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APPENDIX

Variable	Units	
β	—	= parameter in the Rosin-Rammler-Sperling-Weibull distribution (See Ref. 1)
b	m	= radius of core
a	m	= radius of coated pellet
r	m	= radial distance from the center of a sphere
t	sec	= time
s	sec^{-1}	= frequency
s_i	sec^{-1}	= poles in Eq. 22
$C(rt)$	kg/m^3	= concentration of drug in coat at position r and at time t
$C_c(t)$	kg/m^3	= concentration of dissolved drug in the core at time t
$C_e(t)$	kg/m^3	= concentration of drug in extraction medium at time t
C^0	kg/m^3	= initial concentration of drug in core
$\bar{C}(rs)$	kgs/m^3	= time Laplace transform of $C(rt)$ at r
C'	kg/m^3	= constant core concentration in case of a sparingly soluble drug
D	m^2/sec	= diffusion coefficient of drug in coat
k_c	—	= distribution coefficient for drug between core and coat
k_e	—	= distribution coefficient for drug between extraction medium and coat
V_e	m^3	= volume of extraction medium
V_r	—	= the ratio of the volume of extraction medium to total volume of pellets
n	—	= number of pellets of identical size
$N(s_i)$	—	= denominator in Eq. 23 defined in Eq. 24
$M(t)$	kg	= total amount of drug released at time t
N	—	= number of different types of pellets in a sample
$J(t)$	$kg/sec\ m^2$	= flux of drug at time t

Stereoselective Disposition and Glucuronidation of Propranolol in Humans

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Abstract □ Following oral dosing to steady state, the disposition of $S(-)$- and $R(+)$-propranolol and their corresponding glucuronide conjugates was studied in 4 healthy adults using doses from 40 to 320 mg/day of the racemate. Steady-state plasma concentrations of $S(-)$-propranolol and its corresponding glucuronide conjugate were greater than that for $R(+)$-propranolol and its corresponding conjugate. The average steady-state concentration of both enantiomers increased disproportionately to dose. There was a 52 ± 7 (mean ± *SD*) % decrease in the intrinsic clearance (Cl_{int}) of $S(-)$-propranolol and a 65 ± 22% decrease in the Cl_{int} of $R(+)$-propranolol over the dosing range studied. The terminal elimination half-lives of $S(-)$-propranolol and its glucuronide conjugate were longer than for the $R(+)$-enantiomer at all doses. The formation of glucuronide conjugates of $S(-)$- and $R(+)$-propranolol was best described by a saturable process in all subjects. Within individuals, the ratio of V'_{max}/K_m for the glucuronide conjugate of $S(-)$-propranolol was from 2.1- to 4.9-fold greater than for the conjugate of the $R(+)$-enantiomer. These studies demonstrate for the first time, that propranolol undergoes stereoselective disposition in humans.

Keyphrases □ Propranolol—$S(-)$- and $R(+)$-enantiomers and corresponding glucuronide conjugates, stereoselective disposition, humans □ Stereoselectivity—disposition of $S(-)$- and $R(+)$-propranolol, humans □ Glucuronide—conjugates of $S(-)$- and $R(+)$-propranolol in stereoselectivity disposition studies, humans

Propranolol [1-isopropylamino-3-(1-naphthoxy)-2-propanol] is a nonselective beta adrenergic blocking agent used clinically as a racemic mixture of the $S(-)$- and $R(+)$-enantiomers. Because $S(-)$-propranolol is about 100 times more potent as a beta blocker than the $R(+)$-enantiomer, $S(-)$-propranolol is believed to be largely responsible for the clinical effects of racemic drug (1).

Numerous investigators have described the absorption, distribution, metabolism, and elimination of propranolol in humans and animals. Pharmacokinetic studies in healthy volunteers and in patients have demonstrated up to 20-fold variation between individuals in plasma propranolol concentrations after oral doses (2–8). Age (9, 10); cigarette smoking (9, 11); concomitant drug intake (12);

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and renal (13), hepatic (14), and thyroid disease (15–17) have all been shown to affect the disposition of the drug. The disposition of propranolol in humans is highly dose dependent and this previously unrecognized observation may explain much of the apparent differences between individuals (18).

To date, pharmacokinetic studies involving propranolol have conclusions based on total [$S(-)$- plus $R(+)$-] propranolol concentrations. However, no information is available describing the pharmacokinetic behavior of each enantiomer measured simultaneously after administration of the racemate.

Racemic mixtures of drugs are commonly employed clinically. However, there may be large differences between enantiomers in their pharmacological activity, metabolism, and elimination. Warfarin enantiomers, for example, have significantly different anticoagulant properties, are metabolized differently, and have different rates of elimination (19–23).

Reports have indicated that the area under the plasma concentration–time curve (AUC) for $S(-)$-propranolol in the dog was 50% less than the AUC for the $R(+)$-enantiomer after administration of a single oral dose of the racemate (24, 25). Both groups found that the $S(-)$-enantiomer was more extensively glucuronidated and the AUC for the glucuronide conjugate of this enantiomer was over 3 times greater than the AUC for the glucuronide conjugate of $R(+)$-propranolol. However, no differences in the terminal elimination half-life of propranolol enantiomers or their glucuronide conjugates were observed.

The purpose of this study was to investigate, over a wide range of doses in humans, any differences in the disposition of $S(-)$- and $R(+)$-propranolol and formation of their corresponding glucuronide conjugates.

EXPERIMENTAL

Subjects were four healthy male volunteers. Informed consent was obtained and the protocol approved by the University of California, San Francisco, Committee on Human Research. All subjects had a medical history, physical examination, electrocardiogram (ECG), complete blood count with differential, urinalysis, and selected blood chemistries done. There was no evidence of renal or hepatic disease by medical history. Each subject had normal values of blood urea nitrogen, serum creatinine, urinalysis and urine culture, serum glutamic oxloacetic transaminase, lactic dehydrogenase, alkaline phosphatase, bilirubin, prothrombin time, and total serum proteins. All were nonsmokers and abstained from alcohol and marijuana and other medications until completion of the study. On each of 4 days/week that propranolol was taken, subjects were interviewed for side effects, had their pulse and blood pressure measured, and had a 1-min ECG rhythm strip taken.

All subjects were given 40, 80, 160, 240, and 320 mg/day of propranolol in divided doses every 6 hr for a total of 13 doses. No dietary restrictions were imposed, but food was withheld for at least 9 hr before and 3 hr after the 13th dose.

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 butterfly catheter whose patency was maintained by flushing with 1 ml of heparinized saline (10 U/ml) after obtaining each blood sample (26); these small doses of heparin do not affect the plasma protein binding or disposition of propranolol (27). After discarding 0.5 ml of blood, a 7-ml blood sample was collected in a sterile 12-ml disposable syringe. The blood sample was immediately transferred to a 16 × 150-mm polytef-lined 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.

Assay for $S(-)$- and $R(+)$-Propranolol and Their Corresponding Glucuronide Conjugates—The concentrations of $S(-)$- and R(+)-propranolol and their corresponding glucuronide conjugates in plasma were measured in each subject after receiving doses of the racemate by a previously reported method (25). At each dosing rate, plasma concentrations were measured during the 13th dosing interval at steady state. Glucuronide concentrations were determined as the difference between enantiomer concentrations before and after enzymatic hydrolysis (25). One milliliter of plasma was extracted with ether after adding 0.4 ml of 0.2 N KOH by vortexing for 2 min and centrifuging at 2000 rpm (500×g) for 10 min. Ether extracts transferred to conical test tubes were evaporated to dryness under nitrogen. After adding 1 ml of methylene chloride, tubes were placed in a −78° bath. N-trifluoroacetyl-$S(-)$-prolyl chloride (0.3 ml) (the derivatizing agent) and 50 μl of triethylamine were added to the tubes, removed from the bath, and allowed to stand for 15 min. After adding 8 ml of 0.05N NaOH, vortexing for 2 min, and centrifuging at 2000 rpm (500×g) for 10 min, the upper layer was discarded and the lower layer evaporated to dryness under nitrogen after being transferred to culture test tubes. An aliquot of the residue, reconstituted with an equivolume mixture of acetonitrile and water, was injected onto the high-performance liquid chromatograph for analysis.

Data Analysis—The average steady-state concentration ($\overline{C}_{ss}$) of each enantiomer of propranolol and for each of their corresponding glucuronide conjugates during the 13th dosing interval was determined by:

$$\overline{C}_{ss} = \frac{AUC_0}{\tau} \qquad \text{(Eq. 1)}$$

where AUC_0 is the area under the plasma concentration–time curve after the oral dose, and τ is the dosing interval. The AUC of each propranolol enantiomer and for their corresponding glucuronide conjugates was calculated by the trapezoidal rule. The intrinsic clearance (Cl_{int}) of each propranolol enantiomer was calculated by the following (28):

$$Cl_{int} = \frac{D_0}{AUC_0} \qquad \text{(Eq. 2)}$$

where D_0 is the dose of each enantiomer; this is equal to one-half the total dose since the drug is administered as the racemate. This relationship assumes that the drug is totally absorbed and that all blood containing the drug passes through the liver before reaching the systemic circulation. The terminal elimination half-life of each propranolol enantiomer and their corresponding glucuronide conjugates was calculated by 0.693/terminal elimination rate constant (determined by least-squares regression utilizing at least four plasma concentration–time points in the log–linear region).

At steady state, the relationship between the formation of metabolite and its elimination is given by:

$$Cl_{f,M}\, C_{P_{ss}} = Cl_{el,M}\, C_{M_{ss}} \qquad \text{(Eq. 3)}$$

where $Cl_{f,M}$ is the formation clearance and $Cl_{el,M}$ is the elimination clearance for metabolite (M), and $C_{P_{ss}}$ and $C_{M_{ss}}$ are the concentrations of propranolol and metabolite, respectively. Rearrangement of Eq. 3 yields:

$$C_{M_{ss}} = Cl_{f,M}\frac{C_{P_{ss}}}{Cl_{el,M}} \qquad \text{(Eq. 4)}$$

for a first-order process and can be expressed as Eq. 5 for a saturable process:

$$C_{M_{ss}} = \frac{V_{max_M}}{K_{m_M} + C_{P_{ss}}}\frac{C_{P_{ss}}}{Cl_{el,M}} \qquad \text{(Eq. 5)}$$

where V_{max_M} and K_{m_M} are the Michaelis–Menten constants for a saturable metabolic process.

The formation clearance of each glucuronide conjugate (metabolite) can be estimated by substituting steady-state concentrations of propranolol enantiomers, their corresponding glucuronide conjugates, and the elimination clearance for each of the glucuronide conjugates into these equations.

Although the value of $Cl_{el,M}$ for the glucuronide conjugates is not known, in an earlier study (18) the renal clearance (Cl_r) for racemic (total) propranolol glucuronide ($Cl_{r,M}$) has been determined; previous investigators have shown that the clearance of propranolol glucuronide is primarily by the renal route (29). The $Cl_{r,M}$ for racemic propranolol glucuronide was independent of concentration in these same four subjects (18). Therefore, if it is assumed that the renal clearance is equal to the elimination clearance (*i.e.* $Cl_{r,M} = Cl_{el,M}$), then Eqs. 4 and 5 can be rewritten as Eqs. 6 and 7, respectively:

Table I—The Relationship Between the Intrinsic Clearance (Cl_{int}) of Propranolol Enantiomers and the Daily Dose of Racemic Propranolol

Week			3		4		5			
Dose, mg/day; racemate			160		240		320			Percent change in Cl_{int} from week 3 to week 5
Subject	Age, yr	Weight, kg	Cl_{int} of Propranolol Enantiomers (liters/min)[a] $S(-)$	$R(+)$	$S(-)$	$R(+)$	$S(-)$	$R(+)$		$S(-)$ / $R(+)$
MR	25	82	3.69	5.35	3.07	3.89	2.27	3.42		−48 / −46
TB	26	64	3.17	9.74	1.90	3.60	0.89	1.28		−62 / −87
AT	24	68	3.22	5.02	3.00	4.14	1.69	2.65		−48 / −47
TC	28	75	4.25	15.75	2.89	7.44	2.98	3.19		−51 / −80
									$x \pm SD$	-52 ± 7 / -65 ± 22

[a] The intrinsic clearance (Cl_{int}) was calculated according to Eq. 2.

$$C_{M_{ss}} = Cl_{f,M} \frac{C_{P_{ss}}}{Cl_{r,M}} \quad \text{(Eq. 6)}$$

$$C_{M_{ss}} = \frac{V_{max_M}}{K_{m_M} + C_{P_{ss}}} \frac{C_{P_{ss}}}{Cl_{r,M}} \quad \text{(Eq. 7)}$$

Because urinary excretion rate data is available for racemic propranolol glucuronide, the contribution by each glucuronide conjugate to the total renal clearance cannot be precisely estimated. However, the total renal clearance is the sum of the fractional contributions and can be defined by:

$$Cl_{r,M(-)} = \phi_{(-)}\, Cl_{r,M} \quad \text{(Eq. 8)}$$

for the glucuronide conjugate of $S(-)$-propranolol and by:

$$Cl_{r,M(+)} = \phi_{(+)}\, Cl_{r,M} \quad \text{(Eq. 9)}$$

for the glucuronide conjugate of $R(+)$-propranolol. The coefficient, ϕ, may be the same or different for each of the glucuronide conjugates. Therefore, for a saturable metabolic process, the Cl_f can be described by:

$$C_{M_{ss(-)}} = \frac{V_{max_{M(-)}}}{K_{m_{M(-)}} + C_{P_{ss(-)}}} \frac{C_{P_{ss(-)}}}{\phi_{(-)}\, Cl_{r,M}} \quad \text{(Eq. 10)}$$

for the glucuronide conjugate of $S(-)$-propranolol, and by:

$$C_{M_{ss(+)}} = \frac{V_{max_{M(+)}}}{K_{m_{M(+)}} + C_{P_{ss(+)}}} \frac{C_{P_{ss(+)}}}{\phi_{(+)}\, Cl_{r,M}} \quad \text{(Eq. 11)}$$

for the glucuronide conjugate of $R(+)$-propranolol.

To test the hypothesis that the formation of propranolol glucuronide is stereoselective, the predictions of six models were compared: Model 1, same V_{max} and K_m; Model 2, same V_{max}, different K_m; Model 3, different V_{max}, same K_m; Model 4, different V_{max} and K_m; Model 5, similar nonsaturable clearance; Model 6, different nonsaturable clearance.

Because ϕ appears in all equations of Cl_f, the value of V_{max} or Cl_f obtained for saturable or first-order processes, respectively, will have this coefficient incorporated into the estimate.

The formation clearance models listed above were specified (30). The parameters of the models listed previously were estimated by simultaneous unweighted nonlinear least-squares regression (31) of the $C_{M_{ss}}$ for each glucuronide conjugate *versus* the $C_{P_{ss}}$.

Discrimination between the models was made with the Schwarz criterion and Leonard test (32–34).

Mean values are reported with standard deviations; differences between means are evaluated by student's paired t test and are considered to be significant when $p < 0.05$ (35).

RESULTS

The dose-dependent elimination of propranolol and its major metabolites was previously reported in the same four individuals (18). Steady-state conditions were achieved in all subjects at each dosing rate. Steady-state trough concentrations of total [$S(-)$- and $R(+)$-] propranolol were attained at least by the ninth dose and only slight interday variation within subjects was observed.

Because concentrations of propranolol were near the sensitivity limit of the stereospecific assay with doses of 40 and 80 mg/day, enantiomer concentrations were not measured at these dosing rates. Concentrations of $S(-)$- and $R(+)$-propranolol, along with their corresponding glucuronide conjugates, were measured at dosing rates of 160, 240, and 320 mg/day.

Relationship Between the Intrinsic Clearance (Cl_{int}) of Propranolol Enantiomers and the Dosing Rate—The Cl_{int} of $S(-)$- and $R(+)$-propranolol at each dosing rate was determined according to Eq. 2. The Cl_{int} of $S(-)$-propranolol was always lower than the Cl_{int} of the $R(+)$-enantiomer (Table I). On the average, there was a 52 ± 7 (mean $\pm$ SD) % decrease in the Cl_{int} of $S(-)$-propranolol and a 65 ± 22% decrease in the Cl_{int} of $R(+)$-propranolol when the dose was increased from 160 to 320 mg/day.

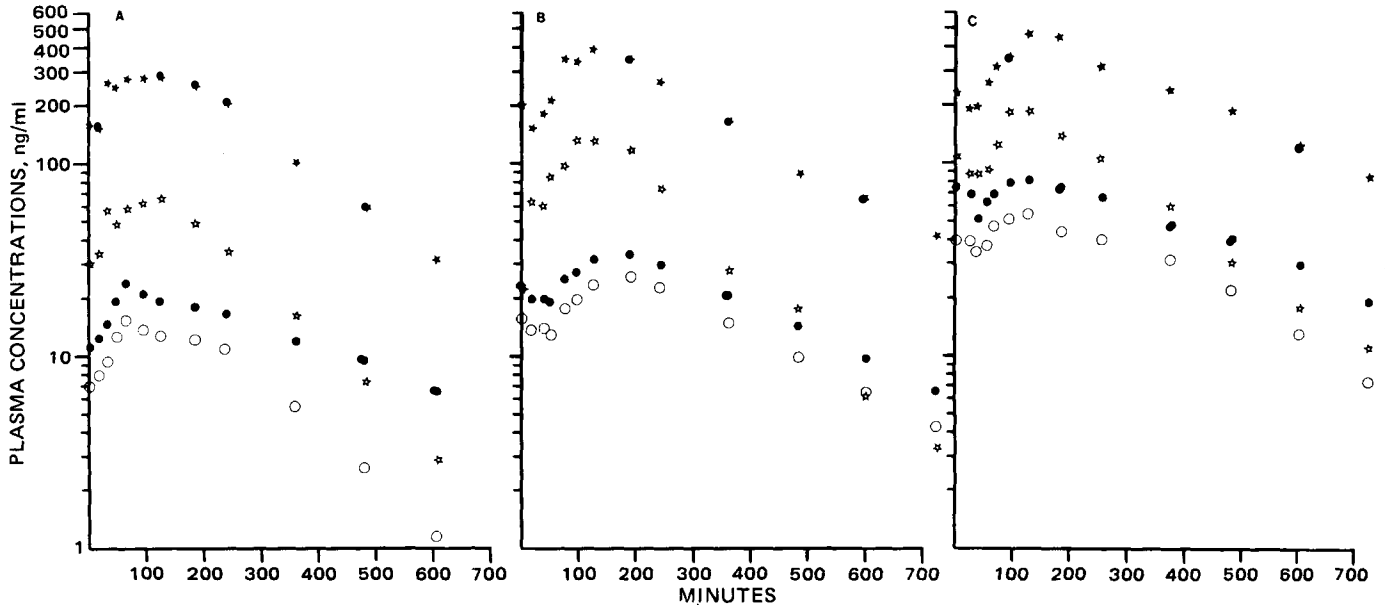

Figure 1—*Plasma concentrations of* S(−)-[●] *and* R(+)-[○] *propranolol and the glucuronide conjugates of* S(−)-[★] *and* R(+)-[*] *propranolol during the 13th dosing interval at steady state after: A, 160; B, 240; and C, 320 mg/day in subject AT. Similar results were seen in the three other subjects studied.*

Table II—Summary of Half-Lives, $t_{1/2}$, for Propranolol Enantiomers and Their Corresponding Glucuronide Conjugates

Dose, mg/day; racemic Subject	160		240		320	
	$t_{1/2}$, hr					
	Propranolol Enantiomer					
	$S(-)$	$R(+)$	$S(-)$	$R(+)$	$S(-)$	$R(+)$
MR	3.8	2.5	4.2	3.3	5.2	3.1
TB	3.5	2.7	4.7	3.2	8.3	5.0
AT	4.3	1.9	3.7	2.9	4.7	3.3
TC	4.5	3.1	4.5	4.2	5.0	3.3
$x \pm SD$	4.0 ± 0.5	2.6 ± 0.5[a]	4.3 ± 0.4	3.4 ± 0.6[a,b]	5.8 ± 1.7	3.7 ± 0.9[a]
	Glucuronide Conjugates of Propranolol Enantiomers					
Subject	$S(-)$	$R(+)$	$S(-)$	$R(+)$	$S(-)$	$R(+)$
MR	3.2	2.2	3.7	2.8	4.3	3.0
TB	2.9	2.2	3.6	3.3	7.3	4.6
AT	2.2	1.8	3.1	2.6	4.0	2.5
TC	2.7	1.8	3.6	3.8	4.3	3.5
$x \pm SD$	2.7 ± 0.4	2.0 ± 0.2[a]	3.5 ± 0.3[b]	3.1 ± 0.5[b]	4.5 ± 1.6	3.4 ± 0.9[a,b]

[a] $p < 0.05$ when compared with the value obtained for the $S(-)$-enantiomer at the same dosing rate. [b] $p < 0.05$ when compared with the value obtained for the same enantiomer at 160 mg/day.

Plasma Concentrations of $S(-)$- and $R(+)$-Propranolol and Their Corresponding Glucuronide Conjugates—Concentrations of $S(-)$-propranolol and its glucuronide conjugate were always greater than those for $R(+)$-propranolol and its glucuronide conjugate in all subjects at all dosing rates. Representative data obtained from subject AT is depicted in Fig. 1*A–C*.

The ratio of steady-state concentrations of $S(-)$- : $R(+)$-propranolol during the 13th dosing interval was 2.45 ± 1.12 at 160, 1.78 ± 0.60 at 240, and 1.51 ± 0.05 and 320 mg/day; and the ratio of the steady-state concentrations of the glucuronide conjugates of $S(-)$- : $R(+)$-propranolol was 4.74 ± 0.96 at 160, 3.64 ± 0.41 at 240, and 2.93 ± 0.17 at 320 mg/day.

There was a disproportionate increase in the $\bar{C}_{ss}$ of $S(-)$- and $R(+)$-propranolol in each subject as the daily dose was increased from 160 to 320 mg/day.

Terminal Elimination Half-Lives of Propranolol Enantiomers and Their Corresponding Glucuronide Conjugates—The half-life of $S(-)$-propranolol was 1.5-fold greater than for the $R(+)$-enantiomer at 160 mg/day ($p < 0.05$) and 1.6-fold greater at 320 mg/day ($p < 0.05$). In addition, the half-life of the glucuronide conjugate of $S(-)$-propranolol was 1.4-fold greater than for the conjugate of the $R(+)$-enantiomer at 160 mg/day ($p < 0.05$) and 1.3-fold greater at 320 mg/day ($p < 0.05$) (Table II). The half-life of both enantiomers and for their corresponding glucuronide conjugates increased with increasing dosing rate.

Estimation of the Formation Clearance (Cl_f) for Glucuronide Conjugates of $S(-)$- and $R(+)$-Propranolol—The Cl_f of glucuronide conjugates of $S(-)$- and $R(+)$-propranolol in all four subjects was best described by a saturable process. However, the model best explaining the data varied among the four subjects. The Cl_f for the glucuronide conjugate of $S(-)$-propranolol estimated by V'_{max}/K_m [$V_{max}/(\phi \cdot K_m)$] ranged from 496 to 1831 ml/min, whereas the Cl_f for the conjugate of $R(+)$-propranolol ranged from 202 to 477 ml/min. The summation of the individual Cl_fs for each individual determined in this investigation was compared with the value for the racemate Cl_f determined in the same individual (Table III).

DISCUSSION

A technique for the simultaneous determination of $S(-)$- and $R(+)$-propranolol along with their corresponding glucuronide conjugates after administration of the racemate was recently developed (25). Utilizing this technique, this investigation has shown that in healthy adults, the intrinsic clearance, plasma concentrations, elimination rate, and the formation clearance of glucuronide conjugates of propranolol enantiomers are substantially different.

The half-life of propranolol in humans after oral administration of the $R(+)$-enantiomer alone was reported to be shorter than after a dose of the racemate (36). In a previous report using a radioimmunoassay, the half-life of propranolol in rats after an intravenous dose of the $R(+)$-enantiomer was found to be shorter than after an intravenous dose of the racemate (37), whereas no difference was observed in mice when the same technique was employed (38).

These studies, however, do not predict the disposition of propranolol enantiomers after administration of the racemate, because $R(+)$-propranolol has no influence on liver blood flow, whereas racemic propranolol results in a decrease in liver blood flow. Because propranolol has a high extraction ratio and its clearance is blood flow limited, racemic propranolol would be expected to have a longer elimination half-life when compared with the half-life after $R(+)$-propranolol (39).

In a previous report (25), it was shown that in a patient with angina pectoris taking 200 mg of propranolol every 6 hr, the area under the plasma concentration–time curve for the $S(-)$-propranolol enantiomer was ~1.4 times greater than that for the $R(+)$-enantiomer. In the present investigation, concentrations of $S(-)$-propranolol were greater than those of $R(+)$-propranolol at each dosing rate (Fig. 1); these results confirm earlier findings but are in contrast to those observed in the dog (24, 25). In addition, the average steady-state concentration of each enantiomer increased disproportionately with doses from 160 to 320 mg/day. Similar to results observed in dogs, glucuronide concentrations of $S(-)$-propranolol were substantially greater than those for the glucuronide conjugate of the $R(+)$-enantiomer (24, 25).

The half-life of $S(-)$-propranolol and its glucuronide conjugate was greater than for the $R(+)$-enantiomer and its corresponding conjugate ($p < 0.05$) (Table II). In contrast, half-lives of propranolol enantiomers and their glucuronide conjugates were identical in the dog (24, 25).

Although the AUC for $S(-)$-propranolol was greater than that for the $R(+)$-enantiomer, it would be invalid to draw conclusions about the relative extent of bioavailability (F). Calculations of F for each enantiomer, based solely on AUCs after oral but not intravenous doses, must assume that the systemic clearances (Cl_s) of each enantiomer are identical. Although Cl_s, was not estimated, the intrinsic clearance of each enantiomer differed sharply and decreased with increasing dose (Table I).

In a previous report involving the same four individuals (18), it was shown that at steady state ~55% of an oral dose of propranolol could be accounted for by formation of three major metabolites: propranolol glucuronide, 4-hydroxypropranolol glucuronide, and α-naphthoxylactic acid. These metabolites are formed by different metabolic pathways and are eliminated by the kidneys in the urine (29). The formation of these metabolites was saturable in the dosage range studied.

The formation clearance (Cl_f) for the glucuronide conjugate of $S(-)$-propranolol (estimated by V'_{max}/K_m) was 2.1–4.9-fold greater than the Cl_f for the glucuronide conjugate of the $R(+)$-enantiomer in a given individual (Table III). However, when the sum of these individual Cl_fs was compared with the Cl_f for the racemate estimated from a previous investigation in the same individuals, a large disparity between estimates was observed. Because both V_{max} and ϕ for each glucuronide conjugate are unknown, it cannot be determined precisely whether the observed difference between $Cl_{f,T}$ and $Cl_{f,T'}$ (Table III) is due to a difference in V_{max}, ϕ, or both.

As previously mentioned, steady-state concentrations of $S(-)$-propranolol and its glucuronide conjugate were much higher than those for the $R(+)$-enantiomer and its conjugate in plasma. It is possible that other metabolites are being formed stereoselectively; at least some of these may be preferential for the $R(+)$-enantiomer. Although stereoselectivity in other metabolic pathways for propranolol has not been demonstrated in humans, a previous study (40) has recently shown that in the rat, $S(-)$-propranolol is preferentially hydroxylated to 4-hydroxypropranolol *in vivo* but not *in vitro*. 4-Hydroxypropranolol is equally active as a beta

Table III—Estimation of Formation Clearance for Glucuronide Conjugates of Propranolol

Subject	$Cl_{r,M}^{a}$, ml/min	Best Model[b]	Glucuronide Conjugate of $S(-)$-Propranolol: V'^{c}_{max}, nmole/min	K_m, nmole/ml	Cl_f $[V'_{max}/K_m]$, ml/min	Glucuronide Conjugate of $R(+)$-Propranolol: V'^{c}_{max}, nmole/min	K_m, nmole/ml	Cl_f $[V'_{max}/K_m]$, ml/min	$Cl_{f,T}^{d}$, ml/min	$Cl_{f,T'}^{e}$, ml/min
MR	79.4	4	344.8	0.37	931.9	10,632.5	38.15	278.7	1210.6	726.8
TB	55.6	3	178.4	0.36	495.6	72.6	0.36	201.7	697.3	723.5
AT	69.4	4	109.8	0.06	1830.0	67.6	0.18	375.6	2205.6	808.3
TC	97.2	3	142.5	0.14	1017.9	66.8	0.14	477.1	1495.0	923.7

[a] Renal clearance for racemic propranolol glucuronide; data from Silber *et al.* (18). [b] See data analysis section for description of models and selection criteria. [c] $V'_{max} = V_{max}/\phi$; see data analysis section for explanation. [d] $Cl_{f,T}$ represents the sum of the Cl_f for the glucuronide conjugates of $S(-)$- and $R(+)$-propranolol. [e] Estimated from V_{max}/K_m for racemic propranolol glucuronide; data from Silber *et al.* (18).

adrenergic blocking agent when compared with propranolol (41); presumably, the $S(-)$-enantiomer of 4-hydroxypropranolol is responsible for the beta blocking effects of a racemic mixture of $S(-)$- and $R(+)$-4-hydroxypropranolol.

Extensive deconjugation of propranolol glucuronide back to propranolol has been reported to occur in the dog (42). The occurrence of such a process in humans could be important since results from this study demonstrate that $S(-)$-propranolol is preferentially glucuronidated and that the resulting conjugate has a slower elimination rate when compared with the glucuronide conjugate for the $R(+)$-enantiomer (Table II). These findings may lend support to the theory proposed by Walle *et al.* (42) that propranolol glucuronide in humans serves as a storage pool or depot for the slow release of propranolol. This may be especially significant since deconjugation of propranolol glucuronide in humans should yield primarily $S(-)$-propranolol, the pharmacologically more active enantiomer. This presumes that the rate and extent of deconjugation, if it occurs, is equivalent for each glucuronide conjugate of propranolol.

The mechanism for the slower rate of elimination of the glucuronide conjugate of $S(-)$-propranolol cannot be explained by this investigation. Previous investigators have shown that the clearance of racemic propranolol glucuronide is primarily by the renal route (29). Further studies will need to establish whether the renal clearance of propranolol glucuronides are equivalent or different.

Because these results clearly demonstrate that the disposition and glucuronidation of $S(-)$- and $R(+)$-propranolol are not the same, and because they are essentially two distinct entities pharmacologically, future pharmacokinetic and pharmacodynamic studies involving propranolol should be focused on the pharmacologically more important $S(-)$-propranolol enantiomer rather than on total [$S(-)$- and $R(+)$-] concentrations of the drug.

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NOTES

Sterility Testing of Fat Emulsions Using Membrane Filtration and Dimethyl Sulfoxide

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Abstract □ A method was described for sterility testing of 10% fat emulsions, which consisted of solubilizing the emulsion in dimethyl sulfoxide and filtering the mixture using a polyester membrane. The procedure was rapid and avoided the problems of turbidity and plugging of the membrane filter encountered with other methods.

Keyphrases □ Dimethyl sulfoxide—sterility testing of 10% fat emulsions using membrane filtration □ Membrane filtration—sterility testing of 10% fat emulsions, dimethyl sulfoxide □ Fat emulsions—sterility testing using membrane filtration and dimethyl sulfoxide

Intravenous fat emulsions are used in peripheral and central vein infusions. Since bacterial contamination might be a problem with this kind of human drug product, a reliable method for sterility testing is necessary to ensure that possible contamination is detected.

The two basic methods for sterility testing (direct inoculation and membrane filtration) described in the USP XX (1) are not suitable to test this product. Direct inoculation of the product into growth media renders the media turbid and makes it impossible to observe microbial growth. To detect growth, various transfers must be made from the original inoculated media to fresh media, thus increasing the possibilities of contamination and extending the analysis time. Only small quantities of the product can be tested with this method. Membrane filtration of the product is difficult because the emulsified fat globules will not pass the commonly used membrane filters, and the filter pores become plugged. The present report describes a method for the sterility testing of fat emulsions in which membrane filtration is combined with the use of a solvent, dimethyl sulfoxide.

As part of the study, dimethyl sulfoxide was tested for bacteriostatic and fungistatic properties because of conflicting reports concerning its antimicrobial effects. It was shown (2) that 10% dimethyl sulfoxide protects and aids in the recovery of heat-shocked *Bacillus subtilis* spores. The high recovery of the bacteria has been linked to the capability of the compound to activate dormant spores (3, 4). At concentrations of 10, 20, and 30% in food transport systems, dimethyl sulfoxide acts as a cryoprotective agent and increases the survival of some bacteria (5). A solution of 10% dimethyl sulfoxide yielded a 100% recovery rate of *B. subtilis* phage after storage of the phage for 25 days at −20° (6). During freezing and thawing of *Aerobacter aerogenes* and red blood cells, 10–20% dimethyl sulfoxide increased the organisms' viability (7, 8). Dimethyl sulfoxide (100%) had no diffusible bacteriostatic activity when tested in disk agar diffusion sensitivity studies with various organisms (9). Normal growth of test organisms was obtained after 15-min exposure to 200,000 ppm (20%) dimethyl sulfoxide (10).

Dimethyl sulfoxide has been classified by other researchers as weakly antibacterial and antifungal (11). Bacterial growth was inhibited when 20% dimethyl sulfoxide was used in media (12, 13). Pottz *et al.* (14) found that 5–10% dimethyl sulfoxide was bacteriostatic and 20–80% bacteriocidal. Dimethyl sulfoxide (25%) was reported to inhibit the growth of bacteria isolated from leukemia and cancer patients (15). Dimethyl sulfoxide affects a wide range of bacteria and fungi in concentrations used in antimicrobial testing programs in the pharmaceutical industry (16).

The described method was designed to test the bacteriostatic and fungistatic capabilities of dimethyl sulfoxide to be used in the sterility testing of fat emulsions. Decimal reduction (D values) were determined using various test organisms.

EXPERIMENTAL

Dimethyl Sulfoxide Preparation—Dimethyl sulfoxide[1] was filter-sterilized using a polyester membrane[2] (0.2-μm pore size, 47-mm diameter) and stored in 25- and 50-ml amounts in sterile glass screw-capped tubes.

Sterility Testing of Fat Emulsions—Aliquots (100 ml) of a 10% intravenous fat emulsion[3] were aseptically transferred to sterile 38 × 200-mm glass screw-capped test tubes. To each tube, 25 ml of dimethyl sulfoxide was added. The mixture was vigorously stirred for 30 sec using a vortex mixer and then filtered through a 0.4-μm polyester membrane[2]. The membrane was rinsed with 100 ml of fluid D (1) and two 100-ml portions of fluid A (1), cut into two sections, transferred to fluid thioglycolate and soybean casein digest broth, and incubated as described in USP XX (1).

Bacteriostatic and Fungistatic Testing of Dimethyl Sulfoxide—Bacterial stock cultures containing <100 organisms/ml of the fol-

[1] Mallinckrodt Chemical Co., St. Louis, Mo.
[2] Nucleopore Corp., Pleasanton, Calif.
[3] Cutter Laboratories, Inc., Berkeley, Calif.

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