Hexane was selected for extraction of ibuprofen from plasma acidified with 1 *M* phosphoric acid. This selection was based on the highest amount of ibuprofen and/or the lowest amount of interfering fluorescent material being extracted. Ethyl acetate, ether, methylene chloride, and chloroform extracted excessive fluorescent substances from plasma (Fig. 2).

When phosphate buffers of pH 3 and 4 were used to acidify plasma, no ibuprofen was extracted. A pH 4 acetate buffer allowed extraction of only minimal amounts of ibuprofen. Phosphoric acid (1.0, 2.0, 3.0, 4.0, and 5.0 *M*) produced maximal extraction of ibuprofen; however, with 1.0 *M* phosphoric acid, minimal interfering constituents were extracted (Fig. 3). The optimum recovery of ibuprofen (74.91 ± 0.56%) from spiked plasma samples was obtained using 1.0 *M* phosphoric acid to acidify the plasma prior to extraction with hexane.

The ratio of the peak height of ibuprofen to the peak height of the internal standard was calculated. Statistical analysis indicated excellent linearity in the range of 1–40 μg of ibuprofen/ml of plasma with a correlation coefficient of 0.993, a slope of 0.0625 ± 0.0016, and an intercept of −0.11743 ± 0.0326 (Table IV).

Plasma levels of volunteers receiving one 600-mg oral dose of ibuprofen were as high as 40 μg/ml and as low as 15 μg/ml at 2 and 6 hr, respectively, after dosing.

The method reported here for the determination of ibuprofen will be a valuable tool for studying pharmacokinetic parameters and for monitoring patients, especially when they receive other nonsteroidal anti-inflammatory drugs.

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Chronic Dog Intestinal Loop Model for Studying Drug Absorption as Exemplified by β-Adrenoreceptor Blocking Agents, Atenolol and Propranolol

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Received December 4, 1979, from the *Pharmaceutical and Biology I Departments, Pharmaceuticals Division, ICI Limited, Alderley Park, Macclesfield, Cheshire, England.* Accepted for publication October 8, 1980.

Abstract □ Chronic *in situ* loops of dog small intestine (jejunum or ileum) were used to investigate the absorption of the β-adrenoreceptor blocking agents atenolol and propranolol. Absorption measurements were made in conscious dogs by monitoring drug disappearance from solution in the loop, with correction for intestinal water absorption. The jejunum had a mean resting pH of 7.3 and a slight net secretion of water into the lumen; the ileum had a resting pH of 7.9 and a strong net absorption of water. Propranolol absorption was rapid and first order in both regions, with the ileum showing faster absorption than the jejunum due to its higher resting pH. In contrast, atenolol absorption was negligible in the jejunum and only moderate in the ileum. The data were quantitatively consistent with the pH–partition mechanism for the absorption of propranolol but not for atenolol. The model was validated for atenolol by showing that, following drug administration into jejunal and ileal loops, drug disappearance rates were similar to absorption rates calculated from systemic blood levels. This technique is useful, realistic, and relatively simple for studying intestinal drug absorption without seriously perturbing normal GI conditions.

Keyphrases □ Atenolol—absorption studied in chronic *in situ* ileal and jejunal loops in dogs □ Propranolol—absorption studied in chronic *in situ* ileal and jejunal loops in dogs □ Absorption—atenolol and propranolol, studied in chronic *in situ* ileal and jejunal loops in dogs □ β-Adrenoreceptor blocking agents—atenolol and propranolol, absorption studied in chronic *in situ* ileal and jejunal loops in dogs

The widespread use of *in situ* intestinal loop preparations for studying drug absorption is partly a result of the difficulty of extracting absorption rate data from blood level measurements. In addition, these techniques allow drug absorption to be measured directly at the absorption site, by monitoring the disappearance of a drug introduced into the intestinal lumen, and require much less experimental effort than blood level studies. Models using rats (1, 2) and dogs (3) have been reported.

However, physiological factors that affect *in vivo* absorption rates (*e.g.*, intestinal pH, water and ion flux, and intestinal blood flow) may themselves be affected by the surgical manipulation and anesthesia involved in preparing the animal for absorption measurements in the acute situation (4–9). In some cases, these effects may render an animal model unsuitable for particular drugs. For instance, it was found (10) that the Doluisio rat *in situ* preparation (2) was a valid model for the *in vivo* absorption of one β-adrenoreceptor blocking agent (propranolol) but not for another (practolol). This result was attributed to the effects of anesthetic and surgical shock on the animal.

A technique designed to overcome some of these problems is described in this paper. It consists of an isolated intestinal loop *in situ* in the conscious dog and is a development of the classical Thiry Vella loop technique (11). The use of this model is illustrated by investigation of the absorption characteristics of two β-adrenoreceptor blocking agents, atenolol and propranolol. This comparison is relevant because of the different extent of absorption of the two drugs in humans and because of the previous observation that the Doluisio *in situ* rat technique is not a good model for this type of compound. Attempts also were made to demonstrate the validity of the technique. Preliminary observations with this model were reported previously (12), and this paper represents an expansion and continuation of this work.

EXPERIMENTAL

Preparation of Intestinal Loops—Adult male beagle dogs, 13–17 kg, were trained to sit unrestrained on a table for 1 hr or more. Thiry Vella

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loops of jejunum or ileum were surgically constructed in each dog (one loop per dog), using a procedure similar to that described by Markowitz *et al.* (11). The dogs were fasted prior to surgery but allowed water *ad libitum*. Anesthesia was induced with thiopental sodium and maintained with halothane[1]–oxygen given by intubation.

A midline abdominal incision was made, and an abdominal retractor was used to expose the small intestine. The section of intestine required for loop formation was identified; it was the midjejunum or terminal ileum (25–50 cm long) and enclosed one complete vascular arch. The mesentery was divided, and each end of the loop was clamped. A second clamp was positioned at each end 2–3 cm from the first clamps. The intestine then was cut between the clamps, and the cut ends were anastomosed to reestablish the integrity of the small intestine. The cut ends of the loop were overstitched, and a small incision was made in the intestinal fold at each end of the loop. A purse-string suture was inserted around this incision, and a titanium cannula[2] (84.5 mm × 9 mm o.d.) was introduced.

The cannula was tied firmly in position and anchored with additional stitches to the flange. The suture lines and insertion of cannulas were covered with a layer of omentum. The mesentery of the small intestine was repaired, and the retractor was removed. The cannulas were exteriorized through stab incisions on the right flank of the animal. The midline incision was repaired, and the animal was allowed to recover.

Postoperatively, the dogs were deprived of food for 24 hr (water *ad libitum*), and a normal diet was introduced gradually over the next 5 days. Antibiotics[3] were administered for 3 days. A recovery period of 2–3 weeks was allowed before any experimental work with the loop was attempted. In addition, the saline washout procedure (see *Absorption Experiments in Loops*) was performed at least once a week during the working life of the loop. Except during absorption experiments, the fistulae were open to the atmosphere.

Histological Examination of Loops—Two dogs were killed ~6 months after construction of the loops. Postmortem examinations were carried out, and several samples of tissue from different regions of the loop and from normal intestine were excised. The tissue was fixed in 10% formaldehyde solution–saline and processed by embedding in paraffin wax, sectioning at 4 μm, and staining with hematoxylin and eosin. Transverse and longitudinal sections prepared in this way were examined microscopically.

Absorption Experiments in Loops—The experiments were carried out in 16 loop dogs (nine jejunal and seven ileal) over ~3 years. A strict crossover design was not possible, but the determination of absorption rates was repeated, both within and between animals, to determine the amount of biological variation in this parameter and to provide an accurate comparison of the absorption of atenolol and propranolol in both the jejunum and ileum.

The dog was allowed to sit or lie quietly and unrestrained on a table, and a balloon catheter[4] was inserted into the proximal fistula. It was secured in position by air inflation of the balloon. With a 50-ml syringe, 30 ml of 0.154 *M* NaCl (warmed to 37°) was flushed through the loop to remove excess mucus and cellular debris. A similar catheter (no extension piece) then was positioned in the distal fistula, and 30 ml of drug solution (37°) was introduced into the loop *via* the proximal catheter. The solution in the loop was sampled immediately after introduction (time zero) and at 2.5- or 5-min intervals up to 1 hr by aspirating the solution into the 50-ml syringe and withdrawing 0.5-ml samples into a 1-ml syringe *via* a three-way tap. At the end of the experiment, the loop was rinsed with 0.154 *M* NaCl.

Drug solutions contained atenolol or propranolol at 0.5 mg/ml (1.9 and 1.7 m*M*, respectively) in 0.154 *M* NaCl. ^{14}C-Labeled polyethylene glycol 4000[5] (0.01 mg/ml) was included as a nonabsorbed marker (13). The solutions were adjusted to pH 7 by addition of 0.1 *N* HCl.

Blood Atenolol Level Experiments—In the blood level experiments, drug was administered either into the intestinal loop [30 ml at 3.33 mg/ml (12.7 m*M*)] or orally (as a rapidly dissolving 100-mg tablet). The blood profile of atenolol after a 40-mg/ml iv bolus was measured in seven nonloop dogs of similar age and weight and from the same colony. In both cases, blood samples (4 ml) were taken from jugular or brachial veins at frequent intervals over 8 hr and collected in sodium oxalate-treated containers.

Analytical Techniques—The pH values of the drug solution samples removed from the loop were measured using a pH meter fitted with a microdual electrode[6]. Measurements were made immediately after withdrawal of the final sample (~70 min after time zero).

The drug content of the intestinal loop samples was measured fluorometrically. The drug concentrations were calculated from peak heights by reference to a calibration curve. For atenolol, aliquots (50 μl) were alkalinized with 2 ml of 0.1 *N* NaOH and extracted with cyclohexane–*n*-butanol (50:50). The drug then was back-extracted from the organic layer into 0.1 *N* HCl (4 ml). The acidic layer was read on a fluorescence spectrophotometer[7] with excitation and emission wavelengths of 235 and 300 nm, respectively. Blood samples of atenolol (1 ml of whole blood) were processed in the same way. For propranolol, intestinal loop samples (100 μl) were diluted to 10 ml in distilled water and read directly on the fluorescence spectrophotometer. Excitation and emission wavelengths were 290 and 340 nm, respectively.

The effective volume of the drug solution in the loop at each time was estimated by measuring the concentration of ^{14}C-labeled polyethylene glycol in the withdrawn samples. Aliquots (0.1 ml) of loop samples were placed in 10 ml of scintillant[8] and counted for 10 min on a scintillation counter[9]. Counting efficiencies were determined by internal standardization, using a spike of 50 μl of ^{14}C-labeled *n*-hexadecane[5] containing 10,000 dpm.

Intestinal Loop Calculations—The polyethylene glycol 4000 concentration in each loop sample (expressed as disintegrations per minute per milliliter) was used to correct the measured drug concentration (milligrams of atenolol per milliliter) for changes in solution volume, occurring as a result of net water absorption or secretion in the intestinal loop, as follows. The ^{14}C-labeled polyethylene glycol 4000 concentration (D_0) at time zero was divided by the value (D_t) at each sampling time to give D_0/D_t. This fraction is an index of the change in drug solution volume. The measured drug concentration (C_t) at each sampling time was divided by the value (C_0) at time zero to give the fraction C_t/C_0. Multiplying C_t/C_0 by D_0/D_t gives f_t, which is the fraction of the initial dose remaining in the loop at any given time, corrected for changes in drug solution volume during the experiment.

The ratio D_0/D_t is equivalent to V_t/V_0, where V_0 and V_t are the actual volumes (in milliliters) of drug solution in the loop at time zero and time t; V_0 is not the same as the volume of drug solution introduced (30 ml) due to dilution by residual fluid in the loop. Comparison of the polyethylene glycol 4000 concentration at time zero (D_0) with that in the drug solution before introduction shows that V_0 is usually about 32 ml, but it varies from experiment to experiment.

The slopes of the plots of log f_t *versus* time were obtained by linear regression using least mean squares. First-order disappearance rate constants ($k_{a(\mathrm{dis})}$) and half-lives ($t_{1/2}$) were calculated from the slopes. With propranolol in both the jejunum and ileum and with atenolol in the jejunum, inspection of the disappearance plots showed that any lag time prior to disappearance was short. Therefore, all time points were used to calculate the disappearance rate constant. With atenolol in the ileum, a distinct lag time was present in nearly all of the disappearance plots, and points obviously not on the first-order part of the plot were excluded from the linear regression. In both cases, the lag time was obtained from the regression equation.

Blood Level Calculations—Absorption rate constants ($k_{a(\mathrm{blood})}$) were calculated from mean blood level results using the instantaneous midpoint-input principle (14).

RESULTS

Conditions of Loop Dogs—Once dogs had recovered from surgery, little special care was necessary to maintain them in good condition. The loops remained viable for up to 9 months, allowing several series of experiments to be carried out in some dogs. Postmortem examination of loop tissue, and comparison with normal intestinal tissue, showed that no gross histological changes occurred over 6 months as a result of the

[1] Fluothane, Imperial Chemical Industries Ltd., Macclesfield, Cheshire, England.
[2] One flange (18.3-mm diameter) was placed at one end, and another (~25-mm diameter) was separated from the first flange by 8.0 mm.
[3] Streptopen (Glaxovet Ltd., Greenford, England); 4 ml (1 g of penicillin G procaine and 1 g of dihydrostreptomycin) was administered by intramuscular injection once daily.
[4] A WSP 4116 two-way (18F9) (Warne Surgical Products Ltd., Andover, England) catheter was fitted with a 10-cm extension piece of 2-mm i.d. Silastic link.
[5] Radiochemical Centre, Amersham, England.
[6] Model 3550 pH meter with Cecar electrode, Beckman Instruments, Irvine, Calif.
[7] Model MPF 43, Perkin-Elmer Ltd., Beaconsfield, England.
[8] Cocktail N, Fisons Ltd., Loughborough, England.
[9] Model 524, Packard Instruments Ltd., Caversham, England, or Intertechnique SL 4000, Kontron Analytical Ltd., St. Albans, England.

Table I—Intestinal Loop Disappearance Parameters for Atenolol [a]

Parameter for Jejunum	Dog					Mean ± *SD*
	349N	190P	141M[b]	514-78	527-78	
$k_{a(dis)}$, min^{-1}	0.0037	0[c]	0[c]	0.0017	0.0007	0.0012 ± 0.0016
$t_{1/2}$, min	187	—	—	408	990	529 ± 414
Lag time, min	12	—	—	19	33	21 ± 11
n	13	13	7	13	13	—
r	0.819	0.408[c]	0.142[c]	0.645	0.585	—
Resting pH of loop	7.25	7.21	7.43	7.34	7.34	7.31 ± 0.009

Parameter for Ileum	Dog									Mean ± *SD*
	337N[b]		397N[b]							
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 3	Exp. 4	352MN	279MP	206-78	
$k_{a(dis)}$, min^{-1}	0.023	0.025	0.019	0.018	0.010	0.021	0.018	0.012	0.019	0.018 ± 0.0048
$t_{1/2}$, min	30	28	36	39	69	33	39	58	36	41 ± 14
Lag time, min	11	15	9	6	13	7	2	11	20	10 ± 5
n	10	6	7	7	11	7	7	8	8	—
r	0.981	0.976	0.948	0.993	0.939	0.949	0.958	0.973	0.921	—
Resting pH of loop	—[d]	7.91	7.67	7.98	7.83	7.64	8.01	7.97	8.03	7.88 ± 0.15

[a] Initial drug concentration was 0.5 mg/ml, unless otherwise shown. [b] Initial drug concentration was 3.33 mg/ml. [c] No significant correlation ($p < 0.05$) between log f_t and time (linear regression). [d] Not recorded.

isolation of the intestinal segment. The only evidence of any difference was a slight reduction in thickness of the muscularis layer of the loop, attributed to its isolation from the passage of normal gut contents.

pH and Volume Changes in Intestinal Loops—Drug solutions, which were adjusted to pH 7 but unbuffered, underwent changes in pH and volume during an experiment. Distinct and characteristic differences were seen between jejunal and ileal loops (Fig. 1). In the jejunum, the pH rose to ~7.5 and then declined gradually back to 7.2 at 60 min (mean ± *SD* was 7.34 ± 0.19). In contrast, the ileum showed an initial rise before the significantly higher "resting" level of 7.88 ± 0.13 was reached. The resting pH in each experiment is recorded in Tables I and II. The pH values recorded for each experiment were measured immediately after completion of the experiment (~70 min after time zero). Control experiments showed that a small increase in sample pH occurred during this time but that it was not significant ($p < 0.05$). There was no difference in the behavior of jejunal and ileal loop samples.

The drug solution volume during a 60-min experiment remained essentially constant in the jejunal loops but showed a marked and consistent decrease in ileal loops. This finding is consistent with other reports (15, 16). Considerable dog-to-dog and experiment-to-experiment variations existed in these volume changes (much more so than with intestinal pH), but the characteristic difference between the jejunum and ileum was nearly always present.

Drug Disappearance Rates—Disappearance profiles for atenolol and propranolol were characteristic of the drug and the type of loop. Mean plots for disappearance of both drugs in the jejunum and ileum are shown in Fig. 2, and the parameters derived from the separate experiments are listed in Tables I and II.

Rapid first-order disappearance of propranolol occurred in both jejunal and ileal loops, with no appreciable lag time before the onset of disappearance. Disappearance half-lives were 17 and 7.1 min for the jejunum and ileum, respectively. The corresponding profiles for atenolol show a slower disappearance. Disappearance in the jejunum was very slow, averaging only 7% of the initial drug concentration in 60 min. Therefore, disappearance parameters are only approximate in this case. Values of $k_{a(dis)}$ and half-life are quoted only if there was a significant linear correlation between log f_t and time over 60 min; where there was no correlation, the rate constant was taken as zero. Atenolol disappearance in the ileum (half-life of 41 min) was first order and much faster than in the jejunum. It had a lag time of ~10 min.

Repeat determinations of $k_{a(dis)}$ for each drug in either the jejunum or ileum showed good reproducibility both between dogs and within the same dog. There was no indication of any progressive change in the rate constant due to loop deterioration during the working life of any dog. Individual values of $k_{a(dis)}$ for each situation varied by a factor of two or less, except for atenolol in the jejunum where the disappearance was so slow that accurate measurements were impossible.

Combined Loop and Blood Level Experiments—Systemic levels of atenolol were measured after administration of drug solution into jejunal or ileal loops and after oral administration (Fig. 3). Blood atenolol levels following jejunal administration were low; after ileal administration, they were similar to those resulting from oral administration of an equivalent dose. This finding indicates that the rate of drug appearance in the systemic circulation was much slower after introduction into the jejunal loop than into the ileal loop, reflecting the pattern of disappearance rates from the loops. Values of $k_{a(blood)}$ in these experiments were calculated from the blood level data in Fig. 3 using intravenous data obtained in a separate group of seven dogs. Actual blood atenolol concen-

Table II—Intestinal Loop Disappearance Parameters for Propranolol [a]

Parameter for Jejunum	Dog							Mean ± *SD*
	349N			135R				
	Exp. 1	Exp. 2	547MR	Exp. 1	Exp. 2	862P	900P	
$k_{a(dis)}$, min^{-1}	0.048	0.048	0.026	0.051	0.028	0.043	0.065	0.044 ± 0.014
$t_{1/2}$, min	14	14	27	14	25	16	11	17 ± 6
Lag time, min	+2.9	−1.6	+0.3	−0.2	−0.8	−6.3	−2.2	−1.1
n	15	14	14	14	12	14	7	—
r	0.997	0.991	0.998	0.997	0.995	0.974	0.992	—
Resting pH of loop	7.50	7.38	7.46	7.34	7.67	7.18	6.92	7.35 ± 0.24

Parameter for Ileum	Dog					Mean ± *SD*
	377N					
	Exp. 1	Exp. 2	479P	31P	206.78	
$k_{a(dis)}$, min^{-1}	0.12	0.095	0.067	0.14	0.096	0.11 ± 0.033
$t_{1/2}$, min	5.8	7.3	10.3	5.0	7.2	7.1 ± 2.0
Lag time, min	+1.1	+0.7	+0.1	+0.4	+4.0	+1.3
n	7	10	12	8	10	—
r	0.976	0.980	0.995	0.995	0.997	—
Resting pH of loop	7.95	7.95	7.98	7.89	7.73	7.90 ± 0.10

[a] See footnotes to Table I.

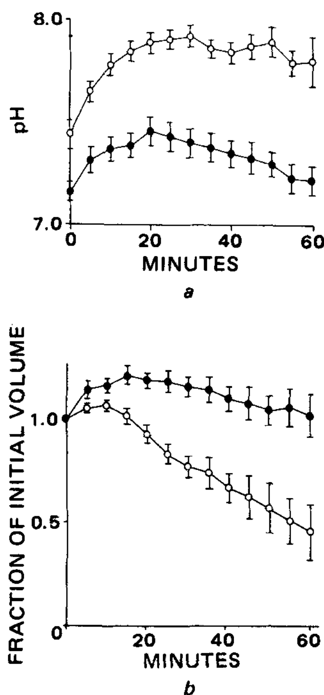

Figure 1—*The pH changes* (a) *and volume changes* (b) *in jejunal* (●) *and ileal* (○) *loops. Each point is the overall mean value* (±SE) *from all atenolol and propranolol experiments detailed in Tables I and II.*

trations used in the calculations are shown in Table III. Plots of the fraction unabsorbed (log scale) against time are shown in Fig. 4, and the derived rate constants are shown in Table IV. Values of $k_{a(\text{blood})}$ for jejunal and ileal absorption were similar to $k_{a(\text{dis})}$ values obtained from experiments in the intestinal loops. In addition, values of $k_{a(\text{blood})}$ for ileal and oral administration were almost identical.

DISCUSSION

Apart from the preliminary article (12), there have been no previous reports in which isolated intestinal loops were used in this way for studying drug absorption in the conscious dog. A similar surgical technique was reported (3), but the animals were anesthetized and absorption was followed by blood sampling. Such an approach does not utilize the full potential of the technique for simulating intestinal conditions *in vivo*.

The histological observations, although limited, suggest that this preparation maintained the integrity of the isolated intestinal segments over the 6–9-month period during which they were used. This concept is supported by the lack of significant change in drug disappearance rates over this period and by reports from other investigators (17, 18). The intestinal loops should be normal functionally as well as histologically. Care was taken during surgery to maintain the mesenteric blood supply intact; with the lack of anesthetic and surgical stress, blood flow to the intestinal tissues during absorption experiments should be the same as in intact gut *in vivo*. Normal function in the intestinal loops was indicated by the return of peristalsis on recovery from the operation, by water absorption in the ileum, and by the strong buffering capacity of the loops toward drug solutions.

The use of buffered drug solutions was avoided so that absorption measurements could be made under intestinal conditions close to normal. Therefore, intestinal pH changed in response to the normal ion absorption and secretion processes of the loops, giving the characteristic pH profiles in the jejunum and ileum (Fig. 1).

To validate such an absorption model, it is necessary to show a correlation between lumenal drug disappearance and appearance on the serosal side of the intestinal membrane. Ideally, this approach involves collecting blood from the mesenteric veins serving the loop to allow direct measurement of drug arrival on the serosal side of the membrane. This study was attempted in other animal models (19, 20), but the techniques are invariably complex and usually necessitate the use of anesthetized animals. To avoid modification of the basic technique, the rate of drug appearance on the serosal side of the membrane was estimated by the less direct method of measuring systemic blood levels and calculating absorption rate constants ($k_{a(\text{blood})}$) for drug administered orally or into jejunal or ileal loops. These experiments were performed only with atenolol since it is largely unmetabolized after oral administration (21, 22). For this drug, there should be a quantitative balance between drug absorbed from the intestinal lumen and that appearing intact in the systemic circulation. This is not the case with propranolol, which is extensively extracted during the first pass through the liver both in humans and in dogs (23, 24).

These experiments showed that $k_{a(\text{dis})}$ and $k_{a(\text{blood})}$ not only correlate well in a relative sense (*i.e.*, ileal *versus* jejunal rates) but are quantitatively almost identical. Therefore, the atenolol disappearance measured in this model was an accurate reflection of true drug absorption into the systemic circulation. In addition, the similarity of $k_{a(\text{blood})}$ values following oral and ileal administration suggests that absorption from the intestinal loops is quantitatively similar to that in the intact small intestine and that the ileum is probably the major site of atenolol absorption in the intestinal tract.

General conclusions concerning the validity of the technique should apply to propranolol as well as to atenolol, although, in this case, the rate of disappearance from the loop corresponds to the appearance of "total" drug (*i.e.*, intact drug and metabolites) in the blood. Significant membrane storage of highly lipophilic drugs (25) destroys the direct correlation between lumenal disappearance of drug and absorption. However, the biexponential disappearance profiles characteristic of this situation were not seen in the dog intestinal loop experiments. In addition, there is some evidence (9) that membrane storage only occurs under conditions of significantly reduced intestinal blood flow (which may be present in an anesthetized *in situ* gut preparation) but not in a relatively stress-free chronic preparation such as the one described here.

Disappearance profiles for atenolol and propranolol in the intestinal loops show distinct and interesting differences between the two drugs and for each drug between jejunal and ileal loops. Both drugs are basic and have pKa values[10] close to 9.5. Therefore, they are more unionized in the ileum (pH 7.88) than in the jejunum (pH 7.34); the theoretical fractions unionized (f_u) are 0.02 and 0.007, respectively. Therefore, the more rapid absorption of both drugs in the ileum than in the jejunum was as expected. The fact that propranolol was absorbed more rapidly than atenolol in both loops is attributable to its much greater lipophilicity (octanol–water log *P* values of 3.65 and 0.23 for propranolol and atenolol, respectively[10]) and, hence, its more ready partitioning between intestinal mucosa and gut contents. Quantitative comparison of $k_{a(\text{dis})}$ values from Tables I and II showed that propranolol was absorbed 37 times more rapidly than atenolol in the jejunum and six times more rapidly in the ileum. The ratios of ileal to jejunal rates were 2.5 for propranolol and 15 for atenolol.

There was an apparent overall adherence to the pH-partition principle (1, 4) since more rapid absorption occurred when a greater proportion of drug was present unionized and the more lipophilic drug was absorbed more rapidly. However, the ileal to jejunal ratios of $k_{a(\text{dis})}$ indicate a basic difference in the behavior of the two drugs. With propranolol, the observed ileal to jejunal ratio of 2.5 was almost exactly that predicted, for both drugs, from the ratio of 2.9 between f_u in the two loops. For atenolol,

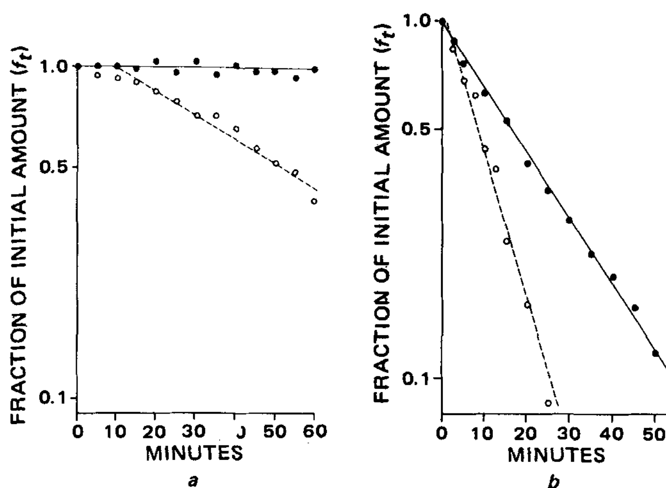

Figure 2—*Disappearance plots for atenolol* (a) *and propranolol* (b) *in jejunal* (●) *and ileal* (○) *loops. Each point is the mean value from experiments detailed in Tables I and II.*

[10] P. J. Taylor, ICI Ltd., Alderley Park, Cheshire, England, personal communication.

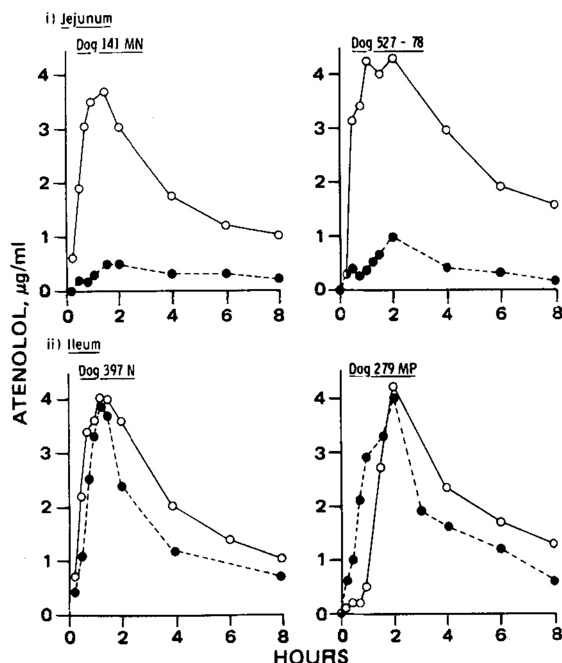

Figure 3—*Atenolol whole blood levels after administration of 100 mg of drug into jejunal (i) or ileal (ii) loops (●) and after oral administration (○). Results are for two dogs with jejunal loops and for two dogs with ileal loops.*

the more slowly absorbed drug, a much larger ratio of 15 was found under the same conditions. This result cannot be explained simply in terms of the difference in the resting pH between the two loops. Even if the absorption rate was controlled by a microclimate pH at the mucosal surface (4, 26) rather than by bulk phase pH, it would be expected to affect both drugs in a similar way.

A fundamental difference in the absorption mechanisms of these two drugs is, therefore, indicated. Propranolol absorption is explicable in simple pH-partition terms, whereas an alternative or additional mechanism is implied for atenolol in the ileum. It is possible that a hydrophilic molecule such as this one is subject to solvent drag or an active transport mechanism, although the current work contains no evidence for it. However, in the ileum the onset of atenolol absorption and net water absorption corresponded closely, both occurring after an initial lag time of ~10 min.

Table III—Atenolol Systemic Blood Concentration Data Used in Calculation of $k_{a(\text{blood})}$ [a]

Hours after Dose	Mean Atenolol Blood Concentration, µg/ml: Intravenous Administration[b] (n = 7)	Oral Administration[c] (n = 4)	Administration into Jejunal Loop (n = 2)	Administration into Ileal Loop (n = 2)
0.08	13.6	—[d]	—[d]	—[d]
0.17	9.2	—[d]	—[d]	—[d]
0.25	7.9	0.43	0.15	0.50
0.33	6.7	—[d]	—[d]	—[d]
0.50	5.4	1.9	0.28	1.1
0.75	4.5	2.5	0.23	2.3
1.0	3.9	3.0	0.33	3.1
1.5	3.2	3.5	0.58	3.5
2.0	2.7	3.8	0.73	3.2
3.0	2.1	—[d]	—[d]	—[d]
4.0	1.7	2.2	0.35	1.4

[a] Dose of 100 mg. [b] Dogs without intestinal loops. n = number of animals. [c] Two dogs with jejunal loops and two with ileal loops. [d] Not measured.

Table IV—Values of $k_{a(\text{blood})}$ for Atenolol Calculated [a] from Systemic Blood Concentrations

Parameter	Oral Administration[b]	Administration into Jejunal Loop[c]	Administration into Ileal Loop[c]
$k_{a(\text{blood})}$, min^{-1}	0.015	0.013	0.014
n	7	7	7
r	0.993	0.988	0.998

[a] Reference 13. [b] Four dogs. [c] Two dogs.

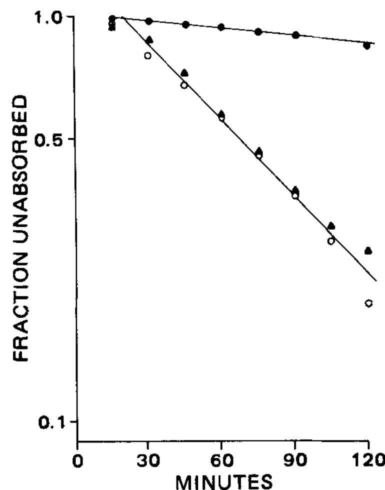

Figure 4—*Plots of fraction unabsorbed (log scale) against time for atenolol, calculated from whole blood levels after administration of 100 mg of atenolol orally (○) and into jejunal (●) and ileal (▲) loops. Mean results are for four dogs dosed orally and for two each with jejunal and ileal loops.*

Much has been published on the pharmacokinetics and metabolism of atenolol and propranolol, but detailed knowledge of their absorption mechanism is lacking. Thus, comparison of the dog intestinal loop data with existing data is possible only in a general sense. Propranolol is absorbed rapidly after oral administration both in dogs and humans, the systemic availability of intact drug being controlled largely by extensive extraction or metabolism at the first pass through the liver (21, 22). In contrast, atenolol is more slowly absorbed. Overall absorption is incomplete (~50%) in humans, rhesus monkeys, and rats but is markedly higher (~90%) in dogs (21, 22, 27). The extent of metabolism of this drug is small. The data obtained for atenolol and propranolol in the dog intestinal loops were consistent with this general picture.

From a clinical viewpoint, both drugs are adequately absorbed in humans, and the fact that atenolol absorption is incomplete is of no therapeutic importance (28). Mechanistically, however, the comparison is of great interest. The intestinal loop experiments suggest that there is not simply a rate difference in the absorption of the two drugs but also a difference in absorption mechanism. They also demonstrate that the animal model used is a useful, realistic, and relatively simple means of studying intestinal drug absorption without seriously perturbing normal GI conditions.

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Bioavailability of Orally Administered Propiram Fumarate in Humans

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Abstract □ Propiram bioavailability was determined in 10 healthy volunteers after a single oral administration of 50 mg (base equivalent) of propiram fumarate in tablet or solution dosage form in a randomized crossover design. The plasma drug concentration–time curve revealed a one-compartment open model with first-order absorption kinetics. There were no statistically significant differences ($p > 0.05$) between all of the measured pharmacokinetic parameters obtained from the tablet and the solution with the exception of the absorption lag time (t_{lag}), where the tablet had a significantly longer t_{lag}. The drug given as a tablet or solution was absorbed rapidly after oral administration with an apparent absorption rate constant of 3.7 hr^{-1} for both dosage forms. The C_{max} value (308 ng/ml for the tablet and 342 ng/ml for the solution) was attained at ~1 hr after oral administration. The elimination half-life was 5.2 hr for the tablet and 4.4 hr for the solution, and the apparent distribution volume was 2.31 liters/kg for the tablet and 1.94 liters/kg for the solution. Total body clearance was much greater than renal clearance, indicating extensive metabolic clearance for both dosage forms. The study showed that propiram administered as the tablet was bioequivalent to the solution.

Keyphrases □ Propiram—bioavailability in humans, tablet *versus* solution □ Hydrolysis—fluorescence analysis, despropionylpropiram □ Bioavailability—orally administered propiram in humans □ Antispasmotics—propiram, tablet *versus* solution bioavailability in humans

Propiram fumarate, *N*-(1-methyl-2-piperidinoethyl)-*N*-2-pyridylpropionamide fumarate, was shown in clinical studies to have analgesic efficacy following oral and parenteral administration. The drug was active in dogs, cats, mice, rats, rabbits, and humans (1, 2) and displayed no physical dependency, carcinogenicity, embryo toxicity, or influence on general reproductive performance (3).

Although pharmacokinetic studies of propiram fumarate in animals (3) and humans (3, 4) have been reported, a detailed pharmacokinetic analysis of the plasma propiram concentration–time data is not available. Thus, the present study was conducted to provide comprehensive information on propiram pharmacokinetics and bioavailability in healthy volunteers after a single oral administration of propiram fumarate in a tablet or solution.

EXPERIMENTAL

Drug Formulation—Two preparations containing propiram fumarate were tested, propiram fumarate tablet[1] and propiram fumarate solution[2] containing 50 mg as propiram.

Study Protocol—Ten healthy male adult volunteers (25–40 years of age) participated. Routine laboratory profiles including a complete blood count, urinalysis, and chemistry panel were obtained prior to the study and 48 hr after drug administration.

The study, a randomized crossover design, was composed of two segments. In the first segment, after an overnight fast, each subject received a single dose of propiram fumarate (equivalent to 50 mg of base) as a tablet or solution with 100 ml of water according to a random code. After at least a 4-day washout period, the subjects were given the other dosage form. No food was allowed until the 4th-hr blood sample was collected, after which a low-fat meal was given.

Blood samples were obtained at 0, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3, 4, 5, 8, 12, 16, and 24 hr. The plasma was separated by centrifugation and stored frozen until it was assayed.

Urine was collected prior to drug administration and for two intervals, 0–24 and 24–48 hr, after drug administration. The total volume of each collection was measured, and an aliquot was frozen until it was assayed.

Assay—A modified method of Pütter and Kroneberg (3) was used. The propionyl group was removed from propiram by acid hydrolysis, and the formed secondary amine (despropionylpropiram) showed a strong fluorescence at specific wavelengths in acidic solution.

Five milliliters of plasma, or 0.5 ml of urine sample plus 4.5 ml of water, was adjusted to pH 11 with 5 *N* NaOH and extracted with 7 ml of toluene. Six milliliters of the toluene phase then was extracted with 3 ml of 1 *N* H_2SO_4. An aliquot of the acidic aqueous phase (2.5 ml) then was transferred to a 3-ml serum bottle[3]. After the air was displaced with argon, the vial was closed with a stopper[4] and aluminum seal and placed in a manifold block[5] heater for 4 hr at 120°. After cooling to room temperature, the fluorescence of the formed despropionylpropiram was measured at 314 (excitation) and 380 (emission) nm in a spectrophotofluorometer[6].

[1] FMR 76228D-06, batch 8747-40, Schering Corp., Bloomfield, N.J.
[2] FMR 77571D, batch 9383-44, Schering Corp., Bloomfield, N.J.
[3] No. CA8290, 3 ml T-1, Kimble Glass, Paramus, N.J.
[4] No. 541, Teflon-lined natural rubber stopper, 13 mm, West Rubber Co., Phoenixville, Pa.
[5] Multi-Temp block heater, No. 2093, Lab-Line Instruments, Melrose Park, Ill.
[6] Aminco-Bowman, American Instruments Co., Silver Spring, Md.

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