The Effect of Fasting on the Rate of Intestinal Drug Absorption in Rats: Preliminary Studies

James M. Orr, PhD, and Leslie Z. Benet, PhD

The absorption rates of two model drugs, salicylate and antipyrine, from the small intestines of rats deprived of food for various periods of time were compared with rats fed *ad libitum*. Fasting reduced the absorption rate constants for both drugs with the salicylate rates being depressed more severely than the rates for antipyrine. Intestinal mass studies showed that the weight/length ratio of the rat intestine is progressively decreased as fasting is prolonged up to 96 hr. The intestinal weight loss was much more pronounced than the total body weight loss. The loss in intestinal weight and the observed decrease in drug absorption rate are believed to be related to the inhibition of intestinal cell proliferation due to fasting, resulting in a decreased absorptive surface and reduced mucosal cell viability.

It has become increasingly evident that the small intestine is the major site of absorption for most orally administered drugs whether these drugs are ionized or nonionized (1, 2). Despite an increased understanding of the structure and function of the various cellular and subcellular units that comprise the intestine as an organ, many details regarding the effects of nutritional lack and starvation on the intestinal absorption process are still unknown. McManus and Isselbacher (3), employing an overnight fast, demonstrated a difference in weight of intestine, with fed rats having heavier intestines than fasted rats, specifically a greater mucosal-cell mass. Fasting has been shown to evoke a continual decrease in the number of cells in the germinal crypts of the intestinal epithelium (4) and to alter the intestinal absorption patterns of compounds such as D-glucose, L-histidine, methionine, and proline (4–7). However, few reports have been published regarding the effects of fasting or nutritional lack on the intestinal absorption of drugs. One such study showed that the absorption rates of certain drugs were adversely affected by fasting for periods of 24 hr or longer (8). Inasmuch as mucosal cells have a turnover time of about 2 days in mature rats (4), these findings would seem to indicate that even short periods of fasting would produce a decrease in absorption rates of drugs if mucosal-cell number were an important factor in absorption.

In order to attempt to delineate the effects of fasting on drug absorption from the intestine, experiments were carried out in which drug solutions were perfused *in situ* through the small intestines of rats fasted for various periods of time.

MATERIALS AND METHODS

Materials

All chemicals and buffer constituents used were of reagent grade. The drug solutions used for perfusion consisted of 17 mM (2.3 mg/ml) salicylic acid or 5.3 mM (1.0 mg/ml) antipyrine made up in glucose-free Krebs-Henseleit

From the Department of Pharmacy, School of Pharmacy, University of California, San Francisco, California.

Dr. Orr's present address is Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada H9R 1G6.

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Address for reprint requests: Dr. Leslie Z. Benet, School of Pharmacy, University of California, San Francisco, California 94143.

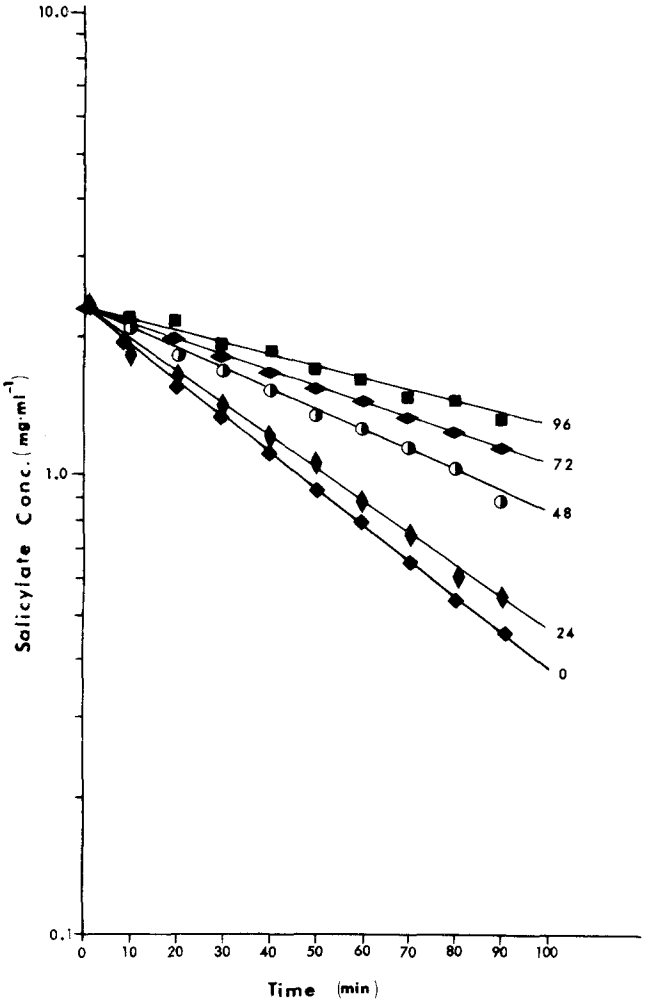

Fig 1. Effect of length of fast on the intestinal perfusate concentration of salicylate. Fasting time in hours is indicated at the right of each semilogarithmic concentration-time plot.

buffer (K-H buffer) adjusted to pH 7.4 (9). These drug concentrations are the same as those used in other studies and were chosen for purposes of comparison (8, 10). Salicylic acid is a small (mol wt 138) weak acid, pK_a 3.0, which should be greater than 99.99% ionized at pH 7.4, while antipyrine (mol wt 188) is a very weak base, pK_a 1.4, which should be essentially completely nonionized at the experimental and physiologic pHs of this study (10). The perfusion solutions were maintained at 37° C by a constant temperature bath (Haake, type R21). Determinations of pH were carried out on a pH meter (Beckman, Research Model).

Surgical Procedures

Male Sprague-Dawley rats (Simonsen) initially weighing 300–350 g were used in all the fasting studies. The animals were housed in wire mesh cages, to prevent coprophagy, in rooms with adequate ventilation and light and were maintained on Purina rat chow (Ralston-Purina Co.). Water was always allowed *ad libitum*. For the fasting studies, rats were continuously fasted for various periods of time beginning at the same time of day in each experiment since the time of day of food withdrawal has been shown to have an effect on the actual length of fasting time for nocturnal animals (3).

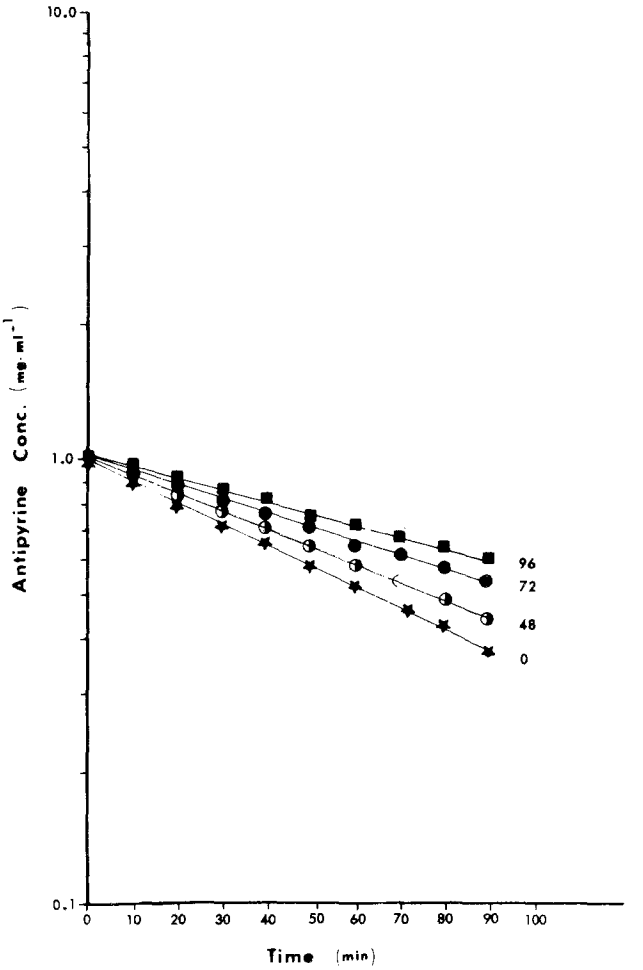

Fig 2. Effect of length of fast on the intestinal perfusate concentration of antipyrine. Fasting time in hours is indicated at the right of each semilogarithmic concentration-time plot.

After a predetermined period of fasting, the animals were weighed, and a 40 mg/kg dose of sodium pentobarbital (Nembutal Sodium®, Abbott Labs.) was injected intraperitoneally to produce anesthesia. A laparotomy following the linea alba was performed, and the small intestine was cannulated with 10-cm lengths of polyethylene tubing (PE 320, Clay-Adams) at the ligament of Treitz and at a point 10 cm proximal to the ileocecal junction so as to form an *in situ* loop of small intestine suitable for perfusion. When the cannulae were inserted, care was taken in placing the ligatures between the intestinal blood vessels so as to maintain intact the blood circulation in the intestinal loop. The segment of intestine was then washed free of chyme using 50 ml of K-H buffer warmed to 37° C. The wash buffer was gently expelled using a bubble of air. The abdominal incision was then closed using clamps and covered with a surgical pad moistened with buffer. The cannulae were attached to an inlet and outlet of a reservoir containing the drug solution.

In Vivo Luminal Perfusion

The recirculation system used was similar to that described by Schanker and coworkers (11). The reservoir contained 50 ml of warmed, stirred, and gassed (95% O_2, 5% CO_2) K-H buffer (pH 7.4) at the appropriate drug concentration. Body temperature was maintained via a small incandescent table lamp, and was monitored by means of a

Table 1. Effect of Fasting on Intestinal Drug Absorption Rate Constants in Rats

Drug studied: Salicylate (17 mM)								
Fasting time (hr)	0	12	24	36	48	60	72	96
Number of studies	7	6	7	5	8	5	4	3
Absorption rate constants	2.68	2.11	1.42	1.28	1.29	1.04	0.91	0.88
$\overline{kap}$ (min^{-1} cm^{-1} 10^4)	(0.68)*	(0.49)	(0.27)	(0.57)	(0.34)	(0.39)	(0.21)	(0.17)
Fraction of nonfasted rate	1.0	.89	.60	.54	.54	.44	.38	.37
Drug studied: Antipyrine (5.3 mM)								
Fasting time (hr)	0	24		48		72	96	
Number of studies	7	6		4		3	3	
Absorption rate constants	1.40	1.32		1.27		1.18	1.16	
$\overline{kap}$ (min^{-1} cm^{-1} 10^4)	(0.10)	(0.22)		(0.30)		(0.02)	(0.04)	
Fraction of nonfasted rate	1.0		.94		.91		.84	.83

*Values in parentheses are standard deviations

rectal thermister thermometer. Perfusion of the loop at a rate of 1.5 ml/min was carried out in the direction of normal gastrointestinal flow by means of a peristaltic pump (Harvard Instrument Co., Millis, Massachusetts). Samples for assay were removed from the reservoir at fixed time periods during the experiment. Intraluminal pressure was monitored using a simple T-tube manometer.

At the end of the experiment, the animal was sacrificed and the intestinal loop was carefully separated from the mesentery. The stretched length of the intestinal loop was measured using a standard weight. The loop was weighed wet and then dried to a constant dry-weight in an 80° C drying oven so as to obtain wet and dry intestinal weights per unit length.

In experiments where blood samples were required, the left femoral vein was exposed. A polyethylene cannula (PE 50), filled with heparinized normal saline (5 units heparin/ml saline) to prevent clotting, was inserted into the femoral vein to the junction of the common iliac vein. In some instances, the right external jugular vein was also exposed and a cannula inserted with the tip positioned in the right precava region.

In one series of fasting experiment the mucosa was separated from the rest of the intestinal mass at the end of each experiment by slitting the perfused loop longitudinally, pinning the flattened intestine on a glass plate, and removing the mucosal fraction by scraping with a microscope slide as described by Schedl and Wilson (12). Wet and dry weights of the mucosal scrapings and underlying tissues were obtained.

In the initial series of experiments, trace [^{3}H]-inulin was added as a volume marker (13). Since the drug–buffer perfusate was in the osmolar range of 310–330 mOsm/liter, it was felt that net water flux would be minimal. In plots of drug concentration vs time, corrected and uncorrected for water flux, the slopes were not significantly different (using a paired t test at a 95% level of significance). Therefore, net water flux was taken to be negligible in its effect in these experiments, and subsequently a volume marker was only employed occasionally to check this assumption.

Assays

Salicylic acid was assayed either spectrophotofluorometrically on a Aminco-Bowman spectrophotofluorometer (SPF) (American Instrument Co.) or by liquid scintillation spectrometry using ^{14}C-labeled salicylic acid (New England Nuclear, Boston). Antipyrine was assayed by liquid scintillation spectrometry using antipyrine-*N*-methyl-^{14}C with a specific activity of 10 μCi/mM (ICN, Irvine, California). Apparent absorption rate constants were calculated from the drug concentration vs time data by means of a weighted log-linear least-squares method.

RESULTS AND DISCUSSION

Drug Absorption Rate Studies

Typical changes in drug concentration over a 90-min time period after various fasting times are shown in Figures 1 and 2. These figures depict concentrations from a single experiment at each fasting time, which is indicated as fasting time in hours to the right of each curve. The slopes can be seen to decrease steadily as the length of fast increases.

The effects of fasting on the average apparent absorption rate constants per unit length ($\overline{kap}$)

Table 2. Intestinal Dry Weight Changes with Fasting

Fasting time (hr)	0 (control)	12	24	36	48	60	72	96
Number of rats	14	6	13	5	12	5	7	6
Average intestinal weight/length (mg/cm)	16.66 (2.05)*	15.45 (1.5)	13.73 (1.26)	13.01 (1.37)	11.47 (1.85)	11.48 (0.43)	10.38 (0.64)	10.16 (1.88)
% change from control	—	7.3	17.6	21.9	31.2	31.1	37.7	39.0

*Values in parentheses are standard deviations

for salicylate and antipyrine are shown in Table 1. There is a continual decrease in the apparent drug absorption rates as the fasting interval is increased to 96 hr. The rates for salicylate decreased by 63% over 96 hr of fasting while the rates for antipyrine decreased by only 14% over the same period of fast. The data show that the average apparent absorption rate constant for salicylate initially decreases rapidly with fasting then tends to level off as the fasting time reaches 96 hr.

The antipyrine rates are less affected by fasting than are salicylate rates. The differences in the effects of fasting on salicylate and antipyrine absorption may be taken as showing that the mucosal cell number or surface area is not as critical for antipyrine absorption as it is for salicylate absorption. This suggestion is also reflected in previous work (10) which indicated that transfer of nonionized antipyrine across the *in vitro* rat intestine yielded equivalent rates for mucosal to serosal and serosal to mucosal fluxes ($J_{m\to s} = J_{s\to m}$). On the other hand the mucosal to serosal flux for salicylate was about two times the flux in the opposite direction ($J_{m\to s} = 2J_{s\to m}$). These workers (10) suggested that the difference in surface area between the mucosal and serosal membranes appeared to be important for ionized compounds like salicylate but not important in the case of antipyrine, and thus the rate-limiting step in intestinal transfer of nonionized drugs may be passage through the membrane barrier rather than passage into or out of the membrane.

Intestinal Mass Studies

The effect of fasting on the total intestinal dry weight is given in Table 2. Intestinal mass/length decreases progressively at a fairly rapid pace as fasting is prolonged, with the largest change occurring over the first 48 hr. These results in Table 2 are in agreement with the data of Hopper et al (4) who noted that with a cell turnover time of 2.1 days for mucosal cells in mature rats (14), even short periods of fasting could be considered to have detrimental effects of mucosal-cell number. The data are also supported by the published reports of Fabry and Kujalova (15) and others (3, 5, 16) who demonstrated that fasting produces a decrease in intestinal mass.

Newey and associates (5) compared fed and 72-hr-fasted rats with respect to their abilities to absorb sugars and amino acids. They found that the transfer rates of glucose and amino acids were decreased in fasted animals. These workers also noted that the intestinal segment average weight decreased by 28% after 72 hr of fasting. The percent decrease in intestinal weight was greater than the percent total body weight loss, which is consistent with the idea that the high rate of proliferation of the germinal cells in the epithelial crypts is rapidly and adversely affected by the nutrient lack caused by fasting. Although McManus and Isselbacher (3) showed no difference in body weight, intestinal length, or lipid content between control and 24-hr-fasted rats, they did show a de-

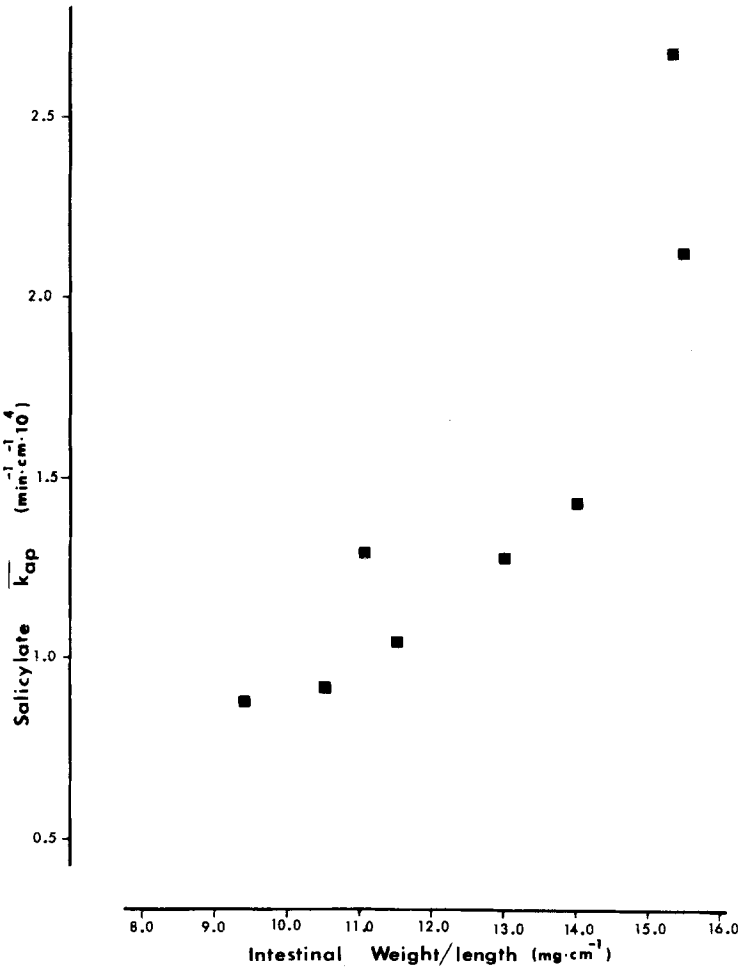

Fig 3. Plot of average apparent absorption rate constants for salicylate vs intestinal weight per unit length at various intervals of fasting.

crease in intestinal weight and a decrease in DNA content, with fasting.

Since both the intestinal mucosal mass and the apparent absorption rates for salicylate decrease as a function of fasting time, it would appear that intestinal mucosal cell mass may be an important factor in the absorption mechanism for drug ions like salicylate. There is a strong postive correlation (0.941) between the average apparent absorption rate constants for salicylate and the intestinal weight/length at various fasting times (Figure 3), indicating that intestinal mucosal cell number may indeed be an important factor in drug absorption as was suggested by Newey and associates (5) for the absorption of amino acids.

Mucosal Separation Studies

In a series of experiments, the intestinal mucosa was separated from the underlying musculature of the rat intestine after various periods

Table 3. Mucosal, Musculature, and Total Intestinal Weight Changes with Fasting

Fasting time (hr)	0 (control)	24	48	72	96
Intestinal dry weights (mg/cm)					
Mucosal weight/length	5.49(1.1)*†	4.74(0.26)	3.32(0.33)	3.07(0.25)	2.68(0.15)
Musculature weight/length	9.42(1.5)	7.13(1.2)	6.98(1.23)	6.64(1.26)	5.37(0.14)
Total weight/length	14.91(2.4)	11.87(1.2)	10.30(1.8)	9.71(1.41)	8.05(0.28)
% change of total weight/ length from control	0	20.4	30.9	34.9	46.0
Mucosa/musculature ratio	0.590(0.10)	0.680(0.11)	0.482(0.05)	0.473(0.09)	0.498(0.02)
Mucosal % of total wt	36.8	40.2	32.5	32.0	33.2

*There were 4 animals in each group.
†Values reported are means ± (SD).

of fasting. Table 3 shows the effect of fasting on the total dry weight and the separated mucosal and musculature fractions of the intestinal weight. The total intestinal dry weight decreased with fasting (Table 3) in a manner similar to that described in Table 2. However, when the mucosa and the musculature are separated, it can be seen that both of these tissue masses loose weight more or less in a parallel fashion as fasting progresses. This indicates that decreased mucosal-cell number may not be totally responsible for the decreased absorption rates observed with fasting. The observed loss in musculature weight that follows fasting may imply that the mesenteric vascular system is decreased in size following fasting and that the decreased absorption rates may be a function of both lowered mucosal cell number and reduced blood flow to the intestine.

Several investigators (17, 18) have noted that fasting causes a decrease in both the number and the size of the mucosal epithelial cells. It has also been observed that following fasting, a significant decrease in *de novo* protein synthesis occurs (19). Newey (5) and others (3, 20, 21) have noted that glucose metabolism in rats, as well as general enzyme activity, was reduced with fasting. Similar studies in humans have related protein–calorie malnutrition with depressed intestinal enzyme activity and reduced absorption of essential amino acids (22, 23). Although many questions regarding basic absorption mechanisms and the effects of fasting remain unanswered, fasting has been shown to reduce the intestinal tissue weight in a fairly linear manner, affecting both the mucosal and musculature fractions about equally. At the same time, the apparent intestinal absorption rate constants of ionized salicylate and nonionized antipyrine were reduced significantly under fasting conditions. The literature provides evidence for reduced carbohydrate and amino acid absorption and reduced enzyme activity with fasting (19, 22).

Thus it seems apparent that there are at least two and probably three factors involved in the intestinal absorption of drugs under the influence of fasting: intestinal mucosal-cell number, mucosal-cell viability, and most likely mesenteric blood flow. If mucosal-cell mass and viability are important factors in absorption, fasting effects could have some clinically important aspects since some disease states, such as tropical sprue and kwashiorkor, often mimic the effects of fasting in terms of morphological changes in mucosal tissue and changes in absorption rate patterns (24–26).

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