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Nitroglycerin pharmacokinetics using specific and sensitive analytical procedures

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Nitroglycerin Pharmacokinetics

Using Specific and Sensitive Analytical Procedures

by

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DISSERTATION

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To

Iris and Sheryl

With Love

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NITROGLYCERIN PHARMACOKINETICS USING SPECIFIC AND SENSITIVE ANALYTICAL PROCEDURES

ABSTRACT

Nitroglycerin (GTN) pharmacokinetics have been poorly understood because the previously used analytical procedures were non-specific and/or insensitive. In this work specific, sensitive and precise capillary GC assays capable of measuring 25 pg/ml nitroglycerin and 100 pg/ml of the 1,2- and 1,3-glyceryldinitrates (1,2-GDN and 1,3-GDN) in human plasma are described. Nitroglycerin metabolism by human blood and plasma, is investigated; half-lives in blood and plasma are 17 to 32 min and 202 ± 5 min, respectively. All four GTN metabolites (1,2-GDN, 1,3-GDN, 1-GMN and 2-GMN) are identified.

Pharmacokinetics and bioavailability of GTN following sublingual, oral and topical administration, relative to intravenous doses, are evaluated in healthy volunteers. A counter-clockwise hysteresis is observed when infusion rate (of 10, 20, 40 and again 10 mcg/min) is plotted versus steady-state concentrations. Nitroglycerin clearance varies not only with dose but also with previous exposure to the drug.

Sublingual GTN bioavailability is low and variable. A large amount of the dose (31.4 ± 18.9 percent) is recovered from the mouth after 8 min. Estimates of sublingual GTN

bioavailability vary depending on the intravenous infusion rate used to calculate clearance. The bioavailability of GTN, administered as a solution, was 0%. Basing transdermal GTN bioavailability on the highest intravenous infusion rate, apparent absorption rates ($mcg/cm^2/hr$) were determined for Nitro-BID^R ointment (5.15 ± 1.07) and sustained release patches (Nitrodur^R (8.09 ± 6.35) and Nitrodisc^R (5.55 ± 3.04). Because ointment and patch transdermal absorption rates are equivalent, it is concluded that the skin is the rate controlling step for transdermal GTN delivery.

The apparent elimination half-lives of 1,2- and 1,3-GDN are 33.2 ± 6.5 and 57.2 ± 30.0 min, respectively. After oral administration of nitroglycerin solutions to volunteers, high concentrations (10 to 30 ng/ml) of 1,2-GDN and 1,3-GDN are detected. The efficacy of oral GTN is most likely related to metabolite concentrations. The ratio of 1,2-GDN to 1,3-GDN varies as a function of route of nitroglycerin administration (oral, 1.96 ± 0.31; intravenous, 7.44 ± 1.55; ointment, 3.92 ± 1.36; sublingual, 4.51 ± 0.63). Metabolite formation depends on the route of administration, thus implying different metabolic specificity of enzymes in the gut, liver, skin, sublingual mucosa and blood vessels.

STATEMENT OF PURPOSE

Nitroglycerin is a potent vasodilator used in the treatment of angina pectoris, congestive heart failure and acute myocardial infarction. Even though nitroglycerin was first synthesized over a century ago, very little is actually known regarding the pharmacokinetics and disposition of this drug. This information has not been available because of the lack of sensitive and specific analytical procedures. We therefore propose the following objectives:

1. To develop a specific and sensitive capillary gas chromatographic assay to measure picogram concentrations of nitroglycerin in plasma.

2. Using this assay, to elucidate the pharmacokinetics of nitroglycerin after intravenous infusion, oral, sublingual and topical doses in healthy volunteers.

3. To investigate the in vitro metabolism of nitroglycerin by human blood.

CHAPTER I

PHARMACOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF NITROGLYCERIN

A. HISTORICAL BACKGROUND

Nitroglycerin was first synthesized in 1846 by Sobrero. The synthesis involves the nitration of glycerol to form the trinitrate-ester of glycerin, i.e. glyceryltrinitrate (GTN, Fig. I-1). Note that nitroglycerin contains nitrate ester groups ($C-O-NO_2$) and not nitro ($C-NO_2$) groups as the name (nitroglycerin) implies. Soon after synthesis, it was noted that after placing a small amount of the oil on the tongue, a severe headache with throbbing temples occurred.

Alfred Nobel, in 1876, used nitroglycerin in the manufacture of dynamite. He found that by modifying mixtures of nitroglycerin and diatomaceous earth or charcoal, dynamite could be modified to serve different types of blasting needs.

William Murrell was the first physician to recognize the usefulness of nitroglycerin for the relief of angina pectoris two decades after nitroglycerin was synthesized (Murrell, 1879). He directed a patient to place 3 drops of a 1% solution of nitroglycerin into one half ounce of water and take it three times daily. He found that both the number and the severity of angina attacks decreased with this regimen. He also found that when the dose was taken

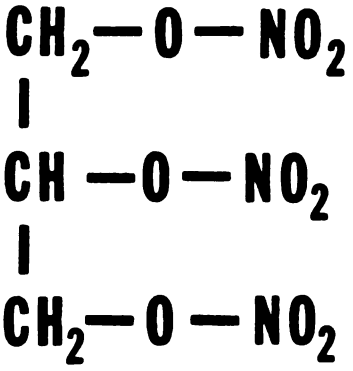


FIG. I-1: Chemical structure of nitroglycerin.

during an anginal episode, the attack duration was shortened. During the hundred years that have elapsed since this study was reported, nitroglycerin has become one of the most widely prescribed drugs used for the treatment of angina pectoris. It is considered the standard against which all newer therapies are measured.

Currently, nitroglycerin is administered sublingually for immediate relief of an acute angina attack. Because the duration of action for a sublingual nitroglycerin dose is short, approximately 20 min, longer-acting dosage forms have been developed. These include sustained release oral tablets and topical ointments, gels and sustained release patches.

B. NITRATE PHARMACOLOGY AND THERAPEUTICS

1. ANTIANGINAL ACTION OF NITRATES

a. ORGANIC NITRATE ACTIONS ON PERIPHERAL CIRCULATION

Nitroglycerin is a member of the organic nitrate class of drugs which dilate vascular (both arterial and venous) smooth muscle. Imhoff et al. (1980) and Packer et al. (1979) found that low concentrations of nitroglycerin dilate predominantly the venous circulation. Higher concentrations produce correspondingly greater effects (i.e., a greater

decrease of pressure) on the arterial circulation in a dose-dependent fashion. The main effect of nitroglycerin is to cause a decrease in venous tone which causes blood to pool in the venous capacitance circulation (Mason and Braunwald, 1965).

The direct effect of organic nitrates on venous capacitance results in a decreased venous return to the heart (decreased preload). A decrease in systemic blood pressure, especially in the standing position, may occur as a result of decreased cardiac output (Williams et al., 1975). In patients with normal left-ventricular function, a reduction in left-ventricular preload is associated with a decreased cardiac output. In patients with congestive heart failure, with elevated left ventricular preload, nitrates may cause an increase in cardiac output which is due to decreased peripheral resistance (Bussman et al. 1977 and Taylor et al., 1976).

Administration of nitroglycerin to subjects with normal left ventricular function may result in a reflex tachycardia response to the decrease in blood pressure (Awan et al., 1978). On the other hand, a change in heart rate is seldomly detected when nitroglycerin is administered to patients with elevated left ventricular end diastolic pressures (Franciosa et al., 1977 and Meister et al., 1976).

b. ORGANIC NITRATE EFFECTS ON THE CORONARY CIRCULATION

Organic nitrates, such as nitroglycerin, produce a prolonged preferential vasodilation of normal large coronary arteries but have only a small, transient effect on the small coronary arteries. Winbury et al. (1969) proposed that nitrates cause a redistribution of the coronary blood supply to ischemic areas. Horowitz et al. (1971) confirmed this hypothesis following sublingual nitroglycerin dosing to ten patients with coronary artery disease. They injected an inert radioactive gas (^{133}Xe) directly into ischemic myocardial tissue and found that sublingual nitroglycerin increased the clearance of radioactivity in 9 of the 10 patients. Aronow (1972) found that, although sublingual nitroglycerin dilated coronary arteries in normal subjects, it did not increase the coronary blood flow in patients with coronary artery disease. Additionally, Ganz and Marcus (1972) found that intra-coronary injections of nitroglycerin to patients with coronary artery disease failed to alleviate pacing-induced angina. Intravenous injections of nitroglycerin, though, were effective in alleviating the angina. They concluded that dilation of the coronary bed played a minor role (if any) in the antianginal effect of nitroglycerin. The major action of nitroglycerin was on the systemic circulation (i.e., on preload).

Recent studies (Feldman et al., 1982; Pepine et al., 1982; Forman et al., 1983) have shown that intracoronary

injections of nitroglycerin increased collateral blood flow to ischemic tissue. Pepine et al. (1982) showed that intracoronary nitroglycerin could reverse coronary artery spasm that did not respond to systemic nitroglycerin administration. These data seem to contradict the earlier study of Ganz and Marcus (1972) which did not show any effect for intracoronary injections of nitroglycerin. However, Ganz and Marcus (1972) did not measure blood flow to ischemic tissue only the response to nitroglycerin. Note, also, that these studies were performed in two different patient populations (coronary artery disease and coronary artery spasm), in which disease etiologies were different. Therefore, direct comparison of these studies is difficult. Although the precise mechanism is not yet known, it appears that nitroglycerin can increase collateral blood flow during acute myocardial ischemia.

c. NITRATE EFFECTS ON MYOCARDIAL OXYGEN DEMAND

In healthy individuals, there is normally a balance between myocardial oxygen demand and supply. Figure I-2 shows a schematic diagram of this relationship between myocardial oxygen supply and demand (Pitt, 1975). When oxygen demand exceeds supply, myocardial ischemia results. As shown in Fig I-2, several factors control myocardial oxygen demand (myocardial oxygen consumption, MVO_2). These factors may be divided into two categories. The major

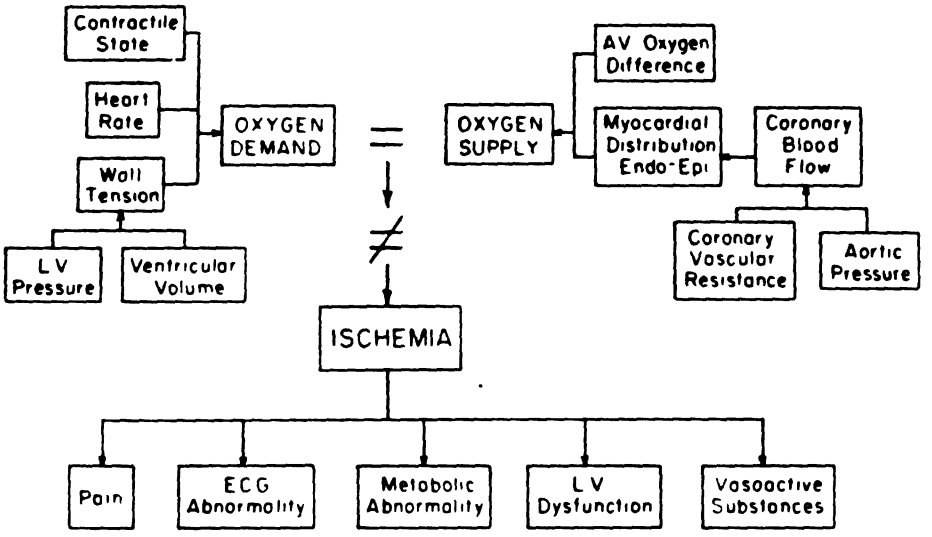


FIG. I-2: Schematic diagram of the relation between myocardial oxygen supply and demand (Pitt, 1975).

factors which control MVO_2 include intramyocardial tension, contractile state and heart rate. Minor factors which determine MVO_2 are the external work (load x shortening), activation energy and basal or resting metabolism. These factors were reviewed in detail by Sonnenblick et al. (1968) and Sonnenblick and Skelton (1971).

The contractile state of the heart is characterized by the force-velocity relationship and the maximal velocity of myocardial contraction. Increases in either the inotropic state of the heart or the heart rate will cause a concomitant increase in MVO_2.

Intramyocardial tension (stress) is the most important determinant of MVO_2. This tension may be defined by the LaPlace relationship. According to this relationship, the tension in the wall of the heart varies as the product of ventricular pressure and the square of the heart cavity radius. Sonnenblick et al. (1968) emphasized that ventricular wall tension (T) is a function of the intraventricular radius (r) and pressure (P), and the ventricular wall thickness (h). These variables are related by the following equation :

$$T = P \times r / 2h$$

The ventricular wall stress is often related to preload and afterload (Needleman and Johnson, 1982). Preload determines the left ventricular end-diastolic pressure

(i.e., the diastolic pressure that distends the relaxed ventricular wall). An increase or decrease in preload results in corresponding changes in ventricular wall tension and MVO_2. Afterload is the force on the ventricular wall during ejection of blood and may be defined as the systolic wall tension. It is related to the aortic pressure (peripheral resistance) and to the radius of the ventricle.

The organic nitrates reduce both preload and afterload as a result of dilatation of both venous capacitance and arterial resistance vessels (Chatterjee, 1980). The net effect of nitrates is to decrease MVO_2 since the primary determinants of oxygen demand (preload and afterload) are reduced. Myocardial ischemia is alleviated when the oxygen supply is sufficient to meet the decreased oxygen demands of the heart muscle.

d. NITRATE EFFECTS ON MYOCARDIAL OXYGEN SUPPLY

When myocardial oxygen demand exceeds supply, an alternative to decreasing demand is to increase the supply (Fig. I-2). Oxygen supply to myocardial tissue is dependent on extraction of oxygen across the myocardium and the blood flow (of oxygen) to the myocardium. Oxygen extraction is already near maximum in myocardial tissues (Pitt, 1965) and can not contribute to increasing myocardial oxygen supply. Therefore, an increase in coronary blood flow is the only mechanism available to meet any increase in oxygen demand.

As previously discussed, nitroglycerin has three major sites of action on the vascular system: the coronary arteries, peripheral arteries and peripheral veins. Nitroglycerin may change coronary blood flow as a direct action on arteriolar resistance (Becker, 1976). Although nitroglycerin dilates coronary arteries, total coronary blood flow is not affected. Rather, nitroglycerin causes a redistribution of blood flow to ischemic areas. The precise mechanism of this blood flow redistribution as related to the antianginal effect is not known.

An alternative mechanism suggests that nitroglycerin reduces preload, thereby reducing myocardial tissue pressure. The reduced pressure increases the effective coronary perfusion pressure which increases subendocardial blood flow (Becker et al., 1971). Note that subendocardial tissues are often ischemic during angina. Also, by reducing preload, nitroglycerin may increase collateral flow to ischemic regions by reducing the mechanical compression of collaterals in the subendocardium (Goldstein et al., 1974).

e. NITROGLYCERIN THERAPY IN ANGINA

Sublingual nitroglycerin is indicated for the acute relief of angina pectoris or for prophylaxis five to ten minutes immediately prior to stressful events to prevent or minimize anginal attacks. The patient places one tablet (0.15, 0.3, 0.4, 0.6 mg) under the tongue and holds it there

until the drug is completely absorbed. This dose may be repeated every 5 min for 15 min, if needed, after which the patient should contact his/her physician if relief has not been attained because this situation might be indicative of myocardial infarction (Warren and Francis, 1978).

Oral and topical nitroglycerin may be administered for prophylaxis against chronic anginal attack, due to coronary artery disease (Winsor and Berger, 1975; Reichek et al., 1974). Patients should take the smallest effective oral dose two to three times daily. Nitroglycerin ointment (2.5 to 5 cm of a 2% ointment) should be spread over a 6 x 6 inch (150 x 150 cm) area of the skin, usually the chest. The dose should then be covered with plastic wrap and held in place with adhesive tape. The usual therapeutic dose is two inches every 8 hr, but some patients may require four to five inches every four hours. The ointment may be useful for controlling nocturnal angina which may develop in some patients, within 3 hours after the patient goes to sleep (Needleman and Johnson, (1980).

2. NITRATE ACTION IN CONGESTIVE HEART FAILURE

Conventional therapy for congestive heart failure patients requires one or more of the following: sodium restriction and diuretics to control edema, positive inotropic agents to increase the force of myocardial contractions, and rest to reduce the demands placed on the

heart (Sloman and Manolas, 1980). Vasodilator drugs may be used in congestive heart failure patients who remain refractory to conventional therapy (Cohn and Franciosa, 1977; Mason, 1978).

Vasodilator action may result from either of two mechanisms. The nitrates are primarily venodilators which act to reduce venous return (i.e., reduce preload) to the heart. Arterial vasodilators, such as hydralazine, act to reduce the resistance to left ventricular outflow (i.e., to reduce afterload). Nitroprusside dilates both arterial and venous smooth muscle and therefore acts by both mechanisms. Nitroprusside has a short half life and must therefore be given as an intravenous infusion. Concomitant use of oral nitrates, such as isosorbide dinitrate, and oral hydralazine, has been shown to be effective in the long term treatment of refractory congestive heart failure (Cohn and Franciosa, 1977; Franciosa et al., 1978).

In patients with congestive heart failure, nitroglycerin should be used close hemodynamic monitoring (Hill et al., 1981). Nies (1978) summarized the use of vasodilators in heart failure: "Selection of patients for vasodilator therapy is critical. Optimum use of vasodilators requires catheterization of the right heart and continuous monitoring of the pulmonary artery pressures. Patients must have elevated pulmonary capillary wedge pressures; those with wedge pressure less than 15 mm Hg will not improve with vasodilators. In selected patients,

vasodilators can improve myocardial performance and have successfully aided managing congestive heart failure that is refractory to standard treatment with digitalis and diuretics." However, there is no evidence for a decrease in mortality due to congestive heart failure when patients chronically use vasodilators.

3. NITRATE ACTION IN ACUTE MYOCARDIAL INFARCTION

An acute myocardial infarction results in decreased stroke volume, ejection fraction, cardiac output and either an increased or decreased arterial pressure. It is also associated with elevated left ventricular end diastolic pressure and volume. Drug therapy is usually directed toward minimizing myocardial damage and controlling life-threatening arrhythmias (Sloman and Manolas, 1980).

Nitroglycerin has been used in attempts to reduce or "salvage" the acutely ischemic myocardium (Warren and Francis, 1978). Vasodilators may reduce the size of an infarct by a reduction of myocardial oxygen demand (via an afterload reduction), thus increasing the perfusion of ischemic tissue. Reduction of myocardial ischemic injury may also be accomplished by increasing the blood flow to the damaged tissue. Sublingual (Dunn et al., 1982; Borer et al., 1975), oral (Bussman et al.,1977) and topical (Armstrong et al., 1976) nitrate therapy have been shown to improve cardiac performance in patients and reduce

myocardial ischemic injury as indicated by S-T segment mapping, gated blood pool scintigraphy and hemodynamic measurements. Jugdutt et al. (1981) found that in a dog model, that myocardial protection with nitroglycerin was due to an increase in collateral blood flow resulting from the direct vasodilating action of nitroglycerin on the coronary bed. Armstrong et al. (1976) found that nitroglycerin both decreased myocardial oxygen demand and preserved "the transmyocardial gradient for coronary blood flow", thus improving cardiac performance in patients with acute myocardial infarction.

C. MECHANISM OF ACTION AT THE RECEPTOR LEVEL

Currently, there are two theories which explain the possible mechanism of action of nitrates at the receptor level. One theory suggests that nitroglycerin oxidizes receptor sulfhydryl groups. The other suggests that nitroglycerin reacts with tissue sulfhydryl groups to form an active intermediate which is ultimately responsible for receptor activation. These theories are presented in the following sections.

1. INTERACTION OF NITROGLYCERIN WITH TISSUE SULFHYDRYL GROUPS: NITRATE RECEPTOR THEORY

The first theory suggests that the interaction of nitroglycerin with tissue sulfhydryl groups is responsible for activity, i.e., smooth muscle relaxation. The organic nitrates were first shown to react with reduced glutathione in the presence or absence of liver "organic nitrate reductase", now known as glutathione S-transferase, by Heppel and Hilmoe (1950). Later studies by Needleman and Krantz (1965) and Needleman and Hunter (1965) provided additional evidence that organic nitrates react with this enzyme and that glutathione is an important metabolic cofactor. These later studies suggested that the action of organic nitrates was mediated via reactions with sulfhydryl groups. Compounds containing sulfhydryl groups have been shown to play important roles in several different biochemical mechanisms. Sulfhydryl-containing compounds are important determinants of mitochondrial structure and are necessary for oxidative phosphorylation. Organic nitrates have been shown to uncouple mitochondrial oxidative phosphorylation (Needleman and Hunter, 1966; Boime and Hunter, 1971) and inhibit monoamine oxidase, another mitochondrial enzyme (Ogawa and Gudbjarnason, 1967). Monoamine oxidase contains sulfhydryl groups which are oxidized in a concentration-dependent fashion by organic

nitrates (Jakschik and Needleman, 1973). Needleman and Johnson (1973) showed a positive correlation between the oxidation of titratable tissue sulfhydryl groups and tissue tolerance to organic nitrates.

Based on these observations, Needleman and Johnson (1973) proposed the existence of the vascular receptor shown in Fig. I-3. They proposed that organic nitrates react with a vascular smooth muscle receptor which contains reduced sulfhydryl groups. The organic nitrates oxidize these sulfhydryls to form disulfide groups and free inorganic nitrite. The disulfide form of the receptor was assumed to have a lower affinity for the organic nitrates than the reduced form based on the following observations. Blood vessels could be made tolerant to the effects of nitroglycerin by initially incubating these tissues in the presence of nitroglycerin (100 μg/ml). After washing excess nitroglycerin from these tissues, the tissues were no longer responsive to nitroglycerin. Tolerant vessels which were incubated with dithiothreitol, a disulfide reducing reagent, responded to nitroglycerin. Dithiothreitol was also found to convert vessels made tolerant in vivo, i.e. those vessels taken from animals made tolerant to nitroglycerin in vivo by chronic subcutaneous administration of nitroglycerin. In vitro incubation of these vessels with dithiothreitol resulted in the reversal of tolerance. The authors concluded that tolerance to nitroglycerin was the result of a chemical modification of the receptor site. These authors

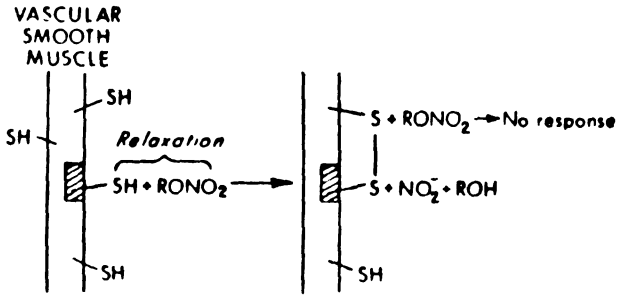


FIG. I-3: Schematic diagram of the reaction of GTN with tissue sulfhydryl groups at its vascular receptor. Abbreviations: $RONO_2$, organic nitrate; ROH, denitrated metabolite (Needleman and Johnson, 1972).

also concluded that this site was specific for the organic nitrates since tolerant tissues were still responsive to other vasodilator compounds (isoproterenol, cyclic AMP, sodium nitroprusside and papaverine).

In a subsequent investigation, Needleman et al. (1973) showed that incubation of aortic strips with ethacrynic acid (which alkylates sulfhydryl groups) reduced or abolished relaxation to all tested vasodilators. Incubation of these strips with ethacrynic acid produced an effect similar to that exhibited in the tolerance studies described above, i.e., a 1000-fold shift (but not a parallel shift) in the nitroglycerin dose response curve. Ethacrynic acid did not affect the ability of norepinephrine to cause tissue contraction. The effect of ethacrynic acid was assumed to be due to alkylation of tissue thiol groups. Alkylation of these groups resulted in reduced responses to not only nitroglycerin but also to other vasodilators, including isoproterenol, sodium nitrite, sodium nitroprusside, cyclic-AMP, papaverine, diamide, hydrogen peroxide and acetaldehyde.

Needleman et al. (1973) evaluated a number of compounds as to their relative ability to relax norepinephrine-contracted aorta strips. These compounds were divided into two groups based on the concentration required for 50% relaxation of the contracted aorta strips. Nitroglycerin, isoproterenol, sodium nitroprusside, cyclic-AMP and sodium nitrite caused relaxation at low concentrations which would

not be expected to be due to nonspecific oxidation of sulfhydryls. Diamide, hydrogen peroxide and acetaldehyde caused relaxation only at high concentrations. Similar high concentrations of the latter three compounds were found to oxidize reduced glutathione in vitro (Needleman et al., 1973). Based on these data, Needleman proposed that there were two distinct vasodilation responses. One response is activated by a direct interaction with a specific receptor site. This interaction does not require sulfhydryl oxidation. The second response is mediated by nonspecific vasodilators, in which the response is correlated with sulfhydryl reactivity.

The schematic diagram of this more complex interrelationship between vasodilators and tissue components is presented in Fig. I-4. Needleman et al., (1973) hypothesized that there is a specific "nitrate receptor" which is responsible for the development of nitrate tolerance after chronic exposure to the organic nitrates. Although these receptors do show cross-tolerance between various organic nitrates, they do not show cross tolerance to other vasodilators such as histamine, isoproterenol, cyclic-AMP, papaverine, diamide and inorganic nitrite (Needleman and Johnson, 1973). Isoproterenol relaxes smooth muscle by activation of the beta-receptor, via activation of adenyl cyclase. This effect is not blocked by chronic nitrate exposure, but may be blocked by specific beta-antagonists such as propranolol (Fleish and Titus, 1972).

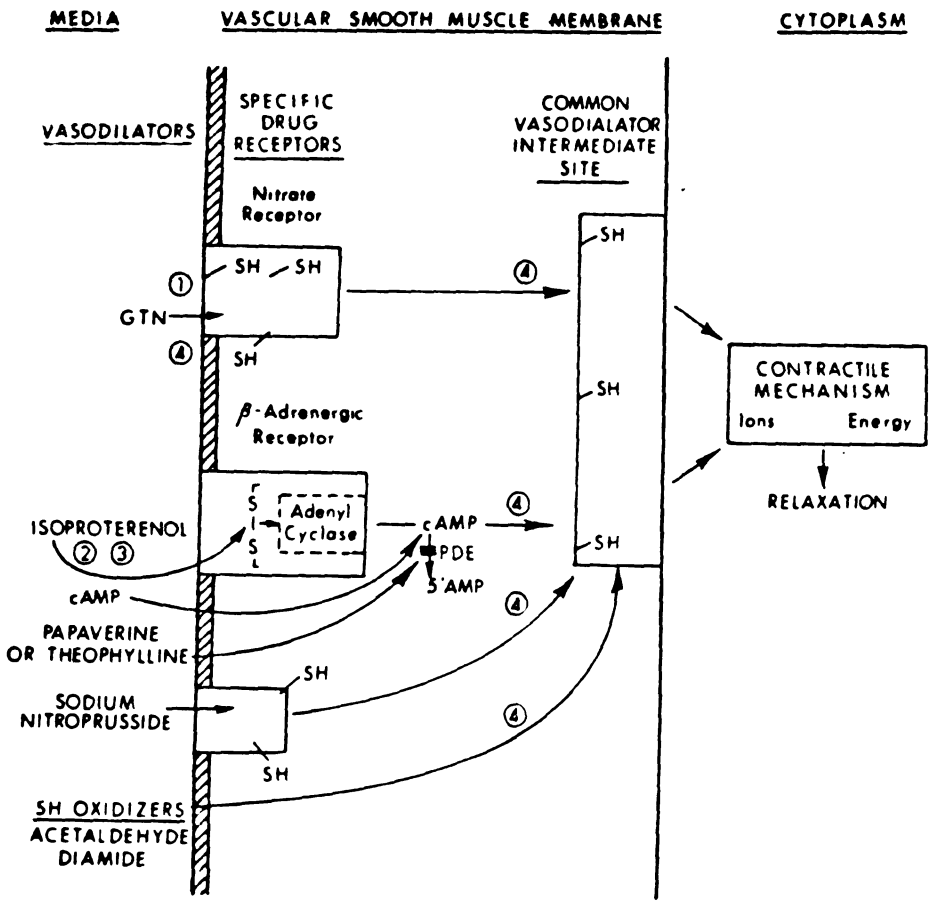


FIG. I-4: Schematic diagram of the interrelationship between vasodilators and tissue components. Abbreviations: GTN, glyceryl trinitrate; cAMP, cyclic 3'5'-adenosine monophosphate; and PDE, phosphodiesterase (inhibited by papaverine or theophylline). The numbers of the scheme indicate the following: 1 blocked by GTN tolerance; 2 blocked in isoproterenol tachyphylaxis; 3 blocked by propranolol; and 4 blocked by ethacrynic acid (Needleman and Johnson, 1973).

Papaverine and theophylline act as phosphodiesterase inhibitors which cause accumulation of cyclic-AMP, which in turn may cause vasodilation. Ethacrynic acid blocks cyclic-AMP induced vasodilation, thus indicating that the blockade is subsequent to the formation of cyclic-AMP and is not at adenyl cyclase. Sodium nitroprusside acts at a receptor which is independent of both the beta-receptor and the nitrate receptor. The sulfhydryl oxidizers, acetaldehyde and diamide, act only at the common vasodilator intermediate site (nonspecific site) and may be blocked by ethacrynic acid. Needleman et al. (1973) could not identify the anatomic location of the nonspecific site but postulated that it might be a regulatory site, part of the specific drug receptors, or even part of the contractile apparatus.

2. NITRATE S-NITROSOTHIOL INTERMEDIATE: RECEPTOR THEORY

The second receptor theory suggests that nitroglycerin reacts with tissue sulfhydryl groups to form an active intermediate (S-NO-thiol) which is responsible for activity. Involvement of cyclic GMP was first suggested in 1975 (Kimura et al., 1975a,b; Mittal et al., 1975) when hydroxylamine, $NaNO_2$ and sodium azide (smooth muscle relaxants) were shown to activate guanylate cyclase and increase tissue c-GMP.

Later studies (Diamond and Holmes, 1975; Diamond and Blisard, 1976; Arnold et al, 1977; Bohme et al., 1978;

Axelsson et al., 1979) reported an increase in c-GMP in certain vascular and nonvascular smooth muscle tissues when exposed to nitroglycerin, nitroprusside, $NaNO_2$, nitric oxide and related agents. Additionally, phosphodiesterase inhibitors increased c-GMP concentrations and/or c-AMP in pig coronary artery and caused relaxation (Kramer and Wells, 1979). These data suggested that both c-GMP and c-AMP may be involved in nitrate induced coronary artery relaxation (Ignarro et al., 1980a,b).

Several studies (Gruetter et al., 1979, 1980a, 1980b; Ignarro and Gruetter, 1980) have shown that nitroprusside and nitroglycerin are capable of releasing NO (nitric oxide) under certain conditions (nitroprusside releases NO spontaneously in aqueous solution while nitroglycerin releases NO in the presence of thiols) and also that thiols enhanced the activation of arterial guanylate cyclase by NO and nitroprusside. Enzyme activation by nitroglycerin did not occur without the addition of cysteine (Ignarro et al., 1980b).

Ignarro et al. (1981) studied S-nitrosothiol formation in more detail. They found that when NO, $NaNO_2$, nitroprusside or nitroglycerin was incubated with cysteine, S-nitrosocysteine was formed. Of the compounds studied, only nitroprusside degrades directly to NO which can react directly with cysteine. Organic nitrates, amyl nitrite (at an alkaline pH) and $NaNO_2$ form NO_2^- which alone does not possess vasodilator activity. At slightly acidic, or neutral

pH to a limited extent, nitrite may react with hydrogen ions (H^+) to form nitrous acid (HONO) which then spontaneously forms NO to react with cysteine to form S-nitrosocysteine.

Ignarro et al. (1981c) also investigated the hemodynamic effects of three S-nitrosothiols, nitroprusside and nitroglycerin on the systemic arterial pressure of the anesthetized cat. They found that all compounds elicited dose-dependent, parallel dose-response curves. These compounds all dilated venous and arterial segments and had rapid onsets and short durations of action, characteristic of organic nitrates.

A schematic diagram of the proposed mechanism by which nitrogen oxide vasodilators relax vascular smooth muscle (via the S-nitrosothiol intermediate) is shown in Figure I-5. This proposed mechanism (Ignarro et al., 1981c) suggests that NO, formed intracellularly from lipophyllic vasodilator drugs, reacts with endogenous sulfhydryl groups to form S-nitrosothiols. These intermediates then activate guanylate cyclase to form c-GMP. The precise mechanism by which c-GMP causes vascular smooth muscle relaxation is not known.

Ignarro et al. (1981c) summarized the evidence supporting this hypothesis as follows: 1) vasodilators elevate tissue c-GMP levels but require addition of thiols to activate guanylate cyclase; 2) organic nitrates and nitrites react with cysteine to form S-nitrosocysteine; 3) S-nitrosothiols (a) activate guanylate cyclase, (b) increase tissue c-GMP levels, (c) relax coronary arterial strips and

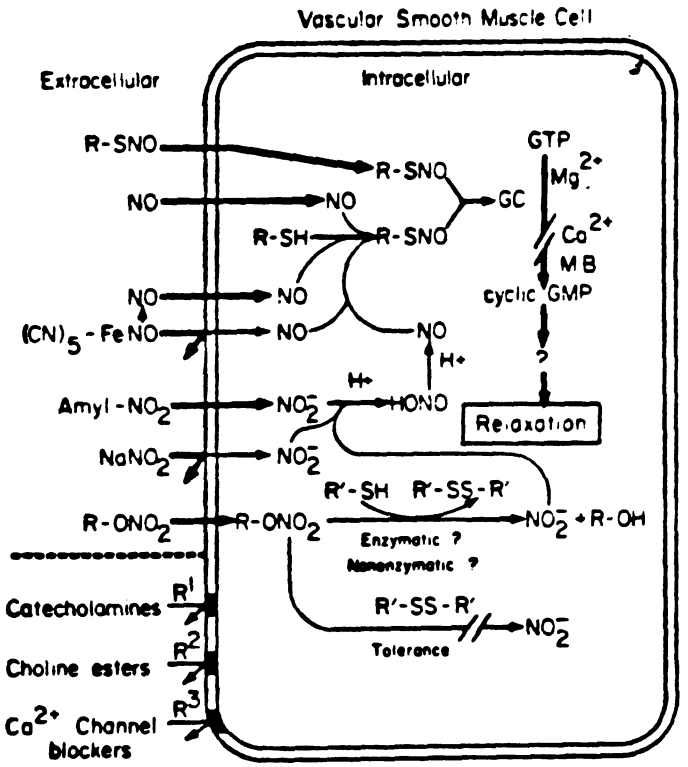


FIG. I-5: Schematic diagram of proposed mechanisms by which nitrogen oxide-containing vasodilators relax smooth muscle. Abbreviations: R-SNO, S-nitrosothiol; NO, nitric oxide; HONO, nitrous acid; $(CN)_5$-FeNO, nitroprusside; R-ONO_2, organic nitrate; R-OH, denitrated organic nitrate; R-SH, low or high molecular weight thiol; R'-SH, thiol that is distinct from R-SH; GC, guanylate cyclase; M.B., methylene blue; $R^{1,2,3}$, extracellular specific receptors. (From Ignarro et al., 1981c).

(d) decrease systemic arterial pressure in the anesthetized cat; 4) methylene blue inhibits the effects produced by S-nitrosothiols (methylene blue inhibits activation of guanylate cyclase and antagonizes nitrate coronary arterial relaxation); 5) the dose-response curves elicited by S-nitrosothiols are parallel to those of nitroglycerin and nitroprusside and are not altered by propranolol; and 6) the short in vitro half-life of S-nitrosothiols (S-nitrosocysteine has a 15 min half-life at 37 ^{o}C in an oxygen atmosphere) is consistent with the short duration of action of nitroglycerin or nitroprusside.

The hypothesis presented in Fig. I-5 has certain parallels to that proposed by Needleman et al. (1973). The two theories are similar in that both proposed at least two different receptors. Needleman and co-workers proposed that one receptor contains sulfhydryl groups specific for organic nitrates. A second receptor interacts with all vasodilators, including the organic nitrates. Ignarro et al. (1981c) postulate that two independent populations of sulfhydryls are involved. One population reacts with NO to form S-nitrosothiols. This population would parallel Needleman's organic nitrate specific site. A second population of sulfhydryl groups are required for the metabolism of organic nitrates to NO_2^-.

D. NITROGLYCERIN TOXICITY

Most of the nitroglycerin side effects are secondary responses to the cardiovascular effects of this drug. Headache, which is due to vasodilatation of meningeal blood vessels, is the most common side effect. Postural hypostension, which is caused by venous pooling and decreased cardiac output, may result in episodes of dizziness and weakness, which are associated with cerebral ischemia. Postural hypotension may be accentuated by alcohol. Nitroglycerin occasionally produces a "drug rash" an occasional response found for all organic nitrates (Needleman and Johnson, 1982).

The following adverse reactions occur in less than one percent of the patients taking nitroglycerin : tachycardia, nausea, vomiting, apprehension, restlessness, muscle twitching, retrosternal discomfort, palpitations and abdominal pain (Physicians Desk Reference, 1982).

The acute toxicity of nitroglycerin in animals has not been documented to any extent in the literature. Lee et al. (1975) studied the acute oral toxicity of nitroglycerin in rats and mice. In mice, LD_{50} values for nitroglycerin were 1.19 and 1.06 g/kg for males and females, respectively. Male and female rats exhibited LD_{50} values of 0.82 and 0.88 g/kg, respectively. Rats and mice treated with toxic doses of nitroglycerin became cyanotic, ataxic and had respiratory depression.

E. PHYSICOCHEMICAL PROPERTIES OF NITROGLYCERIN

1. PHYSICAL PROPERTIES

Nitroglycerin (glyceryl trinitrate), the trinitrate ester of glycerol, is 1,2,3-propanetriol trinitrate. The structure of nitroglycerin is shown in Fig.I-1. The molecular formula for nitroglycerin is $C_3H_5N_3O_9$ and the molecular weight is 227.09. Nitroglycerin is a pale yellow, odorless, oily liquid and has a slightly sweet taste. Application of this compound to sublingual membranes causes a burning sensation (Copelan, 1972).

Selected physical properties of nitroglycerin and its metabolites are presented in Tables I-1 through I-3. The vapor pressure of nitroglycerin has been reported to be 2.6×10^{-4}, 5.5×10^{-4} and 2.2×10^{-3} Torr at 20, 25 and 30 ^{o}C, respectively. (Windholz, 1976, Pikal et al, 1976).

2. CHEMICAL PROPERTIES

a. SYNTHESIS

The synthesis of nitroglycerin is accomplished by esterification of glycerol. Equal volumes of nitric and sulfuric acids are mixed in an ice bath. A small amount of urea or urea nitrate is added to the mixture as a nitrous

TABLE I-1: Some physical properties of nitroglycerin and its degradation products.

Compound	Physical State at Room Temp.	Melting Point (°C)	Boiling Point (°C)	Specific Gravity	Water Solubility (mg/ml)	Ref.
GTN	oil	13.2 (stable) 2.2 (labile)	218 (explosive)	1.60	1.73	2
1,2-GDN	oil	-40	106/0.5 mm	1.47	65.7	2,3
1,3-GDN	oil	-40	115-116/0.6 mm	1.47	78.4	2,3
1-GMN	solid	55	123/0.5 mm	1.47		3
2-GMN	solid	54		1.47		3
Glycerol	oil			1.25		4

1) GDN = glyceryldinitrates, GMN = glyceryl mononitrates

2) Urbanski (1965)

3) Dunstan et al. (1965a,b)

4) Weast and Selby (1968)

TABLE I-2: Nitroglycerin solubility in various solvents[a].

Solvent	Solubility (temp. °C)
Water	1.73 mg/ml (20)
Water	2.46 mg/ml (60)
Ethanol	375 mg/g (0)
Ethanol	540 mg/g (20)
Ethanol	inf[b] ("hot")
Acetone	inf
Benzene	inf
Carbon Disulfide	8.3 mg/g
Chloroform	inf
Ether	inf
Ethyl Acetate	inf
Ethylene Chloride	inf
Methanol	576 mg/g
Nitric Acid	inf
Nitrobenzene	inf
Toluene	inf

[a] Mark (1965); Windholz (1976); McNiff et al. (1980)

[b] Miscible in all proportions.

TABLE I-3: Molecular extinction coefficients for glycerol nitrates in water (Dunstan et al., 1965b).

Wavelength	1-GMN	2-GMN	1,2-GDN	1,3-GDN	GTN
210 nm	2535	2466	4397	4829	5875
215 nm	1693	1765	2799	3072	3298
220 nm	1032	1187	1608	1794	1685
225 nm	607	782	884	985	832
230 nm	348	352	478	531	414

acid scavenger since nitrous acid reacts violently with alcohols. Glycerol is slowly added to the mixture. Both the rate of glycerol addition and the temperature must be controlled to eliminate any alcohol oxidation side reactions. The mixture is poured into cold water, stirred and the nitroglycerin is collected (bottom layer). The oil is first washed with dilute Na_2CO_3 to neutralize any remaining acid and then dried over a desiccant. The reaction scheme is shown in Fig. I-6.

b. CHEMICAL STABILITY

Nitroglycerin is a powerful explosive which is sensitive to shock, heat and friction. The instability of nitroglycerin is enhanced by the presence of contaminants such as nitric acid and nitrogen oxides (Connon, 1970). Nitroglycerin explodes according to the following equation (DiCarlo, 1975):

$$4\ C_3H_5(ONO_2) \longrightarrow 12\ CO_2 + 10\ H_2O + 6\ N_2 + O_2$$

The high energy of a nitroglycerin explosion is due to the high negative heats of formation (CO_2 and water) and to the nitrate group's positive heats of formation, i.e., the nitrates release energy on dissociation (Connon, 1970).

Nitroglycerin undergoes hydrolysis in both acidic

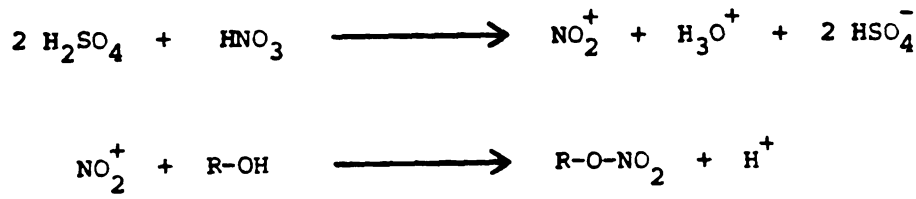


FIG. I-6: Nitroglycerin synthetic scheme.

and basic pH solutions. Hydrolysis in alkaline solution occurs much more rapidly than in acidic solution (Baker and Easty, 1952a, 1952b; Crew and DiCarlo, 1968; Cannon, 1970). Crew and DiCarlo (1968) found that carbon-14 nitroglycerin was completely degraded within 15 min after incubation with 4N NaOH but that 4N HCl degraded only 28% of the nitroglycerin in 6 hr. This study using radio-labeled nitroglycerin allowed the investigators to assay the isomeric glyceryl dinitrates and mononitrates. The reaction sequence involved in HCl hydrolysis is shown in Fig. I-7. These authors found that nitroglycerin is hydrolyzed to form both 1,2-glyceryldinitrate and 1,3-glyceryldinitrate in a 2:1 ratio. This ratio may be expected if the nitrate esters of nitroglycerin were randomly hydrolyzed, since there are two primary nitrate ester functions and only one secondary ester.

Baker and Easty (1952a,b) and Connon (1970) outlined four different modes of reaction for alkaline hydrolysis of nitrate esters (Fig. I-8). Although primary nitrate esters may undergo hydrolysis by any of these four pathways, only two of these are significant. Nucleophilic attack on the carbon or nitrogen (reactions A and B) are the primary pathways of reaction producing the alcohol and inorganic nitrate for primary nitrate esters (i.e., the 1- or 3-position in nitroglycerin). Nucleophilic attack on the alpha and beta- hydrogens (reactions C and D) become more important mechanisms in the hydrolysis of the secondary

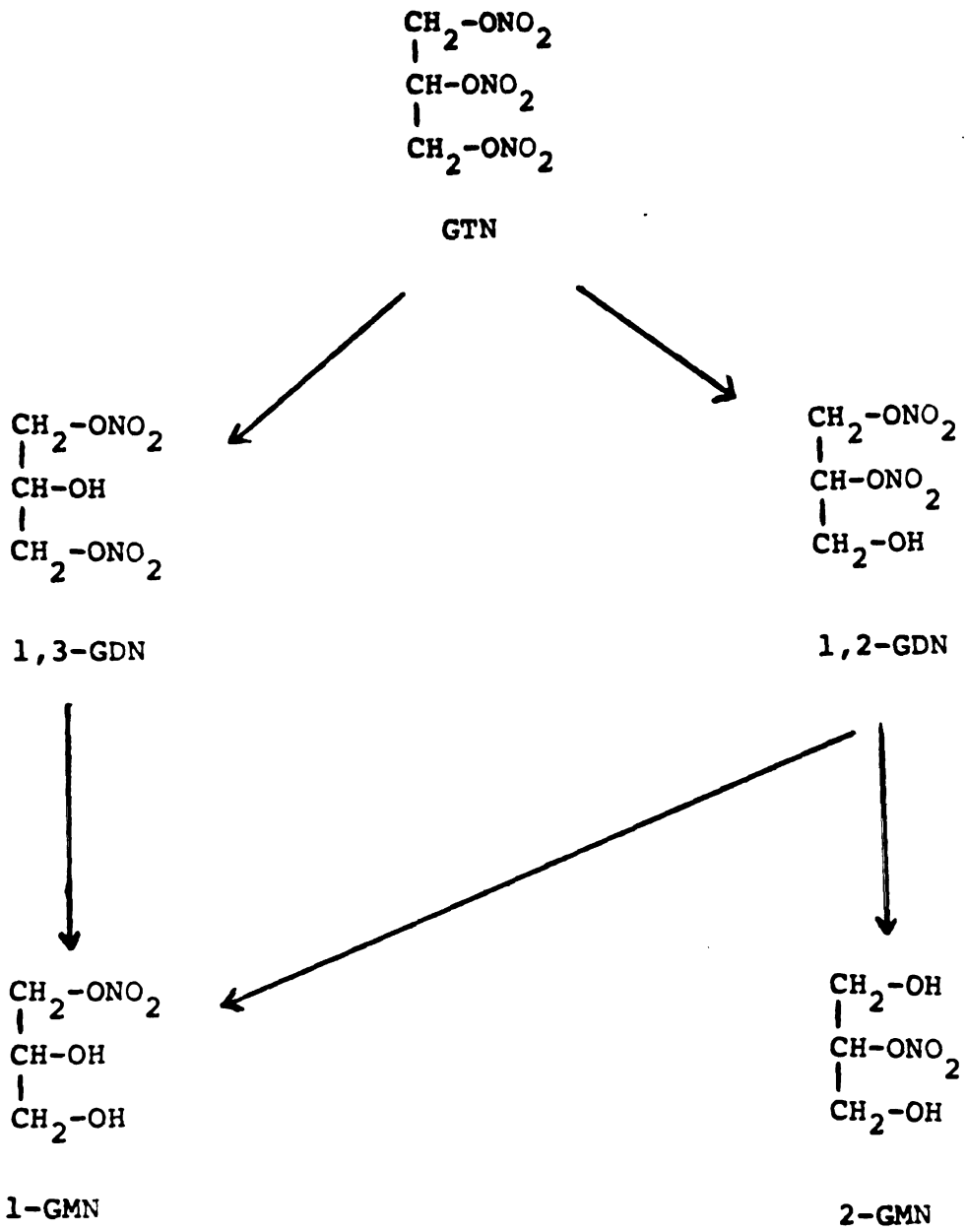
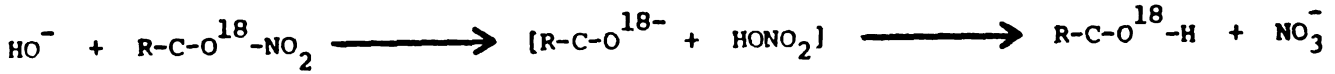


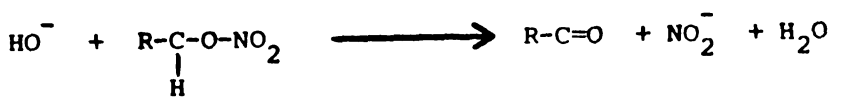
FIG. I-7: Schematic diagram showing the hydrolysis of nitroglycerin to form the mono- and dinitroglycerin metabolites.

A. Nucleophilic attack on carbon:

B. Nucleophilic attack on nitrogen:



C. Nucleophilic attack on α - hydrogen:



D. Nucleophilic attack on β-hydrogen:

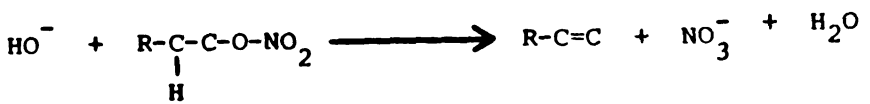


FIG. I-8: Four modes of reaction which may occur on neutral or alkaline hydrolysis of nitrate esters (Connon, 1970).

ester (such as the 2-position of nitroglycerin).

c. PHYSICAL STABILITY

The instability of solid nitroglycerin pharmaceutical dosage forms is due to volatilization, intertablet migration and adsorption (Fusari, 1973a; Page et al., 1975). Fusari (1973a) found that large amounts of nitroglycerin adsorbed onto the cotton stuffing of bottles as well as plastic bottle cap liners. He found that Esterfoil, tinfoil, polyethylene terephthalate and fluorohalo-carbon cap liners were superior to wax vinyl, lubricated vinyl and Excelloseal liners. The problem of nitroglycerin tablet migration and volatility has been corrected by the addition of stabilizers such as povidone and polyethylene glycol 400 (Fusari, 1973b; Fung et al., 1974 and 1976; Goodhart et al., 1976).

Adsorption of nitroglycerin from intravenous solutions to plastic materials, such as plastic intravenous containers and infusion sets, was shown to be an important mechanism by which nitroglycerin may be lost (McNiff et al., 1979; Yuen et al., 1979; Sokoloski et al., 1980; Baaske et al., 1982). Nitroglycerin interacts with polyvinylchloride (Roberts et al., 1980) but does not adsorb to high density polyethylene such as that used in Tridilset, a nitroglycerin administration set made by American Critical Care (Baaske et al., 1982). To avoid stability problems associated with adsorption of nitroglycerin to plastics, only glass

containers and high density polyethylene materials should be used.

CHAPTER II

PHARMACOKINETICS AND PHARMACODYNAMICS OF NITROGLYCERIN

A. NITROGLYCERIN CLEARANCE

1. NITROGLYCERIN BIOCHEMISTRY AND IN VITRO METABOLISM

Nitroglycerin (GTN) is metabolized by a non-specific enzyme, glutathione-S-transferase, previously identified as glutathione-organic nitrate reductase (Habig et al., 1976). This enzyme has been identified in human liver and blood, and in rat liver, kidney, and blood (Habig et al., 1976; Lee, 1973). Nitroglycerin is metabolized to form the dinitrate (1,2- and 1,3-glyceryldinitrates) and mononitrate (1- and 2-glycerylmononitrates) metabolites (Fig. II-1).

Heppel and Hilmoe (1950) found that nitroglycerin undergoes a non-enzymatic reaction with reduced glutathione (GSH), cysteine and cysteinylglycine. Although they were unable to directly measure nitroglycerin metabolites, they measured the production of nitrite (NO_2^-) and oxidized glutathione as products. These investigators also purified an enzyme from hog and rat liver which catalyzed the reaction between nitroglycerin and GSH. In a mass balance study of this reaction, they found that two micromoles of

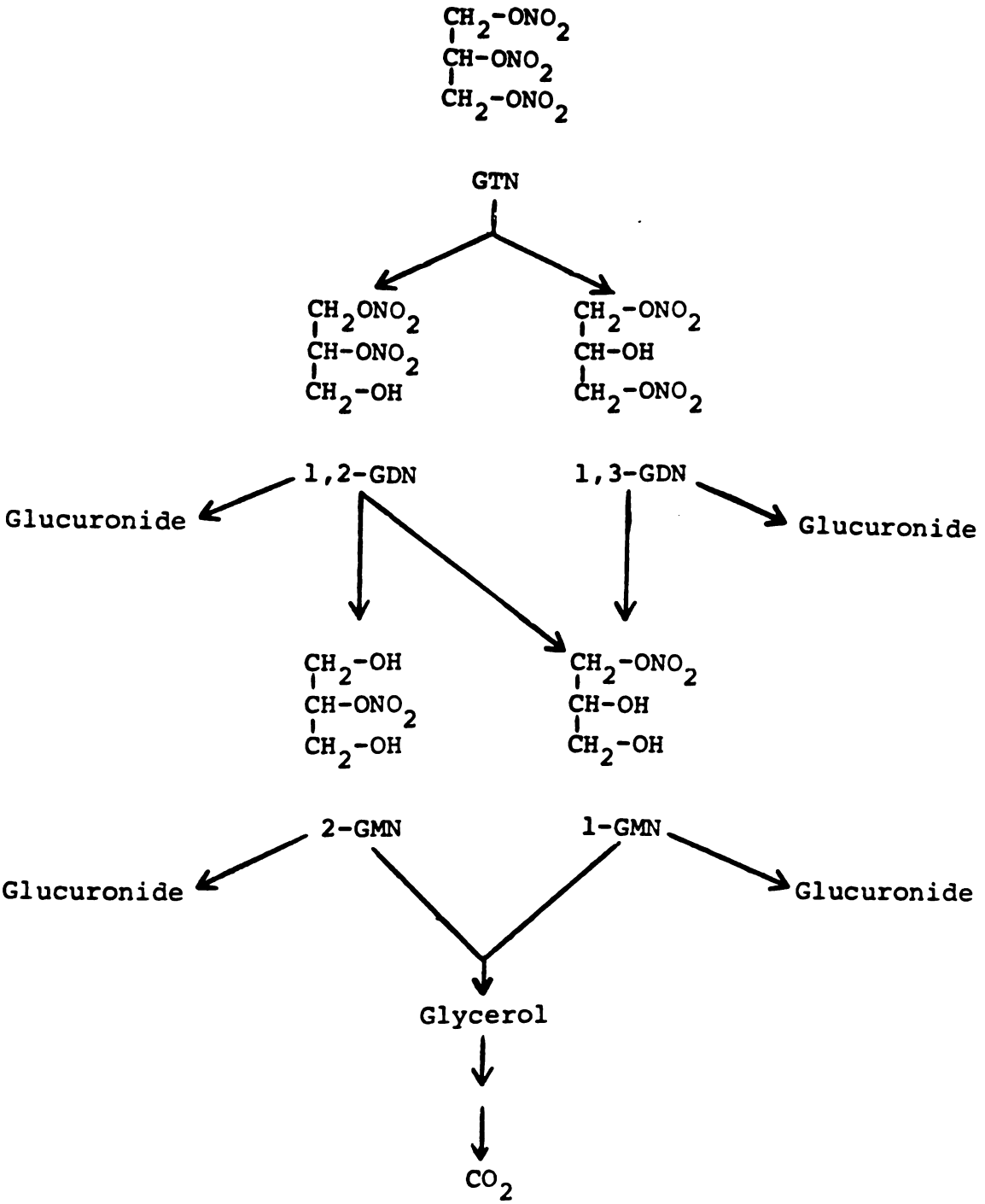


FIG. II-1: Metabolic scheme for nitroglycerin.

GSH were oxidized for each micromole of nitrite produced:

$$GTN + 2\ GSH \longrightarrow GSSG + NO_2^- + \text{"other products"}$$

The metabolism of nitroglycerin requires GSH in the reduced form. Needleman and Hunter (1965) found that the nitroglycerin reaction could be coupled with glutathione reductase to maintain the presence of the reduced form of glutathione:

$$GSSG + NADPH_2 \longrightarrow 2\ GSH + NADP$$

Needleman and Hunter (1965) also investigated the specificity of GSH-organic nitrate reductase. They found that nitroglycerin was metabolized 24 times faster than 1,2-GDN, 48 times faster than 1,3-GDN and 170 times faster than the GMN's. The measured values of V_{max} for GTN, 1,2-GDN, 1,3-GDN and GMN were 120, 5.0, 2.5 and 0.7 mmole/kg protein/min, respectively.

The liver is a significant site of nitroglycerin metabolism. Johnson and co-workers (1972) found that the half-life of nitroglycerin in an isolated perfused rat liver was only two min. This metabolism was found to be saturable (Needleman and Harkey, 1971). These investigators found that metabolism was greatest with low perfusion concentrations and lowest with high nitroglycerin concentrations. Interestingly, they reported that the total

amount of nitroglycerin metabolized was independent of the perfusion concentration, thus suggesting depletion of a cosubstrate. Pretreatment of rats with bromobenzene, which depletes endogenous stores of GSH, resulted in a 90% decrease in nitroglycerin metabolism, thus suggesting that glutathione was the depleted cosubstrate.

Needleman et al. (1969 and 1971) incubated nitroglycerin with rat liver homogenates and found primarily 1,3-GDN, some 1,2-GDN and only a trace of GMN. These investigators could not detect any further metabolism of nitroglycerin (i.e., glycerol or carbon dioxide as metabolites).

Lee and Belpaire (1972) found that hepatic nitroglycerin metabolism could be induced by chronic exposure to phenobarbital. They administered phenobarbital (100 mg/kg, i.p.) to rats daily, for three days. Nitroglycerin (1 mg/ml) was incubated with liver homogenates and the formation of inorganic nitrite was measured. They found that nitroglycerin metabolism was increased by 20% over control. When exogenous GSH was added to the incubation mixture, nitroglycerin metabolism was increased by 43% over control. They concluded that phenobarbital induced both the organic nitrate reductase and GSH generating system (GSH reductase).

As previously mentioned, nitroglycerin may be metabolized at sites in the body other than the liver. DiCarlo and Melgar (1970) found that nitroglycerin was

metabolized by rat serum with a half-life of approximately 20 min (estimated from Fig. 2, DiCarlo and Melgar, 1970). They also found that twice as much 1,3-GDN was produced as was 1,2-GDN. The enzyme responsible for this metabolism showed a pH optimum at 7.8 and was essentially inactive at pH values less than 6 or greater than 10.5. Enzyme activity was inhibited by iodoacetamide, silver nitrate and p-chloromercuribenzoate but not by sodium azide or oxalic acid. Several other investigators have since also shown that nitroglycerin is degraded (in vitro) by blood, plasma or serum (see Chapter III).

Sutton and Fung (1982) studied the metabolism of isosorbide dinitrate and nitroglycerin by rat blood vessels and other tissues. Nitroglycerin metabolic rates (pmoles/mg/hr) were determined for liver (62 ± 5), artery (the abdominal aorta, 26± 9), vein (inferior vena cava, 15 ± 5), lung (25 ± 11) and muscle (6.1 ± 2). Significant amounts of the dinitrates (4 to 27% of the added amount of nitroglycerin) were produced after a one-hour incubation.

2. IN VIVO METABOLISM AND DISPOSITION OF NITROGLYCERIN

Lang et al. (1972) attempted to determine the contribution of liver to the total metabolism of nitroglycerin in vivo. [^{14}C]-Nitroglycerin was administered intravenously to totally eviscerated rats. The elimination half-life of nitroglycerin in these animals was 7-8 min

while in control animals the half-life was 1 min. From these data, the authors concluded that the liver was the primary site of in vivo degradation. This conclusion is not necessarily true since changes in half-life are not always the result of changes in clearance. Since the half- life is a function of both clearance and volume of distribution ($t_{1/2} = 0.693/k = 0.693V/CL$), the increase in the observed half-life may be due to a decrease in clearance, an increase in volume of distribution or both. Assuming that the change in half-life was due entirely to clearance, a 7 - 8 min half-life after evisceration suggests that nitroglycerin is rapidly metabolized at other body sites.

Maier et al. (1980) related the in vivo nitroglycerin metabolism to hepatic in vitro organic nitrate reductase activity in rats. The rats were dosed orally with 100 mg/kg nitroglycerin in 1 ml ethanol:propylene glycol (1:9). They found peak nitroglycerin concentrations of 36 to 475 ng/ml and measured areas under the plasma concentration-time curves. After each pharmacokinetic study, hepatic organic nitrate reductase activity was determined in each rat. Maier et al. (1980) found that the AUC was inversely correlated to the enzyme activity ($r = 0.84$, $p < 0.001$). Thus, they concluded that the oral bioavailability of nitroglycerin was inversely related to hepatic organic nitrate reductase activity (i.e., the extent of first-pass metabolism of nitroglycerin was directly related the metabolic activity of the liver reductase).

Several groups of investigators have determined the in vivo metabolism of nitroglycerin by different animal species. Needleman and Hunter (1965) administered intraperitoneal doses of nitroglycerin to rats (150-175 g). Qualitatively, they found that 1,2-GDN and 1,3-GDN were the major ether extractable urinary metabolites. Only a trace of the GMN metabolites were detected at 24 hr.

Bogaert et al. (1969a) isolated nitroglycerin metabolites from rabbit urine after intravenous doses (1 mg/kg). In control rabbits, only 1,3-GDN and GMN were detectable (i.e., 1,2-GDN was not detectable) which accounted for 10.5 to 17.1% of the administered dose. Phenobarbital treated rabbits were found to excrete metabolites faster, but again, only the 1,3-GDN and GMN's. These rabbits were found to excrete 22 to 50% of the dose. Metabolites were not detectable at all during the first 3 hr after dosing in SKF-525A pretreated animals. These investigators also administered nitroglycerin dinitro-metabolites to rabbits (1.5 mg/kg, intravenously). After administration of 1,2-GDN only GMN was found but after administration of 1,3-GDN, both 1,3-GDN and GMN were detected.

Needleman and co-workers (1971) studied the metabolism of nitroglycerin in more detail after they synthesized [14C]-nitroglycerin (Table II-1). They administered 5 mg/kg nitroglycerin to rats by subcutaneous injection and found that 17% of the dose was metabolized to carbon dioxide and

TABLE II-1: Metabolism of nitroglycerin by rats. Recovery of metabolites (% of dose).

	DiCarlo[a]	Needleman[b]	Hodgson[c]
Dose (mg/kg)	10	5	180
Route	Oral	Subcutaneous	Oral
Study Interval (hr)	4	24	24
Measurement		**% Dose Excreted**	
CO_2	20	17	25.5 ± 1.5
Cumulative Dose Excreted (Urine)	20.95	49 ± 3.5	39.8 ± 2.6
GTN	ND[d]	1.3	ND
1,2-GDN	1.28	6.1	0.7 ± 0.4
1,3-GDN	0.51	1.9	0.4 ± 0.2
1-GMN	2.41	33 (GMN)	10.6 ± 1.3 (GMN)
2-GMN	1.88		
1,2-GDN-Gluc.[e]			10.0 ± 0.7
1,3-GDN-Gluc.[e]			3.5 ± 0.4
GMN-Gluc.[e]			1.5 ± 0.2
Glycerol	6.64		6.9 ± 0.8
Other	8.2	8	4.9 ± 1.5[f]
			1.3 ± 0.3[f]

[a] DiCarlo et al. (1968)

[b] Needleman et al. (1971)

[c] Hodgson and Lee (1975)

[d] Not Detected

[e] Glucuronide Conjugate

[f] Two unidentified peaks isolated

that 49 ± 3.5% of the dose was excreted into the urine. The following compounds were identified in the urine of these animals : GMN's (33%), 1,2-GDN (6%), 1,3-GDN (2%) and nitroglycerin (1%). Eight percent of the dose excreted into the urine was identified only as "non-ether extractable."

DiCarlo et al. (1968) administered 10 mg/kg [^{14}C]-nitroglycerin orally to rats and measured metabolite excretion over a four-hour period. They found that 20 and 21 percent of the dose was eliminated by the lungs (as carbon dioxide) and in the urine, respectively. Both mononitrate and dinitrate metabolites, as well as glycerol, were identified in the urine. Eight percent of the urinary radioactivity was not identified but was more polar than the known nitroglycerin metabolites.

More recently, Hodgson and Lee (1975) found that 80 - 90% of an oral dose of [1,3-^{14}C]-nitroglycerin (180 mg/kg, suspended in peanut oil) was absorbed in the rat in 24 hr. They found that 25.5% of the dose was excreted as carbon dioxide and 40% was excreted in the urine. The urine was found to contain 1,2-GDN (0.7%), 1,3-GDN (0.4) and GMN's (10.6%, isomers not separated). It is interesting to note that the ^{14}C-nitroglycerin used in this study was only 98.3% pure and contained 0.7% 1,2-GDN and 0.4% 1,3-GDN. Coincidentally, these same percentages of these metabolites were isolated in the urine (Table II-1).

Hodgson and Lee (1975) were the first to report that significant amounts of nitroglycerin metabolites were

excreted as glucuronide conjugates. They quantitated urinary excretion of 1,2-GDN, 1,3-GDN and GMN conjugates (10.0%, 3.5% and 1.5% of the total dose, respectively). These conjugates represent 37.5% of the total metabolites excreted in the urine.

DiCarlo et al. (1969b) followed the disposition of [14C]-nitroglycerin in several tissues. They noted that liver concentrations of non-extractable radioactivity increased with time indicating that the nitroglycerin label was covalently bound to this tissue. After administration of 10 mg/kg nitroglycerin to rats, they found [14C]-labeled lipid, protein, glycogen, RNA and DNA in liver tissue. These data may have implications if future investigators wish to use radiolabeled nitroglycerin in human research subjects.

B. NITROGLYCERIN PHARMACOKINETICS

1. INTRODUCTION

Before the development of specific chromatographic techniques, neither nitroglycerin nor its metabolites could be detected in biological fluids. Instead, investigators such as Bogaert et al. (1969b), measured nitrate or nitrite levels in plasma after doses of nitroglycerin or other nitrate ester vasodilator. This method, even when combined with an extraction procedure, will not specifically detect low concentrations of nitroglycerin. With the development of gas chromatographic techniques, the quantitation of

nitroglycerin was somewhat improved. When evaluating nitroglycerin pharmacokinetic literature studies, one must also be able to evaluate the specificity and accuracy of the analytical method used by the authors. These data are critical to the proper interpretation of the pharmacokinetic data.

2. PHARMACOKINETICS OF NITROGLYCERIN IN ANIMALS

a. INTRAVENOUS NITROGLYCERIN ADMINISTRATION

Intravenous nitroglycerin has been administered to at least three different animal species (rat, dog and monkey) during the past decade. Needleman et al. (1972) administered [^{14}C]-nitroglycerin to rats via the jugular vein. Blood was withdrawn through the carotid artery and extracted immediately with petroleum ether (which was assumed to extract only nitroglycerin). Nitroglycerin was found to have a fast distribution half-life of 10 to 14 seconds and a terminal half-life of approximately 1 min. The half life of total ^{14}C in plasma (i.e., total metabolites) was approximately 4 hr. Johnson et al. (1972) also found a 1 min half-life after intravenous administration of 0.5 mg/kg of [^{14}C]-GTN to rats.

Yap and Fung (1978) administered intracardiac doses of 0.7 mg/kg GTN to rats and sampled blood through the orbital sinus. They found that GTN followed one compartment

kinetics (i.e., there was not a distribution phase) and determined the half-life to be 4.2 ± 1.5 min (range = 2.4 to 6.1 min) and the volume of distribution to be 3.1 ± 0.9 L/kg.

Taylor et al. (1980) investigated the possibility of decreased metabolism of GTN due to a possible isotope effect of ^{2}H-labeled GTN. They administered 0.25, 0.5 and 0.75 mg/kg doses (intravenously) of both GTN and deuterated GTN to rats. Arterial blood was sampled via the right common carotid artery and 4 millimolar iodoacetamide was immediately added to inhibit degradation of GTN. The GC assay employed had a reproducibility of 10.6% at 1.9 ng/ml and the authors assumed assay specificity by assuming that the metabolites were not co-extracted with hexane. The authors found that there was no isotope effect for deuterium and that nitroglycerin exhibited dose independent pharmacokinetics. The half-life averaged 4.5 min, similar to that found by Yap and Fung (1978). Although Taylor et al. (1980) claimed that their data exhibited dose independent kinetics, the volumes of distribution (calculated from DOSE / C_{extrap}) showed a trend towards dose dependence. These volumes were 5.4 ± 2.1, 7.3 ± 2.9 and 10.3 (mean of 10.6 and 10.0) for the 0.25, 0.5 and 0.75 mg/kg doses, respectively.

Fung et al. (1981a) administered intravenous infusions, intra-arterial infusions and intra-arterial boluses to rats. These doses all yielded the same plasma clearance from which

the authors concluded that there was a negligible lung first-pass effect. These authors also administered four bolus doses (0.15 to 2.48 mg/kg) into the jugular vein of 22 rats and measured venous plasma concentrations for 90 min. These data were fit to a two-compartment model with a distribution half-life of 2 to 3 min and a terminal half-life of 15 min. Venous plasma clearance was independent of dose (approximately 750 ml/min/kg). The RBC concentration of nitroglycerin was twice that of plasma; venous blood clearance was calculated to be 520 ml/min/kg.

Fung et al. (1981b) measured the arterial/venous (A/V) nitroglycerin concentration gradient in dogs after intravenous infusions. Six dogs received intravenous infusions via the femoral vein in the hind leg. Serial blood samples were drawn from the femoral vein and artery of the opposite leg. Three dogs (not previously exposed to nitroglycerin) received a constant infusion (40 ug/min) of nitroglycerin for 120 min. The steady-state arterial to venous concentration ratio in these animals was 3.8 ± 2.1 (number of samples = 36). The remaining three dogs received a 1.0 mg/min infusion for 60 min followed by a 150 min washout period after which time a 40 ug/min infusion was administered for 120 min. The A/V ratio at 1.0 mg/min was 3.8 but decreased to 1.8 ± 0.7 during the second infusion of 40 ug/min (i.e., upon repeated infusions). The arterial plasma exhibited a shorter half-life after stopping the infusion than the venous plasma. The arterial plasma

clearance was approx. 2.7 times slower than venous plasma clearance (CL_A = 246 and CL_V = 676 ml/min/kg). These latter two observations suggest that the arterial volume of distribution is much smaller than the venous. Based on the equation :

$$t_{1/2} = 0.693 \times V_d / CL$$

and the facts that the arterial half-life is shorter than the venous and that the arterial clearance is slower than venous, then the arterial volume of distribution must be disproportionately lower than the venous.

In a related study, Fung et al. (1981c) infused nitroglycerin into the right femoral veins of rats. Afterwards, they isolated blood vessel segments and assayed them for nitroglycerin concentrations. They found that segments near the infusion site (i.e., the lower and middle inferior vena cava) showed significant concentrations. The lower segment contained about 4-fold more GTN than the middle segment. Additionally, the blood vessel concentrations were about 10-fold higher than plasma concentrations. These results indicated rapid vessel uptake (aorta GTN uptake < inferior vena cava uptake). Nitroglycerin concentrations in blood vessels decreased about 2-fold slower than did the plasma concentrations. These data add even more evidence supporting the theory that the vasculature is affecting the clearance of nitroglycerin.

Using a GC-MS assay capable of detecting 0.1 ng/ml GTN

and 1.0 ng/ml GDN's, Miyazaki et al. (1982) studied the pharmacokinetics of intravenous nitroglycerin in beagle dogs. Three dogs each received a 150 ug intravenous bolus dose of nitroglycerin. Maximum plasma concentrations of 200 ng/ml were obtained (0.5 min). These data were fit to a two compartment model with a distribution half-life equal to 0.45 min and a terminal half-life of 4.25 min. The volume of distribution (V_1) was 3.33 ± 0.32 L (range = 2.99 - 3.63) and the CL was 3.11 ± 0.75 L/min (range = 2.63 - 3.37). One dog received an infusion of 6 ug/min/kg for 30 min. Blood samples were drawn at 15 and 30 min and serial blood samples were drawn after the infusion was stopped. Nitroglycerin steady-state concentrations were 50 - 60 ng/ml (57 ng/ml maximum). Nitroglycerin half-lives were similar to those obtained in the other three dogs (0.50 and 4.95 min). The dinitro-metabolites were measured only in the dog which received the infusion and these plasma concentrations were also fit to a two-compartment model. The 1,2-GDN reached maximum levels of 90 - 100 ng/ml and exhibited half-lives of 8.10 and 40.6 min. The 1,3-GDN reached maximum levels of only about 30 ng/ml but with similar half-lives as those of the 1,2-GDN (8.50 and 48.5 min).

Finally, Wester et al. (1983) administered a 1.92 mg intravenous bolus dose of nitroglycerin to rhesus monkeys and collected serial venous blood samples. Plasma nitroglycerin concentrations were determined using a GC assay capable of detecting 0.1 ng/ml GTN. The data were fit

to a two compartment model with a distribution half-life of 0.8 min and a terminal half-life of 18 min. From the data of these authors, the clearance was calculated and ranged between 1.6 to 2.0 L/min.

b. SUBLINGUAL AND ORAL NITROGLYCERIN

In a novel study, Taylor et al. (1980) determined the sublingual absorption of nitroglycerin in the anesthetized rat. These investigators dosed 5 rats sublingually with 0.75 mg/kg GTN dissolved in less than 100 ul of propylene glycol. Arterial blood samples were obtained as described in the intravenous section. This dose resulted in half-live of 14.3 ± 3.4 min and a concentration at zero time C_o = 20.0 ± 3.5 ng/ml (obtained by extrapolation). Based on the AUC's for these data (calculated from C_o / k_{el}), the bioavailability of sublingual GTN in rats is about 150 ± 50 percent.

After oral doses of [^{14}C]-nitroglycerin to rats, Needleman and associates (1972) found less than 2% of the dose in blood. They noted though, that "these low levels of nitroglycerin radioactivity in blood approach the lower limit of ordinary detection" and concluded that oral doses of nitroglycerin are not systemically available.

These results are in conflict with those by Yap and Fung (1978) and Kamiya et al. (1982). Kamiya et al. (1982) administered oral doses of GTN (3.5 mg/kg) to 5 rats.

Venous plasma from the jugular vein was assayed for unchanged GTN, peak concentrations of 9 ± 5 ng/ml were found. An apparent bioavailability of 1.8 ± 0.9 percent was calculated based on intravenous data in another group of animals.

Yap and Fung (1978) administered 7 mg/kg GTN to rats, sampling blood through the orbital sinus, and were able to fit the data to a one-compartment open model with an absorption step. The bioavailability was calculated based on the AUC's of an intracardiac dose and assuming linear kinetics. They found an availability of 1.61 ± 1.25 percent (range = 0.61 - 3.78), an absorption half-life of 21.4 ± 13.1 min (range = 12.0 - 43.4), a volume of distribution of 2.7 ± 0.3 L/kg (range = 2.3 - 3.0) and T_{max} ranging between 5 to 13 min.

Yap and Fung (1978) also noted that their analytical procedure extracted 10 - 20 percent of the GDN's present in plasma. They were able to resolve these metabolites from the parent drug by GC methods. Qualitatively, they found that the peak height ratios of the GDNs after an oral dose were higher than those for the parent drug (at 10 min) and were much higher than those for the parent after an intracardiac dose (at 6.5 min). This observation substantiates " the conclusion of extensive first-pass metabolism after oral nitrates." This observation of Yap and Fung may be taken a step farther. It is imperative that an analytical procedure for nitroglycerin be specific for

the parent drug. It is not enough to assume that a hexane extraction procedure is specific for GTN, since Yap and Fung have shown that such is not the case. If an extraction procedure is not specific, then the chromatographic procedure must be validated as being able to measure GTN in the presence of metabolites. Otherwise, GTN plasma concentrations are overestimated and the resulting pharmacokinetic parameters are meaningless.

c. TOPICAL AND RECTAL NITROGLYCERIN

The topical administration of nitroglycerin has been studied in some detail in two animal species, rat and monkey. Yap and Fung (1978) applied 7 and 14 mg/kg nitroglycerin as an ointment to a 6.45 cm^2 area on the shaved backs of rats. Serial blood samples were obtained from the tail veins but nitroglycerin was not measureable.

Horhota and Fung (1978) found that nitroglycerin absorption was greater when the dose (20 mg/kg) was applied to a 9 cm^2 area of abdominal skin. After assaying plasma obtained from the jugular vein, they found nitroglycerin concentrations of 10, 20 and 30 to 40 ng/ml when nitroglycerin was applied to shaved back skin, adhesive tape stripped back skin (i.e., stratum corneum removed) and shaved abdominal skin, respectively.

In a later study, Horhota and Fung (1979) applied topical doses (20 mg/kg) of nitroglycerin ointment, 6.9%

nitroglycerin in alcohol and pure nitroglycerin to 9 cm^2 of shaved abdominal skin. They also studied the effect of occlusion on nitroglycerin absorption from the alcoholic solution. They found higher absorption from the nitroglycerin ointment (AUC = 134 ± 75) than both the pure (AUC = 35 ± 13) and alcohol doses (AUC = 27 ± 19). They also found that the alcohol solvent did not affect GTN absorption and that plasma concentrations were unaffected by occlusion.

Noonan and Wester (1980) and Wester et al. (1983) studied the percutaneous absorption of nitroglycerin in the rhesus monkey. Noonan and Wester (1980) measured the amount of total [^{14}C]-nitroglycerin absorbed using only urinary excretion of the carbon-14 label. Nitroglycerin (40 mg) was applied to a 2 cm^2 area of the chest, upper-inner arm, inner thigh and post auricular area. The amount of dose absorbed through this small area varied between 8.9 to 14.8 percent. There was no difference in absorption between any of the sites tested. These data are in contrast to that of Horhota and Fung (1979), but these differences are most likely species related. In another study, this same dose (40 mg) was applied to 50 cm^2 of the chest. A significantly larger percentage (36%) of the dose was absorbed from a larger surface area ($p < 0.02$).

Wester et al. (1983) investigated the pharmacokinetics and bioavailability of topically applied nitroglycerin in the rhesus monkey. Each of three monkeys (4 - 6 kg) was

dosed with [^{14}C]-nitroglycerin ointment (19 mg / 50 cm^2). Plasma concentrations of unchanged GTN were found to peak (6.8 ± 0.3 ng/ml) at 4 hr. The topical bioavailability of unchanged GTN was found to be 56.6 ± 5.8 percent. The bioavailabilities based on plasma total radioactivity and urinary excretion were 77.2 ± 6.7 % and 72.7 ± 5.8%, respectively. The difference in the estimates between unchanged GTN and total radioactivity (20%) was thought to be due to a percutaneous first-pass effect, i.e., skin metabolism of GTN as it was absorbed. Alternatively, this difference may be due to blood vessel clearance before the drug reached the sampling site.

Kamiya et al. (1982) determined the rectal absorption (bioavailability) of nitroglycerin in the rat. These authors sampled venous blood via the jugular vein. When GTN solutions were infused into the unrestricted rectal cavity, the apparent availability was only 26.7 ± 7.0%. When the length of the rectum was restricted (with a septum) to the lower 3.5 and 2.0 cm, the bioavailability of nitroglycerin increased to 83.8 ± 74.5 and 91.2 ± 30.4 percent, respectively. Therefore, when a rectal dose was administered and restricted to the lowest part of the rectum, nearly complete bioavailability was obtained. The authors also demonstrated nearly complete nitroglycerin bioavailability from a rectally administered experimental osmotic minipump.

3. PHARMACOKINETICS OF NITROGLYCERIN IN HUMANS

a. SUBLINGUAL NITROGLYCERIN

Bogaert and Rosseel (1972) were the first investigators to use gas chromatographic methods to measure nitroglycerin plasma concentrations after sublingual doses to humans. They administered sublingual doses of nitroglycerin in 0.12 ml ethanol to 5 volunteers; two individuals received 0.8 mg and three received 1.2 mg. The volunteers were instructed to keep the dose in the sublingual area for 3.5 min after which time each rinsed out his mouth. The saliva and rinsing doses were found to contain 20 - 40 percent of the dose and "fair amounts" of the dinitrate metabolites. The first blood sample drawn from each subject (at 4 min) showed maximal nitroglycerin concentrations. These "peak" concentrations ranged from 3 to 7.5 ng/ml and 6 to 13 ng/ml for the low and high doses, respectively.

Neurath and Dunger (1977) attempted to measure plasma nitroglycerin concentrations after administration of a two-step preparation to 10 volunteers. This preparation contained an outer coating of nitroglycerin (0.56 mg) designed to be sucked off by the patient. The tablet core contained pentaerythritol tetranitrate which was meant to be swallowed after a taste signal. Serial blood samples were collected and hemolyzed by freezing at -18 ^{o}C, and then incubated at 38 ^{o}C overnight with glucuronidase and

extracted with ethyl acetate. These investigators were unable to detect unchanged nitroglycerin (due most likely to in vitro degradation). Instead they reported concentrations of the mono- and dinitrate metabolites. These data show the importance of validating not only the assay chromatographic conditions but also the extraction procedure, to determine extraction efficiencies and potential for in vitro degradation (i.e., during sample preparation).

Armstrong et al. (1979) attempted to estimate volume of distribution and clearance from sublingual doses (0.6 mg) of nitroglycerin in 10 volunteers. They assumed that the sublingual doses were instantaneously and completely absorbed. These parameters were estimated using the following equations:

$$V_d = \text{Dose} / C_{extrap} = 179.6 \text{ liters}$$

$$CL = V_d \times 0.693 / t_{1/2} = 28 \text{ L/min}$$

These volume and clearance parameters are actually composite values since they contain at least one other parameter bioavailability (F), i.e., CL/F and V_d/F. Bogaert and Rosseel (1972) showed that sublingual doses were not completely available, even when dosed in solution. Nitroglycerin peak times in the study by Armstrong et al. (1979) were 2 min and were longer in other studies (Table II-2). Since the nitroglycerin half-life is also 2 min,

TABLE II-2: Selected observations and pharmacokinetic parameters after sublingual doses of nitroglycerin to humans.

Reference	# Subjects	Dose (mg)	C_{peak} (ng/ml)	T_{peak} (min)	Half-life (min)
Bogaert and Rosseel (1972b)	3 2	1.2[a] 0.8[a]	6 - 13 3 - 7.5	4 4	- -
Blumenthal et al. (1977)	1	0.3	1	3	-
Neurath and Dunger (1977)	10	0.56	-	-	-
Armstrong et al. (1979)	10	0.6	2.3 ± 0.4	2	4.4
Wei and Reid (1979)	6	0.6	1.6 ± 0.7	5	5,32[b]
Girre et al. (1980)	1	1.5	1.3 (4.4[c])	10,90[c]	1.8,20[c]
Imhof et al. (1980)	12	1.6	0.6 ± 0.2	5	8b
Maier-Lenz et al. (1980)	6	0.8	2.4 ± 0.5	7	5
Thadani et al. (1980)	2	0.6	0.3, 1.2	3,5	-
Wei and Reid (1981)	12	0.6	1.4 ± 0.6	5	18
Bashir et al. (1982)	6	0.5	1.4 ± 0.1	3	4.2 ± 0.7
Colfer et al. (1982)	10	0.4[d] 0.4[e]	1.4 ± 0.4 1.3 ± 0.2	2 2	- -
Dugger et al. (1983)	18	3 x 0.15	0.95	4-8	-
Weber et al. (1983)	7	0.8[f]	0.2 - 0.4	-	-

[a] Dose in ethanol

[b] Terminal half-life estimated from data in reference

[c] Second absorption peak

[d] Day 1 dose

[e] Day 2 dose

[f] Sublingual spray

these longer peak times are significant and absorption may not be considered to be instantaneous. Therefore, intravenous doses are necessary to accurately determine estimates of volume of distribution and clearance.

Armstrong et al. (1979) and several other investigators have reported similar values for C_{peak}, T_{peak} and half-life after sublingual doses (Table II-2). Peak nitroglycerin plasma concentrations range between 0.2 to 4.4 ng/ml for doses ranging from 0.4 to 1.5 mg. Peak times, when measured, ranged between 2 and 10 min and half-lives ranged between 2 and 5 min. Two studies by Wei and Reid (1979 and 1981) showed longer terminal half-lives (18 to 32 min). These longer half-lives may be assay artifacts since they were estimated from plasma concentrations approaching the assay sensitivity. Also note that the study by Girre et al. (1980) showed a second peak for sublingual nitroglycerin at 90 min. These investigators proposed that this peak was due to oral absorption of a small amount of the dose which was swallowed. Alternatively, since they did not demonstrate assay specificity, this peak may be an artifact due to nitroglycerin metabolites produces after oral absorption.

b. ORAL NITROGLYCERIN

The early work of Needleman et al. (1972) indicated that most if not all nitroglycerin was inactivated on its first pass through the liver. Yet efficacy studies, as

reported by Winsor and Berger (1975), have demonstrated that oral nitrates are efficacious. Very few clinical pharmacokinetic studies have been carried out with oral nitroglycerin preparations. The pharmacokinetic parameters from these few studies are summarized in Table II-3.

A major obstacle in the interpretation of these studies is the lack of information regarding assay validation (as in Blumenthal et al., 1977; Girre et al., 1980; Maier-Lenz et al., 1980; Bashir et al., 1982). These authors do not give any information regarding assay specificity (i.e., regarding potential interference by nitroglycerin metabolites). All four of these groups indicated that unchanged nitroglycerin was present in plasma after oral doses. These authors assume that hexane extraction of plasma was specific for nitroglycerin. Yap and Fung (1978) reported that hexane extracted 10 to 20 percent of the dinitrate (GDN) metabolites resulting in significant peak height ratios after oral dosing.

More recently, Dugger et al. (1983) used a specific GC-MS assay (Settlage et al., 1983), capable of detecting 1.0 pg/ml, quantitating 5 pg/ml and resolving nitroglycerin from the metabolites, to measure nitroglycerin and GDN concentrations after clinical doses of nitroglycerin to volunteers. These investigators found that nitroglycerin concentrations could not be detected (i.e., concentrations less than 5 pg/ml) in the plasma of any of 12 subjects who each received 3 different 2.5 mg sustained release oral

TABLE II-3: Selected observations and pharmacokinetic parameters after oral (sustained release) doses of nitroglycerin to humans.

Reference	# Subjects	Dose (mg)	C_{peak} (ng/ml)	T_{peak} (min)	Terminal $T_{1/2}$ (hr)
Blumenthal et al. (1977)	1	2.5	< 0.1	-	-
	1	6.5	0.2 - 0.3	40	-
Girre et al. (1980)	1	7.5	4.82	180	2.6[a]
Maier-Lenz et al. (1980)	6	2.5	0.30 ± 0.10	30	0.5[b]
Bashir et al. (1982)	6[c]	6.4	2.8 ± 0.6	120	2.5 ± 0.9
	6[d]	6.4	2.5 ± 0.3	240	4.1 ± 0.7
Dugger et al. (1983)	12	2.5	< 0.005	-	-
	12	2.5	< 0.005	-	-
	12	2.5	< 0.005	-	-
	1	50	< 0.005	-	-

[a] Estimated from Table II of Ref.

[b] Estimated from computer generated fit of mean data in Ref.

[c,d] Two different dosage forms

formulations. Even in one individual who received a 50 mg oral dose, unchanged parent drug concentrations were all less than 5 pg/ml.

The data of Dugger et al. (1983) are in direct conflict with the data published by the four other research groups (Table II-3). However, based on the work of Needleman et al. (1972), the data of Dugger and associates are entirely feasable. Alternatively, it might be proposed that Dugger and co-workers used products which were poorly bioavailable. If so, neither parent drug nor metabolites would have been detectable. These authors were able to resolve 1,2-GDN and 1,3-GDN from each other and from nitroglycerin. They found peak concentrations of 1,2-GDN of 500 to 800 pg/ml at 1 to 5 hr. These concentrations declined to 200 to 300 pg/ml at 6 to 8 hr. Even though 1,3-GDN concentrations were not quantitated, the relative ratio of 1,2-GDN to 1,3-GDN was approximately two (calculated from the relative peak heights of these two compounds). These data indicate that nitroglycerin was absorbed but that it was metabolized on first-pass through the liver. Note that had this assay not been specific for nitroglycerin, these authors might have reported plasma concentrations of nitroglycerin after oral doses.

c. TRANSDERMAL ABSORPTION OF NITROGLYCERIN

1. TOPICAL CREAMS AND OINTMENTS

An alternative method of dosing nitroglycerin is to give a transdermal (topical) dose. This mode of administration allows drug to enter the systemic circulation directly, thus bypassing the hepatic circulation and first-pass metabolism.

Experimental and pharmacokinetic results from 10 separate clinical studies using nitroglycerin ointment and creams are summarized in Table II-4. The nitroglycerin dose and surface area were experimental variables which were often not controlled. Peak concentrations and peak times varied between 0 to 32 ng/ml and 0 to 360 min, respectively, depending on the dose and surface area. The duration of measureable plasma concentrations was also affected by these variables and varied between 2 to 7 hr.

Hansen et al. (1979) found that the effectiveness of nitroglycerin ointment may change with the site of application. Note that most of the doses in Table II-4 were applied to the chest but that in several studies the application site was not designated. Iafrate et al. (1982) and Karim et al. (1981) showed that the surface area of application affects the plasma concentrations of nitroglycerin. Note that the surface areas, when mentioned, varied between 25 and 232 cm^2.

TABLE II-4: Selected observations after administration of nitroglycerin ointment to humans.

Reference	# Subjects	Dose (mg)	Surface area (cm^2)	Body Region	C_{peak} (ng/ml)	T_{peak} (min)	Duration of Measurable GTN Levels (min)
Blumenthal et al. (1977)	1	16	-	-	(approx.) 0.2	20 - 60	60[e]
Tristani et al. (1978)	17[a]	12	-	-	0.75	60	60[e]
Wei and Reid (1979)	3[b]	0.27 mg/kg	-	-	1.2 - 3.0	60[f]	-
Armstrong et al. (1980c)	9[c]	0.71 mg/kg	58	-	(approx.) 3 ± 3	60 - 240	240[e]
	5[c]	4 inches	116	-	(approx.) 9 ± 4	60 - 240	240[e]
Girre et al. (1980)	1	20	-	Precordial	3.87	90	420[e]
Imhof et al. (1980)	12	12[d] (#1)	121	Chest	2.24 ± 0.57	60	240[e]
		12 (#2)	121	Chest	1.78 ± 0.35	60	-
		12 (#3)	121	Chest	1.43 ± 0.26	60	-
Maier-Lenz et al. (1980)	6	30.4	-	-	0.4 - 0.7	30 - 60	120
Sved et al. (1981)	3	16	25	Chest	0.15 - 0.18	30	90[e]
	3	16	100	Chest	0.36 - 0.47	45	90[e]
	1	16	100	Chest	0.37	60	390[e]
	1	32	100	Chest	0.88	60	90[e]
Bashir et al. (1982)	6	23(cream)	232	Chest	2.7 ± 0.1	30	360[e]
	6	35(ointment)	232	Chest	2.5 ± 0.2	30	360[e]
Iafrate et al (1983)	10	0.5 inch	25.4	Chest	3.7 ± 1.4	23 ± 125	360[e]
	10	0.5 inch	50.8	Chest	5.4 ± 4.6	204 ± 122	360[e]
	10	1.0 inch	25.4	Chest	11.2 ± 9.4	243 ± 132	360[e]
	10	1.0 inch	50.8	Chest	13.6 ± 8.8	229 ± 112	360[e]

[a] Coronary artery disease patients

[b] Unstable angina patients

[c] CHF patients

[d] Dose = three-times per day

[e] Experiment duration

[f] One sample only

Also important is the variability in dosage. Note (in Table II-4) that the doses were reported as amounts or lengths of nitroglycerin ointment. Kirby and Woods (1980) showed that a 3- to 7-fold variation in ointment doses occurred when doses were measured by the length of ointment squeezed onto an applicator as compared to when the ointment was weighed. All of these factors may account for the variability in nitroglycerin plasma concentrations observed after topical doses.

2. TRANSDERMAL NITROGLYCERIN DELIVERY SYSTEMS

Recently, three new solid state nitroglycerin transdermal delivery systems have been developed. These include Nitrodur (Key Pharmaceuticals, Inc.), Nitrodisc (Searle) and Transderm Nitro (Ciba-Geigy). These systems offer several advantages over ointment doses, including a fixed dose, constant surface area and sustained drug delivery over a 24-hr period.

Table II-5 summarizes several pharmacokinetic studies performed with these products. In each study, the average plasma concentrations of nitroglycerin remained constant for 24 to 32 hr. Furthermore, in each of these studies, average concentrations (mean of several subjects) were used to determine the "steady-state" nitroglycerin concentrations. Figures II-2a and II-2b show that both the intra-subject and inter-subject variability was very large. When these data

TABLE II-5: Pharmacokinetic observations after administration of nitroglycerin transdermal delivery systems to humans.

Reference	# Subjects	Dose	Mean C_p (ng/ml)	Duration (hr)
Gerardin et al. (1981)	14	20 cm^2 TTS[a]	0.21 ± 0.16	24
Karim et al. (1981)	23	8 cm^2 MDD[b]	< 0.1	-
			4.0[d]	32
Colfer et al. (1982)	10	10 cm^2 ND[c]	0.89 ± 0.29	24
	5	20 cm^2 ND	1.81 ± 0.13	24
Gonzalez et al. (1982)	6	20 cm^2 ND	0.2 - 0.3	24
Muller et al. (1982)	6	1 x 10 cm^2 TTS	0.16 ± 0.13	24
	6	2 x 10 cm^2 TTS	0.25 ± 0.04	24
	6	4 x 10 cm^2 TTS	0.57 ± 0.11	24
	10	1 TTS x 10 days	0.18 ± 0.03[e]	-
			0.15 ± 0.02[f]	-
Karim (1983)	12	16 cm^2 MDD	approx. 0.3	32
Mazoyer et al. (1983)	18	20 cm^2 ND	0.24 ± 0.03	24

[a] TTS : Therapeutic Transdermal System, Ciba Geigy Corp.

[b] MDD : Microsealed Delivery System, G.D. Searle & Co.

[c] ND : NITRO-DUR^R, Key Pharmaceuticals, Inc.

[d] Ipsilateral blood samples

[e] mean C_p after 5 days of chronic therapy

[f] mean C_p after 10 days of chronic therapy

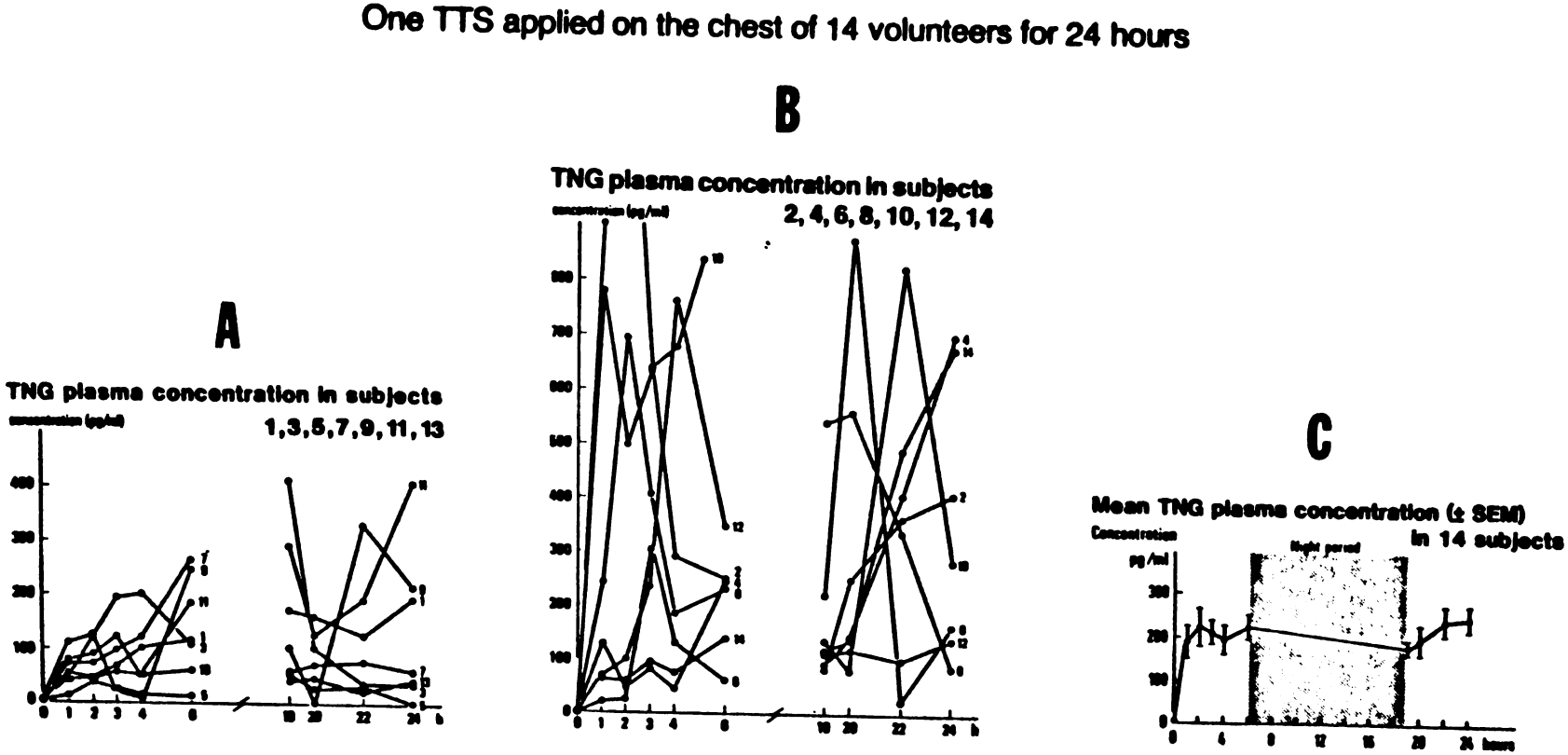


FIG. II-2: Inter- and intrasubject variability in nitroglycerin plasma concentrations after transdermal administration (Gerardin et al., 1981).

were averaged together (Fig II-2c) a "smooth" concentration-time profile was obtained, suggesting an apparent zero-order absorption rate.

d. INTRAVENOUS NITROGLYCERIN

Armstrong et al. (1980b) stated that "the expanding role of intravenous nitroglycerin (GTN) in the management of critically ill hospitalized patients demands a clear knowledge of its pharmacodynamics and kinetics." Until the pharmacokinetic properties of this drug are elucidated, the pharmacodynamics will remain unknown. Armstrong et al. (1975) found that intravenous nitroglycerin doses required to produce similar hemodynamic effects, varied over a 10-fold range. However, knowledge of nitroglycerin pharmacokinetics is required to relate dose to plasma concentrations.

The intravenous nitroglycerin pharmacokinetic parameters reported in the literature are summarized in Table II-6. As was discussed in Chapter I, nitroglycerin adsorbs to many plastics. Baaske et al. (1982) demonstrated that the type of intravenous administration set may significantly influence the amount of "available" nitroglycerin actually delivered intravenously to a patient. Of all the studies summarized in Table II-6, only McNiff et al. (1981) addressed this issue and validated drug delivery from the infusion set which they used. Wei and Reid (1979),

TABLE II-6: Comparison of intravenous nitroglycerin pharmacokinetic parameters reported in the literature.

Parameters	Wei and Reid (1979)	Armstrong et al. (1980b)		McNiff et al. (1981)	Oh and Reid (1981)	Armstrong et al. (1982)		
		Responders	Nonresponders					
Number of Subjects	5	16		8	12	20		
Patient Population	Acute Myocardial Infarction	Congestive Heart Failure		Normal Subjects	Patients undergoing cardiac catheterization	Congestive Heart Failure		
Infusion Rate (mcg/min)	37.5 - 175	15 - 94	59 - 440	15.5 - 21.5	24.5 ± 4.1	15 - 440		
Infusion Duration (min)	One Hour After Blood Pressure Stabilized	Variable[a]		30	20	Variable[a]		
Assay Sensitivity (ng/ml)	0.5	0.2		0.1	0.5	0.3		
Blood Sampling Site	Peripheral Vein	Radial Artery		Peripheral Vein	-	Pulmonary Artery	Artery	Vein
Apparent C_{ss} (ng/ml) (Range)	1.6 ± 0.9 (0.4 - 2.7)	1.2 - 16.1	19.9 - 481	(approx.) 0.5	1.04 ± 0.27	30 ± 53 (1.4 - 139)	25 ± 48 (1.4 - 179)	7 ± 15 (0 - 60)
Plasma Half-life (min) (Range)	-	-	0.9 - 2.7	2.8 ± 0.9 (1.3 - 3.8)	3.30 ± 0.60	-	1.4 ± 0.8 (0.3 - 2.6)	-
Volume of Distribution (L) (Range)	-	-	2.2 - 19.2	232 ± 66 (163 - 334)	112[b]	-	-	-
Apparent Plasma Clearance (L/min) (Range)	82 ± 57 (14 - 146)	13.8 ± 5.8 (7.0 - 24.7)	3.6 ± 1.8 (0.9 - 6.4)	54.5 ± 21.1 (29.8 - 78.3)	23.6	-	-	-

[a] Blood collected 15 min after peak infusion rate was attained.

[b] Estimated from values of CL and $T_{1/2}$ in Ref.

Armstrong et al. (1980b) and Oh and Reid (1981) failed to report such validation. If nitroglycerin was adsorbed by the intravenous sets used in these studies, then clearance and volume of distribution were overestimated.

The blood sampling site is another variable in the experimental design that requires further discussion. One of the basic assumptions in most pharmacokinetic studies is that the drug concentration in venous blood reflects that in arterial blood. Fung et al. (1981a) demonstrated an arterial-venous (A/V) gradient, at steady-state, in dogs during intravenous infusions. Armstrong et al. (1982) demonstrated an A/V gradient in patients receiving nitroglycerin infusions. Table II-7 (adapted from Armstrong et al., 1982) shows that estimates of clearance (infusion rate / C_{ss}) varied as a function of the blood sampling site and that these clearance values exceeded liver blood flow. Note (from Table II-6) that only Armstrong et al. (1980b and 1982) collected blood from the arterial circulation while the remaining investigators sampled blood from the venous circulation. These differences make comparisons of pharmacokinetic parameters difficult. Future intravenous pharmacokinetic investigations should use both arterial and venous blood sampling. These data would not only allow comparisons between literature studies from different sampling sites but may give some indication of the true mechanism of nitroglycerin clearance.

TABLE II-7: Nitroglycerin concentrations at different arterial and venous sampling sites during intravenous infusions of nitroglycerin (Armstrong et al., 1982).

	Infusion Rate	GTN concentration (ng/ml)			AV EXT	$T_{1/2}$	GTN Clearance (L/min)		
Pt.	(mcg/min)	PA	ART	Venous	(%)	(min)	PA	ART	Venous
1	15	2.3	1.8	1.3	27.8	0.7	6.5	8.3	11.5
2	21	1.9	1.4	0.8	42.9	1.2	11.0	15.0	26.2
3	21	2.5	2.9	0.6	79.3	*	8.4	7.2	35.0
4	21	3.9	1.9	0.0	100.0	*	5.4	11.0	*
5	21	-	4.2	2.2	47.6	0.3	-	5.0	9.5
6	30	1.5	1.6	1.7	-6.3	1.9	20.0	18.8	17.6
7	30	4.7	3.2	1.7	46.9	0.8	6.4	9.4	17.6
8	30	9.0	6.5	1.0	84.6	1.5	3.3	4.6	30.0
9	42	9.9	7.5	0.4	94.7	0.8	4.2	5.6	105.0
10	42	5.0	3.7	0.4	89.2	1.2	8.4	11.4	105.0
11	42	4.5	3.0	1.3	58.3	3.7	9.3	14.0	32.3
12	42	1.5	1.4	0.7	50.0	*	28.0	30.0	60.0
13	42	1.4	1.8	0.8	55.6	0.7	30.0	23.3	52.6
14	82	3.8	2.7	1.9	29.6	1.3	21.6	30.4	43.2
15	82	18.2	13.6	7.8	42.6	1.1	4.5	6.0	10.5
16	220	41.7	32.8	7.5	77.1	1.2	5.3	6.7	29.3
17	310	107.8	97.6	12.5	87.2	1.7	2.9	3.2	24.8
18	440	138.9	116.6	60.6	48.0	2.6	3.2	3.8	7.3
19	440	25.9	18.1	2.9	84.0	1.4	17.0	24.3	151.7
20	440	181.8	179.4	40.2	77.6	2.2	2.4	2.4	10.9
Mean	121	29.8	25.1	7.3	60.8	1.4	10.4	12.0	41.0
± SD	156	52.8	48.4	15.4	27.2	0.8	8.6	8.8	39.2

*Subjects in whom plasma GTN was undetectable before enough data could be collected for kinetic analysis.

Abbreviations: PA = pulmonary artery; ART = arterial; AV EXT = arterial venous extraction.

C. PHARMACODYNAMICS OF NITROGLYCERIN

1. PHARMACOLOGICAL ACTIVITY OF NITROGLYCERIN METABOLITES

The pharmacological activity and pharmacokinetics of nitroglycerin metabolites must be evaluated before the pharmacodynamics of nitroglycerin, itself, may be interpreted. It has been generally assumed that nitroglycerin metabolites are inactive and that only the parent drug is responsible for vasodilator/antianginal activity. These assumptions are often based on the work of Needleman and his coworkers (1972) in which intraportal injections of nitroglycerin, in rats, were found to produce insignificant hypotension.

The activity of the dinitrate metabolites (GDN's) has been investigated by Bogaert et al. (1968). They evaluated the activities of the GDN's in three different systems. First, they compared the ability of the GDNs, relative to nitroglycerin, to decrease guinea pig blood pressure by 15 mm. Nitroglycerin was found to be 40- to 50-fold more active than the GDN's. Secondly, they determined that the amount of metabolite required to decrease contraction in rabbit aorta strips (by 50%) was 360- and 600-fold higher than GTN for 1,3-GDN and 1,2-GDN, respectively. Bogaert et al. (1968) also examined the dose required to decrease blood pressure 25 mm upon intra-arterial perfusion into the dog

hind leg. In this system, they found that nitroglycerin was 19- and 28-fold more active than 1,3-GDN and 1,2-GDN, respectively.

Needleman et al. (1969) measured the intravenous dose required to produce a 10 to 30 percent decrease in blood pressure in dogs. In this study, they reported that 1,3-GDN was about 10-fold less potent than nitroglycerin and 1,2-GDN was about 14-fold less potent, although based on the available data shown in this report, nitroglycerin was only 7- and 9-fold more potent than the GDN's. The mononitrate derivatives appeared to lack vasodilator activity. However, the doses used in this study were low and the investigators could only detect a 40-fold difference in mononitrate activity, i.e., had the mononitrates been 50-fold less active, they would have appeared to be totally inactive.

Krantz et al. (1962) examined the activity of a dinitroglycerin derivative, 1-chloro-2,3-propanediol dinitrate. Note the structural similarity to 1,2-GDN, although this derivative is more lipophilic than the GDN. This derivative exhibited equipotent coronary vasodilator activity compared to nitroglycerin.

The measurable pharmacological activity of the nitroglycerin metabolites varies as a function of the test system. In vivo test systems indicate that the GDN's are active and that their activity varies from approximately two to ten percent of that of the parent drug. At very low metabolite concentrations, these activities may be insignif-

icant. However, these active metabolites will show hemodynamic effects if plasma concentrations accumulate (as in chronic oral dosing). Therefore, it is necessary that metabolite and nitroglycerin concentrations be measured during pharmacodynamic studies so that potential metabolite effects can be identified. It is important to consider nitroglycerin metabolite activity when evaluating pharmacologic and pharmacodynamic data in the literature.

2. NITROGLYCERIN PHARMACOLOGICAL ACTIVITY VERSUS THE ROUTE OF ADMINISTRATION

The activity of sublingual nitroglycerin has long been recognized. Krantz (1972) states that "today sublingually administered nitroglycerin maintains its preeminent position in the management of coronary insufficiency." Referring to pharmacological studies, he went on to state that "the gastrointestinal absorption of oral controlled-release nitroglycerin has been adequately demonstrated."

There has been much controversy in the literature, during the past decade, regarding the activity of orally administered nitroglycerin. On the basis of several metabolic studies in animals, Needleman and his coworkers (1972) concluded that there was "no rational basis" for the use of long-acting oral nitrates for the prophylactic therapy of angina pectoris.

Needleman et al. (1972) compared vasodilation and blood

nitrate levels when nitroglycerin was injected into the jugular and portal veins of rats. They found that jugular vein injections produced a typical dose-response curve (Fig. II-3). However, when 0.5 to 500 mcg/kg doses were injected into the portal vein, no change in blood pressure was detected, even at the highest dose, thus indicating that the first-pass effect for nitroglycerin was very high. These investigators also injected ^{14}C-nitroglycerin (500 mcg/kg) directly into the portal vein. They found that "little or none" of the intact nitrate passed through the liver. High blood levels of metabolites were immediately detectable but were incapable of producing vasodepression. From these data they concluded that oral doses of nitroglycerin would not be systemically available nor efficacious.

Two other investigations, similar in design to that by Needleman et al. (1972), have since been published. These studies show results that are different from those reported by Needleman. In the first, Cammarato et al. (1973) showed decreases in blood pressure in dogs, cats and rats after intravenous (i.v.), intraportal vein (p.v.) and intraduodenal (i.d.) administration of nitroglycerin. They determined the potency (relative to i.v. nitroglycerin) of p.v. injections to dogs, cats and rats to be 0.4, 6 and 1 percent, respectively. Intraduodenal administration of 3 mg/kg produced significant decreases in blood pressure in all three species. Maximal hypotension was detected 3 to 5 min after dosing and remained significantly lower for 120

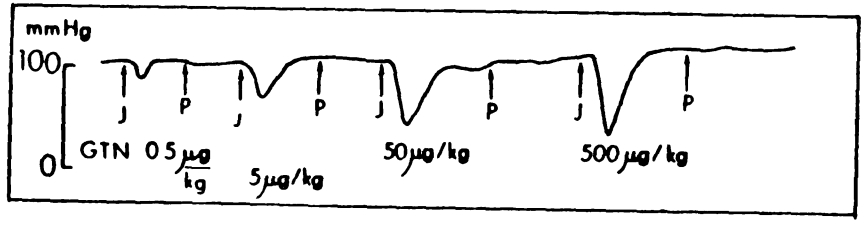


FIG. II-3: Comparisons between portal (P) and jugular (J) vein injections of nitroglycerin on rat blood pressure responsiveness (Needleman et al., 1972).

min in the dog, 60 min in the cat and at least 30 min in the rat. These data indicated that significant hemodynamic responses could be observed even when nitroglycerin was passed through the liver.

A second study, by Heinzow and Zeigler (1981), compared the effects of nitroglycerin administered to rats by different routes, these being: intravenous (i.v.), sublingual (s.l.), intraperitoneal (i.p.), portal vein (p.v.) and intrajejunal (i.j.). The relative potencies of nitroglycerin when administered by i.v., s.l., i.p., p.v. and i.j. routes were 40, 4-8, 2-4, 2 and 1-2, respectively. That is, intravenous nitroglycerin was 5 to 10 times more potent than sublingual, 10 to 20 times more potent than i.p., 20 times more potent than portal vein, and 20 to 40 times more potent than intrajejunal.

It is interesting to note that Heinzow and Ziegler (1981) determined potency by comparing the maximum decrease in blood pressure. However, the time course (and the AUC) of the blood pressure response also varied as a function of the dosing route. The areas under the curve of the blood pressure decrease for the portal and jejunum doses were at least three-fold higher than that for intravenous. Future studies should include an integrated effect relationship in which tne AUC's (not only the absolute magnitude) of the responses are evaluated.

Of these three studies, only the original study by Needleman et al. (1972) evaluated blood samples for the

presence of unchanged nitroglycerin and metabolite concentrations. The work of Cammarato et al. (1973) and Heinzow and Ziegler (1981) are important contributions because these studies show that oral nitroglycerin doses are pharmacologically active. However, the presence of pharmacological activity alone is not sufficient evidence to show availability of unchanged drug. In fact, the longer half-life of the blood pressure response curve, after intraportal doses, may even indicate that metabolites were responsible for apparent drug activity.

Abrams (1980a) indicated that the animal studies of Needleman et al. (1972) were an influential argument against the efficacy of oral nitrates. Additionally, many of the early efficacy studies, in which oral nitroglycerin was judged to be efficacious, were poorly designed and inadequately controlled. The placebo effect has been shown to be a significant factor in the evaluation of antianginal drugs. Beecher (1955) found that 26 to 38 percent of the subjects studied showed anginal relief by placebo. Benson and McCallie (1979) noted a 30 to 40 percent placebo effect. They also noted a study by Aronow and Kaplan (1969) in which 40 pecent of 20 patients had increased exercise tolerance on placebo pills.

Abrams (1980a) admitted that recent studies, using minimum oral dosages, showed negative efficacy; however, numerous recent reports have demonstrated oral nitroglycerin efficacy. These reports indicate that physiologic effects

last one to six hours. Abrams (1980a) summarized the views of many investigators: "It is now clear, however, that large doses of oral nitroglycerin and synthetic nitrate esters produce physiologic effects, probably by overwhelming hepatic nitrate reductase activity."

Several recent evaluations of clinical studies using oral nitroglycerin have appeared over the past eleven years: In carefully controlled clinical studies, oral nitroglycerin has been shown to produce peripheral vasodilatation (Eliakim et al., 1972; Hirshleifer, 1973; Winsor et al., 1972). Gensini et al. (1971) demonstrated that oral nitroglycerin dilated coronary arteries and collateral vessels in man.

Winsor and Berger (1975) studied the effect of oral nitroglycerin in angina patients. Patients received 2.6 mg three times daily. Six to ten days after therapy was begun and 4 hours after the previous tablet was administered, the subjects were tested for exercise tolerance. These authors found that oral nitroglycerin increased exercise tolerance by 64 percent over placebo. Note that a placebo was used in each patient to reduce both the interpatient variability and the 40 percent placebo effect documented by Beecher (1955). At peak exercise, there was no difference in heart rate or blood pressure but the ST segment depression (used as a measure of myocardial ischemia) was significantly reduced. Winsor and Berger concluded that "the data demonstrate that oral nitroglycerin, given as controlled-release tablets, was absorbed from the gastrointestinal tract in quantities

sufficient to provide statistically significant clinical improvement of angina."

Strumza et al. (1979) studied 20 patients with or without congestive heart failure. In patients with congestive heart failure, they found that 5 mg sustained release nitroglycerin significantly decreased preload (as measured by the left and right ventricular filling pressures). There was no effect on heart rate , cardiac output, afterload or contractility.

Davidov and Green (1980) found that oral nitroglycerin significantly reduced the number of anginal attacks and increased exercise capacity. The patients in this study were administered 19.6 to 39 mg nitroglycerin per day. Each patient initially received 3 capsules per day but could increase the dose to 6 per day if angina attacks increased or did not subside. It is interesting to note that 5 of the 12 patients terminated exercise testing due to fatigue or maximum heart rate rather than anginal symptoms. Thus, the actual increase in exercise capacity was underestimated because these patients did not exercise to anginal symptoms. These authors also noted that 5 patients did not complete the study because of severe headaches. Such headaches are a common side effect of nitroglycerin and usually indicate that the dose was absorbed.

All of these studies indicate that oral doses of nitroglycerin are pharmacologically active. However, none of these is sufficient evidence that the parent drug

(nitroglycerin) is absorbed intact after oral doses. In fact, as discussed in Section II.3.b, there is no evidence in the literature that proves definitively that nitroglycerin is orally absorbed unchanged. On the contrary, the discussion in Section II.3.b and the data to be presented in Chapters VI and VII indicate that venous concentrations of nitroglycerin can not be detected after oral administration. Also, the pharmacologically active dinitrate metabolites of nitroglycerin reach relatively high concentrations after oral doses (as will be shown in Chapter VIII). These metabolites, then, may ultimately be responsible for the activity of oral nitroglycerin (functioning as an oral prodrug).

3. NITROGLYCERIN CLINICAL PHARMACODYNAMICS

Attempts to correlate nitroglycerin pharmacokinetics (blood levels) with pharmacological effect have not been conclusive. Most studies attempt to relate nitroglycerin doses to effect but fail to show any relationship. Such attempts have failed because nitroglycerin bioavailability was assumed to be complete. Nitroglycerin bioavailability varies as a function of the route of administration. In the past, sublingual doses were assumed to be completely available (Armstrong et al., 1979). However, as will be shown in Chapter VII, sublingual availability can be quite low and it varies significantly not only between subjects

but also within the same subject. The availability of an intravenous dose may also vary as a function of the type of intravenous tubing used (Baaske et al., 1982). Rather than relating dose to effect, nitroglycerin plasma (or blood) concentrations or the integrated AUC should be related to effect.

Armstrong et al. (1980b,c) have shown that arterial plasma nitroglycerin concentrations, attained in patients with congestive heart failure after intravenous and topical nitroglycerin doses, may be related to pharmacologic effect. Intravenous steady-state plasma concentrations were determined from a single arterial blood sample. This sample was obtained at hemodynamic steady-state at a nitroglycerin infusion rate which was required to decrease pulmonary capillary wedge pressure (PCWP) by 25 percent. These investigators arbitrarily divided their patients into two groups. Group I (responders) included those patients with nitroglycerin plasma concentrations of 11.1 ng/ml or less (1.2 to 11.1 ng/ml) who also showed a reduction in PCWP of 26 to 52 percent (at infusion rates of 15 to 32 mcg/min). Group II (nonresponders) included the remainder of the subjects. Note that these "nonresponders" included not only those patients whose PCWP was not decreased by 25 percent (at infusion rates as high as 440 mcg/min) but also three patients who "responded" to nitroglycerin (with a greater than 25 % reduction in PCWP) at plasma concentrations greater than 11.1 ng/ml. The authors concluded, somehow,

that 1.2 to 11.1 ng/ml nitroglycerin concentrations represented the therapeutic range for nitroglycerin.

In another study on the same groups of patients, Armstrong et al. (1980c) found nitroglycerin concentrations (attained after ointment doses) of 3.1 ± 3.0 ng/ml were effective in reducing PCWP in group I patients. However, concentrations of 8.9 ± 4.0 ng/ml were not effective in group II (non-responding) patients.

The data of Armstrong et al. (1980b) require some additional comments. First, nitroglycerin concentrations were measured in arterial blood samples. The relationship of venous and arterial nitroglycerin concentrations to therapeutic efficacy has not been established. Armstrong et al. (1982) found significant differences in arterial and venous concentrations in congestive heart failure patients during intravenous infusions. The significance of this A/V ratio has not yet been elucidated.

Secondly, the "therapeutic range" was determined somewhat arbitrarily. Armstrong and coworkers did not use infusion rates lower than 15 mcg/min. Five out of the eight "responders" did so at the lowest infusion rate. Two subjects showed decreases in PCWP of 50 percent or more at the lowest infusion rate. Some of these individuals may have responded to lower infusion rates and, therefore, lower nitroglycerin concentrations. The high end of the "therapeutic range" was arbitrarily chosen to be 11.1 ng/ml even though three subjects responded, but at higher

nitroglycerin plasma concentrations (32.8 to 140 ng/ml). The actual therapeutic range of this population was quite large, ranging from less than 1.2 ng/ml to 140 ng/ml. Titration of each patient's dosage is important with such high variability.

Thirdly, the correlation between arterial concentrations and hemodynamics was studied only in congestive heart failure patients. These results may not be able to be extrapolated to other patient populations, such as those with angina.

Wei and Reid (1979) investigated the hemodynamic effects of intravenous nitroglycerin infusions in patients with acute myocardial infarction. They found that infusion rates of 37.5 to 175 mcg/min were required to decrease the mean arterial pressure in this group of patients by 10 percent. Venous plasma concentrations, obtained by analyzing a single blood sample 1 hr after the blood pressure was stabilized, varied from less than 0.5 to 2.7 ng/ml (mean = 1.6 ± 0.9 ng/ml). Note that the assay used by these investigators had a detection limit of 0.5 ng/ml and that one individual plasma concentration was reported to be 0.4 ng/ml. The data reported by these investigators did not show any correlation between infusion rate and nitroglycerin plasma concentrations. However, the comparatively narrow range of nitroglycerin plasma concentrations required to decrease mean arterial pressure indicates that a concentration-response relationship may be applicable to this patient

population. Additional studies are required to define this concentration-response relationship.

Wei and Reid (1981) reported a relationship between sublingual nitroglycerin plasma concentrations and pharmacological effect, as determined by echocardiographic measurements, in normal volunteers. These relationships are shown in Fig. II-4. They found that the time course of the following hemodynamic measurements correlated with plasma nitroglycerin concentrations: heart rate, left ventricular end diastolic dimension, left ventricular end systolic dimension and the velocity of circumferential fiber shortening (V_{cf}). Note, in Fig. II-4 that V_{cf} closely paralleled the nitroglycerin time course.

The nitroglycerin assay of Wei and Reid (1979) used in this (1981) study had a sensitivity and reproducibility of 0.5 ± 0.5 ng/ml. Note in Fig. II-4 that nitroglycerin concentrations remain nearly constant (0.5 - 0.8 ng/ml) between 10 and 30 min after drug administration. These concentrations approach the GC assay sensitivity and very little confidence can be placed in these low values. The plasma profile is unlike other profiles in the literature (e.g., see Colfer et al., 1982) or those to be presented in Chapter VII. Plasma concentrations are generally not detectable at the 20 and 30 min time points. Additionally, Wei and Reid (1979) did not provide information regarding the analytical specificity of their assay with respect to nitroglycerin metabolites. The longer terminal half-life

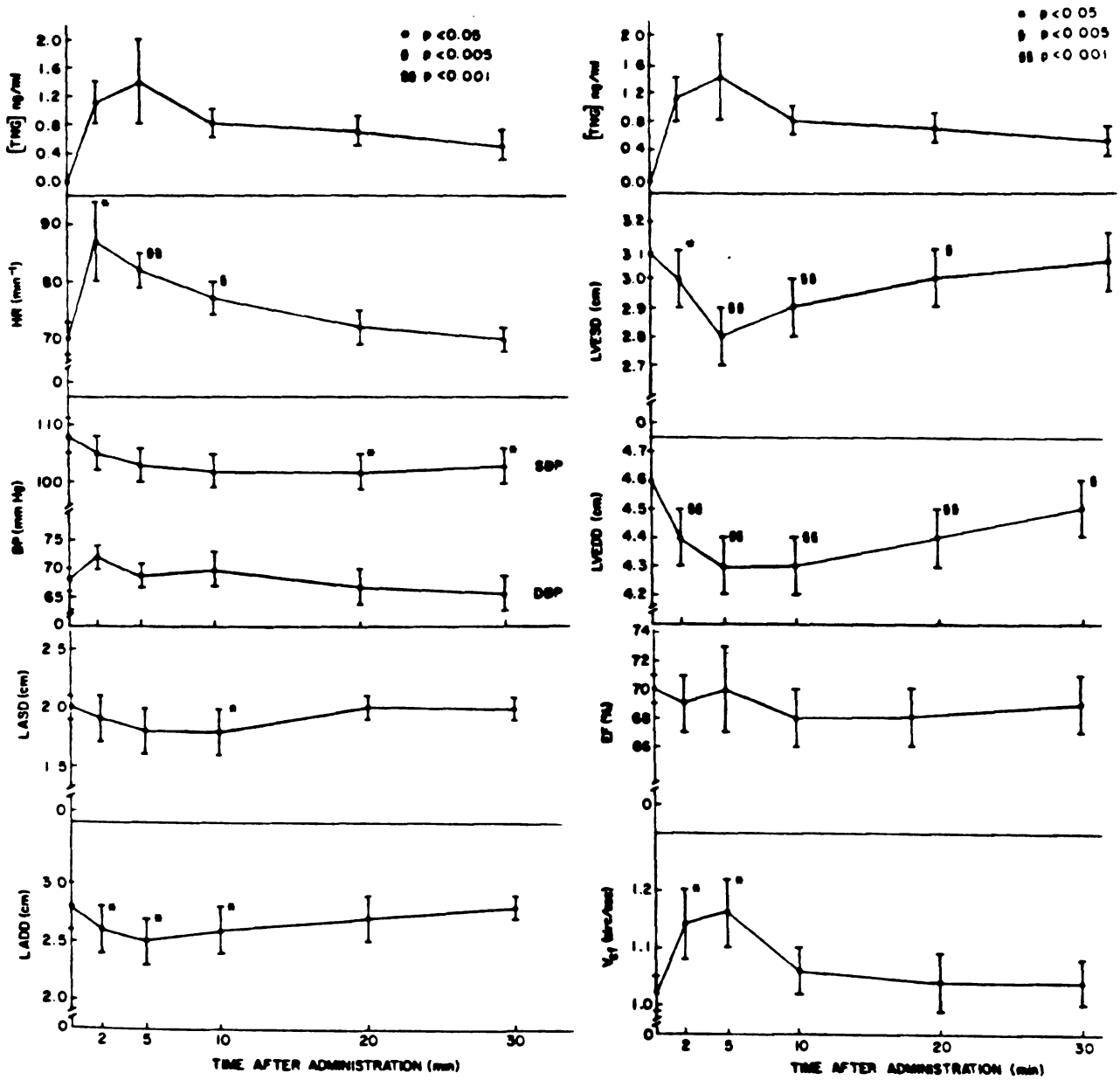


FIG. II-4: Comparison of nitroglycerin plasma concentrations with hemodynamic measurements. Abbreviations: HR, heart rate; SBP and DBP, systolic and diastolic blood pressure; LADD and LASD, left atrial diastolic and systolic dimensions; LVESD and LVEDD, left ventricular end-systolic and end-diastolic dimensions; EF, ejection fraction; Vcf, mean velocity of circumferential fiber shortening. (Wei and Reid, 1981).

(approx. 30 min) shown by Wei and Reid (1979 and 1981) is similar to the GDN half-life to be reported in Chapter VIII. If the assay used by these authors was not specific, then the relationship of nitroglycerin plasma concentrations to pharmacologic activity, developed by Wei and Reid, is highly questionable.

Imhoff et al. (1980) measured differences in the nitroglycerin dose-response in the venous and arterial beds. These studies were performed in 12 healthy volunteers given 1.6 mg doses of sublingual and 12 mg (t.i.d.) doses of topical nitroglycerin. They concluded that venous distensibility was maximal even at low concentrations (less than 0.2 ng/ml, GC detection limit). However they found that peripheral arterial resistance decreased as nitroglycerin plasma concentrations increased. Plasma nitroglycerin concentrations were inversely correlated ($p < 0.01$) with peripheral arterial resistance. It is interesting to note that the authors concluded maximal venous distensibility occurred at less than 0.2 ng/ml, but their data may not support this statement. They did not indicate any significant difference between control venous distensibility and those values attained after nitroglycerin doses.

4. NITROGLYCERIN CLINICAL TOLERANCE

Tolerance to organic nitrates is not well understood.

Abrams (1980b) reported that tolerance to nitroglycerin is not a common clinical problem. In fact, one form of tolerance is beneficial to patients. That is, the nitrate headache (a typical nitrate side-effect) usually disappears after 1 to 2 weeks of therapy. This observation indicates some tolerance in the cranial vasculature (Franciosa, 1980; Abrams, 1980a).

Aronow (1980) concluded that hemodynamic tolerance develops after long-term, high-dose, oral isosorbide dinitrate. However, he emphasized that this tolerance does not impair the antianginal efficacy of high doses of isosorbide dinitrate or of sublingual nitroglycerin.

Thadani et al. (1980) showed a "partial circulatory tolerance" to isosorbide dinitrate (ISDN) after only 1 to 2 days of therapy. They found that heart rate and blood pressure changes were decreased on chronic dosing. They also showed a cross-tolerance to nitroglycerin, i.e., after chronic ISDN doses, the acute response (heart rate and blood pressure) to sublingual nitroglycerin was reduced. They found that ISDN plasma concentrations increased after chronic therapy, suggesting tolerance was due to a decrease in the end-organ response and not to an increase in metabolism.

Georgopoulos et al. (1982) administered topical doses of nitroglycerin via nitroglycerin transdermal therapeutic system to 13 angina patients for 2 weeks. They found that anginal attacks decreased by 67 percent and that there was a

50 percent improvement in ST segment depression. They found that the "development of tolerance was not detected; on the contrary, the anti-anginal effect was more pronounced in the second than in the first week of medication."

In a recent commentary, Hollenberg (1983) addressed the issue of nitrate tolerance. He noted the attenuation of the nitrate-induced fall in blood pressure, ventricular filling pressure and end diastolic volume with chronic nitrate therapy. These changes occurred because the "body probably does not tolerate a state of chronic vasodilation without activating homeostatic mechanisms...". Hollenberg suggested that another potential mechanism for tolerance was the development of a desensitization of the target end organ, but such a mechanism was "difficult to document in man." Hollenberg studied five patients on chronic topical nitroglycerin therapy (Nitro-Dur). After four weeks of therapy, he found that partial tolerance to the hemodynamic effects of the drug had developed but that the drug retained antianginal effects. Although tolerance does not seem to be an important variable in the treatment of angina with nitroglycerin, further research is required to investigate tolerance effects in the treatment of other disorders.

CHAPTER III

FORMATION OF MONO- AND DINITRATE METABOLITES OF NITROGLYCERIN FOLLOWING INCUBATION WITH HUMAN BLOOD

A. INTRODUCTION

Nitroglycerin (GTN) is metabolized by a non-specific glutathione-S-transferase in hepatic tissues (Habig et al. 1976). However, there is controversy regarding the in vitro metabolism or degradation in whole blood. Marcus and coworkers (1978) isolated an isoenzyme of glutathione-S-transferase in human erythrocytes which was different from that found in liver. These authors could not demonstrate measurable metabolism of GTN by this purified enzyme. Lee (1973) demonstrated the conversion of GTN to 1,2- and 1,3-glyceryldinitrates (1,2-GDN and 1,3-GDN) after incubation with blood. However, he used concentrations of GTN that are not clinically obtainable (1 mg/ml). Also, he was not able to detect the formation of glycerylmononitrates (GMNs). Armstrong et al. (1980a) demonstrated the disappearance of pharmacologically relevant concentrations of GTN following incubation with blood. Even though these workers did not measure the appearance of metabolites, they assumed that the loss of GTN was due to metabolism.

More recently, one group of investigators (Wu et al., 1981 and Sokoloski et al., 1983) attempted to measure the

formation of metabolites (GDNs) following incubation of GTN with human erythrocytes. These investigators noted that even though levels of GTN decreased with time, the metabolites could not be detected. They concluded that the disappearance of GTN in blood "can not be enzymatic but probably is physical in nature." The authors did not speculate as to the cause of this "physical" loss. Sokoloski et al. (1983) measured the disappearance of nitroglycerin after in vitro incubation with reconstituted blood, plasma and plasma ultrafiltrate. They found that nitroglycerin disappeared faster in blood than plasma and was essentially stable in plasma water. Again, nitroglycerin metabolites were not detected. The "physical loss" of nitroglycerin was not due to adsorption to glassware because nitroglycerin was stable in plasma ultrafiltrate. These authors did not speculate as to the mechanics of nitroglycerin loss in blood.

The present study was designed to elucidate the mechanism by which GTN might be lost (i.e., either a physical or metabolic loss). [^{3}H]GTN was used in this study to detect drug binding if it occurred.

B. MATERIALS AND METHODS

Heparinized blood was obtained from 3 volunteers immediately before the incubation experiments. Silanized Erlenmeyer flasks containing 50 ml of blood were gently

shaken in a Dubnoff metabolic shaking incubator (Precision Scientific, Chicago, IL) at 37°C. After a 10-min equilibration period, an aliquot of tritiated-GTN (1.36 µg/ml in normal saline, [2-^{3}H]GTN, 150 µCi/mg; radiochemical purity = 97.8%; kindly provided by Dr. Alec Keith of Key Pharmaceuticals, Inc., Miami, FL) was added to the blood to make a final concentration of 17 or 136 ng/ml GTN. One incubation was performed at 17 ng/ml and 3 incubations at 136 ng/ml GTN. Separate 50 ml aliquots of blood from one volunteer were incubated at both concentrations.

Samples of blood were withdrawn from the flasks at the following times: 0, 10, 20, 30, 40, 50, 60, 90 and 120 min. Two samples of blood were treated at each time point: (a) 1 ml of whole blood that was extracted directly and (b) 2 ml of blood was used to obtain 1 ml of plasma which was subsequently extracted. Plasma was obtained by centrifuging (Eppendorf microcentrifuge model 5412, Brinkman Instruments, Westbury, NY) the 2 ml aliquot of blood for 20 seconds at 12,800 g. The plasma and blood samples were first extracted with 10 ml of pentane (all solvents were of pesticide quality, distilled in glass, Burdick and Jackson Laboratories, Muskegon, MI) by shaking on a vortex mixer (Vari-whirl mixer, VWR Scientific, San Francisco, CA) for two minutes in a 16 x 150 mm silanized test tube with a teflon-lined cap (Corning Glass Works, Corning, NY). After transferring the pentane layer to a scintillation vial (Research Products International, Elk Grove Village, IL),

each sample (blood and plasma) was again extracted with 10 ml of ether. The ether was then transferred to a new scintillation vial. Ten ml of cocktail (Aquasol, New England Nuclear, Boston, MA) was added to each vial. Tritium content was determined by scintillation spectrometry (Tri-carb I, Model 3375, Packard Instruments, Downers Grove, IL).

Duplicate aliquots of blood and plasma (obtained from the blood incubation) were extracted at the 120 min time point. The duplicate ether extracts were evaporated under a stream of nitrogen at room temperature. The residues were dissolved in 100 µl ethanol containing unlabeled metabolites (Kindly provided by Midwest Research Institute, Kansas City, MO). A 20 µl aliquot was counted for radioactivity. Another 20 µl aliquot was chromatographed using normal phase HPLC.

HPLC was performed on a liquid chromatograph equipped with a solvent delivery system (Model 8500 pump, Varian Instrument Group, Palo Alto, CA) and manual injector (Model 7125 with silica loop-column, Rheodyne, Berkeley, CA). Detection of GTN metabolites was carried out with a fixed-wavelength UV detector (UV III monitor, model 1203, LDC, Riviera, FL) at 214 nm. The prepacked silica column (4.6 x 250 mm Partisil, 5 µm, Whatman, Clifton, NJ) was eluted with hexane-ethanol (96:4, v/v) at a flow rate of 3.0 ml/min. Under these conditions, the detection limit for the GDNs was 4 ng injected while the GMN detection limit was 15-20 ng

injected (signal/noise ratio=2). All four metabolites (1,2-GDN, 1,3-GDN, 1-GMN and 2-GMN) are resolved from each other and GTN.

After sample injection, fractions of the HPLC effluent were collected into scintillation vials. Those fractions containing metabolites (as indicated by the UV detector) were noted. Each fraction was counted for tritium by scintillation spectrometry as previously described.

As a control experiment, [^{3}H]GTN (136 ng/ml) was incubated both in buffer (Normosol-R, pH 7.4, Abbott Laboratories, North Chicago, IL) and in human plasma. The buffer was chosen so as to mimic both pH and electrolyte composition of blood. Human plasma was obtained from 12 different volunteers and pooled before use. [^{3}H]GTN was incubated (37^{o}C) in triplicate in buffer, and samples (1ml) were withdrawn at 0, 2 and 21 h. Triplicate incubations with plasma were also performed and aliquots (1 ml) were withdrawn at 0, 15, 30, 45, 60, 75, 90, 105, 120, 180, and 240 min, and at 21 h. Each aliquot of buffer and plasma was extracted and tritium content determined as described above. Duplicate aliquots of plasma were extracted at the 21 h time interval. These duplicate extracts were chromatographed (HPLC) as described above to determine metabolite composition.

C. RESULTS AND DISCUSSION

Pentane and ether were chosen as extraction solvents because of their respective partitioning properties. GTN is preferentially extracted into pentane relative to the drug's more polar metabolites. In pentane, 75% of GTN is extracted but only 9% of the GDNs and less than 1% of the GMNs (based on extraction of [^{14}C]-labeled nitroglycerin and metabolites) will be extracted under the conditions described above (i.e. single extraction, 10:1 organic solvent-biological fluid, 2 min contact time). In contrast, 100% of the GDNs and 60% of the GMNs will partition into an ether extract. Thus, GTN and total metabolite levels may be approximated by the tritium content (determined by scintillation spectrometry)in the pentane and ether extracts, respectively.

The levels of radiolabeled compounds (equivalent to GTN) in the ether and pentane extracts as a function of incubation time are shown in Fig. III-1A. Note, that at zero time, significant concentrations of metabolites appeared to be present. However, these apparent concentrations were due to residual nitroglycerin concentrations in the ether extract (because pentane extracted only 75% of the nitroglycerin and ether extracted the remainder). Concentrations of GTN and metabolites, after normalizing for GTN and metabolite extraction efficiencies, are shown in Fig. III-1B. Similar profiles

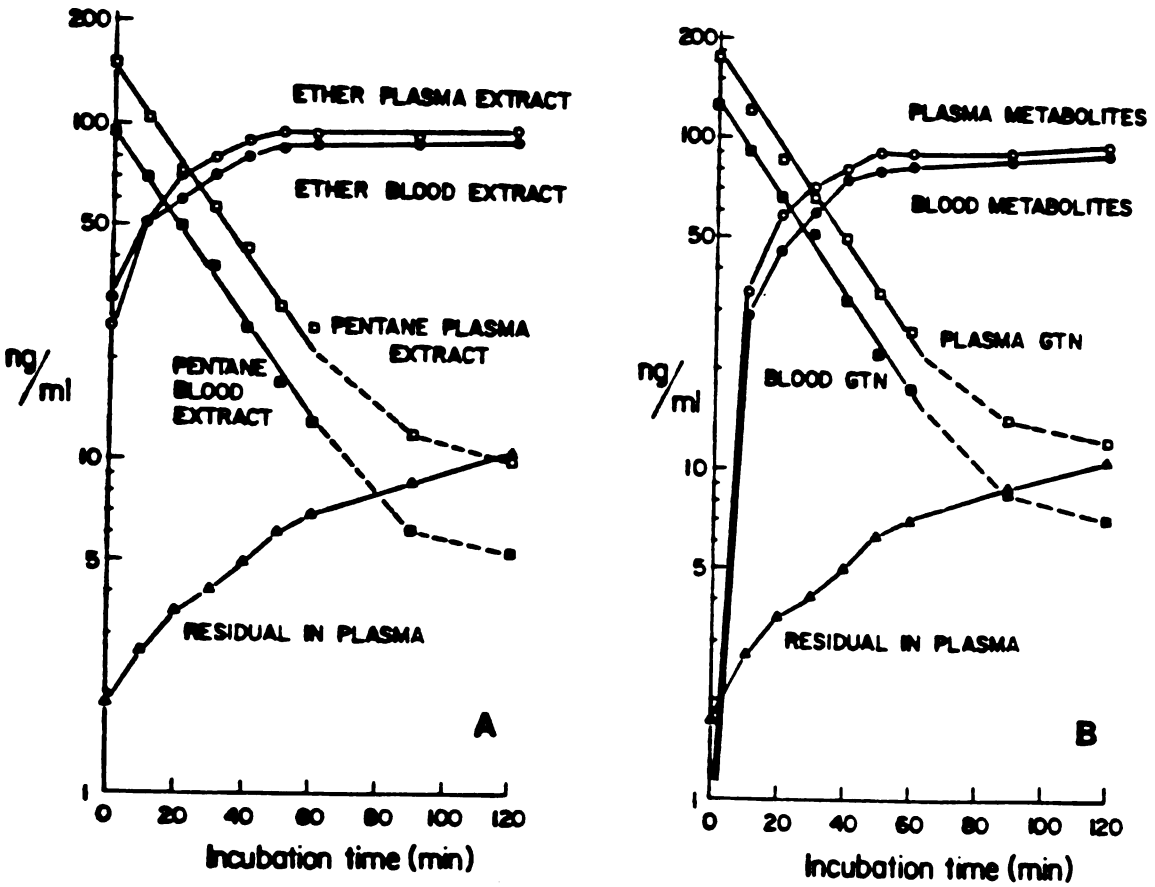


FIG. III-1: In vitro metabolism of ^{3}H-GTN to ^{3}H-metabolites by human blood. Figures show the loss of GTN in both plasma (□) and blood (■) and the accumulation of metabolites (○=plasma, ●=blood) with time. Bottom curve shows the residual concentration of radioactivity (expressed as GTN equivalents) in plasma (▲) after extractions. Figure 1A shows the concentration of radioactive material (as GTN equivalents) found in the extracts. These data were normalized for GTN and metabolite extraction efficiencies to show concentrations of GTN and metabolite (Fig. 1B).

were obtained for each of the four incubations. The disappearance of GTN may be followed in both plasma and whole blood and is apparently a pseudo-first-order-process. At the later time points, GTN concentrations are low with respect to metabolite concentrations. The apparent loss of linearity at these low GTN concentrations may be caused by the partitioning of GDNs into pentane (since pentane can extract approximately 9% of the GDNs). Under these conditions (i.e. very low GTN and high GDN concentrations), the pentane extract may be contaminated with a significant amount of GDN. The GTN half-life was found to vary with the blood source and the initial GTN concentrations (Table III-1). The initial concentrations of GTN used in this study fell within the range of arterial levels reported by Armstrong et al. (1980b) after intravenous infusion. Wu et al. (1981) and Sokoloski et al. (1983) reported a similar observation, that the GTN half-life increased with increasing initial GTN concentration. In a linear pseudo-first- order system, the half-life should be independent of concentration. One might speculate that such behavior (increasing half-life with increasing GTN concentration) may be caused by either substrate or end-product enzyme inhibition.

Figure III-1 also shows that, as the concentration of GTN decreases, the concentration of polar, ether extractable (radiolabeled) compounds increases. These curves reflect the metabolism of GTN and the accumulation of metabolites.

TABLE III-1: Summary of in vitro metabolism of GTN by human blood. Recoveries of metabolites (mass balance) are shown in blood and plasma ether extracts. GTN half-lives and blood/plasma (B/P) ratios were determined between 0-60 min.

Initial GTN Conc.	Subject	Percent of Radioactivity Accounted for in Ether Extract[a]										GTN $t_{\frac{1}{2}}$ (min)	GTN[b] B/P RATIO
		% 1,3-GDN		% 1,2-GDN		% 1-GMN		% 2-GMN		TOTAL			
		Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood		
136 ng/ml	#1	25.4	28.1	49.7	45.8	4.0	6.4	18.3	25.9	97.4	106.3	28	0.66 ± 0.02
	#2	18.5	16.9	60.8	51.0	3.9	4.8	26.6	19.6	109.8	92.3	32	0.66 ± 0.03
	#3	14.1	13.6	48.1	44.0	6.3	7.1	24.1	26.3	92.6	91.0	22	0.62 ± 0.05
	$\bar{X}$	19.3	19.5	52.9	46.9	4.7	6.1	23.0	23.9	99.9	96.5	27	-
	±SD	5.7	7.6	6.9	3.6	1.4	1.2	4.3	3.8	8.9	8.5	5	-
17 ng/ml	#1	11.5	-	52.9	-	5.1	-	23.3	-	92.8	-	15	0.65 ± 0.11

[a] % of each metabolite contained in the initial 120 min ether extract.

[b] ± Standard Deviation, n=7, in 0-60 min measurements.

This accumulation can be measured in both plasma and whole blood. The half-life for the appearance of total metabolites is identical to that for the loss of GTN.

The curve at the bottom of Fig. III-1A shows the relationship between the amount of GTN-related material not extracted from plasma and the time of incubation. Since only 60% of the GMNs are extracted by ether, this curve may reflect the accumulation of GMNs in plasma. The amount of radioactivity remaining in extracted whole blood was not determined.

Table III-2 shows the blood-plasma ratios (B/P) of GTN and total metabolites with time. During the log-linear period of GTN metabolism, the blood-plasma ratio of GTN remained relatively constant. These ratios are similar to that reported by Fung et al. (1981a), i.e., 0.80 ± 0.5. Such a ratio indicates that GTN is not concentrated in the red blood cells. The B/P ratio of total metabolites was initially higher (1.22 ± 0.05) but quickly decreased and remained relatively constant (about 0.9).

The compositions of the 120-min blood incubation ether extracts as determined by HPLC are shown in Table III-1. A sample chromatogram is shown in Fig. III-2. These HPLC conditions resolve GTN (2.4 min), 1,3-GDN (4.0 min), 1,2-GDN (6.1 min), 1-GMN (13.4 min) and 2-GMN (17.5 min). The GTN peak is not shown in Fig. III-2 since GTN is eluted with endogenous extract components which elute prior to the dinitrates. Note from Table III-1 that all four GTN

TABLE III-2: Individual and mean blood/plasma ratios of GTN and total metabolites determined at various incubation times. ^{3}H-GTN (136 or 17 ng/ml) was incubated at 37 °C with fresh human blood.

TIME	136 ng/ml Initial GTN Concentration								17 ng/ml Initial GTN Concentration	
	GTN				METABOLITES				GTN	METABOLITES
(min)	Subject 1	Subject 2	Subject 3	MEAN±SD	Subject 1	Subject 2	Subject 3	MEAN±SD	Subject 1	Subject 1
0	0.68	0.68	0.63	0.66±0.03	1.27	1.18	1.20	1.22±0.05	0.61	0.93
10	0.67	0.68	0.66	0.67±0.01	1.04	0.98	0.99	1.00±0.03	0.65	0.76
20	0.66	0.69	0.68	0.68±0.02	1.09	0.91	0.80	0.93±0.15	0.64	0.80
30	0.66	0.68	0.68	0.67±0.01	1.06	0.88	0.88	0.94±0.10	0.63	0.79
40	0.63	0.63	0.57	0.61±0.03	0.96	1.01	0.93	0.97±0.04	0.53	0.84
50	0.65	0.64	0.58	0.62±0.04	0.93	0.96	0.89	0.93±0.04	0.61	0.82
60	0.64	0.63	0.56	0.61±0.04	0.85	0.90	0.92	0.89±0.04	0.87	(a)
90	0.55	0.56	0.52	0.54±0.02	0.89	0.91	0.95	0.92±0.03	0.76	0.79
120	0.57	0.54	0.52	0.54±0.03	0.91	0.89	0.92	0.91±0.02	0.58	0.75

(a) Sample lost during analysis.

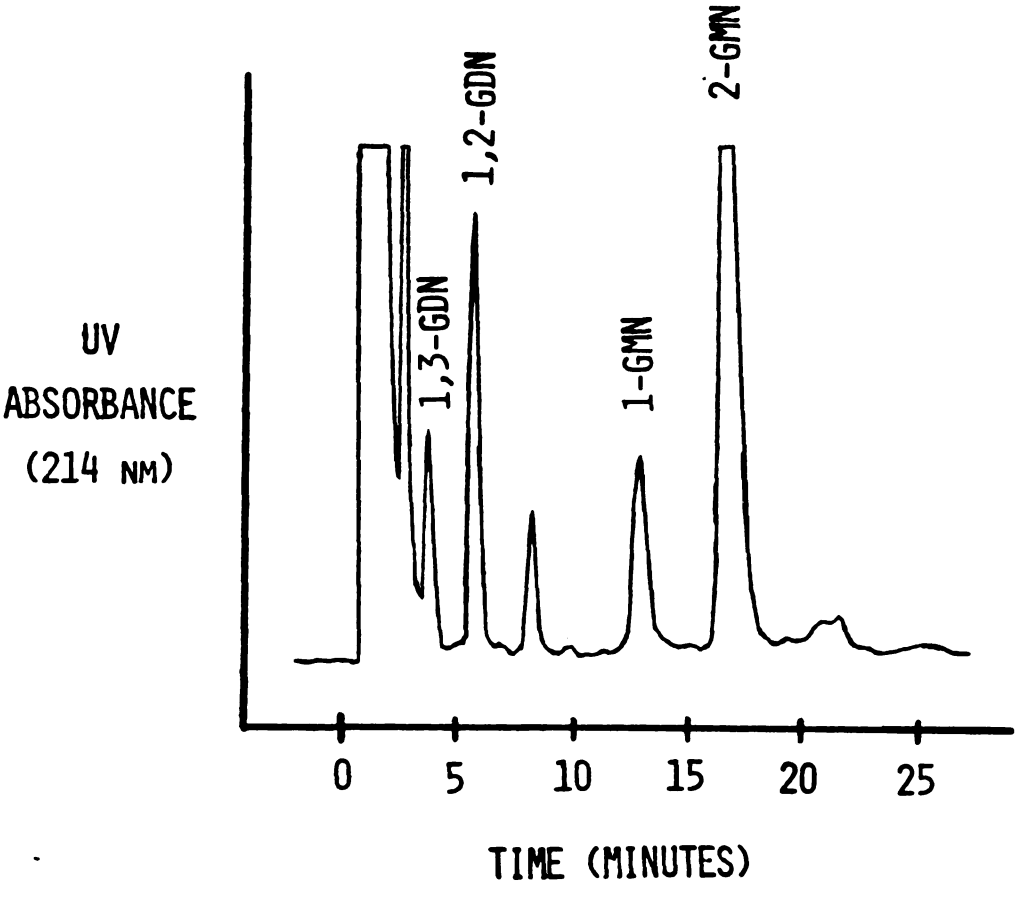


FIG. III-2: HPLC chromatogram of a plasma ether extract containing 1,2-GDN, 1,3-GDN, 1-GMN and 2-GMN.

metabolites were present and that nearly all of the tritium label in the ether extract could be accounted for as these metabolites. It is also of some interest that the relative amounts of these metabolites were similar over an 8-fold concentration range.

The control experiments in which [^{3}H]GTN was incubated with buffer clearly indicated that the degradation of GTN is not due to hydrolysis at physiological pH values. Table III-3 shows that [^{3}H]GTN is essentially stable for 21 h at 37^{o}C. Incubation of [^{3}H]GTN with pooled human plasma, on the other hand, resulted in a first-order loss of GTN. The loss of GTN and accumulation of metabolites are shown in Fig. III-3. [^{3}H]GTN was metabolized significantly slower in plasma than in blood. The mean plasma half-life was 202 ± 5 (± S.D.) min. The rate constant of metabolism was 7.5-fold smaller in plasma than blood. Armstrong et al. (1980a) reported a similar observation, that plasma metabolism in plasma was 8.6-fold slower than in blood.

The metabolite composition in plasma after incubation with [^{3}H]GTN for 21 h is shown in Table III-4. Again, note that all of the radioactivity (tritium label) could be accounted for as the mono- and dinitrates. In fact, 1,3-GDN and 1,2-GDN account for 94% of the total plasma metabolites. DiCarlo and Melgar (1970) also found dinitro-metabolites after nitroglycerin incubation with rat blood serum. Interestingly, they found a similar ratio of 1,3-GDN to 1,2-GDN (ratio=2) as was found in this present

TABLE III-3: Concentrations of extractable ^{3}H-GTN after incubation with pH 7.4 buffer. Note that there are no significant changes in extractable ^{3}H-GTN levels over 21 hours.

Incubation time (hours)	Concentration (ng/ml)	
	Pentane Extract	Ether Extract
0	130 ± 6 [a]	8.0 ± 0.2
2	136 ± 3	8.2 ± 0.2
21	132 ± 7	8.3 ± 0.5

[a] ± standard deviation (n=3)

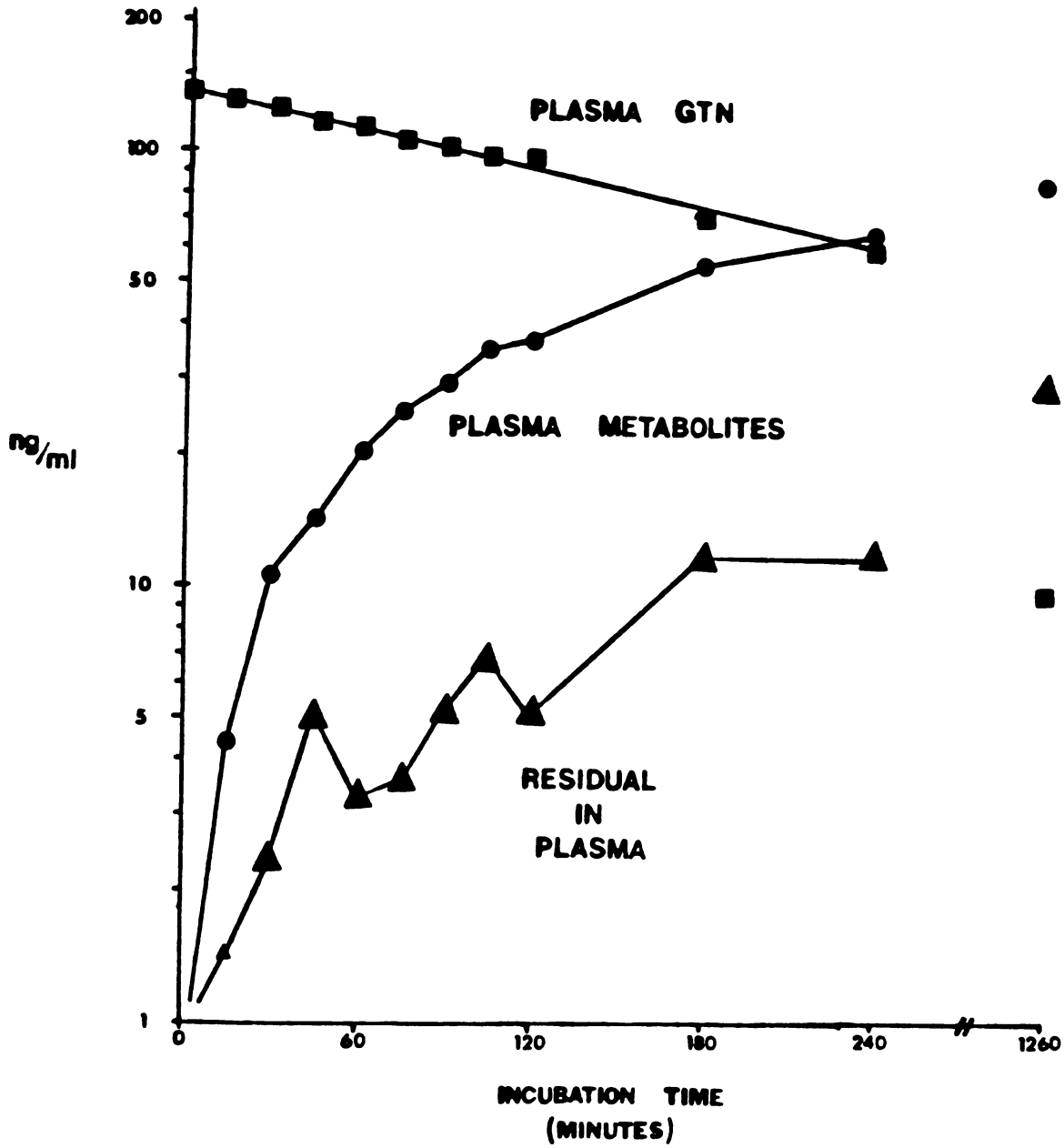


FIG. III-3: In vitro metabolism of ^{3}H-GTN to ^{3}H-metabolites by human plasma. Figure shows the loss of GTN (■), the accumulation of metabolites (●) and the residual concentration (▲) after extractions. All concentrations are expressed as ng/ml GTN equivalents and are normalized as in Fig. III-1B.

TABLE III-4: Metabolite composition in ether extracts of plasma after incubation of ^{3}H-GTN with whole blood or pooled plasma. The incubation times for whole blood and plasma were 2.0 and 21 hours, respectively.

	Metabolite Composition (%)	
Metabolite	**Blood Incubation**	**Plasma Incubation**
1,3-GDN	19.3 ± 5.7 [a]	62.5 ± 5.4
1,2-GDN	52.9 ± 6.9	31.5 ± 0.5
1-GMN	4.7 ± 1.4	3.1 ± 1.0
2-GMN	23.0 ± 4.3	2.9 ± 3.2
Total	99.9 ± 8.9	100.1 ± 8.3

[a] ± standard deviation n=3

investigation.

It is unclear why Sokoloski and co-workers (Wu et al., 1981; Sokoloski et al., 1983) were unable to detect the GDNs after GTN incubation with reconsitiuted red blood cells. Several differences do exist between the two studies: (1) Sokoloski and co-workers used cells from both fresh and outdated blood; (2) they used a one-step ethyl acetate extraction; and (3) they used a GC assay to detect GTN and GDNs.

In the present study, fresh blood was used. Armstrong et al. (1980a), however, did not find a significant difference in the GTN half-life between whole blood and resuspended cells. In the present study, a two-step extraction with pentane and ether was used to remove GTN and metabolites. The single ethyl acetate extraction used by Wu et al. should remove all of the GDNs and 80% of the GMNs, yet the GDNs were not detected. Therefore, neither the blood source nor the difference in solvents can account for the lack of metabolites. The final difference was the use of [^{3}H]GTN which allows the drug to be followed whether eliminated by metabolism or by some other "physical loss". The use of [^{3}H]GTN together with HPLC allowed the detection and quantitation of [^{3}H]GTN metabolites. Also, using [^{3}H]GTN, a mass balance was possible; 90-100% of the tritium label could be accounted for as the four GTN metabolites (Table III-4).

D. SUMMARY

In summary, the loss of GTN during in vitro incubation in blood was shown to be metabolic. Levels of [^{3}H]GTN were found to decrease at a pseudo-first-order rate (log-linearly) with half-lives ranging from 17-32 min. The half-lives varied with the blood source and the initial GTN concentration. Incubation of [^{3}H]GTN in plasma resulted in a much slower rate of metabolism (half-life=202 ± 5 min). However, no degradation of [^{3}H]GTN could be detected after incubation in pH 7.4 buffer for 21 h. Thus, GTN degradation requires other cofactors present in blood and plasma. The decrease of GTN was accompanied by a simultaneous increase in metabolite levels. All four GTN metabolites (1,3-GDN, 1,2-GDN, 1-GMN and 2-GMN) were detected and quantitated after incubation in whole blood and plasma.

E. RELEVENCE TO CLINICAL BLOOD SAMPLING

The results of this study indicate that extreme care must be taken when processing blood samples obtained from patients or volunteers receiving nitroglycerin. The in vitro loss of nitroglycerin must be minimized during the period in which the blood sample is processed to obtain plasma. Serial blood samples are drawn directly into a chilled (in ice water) 7 ml vacutainer through an indwelling

venous catheter (heparin lock) kept patent with heparin. The blood is immediately centrifuged at 12,800 x g for 20 sec. The plasma is then immediately frozen in a dry ice bath. The total amount of time required to withdraw the blood sample and centrifuge to obtain plasma prior to freezing is less than two min.

The quick centrifuging procedure, together with collecting blood in chilled vacutainers, minimizes the in vitro degradation of nitroglycerin by blood. When blood samples were spiked with nitroglycerin at 37°C, 100% recoveries of nitroglycerin were obtained using this quick centrifuging technique. On the other hand, when fortified blood samples were centrifuged at room temperature, using a laboratory centrifuge, only 25% of the fortified nitroglycerin was recovered. Therefore, this quick centrifugation technique was used during all nitroglycerin clinical investigations.

CHAPTER IV

ANALYTICAL METHODOLOGY

A. DETERMINATION OF PICOGRAM NITROGLYCERIN PLASMA CONCENTRATIONS USING CAPILLARY GC WITH ON-COLUMN INJECTION

1. INTRODUCTION

Nitroglycerin administration results in venous plasma concentrations ranging from less than 50 pg/ml to 10 ng/ml, depending upon the route of administration and blood sampling time (Bogaert and Rosseel, 1972b; Armstrong et al., 1982; Bashir et al., 1982). Studies in the rhesus monkey (Wester et al., 1983) indicate that concentrations of ^{14}C-labeled nitroglycerin metabolites are 10 to 300-fold higher than the parent drug. Therefore, an analytical method for nitroglycerin must be both sensitive (25 pg/ml detection limit) and specific (ie., must measure nitroglycerin in the presence of metabolites).

Several different analytical methods have been developed. HPLC methods have been developed to measure nitroglycerin amounts and concentrations in dosage forms (Baaske et al., 1979), waste water (Chandler et al., 1974), trace level explosives (Lafleur and Morriseau, 1980) and blood (Spanggord and Keck, 1980). Of these HPLC methods,

the last (Spanggord and Keck, 1980) used a thermal energy analyzer detector and was the most sensitive. This method was capable of detecting 500 pg (injected on column) and required two ml of plasma (%CV = 12% at 13 ng/ml). None of these methods are capable of detecting nitroglycerin concentrations of 50 pg/ml.

Within the past year, five GC-MS assays for nitroglycerin have been described (Ottoila et al., 1982; Miyazaki et al., 1982; Idzu et al., 1982; Gerardin et al., 1982; Settlage et al., 1983). These assays possess some advantages over HPLC and conventional GC assays. GC-MS assays are selective for the parent drug and quite sensitive (typically 6 - 50 pg/ml). Several disadvantages of GC-MS are readily apparent. The cost and time to set up a GC-MS instrument is often prohibitive for clinical laboratories. Several of these assays (Miyazaki et al., 1982; Idzu et al, 1982; Gerardin et al., 1982) require the use of specially synthesized internal standards labeled with stable isotopes. These internal standards often are not commercially available which may certainly limit the utility of these assays for routine clinical monitoring.

Several GC assays utilizing conventional packed-columns and electron capture detectors have been developed (Rosseel and Bogaert, 1973; Yap et al., 1978; Wu et al., 1982). These were not fully satisfactory due to lack of sensitivity (Rosseel and Bogaert, 1973), complicated extraction procedure and questionable precision (ie., %CV= 23% at 100

pg/ml, Yap et al., 1978) and lack of specificity (Wu et al., 1982). Wu et al. (1982) found interfering chromatographic peaks which varied between individuals and even varied within individuals with time. Another major obstacle to the determination of picogram concentrations of nitroglycerin with conventional packed-column GC is adsorption of nitroglycerin to column components. Several investigators have found that preloading columns, i.e., injecting large amounts of nitroglycerin immediately before analyses of plasma samples, resulted in decreased column adsorption (Armstrong et al., 1979; Taylor et al., 1981; Gonzalez et al., 1982).

Drug adsorption to GC columns may be greatly reduced, if not eliminated, with the use of inert fused-silica capillary columns (Douse, 1981). Douse (1981) reported a fused-silica capillary GC assay (with electron capture detection) which could detect 5 pg and quantify 100 pg nitroglycerin (CV= 13%). Sved et al. (1981) recently reported nitroglycerin plasma levels after a topical dose of nitroglycerin ointment. Although a detection limit of 50 pg/ml (using a fused-silica column) was reported, details of the assay validation have not been reported to date. In the present assay, the use of an on-column injector, a syringe with a fused-silica capillary needle, fused-silica capillary columns and an electron capture detector allows the quantitation of picogram concentrations of nitroglycerin in plasma samples. The assay possesses the precision,

sensitivity and selectivity required to analyze picogram nitroglycerin concentrations in human plasma.

2. MATERIALS AND METHODS

a. CHEMICALS AND REAGENTS

Nitroglycerin was obtained as a 10% triturate on lactose (Key Pharmaceuticals, Inc., Miami, FL) and was extracted from the lactose with diethylether. The internal standard was 2,6-dinitrotoluene (K & K Laboratories, Hollywood, CA). All solvents (Pesticide quality, Glass-distilled, Burdick and Jackson, Muskegon, MI) were used as supplied, with the exception of heptane. Heptane was further purified with activated charcoal. Dimethyldichlorosilane (Aldrich Chemical Co., Milwaukee, WI) was commercially obtained.

b. GLASSWARE SILANIZATION PROCEDURE

All glassware was silanized before use. A 5% dimethyldichlorosilane solution in toluene (v:v) was prepared fresh monthly and stored at $4^{o}C$. Glassware was soaked in this solution for 20 min, rinsed in toluene and soaked in methanol for 20 min to neutralize excess reagent. The glassware was rinsed a final time with fresh methanol, dried at $100^{o}C$ for one hour and baked at $260^{o}C$ for 6 hours.

c. INSTRUMENTS

The gas chromatographs (Varian 3700 and 6000 gas chromatographs, Varian Associates, Los Altos, CA) were equipped with Ni-63 electron capture detectors (ECD) and on-column injectors (On-column injector, J & W Scientific, Inc., Rancho Cordova, CA). Note that this assay has been validated on two separate instruments. The columns are 30 M x 0.25 mm fused silica capillary columns coated with a cross-linked methyl-polysiloxane stationary phase (DB-1, 1.0 micron film thickness, J & W Scientific, Inc., Rancho Cordova, CA). The capillary column should be wrapped in a single sheet of aluminum foil. This technique was found to eliminate peak splitting which occurred due to uneven heat distribution within the GC oven. Helium is used as a carrier gas at a flow rate of 1.5 ml/min (inlet pressure = 40 psi). Nitrogen flow rates to the ECD and column make-up are 5 and 25 ml/min, respectively. Column position inside the ECD was optimized for maximum sensitivity. The oven temperature was programmed from 120 to 180°C at 5°C/min. A second temperature ramp was used to "burn-off" the residual extract. This was accomplished by programming the oven at 50°C/min to 250°C and maintaining this final temperature for 10 min. Chromatograms were recorded and peak height ratios calculated using a recording integrator (HP3390A, Hewlett Packard, Palo Alto, CA).

With routine use (every 100 injections) a 40 cm length of column (at the injector end) should be removed. This procedure, which results in more reproducible standard curves, removes nonvolatile extract components which accumulate and eventually cause chromatographic peaks to broaden and the sensitivity to decrease. An alternative to removing portions of the analytical columnn is the use of a pre-column. The pre-column is a one meter section of column with the same liquid phase as the analytical column. The pre-column should also be wrapped in aluminum foil and is connected directly to the injector. The opposite end of he pre-column is connected to the analytical column using a zero dead-volume union (Zero dead volume union, J & W Scientific, Inc., Rancho Cordova, CA). After approximately 100 injections, the pre-column may be changed and column efficiency is not lost.

d. CLINICAL BLOOD SAMPLING

Serial blood samples are drawn into a chilled 7 ml vacutainer through an indwelling venous catheter kept patent with heparin. The blood is immediately centrifuged at 12,000 x g for 20 sec. The plasma is then immediatedly frozen in a dry ice bath. The total amount of time required to withdraw the blood sample and centrifuge to obtain plasma prior to freezing is less than two min. This procedure minimizes the in vitro degradation of nitroglycerin by

blood (see Chapter III, section E).

e. SAMPLE AND CALIBRATION STANDARDS PREPARATION

Nitroglycerin calibration standards are prepared as follows: Primary stock solutions (1 mg/ml each) of nitroglycerin and 2,6-dinitrotoluene were prepared in ethanol. Secondary stock solutions (10 mcg/ml) were prepared fresh monthly by diluting the primary standards in ethanol (1:100, v:v). Fresh aqueous solutions of nitroglycerin (10 ng/ml) and dinitrotoluene (20 ng/ml) in distilled water were prepared daily from secondary stock solutions. Nitroglycerin calibration standards (0, 25, 50, 100, 200, 300, 400, 500, 750, 1000 and 2000 ng/ml) were prepared by adding the appropriate aliquot of the nitroglycerin standard and 50 ul (1 ng) dinitrotoluene to 1 ml samples of blank human plasma in 16 x 150 mm silanized test tubes with teflon lined screw caps. Clinical plasma samples (1 ml) are transferred to silanized test tubes and mixed with 50 ul dinitrotoluene.

Each calibration standard or clinical sample is extracted with 10 ml pentane by vortexing for two min. Each sample is then centrifuged for 10 min at 2000 x g. The pentane layers are transferred to clean test tubes. The residual plasma layers are extracted and centrifuged a second time, as described above. The combined pentane extracts are evaporated under a stream of nitrogen (at room

temperature) to a final volume of approximately one ml. The extracts are then transferred to one ml reacti-vials (Pierce Chemical Co., Rockford, IL) and evaporated to dryness under a stream of nitrogen. Immediately after each sample goes to dryness, 25 ul of n-heptane is added to each vial and the vials vortexed (approx. 5 sec). A 1.0 ul aliquot of each extract is injected into the gas chromatograph. Sample application to the column is performed via the injector stopcock using a 10 ul gas-tight syringe which was modified to hold a 0.21 x 190 mm fused silica capillary "needle". All extracts were stored at -20^{o}C until chromatographed and on dry ice between GC injections.

f. ASSAY SPECIFICITY

Assay specificity was determined by injecting solutions of several drugs, and some of their known metabolites, on column. The oven temperature was programmed from 120 to 200^{o}C at a rate of 5^{o}C/min, then to 250^{o}C at 50 ^{o}/min. This temperature program is only slightly different from that described earlier and does not affect the retention time of nitroglycerin, nitroglycerin metabolites ,or the internal standard. This temperature program was used to show the relative retention times of a related drug, isosorbide dinitrate, and its metabolites. The analytical method described may be modified in this manner to determine plasma isosorbide dinitrate and metabolite concentrations. All of the compounds tested for possible interferences were tested

using these same conditions.

g. NITROGLYCERIN STABILITY

Nitroglycerin stablility in frozen human plasma was evaluated at -20°C. Drug-free plasma was obtained from four volunteers (3 male, 1 female) and pooled. Nitroglycerin was added to two separate 100 ml aliquots of plasma (at 0°C) to obtain final concentrations of 0.1 and 1.0 ng/ml. Each plasma solution was subdivided into multiple one ml samples, quick-frozen in dry ice and stored at -20°C. Six samples at each concentration were assayed (as described above) after 0, 7, 14, 24, 40, 60, 108 and 200 days of storage.

h. OPTIMIZATION OF THE CAPILLARY COLUMN INSIDE THE ELECTRON CAPTURE DETECTOR

The position of the capillary column exit inside the ECD was optimized for maximum sensitivity. Sensitivity was evaluated from multiple injections of a 1.0 ul aliquot of a nitroglycerin standard solution (1.0 pg/ul nitroglycerin and 40 pg/ul dinitrotoluene in heptane). The position of the column in the detector was adjusted to increase sensitivity (maximum nitroglycerin/dinitrotoluene peak height ratio).

3. RESULTS AND DISCUSSION

a. GC INJECTOR AND DETECTOR OPTIMIZATION

Initial attempts to use a capillary gas chromatographic system to quantitate picogram amounts of nitroglycerin were unsuccessful. Nitroglycerin may adsorb and degrade on active surfaces of capillary injectors, glass columns and detectors. Several injection systems were evaluated. Significant nitroglycerin adsorption occurred with Varian splitless and direct injectors. Use of the split injection technique (90:1 split ratio) decreased the apparent adsorption of nitroglycerin onto the injector. Lower split ratios were necessary to increase assay sensitivity but the coefficient of variation was greater than 20%. Finally, injector precision and assay sensitivity were evaluated using the on-column injection technique. Use of this technique allowed nitroglycerin to be applied directly onto the capillary column, thus avoiding glass and metal surfaces which may adsorb and/or degrade nitroglycerin.

Adsorption and degradation of nitroglycerin may occur not only on surfaces of conventional injectors but also on active surfaces present in the ECD. An advantage of using narrow-bore fused silica capillary columns is that the column may be inserted entirely into the ECD. Optimization of the position of the outlet from the column within the ECD is an important determinant of assay sensitivity. The ECD

contains highly active surfaces which may bind or degrade labile drugs such as nitroglycerin. Therefore, the column must be positioned to both minimize nitroglycerin loss and maximize detector sensitivity.

Figure IV-1 shows that detector sensitivity sharply increases as the column is inserted further into the ECD and peaks at 13.1 cm, i.e., 13.1 cm as measured from the ECD detector insert to the end of the column. Further insertion of the column into the detector results in a sharp decrease in sensitivity. Note that if the column alignment varies by as little as 1 - 2 mm, 50% of the maximum sensitivity was lost.

The position of the column within the ECD may not be as critical in the chromatography of other compounds. For example, lindane and aldrin are easily detected even when the column outlet is placed only 0.5 cm inside the detector insert.

b. EXTRACTION PRECISION AND RECOVERY

Initial assay development utilized an extraction procedure in which 1.0 ml plasma was extracted once with 10 ml pentane. Extraction efficiencies for nitroglycerin and dinitrotoluene (1.0 ng /ml each) were 75 ± 7% and 93 ± 2%, respectively. When biological samples (plasma) were each extracted twice, assay precision, as measured by the decrease in coefficient of variation, was increased by two

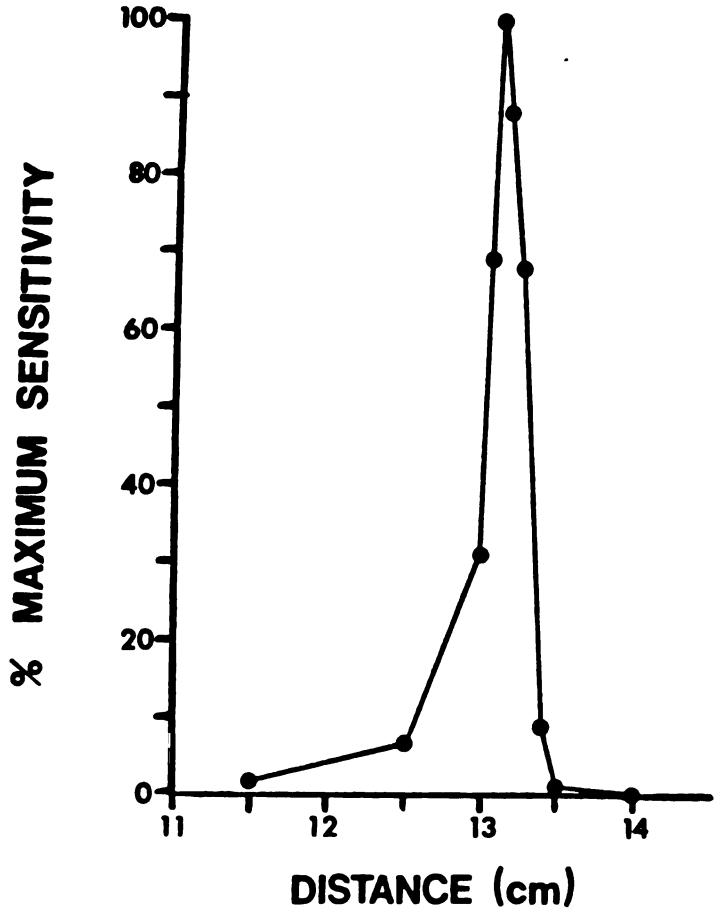


FIG. IV-1: Effect of capillary column alignment inside the GC detector (i.e., distance column is inserted through the detector insert) on nitroglycerin sensitivity.

to three-fold. Coefficients of variation based on six replicates decreased from 13.7% to 6.1%, 15.9% to 2.3% and 9.5% to 2.9% at plasma nitroglycerin concentrations of 50, 100, and 1000 pg/ml, respectively, when single and double extractions were compared.

c. NITROGLYCERIN CHROMATOGRAPHY AND ASSAY SPECIFICITY

Chromatograms of blank and nitroglycerin fortified plasma are shown in Fig. IV-2. Figure IV-2A depicts a chromatogram for a blank plasma extract. The nitroglycerin and dinitrotoluene retention times are indicated by arrows. The small peak, indicated by arrow "a", has the same retention time as nitroglycerin. This peak (which has a signal to noise ratio of three at 250pg/ml) is due to a contaminant present in the heptane and was eliminated by purifying the heptane with activated charcoal. Figure IV-2B shows a representative chromatogram of a plasma sample fortified with 100 pg/ml nitroglycerin and 1 ng/ml dinitrotoluene. The retention times for nitroglycerin and dinitrotoluene were 8.6 and 11.4 min, respectively.

Both nitroglycerin and dinitrotoluene were resolved from nitroglycerin metabolites. The retention times for the metabolites are shown in Table IV-1. Because the capillary GC assay is to be used to measure nitroglycerin plasma concentrations in patient populations, several other drugs

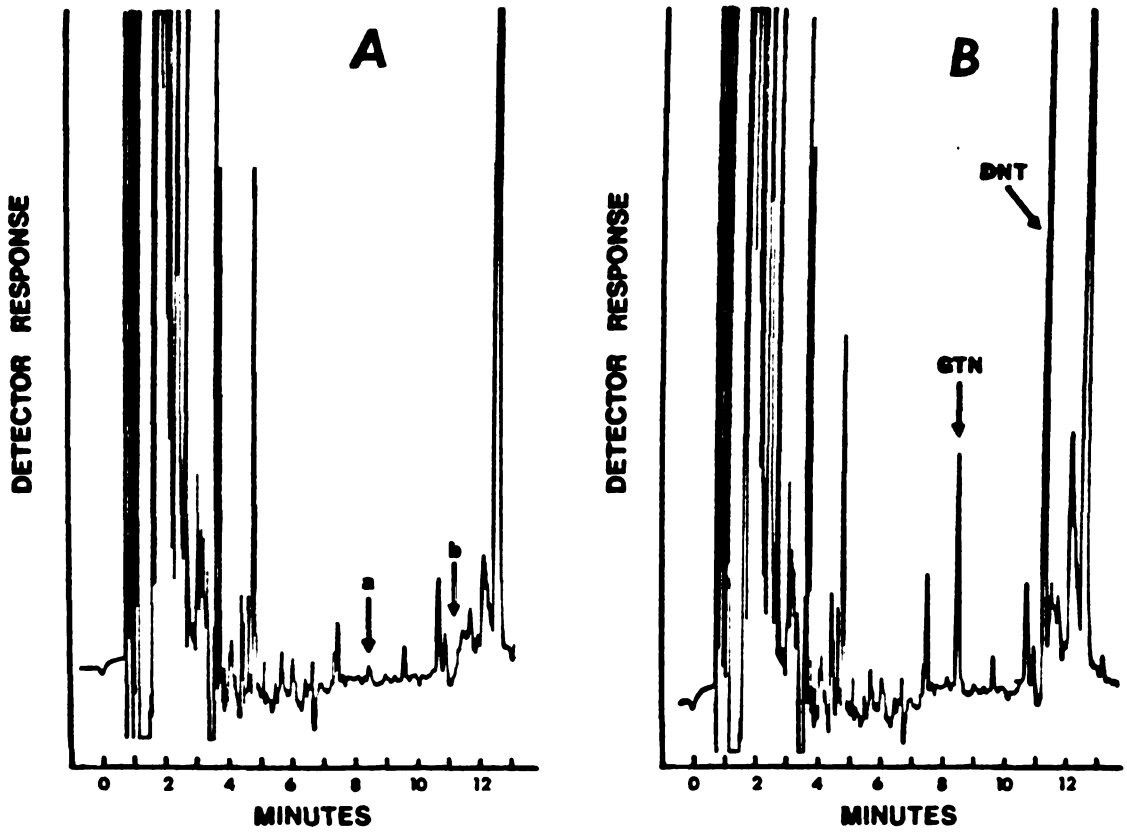


Fig. IV-2: Chromatograms of blank and nitroglycerin spiked plasma extracts. A: Blank plasma extract; nitroglycerin and 2,6-dinitrotoluene retention times are indicated by arrows a and b, respectively. B: Plasma extract at spiked concentrations of 100 pg/ml nitroglycerin and 1 ng/ml 2,6-dinitrotoluene.

TABLE IV-1: Retention times of nitroglycerin, nitroglycerin metabolites and several other commonly prescribed drugs.

Compound	GC Retention Time (min)
Nitroglycerin	8.6
1,3-glyceryldinitrate	7.5
1,2-glyceryldinitrate	7.6
1-glycerylmononitrate	4.5
2-glycerylmononitrate	4.7
2,6-dinitrotoluene	11.4
Isosorbide Dinitrate	14.5
Isosorbide-2-mononitrate	9.8
Isosorbide-5-mononitate	12.2
Acetaminophen	ND[a]
Acetylsalicylate	11.9
Caffeine	ND
Chloral Hydrate	1.0
Furosemide	ND
Hydrochlorothiazide	ND
Metolazone	ND
Procaineamide	ND
N-Acetylprocaineamide	ND
Spironolactone	ND
Triamterene	ND

[a] Not Detectable

were tested for assay interference. Table IV-1 lists the retention times, when detected, of several drugs (and metabolites) which may be prescribed to patients in a hospital cardiac care unit. None of these drugs, nor the metabolites tested, interfere with the nitroglycerin capillary GC assay. Also note that nitroglycerin may be assayed in the presence of isosorbide dinitrate, which is often concurrently administered to patients. No interference is noted for this compound and its two major metabolites, isosorbide-5-mononitrate and isosorbide-2-mononitrate.

d. ASSAY PRECISION, ACCURACY AND LINEARITY

The precision and accuracy of the nitroglycerin assay procedure was assessed by analysis of six replicate plasma samples to which known amounts of nitroglycerin were added. Within-day assay precision and accuracy are summarized in Table IV-2. Coefficients of variation over the concentration range, 25 to 10,000 pg/ml, were less than 10%. The relatively high coefficient of variation at the 10 ng/ml concentration may be due in part to the additional steps involved in sample analysis at higher concentrations, i.e., extracting 100 ul plasma diluted with 900 ul blank plasma. Accuracy for all concentrations, except 25 pg/ml, was within 5% of the actual nitroglycerin concentrations. Between-day assay precision was evaluated over two months (40 standard

TABLE IV-2: Within-day nitroglycerin assay precision and accuracy.

Actual Nitroglycerin Concentrations (pg/ml)	Calculated Nitroglycerin Concentrations (pg/ml)		
	Mean ± SD[a]	CV (%)	Error (%)
25	21.3 ± 1.6	7.6%	-16%
50	49 ± 3	6.1%	-2%
100	104 ± 2	2.3%	+4%
300	288 ± 6	2.1%	-4%
1000	1015 ± 39	3.8%	+2%
10000[b]	9890 ± 670	6.8%	-1%

[a] n = 6

[b] diluted 1 : 10 (v : v) before extraction

curves) . That is, each day for 40 days, nitroglycerin was added to blank plasma samples to yield final concentrations of 0.1 and 1.0 ng/ml nitroglycerin. These samples served as calibration controls until drug stability could be established. Coefficients of variation were 7 and 12% at 1.0 and 0.1 ng/ml, respectively. Accuracy (mean concentrations) were within the percent error noted for these two concentrations in Table IV-2.

Nitroglycerin standard curves were not linear over the entire range of 0 to 3000 pg/ml. At very low concentrations (less than 200 to 300 pg/ml) the standard curve slope was generally 50% greater than that of the high concentration range. Figure IV-3A shows a representative standard curve. The change in slope is not due to extraction efficiencies since the slope change was also present when known concentrations of drug in heptane were injected. The "break point" of the standard curve was consistent and did not change during any given day. Even when six different standard curves were averaged together (Fig. IV-3B), the break point at 200 pg/ml was still present. Therefore, this break point is not due to assay variability.

The break point may vary between different columns, instruments or make-up gas flow rates. The standard curve in Figure IV-3A was analyzed on a different instrument from those averaged in Fig. IV-3B. The break-point in Fig. IV-3A occurred at 200 pg/ml. In each if the six individual curves and the mean curve in Fig. IV-3B, the break point occurred

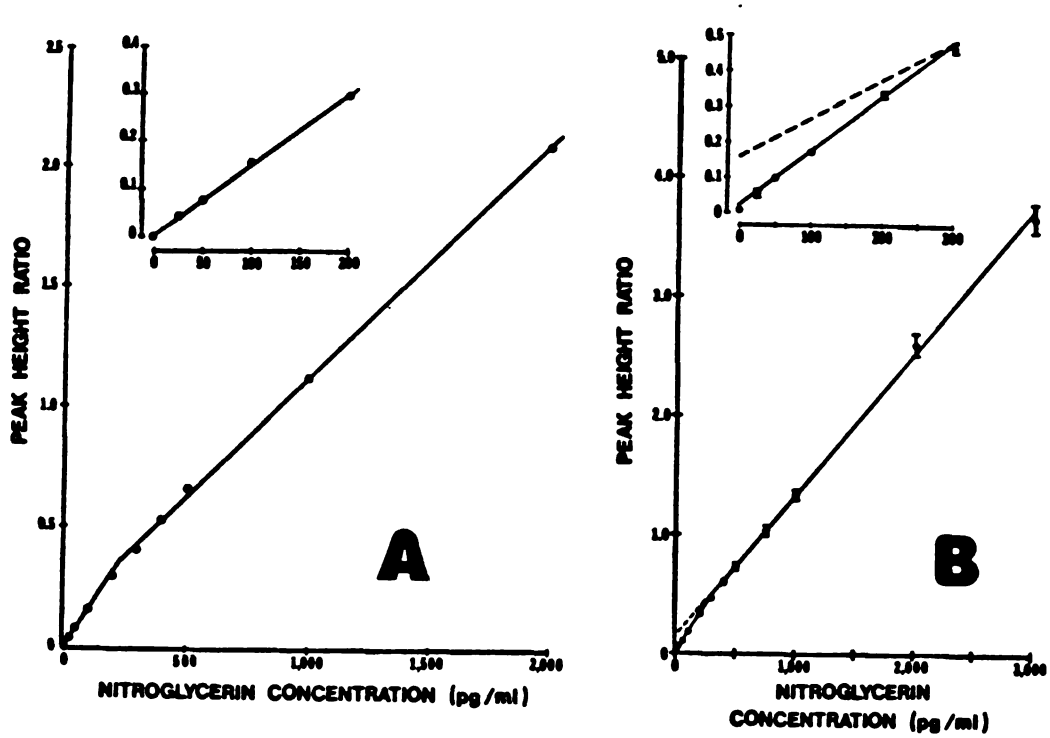


FIG. IV-3: Nitroglycerin standard curves. A: A single representative nitroglycerin standard curve. Curve is linear between 0 - 200 and 200 - 2000 pg/ml. Inset shows the low calibration standard range. B: Mean of six individual nitroglycerin standard curves. Standard error bars are shown except when smaller than the data symbol. Inset shows the low calibration standard range and the extrapolated line (broken line) from the high concentration range.

at 300 pg/ml. The position of the break-point may vary between instruments, but is constant within any one instrument. Although the occurrence of the break-point is quite consistent, the cause has not yet been identified.

e. NITROGLYCERIN STABILITY IN FROZEN PLASMA

Nitroglycerin stablility in frozen plasma has been determined in some detail by Maier et al. (1979). Because these investigators determined nitroglycerin concentrations using a GC assay employing packed columns, it was necessary to validate the plasma storage conditions using the capillary GC assay reported here. The nitroglycerin stability data are shown in Table IV-3. Linear regression of these data yielded half lives of 536 and 546 days for 100 and 1000 pg/ml GTN, respectively. These calculations indicate that 10% of the plasma GTN was degraded in 82 days when stored at -20 ^{o}C.

f. PRELIMINARY CLINICAL STUDIES

As an illustration of the applicability of this assay procedure, nitroglycerin was administered to two healthy volunteers. Subject 1 received a 0.4 mg sublingual dose on two separate occasions (one week apart). The plasma concentrations are shown in Fig. IV-4A. Nitroglycerin peak times were 5 min and terminal half-lives were 2.0 and 3.0

TABLE IV-3: Stability of nitroglycerin in pooled human plasma at 100 and 1000 pg/ml concentrations when stored at -20 °C.

Time (days)	Calculated Concentration (pg/ml)	
	Mean ± SD	Mean ± SD
0	112 ± 7	1015 ± 39
7	102 ± 5	1034 ± 31
14	109 ± 7	987 ± 31
24	102 ± 4	933 ± 33
40	113 ± 6	952 ± 31
60	106 ± 6	955 ± 41
108	97 ± 7	849 ± 28
200	86 ± 12	794 ± 22

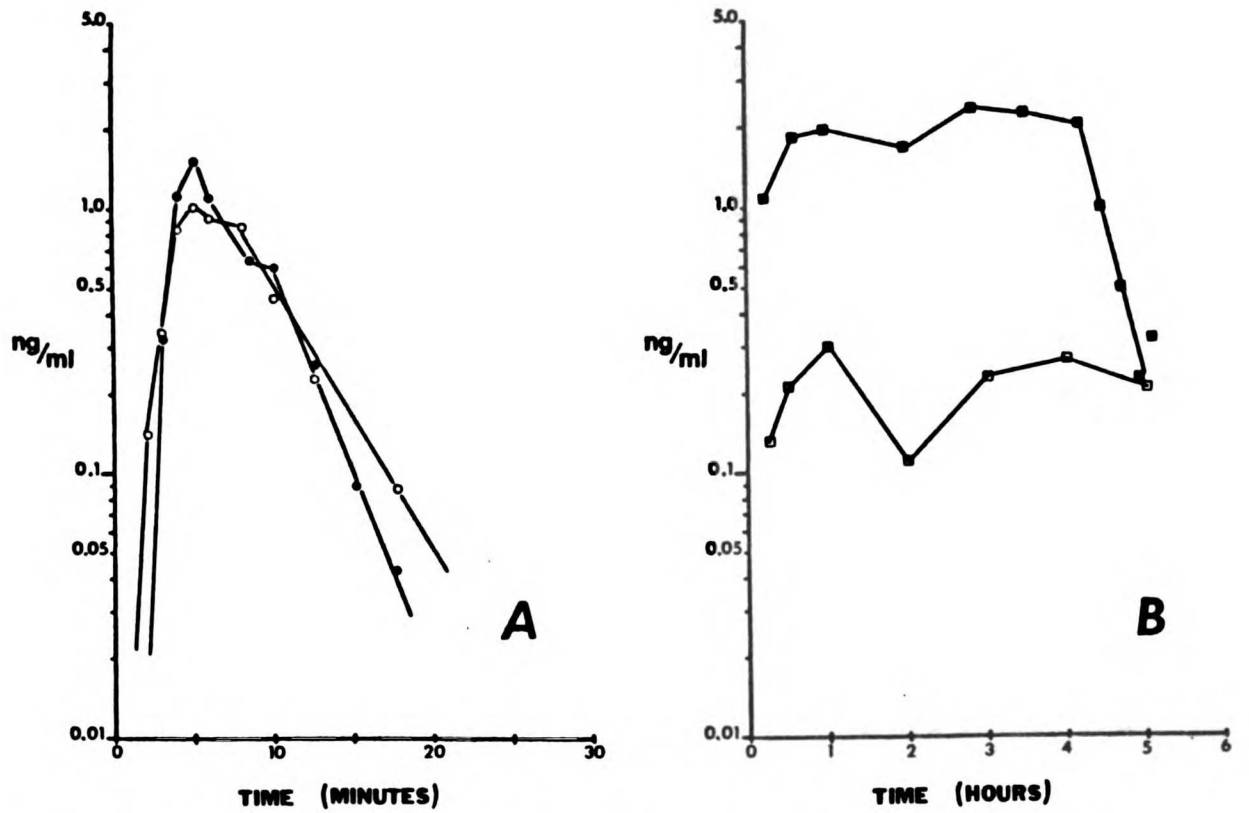


FIG. IV-4: Plasma nitroglycerin concentration vs. time profiles for two subjects. A: Plasma nitroglycerin concentrations after 0.4 mg sublingual doses. B: Plasma concentrations after topical doses of nitroglycerin ointment (■) and a controlled release patch (□).

min. Subject 2 received two topical doses of nitroglycerin on separate occasions. The subject received a one gram dose of 2% nitroglycerin ointment (equivalent to 20 mg nitroglycerin) which was spread evenly over a 200 cm^2 area of the chest and occluded with aluminum foil. The second dose was a sustained- release nitroglycerin patch. Plasma concentrations are shown in Fig. IV-4B. The half-life (15 min) after removing the ointment was longer than after sublingual dosing. The longer half-life is most likely due to continued absorption of nitroglycerin.

4. SUMMARY

In summary, a specific, sensitive and precise capillary GC assay capable of analyzing picogram cconcentrations of nitroglycerin in human plasma is described. The assay described is capable of reproducibly detecting 25 pg/ml nitroglycerin and there is no interference from nitroglycerin metabolites.

B. DETERMINATION OF 1,2- AND 1,3-GLYCERYLDINITRATE IN HUMAN PLASMA

1. INTRODUCTION

Little work has been carried out to characterize the plasma concentration-time profiles for the dinitrate metabolites of nitroglycerin (i.e., 1,2-glyceryldinitrate [1,2-GDN] and 1,3-glyceryldinitrate [1,3-GDN]). This has been primarily due to the lack of specific and sensitive analytical procedures. Neurath and Dunger (1977) and Armstrong et al. (1980d) measured the dinitrate metabolites but were unable to separate the two isomers.

Recently, two methods have been reported in the literature which separate the GDN metabolites and allowed the quantitation in plasma. Wu et al. (1982) reported a packed column GC assay with a limit of quantitation of 7.5 ng/ml in plasma. However, these authors were unable to detect measurable levels of metabolites after in vitro incubation of nitroglycerin with resuspended red blood cells (Chapter III). Miyazaki et al. (1982) reported a detection limit of 1.0 ng/ml in dog plasma using a GC-MS assay.

In this research a capillary GC assay for 1,2-GDN and 1,3-GDN in plasma using on-column injection and electron capture detection was developed. The assay possesses the sensitivity required to analyze 100 pg/ml of the dinitrate metabolites of nitroglycerin in human plasma.

B. MATERIALS AND METHODS

a. CHEMICALS, REAGENTS AND INSTRUMENTS

Nitroglycerin metabolites, 1,2-GDN and 1,3-GDN, were obtained from Marion Laboratories (Kansas City, MO). The internal standard was 2,6-dinitrotoluene (K & K Laboratories, Hollywood, CA). All solvents (Pesticide quality, Glass-distilled, Burdick and Jackson, Muskegon, MI) were used as supplied, with the exception of n-butyl acetate and ether. The n-butyl acetate was further purified with activated charcoal. Ether (anhydrous) required redistillation over lithium aluminum hydride daily. Dimethyldichlorosilane was commercially obtained (Aldrich Chemical Co., Milwaukee, WI).

All glassware was silanized before use as described in Section A.2.b of this chapter. The gas chromatographic instrumentation was identical to that described in Section A.2.c of this chapter. Hydrogen was used as a carrier gas at a flow rate of 1.5 ml/min (inlet pressure = 30 psi). Nitrogen flow rates to the ECD and column make-up were 5 and 25 ml/min, respectively. Column position inside the ECD was optimized for maximum sensitivity. The oven temperature was programmed from 110 to 145°C at 3°C/min. A second temperature ramp was used to "burn-off" the residual extract. This was accomplished by programming the oven 50°C/min to 250°C and maintaining this final temperature for 10 min.

b. CLINICAL BLOOD SAMPLING

Blood samples were processed as previously described for the nitroglycerin assay (Section A.2.d of this chapter).

c. SAMPLE PREPARATION

Dinitroglycerin calibration standards were prepared as follows: Primary stock solutions (1 mg/ml each) of nitroglycerin metabolites and 2,6-dinitrotoluene were prepared in ethanol. Secondary stock solutions (10 mcg/ml) were prepared fresh monthly by diluting the primary standards in ethanol (1:100, v:v). Fresh aqueous solutions of dinitroglycerins (10 and 100 ng/ml) and dinitrotoluene (20 ng/ml) in distilled water were prepared daily from secondary stock solutions. Nitroglycerin metabolite calibration standards (0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0, 20.0 and 25.0 ng/ml) were prepared by adding the appropriate aliquot of the standard and 50 ul (1 ng) dinitrotoluene to 0.5 ml samples of blank human plasma in 16 x 150 mm silanized test tubes with teflon-lined screw caps. Clinical plasma samples (0.5 ml) are transferred to silanized test tubes and mixed with 50 ul dinitrotoluene.

Each calibration standard or clinical sample is extracted with 10 ml pentane:ether (7:3) by gently rotating for 15 min (Sepco Tube Rotator, Scientific Equipment Products, Baltimore MD). Each sample was then centrifuged

for 10 min at 2000 x g. The organic layers were transferred to clean test tubes. The residual plasma layers were extracted and centrifuged a second time, as described above.

The combined organic extracts were evaporated under a stream of nitrogen (at room temperature) to a final volume of approximately one ml. The extracts were then transferred to one ml reacti-vials (Pierce Chemical Co., Rockford, IL) and evaporated to dryness under a stream of nitrogen. Immediately after each sample went to dryness, 50 ul of n-butyl acetate was added to each vial and the vials vortexed (approx. 5 sec). A 0.2 to 1.0 ul aliquot of each extract is injected into the gas chromatograph. Sample application to the column was performed via the injector stopcock using a 10 ul gas-tight syringe which was modified to hold a 0.21 x 190 mm fused silica capillary "needle". All extracts were stored at -20^{o}C until chromatographed and on dry ice between GC injections.

d. NITROGLYCERIN METABOLITE STABILITY IN FROZEN PLASMA

Nitroglycerin metabolite stability in frozen human plasma was evaluated at -20 ^{o}C. Drug free plasma was obtained from healthy volunteers. Two different concentrations (high and low) of each metabolite were evaluated for stability. The high concentration stability standards contained 10.0 ng/ml 1,2-GDN and 5.0 ng/ml 1,3-

GDN. The low concentration stability standards contained 1.0 ng/ml 1,2-GDN and 0.5 ng/ml 1,3-GDN. Each plasma sample was subdivided into multiple aliquots, quick frozen in dry ice and stored at -20 ^{o}C. Six aliquots of each concentration were assayed (as described above) after 0, 1, 3, 4, and 14 weeks of storage.

3. RESULTS AND DISCUSSION

a. NITROGLYCERIN METABOLITE CHROMATOGRAPHY AND ANALYTICAL SPECIFICITY

Chromatograms of blank and dinitroglycerin fortified plasma are shown in Fig. IV-5. Figure IV-5A depicts a chromatogram for a blank plasma extract fortified with internal standard. The nitroglycerin, 1,3-GDN, 1,2-GDN and dinitrotoluene retention times are indicated by arrows. Figure IV-5B shows a representative chromatogram of a plasma extract fortified with 1.0 ng/ml 1,2-GDN, 0.5 ng/ml 1,3-GDN and 1.0 ng/ml dinitrotoluene. The retention times for 1,3-GDN, 1,2-GDN and dinitrotoluene are 6.22, 6.41 and 10.65 min, respectively. Figure IV-5C shows the chromatogram of a plasma sample obtained from a volunteer after a topical dose of Nitro-BID ointment. Both nitroglycerin and dinitrotoluene are resolved from both the 1,3-GDN and 1,2-GDN metabolites. The retention time for nitroglycerin is 7.46 min.

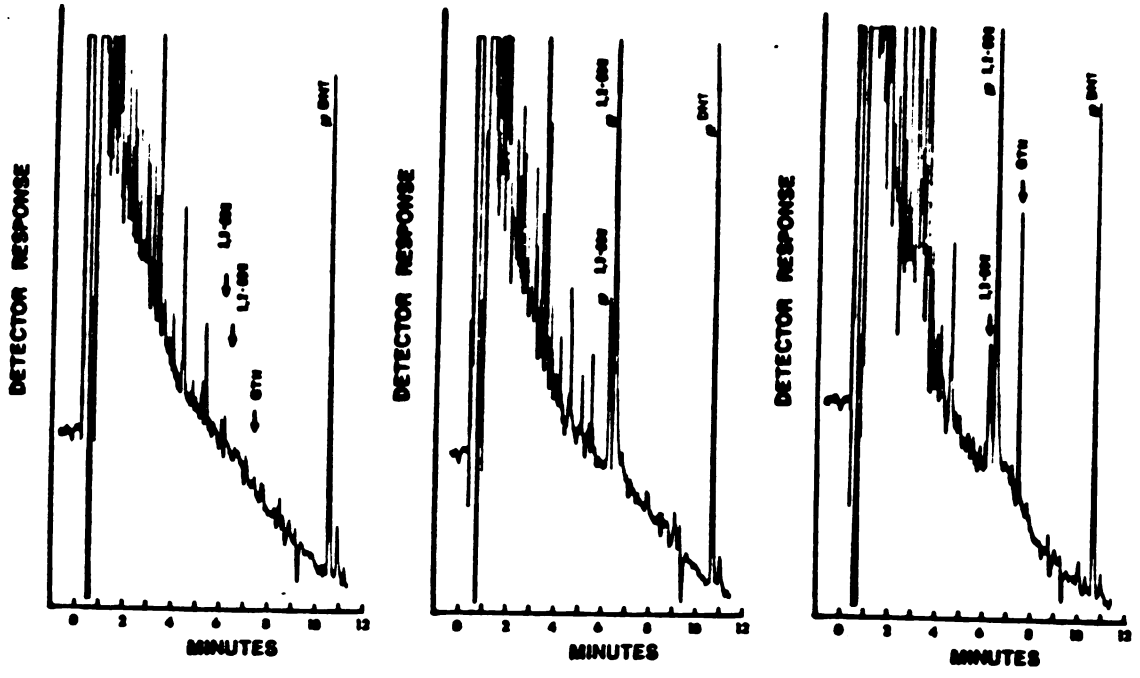


FIG. IV-5: Chromatograms of blank and 1,2-GDN and 1,3-GDN spiked plasma extracts. A: Blank plasma extract spiked with internal standard (DNT); 1,2-GDN, 1,3-GDN, GTN and DNT retention times are indicated by arrows. B: Blank plasma extract spiked with 1.0 ng/ml 1,2-GDN, 0.5 ng/ml 1,3-GDN and 1.0 ng/ml DNT. C: Plasma extract of a plasma sample obtained from a human volunteer who had received a transdermal dose of nitroglycerin oinment.

b. ASSAY PRECISION, ACCURACY AND LINEARITY

The precision and accuracy of the nitroglycerin metabolite assay procedure was assessed by analysis of six replicate plasma samples to which known amounts of 1,2-GDN and 1,3-GDN were added. Within-day assay precision, evaluated at 1.00 ng/ml, was 3.7 and 4.0 percent for 1,2-GDN and 1,3-GDN, respectively. Between-day assay accuracy and precision was evaluated over a two month period and are shown in Table IV-4. Both the assay precision and the bias of the 1,2-GDN assay are within 10%. Assay precision of the 1,3-GDN is within 12% (coefficient of variation) and assay bias ranges between 8.6 to 12%.

Nitroglycerin metabolite standard curves behave similarly to those of the parent drug. Nitroglycerin, 1,2-GDN and 1,3-GDN standard curves are not linear over the entire analytical range. As noted previously (Section A.3.d), at very low nitroglycerin concentrations (less than 200 to 300 pg/ml) the standard curve slope was generally 50% greater than that of the high concentration range. The change in slope was not due to extraction efficiencies. The "break point" of the standard curve was consistent and did not change during any given day. The break point may vary between different columns, instruments or make-up gas flow rates but is not due to assay variability.

The standard curves for 1,2-GDN and 1,3-GDN behaved similarly. The break-points for 1,2-GDN and 1,3-GDN both

TABLE IV-4: Between-day assay precision and accuracy for 1,2-GDN and 1,3-GDN.

Metabolite	Actual Conc. (ng/ml)	Experimental Conc. (ng/ml ± SD)[a]	% Error
1,2-GDN	1.0	0.91 ± 0.07	-9.0
1,2-GDN	10.0	9.04 ± 0.75	-9.6
1,3-GDN	0.5	0.44 ± 0.05	-12.0
1,3-GDN	5.0	4.47 ± 0.49	-8.6

[a] $n = 24$

occurred at 1.0 ng/ml. Representative figures showing GDN peak height ratio as a function of concentration are presented in FIG. IV-6. Because of apparent nonlinearity, individual standard curves were constructed to cover the two different calibration ranges (0 to 1.0 ng/ml and 1.0 to 10.0 ng/ml).

c. STABILITY OF 1,2- AND 1,3-GLYCERYLDINITRATES IN FROZEN PLASMA

Although nitroglycerin stability in frozen plasma has been determined in some detail (see Section A.3.e of this chapter), the stability of the dinitrate metabolites was unknown. The dinitrate metabolites are more stable in plasma than the parent drug at physiological temperatures (Sokoloski et al., 1983). Therefore, one may anticipate that these same metabolites should be even more stable than the parent drug in frozen plasma. The 1,2-GDN and 1,3-GDN stability data are shown in Table IV-5. These metabolites were stable in plasma for a minimum of fourteen weeks when stored at -20 ^{o}C.

d. PRELIMINARY CLINICAL STUDY

As an illustration of the applicability of this assay procedure, nitroglycerin was administered to a healthy volunteer. The subject received a 6.5 mg dose of nitroglycerin dissolved in

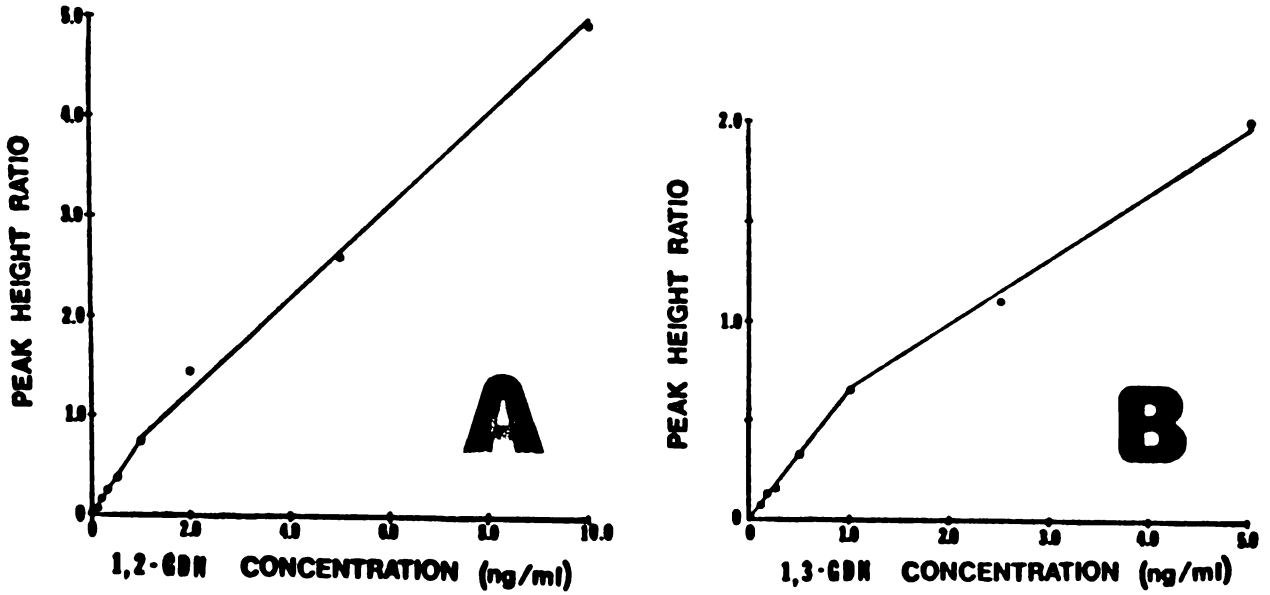


FIG. IV-6: Representative curves showing the relationship of peak height ratio to GDN concentration for 1,2-GDN (A) and 1,3-GDN (B) in human plasma.

Table IV-5: Stability of 1,2-GDN and 1,3-GDN (each over a ten-fold concentration range) in human plasma when stored at -20 °C.

Time (weeks)	Measured Concentration (ng/ml)			
	1,2-GDN		1,3-GDN	
0	9.12 ± 0.53	0.90 ± 0.05	4.42 ± 0.48	0.41 ± 0.07
1	9.68 ± 0.47	0.97 ± 0.08	4.65 ± 0.30	0.46 ± 0.04
3	8.02 ± 0.24	0.89 ± 0.07	3.91 ± 0.31	0.46 ± 0.03
4	9.33 ± 0.43	0.89 ± 0.07	4.88 ± 0.26	0.43 ± 0.04
14	9.32 ± 0.34	0.83 ± 0.03	4.90 ± 0.20	0.41 ± 0.01

50 ml of distilled water. Plasma concentrations of 1,2- and 1,3-GDN are shown in Figure IV-7. The half-lives of 1,2-GDN and 1,3-GDN were 26.9 and 37.5 min, respectively.

4. SUMMARY

In summary, a specific, sensitive and precise capillary GC assay capable of analyzing picogram concentrations of 1,2- and 1,3-dinitroglycerin in human plasma is described. The assay described is capable of detecting 0.1 ng/ml of 1,2-GDN and 1,3-GDN.

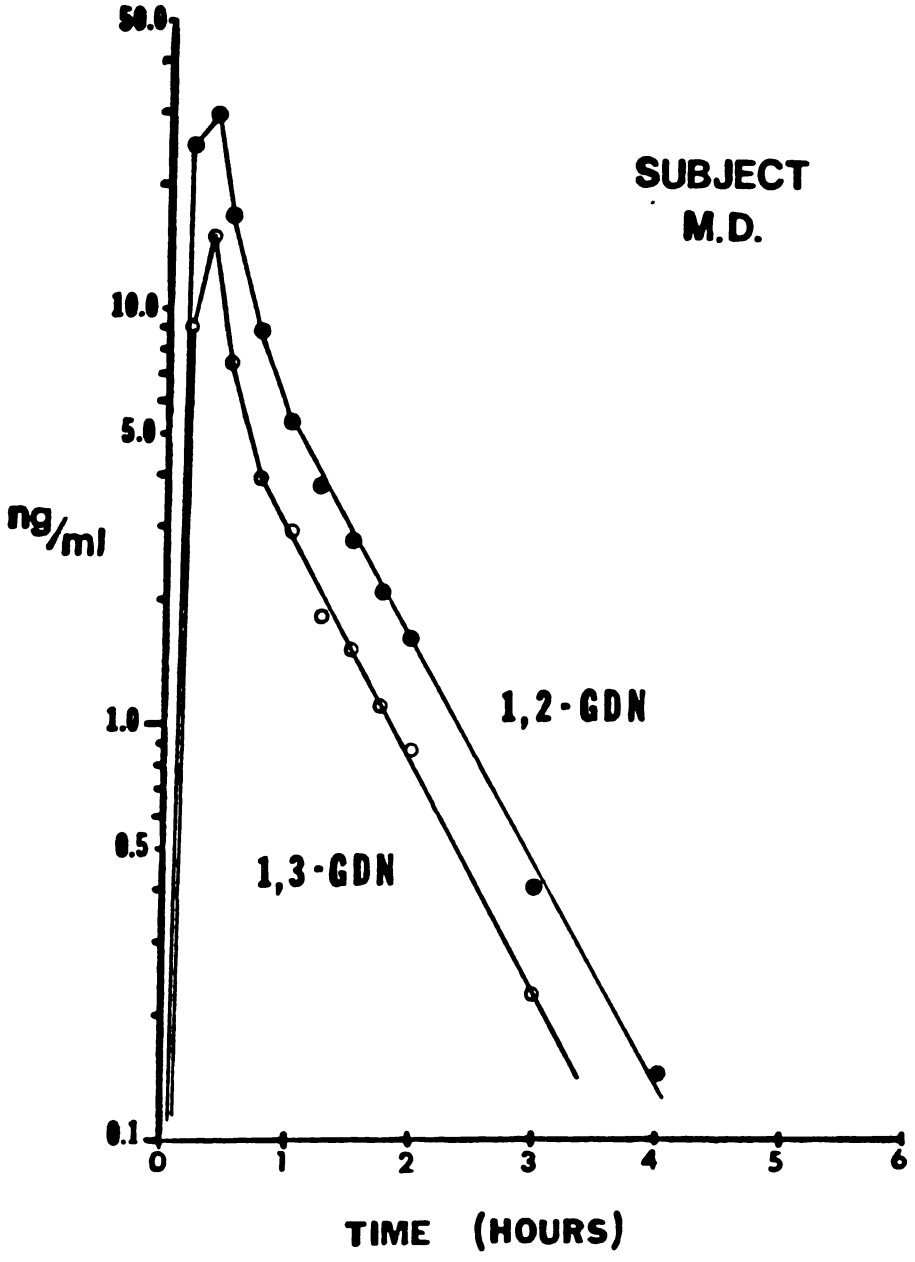


FIG. IV-7: Plasma concentration vs. time profiles for 1,2-GDN and 1,3-GDN after an oral dose of 6.5 mg GTN (in water solution) to a healthy volunteer.

CHAPTER V.

RELATIVE BIOAVAILABILITY OF NITROGLYCERIN FROM TRANSDERMAL AND SUBLINGUAL DOSAGE FORMS

A. INTRODUCTION

Nitroglycerin formulations are used frequently for the treatment of angina pectoris. Although the most common formulation is the sublingual tablet, other formulations are becoming popular. These include sublingual sprays and chewable tablets, oral sustained-release preparations and topical ointments, gels and solid-state devices. The purpose of this study was to evaluate the relative bioavailability of three different nitroglycerin formulations: 1) nitroglycerin spray; 2) a chewable nitroglycerin capsule; and 3) a topical nitroglycerin gel. The products tested are manufactured by Pohl-Boskamp, a West German Pharmaceutical Company. The bioavailabilities of these products were evaluated relative to a sublingual tablet and a topical ointment.

B. METHODS

1. EXPERIMENTAL PROTOCOL

This study was conducted as a randomized crossover study in 12 healthy male volunteers. The 12 subjects were divided into three groups of four volunteers each. Each group received one of the above products on a single occasion and on a second occasion received the reference product. The sublingual reference product was a standard sublingual nitroglycerin product: Nitrostat^{R} (Parke-Davis). The transdermal reference product was Nitro-BID (Marion), a 2% nitroglycerin ointment. Groups one and two received the spray and chewable capsule, respectively, in crossover fashion with Nitrostat^{R} tablets. The third group received the topical gel in crossover fashion with Nitro-BID^{R} ointment. A one week washout period separated the administration of the two products.

2. BLOOD SAMPLING PROCEDURE

Serial blood samples were drawn directly into a chilled (in ice water) 7 ml vacutainer through an indwelling venous catheter (heparin lock) kept patent with heparin. The blood was immediately centrifuged at 12,800 x g for 20 sec. The plasma was then immediately frozen in a dry ice bath. The total amount of time required to withdraw the blood sample

and to centrifuge to obtain plasma prior to freezing was less than two min.

This procedure minimized the in vitro degradation of nitroglycerin by blood. When nitroglycerin was added to blood at 37°C, 100% recoveries of nitroglycerin were obtained using this quick centrifuging technique.

3. STUDY 1

In this study, four individuals received 0.8 mg nitroglycerin as either two 0.4 mg tablets or two actuations of the nitroglycerin spray (0.4 mg per actuation). Each subject was placed in a sitting position (with legs horizontal). At zero time, the dose was either sprayed or placed under the tongue. The subjects were instructed not to swallow for five minutes after drug administration. Blood samples were collected at 0, 0.5, 1, 2, 3, 4, 5, 10, 20, 30, 60, 120, 180 and 240 minutes after dosing. After five minutes, the subjects were allowed to swallow any remaining dose. Following the conclusion of this portion of the study, three subjects received an additional dose of the nitroglycerin spray formulation. Blood samples were drawn as before.

4. STUDY 2

This study was performed as in study 1. Each subject

received 0.8 mg nitroglycerin as a single chewable capsule and two tablets in crossover fashion. The subjects were instructed to bite open the capsule so that the contents could be emptied into the sublingual area. The nitroglycerin tablets were administered as above. After 5 min, the subjects were allowed to swallow any remaining dose. Blood samples were drawn at 0, 0.5, 1, 2, 3, 4, 5, 7.5, 10, 12.5, 15, 20, 25, 30, 60, 120, 180 and 240 minutes after dosing. One subject (#5) received an additional dose of the chewable capsule. This subject was instructed to move the the contents of the capsule around the inside of his mouth to increase the absorptive surface area.

5. STUDY 3

In this study, each subject received either the nitroglycerin gel or ointment which was spread evenly over a 200 cm^2 surface area of the upper inner arm. The dose of each product was 20 mg of nitroglycerin, as two containers of the gel, and a weighed amount (1.00 g) of 2% nitroglycerin ointment. After application of the ointment or gel, the area was occluded with aluminum foil which was taped and secured with gauze strips. Blood samples were collected at 0, 5, 10, 30 and 60 min and at 2, 3, 4 and 8 hours. At the end of the 8-hour period, the dose was removed and the skin was washed with soap and water.

C. RESULTS AND DISCUSSION

1. STUDY 1: NITROGLYCERIN SPRAY VERSUS TABLETS

Volunteers #4, 6, 11 and 12 received nitroglycerin spray and tablets on separate occasions. Plasma concentrations of nitroglycerin as a function of time are shown in Figure V-1. The pharmacokinetic data from these doses are summarized in Table V-1. Note that the area under the plasma concentration-time curve (AUC) of each of the three individuals who received repeated doses of spray, increased by a factor of two to ten. It is important that the spray dosage form of nitroglycerin be administered carefully. The spray canister must not be shaken and care must be taken to direct the dose precisely to the sublingual area. The AUC of the spray dosage form was significantly lower than that of the tablet ($p < 0.05$). The average relative bioavailability of the sublingual spray was 71.1 $\pm$ 100.3 percent. When the abnormally high value for the repeat dose to volunteer #4 was excluded, the average relative bioavailability was reduced to 33.5 $\pm$ 12.9 percent. During the process of actually dosing the volunteers, it was observed that a portion of the "nitroglycerin puff" was blown out of the mouth. The low relative bioavailability for this dosage form may be due to loss of a portion of the dose to the atmosphere during application of the dose.

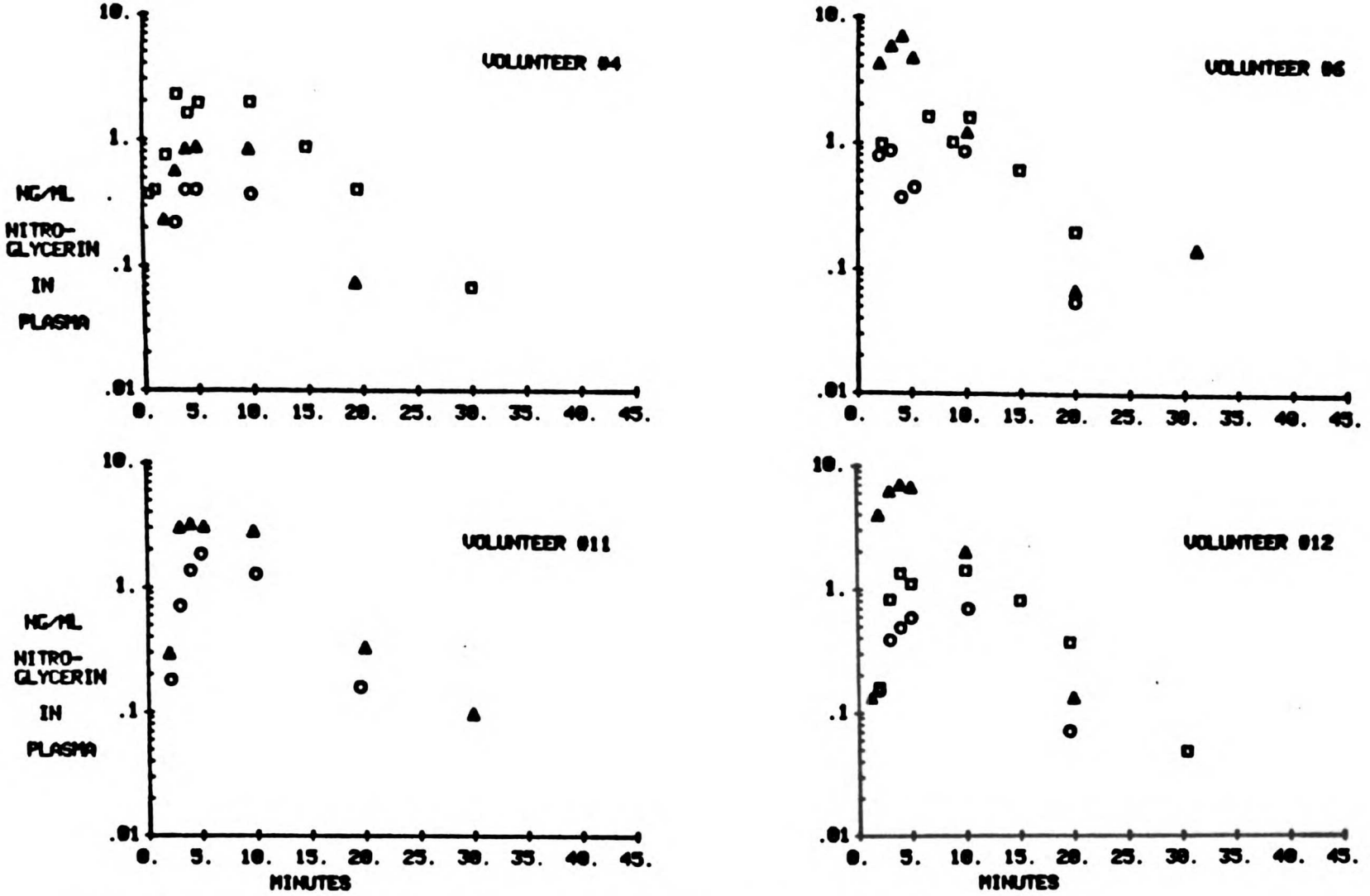


FIG. V-1: Nitroglycerin plasma concentration-time profiles following administration of nitroglycerin spray and tablets to four healthy volunteers. Key: tablet = triangles, circles = spray, squares = repeat spray.

TABLE V-1: Areas under the plasma concentration-time curves and relative bioavailabilities of nitroglycerin in healthy volunteers after sublingual tablet and spray administration.

Dosage Form	Volunteer 4	6	11	12	Mean ± SD	Significance
Areas Under Curves						
Tablet	9.15	37.15	35.82	46.06	**32.04 ± 15.93**	
Spray	2.74	8.24	16.02	7.24	14.00 ± 8.38 (11.80 ± 6.61)[d]	p=0.0470[b]
Spray (R)[a]	27.18	17.57	---	18.98		
Relative Bioavailability[c]						
Spray	29.9	22.2	44.7	15.7	71.1 ± 100.3 (33.5 ± 12.9)[d]	
Spray (R)[a]	297.0	47.3	---	41.2		

[a] Repeat dose

[b] Paired T-Test

[c] Areas under curves, (Spray/Tablet) x 100

[d] Mean ± SD, excluding the repeat dose in volunteer 4.

2. STUDY 2 : NITROGLYCERIN CHEWABLE CAPSULES VERSUS TABLETS.

The second phase of this study tested the bioavailability of the chewable "bite-capsule." The plasma nitroglycerin concentrations for both of these formulations are shown in Figure V-2. Values of the pharmacokinetic parameter (AUC) used to estimate the relative bioavailability of this formulation are shown in Table V-2. The bite-capsule AUC values were lower (although, not significantly) than that of the tablet for each of the four volunteers, including the repeat dose in volunteer #5. Note that the AUC for this repeat dose increased by more than five-fold over the initial dose. The volunteer was instructed to spread this repeat dose over the entire buccal surface of his mouth. This maneuver increased the surface area available for the dose to be absorbed. Figure V-2 (volunteer #5) shows that the plasma concentrations of nitroglycerin in this repeat dose are measureable over a much longer period of time (30 min). These data indicate that drug diffusion from the vehicle used in the bite-capsule may be the rate-limiting step for absorption of nitroglycerin from this capsule.

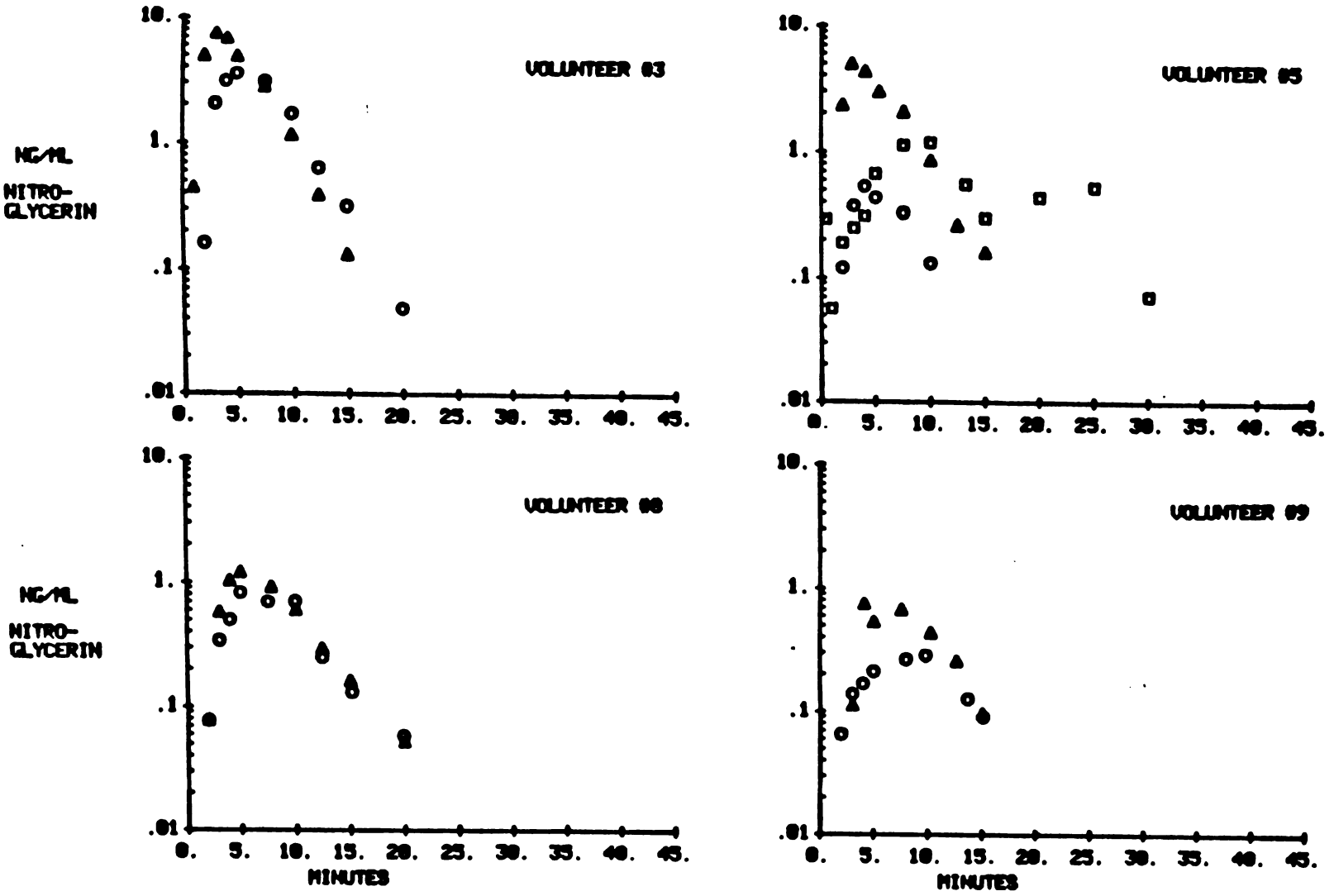


FIG. V-2: Nitroglycerin plasma concentration-time profiles following administration of nitroglycerin capsules and tablets to four healthy volunteers. Key: tablet = triangles, circles = capsule, squares = repeat capsule.

TABLE V-2: Areas under the plasma concentration-time curves and relative bioavailabilities of nitroglycerin in healthy volunteers after sublingual tablet and capsule administration.

Dosage Form	Volunteer 3	5	8	9	Mean ± SD	Significance
Areas Under Curves						
Tablet	37.81	24.56	8.92	5.26	19.14 ± 15.00	p=0.0573[b]
Capsule	25.60	2.72	6.98	2.60	10.61 ± 9.81	
Capsule (R)[a]	---	15.18	---	---		
Relative Bioavailability[c]						
Capsule	67.7	11.1	78.3	49.4	53.7 ± 26.0	---
Capsule (R)[a]	---	61.8	---	---		

[a] Repeat dose
[b] Paired T-Test
[c] Areas under curves, (Capsule/Tablet) x 100

3. STUDY 3 : RELATIVE BIOAVAILABILITY OF NITROGLYCERIN GEL VERSUS OINTMENT.

The final phase of this study involves the comparison of the nitroglycerin gel formulation with the ointment. Figure V-3 depicts the nitroglycerin plasma concentrations after topical doses of the ointment and gel. Nitroglycerin concentrations as high as 3.4 ng/ml were detected after ointment doses. The highest nitroglycerin concentration attained after doses of the gel formulation was only 0.46 ng/ml. Nitroglycerin concentrations in volunteer #1 were all less than 0.05 ng/ml over the entire 8-hr period. This was also true for volunteer #2, with the exception of the 10-min sample (0.088 ng/ml). The AUC values and the relative bioavailabilities for all doses in each volunteer are shown in Table V-3. The relative bioavailabilities for the nitroglycerin gel formulation ranged from 0.00 to 11.2 percent. The AUCs, and therefore the relative bioavailabilities (assuming a constant clearance), for the two topical formulations were significantly different ($p = 0.01$).

D. SUMMARY

The relative bioavailabilities of drug from two sublingual and one topical nitroglycerin formulation were evaluated. The bioavailabilities of drug from sublingual

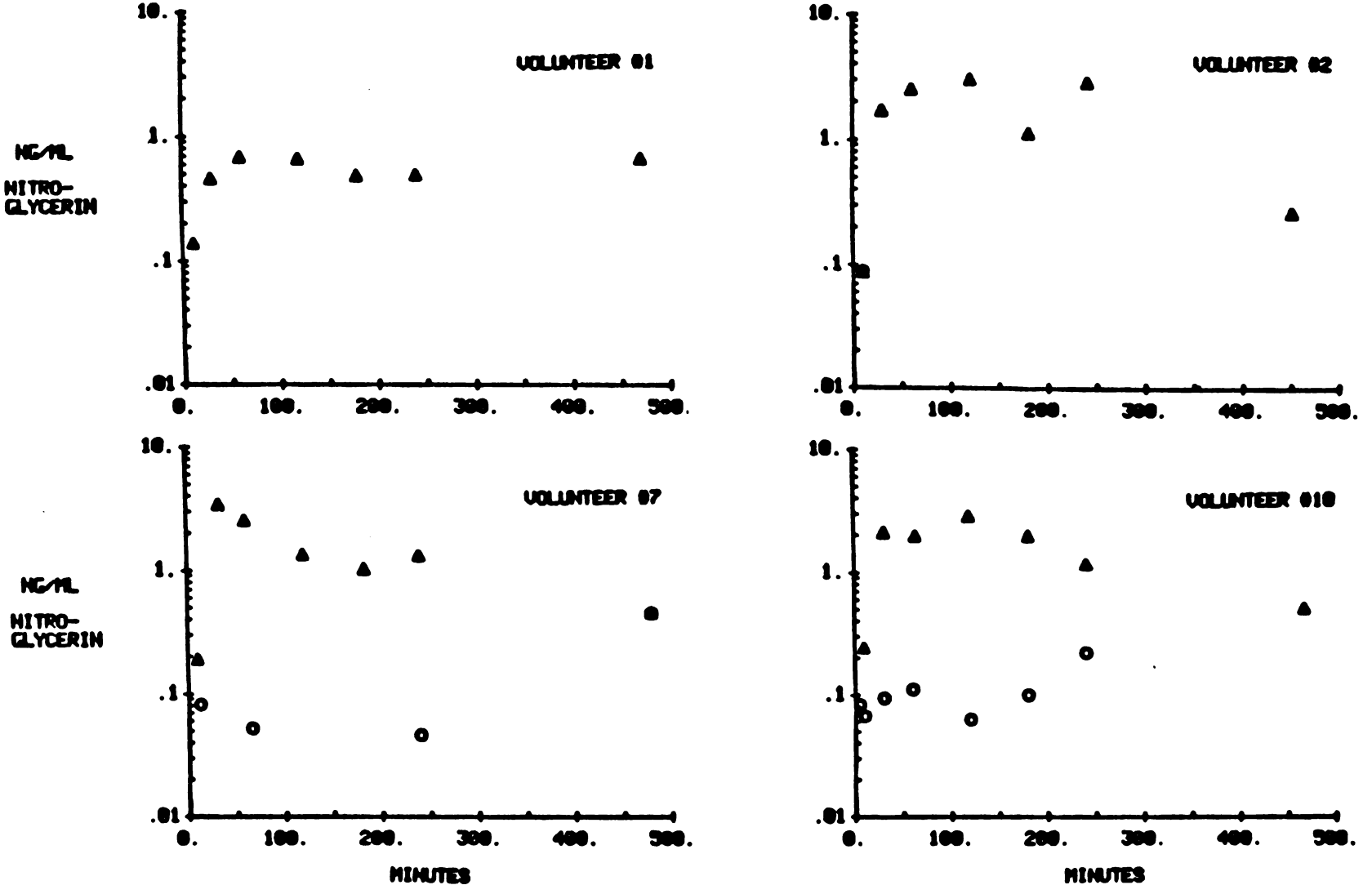


FIG. V-3: Nitroglycerin plasma concentration-time profiles following topical administration of nitroglycerin gel and ointment to four healthy volunteers. Key: ointment = triangles, circles = gel.

TABLE V-3: Areas under the plasma concentration-time curves and relative bioavailabilities of nitroglycerin in healthy volunteers after transdermal administration of nitroglycerin ointment and gel formulations.

Dosage Form	Volunteer 1	2	7	10	Mean ± SD	Significance
Areas Under Curve						
Ointment	260.40	703.06	568.74	644.71	544.23 ± 197.05	p=0.0123[a]
Gel	0.00	0.22	63.96	24.77	22.24 ± 30.15	
Relative Bioavailability[b]						
Gel/Ointment	0.0	0.0	11.2	3.8	3.8 ± 5.3	

[a]Paired T-Test

[b]Areas under curves, (Gel/Ointment) x 100

capsules and spray were determined relative to Nitrostat^{R} sublingual tablets. Bioavailability of drug from topical gel was evaluated relative to Nitro-BID^{R} ointment. The relative bioavailabilities of the nitroglycerin spray formulation ranged from 22.2% to 297%, and was dependent on the dosing technique. The nitroglycerin "bite-capsule" bioavailabilities ranged from 11.1% to 78.3%. As was found in the study with the spray formulation, the bioavailability was dependent on the dosing technique. Although the plasma AUC values of the bite-capsule were not statistically different from those of the tablet formulation, the bite-capsule AUC values were consistently lower than those of the tablet. Finally, the relative bioavailability of the nitroglycerin gel formulation was found to be significantly lower than that for the Nitro-BID^{R} ointment. The relative availabilities ranged from 0.0% in two individuals to 11.2% at the highest.

CHAPTER VI

RELATIVE BIOAVAILABILITY OF ORAL NITROGLYCERIN

A. INTRODUCTION

Based on animal studies and the requirement that patients need a high oral dose of nitroglycerin for antianginal efficacy, it has been assumed that nitroglycerin exhibits a very high first-pass hepatic metabolism. It was further assumed that the oral doses at least partially saturate the metabolic enzymes and that measurable plasma concentrations of unchanged nitroglycerin would be observed. This study was designed to test these assumptions by determining the bioavailability of three oral nitroglycerin formulations relative to a topical ointment formulation. The bioavailabilities were evaluated by measuring plasma nitroglycerin concentrations after oral and topical doses.

B. EXPERIMENTAL

Six healthy male volunteers were used in this study. The study was designed as an open-label, randomized, four-way crossover experiment with three different 6.5 mg nitroglycerin capsule formulations and the ointment administered as a single 20.0 mg topical dose. The three different capsule formulations (encoded A, B, and C) were

tested for potency, dissolution and content uniformity by Marion Laboratories prior to the study. A one-week washout period was allowed between phases.

Each nitroglycerin capsule was administered with eight ounces of room temperature water. The following procedures were performed on the subjects who received nitroglycerin ointment. A 100 cm^2 (10 cm x 10 cm) area was outlined on the surface of the chest of each volunteer (near the sternum) with a marking pen and template. The ointment was uniformly spread over the outlined surface area with the aid of a metal spatula. The area was then covered with a piece of aluminum foil (144 cm^2) and the edges were taped to prevent loss of the dose by evaporation or loss on the volunteers clothing. After 24 hours the remaining ointment was scraped off with a spatula and the area gently washed with soap and water.

Blood samples were collected at 0, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 hours after dosing. A 24-hour blood sample was also drawn from the volunteers receiving Nitro-Bid^R ointment. All plasma samples were assayed for nitroglycerin using the capillary GC assay described in Chapter IV. Note that when this study was performed, the capillary GC assay sensitivity was 50 pg/ml.

C. RESULTS AND DISCUSSION

The resulting nitroglycerin plasma concentrations after

transdermal administration of ointment are shown in Figure VI-1. Peak nitroglycerin plasma concentrations ranged from 400 to 4000 pg/ml and were measurable for at least an eight-hour time period.

After oral administration of formulations A and C, nitroglycerin plasma concentrations at all time points were less than 50 pg/ml. Plasma concentrations in three of the five subjects dosed with oral formulation B were all less than 50 pg/ml and, as such, could not be detected. Concentrations of nitroglycerin were detectable in the plasma of the remaining two subjects, but only at three time points in one subject and at two time points in the second subject. Table VI-1 lists only those nitroglycerin plasma concentrations which were detectable after oral dosing. Both of these subjects received the oral nitroglycerin dose on the same day.

It may be possible that these early time point plasma samples were contaminated during the blood collection process. Such contamination may have occurred when blood samples were processed, to obtain plasma, in the same physical area as the nitroglycerin ointment doses were prepared. This seems reasonable since all plasma concentrations from 13 other separate oral nitroglycerin doses were less than 50 pg/ml, the minimum level of assay sensitivity. Note that subject R.A.M. showed a very high concentration (2720 pg/ml) at 0.25 hr, less than 50 pg/ml at 0.5 hr (not shown in Table VI-1) and 660 pg/ml at 1.0 hr.

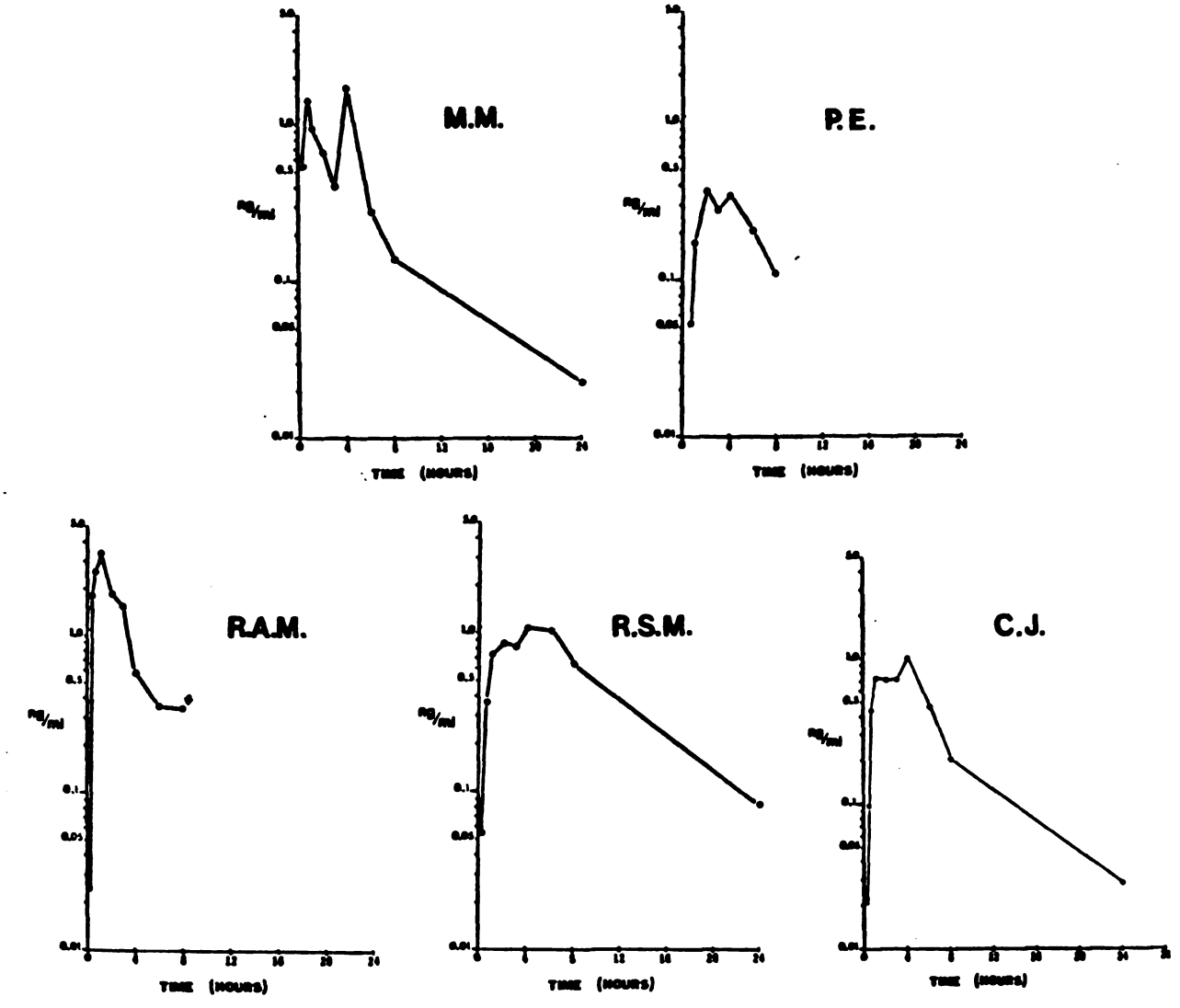


FIG. VI-1: Nitroglycerin plasma concentration-time profiles following topical administration of nitroglycerin ointment to five volunteers.

TABLE VI-1: Plasma samples containing measureable concentrations of nitroglycerin after oral doses.

Subject	Formulation	Time (hr)	GTN (ng/ml)
M.M.	B	0.25	4.12
M.M.	B	0.50	2.04
M.M.	B	1.00	0.09
R.A.M.	B	0.25	2.72
R.A.M.	B	1.00	0.66

It is theoretically possible for nitroglycerin concentrations to decrease from 2720 pg/ml to less than 50 pg/ml in 15 min since the half-life is normally approximately two min. However, we would not expect to observe another high concentration one-half hour later. As will be shown in Chapter VII, the bioavailability of an oral nitroglycerin solution (6.5 mg dose) was zero. During the early time points, this dose produces only very low (150 $\pm$ 100 pg/ml) concentrations of nitroglycerin in plasma. These low concentrations are most likely due to sublingual absorption of a small portion of the oral solution.

The bioavailabilities of these three oral nitroglycerin formulations are essentially zero. These data are quite interesting since two of the dosage forms (A and C) were shown to be pharmacologically active. These two dosage forms were tested by Marion Laboratories using exercise testing in patients and were found to be efficacious. Interestingly, formulation B, which was the only dosage form to show detectable plasma concentrations, was not pharmacologically active.

These data prompted us to evaluate the pharmacokinetics of nitroglycerin administered as an oral solution (Chapter VII). The first pass metabolism of nitroglycerin appears to be quite extensive. Assuming that the first pass effect is saturable, then administration of nitroglycerin in a rapidly absorbed oral solution will maximize the amount of nitroglycerin which escapes

metabolism.

D. SUMMARY

The bioavailability of three different oral nitroglycerin formulations was found to be zero, relative to topical nitroglycerin. Even though plasma concentrations of formulations A and C were less than the lowest measurable concentration (50 pg/ml), these formulations have been demonstrated to be pharmacologically active. From these data we may speculate that nitroglycerin metabolites are responsible for the efficacy of oral nitroglycerin.

CHAPTER VII

PHARMACOKINETICS OF NITROGLYCERIN AFTER INTRAVENOUS INFUSION AND SUBLINGUAL, ORAL AND TOPICAL ADMINISTRATION TO HEALTHY VOLUNTEERS

A. OBJECTIVES OF THIS STUDY

The pain of angina may be treated by administration of sublingual nitroglycerin. Because the duration of action of a sublingual nitroglycerin dose is short, longer-acting dosage forms are necessary for prophylactic treatment. Both oral and transdermal drug dosing offer the advantage of a sustained delivery (and sustained pharmacologic action) over a longer period of time. Because of previous assay limitations, very little information regarding the bioavailability and pharmacokinetics of nitroglycerin is known. The main objective of this study was investigate the pharmacokinetics and to determine the bioavailability of nitroglycerin when administered sublingually, orally and transdermally. Four subjects received these three dosages of nitroglycerin in crossover fashion with intravenous nitroglycerin. This study utilized a latin square four-way crossover design (Table VII-1). Four additional subjects were studied, but these subjects did not participate in the four-way crossover study. These subjects were administered

TABLE VII-1: Latin square design used in the four-way crossover study to investigate the pharmacokinetics and bioavailability of topical (A), oral (B), sublingual (C) and intravenous (D) nitroglycerin. Two subjects received a second sublingual dose (E) on week 5.

	Study Week				
Subject	1	2	3	4	5
T.M.	A	B	C	D	E
M.D.	B	C	D	A	-
J.K.	C	D	A	B	E
D.D.	D	A	B	C	-

sublingual and intravenous dosages of nitroglycerin, to determine if steady-state (intravenous) or peak (sublingual) concentrations varied as a function of body position. Each of these subjects did receive intravenous infusions of nitroglycerin in addition to their other doses of nitroglycerin. The order of dosing for all subjects is summarized in Table VII-2.

B. PHARMACOKINETICS OF NITROGLYCERIN AFTER INTRAVENOUS INFUSION

1. INTRODUCTION

To determine the clearance of nitroglycerin and to calculate the bioavailability of oral, sublingual and topical doses, an intravenous dose of the drug must be given. The primary purpose for this study, then, was to calculate the nitroglycerin clearance so that sublingual, oral and topical bioavailability could be determined. Additionally, steady-state plasma concentrations as a function of the body position were determined.

In some preliminary studies (discussed in section C.3 of this chapter), the body position of the volunteers appeared to have a significant effect on plasma nitroglycerin concentrations obtained after sublingual dosing. This effect may have been caused by changes in the volume of distribution, clearance or bioavailability of sublingual nitroglycerin. Intravenous infusion data was used

TABLE VII-2: Dosing order for each of eight subjects receiving ointment (A-1), Nitrodisc (A-2), Nitrodur (A-3), oral (B), sublingual (C) and intravenous (D) nitroglycerin doses. Note that Dose Number does not correspond to any one particular time or week but only reflects the order in which the doses were administered.

	Dose Number						
Subject	1	2	3	4	5	6	7
R.D.	C	C	C	D	D	A-1	A-2
J.W.	C	C	C	D	-	-	-
P.L.	C	C	D-1	-	-	-	-
S.S.	C	C	D-1	-	-	-	-
T.M.	A-1	A-2	A-3	B	C	D	C
M.D.	A-1	A-2	B	C	D	A-3	-
J.K.	C	D	A	B	C	-	-
D.D.	A-1	A-2	D	A	B	C	-

to elucidate the mechanism by which these changes occurred. If body position altered the steady-state nitroglycerin concentration, then clearance might have changed. If only a transient change in steady-state concentration were detected, then the volume of distribution might have changed with body position. If no changes in nitroglycerin plasma concentrations were detected, then changes in sublingual drug absorption would be indicated.

The final purpose for the intravenous infusion study was to determine whether nitroglycerin pharmacokinetics were dose-dependent. For this study, nitroglycerin was infused at three different infusion rates and steady state plasma concentrations were measured. Nitroglycerin was infused at 10, 20, 40 and again at 10 mcg/min to check the reproducibility of the first infusion rate.

2.EXPERIMENTAL

In this study, a total of eight subjects were administered intravenous nitroglycerin infusions. Tridil^{R} (American Critical Care), used for the infusions, was prepared as a 100 mcg/ml solution in 5% dextrose in water by the I.V. Additive Services of Moffitt Hospital, San Francisco, CA. Nitroglycerin was delivered through a Tridilset (American Critical Care), which adsorbs less than 1% of a nitroglycerin dose (Baaske et al., 1982). The dose was delivered as a constant infusion using a Harvard

infusion pump and a sterile 50 ml glass syringe. The dose was administered through a teflon obturator set placed in a peripheral arm vein. Blood samples were drawn through a heparin lock, placed in a peripheral vein of the opposite arm, kept patent with periodic injections of heparin (67 USP units/ml) in saline.

An in vitro experiment was performed in which a nitroglycerin solution (100 mcg/ml), perfused through an infusion set using a Harvard infusion pump, was assayed to show that the pump was calibrated to deliver the proper amount of nitroglycerin and that nitroglycerin was not being adsorbed to the infusion set. In the first experiment, nitroglycerin was infused through the set at an infusion rate of 40 mcg/min for 105 min. Fractions of the dose were collected with time and analyzed for nitroglycerin using HPLC. In the second experiment, nitroglycerin was infused through a new infusion set at 10, 20, 40 and then again at 10 mcg/min. Fractions were again collected and analyzed for nitroglycerin by reversed phase HPLC. Note that this HPLC assay is different from that described in Chapter III.

HPLC analysis was performed using a Varian 8500 solvent delivery system and Rheodyne manual injector. Detection of nitroglycerin was carried out with a fixed-wavelength UV detector (LDC UV-III Monitor) at 214 nm. The prepacked column (Altex 5 micron octyl (C_8), 4.6 x 150 mm) was eluted with acetonitrile:water (40:60, v/v) at a flow rate of 160 ml/hr. Calibration standards of 0, 10, 25, 50, 75, 100,

200, 300, 400, 600 and 800 mcg nitroglycerin were prepared and chromatographed. Nitroglycerin calibration standards were prepared by adding the appropriate amounts of nitroglycerin stock solution (1.0 mg/ml in ethanol) to scintillation vials. Internal standard (50 mcg 2,6-dinitrotoluene, 1.0 mg/ml in ethanol) was added to each vial. Ten milliliters of mobile phase were added to each vial and the components mixed. Twenty microliters of each calibration standard was injected onto the column.

Fractions collected from the infusion pump were assayed for nitroglycerin as follows. To each fraction was added 50 ul of internal standard and 10 ml of mobile phase. Each fraction was then vortexed for approximately 10 sec. Twenty microliters were chromatographed and the peak height ratio used to calculate the amount of nitroglycerin in each fraction. The infusion rate was calculated from the amount of nitroglycerin and the time interval during which each fraction was collected.

Two types of infusion experiments were performed. Two volunteers received a single constant infusion rate (to study the effect of body position). The remaining six volunteers received various constant infusion rates.

To study the effect of position on plasma nitroglycerin concentrations, two subjects (S.S. and P.L.) received a single constant infusion rate of 40 mcg/min. The subjects remained in a reclining position. After a 30 ml control blood sample was withdrawn, the infusion was begun. An

initial infusion rate of 5 mcg/min was administered. The infusion rate was doubled every three to five minutes until the final rate of 40 mcg/min was achieved. Blood pressure and heart rate were monitored periodically during this period. Zero time was defined as that time at which the 40 mcg/min infusion rate was begun. Seven ml blood samples were withdrawn at 20, 25 and 30 min to establish the steady-state condition.

At 31, 62 and 93 minutes, the subjects changed positions as indicated in Table VII-3. Blood samples were drawn at 1, 3, 5, 20, 25 and 30 min after each position change. Transient changes in steady-state concentrations with position would indicate a change in volume of distribution while a new steady-state plasma level would be indicative of a change in clearance. At the conclusion of the infusion, additional blood samples were drawn at 1, 2, 4, 6 and 10 min to define the nitroglycerin fall-off curve.

The remaining six volunteers received variable infusion rates of nitroglycerin (in a supine position). Generally, at 0, 40, 80 and 120 min, the infusion rates were adjusted to 10, 20, 40 and 10 mcg/min, respectively. The exact dosing schedule for each of these six volunteers is shown in Table VII-4. Blood samples were drawn at 10, 20, 30 and 40 min after an infusion rate was established. At the conclusion of the infusion, blood samples were drawn at 1, 2, 4, 6, 10, 15, 20, 40, 60, 120, 180 and 240 min.

TABLE VII-3: Schedule of blood samples drawn in relation to changes in body position during a constant 40 mcg/min infusion to subjects P.L. and S.S.

Expt. Time (min)	Blood Sample Time (min) After Change	Position	Blood Sample
0	--	Supine	X
20	20		X
25	25		X
30	30		X
31	--	Sitting	-
32	1		X
34	3		X
36	5		X
51	20		X
56	25		X
61	30		X
62	--	Standing	-
63	1		X
65	3		X
67	5		X
82	20		X
87	25		X
92	30		X
93	--	Supine	-
94	1		X
96	3		X
98	5		X
113	20		X
118	25		X
123	30		X
124*	--		-
125	1		X
126	2		X
128	4		X
130	6		X

* Stop infusion

TABLE VII-4: Actual experimental times at which the infusion rate was adjusted.

	Actual Experimental Time (min)					
	Infusion Rate (Event)[a]					
Subject	Low	Med.	High	Off	Low	Off
R.D.#1	0	29.8	60.0	-	90.0	120.0
R.D.#2	0	40.8	80.0	120.0	130.0	170.0
J.W.	0	30.0	60.0	-	90.0	120.0
P.L.	-	-	0	103.0	-	-
S.S.	-	-	0	93.0	-	-
J.K.	0	40.0	80.0	120.5	138.8	178.8
M.D.	0	40.0	80.0	91.0	-	-
T.M.	0	40.0	80.0	120.0	153.0	193.0
D.D.	30.0	60.0	100.0	140.0	162.0	202.0

[a]Nominal infusion rates: Low = 10 mcg/min;

Med. = 20 mcg/min; High = 40 mcg/min.

3. RESULTS AND DISCUSSION

a. NITROGLYCERIN HPLC ASSAY

Nitroglycerin may be analyzed using high pressure chromatography (HPLC), but only at high concentrations. This HPLC assay was developed to analyze nitroglycerin dosage forms and intravenous solutions. Figure VII-1 shows a chromatogram of a standard containing 160 ng of nitroglycerin, 100 ng of each of the dinitrate metabolites (1,2- and 1,3-glyceryldinitrates), 100 ng of 1-GMN and 40 ng DNT. The nitroglycerin, metabolites and internal standard are all resolved from each other. Figure VII-2 shows a nitroglycerin standard curve as determined by HPLC. These standard curves are linear to at least 800 mcg and can detect quantities down to 10 mcg. The coefficient of variation of the HPLC assay was 1.2% at 400 mcg. This assay was used to quantify amounts of nitroglycerin in infusion fluids, sublingual tablets (400 mcg) and mouth rinses containing residual sublingual nitroglycerin doses.

b. CALIBRATION OF INFUSION PUMP

Figure VII-3 depicts the in vitro infusion rates of nitroglycerin as delivered by the Harvard infusion pump through a non-adsorbable infusion set. Figure VII-3 shows that a constant infusion rate (40.6 ± 1.7 mcg/min) can be

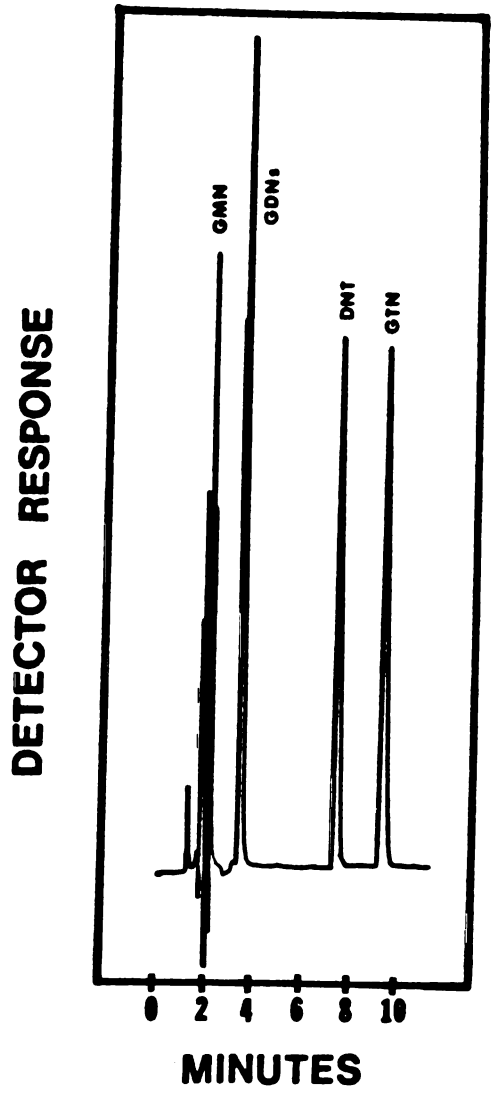


FIG. VII-1: Representative HPLC chromatogram showing the resolution of GTN (160 ng) from 1,2-GDN (100 ng), 1,3-GDN (100 ng), 1-GMN (100 ng) and DNT (40 ng).

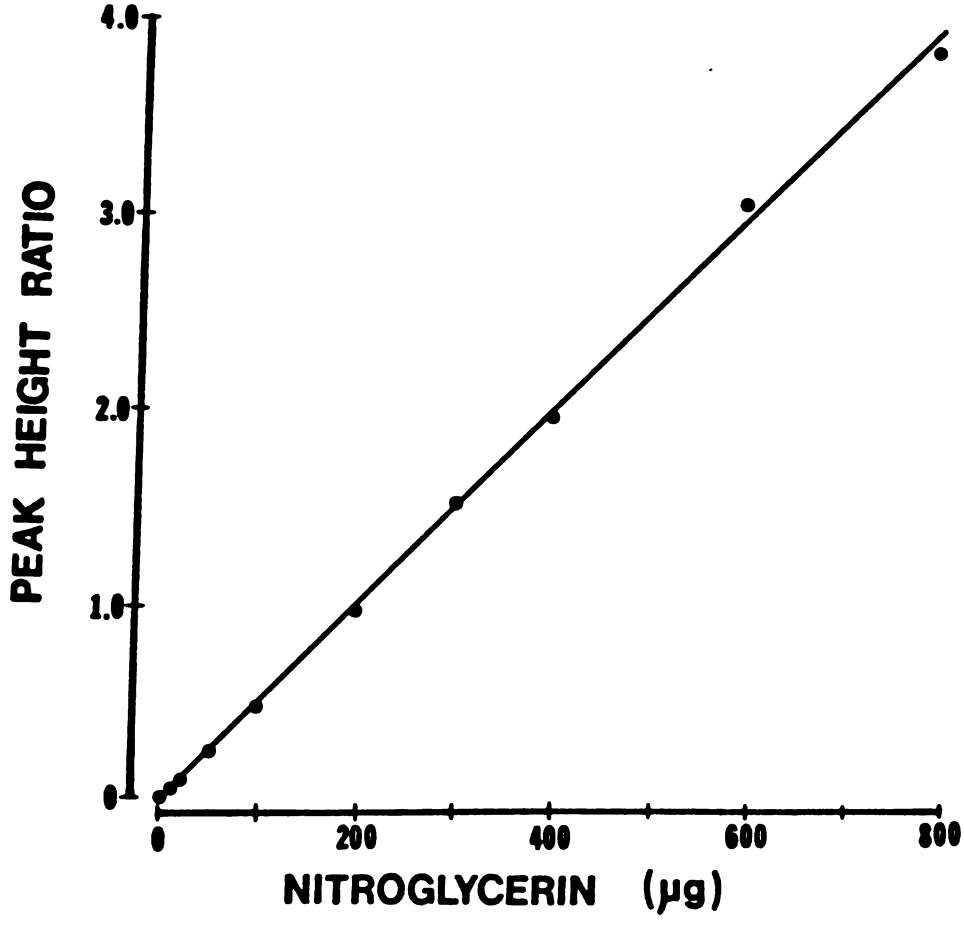


FIG. VII-2: Nitroglycerin HPLC standard curve showing linearity between 0 to 800 mcg nitroglycerin.

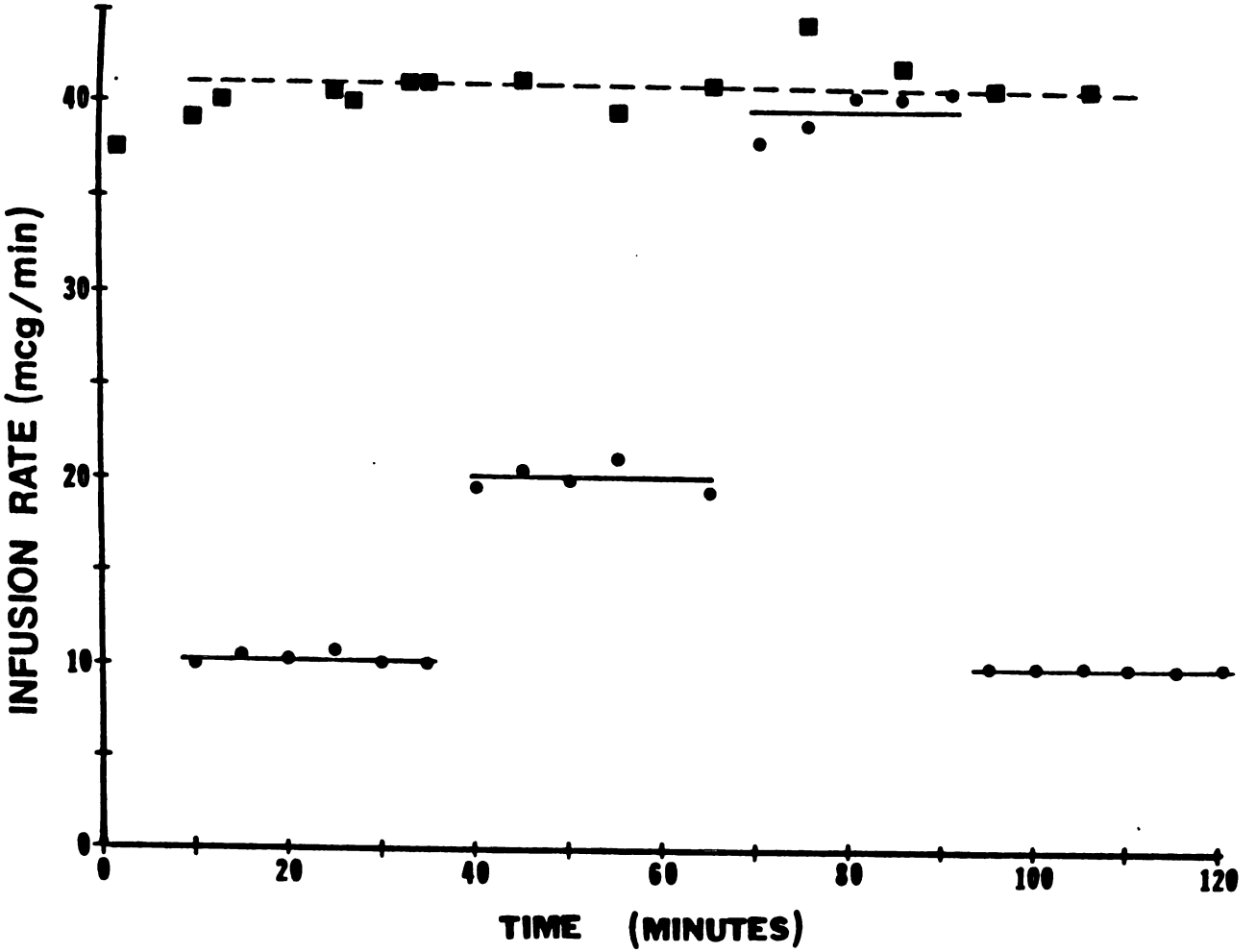


FIG. VII-3: In vitro calibration (delivery) of nitroglycerin through intravenous infusion sets. Key: squares = constant rate of 40 mcg/min, circles = variable rate of 10, 20, 40 and again 10 mcg/min.

maintained for at least 105 min. The figure also shows that the infusion rate can be precisely changed to deliver 10 (10.2 ± 0.3), 20 (20.1 ± 0.7), 40 (39.7 ± 1.2) and then again 10 (10.2 ± 0.1) mcg/min of nitroglycerin.

c. EFFECT OF BODY POSITION

Figures VII-4A and VII-4B show nitroglycerin plasma concentrations during intravenous infusions of 40 mcg/min to two individuals. Plasma concentrations as a function of body position are shown. Steady-state plasma concentrations varied between 1.1 and 3.6 and 1.6 and 3.4 ng/ml in subjects P.L. and S.S., respectively. The sharp decrease in nitroglycerin concentrations, in Figure VII-4B, upon standing, was most likely due to a decrease in the infusion rate. The in vitro infusion rate is affected by the height of the infusion tubing, relative to the pump. When the tubing height is increased, as happened when the volunteer stood, the infusion stops. Interestingly, when subject S.S. stood up, he became hypotensive after 6 min, even though his plasma nitroglycerin concentration had decreased to 30 to 50 percent of steady-state.

These data suggest that body position has no measurable effect on intravenous plasma concentrations. The intra-individual variability of nitroglycerin concentrations is too large to detect small changes in clearance or volume of distribution with change in body position. Therefore, if

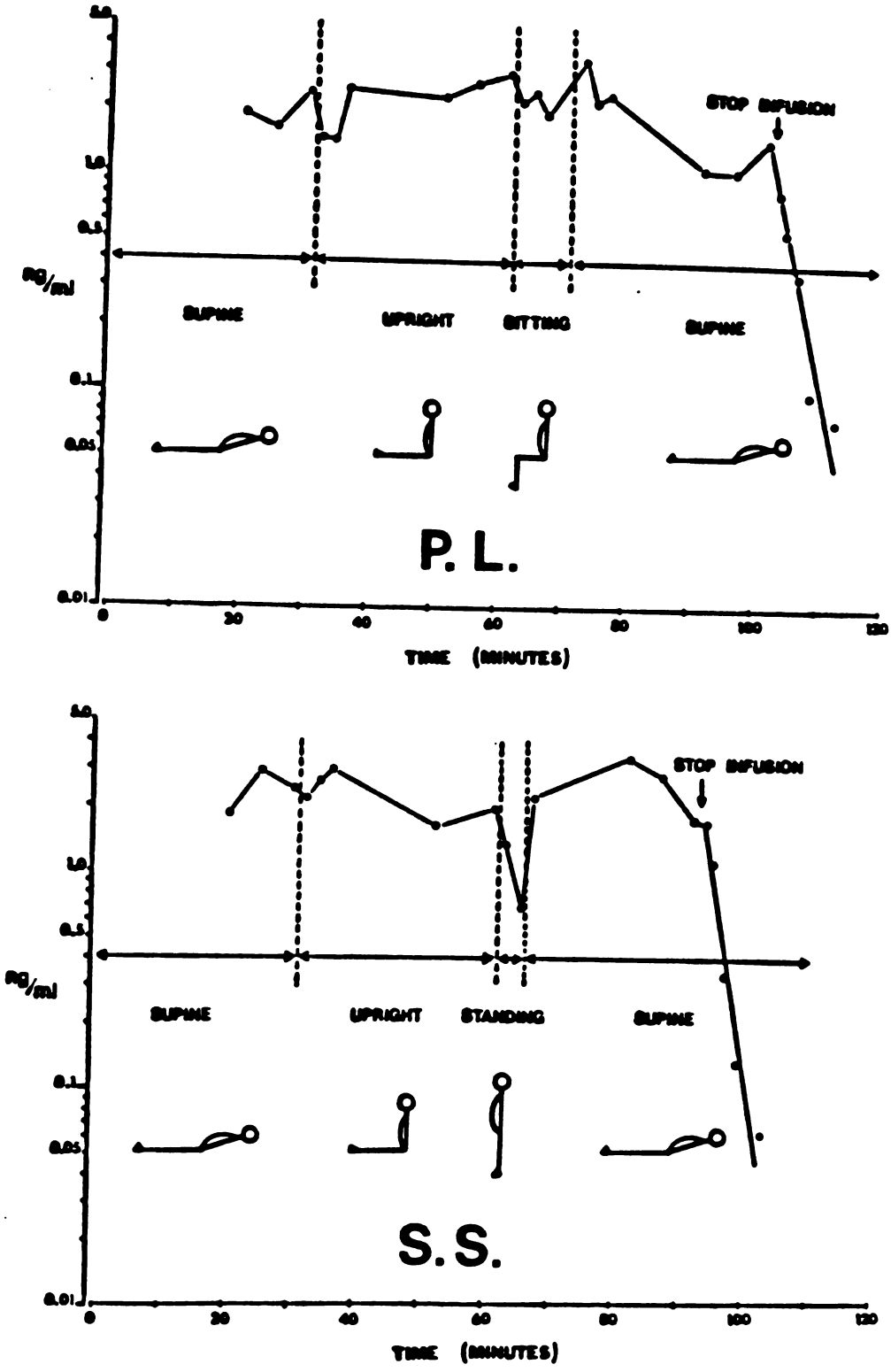


FIG. VII-4: Plasma nitroglycerin concentrations during and after GTN infusions of 40 mcg/min in subjects P.L. and S.S. Position of subject was changed during the infusion as indicated.

nitroglycerin plasma concentrations are affected by body position after sublingual administration (discussed in section C of this chapter), one might conclude that these effects are caused by changes in bioavailability.

d. PLASMA NITROGLYCERIN CONCENTRATIONS AFTER MULTIPLE INTRAVENOUS INFUSIONS

Six subjects each received consecutive nitroglycerin infusions of 10, 20, 40 and then 10 mcg/min. The actual infusion rates as determined by HPLC analysis are shown in Table VII-5. Subject R.D. received an identical series of infusions on two separate occasions. The actual plasma nitroglycerin concentrations for each of the six volunteers are shown graphically in Figures VII-5 and 6.

Plasma samples taken 10 min from the start of an infusion should represent steady-state concentrations since the half-life of nitroglycerin ranges between 1.4 and 3.2 min (Table VII-6). This initial time interval represents three to four half-lives and therefore these concentrations are at least 90% of theoretical steady-state values.

Based on the assumption that steady-state has been achieved, the average steady state concentrations (C_{ss}) and clearances may be calculated using the following equations:

$$C_{ss} = \text{AUC} / \text{Time Interval}$$

$$\text{Clearance} = \text{Infusion Rate} \times \text{Infusion Duration} / \text{AUC}$$

TABLE VII-5: Actual nitroglycerin infusion rates, as measured by HPLC analysis, for each subject administered intravenous nitroglycerin.

	Actual Infusion Rate[a] (mcg/min)			
Subject	Low	Med.	High	Low
R.D.#1	8.33	19.0	38.2	8.33
R.D.#2	9.46	17.8	36.6	9.46
J.W.	9.42	21.5	39.4	9.42
P.L.	-	-	40.6	-
S.S.	-	-	38.3	-
J.K.	12.1	20.6	44.4	12.1
M.D.	10.6	19.8	-	-
T.M.	10.8	21.3	54.0	10.8
D.D.	6.70	19.4	38.5	8.70

[a] Nominal infusion rates: Low = 10 mcg/min; Med. = 20 mcg/min; High = 40 mcg/min.

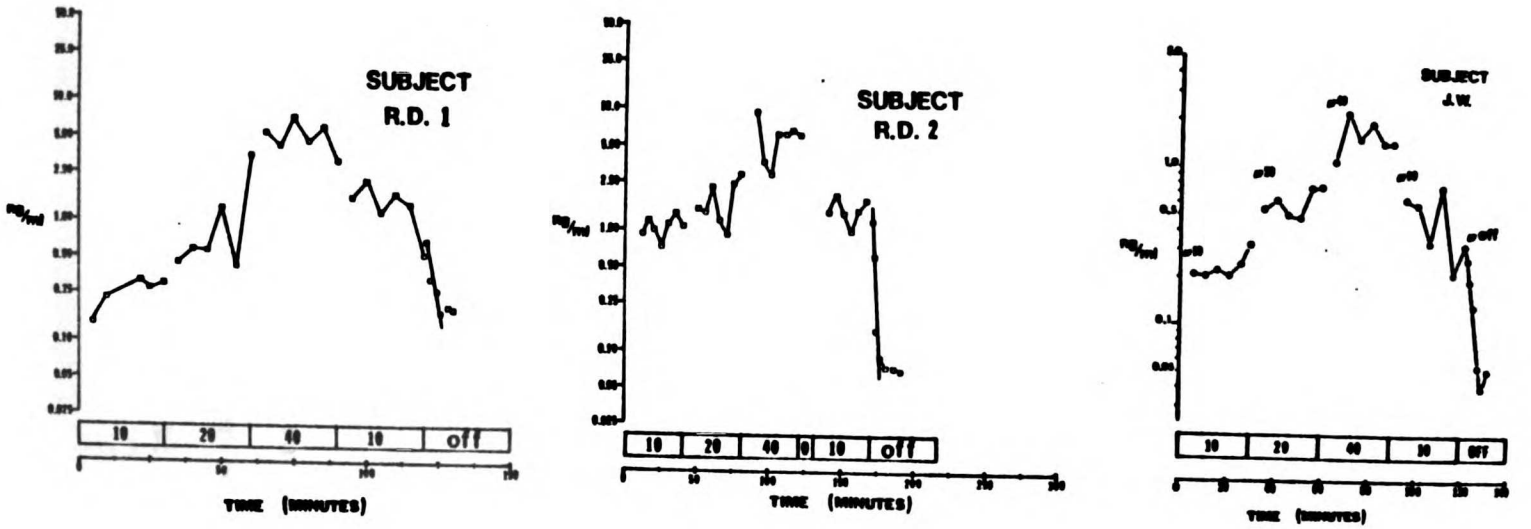


FIG. VII-5: Plasma GTN concentrations in subjects R.D. (two doses) and J.W. during and after consecutive GTN infusions of 10, 20, 40 and 10 mcg/min.

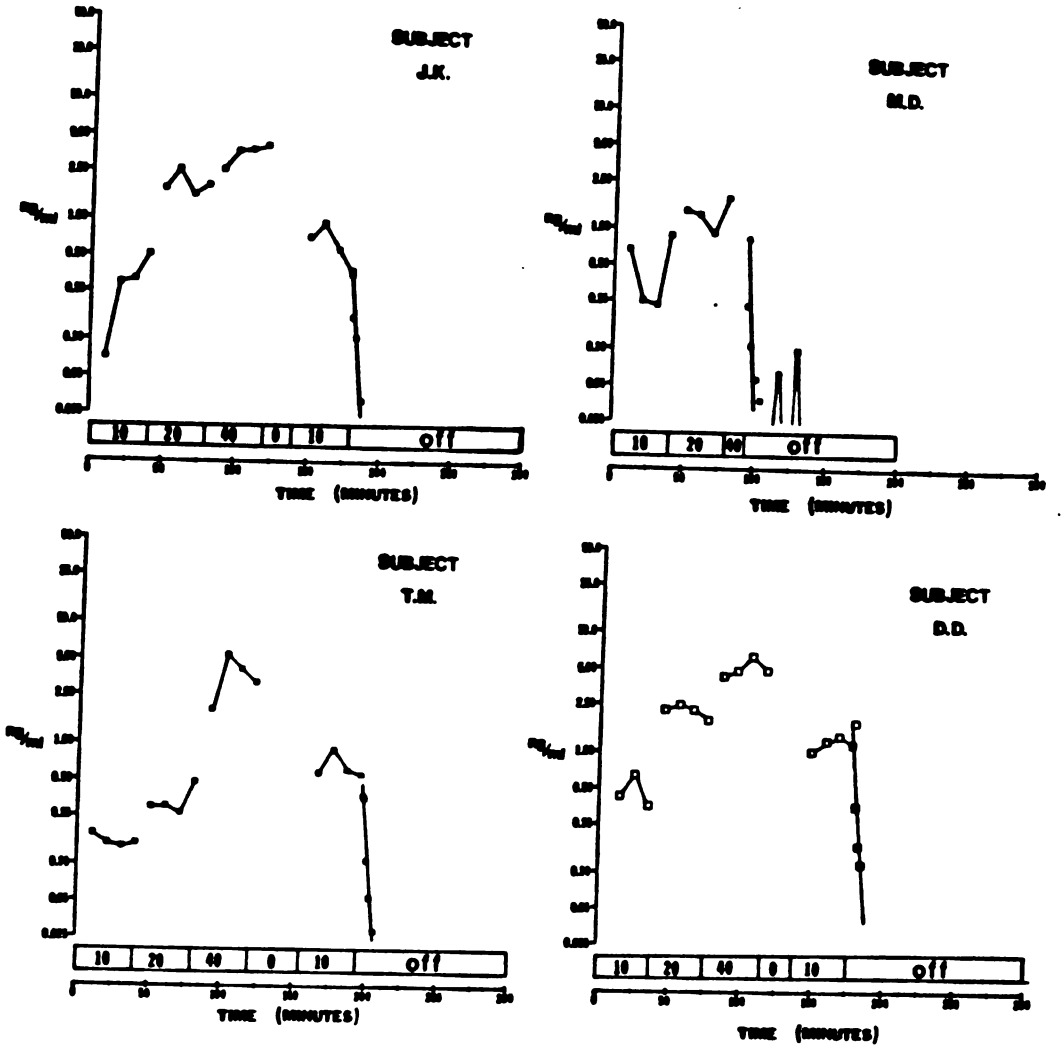


FIG. VII-6: Plasma GTN concentrations in subjects J.K., M.D., T.M. and D.D. after consecutive GTN infusions of 10, 20, 40 and 10 mcg/min.

TABLE VII-6: Steady-state nitroglycerin concentrations measured after intravenous infusion to each subject. Plasma half-life was measured after the final infusion was turned off.

	GTN Steady-State Plasma Conc. (ng/ml)				
	Infusion Rate[a]				
Subject	Low	Med.	High	Low	$T_{1/2}$(min)
R.D.#1	0.26	0.90	5.19	1.52	2.8
R.D.#2	1.07	1.71	5.71	1.48	1.4
J.W.	0.28	0.75	1.64	0.58	2.5
P.L.	-	-	2.08	-	2.4
S.S.	-	-	2.34	-	1.8
J.K.	0.36	2.02	3.6	0.70	1.5
M.D.	0.41	1.25	-	-	2.7
T.M.	0.15	0.32	4.02	0.71	2.2
D.D.	0.55	2.26	5.20	1.27	3.2

[a] Nominal infusion rates: Low = 10 mcg/min; Med. = 20 mcg/min; High = 40 mcg/min.

where AUC is the area under the plasma concentration time curve during the infusion interval. The average steady-state concentrations for each of the 8 volunteers at each infusion rate are shown in Table VII-6. Figure VII-7 shows a plot of the steady-state nitroglycerin concentrations versus infusion rate for each volunteer. Note that in the majority of the subjects, a hysteretic type of response was present. As the dose is increased, these curves are similar to a typical dose-dependent curve (i.e., concave ascending). As the dose is decreased, though, the curves may become linear and intercept at the origin, somewhat representative of dose-independent pharmacokinetics.

Clearance values at each dose were calculated for each volunteer. These values are summarized in Table VII-7. Note that these clearance values (5.5 to 71 L/min) are very high and far exceed the maximum possible hepatic clearance (1.5 L/min). Such high clearance values suggest that nitroglycerin is cleared (metabolized) by organs other than the liver. Clearance does not appear to be directly related to nitroglycerin plasma concentrations. On the average, clearance values at the low and medium infusion rates are significantly higher than those at the high rate. Also, in most subjects, clearance during the initial 10 mcg/min infusion is two to five times higher than that during the final 10 mcg/min infusion. As nitroglycerin concentrations initially increase, the clearance decreases to a constant value. The hysteretic behavior described above can not

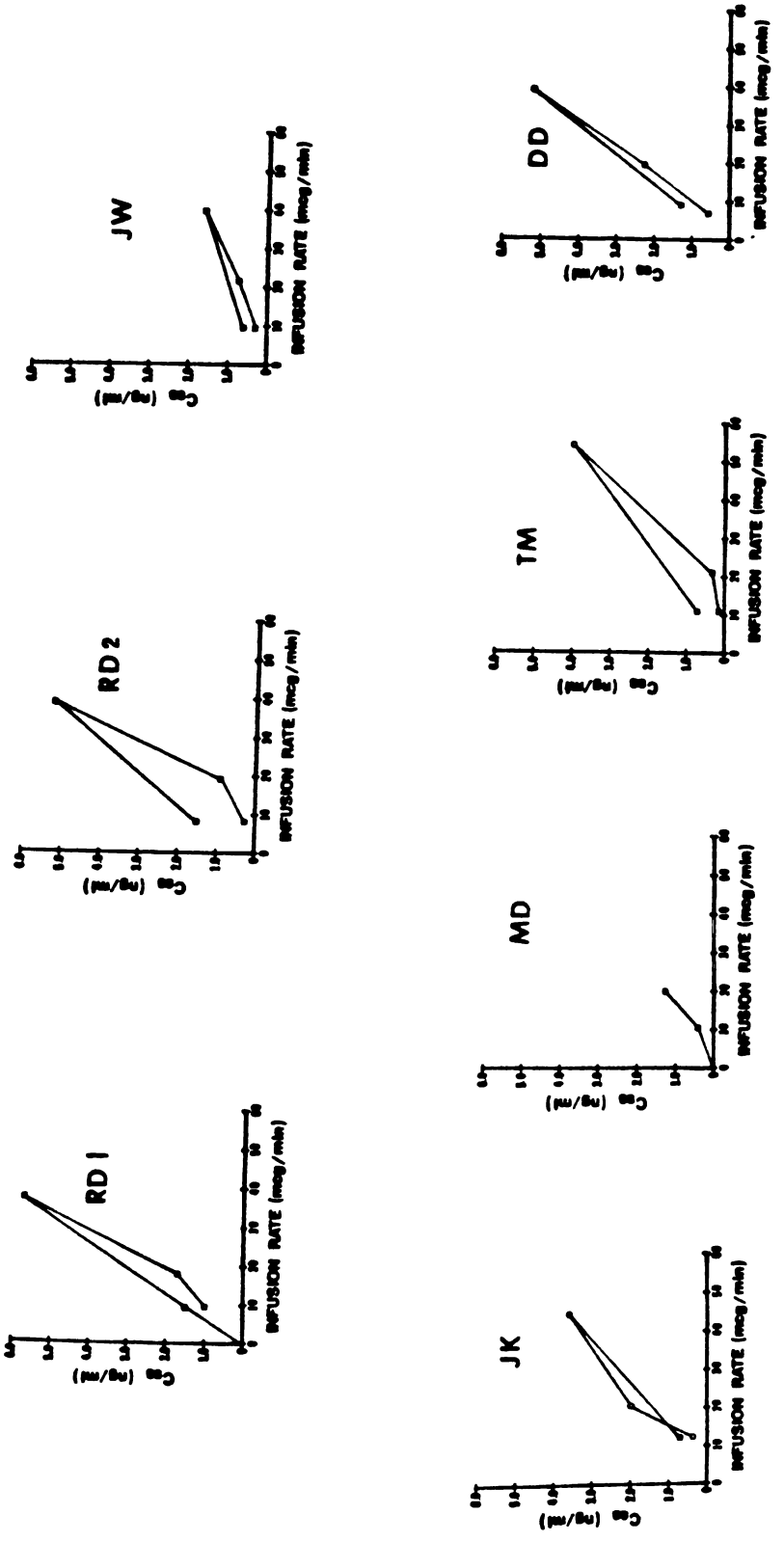


Fig. VII-7: Graphical representation of steady-state nitroglycerin plasma concentrations as a function of infusion rate in six healthy volunteers (subject R.D. studied twice).

TABLE VII-7: Nitroglycerin clearance values for each volunteer at all infusion rates.

	Clearance(L/min)			
	Infusion Rate[a]			
Subject	Low	Med.	High	Low
R.D. #1	32.0	21.0	7.36	5.47
R.D. #2	8.84	10.4	6.41	6.39
J.W.	33.10	28.75	24.10	16.14
P.L.	-	-	19.49	-
S.S.	-	-	16.37	-
J.K.	33.68	10.22	12.33	17.42
M.D.	26.08	15.82	-	-
T.M.	71.22	65.57	13.44	15.24
D.D.	12.28	8.58	7.41	6.86

[a]Nominal infusion rates: Low = 10 mcg/min
Med. = 20 mcg/min
High = 40 mcg/min

be described by first-pass metabolism (saturable kinetics) alone. The potential for first-pass metabolism by blood vessels was described in Chapter II. If this were the only mechanism responsible for nitroglycerin clearance then the curves shown in Figure VII-7 would be concave ascending only. That is, nitroglycerin steady-state plasma concentrations would be the same when the infusion rate was increased or decreased.

The hysteresis observed in the dose versus steady-state concentration curve could be caused by two different mechanisms:

1) End-product inhibition. Sutton and Fung (1982) showed that blood vessels were capable of metabolizing nitroglycerin. Such metabolism may be responsible for a type of "first-pass" effect after intravenous nitroglycerin administration. This first-pass metabolism might be reflected by changes in nitroglycerin clearance in the concave ascending portion of the curves shown in Fig. VII-7. At very high nitroglycerin infusion rates, high concentrations of metabolites (dinitrates) accumulate (to be shown in Chapter VIII). End-product inhibition of nitroglycerin metabolism may decrease nitroglycerin clearance at high metabolite concentrations.

2) Saturable binding (adsorption) of nitroglycerin to blood vessels. Fung et al. (1981c) showed that nitroglycerin concentrations accumulated most in rat blood vessel segments closest to the site of intravenous

nitroglycerin infusion. Adsorption of drug to veins during intravenous infusions to humans would result in a lower apparent bioavailability of nitroglycerin. That is, the infusion rate which is measured in vitro might not be the same as the rate at which the drug reachs the systemic circulation. If this binding phenomenon is saturable, then steady-state nitroglycerin plasma concentrations would appear to be dose dependent.

C. PHARMACOKINETICS OF SUBLINGUAL NITROGLYCERIN

1. Introduction

Sublingual nitroglycerin is used most often for the acute relief of angina pectoris. Many investigators (e.g., Armstrong et al. 1979), in which sublingual nitroglycerin pharmacokinetics were evaluated, have assumed that sublingual nitroglycerin is instantaneously and completely bioavailable. These workers attempted to estimate clearance and volume of distribution of nitroglycerin from a single sublingual dose. Many other groups (e.g., Girre et al., 1980; Colfer et al., 1982) use sublingual nitroglycerin as the relative bioavailability standard for other nitroglycerin dosage forms. The "absolute" bioavailability of sublingual nitroglycerin has not been evaluated to date.

Studies have shown that the pharmacologic action of a sublingual dose of nitroglycerin may be affected by the

patients body position when the dose is taken. Fortuin et al. (1974) used echocardiography to demonstrate that nitroglycerin significantly reduced left ventricular wall stress and cavity size. Interestingly, they found that these effects were significantly greater in the upright (sitting) rather than the supine (reclining) position. One might assume that these effects may be related to changes in nitroglycerin plasma concentrations. The effect of body position on nitroglycerin plasma concentrations after sublingual doses has also not been shown to date.

The purpose of this study was two-fold. The primary purpose was to evaluate the bioavailability of nitroglycerin when administered sublingually to healthy volunteers. Some of our preliminary sublingual nitroglycerin studies indicated that there might be an effect of body position on sublingual plasma concentrations. Therefore, an additional pilot study was designed to evaluate whether or not there was an effect of body position on nitroglycerin plasma concentrations.

2. Experimental

In this study, eight individuals received 0.4 mg of nitroglycerin (Nitrostat, Parke-Davis). All doses were obtained from the same manufactured lot (JBATMA). Each subject was placed in either a reclining or sitting position, where he remained for the first hour of the

investigation. At zero time, a single 0.4 mg nitroglycerin tablet was placed under the subject's tongue. Two drops of distilled water were added to the tablet to insure that the tablet was moistened. The subjects were instructed to maintain the dose in the sublingual area and not to swallow the dose. After eight min, the subjects were instructed to spit out any remaining dose, including saliva, into a glass container. Each subject then rinsed his mouth out further with 50 ml of distilled water. This maneuver would essentially halt the drug absorption process. This water rinse was added to the 8 min saliva collection and assayed by HPLC for residual nitroglycerin content.

Relative to each dose, serial blood samples were collected at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12.5, 15, 17.5, 20, 25, 30, 45, 60, 120, 180, 240 and 360 min. The blood samples were immediately processed and the plasma was frozen in a dry ice bath.

The effect of body position on sublingual nitroglycerin plasma concentrations was studied in four individuals. Two volunteers (R.D. and J.W.) were initially studied in the reclining position and again in the sitting position. Two other subjects (P.L. and S.S.) were dosed in the opposite order (i.e., sitting then reclining). Although tablets from the same lot were always used, tablets were taken from two different bottles . Subjects R.D. and J.W. were dosed a third time in the reclining position.

Four additional subjects (J.K., M.D., T.M. and D.D.)

were studied in crossover fashion with intravenous, topical and oral doses. These subjects received tablets from bottle B and were dosed in the sitting position.

Each of the eight subjects dosed with sublingual nitroglycerin also received intravenous infusions of nitroglycerin (see previous section) so that the absolute bioavailability could be evaluated.

3. RESULTS AND DISCUSSION

Figure VII-8 depicts nitroglycerin plasma concentrations after sublingual administration to each of the four individuals who participated in the "effect of body position" study. Pharmacokinetic parameters for each of these four subjects are summarized in Table VII-8. Initial studies in subjects R.D. and J.W. (doses 1 and 2) indicated that the nitroglycerin AUC increased approximately four-fold (Table VII-8) when nitroglycerin (from bottle A) was administered in the sitting compared to the supine position. Also, nitroglycerin peak concentrations increased by a factor of three to four and the time to peak concentrations decreased by two to four min. Administration of nitroglycerin (from bottle B) to two additional subjects (P.L. and S.S.) showed only a 33% increase in AUC, relative to the supine position, in subject S.S. and no change in subject P.L. Nitroglycerin peak times and peak concentrations also were not significantly different. The

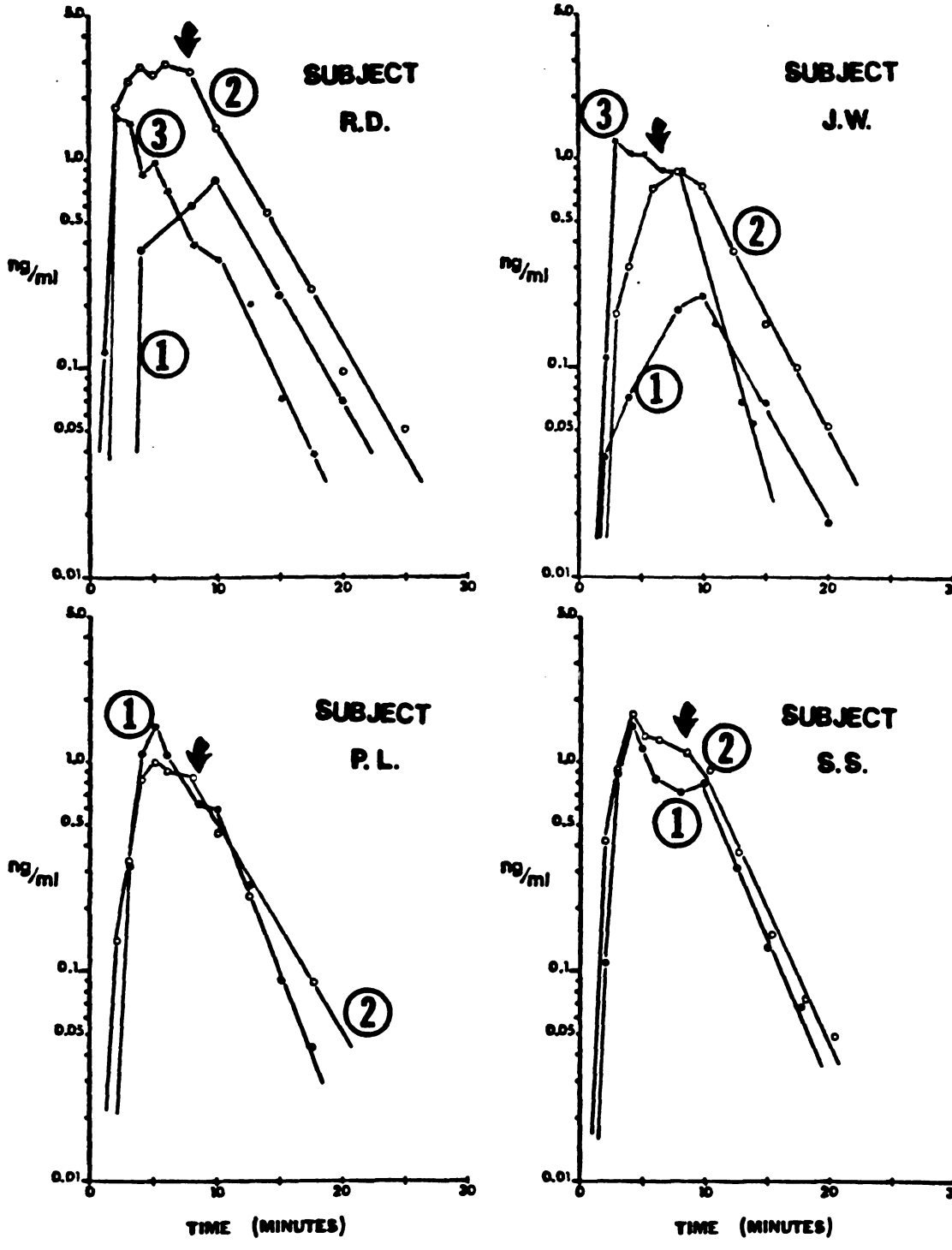


FIG. VII-8: Nitroglycerin plasma concentrations after sublingual GTN (0.4 mg) administration to each of four subjects. Subjects R.D. and J.W. each received two separate doses in the supine position (1 and 2). Dose 3 was administered in the sitting position. Subjects S.S. and P.L. each received a single dose in the sitting (2) and supine (1) positions. Arrows indicate when the residual dose was washed out of the mouth.

TABLE VII-8: Summary of sublingual nitroglycerin pharmacokinetic parameters obtained in each of four subjects who participated in the clinical study in which the effect of body position was investigated.

Parameter	Subject R.D.			Subject J.W.			Subject P.L.		Subject S.S.	
	Supine	Sitting	Supine	Supine	Sitting	Supine	Supine	Sitting	Supine	Sitting
Bottle I.D.	A	A	A	A	A	B	B	B	B	B
Peak Time (min)	10	6	2	10	8	3	5	5	4	4
Half-Life (min)	2.8	3.0	2.3	2.8	2.6	1.4	2.0	3.0	2.2	2.4
Peak Conc (ng/ml)	0.80	2.90	1.61	0.22	0.87	1.23	1.50	1.02	1.52	1.72
AUC (ng·min/ml)	6.87	26.8	8.47	1.97	7.18	7.39	8.18	8.11	9.43	12.5
AUC Ratio (sitting/supine)	3.89	-	3.16	3.65	-	0.97	0.99	-	1.33	-

low bioavailability of sublingual nitroglycerin, administered in the supine position, was reproduced in subject R.D. when a tablet from the original bottle (A) was used but not in subject J.W. when a tablet from bottle B was used.

Nitroglycerin tablets from each bottle were tested for potency using the HPLC assay described in section B.2. These values are shown in Table VII-9. There was no significant difference in the potency or content uniformity of these two bottles of tablets. Therefore, the initial effect observed with bottle A versus B was not due to different nitroglycerin doses.

The nitroglycerin plasma concentrations for the remaining four volunteers are shown graphically in Figure VII-9. Pharmacokinetic parameters for each of these four subjects are summarized in Table VII-10.

The residual amounts of nitroglycerin not absorbed during the 8-min dosing periods are summarized in Table VII-11. The residual amounts of nitroglycerin left after 8 min are not correlated with the bioavailability (AUC's) of the drug.

The bioavailabilities of sublingual nitroglycerin in each volunteer and at all infusion rates is shown in Table VII-11. The clearance of intravenous nitroglycerin varies as a function of the infusion rate and previous exposure to the drug (see intravenous data, section B, this chapter). The sublingual bioavailability values were calculated using

TABLE VII-9: Potency of 0.4 mg nitroglycerin tablets obtained from two different bottles (A and B) of the same manufactured lot.

Sample	Replicate	GTN (mg)	Mean ± SD (% CV)
Bottle A	1	0.51	0.44 ± 0.05 (11.4)
	2	0.44	
	3	0.42	
	4	0.37	
	5	0.44	
Bottle B	1	0.43	0.40 ± 0.045 (11.2)
	2	0.41	
	3	0.39	
	4	0.32	
	5	0.39	
	6	0.45	

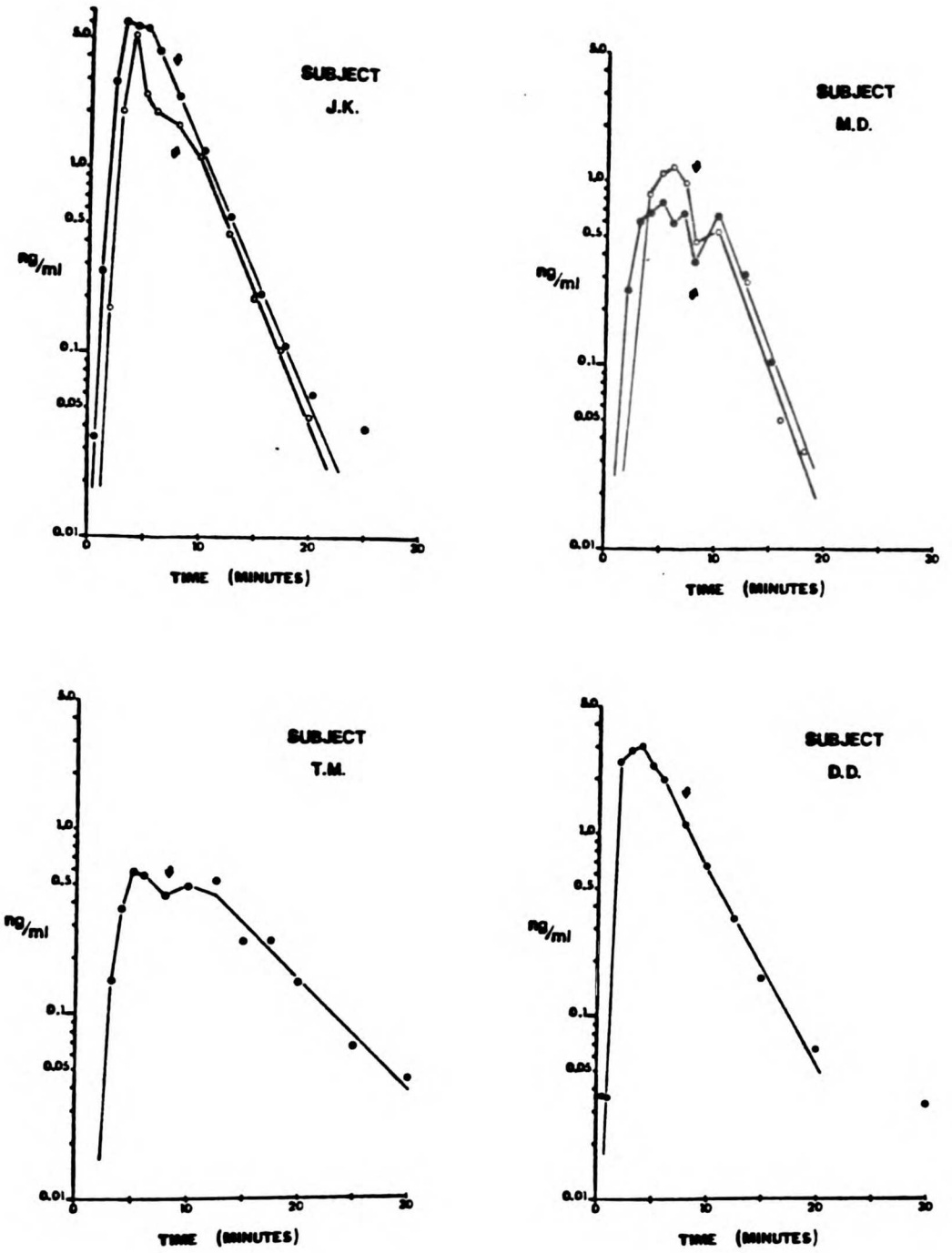


FIG. VII-9: Nitroglycerin plasma concentrations after sublingual GTN (0.4 mg) administration to each of four subjects. Subjects J.K. and M.D. each received a second dose on separate occasions. Arrows indicate when the residual dose was washed out of the mouth.

TABLE VII-10: Pharmacokinetic parameters obtained in four additional volunteers following sublingual nitroglycerin dosing.

Subject	T_{PEAK} (min)	C_{PEAK} (ng/ml)	$T_{1/2}$ (min)	AUC (ng·min/ml)
J.K.#1	3.2	6.10	2.2	36.65
J.K.#2	4.0	5.12	2.4	20.28
M.D.#1	6.0	1.26	1.8	8.39
M.D.#2	5.0	0.78	2.0	6.82
T.M.	5.0	0.60	5.0	7.85
D.D.	4.0	3.01	2.8	19.25

TABLE VII-11: Summary of sublingual nitroglycerin bioavailability. Bioavailability estimates for each sublingual dose were calculated using clearance values obtained at each infusion rate. The amounts of drug (% of dose) found in the mouth rinses are also shown.

	Sublingual Bioavailability (%)				
	Infusion Rate[a]				
Subject	Low Inf.	Med. Inf.	High Inf.	Low Inf.	Mouth Rinse % Dose
R.D. #1	55.0	36.2	12.8	9.4	44.5
#2	214.2	140.7	49.4	36.7	11.8
#3	67.7	44.5	15.6	11.6	41.6
R.D. #1	15.2	17.9	11.0	11.0	44.5
#2	59.3	69.6	43.0	42.8	11.8
#3	18.7	22.0	13.6	13.5	41.6
J.W. #1	16.3	14.1	11.9	7.9	65.8
#2	59.4	51.6	43.3	29.0	60.0
#3	61.2	53.1	44.5	29.8	61.5
P.L. #1	-	-	39.5	-	22.0
#2	-	-	39.8	-	30.5
S.S. #1	-	-	51.2	-	34.8
#2	-	-	38.6	-	29.0
J.K. #1	308.6	93.6	113.0	159.6	18.5
#2	170.7	51.8	62.5	88.3	13.3
M.D. #1	54.7	33.2	-	-	24.3
#2	44.4	27.0	-	-	13.8
T.M.	14.0	12.9	2.6	3.0	29.0
D.D.	59.1	41.3	35.6	33.0	2.7

[a] Sublingual bioavailabilities were calculated based on nominal infusion rates of: 10 mcg/min = Low; 20 mcg/min = Med.; 40 mcg/min = High.

the clearance values obtained from each intravenous infusion rate for each volunteer. Bioavailabilities were calculated using the following equation:

$$\% F = (CLiv * AUC_{sublingual} / DOSE_{sublingual}) \times 100$$

Subject R.D. received intravenous infusions on two separate occasions. Therefore, Table VII-11 shows two sets of values for each sublingual dose. The bioavailability values for R.D. differ by two- to three-fold at the low and medium infusion rates but at the high and second low rates (i.e., the second time the low infusion rate was given) the availabilities are in good agreement. The bioavailabilities for all subjects range from 14-309%, 13-141%, 3-113% and 3-160% for the low, medium, high and second low infusion rates, respectively.

A relatively large amount of the sublingual nitroglycerin dose was not absorbed in most of the subjects. Of the sixteen sublingual doses administered, only once did less than 10% of the dose remain (in the mouth) after 8 min. Subject D.D. had only 3% of the dose remaining after 8 min, yet the calculated bioavailability ranged between 33 and 59 percent. This low recovery may be due to incomplete collection of the residual dose or due to the volunteer swallowing some of the dose before it could be collected. Twelve to sixty-six per cent, of the 0.4 mg sublingual dose was recovered during studies involving the other seven

volunteers (15 doses total). Therefore, the assumption that sublingual nitroglycerin is completely (100%) absorbed is not valid.

The times to peak nitroglycerin concentrations ranged from 2 to 10 min. Since these peak times range from one to five times the half-life of nitroglycerin, the absorption of a sublingual dose can not be assumed to be instantaneous. Therefore, estimates of the volume of distribution of nitroglycerin using V_{extrap}, assuming instantaneous input of a sublingual dose, will certainly be in error.

4. CONCLUSIONS

The effect of body position on nitroglycerin plasma concentrations after sublingual administration to healthy subjects was not consistent. This effect varied both between subjects and bottles of tablets within the same lot number. The differences in bioavailability may be due simply to a large intra-individual variability in the absorption of nitroglycerin. Many factors may play a role in the sublingual absorption of drugs. These factors include blood flow to the sublingual membranes, the ability of the volunteer (patient) to maintain the dose in the sublingual area without swallowing and the hydration state of the sublingual area (i,e., dry mouth) which was reported to affect the sublingual absorption of drugs (Davis et al., 1982).

It may also be concluded that the bioavailability of sublingual nitroglycerin is variable and not complete. The amount of nitroglycerin not absorbed after eight min, as determined from the analysis of the mouth rinses, varied from 2.7% to 65.8% (mean = 31.4 ± 18.9 %) of the administered sublingual dose. Both the inter- and intra-individual variabilities are high for sublingual nitroglycerin absorption. Estimates of sublingual nitroglycerin bioavailability varied not only between the individual doses but also within the same sublingual dose, depending on the infusion rate used. Because nitroglycerin clearance varies with the infusion rate, it follows that estimates of nitroglycerin bioavailability also depend on the infusion rate.

D. PHARMACOKINETICS OF NITROGLYCERIN ADMINISTERED AS AN ORAL SOLUTION.

1.INTRODUCTION

Controversy concerning the efficacy of oral nitroglycerin therapy led us to measure the bioavailability of the parent nitroglycerin after oral administration of this drug as a solution. Nitroglycerin has a high-first pass effect and it is generally assumed that oral doses partially saturate the metabolic enzymes and that measurable concentrations of nitroglycerin are produced at the doses

tested. This does not seem to be the case with the three oral capsule formulations previously described in Chapter VI. Instead of administering a solid dosage form, a 6.5 mg dose as a solution was administered to healthy volunteers. The solution dose eliminates tablet dissolution variables and, as such, may give some indication if hepatic metabolism is saturable.

2. EXPERIMENTAL

Five subjects received oral solutions of nitroglycerin. One subject (P.N.) received a test dose of 3.2 mg nitroglycerin dissolved in distilled water. This dose was used for dose ranging and to test for any potential side effects. The remaining four subjects received 6.5 mg of nitroglycerin dissolved in 50 ml of distilled water. Each subject then drank an additional 50 ml of water. The latter four subjects were also given an intravenous nitroglycerin dose.

Venous blood samples were taken 0, 10, 20, 30 and 45 min and 1, 2, 3, 4, 6 and 8 hours after dosing. The plasma samples were analyzed for nitroglycerin using the capillary GC assay, capable of detecting 25 pg/ml, as described in Chapter IV.

3. RESULTS AND DISCUSSION

A test dose of nitroglycerin in solution was a necessary precaution since only solid formulations of nitroglycerin had been given orally in the past. The absence of nitroglycerin in plasma after oral capsule doses may have been due to a dissolution problem with these formulations. Therefore, a lower test dose of 3.2 mg nitroglycerin in solution was administered to one individual (P.N.) to ascertain that the dose of 6.5 mg would not produce intolerable nitroglycerin plasma concentrations. Nitroglycerin plasma concentrations were all less than the minimum measurable concentration, 25 pg/ml, in this subject.

Plasma concentrations of nitroglycerin in the four subjects receiving the 6.5 mg dose (solution) were less than 25 pg/ml for nearly all samples. Only very low concentrations of nitroglycerin were detected at 10 min in each of the volunteers (J.K., 0.18 ng/ml; M.D., 0.12 ng/ml; T.M., 0.025 ng/ml and D.D., 0.26 ng/ml). The mean nitroglycerin concentration at 10 min was 0.15 ± 0.10 (S.D.) ng/ml. Subject D.D. also showed a very low nitroglycerin concentration (0.049 ng/ml) at 20 min. These low concentrations are most likely due to sublingual absorption of a small amount of the drug during oral dosing. Similar concentrations at early times after dosing with nitroglycerin capsules were not detected, thus supporting the idea that these early drug levels may be due to

sublingual absorption.

Although significant concentrations of nitroglycerin were not present in plasma, peaks with the same retention times as the dinitrate-metabolites were observed in the chromatograms of plasma extracts following oral dosing. Figure VII-10 shows the time dependency of these peaks by plotting peak height ratio (to the nitroglycerin internal standard) versus time for subject P.N. Similar curves were obtained for each of the remaining four volunteers. These data indicate that nitroglycerin is not absorbed unchanged. The drug is extensively metabolized either in the gastrointestinal tract or the liver during first-pass. The pharmacokinetics of the nitroglycerin metabolites are discussed in greater detail in Chapter VIII.

4. SUMMARY

The oral bioavailability of nitroglycerin in solution is 0%. Unchanged nitroglycerin was detectable (0.15 ± 0.10 ng/ml) only at early times, most likely due to sublingual absorption of a small amount of the oral dose.

E. TRANSDERMAL ABSORPTION OF NITROGLYCERIN

1. INTRODUCTION

The pain of angina may be treated by administration of

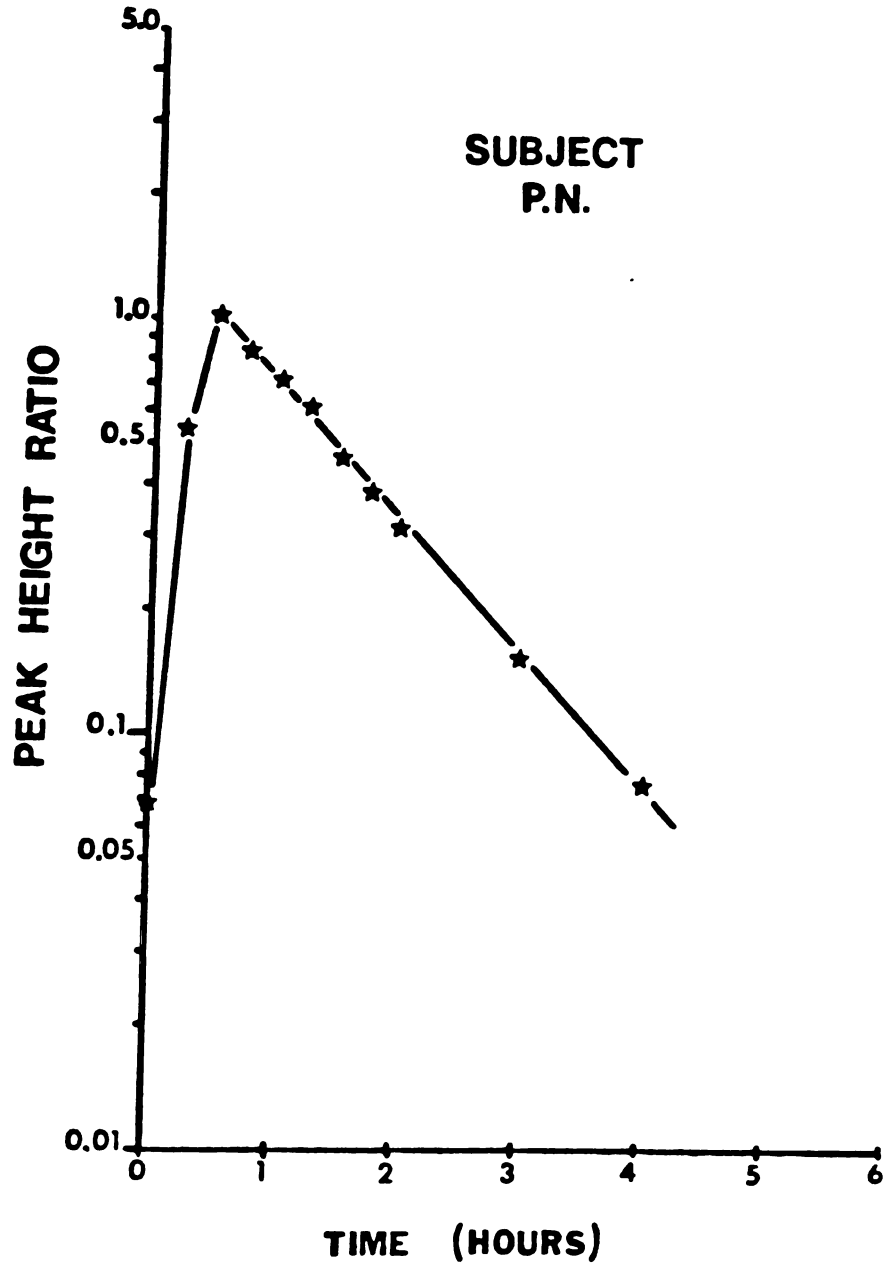


Fig. VII-10: Time dependent GC peak with the same retention time as GDN in plasma obtained from a subject administered 3.2 mg GTN as an oral solution.

sublingual nitroglycerin. Because the duration of action of a sublingual nitroglycerin dose is short, longer-acting dosage forms are necessary for prophylactic treatment. In addition to the oral doses previously discussed, transdermal drug doses offer the advantage of a sustained delivery over a longer period of time. Administration of nitroglycerin ointment has been shown to increase exercise capacity in patients with angina (Reichek et al., 1974; Davidov and Mroczck, 1976; Awan et al., 1978).

Several new nitroglycerin solid state transdermal delivery systems (patches) are currently used for treatment of angina (Dasta and Geraets, 1982). These delivery systems have been reported to yield sustained nitroglycerin concentrations in plasma (Gonzalez et al., 1982; Muller et al., 1982; Karim et al., 1981). However, the systemic bioavailability of transdermal doses (ointment and patches) has not been evaluated. Instead, relative bioavailability studies have been carried out in which transdermal plasma concentrations were compared to either sublingual or other transdermal doses.

This section describes a study in which the absolute bioavailabilities of three transdermal nitroglycerin dosage forms, Nitro-BID^{R}, NITRO-DUR^{R} and Nitrodisc^{R}, were evaluated. Nitro-BID^{R} is a 2% nitroglycerin ointment manufactured by Marion Laboratories. NITRO-DUR^{R} and Nitrodisc^{R} are controlled release nitroglycerin patches designed to release nitroglycerin at a constant rate for 24

hours, and are manufactured by Key Pharmaceuticals, Inc., and G.D. Searle and Co., respectively. The apparent transdermal absorption rates were determined from the amount of nitroglycerin absorbed and the time that the dose was in contact with the skin.

2. EXPERIMENTAL

In this study, each of five healthy male volunteers received topical doses of nitroglycerin. Three subjects (D.D.,T.M. and M.D.) each received Nitro-BID^{R}, Nitrodisc^{R} and Nitro-DUR^{R}. One subject (R.D.) received Nitro-BID and Nitrodisc^{R} and one subject (J.K) received only Nitro-Dur^{R}.

The Nitro-BID^{R} dose (one gram of ointment, equivalent to 20 mg of GTN) was spread evenly over a 200 square centimeter surface area of the chest. One subject (T.M.) received an additional dose (at an earlier time) which was applied to a 200 square centimeter area surface of the upper inner arm. After application of each treatment, a square of aluminum foil was taped over the application site and the entire area was secured with gauze strips. Serial blood samples were usually collected and processed at 0, 0.25, 0.5, 1, 2, 3, 4 and 5 hours. Some samples were taken at times different from the above because the volunteers were exhibiting symptoms of hypotension (one subject was dropped from the study due to severe hypotension and 2 volunteers exhibited dizziness and some postural hypotension). At the

end of five hours, the occlusive dressing was removed and the application site was washed with soap and water. Additional blood samples were drawn at 15, 30, 45 and 60 min after the dose was removed.

The Nitrodisc^{R} patch (32 mg, 16 square centimeters) was applied to the chest. Serial blood samples were collected at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 23 and 24 hours after the patch was initially applied. At the end of 24 hours, the application site was washed with mild soap and water. Additional blood samples were drawn at 15, 30, 45 and 60 min after the dose was removed.

The Nitro-DUR^{R} patch (104 mg, 20 square centimeters) was applied to the anteromedial surface of the upper inner arm. The patient package insert for this product recommends that the patient apply the patch to either the chest or upper-inner arm. Serial blood samples were collected as with the Nitrodisc^{R} dose. Additional blood samples were drawn at 10, 20, 30, 60 and 120 min after the patch was removed.

Each subject also received a nitroglycerin intravenous infusion. All four NITRO-DUR^{R} doses were given as part of the four-way random crossover study. The order of dose administration is shown in Table VII-12. The intravenous infusions are discussed in detail in Section B of this chapter.

TABLE VII-12: Order of drug (topical and intravenous) administration.

Subject	Nitro-BID	Nitro-Dur	Nitrodisc	I.V.
R.D.	2	-	3	1
J.K.	-	2	-	1
M.D.	1	4	2	3
T.M.	1,2	4	3	5
D.D.	1	4	2	3

3. RESULTS AND DISCUSSION

After administration of NITRO-BID ointment, plasma concentrations of 0.35 to 4.40 ng/ml were detected (Figs. VII-11). Plasma concentrations ranged from 0.025 to 0.84 ng/ml after administration of NITRO-DUR and Nitrodisc patches (Figs. VII-12 and 13). Bioavailabilities could be calculated from these data, using the intravenous dose as a reference. However, bioavailability is less meaningful since these dosage forms are designed to contain a large reservoir of drug which is not absorbed. Instead, the amount of nitroglycerin absorbed was determined using the following basic pharmacokinetic relationship:

$$\text{Amount Absorbed} = F \times \text{Dose}_{topical} = CL_{iv} \times AUC_{topical}$$

where the amount of drug absorbed is directly proportional to the clearance, determined from the intravenous infusion, and the transdermal AUC.

The amount of nitroglycerin absorbed (Table VII-13) after administration of Nitro-BID, NITRO-DUR and Nitrodisc was 4.68 ± 1.99, 3.88 ± 3.04 and 1.65 ± 0.41 mg, respectively, based on the clearances determined from the highest infusion rate. It is difficult to compare the amounts of drug absorbed since the doses were left in contact with the skin for varying periods of time. This time variable was used to determine the average in vivo

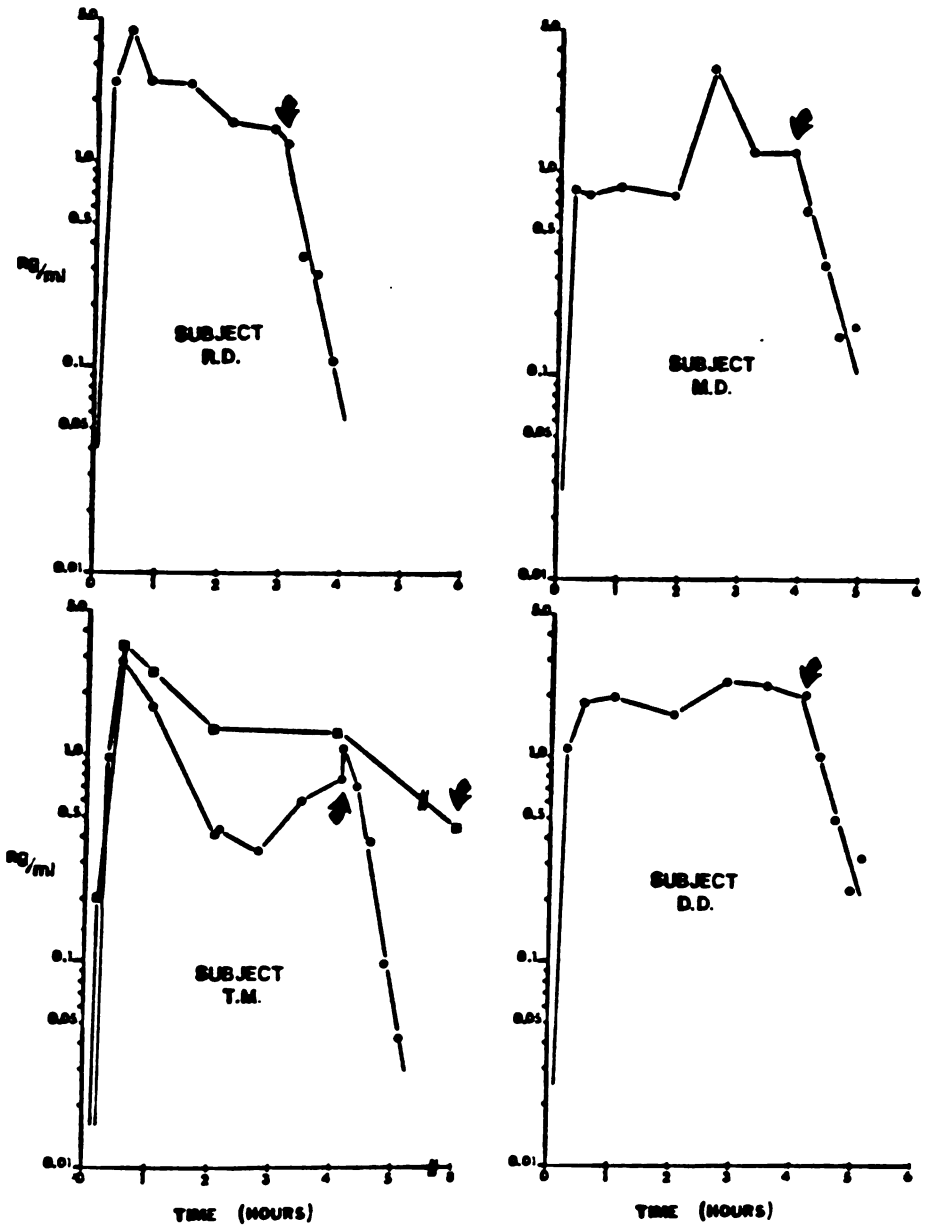


Fig. VII-11: Plasma nitroglycerin concentrations in four subjects administered 20 mg GTN as a topical ointment, spread over over 200 sq cm. Arrows indicate the time at which the ointment was removed. On another occasion subject T.M. received an identical dose which was left on the skin for 8 hours.

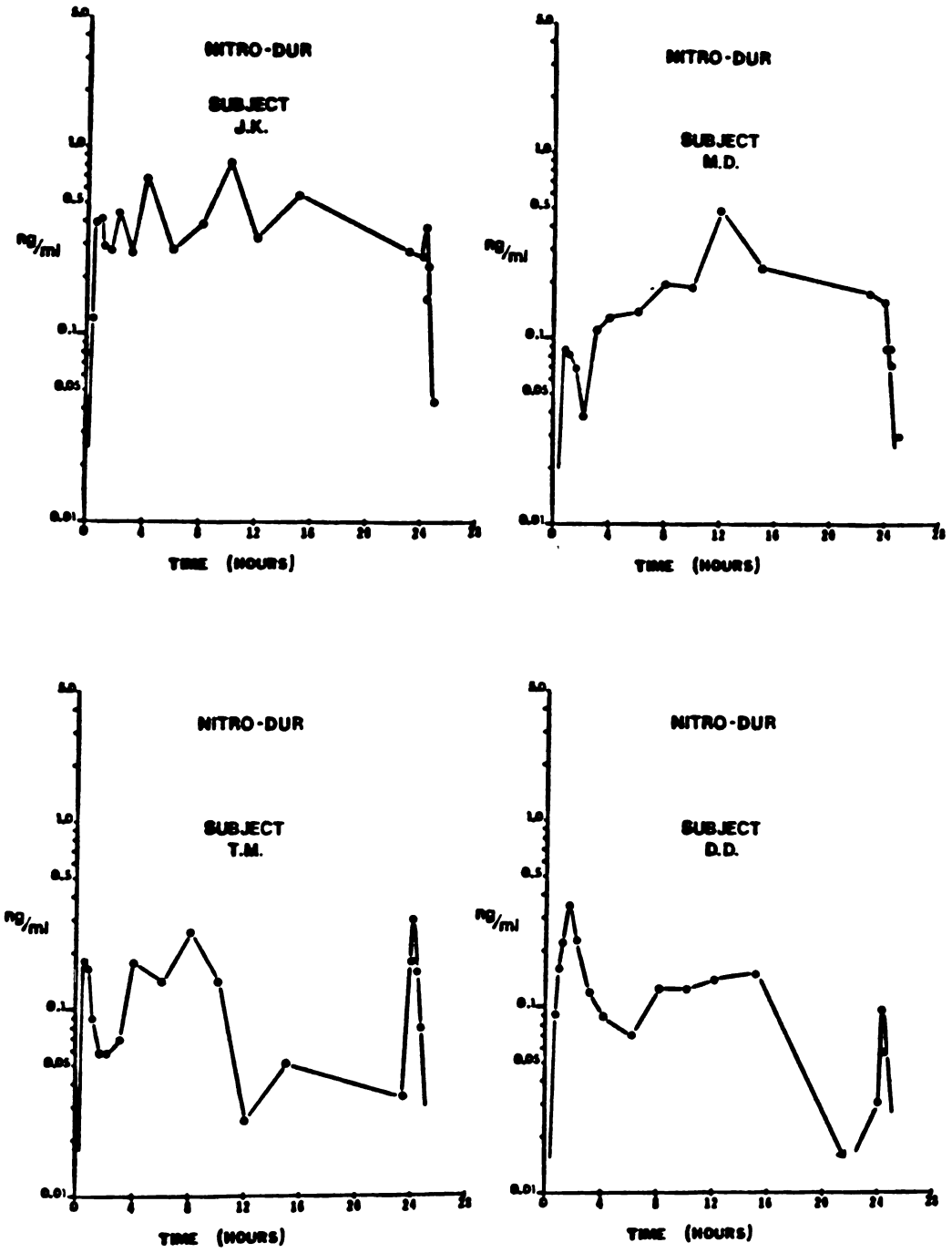


FIG. VII-12: Plasma nitroglycerin concentrations after administration of Nitro-dur^{R} (20 cm^{2}) to four subjects. The dose was left in place for 24 hours.

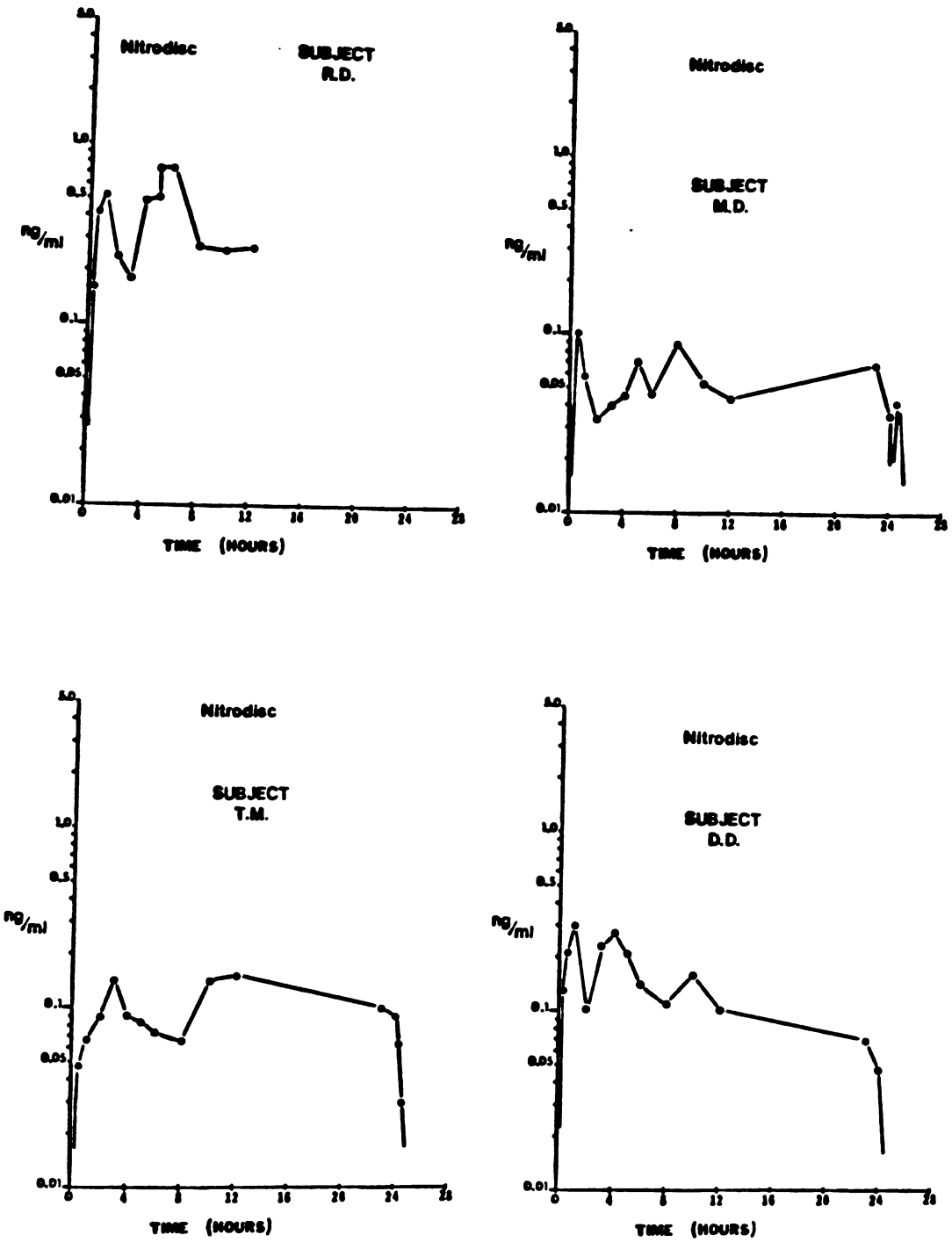


FIG. VII-13: Plasma nitroglycerin concentrations after administration of Nitrodisc^{R} (16 cm^{2}) to each of four volunteers. Doses were left in place for 24 hours, except for subject R.D. who withdrew from the study at 14 hours.

TABLE VII-13: **Amount of nitroglycerin absorbed through the skin after administration of ointment and two transdermal products (Nitro-Dur and Nitrodisc). The time intervals during which the dose was in contact with skin is shown in parentheses.**

	Amount GTN Absorbed (mg)		
Volunteer	Nitro-BID[a]	Nitro-Dur[b]	Nitrodisc[c]
D.D.	3.94 (4.2)	1.17 (24.0)	1.32 (24.0)
M.D.	5.45 (3.9)	4.76 (24.0)	1.29 (24.0)
T.M.	3.38 (4.1)	1.82 (24.0)	2.12 (24.0)
R.D.	2.86	-	1.89 (24.0)
J.K.	-	7.79 (24.0)	-

[a] 20 mg, 200 cm^2 , occluded with aluminum foil.

[b] 104 mg, 20 cm^2

[c] 32 mg, 16 cm^2

absorption rates for transdermal nitroglycerin in each individual. These rates, normalized for surface area, are shown in Table VII-14. The mean absorption rates, for these three transdermal dosage forms, are not significantly different from each other.Note from Table VII-14 that only 3 subjects received all 3 doses. The differences in mean absorption rates of only these 3 subjects were even smaller. These rates were 5.26 ± 1.49, 5.37 ± 3.98 and 4.11 ± 1.21 for Nitro-BID, NITRO-DUR and Nitrodisc, respectively.

Although only a limited number of subjects were studied, it may be concluded that absorption of nitroglycerin through the skin is the rate limiting step of the transdermal delivery process, whether delivered from an ointment or a "controlled-release patch." It is also interesting to note that the transdermal patches show a higher intersubject variability (54.8% and 78.4% C.V.) than does the ointment (20.8% C.V.). The higher variability would be expected since the dose was released over a much longer period of time with the solid state delivery systems.

Figure VII-14 shows the average plasma concentrations, as reported by Karim (1983), which seem to remain constant over at least a 24-hour period. These data suggest that the transdermal absorption of nitroglycerin will yield steady-state concentrations of 300 pg/ml, similar to that which would be obtained from a zero-order intravenous infusion. Note that the figure shows average concentrations of 300 ± 100 pg/ml (standard error of the mean). If standard

TABLE VII-14: Nitroglycerin transdermal absorption rates, normalized for skin surface area to which the doses were applied.

	Absorption Rate (mcg/hr/cm2)		
Volunteer	Nitro-BID	Nitro-Dur	Nitrodisc
D.D.	4.72	2.43	3.45
M.D.	6.95	9.90	3.37
T.M.	4.12	3.78	5.51
T.M.	4.87		
R.D.	5.08	-	9.86
J.K.	-	16.3	-
Mean	5.15	8.09	5.55
± SD	1.07	6.35	3.04
% CV	20.8%	78.4%	54.8%

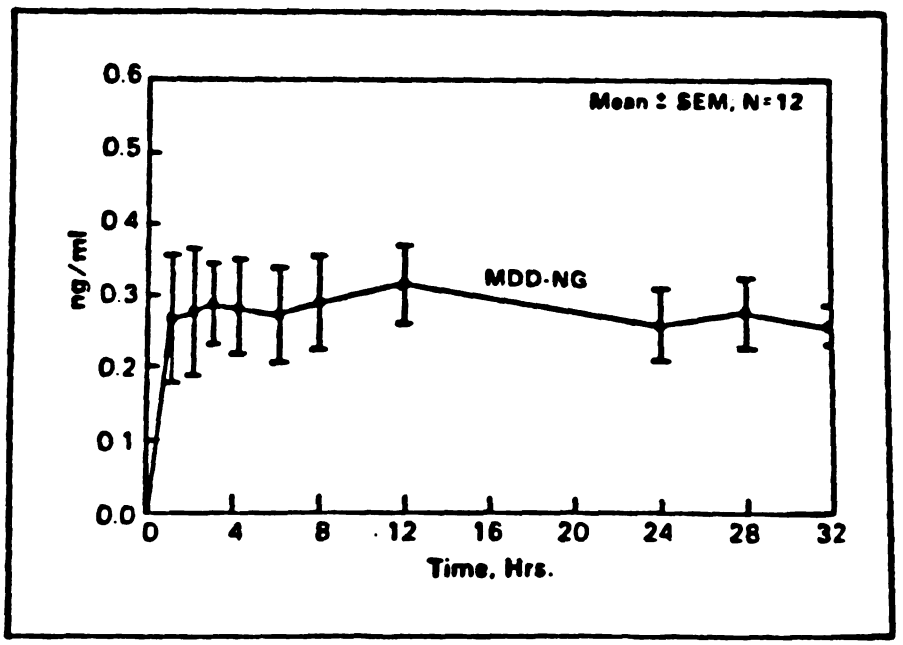


FIG. VII-14: Average Nitrodisc nitroglycerin plasma concentrations reported by Karim (1983).

deviations for these data were calculated and plotted, the average concentration would be 300 ± 300 pg/ml. Thus, the presentation of only mean data can be quite misleading since both inter- and intraindividual variability are lumped together. Although average nitroglycerin concentrations over 24 hours may be constant, the concentrations in individual patients may potentially fluctuate during the day to levels below some "minimum therapeutic concentration." As can be seen in Figures VII-12 and 13, both the inter- and intrasubject variablility can be quite significant and plasma nitroglycerin concentrations may decrease to very low concentrations during the day (or night). Figure VII-15 shows the average nitroglycerin plasma concentrations (and standard error bars) of NITRO-DUR and Nitrodisc. The intra-subject variability in plasma concentrations is obscured when the data are plotted in this manner. Data should be presented for individual subjects rather than as mean data.

4. CONCLUSIONS

The bioavailability of transdermal nitroglycerin was determined in healthy human volunteers. Based on the highest intravenous infusion rate, the amounts of nitroglycerin absorbed over the dosing interval were determined. The apparent absorption rates ($mcg/hr/cm^2$) were determined for Nitro-BID ointment (5.15 ± 1.07), NITRO-DUR (8.09 ± 6.35) and Nitrodisc (5.55 ± 3.04). Since the

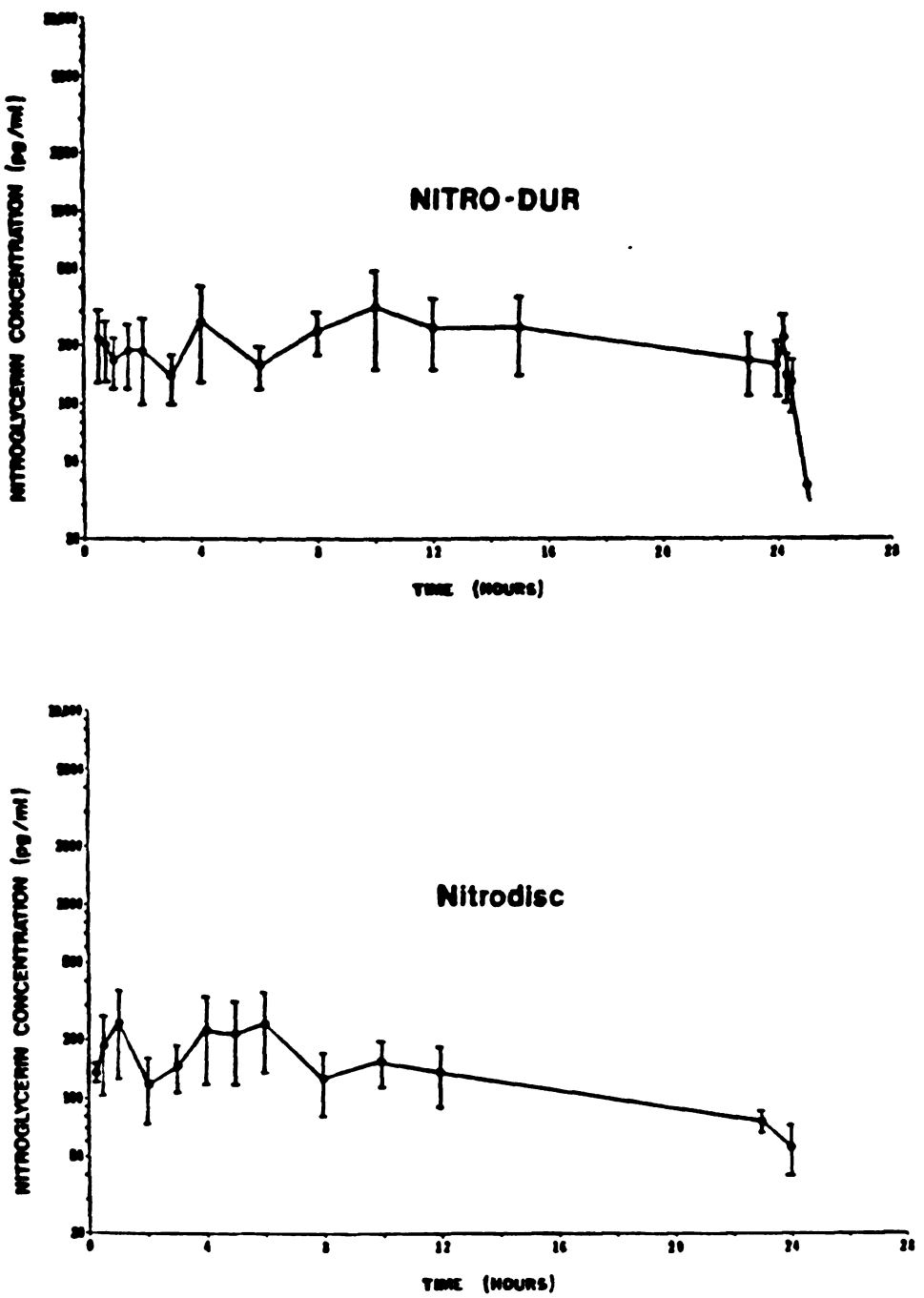


FIG. VII-15: Average nitroglycerin plasma concentrations from doses of Nitro-dur (n=4) and Nitrodisc (n=4).

transdermal absorption rates of the three nitroglycerin formulations were equivalent, it was concluded that drug absorption through the skin is the rate-limiting step in the transdermal delivery process. Because significant intra-individual variations in plasma concentrations, during a single transdermal dose, were observed, individual transdermal data, rather than mean data, should be presented.

CHAPTER VIII

PHARMACOKINETICS OF 1,2- AND 1,3-GLYCERYLDINITRATES AFTER INTRAVENOUS INFUSION AND ORAL, TRANSDERMAL AND SUBLINGUAL NITROGLYCERIN ADMINISTRATION.

A. INTRODUCTION

In Chapter VII the pharmacokinetics of nitroglycerin in healthy volunteers after intravenous, oral, transdermal and sublingual administration of nitroglycerin were described. The data presented in Chapter VII (and Chapter VI) showed that oral nitroglycerin is not bioavailable. However, the efficacy of oral nitroglycerin has been demonstrated (Chapter II). After oral doses of nitroglycerin, relatively high concentrations of the dinitrate metabolites are present in plasma. Although the pharmacologic activity of these metabolites is lower than that of the parent drug, the relatively high concentrations of the GDNs present in plasma may be responsible for the oral activity of nitroglycerin and may also contribute to the activity of nitroglycerin when administered by other routes.

In the present chapter the pharmacokinetics of the dinitro-metabolites following nitroglycerin administration in some of these subjects is presented. Plasma samples, previously analyzed for nitroglycerin, were again analyzed to determine concentrations of 1,2-glyceryldinitrate (1,2-

GDN) and 1,3-glyceryldinitrate (1,3-GDN) using the capillary GC assay described, in detail, in Chapter IV. Plasma concentrations of 1,2-GDN and 1,3-GDN were assayed for four individuals who received intravenous infusions, and topical and oral doses of nitroglycerin. Plasma concentrations of dinitrate metabolites were also measured in one individual who received a sublingual dose of nitroglycerin.

B. RESULTS

1. PHARMACOKINETICS OF 1,2-GDN AND 1,3-GDN AFTER INTRAVENOUS INFUSIONS TO HEALTHY VOLUNTEERS.

Nitroglycerin was administered as an intravenous infusion to four subjects (J.K., M.D., T.M. and D.D.). Each subject received four consecutive infusions of nitroglycerin. The infusion rates administered were 10, 20, 40 and again 10 mcg/min (described in detail in Chapter VII).

Figures VIII-1 to VIII-4 show plasma concentrations of nitroglycerin, 1,2-GDN and 1,3-GDN during and after each infusion. Concentrations of 1,2-GDN were 5- to 15- fold greater than the parent drug in the plasma samples. Concentrations of 1,3-GDN were generally lower than nitroglycerin (10 to 90 percent of the parent drug). The concentrations of 1,2-GDN were always higher than those of 1,3-GDN. The ratio of 1,2-GDN to 1,3-GDN was constant

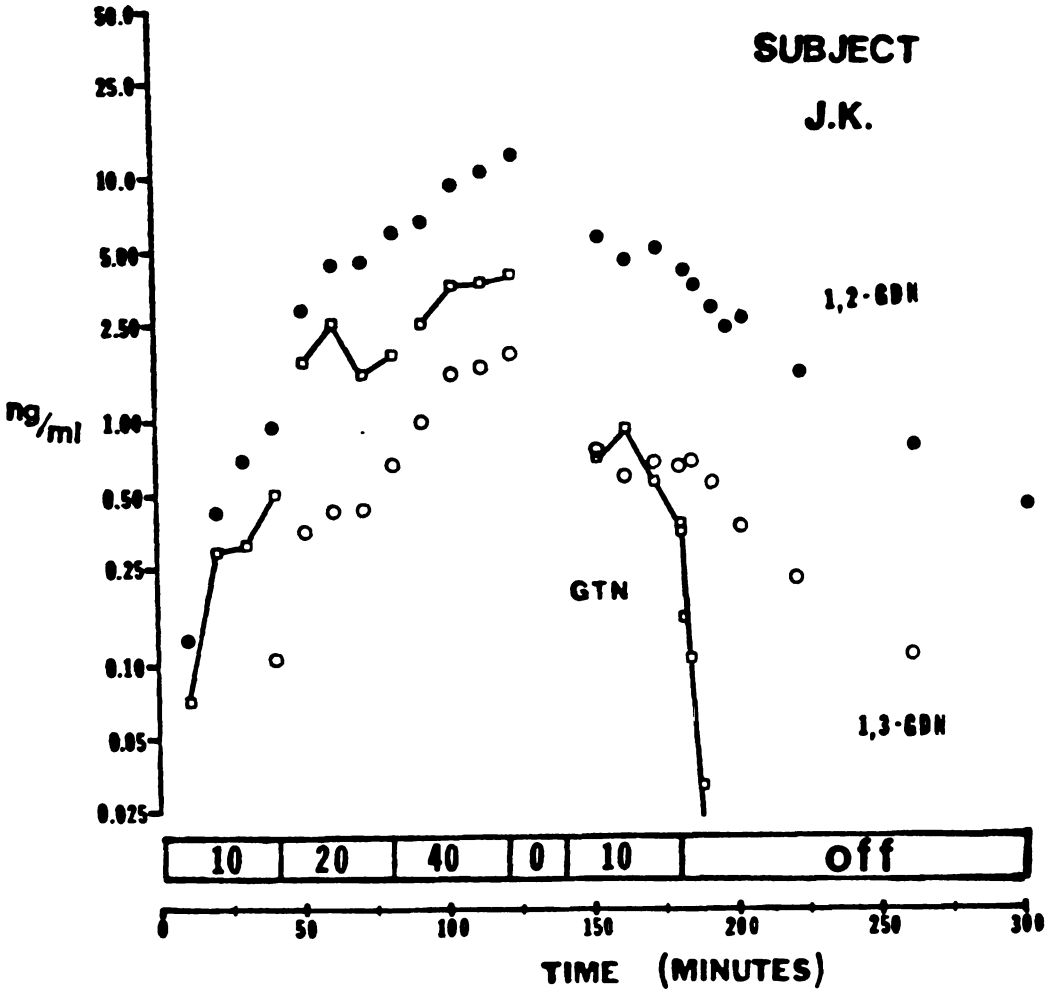


Fig. VIII-1: Plasma concentrations of nitroglycerin, 1,2-GDN and 1,3-GDN in subject J.K. during and after intravenous nitroglycerin infusions.

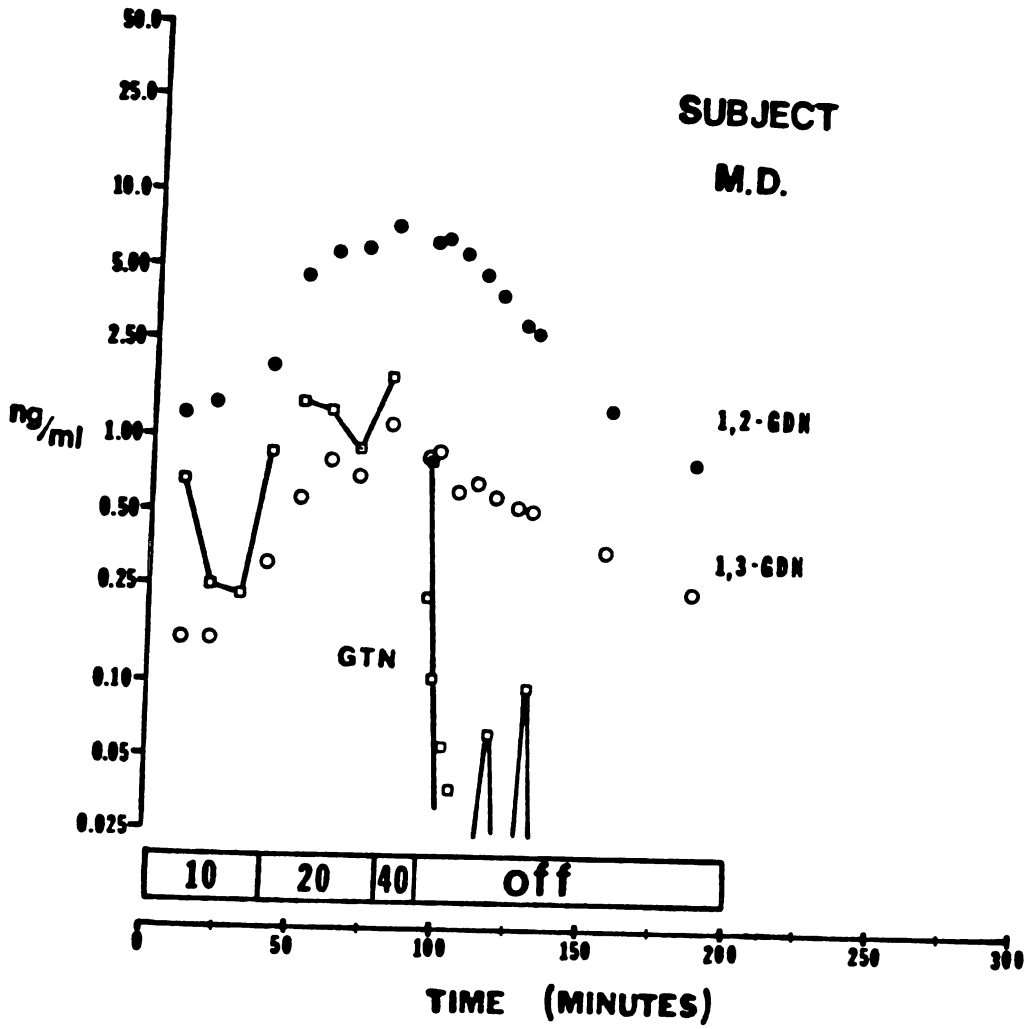


Fig. VIII-2: Plasma concentrations of nitroglycerin, 1,2-GDN and 1,3-GDN in subject M.D. during and after intravenous nitroglycerin infusions.

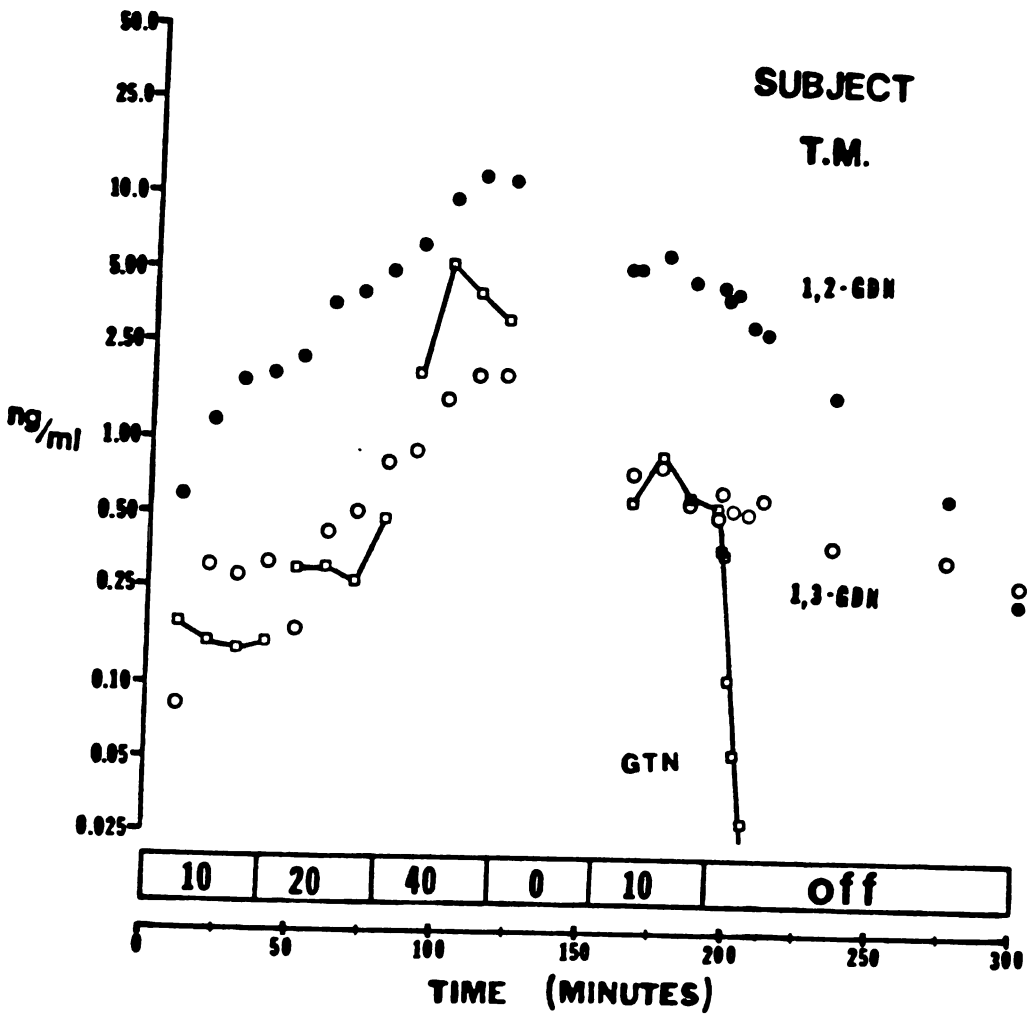


Fig. VIII-3: Plasma concentrations of nitroglycerin, 1,2-GDN and 1,3-GDN in subject T.M. during and after intravenous nitroglycerin infusions.

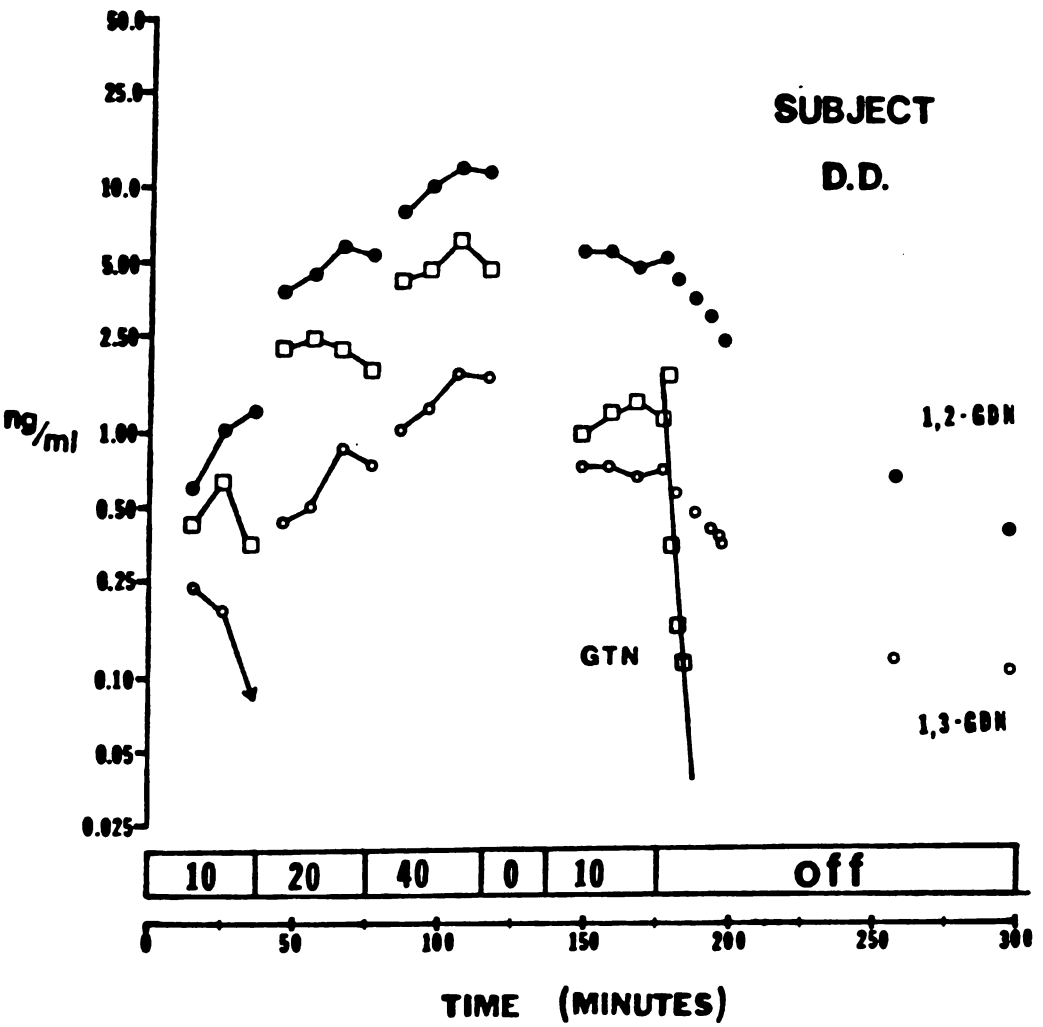


Fig. VIII-4: Plasma concentrations of nitroglycerin, 1,2-GDN and 1,3-GDN in subject D.D. during and after intravenous nitroglycerin infusions.

during the infusion period. The ratios for the individual subjects (mean ± standard deviation) were 7.83 ± 1.34, 7.76 ± 1.12, 7.34 ± 1.89 and 7.02 ± 1.51 for subjects J.K., M.D., T.M. and D.D., repectively, during the infusion periods.

Table VIII-1 shows the ratios of 1,2-GDN to 1,3-GDN for each subject at all sampling times during the infusion. The 1,2-GDN/1,3-GDN ratio decreased as a function of time (in three of the four volunteers) after the nitroglycerin infusion was terminated. These ratios, plotted as a function of time, are shown in Fig. VIII-5. Although the relationship between the 1,2-GDN/1,3-GDN ratio and time may not be linear, these ratios do appear to be decreasing with time.

After intravenous administration of nitroglycerin, the apparent elimination half-lives of 1,2- and 1,3-GDN were calculated to be 33.2 ± 6.5 and 57.2 ± 30.0 min, respectively. Because the half-life of nitroglycerin was 10-fold shorter than these half-lives, estimated from the terminal portion of the plasma concentration-time curve, these longer half-lives represent the true elimination half-lives of the metabolites.

Figures VIII-1 to 4 also show that dinitrate metabolite concentrations did not reach steady-state during the first three infusion periods. However, the dinitrate metabolites reached an apparent steady-state during the final 10 mcg/min infusion. The concentration ratio of each dinitrate metabolite (1,2-GDN and 1,3-GDN) to nitroglycerin was

TABLE VIII-1: Ratios of 1,2-GDN to 1,3-GDN plasma concentrations during intravenous infusions to healthy volunteers.

Infusion Rate (mcg/min)	Plasma Sample	(1,2-GDN/1,3-GDN)Ratio			
		J.K.	M.D.	T.M.	D.D.
10	1	-	8.40	7.28	-
10	2	-	9.20	4.03	2.50
10	3	-	-	6.25	5.49
10	4	9.00	6.28	5.94	-
20	5	7.97	8.07	12.82	8.64
20	6	9.96	7.22	8.71	8.44
20	7	10.13	8.67	7.87	6.65
20	8	9.01	6.50	6.17	7.11
40	9	6.64	-	7.02	7.72
40	10	6.11	-	6.66	7.80
40	11	6.49	-	6.71	6.98
40	12	6.79	-	6.32	7.03
10	13	7.60	-	6.90	7.60
10	14	7.83	-	7.53	7.64
10	15	7.71	-	8.23	7.41
10	16	6.58	-	8.96	7.24
	Mean	7.83	7.76	7.34	7.02
	± SD	1.34	1.12	1.89	1.51

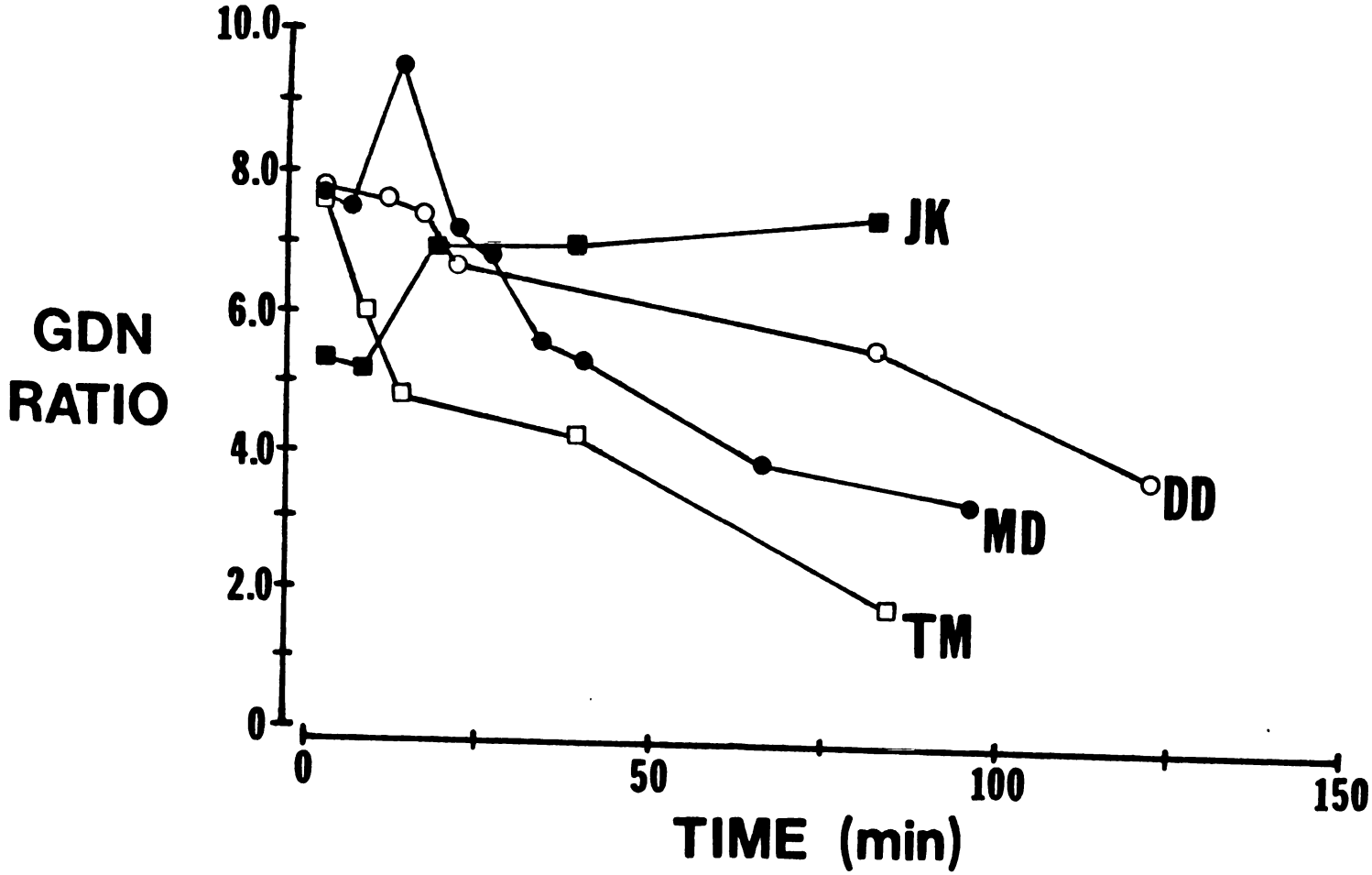


Fig. VIII-5: Relationship of the 1,2-GDN/1,3-GDN ratio to the time after cessation of intravenous infusions in four subjects.

calculated only during this period of apparent steady-state. These ratios are shown in Table VIII-2. The 1,2-GDN/GTN ratios varied between 4.58 - 8.27, while the 1,3-GDN/GTN ratios varied between 0.62 - 1.13, during this same time interval.

2. PHARMACOKINETICS OF 1,2-GDN AND 1,3-GDN AFTER ORAL ADMINISTRATION.

Nitroglycerin was administered as an oral solution containing 6.5 mg nitroglycerin dissolved in distilled water (see Chapter VII for details). Plasma samples were assayed to determine concentrations of 1,2-GDN and 1,3-GDN.

Figure VIII-6 shows plasma concentrations of these dinitrate metabolites in four subjects after oral dosing. Plasma concentrations of 1,2-GDN were always greater than 1,3-GDN (except in subject T.M., at later time points, e.g., 4.0 hr). The ratio of 1,2-GDN to 1,3-GDN was approximately 2.0 after oral doses (2.12 ± 0.20, 2.07 ± 0.29, 1.70 ± 0.34 and 1.92 ± 0.27 in subjects J.K., M.D., T.M. and D.D., respectively). The individual 1,2-GDN to 1,3-GDN ratios are summarized in Table VIII-3.

It is important to note that the parent drug (nitroglycerin) was not systemically available after oral doses, but, high concentrations of the dinitrate metabolites were detectable in plasma. Peak 1,2-GDN and 1,3-GDN concentrations of 19.8 to 31.0 and 8.6 to 16.0 ng/ml,

Table VIII-2: Summary of the 1,2-GDN/GTN and 1,3-GDN/GTN ratios at apparent steady-state GDN concentrations during the second 10 mcg/min (GTN) intravenous infusion to healthy volunteers.

	1,2-GDN/GTN Ratio			1,3-GDN/GTN Ratio		
	Subject			Subject		
	J.K.	T.M.	D.D.	J.K.	T.M.	D.D.
	8.01	9.00	5.50	1.05	1.31	0.72
	4.90	6.79	4.59	0.62	0.90	0.60
	9.03	7.84	3.62	1.17	0.95	0.49
	11.13	8.18	4.59	1.69	0.91	0.67
Mean	8.27	7.95	4.58	1.13	1.02	0.62
± SD	2.59	0.92	0.77	0.44	0.20	0.10
% CV	31%	12%	17%	39%	20%	16%

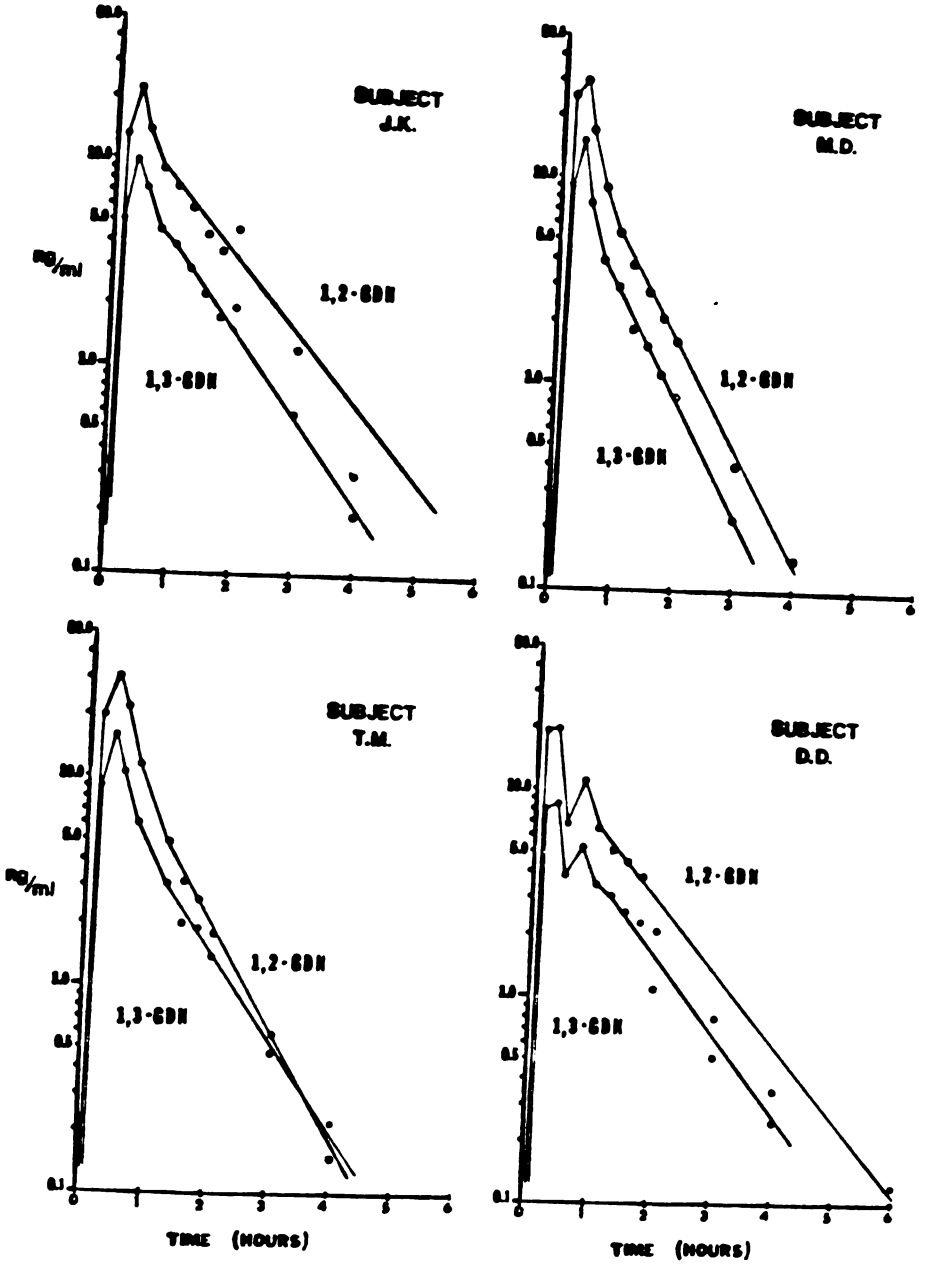


Fig. VIII-6: Plasma concentrations of 1,2-GDN and 1,3-GDN after oral administration of 6.5 mg nitroglycerin to four healthy volunteers.

TABLE VIII-3: Ratios of 1,2-GDN/1,3-GDN attained after oral GTN doses to volunteers.

Time (min.)	SUBJECT J.K.	M.D.	T.M.	D.D.
10	2.53	2.71	2.17	2.36
20	2.28	2.09	1.94	2.31
30	1.95	2.29	2.03	1.79
45	1.98	2.25	1.95	2.16
60	1.95	1.84	-	1.89
75	2.04	2.14	1.63	1.66
90	1.94	1.88	1.58	1.80
105	2.10	1.88	1.38	1.72
120	2.36	1.83	1.25	1.91
180	2.07	1.82	1.33	1.56
Mean	2.12	2.07	1.70	1.92
± SD	0.20	0.25	0.34	0.27

respectively, were found at 20 min in all subjects (Table VIII-4).

The apparent elimination half-lives, estimated from the terminal portion of the plasma concentration-time curves, were 44.1 ± 8.3 (range: 36.4 - 52.4) and 42.6 ± 10.6 (range: 26.9 - 50.0) min for 1,2-GDN and 1,3-GDN, respectively (Table VIII-4). There was no significant difference between the half-lives obtained after oral and after intravenous nitroglycerin doses. Therefore, these half-lives may represent the true elimination half-lives of the dinitrate metabolites.

3. PHARMACOKINETICS OF 1,2-GDN AND 1,3-GDN AFTER TOPICAL ADMINISTRATION OF NITROGLYCERIN OINTMENT.

Nitroglycerin was administered as a topical ointment dose to each of four subjects (see Chapter VII for dosing details). Plasma samples were assayed for both 1,2-GDN and 1,3-GDN.

Generally, concentrations of both dinitrate metabolites were greater than that of the parent nitroglycerin. Concentrations of 1,2-GDN were always greater than those of 1,3-GDN. The plasma concentration versus time curves for the metabolites are shown in Figure VIII-7. Table VIII-5 summarizes the ratios of 1,2-GDN to 1,3-GDN in these four subjects after topical administration of nitroglycerin. Because two of the four subjects experienced postural

TABLE VIII-4: Summary of GDN pharmacokinetic parameters after oral administration to volunteers.

Subject	Peak Time (min)	1,2-GDN C_{peak} (ng/ml)	1,2-GDN $t_{1/2}$ (min)	1,3-GDN C_{peak} (ng/ml)	1,3-GDN $t_{1/2}$ (min)
J.K.	20	22.36	52.4	9.79	46.3
M.D.	20	30.28	37.5	14.48	26.9
T.M.	20	31.00	36.4	16.02	50.0
D.D.	20	19.71	50.2	8.55	47.0
Mean	20	25.84	44.1	12.21	42.6
± SD	0	5.66	8.3	3.60	10.6

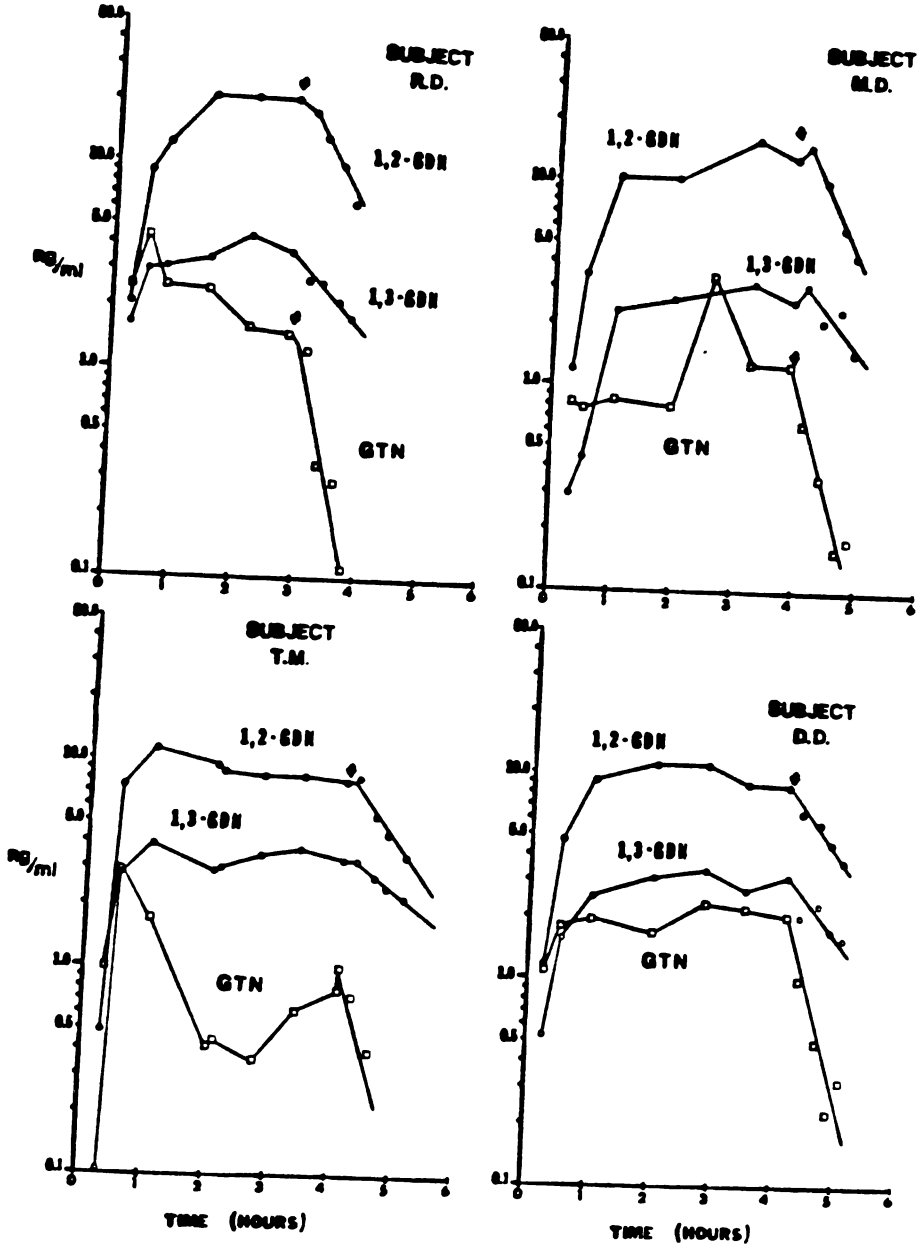


Fig. VIII-7: Plasma concentrations of 1,2-GDN and 1,3-GDN after topical administration of 20 mg nitroglycerin in ointment to four healthy volunteers.

TABLE VIII-5: Ratios of 1,2-GDN/1,3-GDN after topical ointment doses of GTN to healthy volunteers at variable times during dose application and at four uniform time points after the dose was removed.

	SUBJECT			
	R.D.	M.D.	T.M.	D.D.
	3.02	4.00	4.80	3.10
	4.06	7.93	2.67	3.67
	6.28	4.60	2.96	3.56
	4.69	3.97	3.25	3.33
	5.65	5.16	2.91	3.32
		5.18	2.44	2.81
			2.30	
			2.43	
Mean	4.74	5.14	2.97	3.30
± SD	1.29	1.47	0.81	0.31
Time After Dose Removed (min)				
15	6.67	4.98	2.56	3.25
30	5.21	4.87	1.98	2.52
45	4.70	2.53	1.84	2.70
60	3.57	3.09	1.59	2.38

hypotension (and a fifth subject dropped out of the study due to severe hypotension), the duration of this study was reduced to 4 hours. Blood samples were drawn at variable times, and therefore, common sample times are not available for these subjects. The 1,2-GDN/1,3-GDN ratios (3.0 to 5.1) were intermediate between those for the oral dose (i.e., 2.0) and the intravenous dose (i.e., approx. 7.0). It is also interesting to note that the 1,2-GDN/1,3-GDN ratio apparently decreased with time after the nitroglycerin dose was removed from each subject (Table VIII-5). This apparent decrease was similar to that observed during the elimination of the intravenous nitroglycerin dose.

The apparent half-lives of 1,2-GDN and 1,3-GDN were 35.8 ± 10.3 and 67.3 ± 11.7 min, respectively, after topical doses of nitroglycerin ointment. These half-lives were not significantly different from those after intravenous administration of nitroglycerin.

4. PHARMACOKINETICS OF 1,2-GDN AND 1,3-GDN AFTER SUBLINGUAL NITROGLYCERIN ADMINISTRATION.

Plasma concentrations of 1,2-GDN and 1,3-GDN were determined in one subject who received a 0.4 mg sublingual dose of nitroglycerin. The plasma concentrations of nitroglycerin and the two dinitrate metabolites are shown in Figure VIII-8. Note that metabolite concentrations peaked at 8 min (when the dose was washed out of the subject's

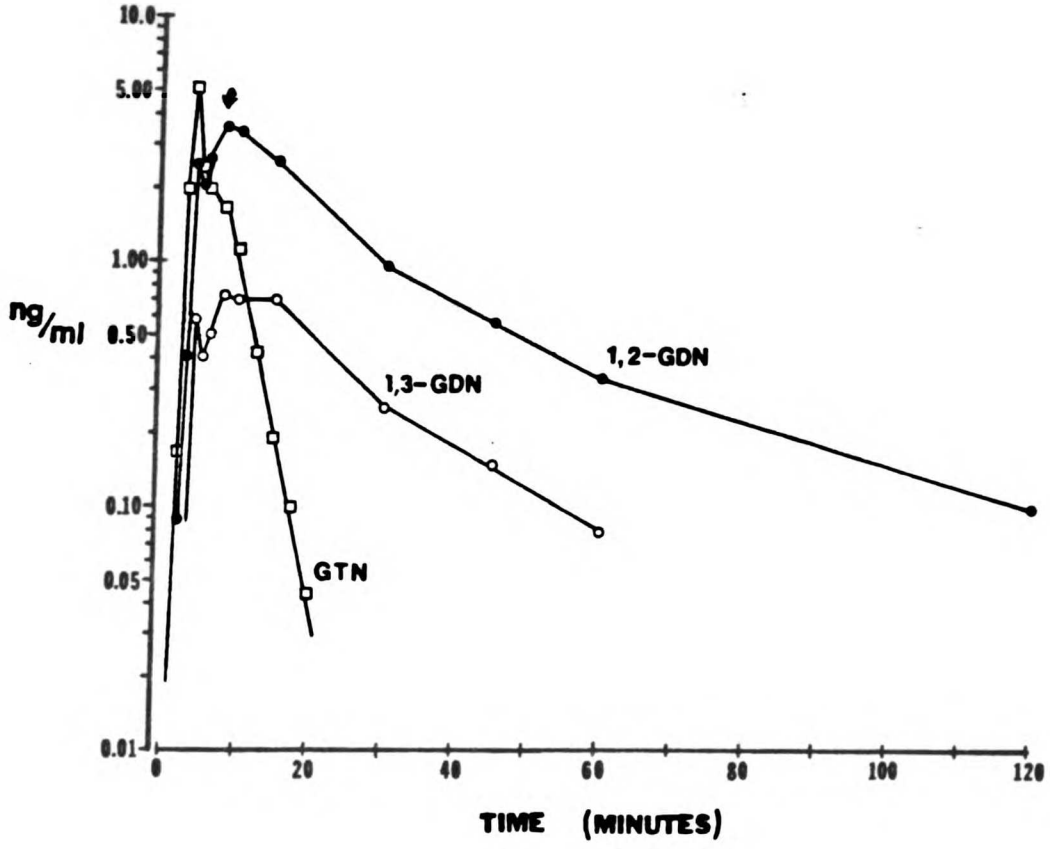


FIG. VIII-8: Plasma concentrations of nitroglycerin, 1,2-GDN and 1,3-GDN after sublingual administration of 0.4 mg nitroglycerin to one volunteer.

TABLE VIII-6: Ratio of plasma concentrations of 1,2-GDN/1,3-GDN after sublingual GTN administration to subject J.K.

Time(min)	Ratio
4.0	4.38
5.0	5.12
6.0	5.46
8.0	4.89
10.0	4.93
15.1	3.73
30.6	3.74
45.1	3.97
60.0	4.33
Mean	4.51
±SD	0.63

mouth), compared to the 4 min peak time for the parent drug. Peak concentrations for 1,2-GDN and 1,3-GDN were 3.58 and 0.73 ng/ml, respectively.

The ratios of 1,2-GDN to 1,3-GDN, after sublingual administration of nitroglycerin to subject J.K. are shown in Table VIII-6. The mean ratio for these two metabolites was 4.51 ± 0.63, which was similar to ratios obtained after ointment doses but higher than after oral doses. Interestingly, this ratio did not decrease with time as seen after oral and topical doses. The terminal half-lives for 1,2-GDN and 1,3-GDN, determined from the last three data points in each curve, were 32.3 and 17.4 min, respectively. These estimates may be underestimates of the true half-lives of the metabolites due to sampling during the distribution phase (i.e., the metabolite pharmacokinetics may be described by an apparent two-compartmental model.

C. SUMMARY AND CONCLUSIONS

It is important to emphasize that these pharmacokinetic studies, in which the dinitrate metabolites of nitroglycerin were measured, were not designed to elucidate the pharmacokinetic parameters of the metabolites but rather nitroglycerin pharmacokinetics. Metabolite concentrations in plasma were measured only after we realized that our method allowed us to measure these compounds.

The pharmacokinetic parameters of nitroglycerin

metabolites in humans can not be determined without administering these metabolites in pure form to human volunteers or patients. Such clinical studies require the approval of government regulatory agencies (i.e., the Food and Drug Administration). With these restrictions in mind, however, several further comments may be made.

First, after oral administration of nitroglycerin solutions to volunteers, high concentrations of 1,2-GDN and 1,3-GDN were detectable. The metabolite concentrations peaked at 20 min and were measureable for 4 hours. These data are quite interesting because oral nitroglycerin was not bioavailable, yet high concentrations of potentially active metabolites were present. The efficacy of oral nitroglycerin doses has been established (Winsor and Berger, 1975). However, this efficacy has not yet been correlated with plasma concentrations of either nitroglycerin or the dinitratemetabolites. Based on the data presented in Chapters VII and VIII, the efficacy may be related to metabolite concentrations.

Secondly, estimates of the half-lives of 1,2- and 1,3-GDN were obtained (Table VIII-7). After intravenous administration of nitroglycerin, the apparent elimination half-lives of 1,2- and 1,3-GDN were determined to be 33.2 ± 6.5 and 57.2 ± 30.0 min, respectively. The half-life of nitroglycerin was 10-fold shorter. The long half-lives represent the true elimination half-lives of the metabolites. The longer half-life implies a longer time to

Table VIII-7. Summary of 1,2-GDN and 1,3-GDN half-lives after intravenous, oral, topical and sublingual nitroglycerin administration to healthy volunteers.

		Metabolite Half-Lives (min)			
Subject	Metabolite	Oral	I.V.	Topical	Sublingual
J.K.	1,2-GDN	52.4	42.0	-	32.3
	1,3-GDN	46.3	30.2	-	17.4
M.D.	1,2-GDN	37.5	29.4	25.9	-
	1,3-GDN	26.9	52.8	51.2	-
T.M.	1,2-GDN	36.4	27.2	36.5	-
	1,3-GDN	50.0	99.8	76.8	-
D.D.	1,2-GDN	50.2	34.1	49.8	-
	1,3-GDN	47.0	45.8	66.4	-
R.D.	1,2-GDN	-	-	30.9	-
	1,3-GDN	-	-	74.9	-
Mean	1,2-GDN	44.1	33.2	35.8	32.3
(± SD)		8.3	6.5	10.3	-
Mean	1,3-GDN	42.6	57.2	67.3	17.4
(± SD)		10.6	30.0	11.7	-

OVERALL MEAN (± SD)

1,2-GDN = 37.3 (8.9)

1,3-GDN = 52.7 (22.4)

reach steady-state concentrations for the metabolites compared to nitroglycerin. Figures VIII-1 to 4 show that the dinitrates did not reach steady-state during the first three infusions, indicating much longer half-lives. There does appear to be a tendency for the 1,3-GDN to exhibit a longer half-life than the 1,2-GDN, but the variability is high. These longer metabolite half-lives may have pharmacological significance since the effects of nitroglycerin often last longer that the duration of measurable GTN plasma concentrations.

Finally, the ratio of 1,2-GDN to 1,3-GDN was found to vary as a function of route of nitroglycerin administration (Table VIII-8) suggesting enzymes in several tissues with different metabolic specificities. After oral administration of nitroglycerin, the ratio of 1,2- to 1,3-GDN was 1.96 ± 0.31 and was constant over a three-hour period after dosing. This ratio is approximately what one would expect from a nonspecific or random metabolism. Metabolism of nitroglycerin, by a nonspecific enzyme, would result in twice as much 1,2-GDN as 1,3-GDN being produced. The 1,2-GDN metabolite may be formed by the reduction of the nitrate ester group at either the 1- or 3-position. In contrast, the 1,3-GDN may be formed only by reduction of the nitrate ester at the 2-position.

After intravenous infusion, the ratio of 1,2- to 1,3-GDN was 7.44 ± 1.55. This high ratio indicates that the metabolism of nitroglycerin was much more specific. One

TABLE VIII-8: Summary of 1,2-GDN/1,3-GDN ratios after intravenous, oral, topical and sublingual nitroglycerin administration.

Subject	1,2-GDN/1,3-GDN Ratios			
	Infusion	Oral Solution	Ointment	Sublingual
J.K.	7.83±1.34	2.12±0.20	-	4.51±0.63
M.D.	7.76±1.12	2.07±0.29	5.14±1.47	-
T.M.	7.34±1.89	1.70±0.34	2.97±0.81	-
D.D.	7.02±1.51	1.92±0.27	3.30±0.31	-
R.D.	-	-	4.74±1.29	-
Mean	7.44	1.96	3.92	4.51
±SD	1.55	0.31	1.36	0.63
n	50	39	25	9

possible mechanism for such specificity is that the responsible enzyme may have a higher specificity towards primary nitrate esters (1-position) than secondary nitrate esters (2-position). This may be due to steric hindrance in the secondary position. After ointment and sublingual administration, the ratio of 1,2- to 1,3-GDN was 3.92 ± 1.36 and 4.51 ± 0.63, respectively. These lower ratios are indicative of somewhat lower metabolic specificity. These results indicate that metabolite formation does depend on the route of administration, thus implying different metabolic specificity of enzymes in the gut, liver, skin, sublingual mucosa and blood vessels.

An alternate explanation of these higher ratios might be that the 1,2- and 1,3-GDN are always produced in a 1:2 ratio, but that the 1,3-GDN is preferentially metabolized to the GMN. Because plasma GMN concentrations were not measured, this explanation can not be completely discounted. However, the preferential metabolism of one of the dinitrates would yield a shorter half-life for that metabolite. As explained above, the 1,3-GDN actually has a longer half-life than the 1,2-GDN, although this difference is not significant.

CHAPTER IX

SUMMARY

The preceding chapters have described the development of the analytical methods necessary to measure nitroglycerin plasma concentrations following clinically-relevant doses and the clinical pharmacokinetics of nitroglycerin using these methods. These chapters have described :

A) The development of specific and sensitive assay methods capable of quantifying picogram concentrations of nitroglycerin and the two dinitrate metabolites (1,2- and 1,3-glyceryl-dinitrates).

B) The in vitro metabolism of nitroglycerin by human plasma and blood.

C) The pharmacokinetics of nitroglycerin after intravenous infusion, oral, sublingual and topical doses to healthy volunteers.

D) The pharmacokinetics of the 1,2- and 1,3-glyceryl-dinitrate metabolites of nitroglycerin in some of these studies.

The specific, sensitive and precise capillary GC assay method described is capable of reproducibly detecting 25 pg/ml nitroglycerin; there is no interference from nitroglycerin metabolites. A similar GC assay capable of analyzing picogram concentrations of 1,2- and 1,3-glyceryldinitrates in human plasma is also described. This assay is capable of detecting 100 pg/ml of 1,2- and 1,3-glyceryldinitrates.

The loss of nitroglycerin during in vitro incubation in blood and plasma is due to metabolism. Concentrations of nitroglycerin were found to decrease at a pseudo-first-order rate with a half-life ranging from 17 to 32 min. The half-life varied with the blood source and the initial nitroglycerin concentration. Incubation of nitroglycerin in plasma resulted in a much slower rate of metabolism (half-life = 202 ± 5 min). No nitroglycerin degradation could be detected after incubation in pH 7.4 buffer for 21 hours. Thus, nitroglycerin degradation requires other cofactors present in blood and plasma. The loss of nitroglycerin was accompanied by a simultaneous increase in metabolite levels. All four nitroglycerin metabolites (1,2-GDN, 1,3-GDN, 1-GMN and 2-GMN) were detected and quantitated after incubation in whole blood and plasma. These data indicate that extreme care must be taken when processing blood samples obtained from patients or volunteers receiving nitroglycerin. The in vitro loss of nitroglycerin must be minimized during the period in which the blood sample is processed to obtain

plasma.

Using the assay procedures developed above, the pharmacokinetics of nitroglycerin were evaluated. The relative bioavailabilities of two sublingual (spray and bite-capsule), one topical (gel) and three oral (capsule) formulations were evaluated. The sublingual bioavailabilities were determined relative to standard nitroglycerin tablets while the bioavailabilities of the topical and oral doses were evaluated relative to a standard nitroglycerin ointment. The relative bioavailabilities of the spray and bite-capsule formulations were 22.2 - 297 and 11.1 - 78.3 percent, respectively. Bioavailability was found to be dependent on the dosing technique. The bioavailability of the nitroglycerin gel compared to the ointment ranged from 0.0 to 11.2 percent. The bioavailability of the oral capsules was zero since plasma concentrations of nitroglycerin could not be detected after this dosing route.

Nitroglycerin pharmacokinetics and bioavailability, relative to intravenous doses, were evaluated in healthy volunteers. Steady-state concentrations of nitroglycerin were determined at infusion rates of 10, 20, 40 and again 10 mcg/min. Steady-state nitroglycerin concentrations ranged from 0.15 to 1.07, 0.32 to 2.26, 1.64 to 5.71, and 0.58 to 1.52 ng/ml at the low, medium, high and repeat low infusion rates, respectively. A dose versus steady-state concentration curve with counter-clockwise hysteresis was detected. indicating that nitroglycerin clearance varied not

only with dose but also with previous exposure to the drug. Two possible causes of such behavior are end-product inhibition and saturable binding of nitroglycerin to blood vessels.

The bioavailability of sublingual nitroglycerin was found to be low and variable. A significant amount of the dose (31.4 ± 18.9 percent) was recovered in the mouth after maintaining the dose in the sublingual area for 8 min. Both the inter- and intra-subject variabilities were high for sublingual nitroglycerin absorption. Estimates of sublingual nitroglycerin bioavailability varied not only between individual doses (within the same person) but also within the same sublingual dose, depending on infusion rate. Since it was found that estimates of nitroglycerin clearance varied with the infusion rate, it follows that estimates of nitroglycerin availability will depend on the clearance value.

The bioavailability of oral nitroglycerin was evaluated. Nitroglycerin was administered as a solution, to each of four subjects, in an effort to saturate the extensive first-pass metabolism of this drug. The oral bioavailability of a clinically efficacious dose (6.5 mg) of nitroglycerin in solution was 0%. Unchanged nitroglycerin was detected only at early time points (0.15 ± 0.10 ng/ml). These low concentrations were most likely due to sublingual absorption of a small amount of the dose in solution as it was being swallowed.

The bioavailability of transdermal nitroglycerin was determined in healthy volunteers. Based on the highest infusion rate, the amounts of nitroglycerin absorbed over the dosing interval were determined. The apparent absorption rates ($mcg/cm^2/hr$) were determined for Nitro-BID^R ointment (5.15 ± 1.07), Nitrodur^R patch (8.09 ± 6.35) and Nitrodisc^R patch (5.55 ± 3.04). Since the transdermal absorption rates of nitroglycerin were equivalent, it may be concluded that the skin, and not the physical formulation, is the rate-limiting step in the transdermal delivery process for the preparations studied. Significant intra-individual variations in plasma concentrations, during a single transdermal dose, were observed.

Nitroglycerin metabolites were measured in plasma samples obtained during the studies summarized above, which were designed to elucidate the pharmacokinetics of the parent drug only. However, several observations regarding metabolite pharmacokinetics could be made. After oral administration of nitroglycerin solutions to volunteers, high concentrations of 1,2-GDN and 1,3-GDN were detectable. The metabolite concentrations peaked at 20 min and were measureable to 4 hours. These data are quite interesting because oral nitroglycerin was not bioavailable, yet high concentrations of potentially-active metabolites were present in the plasma. The efficacy of oral nitroglycerin has not yet been correlated with plasma concentrations of either nitroglycerin or the dinitrate metabolites. Based on

the data presented in Chapters VII and VIII, the efficacy may be related to metabolite concentrations.

After intravenous administration of nitroglycerin, the apparent elimination half-lives of 1,2- and 1,3-GDN were determined to be 33.2 ± 6.5 and 57.2 ± 30.0 min, respectively. There does appear to be a tendency for the 1,3-GDN to exhibit a longer half-life than the 1,2-GDN, but the high variability lends no confidence to this assumption.

Finally, the ratio of 1,2-GDN to 1,3-GDN was found to vary as a function of route of nitroglycerin administration. After oral administration of nitroglycerin, the ratio of 1,2- to 1,3-GDN was 1.96 ± 0.31 and was constant over a three-hour period after dosing. This value is approximately that which one would expect from a nonspecific or random metabolism. After intravenous infusion, the ratio of 1,2- to 1,3-GDN was 7.44 ± 1.55. This high ratio indicates that the metabolism of nitroglycerin was much more specific. After ointment and sublingual administration, the ratio of 1,2- to 1,3-GDN was 3.92 ± 1.36 and 4.51 ± 0.63, respectively. These lower ratios are indicative of somewhat lower metabolic specificity. These results indicate that metabolite formation does depend on the route of administration, thus implying different metabolic specificity of enzymes in the gut, liver, skin, sublingual mucosa and blood vessels.

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