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Percutaneous Nitroglycerin Absorption in Rats

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Abstract □ Percutaneous nitroglycerin absorption was studied in shaved rats by monitoring unchanged plasma drug concentrations for up to 4 hr. Drug absorption from the neat liquid state or from an alcoholic solution was considerably poorer than that from a commercial ointment. This observation was unanticipated since the driving force for percutaneous drug absorption was assumed to be drug thermodynamics. Potential artifacts such as drug volatilization from the skin, reduction of surface area through droplet formation, and vehicle occlusion were investigated, but they did not appear to be responsible for the observed results. Two experimental aqueous nitroglycerin gels were prepared with polyethylene glycol 400. One gel contained just sufficient polyethylene glycol to solubilize the nitroglycerin; the other had excess polyethylene glycol to solubilize nitroglycerin far below saturation. Both gels gave extremely low plasma nitroglycerin levels. The composite data suggested that percutaneous nitroglycerin absorption is highly vehicle dependent and that this dependency cannot be explained by simple consideration of drug thermodynamic activity.

Keyphrases □ Absorption, percutaneous—nitroglycerin, various topical dosage forms, rats □ Nitroglycerin—percutaneous absorption, various topical dosage forms, rats □ Dosage forms, topical—nitroglycerin, percutaneous absorption, rats

Topical nitroglycerin ointments produce sustained and clinically beneficial hemodynamic responses in patients with various cardiovascular ailments. Application of a 2% nitroglycerin preparation on 150–230 cm^2 of skin surface reduced the frequency and severity of exercise-induced angina attacks (1, 2) and decreased heart workload and myocardial oxygen consumption (3).

Systemic availability is the primary goal of topical nitroglycerin application. However, there is little information about the physical and physiological factors influencing its percutaneous absorption. The relatively large clinical nitroglycerin ointment doses and the extensive surface area needed for therapeutic effect suggest that transdermal delivery of nitroglycerin, at present, is inefficient and that attempts to increase percutaneous nitroglycerin absorption may be worthwhile.

The purpose of this investigation was to examine various factors that may affect the rate and extent of topical nitroglycerin absorption. *In vivo* and *in vitro* models were used to gather data to form a rational basis for improving transdermal drug delivery. The relationship between thermodynamic activity and *in vivo* nitroglycerin absorption was examined.

BACKGROUND

The skin is one of the most impermeable tissues of the body. It functions as a barrier against attack by microorganisms, viruses, and many toxic chemicals. At the same time, it limits the loss of physiologically essential components, such as water. The extensive skin barrier properties have caused it to be regarded as a poor route for systemic drug administration. Recently, serious attempts have been made to fabricate devices for controlled, sustained delivery through the dermal route. A tape capable of delivering a constant rate of transdermal scopolamine for up to 3 days was described (4).

Several factors influence percutaneous drug absorption (5, 6) including drug permeability through the stratum corneum, the drug's physical–chemical properties, the vehicle in which the drug is incorporated, the drug's ionization state, the skin's hydration state, the skin's lipid content, and regional variations in skin properties. The quantitative relationship between drug transfer and several of these factors is (7):

$$dQ/dt = KC_vDA/T \qquad \text{(Eq. 1)}$$

where dQ/dt is the steady-state drug transfer rate across a skin barrier, K is the effective skin–vehicle drug partition coefficient, C_v is the drug concentration dissolved in the vehicle, D is the drug diffusivity through the barrier, A is the surface area of application of the vehicle, and T is the effective barrier thickness.

The model assumes that the skin represents a simple diffusional barrier to drug transfer. When the surface area of application, drug diffusivity through the skin, and barrier thickness are constant, the equation addresses the role of thermodynamics in determining percutaneous absorption rates. Specifically, absorption will be enhanced from vehicles with a low affinity for the drug and in which the drug concentration approaches saturated solubility. Therefore, approaches toward optimizing drug delivery should maximize the drug's thermodynamic activity in the vehicle, as represented by the product term KC_v in Eq. 1. The validity of the thermodynamic relationship has been confirmed qualitatively (8, 9), and Eq. 1 has provided valuable guidance in the development of improved vehicles for topical application of new drug entities.

While the thermodynamic approach does include skin characteristics—*viz.*, D and T, in determining absorption rate, these parameters are usually difficult to measure as functions of vehicle changes. Therefore, they are often assumed to be independent of vehicle effects. This assumption is not always valid since topical formulations are known to affect these parameters (5, 6, 10). Vehicle-induced changes in these variables may be of sufficient magnitude to override the contribution of the thermodynamic term, KC_v. In such cases, thermodynamic considerations alone, although predictive for *in vitro* drug release, may not explain absorption differences in animals or humans.

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Nitroglycerin is a liquid at room temperature. Absorption of pure drug applied to the skin would be uncomplicated by vehicle and/or particle dissolution effects. Unless a positive deviation from Raoult's law results when nitroglycerin is formulated in topical vehicles, the neat liquid state should give the maximal thermodynamic activity for drug penetration. This mode of application could serve as a convenient thermodynamic reference point for trials of experimental formulations.

In this study, *in vivo* nitroglycerin absorption from the neat liquid was compared to that from a clinical ointment[1]. The latter contains 2% nitroglycerin formulated in an oleaginous vehicle. Because of the favorable solubility of nitroglycerin in nonpolar solvents, a strict interpretation of Eq. 1 would suggest reduced nitroglycerin absorption from the ointment when compared to the neat state. This hypothesis was tested.

Followup experiments explored other factors, *e.g.*, evaporative loss and occlusion, that may influence nitroglycerin absorption. Aqueous polymer gels reflecting widely divergent thermodynamic activities of nitroglycerin, as estimated from partitioning and solubility data, were also prepared. The *in vitro* release and *in vivo* absorption of nitroglycerin from these formulations were compared to those of the ointment. The importance of thermodynamic considerations in optimizing transdermal nitroglycerin delivery was evaluated.

EXPERIMENTAL

Materials—All reagents were analytical grade unless otherwise noted.

Isolation and Assay of Nitroglycerin Raw Material—Nitroglycerin lactose triturate[2] (10% w/w) was packed as a column and eluted with anhydrous ether[3]. The solvent was evaporated from the eluate under a stream of dry compressed air for 6–8 hr. Nitroglycerin was recovered as a liquid, and its purity was determined by the USP XIX (11) procedure or by GLC (12).

Partitioning of Nitroglycerin between Isooctane and Polyethylene Glycol 400–Water—Solutions containing varying concentrations of polyethylene glycol 400 in distilled water were equilibrated with an equal volume of isooctane[4] containing 2.60 μg of nitroglycerin/ml. The mixtures were rotated end-over-end for 24 hr in a 20 ± 0.5° water bath. Duplicate samples were prepared for each composition. After centrifugation, aliquots of the two phases were diluted with absolute methanol and assayed by the kinetic procedure (13, 14).

In the concentrations used, polyethylene glycol did not affect the calibration curve in this kinetic assay. With 100% polyethylene glycol 400, nitroglycerin was overwhelmingly distributed into the polyethylene glycol phase. Therefore, assay of the organic nitrate in the isooctane phase was conducted by GLC.

***In Vitro* Nitroglycerin Release from Topical Preparations**—Aqueous polymer gels containing 2% nitroglycerin were prepared by dissolving raw nitroglycerin (96% purity) into polyethylene glycol–water solutions with stirring. Microcrystalline cellulose[5] (~29% w/w) was added to gel these solutions. All blends were assayed and used within 24 hr of preparation.

A modification of the *in vitro* release model described by Poulsen *et al.* (8) was used. The formulation was introduced into a tared 60-mm diameter × 15-mm petri dish cover[6], leveled to the top edge with a microscope slide, and weighed accurately. The cover was lowered into a 250-ml beaker, which was then placed in a 20 ± 0.5° water bath. A three-blade propeller-stirrer, 27 mm in radius, was positioned 1.5 cm above the test material surface. A variable-speed motor drive[7] was set for 10 rpm. Isopropyl myristate[8] (100.0 ml) previously equilibrated at 20° was added with care over the formulation. Samples of isopropyl myristate (1.0 ml) were taken at specified intervals, and the volume was replaced by fresh solvent equilibrated at the same temperature.

The nitroglycerin concentration in the isopropyl myristate receptor phase was determined by GLC after appropriate dilution with hexane. The total amount of drug released into the medium at each point was corrected for sampling. The area under the percent nitroglycerin released *versus* time curve (0–60 min) was calculated by the trapezoidal rule.

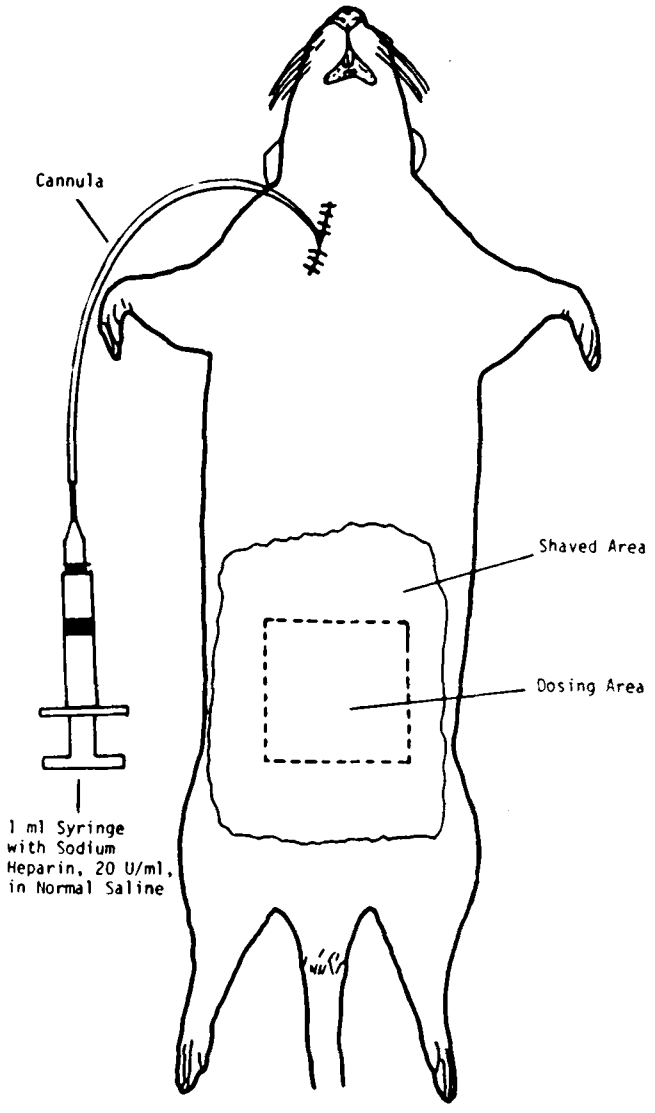

Figure 1—*Schematic representation of animal preparation and abdominal dosing area used in topical absorption studies.*

***In Vivo* Absorption Studies**—An abdominal dosing site (Fig. 1) on male Sprague–Dawley rats[9], 280–390 g, was prepared as described previously (15). The following dosage forms were investigated at 20 mg/kg: A, 2% nitroglycerin ointment[1]; B, 6.9% nitroglycerin solution in alcohol; C, neat nitroglycerin, isolated by evaporating a known volume of the alcoholic solution on a microscope slide and uniformly transferring the material to the dosing area; D, occlusion of the dosing site with polytef tape[10] 1 hr after application of the alcoholic solution; and E, 2% nitroglycerin experimental formulations, described previously. The area under the plasma concentration–time curve was evaluated from 0 to 4 hr after dosing (AUC_{0-4}) using the trapezoidal rule.

Nitroglycerin Recovery from Tissue Sections—Rat abdominal tissue sections from Treatments B and C were excised at the end of the experiments and minced. These tissue preparations were shaken with 25.0 ml of isomeric hexanes for 1 hr and allowed to sit overnight at room temperature. Appropriate dilutions with hexanes produced concentrations of 50–150 ng of nitroglycerin/ml. The internal standard was added to a final concentration of 250 ng/ml, and nitroglycerin was assayed by GLC. The extraction efficiency for this procedure was determined by excising and treating skin sections immediately after nitroglycerin application. In two experiments, a mean recovery of 75.8% was obtained.

RESULTS AND DISCUSSION

Results from trials involving abdominal dosing of 18 animals, allocated

[1] Nitro-Bid Ointment, lot T4808, Marion Laboratories, Kansas City, MO 64137.
[2] SDM 17, lot D-17-H2, ICI Americas, Atlas Explosives Division, Tamaqua, PA 18252.
[3] Mallinckrodt, St. Louis, MO 63147.
[4] J. T. Baker Chemical Co., Phillipsburg, NJ 08865.
[5] Avicel PH-105, FMC Corp., Marcus Hook, PA 19061.
[6] Corning Glass 3160, supplied through VWR Scientific, Buffalo, N.Y.
[7] Model 4440, Cole Parmer, Chicago, IL 60648.
[8] Technical grade, Pfaltz & Bauer, Flushing, NY 11368.
[9] Blue Spruce Farms, Altamont, N.Y.
[10] Chemfluor Lab-Tape, Chemplast, Inc., Wayne, NJ 07470.

Table I—Comparison of Areas under the Plasma Nitroglycerin *versus* Time Curve (0–4 hr) after Different Treatments

	Treatment		
	Ointment	Solution	Neat
	252.1[a]	2.2	36.9
	200.1	49.3	43.2
	72.3	49.3	29.3
	121.6	31.0	24.6
	71.1	15.3	20.1
	88.6	17.0	55.7
Mean	134.3	27.3	35.0
SD	75.2	19.3	13.1
	$p < 0.01$	$p > 0.05$	
	$p < 0.02$		

[a] In ng-hr/ml.

randomly to three treatment groups, are shown in Fig. 2. The clinically used nitroglycerin ointment yielded average 30–40-ng/ml plasma levels within 1 hr, while neat and alcohol applications gave mean concentrations below 10 ng/ml. The wide standard error ranges (Fig. 2) indicate that there was a large degree of individual variability in percutaneous nitroglycerin absorption. Comparison of AUC_{0-4} is presented in Table I. Despite the observed variability, analyses of variance [Kruskall–Wallis rank method (16)] with multiple comparisons indicated the ointment treatment to be significantly different from both the neat ($p < 0.02$) and the alcohol ($p < 0.01$) administrations. The latter cases were statistically indistinguishable, suggesting that they represent equivalent topical dosing modes.

Both neat and alcohol solution treatments were studied because neat nitroglycerin represented a small volume of drug, which may have been subjected to significant transfer errors, while the alcohol solution represented a more manageable volume for uniform dosing, but the effect of the solvent on the skin could not be predetermined. The results show that the application appeared adequate in both cases and that the alcohol solvent did not significantly alter skin characteristics *vis-à-vis* nitroglycerin absorption.

The finding of reduced absorption from neat nitroglycerin was unanticipated from thermodynamic considerations of Eq. 1. Possible explanations include: (*a*) evaporative loss of neat drug from the absorption site, (*b*) alteration in skin permeability by an occlusion effect caused by the ointment, (*c*) artifactual reductions in total surface contact in the case of neat nitroglycerin, (*d*) positive deviations from Raoult's law (*i.e.*, increases in thermodynamic activity) due to a specific interaction between the drug and some vehicle component, and (*e*) nonspecific effects produced by certain components of the ointment vehicle that increase nitroglycerin penetration.

Experiments were carried out to assess each of these possible factors.

Nitroglycerin Loss from Absorption Site—The volatility of nitroglycerin sublingual tablets has been documented extensively (17–19). While the thermodynamic activity of nitroglycerin and, hence, its potential for absorption were expected to be maximized in the neat state,

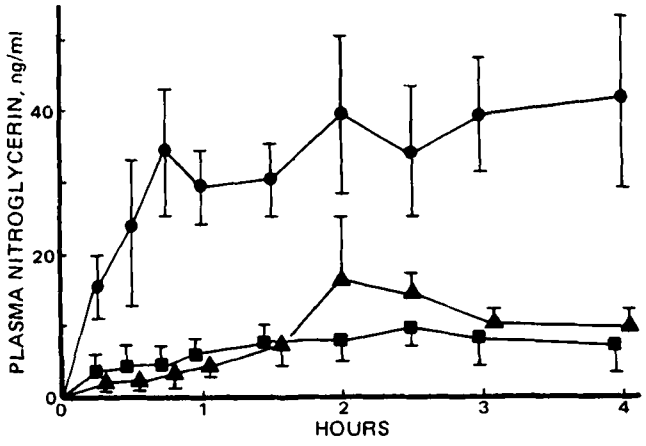

Figure 2—In vivo *absorption of nitroglycerin after topical application (20 mg/kg) to the abdominal skin surface of rats. Key:* ●, *2% nitroglycerin commercial ointment;* ■, *6.9% nitroglycerin alcoholic solution; and* ▲, *neat nitroglycerin. Each point represents the mean* (±SEM) *of six animals.*

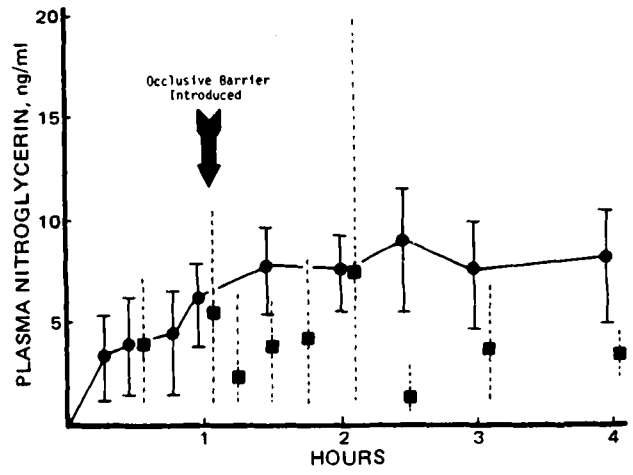

Figure 3—*Effect of polytef tape occlusion on topical absorption of nitroglycerin from alcoholic solution. Key:* ●, *no occlusion, bars indicate standard errors; and* ■, *occlusion with polytef tape after 1 hr. Each point represents the mean of three animals. Broken lines indicate experimental range.*

the energy balance would simultaneously favor nitroglycerin escape to the atmosphere. This phenomenon might lead to reduced absorption because of volatilization of drug from the dosing site.

To investigate this possibility, tissue sections representing the entire dosing area were excised after the 4-hr absorption experiments and the amount of nitroglycerin remaining was determined. An average of nearly 60% of the applied drug could be recovered from the surface skin sections (Table II). The unrecovered 40% of the dose might have been in the systemic circulation or still moving through the epidermal layers. Nevertheless, the evidence suggests that there was no extensive evaporative loss of drug at the absorption site after application of neat nitroglycerin or the alcoholic solution. Significant quantities of drug were still available for absorption at the dosing site, even at the end of the experimental period. This conclusion is supported by results from the occlusion experiment.

Effect of Skin Occlusion—Skin hydration is often considered to be the most crucial parameter affecting percutaneous absorption of chemical compounds (5). Increasing the water content of the stratum corneum has been shown to alter the rate and extent of transdermal drug passage (20). Oleaginous vehicles and occlusive dressings offer certain advantages by inhibiting transepidermal water loss. Thus, the petrolatum–lanolin ointment base possibly increased percutaneous nitroglycerin absorption primarily through an occlusive effect.

To test this hypothesis, an alcoholic solution of nitroglycerin was applied to the shaved abdominal surfaces of three rats. The dosing site remained exposed for the 1st hr during which blood samples were collected to establish control values for plasma nitroglycerin concentrations. At 1 hr, the area was covered with polytef tape and sampling was continued. Enhanced nitroglycerin absorption after this time point would suggest a positive influence of an occlusive effect. The results showed, however, that the plasma concentrations were unaffected by occlusion (Fig. 3). Thus, it is unlikely that occlusion was responsible for the greater absorption of nitroglycerin from the ointment preparation.

Polytef tape presents a barrier to water mass transfer (21), and preliminary studies demonstrated a resistance to nitroglycerin transfer. The polytef tape not only prevented water loss from the skin but should have retarded significant volatilization of nitroglycerin from the dosing site.

Table II—Percent Recovery [a] of Nitroglycerin from Rat Tissue Sections 4 hr after Abdominal Application of a 20-mg/kg Dose

Application[b]	Recovery, %
Neat	50.8
Neat	64.1
Neat	55.2
Neat	49.7
Alcohol solution	60.9
Alcohol solution	74.1
Mean	59.1

[a] Corrected for extraction. [b] Each listing represents an individual tissue section from separate animals.

Because there was no increase in plasma nitroglycerin levels after enclosure of the dosing site with polytef tape, evaporative drug loss after application of neat nitroglycerin and its alcoholic solution could not have been extensive.

Surface Area Considerations—As clearly stated in Eq. 1, the conditions required for testing the thermodynamic concept must include a constant surface area for drug application. The total abdominal dosing area was controlled at 9 cm^2 for all experiments. This region could, however, represent only an apparent dosing area if the interfacial tension between nitroglycerin and the skin was of sufficient magnitude to cause poor "wetting," reducing the actual contact area through droplet formation.

Gross examination of the skin after dosing showed nothing to suggest that uniform film formation had not taken place. Microscopic examination and photographic documentation proved difficult because of the low level of contrast between the deposited drug, a clear to slightly yellowish liquid, and the skin background. Attempts to visualize the drug on the skin using oil-soluble dyes or fluorescent markers were also inconclusive. Thus, within the visualization capability of the experiment, surface area artifacts arising from droplet formation from neat nitroglycerin appeared to be absent. This conclusion will be substantiated further by comparison of *in vivo* absorption of nitroglycerin from ointment and experimental gels.

Comparison of *In Vitro* Nitroglycerin Release between Experimental Gels and Ointment—Aqueous nitroglycerin solubility at 20° was determined to be 1.77 mg/ml, in close agreement with literature reports (22). Nitroglycerin partitioning between isooctane and polyethylene glycol 400–water combinations is shown in Fig. 4. Because of extensive nitroglycerin solubility in alcohols, the partition coefficients (oil-water) were reduced drastically in the presence of significant concentrations of polyethylene glycol 400. The observed partition coefficients and measured water solubility value were used to estimate nitroglycerin solubility in the different water-polyethylene glycol 400 mixtures (Fig. 4). This method of solubility estimation was used instead of the classical excess compound technique to avoid handling large quantities of pure nitroglycerin.

Gels were prepared using polyethylene glycol 400–water mixtures and microcrystalline cellulose. Simple mixing of components yielded pharmaceutically suitable preparations of uniform texture and acceptable viscosity. Preliminary experiments involving gels prepared with different concentrations of microcrystalline cellulose showed that this material did not inhibit nitroglycerin diffusion out of the gels. This finding suggests that drug binding or adsorption to microcrystalline cellulose is insignificant in the presence of polyethylene glycol 400.

From the calculated solubility profile, it was determined that a 2% nitroglycerin gel could be prepared near saturated solubility using 70% polyethylene glycol 400 in water (Gel A). This formulation presented nitroglycerin at a near maximal thermodynamic state when compared to similar formulations in which 2% nitroglycerin did not represent saturated solubility. In 100% polyethylene glycol 400, the estimated nitroglycerin solubility was approximately 150-fold greater than in 70% polyethylene glycol 400. Thus, a 2% nitroglycerin gel prepared with 100% polyethylene glycol 400 (with microcrystalline cellulose added for viscosity) (Gel B) presented nitroglycerin at a 150-fold reduction in thermodynamic activity compared to that produced by Gel A.

Estimation of nitroglycerin thermodynamic activity in the ointment could not be made because of solubility problems. Information on the composition of this ointment was not available, and the saturated solubility of nitroglycerin in the ointment could not be determined easily. The relative thermodynamic activity of nitroglycerin in the gels and the ointment could be determined by the relative *in vitro* release rates.

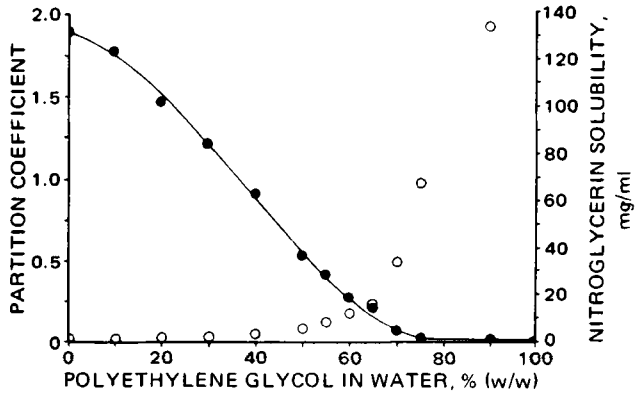

Figure 4—*Partitioning and estimated solubility of nitroglycerin at 20°. Key: ●, observed partition coefficient (isooctane–aqueous–polymer) for nitroglycerin; and ○, calculated solubility of nitroglycerin in water–polymer mixtures.*

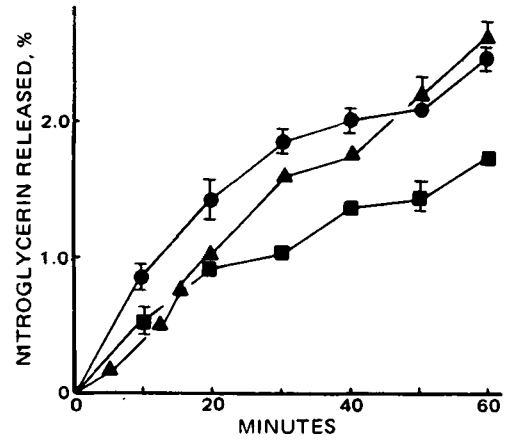

Figure 5—*In vitro release of nitroglycerin from 2% topical preparations into isopropyl myristate. Key: ●, Gel A; ■, Gel B; and ▲, commercial ointment. Each point represents the mean of two trials. Bars indicate the range of observed values. Where bars are not shown, the observed range was within the area covered by the symbols used.*

Comparative *in vitro* release rates from Gels A and B and the ointment into isopropyl myristate were evaluated. A hydrocarbon solvent such as isooctane could not be used because it caused rapid dissolution of the oleaginous vehicle. On the other hand, higher molecular weight alcohols such as octanol brought about disintegration of the polyethylene glycol-based gels. Isopropyl myristate was chosen as a compromise because both polyethylene glycol and water were immiscible with it while ointment dissolution, although present, was considerably less rapid. To avoid further artifactual results from ointment dissolution, a very slow stirring rate for the receptor phase was used. Sampling was conducted for 1 hr to compare the initial release characteristics from the three preparations (Fig. 5).

The areas under the percent release–time curves (in percent release-min) were 94.2 and 85.3 for Gel A, 55.6 and 55.1 for Gel B, and 80.2 and 75.9 for the ointment. The data suggested that Gel A released nitroglycerin about two times faster than Gel B. This result is in qualitative agreement with a prediction based on solubility arguments, but Gel A did not release nitroglycerin 150 times faster than Gel B, as would be expected if the drug solubility in the vehicle is inversely related to release. Isopropyl myristate, however, is a much better solvent for nitroglycerin than is isooctane. Thus, differences in partitioning with the latter solvent may be greatly reduced because of the solvent "leveling" effect of isopropyl myristate. At any rate, nitroglycerin release from the ointment was not statistically different from that produced by Gel A. This finding suggests that nitroglycerin is not present in the ointment at an abnormally high thermodynamic activity. Thus, a positive deviation from Raoult's law cannot be invoked for the increased nitroglycerin absorption from the ointment over the neat liquid. The absence of an enhanced "escaping tendency" of nitroglycerin in the ointment was further indicated by the following observation: when several ointment samples were placed unmixed and allowed to equilibrate with water at 20°, the resultant aqueous concentrations measured at 1 and 2 weeks did not exceed the aqueous solubility of nitroglycerin.

The lack of quantitative correlation between the *in vitro* release rate and solubility in Gels A and B might have been due to the slow stirring rate used. Release rates from topical steroid preparations using a similar *in vitro* test system were reported to be sensitive to stirring rates of less than 30 rpm (8). This degree of agitation could not be accommodated in the present study without erosion of the ointment base by the solvent. An alternative approach using a dialysis membrane[11] to separate the vehicle and isopropyl myristate was unsuccessful because polyethylene glycol 400 rapidly diffused through the artificial membrane.

***In Vivo* Nitroglycerin Absorption from Polymer Gels**—The *in vivo* study of percutaneous absorption of Gels A and B produced unan-

[11] Spectrapor membrane tubing, mol. wt. cutoff 6000–8000, Spectrum Medical Industries, Los Angeles, CA 90054.

ticipated results. In contrast to the ointment, where a plateau drug level of 30–40 ng/ml was achieved, both gels (three animals in each trial) gave negligible (<1 ng/ml) plasma nitroglycerin concentrations over 4 hr. The *in vivo* absorption data did not correlate with the *in vitro* release results.

The composite data suggest a specific skin–vehicle interaction with the ointment, which has the net effect of increasing nitroglycerin permeability or decreasing the effective skin barrier thickness. Alternatively, poor nitroglycerin absorption from the neat material, alcoholic solution, and polyethylene glycol gels may have arisen from unfavorable interactions with the skin. A recent study showed that benzocaine diffusion through human stratum corneum decreased in the presence of relatively high amounts of low molecular weight polyethylene glycol (23). These researchers also showed that polyethylene glycol significantly affected the surface structure of the stratum corneum. These effects may be tested by comparing the absorption of a marker chemical following application of neat nitroglycerin or polyethylene glycol gels on the skin to absorption through untreated skin.

The ointment may increase transdermal nitroglycerin delivery by inhibiting skin metabolism. Enzymatic process in the skin and considerations of the skin as an active metabolizing barrier were discussed recently (24, 25). Previous studies also showed a total body nitroglycerin clearance from the rat in excess of reasonable hepatic clearance (26), suggesting extensive tissue degradation of this drug. The skin may represent a first-pass metabolic site for topically applied nitroglycerin. Interference with this process can affect the amount of intact nitroglycerin reaching the systemic circulation. This aspect of nitroglycerin transdermal delivery will be investigated.

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Kinetics of Dopamine Oxidation by Dialkylaminoalkylphenothiazine Cation Radicals

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Abstract □ The kinetics of dopamine oxidation by dialkylaminoalkylphenothiazine cation radicals (with two- or three-carbon side chains) were investigated. The two-carbon side-chain derivatives have reaction rates higher than the three-carbon ones. For chlorpromazine and promazine, extrapolation of pH 1–6 data shows that reaction rates become very fast at physiological pH.

Keyphrases □ Dopamine—oxidation by phenothiazine cation radicals, kinetics, structure–activity relationships □ Phenothiazine derivatives—oxidation of dopamine, kinetics, structure–activity relationships □ Structure–activity relationships—phenothiazine cation radicals, oxidation of dopamine, kinetics

Several reports (1–4) pointed out that transformation of chlorpromazine [2-chloro-*N*,*N*-dimethyl-10H-phenothiazine-10-propamine] (I) is necessary for some *in vitro* enzyme inhibitions. A I intermediate, the cation radical [obtained also *in vivo* (5)], was shown to inhibit (Na^+, K^+) adenosine triphosphatase (6). The cation radical also inhibited microsomal brain enzyme (7) and uridine diphosphate glucose dehydrogenase (8).

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