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Drug Absorption VII: Influence of Mesenteric Blood Flow on Intestinal Drug Absorption in Dogs

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Abstract □ Intestinal absorption of sulfaethidole and haloperidol was determined using an *in situ* canine intestinal preparation. Intestinal absorption of sulfaethidole was determined at three or four mesenteric blood flow rates in each dog, ranging from unaltered flow (100%) to no flow (0%). A relatively small change in absorption rate occurred when the splanchnic blood flow rate was decreased about 35%. Further reductions in mesenteric blood flow resulted in progressive impairment of sulfaethidole absorption. The simultaneous measurement of sulfaethidole intestinal disappearance and appearance in blood indicates that sulfaethidole disappearance is equivalent to absorption. Haloperidol absorption also decreased with decreased intestinal perfusion but differed from sulfaethidole in that membrane storage of haloperidol appeared to take place during its absorption.

Keyphrases □ Absorption, drug—influence of mesenteric blood flow on intestinal absorption of sulfaethidole and haloperidol, *in situ* canine intestinal preparation □ Drug absorption—influence of mesenteric blood flow on intestinal absorption of sulfaethidole and haloperidol, *in situ* canine intestinal preparation □ Sulfaethidole—influence of mesenteric blood flow on intestinal absorption, *in situ* canine intestinal preparation □ Haloperidol—influence of mesenteric blood flow on intestinal absorption, *in situ* canine intestinal preparation □ Blood flow, mesenteric—influence on intestinal absorption of sulfaethidole and haloperidol, *in situ* canine intestinal preparation

Interest in the influence of blood flow on the GI absorption rate of drugs was stimulated by the observation (1) that periods of fasting greater than 17 hr in rats resulted in decreased rates of drug absorption. This decrease in absorption was accompanied by a concomitant blanching of the intestine, which became more pronounced with longer fasting periods. The observed changes appeared to be due to changes in intestinal blood perfusion (2).

There are many documented cases of pathological conditions leading to decreased intestinal blood perfusion. One study (3) showed that experimentally induced ventricular and supraventricular tachycardias can cause mean superior mesenteric blood flow to drop nearly 40%. Other investigators (4) reported that hemorrhagic necrosis, infarction, and gangrene of the intestinal tract secondary to decreased splanchnic perfusion may result from rapid tachycardia. A high association was reported (5) between congestive heart failure and nonobstructive intestinal ischemia. In one case, intestinal ischemia resulting from congestive heart failure produced digoxin malabsorpion severe enough to make the patient's clinical condition difficult to control (6). Intramuscular administration of digitoxin produced marked improvement in the patient. These reports suggest that the absorption of vital drugs in patients with circulatory pathology may be dangerously reduced.

BACKGROUND

It has long been known that heat and exercise produce a significant reduction in splanchnic blood flow (7–9). A study with five volunteers found that light exercise for 7–8 min decreased splanchnic blood flow by 20% (8). A fall in hepatic and splanchnic blood flow, sometimes in excess of 80%, during severe exertion was clearly demonstrated (9). Thus, it is conceivable that drug absorption following three sets of tennis or during strenuous exercise on the job could be quite different from that encountered under more restful circumstances. In this regard, exercise in a hot environment significantly decreased the absorption of 3-*O*-methylglucose but not D-xylose (10). Thus, exercise or the lack of it during bioavailability studies (studies of drug product performance) could greatly influence the apparent availability and rate of absorption of the product.

The interaction between the circulation and intestinal absorption is complex; blood flow influences absorption, but the reverse also holds true. Digitalis has been shown to be a potent splanchnic vasoconstrictor, and cardiac glycosides have precipitated intestinal necrosis (11). A reduction in splanchnic blood flow of 30–40% was found in human volunteers following a 0.25-mg dose of ouabain (12). The same effect appears to be true for digitalis (12).

The use of oral contraceptives in women also appears to affect significantly the mesenteric blood flow. Numerous reports have appeared in the last 10 years (13–15) linking the use of oral contraceptives in women to decreased intestinal blood flow. Of 24 collected cases of ischemic disease of the colon in younger persons, six were women taking oral contraceptives (13). The role of oral contraceptives in vascular diseases is unclear, and possible associated

factors include alterations in the clotting mechanism, thickening of the vessel wall (16), and decreased blood flow (17). In some of the cases reported (14, 15), the clinical symptoms disappeared upon discontinuance of the medication but returned following its reinstitution. Therefore, changes in splanchnic blood flow produced by one drug could significantly influence the rate of absorption of a concurrently administered drug.

Despite the paucity of information available concerning the influence of intestinal blood flow on drug absorption, several recent studies have appeared concerning this topic. Most of these studies dealt with the absorption of nutrients and entailed interruption of the circulation either by collection of the mesenteric venous blood or by excorporeal perfusion of the intestine. By far the most complete study was performed by Winne and coworkers (18, 19), in which intestinal blood flow was regulated by varying the rate of blood infusion through the jugular vein of the rat, with absorption being determined by mesenteric venous collection. These investigators found that the intestinal absorption of aminopyrine, antipyrine, benzoic acid, salicylic acid, and ethanol was dependent on intestinal blood flow while ribitol was independent of blood flow (18, 19). These investigators also demonstrated the influence of blood flow on intestinal water flux (20) and on the absorption of an actively absorbed amino acid, phenylalanine (21).

A study of the absorption and metabolism of salicylamide in the rabbit found both of these processes to be profoundly influenced by intestinal blood flow (22). In an interesting study (23), partial ligation of the canine superior mesenteric artery *in vivo* resulted in the induction of a chronic malabsorption state characterized by decreased absorption of D-xylose and triolein. The intestinal absorption of the cardiac glycosides digitoxin and digoxin in the guinea pig was strongly dependent on portal blood flow (24). Williams *et al.* (10) reported that the absorption of glucose and xylose was significantly reduced when mesenteric blood perfusion *via* a pump was reduced 50%.

This report discusses in detail studies of the effects of an induced reduction in mesenteric blood flow on the rates of absorption of sulfaethidole and haloperidol from the lumen of intestinal loops, *in situ*, in the anesthetized dog.

EXPERIMENTAL

Test Animals—Mongrel dogs of either sex, 10–19 kg, were rendered free of parasites and held under the care of a veterinarian for 24 days prior to use. All animals were fasted for 16 hr prior to surgery, with water freely available.

Surgical Preparation—General anesthesia was induced 1 hr prior to surgery with 0.6 ml/kg iv allobarbital–urethan[1] (100 mg of allobarbital and 400 mg of urethan/ml). If additional anesthesia was needed during the surgical procedure, the animal received one-fourth to one-half of the initial anesthetizing dose. Following the onset of anesthesia, an endotracheal tube was inserted. Respiration was normally spontaneous and rhythmical but was supported mechanically when necessary.

A polyethylene catheter was inserted into the femoral artery to monitor arterial pressure. A venous catheter was inserted into the vena cava *via* the femoral vein for the removal of blood samples and administration of drugs or replacement solutions. Core body temperature was monitored rectally and maintained constant with a heating pad directly under the animal. Arterial blood pressure was measured using a linear core pressure transducer and recorded by a direct writing polygraph. Mean arterial blood pressure was normally maintained above 80 mm Hg. When mean arterial blood pressure dropped below this value for a significant period, the experiment was terminated and the animal was sacrificed. In cases where blood specimens were taken, blood volume was replaced with saline or dextran solution. This amount was usually 7% or less of total blood volume.

Implantation of Blood Flow Probe—Following a midline laparotomy, the entire small intestine was gently removed from the abdominal cavity, with care being taken not to disturb intestinal blood flow. The intestine was kept moist and warm during the remainder of the procedure. The superior mesenteric artery, the only source of blood in the section of the intestine used, was exposed from its origin at the aorta to the first branch, and a 3- or 4-mm noncannulating electromagnetic flow transducer[2] was then placed on the exposed artery proximal to any of its branches. The transducer was connected to a gated sign wave electromagnetic flowmeter[3] for determination of blood flow. A hydraulic blood vessel occluder[4] was placed distal to the transducer.

This arrangement allowed blood flow to the intestines to be reduced as needed and allowed constant monitoring of the flow rate. The intestines were then gently returned to the peritoneal cavity. Normally this entire isolation and implantation procedure took no more than 20 min. Following its completion, the abdomen was closed and a 1-hr stabilization period was allowed.

Preparation of Intestinal Absorption Loop—Following the 1-hr stabilization period, part of the small intestine was again exposed and the ligament of Treitz was visualized. An incision in the jejunum 30.5 cm (12 in.) distal to the ligament of Treitz was made, and a second incision was made 30.5 cm (12 in.) distal to the first. The incisions were made on the side of jejunum opposite the mesenteric attachment, and care was taken to reduce bleeding as much as possible. Two polyethylene cannulas with inflatable rubber tracheal cuffs slipped over the intestinal ends were inserted into the incisions and ligated in place. The tracheal cuffs were then inflated to prevent movement of the cannulas.

Care was taken during the entire surgical procedure to keep trauma to the intestine at a minimum. The intestine was returned to the abdominal cavity, covered with gauze, and allowed to recover for several minutes. In studies in which blood flow was altered, two or three additional intestinal segments were measured distally from the first and prepared in a similar fashion.

Determination of Drug Absorption Rates from Intestinal Loops—Following the attachment of a 60-ml syringe barrel to each cannula, perfusion fluid (25) warmed to 37° was slowly passed through the cannulated segment until the effluent was clear. Several aliquots of drug solutions (2.7 g/liter sulfaethidole or 0.7 g/liter haloperidol buffered to pH 6.0 with 0.14 *M* phosphate buffer and adjusted to isotonicity with sodium chloride) were passed through the cannulated intestinal segment and removed. Following this washing, 30 ml of fresh drug solution was introduced into the segment. Serial samples of 0.5 ml were removed as needed and assayed for sulfaethidole as described by Bratton and Marshall (26) or for haloperidol as described previously (25). Venous blood samples were obtained as needed and assayed for sulfaethidole (26).

In experiments where absorption was determined only at the control blood flow rate, a single jejunal segment was used in each dog. In experiments where blood flow was altered, a total of three or four jejunal segments was studied in each dog. At the end of a study on a given jejunal segment, the remaining drug solution was washed out of the lumen with perfusion solution, the cannulas were removed, and another jejunal segment was prepared immediately distal to the preceding segment.

Determination of Mesenteric Blood Flow Rate during Drug Absorption—To determine absolute blood flow rates, a standard curve was constructed each day by perfusing the transducer with known flow rates of saline. After implantation of the transducer, it was also necessary to close completely the hydraulic occluder on the mesenteric artery to obtain a transient recording of zero flow.

When several flow rates were to be studied in the same animal, unrestricted blood flow (100% of control) was measured and utilized for the first jejunal segment. Then flow rates of approximately 65, 35, and 0% of control were created by adjusting the hydraulic occluder, and subsequent segments were studied. Following each reduction in blood flow rate, several minutes was allowed for the animal to reequilibrate at the new condition before the absorption rate study was begun.

Although the intestinal blood flow was generally constant over the absorption period, small fluctuations did occur in a few cases. The flow rates reported are the actual observed mean flow rates rather than predetermined values.

Pharmacokinetics of Sulfaethidole in Anesthetized Dogs following Intravenous Administration—The animals were pre-

[1] Dial-Urethane, Ciba Laboratories.

[2] Medicon model Q-2030A, Medicon Instruments, Los Angeles, Calif.

[3] Medicon microflow model K 2000, Medicon Instruments, Los Angeles, Calif.

[4] Model VO-3, In Vivo Metric System, Los Angeles, Calif.

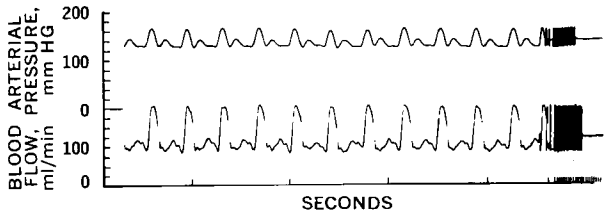
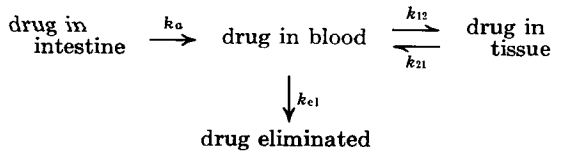


Figure 1—*Physiograph tracing of mesenteric blood flow and arterial blood pressure. Horizontal lines on right side of figure are the mean values. Tracings on left and center of figure are pulsatile values.*

pared in exactly the same way as already described except that isotonic buffer instead of drug solution was placed in the jejunal segments. An intravenous bolus injection of either 350 or 500 mg of sulfaethidole dissolved in isotonic saline was administered to each dog, and the concentration in blood at various times postadministration was determined by the method of Bratton and Marshall (26). The buffer in the jejunal lumen was also analyzed for sulfaethidole.

Pharmacokinetic Evaluation of Sulfaethidole Data—Pharmacokinetic analysis of sulfaethidole blood levels following intravenous and intestinal administration was accomplished using the SAAM25 program[5] (27). This program uses the differential equations implied by the compartmental model chosen and adjusts the model parameters until the best least-squares fit to the experimental results is obtained. In this case, computer fitting took place between the theoretical calculated levels of sulfaethidole in the blood compartment and the experimentally determined sulfaethidole blood levels using the two-compartment open model (Scheme I).



Scheme I

The rate constant k_a is the rate constant for absorption, k_{12} is the rate constant for transfer from the blood to the tissue compartment, k_{21} is the rate constant for transfer from the tissue compartment to the blood compartment, and k_{el} is the rate constant for elimination. Assumptions made in using this program include the assumption of approximate first-order kinetics for the transfer of drug between compartments and the assumption of constancy of the values of the rate constants and apparent volumes of the compartments during the experiment. The computer, in fitting the data to the theoretical curve, calculates the amount of drug in the various compartments of the model. Thus, it is possible to estimate from the blood level data the amount of sulfaethidole remaining unabsorbed from the intestinal compartment as a function of time following an intestinal dose of the drug. All data analyzed by the computer were individual data, but good agreement was found between average blood concentrations and theoretical curves predicted from the average of the individual rate constants. Initial estimates of the rate constants were determined by graphical analysis of the data.

The SAAM25 program can also be used as a digital analog computer by locking the rate constants and the volume of distribution into the program and using it to predict blood level profiles based on these values. In experiments where multiple doses of sulfaethidole were given, the rate constants and the volume of distribution for all processes except absorption were locked in. The rate constant for absorption was adjusted in conjunction with the observed absorption rate, and the calculated blood level curve was determined for each absorption period.

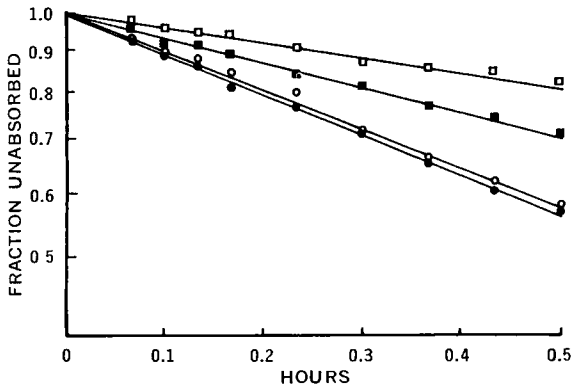


Figure 2—*Intestinal disappearance of sulfaethidole in Dog 30 at 100 (●), 64 (○), 35 (■), and 0 (□) % of control blood flow. Corresponding half-lives are 0.60, 0.626, 1.01, and 1.73 hr, respectively.*

[5] On an IBM 360-75 computer.

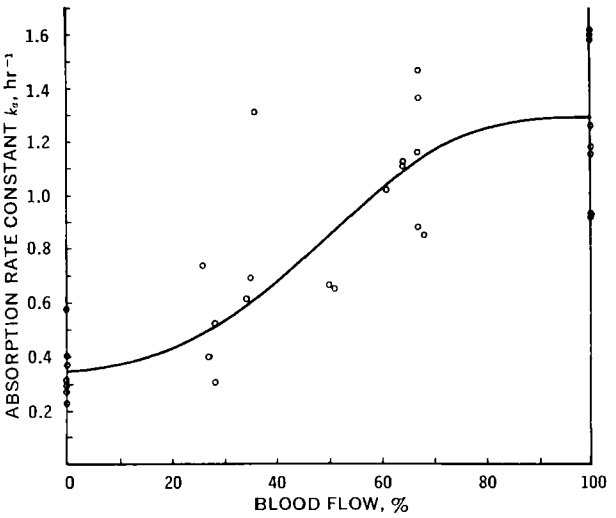


Figure 3—*Relationship between the absorption rate constant, k_a, and superior mesenteric blood flow. Each point represents one intestinal absorption experiment at one blood flow. Data are from Table I.*

RESULTS AND DISCUSSION

Intestinal Absorption of Sulfaethidole—Superior mesenteric

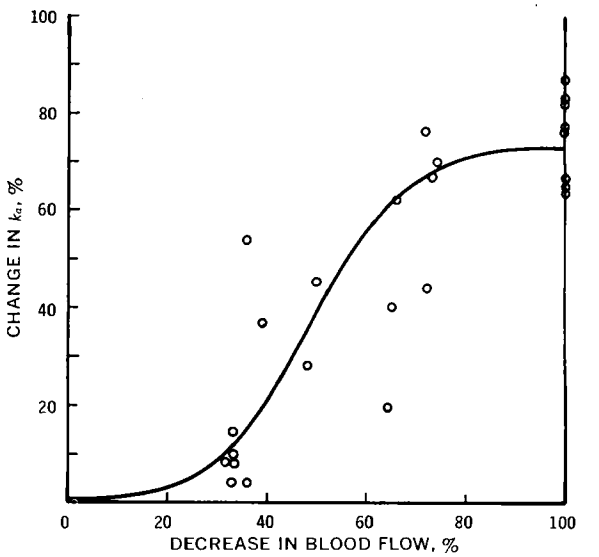


Figure 4—*Relationship between the percent change in the absorption rate constant, k_a, and the percent change in mesenteric blood flow. Data are from Table I.*

Table I—Comparison of Mesenteric Blood Flow and Sulfaethidole Absorption

Dog	Experiment	Blood Flow				Sulfaethidole Absorption		
		ml/min	ml/min/kg	% Flow	% Change in Flow	k_a[a], hr^{-1}	% Change in k_a	Half-Life, hr
13	1	165	12.0	100	0	0.924	0	0.750
	2	85	6.2	50	48	0.660	29	1.05
	3	113	8.2	68	32	0.848	8	0.817
14	1	248	20.2	100	0	1.188	0	0.583
	2	126	10.2	51	49	0.650	45	1.067
	3	68	5.5	27	73	0.392	67	1.767
	4	0	0	0	100	0.267	78	2.60
15	1	116	11.6	100	0	1.599	0	0.433
	2	71	7.1	61	39	1.014	37	0.680
	3	0	0	0	100	0.371	77	1.867
17	1	210	12.8	100	0	1.599	0	0.433
	2	141	8.6	67	33	1.363	15	0.508
	3	72	4.4	34	66	0.611	62	1.133
	4	0	0	0	100	0.291	82	2.383
20	1	112	9.8	100	0	0.924	0	0.750
	2	68	6.0	67	33	0.885	4	0.783
	3	22	1.9	28	72	0.520	44	1.333
	4	0	0	0	100	0.310	67	2.233
21	1	122	7.9	100	0	1.260	0	0.550
	2	82	5.3	67	33	1.155	8	0.600
	3	37	2.4	28	72	0.297	76	2.333
	4	0	0	0	100	0.222	82	3.116
29	1	241	15.6	100	0	1.624	0	0.427
	2	162	10.5	67	33	1.464	10	0.473
	3	87	5.6	36	64	1.308	20	0.530
	4	0	0	0	100	0.571	65	1.213
30	1	159	8.5	100	0	1.155	0	0.600
	2	107	5.8	64	36	1.106	4	0.626
	3	56	3.0	35	65	0.687	41	1.008
	4	0	0	0	100	0.400	65	1.733
34	1	160	16.0	100	0	2.445	0	0.280
	2	102	10.2	64	36	1.124	54	0.617
	3	42	4.2	26	74	0.736	70	0.942
	4	0	0	0	100	0.310	87	2.33

[a] k_a = absorption rate constant.

blood flow rates were monitored during the entire experiment; a typical recording of mesenteric blood flow is shown in Fig. 1. Although very little fluctuation generally occurred, the blood flow rates reported in Table I are the average flow rates recorded over the entire absorption experiment. The observed control blood flow rates found here compare favorably with those reported in the literature. Mean superior mesenteric blood flow rates of 12.7 ± 4.0 ml/min/kg (mean ± *SD*) (listed individually in Table I) compare closely to the 14.7 ± 4.0 ml/min/kg (mean ± *SD*) reported (28) for fasted, nonanesthetized dogs with chronically implanted flow probes.

Absorption of sulfaethidole appeared to follow first-order kinetics at all blood flow rates studied. Figure 2 shows typical intestinal disappearance curves for four different blood flow rates in a single dog (Dog 30). Absorption half-lives of 0.60, 0.626, 1.01, and 1.73 hr were found for mesenteric blood flow rates of 100, 64, 35, and 0% of control flow, respectively. Similar results for eight additional dogs are listed in Table I. The data are plotted in Fig. 3 as the absorption rate constant, k_a, *versus* the percent of control blood flow and in Fig. 4 as the percent change in the absorption rate constant *versus* the percent change in mesenteric flow. The scatter in the data reflects the animal to animal variation due to using mongrel dogs of various weights, ages, and sexes.

The results suggest that the rate of intestinal absorption of sulfaethidole is not linearly related to the rate of mesenteric blood perfusion. A decrease of about 35% in perfusion rate caused a relatively small decrease in intestinal absorption rate. Following this initial period, the absorption rate appeared to decline linearly with decreasing perfusion rate until reaching approximately 30% of control flow, where the curve again became nonlinear. There was no statistically significant difference ($p \geq 0.05$) between the absorption rate constants found at 100% blood flow and those at 61–74% blood flow. However, there was a statistically significant difference between the absorption rate constants obtained at 61–74% and 26–36% blood flow ($p \leq 0.01$) and between 26–36% and zero blood flow ($p \leq 0.05$).

The terminal nonlinearity in Figs. 3 and 4 is most likely not directly related to mesenteric blood flow since the linear portion of the curve in Fig. 3 can be extrapolated to approximately zero absorption rate at zero blood flow. Although it is generally assumed for most drugs that transfer from the intestine into the general circulation by routes other than transport by mesenteric blood is minimal (29), it is possible that alternative pathways became more

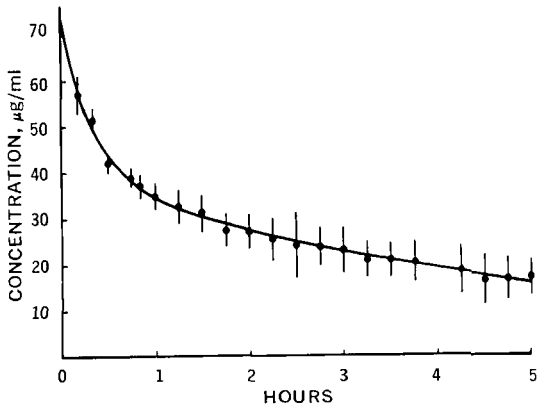


Figure 5—*Mean sulfaethidole blood levels following intravenous administration of 500 mg in three dogs. The circles (●) represent experimental data points, and the line represents the SAAM25 computer-fit curve based on the two-compartment open model. The rate constants used to draw this line are the means of individual fits found in Table II.*

Table II—Rate Constants following Intravenous Sulfaethidole Administration[a]

Dog	V_p, liters	$V_{p/kg}$, liters	k_{12}, hr^{-1}	k_{21}, hr^{-1}	k_{el}, hr^{-1}
		350-mg Dose			
23	3.23	0.222	3.32	1.41	0.209
24	5.92	0.362	2.52	1.94	0.349
25	5.62	0.413	2.32	2.67	0.329
Mean	4.93	0.332	2.72	2.01	0.296
SD	±1.49	0.099	0.53	0.63	0.076
		500-mg Dose			
40	8.93	0.633	0.706	1.281	0.249
41	6.58	0.603	0.722	1.346	0.540
48	5.92	0.290	2.64	3.06	0.217
Mean	7.14	0.509	1.36	1.89	0.335
SD	±1.58	0.190	1.11	1.01	0.178

[a] See Scheme I for explanation of terminology.

important under the conditions of low blood flow or no blood flow. The most likely explanation for this absorption at zero blood flow relates to drainage of the intestines by the lymphatic system, although other possible mechanisms have not been ruled out. In this regard, DeMarco and Levine (29) found that a significant amount (20%) of *p*-aminosalicylic acid was transported by the lymphatic system when the intestinal blood flow was reduced to zero, and Williams *et al.* (10) reported that approximately 27% of control D-xylose absorption occurred at zero blood flow. This value compares favorably with the 27% of control sulfaethidole absorption found in this study at zero blood flow.

Implantation of the flow probe appeared to have little effect on the intestinal absorption rate. Half-lives for intestinal absorption in a single dog before and after implantation of the flow probe were 0.54 and 0.58 hr, respectively. Intestinal viability at most blood flow rates did not appear to be a problem during the time period covered by the experiment. Identical absorption half-lives of 0.63 hr were found for two repetitive absorption experiments carried out in the same intestinal segment. In another experiment in which blood flow was decreased and then later increased, absorption half-lives of 0.75, 1.05, and 0.82 hr were found for blood flow rates of 100, 50, and 68%, respectively.

Little difference in absorption rate was found between the four intestinal segments used. For example, absorption half-lives of 0.45, 0.45, and 0.49 hr were found in intestinal segments 1, 3, and 4, respectively, in one dog with unaltered blood flow. These results are in agreement with other data from these studies indicating that no change occurred in the intrinsic intestinal absorption capacity or in jejunal viability with decreased blood flow.

Pharmacokinetics of Sulfaethidole after Intravenous Administration—The pharmacokinetic profile of sulfaethidole was characterized by intravenous and intestinal administrations under normal intestinal blood flow and by intestinal administration under decreased blood perfusion. Individual blood concentration–time profiles following intravenous administration of 350- and 500-mg doses of sulfaethidole were fit to the two-compartment open model (Scheme I) using the SAAM25 program (27). The rate constants k_{12}, k_{21}, and k_{el} and the volume of distribution, V_p, for the intravenous data are shown in Table II. No significant difference ($p \geq 0.05$) in the rate constants was found between the 350- and 500-mg doses.

Figure 5 shows the results for the 500-mg dose where the data points are the mean blood levels for three dogs and the line is the computer-estimated blood level based on the average of the individual rate constants for the three dogs (Table II). The data appear to be well described by the two-compartment open model. In the case of sulfaethidole, the intestine does not appear to be a preferential volume of distribution, as has been indicated for some other drugs. Prior to the intravenous injection of sulfaethidole, an intestinal segment similar to the one used in the absorption experiment was prepared but filled with buffer rather than drug solution. Less than 5% of the injected dose returned to the intestine during the experiment, with sulfaethidole intestinal levels consistently below blood levels.

Pharmacokinetics of Sulfaethidole after Intestinal Administration—*Control Blood Flow*—In the described experiments, disappearance from the intestine rather than absorption (*i.e.*, appearance in blood) was measured. To show that the disappearance rates determined in these experiments were equivalent to the absorption rates, sulfaethidole was administered to 10 dogs by a single *in situ* jejunal segment prepared as described in the *Experimental* section. Disappearance of sulfaethidole from the intestine as well as appearance in blood was determined; individual blood concentration–time data following sulfaethidole administration were analyzed by the SAAM25 program (27) using the two-compartment open model. Initial estimates of the rate constants were

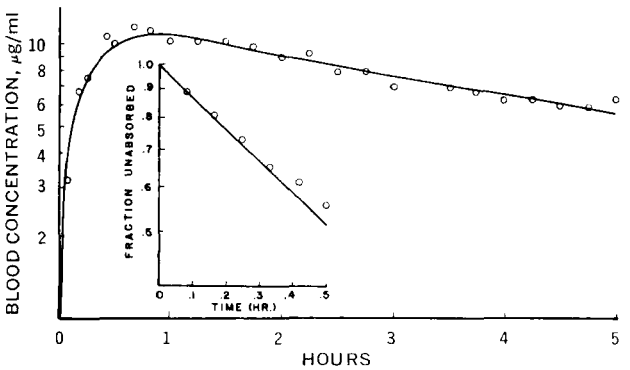


Figure 6—*Blood concentration of sulfaethidole following intestinal administration in Dog 37. The line represents the computer-calculated blood concentrations, and the circles (○) represent experimental data points. Rate constants used in generating the curve are listed in Table III. In the inset, the open circles represent the experimentally determined fraction of sulfaethidole unabsorbed from the intestine and the line represents the computer-predicted values based on the blood concentrations in this dog.*

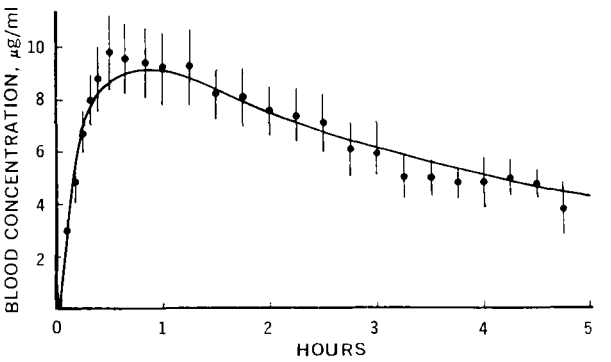


Figure 7—*Mean blood levels of sulfaethidole in 10 dogs following intestinal administration. The bars represent standard errors of the mean. The line represents the computer-calculated blood concentrations based on the mean rate constants (Table III) for these 10 dogs.*

Table III—Calculated and Observed Rate Constants for Sulfaethidole following Intestinal Administration[a]

Dog	V_p, liters	$V_{p/kg}$, liters	k_{12}, hr^{-1}	k_{21}, hr^{-1}	k_{el}, hr^{-1}	k_a (calc), hr^{-1}	k_a (obs), hr^{-1}	Sum of Squares, $\times 10^{-4}$
28	6.25	0.431	3.63	0.569	0.103	0.980	0.950	0.0017
37	1.35	0.123	2.58	0.716	0.751	0.699	0.690	0.035
39	3.55	0.300	3.20	0.893	0.570	1.280	1.07	0.014
42	0.606	0.041	1.98	0.000	0.909	0.217	0.385	0.042
43	3.33	0.272	0.543	0.222	0.373	0.853	0.790	0.480
44	2.39	0.128	1.43	3.050	0.975	2.050	1.88	0.120
45	2.30	0.120	0.949	1.220	0.900	1.000	0.885	0.062
46	2.10	0.121	1.39	0.443	0.798	1.280	1.070	0.077
47	2.53	0.164	1.78	1.030	0.379	1.350	1.150	0.220
49	1.47	0.104	2.35	0.964	0.506	0.850	0.730	0.690
Mean	2.58	0.180	1.98	0.910	0.626	1.05	0.960	
SD	± 1.56	0.117	0.973	0.841	0.286	0.482	0.394	

[a] See Scheme I for explanation of terminology.

obtained from the intravenous data. Typical data showing how the observed blood concentration–time profile is used to calculate the absorption rate in a single dog (Dog 37) are shown in Fig. 6. The rate constants calculated in this fit are found in Table III. The solid line indicates the SAAM25 estimated least-squares regression fit. The inset in this figure shows as the data points the observed intestinal disappearance rate with the solid line indicating the disappearance rate estimated by SAAM25 from the blood concentration–time data in this figure. Observed and calculated absorption half-lives of 0.99 and 1.0 hr, respectively, were found. The rate constants obtained by a similar treatment for nine additional dogs are shown in Table III.

Figures 7 and 8 show this same general relationship for all 10 dogs studied. In Fig. 7 the data points are the average blood levels for the 10 dogs and the solid line represents the blood levels generated by the SAAM25 program from the average of the individual rate constants for these dogs (Table III). Figure 8 compares the average observed fraction of the dose remaining unabsorbed in the jejunum with a line with a slope corresponding to the average absorption rate constant estimated by fitting with SAAM25 the individual blood concentration–time data in the 10 dogs. The observed and calculated absorption half-lives were 0.72 and 0.66 hr, respectively, which were not significantly different ($p \geq 0.05$). This excellent agreement between observed intestinal disappearance rates and absorption rates calculated from blood level data indicates that disappearance of sulfaethidole from the intestine is equivalent to absorption into the systemic circulation under the experimental conditions. It also indicates that the method of calculation yields results that truely reflect events in the GI lumen.

Two of the 10 dogs listed in Table III, Dogs 42 and 44, showed unusual absorption profiles. Although the reason for these unusual absorption rates was not determined, the SAAM25 program estimated the absorption rates fairly accurately from the blood level data. This further substantiates the validity of this method.

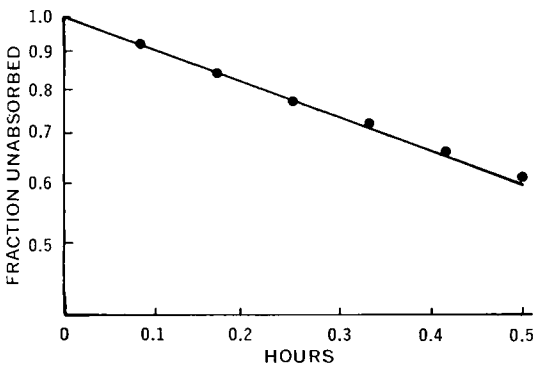


Figure 8—*Comparison of the fraction of sulfaethidole unabsorbed from the intestine and that predicted by SAAM25 from blood concentrations. The points represent the mean of 10 dogs (Table III), and the line represents the computer-calculated fraction based on the average rate constants (Table III) for the 10 dogs.*

Reduced Blood Perfusion—To verify that the kinetics of sulfaethidole described previously are valid in the experiments involving artificial reduction in blood flow and repeated administration of drug, the observed blood concentration–time profile in individual dogs was compared with that predicted by the SAAM25 program with the two-compartment open model. Therefore, the blood level data following the first administration of sulfaethidole (control blood flow) were fit by SAAM25, using the average rate constants from Table III as initial estimates. These calculated rate constants were then fixed and only the absorption rate corresponding to the disappearance of sulfaethidole from the jejunal segments at each blood flow rate was varied to generate the blood concentration–time profiles for all four absorption experiments in each dog. The results of this treatment for Dog 29 are shown in Fig. 9, where the data points are the observed blood levels and the continuous line represents the SAAM25 estimated blood levels based on the intestinal disappearance data at each blood flow. The generally good agreement here indicates that the same relationship described previously between intestinal disappearance and absorption holds even at decreased blood flow rates, and it also confirms that the pharmacokinetic model is accurately reflecting both disposition of the drug and absorption behavior from the jejunal loop.

In the last absorption experiment (IV) in Fig. 9, an increase in the blood level is apparent even though there is no mesenteric blood flow. In the SAAM25 prediction of the blood levels at zero flow, the observed intestinal disappearance rate constant (k_a = 0.571 hr^{-1}) resulted in a much better fit of the data than a rate constant of zero and is the one used in this figure. This observation is in agreement with the observed disappearance data at no flow in the previous experiments, reinforcing the concept that some sulfaethidole absorption does take place when no mesenteric blood flow is present.

Haloperidol Absorption in Reduced Intestinal Blood Perfusion—Sulfaethidole appears to be absorbed by passive diffusion of both ionized and nonionized drug species (31), but it probably has very little potential for membrane storage as described by Doluisio *et al.* (30). On the other hand, haloperidol has been shown to have a potential for membrane storage (30), and it was of interest to determine how this property might affect its absorption at decreased

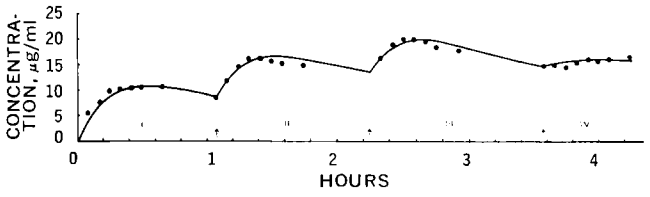


Figure 9—*Blood levels of sulfaethidole for Dog 29 under 100 (I), 65 (II), 35 (III), and 0 (IV) % flow. The circles indicate experimental data points, and the line represents the computer-estimated blood concentrations based on the intestinal disappearance rate constants for Dog 29 as described in the text. The arrows indicate when each dose was administered;* k_{12} = *1.44* hr^{-1}, k_{21} = *1.34* hr^{-1}, k_{el} = *1.26* hr^{-1}, *and* V_p = *2.30 liters.*

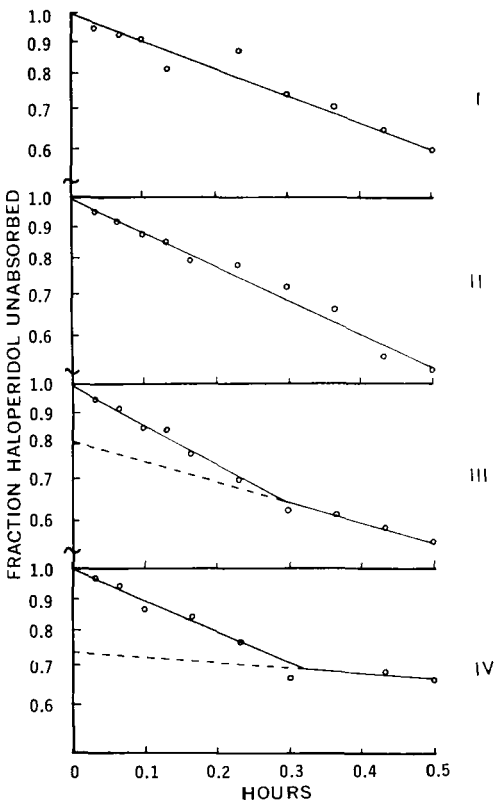


Figure 10—*Disappearance of haloperidol from the intestinal lumen at 100 (I), 70 (II), 37 (III), and 0 (IV) % flow.*

blood flow. The methodology used was similar to that of the previous studies with sulfaethidole, and the results are shown in Fig. 10. Blood levels of haloperidol were not measured because they were much too low for accurate determination. Disappearance of haloperidol from the intestine was linear at blood flow rates of 100 and 70% of control but became biphasic at lower rates of mesenteric blood flow. This finding suggests that not only the rate of intestinal disappearance of haloperidol changes with decreased blood perfusion but that a change in the absorption characteristics also occurs. The change may be due to the mechanism of absorption of haloperidol which appears to involve two steps: *(a)* transfer of haloperidol from the intestinal solution into the membrane (*i.e.*, membrane storage) and *(b)* transfer of haloperidol from the membrane into blood.

Thus, at higher rates of blood flow, *i.e.*, above 70% of control, transfer from the intestinal solution into the membrane would be the slower or rate-limiting step and would determine the overall rate of disappearance from the intestine. As blood flow decreases below 70% of control, however, the transfer of haloperidol from the membrane to blood becomes rate limiting; following the initial partitioning into the membrane, the rate of disappearance from the intestine is determined by the membrane to blood transfer rate. This hypothesis is strengthened by the observation that the initial rate of disappearance of haloperidol from the intestine remains relatively constant with decreasing blood flow while the later biphasic part of the curve appears to decrease with decreasing blood flow (Fig. 10). This was particularly apparent in the last absorption experiment where no blood flow was present, and the terminal biphasic tail of the curve is nearly flat. Membrane storage of the amino acid phenylalanine at several blood flow rates has been reported (21). In these experiments (21), disappearance from the lumen did not equal appearance in the blood and a computer program assuming a disappearance rate equal to an appearance rate did not converge.

CONCLUSION

The absorption rate of sulfaethidole is reduced by a reduction in mesenteric blood flow to the intestines. This effect does not appear to be linear. For reductions in intestinal perfusion of about 30%, a relatively small decrease in the intestinal absorption rate occurs. Changes greater than a 30% reduction in intestinal perfusion result in decreased rates of drug absorption. Measurement of sulfaethidole disappearance from the intestine and appearance in blood indicates that disappearance from the intestine is equivalent to absorption into the circulation. Haloperidol disappearance from the dog jejunal lumen occurs by a two-stage process, and a large amount of haloperidol appears to accumulate in the membrane during absorption.

Abnormal intestinal blood flow rates may be the result of pathological conditions, strenuous exercise, or the presence of other drugs. Changes in the intestinal blood flow rate during therapy could result in the increased or decreased potency of certain vital drugs. Decreased blood flow rates in certain pathological conditions could be responsible for therapeutic failures of some drugs, and differences in exercise or bedrest could be responsible for differences among subjects or poor performance of certain drug products in bioavailability studies.

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In Vivo Method for Monitoring Polysorbate 85 Effect on Epidermal Permeability

KEVIN J. RYAN and MICHAEL MEZEI ˣ

Abstract □ An *in vivo* method of monitoring the rate of water desorption from human forearms, using "dry" nitrogen gas passed over approximately 1 cm^2 of skin was investigated with the aid of a commercial electrolytic moisture analyzer. The assembled apparatus was used to evaluate the differences in water loss rates from treated and untreated (control) forearms following surfactant application. The changes in the differences were also monitored after cessation of treatment, *i.e.*, during the healing process. The apparatus provided an accurate, rapid, and painless method of monitoring relative water loss rates and, as such, could prove a useful tool in routine testing in experimental dermatology and cosmetology. The results confirm the earlier finding from an *in vitro* method with excised rabbit skin that the tested surfactant increases the permeability of the epidermis.

Keyphrases □ Permeability, epidermal—*in vivo* method for monitoring effect of polysorbate 85, relative water loss rates □ Epidermis—permeability, *in vivo* method for monitoring effect of polysorbate 85, treated and untreated human epidermis □ Polysorbate 85—effect on epidermal permeability, *in vivo* method for determining relative water loss rates □ Water loss rates—human epidermis, *in vivo* method

An *in vitro* method (1) proved satisfactory for quantitating the water content and rate of water desorption from 1.0-cm^2 samples of excised rabbit skin. The method could be applied to human skin; however, the excision of the skin sample, although virtually painless, is not readily accepted by most human subjects. In addition, an *in vitro* method can monitor the desorption of only a finite amount of water and may be, at best, unpredictably extrapolatable to the *in situ* conditions where the supply of water is essentially inexhaustible.

It is well known (2–8) that surfactants generally increase the permeability of the skin, which can easily be studied by measuring the rate of the transepidermal water loss. Accurate quantitative *in vivo* measurements from human forearms have been reported (9–13). The actual water loss under defined conditions has been determined for areas as small as 0.1 mm^2, although at least 1 mm^2 should be the minimum area considered "a representative sample for forearm skin generally" (12). These quantitative methods required considerable effort to minimize the instrumental baseline. The purpose of this study was to design a relative method that requires less stringent ambient and instrumental manipulation and, therefore, is more practical and rapid.

EXPERIMENTAL

An electrolytic moisture analyzer[1] provided the nucleus for the assembled apparatus. The "heart" of the instrument is a horizontal glass "cell" containing two platinum wires across which there is a potential difference of 75 v dc. The medium between these wires is phosphorus pentoxide, which is converted into phosphoric acid following the introduction of water into the cell by the carrier gas. Upon completion of the circuit, the water is electrolyzed and the phosphorus pentoxide is regenerated. There is a direct relationship between the amount of water in the gas and the current used. The instrument is so designed that there is a direct readout on the instrument meter in parts per million of water for a specific gas flow. The signal to the meter is connected to a recorder[2], so a continuous graph of moisture content *versus* time is obtained.

The carrier gas, ultra high purity nitrogen (containing less than 3 ppm of water), was passed *via* Teflon tubing (1 mm i.d., 4 mm o.d.) into a sampling "cup" sealed against the skin of the forearm by 400 g weight. The "dry" gas inlet was so positioned as to direct the stream of gas onto the skin surface, where it readily picked up the surface water; the "wet" gas exited from the cup *via* Teflon tubing into the instrument. The sampling cup was turned down from a 2-cm diameter brass rod, 3 cm in length. It was plastic coated to decrease porosity and heat transfer. The apparatus was in operation for several hours prior to each day's use to remove moisture that had permeated the apparatus, so that a constant baseline could be achieved.

As a test of the reproducibility of the apparatus, readings were taken on symmetrical sites on 20 subjects' left and right forearms. Prior to their participation in the study, the subjects' arms were visually inspected to ensure that no dry skin condition or other dermatological irregularities existed. The subjects were university

[1] MEECO, model W, type SPR, Manufacturers Engineering & Equipment Corp., Warrington, PA 18976
[2] Coleman 165.