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Dose-Dependent Elimination of Propranolol and its Major Metabolites in Humans

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Abstract □ The disposition of propranolol and the formation of its major metabolites, propranolol glucuronide (I), 4-hydroxypropranolol glucuronide (II), and α-naphthoxylactic acid (III), were examined at steady state in four healthy volunteers given oral doses of 40–320 mg/day. The blood to plasma ratio of propranolol was 0.85 ± 0.11 (*SD*). In all subjects, the average steady-state concentration ($\bar{C}_{ss}$) of propranolol in plasma increased disproportionately with dose. There was a 1.8- to 2.6-fold difference in the $\bar{C}_{ss}$ between subjects, a 56 ± 20% reduction in the intrinsic clearance, and a 175% increase in the half-life of propranolol over the range of doses administered. The renal clearance was 75.4 ± 17.5 ml/min for I, 130.6 ± 28.3 ml/min for II, and 56.8 ± 13.3 ml/min for III. The formation of I was saturable in three subjects; the V_{max} and K_m were 103 ± 43 mg/day and 124 ± 46 ng/ml, respectively. In the remaining subject the nonrenal clearance of I was 496 ml/min. The formation of II and III was saturable in all subjects. The V_{max} and K_m were 71 ± 25 mg/day and 46 ± 22 ng/ml, respectively, for II and 92 ± 35 mg/day and 35 ± 24 ng/ml, respectively, for III. In each subject, the formation clearance associated with the unidentified metabolic pathway(s) (accounting for ~45% of the dose) was best described by a saturable process. The V_{max} and K_m estimated for this pathway were 212 ± 34 mg/day and 40 ± 12 ng/ml, respectively. These results suggest that the elimination of propranolol is saturable in the human at doses from 40 to 320 mg/day and can be explained only partly by saturability in the metabolic pathways resulting in the formation of I, II, and III.

Keyphrases □ Propranolol—formation of metabolites, dose-dependent elimination in humans, metabolic pathways □ Metabolites—of propranolol in humans, dose-dependent elimination, metabolic pathways □ Metabolism—of propranolol in the human, elimination, metabolic pathways

Propranolol is used extensively in the treatment of angina pectoris, hypertension, cardiac arrhythmia, and other disease states in which β-adrenergic blockade is desirable. The β-blocking activity of propranolol approaches a maximal therapeutic effect at a plasma concentration of ~80–100 ng/ml (1).

The absorption of propranolol following its oral administration is complete in the human (2); gut-wall metabolism has not been found in the dog (3). The drug has a high extraction ratio and is metabolized virtually completely in the liver (4, 5): <1% of the intact drug is found in the urine (6).

The disposition of propranolol can be affected by age (7, 8); cigarette smoking (6, 7); concomitant drug administration (9); and renal (10), hepatic (11), or thyroid disease (12–14). Pharmacokinetic studies in healthy adults and in patients with various disease states have demonstrated as much as 10- to 20-fold variation in the plasma concentrations of propranolol between individuals after oral, but not intravenous, doses of the drug (15–21). However, Walle *et al.* (22) reported only a threefold intersubject variability in peak concentrations of propranolol with doses of 40–320 mg/day and suggested that their findings resulted from careful study design, control of factors such as concomitant drug intake, and use of specific analytical procedures.

The metabolism of propranolol is complex. More than 18 metabolites have been identified (22–24), with at least four of these having pharmacological activity (25–27). Walle *et al.* (28–30) reported that at steady state, ~60% of an oral dose can be accounted for by metabolites detectable in both plasma and urine. The major metabolites are propranolol glucuronide (I), 4-hydroxypropranolol glucuronide (II), and α-naphthoxylactic acid (III). To our knowledge, no one has investigated whether the formation of these metabolites occurs by first-order or saturable processes.

Kornhauser *et al.* (31) reported an average intrinsic clearance (CL_{int}) of 2.71 liters/min for propranolol (based

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Table I—Blood to Plasma Ratio (B/P) of Propranolol

Subject	Concentration of Propranolol in Blood, ng/ml 4.9	9.8	24.6	49.1	73.7	98.2	280.4	Mean ± SD
A	1.17	0.82	0.83	0.78	0.82	0.78	0.80	0.86 ± 0.14
B	0.75	0.84	0.83	0.88	0.80	0.76	—[a]	0.81 ± 0.05
C	0.91	0.75	0.77	1.09	0.82	0.87	—	0.87 ± 0.12
D	0.96	0.99	0.69	0.90	0.76	0.88	—	0.86 ± 0.12
							Mean ± SD	0.85 ± 0.11

[a] — Not determined.

Table II—Relationship Between the Intrinsic Clearance and the Rate of Propranolol Dosing

Subject	Age, year	Weight, kg	Intrinsic Clearance (CL_{int})[a] liter/min Week 1	Week 2	Week 3	Week 4	Week 5	Change[b], %
A	26	64	4.59	4.93	4.64	2.43	1.03	−78
B	28	75	6.62	6.08	6.93	4.16	2.57	−61
C	24	68	4.51	3.82	3.78	3.44	2.01	−55
D	25	82	3.85	3.65	4.51	3.42	2.69	−30
							Mean ± SD	−56 ± 20

[a] The intrinsic clearance (CL_{int}) was calculated according to Eq. 4 as described in *Theoretical*. The dosage of propranolol given was 40, 80, 160, 240, and 320 mg/day during weeks 1, 2, 3, 4, and 5, respectively. [b] Percentage difference between weeks 1 and 5.

on whole blood concentrations) at a dose of 240 mg/day. Because urinary recovery of I, II, and III accounts for about one-half of the oral dose at steady state, the CL_{int} accounted for by these metabolites is ~1.36 liters/min. The renal clearance (CL_r) is 57 ml/min for I (30), 60 ml/min for II (29), and 37 ml/min for III (28). Therefore, the CL_{int} for each of these metabolites is much greater than their CL_r. Because the apparent volume of distribution of these metabolites is unknown, the rate constant of formation (k_f) and elimination (k_{el}) for each cannot be determined from administration of propranolol alone. However, if $k_f \gg k_{el}$ for each metabolite, the formation clearances can be determined from steady-state experiments only.

The results of several investigations suggest that an increase in the rate of propranolol dosing results in a disproportionate increase in the observed concentrations of the drug (4, 5, 22, 32). However, these results have been obtained from single-dose studies, steady-state studies spanning a narrow range of doses in a given individual, or steady-state studies employing analytical techniques having questionable specificity. No studies have examined the relationship between the average steady-state concentrations ($\bar{C}_{ss}$) of propranolol over a wide range of doses in the same individual, the variability between individuals under these conditions, or the mechanisms responsible for the nonlinear relationship between the dosing rate and $\bar{C}_{ss}$. This study was designed to examine these points in healthy adult volunteers. Urinary excretion rates of I, II, and III were measured simultaneously to identify the metabolic pathways responsible for differences between individuals.

THEORETICAL

After a sufficient number of oral doses of a drug have been given to reach steady state, the average steady-state concentration of a drug ($\bar{C}_{ss}$) can be related to the dose (D_0) by:

$$\frac{D_0}{\tau} = \frac{CL}{F} \cdot \bar{C}_{ss} \qquad \text{(Eq. 1)}$$

where CL is the systemic clearance of the drug, F is the fraction of the dose reaching the systemic circulation (bioavailability), and τ is the dosing interval. The $\bar{C}_{ss}$ can be defined by:

$$\bar{C}_{ss} = \frac{\int_{t_1}^{t_2} C(t)dt}{\tau} = \frac{AUC_0}{\tau} \qquad \text{(Eq. 2)}$$

where $C(t)$ is an expression describing concentration as a function of time (t) and AUC_0 is the area under the blood or plasma concentration time curve during one dosing interval. Therefore, $\bar{C}_{ss}$ can be estimated from the AUC_0 at steady state and τ; this does not require explicit knowledge of F even though F may change with a change in the dose.

Based on the venous equilibration model described by Rowland *et al.* (33), the intrinsic clearance (CL_{int}), which estimates the activity of the drug metabolizing enzymes in the liver, may be defined. Wilkinson and Shand (34) have shown that the apparent oral clearance (CL_o) of a drug is equivalent to CL_{int} and is defined by:

$$CL_{int} = \frac{D_0}{AUC_0} = \frac{CL}{F} \qquad \text{(Eq. 3)}$$

This relationship assumes that the drug is totally absorbed from the GI tract, that all blood containing the drug passes through the liver before reaching the systemic circulation, and that the liver accounts for all loss processes. Therefore, a change in F results from a change in the hepatic extraction of propranolol by the liver.

A basic presumption in the derivation of CL_{int} (33) is that the drug is undergoing metabolism by a first-order process. If, however, metabolism involves capacity-limited kinetics in the concentration range involved, then the CL_{int} calculated by Eq. 3 will be an average of the values over the concentration range. Substituting CL_{int} for CL/F in Eq. 1 yields:

$$\frac{D_0}{\tau} = CL_{int} \cdot \bar{C}_{ss} \qquad \text{(Eq. 4)}$$

The relationship between the dose rate and C_{ss} may be defined by a series of potential models for propranolol elimination processes symbolized by CL_{int} in Eq. 4:

$$\frac{D_0}{\tau} = f_x \cdot \sum_{i=1}^{n} dAe_{M_i}/dt \qquad \text{(Model 1)}$$

Model 1 assumes that the measured pathways (forming I, II, and III) account for all of the dose. Based on urinary recovery, these major metabolites of propranolol account for <100% of the parent drug. The term $\Sigma_{i=1}^{n} dAe_{M_i}/dt$ is the sum of metabolite formation only accounted for by urinary excretion of I, II, and III. It is possible that these metabolites are also eliminated by extrarenal routes (*e.g.*, biliary). In this model, the term f_x is a unitless number >1.0 which attempts to correct for these extrarenal losses.

$$\frac{D_0}{\tau} = CL_x \cdot \bar{C}_{ss} + \sum_{i=1}^{n} dAe_{M_i}/dt \qquad \text{(Model 2)}$$

In Model 2, the term Cl_x identifies the sum of all first-order metabolic pathways of propranolol to unknown or unidentified metabolites.

Table III—Time[a] to Peak Concentration (t_{peak}) and the Terminal Half-Life ($t_{1/2}$) at Increasing Dosing Levels of Propranolol

	Daily Dose of Propranolol, mg/day									
	40		80		160		240		320	
Subject	t_{peak}	$t_{1/2}$	t_{peak}	$t_{1/2}$	t_{peak}	$t_{1/2}$	t_{peak}	$t_{1/2}$	t_{peak}	$t_{1/2}$
A	4.0	2.6	3.3	3.6	3.5	3.5	3.5	4.7	4.0	8.3
B	2.2	1.6	2.0	2.8	2.0	3.5	2.5	3.8	3.0	4.7
C	2.0	2.0	2.0	3.0	2.0	3.0	3.0	3.5	2.0	4.8
D	2.0	1.8	1.8	2.0	3.2	2.7	2.0	3.7	2.0	4.3
Mean	2.5	2.0	2.3	2.9	2.7	3.2	2.8	3.9[b]	2.8	5.5[b,c]
(SD)	(1.0)	(0.4)	(0.7)	(0.6)	(0.8)	(0.4)	(0.6)	(0.5)	(1.0)	(1.9)

[a] In hours. [b] Significantly different at $p < 0.05$ when compared with the value obtained at the 40-mg/day dose. [c] Significantly different at $p < 0.05$ when compared with the value obtained at the 240-mg/day dose.

Table IV—Akaike Information Criterion (AIC) Calculated for Each Model Describing the Elimination Rate of Propranolol[a]

Subject	Model 1	Model 2	Model 3	Model 4	Model 5
A	187.5	177.7	174.6[b]	181.9	275.9
B	174.2[b]	177.4	176.4	177.4	230.3
C	197.9	191.6	189.6[b]	200.2	200.2
D	214.0	213.9	211.8[b]	215.6	228.2

[a] See *Data Analysis* for a description of the AIC calculation procedure. [b] Model with minimum AIC.

$$\frac{D_0}{\tau} = \frac{V_{max_x} \cdot \bar{C}_{ss}}{K_{m_x} + \bar{C}_{ss}} + \sum_{i=1}^{n} dAe_{M_i}/dt \qquad \text{(Model 3)}$$

In Model 3, the first term represents a saturable clearance pathway for unidentified metabolites with parameters V_{max_x} and K_{m_x}; added to these unidentified metabolites is the sum of the elimination accounted for by known metabolites.

$$\frac{D_0}{\tau} = CL_x \cdot \bar{C}_{ss} + f_x \cdot \sum_{i=1}^{n} dAe_{M_i}/dt \qquad \text{(Model 4)}$$

Model 4 is a combination of Models 1 and 2.

$$\frac{D_0}{\tau} = CL_x \cdot \bar{C}_{ss} + \frac{V_{max_x} \cdot \bar{C}_{ss}}{K_{m_x} + \bar{C}_{ss}} + \sum_{i=1}^{n} dAe_{M_i}/dt \qquad \text{(Model 5)}$$

Model 5 is a combination of Models 2 and 3.

The known (or measured) excretion rate for each metabolite in these models may be calculated from:

$$dAe_{M_i}/dt = \frac{V_{max,M_i} \cdot \bar{C}_{ss}}{K_{m,M_i} + \bar{C}_{ss}} \qquad \text{(Eq. 5)}$$

where V_{max,M_i} and K_{m,M_i} are parameters for each known metabolite formation pathway for propranolol. At steady state, dAe_{M_i}/dt is equal to the formation rate for that particular metabolite.

When the apparent $K_m \gg \bar{C}_{ss}$, the relationship between dAe_{M_i}/dt and $\bar{C}_{ss}$ is linear. The slope of this line is equal to the metabolic clearance for this pathway.

EXPERIMENTAL

Subjects—The subjects were four healthy adult volunteers. Informed consent was obtained, and the protocol approved by the University of California, San Francisco, Committee on Human Research. A medical history, physical examination, electrocardiogram (ECG), complete blood count with differential, urinalysis, and selected blood chemistries were completed for all subjects. There was no evidence of renal or hepatic disease in the medical history, and values of blood urea nitrogen, serum creatinine, urinalysis and urine culture, serum glutamic oxaloacetic transaminase, lactic dehydrogenase, alkaline phosphatase, bilirubin, prothrombin time, and total serum proteins were normal. All were nonsmokers and abstained from alcohol, marijuana, and other medications until completion of the study. On each of the four days per week that propranolol was taken, the subjects had their pulse and blood pressure measured, underwent a 1-min ECG, and were interviewed to determine side effects.

Propranolol Administration—All subjects were given 40, 80, 160, 240, and 320 mg/day of propranolol in divided doses over a 5-week period. Each subject took one-fourth of the daily dose every 6 hr for a total of 13 doses at each dosing rate. No dietary restrictions were imposed, but food was withheld for at least 9 hr before and 3 hr after the 13th dose.

Blood Sampling—Blood samples were obtained at the end of the 8th, 9th, and 12th dosing intervals (trough concentrations) and at 0, 15, 30, 45, 60, and 90 min and 2, 3, 4, 5, 6, 8, 10, and 12 hr following the 13th dose. Venous blood samples were obtained using an indwelling catheter[1]; patency was maintained by flushing with 1 ml of heparinized saline (10 U/ml) after obtaining each blood sample (35). It was shown that these small doses of heparin do not affect the plasma protein binding or disposition of propranolol (36). After discarding 0.5 ml of blood, a 7-ml blood sample was transferred immediately to a 16 × 150-mm polytef-lined[2] screw-cap test tube to which had been added 100 U of aqueous sodium heparin. After gentle mixing, blood samples were centrifuged at 2000 rpm (500×*g*) for 10 min. Plasma was transferred with a disposable pasteur pipet to glass screw-cap vials and stored at −20° until assay.

Urine Sampling—Urine samples were collected during the 5th, 9th, and 12th dosing intervals and at frequent times for up to 12 hr after the 13th dose each week. Urine volumes were recorded and 10 mg of (−)-ascorbic acid was added to prevent oxidation of phenolic metabolites (27); aliquots were stored at −20° until assayed.

Assay Procedures—Propranolol and 4-hydroxypropranolol concentrations were measured in plasma before and after enzymatic hydrolysis of the glucuronide conjugate[3] and in urine after enzymatic hydrolysis (37). The limits of sensitivity are 0.4 and 1.0 ng/ml, respectively. α-Naphthoxylactic acid concentration in plasma and urine were measured directly after protein precipitation by a method previously described (38).

Blood to Plasma Ratio of Propranolol—The equilibrium time for propranolol between human erythrocytes and plasma was reported to be ~5 min (39). The blood to plasma ratio (B/P) was determined over the range of measured concentrations by adding propranolol to test tubes containing 2 ml of fresh blood obtained from each individual and 100 U of heparin (to prevent clotting). After allowing each sample to stand for 2 hr (with gentle agitation every 15 min), the test tubes were centrifuged, and the plasma was measured. These measurements were compared with 2 ml of plasma containing the same amount of drug, to account for possible *in vitro* degradation of propranolol by plasma.

Data Analysis—The CL_{int} of propranolol at each C_{ss} was calculated according to Eq. 4. The AUC of propranolol, I, II, and III during the 13th dosing interval were calculated by the trapezoidal rule. The half-life of propranolol was calculated as 0.693/terminal elimination rate constant (determined by least-squares regression utilizing at least four plasma concentration time points in the log-linear region). The renal clearance (CL_r) of each metabolite was calculated as the slope of the line determined by least-squares regression of the rate of urinary excretion *versus* C_{ss} for each metabolite in plasma. The C_{ss} of propranolol and its metabolites at each dosing rate was calculated from the data obtained during the 13th dosing interval using Eq. 2.

The elimination rate models described in *Theoretical* were specified using MKMODEL (40). The predicted value for C_{ss} was calculated for each rate model using the ROOT function in MLAB (41). This function determines the value of C_{ss} that satisfies the rate model equation. The

[1] Butterfly Catheter, Abbott Laboratories, North Chicago, IL 60064.
[2] Teflon.
[3] Glusulase, Cat. No. GD 751, Sigma Chemical Co., St. Louis, MO 63178.

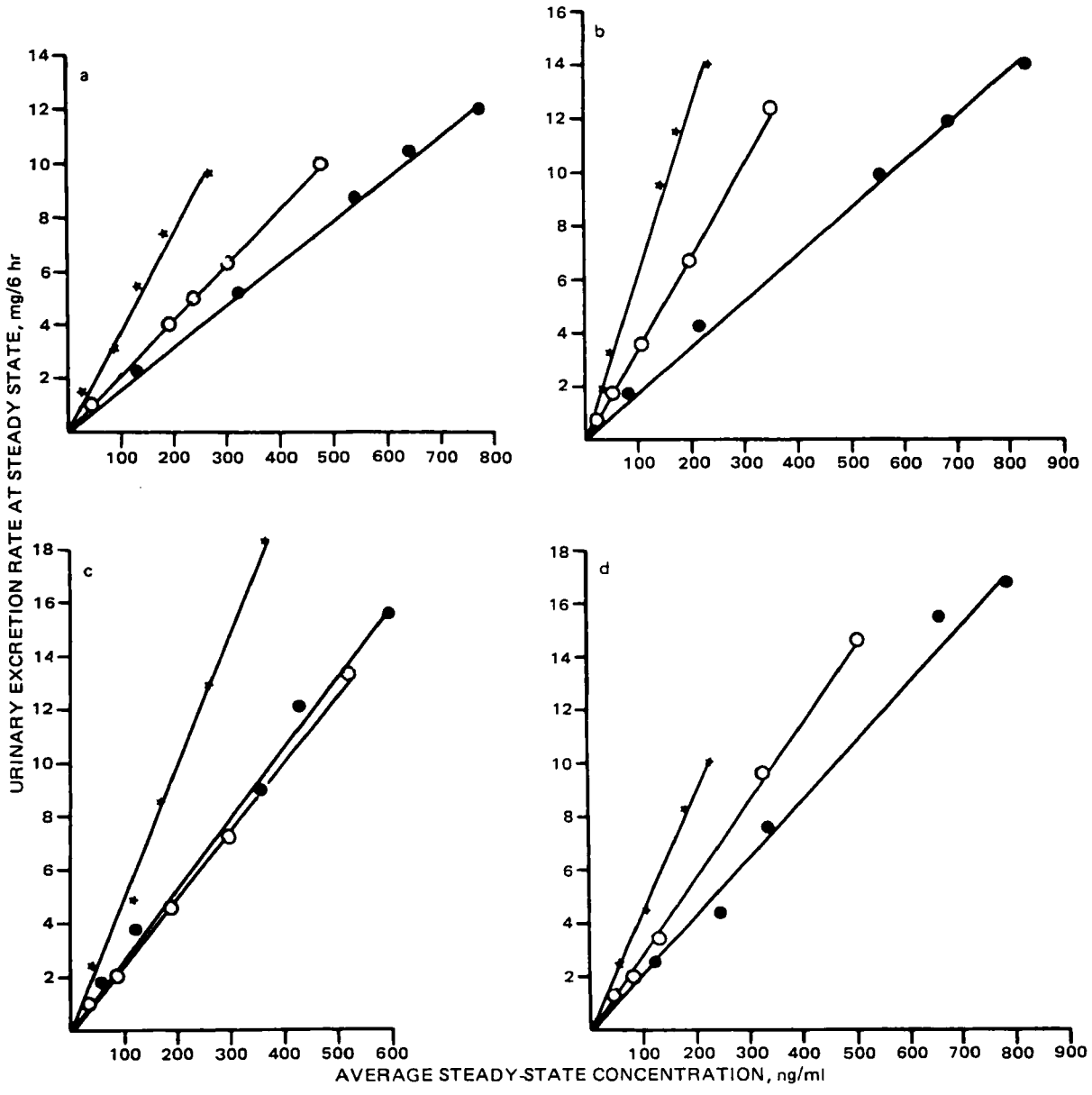

Figure 1—*Urinary excretion rate of each measured metabolite for each subject (a–d) as a function of the average steady-state concentration of the corresponding metabolite in plasma. Key: (○) propranolol glucuronide; (★) 4-hydroxypropranolol glucuronide; (●) α-naphthoxylactic acid.*

parameters of the models were estimated by simultaneous unweighted nonlinear least-squares regression (41) of the dose rate *versus* the measured C_{ss} and metabolite excretion rates.

Discrimination between the models was made with the Akaike information criterion (AIC) (42–44):

$$\text{AIC} = N \ln \text{RSQ} + 2p \qquad \text{(Eq. 6)}$$

where N is the number of observations, p is the number of parameters, and RSQ is the residual sums of squares of the observed points. The model yielding the lowest AIC value was considered to be the best representation of the experimental data for each subject.

Mean values are reported with standard deviations; differences between means were evaluated by one-way analysis of variance and the Newman-Keuls multiple-range test (45). Data anlaysis and graphical examination of the results were performed with the PROPHET (46) computer system[4].

RESULTS

Side Effects—No major adverse effects resulted from the administration of propranolol. The only side effect noted was in subject A while receiving 320 mg/day. Five hours after the 13th dose, he developed weakness and dizziness. After resting supine for 1 hr, he was able to resume his usual activities.

Validation of Steady-State Conditions—In each subject, steady-state trough concentrations were attained by the ninth dose (or less) and, once achieved, were associated with only slight interday variation.

Quantitation of 4-Hydroxypropranolol in Plasma—Because 4-hydroxypropranolol concentrations in plasma were <20 ng/ml and were ~25- to 30-fold greater after enzymatic hydrolysis, 4-hydroxypropranolol glucuronide concentrations were considered to be equivalent to the total 4-hydroxypropranolol concentration posthydrolysis.

Blood to Plasma Ratio of Propranolol—The blood to plasma ratio (B/P) of propranolol measured over the observed concentration range is shown in Table I. The B/P was independent of concentration, and only slight interindividual variability in the B/P was observed. In our subjects, the B/P was 0.85 ± 0.11.

Relationship Between the Intrinsic Clearance and the Dosing Rate of Propranolol—The CL_{int} of propranolol was determined at each of five dosing rates using Eq. 4. There was a 56 ± 20% reduction in CL_{int} on average over the eightfold range in doses (Table II).

Disposition of Propranolol During the 13th Dosing Interval—The time to peak concentration of propranolol during the 13th dosing interval ranged from 2.5 ± 1.0 to 2.8 ± 1.0 hr at doses from 40 to 320 mg/day. As

[4] A specialized resource developed by the Chemical Biological Information Handling Program of the National Institutes of Health.

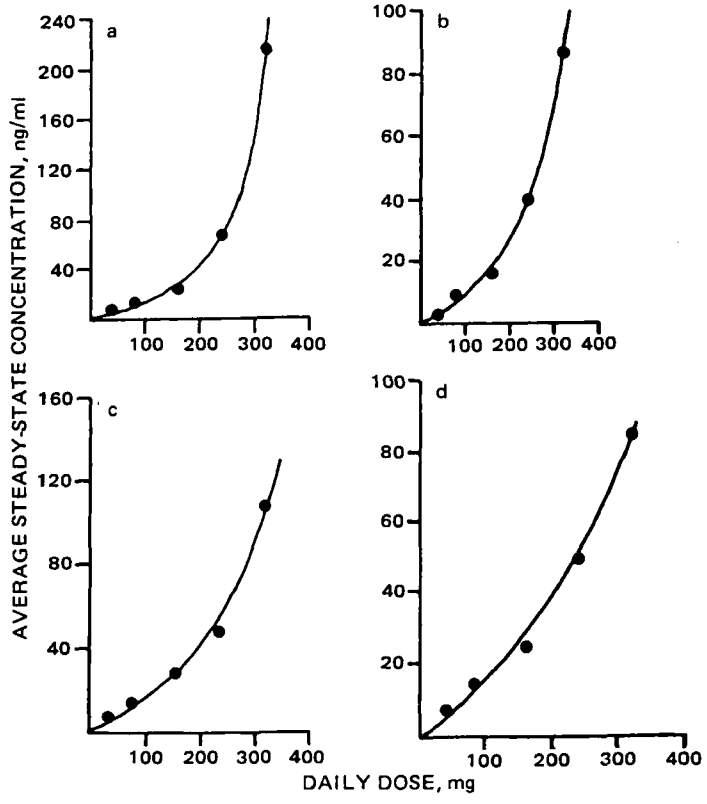

Figure 2—*Average steady-state concentration of propranolol in plasma during the 13th dosing interval at dosing rates from 40 to 320 mg/day. The line is the concentration predicted by the best elimination-rate model for each subject (a–d).*

shown in Table III, the terminal half-life of propranolol increased significantly, from 2.0 ± 0.4 to 5.5 ± 1.9 ($p < 0.05$), as the dose was increased from 40 to 320 mg/day.

Renal Clearance of Propranolol Metabolites—Less than 2% of propranolol was recovered in urine as free propranolol or 4-hydroxypropranol (unconjugated); no α-naphthoxylactic acid glucuronide was detected in the urine. There was a linear relationship between the urinary excretion rate of propranolol glucuronide (I), 4-hydroxypropranolol glucuronide (II), and α-naphthoxylactic acid (III) *versus* $\bar{C}_{ss}$ of each metabolite in plasma over the entire range of observed concentrations. The CL_r was 75.4 ± 17.5 ml/min for I, 130.6 ± 28.3 ml/min for II, and 56.8 ± 13.3 ml/min for III (Fig. 1).

Fraction of Propranolol Excreted in Urine as Propranolol Metabolites—The percentage of propranolol (on a molar basis) excreted in urine increased from 9.6 ± 2.5 to 16.2 ± 2.0 (significant at $p < 0.05$) for I, decreased from 17.7 ± 2.7 to 14.7 ± 3.3 for II, and decreased from 25.1

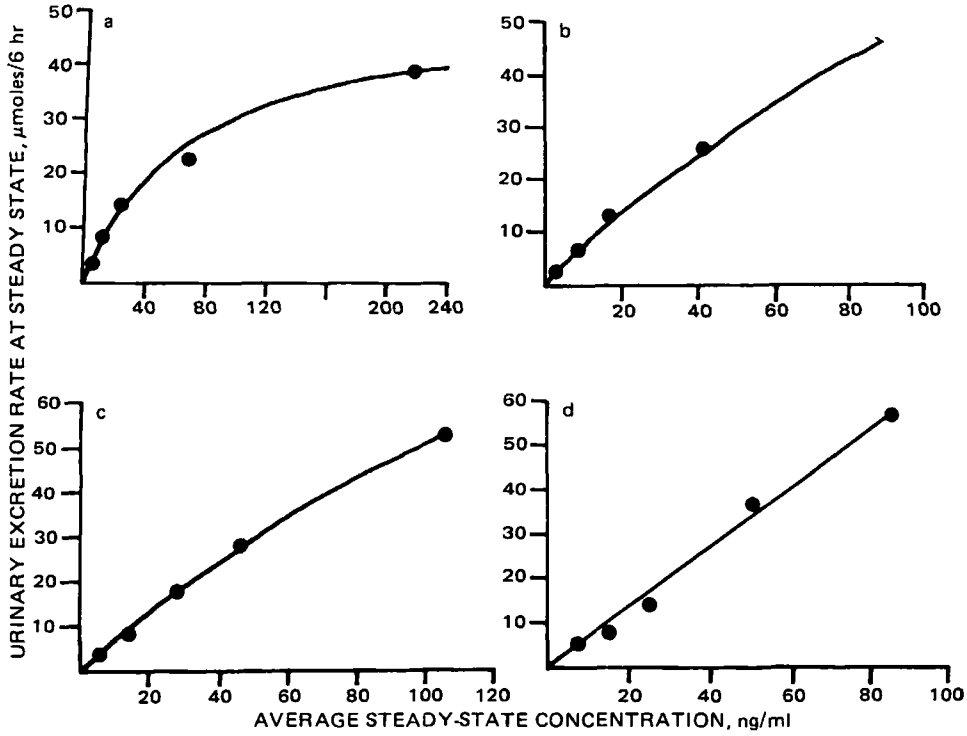

Figure 3—*Urinary excretion rate of propranolol glucuronide during the 13th dosing interval as a function of the average steady-state concentration of propranolol in plasma for each subject (a–d).*

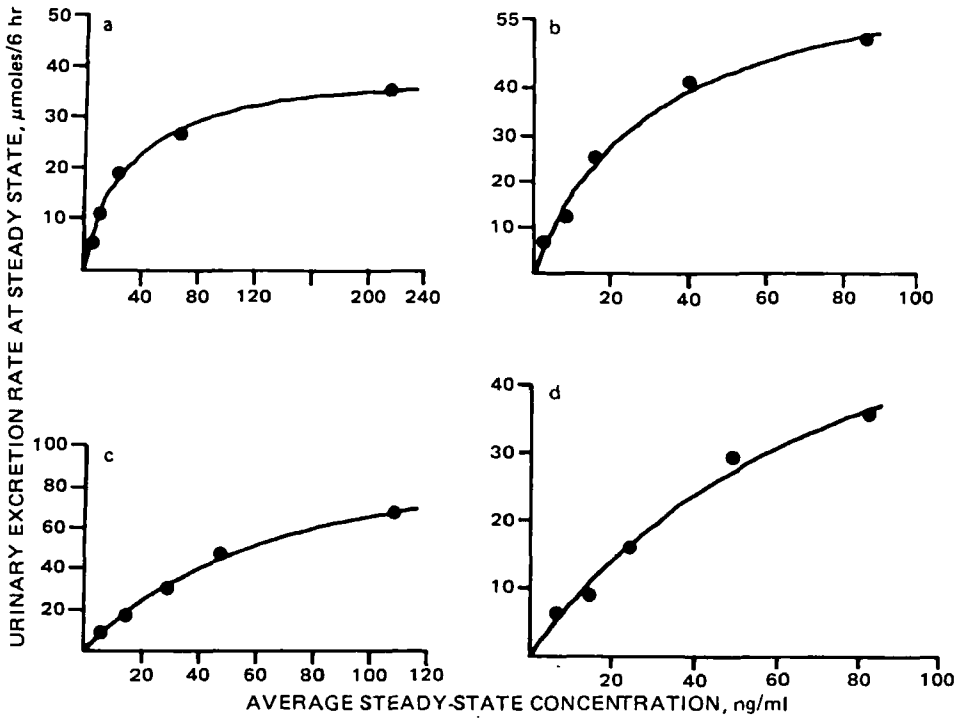

Figure 4—*Urinary excretion rate of 4-hydroxypropranolol glucuronide during the 13th dosing interval as a function of the average steady-state concentration of propranolol in plasma for each subject (a–d).*

± 3.7 to 18.6 ± 3.3 (significant at $p < 0.05$) for III as the dosing rate of propranolol was increased from 40 to 320 mg/day.

Relationship Between the $\bar{C}_{ss}$ of Propranolol and the Daily Dose—There was a disproportionate increase in the $\bar{C}_{ss}$ of propranolol as the daily dose was increased in each subject. There was a 1.8–2.6-fold difference in the $\bar{C}_{ss}$ of propranolol between subjects as the dose was increased from 40 to 320 mg/day (Fig. 2). The clearance associated with the unidentified metabolic pathway(s) was best described by a saturable (Models 1 and 3) rather than a first-order (Model 2) process (Table IV). In three subjects, the parameters of this saturable process were clearly different from those of the measured metabolites (Model 3), but in the remaining subject (B) they were indistinguishable (Model 1). These models were superior to either combination model (Models 4 and 5). The f_x-values estimated from Model 1 were 2.4, 2.0, 1.7, and 1.9 in subjects A, B, C, and D, respectively.

Formation Clearance Estimates for Known and Unknown Metabolic Pathway(s)—The formation of I was best described by a saturable process in three of four subjects (A, B, C) (Fig. 3). Using the estimates from Model 3 for all subjects, the V_{max} for propranolol glucuronide was 114 ± 51 mg/day and the K_m was 140 ± 55 ng/ml. In subject D, the apparent K_m for I was much greater than the C_{ss} of propranolol. The corresponding first-order metabolic clearance estimated from the slope of *dAe/dt versus* C_{ss} of propranolol was 496 ml/min. The formation of II and III was saturable in all four subjects over the dosing range studied (Figs. 4 and 5). The V_{max} for II was 72 ± 25 mg/day, and the K_m was 46 ± 21 ng/ml; the V_{max} for III was 92 ± 35 mg/day, and the K_m was 35 ± 234 ng/ml. The V_{max} for the unidentified metabolic pathway(s) was 212 ± 34 mg/day, and the K_m was 40 ± 12 ng/ml. These results are summarized in Table V.

DISCUSSION

The results from the present investigation demonstrate that the elimination of propranolol is nonlinear with doses of 40–320 mg/day and can be explained by capacity limitation in the major metabolic pathways that result in the formation of propranolol glucuronide (I), 4-hydroxypropranolol glucuronide (II), α-naphthoxylactic acid (III), and unidentified metabolite(s).

The 4-hydroxy metabolite of propranolol (4-hydroxypropranolol, IV) has similar β-blocking activity to the parent drug (25). Free (unconjugated) concentrations of IV reported by other workers following oral doses of propranolol have varied greatly. The ratio of IV to propranolol reported by others has ranged from 0.03 to 1.07, but was generally ~0.2 (29, 47, 48). By employing an analytical technique having a sensitivity limit for IV of ~1.0 ng/ml (37), it was found that concentrations of this metabolite were <20 ng/ml even at propranolol doses of 320 mg/day; the ratio of IV to propranolol was ~0.08. The lower ratio of IV-propranolol observed in this study relative to those by other workers may be due to differences between patients and healthy volunteers. Virtually all (>97%) of IV in the plasma was present as the glucuronide conjugate (II). Therefore, concentrations of II were estimated as the total IV concentration following enzymatic hydrolysis. Unlike propranolol, which is known to undergo conjugation at the secondary hydroxyl group on the side chain yielding an ether linkage, the specific location of the glucuronide on IV in the human is unknown; the conjugate may involve an ether or phenolic linkage, or may be a mixture of both. Fenseleau and Johnson have previously stressed that enzymatic hydrolytic techniques cannot discriminate between potential glucuronide conjugates when multiple sites of glucuronidation are possible (49).

The blood to plasma ratio (B/P) of propranolol was determined for each subject, and, in contrast to previous results (39), it was found that the B/P was unchanged over the 20–50-fold range of observed concentrations (Table I).

In the present study, the intrinsic clearance (CL_{int}) of propranolol varied from 1.8- to 2.6-fold between subjects at doses of 40 and 320 mg/day, respectively (Table II). On average, there was a 56% reduction in the CL_{int} over the eightfold range in daily doses of the drug. Makichan *et al.* (32) reported a 44% decrease in CL_{int} after doses from 10 to 80 mg, and Schneck *et al.* (4) reported a 53% decrease in CL_{int} after 160- and 320-mg doses. These results, however, were obtained from single-dose rather than steady-state experiments. Because CL_{int} is concentration dependent, estimates of its value are best obtained at steady state.

The time to peak concentration was similar, whereas the half-life of propranolol increased approximately threefold as the dose was increased from 40 to 320 mg/day (Table III). If it is assumed that the apparent volume of distribution of propranolol remains unchanged throughout the study, then the observed increase in the half-life must be due to a change in the systemic clearance.

The renal clearance (CL_r) of I, II, and III was linear over the range of observed metabolite concentrations (Fig. 1). The CL_r of II in the present study was about twice that previously reported [130 *versus* 60 ml/min (29)]; the CL_r of I and III were closer to those in previous reports [75 *versus* 57 ml/min (30), and 57 *versus* 37 ml/min (28), respectively]. Stone and Walle recently reported that the plasma concentrations of I, II, and III were ~20-fold greater in uremic patients when compared with patients having normal renal function (50). However, because the pharmacological and toxicological activities of these compounds are unknown, the clinical significance of these findings is uncertain.

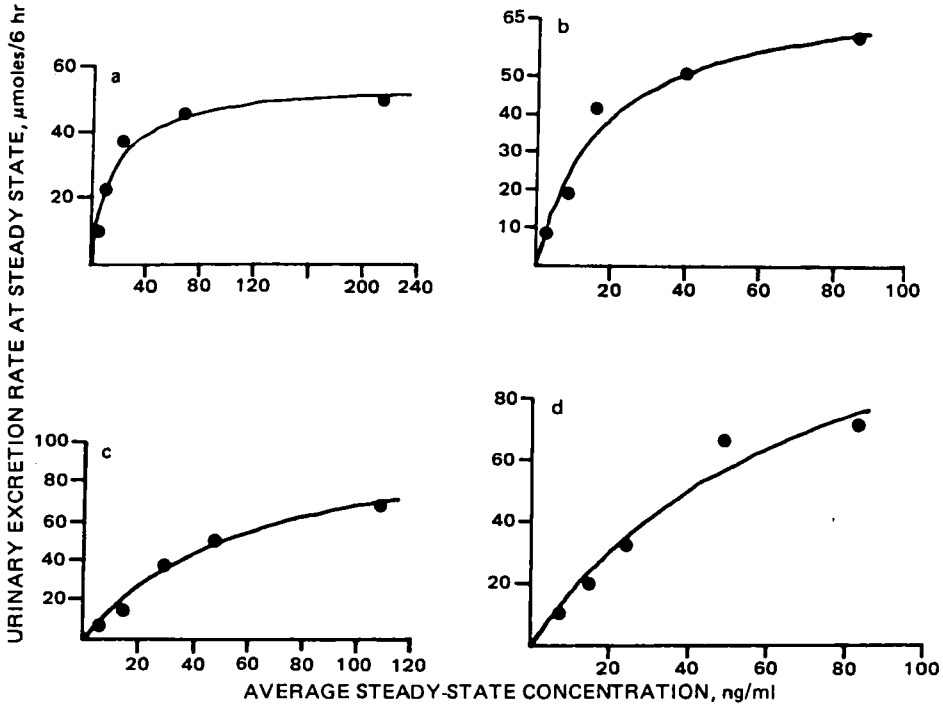

Figure 5—*Urinary excretion rate of α-naphthoxylactic acid during the 13th dosing interval as a function of the average steady-state concentration of propranolol in plasma for each subject (a–d).*

The formation of I from propranolol appeared to be saturable in three of four subjects (Fig. 3). In subject D, the metabolic clearance of I (496 ml/min) was ~6.4 times greater than its renal clearance (77.3 ml/min). Saturable glucuronidation has been reported previously for salicylamide and salicylic acid (51–53).

The V_{max} and K_m for II rather than for IV were estimated (Table V); there was essentially no free IV excreted in the urine. Therefore, saturability in the formation of II could be due either to saturation in the formation of IV from propranolol or in the formation of II from IV. If formation of IV from propranolol was a first-order process and formation of II from IV was saturable, one would expect to see disproportionate increases in IV concentrations (because it would accumulate) as the dose of propranolol was increased. That this was not observed suggests that saturability in the formation of II results from saturation in the formation of IV from propranolol.

In contrast to previous findings (28, 47), it was observed that the formation of III from propranolol was saturable (Fig. 5). However, this contention is predicated on several assumptions, because propranolol is not converted directly to this metabolite. Side-chain oxidation of propranolol first results in the formation of *N*-desisopropylpropranolol (V). Further oxidation of V yields a reactive aldehyde intermediate that can undergo either reduction to propranolol glycol or oxidation to III. In addition, III can be further oxidized to α-naphthoxyacetic acid (VI). Measurable concentrations of V, propranolol glycol (mainly as the glucuronide), and VI were detected in the urine of our subjects. As in previous studies (47), these metabolites accounted for <2% of the dose. Lo observed a similar urinary excretion profile for propranolol metabolism in the dog, but found significant concentrations of propranolol glycol (as the glucuronide) in the bile (3). Because only trace amounts of V, propranolol glycol (glucuronide), and VI are excreted in the urine at steady state (whereas up to 25% of propranolol can be accounted for as III), saturability in the formation of III is probably a reflection of the primary metabolic step in the *N*-dealkylation of propranolol to V. However, the value of V_{max} estimated for III is probably an underestimate of the total V_{max} for this metabolic pathway, since it is likely that other unidentified metabolism *via* this pathway is occurring and not being detected in the urine.

Elimination Models 1 and 3 adequately described the urinary excretion of the three measured metabolites in all individuals (Table IV). There was, however, a consistent underestimation of C_{ss} at the 40- and 80-mg dose rates in each individual (Fig. 2). This error was small (5% of C_{ss}) and does not detract from the overall ability of these models to describe C_{ss}

Table V—Estimated Michaelis–Menten Parameters for Propranolol Metabolism [a]

Subject	Propranolol Metabolite			
	Propranolol Glucuronide	4-Hydroxy-propranolol Glucuronide	α-Naphthoxylactic Acid	Unidentified Metabolic/ Pathway(s)
	V_{max}, mg/day			
A	55	41	56	230
B	142	72	77	205
C	146	101	99	167
D	4,577	72	137	244
Mean	114[b]	72	92	212
(*SD*)	(51)	(25)	(35)	(34)
	K_m, ng/ml			
A	76	29	14	45
B	175	31	18	28
C	168	51	43	33
D	6,297	74	63	55
Mean	140[b]	46	35	40
(*SD*)	(55)	(21)	(23)	(12)

[a] All values of V_{max} and K_m were estimated from Model 3. [b] Excludes values from subject D. Since the apparent K_m for propranolol glucuronide for subject D was much greater than C_{ss}, a metabolic clearance for this pathway of 496 ml/min was estimated from the slope of dAe/dt *versus* C_{ss}.

and metabolite excretion over a wide range of doses. However, it does suggest the existence of some time-related change in one or more clearance pathways. There was no evidence of a first-order elimination process for propranolol distinguishable from the saturable pathways.

To obtain a relationship between the C_{ss} of propranolol and the dosing rate (D_0/τ) using Eq. 4, the fraction of the dose not explained by measurement of I, II, and III had to be accounted for. The metabolic pathway(s) responsible for the formation of these unidentified metabolites was best described by a saturable rather than a first-order process. This may reflect either elimination of I, II, and III by routes other than in urine or in the formation of metabolites not measured (*e.g.*, propranolol glycol glucuronide in bile). The improved fit of Model 3 compared with Model 1 in three of four subjects supports the latter explanation.

The finding of saturable propranolol elimination in each individual studied suggests that clinicians should not presume linear pharmacokinetics when dosing patients with propranolol. It should be anticipated, therefore, that dosage increases may result in disproportionate increases in the plasma concentration of the drug. Although a 2.6-fold variation in the C_{ss} of propranolol between individuals was observed (Fig. 2), there was up to a fourfold difference in K_m for the various metabolic pathways (Table V). This latter finding may be of particular importance if one or more metabolites arising from the various metabolic pathways contribute to the clinical effects of propranolol.

REFERENCES

(1) A. S. Nies and D. G. Shand, *Circulation*, **52**, 6 (1975).

(2) J. W. Paterson, M. E. Conolly, C. T. Dollery, A. Hayes, and R. G. Cooper, *Pharmacol. Clin.*, **2**, 127 (1970).

(3) M. Lo, PhD thesis, University of California at San Francisco, 1981.

(4) D. G. Shand, E. M. Nuckolls, and J. A. Oates, *Clin. Pharmacol. Ther.*, **11**, 112 (1970).

(5) D. G. Shand and R. E. Ragno, *Pharmacology*, **7**, 159 (1972).

(6) R. E. Vestal, A. J. J. Wood, R. A. Branch, D. G. Shand, and G. R. Wilkinson, *Clin. Pharmacol. Ther.*, **26**, 8 (1979).

(7) C. M. Castleden, C. M. Kay, and R. L. Parsons, *Br. J. Clin. Pharmacol.*, **2**, 303 (1975).

(8) C. M. Castleden and C. F. George, *Br. J. Clin. Pharmacol.*, **7**, 49 (1979).

(9) R. E. Vestal, D. M. Kornhauser, J. W. Hollifield, and D. G. Shand, *Clin. Pharmacol. Ther.*, **25**, 19 (1979).

(10) G. Bianchetti, G. Grazini, and D. Branacaccio, *Clin. Pharmacokinet.*, **1**, 373 (1976).

(11) R. A. Branch and D. G. Shand, *Clin. Pharmacokinet.*, **17**, 264 (1976).

(12) J. Feely and I. H. Stevenson, *Br. J. Clin. Pharmacol.*, **6**, 446 (1978).

(13) J. Feely, I. H. Stevenson, and J. Crooks, *Clin. Pharmacol. Ther.*, **28**, 40 (1980).

(14) J. G. Riddell, J. D. Neill, J. G. Kelly, and D. G. McDevitt, *Clin. Pharmacol. Ther.*, **28**, 565 (1980).

(15) W. A. Briggs, D. T. Lowenthal, W. J. Cirksena, W. E. Price, T. P. Gibson, and W. Flamenbaum, *Clin. Pharmacol. Ther.*, **18**, 606 (1975).

(16) C. A. Chidsey, P. Morselli, G. Bianchetti, A. Morganti, G. Lenonetti, and A. Zanchetti, *Circulation*, **52**, 313 (1975).

(17) M. Esler, A. Zweifler, O. Randall, and V. DeQuattro, *Clin. Pharmacol. Ther.*, **22**, 299 (1977).

(18) A. Lehtonen, J. Kanto, and T. Kleimola, *Eur. J. Clin. Pharmacol.*, **11**, 155 (1977).

(19) M. Pine, L. Favrot, S. Smith, V. McDonald, and C. A. Chidsey, *Circulation*, **52**, 886 (1975).

(20) D. G. Shand, *Med. Clin. North Am.*, **58**, 1063 (1974).

(21) E. Vervolet, B. C. M. J. Takx-Kohlen, B. F. M. Pluym, and F. W. H. M. Merkus, *Clin. Pharmacol. Ther.*, **23**, 133 (1978).

(22) T. Walle, E. C. Conradi, U. K. Walle, T. C. Fagan, and T. E. Gaffney, *Clin. Pharmacol. Ther.*, **24**, 668 (1978).

(23) T. Walle and T. E. Gaffney, *J. Pharmacol. Exp. Ther.*, **182**, 83 (1972).

(24) T. Walle, J. I. Morrison, and G. L. Tindell, *Res. Commun. Chem. Pathol. Pharmacol.*, **9**, 1 (1974).

(25) J. D. Fitzgerald and S. R. O'Donnell, *Br. J. Pharmacol.*, **45**, 207 (1972).

(26) T. Ishizaki, P. J. Privitera, T. Walle, and T. E. Gaffney, *J. Pharmacol. Exp. Ther.*, **189**, 626 (1974).

(27) D. A. Saelens, T. Walle, T. E. Gaffney, and P. J. Privitera, *Eur. J. Pharmacol.*, **42**, 39 (1977).

(28) T. Walle, E. C. Conradi, U. K. Walle, T. C. Fagan, and T. E. Gaffney, *Clin. Pharmacol. Ther.*, **26**, 548 (1979).

(29) T. Walle, E. C. Conradi, U. K. Walle, T. C. Fagan, and T. E. Gaffney, *Clin. Pharmacol. Ther.*, **27**, 22 (1980).

(30) T. Walle, T. C. Fagan, E. C. Conradi, U. K. Walle, and T. E. Gaffney, *Clin. Pharmacol. Ther.*, **26**, 167 (1979).

(31) D. M. Kornhauser, A. J. J. Wood, R. E. Vestal, G. R. Wilkinson, R. A. Branch, and D. G. Shand, *Clin. Pharmacol. Ther.*, **23**, 165 (1978).

(32) J. J. Makichan, D. R. Pyzcznski, and W. J. Jusko, *Biopharm. Drug Dispos.*, **1**, 159 (1980).

(33) M. Rowland, L. Z. Benet, and G. G. Graham, *J. Pharmacokinet. Biopharm.*, **1**, 123 (1973).

(34) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).

(35) N. H. G. Holford, S. Vozeh, P. Coates, J. R. Powell, J. F. Thiercelin, and R. Upton, *N. Engl. J. Med.*, **296**, 1300 (1977).

(36) B. Silber, M. Lo, and S. Riegelman, *Res. Commun. Chem. Pathol. Pharmacol.*, **27**, 419 (1980).

(37) M. Lo, B. Silber, and S. Riegelman, *J. Chromatogr. Sci.*, **20**, 126 (1982).

(38) M. Lo and S. Riegelman, *J. Chromatogr.*, **183**, 213 (1980).

(39) G. Sager and S. Jacobsen, *Acta Pharmaceut. Suec.*, **17**, 86 (1980).

(40) N. H. G. Holford, "MKMODEL—A Mathematical Modeling Tool, PROPHET Public Procedures Notebook," Bolt Beranek and Newman, Cambridge, Mass., 1980.

(41) A. G. Knott, *Comp. Progr. Biomed.*, **10**, 271 (1979).

(42) H. Akaike, *I.E.E.E. Tr. Automat. Contr.*, **19**, 716 (1973).

(43) H. Akaike, *Biometrika*, **66**, 237 (1979).

(44) A. C. Atkinson, *Biometrika*, **67**, 413 (1980).

(45) J. H. Zar, "Biostatistical Analysis," Prentice-Hall, Englewood Cliffs, N.J., 1974, p. 130.

(46) W. F. Raub, *Fed. Proc.*, **33**, 2390 (1974).

(47) D. W. Schneck, J. F. Pritchard, T. P. Gibson, J. E. Vary, and A. H. Hayes, *Clin. Pharmacol. Ther.*, **27**, 744 (1980).

(48) A. M. Taburet, A. A. Taylor, J. R. Mitchell, D. E. Rollins, and J. L. Pool, *Life Sci.*, **24**, 209 (1979).

(49) C. Fenseleau and L. P. Johnson, *Drug. Metab. Dispos.*, **8**, 274 (1980).

(50) W. J. Stone and T. Walle, *Clin. Pharmacol. Ther.*, **28**, 449 (1980).

(51) W. H. Barr and S. Riegelman, *J. Pharm. Sci.*, **59**, 164 (1970).

(52) G. Levy and J. A. Procknal, *J. Pharm. Sci.*, **57**, 1330 (1968).

(53) G. Levy, *Chem. Biol. Interact.*, **3**, 291 (1971).

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