trate level in the same plasma sample. This estimation apparently cannot be obtained directly from the Rosseel–Bogaert assay because of large interference peaks early in the chromatogram.

Linear calibration plots for nitroglycerin in rat and human plasma were obtained with the present procedure. The correlation coefficients of the lines were greater than 0.99. The precision and reproducibility of the present assay applied to plasma samples are shown in Table I. As expected, the relative standard deviations were higher (≃20%) for samples containing low concentrations of nitroglycerin (0.1 and 0.5 ng/ml) than for those with high concentrations. At 2 and about 40 ng/ml, the relative standard deviations were determined to be 8.6 and 4.2%, respectively. Saturation of the electron-capture detector occurred when the on-column amount of nitroglycerin exceeded about 0.5 ng.

Applications of the present technique in pharmacokinetic studies of nitroglycerin in a human subject (6) and in rats (7) are described elsewhere.

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Pharmacokinetics of Nitroglycerin in Rats

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Abstract □ The plasma nitroglycerin levels obtained after intracardial (0.7 mg/kg), oral (7 mg/kg), and topical (7–14 mg/kg) doses of nitroglycerin in rats are reported. Nitroglycerin followed essentially one-compartment kinetics after intracardial administration, showing a mean half-life of about 4 min and a mean apparent volume of distribution of about 3 liters/kg. After oral drug administration, "flip-flop" kinetics were evident. The mean oral bioavailability was determined to be 1.6%, firmly supporting the contention that nitroglycerin is extensively metabolized during first passage through the liver. Under the experimental conditions studied, no detectable levels of nitroglycerin were observed after topical application.

Keyphrases □ Nitroglycerin—pharmacokinetics after intracardial, oral, and topical administrations in rats □ Pharmacokinetics—nitroglycerin after intracardial, oral, and topical administrations in rats □ Vasodilators, coronary—nitroglycerin, pharmacokinetics after intracardial, oral, and topical administrations in rats

Recent studies dealt with the analytical (1–3), stability (4–7), and formulation (6, 8–12) aspects of nitroglycerin. These studies showed definitively that nitroglycerin in sublingual tablets can be stabilized effectively against volatilization by the inclusion of macromolecules such as povidone (8–10), polyethylene glycol (6), and microcrystalline cellulose (8, 12).

BACKGROUND

Very little information is available, however, on the pharmacokinetics of nitroglycerin in animals and humans. The analytical difficulty associated with the determination of nanogram or subnanogram concentrations of the intact drug in biological fluids is a primary reason for the paucity of quantitative information regarding nitroglycerin absorption and disposition. Recently, a GLC assay (13) quantifying nitroglycerin in plasma at these low concentrations was developed. This assay offers a unique opportunity to initiate rigorous *in vivo* pharmacokinetic studies in animals and humans.

Nitroglycerin is used in oral sustained-release dosage forms for the prophylaxis of angina. The effectiveness of this mode of nitroglycerin administration was seriously questioned by Needleman *et al.* (14) who showed that the drug undergoes extensive first-pass metabolism after oral dosing. The intact nitroglycerin levels were much lower after oral administration than after the intravenous route. Their data, however, were not reported in sufficient detail to allow for the estimation of the bioavailability of an oral dose of nitroglycerin.

Recently, nitroglycerin was used in ointment form for the hemodynamic management of patients with chronic congestive heart failure (15). This administration route for nitroglycerin was reported to produce beneficial effects lasting for 3–6 hr after a single application. The therapeutic efficacy and duration of effect of nitroglycerin, therefore, appear to be highly dependent on the route of drug administration.

The present study deals with the effect of the administration route on nitroglycerin pharmacokinetics in the rat. A pilot, collateral study in humans after therapeutic sublingual, oral, and topical doses of nitroglycerin is reported elsewhere (16).

EXPERIMENTAL

Materials—A 1.16-mg/ml aqueous solution of nitroglycerin was prepared by dissolving the appropriate amount of 10% nitroglycerin–lactose adsorbate[1] in distilled water. Solution was effected by overnight mechanical agitation and confirmed by the kinetic assay (2). The internal standard, isosorbide dinitrate, was obtained by acetone extraction from a 25% (w/w) isosorbide dinitrate–lactose powder[2], followed by evaporation of the organic solvent. Male Sprague–Dawley rats, 270–340 g, were used.

Intracardial Experiments—Six rats were fasted overnight and given an intracardial dose of nitroglycerin (0.7 mg/kg) under ether anesthesia. The dosing volume was approximately 0.2 ml. Blood samples (0.5–0.8 ml) were obtained through orbital sinus puncture using heparinized capillary tubes. Half of the rats (A, B, and C) were anesthetized during blood

[1] Nitroglycerin 10% (w/w) in lactose, ICI America, Atlas Chemical Division, Wilmington, DE 19899.

[2] Stuart Pharmaceuticals, Division of ICI United States, Wilmington, DE 19897.

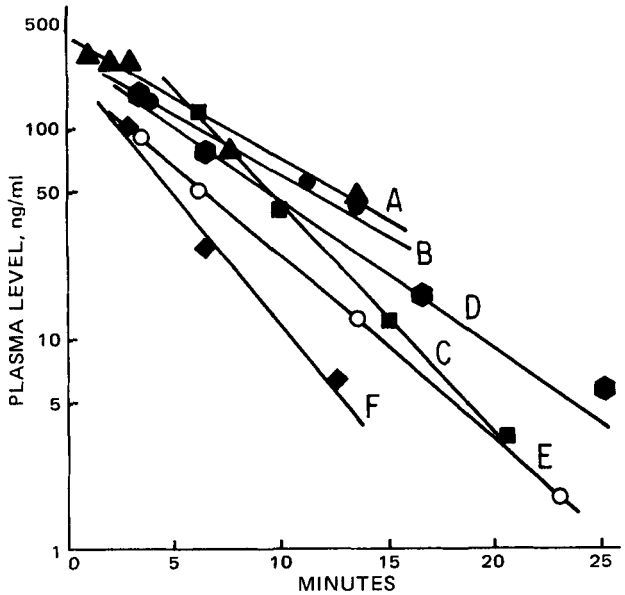

Figure 1—*Plasma nitroglycerin concentrations after intracardial administration of 0.7 mg of nitroglycerin/kg. The letters denote the different rats in the experiment.*

sampling while the others were not. The animals were not restrained except when blood samples were taken.

Oral and Topical Experiments—Five rats were each given a 7-mg/kg po dose of nitroglycerin in about 2 ml of aqueous solution by intubation under ether anesthesia. The animals were then placed in restraining cages. Topical doses of 7 and 14 mg of nitroglycerin/kg were administered to four and two animals, respectively, using a commercial nitroglycerin ointment[3]. The rats were placed in restraining cages after ether anesthesia, and the ointment (about 100–200 mg) was applied onto a 6.452-cm^2 area on the shaved back of the animals. Blood samples were obtained from the tail vein. All animals were fasted overnight with free access to water prior to drug administration.

Nitroglycerin Assay—Blood samples were centrifuged, and 0.2-ml plasma samples were removed. A 10-μl aliquot of 1 *M* silver nitrate immediately was added to the plasma to prevent enzyme degradation of the drug. Nitroglycerin was determined using the GLC assay recently developed (13).

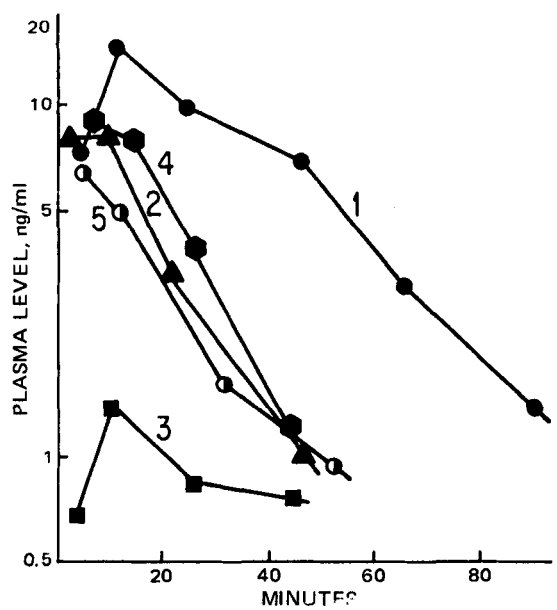

Figure 2—*Plasma nitroglycerin concentrations after oral administration (7 mg/kg) in rats. The numbers denote the different rats in the study.*

[3] Nitrobid ointment (2% nitroglycerin), Pharmaceutical Division, Marion Laboratories, Kansas City, MO 64137.

Table I—Pharmacokinetic Parameters[a] Obtained after Intracardial Administration of 0.7 mg of Nitroglycerin/kg

Rat	C_0, ng/ml	k_e, min^{-1}	$t_{1/2}$, min	V_d, liters/kg	$AUC_{0\to\infty}$
A	242	0.13	5.4	2.9	2024
B	200	0.11	6.1	3.5	1813
C	508	0.25	2.8	1.4	2421
D	198	0.14	4.9	3.5	1844
E	179	0.20	3.5	3.9	957
F	211	0.29	2.4	3.3	814
Mean	257	0.19	4.2	3.1	1646
SD	125	0.07	1.5	0.9	629

[a] Symbols are defined in the text.

RESULTS AND DISCUSSION

Kinetics following Intracardial Administration—The plasma nitroglycerin concentration–time curves obtained following intracardial injection of 0.7 mg of nitroglycerin/kg in Rats A–F are shown in Fig. 1. The disappearance of nitroglycerin in plasma followed first-order kinetics. Least-squares linear regression of the semilog plot of concentration *versus* time yielded a correlation coefficient greater than 0.99 for each animal. Using radioactive drug and cannulation techniques in rats, Needleman *et al.* (14) found that blood clearance of nitroglycerin was biphasic, with a distribution $t_{1/2}$ of less than 20 sec. Under the present experimental conditions, the distribution phase was not detected.

Table I lists the pharmacokinetic parameters calculated from the intracardial data. The plasma drug concentration at time zero, C_0, and the elimination rate constant, k_e, of the drug in each rat were obtained from the *y*-intercept and slope, respectively, of the least-squares fits of the respective log plasma nitroglycerin concentration–time curve. The biological half-life, $t_{1/2}$ (mean ± *SD*), was 4.2 ± 1.5 min. This value agrees with the values found using radioactive nitroglycerin (14, 17). An apparent volume of distribution, V_d, of 3.1 ± 0.9 liters/kg also was calculated. This rather high volume indicated that nitroglycerin is extensively distributed in the rat and is consistent with literature data (18, 19), which showed that, after administration of radioactive nitroglycerin, the radioactivity in blood constituted only a small fraction of the total radiolabeled dose.

Kinetics following Oral Administration—The plasma concentrations, C_p, as a function of time t after 7 mg of nitroglycerin/kg po are shown in Fig. 2. Biexponential plasma nitroglycerin concentration–time curves were observed in all animals after oral dosing. Peak plasma drug concentrations were generally detected within 12 min after drug administration. A one-compartment open model with an absorption step (absorption rate constant, k_a) was utilized in the kinetic analysis of these data. The integrated expression for the model is of the familiar form:

$$C_p = \frac{k_a FD}{V_d(k_e - k_a)}(e^{-k_a t} - e^{-k_e t}) \qquad \text{(Eq. 1)}$$

"Flip-flop" pharmacokinetics (20) appeared to be in operation upon oral nitroglycerin administration. This effect is to be expected for nitroglycerin because of its short biological half-life. Graphical calculation of pharmacokinetic parameters (using the mean value of k_e obtained previously) showed that the mean absorption rate constant was about 0.04 min^{-1}, about 20% of the magnitude of the elimination rate constant (0.19 min^{-1}) (Table II). These values were statistically different ($p < 0.02$) using the Student t test. Examination of the areas under the plasma concentration–time curve, $(AUC)_{0\to\infty}$, after oral administration revealed

Table II—Pharmacokinetic Parameters[a] Obtained after Oral Administration of 7 mg of Nitroglycerin/kg

Rat	k_a, min^{-1}	Absorption $t_{1/2}$, min	$AUC_{0\to\infty}$	F, %	V_d, liters/kg	t_{max} Obs.	t_{max} Calc.
1	0.030	23.0	622	3.78	2.3	13	12
2	0.058	12.0	204	1.24	3.0	5	6
3	0.016	43.4	100	0.61	2.7	11	10
4	0.058	12.0	234	1.42	2.7	7	8
5	0.042	16.5	167	1.01	2.7	5	5
Mean	0.041	21.4	265	1.61	2.7		
SD	0.018	13.1	205	1.25	0.3		

[a] Symbols are defined in the text.

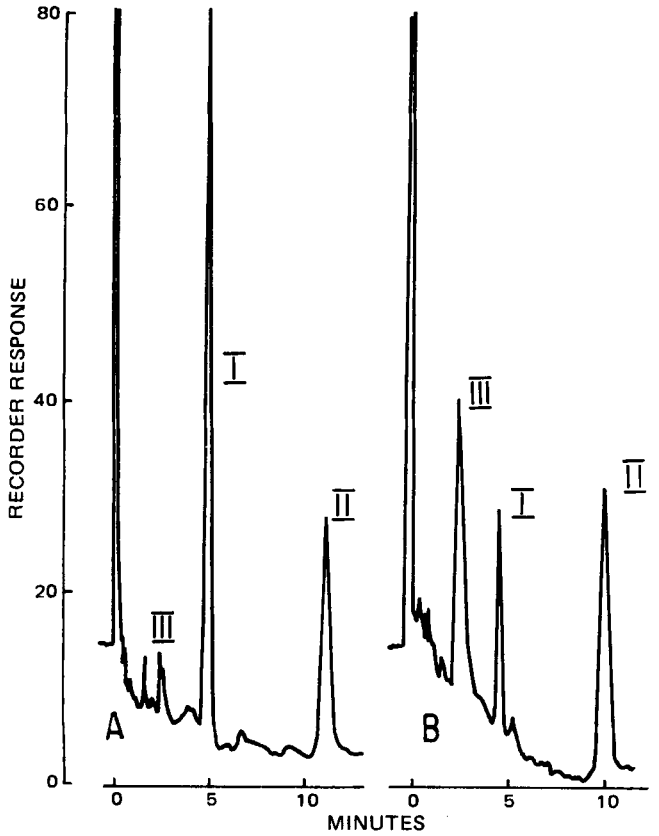

Figure 3—*Chromatograms of rat plasma 6.5 min after intracardial administration of 0.7 mg of nitroglycerin/kg (A) and 10 min after oral administration of 7 mg of nitroglycerin/kg (B) in rats. Key: I, nitroglycerin; II, isosorbide dinitrate; and III, isomeric glyceryl dinitrates.*

that they were only about 2% of those found after intracardial dosing. In the absence of dose-dependent elimination, the ratio $AUC_{oral}/AUC_{intracardial}$ after correction for dose could be equated to F, the fraction of the oral dose absorbed. The low magnitude of F and its considerable variability (Table II) support the general belief that nitroglycerin is extensively metabolized during its first passage through the liver (14).

The consistency of this pharmacokinetic treatment was examined *via* two parameters: the apparent volume of distribution and the time of peak plasma concentration, t_{max}. Equation 2 allows for the calculation of V_d from the oral data, using I, the extrapolated y-intercept, and other previously determined parameters:

$$V_d = \frac{k_a FD}{(k_e - k_a)I} \qquad \text{(Eq. 2)}$$

The V_d value calculated in this manner was 2.7 ± 0.3 liters/kg (mean ± *SD*). This value is not significantly different from that obtained after intracardial administration (3.1 ± 0.9 liters/kg). Equation 3 allows for the calculation of t_{max} after oral dosing:

$$t_{max} = \frac{2.303}{(k_a - k_e)} \log \frac{k_a}{k_e} \qquad \text{(Eq. 3)}$$

The calculated values were close to those observed (Table II).

Topical Nitroglycerin Administration in Rats—No detectable plasma nitroglycerin levels were observed after topical application of 7–14 mg of nitroglycerin/kg in the rat. This finding may be a result of very slow percutaneous absorption of the organic nitrate, leading to plasma drug concentrations beyond the assay detection limit. Since the nitroglycerin ointment used was petrolatum based, the release of the oil-soluble nitroglycerin was expected to be very slow. It is also possible that the rat skin (at the animal's back) is very impermeable to nitroglycerin penetration. The present animal model for skin absorption of nitroglycerin does not appear to reflect the situation with humans in whom topical nitroglycerin administration has been shown to be clinically effective (15).

Plasma Isomeric Glyceryl Dinitrate Levels—Figure 3 shows the typical chromatograms observed after intracardial (Fig. 3A) and oral (Fig. 3B) nitroglycerin administrations. Glyceryl dinitrates are the major metabolites of nitroglycerin in the rat and in other animals (14). Even though the recovery of isomeric glyceryl dinitrates from rat plasma is rather poor (~10–20%) in the assay of nitroglycerin, qualitative information regarding the metabolites may still be obtained from the chromatograms. In general, the peak height ratios of the dinitrates *versus* nitroglycerin were much higher at the early time points after oral administration, substantiating the conclusion of extensive first-pass metabolism after oral administration.

Very low levels of dinitrates were found after topical administration, suggesting again that the lack of nitroglycerin availability from this route of administration is due to poor absorption rather than extensive skin metabolism of the drug.

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