Effect of Mesenteric Blood Flow on Intestinal Drug Absorption

Keyphrases □ Drug absorption, intestinal—mesenteric blood flow effect □ Mesenteric blood flow—drug absorption □ Sulfaethidole half-life—mesenteric blood flow

Sir:

A previously published report from our laboratory indicated that drug absorption rates in rats slowed for several drugs when the animals were fasted for periods beyond 20 hr. (1). These results suggested that some nonspecific phenomenon was occurring during prolonged fasting which affected absorption rates about equally for each drug. Although a multitude of physiological and biochemical changes are known to occur during fasting (1), particular interest is the possibility that intestinal blood flow decreases during periods of inanition. In our previous studies, subjective observation of rat intestine, following prolonged periods of fasting, supported the view that the decreased drug absortion rate was, at least in part, the consequence of reduced intestinal blood perfusion. In the absence of prolonged fasting, the color of the intestines was reddish pink; but after prolonged fasting, the intestines became blanched.

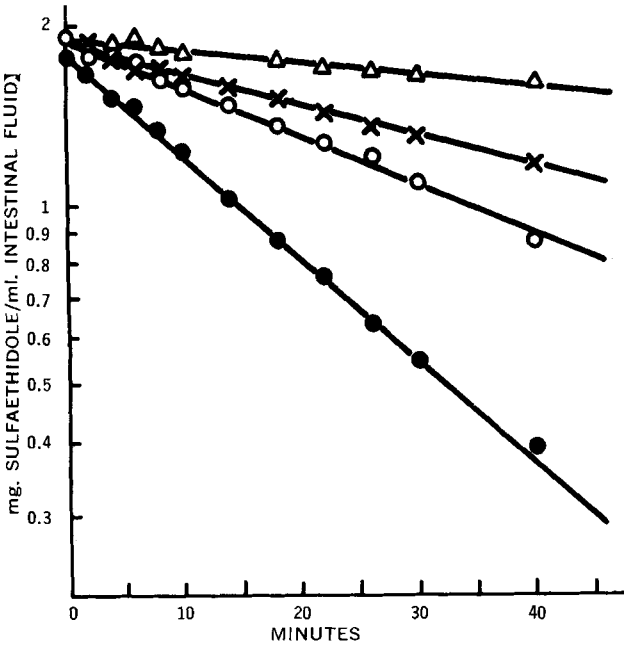

Figure 1—*Semilogarithmic plot of the disappearance of sulfaethidole from the dog intestinal lumen of Dog No. 1 as a function of time for four intestinal blood flow rates. Key:* ●, *100%;* ○, *64%;* ×, *26%; and* △, *0%.*

Table I—Effect of Mesenteric Blood Flow on the Absorption of Sulfaethidole

Dog No.	Approximate Blood Flow Rate, % 100	65	30	0
	Absorption Half-Life, min.			
1	17	37	57	134[a]
2	26	31	76	143[a]
3	26	39	78	122[a]
Mean	23	36	70	133[a]
SD	5.2	4.1	11.6	10.5

[a] Approximate values obtained by extrapolation of disappearance curve.

The possibility that the unusual drug absorption pattern, which was encountered in fasted animals, was the result of an alteration in intestinal blood perfusion led us to investigate the effects of changes in intestinal blood flow on the kinetics of drug absorpion. Three mongrel dogs (8–19 kg.) were anesthetized with allobarbital–urethane, and the small intestine was exposed by midline laparotomy. A segment of the jejunum was cannulated, and approximately 12 luminal samples were withdrawn over a 40-min. period using a double-syringe method for absorption determination (2). The cranial mesenteric artery was exposed from its origin at the aorta to the first branch, a blood flow probe was implanted on the artery, and blood flow was measured by means of an electromagnetic flowmeter (Medicon). Carotid arterial blood pressure, measured by means of a pressure transducer (E & M Linear Core), and cranial mesenteric blood flow were recorded simultaneously on a direct-writing polygraph. Intestinal solutions were buffered at pH 6.0 and warmed to 37° (2).

Initially, serial luminal samples were collected from animals with intestinal blood flow unaltered. The blood supply to the intestines was then gradually diminished by means of an hydraulic occluder located immediately distal to the flow probe. In this way, we determined the effect of reducing the rate of volume flow to approximately 65, 30, and 0% of control values on the absorption profile of sulfaethidole. Sulfaethidole concentrations were determined by the method of Bratton and Marshall (3).

Figure 1 shows the results of a typical experiment using the *in situ* dog intestinal preparation, in which the rate of disappearance of sulfaethidole from the jejunum was followed at several intestinal blood flow rates. Each line represents a separate gut segment, but all four determinations are from the same dog. Half-lives of 17, 37, 56, and 134 (approx.) min. were found for 100, 64, 26 and 0% of control blood flow rate, respectively. In these experiments, the absorption

process obeyed apparent first-order kinetics. The results obtained from three animals are shown in Table I.

Preliminary experimentation indicated that half-lives obtained from different jejunal segments in the same dog did not vary from one another by more than 15%. These results are thus suggestive of a meaningful relationship between intestinal blood perfusion and drug absorption rate. In most instances, a 40–60% reduction in mesenteric blood flow resulted in a dramatic increase in the absorption half-life for sulfaethidole.

Mesenteric circulation is subject to alteration from a wide variety of sources, and some of these will be examined and discussed in a future publication. However, it is obvious that adequate precautions should be taken to assure that significant differences in intestinal blood perfusion rates do not exist among the different animals used in a particular absorption study. Unless such precautions are taken, comparisons of the drug absorption data obtained from the various animals should be viewed with a degree of caution.

Although the data reported herein do confirm that the intestinal drug absorption process is hindered by a decrease in vascular perfusion, additional studies must be designed to quantitate the effects of fasting on intestinal blood flow before ascribing a causative role to this factor in our previous experiments.

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Spectrophotometric Analysis of Acetylcholine Levels in Plasma

Keyphrases ☐ Acetylcholine levels, plasma—determination ☐ Plasma—acetylcholine determination ☐ Colorimetric analysis—spectrophotometer

Sir:

The interest in determining acetylcholine levels in biological media before and after angiotensin-II administration parenterally in turtles led to the search for an acceptable method of analysis. The bioassay of isolated guinea pig ileum contractions against known acetylcholine concentrations was initially considered (1), but the sensitivity of the test was proven unacceptable. The bromocresol purple method of Woods (2) and the methyl orange method of Brodie (3, 4) are general methods for determining organic bases and are limited in their usefulness for biological media, which normally contain interfering organic bases.

The bromophenol blue method of Auerbach (5), modified by Mitchell and Clark (6), was chosen because it bypassed this difficulty of interfering bases and increased the sensitivity of the test 10-fold.

The interaction involves the formation of a color complex between anionic bromophenol blue and the quaternary cation, acetylcholine. The reaction occurs in an alkaline medium with the resulting color complex formed being quantitatively extracted, utilizing organic solvents. The advantage of this method is that organic bases and unreacted dye do not interfere with the extraction. Auerbach (5) also has tested 50 tertiary amines with negative results.

Procedure—Three map turtles heparinized with 100 USP units were utilized for the analysis. Each turtle was treated with 20 mg./kg. of physostigmine salicylate 5 min. prior to withdrawal of the blood sample. One milliliter of blood was removed from each turtle and added to 0.5 ml. of a 0.5% physostigmine solution. The resulting mixture was centrifuged for 15 min. at 2000 r.p.m., and a 0.5-ml. plasma sample was used for analysis. Following the addition of a buffer (0.3 g. of K_2HPO_4 and 0.3 g. of Na_2CO_3) to pH 9, the indicator, bromophenol blue, was added in a 0.5-ml. volume (0.08% in 30% K_2HPO_4). Fifteen minutes of shaking with organic solvents (washed ethylene dichloride and 4% isoamyl alcohol) completed the extraction of the dye–acetylcholine complex. The organic phase was read at 600 mμ against an ethylene dichloride–isoamyl alcohol blank because none of the other reagents absorbs. It is mandatory that the analysis take place within a 1-hr. time span because of the rapid fading of the indicator after this period. All absorbances were read on the Coleman (Hitachi 124) double-beam spectrophotometer. The procedure was then repeated following angiotensin-II administration. The differences in absorbance are due to increased acetylcholine levels. This was again repeated, using turtles with both vagi surgically severed. In the turtles with intact vagi, the following values were read: plasma sample plus physostigmine, 0.040, 0.055, and 0.043—mean = 0.046 ± 0.008 *SD;* plasma sample plus physostigmine after administration of angiotensin, 0.395, 0.410, and 0.380—mean = 0.395 ± 0.014 *SD*. The difference in absorbance, 0.349 mμ, is attributed to increased acetylcholine blood levels and corresponds to a concentration of 9.1 mcg./0.5 ml. on the standard curve. When this was repeated in vagotomized turtles, the following values for absorbance were read: plasma sample plus physostigmine, 0.050, 0.040, and 0.045—mean = 0.045 ± 0.004 *SD;* plasma sample plus physostigmine in angiotensin-treated turtles, 0.290, 0.285, and 0.305—mean = 0.293 ± 0.010 *SD*. The difference in absorbance between the two means, 0.248 mμ, is attributed to increased acetylcholine blood levels and corresponds to