Because of the nonselectivity of the fluorometric method, previously reported dissolution profiles in water and acidic media (11, 12) were almost identical. In the present work, however, this was not the case when the modified xanthydrol method was used. As shown in Fig. 3*b*, the percentage of digoxin in solution was significantly reduced in the USP medium. After 1 hr, only about 26% of the labeled digoxin remained. This result is in agreement with the value obtained from the stability studies at pH 1.3 where 27.5% hydrolysis occurred after 1 hr (Fig. 1). In water (the BP medium), no apparent hydrolysis took place.

Table III compared the amounts of digoxin recovered after 1 hr of dissolution of the tablets following the BP and USP monographs. The brands of digoxin tablets used had differing release profiles since their dissolution plots in water and the calculated $t_{90\%}$ (Table III) varied significantly. The extent of hydrolysis in the USP medium was dependent on the brand used; the fast dissolving Brand A showed more hydrolysis than Brand C (Table III).

Although the type of adjuvants used in the tablet formulations might have an effect, it is believed that the digoxin release rate from the tablets is the rate-determining factor in controlling the *in vitro* hydrolysis of the released digoxin. From the foregoing, it is concluded that digoxin undergoes rapid hydrolysis in acid solutions. As was shown by TLC separation (6), the main hydrolysis product is digoxigenin. Since the latter possesses only about one-tenth the cardioactivity of the parent glycoside (13), it follows that some loss of therapeutic efficacy may result if digoxin is exposed for a sufficient time to the acidic pH of the gastric juice. Normally, gastric pH may reach 1–2 (14, 15); but in hyperacidic patients as in Zollinger–Ellison syndrome (16), gastric pH may fall below 1. Under such conditions, some hydrolysis may occur.

Clark and Kalman (17) found 32 and 40% digoxin breakdown products in the urine of two patients but only 0–1% in four others. This result may be attributed to variations in the gastric pH since a decrease in the pH from 2.2 to 1.1 produced about a 13-fold increase in the value of K (Table II). Some reports indicated significant differences in serum digoxin levels between volunteers (18, 19). Since the gastric pH values were not given, the observed differences could be partly due to variations in the gastric pH of the volunteers.

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Plasma Propranolol Levels in Beagle Dogs after Administration of Propranolol Hemisuccinate Ester

Y. GARCEAU, I. DAVIS, and J. HASEGAWA ˣ

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Abstract □ The hemisuccinate ester of propranolol was administered to beagle dogs to test its applicability as a potential prodrug of propranolol. Following oral administration of propranolol hemisuccinate, plasma propranolol levels were eight times higher than after an equivalent dose of propranolol hydrochloride. The hemisuccinate was absorbed rapidly, with peak plasma levels observed at 0.5–1 hr. Following intravenous dosing, the disappearance half-life of the prodrug from the plasma was 0.5 hr while the propranolol half-life was 1.7 hr. This study demonstrated the potential usefulness of the prodrug approach when a highly metabolized drug such as propranolol is protected from first-pass elimination.

Keyphrases □ Propranolol—plasma levels after administration of hemisuccinate in dogs □ Plasma levels—propranolol after administration of hemisuccinate in dogs □ Prodrugs, potential—propranolol hemisuccinate, plasma levels in dogs □ Cardiac depressants—propranolol, plasma levels after administration of hemisuccinate in dogs

Propranolol (I) is a commonly used β-adrenergic receptor blocking drug. When compared to intravenous administration, the bioavailability of an oral propranolol dose is low and varies widely from patient to patient. This variation has been attributed to extensive first-pass elimination of the drug (1).

Several metabolites of propranolol have been identified (2–8). The major metabolites are propranolol *O*-glucuronide, *p*-hydroxypropranolol and its glucuronide conjugate, and naphthoxylactic acid. One major site of propranolol metabolism is the GI tract since *p*-hydroxypropranolol is detected after oral, but not after intravenous, adminis-

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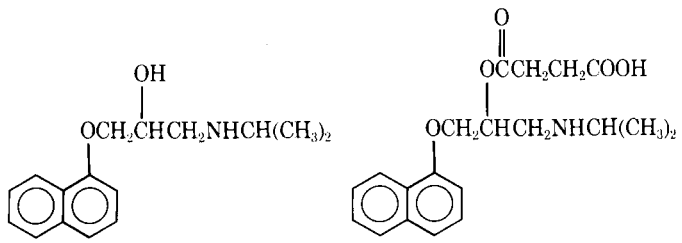

I II

tration. There is evidence that glucuronide conjugation of some drugs occurs, at least in part, in the gut wall (9, 10).

The use of a propranolol prodrug could lead to improved bioavailability. The hemisuccinate ester of propranolol (II) was selected as a potential prodrug and tested in beagle dogs. The hypothesis was that propranolol hemisuccinate administration would make it possible to bypass glucuronide formation during absorption. Propranolol would subsequently be released in the blood by prodrug hydrolysis. The results of this investigation are reported in this paper.

EXPERIMENTAL

Study Protocols—Four blood level studies were conducted. In each study, propranolol or its hemisuccinate ester was administered as the hydrochloride salt. The same beagle dogs were used in all studies.

Study 1—Four female beagle dogs, ~10 kg, were given 80 mg po of propranolol hydrochloride (8 mg/kg) or an equivalent dose of the ester prodrug. The animals were divided into two groups of two, each group receiving the drugs according to a crossover design. The entire experiment was repeated twice. At least 1 week elapsed between each dosing. At regular intervals after dosing, blood samples (10 ml) were obtained in tubes containing edetic acid (9 mg/tube) and centrifuged. The plasma was frozen immediately after preparation, and the samples were analyzed for free propranolol within 1 week.

Study 2—The same four beagle dogs were given a 20-mg iv bolus dose of propranolol hydrochloride on 1 experimental day and an equivalent dose of the prodrug 1 week later. Blood samples were processed as in Study 1, and plasma was analyzed for free propranolol.

Study 3—Two female beagle dogs were given 13.4 mg po of propranolol hemisuccinate ester (equivalent to 10 mg of propranolol hydrochloride). Blood samples were obtained and treated as in Study 1.

Study 4—Four female beagle dogs were given propranolol hemisuccinate intravenously and orally. The dogs were divided into groups of two, each group receiving each dosing regimen according to a crossover design. The oral dose consisted of 107 mg, and the intravenous dose consisted of 26.7 mg of propranolol hemisuccinate. Blood samples were collected as in Study 1, and plasma was analyzed for free propranolol and unchanged propranolol hemisuccinate.

Dosage Forms—Propranolol hydrochloride was given orally as two 40-mg tablets[1]. Propranolol hydrochloride injectables were prepared in saline at 3 mg/ml. Propranolol hemisuccinate was given in capsules of 107 or 13.4 mg (equivalent to 80 or 10 mg of propranolol hydrochloride, respectively). Propranolol hemisuccinate injectables were prepared in saline at 9 mg/ml.

Analytical Methods—Free propranolol was analyzed by a TLC–fluorometric procedure (11). Plasma samples of 2 ml were mixed with 2 ml of pH 10 buffer[2] and extracted with 25 ml of ether. A 20-ml aliquot from the ether phase was evaporated to dryness, and the dry residue was dissolved in 100 μl of ethanol. Aliquots of 25 μl were spotted on silica gel[3] TLC plates along with standard solutions of propranolol.

The plates were developed in a saturated tank containing methanol–concentrated ammonium hydroxide (100:0.4). After development, the plates were dried and sprayed with 50% propylene glycol in water. They were then read in the fluorescence mode of a spectrodensitometer[4], with excitation at 290 nm and emission at 365 nm. The limit of reliable quantitation was 2 ng of propranolol/ml of plasma. The R_f value of propranolol was 0.34, and the method was free of interference from all known metabolites of propranolol and from propranolol hemisuccinate.

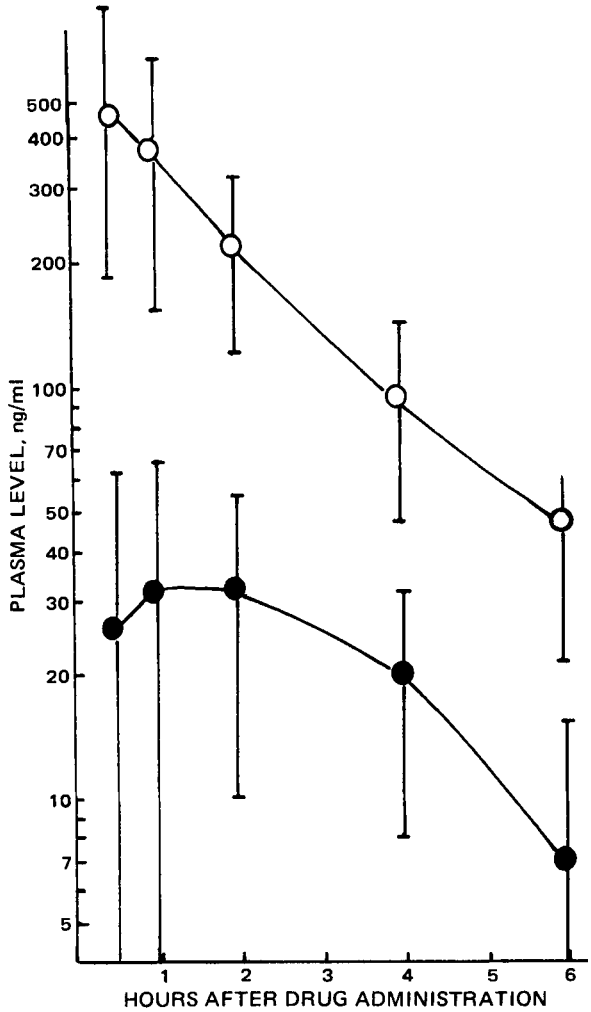

Figure 1—*Mean plasma propranolol levels* (±SD, n = 8) *following 80 mg po of propranolol hydrochloride* (●) *and 107 mg po of propranolol hemisuccinate* (○) *to beagle dogs.*

Propranolol hemisuccinate also was analyzed by a TLC–fluorometric procedure. Plasma samples, 2 ml, were mixed with 2 ml of pH 4 buffer[5] and extracted with 40 ml of methylene chloride. A 30-ml aliquot from the organic phase was evaporated to dryness, and the residue was dissolved in 100 μl of ethanol. Aliquots of 25 μl were spotted on silica gel[6] TLC plates along with standard solutions of propranolol hemisuccinate.

The plates were developed in a saturated tank containing isopropyl alcohol–benzene–water–formic acid (55:40:5:3). After development, the plates were dried and sprayed with 50% propylene glycol in water. They were read in the fluorescence mode of a spectrodensitometer[4], with excitation at 290 nm and emission at 365 nm. The limit of reliable quantitation was 25 ng/ml of plasma. The R_f value of propranolol hemisuccinate was 0.50 while that of propranolol was 0.37. The method was free of interference from propranolol metabolites.

Stability of Propranolol Hemisuccinate—The hemisuccinate hydrolyzed slowly to propranolol in stored plasma. The hydrolysis half-life was 14 hr at room temperature (21°) and 34 days at −20°. The time required to process and extract plasma samples in the assay varied from 30 to 45 min.

To determine how much propranolol was obtained from *in vitro* hydrolysis, plasma samples were spiked with propranolol hemisuccinate and subjected to the analytical procedure for free propranolol; 5% of the ester hydrolyzed to propranolol during sample preparation and analysis.

[1] Inderal, Ayerst Laboratories.
[2] Anachemia, R-1280.
[3] E. Merck.
[4] Schoeffel, SD 3000.
[5] Anachemia, R-1180.
[6] Macherey-Nagel.

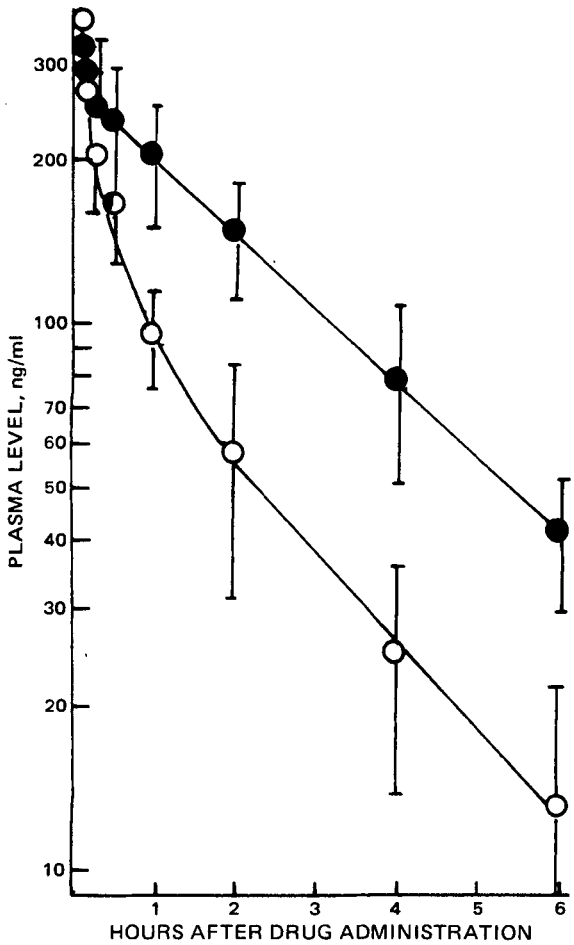

Figure 2—*Mean plasma propranolol levels* (±SD, n = 4) *following 20 mg iv of propranolol hydrochloride* (●) *and 26.7 mg iv of propranolol hemisuccinate* (○) *to beagle dogs.*

Therefore, to ensure that analytical data would not be biased significantly as a result of *in vitro* hydrolysis, plasma samples were processed and analyzed promptly in all experiments.

The hydrolysis rates of propranolol hemisuccinate were determined in buffer at 37°. In pH 7.3 buffer, the hydrolysis half-life was 4.7 hr. In pH 2 buffer, no hydrolysis was observed after 5 days of incubation.

Synthesis of Propranolol Hemisuccinate—The hemisuccinate of propranolol was synthesized from propranolol hydrochloride and succinic anhydride, mp 112–114°; IR (mineral oil mull): 1750 (ester function) and 1720 (free carboxyl function) cm^{-1}. The NMR scan of propranolol hemisuccinate was similar to that of propranolol except for a broad peak at 2.5 ppm which corresponded to the succinate group.

Anal.—Calc. for $C_{20}H_{25}NO_5 \cdot HCl$: C, 60.67; H, 6.61; N, 3.53. Found: C, 60.97; H, 6.96; N, 3.51.

RESULTS AND DISCUSSION

Study 1—Mean plasma levels (n = 8) of propranolol after 80 mg po of propranolol hydrochloride and an equivalent dose of hemisuccinate to beagle dogs are given in Fig. 1. Propranolol concentrations were considerably higher following prodrug administration. The mean area under the plasma level–time curve (*AUC*, 0–6 hr) was 1075 ng/ml × hr (*SD* 445, *CV* 41%) after the hemisuccinate ester and 132 ng/ml × hr (*SD* 87, *CV* 65%) after propranolol hydrochloride. Analysis of variance revealed that difference to be highly significant ($p < 0.01$). In addition, intersubject variation in plasma propranolol levels was reduced following prodrug administration, as evidenced by the smaller coefficient of variation calculated from the *AUC* values.

Absorption of the prodrug and its conversion to propranolol were rapid, peak plasma levels of propranolol being reached between 0.5 and 1 hr after administration of the hemisuccinate. Since blood sampling was terminated at 6 hr after dosing, only a few points were available for calculating half-lives. Based on these values, the disappearance half-life of propranolol was 1.8 hr after oral propranolol hydrochloride and 1.7 hr after oral prodrug administration.

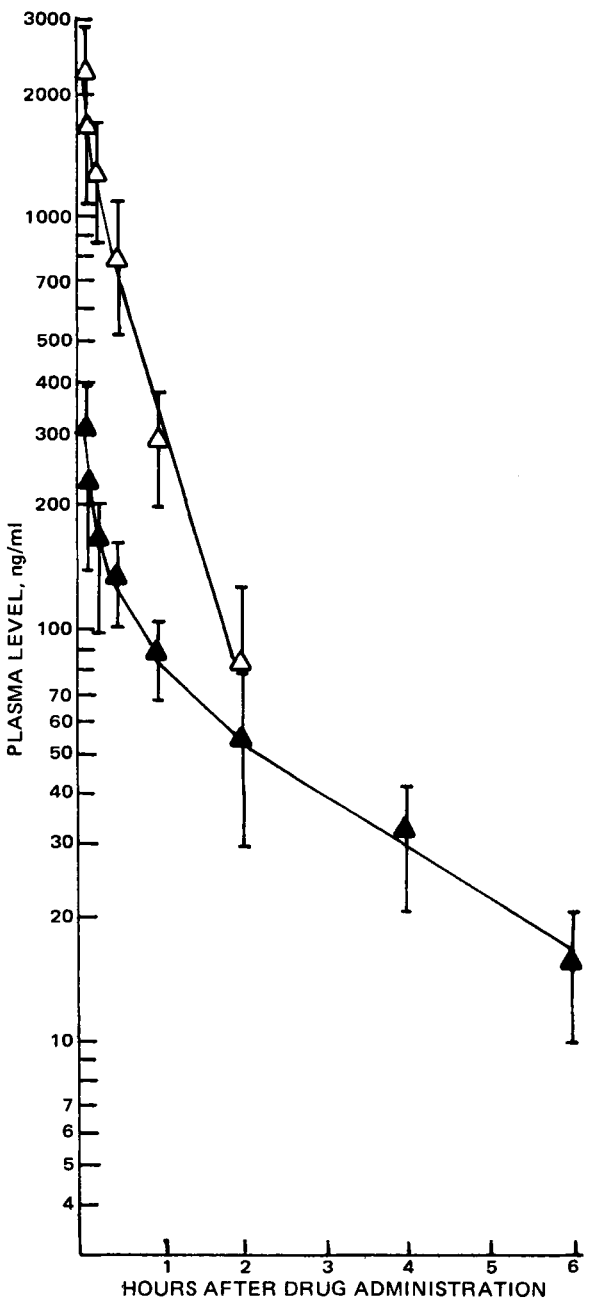

Figure 3—*Mean plasma levels* (±SD, n = 4) *of propranolol hemisuccinate* (△) *and propranolol* (▲) *following 26.7 mg iv of propranolol hemisuccinate to beagle dogs. Levels of propranolol hemisuccinate were below the limit of reliable quantitation (25 ng/ml) in the 4- and 6-hr postdosing plasma samples.*

Study 2—Mean plasma propranolol levels (n = 4) following 20 mg iv of propranolol hydrochloride and an equivalent dose of hemisuccinate to beagle dogs are shown in Fig. 2. Again, conversion of the prodrug to propranolol was fast, high levels of propranolol being found 5 min after intravenous administration. The mean plasma level–time curve of propranolol after intravenous prodrug showed two phases: an initial phase with a half-life of 0.3 hr and a late phase with a half-life of 1.9 hr. The plasma level–time curve after intravenous propranolol was also biphasic, with a late phase half-life of 2.3 hr.

Plasma propranolol levels were lower following intravenous prodrug administration. The mean *AUC* was 367 ng/ml × hr (*SD* 70, *CV* 19%) after propranolol hemisuccinate and 746 ng/ml × hr (*SD* 192, *CV* 27%) after propranolol hydrochloride. It is difficult to explain this observation because too little is known of the distribution, elimination, and metabolism of the prodrug.

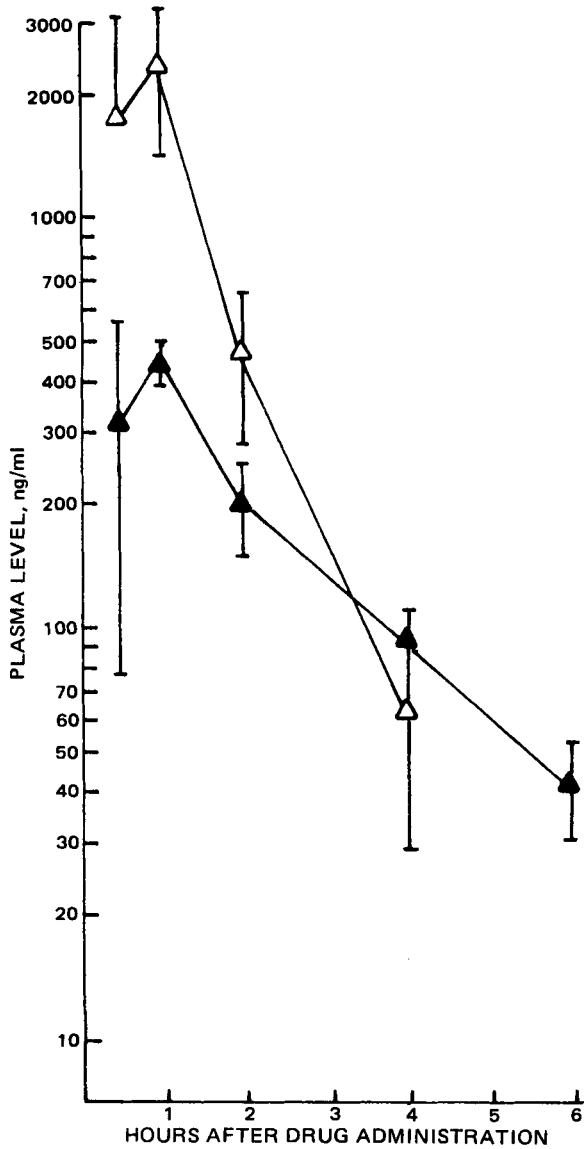

Figure 4—*Mean plasma levels* (±SD, n = 4) *of propranolol hemisuccinate* (△) *and propranolol* (▲) *following 107 mg po of propranolol hemisuccinate to beagle dogs. Levels of propranolol hemisuccinate were below the limit of reliable quantitation (25 ng/ml) in the 6-hr postdosing plasma samples.*

Study 3—Two dogs were given 13.4 mg po of propranolol hemisuccinate (equivalent to 10 mg of propranolol hydrochloride), and their plasma propranolol levels were compared to those obtained after 80 mg of propranolol hydrochloride (Table I). In Dog C, the *AUC* values were 101 and 92 ng/ml × hr after administration of the prodrug and propranolol hydrochloride, respectively; in Dog B, the *AUC* values were 114 and 168 ng/ml × hr, respectively. Although only two dogs were tested, results indicate that a dose of prodrug one-eighth that of propranolol hydrochloride gave comparable levels of free propranolol.

Study 4—In this experiment, plasma unchanged propranolol hemisuccinate levels as well as plasma propranolol levels were measured following intravenous and oral administrations of the prodrug to dogs. The intravenous dose was equivalent to 20 mg, and the oral dose was equivalent to 80 mg of propranolol hydrochloride. Results are given in Figs. 3 and 4, respectively.

Table I—Plasma Propranolol Levels (Nanograms per Milliliter) following 80 mg po of Propranolol Hydrochloride and 13.4 mg po of Propranolol Hemisuccinate to Beagle Dogs

Dog	Hours after Dosing[a] 0.5	1	2	4	6	*AUC*, 0–6 hr, ng/ml × hr
	After Propranolol Hydrochloride					
B	10	38	51	26	9	168
C	18	44	24	7	0	92
	After Propranolol Hemisuccinate					
B	84	42	15	9	0	114
C	10	50	27	9	0	101

[a] No drug was detected at 0 hr.

Following intravenous dosing, the disappearance of propranolol hemisuccinate was biphasic, with a late phase half-life of 0.5 hr; that of propranolol was also biphasic, with a late phase half-life of 2.3 hr. Virtually no prodrug was detected in the blood 4 hr after administration. In contrast, significant propranolol levels were still present 6 hr after dosing with the prodrug.

Following oral administration, plasma unchanged propranolol hemisuccinate levels peaked early (0.5–1 hr postdosing), and levels were negligible 6 hr after dosing. This result suggested rapid absorption and conversion of the prodrug. The half-life of propranolol hemisuccinate disappearance was 0.7 hr, and that of propranolol was 2.0 hr. Plasma propranolol levels peaked at 1 hr and were still substantial 6 hr after dosing. As an index of the amount of the prodrug absorbed orally, the area under the mean plasma level–time curve of propranolol hemisuccinate (3474 ng/ml × hr) after oral dosing was compared to that after intravenous dosing (1234 × 4 = 4936 ng/ml × hr corrected dose). About 70% of the oral dose of the prodrug was absorbed.

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