Biochemical Pharmacology, Vol. 34, No. 15, pp. 2679–2683, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00
Pergamon Press Ltd.

REDUCED HEPATIC UPTAKE OF PROPRANOLOL IN RATS WITH ACUTE RENAL FAILURE

RYOHEI HORI,* KATSUHIKO OKUMURA, MASATO YASUHARA and HIROKAZU KATAYAMA
Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan

(*Received* 12 *December* 1984; *accepted* 1 *March* 1985)

Abstract—The effect of acute renal failure (ARF) on the hepatic uptake and metabolism of propranolol was investigated in relation to the hepatic clearance of the drug. ARF was induced by the subcutaneous injection of uranyl nitrate to rats. The uptake rate of propranolol in the isolated perfused liver was determined by the multiple-indicator dilution method and was found to decrease from $43.6 \pm 2.0\ min^{-1}$ (mean ± S.E.) in control to $29.4 \pm 1.7\ min^{-1}$ in ARF ($P < 0.001$). The recovery fraction of propranolol in effluent venous blood increased about twofold in ARF compared to control ($P < 0.05$). The metabolic activity for propranolol was examined using the hepatic microsomal fraction prepared from control and ARF rats. There was no significant difference in the kinetics of oxidative metabolism of propranolol between two groups. These results suggest that the previously reported decrease in the hepatic clearance of propranolol in ARF is due to decreased hepatic uptake of the drug from the blood into the liver cells.

Evidence has accumulated that the oral availability of drugs such as propranolol [1], bufralol [2] and dextropropoxyphene [3] is increased in patients with renal failure. Recent studies in rats with acute renal failure (ARF)† have shown the decreased presystemic elimination of propranolol [4, 5], but the reason for this change is unknown. In the isolated liver recirculation study, we found a reduced hepatic clearance of propranolol in ARF [5]. Hence, the effect of blood flow rate, plasma protein binding and nervous system could be ruled out.

Hepatic extraction of propranolol consists of hepatic uptake and sequential metabolism. The purpose of this study was to clarify which process was affected in the hepatic extraction of propranolol by ARF. We have investigated the effect of ARF on these two processes by separate experiments respectively: the hepatic uptake rate of propranolol was determined by the multiple-indicator dilution method [6] and the metabolic activity of ARF rat liver was compared to that of control using the microsomal fraction.

MATERIALS AND METHODS

Chemicals. DL-Propranolol hydrochloride and D-glucose-6-phosphate disodium salt were obtained from Nakarai Chemicals Co. (Japan). Sodium phenolsulfonphthalein (PSP) was supplied from Tokyo Kasei Ind. Co. (Japan). NADP and glucose-6-phosphate dehydrogenase were purchased from Kohjin Co. (Japan) and Sigma Chemical Co. (U.S.A.), respectively. All other reagents were of the finest grade available.

Induction of acute renal failure. Male Wistar albino rats, weighing 200–300 g, were used. For the induction of ARF, uranyl nitrate (10 mg/kg) was administered subcutaneously [5]. The studies on rats with ARF were performed 3 days after the injection of uranyl nitrate [5].

Multiple-indicator dilution method. The hepatic uptake rates of drugs were measured by the multiple-indicator dilution method [6, 7]. The experiments were carried out on perfused rat liver by the method of Mortimore *et al.* [8]. The liver was perfused via the hepatic portal vein with 20% (v/v) bovine blood cell, 5% (w/v) bovine serum albumin in Krebs–Henseleit buffer solution, equilibrated with 95% O_2 + 5% CO_2 to maintain a pH of 7.4 at 37°. A constant flow rate of 20 ml/min and a constant temperature of 37° were maintained and the liver was allowed to equilibrate with the buffer solution for 10–20 min before the injection of test substances. During the perfusion period, there was no sign of physiological damage such as swelling of the liver, bleeding and reduction of bile flow. The injection mixture contained a test substance (propranolol (1 mg/ml) or PSP (0.67 mg/ml)) and a reference substance (inulin (10 mg/ml)) in the liver perfusate. After the rapid injection (less than 0.5 sec) of 0.3 ml of the mixture into the hepatic portal vein, total hepatic vein effluent was collected in Eppendorf polypropylene microtest tubes at 1 sec intervals for about 30 sec and thereafter collected in a 50 ml test tube up to 2 min 15 sec. 0.1 ml of effluent was used for the determination of propranolol concentrations in whole blood and the effluent plasma was used for the assay of inulin and PSP.

Preparation and incubation of hepatic microsomes. The microsomal fraction was prepared by a modification of the method of Omura and Sato [9]. Rats were fasted for about 16 hr before experiments with free access to water. Under ether anesthesia, the abdomen was opened and the liver was perfused *in situ* with the ice-cold saline solution. The liver was excised, weighed and homogenized with 4 vol. of 0.15 M KCl solution in a Potter glass homogenizer

* Author to whom correspondence should be addressed.

† Abbreviations used: ARF, acute renal failure; PSP, phenolsulfonphthalein.

equipped with a Teflon pestle. The homogenate was centrifuged at 10,000 g for 15 min at 4°, and the precipitate was discarded. The microsomal fraction was obtained by centrifugation at 100,000 g for 60 min at 4°. The pellet of microsomes was resuspended in 0.15 M KCl–0.025 M Tris–HCl buffer (pH 7.4), at a concentration of 0.5–2.0 mg protein per ml.

Incubation were performed at 37° by shaking the mixtures of microsomal suspensions (0.5 ml), 0.1 M Tris–HCl buffer (pH 7.4, 0.5 ml), 0.15 M KCl–0.025 M Tris–HCl buffer (pH 7.4, 0.5 ml) and NADPH generating system (0.3 ml) consisting of 5 mM NADP, 50 mM $MgCl_2$, 50 mM D-glucose-6-phosphate and 5 unit/ml glucose-6-phosphate dehydrogenase. Control tubes contained no NADPH-generating system. After preincubation for 5 min, the reaction was initiated by the addition of propranolol dissolved in 0.15 M KCl–0.025 M Tris–HCl buffer (1–30 μM; 0.2 ml) and the tubes were incubated for a suitable time period. The reaction was then terminated by the addition of 2.0 ml of ice-cold acetonitrile and cooling to 0°. After centrifugation, the supernatant was stored at 4° for the assay of propranolol. The extent of propranolol metabolism was obtained by subtracting the experimental reading from the control reading.

Analytical method. Propranolol concentrations were determined by the method previously described [5]. A Shimadzu Model LC-3A high performance liquid chromatograph equipped with a Shimadzu RF-500LC fluorescence spectromonitor was used. Plasma concentrations of inulin were determined by a modification of the method of Dishe and Borenfreund [10] after the precipitation of protein by $ZnSO_4$ and NaOH [11]. PSP concentrations in plasma were determined by the addition of 1 N NaOH (1 ml) to 0.1 ml of the plasma with Hitachi Model 200-20 spectrophotometer at 560 nm. Protein concentrations were determined by the Bio-Rad Protein Assay (Bio-Rad, U.S.A.) with bovine plasma gamma globulin as the standard.

Data analysis. The apparent rate constant for hepatic uptake of a test drug ($k_{1,app}$) was obtained from multiple-indicator dilution experiments by using the following equation [6].

$$\ln\left(\frac{C(t)_{inulin}}{C(t)_{drug}}\right) = \frac{k_1 \lambda t}{1+\gamma} = k_{1,app} \cdot t \qquad (1)$$

In this equation, $C(t)_{inulin}$ and $C(t)_{drug}$ are fractions of dose that appeared in 1 ml of perfusate effluent at time t for inulin and a test drug (propranolol or PSP), respectively, k_1 is the net rate constant for hepatic uptake, λ is the ratio of the cellular space to the sinusoidal space, and γ is the ratio of the Disse space to the sinusoidal space. Thus, $\lambda/(1+\gamma)$ represents the ratio of cellular volume to extracellular (Disse + sinusoid) volume. According to Eqn 1, the plot of the logarithm of the ratio of the outflow fraction per ml for inulin to that for a test drug should yield a straight line and $k_{1,app}$ was determined from the slope of this line by a least squares regression analysis.

Mean values were reported with standard errors. Statistical analysis was performed using Student's t-test with $P = 0.05$ as the minimal level of significance.

RESULTS

Hepatic uptake of propranolol

Figures 1(a) and (b) show the representative hepatic venous outflow curves for propranolol and inulin after bolus injection into the hepatic portal vein. There was no significant difference in the outflow pattern of inulin between control and ARF rats. On the other hand, the outflow fraction of propranolol significantly increased in ARF compared to control (Table 1). Figures 1(c) and (d) show the logarithmic plots of the ratio of the outflow fraction per ml for inulin to that for propranolol vs. time. The plot yielded a straight line for about 7 sec after bolus injection and $k_{1,app}$ for propranolol, calculated from the slope of this line, showed a significant decrease in ARF compared to control rats.

As is evident from Eqn 1, $k_{1,app}$ consists of the net uptake rate constant and the volume ratio between the cellular and extracellular space. In order to investigate the effect of ARF on cellular and/or extracellular volume of rat liver, the multiple-indicator dilution experiments were performed on PSP, whose hepatic clearance was found to be not affected by ARF [12]. No significant change was found in either hepatic venous outflow pattern or $k_{1,app}$ for PSP between control and ARF (Fig. 2, Table 1).

Metabolic activity for propranolol

Preliminarily, the metabolism of propranolol was examined using whole liver homogenate at the protein concentration of about 5 mg/ml in the same condition as in the microsomal experiment. Both groups of rat liver showed similar metabolic rates of propranolol at the initial drug concentration of 1.5 μM for 2 min (0.070 ± 0.018 and 0.072 ± 0.010 nmole/min/mg protein for control and ARF, respectively, $N = 3$), and the protein contents of rat liver were 251 ± 5 mg/g liver in control and 246 ± 9 mg/g liver in ARF.

Figure 3 shows the time course of propranolol metabolism in microsomal fraction of rat liver at the initial drug concentration of 1.5 μM. Propranolol was metabolized completely at 15 min after the initiation of the reaction and no marked difference was found between control and ARF. Then, the microsomal fraction was incubated for 2 min at various concentrations of propranolol ranging from 0.1 to 3.0 μM and the metabolic activity for propranolol was determined. As shown in Table 2, neither apparent Michaelis–Menten constant (K_m) nor the apparent maximum rate of metabolism (V_{max}) differed between control and ARF. In addition, no difference was found in the total recovery of microsomal protein from each groups of rat liver (7.3 ± 1.4 and 8.4 ± 2.2% of liver protein for control and ARF, respectively).

DISCUSSION

Propranolol has a high hepatic extraction ratio and is metabolized virtually completely in the liver [13] and less than 1% of the intact drug is found in the urine [14]. Consequently, assuming the well-stirred model, the clearance of propranolol after intravenous dose depends on the hepatic blood flow and

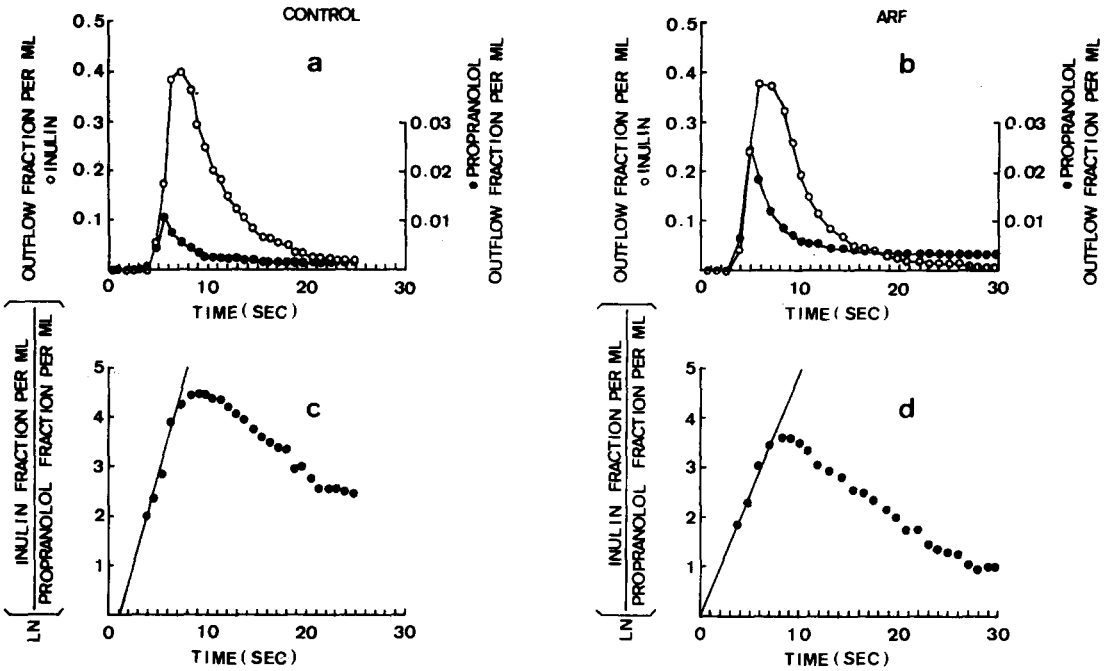

Fig. 1. Hepatic venous dilution curves of propranolol. The mixture of propranolol (300 μg) and inulin (3 mg) was rapidly injected into the portal vein of the perfused liver and the total hepatic vein effluent was collected at 1 sec intervals. The amounts of drugs in effluent were expressed as the fraction of dose per ml of blood. In the upper panels (a, b), the dilution curves of propranolol (●) and inulin (○) are plotted against times. In the lower panels (c, d) the logarithm ratios of the outflow fraction per ml for inulin to that for propranolol are plotted against times. a, c, control; b, d, ARF.

its oral kinetics depends on both the drug elimination function of the liver and the degree of plasma protein binding [15]. Though these determinants apparently seem to be not affected by ARF, malfunction of the kidney is known to result in an array of symptoms which reflect pathological alterations in every organ system of the body [16]. In fact, there are some clinical reports suggesting the altered pharmacokinetics of propranolol in renal disease [1, 17]. In our previous study, the hepatic extraction of propranolol was found to decrease in rats with uranyl nitrate-induced ARF without affecting the hepatic blood flow and plasma protein binding [5]. These previous results suggested the ARF-induced impairment of the drug elimination function of the liver.

Hepatic extraction of drugs consists of hepatic uptake and sequential metabolism or biliary excretion. Yates *et al.* showed that the hepatic uptake and plasma clearance of indocyanine green were decreased in rats with acute [18] and chronic [19] renal failure, and suggested an impaired movement of dye into the hepatocyte in renal failure. On the other hand, investigations of experimental acute and chronic renal failure in animals have shown alterations in certain pathways of drug metabolism [20–23]. In the present study, therefore, we have extended our investigation of the pharmacokinetics of propranolol in ARF to clarify which process was affected by ARF in the hepatic disposition of the drug. ARF was induced by the subcutaneous injec-

Table 1. Effect of ARF on the apparent rate constants for hepatic uptake of propranolol and PSP and on the recovery fractions of drugs in effluent hepatic venous blood

	Propranolol			PSP		
	$k_{1,app}$ (min^{-1})	Recovery (% of dose)	Recovery ratio (propranolol/inulin)	$k_{1,app}$ (min^{-1})	Recovery (% of dose)	Recovery ratio (PSP/inulin)
Control	43.6 ± 2.0	8.9 ± 1.3	0.095 ± 0.015	1.22 ± 0.13	81.2 ± 4.4	0.901 ± 0.025
ARF	29.4 ± 1.7*	16.0 ± 2.9*	0.183 ± 0.034†	1.03 ± 0.13	81.3 ± 1.9	0.915 ± 0.016

The hepatic uptake rates of drugs in the isolated perfused rat liver were determined by the multiple-indicator dilution method. After the rapid injection of the mixture of propranolol (300 μg) or PSP (200 μg) with inulin (3 mg) into the hepatic portal vein, total hepatic vein effluent was collected at 1-sec intervals. The recovery of a test drug in hepatic vein effluent for 2 min 15 sec was expressed as a percent of dose, and its ratio to that of inulin was also shown. Results are given as the mean ± S.E. of 6 animals for propranolol and 4 animals for PSP, respectively.

* $P < 0.001$, † $P < 0.05$ when compared to control.

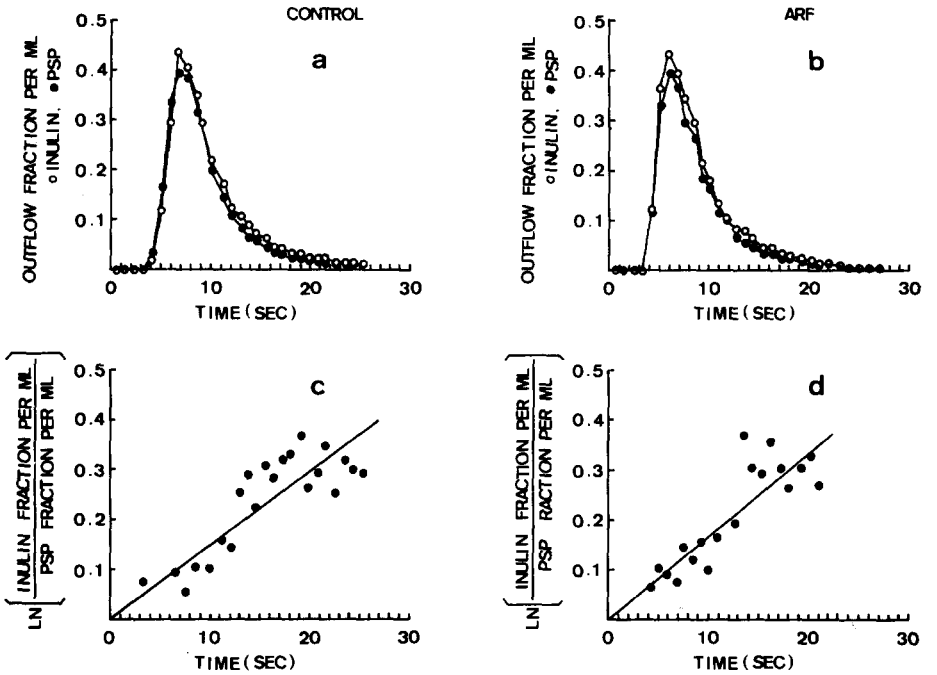

Fig. 2. Hepatic venous dilution curves for PSP. The mixture of PSP (200 μg) and inulin (3 mg) was rapidly injected into the portal vein of the perfused liver. In the upper panels (a, b), the dilution curves of PSP (●) and inulin (○) are plotted against times. In the lower panels (c, d), the logarithm ratios of the outflow fraction per ml for inulin to that for PSP are plotted against times. a, c, control; b, d, ARF.

tion of uranyl nitrate (10 mg/kg) to rats. The validity of this disease model has been already demonstrated [5, 24, 25]. In our model, plasma concentrations of urea nitrogen and creatinine increased about three-fold respectively at 3 days after the injection of uranyl nitrate compared to these values before dose [5].

It is clear from the results that the hepatic uptake rate of propranolol was decreased in rats with ARF. The apparent rate constant for hepatic uptake of propranolol was determined by the multiple indicator dilution method and this is a hybrid constant of the net uptake rate constant and the volume ratio between the cellular and extracellular space. The latter seems to be not affected by ARF since no significant change in $k_{1,app}$ for PSP was found between control and ARF. Consequently, ARF can be considered to decrease the uptake rate of propranolol from the blood into the liver cells.

In addition, a marked difference was found in shape between the graphs for propranolol (Figs 1c and d) and PSP (Figs 2c and d). In contrast to the monophasic straight line for PSP, propranolol yielded a biphasic curve; an upward straight line for about 7 sec after bolus injection, and thereafter a downward curve. According to the hepatic transport model proposed by Goresky *et al.* [6], the second phase seems to represent the efflux of propranolol

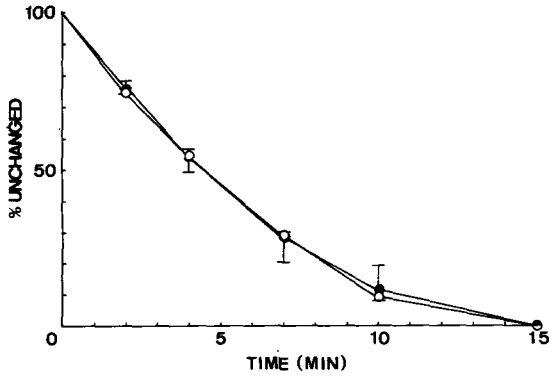

Fig. 3. Time course of propranolol metabolism in rat liver microsome fraction. The microsome fraction prepared from control (○) and ARF (●) rat liver was incubated with propranolol at the initial drug concentration of 1.5 μM (pH 7.4, 37°). Results are given as the mean ± S.E. of three separate experiments.

Table 2. Effect of ARF on the metabolic activity for propranolol in rat hepatic microsomal fraction

	K_m (μM)	V_{max} (nmole/min/mg protein)
Control	0.163 ± 0.053	0.397 ± 0.061
ARF	0.184 ± 0.024	0.405 ± 0.071

The microsomal fraction prepared from control and ARF rats was incubated for 2 min at various concentrations of propranolol ranging from 0.1 to 3.0 μM and the metabolic rate for propranolol was determined. The apparent maximum rate of metabolism (V_{max}) and apparent Michaelis–Menten constant (K_m) were obtained from the Lineweaver–Burk plot. Results are given as the mean ± S.E. of four separate experiments.

from the liver cells into the extracellular space. The monophasic straight line for PSP suggests that the efflux of PSP may be negligible in our experimental system.

No evidence was obtained to indicate the altered metabolic activity for propranolol in ARF. The major pathways for propranolol metabolism described to date are *O*-dealkylation, side chain oxidation, ring oxidation and glucuronic acid conjugation [26]. The most important enzymes for oxidative metabolism are the hepatic microsomal mixed-function mono-oxygenases. Van Peer and Belpaire [21] produced ARF in rats by ligation of the ureters or by intravenous injection of uranyl nitrate and showed that aminopyrine *N*-demethylase was decreased while aniline hydroxylase remained unaltered in ARF compared to control rats. Patterson and Cohn [23] used a rat with surgically produced chronic renal failure and reported that the changes brought about by renal failure on microsomal enzymes are not generalized but show some apparent specificity. No delay in the conversion of propranolol to the 4-hydroxymetabolite nor in the formation of the acid labile conjugates of propranolol and 4-hydroxypropranolol was found in patients with renal failure [27]. In the present study, the metabolic activity was determined using the hepatic microsomal fraction with enough amount of cofactors for oxidative metabolism. The possibilities that some cofactors may be deficient in ARF or a kind of uremic toxins accumulated in the blood may exert an enzyme inhibitory action remain to be investigated. In addition, conjugational biotransformations of propranolol were not determined in the present study.

Recently, Iwamoto *et al.* [28] examined the uptake kinetics of aspirin and salicylamide into isolated rat hepatocytes and showed that the greater hepatic first-pass effect of salicylamide is directly related to more extensive and rapid transport of the drug into the liver as compared with that of aspirin. Since the first and prerequisite step for the hepatic metabolism of drugs is the transport of the compounds into the liver cells, the decreased uptake rate of propranolol observed in the present study could explain at least in part the decreased hepatic clearance of the drug in ARF.

REFERENCES

1. G. Bianchetti, G. Graziani, D. Brancaccio, A. Morganti, G. Leonetti, M. Manfrin, R. Sega, R. Gomeni, C. Ponticelli and P. L. Morselli, *Clin. Pharmacokin.* **1**, 373 (1976).
2. L. Balant, R. J. Francis, T. N. Tozer, A. Marmy, J. M. Tschopp and J. Frabe, *J. Pharmacokin. Biopharm.* **8**, 421 (1980).
3. G. Levy and K. M. Giacomini, in *Drug Absorption* (Eds. L. F. Prescott and W. S. Nimmo), p. 115. ADIS Press, Australia (1981).
4. N. Terao and D. D. Shen, *J. Pharmac. exp. Ther.* **227**, 295 (1983).
5. H. Katayama, J. Fujiwara, M. Yashuhara, K. Okumura and R. Hori, *J. Pharmac. Dyn.* **7**, 536 (1984).
6. C. A. Goresky and G. G. Bach, *Ann. N.Y. Acad. Sci.* **170**, 18 (1970).
7. N. Itoh, Y. Sawada, Y. Sugiyama, T. Iga and M. Hanano, *Jpn. J. Physiol.*, in press.
8. G. E. Mortimore, F. Tietze and D. Stetten, Jr., *Diabetes* **8**, 307 (1959).
9. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
10. Z. Dishe and E. Borenfreund, *J. biol. Chem.* **192**, 583 (1951).
11. M. Somogyi, *J. biol. Chem.* **86**, 655 (1930).
12. M. Yasuhara, H. Katayama, J. Fujiwara, K. Okumura and R. Hori, *J. Pharmac. Dyn.* **8**, 375 (1985).
13. D. G. Shand and R. E. Rangno, *Pharmacology* **7**, 159 (1972).
14. T. Walle, E. C. Conradi, U. K. Walle, T. C. Fagan and T. E. Gaffney, *Clin. Pharmac. Ther.* **24**, 668 (1978).
15. G. R. Wilkinson and D. G. Shand, *Clin. Pharmac. Ther.* **18**, 377 (1975).
16. J. P. Knochel and D. W. Seldin, in *The Kidney*, 2nd Edn (Eds. B. M. Brenner and F. C. Rector), p. 2137. W. B. Saunders, Philadelphia (1981).
17. D. T. Lowenthal, W. A. Briggs, T. P. Gibson, H. Nelson and W. J. Cirksena, *Clin. Pharmac. Ther.* **16**, 761 (1974).
18. M. S. Yates, C. J. Bowmer and J. Emmerson, *Biochem. Pharmac.* **32**, 3109 (1983).
19. M. S. Yates, J. Emmerson and C. J. Bowmer, *J. Pharm. Pharmac.* **35**, 593 (1983).
20. H. W. Leber and G. Schutterle, *Kidney Int.* **2**, 152 (1972).
21. A. P. Van Peer and F. M. Belpaire, *Arch. int. Pharmacodyn.* **228**, 180 (1977).
22. U. K. Terner, L. I. Wiebe, A. A. Noujaim, J. B. Dossetor and E. J. Sanders, *Clin. Biochem.* **11**, 156 (1978).
23. S. E. Patterson and V. H. Cohn, *Biochem. Pharmac.* **33**, 711 (1984).
24. W. Flamenbaum, M. L. Huddleston, J. S. McNeil and R. J. Hamburger, *Kidney Int.* **6**, 408 (1974).
25. K. M. Giacomini, S. M. Roberts and G. Levy, *J. Pharm. Sci.* **70**, 117 (1981).
26. P. A. Routledge and D. G. Shand, in *Applied Pharmacokinetics* (Eds. W. E. Evans, J. J. Schentag and W. J. Jusko), p. 464. Applied Therapeutics, San Francisco (1980).
27. F. D. Thompson, A. M. Joekes and D. M. Foulkes, *Brit. Med. J.* **2**, 434 (1972).
28. K. Iwamoto, Y. Furune and J. Watanabe, *Biochem. Pharmac.* **33**, 3089 (1984).