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Wu, Lei-Shu Chang

BIOTRANSFORMATION OF NITROGLYCERIN IN BLOOD AND ITS BIOAVAILABILITY AND PHARMACOKINETICS DURING CARDIOPULMONARY BYPASS

The Ohio State University

Ph.D. 1984

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BIOTRANSFORMATION OF NITROGLYCERIN IN BLOOD AND
ITS BIOAVAILABILITY AND PHARMACOKINETICS
DURING CARDIOPULMONARY BYPASS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By
Lei-Shu Chang Wu, B.S., M.S.

The Ohio State University
1983

Reading Committee:
Theodore D. Sokoloski
Allan M. Burkman
Robert E. Notari
Sylvan G. Frank

Approved by

Theodore D. Sokoloski
Adviser
College of Pharmacy
DEDICATION

To my parents, my mother-in-law and my husband, Chin, for their love and constant encouragement.
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VITA

March 11, 1952 ....... Born in Taiwan, Republic of China

1970-1974 ....... B.S. Pharmacy
Taipei Medical College
Taipei, Taiwan

1974-1975 ....... Teaching Assistant
Taipei Medical College
Taipei, Taiwan

1975-1977 ....... M.S. Molecular Biology
National Tsing-Hua University
Hsin-chu, Taiwan

1977-1978 ....... Research Associate
National Tsing-Hua University
Hsin-Chu, Taiwan

1978-1979 ....... Research Associate
National Institute of Nuclear Energy, Taiwan

1979-1982 ....... Research Associate
College of Pharmacy
The Ohio State University
Columbus, Ohio

1982-1983 ....... Teaching Associate
College of Pharmacy
The Ohio State University
Columbus, Ohio

PUBLICATIONS


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PART I

BIOTRANSFORMATION OF NITROGLYCERIN IN BLOOD
Chapter I

INTRODUCTION

Nitroglycerin has been used in the treatment of angina pectoris for over one hundred years. It was first synthesized by Sobrero in 1847 and used initially in explosives by the Swedish engineer Alfred Nobel. In 1879, a distinguished English physician William Murrell(1) successfully gave nitroglycerin to a patient for the treatment of severe angina pectoris(2), and since then, nitroglycerin has been the drug of choice for this disease(3).

Over the past decade, investigations involving nitroglycerin have resurfaced in the literature. This may be partially because of the availability of new analytical techniques(4-12) and partially because of an extension of its clinical applications. The therapeutic concentration of nitroglycerin in blood has been defined to be in range of 1.2-11.1 ng/ml(30). The development of sensitive gas chromatographic methods now allow investigators to quantitate nano or pico gram levels of nitroglycerin and its degradation or metabolic products. For the first time then, investigators are able to take a close look at the drug's pharma-
ookinetic behavior and study its metabolic pathways in human subjects. The availability of sensitive assay methods also make a study of the bioavailability of nitroglycerin preparations feasible. In recent clinical studies, it has also been demonstrated that nitroglycerin is useful in congestive heart failure, reducing infract size, treatment of heart failure following acute myocardial infarction and blood pressure reduction during either coronary artery bypass or general surgery. For convenience in clinical use, nitroglycerin is available in different dosage forms including sublingual tablets, percutaneous ointments, transdermal dosage forms, sustained release capsules, intravenous solutions and an inhalation aerosol.

The biological half life of nitroglycerin in blood is uncommonly short, about 2 minutes(30). Tremendous interest has focused on the distribution and metabolism of nitroglycerin. Numerous studies have been reported in this area. However, major routes of distribution and biotransformation of nitroglycerin in humans remain incompletely understood. Using radioisotope labelled nitroglycerin and thin layer chromatographic analysis of ether extracts from reaction medium of nitroglycerin in rat liver homogenates, Needleman(14-15) reported that nitroglycerin can be denitrated rapidly in the presence of a liver enzyme, glutathione transferase. One molecule of nitroglycerin reacts with
glutathione to release one inorganic nitrate ion from either the 2 or 3 position to form 1,2- and 1,3-dinitroglycerin. Denitration of dinitroglycerin proceeded at only 2 to 5% the rate for nitroglycerin to form 1- and 3-mononitroglycerin. Mononitroglycerin is practically unaffected by liver enzyme. In order to establish that hepatic denitration is the main metabolic pathway, studies of denitration of nitroglycerin were performed in totally eviscerated rats(16). After intravenous administration of $^{14}$C-labelled nitroglycerin in control animals, a rapid disappearance of nitroglycerin from rat blood was observed. The biological half life was less than 1 minute. There is also a simultaneous rapid appearance of nitroglycerin metabolites in the blood which reached peak concentration at 2-5 minutes and disappeared with a biological half life of 3-4 hours. In contrast with these studies, the time course of disappearance of $^{14}$C-labelled nitroglycerin from the blood of eviscerated animals proceeds much slower than that of controls. The apparent biological half life of $^{14}$C-nitroglycerin was 7-8 minutes. Furthermore, there was no increase in the concentration of nitroglycerin metabolites in the blood with time. These data indicated that the in vivo degradation of nitroglycerin in rat takes place primarily in the liver and presumably results from reaction with glutathione organic nitrate transferase.
Recently, liver glutathione s-transferases which play an important role in the biotransformation of nitroglycerin have been isolated and purified from human liver(17-18). They are made up of a group of related enzymes that catalyze the reaction of glutathione with a large group of hydrophobic compounds bearing an electrophilic center(19-21). These enzymes comprise approximately 10% and 3%, respectively, of the soluble protein in rat or human liver. Although they have the same amino acid composition, the predominate metabolic activity for nitroglycerin was located in glutathione transferase 6. For nitroglycerin this is 72,600 ng per minute per milligram of liver protein(18). When glutathione s-transferases catalyze the reaction of glutathione with nitroglycerin, the alcohol derivatives of the parent compound and oxidized glutathione are found in the reaction medium. This enzymatic reaction can be described as follows:

\[ \text{H}_2\text{C-NO}_3 + 2 \text{GSH} \rightarrow \text{H}_2\text{C-OH} + \text{H}_2\text{C-NO}_3 \text{ or H}_2\text{C-OH}^3 + \text{HNO}_2 + \text{GSSH} \]

This result indicated that liver enzymes could metabolize all the nitroglycerin reaching this organ. Because the blood concentration of nitroglycerin in most clinical studies was in a range of nano grams per milliliter, the metabolism of nitroglycerin therefore could be limited by liver blood flow.
In 1973, Lee(22) reported studies on interactions of nitroglycerin in human blood. In order to locate the metabolic compartment of blood active toward nitroglycerin, nitroglycerin was incubated with whole blood, resuspended red cells, and plasma. In this study, $^{14}$C-labelled nitroglycerin at a concentration of 1000 µg per ml was used. Nitroglycerin and its degraded products were quantitated by a thin layer chromatographic method. Metabolites were found after incubating nitroglycerin with either whole blood or red cells. When nitroglycerin was incubated with human plasma for 30 minutes at 37°C, nitroglycerin concentration in plasma remained the same and no metabolite was found in the reaction medium. Although this study demonstrated that nitroglycerin can be converted to its metabolites by whole blood or red blood cells, the concentration of nitroglycerin used is much higher than therapeutic concentrations and a single time for incubation was performed. Therefore, further studies were needed to identify the mechanism of loss of nitroglycerin under physiological concentrations.

Marcus et al.(23) have also isolated and purified a glutathione transferase $\rho$ from human erythrocytes. The enzyme has a molecular weight of 47,500 and is present at a concentration of about 1.2 ng per 100 ml of packed human erythrocytes. The amino acid composition of the erythrocyte enzyme is different from that of glutathione transferase
from human liver. In addition, the erythrocyte enzyme is also different from the liver enzyme in its inability to catalyze the denitration of organic nitrates. This data suggested that short half life of nitroglycerin may result from the rapid distribution of nitroglycerin in tissue or in blood cells or enzymatically degraded by plasma enzyme or red cell enzymes other than glutathione s-transferases, or perhaps by other biotransformations.

In 1978, Maier et al. (24) examined the effect of concentration, temperature and silver nitrate concentration on nitroglycerin stability in human plasma. When nitroglycerin at an initial concentration of 141, 25 and 7 ng/ml was incubated with human plasma at 24°C, drug loss followed an apparent first order process and was concentration independent. The half-lives of disappearance of nitroglycerin from plasma were 2.9 hours at 37°C and 12 hours at 24°C. A GC assay method was used in this study, and formation of metabolites was not determined. An approximate 17 fold increase in stability of nitroglycerin was observed after adding silver nitrate to human plasma. Silver nitrate forms a complex with SH groups on proteins. Thus, the author concluded that the mechanism of the inhibition of the nitroglycerin degradation by silver nitrate could be explained as a denaturing of the plasma enzyme proteins which are responsible for nitroglycerin degradation.
In 1980, Armstrong et al.(13) studied the pharmacokinetics of nitroglycerin in patients with congestive heart failure. Intravenous nitroglycerin was used in this study. They noticed that when the infusion rate of nitroglycerin was increased progressively through 59, 220 and 440 µg/min, there was a corresponding rise of nitroglycerin concentration in blood. When the infusion was stopped, the drug concentration in blood fell off rapidly with a half life of 1.9 minutes. The clearance of nitroglycerin was estimated as 13.8 liters per minute. This demonstrated that nitroglycerin clearance was well in excess of hepatic blood flow. Therefore it was suggested that liver may not be the sole elimination site.

In a separate study, Armstrong reported a rapid disappearance of nitroglycerin following incubation with human blood. The half-life was 6.2 minutes when nitroglycerin at a concentration of 50 ng/ml was incubated aerobically with whole blood at 37°C, and the half-lives in resuspended red cells and plasma were 6.6 min and 53.4 min, respectively. When glutathione was added to give a final concentration of 1.09 µmole/ml in plasma, the rate of nitroglycerin disappearance remained the same as that in plasma. This result precluded the possibility that the plasma might contain glutathione S-transferase but that it lacks sufficient glutathione for the enzyme to be active. Although the mechanism
of nitroglycerin disappearance was assumed to be an enzymatic process, no effort was made to locate the metabolites in this study.

Recently, Noonan et al. (25) reported a study on nitroglycerin loss in fresh human blood and pooled plasma. Tritium labelled nitroglycerin was used and the nitroglycerin and its degraded products were determined by selective extractions and normal phase high pressure liquid chromatography. After incubation of nitroglycerin with fresh human blood at the concentration of 17 and 136 ng/ml, the decrease of labelled nitroglycerin in fresh human blood and plasma was accompanied by a simultaneous increase in metabolite levels. This study suggested that nitroglycerin loss in whole blood and plasma could be enzymatic and that the reaction rate could depend on the blood source and the initial nitroglycerin concentration.

As a result of all of these previous studies, it was apparent that the biological fate of nitroglycerin in human body remained a mystery. Since the rate of loss of nitroglycerin in blood is rapid, it would appear that a knowledge of the kinetics and mechanism of its loss would have significant impact on an analysis of the disposition kinetics of the drug and possibly its physiological activity as well. Therefore, these studies was designed to further elucidate the kinetics and mechanism of drug loss in blood components.
Nitroglycerin stock solutions were prepared from an alcoholic extract of a 10% of lactose adsorbate. The major metabolites of nitroglycerin, 1,2-dinitroglycerin and 1,3-dinitroglycerin, were prepared from 2,3-dibromo-1-propanol and 1,3-dibromo-2-propanol using the method of Dunstan et al. The purity of the compounds was determined by high-performance liquid chromatography (HPLC) and TLC and the solutions standardized as reported by Yuen et al. All other chemicals used were obtained commercially and were reagent grade or better.

**Blood samples**

The loss of nitroglycerin or metabolite was followed in a number of systems; whole blood, resuspended red blood cells, plasma, protein-free plasma, and normal saline solution. The majority of the studies reported used resuspended cells, since the loss of nitroglycerin is the same in these systems as in whole blood and the resuspended cells represent a more clearly defined systems that yields trouble-free reference blanks.
The various systems used in the several studies were obtained from healthy normal subjects. Blood was centrifuged at 1200 rpm at 5°C for 20 minutes, and then the plasma was separated. A portion of this plasma was used for one set of experiments. The remainder of the plasma was rendered protein free by ultrafiltration at 30 psi using a suitable membrane. The cells, obtained by centrifugation, were washed three times with saline solution and reconstituted after centrifugation to 100 ml with saline solution. The cell count in such preparations was measured and adjusted to the appropriate number of cells by using a particle size analyzer. Two ml of a particular preparation (whole blood, resuspended cells, plasma, or saline) was transferred to each of a series of 12 ml silanized tubes covered with teflon-lined caps.

Comparison studies in whole blood, washed and resuspended cells, plasma, protein free plasma, and saline solution at the same initial concentration of nitroglycerin

Two ml of a particular preparation (whole blood, resuspended cells at normal erythrocyte level, plasma, protein-free plasma, or saline) was equilibrated at 37°C for 20 minutes, then an aliquot of a stock solution of nitroglycerin in normal saline was added to each tube to give a final concentration in each set of tubes of 60 ng/ml. The tubes were gently stirred using a rotator submerged in a water bath at 37°C. Periodically over a given time interval, a tube was
removed, the internal standard was added (if necessary), and the preparation was immediately extracted twice with 3 ml of pentane. Both extracts were combined and concentrated, if needed, for GC analysis. A portion of this solution (8 μl) was injected into the gas chromatograph using a 3% carbowax 20M-TPA column and a $^{63}$Ni-detector for determination of nitroglycerin.

**The effect of various initial concentrations of nitroglycerin and metabolite on the rate of their loss in human red blood cells**

The same procedure was carried out as in the comparison study, except, an aliquot of a solution of nitroglycerin or metabolite in normal saline was added to each tube of resuspended cells to give a final concentration in each set of tubes of 10, 20, 60, 120, 180 or 480 ng/ml for one subject. Concentration of nitroglycerin was measured using gas chromatography with a 3% carbowax 20M-TPA column. Concentrations of 15, 60, 120, 240, 600, 1220 ng/ml were used for another subject and in this case the concentration of nitroglycerin was measured using gas chromatography, an electron capture detector and a 9% QF-1 column.

**The effect of metabolite pretreatment on the rate of nitroglycerin loss**

Two ml of resuspended cells at a normal erythrocyte level was equilibrated at 37°C for 20 minutes, then an ali-
quot of a solution of 1,2-dinitroglycerin in normal saline was added right before the addition of nitroglycerin solution giving a final concentration of 1.1 μg/ml of 1,2-dinitroglycerin and 120 ng/ml of nitroglycerin. The tube was gently stirred using a rotator submerged in a water bath at 37°C. After incubating for 5 minutes, the preparation was immediately extracted with 3 ml of ethylacetate. Nitroglycerin concentration was measured by GC analysis. The same procedure was carried out for a control study except no 1,2-dinitroglycerin was added.

In a separate study, the same procedure was carried out as in the comparison study, except, 300 ng of 1,2-dinitroglycerin was added to 2 ml of resuspended cell solution before adding 240 ng of nitroglycerin.

The effect of temperature on the rate of nitroglycerin loss in human red blood cells

The same procedure was used as in the comparison study, except, each set of 2 ml of resuspended cells was equilibrated and incubated at 8°C, 22°C, and 37°C.

The effect of number of cells on the rate of nitroglycerin loss

The same procedure was used as in the comparison study, except, five sets of 2 ml of resuspended cell with cell number of 4.85 x 10^6, 3.83 x 10^6, 3.39 x 10^6, 2.36 x 10^6, 2.13
x $10^6$ cells per micro liter was incubated with nitroglycerin at a final concentration of 60 ng/ml.

**Gas chromatographic analysis**

Method(1): 9% QF-1 on 60-80 mesh Supelcoport was used as a stationary phase(28). The coated material was packed into a 2 mm x 0.915 m silaranized glass column. A Varian 3700 series dual column gas chromatograph equipped with an $8 \text{ mCi} \mathrm{^{63}Ni}$ electron capture detector coupled with a Shimadzu Chromatopac C-RIA recording data processor was used. The temperatures of the injection port, the column and the detector were 150°, 135° and 200°C, respectively. The carrier gas was 5% methane in argon used at a flow rate of 30 ml/min. The gas was passed through a Dow gas purifier before entering the gas chromatograph. Under these conditions the retention times were: mixture of 1,2-dinitroglycerin and 1,3-dinitroglycerin, 2.15 min; nitroglycerin, 4.59 min. The system does not separate the two isomeric metabolites but does optimize their combined detection. The metabolites had a detector response of about one half that of an equal amount of nitroglycerin. Because of the thermal decomposition of nitroglycerin on a GC column a calibration curve is needed in order to obtain an accurate measure of dinitroglycerins produced from the degradation of nitroglycerin in blood cell preparations.
Method(2): The same electron capture gas chromatograph was used with a data processor. The column (silanized glass, 2 mm x 50 cm) consisted of 3% carbowax 20M-TPA\textsuperscript{9} on 60-80 mesh Supelcoport\textsuperscript{(29)}. The temperatures of the injection port, the column and the detector were $150^\circ$, $135^\circ$ and $200^\circ$C, respectively, and the flow rate of the carrier gas was 22 ml/min. Using these conditions the two isomeric dinitroglycerin could be separated from each other, and from nitroglycerin, but the sensitivity for dinitroglycerin is not as good as that of method(1).
Chapter III
RESULTS AND DISCUSSION

When nitroglycerin at a concentration of 60 ng/ml is incubated with whole blood or red blood cells that are washed and resuspended at a normal erythrocyte levels in normal saline solution at 37°C for various periods of time, the loss of nitroglycerin in either preparation follows a first-order process after an apparent initial rapid step. This is demonstrated in Figure 1.1. The half-lives for the first order drug loss in whole blood and in red blood cells are 7 and 6.7 minutes respectively. These results are similar to those found by Armstrong et al. (13). In their studies, half-lives for nitroglycerin loss in whole blood and resuspended cell preparations at an initial nitroglycerin level of 50 ng/ml and at 37°C are 6.2 and 6.6 minutes respectively. Figure 1.1 also shows that the loss of nitroglycerin in human plasma at the same initial concentration as that in whole blood or in red cell suspension follows an apparent first-order process without a detectable rapid initial phase, but the reaction occurs at a much slower rate in
Figure 1.1 Relationship between the logarithm of the amount of nitroglycerin (ng) extracted from 2 ml of several preparations initially at 60 ng/ml and time in minutes. The preparations used at 37°C together with their interpolated half-lives (in parentheses) were: (○) normal saline and protein free plasma (60); (□) plasma (130); (△) red cells at normal erythrocyte count (7); (●) whole blood (6.7).
Figure 1.1

AMOUNT NITROGLYCERIN, ng vs TIME, minutes
comparison with the blood cell studies: half-life 130 minutes. This slower rate in human plasma was also observed by Armstrong et al.(13) who reported a half-life of 53 minutes and by Maier et al.(24) who reported a half-life of 175 minutes. The latter group used a slightly diluted sample. On the other hand, protein-free plasma and normal saline containing the same initial concentration of nitroglycerin as that in the blood studies show no drug loss. This indicates that the decomposition of nitroglycerin in plasma may be due to interaction with protein. These results also suggest that reaction with blood cells is the major contributor to the rapid loss of nitroglycerin.

It is known that nitroglycerin can be degraded to dinitroglycerin. When nitroglycerin is incubated with red blood cells obtained from five males and two females, the same degradation pattern is seen again where decreasing nitroglycerin concentration is accompanied by a simultaneous increase in its metabolites, predominantly, 1,2- and 1,3-dinitroglycerin (Figure 1.2). This result is also in general agreement with the work of Noonan and Benet(25).

The effect of various initial nitroglycerin concentration on drug loss following its incubation with a constant level of red blood cells at 37°C is given in Figures 1.3 and 1.4. When the initial concentration of drug is varied and the rate of loss of nitroglycerin is followed, it is seen
Figure 1.2 Relationship between the logarithm of the concentration of nitroglycerin (●) and of metabolites (★) extracted after incubation with washed red blood cells in normal saline and the time in minutes. Decrease of nitroglycerin is accompanied by simultaneous increase of metabolites.
Figure 1.2
that at each nitroglycerin level there is an initial very rapid loss followed by an apparent first order process. Interpolated first order half-lives varied with the initial concentration. As the initial concentration increases, the half life increases. In one male subject's blood (Figure 1.3), these value were 45.6 minutes at 1220 ng/ml, 42.0 minutes at 600 ng/ml, 30.3 minutes at 240 ng/ml, 10.1 minutes at 120 ng/ml, 5.6 minutes at 60 ng/ml and 5.3 minutes at 15 ng/ml. In another study involving a different subject's blood, a similar trend was found (Figure 1.4). Here the half lives found were: 52 minutes at 480 ng/ml, 20 minutes at 180 ng/ml, 16 minutes at 120 ng/ml, 7 minutes at 60 ng/ml and 4 minutes at 10 ng/ml. When the formation of 1,2- and 1,3-dinitroglycerin was monitored concomitant with nitroglycerin loss, results showed that the dinitro compound formed rapidly (Figure 1.5). Conversion to other metabolites (mononitroglycerins and glycerol) occurred very slowly.

Since dinitroglycerin is the predominant metabolite formed, the rate of disappearance of dinitroglycerins incubated with red blood cells was studied. Dinitroglycerins at various initial concentrations were incubated with a constant level of red blood cells in normal saline at 37°C. The amount of metabolite extracted from samples incubated for various lengths of time is shown in Figure 1.6. After a possible initial rapid phase the loss of metabolite followed
Figure 1.3 Relationship between the logarithm of the concentration of nitroglycerin extracted from 2 ml of washed red blood cells suspended in normal saline and the time in minutes. The cells used are from a single individual. The suspensions at normal erythrocyte level and at 37°C were incubated with varying initial concentrations of nitroglycerin. The concentrations used and the interpolated half-lives were: (●) 1220 ng/ml, 45.6 min; (○) 600 ng/ml, 42.0 min; (□) 240 ng/ml, 30.3 min; (■) 120 ng/ml, 10.1 min; (◇) 60 ng/ml, 5.6 min; (◇) 15 ng/ml, 5.3 min.
CONC. NITROGLYCERIN, mg/ml

Figure 1.3

TIME, min

Graph showing concentration of nitroglycerin over time.
an apparent first order process at each level of 1,3-dinitroglycerin studied and the single level of 1,2-dinitroglycerin used. The half-lives for the 1,3-isomer interpolated from the plots increased as the initial concentration increased: at 10 ng/ml, 33 minutes; at 60 ng/ml, 40 minutes, at 180 ng/ml, 141 minutes; and at 480 ng/ml, 228 minutes. Although the same trend is found for the dependence of half-life on initial concentration as for nitroglycerin, the reaction at comparable concentrations is much slower for the dinitro compound than for nitroglycerin. The rate difference between dinitroglycerin and nitroglycerin may be explained by (a) The biotransformation of organic nitrates by red blood cell components is more active for nitroglycerin than for dinitroglycerin. (b) Chemically, dinitroglycerin is more stable than nitroglycerin because the removal of one nitroxy group can relieve the steric strain existing in the more crowded nitroglycerin molecule.

Figure 1.6 also shows that the first-order rate of loss of the 1,2-isomer is about the same as the 1,3-isomer. When both are initially present at 60 ng/ml, the half-life interpolated for 1,2-dinitroglycerin is 51 minutes and that for 1,3-dinitroglycerin 40 minutes. Since the same general kinetic behavior is found for metabolites as with nitroglycerin, it would seem that whatever the mechanism of loss of nitroglycerin in the presence of red blood cells is, the loss of metabolite follows a similar mechanism.
Figure 1.4 Relationship between the logarithm of the amount of nitroglycerin (ng) extracted from 2 ml of washed red cells suspended in normal saline and the time in minutes. The cells used are from a subject different from that in Fig. 1.3. The suspensions at normal erythrocyte count and at 37°C were incubated with varying initial concentrations of nitroglycerin. The concentrations used and the interpolated half-lives were: (Δ) 480 ng/ml, 52 min; (O) 180 ng/ml, 20 min; (□) 120 ng/ml, 16 min; (◇) 60 ng/ml, 7 min; (O) 10 ng/ml, 4 min.
Figure 1.4
Figure 1.5 Relationship between the logarithm of the concentration of metabolites extracted from 2 ml of red cell suspensions at normal erythrocyte count and time in minutes. The suspensions at 37°C were incubated with varying initial concentrations of nitroglycerin: (★) 1220 ng/ml; (●) 600 ng/ml; (○) 240 ng/ml; (◇) 120 ng/ml; (☐) 60 ng/ml; (●) 15 ng/ml.
Figure 1.6  Relationship between the logarithm of the amount of nitroglycerin metabolites extracted from 2 ml of red cell suspensions at normal erythrocyte count and time in minutes. The suspensions at 37°C were incubated with varying initial concentrations of metabolite. The concentrations of the metabolite (I = 1,2-dinitroglycerin; II = 1,3-dinitroglycerin) used, and the interpolated half-lives were: (○) II, 480 ng/ml, 228 min; (□) II, 180 ng/ml, 141 min; (△) I, 60 ng/ml, 51 min; (■) II, 60 ng/ml, 40 min; (●) II, 10 ng/ml, 33 min.
Figure 1.6

AMOUNT OF METABOLITE, ng

TIME, minutes
The kinetic behavior of nitroglycerin (Figures 1.2 and 1.3) suggested that it was possible that the loss of drug could be described by Michaelis-Menten kinetics with competitive product inhibition. This kind of behavior has been demonstrated in intact animals (33-35) for some other drugs and its nature demonstrated by computer simulation procedures (36). For example, product inhibition can cause dose dependent effects where drugs at large doses essentially become more slowly eliminated than when given in small doses. Whereas the rate of decline of drug concentration in the post-distributive phase at any given concentration of drug in the body tends to be independent of dose in Michaelis-Menten kinetics (31-32), the rate will tend to decrease with increasing dose in the case of product inhibition. Moreover, drug elimination may appear to be first order with half-lives increasing with increased dose.

To test the effect of metabolite on the rate of loss of nitroglycerin, rate studies using blood cells pretreated with 1.1 µg/ml of 1,2-dinitroglycerin before adding 120 ng/ml of nitroglycerin were compared with those rates having the same initial concentration of nitroglycerin but no metabolite. Figure 1.7 shows a chromatogram obtained from a 5 minute sample. The nitroglycerin level (designated as I in figure 1.7) with metabolite added (Figure 1.7A) is 25% higher than the corresponding level (Figure 1.7B) in the sample.
without metabolite initially added. This strongly supports a product inhibition mechanism for the nitroglycerin degradation.

It is interesting to note that the rapid initial decrease of nitroglycerin might ordinarily be interpreted as being due to drug distribution in a multicompartmental system. However, in separate studies, where 300 ng of 1,2-dinitroglycerin was added to 2 ml of a cell solution before 240 ng of nitroglycerin was added, there is no initial rapid phase with the metabolite pretreated set in comparison with the control (Figure 1.8). Therefore, the initial rapid decrease of nitroglycerin level is most likely due to the metabolite not reaching an observable inhibitory level.

The effect of temperature on the disappearance of nitroglycerin is given in Figure 1.9. The loss of nitroglycerin initially at 60 ng/ml incubated with resuspended red cell at 37°C, 22°C and 8°C follows an apparent terminal first-order process at each temperature. The first order half-life increases as the temperature decreases. The apparent half-lives as determined by graphical interpolation are: 6.7 minutes at 37°C, 23.9 minutes at 22°C and 100.6 minutes at 8°C. The relationship between rate constant and temperature is treated in an Arrhenius fashion and shown in Figure 1.10. The calculated activation energy for the reaction is 16 kcal/mole. Because the interaction rate itself
Figure 1.7 Chromatograms for nitroglycerin(I) and its metabolite extracted from 2 ml of red cell suspensions which were incubated with 120 ng/ml of nitroglycerin for 5 min. (A) with metabolite pretreatment (B) without metabolite pretreatment.
Figure 1.8 Relationship between the logarithm of the concentration of nitroglycerin (ng/ml) extracted from 2 ml of washed red cells in normal saline and the time in minutes. The suspensions at normal erythrocyte count and at 37°C were incubated with nitroglycerin at a concentration of 120 ng/ml. (●) the concentration-time profile for systems pretreated with 150 ng/ml of 1,2-dinitroglycerin; (♦) the concentration-time profile for the control study in which no metabolite was added.
Figure 1.8
Figure 1.9 The time course for nitroglycerin concentration, at different temperatures. The temperature used and interpolated half-lives were: (♦) 37°, 6.7 min; (□) 22°, 23.9 min; (○) 8°C, 100.6 min.
Figure 1.10 Relationship between logarithm of rate constant and reciprocal of absolute temperature for the interaction between human red blood cells and nitroglycerin. The activation energy for the interaction is 16 kcal per mole using 60 ng/ml of nitroglycerin initially and incubating it with suspended blood cell at different temperatures.
Figure 1.10

RATE CONSTANT × 10^3, min⁻¹

T × 10^3, °K⁻¹

Figure 1.10
is dependent on the initial concentrations of nitroglycerin, the activation energy may be also dependent on the initial nitroglycerin concentration.

The effect of the number of red blood cells on the rate of nitroglycerin loss is shown in Figure 1.11. When nitroglycerin is incubated with various numbers of red cells, the rate of drug loss increases as the number of cells increases. The apparent half-lives as determined by graphical interpolation at the several cell levels are (half-life, number of cells/μl): 5.3 at 4.85 × 10^6, 7.6 at 3.83 × 10^6, 15.3 at 3.39 × 10^6, 36 at 2.36 × 10^6 and 48.0 at 2.13 × 10^6. The relationship between rate constant and cell number (Figure 1.12) also shows the saturable nature of the loss of nitroglycerin. This relationship between rate and number of red cells can explain why the rate of loss of some organic nitrates is faster in males than in females; the number of blood cells per unit volume in males is greater than that in females.

The dose dependence of nitroglycerin loss suggested that what is occurring is the result of a saturation of some biochemical pathway. Assuming that the process is metabolism, it might be concluded that metabolites inhibit the biotransformation of nitroglycerin (Figures 1.3, 1.4). Direct evidence for involvement of metabolite is seen in the metabolite pretreatment studies (Figure 1.6). Support for
Figure 1.11 The time course for nitroglycerin loss incubated with different numbers of red cells. The cell number (in cell per ul) used and the interpolated half-lives were: (*) $4.85 \times 10^6$, 5.3 min; (●) $3.83 \times 10^6$, 7.6 min; (◎) $3.39 \times 10^6$, 15.3 min; (□) $2.36 \times 10^6$, 36 min; (○) $2.13 \times 10^6$, 48 min.
Figure 1.11
Figure 1.12 Relationship between logarithm of rate constant and logarithm of number of cells.
Figure 1.12

RATE CONSTANT \( \times 10^2 \), mm\(^{-1} \)

NUMBER OF CELLS \( \times 10^{-6} \)/mm

Graph showing the relationship between rate constant and number of cells.
the existence of a microsomal metabolizing system are the
circulatory effects observed for some organic nitrates,
especially isosorbide dinitrate(37). Acute and sustained
therapy using the drug has been studied where the authors
found that plasma isosorbide dinitrate concentration after
any given dose of isosorbide was higher with sustained dos­
ing in contrast with acute therapy. Kinetic studies using
metabolite (Figure 1.6) show a slower rate of disappearance
for metabolite in comparison with nitroglycerin. This sug­
gests that patients on sustained therapy will have a high
level of circulating metabolites which may compete with ni­
troglycerin for the receptor, therefore, causing tolerance
to the drug.

These and earlier studies strongly suggest the follow­
ing: (1)The metabolism of nitroglycerin in blood is rapid
and predominantly takes place in the cellular compartment.
(2)Decrease in nitroglycerin concentration is accompanied by
increase in metabolite level. (3)Nitroglycerin levels de­
crease by an apparent first order process, and rate constant
of the process varies with initial nitroglycerin concen­
tration and possibly blood source as well. (4)The biotransfor­
mation may be described by Michaelis-Menten kinetics with
competitive product inhibition. (5)In accordance with pre­
vious studies, the whole blood volume can be considered a
metabolically active system.(6)The biotransformation of ni-
triglycerin in blood is a rapid enough process so that it contributes significantly to the short circulating half-life of the drug in humans.
FOOTNOTES


2. Eastman Kodak, lot A5A, Rochester, NY 14650.


5. Analabs, No. Haven, CT 06473.


PART II

BIOAVAILABILITY AND PHARMACOKINETICS OF NITROGLYCERIN
DURING CARDIOPULMONARY BYPASS
Nitroglycerin is a potent vasodilating agent (38) and it is the drug of choice for the treatment of angina pectoris (3). It is widely used in patients with congestive heart failure, myocardial infarction, and during and after cardiac surgery. Although nitroglycerin can elicit systemic and coronary arterial vascular relaxation, its major hemodynamic effect appears to result from systemic venous dilation with an associated pooling of blood, decreased cardiac blood return, and diminished left ventricular filling volume and pressure (6,39,40). Antianginal effectiveness, therefore, appears to be mediated predominantly by alteration in myocardial oxygen demand rather than augmentation of coronary arterial blood flow or oxygen supply.

The mechanisms for vasodilation of nitroglycerin remain largely unknown. It has been suggested that nitroglycerin may bind to a specific peripheral nitrate receptor through the formation of disulfide bonds (41). More recently, postulates for the mechanism of activity have included inhibition
of calcium mobilization from intracellular storage sites (42), alteration in the products of cyclooxygenase metabolism (43-45), and induction of cyclic GMP formation (46). Studies involving the latter two mechanisms have been extensive. The prostaglandins and related compounds are important mediators of vascular events. After activating phospholipase, phospholipid in endothelial cell membranes or from platelets can be converted to arachidonic acid which can be converted to prostaglandin G_2 and transformed to prostaglandin H_2 by activating cyclooxygenase. Thromboxane synthetase catalyzes the conversion of prostaglandin H_2 to the potent constrictor thromboxane A_2. Another pathway is via the conversion of prostaglandin H_2 to the potent vasodilator prostacyclin by prostacyclin synthetase. Nitroglycerin can inhibit thromboxane synthetase leading to the production of prostacyclin. Prostacyclin stimulates the formation of cyclic AMP which promotes vascular smooth muscle relaxation by reducing intracellular calcium ion and by promoting the phosphorylation of myosin light chain kinase thereby reducing its ability to be activated by calcium.

The second mechanism for nitrate induced vasodilation is through the production of nitric oxide in the endothelium and subsequent stimulation of guanylate cyclase. There appears to be tight coupling between the extent of relaxation and the nitrate-induced cyclic GMP formation. When guany-
late cyclase is inhibited by methylene blue, the relaxing capacity of the nitrates is decreased.

Despite favorable reports of the antihypertensive effects of nitroglycerin during cardiac surgery, two recent studies suggest a less than optimal response to the drug during cardiopulmonary bypass\(^{(47,48)}\). One explanation for this finding is a change in its pharmacokinetics since pharmacokinetic properties of several other drugs, for example, digoxin\(^{(49-52)}\), digitoxin\(^{(53)}\), fentanyl\(^{(54-56)}\), cephalosporins\(^{(57)}\), and propranolol\(^{(58-61)}\) are altered during cardiopulmonary bypass. Primary uses of cardiopulmonary bypass are to supply the body tissue cells with nutritional materials such as oxygen and to remove metabolic waste products in the absence of cardiac function. It makes no difference to the cell whether these things are done by the natural heart or by a mechanical pump. However, the extracorporeal pump oxygenator at this moment still does not completely simulate the entire heart and lung function. There are several differences between normal circulation and cardiopulmonary bypass: different circulation patterns in the vascular system for venous return, different circulation patterns in the arterial system, and nonpulsatile blood flow. Physiological effects resulting from the bypass and some of their pharmacokinetic sequelae has been demonstrated. At the initial stage of cardiopulmonary bypass, hemodilation occurs sudden-
ly when the patient's blood volume is mixed with the cardiopulmonary bypass-priming solution. The resultant decrease in blood viscosity cause a reduction in systemic vascular resistance, thus hypotension occurs. A more direct influence on pharmacokinetic parameters occurs as plasma drug concentration is reduced proportionately: The apparent volume of distribution increases acutely with hemodilution. During cardiopulmonary bypass, the patient's body temperature is lowered to 27°-32°C using an heat exchanger. This hypothermia may reduce metabolic clearance, reduce enzymatically active secretion, and/or reabsorption of certain drugs.

During cardiopulmonary bypass, pulmonary circulation is abolished. Lung isolation may significantly affect basic drugs, such as lignocaine and propranolol, which are thought to distribute significantly into the lungs(62). During bypass, the lung may serve as a reservoir for drug which can not be rapidly eliminated or distributed. After termination of bypass, when the lungs are reperfused by the pulmonary circulation, significant amounts of drug may be eluted into the systemic circulation raising the plasma concentration of the drug relative to that during bypass.

As cardiopulmonary bypass is continued, destruction of blood components can become significant. Hemolysis occurs, with release of intracellular proteins into plasma. Furth-
ermore, Stanley(63) found that muscle pO$_2$ falls and muscle pCO$_2$ rises significantly which may affect the tissue distribution of drugs whose tissue binding is sensitive to pH.

Furthermore, the stress of surgery and bypass might cause hormonal changes that could affect drug disposition. The kinin system is activated(64) and plasma catecholamine concentrations rise, as is also true of vasopressin, a potent vasoconstrictor(65).

Physiological effects at the termination of cardiopulmonary bypass are similarly striking. The body temperature rises back to normal, affecting both regional blood flow and enzymatic activity. As the blood pressure and cardiac output increase, perfusion of all tissues improves and the blood flow in peripheral tissues increases greatly.

Another possibility for a lessened effect of nitroglycerin during cardiosurgery is the possibility of adsorption of nitroglycerin to certain plastics and its extraction by the blood oxygenator. During cardiopulmonary bypass, nitroglycerin is transported via polyvinylchloride surgical tubing which connects to the blood oxygenator and to the patient. The blood oxygenator itself is another possible source of extraction for nitroglycerin. It has been reported that nitroglycerin can be adsorbed by iv injection sets made of polyvinylchloride or materials like cellulose propionate, polyester, polyethylene cellulose, or polyurethane.
The loss of nitroglycerin to PVC bags has been characterized as a rapid adsorption of drug to the surface followed by a slow diffusional absorption process. Therefore, adsorption studies of nitroglycerin to surgical tubing and to the oxygenator were needed to provide information exploring the possibility of inducing changes in the pharmacokinetics of nitroglycerin during cardiopulmonary bypass.

Although nitroglycerin has been used for a hundred years, its pharmacokinetic behavior is not completely understood. The pharmacokinetics of nitroglycerin are unusual in two ways. First, it has an extremely large systemic clearance and even then the values are variable and dependent on the site of blood sampling, even in the same individual. Secondly, there is an arterial-venous gradient in plasma concentration, but this arterial-venous concentration gradient diminishes with long term dosing. These characteristics created complications in explaining the pharmacokinetics of nitroglycerin. Serum concentrations of nitroglycerin have not been reported in cardiac surgery patients. Hence the current studies investigated the pharmacokinetics of nitroglycerin before and during cardiopulmonary bypass and thus provide another dimension to a completion of understanding the effects of the body on the disposition and kinetics of nitroglycerin.
Chapter II

EXPERIMENTAL

Study of the loss of nitroglycerin to central venous pressure catheters

An iv admixture of nitroglycerin\(^1\) was prepared in a 1000 ml glass container containing NaCl 0.45%\(^2\) at a final concentration of 100 µg/ml. Polyethylene iv tubing was connected to the bottle. Flow rate through the tube was maintained at 30 ml/h (50 µg/min) with an IVAC 230 infusion controller\(^3\).

Samples (3 ml) for measurement of nitroglycerin content were obtained from the bottle at the time of preparation and from the tubing effluent at 0 (first pass of solution through tubing), 30 and 60 minutes. A CVP catheter\(^4\) was then attached to the iv tubing, and effluent samples were collected at 0 (first pass of solution through catheter), 5, 10, 25, 60 and 90 minutes. All samples were collected in silanized glass vials and frozen until analyzed. Nitroglycerin content was determined by electron capture gas chromatography\(^8\) and results were reported as percent of nitroglycerin remaining, using the bottle concentration as 100 percent.
Study of the loss of nitroglycerin to a bubble oxygenator

An IV admixture of nitroglycerin was prepared by adding the contents of two nitroglycerin ampules (100 mg) to a 1000 ml glass IV bottle of normal saline. The bottle was connected to polyethylene tubing. An IVAC 230 controller was used to control the flow rate at 90 ml/h. Samples for analysis of nitroglycerin were obtained from the IV bottle and tubing effluent at 0, 30 and 60 minutes, the tubing was then attached to the accessory port at the top of an isolated bubble oxygenator and the solution permitted to flow at 90 ml/h. Samples for nitroglycerin analysis were obtained from the arterial outlet at 0 (first-pass of solution through the oxygenator), 5, 10, 25, 45 and 60 minutes. All nitroglycerin samples were collected in silanized glass vials and frozen until time of analysis.

Study of the loss of nitroglycerin to a cardiopulmonary bypass apparatus with a bubble oxygenator

A solution of nitroglycerin was prepared by adding an aliquot of a commercial nitroglycerin product to a 2 liter solution of normal saline to achieve a final concentration of 100 μg/ml. This solution was placed in the arterial reservoir of a bubble oxygenator with the temperature maintained at 32°C. Samples (3 ml) for nitroglycerin analysis were obtained from the nitroglycerin solution just after preparation (reference standard) and immediately after addi-
tion to the arterial reservoir. Twenty feet of PVC surgical tubing were connected to the oxygenator at the arterial outlet and venous inlet to form a continuous circuit (Figure 2.1). A roller pump was set to generate a flow of 5 l/min. A sample for nitroglycerin analysis was obtained from the venous inlet during the first pass of solution through the tubing. Further samples were obtained simultaneously from the arterial outlet and venous inlet at 5, 10, 25, 45 and 60 min. All nitroglycerin samples were collected in silanized glass vials and frozen prior to analysis.

Study of the loss of nitroglycerin to a cardiopulmonary bypass apparatus with a membrane oxygenator

A stock solution of nitroglycerin was prepared by adding an aliquot of a commercial nitroglycerin product to 2 liters of normal saline solution to obtain a concentration of 150 ng/ml. This solution was placed in a silanized glass container. An in vitro cardiopulmonary bypass circuit was constructed to mimic clinical conditions (Figure 2.2). A glass cardiotomy reservoir was the initial receptacle for the nitroglycerin solution. Twenty feet of PVC surgical tubing were connected to the reservoir to form a complete circuit. A membrane oxygenator was connected to the tubing ten feet beyond the reservoir.

A roller pump generated a flow of 5 l/min. Samples (3 ml) for nitroglycerin analysis were obtained from the stock
Figure 2.1 Experimental cardiopulmonary bypass apparatus circuit using a bubble oxygenator. Nitroglycerin solution at a concentration of 100 μg/ml was placed in the arterial reservoir of a bubble oxygenator and was infused through twenty feet of polyvinylchloride surgical tubing at a flow rate of 5 l/min. Samples for nitroglycerin analysis were obtained from the venous inlet and arterial outlet simultaneously at 0, 5, 10, 25, 45, 60 min.
Figure 2.2 Experimental cardiopulmonary bypass apparatus circuit using a membrane oxygenator. Nitroglycerin solution at a concentration of 150 ng/ml was placed in the glass cardiotomy reservoir. The solution was infused through twenty feet of PVC surgical tubing at a flow rate of 5 l/min. Samples were obtained simultaneously from the inflow and outflow ports at 0, 2, 5, 10, 25, 45 and 60 min.
Figure 2.2
solution immediately after mixing and after the solution was placed in the reservoir. Just as the roller pump was started, a sample was taken from the inflow port prior to entrance of solution into the oxygenator. And a sample was taken from the outflow port after initial passage through the oxygenator. Additional samples were obtained simultaneously from the inflow and outflow ports at 2, 5, 10, 25, 45 and 60 minutes. Samples were collected via a three way stopcock placed in the tubing 3-4 cm before and after the oxygenator. All nitroglycerin samples were collected in silanized glass vials and frozen at -20°C until time of analysis. An appropriate volume of sample was extracted twice with spectrograde pentane.

Nitroglycerin pharmacokinetics in patients during cardiopulmonary bypass

The study was approved by the Ohio State University Biomedical Science Human Subject Review Committee and informed consent was obtained from seven patients (four men and three women) with a mean (± SD) age of 54.7 ± 10 years and mean weight of 85.3 ± 16 kg. All had normal hepatic and renal function and none suffered from significant hypertension or congestive heart failure. Patients were scheduled for elective coronary artery surgery by the same surgeon. All were premedicated with lorazepam 0.03 mg/kg orally and 0.15 mg/kg morphine intramuscularly. Anesthesia was induced
with fentanyl (50 µg/kg) while the patient was breathing 100% oxygen. Patients were given pancuronium 0.1 mg/kg intravenously concurrent with fentanyl. Anesthesia was maintained with additional doses of fentanyl to a total dose of 100 µg/kg.

Nitroglycerin was freshly prepared using a Tridil\textsuperscript{R} ampule, 50 mg/10 ml. A glass bottle of dextrose 5% in water was used and the nitroglycerin added to give a final concentration of 200 µg/ml. The bottle was connected to polyethylene intravenous tubing. The dosage was controlled using an IVAC-530 infusion pump and the drug was infused into the patient's central pressure catheter throughout the study.

The apparatus for cardiopulmonary bypass basically consists of (1) A pump, which is required to return the blood to the arterial system. It has the capacity to provide the equivalent of a basal cardiac output under the circumstances of perfusion. It also creates a reasonably high pressure in the arterial vascular tree. (2) Tubing and other circuit components: connectors, filters, heat exchangers, reservoirs. When the blood is exteriorized and exposed to the process of oxygenation, heat loss occurs. It is necessary to add heat to maintain the temperature of the blood at about 37°C. A heat exchanger is also necessary when the body must be kept at a controlled low temperature (hypothermia). (3) An oxygenator, in which the blood is exposed to
oxygen and through which carbon dioxide is eliminated. (4) Sensors: Transducers and monitors that permit assessment and control of the process.

The main circuit for cardiopulmonary bypass is shown in Figure 2.3. The blood is drained from the venous system through a venous catheter(1) and blood conduit(2). It then reaches the reservoir(3) and passes through the oxygenator(4) and heat exchanger(5). The blood then is pumped through a bubble catcher(7) and a blood filter(8) into an arterial catheter. It then returns into patient's total circulation.

Before the extracorporeal circuit is connected to the patient, the circuits are filled with a priming solution which was the same for each patient. Perfusion was started as a partial bypass where blood is still flowing into the heart and through the lungs. Tourniquets put around the venous catheters are then closed. This directs all the blood into the oxygenating device. During such a total bypass the device has to totally support the body circulation and metabolism, maintaining a reasonable blood flow and pressure and oxygenating the total cardiac output. The average blood flow was 5 l/min and patients were cooled to an average body temperature of 27°C.

All blood samples were collected in disposable plastic syringes shown not to adsorb nitroglycerin and was immedi-
Figure 2.3 The main circuits used in cardiopulmonary bypass surgery. (1) venous catheter, (2) blood conduit, (3) reservoir, (4) oxygenator, (5) heat exchanger, (6) roller pump, (7) bubble catcher, (8) blood filter, (9) meter, (10) arterial catheter.
ately transferred into cold, silanized glass tubes. The tubes were placed in a centrifuge within one minute of sample collection, and spun at 5°C. Sera were frozen at -20°C and analyzed by gas chromatography within two weeks of collection.

**Data analysis**

Apparent nitroglycerin clearance (Cl) was determined using the arterial serum nitroglycerin concentration at steady state (Css) and infusion rate (R) by the relationship:

\[
Cl = \frac{R}{Css}
\]

Steady state nitroglycerin concentrations were defined as those samples obtained after at least 20 min (5 half-lives) of a constant nitroglycerin dosage. In the patients where simultaneous arterial and venous samples were taken, the arterial-venous extraction was calculated using nitroglycerin concentrations from the arterial (Ca) and venous (Cv) sites by the expression:

\[
\text{Extraction Ratio} = \frac{Ca - Cv}{Ca} \times 100
\]
Nitroglycerin dosage and apparent nitroglycerin clearance before and during bypass were compared by the paired t-test. Differences were considered significant at $p < 0.05$.

**Gas chromatographic assay method**

All reagents used were analytical grade unless otherwise indicated. Three percent carbowax 20M-TPA was used as a stationary phase on 60/80 mesh supelcoport. The coated material was packed into a 42 cm x 2 mm silanized glass column. A Varian 3700 series dual column gas chromatograph equipped with an 8 mCi $^{63}$Ni electron capture detector coupled with a Shimadzu Chromatopac C-RIA recording data processor was used. The temperature of the injection port, column and detector were 150°, 135° and 200°, respectively. The carrier gas was 5% methane in argon used at a flow rate of 20 ml/min. The gas was passed through a Dow gas purifier before entering the gas chromatograph. Variation in retention times was ± 1.5%, when studies are run on the same day, and the day to day variation is about 5%. An internal standard (o-iodobenzylalcohol) was added to the plasma sample or to an appropriate dilution of sample. The sample was extracted with 4 ml of pentane twice right after it had been thawed to room temperature. Both extracts were combined and concentrated or diluted for GC analysis.
Chapter III

RESULTS AND DISCUSSION

Loss of nitroglycerin to the central venous pressure catheter

An increasing number of cardiovascular disordered patients undergo surgery. In many instances, nitroglycerin may not be infused directly into a peripheral vein but through a central venous pressure (CVP) catheter, which is made from PVC. The solution may be transported via surgical tubings which connect the blood oxygenator to the patient during cardiopulmonary bypass (CPB). Any of these routes can potentially remove nitroglycerin from solution. The loss of nitroglycerin to a central venous pressure catheter was studied to determine the impact of this component on drug therapy.

The loss of nitroglycerin to polyethylene iv tubing is summarized in Figure 2.4. It is seen that the first pass of solution delivered 93.6 percent of the bottle concentration and at 30 and 60 minutes, 97.5 and 99.7 percent were delivered. This shows the nonadsorptive character of polyethylene tubing toward nitroglycerin.
Figure 2.4 The loss of nitroglycerin as a function of time after drug infusion through polyethylene plastic tubing.
Figure 2.4
With a CVP catheter attached substantial loss of nitroglycerin occurred initially (Figure 2.5). There was maximal loss during the first pass of solution. The amount of nitroglycerin delivered increased with time and at 90 minutes, 97 percent of the bottle concentration was delivered.

Nitroglycerin is a lipo soluble drug that has been shown to adsorb to plastic iv bags and infusion sets composed of polyvinylchloride (PVC)(8-12), but this is the first attempt that follows the loss of nitroglycerin to a central venous pressure catheter.

Initial potency in the plastic tube was much lower than the theoretical potency (Figure 2.5) showing that the adsorption process is very fast. In the time the solution traverses the tube drug adsorption has started. The mechanism of loss of nitroglycerin to the PVC tube has been studied by Sokoloski et al.(66), who demonstrated that a type III adsorption behavior happened upon initial contact of nitroglycerin and the PVC tube. Equilibrium studies where nitroglycerin was incubated with strips of a PVC intravenous container were done by Yuen et al.(27). They showed that nitroglycerin is removed from aqueous solution by the plastic through an absorption process over long term incubation, and that the time course for the absorption can be described as diffusion through the plastic matrix. Thus initial drug loss is due to rapid adsorption. Once the adsorption sites
Figure 2.5 The loss of nitroglycerin as a function of time after drug infusion through a central venous pressure catheter.
are saturated, absorption by the polymer matrix becomes the limiting factor. Because absorption processes are slow, little drug is lost from solution as it flows through tubing, only adsorptive loss is important at this phase of administration. With time the amount of nitroglycerin delivered increases as adsorption sites become saturated, as shown in Figure 2.5. Thus, the most likely mechanism for the loss of nitroglycerin to PVC bags is a rapid adsorption of drug to the surface followed by a slow diffusional controlled absorption process, this can be described diagrammatically as in Scheme I(67).

\[
\begin{array}{ccc}
\text{nitr
glycerin} & \xrightarrow{k_1} & \text{nitr
glycerin} \\
\text{in aqueous} & & \text{adsorbed on} \\
\text{solution} & \xrightarrow{k_2} & \text{plastic surface} \\
\end{array}
\]

\[
\text{nitr
glycerin} \rightarrow \text{dissolved in} \\
\text{plastic matrix}
\]

**Scheme I**

Scheme I can be expressed in terms of the instantaneous amounts of nitroglycerin in solution(A), nitroglycerin adsorbed to the plastic surface(B), and nitroglycerin dissolved in the plastic matrix(C), as in Scheme II.

\[
\begin{array}{ccc}
A & \xrightarrow{k_1} & B \\
& \xrightarrow{k_2} & C \\
\end{array}
\]

**Scheme II**
The partition coefficient of nitroglycerin between these plastic and aqueous solutions is about 10⁴ at 30°C(27), therefore the affinity of nitroglycerin is expected to be much greater for the nonpolar plastic matrix than the aqueous phase. It is expected that $k_3 \gg k_2$, thus the loss of nitroglycerin to PVC bags can be described mathematically by equation 1(67).

$$A = \beta e^{-k_3 t} + (A - \beta) e^{-k_1 t}$$

(eq. 1)

where

$$\beta = \frac{A_0}{k_3^2 + \frac{k_1^2}{k_1} - \frac{2k_3}{k_1 k_2}}$$

The polyvinylchloride plastic used is a homogeneous gel that is essentially a complex mixture of polyvinylchloride, plasticizers, fillers, and other ingredients. Thus, the sorption process is dependent on (1) The formulation of the plastic material. (2) The concentration of nitroglycerin in solution. (3) The ratio of the volume of nitroglycerin solution to the area of the plastic surface in contact with the solution. (4) The solubility of nitroglycerin in plastic matrix. (5) The partition coefficient of nitroglycerin between
plastic and the aqueous phase, and (6) the temperature. The constant $k_1$ is a function of the amount of drug in solution, the surface area available for adsorption, and the difference between solution and plastic matrixes. The constant $k_3$ is a function of the volume of the plastic matrix and the solubility of nitroglycerin in this matrix.

In this study, because of the short length of the catheter used, the limited area for adsorption appears to be saturated quickly. Although drug continuously diffuses through the plastic matrix, the diffusion rate is comparatively slow. Therefore, the amount of drug delivered increases to 90 percent of the true infusion solution within 90 minutes. It should be noted that nitroglycerin may also be infused through longer catheters, such as flow-directed pulmonary artery catheters. Since these catheters are also made of PVC and are as long as IV tubing, the rate and extent of drug loss may be quite significant.

**The loss of nitroglycerin to the bubble oxygenator**

The nitroglycerin concentration of the solution in the arterial reservoir was 100% of that of the stock solution. When the solution was infused into the bubble oxygenator, it took one hour and 15 minutes for a sufficient volume (3 ml) of solution to be recovered from the arterial outlet for analysis. The solution then flowed freely and nitroglycerin
analysis revealed substantial removal of drug (Table 2.1). The first pass extraction is very high (> 90%). After 60 minutes, less than 15% of delivered drug concentration appeared in the arterial outlet. The bubble oxygenator consists of a gas exchange column where oxygen is transferred to the blood and carbon dioxide is removed. It also has an integral venous heat exchanger, a bubble defoaming chamber, and an arterial reservoir. The unit also includes an integral 0.2 micron hydrophobic bacterial gas filter. When venous blood enters the oxygenator, it ascends up a heat exchanger and is mixed with oxygen. Blood then flows into a polyurethane sponge covered with nylon tricot and enters into a polycarbonate arterial reservoir. Since nitroglycerin can be adsorbed to polyurethane(68), it appears that this defoaming device is primarily responsible for the extraction. The amount of adsorbed drug decreased with time probably due to saturation of adsorption sites in the oxygenator. The data presented here may not quantitatively represent the clinical situation because blood carrying nitroglycerin would rapidly flow through the oxygenator at a rate approximating cardiac output. The rate of infusion (90 ml/h) in this study is much slower than that of cardiac output, but the adsorption of nitroglycerin to a bubble oxygenator, the most essential component of a cardiopulmonary bypass apparatus, does happen and must have impact in clinical settings.
Table 2.1. Loss of nitroglycerin to a bubble oxygenator

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Arterial outlet sample (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.30</td>
</tr>
<tr>
<td>5</td>
<td>0.55</td>
</tr>
<tr>
<td>10</td>
<td>3.60</td>
</tr>
<tr>
<td>25</td>
<td>8.21</td>
</tr>
<tr>
<td>45</td>
<td>10.32</td>
</tr>
<tr>
<td>60</td>
<td>14.31</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as % Nitroglycerin remaining in an iv admixture.

<sup>b</sup> First pass of solution through oxygenator.
Loss of nitroglycerin to CPB apparatus with a bubble oxygenator

The loss of nitroglycerin to a CPB apparatus is given in Table 2.2. As the solution circulated through the system, there was substantial removal of nitroglycerin. The loss of nitroglycerin to the surgical tubing was minimal (5% loss) upon first-pass of solution; however, significantly less nitroglycerin was available as the solution continued to circulate through the system. The loss continued throughout the study period. After 60 minutes only 18% of the initial nitroglycerin concentration was available. The major loss occurred during the first pass of drug through the oxygenator; an extraction of 65%. Subsequent loss to the device was minimized due to possible saturation of extraction sites on the surface. The data in Table 2.1 showing a higher extraction ratio (>90%) than that in this experiment (65%) can be explained by a longer transit time through the system using a flow rate of 90 ml/h compared to that of 51/min in this experiment.

The initial flow of nitroglycerin through the surgical tubing resulted in only a 5% loss of drug (Table 2.2). This is in contrast to up to a 50% loss of drug after first-pass of solution through catheter (Figure 2.5). These findings can be explained, at least partly, by the fact that the extent of drug adsorption is dependent on the ratio of surface
Table 2.2. Loss of nitroglycerin to a cardiopulmonary bypass circuit with a bubble oxygenator

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>outflow port sample (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>inflow port sample (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.8</td>
<td>95.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>30.5</td>
<td>43.0</td>
</tr>
<tr>
<td>10</td>
<td>28.7</td>
<td>28.7</td>
</tr>
<tr>
<td>25</td>
<td>25.3</td>
<td>22.3</td>
</tr>
<tr>
<td>45</td>
<td>20.4</td>
<td>19.8</td>
</tr>
<tr>
<td>60</td>
<td>17.8</td>
<td>18.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as % nitroglycerin stock solution remaining.

<sup>b</sup> First pass of solution through tubing.
area of plastic to the volume of solution and also on the rate of drug flow through the system. Contact time between nitroglycerin solution and the tubing affects the extent of drug loss as has been shown by Baaske et al. (75). The surface area to volume ratio of surgical tubing with a 1/2 inch internal diameter is 8 while that of an equal length of iv tubing with an internal diameter of 1/10 inch is 40; a 5-fold difference. More drug, therefore, would be expected to be lost when solution flows through plastic iv tubing with a large surface area to volume ratio at slower rates.

Extraction of nitroglycerin by a CPB apparatus with a membrane oxygenator

The in vitro CPB circuit used in this study was designed to mimic clinical conditions. The solution flowed at a rate similar to normal cardiac output and was cooled to hypothermic conditions. The length of surgical tubing was similar to that used during cardiac surgery. The nitroglycerin measured in the cardiotomy reservoir was 97.4% of the stock solution and all subsequent measurements are reported using this value as the reference. As the solution circulated through the circuit, there was substantial loss of nitroglycerin (Table 2.3). A first pass of solution through the surgical tubing resulted in minimal (11.3%) loss, whereas the oxygenator extracted 70% of the drug concentration entering the inflow port. Drug concentration in-
Table 2.3. Loss of nitroglycerin to a cardiopulmonary bypass circuit with a membrane oxygenator

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Inflow port sample (%)</th>
<th>Outflow port sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>88.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>48.1</td>
<td>35.2</td>
</tr>
<tr>
<td>5</td>
<td>30.8</td>
<td>30.1</td>
</tr>
<tr>
<td>10</td>
<td>27.2</td>
<td>26.0</td>
</tr>
<tr>
<td>25</td>
<td>19.9</td>
<td>23.5</td>
</tr>
<tr>
<td>45</td>
<td>22.5</td>
<td>23.3</td>
</tr>
<tr>
<td>60</td>
<td>20.2</td>
<td>21.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expression as % nitroglycerin relative to stock solution.

<sup>b</sup> First pass of solution through tubing.

<sup>c</sup> First pass of solution through oxygenator.
creased transiently for the first 10 minutes then remains constant throughout the remainder of the study.

The first pass of solution through the tubing resulted in only an 11.3% loss of drug which is consistent with a previous finding (Table 2.2). Minimal loss is believed a result of the rapid flow rate of drug and the small surface area of tubing relative to the volume of solution passing through the tubing.

The membrane oxygenator consists of a silicone rubber sleeve in which a homogenous silicone rubber membrane is wound in a spiral coil upon a polycarbonate spool. The interior of the silicone rubber membrane envelope is the gas compartment. A spacer screen inside this envelope permits gas flow. Blood flows between turns of the envelope in a thin film. Oxygen from the gas compartment diffuses through the membrane into the blood stream. Carbon dioxide diffuses through the membrane into the gas compartment and is flushed from the oxygenator by the oxygen flow. During CPB, venous blood flows into the inflow port, around the membrane for gas exchange and exits through the blood outflow port. The most likely site of loss of nitroglycerin is the membrane since nitroglycerin can be adsorbed to silicone rubber(69).

After the initial loss of drug during the first pass through the oxygenator, there was a transient increase in concentration followed by a plateau where only about 20% of
the initial concentration remained (Table 2.3). The increased concentration observed during the first ten minutes of the study was apparently due to the incomplete emptying of solution from the cardiotomy reservoir during the initial passage. The solution from the outflow port of the oxygenator then mixed with the original stock solution remaining in the reservoir. After the first pass of solution, only 26.5% of the original nitroglycerin concentration remained. This magnitude of drug loss, however, must be cautiously extrapolated clinically due to possible sequestration of drug from the oxygenator by plasma proteins and saturation of oxygenator adsorption sites during prolonged administration.

In summary, significant nitroglycerin is extracted by an oxygenator at flow rates comparable to those of cardiac output. Thus, even if precautions are taken to prepare the nitroglycerin solution in a glass bottle and the drug is infused through nonadsorbing iv tubing, the patient may not receive a constant dose of nitroglycerin during CPB despite use of a constant drip rate. This may explain, at least in part, the increased nitroglycerin requirement noted during CPB in comparison with the prebypass period.

Nitroglycerin pharmacokinetics in patients during cardiopulmonary bypass

The metabolism of nitroglycerin after oral administration of $^{14}$C-labelled drug has been studied. Nitroglycerin
is converted in the body to 1,2- and 1,3-dinitroglycerin, which are further metabolized to 1- and 2-mononitroglycerin. The latter metabolites are converted to glycerol, and ultimately, excreted in the urine or expired as carbon dioxide (3). In the past, the biotransformation of nitroglycerin was suggested as being a result of enzymatic reaction involving glutathione-organic nitrate reductase in the liver (20). Reduced nitroglycerin molecules are either conjugated to form the glucuronides or are further denitrated. The conjugates are excreted in the urine as depicted in Scheme III.
Although nitroglycerin has been used for many years, information on its pharmacokinetics has only recently become available. Pharmacokinetic characteristics of nitroglycerin after intravenous, sublingual, and intranasal administration in man have been reported. The drug has a brief duration of action with an average elimination half-life of 2-4 minutes, an apparent volume of distribution of 4-210 l and a clearance of 4-146 l/min(71). This suggests a wide distribution in the body and a rapid elimination. The reported clearance values for nitroglycerin are much greater than average liver blood flow (1.5 l/min). This suggests either metabolism by organs not commonly thought to degrade drug or an uptake or storage of drug within the body. In previous studies, it shown that metabolism of nitroglycerin in human blood is rapid and predominately takes place in the cellular compartment of the blood. Decrease of nitroglycerin followed a pseudo-first order process and the interaction rates varied with the initial concentration. This is accompanied by a simultaneous increase in metabolite levels. This kind of biotransformation process is describable by Michaelis-Menten kinetics with competitive product inhibition. If whole blood volume can be considered as a metabolically active system, then the blood system may be an important in vivo extrahepatic metabolic site for nitroglycerin.
In this study, nitroglycerin dosages required to maintain normal hemodynamics ranged from 7-100 µg/min for different patients. The average dosage before and during cardiopulmonary bypass for the same individual was not significantly different (Table 2.4). Arterial nitroglycerin concentration varied widely among patients and ranged from 2.5 to 23.8 ng/ml before bypass and 2.0 to 18.3 ng/ml during bypass (Table 2.4) which is probably due to rapid elimination and extensive tissue distribution. Plasma nitroglycerin concentrations can be greatly affected by minor changes in tissue binding of the drug. In four patients where simultaneous arterial and venous samples were obtained, the venous nitroglycerin concentrations were always lower than the arterial concentration with an average arterial-venous extraction of 75.5% (Table 2.4). Armstrong et al. also reported considerably lower serum nitroglycerin concentration when samples were obtained from a peripheral vein compared to those simultaneously drawn from the radial or pulmonary artery(72). Their average arterial venous extraction of 61% was similar to our results of 75.5% (Table 2.4). Site of sampling is therefore important in interpreting nitroglycerin pharmacokinetics. This preferential uptake of nitroglycerin by veins may explain the greater sensitivity of the veins to vasodilatory action of nitroglycerin(73-74), or the vasculature may be an in vivo distributive site for nitroglycerin.
Table 2.4. Nitroglycerin pharmacokinetic data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Nitroglycerin dose (µg/ml) before bypass</th>
<th>Nitroglycerin concentration (ng/ml) before bypass</th>
<th>Nitroglycerin concentration (ng/ml) during bypass</th>
<th>Arterial-Venous extraction (%)</th>
<th>Nitroglycerin clearance (1/min) before bypass</th>
<th>Nitroglycerin clearance (1/min) during bypass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33(34)a</td>
<td>16(25)a</td>
<td>6.8(2.3)b</td>
<td>66.2</td>
<td>4.85</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>16(27)</td>
<td>16(35)</td>
<td>2.7</td>
<td>3.1</td>
<td>--</td>
<td>5.92</td>
</tr>
<tr>
<td>2</td>
<td>66(21)</td>
<td>16(22)</td>
<td>10.5(2.2)</td>
<td>2.5</td>
<td>79.0</td>
<td>6.28</td>
</tr>
<tr>
<td></td>
<td>33(41)</td>
<td>16(23)</td>
<td>6.8(0.4)</td>
<td>3.6</td>
<td>94.1</td>
<td>4.85</td>
</tr>
<tr>
<td>3</td>
<td>100(47)</td>
<td>100(19)</td>
<td>20.1(4.5)</td>
<td>16.6</td>
<td>77.6</td>
<td>4.97</td>
</tr>
<tr>
<td></td>
<td>100(23)</td>
<td>100(24)</td>
<td>16.3(2.2)</td>
<td>16.2</td>
<td>88.0</td>
<td>5.46</td>
</tr>
<tr>
<td></td>
<td>100(35)</td>
<td>100(27)</td>
<td>23.8(5.2)</td>
<td>18.3</td>
<td>78.1</td>
<td>4.20</td>
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<tr>
<td>4</td>
<td>7(44)</td>
<td>7(18)</td>
<td>2.5</td>
<td>1.4</td>
<td>--</td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td>13(40)</td>
<td>7(40)</td>
<td>4.7</td>
<td>2.0</td>
<td>--</td>
<td>2.76</td>
</tr>
<tr>
<td>5</td>
<td>16(63)</td>
<td>47(60)</td>
<td>15.7(8.5)</td>
<td>12.2</td>
<td>45.6</td>
<td>1.02</td>
</tr>
<tr>
<td>6</td>
<td>16(31)</td>
<td>16(24)</td>
<td>4.9</td>
<td>3.6</td>
<td>--</td>
<td>3.26</td>
</tr>
<tr>
<td></td>
<td>16(43)</td>
<td>16(28)</td>
<td>6.1</td>
<td>2.9</td>
<td>--</td>
<td>2.62</td>
</tr>
<tr>
<td>7</td>
<td>33(41)</td>
<td>16(26)</td>
<td>7.5</td>
<td>2.9</td>
<td>--</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>33(46)</td>
<td>16(25)</td>
<td>6.7</td>
<td>2.3</td>
<td>--</td>
<td>4.92</td>
</tr>
</tbody>
</table>

**MEAN ± SD**

| 33 ± 29 | 31 ± 33 | 9.8 ± 6.8 | 6.7 ± 6.7 | 75.5 ± 15.8 | 4.16 ± 1.5 | 5.12 ± 0.94 |

*a* The number in parenthesis is duration of infusion (min).

*b* The number in parenthesis is concentration of nitroglycerin in venous blood.

*c* The value includes total body clearance and that caused by oxygenator during cardiopulmonary bypass.
During bypass, the average arterial nitroglycerin concentration was 6.7 ± 6.7 ng/ml (in a range of 1.4-18.3). Individual serum nitroglycerin concentrations varied widely among patients and were influenced by dose, cardiopulmonary bypass, and sampling site. However, the values were within the reported range in normal and stable congestive heart failure patients receiving intravenous nitroglycerin. In a group of congestive heart failure patients, Armstrong et al reported a therapeutic range of arterial serum nitroglycerin concentrations of 1.2-11.1 ng/ml in those patients responding to the drug with a 25% fall in pulmonary capillary wedge pressure. Except for patients 3 and 5 in the present study all patients had arterial nitroglycerin concentration within this range (Table 2.4).

Apparent nitroglycerin clearance increased an average of 23% during bypass (Table 2.4). The increased clearance of nitroglycerin seen in this study during cardiopulmonary bypass could be rationalized in several ways. Upon initiating cardiopulmonary bypass, the patients' hematocrits fell an average of 30% as the patients' blood is mixed with the priming solution. Serum drug concentrations reduced in some patients. Furthermore, the oxygenator may have functioned as an external organ of clearance since previous studies have shown a significant loss of nitroglycerin during passage through membrane and bubble oxygenators in a simulated
cardiopulmonary bypass apparatus (76). The magnitude of the reported drug loss is sufficient to cause the changes in pharmacokinetics observed in this study.

There are several factors, however, that may reduce the clearance of drugs during cardiopulmonary bypass. Liver blood flow as measured by indocyanine green clearance is reduced 30% during cardiopulmonary bypass (56). This would reduce hepatic clearance of drugs having a high hepatic extraction since their hepatic clearance is dependent on liver blood flow (77). Hypothermia is reported to slow metabolic enzyme processes. However it appears that the factors leading to an increased clearance of nitroglycerin predominate during cardiopulmonary bypass.

The increased apparent nitroglycerin clearance in this study, therefore only partially accounts for the previously reported increased nitroglycerin dosage needed to treat hypertension that develops during cardiopulmonary bypass (47-48). Despite this observation, nitroglycerin dosages were not significantly different in our patients during bypass compared to the pre-bypass period. This finding was also seen by Hempelmann et al. who reported a similar decrease in blood pressure before and during cardiopulmonary bypass at the same relatively low nitroglycerin dosage (33 μg/min) (78). Our patients and those in the Hempelmann study did not develop hypertension during cardiopulmonary bypass.
while those in the papers by Kaplan(47) and Townsend(48) did. Thus, the extent of increased nitroglycerin clearance during cardiopulmonary bypass does not apparently affect the hemodynamic response to the drug when administered to maintain normal hemodynamics since the venous system responds to very low concentrations of the drug(73).

For the first time, this study provided insight into the pharmacokinetics of nitroglycerin during cardiac surgery. We conclude that arterial nitroglycerin concentrations within the reported therapeutic range are achieved during cardiac surgery, however individual pharmacokinetics of the drug are variable. Apparent nitroglycerin clearance is increased during cardiopulmonary bypass, however the magnitude of change only partially explains the increased dosage requirement to treat hypertension during bypass.
FOOTNOTES

1. Tridil, American Critical Care, McGaw Park, IL.
2. Travenol, Deerfield, IL.
3. IVAC Corp., San Diego, CA.
4. Intracath, 12 inch 14 gauge, Deseret Labs, Sandy, UT.
5. Cobe Laboratories, Inc., Lakewood, CO.
6. Tygon, Norton Company Health Care Products, Akron, OH.
7. Sci-med Kobolow membrane lung, model 3500-3B (3.5 M )
Sci-med life systems, Inc., Minneapolis, Minn.
8. Bently Spiraflo BOS-10S, Bently Laboratories Inc.,
Irvine, Ca.
10. Supelco, Bellefonte, PA 16823.
REFERENCES


