Br. J. Pharmac. (1982), **77**, 083–088

ELIMINATION OF GLYCERYL TRINITRATE: EFFECTS OF SEX, AGE, SPECIES AND ROUTE OF ADMINISTRATION

C. IOANNIDES, D.V. PARKE & I.W. TAYLOR[1]

Biochemistry Department, University of Surrey, Guildford, Surrey, GU2 5XH

1 Orally administered glyceryl trinitrate to rats undergoes extensive first pass metabolism leading to low bioavailability.

2 Sex differences in the plasma elimination of glyceryl trinitrate were seen in the rat, the female exhibiting the longer plasma half-life. No sex differences in this respect were detected in the rabbit.

3 The plasma half-life of glyceryl trinitrate was longer and the volume of distribution larger, in older animals.

4 The plasma elimination of glyceryl trinitrate was different in various animal species. There was a good correlation between plasma half-life and animal bodyweight.

Introduction

Glyceryl trinitrate (nitroglycerine, GTN) enjoys wide-spread use because of its effectiveness in the treatment of angina and other heart conditions (Aronow, 1965; Parratt, 1979). Although first introduced more than a century ago, still very little is known about its rates of metabolism and pharmacokinetics, essentially because of the lack of a sensitive analytical method for its determination in biological fluids at the subnanogram level. A suitable technique has now been developed in our laboratory (Taylor, Ioannides, Turner, Koenigsberger & Parke, 1981) which permits the study of factors affecting the rates of metabolism and elimination, and therefore the pharmacological effect, of GTN.

Earlier studies have suggested that GTN administered orally undergoes extensive first pass metabolism (Needleman, Lang & Johnson, 1972; Yap & Fung, 1978) so limiting the usefulness of this route for administration of the drug. As a result, in clinical practice, GTN is often administered sublingually as this mainly avoids hepatic first pass metabolism and peak drug levels are achieved within 2 min (Armstrong, Armstrong & Marks, 1979).

The metabolism and elimination of drugs may also be affected by physiological factors such as sex and age, and may be different in various animal species. Sex differences in the activity of the hepatic microsomal drug metabolizing enzymes in the rat have been extensively documented (Schenkman, Frey, Remmer & Estabrook, 1967; Hietanen, 1974), and some sex differences have also been seen in the albino ferret (Ioannides *et al.*, 1977). However, no studies have been reported on sex differences in GTN metabolism, which involves denitration by glutathione-dependent nitrate reductase (Needleman, 1975; 1976) and/or glutathione S-transferases (Habig, Keen & Jakoby, 1975).

Foetal and newborn animals have very low levels of hepatic microsomal enzyme activity which steadily increase to reach adult levels within a few weeks of birth (Jondorf, Maickel & Brodie, 1959; Basu, Dickerson & Parke, 1971; Ioannides, Sweatman, Richards & Parke, 1977), but later decline again in old age (Kato & Takanaka, 1968; Rikans & Notley, 1981).

The metabolism of drugs proceeds at different rates in different animals and, in general, the rates of microsomal mixed function oxidation have been shown to vary inversely with the bodyweight of the animal species (Walker, 1978). The present work concerns the study of the bioavailability of GTN administered by different routes, and the effect of sex, age and species on the elimination of the compound from the plasma.

Methods

Adult Wistar albino rats (240–320 g, Animal Breeding Unit, University of Surrey) were anaesthetized with pentobarbitone sodium (Sagatal: BDH, Poole, Dorset; 60 mg/kg) given intraperitoneally prior to the experiment. The right external jugular vein and right common carotid artery were cannulated using Portex tubing (800/200/100/100; Portex Limited, Hythe, Kent). GTN, (0.75 mg/kg), synthesized by nitration of glycerol (Taylor *et al.*, 1981) was ad-

[1]Present address: Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre, Huntingdon, Cambs., PE18 6ES.

0007-1188/82/090083-06 $01.00

ministered intravenously via the jugular vein. The drug was dissolved in the minimum amount of propylene glycol and then diluted with saline. Blood samples (0.5 ml) were withdrawn at regular intervals from either the carotid artery cannula or by serial cardiac puncture; iodoacetamide (Sigma Chemicals Limited, Poole, Dorset), at a final concentration of 4 mM, was added to inhibit the plasma degradation of nitroglycerine. GTN (0.75 mg/kg) was also administered sublingually in <0.1 ml propylene glycol and blood samples were obtained from the carotid artery cannula. GTN (7.5 mg/kg) was also administered orally to unanaesthetized rats and blood samples obtained by 'tail-bleeding'. The method of blood withdrawal did not affect the pharmacokinetics of GTN, and no significant differences were seen in the different pharmacokinetic parameters following intravenous administration of the drug ($V_D = 4.6 \pm 2.0$ for carotid artery; 4.8 ± 1.9 for cardiac puncture).

Adult male, DNS-golden hamsters (118–135 g), Dunkin Hartley guinea-pigs (445–600 g, Animal Virus Research Institute, Pirbright, Surrey) and albino ferrets (1050–1600 g) (Porcellus Animal Breeding Ltd., Heathfield) were used. Each was anaesthetized with Sagatal (40–120 mg/kg) given intraperitoneally and both the right external jugular vein and right common carotid artery were cannulated. GTN (0.75 mg/kg) was administered intravenously and blood samples (0.25–0.5 ml) were obtained from the carotid artery. Since 4 mM iodoacetamide did not satisfactorily inhibit plasma degradation of GTN in the ferret, blood samples were kept at 0°C and plasma samples were frozen to −40°C. Each sample was extracted immediately upon thawing.

Male and female, miniature Dutch rabbits (Dutch Fl hybrid; 1400–1900 g, Animal Breeding Unit, University of Surrey) were used. GTN (0.75 mg/kg) was administered through the marginal vein of the left ear, by means of a butterfly infusion cannula (23 gauge; Abbott Laboratories Limited), and blood samples (0.5 ml) removed from the marginal vein of the right ear. Blood samples were kept at 0°C and plasma samples frozen as 4 mM iodoacetamide does not satisfactorily inhibit GTN degradation in rabbit plasma.

Aliquots of plasma (100 μl for hamster, 200 μl for other species) were analysed for GTN content by extraction into hexane followed by gas liquid chromatography using a 5% SP 2401 glass column (1.5 m × 4 mm) and a heated electron capture detector (^{63}Ni) with isosorbide dinitrate as internal standard (Taylor *et al.*, 1981). Recoveries of GTN from blood plasma were 77% for the rat, 35% for the hamster, 70% for the rabbit and guinea-pig, and 80% for the ferret, and standard curves varied with the animal species concerned.

The pharmacokinetic parameters for GTN given intravenously were calculated using the following equations:

$$V_D = \frac{X_0}{C_0}, \quad K_{el} = \frac{0.693}{T_{\frac{1}{2}}},$$

$$AUC_{tot} = \frac{C_0}{K_{el}}, \quad Cl = \frac{X_0}{AUC_{tot}}$$

where V_D = volume of distribution, X_0 = administered dose, C_0 = plasma GTN concentration at zero time, K_{el} = elimination rate constant, $T_{\frac{1}{2}}$ = plasma half-life. AUC_{tot} = total area under the curve, and Cl = total systemic clearance. For oral and sublingual administration the 'flip-flop' model is used since the rate of elimination is faster than the rate of absorption. In such a case:

$$V_D = \frac{FX_0}{K_{el}\, AUC_{tot}},$$

$$\text{where } AUC_{tot} = I\left[\frac{1}{K_a} - \frac{1}{K_{el}}\right], \quad Cl = \frac{FX_0}{AUC_{tot}}$$

$$\text{and } F(\%) = \frac{AUC_{tot} \text{ for oral administration (dose corrected)}}{AUC_{tot} \text{ for i.v. administration}} \times 100,$$

$$K_a = \frac{0.693}{t_{\frac{1}{2}}},$$

where K_a = absorption rate constant, and I = intercept on concentration axis.

Statistical evaluation was carried out by Student's *t* test.

Results

The pharmacokinetic parameters describing the elimination of GTN are markedly affected by the route of administration of the drug (Table 1). The plasma half-life of GTN is 2 and 4 fold greater when the drug is administered sublingually and orally, respectively than when it is given intravenously. This is because after intravenous administration the measured half-life is an elimination half-life, while after oral dosing it is an absorption half-life, and after sublingual administration it is a mixture of both. Extrapolated plasma peak levels (C_0) are lower following sublingual administration than intravenous dosage, and when orally administered are less than 10% of those achieved by intravenous administration. The absolute bioavailability of orally administered GTN is very low (about 5%).

Sex differences in the elimination of GTN from plasma occur in the rat but not in the rabbit (Table 2).

Table 1 Effect of route of administration on the pharmacokinetics of glyceryl trinitrate in the rat

Route	*Dose* ($mg\,kg^{-1}$)	$T_{\frac{1}{2}}$ (min)	C_0 ($ng\,ml^{-1}$)	V_D (l)	K_{el} (min^{-1})	*Cl* ($l\,kg^{-1}\,min^{-1}$)	AUC_{tot} ($ng\,min\,ml^{-1}$)	*F%*
Intravenous (6)	0.75	6.7±1.0	53±21	4.6±2.0	0.10 ±0.02	1.7±0.6	506±164	—
†Sublingual (5)	0.75	14.3±3.4**	20±4	4.5±0.3**	0.052±0.018	2.4±0.0*	301±126*	96±40
†Oral (4)	7.5	30±11**	3.9±1.5	3.7±0.3**	0.027±0.011	2.4±0.0	143±109**	††4.6±3.0**

Results are presented as mean values ± s.d. for the number of animals shown in parentheses.
†Pharmacokinetic parameters were determined using the 'flip flop' model.
††This value has been adjusted to account for the larger dose.
*$P<0.05$; **$P<.001$ when compared to the intravenous route.

The female rat exhibited a longer biological half-life, lower extrapolated plasma levels and larger volume of distribution than the male. Similarly the plasma half-life GTN was longer in older animals and plasma levels were lower due to the larger volume of distribution (Table 3). No age or sex differences were observed in total systemic clearance values.

Table 4 shows that the elimination characteristics of GTN vary in different animal species. Generally, there is an increase in plasma half-life with increasing body weight (Figure 1).

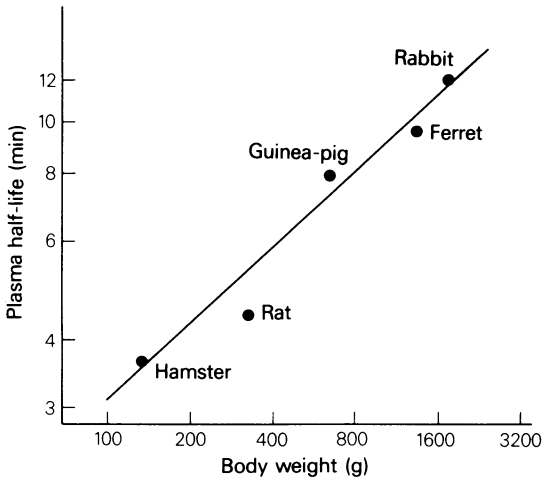

Figure 1 Correlation between animal body weight and plasma half-life of glyceryl trinitrate.

Discussion

Glyceryl trinitrate, because of its lipophilic nature is rapidly absorbed and extensively distributed, its plasma elimination obeying first order kinetics for a single compartment open model (Di Carlo, Crew, Haynes, Melgar & Gala, 1968; Armstrong *et al.*, 1979; Taylor *et al.*, 1981). Studies employing radiolabelled GTN showed that, following intravenous administration, very little GTN remained in the blood, the majority being extensively distributed (Johnson, Harkey, Blehm & Needleman, 1972). In the present study, only the parent compound was determined by using a sensitive g.l.c. technique specific for GTN (Taylor *et al.*, 1981). Large volumes of distribution were found for all species, although the analytical technique used determines both the free and protein-bound drug, indicating that as GTN is highly lipophilic it is probably localised in the fat.

The therapeutic efficiency of orally administered GTN is a controversial issue. It has been suggested (Needleman, 1970) that, as orally administered GTN undergoes such an extensive first pass metabolism in the liver, thereapeutic levels in the plasma are never achieved. This is supported by some clinical studies

Table 2 Sex differences in the pharmacokinetics of glyceryl trinitrate

Species	*Sex*	$T_{\frac{1}{2}}$ (min)	C_0 ($ng\,ml^{-1}$)	V_D (l)	K_{el} (min^{-1})	*Cl* ($l\,kg^{-1}\,min^{-1}$)	AUC_{tot} ($ng\,min\,ml^{-1}$)
Rat	Male (6)	4.4±0.7	111±59	2.6±2.0	0.16±0.03	1.59±1.49	689±306
	Female (9)	**7.7±1.2	*45±20	4.7±1.9	0.09±0.016	1.71±0.47	487±217
Rabbit	Male (4)	12.2±2.5	21±12	71±34	0.06±0.015	2.4 ±0.8	336±104
	Female (4)	11.5±1.3	14±2	101±17	0.06±0.007	3.4 ±1.0	237±59

Results are presented as mean values ± s.d., with the number of animals given in parentheses.
Animals received a single intravenous dose (0.75 mg/kg) of GTN.
*$P<0.01$; **$P<0.001$.

Table 3 Effect of age on the pharmacokinetics of glyceryl trinitrate in the rat

Age (weeks)	$T_{\frac{1}{2}}$ (min)	C_0 (ng ml^{-1})	V_D (l)	K_{el} (min^{-1})	*Cl* (l kg^{-1} min^{-1})	AUC_{tot} (ng min ml^{-1})
8	4.4±0.7	111±59	2.6±2.0	0.16±0.03	1.59±1.49	689±306
26	5.5±0.5	86±39	5.1±2.3	0.13±0.01	1.36±0.67	676±309

Results are presented as mean values ± s.d. for 6 animals.
Animals received a single intravenous dose (0.75 mg/kg) of GTN.

(Stipe & Fink, 1973), but other workers reported that sustained release oral preparations were beneficial to patients suffering from angina (Winsor & Berger, 1975). In the present study, oral administration of GTN resulted in low bioavailability, less than 5% of the dose apparently being absorbed systemically. Similarly Yap & Fung (1968) reported that only 1.6% of the dose of the unchanged drug reaches systemic circulation. Plasma peak levels were less than 10% of those achieved when the drug was administered intravenously even when the oral dose was ten times larger. First pass metabolism may occur in the liver, gastrointestinal tract or other extrahepatic sites. As the fraction of the dose absorbed (F) following sublingual administration was 96%, it is likely that there are no other sites of substantial first pass metabolism than the liver and gastrointestinal tract. Considerable organic nitrate reductase activity has been demonstrated in gastrointestinal cell preparations, being 40% of the hepatic specific activity, so the gastrointestinal tract may make a significant contribution to the first pass metabolism of GTN (Taylor, Ioannides and Parke, unpublished observations). The sublingual route of administration gave rise to higher blood levels and bioavailability, since this route mainly avoids hepatic and gastrointestinal first pass metabolism. Following sublingual administration to man, GTN appears in the blood within 30 s, reaches peak levels within 2 min (Armstrong *et al.*, 1979).

Sex differences in GTN metabolism were observed in the rat, the female having a slower rate of elimination and a longer biological half-life. Organic nitrate reductase has been demonstrated in rat, cat, rabbit, dog and man (Lee, 1973), and has been isolated, purified and shown to be indistinguishable from the glutathione S-transferases (Habig *et al.*, 1975). Sex differences in glutathione S-transferases have been reported for the rat, the male being more active (Chasseaud, 1979) and these may account for the observed sex differences in the plasma elimination of GTN. Similar sex differences in the rat are also known for microsomal enzymes, such as the mixed function oxidases (Schenkman *et al.*, 1967; Hietanen, 1974), to the extent that in toxicity studies one may consider the male and female rats as being two different species (Williams, 1974). No sex differences in GTN pharmacokinetics were noted in the rabbit except for a small increase in the volume of distribution in the female which may be attributed to its greater proportion of body fat.

The activities of the hepatic microsomal mixed function oxidases are low at birth, increasing with age and reach maximum levels usually within a few weeks (Jondorf *et al.*, 1959; Basu *et al.*, 1971; Ioannides *et al.*, 1977). However, the levels of enzyme activity start declining again as the animal approaches old age (Kato & Takanaka, 1968; McMartin, O'Connor, Fasco & Kaminsky, 1980; Rikans & Notley, 1981). In the present study older animals exhibited a longer plasma half-life and larger volume of distribution for GTN, the latter possibly reflecting their higher body fat content.

In all animal species studied, the elimination of

Table 4 Species variation in the pharmacokinetics of glyceryl trinitrate

Species (males)	*Body weight* (kg)	$T_{\frac{1}{2}}$ (min)	C_0 (ng ml^{-1})	V_D (l kg^{-1})	K_{el} (min^{-1})	*Cl* (l kg^{-1} min^{-1})	AUC_{tot} (ng min ml^{-1})
Hamster (6)	0.12	3.7±0.4	71±21	11.4±3.2	0.19±0.02	2.2 ±0.7	384±145
Rat (6)	0.28	4.4±0.7	111±59	9.5±7.4	0.16±0.03	1.59±1.49	689±306
Guinea Pig (6)	0.52	8.0±2.3	26±10	34±15	0.10±0.03	2.9 ±0.4	263±38
Ferret (4)	1.4	9.5±2.8	87±26	9.2±2.1	0.08±0.03	0.72±0.31	1212±517
Rabbit (4)	1.7	12.2±2.5	21±12	41±19	0.06±0.015	2.4 ±0.8	336±104

Results are presented as mean values ± s.d., with the number of animals given in parenthesis.
Animals received a single intravenous dose (0.75 mg/kg) of GTN.

GTN followed first-order kinetics for a one-compartment open model. Marked differences in the plasma elimination of GTN were seen, with the smaller animals exhibiting shorter plasma half-lives, i.e. faster elimination rates (Figure 1). The activities of the hepatic microsomal mono-oxygenases have also been shown to vary inversely with body weight (Walker, 1978; 1980; Parke & Ioannides, 1981; Parke, 1982). This is most probably due to species differences in tissue oxygen concentration, since tissue O_2 tension varies inversely with body weight (Booth, Boyland & Cooling, 1967). Although the metabolism of GTN does not involve O_2 directly, the correlation may result from the indirect involvement of O_2 in the metabolic formation of NADPH required to regenerate glutathione which is necessary for the denitration of GTN. Furthermore, species differences were also seen with respect to glutathione S-transferases (Chasseaud, 1979) and there is a good correlation between this activity and the half-life of GTN determined in the various species. The ferret, the only non-rodent animals used, exhibited a much lower volume of distribution than expected. This may be due to the ferret being a muscular animal having a smaller proportion of body fat in which the GTN may be localised.

In conclusion the present study confirms that GTN undergoes extensive first pass metabolism and that the organic nitrate reductases and/or glutathione S-transferases exhibits species, age and sex differences of the sort reported for the mixed function oxidases, indicating that both groups of enzymes may be under similar hormonal and genetic control.

The authors thank the Science Research Council and Biorex Laboratories, London for financial support for this work.

References

ARMSTRONG, P.W., ARMSTRONG, J.A. & MARKS, G.S. (1979). Blood levels after sublingual nitroglycerin. *Circulation*, **59**, 585–588.

ARONOW, W.S. (1975). Use of nitrates as antianginal drugs. *Handb. exp. Pharmak.*, **40**, 163–174.

BASU, T.K., DICKERSON, J.W.T. & PARKE, D.V. (1971). Effect of development on the activity of microsomal drug metabolising enzymes in rat liver. *Biochem. J.*, **124**, 19–24.

BOOTH, J., BOYLAND, E. & COOLING, C. (1967). The respiration of human liver tissue. *Biochem. Pharmac.*, **16**, 721–724.

CHASSEAUD, L.F. (1979). The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. *Adv. Cancer Res.*, **29**, 175–274.

DICARLO, F.J., CREW, M.C., HAYNES, L.J., MELGAR, M.D. & GALA, R.L. (1968). The absorption and biotransformation of glyceryl trinitrate-1,3-^{14}C by rats. *Biochem. Pharmac.*, **17**, 2179–2183.

HABIG, W.H., KEEN, J.H. & JAKOBY, W.B. (1975). Glutathione S-transferase in the formation of cyanide from organic thiocyanates and as an organic nitrate reductase. *Biochem. biophys. Res. Commun.*, **64**, 501–506.

HIETANEN, F. (1974). Effect of sex and castration on hepatic and intestinal activity of drug-metabolising enzymes. *Pharmacology*, **12**, 84–89.

IOANNIDES, C., SWEATMAN, B., RICHARDS, R. & PARKE, D.V. (1977). Drug metabolism in the ferret: Effects of age, sex and strain. *Gen. Pharmac.*, **8**, 243–249.

JOHNSON, E.M., HARKEY, A.B., BLEHM, D.J. & NEEDLEMAN, P. (1972). Clearance and metabolism of organic nitrates. *J. Pharmac. exp. Ther.*, **182**, 56–62.

JONDORF, N.R., MAICKEL, R.P. & BRODIE, B.B. (1959). Inability of newborn mice and guinea pigs to metabolise drugs. *Biochem. Pharmac.*, **1**, 352–354.

KATO, R. & TAKANAKA, A. (1968). Metabolism in old rats II. Metabolism *in vivo* and effect of drugs in old rats. *Jap. J. Pharmac.*, **18**, 389–396.

MCMARTIN, D.N., O'CONNOR, J.A. JR., FASCO, M.J. & KAMINSKY, L.S. (1980). Influence of aging and induction on rat liver and kidney microsomal mixed function oxidase systems. *Toxic. appl. Pharmac.*, **54**, 411–419.

LEE, N.H. (1973). The metabolism of glyceryl trinitrate by liver and blood from different species. *Biochem. Pharmac.*, **22**, 3122–3124.

NEEDLEMAN, P. (1970). Tolerance to the vascular effects of glyceryltrinitrate. *J. Pharmac. exp. Ther.*, **171**, 98–102.

NEEDLEMAN, P. (1975). Biotransformation of organic nitrates. *Handb. exp. Pharmak.*, **40**, 57–95.

NEEDLEMAN, P. (1976). Organic nitrate metabolism. *A. Rev. Pharmac. tox.*, **16**, 81–93.

NEEDLEMAN, P., LANG, S. & JOHNSON, E.M. JR. (1972). Organic nitrates: Relationship between biotransformation and rational angina pectoris therapy. *J. Pharmac. exp. Ther.*, **181**, 489–497.

PARKE, D.V. (1982). Species differences in pharmacokinetics. *Vet. Res. Commun.*, (in press).

PARKE, D.V. & IOANNIDES, C. (1981). Role of mixed-function oxidases in the formation of biological reactive intermediates. In *Biological Reactive Intermediates* II: Chemical Mechanisms and Biological Effects. New York: Plenum Press, (in press).

PARRATT, J.R. (1979). Nitroglycerin – the first one hundred years: new facts about an old drug. *J. Pharm. Pharmac.*, **31**, 801–809.

RIKANS, L.E. & NOTLEY, B.A. (1981). Decline in hepatic microsomal mono-oxygenase components in middle-aged Fischer 344 rats. *Exp. Geront.*, **16**, 253–259.

SCHENKMAN, J.B., FREY, I., REMMER, H. & ESTABROOK, R.W. (1967). Sex differences in drug metabolism by rat liver microsomes. *Molec. Pharmac.*, **3**, 516–525.

STIPE, A.A. & FINK, G.B. (1973). Prophylactic therapy of angina pectoris with organic nitrates. Relationship of drug efficacy and clinical experimental design. *J. clin.*

Pharmac., **13,** 244–250.

TAYLOR, I.W., IOANNIDES, C., TURNER, J.C., KOENIGSBERGER, R.U. & PARKE, D.V. (1981). Microdetermination of glyceryl trinitrate in biological fluids; effect of deuteration of glyceryl trinitrate on the pharmacokinetic properties. *J. Pharm. Pharmac.*, **33,** 244–246.

WALKER, C.H. (1978). Species differences in microsomal mono-oxygenase activity and their relationship to biological half-lives. *Drug Metab. Rev.*, **7,** 295–323.

WALKER, C.H. (1980). Species variations in some hepatic microsomal enzymes that metabolize xenobiotics. *Progress in Drug Metabolism*, **5,** 113–164.

WILLIAMS, R.T. (1974). Interspecies variations in the metabolism of xenobiotics. *Biochem. Soc. Trans.*, **2,** 359–377.

WINSOR, T. & BERGER, H.J. (1975). Oral nitroglycerin as a prophylactic antianginal drug: Clinical, physiologic and statistical evidence of efficacy based on a three-phase experimental design. *Am. Heart J.*, **90,** 611–626.

YAP, P.S.K. & FUNG, H.L. (1978). Pharmacokinetics of nitroglycerin in rats. *J. Pharmac. Sci.*, **67,** 584–586.

(*Received December 29, 1981.*
Revised April 2, 1982.)