability with the coadministration of mannitol hexanitrate was tested. Seven rats received an oral dose of nitroglycerin or a combination dose of mannitol hexanitrate and nitroglycerin, in a randomized crossover fashion. Essentially the same procedure was used as in the oral dosing experiments described previously. The molar ratio of mannitol hexanitrate to nitroglycerin was ~1.6. Preliminary studies showed that mannitol hexanitrate did not interfere with the assay: mannitol hexanitrate did not alter the plasma recovery of nitroglycerin; after an intravenous dose of mannitol hexanitrate at 60 μg/kg, no noticeable peaks were observed in the chromatogram which would interfere with the determination of nitroglycerin.

**Nitroglycerin Assay**—Blood samples were centrifuged immediately, and 0.1–0.2 mL of plasma was removed. A 10–20-μL aliquot of silver nitrate was added to each plasma sample to prevent degradation of the drug. Plasma samples were stored overnight at −20°C and nitroglycerin levels were determined according to the GC assay developed by Yap *et al.* (21). Isosorbide dinitrate was added as the internal standard. In this study, the average recovery of nitroglycerin from plasma was ~92% with a coefficient of variation of 10%.

**Pharmacokinetic Calculations**—*Parenteral Doses (Model-Independent Parameters)*—The plasma concentration *versus* time profiles after various bolus intravenous doses exhibited biexponential characteristics. The composite data were therefore fitted (NONLIN[2]) to the biexponential equation $C_t$ =

[1] Nitroglycerin 10% (w/w) in lactose; ICI Americas, Atlas Chemical Div., Wilmington, Del.

[2] See, *e.g.*, C. M. Metzler, G. L. Elfring, and A. J. McEwen, *Users Manual for NONLIN and Associated Programs*, 1974. The Upjohn Co., Kalamazoo, Mich.

**Table I—Mean Estimated Values of Model-Independent Pharmacokinetic Parameters of Nitroglycerin at Various Intravenous Bolus Doses [a]**

| Parameter [b] | Dose, mg/kg | | | |
|---|---|---|---|---|
| | 0.15 | 0.35 | 1.75 | 2.48 |
| $A_1$ (ng/mL) | 69.8(4.9) | 160(9) | 506(54) | 809(49) |
| $\lambda_1$ ($min^{-1}$) | 0.41(0.01) | 0.35(0.01) | 0.24(0.02) | 0.27(0.02) |
| $A_2$ (ng/mL) | 1.7(0.2) | 3.0(0.3) | 33.1(3.9) | 43.6(8.0) |
| $\lambda_2$ ($min^{-1}$) | 0.045[c] | 0.045[c] | 0.036(0.002) | 0.044(0.0004) |
| Venous plasma clearance (mL/min/kg) | 669(46) | 642(40) | 528(60) | 654(49) |
| $Vd_{ss}$ (L/kg) | 3.9(0.3) | 4.4(0.3) | 6.1(0.6) | 6.2(0.5) |

[a] Expressed as mean (*SE*). [b] Typical parameters used to describe a biexponential equation, $C_t = A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t}$. [c] Set as corresponding value obtained from curve-fitting of normalized, composite data of the two high doses.

$A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t}$, where $C_t$ is the plasma concentration of nitroglycerin at time $t$ and $A_1$, $A_2$, $\lambda_1$ and $\lambda_2$ are the pharmacokinetic constants used to describe a biexponential decay curve. Initial estimates for the NONLIN computer program were either obtained graphically (method of residuals) or from CSTRIP[3]. Various weighting factors (1, $C_t^{-1/2}$, $C_t^{-1}$, and $C_t^{-2}$) were used to obtain the best fit, as determined by the residual sum of squares, parameter standard deviations, and $r^2$ values.

The apparent systemic plasma clearance was calculated by dividing the intravenous dose by the area under the plasma concentration *versus* time curve (AUC) from time zero to infinity. The individual concentration at time zero ($C_0$) for each animal in the low-dose group was estimated by extrapolation of the linearly regressed line composed of the first three plasma concentration *versus* time points. For the two high-dose groups $C_0$ was obtained either by linear extrapolation, as described, or from computer estimates, whichever was considered more accurate. The AUC from time zero to the last concentration-time point was calculated by the Spline Method[4]. The residual AUC was calculated from $C^*/\lambda_2$ where $C^*$ is the last concentration-time point determined experimentally. For the two small doses, where the $\lambda_2$ phase could not be characterized fully because of assay limitations, the residual AUC was calculated using the mean $\lambda_2$ obtained from the higher doses. The volume of distribution at steady state was obtained by a model-independent approach which relied on AUC calculations (22). Thus:

$$Vd_{ss} = \frac{\text{Dose}_{iv} \cdot \text{AUMC}}{\text{AUC}^2} \qquad \text{(Eq. 1)}$$

where AUMC is the area under the moment curve, obtained *via* Eq. 2:

$$\text{AUMC} = \int_0^{t^*} tC_t dt + t^*C^*/\lambda_2 + C^*/\lambda_2^2 \qquad \text{(Eq. 2)}$$

where $t^*$ is the time at which the last data point was collected. All other terms were defined previously.

*Oral Doses*—AUC from time zero to $t^*$, $AUC_{0\text{-}t^*}$, was estimated by the Spline Method[4]. The residual area from time $t^*$ to $\infty$ was calculated by $C^*/\lambda_t$ where $\lambda_t$ was the terminal rate constant. In several cases, only two or three data points could be identified in the terminal phase. Nevertheless, the residual portion of the curve accounted for ≤10% of the total area and would not be expected to bias the overall analysis seriously.

The apparent bioavailability ($F$), defined as the fraction of the dose reaching the systemic circulation intact, was calculated from $CL \times AUC_{0\text{-}\infty}/\text{dose}$, where $CL$ is the plasma clearance observed after intravenous dosing, set here at a value of 650 mL/min/kg. The paired $t$ test was used to determine statistical differences in the two crossover studies, and the Kruskal-Wallis test was used in the study in which the effect of dose was examined in separate animals.

## RESULTS AND DISCUSSION

**Parenteral Dosing**—Figure 1 shows the plasma nitroglycerin concentrations obtained after the highest and lowest intravenous bolus doses (*i.e.*, 2.48 and 0.15 mg/kg, respectively). Because of the relatively small plasma sample size that could be obtained from the rat (0.1-0.2 mL), the detection limit of nitroglycerin was ~0.5-1 ng/mL of plasma in these studies. Consequently, with the two lower doses, measurable concentrations were only recorded up to 20 min after injection. At the two higher doses, sample collection (and detectable concentrations) could be extended to 90 min after dosing. In fitting these data with the NONLIN program, a weighting factor of $C_t^{-2}$ was found to produce the best fit in the groups given the two high doses. This weighting factor was then used in all other curve fittings.

Table I shows the pharmacokinetic parameters obtained from these fittings. The fit of data from all four doses appeared quite reasonable, since, for each dose, the $AUC_\infty$ calculated directly from the experimental data agreed well with that estimated from the curve fit. A striking feature observed from Table I is that the apparent plasma clearance is independent of dose and is on the order of 500-700 mL/min/kg, well in excess of the normal cardiac output of ~300 mL/min/kg in this species (23). This large plasma clearance, which is now documented for several doses and from more detailed sampling, is consistent with the preliminary observation in the rat reported by Yap and Fung (10) and also with all the available human data (7-9, 24). The apparent volume of distribution at steady state, $Vd_{ss}$, was 4-6 L/kg. This large volume is consistent with the moderate binding (~60%) of nitroglycerin to rat plasma protein (25), and the location of rather high radioactivity in the carcass and other soft tissues after oral administration of [$^{14}$C]nitroglycerin (26).

After intravenous doses of 1.75 and 2.48 mg/kg, nitroglycerin disappearance from plasma was clearly biphasic. At lower doses, this biexponential decay was less evident because assay limitations did not permit characterization of the second disappearance phase. The $\lambda_1$ half-life observed here, at 2-3 min, is consistent with values reported previously in rats (10) and in humans (7-9). The second disappearance phase, with a half-life of 15-20 min, has not been reported previously in the literature.

Recent experiments by Kamiya *et al.*[5] showed that when nitroglycerin was incubated in whole blood, the *in vitro* degradation half-life was ~11 min. This half-life could be assumed to represent the slowest possible elimination half-life of nitroglycerin in the systemic circulation. Interestingly, however, this half-life is somewhat shorter than the observed *in vivo* plasma $\lambda_2$ half-life of 15-20 min. This would seem to indicate that tissue redistribution rather than elimination is an important factor in describing the second disappearance phase.

Although there was no apparent dose dependency in the venous plasma clearance of nitroglycerin, examination of the data presented in Table I suggested that there are possibly minor differences in the $\lambda_1$ and $Vd_{ss}$ terms between the two lower doses (0.15 and 0.35 mg/kg) and the two higher ones (1.75 and 2.48 mg/kg). There are some theoretical reasons to anticipate possible dose dependency in nitroglycerin disposition kinetics. The venous plasma clearance is so large that flow limitation may be expected to play an important role. The higher bolus doses administered were close enough to an observed toxic dose of 3.5 mg/kg that dose-dependent changes in blood perfusion to various tissues are likely. Recently, Wu *et al.* (27) suggested that the rate constant for the loss of nitroglycerin from human blood decreases as the initial concentration of nitroglycerin increases. The implication of this finding to the present situation is unclear.

A possible contribution to the large plasma clearance observed for nitroglycerin is the existence of pharmacokinetic phases that were undetected because of the methodological difficulties involved. Johnson *et al.* (28) and recently Stein *et al.* (29) suggested the presence of a rapid disappearance phase of nitroglycerin in blood that exhibited a half-life of ~15 s. In both of these studies, however, the drug ([$^{14}$C]nitroglycerin) was introduced into the jugular

**Table II—Normalized AUC of Nitroglycerin in Rats Given Both 20-mg/kg and 100-mg/kg Oral Doses**

| Animal | AUC/Dose × $10^4$ min·kg·$mL^{-1}$ | |
|---|---|---|
| | 20 mg/kg | 100 mg/kg |
| 1 | 1.11[a] | 3.35 |
| 2 | 1.16 | 2.10[a] |
| 3 | 1.53 | 2.07[a] |
| 4 | 1.76[a] | 2.09 |
| 5 | 1.76[a] | 2.77 |
| 6 | 0.91 | 3.37[a] |
| Mean | 1.37 | 2.63[b] |
| *SE* | 0.15 | 0.26 |

[a] Animal given this dose first. [b] $p < 0.01$ compared with AUC/Dose at 20 mg/kg.

[3] "CSTRIP—A Fortran IV computer program for obtaining initial polyexponential parameter estimates," J. G. Wagner, College of Pharmacy, University of Michigan, Ann Arbor, Mich.

[4] Desktop computer, general utility program tape #9825; Hewlett-Packard, Fort Collins, Colo.

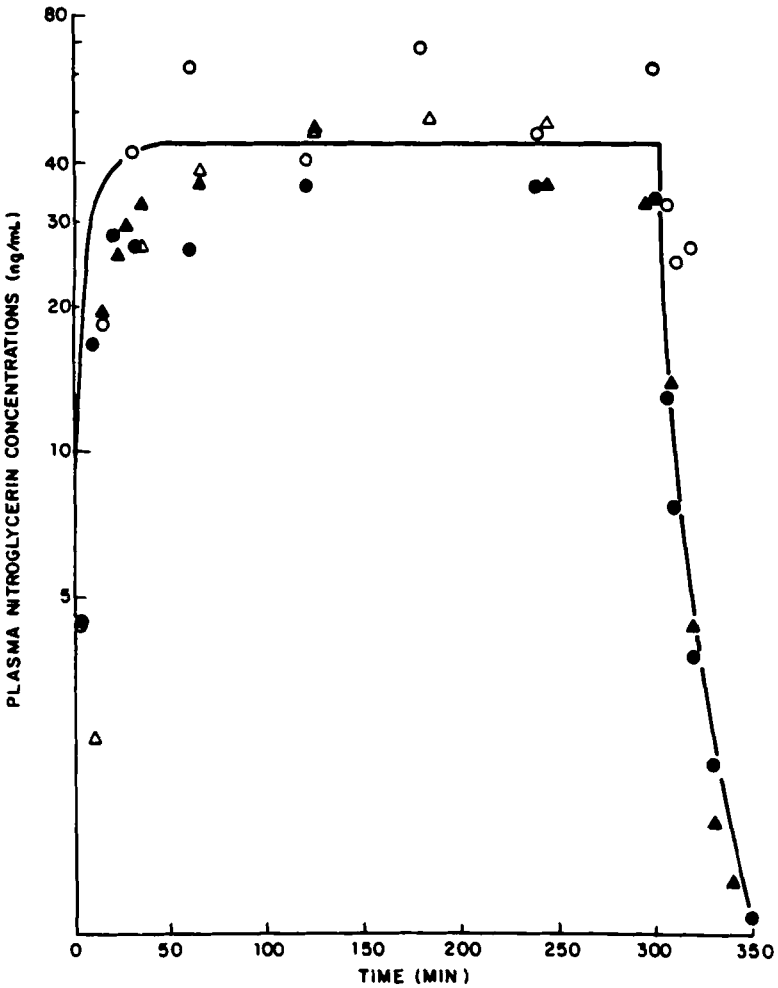


**Figure 2**—*Venous plasma nitroglycerin concentrations obtained after intra-arterial infusion at 8.2 μg/min. Symbols represent different animals (250–300 g). The line was drawn using the pharmacokinetic parameters generated compositely from the two high intravenous bolus doses.*

vein and blood was collected from the carotid artery. Thus, the early blood samples might have been collected before the dose was distributed throughout the systemic circulation, and the existence of a true pharmacokinetic phase with such a short half-life is uncertain. On the other hand, if such a fast "distributional" phase does exist and the sampling protocol adopted here was incapable of detecting it, an erroneously large plasma clearance could have resulted because a significant portion of the AUC was not included in the calculation. This problem also can exist if a slow terminal-phase $\lambda_3$ is present, but it, too, could not be detected because of assay limitations.

Figure 2 shows the venous plasma concentrations of nitroglycerin in four rats infused intra-arterially for 5 h at 28.5 μg/min/kg. Since the drug was infused *via* the carotid artery and blood was collected from the jugular vein [reverse of Johnson *et al.* (28) and Stein *et al.* (29)], drug mixing in the systemic circulation was unlikely to be a complicating factor. Under these conditions an apparent steady-state in nitroglycerin concentration was observed. The plasma concentrations observed from this infusion were generally consistent with those predicted using the pharmacokinetic parameters generated from the composite data of the two high-dose groups (Table I). It would thus appear unlikely that a rapid disappearance phase had been missed in the bolus injection study. The data also suggested that if a hidden $\lambda_3$ phase existed, its half-life would be considerably longer than 1–2 h, since infusion for 5 h failed to detect its presence. Since the pharmacokinetic parameters obtained are similar after bolus injection and after slow infusion, it would appear that the errors incurred in assuming instantaneous input after bolus injection (30, 31) are not large for nitroglycerin.

**Table III—Effect of Solvent on the Area Under the Plasma Nitroglycerin Concentration *versus* Time Curve after a 7-mg/kg Oral Dose**

| Animal | $AUC_{0-\infty} \times 10^{-2}$, ng·min·mL$^{-1}$ | | AUC Ratio (Alcohol Co-solvent/Water) |
|---|---|---|---|
| | Water | Alcohol Cosolvent[a] | |
| 7 | 5.2[b] | 7.8 | 1.5 |
| 8 | 6.4 | 7.2[b] | 1.1 |
| 9 | 3.2 | 3.7[b] | 1.1 |
| 10 | 1.5[b] | 2.8 | 1.9 |
| 11 | 2.0[b] | 2.2 | 1.1 |
| 12 | 1.3 | 2.2[b] | 1.8 |
| Mean | 3.3 | 4.3[c] | 1.4 |
| *SE* | 0.9 | 1.0 | 0.2 |

[a] 10% Ethanol in propylene glycol. [b] Animal given this vehicle first. [c] $p < 0.05$ compared with animals dosed with aqueous vehicle.

Another consideration in the interpretation of the observed plasma clearance is how it may be related to the blood degradation clearance of nitroglycerin. Our group, in a separate study[5], has examined the *in vitro* partitioning of nitroglycerin between red blood cells and plasma and the rate of *in vitro* degradation of nitroglycerin in these components of rat blood. It was found that the partitioning of nitroglycerin into red blood cells was nearly instantaneous; the ratio of nitroglycerin concentration in red blood cells *versus* that in plasma was a constant, $\simeq 2.6$, over an incubation period of 30 s to 30 min at 37°C. The rate of degradation of nitroglycerin in red blood cells was approximately the same as that found in plasma. If a hematocrit value of 0.45 for the rat (23) is used, blood nitroglycerin concentrations could be estimated to be 1.72 times the corresponding plasma concentrations. Thus, *in vivo* venous blood nitroglycerin clearance could be estimated by dividing the venous plasma clearance by 1.72; *i.e.*, $650/1.72 \simeq 378$ mL/min/kg. This value is near that of cardiac output.

The extent of contribution of blood degradation *per se* to the total *in vivo* venous blood clearance of nitroglycerin could also be estimated. Assuming a blood volume of 65 mL/kg in the rat and a degradation half-life of 11 min, the clearance due to blood degradation is $65 \times 0.693/11 \simeq 4.1$ mL/min/kg, which accounts for <1% of the total *in vivo* venous blood clearance.

A further complication in interpreting nitroglycerin pharmacokinetics is the existence of concentration differences in the systemic circulation depending on the sampling site. Thus, Hill *et al.* (32) showed that after intranasal administration of nitroglycerin in humans, the apparent clearance was about twice as large in peripheral venous blood (~37 L/min) than in the arterial circulation (~19 L/min). Armstrong *et al.* (24) recently showed that arterial plasma clearance of nitroglycerin in 20 patients with heart failure was ~12

[5] A. Kamiya, T. Schmitt, and H.-L. Fung, unpublished results.

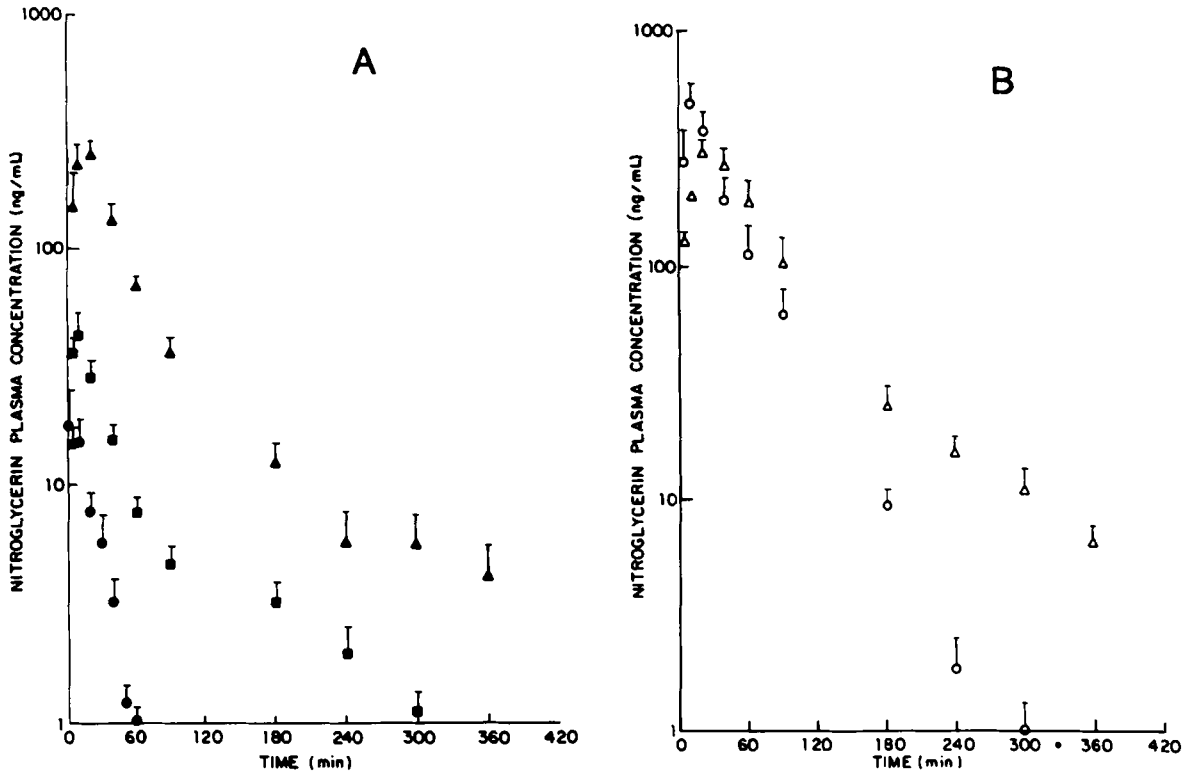


**Figure 3** *Plasma nitroglycerin concentrations (mean ± SE) at various times after oral dosing at 7 (●), 20 (■), 50 (▲), 75 (○), and 100 (△) mg/kg.*

L/min compared with a venous clearance of 39 L/min. These authors calculated an extraction ratio of 61% for nitroglycerin across the arterial-venous bed. The existence of arterial-venous differences in blood concentrations have been shown for several drugs by Chiou and co-workers (31, 33). In these examples, however, the arterial drug clearance was always found to be similar to its venous clearance, suggesting that distributional factors were primarily responsible for the observed arterial-venous difference in concentration. In contrast, metabolic factors may be involved in the case for nitroglycerin, because an arterial-venous difference in systemic clearance has been demonstrated and *in vitro* metabolic activity toward nitroglycerin by isolated blood vessels has also been shown (34).

**Oral Dosing**—Table II shows the results of the randomized crossover study in which six rats were given both 20 and 100-mg/kg oral doses. The normalized AUC was statistically different between the two doses, at $p < 0.01$, using the paired $t$ test. Since the apparent venous plasma clearance of nitroglycerin was not concentration dependent, the increase in normalized AUC after the 100-mg/kg dose is probably due to an increase in bioavailability at this dose. Using an average value of 650 mL/min/kg for the apparent plasma clearance of nitroglycerin, the mean apparent bioavailability at 20 and 100 mg/kg could be estimated as 10.3 and 19.4%, respectively.

These apparent bioavailability values were considerably higher than those suggested by Needleman *et al.* (14) and by Yap and Fung (10) at lower doses. The former group of authors estimated the bioavailability of nitroglycerin to be ≃2% at a dose of 0.5 mg/kg while the latter workers, in a preliminary study, reported a bioavailability estimate of ~2% after an oral dose of nitroglycerin at 7 mg/kg. It must be pointed out that in the second study (10), an aqueous vehicle was used, while in the present work a vehicle containing 10% ethanol in propylene glycol was administered. The possible effect of solvent was examined in a randomized crossover study in which six rats were dosed alternately with nitroglycerin dissolved in water or in 10% ethanol in propylene glycol. The AUC data can be found in Table III. It is apparent that there is a substantial degree of variability in each of the treatment groups, but this observation was quite typical of other kinetic studies of this high-clearance drug (7-10). Nevertheless, there is a modest, but significant ($p < 0.05$), increase in AUC when nitroglycerin administration was changed from the aqueous to the alcoholic vehicle (Table III). It is not known, at present, how much of this increase was influenced by the effect of the vehicle on the systemic clearance and how much was due to a change in presystemic metabolism. Oguma and Levy showed recently that ethanol infusion into the portal vein, from −3 to +3 h of propoxyphene dosing, increased both the systemic clearance and the systemic availability of this analgesic (35). Since the alcohol mixture was administered with the drug, it might be speculated that the apparent plasma clearance of nitroglycerin, which is well in excess of liver blood flow (23), might not be greatly affected by the organic solvents. Regardless of the mechanism involved, the effect of solvent on the apparent systemic availability of nitroglycerin at 7 mg/kg appeared to be relatively small.

An expanded study was carried out to examine, in further detail, the relationship between oral nitroglycerin dosage and bioavailability. Since five to six different doses were involved, a crossover design was not practical and, therefore, was not attempted.

Figure 3A and B shows the mean plasma concentrations of nitroglycerin obtained in different rats after oral doses of 7, 20, 50, 75, and 100 mg/kg administered in the alcohol solvent mixture. Peak plasma drug concentrations were observed generally between 2-20 min after dosing for all doses; however, the values of peak concentrations were not proportional to dose. The profiles of these curves were generally biphasic, except in the case of the lowest dose, 7 mg/kg, where characterization of plasma nitroglycerin concentrations beyond 60 min was impossible due to assay limitations. The apparent terminal half-life observed after oral dosing at 20, 50, and 100 mg/kg ranged from 60-140 min, considerably longer than the $\lambda_2$ half-life of 15-18 min observed after intravenous administration. The data suggested, therefore, that absorption "flip-flop" kinetics (36) is operative here and that the apparent terminal half-life observed reflected more of absorption than of elimination. After a dose of 75 mg/kg, the apparent terminal half-life was shorter ($t_{1/2}$ at ~30 min) than those observed for doses >20 mg/kg. Interestingly, after this dose, peak concentration was higher and the time to peak was shorter than found with the highest dose, 100 mg/kg. These observations suggest that the absorption rate might be faster at 75 mg/kg than at other doses; however, the reasons for these observations are unknown at present.

The relationship between dose and dose-normalized AUC is shown in Table

**Table IV—Effect of Dose on the Normalized AUC and Apparent Bioavailability ($F$)**

| Dose | $n$ | AUC/Dose × $10^4$ | $F$, %[e,f] |
|---|---|---|---|
| 3.5[a] | 5 | 0.25 ± 0.05[d] | 1.6 ± 0.3 |
| 7[a] | 6 | 0.47 ± 0.12 | 3.2 ± 0.8 |
| 7[b] | 11 | 0.64 ± 0.05 | 4.2 ± 0.3 |
| 14[b,c] | 7 | 0.51 ± 0.19 | 3.3 ± 1.2 |
| 20[b] | 13 | 1.59 ± 0.20 | 10.3 ± 1.3 |
| 50[b] | 9 | 3.05 ± 0.50 | 19.8 ± 3.2 |
| 75[b] | 4 | 2.86 ± 0.07 | 18.6 ± 0.4 |
| 100[b] | 22 | 2.98 ± 0.45 | 19.4 ± 2.9 |

[a] Aqueous vehicle. [b] Vehicle of 10% ethanol in propylene glycol. [c] From interaction study with mannitol hexanitrate. [d] Mean ± $SE$, unit of min·kg·mL$^{-1}$. [e] Mean ± $SE$; $F = (AUC_{0-\infty}/Dose) \times CL$; $CL$ = 650 mL · min$^{-1}$ · kg$^{-1}$. [f] $F$ is found to be dose dependent (Kruskal-Wallis test, $p < 0.001$).

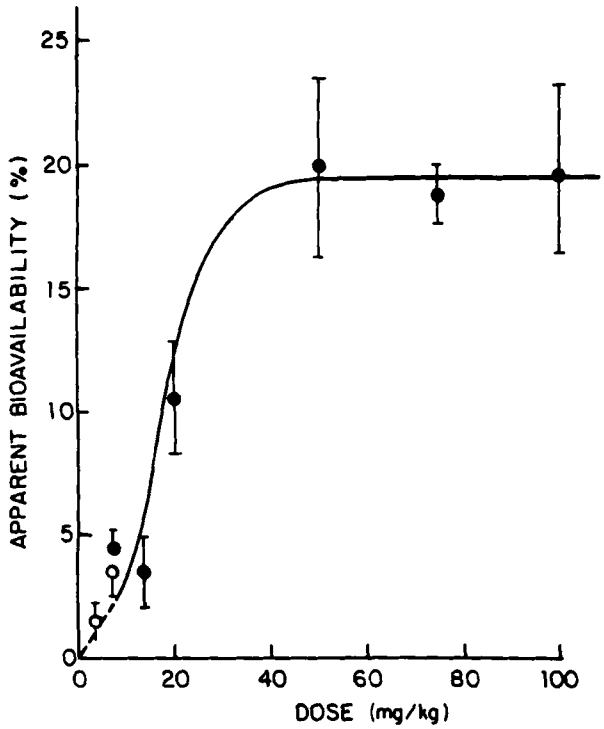


**Figure 4**—*Apparent oral bioavailability of nitroglycerin (mean ± SE) as a function of dose. The drug was administered either in water (○) or alcohol cosolvent (●).*

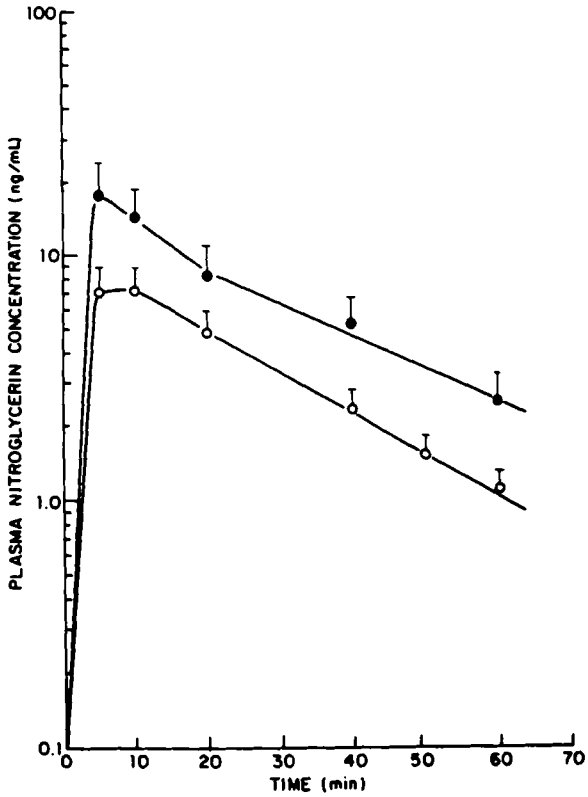


**Figure 5**— *Plasma nitroglycerin concentrations (mean ± SE) after a 14-mg/kg oral dose of nitroglycerin by itself (●) or in combination with 47 mg/kg of mannitol hexanitrate (○).*

IV. Similar data obtained with the aqueous vehicle at 3.5 mg/kg (37) and 7 mg/kg (10) are included for comparison. It is evident that, as the dose increased, the normalized AUC increased and then plateaued. If the systemic clearance obtained after intravenous administration of nitroglycerin in aqueous solution is assumed to be applicable under these experimental conditions, then the apparent bioavailability obtained after these oral doses may be estimated (Table IV). Thus, the apparent bioavailability appeared to increase with dose between 0–50 mg/kg. Between doses of 50–100 mg/kg, however, the apparent bioavailability remained constant at ~19%.

The interesting relationship between oral dose and apparent bioavailability, as observed here, supports the following conclusions. First, consistent with previous reports (14, 17, 21, 38), the oral bioavailability of nitroglycerin in the rat is indeed very small (< 3%) at doses <5 mg/kg. This poor bioavailability is probably a result of extensive first-pass metabolism (10, 14) rather than of lack of absorption *per se*, since radioactivity studies (39) showed absorption of the dose to be essentially complete. Second, the oral bioavailability of nitroglycerin is a rather complex function of dose. A possible model consistent with the data is that nitroglycerin is metabolized during absorption by at least two enzyme systems (or sites) which have different affinities and/or capacities for the drug substrate. This model is consistent with the observation by Jacoby (40) who suggested that organic nitrate reductase may, in fact, be one of several activities inherent to the glutathione-*S*-transferase system, which may consist of several "enzyme units." One could speculate that these different "enzyme units" may possess different substrate affinities and maximal velocities. Third, the bioavailability of oral nitroglycerin is highly variable at any dose. We have examined the source of this inter-animal variability and have shown (41) that it was due, in part, to the variability of the intrinsic hepatic organic nitrate reductase activity in individual rats. In view of the observed dose-dependent increase in bioavailability and the high inter-animal variability, some animals could have substantial or near complete bioavailability when given high doses of oral nitroglycerin. Thus, the general statement that nitroglycerin is poorly bioavailable has to be qualified by consideration of the dose administered and the individual animal being examined.

The dependency of $F$ on dose (and thus also on hepatic drug concentration) points out a possibly important consideration in designing oral controlled-release dosage forms of nitroglycerin. If a similar relationship exists in humans, the dependency of $F$ on drug release rate will have the same temporal pattern as is shown for dose (Fig. 4).

Figure 5 shows the plasma concentration of nitroglycerin after coadministration with mannitol hexanitrate. Surprisingly, the mean plasma concentration profile and AUC were actually smaller in the presence of the hexanitrate. However, despite a threefold difference in the mean AUC, statistical significance was not achieved due to the large coefficients of variation (100 and 50%, respectively, for nitroglycerin alone and combined administration). The effect of mannitol hexanitrate (if any) on bioavailability was extremely variable. For example, one rat exhibited almost a 10-fold decrease in AUC, whereas another showed a 1.6-fold increase in AUC in the presence of mannitol hexanitrate. In any event, mannitol hexanitrate did not apparently improve the bioavailability of nitroglycerin under the conditions tested.

There are several possible explanations for the apparent lack of effect. One possibility may be that an insufficient amount of mannitol hexanitrate was absorbed due to its poor aqueous solubility. Consequently, the organ concentration of mannitol hexanitrate would be too small to exert an inhibitory effect on nitroglycerin metabolism by organic nitrate reductase. Second, because of permeability and flow considerations, the relative affinity of mannitol hexanitrate *versus* nitroglycerin for the enzyme site *in vivo* may not be as favorable as *in vitro* determinations have suggested. Third, it is possible that mannitol hexanitrate coadministration may have altered the systemic clearance of nitroglycerin. Because of the very poor aqueous solubility of mannitol hexanitrate, a rigorous experiment involving intravenous administration of mannitol hexanitrate at the same dose was not carried out. Whatever the mechanism(s), however, oral coadministration of mannitol hexanitrate with nitroglycerin, under the conditions described, did not lead to any apparent increase in nitroglycerin bioavailability.

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# Phase Separation Induced in Gelatin–Base Coacervation Systems by Addition of Water-Soluble Nonionic Polymers I: Microencapsulation

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**Abstract** □ A microencapsulation procedure in which water-soluble nonionic polymers (especially, polyethylene oxide or polyethylene glycol) were added to gelatin–base coacervation systems is described. The advantages of this method are: (*a*) The addition of a small amount of polyethylene glycol (PEG) or polyethylene oxide (PEO) to a complex coacervation system (*e.g.*, gelatin–acacia) allows microencapsulation to occur over an expanded pH region (pH 2–9 in gelatin–acacia). (*b*) These polymers induce phase separation in an aqueous solution of gelatin alone and enable the preparation of gelatin-coated microcapsules not only in the vicinity of the isoelectric point (pH 9.0), but over a wide pH range (pH 5.5–9.5). (*c*) Spherical single-seeded microcapsules can be obtained.

**Keyphrases** □ Microencapsulation—gelatin–base coacervation systems, water-soluble nonionic polymer □ Phase separation—coacervation induced by the addition of water-soluble nonionic polymer □ Coacervation—gelatin–acacia, polyethylene glycol, polyethylene oxide, phase separation, microencapsulation

Microencapsulation using coacervation of gelatin or gelatin–acacia is the most popular of the aqueous media procedures. The practicality of the gelatin–acacia complex coacervation method (1–3) is evidenced by products such as no-carbon paper and liquid crystal thermometers. Complex coacervation is the phase separation caused in a mixture of anionic and cationic polymers (*e.g.*, acacia and gelatin) under restricted pH conditions and colloid concentration. This limitation is the disadvantage of the conventional microencapsulation procedure by complex coacervation.

On the other hand, under certain conditions the addition of a water-miscible organic solvent (*e.g.*, ethanol) or salt (*e.g.*, sodium sulfate) to the aqueous solution of a polymer (*e.g.*, gelatin) causes phase separation. This phenomenon has been used in microencapsulation as a simple coacervation method (4–8). The disadvantages of the simple coacervation method are the requirement of a large quantity of the organic solvent, the necessity of desalting, and the difficulty of setting the optimum conditions to obtain satisfactory microcapsules. Therefore, in this work, improvement of the conventional coacervating techniques was attempted.

## EXPERIMENTAL SECTION

**Materials**—The gelatin used has the following specifications as provided by the manufacturer[1]: bloom, 300; viscosity, 60.9 millipoises; pH 4.2; moisture, 9.9%; and isoelectric point, 9.0. The anionic polymers were acacia[2], carboxymethylcellulose (CMC)[3], and ethylene–maleic anhydride copolymer

[1] Miyagi Kagaku Kogyo.
[2] Spray-dried powder; Gokyo Sangyo.
[3] Serogen PR; Daiichi Kogyo Seiyaku.

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