Drug Absorption I: An *In Situ* Rat Gut Technique Yielding Realistic Absorption Rates

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Abstract □ A method is reported for studying gastrointestinal drug absorption from isolated gut segments of the anesthetized rat *in situ*. The experimental technique is simple and utilizes readily available laboratory equipment. The results are closely reproducible and yield absorption rates which are realistic in terms of the known absorption behavior of drugs in humans and intact animals. Disappearance of the drugs from the lumen of the small intestine followed apparent first-order kinetics; and the following half-lives were determined at pH 6: aminopyrine, 32 min.; barbital, 19 min.; haloperidol, 24 min.; prochlorperazine, 23 min.; salicylic acid, 8 min,; and sulfaethidole, 32 min. These absorption rates are much faster than those normally observed in *in situ* intestinal preparations. Absorption rates from rat stomach *in situ*, and preliminary data showing a relationship between fasting time and intestinal absorption rates in the rat are also presented.

Keyphrases □ Gastrointestinal absorption rate determination—methodology □ Absorption, drug—isolated gut segments, stomach, *in situ* □ Drug diffusion—blood to gut lumen □ Fasting effect—drug absorption □ UV spectrophotometry—analysis

The current basic concepts of the processes involved in gastrointestinal drug absorption are based largely upon experiments in rats (1–5). For example, Brodie *et al.* using rats, employed an *in situ* technique to determine the equilibrium ratios of drug concentrations in blood and the gut lumen by simultaneously injecting the drug into the blood stream and perfusing drug solutions through the gut (3, 4). Intravenous dosages and drug concentrations in the gut perfusion fluids were adjusted until no net transfer of drug between gut and blood occurred. The drug concentrations were corrected for protein binding, and the ratios of unbound drug in each compartment were calculated. In the stomach, the experimental values compared favorably with ratios predicted from equations derived on the assumption that transfer occurred by passive diffusion of the unionized drug through the gut wall. In the small intestine, favorable correlations were obtained if the additional assumption was made that the "virtual" pH of the intestinal membrane surface, which controls drug partitioning, is about 5.3 regardless of the pH of the lumen fluid.

These equilibrium experiments yielded useful information and eliminated the need to consider such processes as metabolism, excretion, and tissue distribution; but they did not deal directly with the dynamics of the absorption process. Schanker *et al.* (1), Kakemi (6), and others have studied the dynamics of the absorption process by measuring drug disappearance from gastric or intestinal perfusion solutions following a single pass or when the solution was circulated. In the single pass experiments, a drug solution was perfused through the gut; and the entering and leaving concentrations were determined. The assumption made in this approach is that first-order absorption kinetics occurs. Also, each animal produces only one datum point. In the circulation experiments, the decrease in drug concentration in circulating perfusion fluid was followed. This approach has the disadvantage that the volume of the perfusion fluid must be much larger than the volume of the gut lumen. Consequently, only a fraction of the perfusion fluid is exposed to the absorption site at any given time.

Still other investigators (7–9) have attempted to study gastrointestinal drug absorption rates by techniques utilizing isolated gut segments *in vitro*. Although these techniques control many of the variables encountered in the absorption process, they suffer from the major disadvantage that the gut segments lack a blood supply. The drug must diffuse not only through the membrane barrier but also through the muscle, connective tissue, fatty tissue, and other tissue structures which comprise the entire intestinal wall. As a result, absorption rates measured by these *in vitro* techniques are unrealistically slow; and storage of drug in the "membrane" is unrealistically large. In addition, the *in vitro* preparations tend to deteriorate rapidly due to cellular death.

Previous publications (10, 11) have dealt with the use of an *in vitro* model for the drug absorption process which employs an artificial lipid-like barrier separating two aqueous buffered phases representing gut lumen fluid and blood, respectively. The results of these studies have served largely to establish kinetic models in a three-compartment (water–oil–water) system and to screen various agents that might later be studied for their influences on the drug absorption process in living systems. In most cases, a lipid-like barrier (cyclohexane) which permits passage of only unionized drugs has been utilized; and the results are in agreement with the predictions of the pH-partition hypothesis (1–4).

Despite the fact that much has been learned about the drug absorption process through experiments utilizing the techniques discussed above, no single technique or combination of techniques reported to date allows the researcher to do more than make rank order comparisons among drugs with respect to oral absorption rates. In most *in vitro* and *in situ* gut preparations, the apparent rates of drug absorption are relatively slow; whereas in humans and intact animals, drug absorption as calculated from blood level data is usually very rapid. For example, Levy *et al.* (12) reported that in humans the half-life for oral absorption of aspirin from solutions is about 5 min.; and a half-life of 15–30 min. for oral absorption of sulfaethidole from fine particle suspensions in humans can be estimated from

the data of Swintosky *et al.* (13). The data of Rosen *et al.* (14) for dextroamphetamine-^{14}C sulfate capsules in dogs suggests that the half-life for absorption is about 20 min., and the data of Dittert *et al.* (15) for 4-acetamidophenyl 2,2,2-trichloroethyl carbonate suspensions in mice suggests that the half-life for absorption is about 10 min.

This paper reports upon a method for determining drug absorption rates from segments of the gastrointestinal tracts of rats, *in situ*, which offers significant advantages over other methods. The main advantage is that the technique produces absorption rates which are realistic and comparable to those calculated from blood concentration data following oral drug administration to humans and intact animals. In addition (*a*) the experimental technique is simple (*b*) the apparatus is inexpensive and readily available (*c*) a single animal yields experimental data suitable for complete quantitative kinetic analysis (*d*) animal to animal variation of the kinetic results is minimal, and (*e*) the time required to conduct an experiment is moderate.

EXPERIMENTAL

Equipment and Reagents—All of the chemicals employed in this study were reagent grade unless otherwise specified. All solutions were prepared with distilled, deionized, boiled water. A pH meter (Beckman Zeromatic II), a constant temperature water bath (Haake type FBE), and a spectrophotometer (Cary model 15) were utilized.

Perfusion and Drug Solutions—The perfusion solution used for irrigating the gastrointestinal tract contained the following molar concentrations of salts: 1.45×10^{-1} *M* NaCl, 4.56×10^{-3} *M* KCl, 1.25×10^{-3} *M* $CaCl_2$, and 5.00×10^{-3} *M* NaH_2PO_4. Drug solutions were made isotonic and were buffered at pH 6.0 with a Sorensen buffer, or pH 3.0 with a citrate-phosphate buffer. All solutions were maintained at 37° during administration.

Test Animal—Male Sprague-Dawley albino rats weighing 220–260 g. were fasted 16–24 hr. prior to surgery; however drinking water was readily accessible. They were kept in cages having wide mesh floors to prevent coprophagy. The rats were anesthetized approximately 1 hr. prior to surgery with ethyl carbamate (urethan) using an intraperitoneal injection of 1 mg./g. body weight.

Intestinal Absorption Procedure—The small intestine was exposed by a midline abdominal incision, and two *L*-shaped glass cannulae were inserted through small slits at the duodenal and ileal ends, as illustrated in Fig. 1. Care was taken to handle the small intestine gently and to reduce surgery to a minimum in order to maintain an intact blood supply. The cannulae were secured by ligation with silk suture, and the intestine was returned to the abdominal cavity to aid in maintaining its integrity. Four-centimeter segments of Tygon tubing were attached to the exposed ends of both cannulae, and a 30-ml. hypodermic syringe fitted with a three-way stopcock and containing perfusion fluid warmed to 37° was attached to the duodenal cannula (see Fig. 1). As a means of clearing the gut, perfusion fluid was then passed slowly through it and out the ileal cannula and discarded until the effluent solution was clear. The remaining perfusion solution was carefully expelled from the intestine by means of air pumped through from the syringe, and 10 ml. of drug solution was immediately introduced into the intestine by means of the syringe. The stopwatch was started, and the ileal cannula was connected to another 30-ml. syringe fitted with a three-way stopcock. This arrangement enabled the operator to pump the lumen solution into either the ileal or the duodenal syringe, remove a 0.1-ml. aliquot, and return the remaining solution to the intestine within 10–15 sec. To assure uniform drug solution concentrations throughout the gut segment, aliquots were removed from the two syringes alternately.

Stomach Absorption Procedure—In a manner similar to that described for the intestinal procedure, the stomach was exposed, cannulated at the cardiac and duodenal ends, and washed from the cardiac end with perfusion solution until the effluent was clear.

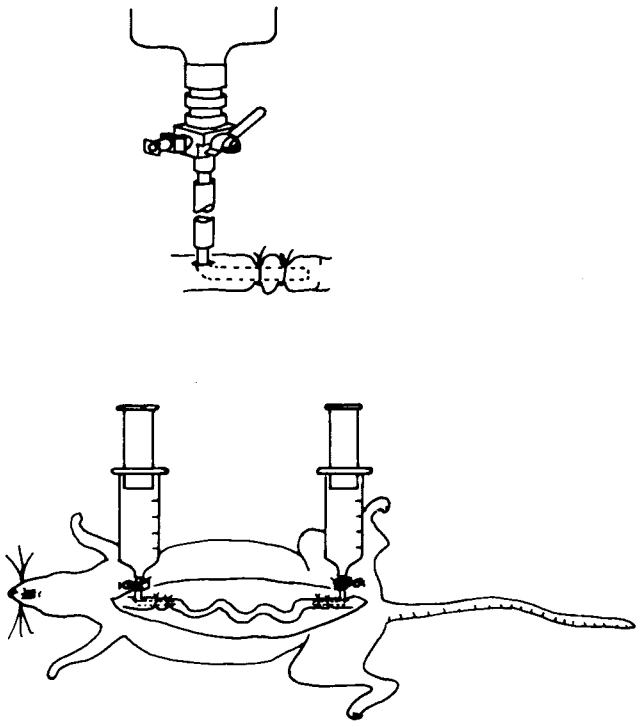

Figure 1—*Experimental arrangement for determining rates of absorption of drugs from rat intestinal lumen* in situ.

The perfusion solution was expelled with air, and 5 ml. of drug solution was introduced through the cardiac cannula. The stopwatch was started; and a 15-cm. section of 0.011-i.d. polyethylene tubing, connected to a 2-ml. syringe fitted with a three-way stopcock, was inserted through the duodenal cannula. The stomach contents were sampled by withdrawing about 0.5 ml. of the solution into the syringe, removing a 0.1-ml. aliquot, and returning the remainder.

Analytical Methods—The initial concentrations of the drugs were chosen on the basis of drug solubility and assay sensitivity. For sulfaethidole, a concentration of 0.4 mg./ml. was employed. For all other drugs, approximately 1 to 2 mg./ml. was employed. In the latter cases, the lowest concentration consistent with assay sensitivity was chosen.

The lumen samples were made appropriately basic with sodium hydroxide or acidic with hydrochloric acid and the unionized drugs extracted into chloroform. The absorbances of the chloroform solutions of the following drugs were determined at the indicated spectral peaks: aminopyrine, NF, 270 mμ; haloperidol (McNeil), 240 mμ; prochlorperazine (Smith Kline & French) 252 mμ; and salicylic acid, USP, 305 mμ.

Barbital was extracted from the samples with chloroform, then from the chloroform into a pH 9.4 borate buffer; and the absorbance was determined at 242 mμ. Sulfaethidole was analyzed by the method of Bratton and Marshall (16).

RESULTS AND DISCUSSION

Before beginning extensive studies utilizing the *in situ* gut preparation described in this report, it was necessary to establish (*a*) to what extent the volume of the solution in the gut lumen might change during an experiment, (*b*) what influence the initial volume of this solution might have on the absorption rate, and (*c*) to what extent return of the absorbed drug from blood to gut might occur during an experiment.

The volume of the lumen solution was followed by adding 5 mg./ml. of phenol red dye to the drug solutions. This dye is reported to be virtually unabsorbed (1), and it acts as a marker to detect gain or loss of water by the lumen. The dye did not appear to influence the absorption rates of any of the drugs studied, and in no case did the volume of the lumen solution decrease more than 10%. These results indicated that water absorption was not significant in these experiments, and the great majority of the results reported here were

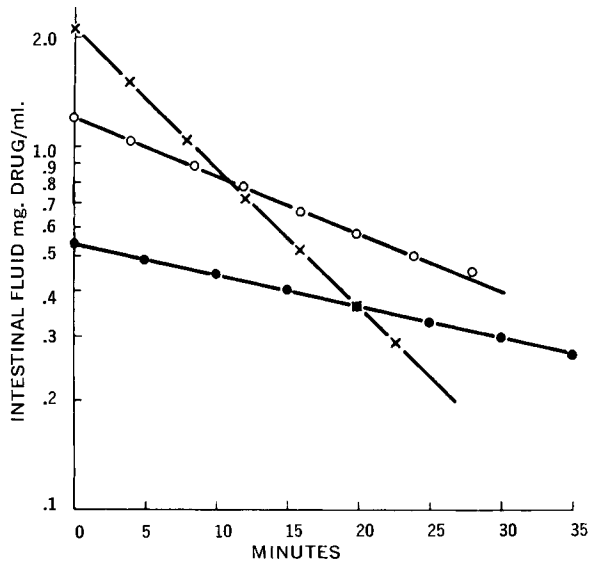

Figure 2—*Semilog plots of rat intestinal lumen concentrations (mg./ml.)* versus *time for weakly acidic drugs (pH 6.0, 10-ml. lumen solution volume).* ×—×, *salicylic acid;* ○—○, *barbital;* ●—●, *sulfaethidole.*

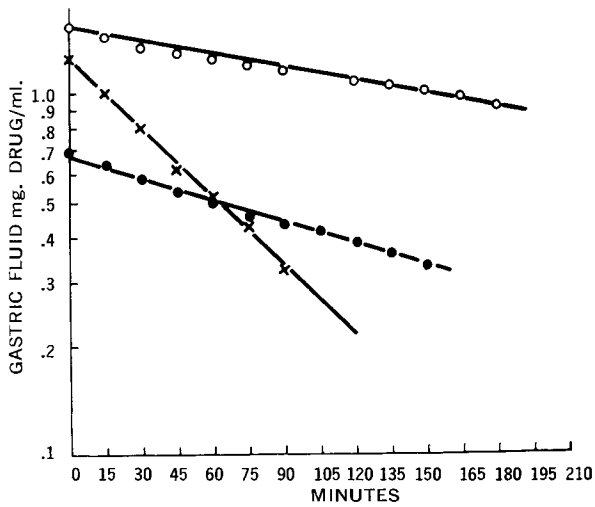

Figure 4—*Semilog plots of rat gastric lumen concentrations (mg./ml.)* versus *time for weakly acidic drugs (pH 3.0, 5 ml. lumen solution volume).* ○—○, *barbital;* ×—×, *salicylic acid;* ●—●, *sulfaethidole.*

obtained without the use of the dye marker. In the absence of phenol red, the lumen volume could be monitored satisfactorily by simply noting the volumes in the syringes each time the solutions were removed from the gut for sampling. In these experiments, this volume never changed more than 10%. In virtually all intestinal absorption experiments, the initial volume of the lumen solution was 10 ml. Identical absorption rates were obtained if initial volumes as low as 8 ml. or as high as 12 ml. were used. Similarly, in the stomach absorption experiments, initial volumes between 4 and 6 ml. gave identical absorption rates.

Diffusion of the drugs from blood to gut lumen was studied by intravenously injecting 1.5 to 5 times the amount of drug normally placed in the intestinal lumen and by placing 10 ml. of perfusion solution (no drug) into the lumen. Lumen samples were taken periodically for 3 hr. In no case was more than 5% of the i.v. dose detected in the lumen solutions; therefore it was concluded that return of previously absorbed drug to the lumen could be ignored for the drugs employed in this report.

Drug Disappearance from Stomach or Intestinal Lumen—Typical plots showing the disappearance of several drugs from intestinal lumen solutions are shown in Figs. 2 and 3, and from gastric lumen solutions in Figs. 4 and 5. The results show that aminopyrine, barbital, salicylic acid, and sulfaethidole absorptions follow apparent first-order kinetics for the entire experiment in both gastric and intestinal preparations. Haloperidol and prochlorperazine, however, disappeared more rapidly in the early minutes of the experiments than at later times; however. after approximately 8 min. in the intestine and 45 min. in the stomach, the absorptions of these drugs also followed apparent first-order kinetics. The reasons for this behavior will be dealt with in a future publication. For the purposes of this report, half-lives for haloperidol and prochlorperazine were calculated from the straight "tails" of the semilog plots.

Figures 2–5 illustrate the precision of the data obtained using the *in situ* technique. The semilog plots are linear. The points are all quite close to the line, and no difficulty was encountered in fitting the line and determining the half-lives. Table I summarizes all the results obtained with the intestinal preparation and illustrates the very low rat-to-rat and day-to-day variation obtained. In all experiments, the pH of the lumen solutions remained within the range of 6.0–6.2 during the entire period of data collection.

The half-lives for drug absorption determined by following drug concentration in the rat gut lumen (Table I) are similar in magnitude

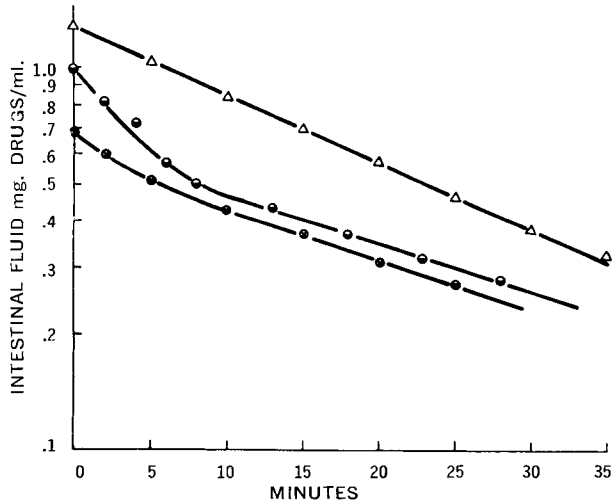

Figure 3—*Semilog plots of rat intestinal lumen concentrations (mg./ml.)* versus *time for weakly basic drugs (pH 6.0, 10 ml. lumen solution volume).* △—△, *aminopyrine;* ◒—◒, *prochlorperazine;* ⊗—⊗, *haloperidol.*

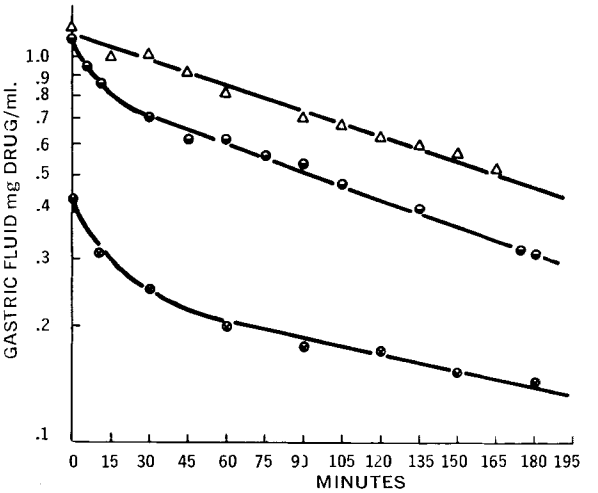

Figure 5—*Semilog plots of rat gastric lumen concentrations (mg./ml.)* versus *time for weakly basic drugs (pH 6.0, 5 ml. lumen solution volume).* △—△, *aminopyrine;* ◒—◒, *prochlorperazine;* ⊗—⊗, *haloperidol.*

Table I—Half-Lives and First-Order Rate Constants for the Disappearance of Drugs from Rat[a] Small Intestinal Lumen, *In Situ*

Drug	Initial pH 6.0[b] Half-Lives (min.)	Average Half-Life (min.)	Average k_a (min.$^{-1}$)
Weak Acids			
Salicylic acid	8, 8, 8, 8, 8	8	0.085
Barbital	19, 19, 17, 19	19	0.037
Sulfaethidole	34, 30, 31, 33, 30, 34	32	0.022
Weak bases			
Prochlorperazine	24, 19, 24, 24	23	0.030
Haloperidol	24, 24, 26, 24	24	0.028
Aminopyrine	36, 28, 33	32	0.022

[a] The rats weighed between 220 and 260 g. A 10-ml. volume of lumen solution was employed. [b] In no case did the pH shift more than 0.2 units during the experiment.

to absorption rates determined by following blood concentrations after oral administration of drugs to humans and intact animals (12–15). Swintosky *et al.* (17) also present views which indicate that the absorption rates reported in this publication are realistic values. They state that absorption rates may be estimated from the time at which the peak blood concentration is reached following oral administration of a drug. The half-life for absorption by this method of estimation is about $^1/_5$ to $^1/_6$ the time for attainment of peak blood concentration when a first-order absorption process is occurring and the rate of absorption is at least five times the rate of elimination. Thus, drugs which show peak blood concentrations between 30 and 150 min. following oral administration are exhibiting absorption half-lives of approximately 6 to 30 min. Since many drugs show peak blood concentrations in the first few hours after oral administration, the absorption rates reported in this paper correlate well with those observed in humans. The realistic absorption rates determinable by this *in situ* technique, coupled with its simplicity, is one of the most cogent arguments for recommending it in preference to earlier experimental variations of this technique.

Aminopyrine and sulfaethidole exhibited the slowest absorption rates in the intestinal preparation. In both cases, half-lives of 32 min. (k_a = 0.022 min.$^{-1}$) were obtained. On the other hand, salicylic acid was the most rapidly absorbed drug exhibiting a half-life of 8 min. (k_a = 0.087 min.$^{-1}$). This is somewhat surprising since salicylic acid, which has a pKa of approximately 3, is essentially totally ionized at pH 6. If it is assumed that salicylic acid is absorbed by passive diffusion of the unionized acid, it would be necessary for this species to diffuse through the intestinal wall at a phenomenal rate. Assuming a "virtual" pH of 5.3 for the mucosa controlling drug partitioning, the required rate of diffusion of unionized salicylic acid would still be extremely rapid. Thus, the salicylic acid data reported here lends support to the concept that this drug is absorbed by mechanisms other than, or in addition to, passive diffusion of the unionized form.

Table II summarizes the results obtained with the stomach preparation. Again, rat-to-rat and day-to-day variation was low; but

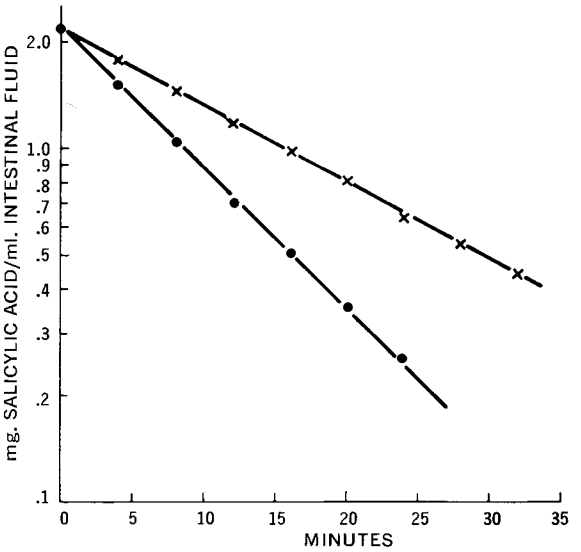

Figure 6—*Plots showing the effect of fasting on the half-life for absorption of salicylic acid from rat intestinal lumen* in situ *(pH 6.0, 10 ml. lumen solution volume). Key:* ●—●, *rats fasted 7 hr.,* $t_{1/2}$ = *8 min.;* k_a = *0.085 min.*$^{-1}$; ×—×, *rats fasted 62 hr.,* $t_{1/2}$ = *13 min.;* k_a = *0.053 min.*$^{-1}$.

absorption from the stomach at pH 3 was much slower for both weakly acidic and weakly basic drugs than from the small intestine at pH 6. In the case of the weakly basic drugs, these results could be rationalized in terms of the greater fraction of the drug present in the lipid insoluble ionized form at the lower pH. But, in the case of the weakly acidic drugs, the results must be rationalized in terms of the larger absorbing surface of the intestine and its better physiological organization for absorption compared to those of the stomach. In addition, the high ratio between the absorbing surface area and the lumen solution volumes probably contributed to more rapid absorption in the intestinal experiments.

When the pH of the gastric lumen was shifted to 6, the weakly acidic drugs were absorbed more slowly while the weakly basic drugs were absorbed more rapidly than at pH 3. However, in no case was the stomach absorption rate at pH 6 as fast as the intestinal absorption rate at pH 6, again reflecting the relative surface areas and physiological organizations of the two sites, as well as the relative surface-to-volume ratios in the two experiments.

In addition to allowing determination of absorption rates from various segments of the gastrointestinal tract, the *in situ* technique offers many opportunities for studying various aspects of the drug absorption process under conditions in which realistic absorption rates are obtained. An example of one type of study that can be performed is illustrated in studies of the effect of fasting on drug absorption rates. The rats used in the previously described studies were routinely fasted for 16–24 hr. prior to surgery in order to clear the gut lumen as much as possible. When rats were fasted for

Table II—Half-Lives and First-Order Rate Constants for the Disappearance of Drugs from Rat[a] Gastric Lumen, *In Situ*

Drug	Initial pH 3.0[b] Half-Lives (min.)	Average Half-Life (min.)	Average k_a (min.$^{-1}$)	Initial pH 6.0[b] Half-Lives (min.)	Average Half-Life (min.)	Average k_a(min.$^{-1}$)
Weak acids						
Salicylic acid	46, 47	46.5	0.015	130, 130, 130	130	0.0053
Barbital	220, 250, 240	237	0.0029	280, 260, 270	270	0.0026
Sulfaethidole	160, 190	175	0.004	280, 320	300	0.0023
Weak bases						
Prochlorperazine	>350	>350	<0.002	120, 102	111	0.0062
Haloperidol	250, 250	250	0.0028	168, 144, 190	167	0.0041
Aminopyrine	>350	>350	<0.002	150, 154	152	0.0046

[a] The rats weighed between 220 and 260 g. A 5-ml. volume of lumen solution was employed. [b] In no case did the pH shift more than 0.4 units during the experiment.

longer periods, the rate of absorption of salicylic acid was found to be much slower (see Fig. 6). This has lead us to study the influence of fasting on drug absorption rates, and the results of that study are to be reported separately (18).

Future publications will deal with the influence of the physical-chemical properties of drugs on their absorption rates from the *in situ* rat intestine and with correlations between drug transfer kinetics in a three phase *in vitro* model for drug absorption (11) and drug transfer kinetics in the *in situ* rat intestinal preparation.

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Drug Absorption II: Effect of Fasting on Intestinal Drug Absorption

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Abstract □ The effects of fasting on the intestinal absorption profiles of salicylic acid, barbital, haloperidol, and chlorpromazine were studied in anesthetized rats. The *in situ* technique employed in this study yielded absorption rate constants which were realistic and comparable to those observed following oral drug administration. The weak acids, barbital and salicylic acid, were found to obey first-order kinetics throughout the experiments. The highly lipid-soluble weak bases, haloperidol and chlorpromazine, obeyed first-order kinetics after the first 10 min. of experimentation. No apparent deviation in absorption patterns occurred when fasting periods were less than 20 hr. However, when the period of inanition exceeded 20 hr., absorption rates were found to decrease significantly and the decrease was dependent on the duration of the fasting period. It is possible that the unusual drug absorption patterns noted in these studies could be accounted for by one or more of the various physiological and/or biochemical changes which occur within an organism subjected to conditions of prolonged fasting.

Keyphrases □ Drug absorption—intestinal □ Intestinal absorption, drug—fasting effect □ Perfused intestine—drug absorption □ Fasting—intestinal effect

A previously published report from the authors' laboratory described an improved *in situ* method for the quantitative determination of realistic absorption rate values for drugs from isolated segments of the gastrointestinal tract of anesthetized rats (1). The technique yielded reproducible data from which kinetic values for drug absorption could be calculated. The experimental protocol employed in these studies called for routine periods of fasting (16–24 hr.) prior to experimentation. On occasion, when the experiments could not be conducted during the intended time period, the animals were fasted for an additional period of time. Results obtained from animals subjected to these prolonged starvation periods revealed unexpected and unusual drug absorption patterns. Consequently, studies were designed specifically to determine the effect of fasting on intestinal drug absorption. This paper reports on the results of those studies.

EXPERIMENTAL

Reagents and Equipment—All of the chemicals employed in this study were reagent grade unless otherwise specified. The perfusion solution consisted of 1.45×10^{-1} *M* NaCl, 4.56×10^{-3} *M* KCl, 1.25×10^{-3} *M* $CaCl_2$, and 5.0×10^{-3} *M* NaH_2PO_4 prepared with distilled, deionized, and boiled water. The drug solutions consisted of 2.3 g./l. salicylic acid, 0.80 g./l. haloperidol, 0.80 g./l. chlorpromazine, or 1.20 g./l. barbital made isotonic with NaCl and buffered with Sorensen phosphate buffer at a pH 6.0. A constant temperature water bath (Haake type FBE) was used to maintain the perfusing solutions at 37°. The pH determinations were carried out with a pH meter (Beckman Zeromatic II). A spectrophotometer