Rectal Absorption of Nitroglycerin in the Rat: Avoidance of First-Pass Metabolism as a Function of Rectal Length Exposure

A. KAMIYA, H. OGATA*, and HO-LEUNG FUNG ˣ

Received June 8, 1981, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication September 2, 1981. * Present address: National Institute of Hygienic Sciences, Tokyo, Japan.

Abstract □ Nitroglycerin administered orally undergoes substantial presystemic elimination. It was shown recently that first-pass hepatic metabolism of high clearance drugs can be substantially avoided *via* rectal administration. In applying this concept to nitroglycerin in rats, it was found that unrestricted rectal instillation of nitroglycerin (at 3.5-mg/kg dose) gave a mean ±*SD* bioavailability of 26.7 ± 7.0% (n = 6) compared to 1.8 ± 0.9% (n = 5) from oral dosing. This mode of dosing did not lead to complete avoidance of first-pass metabolism of nitroglycerin in rats. When the rectal exposure length to nitroglycerin was restricted to 3.5 cm from the anus, the mean ±*SD* bioavailability increased to 83.5 ± 74.5% (n = 14). However, the variability in bioavailability was extremely large. When the rectal exposure length was restricted to 2.0 cm from the anus (at 1.75-mg/kg dose), nitroglycerin bioavailability was estimated at 91.2 ± 30.4% (n = 6). The plasma nitroglycerin concentrations (>5 min) obtained after this mode of administration were similar to those achieved after intravenous dosing. The data showed that substantial avoidance of presystemic nitroglycerin metabolism can be achieved *via* rectal administration. This avoidance can be nearly complete if nitroglycerin is limited in exposure to only the lower rat rectum. It was also demonstrated that sustained (at least 24 hr) nitroglycerin delivery *via* the rat rectal route was feasible with an experimental osmotic minipump. This delivery system also produced nearly complete bioavailability for nitroglycerin in the rat.

Keyphrases □ Nitroglycerin—rectal absorption, avoidance of first-pass metabolism as a function of rectal length exposure, rats □ Absorption, rectal—nitroglycerin, avoidance of first-pass metabolism as a function of rectal length exposure, rats □ First-pass metabolism—avoidance as a function of rectal length exposure, nitroglycerin, rats

Nitroglycerin undergoes extensive and variable first-pass metabolism when administered orally in rats (1–3) and in humans (4). Recent studies showed that rectal administration of lidocaine, propranolol, and salicylamide in rats led to substantial avoidance of first-pass metabolism of these drugs (5–7). The possibility of similar improvement in bioavailability of nitroglycerin, another high-clearance drug, through the rectal route has been suggested (5). This hypothesis, however, has not been tested.

The studies reported here were designed, therefore, to determine the bioavailability of nitroglycerin after rectal administration in rats. Since drug spreading in the colon and rectum may lead to reduction of avoidance of first-pass metabolism (5), rectal dosing of nitroglycerin was carried out with three different lengths of rectal exposure to examine this effect. Finally, an experimental rectal delivery system was tested to explore whether sustained and complete absorption of nitroglycerin was feasible *via* the rectal route.

BACKGROUND

Since its introduction into clinical practice by Murrell (8) about 100 years ago, nitroglycerin has been the drug of choice for the acute relief and prophylaxis of angina pectoris. Interest in nitroglycerin has increased substantially in the past few years because new important clinical use for this drug has been documented. For example, nitroglycerin improved hemodynamics in patients with congestive heart failure (9), myocardial infarction (10), and unstable angina (11). Recently, this drug has been also used for the induction of hypotension during anesthesia and surgery (12). In spite of the long history of use and the increasing therapeutic scope of nitroglycerin, however, many basic aspects of the pharmacokinetics of this drug have not been investigated. The recent availability of sensitive analytical methods (13, 14) have made initiation of these studies (1–4) possible.

Oral sustained-release dosage forms of nitroglycerin are used clinically for prophylaxis of angina. The effectiveness of these dosage forms, and indeed the oral route itself, have been a subject of considerable debate in the literature (15). The pioneering work of Needleman *et al.* (1) showed that oral dosing of nitroglycerin in rats gave negligible blood concentrations of intact drug compared to those found for metabolites. Since human liver biopsy samples were shown to have an enzyme capacity similar to that found in rats, these authors concluded that there is no rational basis for oral nitroglycerin use. However, this suggestion had been refuted by clinical experience (16) and also by a well-conducted clinical study (17) which showed oral nitroglycerin to have significant benefits in controlling angina. Recent pharmacokinetic data (4, 18) also showed that intact nitroglycerin could be detected in plasma after oral admin-

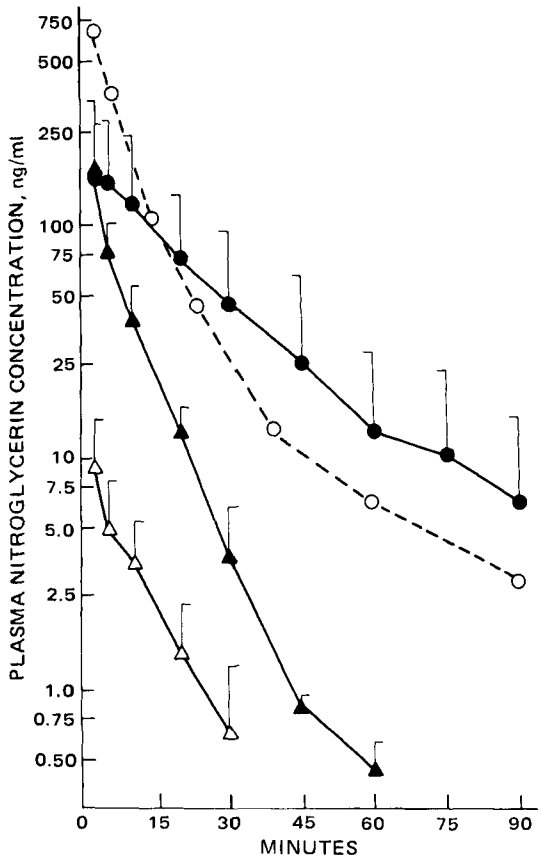

Figure 1—*Plasma nitroglycerin concentrations following different routes of administration at 3.50 mg/kg. Data represent mean ± SD of restricted rectal (3.5 cm) (●); unrestricted rectal (▲); and oral (△) administration. Plasma concentration curve after intravenous dosing (○) represents the predicted mean values calculated from a 1.75-mg/kg nitroglycerin dose*[8].

0022-3549/82/0600-0621$01.00/0

Table I—Bioavailability of Nitroglycerin following Different Routes of Administration

Route of Administration	Dose, mg/kg	Number of Animals	Peak Plasma Concentration[a], ng/ml	*AUC*[b], ng min/ml	Apparent Bioavailability[c], %
Oral	3.50	5	9 ± 5*	0.09 ± 0.04*	1.8 ± 0.9**
Unrestricted rectal	3.50	6	170 ± 58	1.28 ± 0.34	26.7 ± 7.0+
Restricted rectal (3.5 cm)	3.50	14	189 ± 181	4.02 ± 3.57	83.8 ± 74.5
Restricted rectal (2.0 cm)	1.75	6	129 ± 56	2.19 ± 0.73	91.2 ± 30.4

[a] Mean ± *SD*. [b] Area under plasma concentration *versus* time curve. [c] See *Experimental*. Symbols indicate significant difference from all other groups (*); from 100% and all other groups (**); and from 100% and restricted rectal (2.0 cm) dosing group (+).

istration of sustained-release capsules in humans. A report has appeared which suggests the bioavailability of a retard capsule to be ~20% of an equivalent dose of a sublingual tablet (4). In rats, the bioavailability of an oral nitroglycerin dose in aqueous solution was ~2% after a nitroglycerin dose of 7 mg/kg of body weight (2). Interanimal variability in oral bioavailability was also large. This variation has been found to be primarily due to the difference in the intrinsic activity of liver organic nitrate reductase in each individual animal (3). The composite evidence to date suggests, therefore, that although oral nitroglycerin may be bioavailable in part, first-pass metabolism (primarily hepatic in nature) is both extensive and variable.

A recent study has shown that the first-pass hepatic metabolism of lidocaine, another high clearance drug, could be avoided *via* rectal administration. This avoidance was partial in humans (5) but almost complete in rats (6). Nitroglycerin may exhibit a similar dependency. It was pointed out (7) that at least two sets of rectal veins (upper and lower) carry blood from the rat rectum: the upper rectal vein feeds into the portal vein, while the lower rectal vein empties into the inferior vena cava directly without first passing through the liver. Thus, drugs absorbed rectally and carried into the body *via* the upper rectal vein can be, in principle, subjected to first-pass elimination, while drug carried through the lower rectal vein is not. It is therefore anticipated that avoidance of first-pass metabolism may be dependent on the length of the rectum which is exposed to the drug solution. It is believed that there has not been any documentation of this phenomenon in the literature. Therefore, the bioavailability of rectally administered nitroglycerin as a function of rectal length exposure was examined to determine whether this hypothesis can be substantiated.

A major disadvantage of rectal delivery of drugs is that the bioavailability is highly variable. This variability may be caused by the difference in the time elapsed between rectal drug administration and bowel movement in individual subjects (19). If a rectal delivery device can be fabricated which produces ideal zero-order release at all times, then removal of the device for bowel movement and reinsertion of the same device (or for aesthetic reasons, a fresh one) afterwards will potentially allow the drug to be delivered at the same rate before and after bowel movement. For nitroglycerin, which has an extremely short half-life ($t_{1/2}$ = ~2–3 min) (2, 20, 21), zero-order delivery through the appropriate part of the rectum may also produce sustained plasma drug concentrations leading to potential prolongation of the therapeutic effects of the drug. The last part of this report deals with studies that explored the possibility of using an experimental osmotic pump system to produce sustained delivery and improved bioavailability of nitroglycerin in the rat.

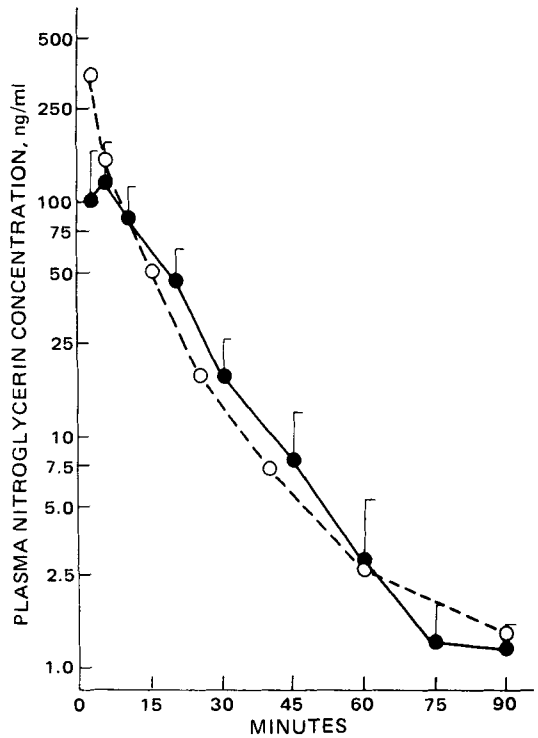

Figure 2—*Plasma nitroglycerin concentrations following restricted rectal (2.0 cm) administration at 1.75 mg/kg. Data represent mean ± SD after restricted rectal (2.0 cm) administration, (●); and mean after intravenous dosing*[8] (○).

EXPERIMENTAL

Materials—Nitroglycerin and isosorbide dinitrate solutions in hexane were prepared according to a previous report (14). A commercial 10% (w/w) nitroglycerin–lactose adsorbate[1] was used to prepare the aqueous and propylene glycol nitroglycerin solutions. To obtain the lactose-free nitroglycerin, an aqueous solution of the powder was extracted with ether, which was then evaporated under a gentle nitrogen stream. The resultant oil was dissolved in distilled water or propylene glycol to yield the appropriate concentration, which was then confirmed by gas chromatographic assay (14).

Animals—Male Sprague-Dawley rats[2] (300—360 g) were used. The right jugular vein was cannulated chronically (22). Animals were used after at least 16 hr of fasting prior to nitroglycerin administration and allowed free movement in a metabolic cage after dosing. Blood (~0.5 ml) was withdrawn *via* the jugular vein cannula at appropriate times.

Bolus Administration Studies—Nitroglycerin in normal saline solution (1.22 mg/ml) was used. For unrestricted rectal dosing, a septum plug[3] was affixed to the anus with glue[4]. Nitroglycerin was then injected into the rectum with a syringe through the septum. For restricted rectal

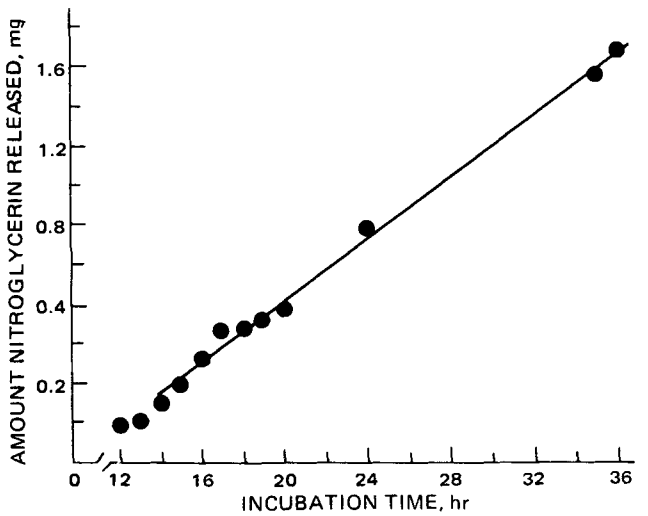

Figure 3—*A representative plot of* in vitro *nitroglycerin release from osmotic minipump.*

[1] Nitroglycerin 10% (w/w) in lactose, ICI America, Atlas Chemical Division, Wilmington, DE 19899.
[2] Blue Spruce Farms, Altamont, NY 12009.
[3] F-145 Septum plugs, 6.5-mm diameter, Alltech Associates, Arlington Heights, IL 60004.
[4] New Elmer's Wonder Bond, Borden, Inc., Columbus, OH 43215.

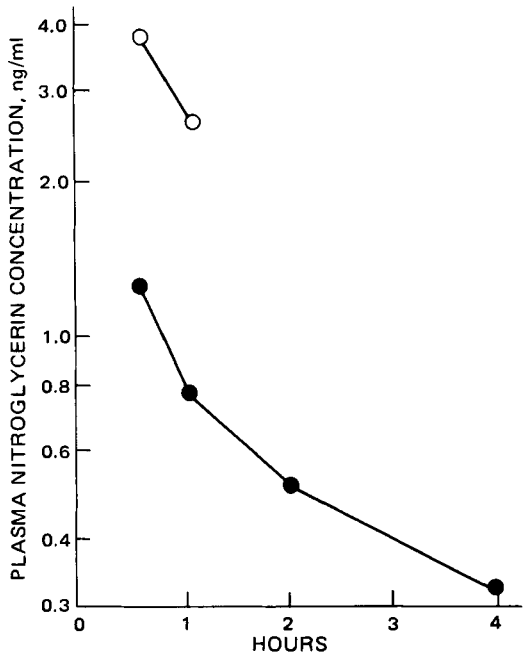

Figure 4—In vivo *nitroglycerin rectal delivery* via *osmotic minipump (upright placement). Different symbols indicate different animals. In the rat denoted by open circles, the plasma concentrations were indistinguishable from blank values at times beyond 1 hr.*

dosing, a device was constructed which connected two septum plugs at a fixed distance (either 2.0 or 3.5 cm) with a stainless steel wire. This device was inserted into the rectum from the anus while the animal was under slight ether anesthesia. The upper septum plug[5] was used to avoid upward spreading of nitroglycerin solution. The lower septum plug[3] was glued to the anus. Drug solution was injected into the rectum following the withdrawal of a volume of air from the rectum identical to that of the rectal drug solution. For oral administration, nitroglycerin solution was introduced by intubation to rats under slight ether anesthesia. Nitroglycerin doses were 3.50 mg/kg for unrestricted rectal dosing, restricted rectal dosing at 3.5-cm rectal length exposure, and oral dosing; and 1.75 mg/kg for restricted rectal dosing at 2.0-cm rectal length exposure. The lower dose was used in the last case because of limitations in the aqueous solubility of nitroglycerin and in the volume of dosing solution which could be injected at that specified rectal length exposure.

Sustained Rectal Delivery Studies—Osmotic pumps[6] were used as possible drug delivery systems for sustained rectal delivery of nitroglycerin. Nitroglycerin was dissolved in propylene glycol at 89.0 mg/ml. Each minipump was filled with ~0.25 ml of drug solution. The pump was placed at the bottom of a round-bottom flask in 200 ml of normal saline at 37°. The solution was stirred at 60 rpm and sampled from 12 to 36 hr (0.5 ml/sample) to determine the *in vitro* nitroglycerin release rate.

After this *in vitro* experiment, the minipump was washed with saline, blotted dry, and inserted into the rat. The osmotic pump was inserted into the rectum either in the upright (release hole pointing toward the intestine) or inverted position under slight ether anesthesia. For upright placement, the bottom of the osmotic pump was glued directly in place at the anus. For inverted placement, the neck plug of the pump was connected to a septum[3] by a stiff wire. This delivery system was inserted into the rectum and the septum was secured at the anus with glue. Blood samples were withdrawn up to 24 hr after insertion of these devices.

Assay of Nitroglycerin—After collection, blood samples were immediately transferred to chilled centrifuge tubes and centrifuged. Aliquots of plasma (0.2 ml) were mixed with 10 μl of 1 *N* silver nitrate and stored at −20° until assay. In the *in vitro* release experiment, 0.1-ml aliquots were assayed. The samples were extracted with hexane and nitroglycerin concentrations were determined by a previously reported gas chromatograph procedure (14) using isosorbide dinitrate as the internal standard.

The glue and septum plugs used were shown not to produce any interference in the assay of nitroglycerin. Sham experiments conducted using these devices showed also that the apparent clearance of nitroglycerin after intravenous injection was unaltered.

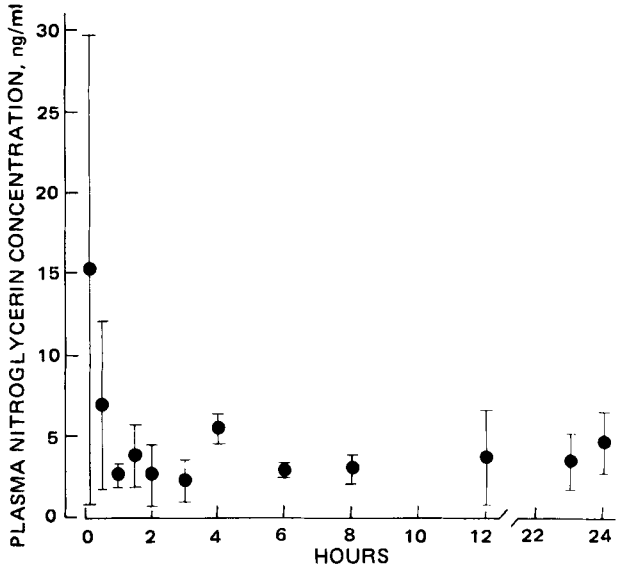

Figure 5—In vivo *nitroglycerin rectal delivery* via *osmotic minipump (inverted placement). Data represent mean* ± SD.

Calculation of Statistics—The area under the plasma concentration *versus* time curve (*AUC*) was computed using the spline method from a commercially available program with a desk top computer[7]. Residual area of *AUC* ($AUC_{t\to\infty}$) was estimated by C_t/k, where k was the first-order kinetic rate constant derived from linear least-square regression using the last three plasma concentrations of each experiment and C_t was the plasma nitroglycerin concentration at the last sampling time. The apparent bioavailability (F) after oral or rectal dosing was estimated by the traditional method of comparing the $AUC_{0\to\infty}$ to that of an equivalent intravenous dose. It is assumed here that the systemic clearance is independent of the route of administration. The apparent systemic clearance after intravenous bolus administration in rats has been found to be independent of dose from 0.15 to 2.5 mg/kg and has a value of 730 ± 241 ml/min/kg (mean ±*SD*, n = 26)[8].

Statistical analysis was performed using the unpaired t test and $p < 0.05$ was employed to establish statistical significance.

RESULTS AND DISCUSSION

Plasma nitroglycerin concentrations observed at various times after oral, rectal, and intravenous administration are shown in Fig. 1 (3.50-mg/kg dose) and Fig. 2 (1.75-mg/kg dose). Intravenous bolus doses of nitroglycerin at 3.50 mg/kg were found to be toxic to the animals, many of which exhibited immediate severe convulsions, presumably because of the extremely high initial concentrations. The intravenous curve shown in Fig. 1 was constructed, therefore, from the mean data of 1.75 mg/kg iv dose after correction for dose. It will be shown elsewhere[8] that the systemic intravenous nitroglycerin clearance in the rat is essentially concentration independent below its toxic dose.

Oral bioavailability of nitroglycerin at 3.50 mg/kg in aqueous solution was shown to be poor (Fig. 1). The apparent bioavailability was found to be ~2% at this dose (Table I), which agrees with data obtained previously at 7 mg/kg (2).

Three rectal lengths of exposure were used to determine the degree of avoidance of first-pass metabolism of nitroglycerin in the rat. In the first case, only one septum was affixed at the anus and the administered drug was allowed to spread unrestricted into the lower and upper intestines. In a preliminary experiment using a dye as a marker, it was noted that this mode of administration led to significant upward spreading of the rectal dose, presumably due to peristaltic movement of the GI tract. When nitroglycerin was given in this manner, the bioavailability was found to be 26.7 ± 7.0% (Table I) of a comparable intravenous dose. This bioavailability was ~15 times that of an oral dose, indicating that rectal dosing led to some avoidance of first-pass metabolism.

When the rectal dosing solution was restricted to a region no further than 3.5 cm from the anus, absorption of nitroglycerin was considerably improved but rather variable (Fig. 1). The terminal half-life obtained

[5] F-174 Septum, 9.0-mm diameter, Supelco, Inc., Bellefonte, PA 16823.
[6] ALZET Osmotic minipump, Model 2001, Alza, Palo Alto, CA 94304.
[7] Hewlett-Packard 9825A Calculator, Hewlett-Packard, Loveland, CO 80537.
[8] H.-L. Fung, G. A. Maier, H. Ogata, and A. Kamiya, unpublished data.

Table II—Individual Data on *In Vivo* Nitroglycerin Rectal Delivery *via* Osmotic Pump (Inverted Placement)

Rat	*In Vitro* Release Rate, μg/hr	Mean Plasma Nitroglycerin Concentration[a] (1 → 24 hr), ng/ml	Estimated[c] Mean *In Vivo* Release Rate, μg/hr
1	65.8	3.32 ± 2.14	48.0
2	38.7	3.46 ± 1.66	50.0
3	30.1	3.11 ± 1.41[b]	45.0
4	76.5	3.55 ± 1.76	51.3
Mean ± *SD*	52.8 ± 21.9	3.40 ± 1.72	48.6 ± 2.7

[a] Mean ± *SD*. [b] 1 → 4 hr only, rectal septum bitten off by animal. [c] $k_0 = C_{ss}Cl$.

after this mode of administration (16.5 ± 8.4 min, mean ± *SD*) appeared larger than, though not statistically different from, those observed after unrestricted rectal ($t_{1/2}$ = 10.1 ± 3.2 min) and intravenous administration (18.3 ± 6.1 min). Since the plasma clearance of nitroglycerin was shown to be independent of intravenous dose[8], it is unlikely that the systemic clearance of nitroglycerin has been altered with this mode of dosing. Any potential divergence in apparent plasma half-life in the present case might have arisen instead from differences in absorption rates among the treatments, since flip-flop kinetics might be operative after nonintravenous administration (2). Using these assumptions, the apparent bioavailability of nitroglycerin from this mode of rectal dosing (restricted, 3.5 cm) could be estimated as 83.8 ± 74.5% (Table I), which was ~3 times higher than that found in the case where the rectal solution was unrestricted.

Figure 2 shows the case in which the dose was restricted to the lowest 2.0 cm of the rectum. It is apparent that, except for the first time point, the rectal dose yielded similar plasma nitroglycerin concentrations compared to an equivalent intravenous dose. Indeed, the *AUC*'s calculated for these two routes of administration were not statistically different ($p > 0.05$). The bioavailability of this rectal mode of dosing was estimated at 91.2 ± 30.4% (Table I). The interanimal variability was noticeably smaller here than in the previous case where rectal exposure was set at 3.5 cm.

It is quite clear, then, that rectal administration of nitroglycerin in the rat leads to substantial avoidance of the first-pass metabolism of nitroglycerin. Consistent with anatomical prediction, this avoidance decreases when the rectal dose is exposed to the upper rectal area where drug can be adsorbed through the upper rectal veins and transported *via* the portal vein to the liver. It is believed that this is the first instance in which this suspected relationship of drug rectal length exposure to avoidance of first-pass metabolism has been clearly demonstrated.

A previous study has shown that instillation of lidocaine into the unrestricted rectum of rats led to near complete bioavailability of this drug (6). As far as can be determined, the present experimental procedure in the unrestricted rectum case was similar to that used by these authors, with the possibly important difference in the volume of rectal solution used. This study (6) used an instillation volume of 0.4 ml, whereas a volume of ~1.0 ml was used in the present study. The smaller volume used in the lidocaine study might permit less upward spreading of the dose, thus allowing more complete avoidance of first-pass metabolism. It is also possible that this apparent discrepancy may have arisen from the difference in the permeability and metabolic properties of the drugs themselves.

The osmotic minipump has been reported to produce sustained zero-order drug delivery *in vivo* (23). This particular experimental device was tested to explore whether rectal delivery of nitroglycerin can produce almost complete bioavailability as well as sustained plasma drug concentrations over a 24-hr period. When these minipumps were filled with a propylene glycol solution of nitroglycerin (an aqueous solution was impossible because of solubility limitations), the *in vitro* release was shown to be zero-order in nature (Fig. 3). Preliminary trials of these minipumps inserted into the animal in the upright position showed them to be ineffective in producing sustained plasma nitroglycerin concentrations (Fig. 4). Since zero-order delivery of drugs from the osmotic minipump depends, among other factors, on an unobstructed aperture in the pump itself, fecal materials which might have been deposited on the aperture could interfere with drug delivery. When the osmotic minipump was inverted so that the pump aperture now pointed toward the anus (which was sealed with a septum), the desired pharmacokinetic release profile was observed. Figure 5 shows the mean (±*SD*) plasma concentrations of nitroglycerin over 24 hr after insertion of the rectal osmotic minipumps in the inverted position in four rats. Although these pumps had been pre-equilibrated in saline at 37° for at least 36 hr (during which time the *in vitro* release rate was determined), an initial burst of nitroglycerin release was observed upon insertion of those devices. However, the plasma nitroglycerin concentrations from 1 to 24 hr after rectal pump insertion were quite stable (Fig. 5 and Table II).

It was noted that the *in vitro* drug release rate of the minipumps was quite variable. The reasons for this variability are not known presently. The *in vivo* release rate of each minipump could not be measured directly but could be approximated by the equation $k_0 = C_{ss}Cl$ where C_{ss} is the measured mean steady-state plasma nitroglycerin concentration in each animal and Cl is the systemic clearance of nitroglycerin (which averages 241 ml/min/300-g rat). Based on the mean values (Table II) the estimated *in vivo* release rates for the osmotic pumps appeared similar to the measured *in vitro* rates. This agreement suggests that near complete bioavailability can be achieved with this mode of rectal dosing. It would appear then that the experimental osmotic pump, when placed in the inverted position, can provide both sustained and complete delivery of nitroglycerin *via* the rectal route.

CONCLUSIONS

The present studies showed that rectal dosing of nitroglycerin in rats can lead to substantial avoidance of first-pass metabolism. If the nitroglycerin dose can be restricted to the lower rectum, this avoidance can be made complete. It has been shown that an experimental osmotic pump is capable of producing sustained and apparent zero-order release of nitroglycerin over a 24-hr period. Since the degree of avoidance of first-pass metabolism from rectal administration is somewhat less in humans when compared to the rat (5, 6), the applicability of the present findings to rectal absorption of nitroglycerin in humans has to be validated.

REFERENCES

(1) P. Needleman, S. Lang, and E. M. Johnson, Jr., *J. Pharmacol. Exp. Ther.*, **181,** 489 (1972).

(2) P. S. K. Yap and H.-L. Fung, *J. Pharm. Sci.*, **67,** 584 (1978).

(3) G. A. Maier, C. Arena, and H.-L. Fung, *Biochem. Pharmacol.*, **29,** 646 (1980).

(4) V. H. Maier-Lenz, L. Ringwelski, and A. Windorder, *Arzneim.-Forsch.*, **30,** 320 (1980).

(5) A. G. de Boer, D. D. Breimer, H. Mattie, J. Pronk, and J. M. Gubbens-Stibbe, *Clin. Pharmacol. Ther.*, **26,** 701 (1979).

(6) A. G. de Boer, D. D. Breimer, J. Pronk, and J. M. Gubbens-Stibbe, *J. Pharm. Sci.*, **69,** 804 (1980).

(7) A. G. de Boer and D. D. Breimer, in "Drug Absorption," L. F. Prescott and W. S. Nimmo, Eds., Adis Press, Balgowlah, Australia, 1981, pp. 61–72.

(8) W. Murrell, *Lancet*, **I,** 80, 113, 225, 642 (1879).

(9) E. Mikulic, J. A. Franciosa, and J. N. Cohn, *Circulation*, **52,** 477 (1975).

(10) P. W. Armstrong, D. C. Walker, J. R. Burton, and J. O. Parker, *ibid.*, **52,** 1118 (1975).

(11) P. B. Oliver, D. E. Plots, and R. G. Pluss, *N. Engl. J. Med.*, **288,** 745 (1973).

(12) H. A. Kaplan, R. W. Dunbar, and E. L. Jones, *Anesthesiology*, **45,** 14 (1976).

(13) M. T. Rosseel and M. G. Bogaert, *J. Pharm. Sci.*, **62,** 754 (1973).

(14) P. S. K. Yap, E. F. McNiff, and H.-L. Fung, *ibid.*, **67,** 582 (1978).

(15) J. C. Krantz, Jr., and C. D. Leake, *Am. J. Cardiol.*, **36,** 407 (1975).

(16) I. Hirshleifer, *Curr. Ther. Res.* **15,** 158 (1973).

(17) T. Winsor and H. J. Berger, *Am. Heart J.*, **90,** 611 (1975).

(18) H. P. Blumenthal, H.-L. Fung, E. F. McNiff, and S. K. Yap, *Br. J. Clin. Pharmacol.*, **4,** 241 (1977).

(19) M. M. Nowak, B. Grundhofer, and M. Gibaldi, *Pediatrics* **54,** 23 (1974).

(20) S. Lang, E. M. Johnson, Jr., and P. Needleman, *Biochem. Pharmacol.*, **21,** 422 (1972).

(21) R. L. Stein, J. K. O'Brien, C. Irwin, J. K. Townsend-Parchman, and F. E. Hunter, Jr., *ibid.*, **29,** 1807 (1980).

(22) J. R. Weeks and J. D. Davis, *J. Appl. Physiol.*, **19,** 540 (1964).

(23) H. A. J. Struyker-Boudier and J. F. Smits, *J. Pharm. Pharmacol.*, **30,** 576 (1978).

ACKNOWLEDGMENTS

Supported in part by NIH grants HL 22273 and GM 20852.