Biochemical Pharmacology, Vol. 33, No. 16, pp. 2681–2686, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

ROLE OF THE LIVER IN THE DISPOSITION OF INTRAVENOUS NITROGLYCERIN IN THE RAT

ANDRES T. BLEI,* JEANNE GOTTSTEIN and HO-LEUNG FUNG

Department of Medicine, Lakeside VA Medical Center and Northwestern University, Chicago, IL, and Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY, U.S.A.

(*Received* 16 *July* 1983; *accepted* 23 *December* 1983)

Abstract—Previous studies have indicated that the liver is the main site of nitroglycerin (NTG) elimination when the drug is systematically infused. To examine this hypothesis, we measured the apparent systemic clearance (Cl_s) of nitroglycerin in anesthesized rats receiving a constant intravenous infusion at a dose of 100 μg per kg per min. Animals were divided into shunt and sham groups; the former had undergone a portal vein ligation 10 days prior to the study, while the latter was subjected to a sham operation. On the study day, half of the animals of each group also received probenecid at 200 mg/kg, i.v., a drug previously reported to inhibit organic nitrate ester reductase (ONER) activity in rat liver. Arterial NTG samples were obtained at 41, 43 and 45 min of infusion in all four experimental groups; Cl_s was 439 ± 32 ml per kg per min ($\bar{x}$ ± S.E.) in sham, 460 ± 44 in sham and probenecid, 477 ± 39 in shunt, and 461 ± 34 in shunt and probenecid animals. During NTG infusion, hepatic blood flow (measured with a constant infusion of indocyanine green) was decreased markedly in shunted rats as was liver/body weight, indicating hepatic atrophy. The specific activity of hepatic ONER was similar in all four groups. In spite of marked differences in hepatic blood flow and hepatic mass, the Cl_s was similar in all four groups. The liver does not appear to be a major site for the elimination of systemic nitroglycerin as hitherto assumed.

In spite of recent advances in our understanding of the disposition of nitroglycerin (NTG) [1, 2], several areas of controversy persist, one of which is the role of the liver in the systemic elimination of this drug. When NTG is administered orally, the high first-pass extraction by the hepatic tissue results in a minimal bioavailability [3]. Lang *et al.* [4] proposed that the liver was also the elimination site of systemic nitroglycerin; the supporting evidence was that the elimination half-life of intravenously administered [^{14}C] NTG was markedly prolonged in hepatectomized rats, approaching the values seen when [^{14}C]NTG was incubated with whole blood. This hypothesis received additional support with the recent work of Stein *et al.* [5]. These authors administered probenecid, an inhibitor of organic nitrate ester reductase (ONER, a glutathione-*S*-transferase activity responsible for the metabolism of NTG in the liver [6]), to anesthesized rats and showed that the elimination half-life of [^{14}C]NTG was prolonged when compared to controls.

The high apparent plasma systemic clearance of NTG reported in the experimental animal [7] and in man [1, 2, 8], which is far in excess of hepatic blood flow values, appeared to contradict the previous observations. We therefore designed experiments to study the relation between hepatic blood flow, hepatic metabolic activity, and the clearance of intravenous NTG in the rat. For this purpose we used the portal vein-ligated model, a preparation that has been well characterized as exhibiting decreased hepatic blood flow and marked portal systemic collaterals [9, 10] and in which cytochrome P-450 activity, among others, was shown to be decreased [11]. Our results indicate that in the rat the liver can only account for a small fraction of the apparent systemic clearance of NTG.

MATERIALS AND METHODS

This study was performed in male Sprague–Dawley rats, weighing 250–400 g (L. Erickson & Co., Chicago, IL), which were studied 10 days after portal vein ligation. Under ether anesthesia, the portal vein was exposed and a 3-0 silk ligature tied around a 21 gauge needle placed parallel to the vein; the needle was removed and the abdomen closed in two layers. Extensive portal systemic collaterals develop rapidly in this model [9, 10]. Sham-operated animals received the same procedure except the ligature was not tied.

Sham and shunted rats were housed in individual cages with meshed wiring in order to decrease coprophagia [12]. After an overnight fast, but with access to water, the animals were anesthesized with intraperitoneal sodium pentobarbital (50 mg/kg). The external jugular vein and a carotid artery were cannulated with PE-50 catheters for saline or drug administration and arterial pressure monitoring respectively. Physiological saline was administered at a rate of 0.068 cc/min via a calibrated Harvard pump (model 600-900, Dover, MA). Rectal temperature was monitored with a thermistor probe and maintained at 37.5 ± 0.5° with a heating blanket. A tra-

* Address correspondence and reprint requests to: Andres T. Blei, M.D., Staff Physician, Gastroenterology Section (111G), VA Lakeside Medical Center, 333 East Huron St., Chicago, IL 60611.

cheostomy was performed on all animals to ensure adequate ventilation.

One-half of twelve sham and twelve shunted animals received probenecid (200 mg/kg) as an i.v. bolus once the surgery had been completed. Probenecid (Merck Sharp & Dohme, West Point, PA) was freshly prepared by dissolving the powder in 8.4% $NaHCO_3$ to a pH of 6.7; a volume of 1.0 to 1.5 cc was injected. The infrared spectrum of probenecid at our laboratory confirmed the identity of the drug. Animals not receiving probenecid (six sham and six shunt) were injected with a similar volume of physiological saline.

Nitroglycerin was administered, therefore, to four experimental groups: sham, sham and probenecid, shunt, and shunt and probenecid. Twenty to thirty minutes after the administration of probenecid, NTG (Tridil, American Critical Care Co., McGaw Park, IL) was infused intravenously at a dose of 100 μg per kg per min and an infusion rate of 0.068 cc/min. The NTG vehicle was 30% (v/v) alcohol USP and 30% propylene glycol with water. Glass syringes and a short length of polyethylene tubing were used to minimize adsorption of NTG [13]. The tubing was flushed with 500 μl of NTG to eliminate dead space. The drug infusion was started and maintained for 45 min. Carotid arterial samples were obtained at 41, 43 and 45 min of infusion and at 1, 3, 5, 8 and 20 min thereafter. The animal was killed with i.v. KCl, the abdomen opened, and the liver removed and weighed.

Hemodynamic measurements. Arterial pressure was monitored via the carotid artery catheter connected to a transducer (Altech, City of Industry, CA), calibrated with a mercury manometer with the zero reference point at the level of the heart; values were inscribed on a continuous recorder (Atlan-Tol, model 40, West Warwick, RI). Measurements of mean arterial pressure (MAP) were obtained every minute for 5 min prior to the NTG administration (baseline period) and at 40 min of infusion.

In an additional group of twelve rats, similarly divided into the four experimental groups, hepatic blood flow was measured during NTG infusion. Through a midline incision, the hepatic vein draining the left lateral lobe was cannulated with a pre-curved needle [14]. Through another external jugular vein, a constant infusion of indocyanine green (ICG) (0.02 mg/min at 0.069 cc/min) was simultaneously begun with the NTG infusion. At 45 min of infusion, samples from the carotid artery and hepatic vein were obtained for plasma ICG determinations via spectrophotometry at 800 nm (Coleman Junior II, Maywood, IL). A shift of the maximum peak wavelength to 800 nm occurred because of dilution of plasma samples with deionized water [14]. Hematocrit (Hct) values were measured in capillary tubes, and hepatic blood flow was calculated as $[IR/(A\text{-}V)] \times [1/(1\text{-Hct})]$, where IR = ICG infusion rate, and A and V = arterial and hepatic venous ICG concentrations respectively [15]. The extent of portal systemic shunting in these animals was measured after the injection of 20,000–30,000 microspheres labeled with ^{85}Sr into the splenic tissue. Details of this procedure have been reported previously [9, 16]. Once the animals were killed, radioactivity in the lungs and in the liver was measured in a gamma counter (1185 Series, Nuclear-Chicago, Des Plaines, IL) and shunting calculated as the ratio of lung cpm/(lung + liver) cpm [9].

ONER activity in rat liver cytosol. In an additional group of twenty animals, similarly divided into the four experimental groups, the activity of hepatic ONER was determined by the method of Needleman and Hunter [17]. Ten days after the original operation and under pentobarbital anesthesia, the abdomen was opened 60 min after the administration of probenecid and the liver was flushed with cold 0.15 M NaCl via the portal vein. After excision, the hepatic tissue was homogenized with 3 vol. of 0.25 M sucrose and the 100,000 g supernatant fluid was used for the ONER determination. Details of this assay have been published [18]; in brief, the measured disappearance of NADPH reflects two coupled enzymatic reactions: denitration of NTG by ONER with reduced glutathione (GSH) as a cofactor and regeneration of GSH by gluthatione reductase (GR) with NADPH serving as hydrogen donor. The initial rate of disappearance of NADPH parallels the production of nitrite derived from NTG metabolism [17].

The final reaction was started with the addition of 0.2 ml of the 100,000 g supernatant fluid to a solution containing GSH (Aldrich), GR (Sigma), NADPH (Sigma) and other cofactors [18] and 1.0 ml of a 5.5 mM NTG solution. For the latter, NTG was extracted from 1:10 lactose adsorbate and assayed photocolorimetrically with the USP assay [19]. The rate of NADPH disappearance was measured at 340 nm for 3–5 min (Beckman model 25). Protein concentration in the supernatant fluid was determined by the method of Lowry *et al.* [20]. ONER specific activity was expressed as μmoles per min per mg protein. Correction for concurrent enzymatic or chemical oxidation of NADPH was made in every determination [18].

Nitroglycerin assay. Five microliters of 0.1 M $AgNO_3$ was added to 0.1 to 0.15 ml of plasma to prevent degradation of NTG. Tared-weighed samples were coded, frozen at −20°, and shipped in dry ice. Plasma NTG was assayed with the gas chromatographic method of Yap *et al.* [21]; the lower limit of the assay is 0.5 ng/ml and the coefficient of variation at concentrations about 10 ng/ml was less than 2% with a recovery about 95% [21]. Samples were assayed 1–6 months after collection; a group of nine samples selected from all four groups was refrozen after the first assay and reassayed 6–12 months later. The difference in NTG concentration from the two determinations was plus $5 \pm 3\%$ (mean ± S.E.), indicating that the sample handling procedures were satisfactory, and that NTG degradation did not occur during storage. In eight samples, obtained from sham and shunted rats at 45 and 65 min after the administration of probenecid alone (no NTG), no interference with the gas chromatographic peaks of NTG and the internal standard was detected.

Pharmacokinetic calculations. The apparent steady-state NTG concentration (C_{ss}) was obtained from the mean values at 41, 43 and 45 min of infusion. Apparent systemic plasma arterial NTG clearance

Table 1. Body and liver weights*

	N	Preoperative body weight	Experimental body weight	Experimental liver weight	Experimental liver/body ratio
Sham	9	342 ± 6	346 ± 9	11.22 ± 0.50	3.18 ± 0.09
Sham + PBN	9	349 ± 14	349 ± 16	11.28 ± 0.72	3.16 ± 0.13
Shunt	9	314 ± 14	306 ± 14†	8.10 ± 0.58‡	2.68 ± 0.18†
Shunt + PBN	9	337 ± 12	323 ± 13	8.04 ± 0.55‡	2.52 ± 0.18†

* All results are $\bar{x}$ ± S.E. in grams. Liver/body ratio (×100). PBN = probenecid. Preoperative = prior to portal vein ligation. Experimental = studied 10 days thereafter.
† $P < 0.05$ vs either sham group.
‡ $P < 0.01$ vs either sham group.

(Cl_s) was calculated as NTG infusion rate ÷ C_{ss} and expressed as ml per kg per min. The terminal half-life ($T_{\frac{1}{2}}$) was estimated by a linear regression plot of the log NTG plasma concentration versus time for the last four post-infusion samples.

All results are expressed as mean ± S.E. The groups were compared using parametric log scale one-way analysis of variance and Tukey's test [22].

RESULTS

Body and liver weight (Table 1). The pre-operative weight of all four groups was similar; 10 days after surgery, shunted rats had lost 3–4% of body weight, while sham animals remained unchanged. More conspicuous were the differences in liver weight, and hence in liver weight/body weight ratios; shunted animals exhibited a smaller liver without macroscopic abnormalities. Histological examination of four of these livers did not reveal abnormalities on light microscopy.

Hemodynamic measurements (Table 2). Sham-operated rats exhibited significantly higher baseline mean arterial pressures than shunted animals, without differences in pulse pressure (Table 2). Among the animals that received probenecid, baseline MAP was lower and the pulse pressure had widened, suggesting a vasodilatory effect. This widened pressure did not reflect the effect of the probenecid vehicle ($NaHCO_3$); in five additional shunted animals, no changes in blood pressure were seen 10 min after the injection (1.0 to 1.5 cc) of 8.4% $NaHCO_3$ adjusted to a pH of 6.7.

The decrease in MAP seen with NTG was less in only one of the probenecid groups, and a significant difference was observed between the shunt vs shunt + probenecid group (Table 2). Stable pressure values were observed at 5 min of NTG administration in all four groups.

Hepatic blood flow during NTG infusion was clearly decreased in the shunted animals (Table 2). Probenecid resulted in a small decrease in total hepatic perfusion; this reduction did not reach statistical significance in this small series. Probenecid (without NTG) has been shown to decrease portal venous blood flow in the dog [23].

Portal systemic shunting was 98.8 ± 0.3% in the three shunted animals and 99.8 ± 0.13% in the three shunt and probenecid experiments; sham animals have been demonstrated repeatedly to show values between 0 and 1% [9, 16].

ONER activity (Table 3). The values of ONER specific activity in the sham group were in the range of previously described levels [18]. When all four groups were compared, no differences in ONER activity were detected. Although sham animals (as a group) exhibited higher mean values than the shunted rats, the overlap was substantial. Similarly, probenecid did not appear to reduce ONER activity.

Pharmacokinetic results (Table 3 and Fig. 1). The apparent $\bar{C}_{ss}$ and hence the apparent systemic NTG clearance values were similar in all four groups. The estimated Cl_s of all sham animals with or without probenecid was not significantly different from the shunted groups [(N = 12) 450 ± 26 vs 469 ± 25 ml per kg per min]. The terminal elimination half-life was also not apparently affected by the presence of shunts or probenecid; an earlier disappearance rate

Table 2. Hemodynamic measurements*

	N	Baseline MAP (mm Hg)	Baseline pulse pressure (mm Hg)	NTG-MAP (mm Hg)	NTG-HBF (ml/min/g)
Sham	9	123 ± 6	40 ± 2	69 ± 4	2.24 ± 0.30
Sham + PBN	9	108 ± 4†	51 ± 6†	77 ± 4	1.71 ± 0.05
Shunt	9	108 ± 3†	41 ± 1	68 ± 5	1.14 ± 0.14†
Shunt + PBN	9	105 ± 3†	52 ± 4†	81 ± 3‡	1.15 ± 0.32†

* Values are $\bar{x}$ ± S.E. All pressure values are in mm Hg. Abbreviations: MAP, mean arterial pressure; NTG-MAP, pressure values at 40 min of nitroglycerin infusion; NTG-HBF, hepatic blood flow during nitroglycerin administration (N = 3 in each group for HBF measurements); and PBN, probenecid.
† $P < 0.05$ vs sham.
‡ $P < 0.05$ vs shunt.

in the first minute after stopping the infusion could not be analyzed due to the lack of appropriate samples.

DISCUSSION

We have shown in the rat that the apparent systemic plasma clearance of nitroglycerin remains unchanged in the presence of alterations in hepatic mass and hepatic blood flow. Administration of probenecid, a drug reported to inhibit guinea pig or rat ONER activity [5], resulted in similar clearance values. Furthermore, the high systemic plasma clearance seen in all four experimental groups (mean $\cong$ 460 ml per kg per min) far exceeds the values of hepatic blood flow (mean range = 30–72 ml per kg per min).

The model used in our study, the portal vein-ligated rat, was originally described 50 years ago [10]; recently it was hemodynamically characterized [24] and used for hepatic drug metabolism studies [11]. The relatively simple surgery and predictable changes seen 10 days after the initial operation, combined with the similarity of several features in this model that are present in chronic liver disease (altered hepatic blood flow, reduced hepatic mass and presence of portal systemic collaterals), make this a valuable model for the study of the role of the liver in drug disposition.

Rats undergoing portacaval anastomosis lose weight after the procedure, especially if allowed to continue with coprohagic habits [12]. Also, a decrease in hepatic mass is observed which appears to be related to the shunting of "hepatotrophic factors" normally present in portal venous blood [25]. The liver weight was clearly decreased in our portal vein-ligated animals, in which the degree of shunting approximated those of total bypass.

Shunted rats showed lower baseline MAP values. Although we had not seen this difference in a previous study [16], other investigators have shown that this model exhibits an increased cardiac output and decreased peripheral vascular resistance [26], characteristics that explain a lower MAP value. Probenecid, administered i.v., also produced a fall in MAP that may have been related to peripheral vasodilatation, as suggested by the widened pulse pressure. When NTG was administered with probenecid, the decrease in MAP was of a lesser magnitude. Whether this diminished effect represented an effect on the metabolism of NTG at the vascular smooth muscle level or simply reflected a hemodynamic interaction is unclear at this time.

The approximately 40% reduction in estimated hepatic blood flow exhibited by shunted rats is in accordance with previous investigations [24], although NTG was not administered in the latter studies [24]. Our measurements were made while NTG was infused at a high dose; in our laboratory, the value of hepatic blood flow in normal rats using the ICG infusion method was 1.14 ± 0.04 ml per min per g with a 1 μg per kg per min infusion of NTG.

Total cytochrome P-450 activity is reportedly decreased in portal vein-ligated rats [11]. We did not measure total glutathione content in our animals; however, ONER specific activity, a GSH-*S*-transfer-

Table 3. ONER activity and pharmacokinetic measurements*

	ONER activity (μmoles/min/mg protein × 10^{-2})	NTG C_{ss} (ng/ml)			Cl_s NTG (ml/kg/min)	Terminal half-life (min)
		41 min	43 min	45 min		
Sham	5.76 ± 0.46 (5)	228 ± 22 (5)	239 ± 19	251 ± 22	439 ± 32	10.51 ± 2.6
Sham + PBN	5.09 ± 0.33 (5)	224 ± 27	233 ± 27	228 ± 24	460 ± 44	7.68 ± 0.5
Shunt	5.09 ± 0.16 (5)	221 ± 21	217 ± 16	214 ± 20	477 ± 39	6.70 ± 0.96
Shunt + PBN	5.33 ± 0.51 (5)	203 ± 13	238 ± 17	227 ± 17	461 ± 34	7.64 ± 0.52

* All results are $\bar{x}$ ± S.E. Numbers in parentheses represent the number of experiments: when not stated, N = 6. Statistical analysis revealed no differences between groups.

Abbreviations: C_{ss}, concentration at steady state; Cl_s NTG, infusion rate (100 μg/kg/min) ÷ mean C_{ss}; ONER, organic nitrate ester reductase; and PBN, probenecid.

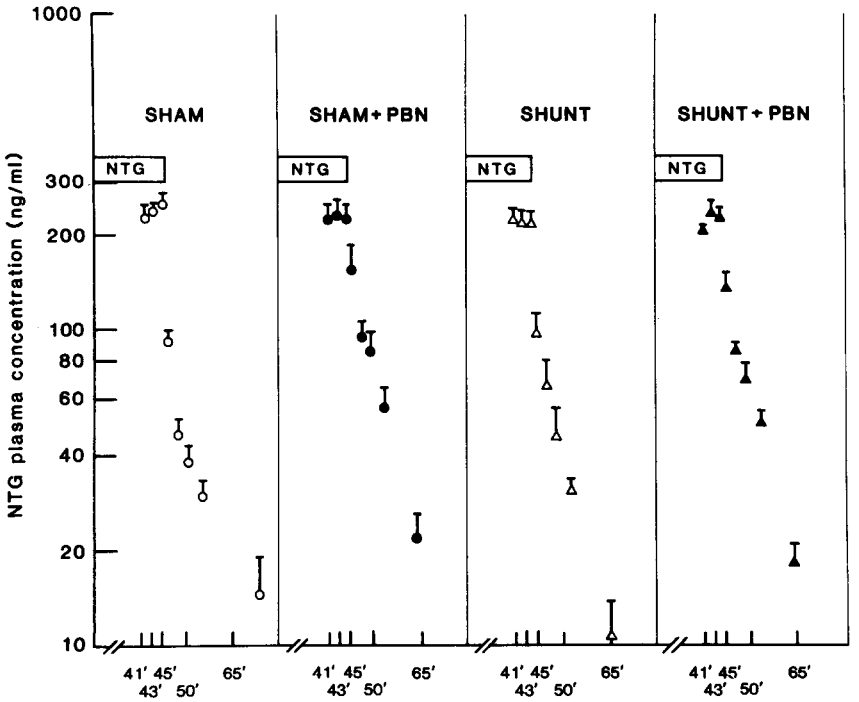

Fig. 1. Plasma level during a continuous infusion of nitroglycerin. The NTG infusion (100 μg per kg per min) was maintained for 45 min. Arterial samples were obtained to determine steady-state levels and terminal half-life (see Table 3). PBN = probenecid.

ase activity that metabolizes NTG in the liver [6], was apparently not decreased in our shunted rats. It would be of interest to study whether enzymatic activity is selectively lost in this model, as we cannot conclude from the design and the limited number of our experiments that this is the case. Neither did we demonstrate a decrease in ONER activity with probenecid. In our studies, we did not determine the K_m of NTG nor the K_i of probenecid; thus we can neither confirm nor refute the findings of Stein *et al.* [5], who had shown competitive inhibition of NTG with probenecid in the guinea pig. Furthermore, total ONER activity was not measured. In our *in vivo* experiments, however, we did not observe any changes in the terminal elimination half-life of NTG using the same probenecid dose and time interval as reported earlier [5]. It is recognized, however, that the NTG dose used here was markedly greater and a different elimination phase was examined compared to the work of Stein *et al.* [5].

In spite of the difference in hepatic blood flow and hepatic mass, similar apparent plasma NTG clearance values were obtained in all four groups. We measured NTG in arterial blood samples; different investigators have now shown a 2- to 4-fold higher value of arterial compared to venous concentrations of NTG [2, 27]. Since it is likely that the vasculature represents a "clearing" structure for NTG, estimation of systemic NTG clearance using arterial blood is appropriate.

Regardless of the sampling site, the apparent plasma clearance values seen in our experiments exceeded the values of normal cardiac output in the rat. However, partitioning of NTG between red blood cells and plasma might explain some of this discrepancy. One of us has shown (H-L. Fung, unpublished results) that, in the rat, blood NTG concentration was 1.72 times the corresponding plasma value. Thus, blood NTG clearance could be estimated in our experiments by dividing the overall mean plasma clearance by 1.72, 460/1.72 = 280 ml per min per kg. The cardiac output in the rat with a similar NTG dose was found to be 278 ± 25 ml per min per kg (N = 6, A.T. Blei, S. Friedman, J. Gottstein and H.-L. Fung, unpublished results). Thus, one could hypothesize that the clearance of NTG could be limited by the values of cardiac output. If indeed this is the case, the profound hemodynamic changes seen in the eviscerated rat could explain the differences in nitroglycerin elimination seen in the experiments of Lang *et al.* [4].

There is still considerable uncertainty regarding the number of disposition phases of NTG. Two papers [5, 28] have reported a rapid distribution phase of NTG after an i.v. bolus, with a half-life of 10–30 sec. Samples at 46 min in our study suggest the presence of a rapid elimination phase than cannot be assumed to reflect distribution after a steady-state had been achieved. The precise half-life of this phase could not be calculated due to lack of appropriate samples, but it appeared to be about 1 min. The terminal elimination $T_{\frac{1}{2}}$, determined between 6 and 10 min after the end of infusion, although not different among the four groups, was more prolonged than the value of 4 min reported previously in the unanesthesized and less surgically complicated rat

[7]. Another study [29] showed NTG disposition in the rat to be characterized by disappearance half-lives of approximately 3 and 15 min. The reasons for this discrepancy are unclear but could be due to animal preparation (anesthesia and surgery), the dosing regimen, and the sampling protocol used. In this experiment, the apparent terminal elimination half-life that could be determined was between 7 and 10 min. The observation of relatively steady plasma NTG concentrations from 41 to 45 min of infusion (about four to five half-lives) was internally consistent.

Although liver disease results in a marked enhancement of the bioavailability of oral NTG in man [30], the disposition of systemic NTG may not be affected by hepatic abnormalities. Confirmation in man of our observations in the rat is important in view of the recent interest in the use of nitrates in the treatment of portal hypertension [31].

Acknowledgements—Excellent technical support was received from Anna Poliszczuk and Brian Neese. Supported in part by NIH grant HL-22273 and the Veterans Administration Research Service. We thank Carl-Bertil Walllemark of the Northwestern Cancer Biometry Section for statistical advice and Celesta M. Harvey for expert secretarial assistance.

REFERENCES

1. E. F. McNiff, A. Yacobi, F. M. Young-Chang, L. H. Golden, A. Goldfarb and H.-L. Fung, *J. pharm. Sci.* **70**, 1054 (1981).
2. P. W. Armstrong, J. A. Moffat and G. S. Marks, *Circulation* **66**, 1273 (1982).
3. P. Needleman, S. Lang and E. M. Johnson, Jr., *J. Pharmac. exp. Ther.* **181**, 489 (1972).
4. S. Lang, E. M. Johnson, Jr. and P. Needleman, *Biochem. Pharmac.* **21**, 422 (1972).
5. R. L. Stein, J. K. O'Brien, C. Irwin, J. K. Townsend-Parchman and F. E. Hunter, Jr., *Biochem. Pharmac.* **29**, 1807 (1980).
6. P. Needleman and J. C. Krantz, Jr., *Biochem. Pharmac.* **14**, 1225 (1965).
7. P. S. K. Yap and H.-L. Fung, *J. pharm. Sci.* **67**, 584 (1978).
8. J. T. Wei and P. R. Reid, *Circulation* **59**, 588 (1979).
9. M. Chojkier and R. J. Groszmann, *Am. J. Physiol.* **240**, G371 (1981).
10. L. R. Dragstedt, *Science* **73**, 315 (1931).
11. G. Farrell and L. Zaluzny, *Gastroenterology* **85**, 275 (1983).
12. R. Herz, V. Sautter, F. Robert and J. Bircher, *Eur. J. clin. Invest.* **2**, 390 (1972).
13. B. L. McNiff, E. F. McNiff and H.-L. Fung, *Am. J. Hosp. Pharm.* **37**, 201 (1980).
14. M. Yokote, T. Lga, S. Awazu and M. Hanano, *J. appl. Physiol.* **41**, 439 (1976).
15. S. E. Bradley, F. J. Ingelfinger, G. P. Bradley and J. J. Curry, *J. clin. Invest.* **24**, 890 (1945).
16. A. T. Blei, D. J. O'Reilly and J. Gottstein, *J. Lab. clin. Med.*, in press.
17. P. Needleman and F. E. Hunter, Jr., *Molec. Pharmac.* **1**, 77 (1965).
18. G. A. Maier, C. Arena and H.-L. Fung, *Biochem. Pharmac.* **29**, 646 (1980).
19. *United States Pharmacopeia*, Vol. 20. Mack Publishing Co., Easton, PA (1980).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. S. K. Yap, E. F. McNiff and H.-L. Fung, *J. pharm. Sci.* **67**, 582 (1978).
22. A. Haber and R. P. Runyon, *General Statistics*, 2nd Edn, pp. 265–75. Addison-Wesley, Reading, MA (1973).
23. M. Barja, J. Brusch, M. G. Bergeron, O. Kenmotsu and L. Weinstein, *J. infect. Dis.* **131**, 586 (1975).
24. R. J. Groszmann, J. Vorobioff and E. Riley, *Am. J. Physiol.* **242**, G156 (1982).
25. T. E. Starzl, K. Watanabe and K. A. Porter, *Lancet* **1**, 821 (1976).
26. J. Vorobioff, J. E. Bredtfeldt and R. J. Groszmann, *Am. J. Physiol.* **244**, G52 (1983).
27. J. F. Bymer, P. L. Stetson, J. A. Walton, Jr., B. Lucchesi and B. Pitt, *Clin. Res.* **17**, 229A (1979).
28. E. M. Johnson, Jr., A. B. Harkey, D. J. Blehm and P. Needleman, *J. Pharmac. exp. Ther.* **182**, 56 (1972).
29. G. A. Maier, *Ph.D. Thesis*. State University of New York at Buffalo (1981).
30. H. Porchet and J. Bircher, *Gastroenterology* **82**, 629 (1982).
31. R. J. Groszmann, D. Kravetz, J. Bosch, M. Glickman, J. Bruix, J. Bredfeldt, H. O. Conn, J. Rodes and E. H. Storer, *Hepatology* **2**, 757 (1982).