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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ACKNOWLEDGMENTS AND ADDRESSES

Received January 21, 1969 from the *College of Pharmacy, University of Kentucky, Lexington, KY 40506*

Accepted for publication June 19, 1969.

This research was supported, in part, by a grant from Smith Kline & French Foundation, Philadelphia, PA 19101

The authors wish to acknowledge the technical assistance of Mrs. Gin Heng Tan in carrying out a portion of the laboratory work.

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Drug Absorption II: Effect of Fasting on Intestinal Drug Absorption

J. T. DOLUISIO, G. H. TAN, N. F. BILLUPS and L. DIAMOND

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

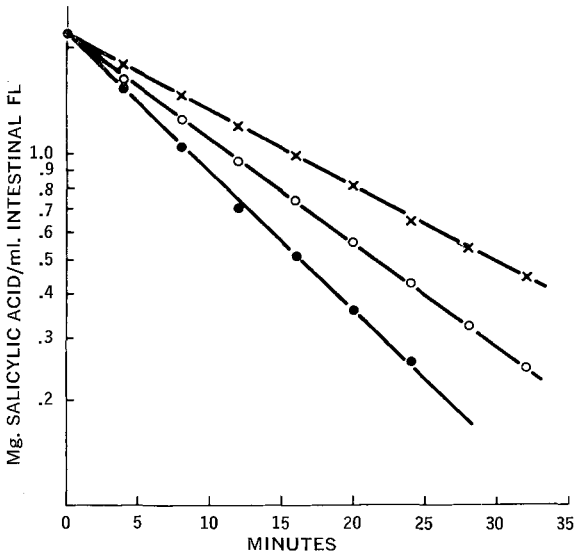

Figure 1—*Semilogarithmic plots of salicylic acid disappearance from 10-ml. solution buffered at pH 6 in rat small intestines after fasting periods of either 7 hr. (●), 40 hr. (○), or 62 hr. (×).*

(Cary model 15) was utilized for analyzing the drug solutions.

Test Animal—Male Sprague-Dawley albino rats weighing between 220 and 260 g. were selected for use in this study. Food was withheld from the animals prior to experimentation but water was allowed *ad libitum*. The duration of the fasting periods ranged from 7–62 hr. The animals were individually housed in cages having wide mesh floors to prevent coprophagy.

Surgical Procedures—The animals were anesthetized approximately 1 hr. prior to surgery with urethan (1 g./kg. intraperitoneally). Laparotomy was performed through a midline incision and the entire length of the small bowel was identified. Two L-shaped glass cannulae were inserted into the intestine, one isoperistaltically at the proximal end of the duodenum, the other antiperistaltically at the distal end of the ileum. Perfusion solution was passed through the intestinal segment continuously until a clear perfusate was obtained. The perfusion solution was then expelled and drug solution was introduced into the system. Samples were withdrawn at periodic intervals and held for subsequent analysis. Details of the aborption technique and procedure employed in drug analyses were presented in a previous paper (1).

RESULTS

This paper reports the effect of fasting on the intestinal absorption profiles of salicylic acid, barbital, haloperidol, and chlorpromazine in rats. A previous report (1) has established that the technique employed yields absorption rate constants that are realistic

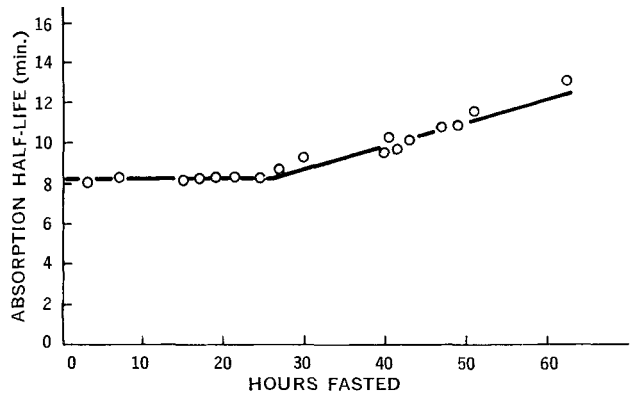

Figure 2—*Intestinal absorption half-lives of salicylic acid after periods of fasting.*

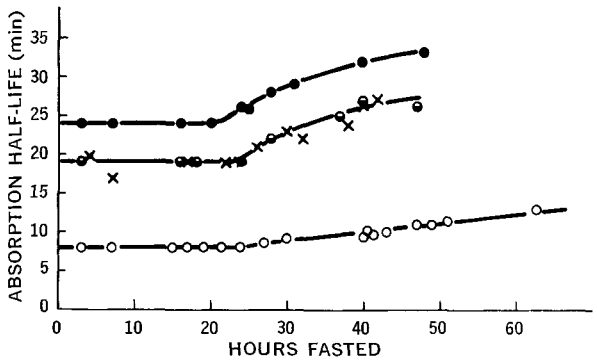

Figure 3—*Intestinal absorption half-lives of salicylic acid (○), barbital (×), haloperidol (●), and chlorpromazine (◒) following periods of fasting. Intestinal solutions were buffered at pH 6.*

and comparable to those observed following oral drug administration. Another advantage of this absorption technique is that reduced time is required for obtaining accurate, reproducible, and quantitative kinetic absorption data.

Treatment of Data—In these experiments, semilogarithmic plots of the amount of drug unabsorbed *versus* time were utilized to determine if drug absorption obeyed simple first-order kinetics and to determine the absorption half-lives. 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. The significance of the initial nonlinear segment of the curve will be discussed in detail in a future publication. In this report the absorption half-lives were determined from the linear portion of the curve.

The intestinal absorption profiles for salicylic acid in rats fasted either 7, 40, or 62 hr. are presented graphically in Fig. 1. From this graph it can be seen that excellent first-order plots were obtained following each period of fasting; allowing absorption half-lives of 8.2, 9.5, and 13.0 min., respectively, to be calculated from these plots.

Figure 2 summarizes the effect of fasting on the intestinal absorption of salicylic acid in rats. No apparent deviation in absorption rate occurred when the fasting period was less than 20 hr. However, when fasting exceeded 20 hr., absorption rates were found to decrease significantly as the fasting period was increased. Figure 3 illustrates comparable results for barbital, haloperidol, and chlorpromazine. Figure 4 is a plot of the composite absorption rate data showing the percent increase of absorption half life as a function of fasting time. This figure illustrates that beyond a fasting period of 20 hr. a similar slowing of absorption rate occurs for each drug. This similarity suggests that some general physiologic phenomena are affecting the absorption rate equally for all the drugs studied.

DISCUSSION

It is generally accepted that the presence of food in the gastrointestinal tract impairs drug absorption. This impairment may be

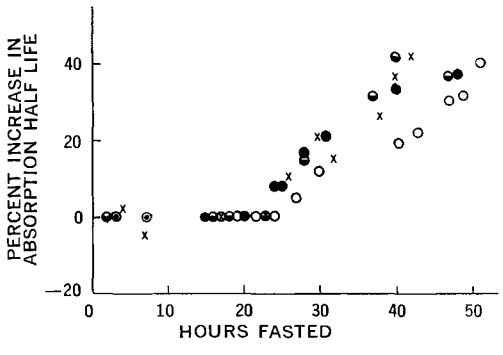

Figure 4—*Percent increases in absorption half-lives for salicylic acid (○), barbital (×), haloperidol (●), and chlorpromazine (◒) following periods of fasting. Data is from Fig. 3.*

due to the binding of the drug to foodstuffs, to poor gastrointestinal mixing, or to other physical processes (*e.g.*, metal complexation and precipitation) that reduce the availability of the drug for absorption. In these instances, fasting improves absorption. However, in the authors' experiments the intestines were cleansed prior to experimentation; moreover, fasting exceeding 20 hr. hindered, rather than enhanced, the absorption process. Therefore, it appears that some physiologic and/or biochemical change that occurs during periods of starvation might be responsible for slowing the absorption rates as observed in these studies.

There are numerous physiologic and biochemical changes which have been reported to occur during periods of starvation and which could possibly account for the drug absorption patterns observed in these studies. It is well known that the development and growth of the body as a whole is affected by starvation. However, various organs appear to be affected more profoundly than others. The small intestine has been shown to be exceptionally susceptible to the influences of prolonged fasting. Ju and Nasset (2) demonstrated a disproportionately greater and more rapid loss of weight from the small intestine than from the body as a whole during total starvation. Steiner *et al.* (3) also noted that during starvation the weight loss of the small intestine was out of proportion to body weight loss.

In considering the effects of a decrease in intestinal weight on its absorptive capacity, it is necessary to take into account which part of the gut contributes most to the weight loss. If this is due primarily to a reduction in epithelial tissue, then a decrease in absorptive capability might be anticipated as a result of a reduction in the amount of mucosal tissue capable of participating in the absorptive process.

In this connection, some of the histologic evidence which has appeared in the literature concerning the acute effects of fasting on gastrointestinal structure merits discussion. As early as 1929, Sun (4) observed destruction of the villus tips and marked shortening of villi in mice fasted for 48 hr. Achord (5) points out that there are at least two factors involved in keeping the intestinal villus adequately covered by new epithelial cells. One of these is the rate of cell proliferation in the intestinal crypts and the other is the rate at which the newly formed cells migrate up the sides of the villi. Both of these factors have been shown to be adversely affected by fasting. Diller and Blauch (6) demonstrated a marked reduction in the number of mitotic figures in the gut of acutely starved mice and their observation was confirmed by Hooper and Blair (7) who noted a reduction in the actual quantity of cells lining the crypts and villi. In 1963, Brown *et al.* (8) used autoradiography to again confirm the inhibition of gut epithelial cell renewal during starvation in mice and further demonstrated that the migration rate was also reduced. Of particular interest along this line is the paper of Thaysen and Thaysen (9) who reported observing atrophy of the villus epithelium, the epithelial cells in the crypts, and the intestinal submucosa and muscularis in rats fasted for 4 days and the recent report by Pittman (10) of a mucosal biopsy of complete villus atrophy in a patient attempting to lose weight by total starvation. Finally, Levin *et al.* (11) reported that a decrease in the intestinal weight of rats, due to adrenalectomy or a 3-day fast, was accompanied by a thinning of the lamina propria and a reduction in the ability to absorb both actively and passively absorbed substances. The authors attributed the latter finding to some nonspecific factor such as a reduction in surface area or blood flow to the intestinal mucosa.

The mechanism by which fasting produces its deleterious effects on the intestines remains obscure. However, Pfeiffer and Debro (12) have established that one of the effects of inanition in rats is a significant depression of hexosemonophosphate shunt activity. These authors point out that the large energy demands required for the absorptive and synthetic processes of the gut mucosa are supplied primarily by carbohydrate metabolism *via* the hexosemonophosphate shunt and they suggest that inhibition of specific enzymes involved in this metabolic pathway may play some role in the pathogenesis of mucosal lesions.

The precise tissue constituents involved in the intestinal weight loss during starvation have not been completely identified. Steiner *et al.* (3) reported a 50% reduction in the water and protein content of the intestinal mucosa of rats fasted for 4 days. The possibility that a reduction in the circulating blood volume of the small intestine is at least partially responsible for the loss of intestinal weight during starvation has not been investigated.

No direct and unequivocal evidence has appeared in the literature with respect to the effect of fasting on intestinal blood flow. However, evidence has been reported which indicates that fasting may serve as a stressful experience (13, 14). One of these reports (14) demonstrated thay a 3- day fast in humans caused an increase in urinary excretion of catecholamines (free adrenaline, noradrenaline, and 3-methoxy-4-hydroxy mandellic acid). This finding certainly suggests an augmented activity of the sympatho-adrenal system. It would not be unreasonable then to expect that some constriction of the splanchnic vasculature with concomitant reduction in intestinal blood flow might occur during starvation. That a decrease in intestinal blood flow can hinder the absorption of certain drugs is borne out by the recent work of Ochsenfahrt and Winne (15).

It would seem appropriate at this point to speculate as to how a diminution in intestinal blood flow might be expected to retard the intestinal absorption of drugs. A decrease in the blood perfusion of the mucosa and submucosa could result in the accumulation of absorbed solute in the mucosal and submucosal cells. Since many drugs are believed to be absorbed by the simple process of passive diffusion, it would seem rational to assume that decreased blood flow, by virtue of its diminishing the concentration gradient across the mucosal membrane, could interfere with the intestinal absorption of drugs.

Before concluding, a final comment should be made regarding the possible effects of fasting on mesenteric blood flow. Achord (5) points out that in humans during the first 48 to 72 hr. of a fast there occurs a reduction in plasma volume and as a result the systemic blood pressure characteristically falls. It would not be unreasonable then to speculate that the decreased intestinal drug absorption reported in the present study was, at least in part, the consequence of reduced intestinal blood perfusion. Subjective observation of the intestines after prolonged periods of fasting in these studies supports this view. In absence of prolonged fasting the color of the intestines is a reddish pink; after prolonged fasting the intestines are blanched.

It is obvious from the above discussion that fasting can produce a multitude of fundamental physiologic and biochemical changes within the organism. Further studies will be required to ascertain whether one or more of these factors was responsible for the intestinal absorption profiles encountered in the course of this investigation. Initial experimentation will evaluate the effect of mesenteric blood flow on drug absorption.

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 21, 1969, from the *University of Kentucky, College of Pharmacy, Lexington, KY 40506*

Accepted for publication February 25, 1969.

This investigation was supported, in part, by a research grant from McNeil Laboratories, Fort Washington, PA 19034